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EURATOM

JAHRESBERICHT 1971

Programm Biologie - Gesundheitsschutz

RAPPORT ANNUEL 1971

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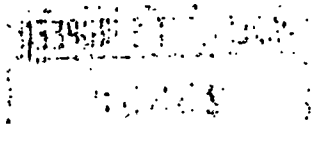

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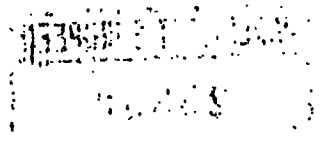

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The annual reports in this volume were prepared under the responsibility of the heads of the research teams, set up under the various contracts, and were submitted in this form to the Commission and its contractual partners.

INHALTSVERZEICHNIS

TABLE DES MATIERES

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INHOUDSOPGAVE

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* Der Jahresbericht 1971 war noch nicht verfügbar.
Le rapport annuel 1971 n'était pas encore disponible.
Il relazione annuale 1971 non era ancora disponibile.
Het jaarverslag 1971 was nog niet beschikbaar.
The annual report 1971 was not yet available.

KURZZEITWIRKUNGEN (AKUTES STRAHLENSYNDROM UND SEINE BEHANDLUNG)

EFFETS A COURT TERME (SYNDROME AIGU D'IRRADIATION ET DE SON TRAITEMENT)

EFFETTI A BREVE SCADENZA (SINDROME ACUTA DA IRRADIAZIONE E SUO TRATTAMENTO)

EFFECTEN OP KORTE TERMIJN (ACUUT STRALINGSSYNDROOM EN BEHANDELING)

SHORT-TERM EFFECTS (ACUTE IRRADIATION SYNDROME AND ITS TREATMENT)

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

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

Altri lavori di ricerca al riguardo vengono descritti anche nelle seguenti relazioni annuali:

Verdere publikaties over dit thema zijn ook in de volgende jaarverslagen opgenomen:

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

007-BIAB	ULB Bruxelles (Brachet)
078-BIAC	CEN Mol (Maisin)

CONSEQUENCES OF RADIATION EXPOSURE :

PREVENTION AND TREATMENT OF PATHOLOGICAL EFFECTS

Scientific Report 1971

of a

Collaborative Research Program

of the European Atomic Energy Community (EURATOM)
and the following Institutions :

- University of Ulm, Department of Clinical Physiology
- Claude-Bernard Association, Institute of Cancerology and Immunogenetics, Villejuif
- Nederlandse Centrale Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO), Radiobiological Institute TNO, Rijswijk (ZH)
- Istituto di Ricerche Farmacologiche "Mario Negri", Milano
- Institut Jules-Bordet, Bruxelles

(Contract 079-69-1 BIAC)

TABLE OF CONTENTS :

1. General Introduction

The collaborative research program, supported by the Association Contract 079-69-1 BIAC, between the European Atomic Energy Community and 5 European research institutes is concerned with

1. the evaluation of damage of ionizing radiation mainly to the hematopoietic tissue;
2. the design and perfection of treatment of bone marrow failure as seen after whole body irradiation; and
3. with selected studies on the physiology and pathophysiology of cell systems relevant to the understanding of radiobiological processes.

In 1971, considerable progress has been made in these various areas in all participating laboratories. The success of the research work has been strongly influenced by the fact, that the contributing laboratories are core members of the EORTC (European Organization for Research on Treatment of Cancer) and have initiated Clubs and Project Groups in which the ongoing research work is continuously monitored and developed by mutual exchange of information and by actual coordinated research. By means of the Association Contract it has also been possible to launch a new collaborative group : the European Late Effects Project Group. In this group, the primary attention will be on the evaluation of the pathogenesis of late effects after radiation exposure. This group, from 1972 onward, will be supported by a separate association contract but close ties will continue between the "early effects work" covered in this contract and the "late effects work" supported in the new group.

In this report, the scientific results of the work 1971 will be summarized in chapter 2, 3 and 4. Detailed reports about the work in the participating institutes will be given in chapter 5.1 to 5.5.

2. Evaluation of damage to the hematopoietic system

In 1971, the emphasis of the work to evaluate mechanisms of damage to the hematopoietic systems by ionizing radiation has been on the effects of incorporated radionuclids. The Ulm group (5.1) has investigated the consequences of thorotrast incorporation in man on lymphocyte chromosomes 20 - 30 years after thorotrast application. Of particular importance are the studies on the pathogenetic mechanisms of tritium toxicity (5.1). This problem has been studied in adult rats and in rats exposed to tritium during the in-utero development. Tritium was administered in the form of tritiated thymidine and of tritiated water. It has been of interest to note the dose-effect relationships in adult rats as well as in newborn rats. These studies will provide an experimental basis for the estimation of toxicity levels after tritium administration in various forms.

3. The design and perfection of treatment of bone marrow failure

The major work of the institutes of this association contract has been in the area of the treatment of bone marrow failure as seen after radiation exposure. The work in man has been concerned with the restoration of hemopoietic tissue after bone marrow transplantation and means and ways to prevent "secondary disease" and its various phases (see 5.3). The major objective of these clinical bone marrow transplantation studies in 39 patients (see 5.3) was to investigate the effectiveness of antilymphocytic serum as a means to condition the marrow recipient for a take. It could be demonstrated that ALS administration prevented the typical manifestation of severe secondary disease. Other clinical studies had been directed to develop methods to prevent bacterial infection in patients with lowered resistance during phases of hematopoietic failure by means of the induction of a "gnotobiotic state" and maintenance of it whithin an isolation system (see 5.1). These gnotobiotic studies are carried out within the EORTC Gnotobiotic Project Group in a randomized way in which about 10 European hospitals participate. This group receives catalytic support by this association contract.

However, this clinical work finds its experimental basis in studies in monkeys (5.4), in dogs (5.1) and in rodents (5.1; 5.2; 5.3; 5.4).

In monkeys (see 5.4), the studies were directed towards the improvement of methods for tissue typing as a basis for allogeneic marrow transplantation. Other investigations were concerned with the problem of the mitigation of secondary disease following bone marrow transfusion. It was concluded that the acute form of secondary disease is initiated by lymphocytes present in the grafted material, while the delayed secondary disease is initiated by lymphoid cells developing from precursor cells after grafting.

In dogs the major emphasis has been on the development of techniques to identify and separate hemopoietic stem-cells from the peripheral blood and to store them at ultralow temperatures in the process of the establishment of a model for a blood-stem-cell-bank that could be transscribed to the clinical level (see 5.1).

In rodents, the work performed may be classified in studies on the identification, cloning and storage of stem-cells from bone marrow (see 5.4), studies on the mechanism and control of secondary disease (see 5.3 and 5.4) and studies on the study and development of immune-suppressive agents (see 5.2). In these pharmacological studies, the emphasis was on the metabolism of agents depressing immune reactivity and on drug interactions in immunosuppressive treatment. Additional investigations were concerned with the development of methods to determine in plasma and tissue radiomimetic drugs and metabolites.

The results of experimental and clinical studies to develop methods for the treatment of bone marrow failure indicate that

a. Hemopoietic stem-cells can be derived from bone marrow as well as from blood, can in part, be separated from immunocompetent lymphocytes (thus reducing the severity of "secondary disease"), and can be stored by suitable cryobiological methods.

b. Infections as a consequence of hemopoietic failure can be controlled by bacterial decontamination and maintenance of the individual in an germfree environment.

c. Secondary disease can be mitigated by several means, such as by tissue typing between donor and recipient, by pre-treatment of recipient and possibly of the donor by antilymphocyte serum, and by the application of immunosuppressive drugs.

All three areas will be developed further in the years to come.

4. Physiology and pathophysiology of cell systems as relevant to the understanding of radiobiological processes

In the participating laboratories, research has been carried out that covers certain basic problems which are relevant to the investigation of the mechanisms of radiation damage and of the treatment of radiation induced bone marrow failure.

In one of the laboratories (see 5.5), the basic mechanisms of DNA repair of normal and leukemic cells were studied after UV-irradiation under the influence of corticosteroids. It was found that prednisone decreases the capability of UV-irradiated cells to repair their DNA. Other studies included the investigation of the mechanisms of the action of a radiomimetic drug (cyclophosphamide) on cells labeled with tritiated thymidine in their DNA. There was evidence, that cells may loose radioactive DNA breakdown products after the action of a radiomimetic drug.

Other laboratories are concerned intensively with the identification and functional characterization of hemopoietic stem-cells. Of great interest has been the question, whether there is a possibility to culture stem-cells in-vitro (see 5.4). Preliminary data indicate, that there are possibilities to increase the yield of stem-cells in in-vitro cultures. In the same laboratory, the techniques have been improved to separate stem-cells from other nucleated cells and there has been evidence for the morphological identification of stem-cells at the electronmicroscopical level contained in stem-cell rich cell suspensions (see 5.4). For the treatment of bone marrow failure as well as for the understanding of the mechanisms of radiation injury of the hematopoietic tissues, the characterization of the stem-cell pools and

their interrelationship remains of greatest interest. One of the laboratories (see 5.1) executes a program to study the interrelationship between uncommitted and committed stem-cell pools. The first data indicate a kinetic relationship between the erythropoietic and myelocytic committed stem-cell pools. In this context, it has been of interest to investigate the embryogenesis of the stem-cell pools, especially of the responsiveness to erythropoietin. This has been the subject of a program executed in a sub-contract with a research laboratory in Italy (see 5.1).

5.1. ASSOCIATION CONTRACT NO. 079-69-1 BIAC

EUROPEAN ATOMIC ENERGY COMMUNITY (EURATOM)

and

LAND BADEN-WÜRTTEMBERG FOR THE
SCHWERPUNKTGRUPPE HÄMATOLOGIE
OF THE UNIVERSITY OF ULM

REPORT ABOUT THE RESEARCH ACTIVITIES 1971

presented by

Professor Dr. Theodor M. Fliedner
Abteilung für Klinische Physiologie
Universität Ulm

1. GENERAL INTRODUCTION

The research activities of the Research Group in Ulm concentrated on the study of the mechanisms of radiation effects produced by incorporated radioactive nuclides, on studies related to the treatment of hematopoietic failure such as is seen after whole body radiation exposure, and on studies that are relevant to the understanding of the pathophysiological mechanism of radiation induced hematopoietic failure. These studies represent a continuation of research work initiated in previous EURATOM contracts (No. 031-64-1 BIAD, 072-68-1 BIOD, 079-69-1 BIAC) and contribute to the improvement of existing methods and the development of new approaches to prevent, recognize and treat radiation effects in man such as are seen after single, repeated or continuous exposure to ionizing radiation from internal or external sources.

2. STUDIES ON THE MECHANISMS OF RADIATION EFFECTS OF INCORPORATED RADIONUCLIDES

2.1 THOROTRAST STUDIES

(Contribution by J.P. Chaudhuri and H. Knörr-Gärtner)

During the last year, chromosome aberrations were studied in 40 persons who had received varying quantities of thorotrast between 1940 and 1949. In general, a high accumulation of all kinds of chromosomal abnormalities has been observed in blood lymphocyte cultures. In each cell preparation, in the order of 100 mitotic figures were analysed. The frequency of aberrant cells varied in this group of patients between 1.1 and 17.1 % in the 48 hour cultures. These aberrant cells are those harbouring chromosome type aberrations only and include chromosome breaks and fragments, as well as dicentrics and rings. If the percent values of aberrant cells and the chromosome breaks of the persons studied is listed in the diminishing order of their thorotrast burden, the data indicate a reasonable correlation between body burden and frequency of chromosomal aberrations. This correlation is better for the data obtained in 48 hour cultures than for those in the 72 hour cultures. Cytogenetic screening has been performed in some of these cases already in 1965 - 66, using a culture time longer than 60 hours. A comparison of the aberration frequencies then and now indicates a general tendency of progressive accumulation of aberrations. None of the cases developed a recognizable cytogenetic clone of lymphocytes. Only one case showed a small deletion in each of the cells studied, indicating an inborn cytogenetic abnormality. These studies are extremely time consuming, but their value lies in the fact that this is one population of human beings in which an example of radioactive body burden can be followed over many years in its somatic consequences as expressed in chromosomal aberrations.

2.2 STUDIES ON THE RADIOTOXIC EFFECTS OF TRITIATED THYMIDINE ON ADULT RATS

(Contribution by W. Calvo, J. Forteza-Vila, R.J. Haas and E.B. Harriss)

The morphological changes produced in the bone marrow by a continuous intravenous infusion of ^{3}H-thymidine were studied in 12 four-month-old female Wistar rats. The dose injected was 864 mCi per day, per animal. The specific activity was 6 mCi/mMol. The animals were sacrified in pairs 3,6,9,12,15 and 18 days after beginning the administration of thymidine and studied with cytological, histological and electron-microscopical techniques. The marrow of two animals, sacrified at the 9th and the 18th day was aplastic. The aplasia is believed to be due to the incorporation of ^{3}H-thymidine at a lethal level in the nuclei of rapidly proliferating cells during the synthesis of DNA. These cells disappeared, while the elements that did not duplicate their DNA during the infusion of the radioactive isotope remained in the marrow.

The cells found in the aplastic bone marrow were classified, according their ultrastructural characteristics, in the following five groups :

a) Small lymphocytes with scanty cytoplasm, few small mitochondria and ribosomes.

b) Undifferentiated cells of medium size, with little ergastoplasmic reticulum.

c) Large undifferentiated cells with abundant organelles.

d) Differentiated cells, comprising mature granulocytes and plasma cells.

e) Stroma cells, namely fibrocytes, fat cells, endothelial cells, macrophages, cells of Schwann and reticulum cells.

In all the other animals (ten) the marrow showed different degrees of hypoplasia.

Corresponding to the bone marrow changes, the blood cell alterations were studied. It was of interest to note the pattern of the development of pancytopenia in these animals which differed in certain respects from that of pregnant female rats infused for the same time with equal amounts of tritiated thymidine. The results of these studies indicate the value and the need to investigate the radiation effects of tritium, incorporated into different molecules of biological importance. It is planned to continue these studies of the tritium effects in adult rats and to initiate studies in mice in order to explore further the mechanisms of action at the stem cell level using modern stem cell assay techniques. In order to establish the relative biological effectiveness of tritium in its various metabolic forms, studies using the same endpoints need to be performed with continuous external radiation.

2.3 STUDIES ON THE RADIOTOXIC EFFECTS OF TRITIATED THYMIDINE IN DEVELOPING RATS

2.3.1 MORPHOLOGICAL STUDIES

(Contribution by R.J. Haas, W. Schreml and W. Calvo)

The production of tritium by neutron bombardment of various elements in reactors and the release of tritium to the reactor effluent in the form of tritiated water raises the question of biologic effects of tritium on the mammalian organism. Since the most vulnerable phases of mammalian life are the embryo and fetus an investigation with particular reference to the effect of a continuous application of ^{3}H-thymidine (^{3}H-TdR) or tritiated water (HTO) has been carried out. We used wide range of dose levels continuously administered to pregnant Wistar-rats with an average body weight of 180 g. From the 9th day of pregnancy (the time when organogenesis commences) until parturition the following doses of ^{3}H-thymidine or tritiated water respectively were given by a continuous i.v. infusion via a polyethylene catheter using a Harvard pump delivering 1 ml/day.

^{3}H-thymidine dose/day		Specific activities	
72	µCi	0,5	Ci/mM
144	µCi	1	Ci/mM
288	µCi	2	Ci/mM
576	µCi	4	Ci/mM
864	µCi	6	Ci/mM
1152	µCi	8	Ci/mM
1440	µCi	10	Ci/mM

Tritiated water doses administered :
288 µCi, 1440 µCi, 2880 µCi, 5760 µCi.

The following parameters were studied in the developing animals for a total of 3 weeks after birth at 2 day intervals :

Litter size, survival data, malformations, general development and development of thymus, spleen and liver, bone marrow structure and composition, number of granulocytes, lymphocytes, platelets, reticulocytes, erythrocytes and packed cell volume, chromosome aberrations and finally, as a most sensitive parameter, determination of remaining oocytes in histological section.

In the ^{3}H-thymidine experiments dose levels up to 288 µCi of ^{3}H-TdR produced only chromosome aberrations in liver cells of the newborn animals and a severe effect on oocyte number. The reduction of total oocyte number was about 30% after 72 µCi, 70% after 144 µCi and more than 99% after 288 µCi (for details see Int. J.Rad.Biol. 19, 197, 1971). In contrast, significant alterations of the other parameters could only be noticed when doses higher than 288 µCi ^{3}H-TdR were applied. Here a dose dependent bone marrow destruction was observed up to the dose of 864 µCi which was followed by regeneration. The litter size was reduced as compared to controls proportional to the dose given. The general development and organ

development were also severely affected in a linear mode depending on the dose delivered. 1152 µCi and 1440 µCi allowed no survival of animals beyond 12 days after the commencement of the ^{3}H-TdR infusion. Severe malformations could be noted in the offspring.

When HTO was given similar effects on biological parameters as described above could only be observed when the dose administered was 10 times higher than the dose of ^{3}H-TdR. Only the dose of 5760 µCi produced effects on bone marrow composition, hematological data, litter size, general development and organ development. These effects are very similar to that seen after 576 µCi ^{3}H-TdR.

The reduction of oocyte number was about 2% after 288 µCi, 70% after 1440 µCi and 100% after 2880 µCi of tritiated water, indicating again that decays originating from ^{3}H-TdR appear to be 10 times as effective in reducing the oocyte number as those from HTO. The effects observed after administration of HTO are in agreement with those observed by CAHILL and YUILE (9th Hanford Symposium, Richland/Washington 1969) who calculated the radiation doses in rads received by the developing rats to be about 3 rads/day, when 1450 µCi HTO were given to rats during pregnancy and about 6 rads/day when 2900 µCi were given.

2.3.2 BIOCHEMICAL STUDIES

(Contribution by W. Schreml, M. Spoljar, R.J. Haas and E.B. Harriss)

For evaluation of the biological parameters, data were obtained by biochemical methods on the incorporation pattern, distribution and biochemical characteristics of the incorporated activity. From these data, attempts are made for a calculation of dose delivered to the organism and to the target organs.

The total activity incorporated into newborn animals at the day of birth showed a direct relationship to the activity administered to the mothers (see following table) :

Total dose administered to mothers	Isotope	Activity incorporated into babies (µCi/g body weight) exp. series 1	series 2
2 mCi	^{3}H-TdR		3,1
4 mCi	^{3}H-TdR	7,4	6,3
8 mCi	^{3}H-TdR	12,9	
12 mCi	^{3}H-TdR	22,6	
16 mCi	^{3}H-TdR	32,7	
20 mCi	HTO		34,9
40 mCi	HTO		81,1

The direct relationship between administered and incorporated activity was found to be consistent in different experimental series. Also, very similar incorporation efficiencies were observed for ^{3}H-TdR and HTO. Therefore, the observed difference in biological efficiency for ^{3}H-TdR and HTO cannot be explained on the basis of differences in the net incorporation of the different compounds.

The distribution of the activity in different organs and in extracted fractions showed, that ^{3}H-TdR appears almost exclusively in the acid soluble pool of low molecular weight compounds and in DNA. The distribution between these fractions is organ-specific, ranging from 70% in DNA for the spleen to 25% in DNA for the brain. In contrast, tritiated water is mostly contained in the soluble fraction (96%), while the remaining 4% appear in the protein fraction. Only minimal HTO-activity is associated with RNA and DNA. The protein-bound activity has a low disappearance rate. During the first 6 days after birth, i.e. after cessation of HTO-administration, the total activity falls to 20% owing to loss from the soluble pool, while the activity in the protein fraction remains nearly constant.

These biochemical data indicate that a great number of factors influence the final position of the tritium isotope from which it exerts its radiobiological effect. Dose-calculations can therefore only be tentative. For HTO, the assumption of uniform distribution throughout the tissue may be made. In the experiments showing a 50% reduction of oocyte number at a total dose of 20 mCi delivered to the mother as tritiated water, the specific activity in the ovaries of babies was 48 µCi/g wet weight, corresponding to a dose of 15 rad/day delivered to the ovarian tissue. Since uniform distribution is assumed, this dose is also delivered to the nuclei. For ^{3}H-TdR, the distribution is not uniform. The proportion of 25% extracted in the DNA fraction from ovaries is restricted to the nuclei. From the specific activity of ovarian DNA (0,196 µCi/mg DNA) extracted from the group which showed 50% reduction of oocytes (2 mCi to mothers) the dose delivered to the nuclei is calculated by the formula of APELGOT and DUQUESNE. The calculated dose, 1,9 rad/day, is the dose delivered to the nuclei by the activity incorporated into DNA. Assuming a homogeneous distribution for the remaining 75% of activity, i.e. 2,4 µCi/g wet weight, the dose delivered is 0,75 rads/day. Adding these two fractions, a dose of 2,65 rads/day to the oocyte nuclei is calculated, which produces an effect similar to a dose of 15 rads delivered by HTO. Further studies will be aimed to clarify the mechanism of this difference in relative efficiency.

STUDIES ON THE TREATMENT OF HEMATOPOIETIC FAILURE AS SEEN AFTER WHOLE BODY IONIZING RADIATION

3.1 BACTERIAL DECONTAMINATION IN HUMAN BEINGS SUFFERING FROM VARIOUS FORMS OF HEMATOPOIETIC FAILURE

(Contribution by M. Dietrich, D. Krieger)

One of the important aspects of hematopoietic failure in the acute radiation syndrome is granulocytopenia and bacterial infection. It has been the purpose of the clinical gnotobiotic studies to examine the possibilities of bacterial decontamination of patients and the maintenance in a sterile environment and its value in the treatment of granulocytic failure.

Thirteen patients have been treated under gnotobiotic conditions during 1971. Those patients suffering from acute leukemia have been observed during a period of 388 days. Since March 1971 all acute leukemia patients have been randomized accordingly to the evaluative study designed by the Gnotobiotic Project Group of the EORTC. Thus, five patients were treated in isolation, eight patients in isolation under decontamination procedures to eliminate or suppress the endogenous microflora and six patients were followed during their treatment on the normal ward. Final conclusions cannot be drawn from this study at the present time concerning the beneficial effect of the measures undertaken to prevent infection and lethality.

Based on the clinical observation that the patients in a decontaminated state showed less pronounced bleeding tendency than the others, detailed investigations of the coagulation system have been initiated, In cooperation with Dr. Rasche (Service of hemostaseology) laboratory findings supported the clinical observations. Thrombelastographic studies showed marked differences between these patient groups. Psychological studies in these patients who have been informed about the diagnosis and prognosis of their disease revealed that any severe psychopathological complications could not be seen in the isolated patients. Depressive reactions could be correlated with complications of the underlying disease, e.g. stomatitis or febrile episodes. These studies have been done on cooperation with Dr. Köhle and Dipl.-Psych. Simons.

So far, the clinical studies on the bacterial decontamination in the treatment of hemopoietic failure must be continued to obtain significant results which may answer the basic question whether gnotobiotic techniques are superior to the ordinary therapy or not. Within the above named evaluative study of the EORTC Gnotobiotic Project Group hard data are to be expected in 1974.

3.2 STUDIES ON THE PHYSIOLOGY AND PATHOPHYSIOLOGY OF GERMFREE AND DECONTAMINATED MICE

(Contribution by H. Heit)

Studies at the Radiobiological Institute in Rijswijk have clearly established the possibility of decontaminating various mammalian species by means of antibiotic treatment and maintenance in a germfree environment. In the Ulm group, these principles have been conformed. Mice with a common bacterial flora were treated with antibiotics to render them bacteriafree. The treatment with non-resorbable antibiotics such as Bacitracin, Neomycin and Anabactyl in a concentration of 4g/l drinking water makes it possible to sterilize at least the intestinal tract within 3 days. A strict isolation of the antibiotic-treated animals in specially designed isolation cages or in a laminar flow bench maintained the induced sterility. In 1971 studies were performed in 100 CBA mice and in all cases, the antibiotic treatment resulted in a "germfree" state. This method of bacterial decontamination is being employed to investigate the importance of the microbial flora for the physiological state of different organ functions.

Of particular importance are studies on the effect of decontamination on secondary disease after bone marrow transplantation in whole body irradiated mice. Preliminary results confirm in decontaminated mice the importance of the microbial flora for the course of graft-versus-host disease first established in axenic mice. In addition, studies are in progress to elucidate the observations made earlier, that in germfree mice given whole body X-irradiation the recovery of the myelopoiesis is markedly retarded as compared to erythropoiesis and megakaryocytopoiesis. The preliminary results (employing in-vivo and in-vitro stem cell assays) indicate that the reason for this observation may be sought in functional responses of the stem cell pools. Future studies in 1972 will be directed to establish further the possibilities of improving the survival conditions of whole body irradiated mammals by bacterial decontamination, with and without stem cell transplantations of isologous and allogeneic types and to investigate physiological and pathophysiological aspects of cell renewal systems in germfree and decontaminated animals.

3.3 INFLUENCE OF ANTIBIOTIC DECONTAMINATION ON THROMBOCYTOPENIC BLEEDING IN IRRADIATED RATS

(Contribution by R. Hohage and H. Meyer)

It has been the purpose of these studies to investigate the significance of the presence or absence of microbial flora on the intensity of bleeding caused by thrombocytopenia in lethally irradiated rats. This problem appeared of interest since clinical evidence had indicated that thrombocytopenic patients may tolerate a low platelet count without signs of bleeding as long as there is no manifestation of microbial infection. In germfree mice, the incidence of hemorrhage was found to be markedly reduced as compared to conventional controls when given doses of irradiation lethal to the conventionally kept animals. In this study, the extent of hemorrhage was studied after whole body X-irradiation with 700 R (250 kvp., 15 mA) in rats whose gut was rendered bacteria-free by oral antibiotics (given in drinking water). The gnotobiotic state was maintained in a sterile environment provided by a laminar flow bench. Non-decontaminated rats served as controls. In order to quantitate the extent of hemorrhage in the experimental and control group, the thoracic duct was canulated and the red cell content of the lymph measured. In the normal control animals, the concentration of erythrocytes in the thoracic duct lymph is below $2000/mm^3$. In addition, the hemoglobin content of mesenteric lymph nodes was determined. In unirradiated controls it is below 0.1 percent of the lymphnode tissue. In the irradiated conventional rats, the erythrocyte concentration increased slightly on the 7th day, reached a peak on the 9th day ($200,000/mm^3$) and decreased thereafter. There was a concomitant rise and fall in the hemoglobin content of the mesenteric lymphnodes, reaching a peak at 9 days (3.0% of lymph node tissue). In the decontaminated rats, there was a markedly lower red cell concentration in the lymph and hemoglobin concentration in the lymph nodes. The values at the 9th and 12th day after irradiation were reduced to 40 - 60 % of those observed in the controls. This reduction in the bleeding tendency in gnotobiotic rats cannot be explained on the basis of the platelet level and its time course after irradiation. This pattern

was similar in both irradiated animals groups. Thus, it is suggestive to assume that the bacterial flora is at least partially responsible for enhancement of thrombocytopenic bleeding. This may be due to the manifestation of infection by bacteremia or by absorption of bacterial toxins or breakdown.

.4 STUDIES ON THE ISOLATION AND TRANSFUSION OF HEMATOPOIETIC STEM CELLS FROM THE PERIPHERAL BLOOD OF DOGS

In 1971 a number of studies have been performed in dogs with the final goal of establishing a model for the setting-up and use of a stem cell bank. It is obvious that the practical use of transplantation of stem cells derived from bone marrow will always be limited in man, owing to the technical and ethical difficulties of obtaining unlimited numbers of stem cells of one type. The Ulm research group has therefore launched a systematic investigation in dogs to try to overcome these problems by isolating stem cells for hematopoietic restoration from the circulating blood and by improving the methods of storing these cells at ultra-low temperatures before they are transfused into lethally irradiated recipients. These studies require also a large scale attempt to establish histocompatibility patterns in dogs so that the blood stem cell transfusions can be performed in relation to the degree of histocompatibiligy between donor and recipient. Thus, in 1971 the emphasis has been on the establishment of blood leukocyte isolation procedures, tested in autologous situations, on the establishment of methods of transfusing large quantities of frozen and thawed leukocytes and of investigating histocompatibility pattern in dogs.

3.4.1 LETHAL DOG IRRADIATION AND TRANSFUSION OF FRESH, AUTOLOGOUS LEUKOCYTES

(Contribution by E. Herbst, C. Bruch, P. Kovács, H.P. Schnappauf, W. Calvo)

In order to test hematopoietic functions of blood leukocytes in principle, an in-vivo system has to be established. In this group, the lethally irradiated dog is used and the degree of bone marrow regeneration is estimated at day 10 after a total body dose of 1200 R using 250 kvp X-rays. The control dogs without leukocyte transfusions are kept alive during the first 9 - 10 days after this dose of irradiation by extensive electrolyte and antibiotic therapy as well as starvation during the first 5 - 7 days. At 9 - 10 days, these control dogs showed complete marrow aplasia in all bone marrow sites throughout the body. This pattern has been established so far in 8 control dogs.

In a second series of experiments, the effect of autologous leukocyte transfusions on bone marrow regeneration has been studied in 5 dogs. 20 x 10^9 leukocytes were removed from the peripheral blood by means of the IBM continuous cell

separation centrifuge. These cells were stored at 4°C for a few hours until the dog had received 1200 R whole body X-irradiation and immediately afterwards autotransfused. It was clearly established in all 5 dogs, that 7 x 10^9 mononuclear leukocytes contain a sufficient number of hematopoietic stem cells to bring the bone marrow within 10 days to a hemopoietic cellularity of about 50% of normal.

.4.2 LETHAL DOG IRRADIATION AND TRANSFUSION OF FROZEN AUTOLOGOUS LEUKOCYTES

(Contribution by C. Bruch, E. Herbst, P. Kovács, H.P. Schnappauf, W. Calvo)

The next step was to separate leukocytes from the peripheral blood as before, but to freeze them at ultralow temperatures (liquid nitrogen) and to transfuse them after thawing.

Sufficiently high numbers of stem cells, isolated by the IBM cell separator, for the transplantation of lethally irradiated dogs in the homologous system can only be collected by multiple (~5) centrifugations. This means freezing and storage of cells from each centrifugation.

Although stem cells of different species have been frozen in 10% Dimethylsulphoxide (DMSO) and stored at -196°C for years, the application of these routine methods for the above mentioned purpose proved difficult for the following reasons :

1. The large volume of packed cells from one centrifugation (30 - 90 ml total, 10 - 15 ml white cells) led to volume problems.
2. The amount of DMSO to freeze these cells led to toxic problems.
3. The high number of erythrocytes which were destroyed by the freezing and thawing procedure led to high amounts of free hemoglobin and renal failure.

We therefore established the following method :

100 ml of a cold 20% DMSO / Hanks solution was transferred in a 600 ml Fenwal plastic bag containing the isolated peripheral blood cells suspended in 100 ml of cold ACD-plasma. The bag was clamped between two thin copper plates cooled down 1°C/min to -40°C, 7°C/min from -40 to -130°C and stored in the gas phase of liquid nitrogen. Thawing was performed within 1 1/2 min by shaking the bag in a water bath at 50°C. 400 ml of Hanks - ACD plasma solution was added in order to dilute the DMSO to non-toxic levels. The free hemoglobin (erythrocytes destroyed by the dilution procedure) and the DMSO were removed by two washings and centrifugations. The heavy clumping of cells after the last centrifugation, caused by destroyed granulocytes (~60% of the total white cells) could be eliminated by the use of desoxyribonuclease (15,000 Donase units/ml).

Cells of one centrifugation obtained by the procedure outlined above could be resuspended in a volume of 15 - 20 ml (loss of mononuclear cells 10 - 20%).

In 5 experiments, cells handled in this way were transfused intravenuously into lethally irradiated dogs in the autologous system and were able to repopulate the bone marrow similarly to unfrozen fresh cells within 10 days.

3.4.3 SEPARATION OF SUFFICIENT NUMBERS OF BLOOD LEUKOCYTES FOR ALLOGENEIC STEM CELL TRANSFUSIONS

(Contribution by E. Herbst, P. Kovács, H.D. Flad, H.P. Schnappauf, C. Bruch, W. Calvo)

From bone marrow transfusions in dogs it is known that about 10 times as many cells are necessary to achieve a bone marrow repopulation in the allogeneic situation as compared to the autologous situation. Thus, if one wants to achieve bone marrow repopulation by blood stem cells, one has to obtain much larger numbers for the allogeneic grafting than for the autologous situation - not withstanding the unsolved immunological problems. Thus, studies are under way to establish the methodology of repeated blood cell separation in dogs. The first attempts along this line have shown that it is possible to perform from one shunt at least 5 cell separations, obtaining in each session (about 4 hours each) 20 x 10^9 cells. These studies will be continued, expecially to answer the question in what way the number of repopulating cells may vary from session to session. The agar colony-forming unit assay as well as in-vivo marrow repopulation will be used to test this point.

3.4.4 PREPARATIONS FOR ALLOGENEIC BLOOD STEM CELL TRANSFUSIONS IN DOGS

(Contribution by K. v. Loringhoven, H.D. Flad, H.P. Schnappauf)

Meaningful allotransplantation of hematopoietic stem cells in the dog requires typing of donor recipient pairs for Dog Lymphocyte Antigens as a first step towards reproducible conditions for modifying host-versus-graft and graft-versus-host reactions. 90 beagles of 11 families from a closely bred colony were typed using 21 sera. 4 sera originally provided by the Thomas-Storb group (Seattle, Washington) proved to be rather polyspecific; conditions were shown to be considerably more complicated than predicted by this group. Very recently, sera for DLA typing have been exchanged with Dr. Vriesendorp, University of Rotterdam. To some extent our family studies fit a so far unpublished system for DLA, developed by Dr. Vriesendorp.

4. STUDIES ON THE PHYSIOLOGY AND RADIATION - PATHOPHYSIOLOGY OF THE STEM CELL POOLS

The tritium toxicity studies as well as the studies on the improvement of the treatment of bone marrow failure as seen after whole body irradiation indicate clearly the importance of the stem cell pools for the understanding of the hematological consequences of radiation exposure and their treatment. This understanding requires the advancement of the general knowledge in this field. Thus, the Ulm Research Group tries to contribute to the investigations of the physiology and pathophysiology of the hematopoietic stem cell pools.

4.1 STUDIES ON THE INTERRELATIONSHIP BETWEEN UNCOMMITTED AND COMMITTED STEM CELL POOLS

(Contribution by B. Kubanek, O. Bock, W. Heit, E.B. Harriss)

The changes in size of the pluripotent stem cell (CFU), the granulocytic committed stem cell (ACU) and the erythroid committed stem cell (ERC) compartments under the influence of plethora have been investigated. CFU were measured by the spleen colony forming assay, ACU by the agar colony-forming assay and ERC by the 59-Fe incorporation after a standard dose of 6 units of erythropoietin.

Mice were made polycythaemic during 3 weeks exposure to intermittend hypoxia in a high altitude chamber (23,000 feet). After removal from the chamber, plethora was maintained by twice weekly intraperitoneal injections of washed red cells over a period of 4 weeks. Assays were performed at weekly intervals on spleen and femoral bone marrow in groups of 8 mice each.

The data obtained showed that the absolute numbers and concentration of CFU's in femoral bone marrow did not change during the 4 weeks of plethora, whereas in the spleen both content and concentration of CFU's began to increase after 2 weeks and were significantly increased after 4 weeks. The erythropoietic response to 6 units of erythropoietin was significantly decreased by the end of the second week and then increased again, but at 4 weeks was still significantly lower than the value 6 days after removal from the high altitude chamber. In contrast, the ACU's in spleen and femur increased and remained elevated throughout the period of study.

The data indicate that the decreased demand for erythropoiesis in the plethoric animal leads to changes in the relative sizes of the uncommitted and committed stem cell compartments. The decrease in the response to erythropoietin and increase in ACU's could perhaps be due to some extent to a transformation of ERC's into the ACU compartment or it could be that the decreased demand for ERC's allows more CFU's to be available for the granulocytic committed ACU compartment. The latter explanation is compatible with the interpretation that, owing to a prolongation of the G_1 phase of the cell cycle, a reduction of sensitivity of the ERC's to erythropoietin occurs, according to Kretchmar's hypothesis.

Further investigations are necessary in order to decide which of the possible explanations is applicable.

4.2 STUDIES ON THE PHYSIOLOGY OF ERYTHROPOIETIC STEM CELLS IN THE NEONATAL DEVELOPMENT OF RATS

(Contribution by G. Lucarelli -
subcontract with the Hematology Group in Pesaro -)

The hemopoietic conditions obtained by means of starvation in the newborn 10-day-old rat (S-10), can be considered as a good experimental tool to evaluate the effect of erythropoietin at the stem cell level (differentiation) and at the erythroid cell level (metabolism or Hb-synthesis). The reasons for this are the following :

1. Starvation in the newborn rat does not result either in depletion of erythroid cells in the bone marrow, nor in the disappearance of reticulocytes from the peripheral blood, as is usually seen in the adult rat.
2. 59-Fe incorporation into red blood cells and the in-vitro 59-Fe uptake by reticulocytes are reduced in the S-10.

It appears that starvation in the newborn rat is followed by a state of "suspended animation" of the erythroid cells, while a certain degree of stem cell differentiation toward erythroid cells is maintained. That erythropoietin could be responsible for this impairment of erythropoiesis seems to be indicated by the fact that administration of erythropoietin (2 units daily) to the newborn rat throughout the time of starvation, brings the red blood cell-producing machinery back to work. Repeated experiments have failed to detect erythropoietin in the plasma of S-10, while the amount of circulating erythropoietin has been found to be of the order of 0.03 units/ml in the normal 10-day-old rat. Radioiron distribution into hemopoietic organs after tail vein administration of 59-Fe have been studied in the S-10. The 59-Fe plasma clearance is lower in S-10 than in normal. The radioiron enters the bone marrow (femur) at the same rate as normal, but the curve of disappearance of 59-Fe is decreased in the S-10, in accordance with depletion of erythroid cells in this organ during starvation. The reason for the different behaviour of erythropoiesis of the bone marrow and the spleen in the S-10 is unknown and deserves further study.

5. STUDIES TO BE EXECUTED IN 1972 AND LATER

The Ulm Research Group would like to continue its work from 1972 onward with emphasis on the following projects, which have been submitted to the commission in December 1971 :

1. 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 radiations of other qualities.

2. Investigations on the pathogenesis of ^{3}H-thymidine toxicity in mammals following single, repeated or continuous doses and the mechanism of regeneration.

3. Comparative investigations on the damage and repair of hemopoietic cells by ^{3}H-thymidine or radiomimetic substances, with particular reference to the uncommitted and committed stem cells.

4. Investigations on the possibilities of isolating hemopoietic stem cells from the peripheral blood in dogs.

5. Investigations on the possibility of allogeneic blood stem cell transplantation as a model for the therapy of the acute radiation syndrome.

6. Investigations on the possibility of bacterial decontamination in experimental animals and human beings in the treatment of consequences of granulocytopenia.

7. Investigations on the functional relationship between the hemopoietic stem cell pools as a basis for the understanding of hemopoietic regeneration following radiation damage.

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5.3. RAPPORT D'ACTIVITE

Contrat n° : 079-69-1 BIAC

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G. MATHE

RECHERCHE SUR LE TRAITEMENT DES SUJETS IRRADIES : TRAITEMENT SYMPTOMATIQUE ET GREFFE DE MOELLE OSSEUSE.

Experimental investigations and clinical trials with investigations have shown : a) that if secondary disease (SD) which complicates bone marrow transplantation (BMT) when the recipient is conditioned by total body irradiation (TBI) or cyclophosphamide (CPM) is induced by graft-versus-host reaction (GVHR), its prognosis depends on the conditioning, TBI or CPM, and higher the doses, the more severe is the SD. In antilymphocyte serum (ALS) conditioning animals GVH does not induce SD.

SD can be prevented by administration to the donors or the recipient (recent experiments) of inhibitors extracted from the lymphoid tissue and possessing the characteristics of chalones. Such inhibitors have been prepared from the thymus as well as from the spleen.

Our clinical trials have been confined to the conditioning by antilymphocytic serum : 51 trials have been conducted in 39 patients : a) 24 patients suffering from a chronic idiopathic or chemical induced marrow aplasia ; b) 14 patients were leukemic and suffering from subacuté aplasia, due either to the leukemic process by itself, or to chemotherapy or to both factors ; c) one patient was suffering from thalassemia.

The overall results were as follows : a blood and bone marrow (BM) restoration was observed following BM trans-

fusions in 12 patients out of 39 patients. Out of 12 patients who were restored haematologically,the engraftment was proven in ten.

The most important observation is undoubtely the absence of any typical manifestations of severe secondary disease, well known in human radiochimeras and cyclophosphamide chimeras.

The following finding offers the basis for the most fruitful explanation : in one patient, it was demonstrate that while immunoglobulins and red cells were of the donor type, blood lymphocytes transformed by PHA in vitro had a chromosome constitution indicating they were of host origin. In other words, B-lymphocytes were produced by the graft, while T-lymphocytes were produced by the recipient.

Projet n°1

1) NEW DATA ON MECHANISM AND CONTROL OF SECONDARY DISEASE

1,1) PHYSIOPATHOLOGY

G. Mathé, M. Gutierrez-Romero, J.L. Amiel, L. Schwarzenberg and M. Schneider

Although it has been shown that the secondary disease (SD) after allogeneic bone marrow grafts (BMG) can be induced by the reaction of the graft-versus-host (GVH) (Van Bekkum and Vos, 1957), then it is important to realize that SD and GVH are not synonymous.

In experiments conducted some years ago (Mathé and Amiel, 1960), we demonstrated that SD occurs in two phases : the first phase, more or less of short duration, is dominated by lymphoid hyperplasia, bound to the proliferation and transformation of lymphocytes of the donor and accompanied by a proliferation of the recipient's macrophages. The second phase is characterized by lymphoid aplasia, causing loss of the immune reactions and of immune memory (Mathé et al., 1961). It is this second phase with its immune insufficiency that mainly influences the prognosis in our clinical experience of allogeneic BMG in patients conditioned by total body irradiation (TBI), as all the deaths from SD were directly related to infection (Mathé et al., 1971). This also seems to be much the same in patients conditioned with cyclophosphamide (CPM) (Santos et al., 1970 ; Graw et al., in press). As TBI and CPM produce a marked aplasia of the lymphoid tissue, we have questioned whether the methods of conditioning that produce aplasia might play a major role in the immune insufficiency that is the essential feature of late SD.

To try to answer this question, we have induced a GVH reaction by transfusing bone marrow and lymphoid cells under conditions that do not require the recipient to be pre-treated for the graft to take. These conditions are present when parental cells are transfused into F1

hybrids. We have studied the effect of an additional conditioning prior to grafting these F1 hybrids, on the immune insufficiency. The hosts were pretreated with TBI or CPM, or given antilymphocyte serum (ALS), each in various doses (Gutierrez-Romero and Mathé, in press). The injection of parental cells into untreated F1 hybrids did not produce any detectable immune insufficiency, as measured by counting the number of hemolytic plaque forming cells (HPFC) in the spleen after immunisation with sheep erythrocytes. On the other hand, a similar injection of parental cells increased the immune insufficiency induced by TBI or CPM and kindered the restoration of immunity when these agents had been given in high doses. The parental cell transfusion increased the transitory immune insufficiency in animals treated with ALS, and did not impair the restoration of immunity (Figs. 1-3).

It is this major difference in the effect of these various conditioning methods that justified trying allogeneic BMG in human beings conditioned with ALS alone. The clinical result is in agreement with the experimental findings. Grafts have been established in 20 % of these patients, they are of a transitory nature and have never been complicated by SD.

The reason for the transitory chracter of the graft and the absence of SD seems to be related to the incomplete or transitory nature of the aplasia of the thymic dependent lymphocytes. Fig. 4 shows the course of a patient whose chimeric state was proved by his production of red cells and immunoglobulins of donor origin, whilst lymphocytes transformed by phytohemagglutinin (PHA) were of recipient origin. These clinical findings have recently been confirmed by Speck and Kissling (1971) in their study of BMG in rabbits rendered aplasic by benzene treatment.

Unfortunately this absence of SD in leukemic patients goes in hand with the lack of an antileukemic effect derived from the GVH reaction (Fig. 5), that we have described previously in mice and observed in leukemic patients grafted after TBI (Mathé et al., 1967).

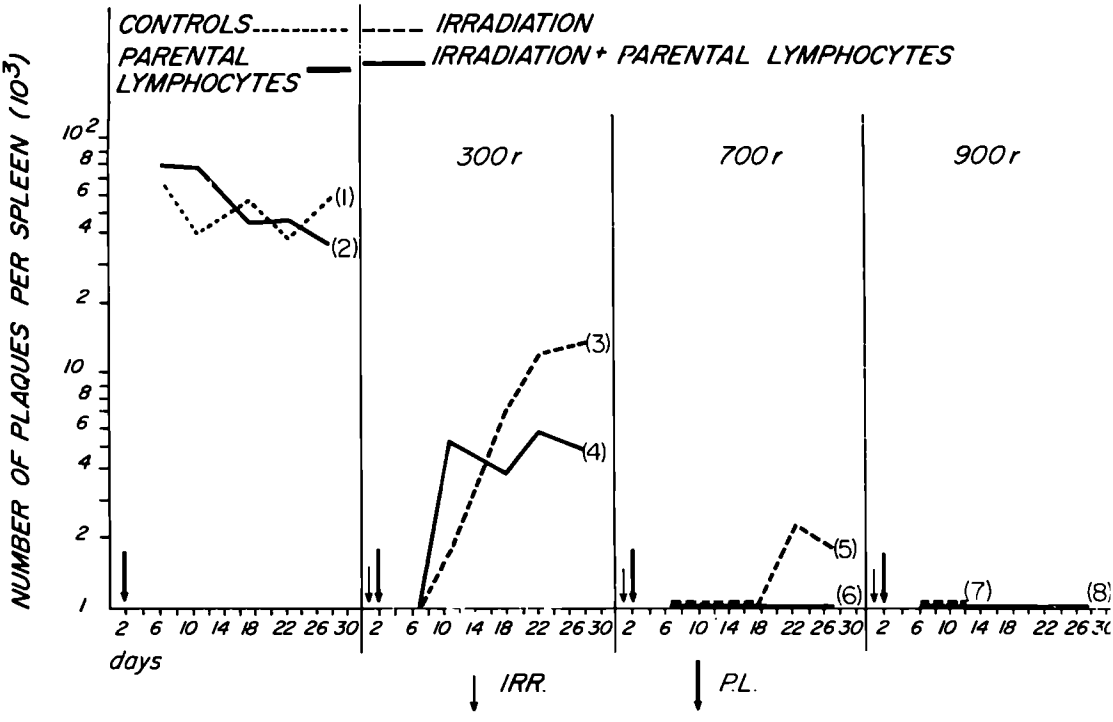


Fig. 1 Changes in the number of haemolytic plaques in control animals (1) ; animals given a transfusion of parental marrow and lymph node cells (2) ; animals given 300 rads 5BI (3) ; animals given 300 rads TBI + a transfusion of parental marrow and lymph node cells (4) ; animals given 700 rads TBI (5) ; animals given 700 rads TBI + parental marrow and lymph node cells (6) ; animals given 900 rads TBI (7) ; animals given 900 rads TBI + parental marrow and lymph node cells (8). The curves represent the arithmetic means.
Statistical study. The difference between (1) and (3) at day 25 after the grafts is significant ($t = 2.38$, $p < 0.05$) as the difference between (1) and (5) ($t = 2.76$, $p < 0.05$). The difference between (2) and (4) at day 25 after the graft is not significant ($t = 2.01$), but the difference between (2) and (6) and (2) and (3) is significant ($t = 2.42$, $p < 0.05$) as the difference between (4) and (6) ($t = 2.61$, $p < 0.05$). The difference between (3) and (4) is significant ($t = 6.66$, $p < 0.01$).

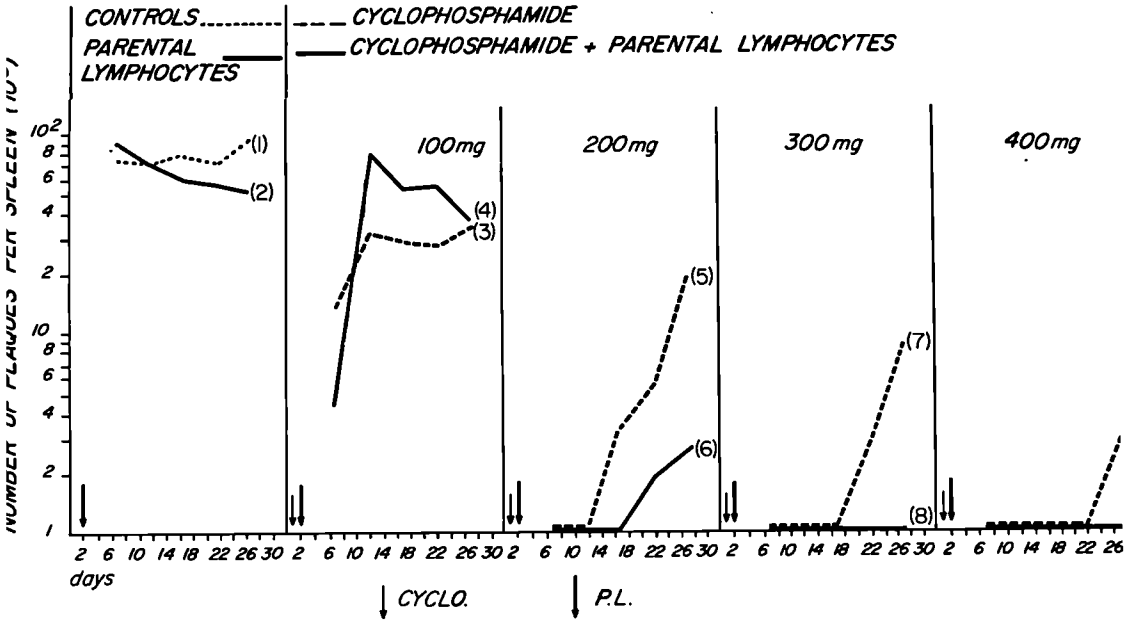


Fig. 2 Change in the number of haemolytic plaques in control animals (1) ; animals given a transfusion of parental marrow and lymph node cells (2) ; animals given 100mg/kg cyclophosphamide (3) ; animals given 100mg/kg cyclophosphamide + parental marrow and lymph node cells (4) ; animals given 200mg/kg cyclophosphamide (5) ; animals given 200mg/kg cyclophosphamide + parental marrow and lymph node cells (6) ; animals given 300mg/kg cyclophosphamide (7) ; animals given 300mg/kg cyclophosphamide + parental marrow and lymph node cells (8) ; animals given 400mg/kg cyclophosphamide (9) ; animals given 400mg/kg cyclophosphamide + parental marrow and lymph node cells (10).
Statistical study. The difference between (1) and (5) (7) (9) is significant ($t = 6.49$, $p < 0.001$). The difference between (2) and (6) is significant ($t = 6.79$, $p < 0.01$) as well as the difference between (4) and (6) ($t = 5.88$, $p < 0.01$). The difference between (5) and (6) is significant ($t = 5.84$, $p < 0.01$).

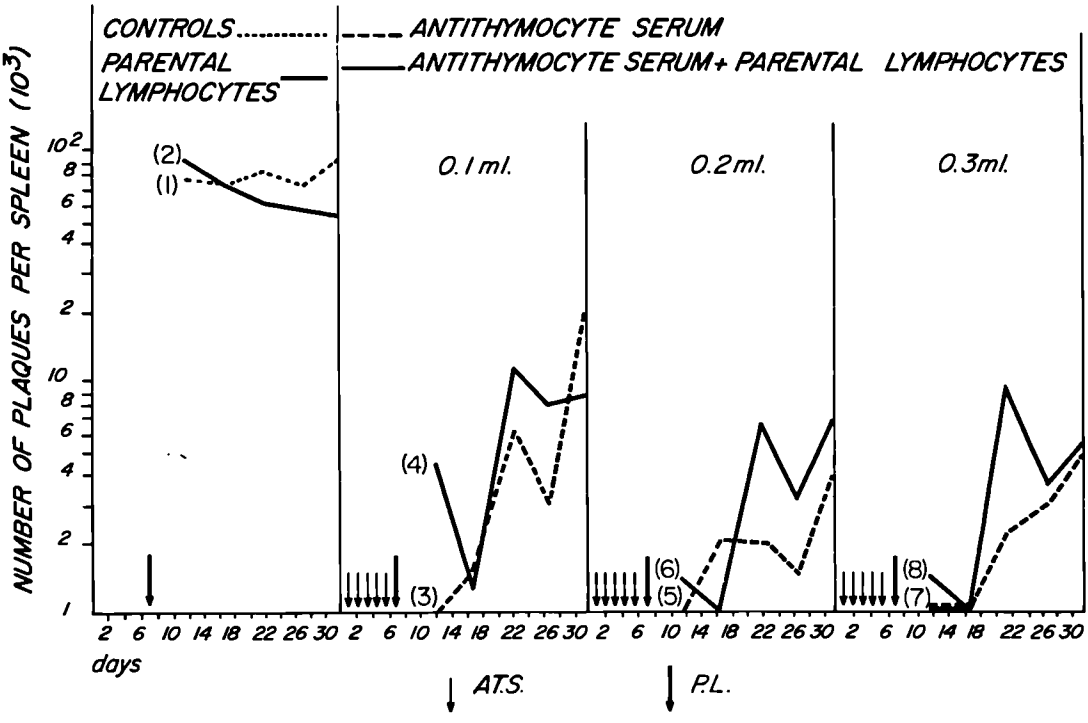


Fig. 3 Change in the number of haemolytic plaques in control animals (1) ; animals given parental marrow and lymph node cells (2) ; animals given 0.1ml ATS (3) ; animals given 0.1ml ATS + parental marrow and lymph node cells (4) ; animals given 0.2ml ATS (5) ; animals given 0.2ml ATS + parental marrow and lymph node cells (6) ; animals given 0.3ml ATS (7) ; animals given 0.3ml ATS + parental marrow and lymph node cells (8). Statistical study. Between (3) (5) and (7) at day 25 after the graft, there is no difference. Between (1) and (3) (5) (7), the difference is significant ($t = 8.64$, $p < 0.0001$). Between (4) (5) and (8) at day 25 after the graft, there is no difference. Between (2) and (4) (6) (8) the difference is significant ($t = 2.99$, $p < 0.02$). Between (3) and (4), (5) and (6), (7) and (8) there is not difference.

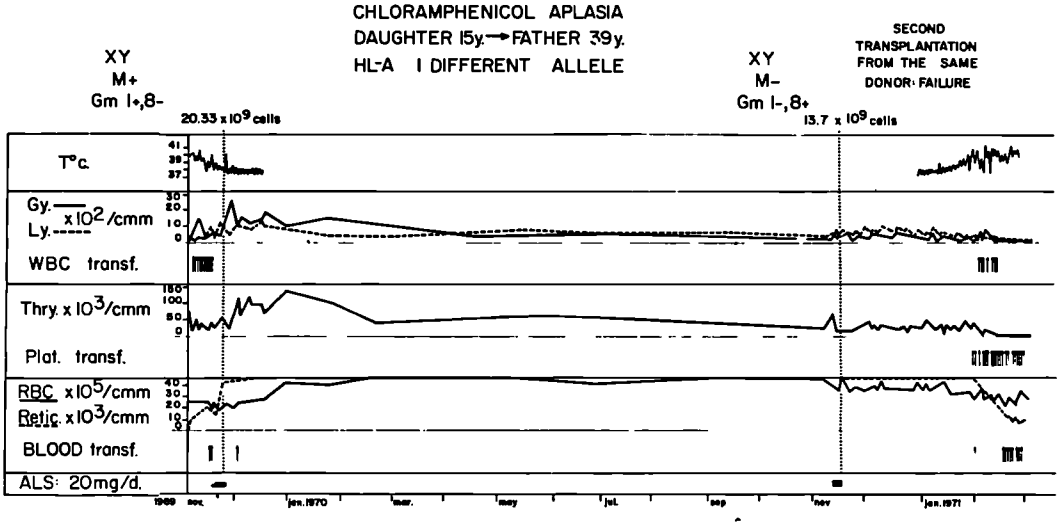


Fig. 4 Example of bone marrow allograft with a dissociated chimerism : the red blood cells and immunoglobulins are of the donor type, while the blood lymphocytes, transformed by PHA in vitro, are of the recipient's sex.

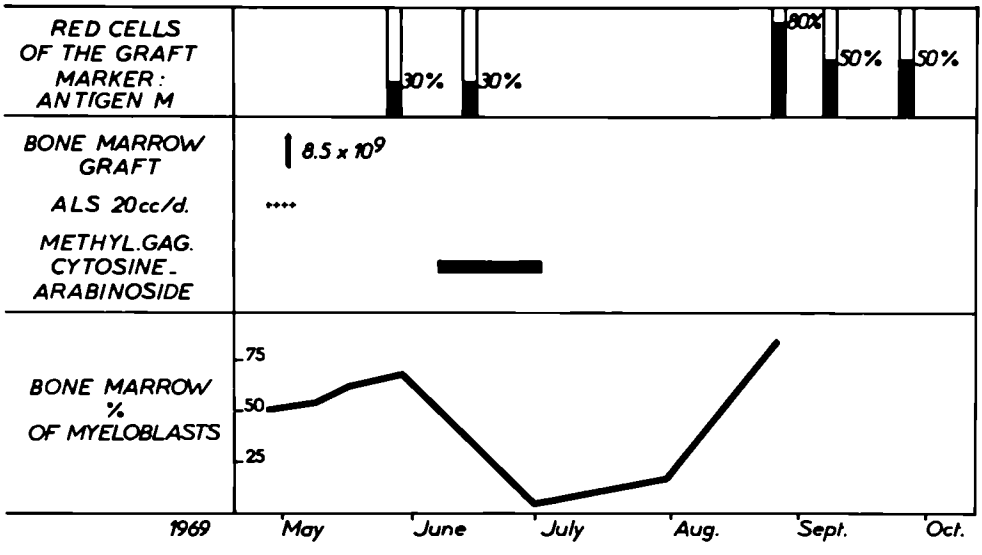


Fig. 5 Allogeneic bone marrow graft after antilymphocytic serum in a patient with acute myeloid leukemia in an overt phase of the patient. The graft was successful but without any antileukemic effect.

These studies need to be continued to try to find means to control the SD which complicated BMG after treatment with immunosuppressives that produce aplasia.

The addition of ALS to an aplasic conditioning agent such as CPM does not prevent SD. In a series of patients conditioned by a combination of CPM and ALS, we lost 3 patients with SD (not published).

It should be remembered that the choice of the best possible donor from HL-A typing has not protected them from SD in our experience (Amiel et al., 1971) ; this has been confirmed by several publications (Graw et al., 1970) : grafts of bone marrow in HLA identical siblings can induce a fatal SD (Graw, Rogentine et al., 1970).

These clinical observations have encouraged us to return to experimental studies of the control of SD that complicated BMG in patients pretreated with cytostatic agents. We are now investigating lymphocyte chalones that we have discovered in lymphoid tissue (Garcia-Giralt et al., 1970 ; Kiger, 1971) and the possibility of making the donors tolerant by giving them soluble histocompatibility antigens (Halle-Pannenko et al., 1971).

1,2) CONTROL OF GVH BY LYMPHOCYTE INHIBITOR(S) (CHALONE ?) AND BY DONOR TOLERANCE INDUCED BY SOLUBLE H-2 ANTIGEN

G. Mathé, E. Garcia-Giralt, N. Kiger, I. Florentin, O. Halle-Pannenko and M.C. Martyré

Our experimental research is orientated into two directions : a) to find non-specific methods of controlling secondary disease (SD) ; b) to find specific methods. A non-specific method means that graft-versus-host reaction is depressed by a measure which reduces all immune reactions. A specific approach means that only the reactions against the recipient histocompatibility antigens are depressed (Mathé et al., 1971).

The non-specific methods we are working on consist of the use of factor(s) that two groups working in our institute have extracted from the lymphoid tissue, either from the spleen (Garcia-Giralt et al., 1970), or from the thymus (Kiger, 1971).

The methods for preparation and purification are given on Table I [the active fractions are S (spleen) or T (thymus) 4]. These factors possess the same lymphocyte proliferation inhibiting effect as indicated on Table II.

S_4 has been shown to be able to reduce the mortality induced in (C57Bl/6 x DBA/2)F1 by the I.V. injection of 2.5×10^7 lymph node cells and 1×10^6 bone marrow cells from C57Bl/6 mice 24 hours after a dose of 500 rads total body irradiation (TBI). a) It reduces mortality when administered to the donors (Fig. 6) : the C57Bl/6 donor mice were treated daily with I.V. injections of 0.3ml of S_4 fraction from day -8 to day 0. b) This fraction reduces also the mortality when administered to the recipient after the I.V. injection of the allogeneic lymph node and bone marrow cells. In the experiment described on figure 7, recipients were treated either from day 5 to day 10 or from day 6 to day 10 with one milligram of the bovine S_4 per mouse, per day. The controls received either the same dose of a liver extract or injections of a saline solution. Fourty days after the beginning of the experiment, 85 % of the animals of the two groups of mice which had been treated with S_4 survived, while there was no survival in the controls which had received the liver extract or the saline solution.

We have described previsouly that S_4 is a protein which can be inactivated by 80 % by incubating at 70°C for 15 min. The data shown on Figure 8 indicate that recipients treated either with two mg of S_4 daily from day 6 to day 10 after the injection of allogeneic lymph node and bone marrow cells, or with injections of four mg of S_4 from day 6 to day 14 each second day, had a survival of 88 and 76 % respectively. To the contrary, the animals which had received, from day 6 to day 10, two mg/day of heated inactivated S_4 the saline solution showed no significant difference in their rate of mortality when compared with the controls and none of these recipients were alive on day 40.

Progress has been achieved in the purification of the active factor extracted from the thymus (T_4). In this

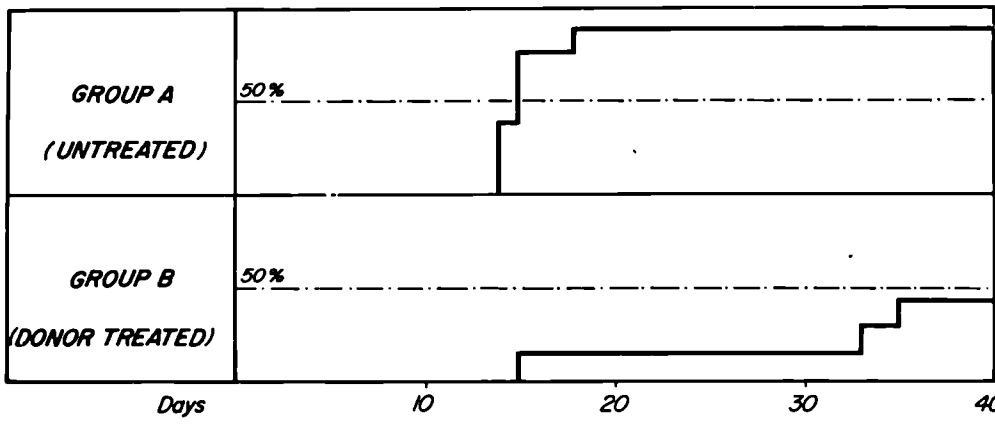


Fig. 6 Cumulative survival curve of (DBA/2 x C57Bl/6) F1 mice submitted to a radiation dose of 500 rads and 10^7 bone marrow cells. Group A received 2.5 x 10^7 lymph node cells C57Bl/6 ; group B, 2.5 x 10^2 lymph node cells from donors treated with S_4.

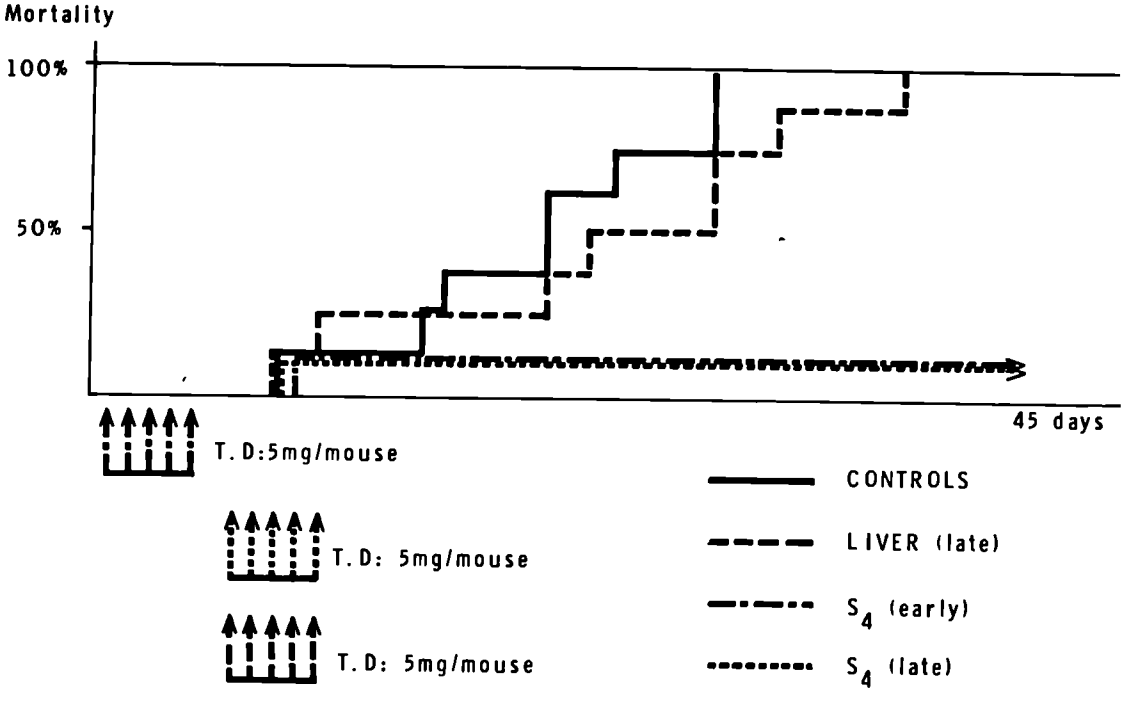


Fig. 7 Acute GVH syndrome : comparative survival of animals submitted five days to a simple course of liver extra spleen extract (S_4) or saline.

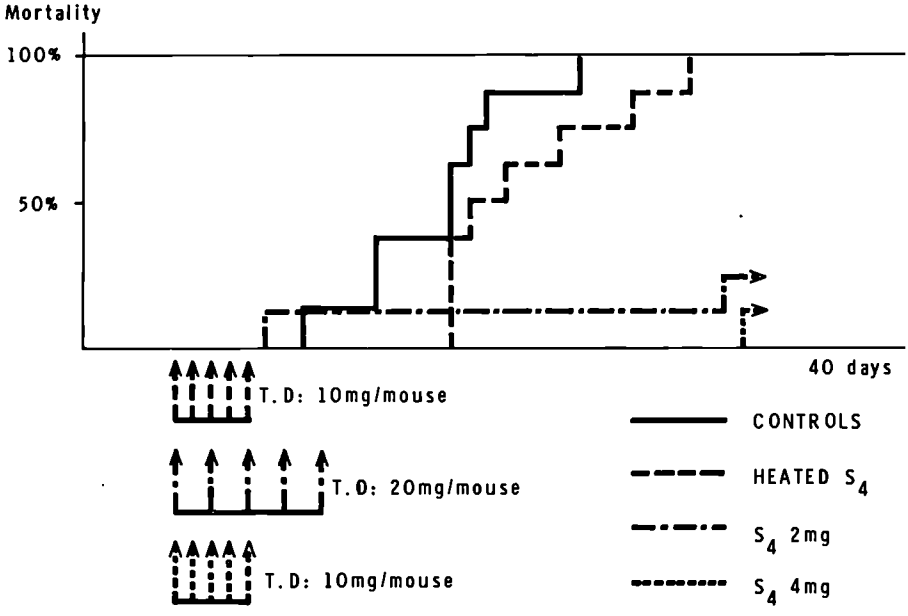


Fig. 8 Acute GVH syndrome : comparative survival of animals submitted to different doses and courses of spleen extract (S_4), heat inactivated S_4, or saline.

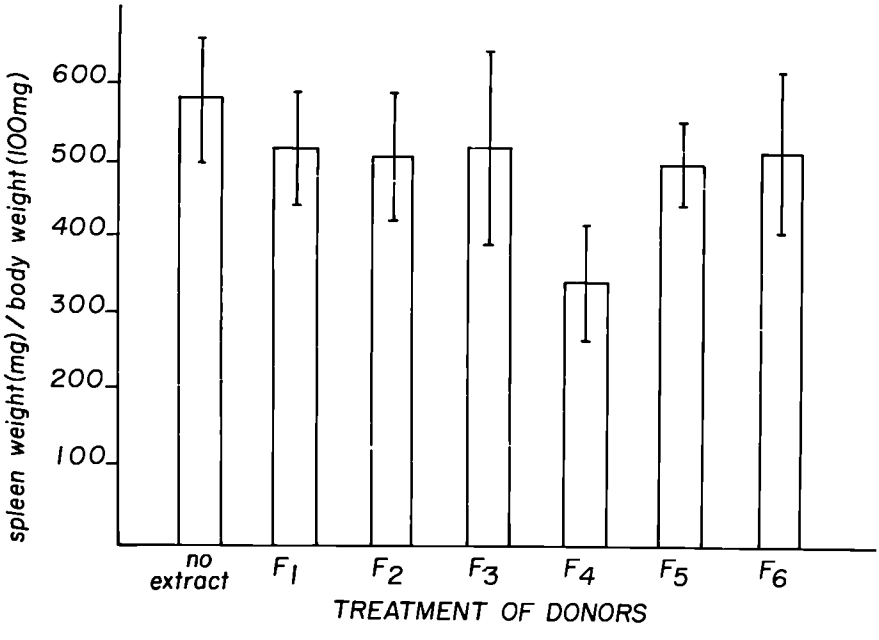


Fig. 9 Spleen index of (DBA/2 x CBA)F1 mice receiving parental DBA/2 spleen cells treated with various fractions.

purification, the activity of the fraction on GVHR has been measured by Simonsen's splenomegaly assay (1958). As shown in Figure 9, the active fraction after chromatography on DEAE-Sephadex A50 equilibrated in Tris HCl buffer 0.01 M pH 7.0 and stepwise elution with increasing NaCl molarities was T_4d (0.6 M NaCl) (T_4d).

It is evident that these factors only act in a non-specific fashion on lymphocyte proliferation and therefore their help in controlling SD complicating bone marrow allografts could only be partial and in any case only useful in the treatment of marrow aplasia by bone marrow grafting.

The most ideal solution would only seem to be a specific conditioning of the lymphocytes of the graft. With Halle-Pannenko and Martyré (1971), we are studying this approach in mice, using soluble H-2 antigens. The preparation of these antigens is shown in Table III. Giving variable doses to the donors, doses which in skin grafting experiments have given a high or low zone tolerance or immunization (Halle-Pannenko et al., 1971), we have been able with three doses (8000 γ, 30.4 γ, and 1.9 γ) (Halle-Pannenko et al., 1971) (Fig. 10) to reduce significantly the mortality from the acute GVHR induced by the I.V. injection of lymphocytes and bone marrow from C57Bl/6 donors into (C57Bl/6 x DBA/2)F1 irradiated with 500 rads TBI. These results are of further interest as they suggest and approach to be used in attempts to produce an adoptive immunotherapy of leukemia. Donors specifically tolerant to the H-2 histocompatibility antigens of the recipient, would still keep their reactivity against the recipients tumour antigens. We have obtained preliminary encouraging results using this system in mice.

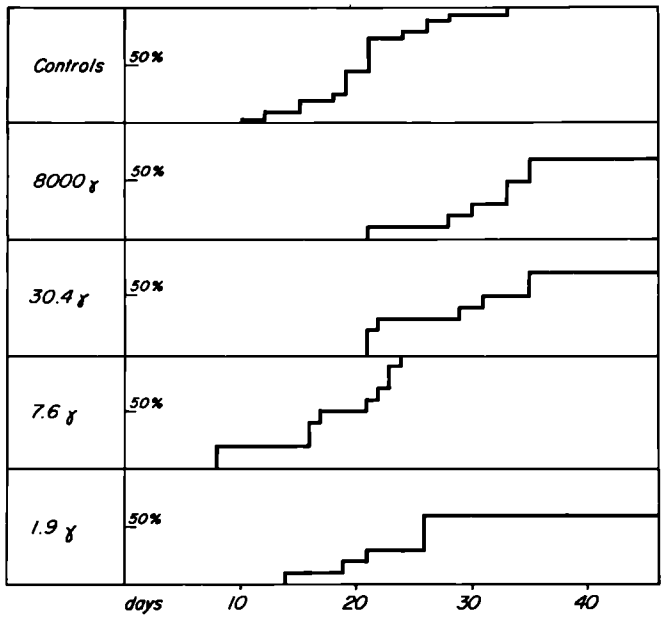


Fig.10 Cumulative mortality and survival time of irradiated F1 mice grafted with parental bone marrow and lymph node cells, the donors being normal, or treated with 8,000 γ , 30.4 γ , 7.6 γ or 1.9 γ of a preparation of soluble recipient histocompatibility antigens.

Projet n° 2

2) MARROW TRANSPLANTATION IN APLASTIC STATES EMPLOYING ANTI-LYMPHOCYTE GLOBULIN (ALG). ABSENCE OF SECONDARY DISEASE. SPLIT LYMPHOCYTE CHIMERISM

L. Schwarzenberg, G. Mathé, J.L. Amiel, M. Schneider, D. Belpomme, C. Jasmin, C. Rosenfeld, M. Hayat, F. de Vassal and M. Steresco

It has been shown since 1958 (Mathé, 1969 ; Mathé et al., 1959 ; Mathé et al., 1959) that bone marrow graft (BMG) is possible in man, after conditioning by total body irradiation (TBI), and since 1968 (Santos et al., 1970), that it is possible after conditioning by treating the recipient with cyclophosphamide (CPM). But the take, if conditioned by these cytostatic agents, is very often followed by a complication called secondary disease (SD), which can be lethal from early to very late according to whether it is hyperacute, acute, subacute or chronic (Mathé, 1961 ; Mathé et al., 1971).

Though it has been shown that graft-versus-host reaction (GVHR) is able to induce SD (Van Bekkum and Vos,1957), this disease can occur under conditions where GVHR appens, the GVHR is not to the sole factor determining the prognosis of SD. Experimental observations Gutierrez-Romero and Mathé, in press) have shown that the conditioning of the recipient with cytostatic agents such as TBI or CPM play a more important role in determining the degree of immune insufficiency which is the key factor in the prognosis SD in man as most patients dye from infections (Mathé et al., 1971) : the GVHR in animals conditioned by cytostatics leads to an immune insufficiency which is reversible when the dose of the cytostatic is small or moderate, and irreversible when the dose is high ; and it is these high dose which is needed for a BMG to take. We were considerably interested in this experiment by the observation that the GVHR in animals pretreated by antilymphocytic serum (ALS), did not add to the immunosuppression induced by the ALS, even at a maximum dose, and did not cause the immunosuppression to become irreversible.

Hence we tried to conduct clinical trials in which the patients would be conditioned for bone marrow transplantation exclusively by ALS (Amiel et al., 1970 ; Mathé et al., 1970).

51 trials have been conducted in 39 patients (ten patients having been submitted to two trials and one to three trials) ; a) 24 patients were suffering from a chronic idiopathic or chemical induced marrow aplasia ; b) 14 patients were leukemic and suffering from subacute aplasia, due either to the leukemic process by itself, or to chemotherapy or to both factors ; c) one patient was suffering from thalassemia. Nine aplastic patients and one leukemic were submitted to two trials, one aplastic patient to three trials.

ALS was prepared from horses immunized (a) either by human fresh lymphocytes obtained from the blood of chronic lymphocytic leukemia patients or of healthy volunteer donors by means of the IBM continuous blood cell separator (ALS 1) (Schwarzenberg and Choay, 1971; Schwarzenberg et al., 1968), (b) or by cells from an established cell line from the blood of a patient with acute lymphoid leukemia (ALS 2) (Belpomme et al., 1969 ; Belpomme et al., in press ; Rosenfeld et al., 1969). The horses were immunized by eight weekly sc injections given in various sites. The first three injections each contained 5×10^9 cells, the next three 10×10^9, the seventh 15×10^9 and the final injection 30×10^9. The serum was prepared at the end of the ninth week, decanted from the blood after storage for 24 hours at +4°C, and absorbed three times with pooled human red cells for two hours at +4°C. The globulins were precipitated with ammonium sulphate, fractionated by chromatography on diethylamino-ethanol cellulose and the IgG globulins were isolated. The IgG fraction was filtered through a 0.2μ diameter Millipore filter.

The antilymphocyte glubulins (ALG) were submitted to the following tests. The absence of cold anti-erythrocyte agglutinins was confirmed. The titre of the cytotoxic activity against human lymphocytes was estimated (Amiel, 1969), and found usually to be about 1/4,000. The inhibition of rosette formation by sheep red cells on human lymphocytes

(Bach et al., 1968) was tested : the titres were of the order of 1/16,000. After these tests the sera were adjusted to have a cytotoxicity of 1/1,000 in vitro.

The conditioning regimens were of the three types : a) in the first type 26 patients (19 aplastic and seven leukemic) were given 200 to 400mg ALG IM or IV for fourth to 12 days (depending on their tolerance of this antiserum) before a BM transplantation ; b) in the second type, 11 patients (three aplastic, seven leukemic and one thalassemic) were pretreated in the same way with ALG and their BM donors received the same dose of ALG for ten days ; c) in the third type, four aplastic patients (two of them being submitted to a second trial) were given ALS 2, IV, in the following manner : 200mg of ALG the first day, 400mg the second day, and so on, the total amount being 200mg of ALG per kg of body weight. The tolerance of ALG 2 was perfect.

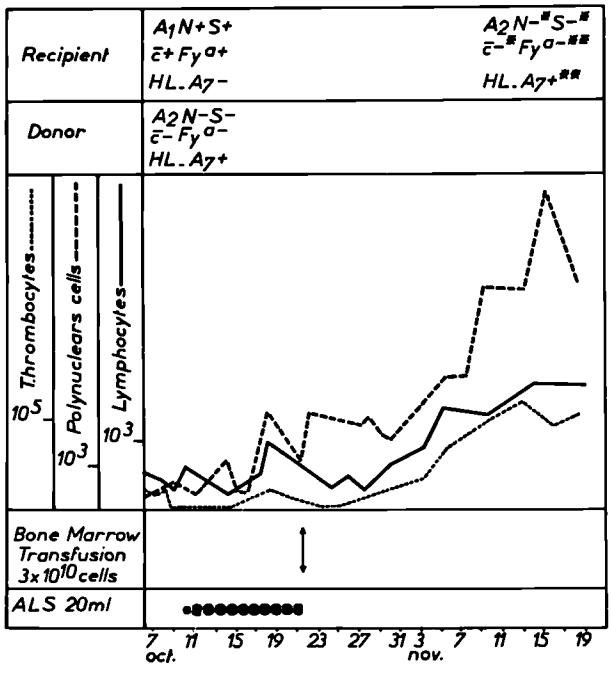
The method of BM transfusion has been described previously (Mathé et al., 1959). The donor-recipient histocompatibility relationship is indicated on Tables IV and V.

No prophylactic treatment for possible SD was given. The proliferation of the transfused allogeneic marrow was determined by means of erythrocyte, leukocyte and immunoglobulin markers (Table V).

2,1. The engraftment

The overall results were as follows : a blood and BM restoration was observed following BM transfusions in 12 patients out of 39 patients. It appeared 15 to 150 days after transplantation.

In five patients, blood restoration was a fast (15 to 30 days) as in radiochimeras and the blood count returned to normal (Fig. 11). Symptomatic effect in such cases was spectacular. In seven patients, it was slow and the blood counts remained below normal (Fig. 12) though a symptomatic improvement was evident in most of them. Eight patients still needed whole blood transfusions, within 30 days after transplantation ; two patients white cell (WC) transfusions and



\# 50 p.100 on nov. 19 and 80 p.100 on dec. 22

\#\# Not quantified

Fig.11 Allogeneic bone marrow graft after antilymphocytic serum, in a patient with acute myeloid leukemia, in a latent phase of the disease and an aplastic marrow

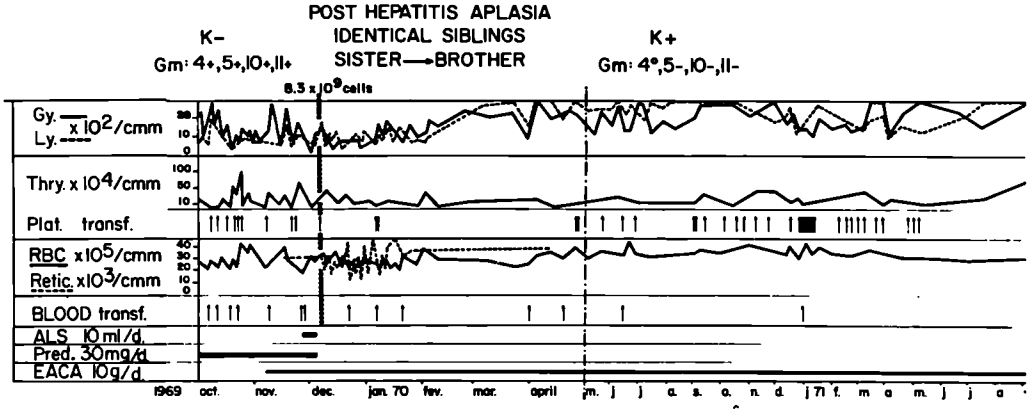


Fig 12 Allogeneic bone marrow graft after antilymphocytic serum, in a patient with post hepatitis aplasia ; dissociation of restoration for the different series.

seven patients platelet transfusions ; these transfusions tended to make more difficult the estimation of the take by study of thermarkers. Sometimes red cells, white cells and thrombocyte restorations are dissociated (Fig. 12).

Out of 12 patients who were restored haematologically the engraftment was proven in ten. Table VI summarizes the available data supporting this conclusion.

Several factors have to be considered as possibly important for determining the success of the take.

2,11. The disease and other recipients. Table VII indicated the different etiological varieties of aplasia and the incidence of takes in each variety. There is no paramount differences, though the results appear to be the best in leukemia and the worst in idiopathic aplasia.

Table VIII classifies the patients according to the number of blood transfusions they had received before ALS treatment : it shows that a high number of transfusions may defavorize the take of the graft.

2,12. The donor and recipient histocompatibility relationship. Table IX shows the donor and recipient histocompatibility relationship and the incidence of the take according to this relationship. It shows that the better results are obtained when siblings are used as donors, specially when both alleles are identical.

2,13. The preparation of ALS. All takes has been obtained in recipients conditioned by ALS 1 and no take as been observed in patients treated by ALS prepared from cultured leukemic cells.

2,2. The absence of secondary disease

The most important and exciting observation is undoubtely the absence of any typical manifestations of severe SD, well known in human radiochimeras (Mathé, 1961 ; Mathé et al., 1960 ; Mathé et al., 1965), cyclophosphamide chimeras (Graw et al., 1970 ; Santos et al., 1970), and patients treated with WC transfusions (Schwarzenberg et al., 1967). The severe SD was absent in all groups, whether both donor and

recipient were treated by ALS or when recipient alone was treated.

Nevertheless, one must mention two patients. 1) One died of a Pseudomonas pyocyaneus cerebral abcess two months after grafting. He had had a perineal fistula infected with this microorganism before the graft, which later gave rise to metastatic abcesses in the joints and the brain, despite an apparently complete hematological restoration. This patient is the exception to the generel rule : in other patients with infection, even septicemia, resistant to antibiotics and possibly to WC transfusions, all signs of infection disappeared after a successful BMG. So far, no signs of secondary immune insufficiency which would indicate a GVHR have developped in these patients. 2) The other patient presented a few days after BM transfusion a general rash, arthritis, diarrhoea, asthenia, a syndrome evocating SD which disappeared after two methotrexate injections.

One can wonder if the absence of severe SD is due to the possible protective effect of ALS as suggested by some experimental data from Van Bekkum et al. (1971). The very severe SD (lethal in three cases) we have seen in leukemic patients conditioned by a combination of ALS and cyclophosphamide suggests this is not the correct explanation.

On the other hand, the following findings offer the basis for a more fruitful hypothesis : in one patient (Fig. 4), we could demonstrate that while immunoglobulins and red cells were of the donor type, blood lymphocytes transformed by PHA in vitro had a chromosome constitution indicating they were of host origin. In other words, B-lymphocytes were produced by the graft, while T-lymphocytes were still produced by the recipient. Skin graft of the donor was normally rejected. This condition does not seem to be exceptional, since it has been reproduced by Speck and Kissling (1971) in rabbits made aplastic by benzene then restored haematologically by BMG after treatment with ALS.

This state of affairs seems at first to be paradoxical as the target cells of ALS are T-lymphocytes, however

in reality, this idea is very acceptable when one considers the experimental observations we have made with Gutierrez-Romero (in press), showing that a GVHR in ALS conditioned mice does not aggravate ALS induced immune insufficiency and does not make it irreversible.

Two explanations for this occurence can be proposed : a) ALS does not destroy T-lymphocytes ; it only "paralyses" these cells ; hence it does not make as much "space" as irradiation or chemocytostatic drugs in peripheral lymphocytic tissues ; so T-lymphocytes of the donor have not enough space to proliferate ; b) ALS affects T-lymphocytes but does not kill the lymphocyte stem cells which, in aplasia, may be normal, since this pathological condition is not usually complicated by immune insufficiency.

Although this absence of clinically detectable GVHR is very precious in treatment of aplasias, it has a disadvantage : getting rid of it, we have lost, as illustrated by Figure 5, the antileukemic effect of BMG that we had experimentally demonstrated (Mathé et al., 1960, 1962, 1962, 1964) and clinically described (Mathé et al., 1965 ; Mathé et al., 1970) in radiochimeras and that has been found in cyclophosphamide conditioned chimeras but at the price of lethal SD in some patients (not published).

The absence of GVHR may reside in the transitory character of the graft. In leukemia, this could be explained by the persistance of the myeloid stem cells of the host, and this interpretation is illustrated by the frequent coexistence of host and donor blood cell populations. In aplasia, a disease in which the myeloid stem cells are exceptional or absent and in which, when the graft disappears, the patient became pancytopenic again, it is more satisfactorily explained by the persistence of host T-lymphocytes which may tolerate the graft for a given time only : the limitation of this time could be due to the replacement of ALS conditioned T-lymphocytes by new T-lymphocytes produced by lymphocyte stem cells. This hypothesis suggests the possible interest of prolonging treatment with ALS in hematochimeras, that have been

established as the result of conditioning with ALS.

In conclusion, the experimental and clinical realisation of BMG after exclusive preparation of the host by ALS, represents an indiscutable step ahead in the BM transplantation : a) because, it is as seen in Figure 13 the first efficient treatment of the various chronic and irreversible aplasias of the bone marrow ; b) because it allors BM transplantation to be used in clinical practice without subjecting the patients to a potentially, which is unacceptable ethically.

Now we must try to increase the incidence of the takes ; to try to prolong the duration of the graft and possibly to make it permenant and to try to recover the antileukemic effect, produced by the adoptive immunotherapy.

Our experimental and clinical observations suggest several directions that we are now following, mainly the combination of ALS with a semicorporal irradiation, and the combination of ALS with non-intensive chemotherapy made specifically immunosuppressive by virtue of lymphocyte synchronization.

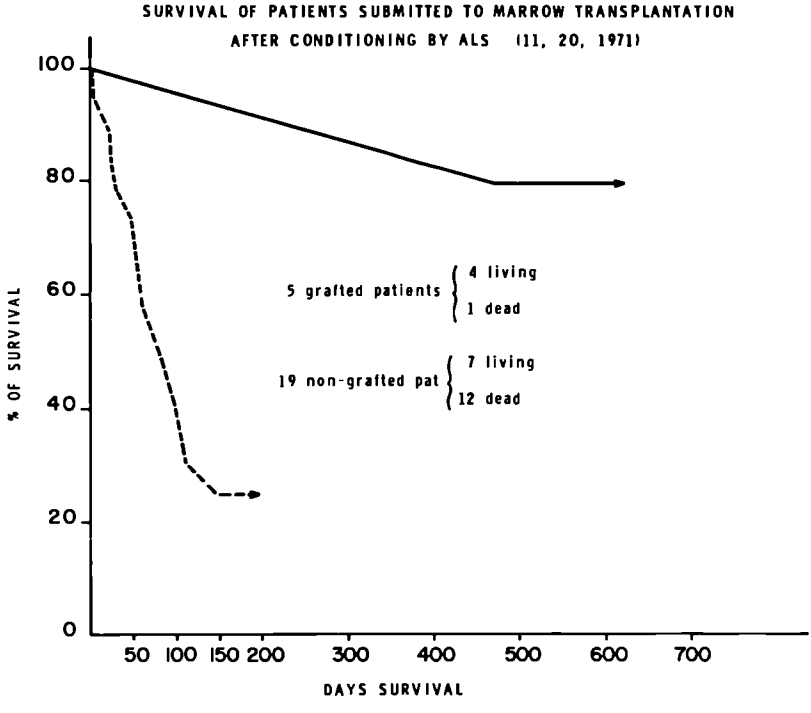


Fig.13 Survival of patients submitted to marrow transplantation after conditioning by antilymphocytic serum (November 20th, 1971).

TABLE I

PURIFICATION OF THE LYMPHO-INHIBITORY FRACTION FROM SPLEEN OR THYMUS

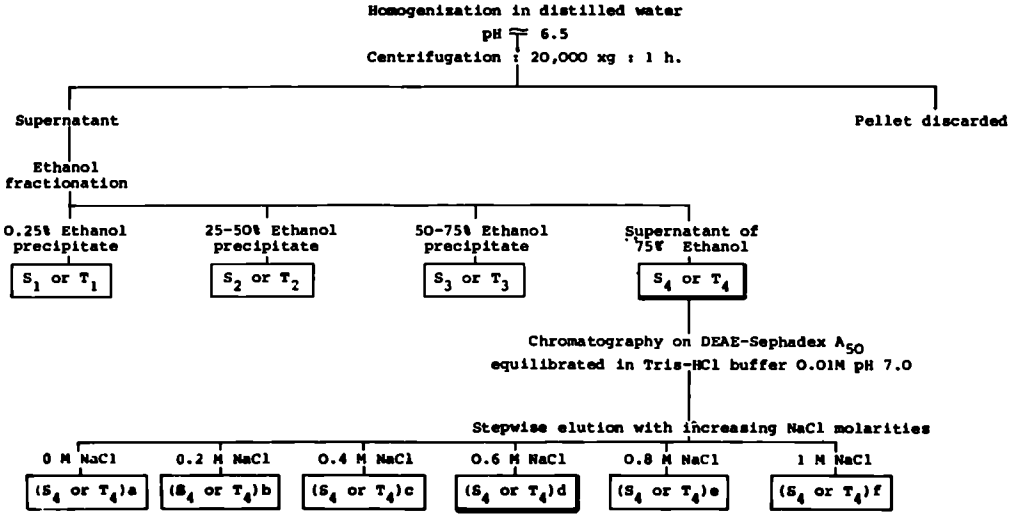


TABLE II

COMPARATIVE EFFECTS OF THE FACTORS EXTRACTED FROM THE SPLEEN (S_4) AND THE THYMUS (T_4)

TESTS		S_4	T_4
INHIBITION OF LYMPHOCYTE TRANSFORMATION IN THE PRESENCE OF PHYTOHEMAGGLUTININE	IN HUMANS	+	+
	IN MICE	+	+
INHIBITION OF LYMPHOCYTE TRANSFORMATION IN THE PRESENCE OF PPD	IN HUMANS	+	?
INHIBITION OF LYMPHOCYTE TRANSFORMATION IN MIXED LYMPHOCYTE CULTURES	IN HUMANS	+	?
	IN MICE	+	?
INHIBITION OF THYMO-DEPENDENT SPONTANEOUS ROSETTE FORMING CELLS AGAINST SRBC	IN MICE	?	+
INHIBITION OF GRAFT-VERSUS-HOST REACTION	IN MICE	+	+
BONE MARROW GRAFT	IN MICE	+	
INHIBITION OF ALLOGENEIC SKIN GRAFT REJECTION	IN MICE	?	+
INHIBITION OF THE PRODUCTION OF HEMOLYSIN ANTI-SRBC	IN MICE	+	+

TABLE III

EXTRACTION AND SOLUBILIZATION OF H-2 ANTIGENS

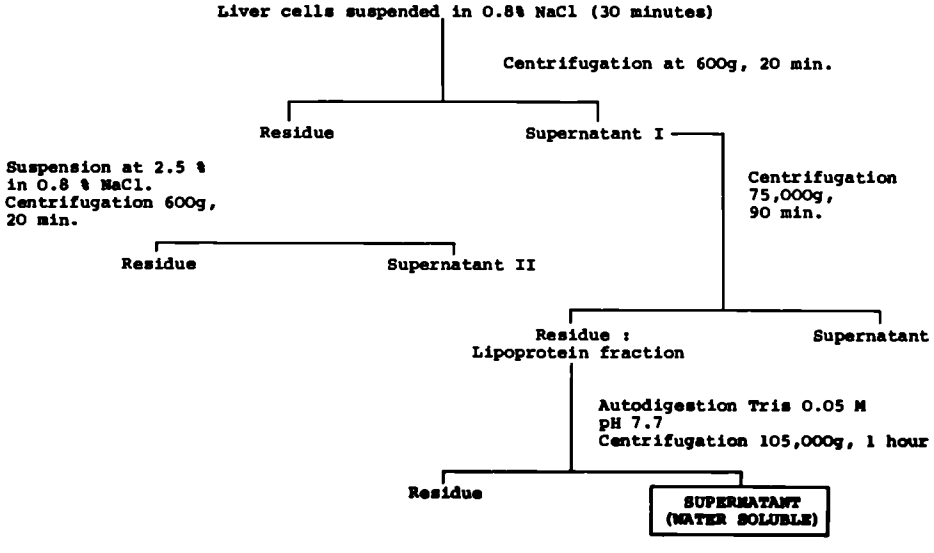


TABLE IV

DONOR-RECIPIENT HISTOCOMPATIBILITY RELATIONSHIP

RECIPIENT		DONOR	HL-A HAPLOTYPE: ALLELES			RECIPIENT		DONOR	HL-A HAPLOTYPE: ALLELES		
N°	DISEASE	RELATIONSHIP TO RECIPIENT	BOTH SAME	ONE or TWO DIFFERENT	UNKNOWN	N°	DISEASE	RELATIONSHIP TO RECIPIENT	BOTH SAME	ONE or TWO DIFFERENT	UNKNOWN
1	APLASIA	NOT RELATED			+	19	APLASIA	BROTHER			+
2a	"	BROTHER			+	20	"	UNCLE			+
2b	"	"			"	21a	"	BROTHER			+
3	"	BROTHER			+	21b	"	SISTER			+
4	"	SON		1		21c	"	"			"
5a	"	DAUGHTER		1		22a	"	SISTER			+
5b	"	"		"		22b	"	BROTHER			+
6	"	SISTER	+			23	"	SON		1	
7a	"	NOT RELATED			+	24	"	BROTHER			+
7b	"	MOTHER		1		25	A.M.L	BROTHER			+
8a	"	SON		1		26	"	BROTHER			+
8b	"	"		"		27	"	FATHER		1	
9	"	SISTER			+	28	"	1/2 BROTHER			+
10	"	NOT RELATED			+	29	"	SISTER			+
11a	"	SISTERS (2)	+			30	"	1/2 BROTHER			+
11b	"	SISTER	"			31	"	DIZYGOTIC TWIN			+
12	"	BROTHER			+	32	A.L.L	SISTER			+
13	"	MOTHER		1		33	"	MOTHER		1	
14	"	SISTER	+			34	"	BROTHER			+
15a	"	SISTER			+	35	"	MOTHER		1	
15b	"	"			"	36	"	MOTHER		1	
16a	"	FATHER		1		37a	"	MOTHER		1	
16b	"	BROTHER		2		37b	"	BROTHER			+
17	"	BROTHER	+			38	POLYCYTHAEMIA	SON		1	
18a	"	BROTHER			+	39	THALASSEMIA	FATHER		1	
18b	"	"			"						

a) 1st graft trial)
b) 2nd graft trial) in the same patient
c) 3rd graft trial)

TABLE V

CHROMOSOME, ERYTHROCYTE, LEUKOCYTE and IMMUNOGLOBULIN MARKERS

N°	CHROMOSOME		ERYTHROCYTE TYPING		LEUKOCYTE TYPING (HL-A)		IMMUNOGLOBULIN TYPING	
	D+	R-	D+ R-	D- R+	D+ R-	D- R+	D+ R-	D- R+
1	XX			M, S, Fy^a		2,5		
2	XY			C			Isf 1	Gm 1°,17°,21°
3	XY			D, C, E				
4				E, Fy^a	3,5		Isf 1	Gm 4
5	XX		Le^b	M (50%)		7,9	Gm 8	Gm 1
6			K,Le^a	M, P, E, Fy^a				Gm 5, 10, 11
7a			K	M, Le^a	7,8		Gm 8	Inv 1,2
7b	XX		O, D	A1, Fy^a		3,5	Gm 5,8	Gm 4,21
8				M, Fy^a	5			Inv 1,2
9	XX					5		Inv 1,2
10	XX			M,s,C,Le^a	2,3,5,7	9	Gm 2	Gm 4,5,8,10,11
								Inv 1,2
12	XY		E	C	2,7			
13			Le^a	M,Fy^a	10	3	Isf 1	Gm 1,2,21
								Inv 1,2
14	XX			Jk^a				Gm 2,4,5,8,10,11
15	XX					7		Gm 1,2,21
16a	XY			M,S,C,E		2,8		Gm 2
16b	XY			E	7	5,8,9		
17				D				Gm 1,2,21
								Inv 1,2
18				M,P,Le^a,Fy^a	3,8			Gm 2,21 ;Inv 1
19				s, Le^b	8			Gm 1,21
20				D	12			Gm 1,21 ;Inv 1
21a	XY		Fy^a		2			Gm 1,21 ;Inv 1
21b			Le^b	P				Gm 4,5,8,10,11
22a	XX			s	2,9,7			
22b			Le^a	S	2,9,7			
23				M,S,Le^a				Gm 1
24	XY			M,S,P,C,Fy^a		3,5,7		"
25			C, Le^b	A,M,S,Fy^a,Le^a	7			
26			Le^b	M,S,Le^a			Inv 1,2	Gm 1,2,17,21
27			E		2	3	Gm 2	Inv 1
28	XY		K	M				
29	XX			D,C				
30	XY		$A2^2$	A1,M		3		Isf 1
31	XY		Le^b	P,D,C,E	1	3		Gm 4,5,8,10,11
32	XX		A2	A1,P		1,5,7	Gm 2,21	
33			O	A1,P	3			Gm 8,21
34	XY		K					
35				M				Gm 1,21; Inv 1,2
36				D,C			Gm 2	Gm 4,5,10,11
37a			Le^b					Gm 1 ; Inv 1,2
37b	XY		Le^b	E	2			Inv 1,2
38	XY		E					Gm 8
39			P		2			

PATIENT N° 11 : TOTAL IDENTITY

TABLE VI

PROOFS OF THE GRAFT IN RESTORED PATIENTS

N° PATIENTS	CARYOTYPE	ANTIGENS			DONOR' S SKIN GRAFT TOLERANCE
		ERYTHROCYTE	LEUKOCYTE	IMMUNO-GLOBULINS	
5		+		+	
6		+		+	
7			+		
9					+
11		NO DIRECT PROOF			
12	+				+
14		NO DIRECT PROOF			
25		+	+		
28		+			
30		+			
32		+			
33		+			

TABLE VII

RESULTS ACCORDING TO THE ETIOLOGICAL VARIETIES OF APLASIA

		N° TRIALS	N° RESTORATION	N° TAKES	N° FAILURES
APLASIA	TOXIC: CHLORAMPHENICOL	5	1	1	4
	TOXIC: CHEMICAL MANURE	1	1	1	
	TOXIC: BENZENE	1			1
	TOXIC: 6-MERCAPTOPURINE (Kidney homotransplant)	1	1		
	POST-HEPATITIS	1	1	1	
	"IDIOPATHIC"	26	3	2	23
LEUKEMIA	CHEMOTHERAPY	8	3	3	5
	NO CHEMOTHERAPY	7	2	2	5

TABLE VIII

CLASSIFICATION OF PATIENTS ACCORDING TO THE TRANSFUSIONS RECEIVED BEFORE ALS TREATMENT

PATIENT	NUMBER OF BLOOD DONORS FOR EACH PATIENT	MEDIAN NUMBER OF BLOOD DONORS
GRAFTED	0 - 38	16.7 ($\pm$ 7.4)
NON GRAFTED	0 - 300	67.7 ($\pm$ 21.1)

One Whole blood transfusion = One donor
One Platelet transfusion = Four donors
One white cell transfusion = One donor

TABLE IX

INCIDENCE OF THE RESTORATION ACCORDING TO THE HISTOCOMPATIBILITY RELATIONSHIP

	RESULTS OF THE TRIALS	
	TAKE	FAILURE
BOTH ALLELES SAME : SIBLINGS AS DONORS	3	1
ONE ALLELE DIFFERENT : PARENTS AS DONORS	2	7
ONE ALLELE DIFFERENT : CHILDREN AS DONORS	1	4
HAPLOTYPE NOT ESTABLISHED : SIBS AS DONORS	6	17
HAPLOTYPE UNKNOWN : NOT RELATED DONORS	0	3

FIVE PATIENTS HAVE RECEIVED SEVERAL TRANSPLANTS FROM DIFFERENT DONORS (SEVEN PATIENTS HAVING RECEIVED SEVERAL TRANSPLANTS FROM THE SAME DONOR ARE QUOTED ONCE)

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5.2. REPORT ON STUDIES

carried out during 1971 at the

ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI"

Supported by: EURATOM

Contract No.: 079-69-1 BIA C

1. STUDIES ON THE METABOLISM OF AGENTS DEPRESSING IMMUNE REACTIVITY

Metabolism and Comparative Immunosuppressive Activity of 6-Mercaptopurine and Azathioprine

Azathioprine (6-(1-methyl-4-nitroimidazolyl)-thiopurine = Imuran) is perhaps the most widely employed agent in clinical immunosuppressive therapy after early reports (1,2,3,5,6) which concluded that this agent was more effective and less toxic than the parent compound 6-mercaptopurine (6-MP). As a part of a long-term interest of this Institute, in a further elucidation of some aspects of the pharmacology of radiomimetic immunosuppressive agents, studies have been carried out on the comparative metabolism and *in vivo* immunosuppressive activity of Imuran and 6-MP.

Methodology

Swiss Albino mice (25± 2 g body weight) were used; they had free access to food (diet ALAL 56 of Alal S.p. A., Milan) and water during the experiments except in the case of oral administration of the agents when food was removed 12 hr before drug administration. The animals were kept at room temperature of 22°C with relative humidity of 60% , in Makrolon cages. Imuran and 6-MP in equimolar doses, were dissolved in 10% NaOH 0.1 N and 90% phosphate buffered saline (pH 7.2) for parenteral administrations, or suspended in 1% of carboxymethylcellulose for oral administrations. 6-MP was measured in blood according to the method of Finkel (4) and in spleen according to a spectrophotometric method originally developed in this Institute and previously described (see Final Scientific Report, 1970, Contract No.: 079-69-1 BIA C).

Since no method is presently available for the chemical determination of Imuran as such,and since this agent is believed (3,5) to act mainly by releasing 6-MP,so,after Imuran administration, the release amounts of 6-MP were measured in plasma and spleen at various times after injection of different dosages of the compound.
In order to measure the immunosuppressive activity of either agent, the animals were injected on day 0 with $4x10^8$ sheep erythrocytes (SRBC) intraperitoneally, either agent in the doses listed below was given on day +2 and the number of spleen hemolytic plaque-forming cells assessed on day +4 by Jerne's technique (7) with minor modifications.

Results

The results presented in Tables 1 and 2 show that soon after intravenous injections azathioprine is almost entirely splitted into 6-MP, and that during the period of observation the levels of 6-MP in blood and spleen measurable after administration of 6-MP or Imuran, are very similar. Half-lives of 6 MP in blood are 6'15" , 22'05" and 22'00" after 6-MP injection (50, 100 and 150 mg/kg ,respectively) and 6'50", 19'00" and 19'10" after injection of equimolar doses of Imuran (81, 163 and 244 mg/kg ,respectively); the half-life of free-6-MP in the spleen are 17'40" and 18'20" after the doses of 100 mg/kg and 150 mg/kg 6-MP and 14'20" and 18'20" after equivalent doses of Imuran (Table 2). Tables 3 and 4 show the comparative immunosuppressive activity of 6-MP and Imuran given in equimolar doses by intravenous route: it can be observed that no significant differences in activity are recognizable when the 50 mg/kg 6-MP dose and the equivalent 81 mg/kg Imuran are used. However, 6-MP, given intravenously in higher doses (100 and 150 mg/kg) , was consistently found

to be significantly more active than Imuran in reducing the number of spleen hemolytic plaque-forming cells (the difference in activity being a factor of approximately 3), in spite of essentially analogous blood and organ levels. Conversely, when the two drugs are given by oral route (Table 5), Imuran is more immunodepressant than 6-MP. Results, not reported here, indicate that the toxicity of the two drugs in mice is not significantly different when they are given by intravenous or intraperitoneal route, and that the immunosuppressive activity of 6-MP is higher than that of Imuran also by subcutaneous and intraperitoneal routes of administration. Experiments in progress are showing that results analogous to those observed in mice can be obtained also in rats (Table 6); in this species again, 6-MP was found to be more immunosuppressive than Imuran when given parenterally.

2. DRUG INTERACTIONS IN IMMUNOSUPPRESSIVE TREATMENT

An expanding number of pathological conditions are being recognized to benefit from treatment with immunodepressant agents, i.e. drugs which are or resemble radiomimetic agents for having common properties. Current therapeutical treatments in human immunosuppressive therapy commonly employ mixtures of immunodepressants in patients who are also very frequently given other types of medications(hormones, antibiotics , etc..). Since many of the immunodepressant radiomimetics are metabolized through the same system (the liver microsomal enzymes) also adopted for the metabolism of many other compounds (8), thus radiomimetics may alter the distribution, metabolism and ultimately the activity of other immunosuppressants or unrelated drugs concomitantly administered and, conversely, concurrent or antecedent medication may influence the metabolism and activity of the very radiomimetics. A number of examples of the changes induced by radiomimetics on the metabolism of other drugs have been described by members of this Institute (11,12),and a further example is presented in Table 7 which shows the effect of cyclophosphamide treatment on pentobarbital (sodium 5-ethyl--5(1-methylbutyl)barbiturate) distribution. It can be seen that the treatment with cyclophosphamide markedly decreases the disappearance rate of pentobarbital in blood and brain. The studies , below reported, were carried out to investigate the inverse situation, namely to ascertain whether antecedent treatment with commonly used drugs can or not exert influence on the metabolism and activity of radiomimetic immunodepressants. The first phase of this study considered drug interaction between phenobarbital (5-ethyl-5-phenylbarbituric acid) -a barbituric acid derivative very commonly given for sedation of hospitalized patients and known to profoundly modify the acti-

vity of the liver microsomal enzymatic system (9,10) - and a number of immunodepressant radiomimetics.

Methodology

Swiss Albino male mice (23±2 g body weight) were used. The animals were housed in standard conditions and had free access to food and water; they were given $4x10^8$ sheep erythrocytes (SRBC) i.p. on day 0 and were then schematically divided into three groups: group A served as control, group B received the immunosuppressive agents alone, and group C received a treatment with phenobarbital (80 mg/kg i.p.) on the two days preceeding the injection of the immunosuppressive agent (details of treatment are listed under the corresponding tables). The animals were then killed 4 days after the injection of SRBC, their spleens removed and the number of hemolytic plaque-forming cells evaluated by Jerne's technique (7) with minor modifications.

Results

Results are presented in Tables 8-15. It can be seen that the pretreatment of mice with 2 phenobarbital injections markedly modified the immunosuppressive activity of a number of the agents investigated; specifically the immunosuppressive activity of 6-mercaptopurine (6-MP), 1,3 bis(2-chloroethyl)-1-nitrosourea (BCNU), was enhanced by phenobarbital pretreatment at the dosage employed, while the activity of hydrocortisone, cyclophosphamide and mechlorethamine was decreased.On the other hand, the immunosuppressive activity of other agents, such as methotrexate, procarbazine, azathioprine, daunomycin, ALS (antilymphocytic serum), DIC (5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide) , 5-fluorouracil, triethylenmelamine was essentially unchanged by the phenobarbital treatment

employed in these experiments.
Appropriate controls showed that the phenobarbital treatment which was able to modify the immunosuppressive activity of the agents listed above, had no immunosuppressive effect by itself, as judged by the number of plaque-forming cells countable in the spleen of treated animals. A similar increase in immunosuppressive activity was observed with phenobarbital treatment when the doses of 50 and 100 mg/kg of 6-MP were used, the difference in activity as compared to non-phenobarbital pretreated animals being in the order of 2.5-3; quantitatively similar modifications were also observed for BCNU and in the case of cyclophosphamide.
An increase in 6-MP activity by phenobarbital could be observed irrespective of the routes (i.p., i.v.) employed for administering the immunosuppressant, provided that the phenobarbital treatment was adequate, in fact a treatment with 40 mg/kg of phenobarbital given on the two days preceeding the 6-MP injection, was unable to significantly modify the activity of the drug, nor was the number of antibody producing cells diminished if only a single injection of 80 mg/kg was given in various days or subsequent to the injection of 6-MP. The effects of SKF 525-A (2-diethylaminoethyl-2,2-diphenyl valerate),a known inhibitor of the hepatic microsomal enzyme activty , on the immunosuppressive aetivity of 6-MP and of cyclophosphamide was also investigated: an i.p. injection of 50mg/kg of SKF 525-A one hour before the injection of the agents, failed to exert any significant effect on their immunosuppressive activity. Studies were also carried out to investigate the effect of phenobarbital pretreatment, at dosages able to modify its in vivo activity, on 6-MP plasma and spleen levels: as evidenced in Tables 13 and 14, no significant changes in the levels of the drug could be found in both compartments between phenobarbital pretreated and control animals.

Other investigations were also performed to establish whether liver microsomal preparations of phenobarbital pretreated animals did show any increased metabolic capacity for 6-MP in respect to untreated animals; the results obtained permitted to draw the conclusion that there was no significant difference in the metabolic degradation of 6-MP in animals given phenobarbital in respect to controls. Of possible interest are also results obtained in the course of these studies showing that while the immunosuppressive activity of cyclophosphamide was reduced by phenobarbital as employed in our system, no reduction in its anti-tumor efficacy was observed.

Studies are in progress to elucidate the possible mechanisms of the phenobarbital effects above described, as well as to extend our knowledge about the possible interactions of radiomimetic drugs with other frequently employed therapeutic agents.

ANALYTICAL METHODS

Of paramount importance for the studies on the pharmacology of radiomimetic agents included in this program, is obviously the availability of analytical methods combining high sensitivity and specificity for the determination in plasma and tissues of radiomimetic drugs and their possible metabolites. In the course of the last year , methods were developed or applied at this Institute for the determination of the following compounds - 6-aminochrysene, nitroso derivatives, procarbazine, adriamycin, cyclophosphamide and other alkylating agents - in the body.

6-Aminochrysene is measured in blood and tissues according to a spectrophotometric method set up at this Institute The estimation of 6-aminochrysene in biological material involves an extraction at neutral pH in n-benzol. The drug absorbed in the organic phase (98% recovery from an aqueous solution) is measured spectrophotometrically at 280 mμ where the compound exhibits a pronounced peak.
0.5 ml of urine or 2 ml of tissue homogenate 1/3 in KCl solution 1.15% are tranferred into a 15 ml glass tube. Sodium phosphate buffer (0.2 m pH 7.3) is added to the tube, to a final volume of 3 ml. The extraction is performed by the addition of 3 ml of n-benzol. The tubes are shaken for 20 min. and centrifuged. The supernatant organic phase is removed by aspiration and dried over anhydrous sodium sulphate. 2 ml of the dried extract are transferred into quartz cuvette and the optical density is recorded at 280 mμ. The optical densities are proportional between the concentrations of 0.1 and 5 μg/ml of n-benzol.

An optical density of approximately 0.100 is obtained when 1 µg of 6-aminochrysene is run through the procedure. The addition of 6-aminochrysene to biological material gives recovery of 89±5% from blood, 88±4 from urine and 70±5 from tissues.

Nitroso-derivatives (bischloroethylnitrosourea and methylnitrosourea) are measured in blood and tissues according to a spectrophotometric method described by Forist (13) and adapted to biological fluids. 0.5 ml of serum or 1 ml of tissue homogenate in 3 volumes KCl 1.15%, added with acetate buffer solution, are deproteinized with 0.2 ml of trichloroacetic acid 40% and treated as described by Forist.
Optical densities of approximately 0.252 for MNU and 0.110 for BCNU are obtained when 5 µg of each compound are run through the procedure.
The addition of these compounds to biological material, either blood or tissue homogenates, gives a recovery of 95±5% for MNU and 60±2% for BCNU.

Cyclophosphamide and other alkylating agents are measured in blood and tissues according to a spectrophotometric method as described by Friedman and Boger (14). The sensitivity is about 1 µg and the overall recovery 90±5 %.

Procarbazine is measured in blood and tissues according to a spectrophotometric method described by Oliverio et al. (15). The sensitivity is about 5 µg and the overall recovery 80±4%.

Adriamycin is measured in blood and tissues according to a spectrofluorimetric method described by Dusonchet et al. (16). After extraction with ethylacetate, the samples are read by exciting at 465 mμ and recording in emission the fluorescence values of 540 and 584 mμ. The results are linear between 1.6 and 3.2 ng of adriamycin, and the lowest limit of sensitivity is about 0.1 μg. The recovery for blood is 60±4% , while for tissues 80±3%.

Examples of the application of the above-mentioned methods are given in Tables 16 and 17 which show the blood and tissue levels of cyclophosphamide and methylnitrosourea in tumor-bearing mice.

In addition, a methodological approach allowing the in vitro discrimination of direct or metabolite-mediated cytotoxic activity of radiomimetics has been developed. Conventional in vitro methods, in fact, are not used in cases in which a transformation product of the drug, and not the drug itself -just like in the case of cyclophosphamide -, is responsible for the activity; this approach, on the contrary, gives a system more representative of the in vivo situation where the net final result can be the composite balance of the activities of the very drug and its metabolites. The method set up consists of adding actively metabolizing liver fractions to in vitro cultured cells in the presence of the drug under examination. In short, liver is homogenized 1/5 w/v in phosphate buffered solution (pH 7.4) and centrifuged at 9000xg for 20 min. in order to separate the liver microsomal fraction; the microsome-containing supernatant (1 ml is equivalent to 200 mg of liver homogenate) is added to test tubes containing the cell under test, be it cancer or normal cells, and microsomal cofactors are added (glucose 6-phosphate 10 mg;

TPN 1.2 mg, $MgCl_2 \cdot 6H_2O$ 2 mg; glucose 6-phosphate dehydrogenase 0.5 units). The total volume of the incubation mixture is 3 ml. Tubes are placed in a rotating apparatus at 37°C and after different lengths of incubation, the supernatant is removed sterilly and the cells washed once with PBS; 1 ml of medium 199 (with 1% antibiotics and 10% calf serum) is then added and the tubes incubated for a further period of 24 hr at 37°C. After this period, cells are removed with trypsin (0.25% in PBS), and their viability assessed. In Table 18, an indication of the liver microsomes metabolizing activity is given, in conditions where known substrates of liver microsomal enzymes, such as aniline and cyclophosphamide, are employed. Table 19 shows that cytotoxicity can be traced when the drug is incubated with liver homogenate although cyclophosphamide alone is not cytotoxic for KB cells in vitro.

Studies are in progress for the application of this approach in studying the immunosuppressive activity of radiomimetic agents in in vitro systems which are more similar to in vivo situation.

TABLE 1 Serum Levels of 6-Mercaptopurine (6-MP) after Intravenous Injections of Equimolar Doses of 6-MP or Azathioprine (Imur) in Mice

DRUG	DOSE (mg/kg)	6-MP (µg/ml ±S.E.) AFTER MINUTES				$T_{\frac{1}{2}}$ (min)[a]	Vd (l/kg)[a]
		1	5	15	30		
6-MP	50	79.1±2.4	35.4±1.8	14.1±2.3	2.2±1.0	6'15(4'45-7'45)	0.78(0.46-1.34)
Imur	81	57.4±2.5°	37.9±2.1	18.5±0.8	2.8±1.1	6'05(4'45-7'25)	0.65(0.46-0.89)
6-MP	100	120.6±3.7	59.1±2.2	47.5±1.6	31.5±1.6	22'05(18'40-25'30)	1.34(1.24-1.46)
Imur	163	113.9±4.1	96.3±3.3°	68.5±3.9°	35.7±2.9	19'00(17'15-20'45)	0.85(0.78-1.02
6-MP	150	267.8±5.8	116.1±4.0	80.5±1.4	75.5±5.4	22'00(17'50-26'10)	1.05(0.95-1.20)
Imur	244	203.9±0.1°	149.7±8.9°	111.3±7.0°	60.4±2.6	19'10(16'00-29'20)	0.83(0.74-0.93)

° $p < 0.05$ relative to corresponding levels of 6-MP

a= in parenthesis 95% confidence limits

Each figure is the average of 5 animals (Swiss male mice); results presented are the average of 3 successive experiments.

TABLE 2 Spleen Levels of 6-Mercaptopurine (6-MP) after Intravenous Administration of Equimolar Doses of 6-MP or Azathioprine (Imur) in Mice

DRUG	DOSE (mg/kg)	6-MP (μg/g±S.E.) AFTER MINUTES							$T_{\frac{1}{2}}$ (min)[a]	Vd l/kg[a]
		1	5	15	30	60	90	120		
6-MP	50	21.6±3.6	13.3±1.6	<5	<5	-	-	-	-	-
Imur	81	12.7±3.2	16.1±3.2	<5	<5	-	-	-	-	-
6-MP	100	44.3±5.2	84.6±4.4	39.6±3.3	22 ±2.3	11 ±2.7	<5	-	17'40(13'55-21'25)	1.17(0.99-1.38)
Imur	163	35.1±2.5	40.6±1.6	78.3±4.1°	20.9±4.5	6.7±2.1	<5	-	14'20(11'25-17'15)	0.76(0.50-1.13)
6-MP	150	75 ±8.1	100.4±3.2	40.7±1.0	23.5±1.7	14.3±2.1	11.1±2.3	8.3±2.6	21'20(20'00-22'20)	1.67(1.29-2.18)
Imur	244	32.7±1.1	57 ±1.5°	80.7±1.4°	38.0±1.9°	13.9±0.9	9.2±1.5	8.1±1.5	18'20(17'05-19'35)	1.12(1.01-1.24)

° $p < 0.05$ relative to the corresponding levels of 6-MP

a=in parenthesis 95% confidence limits

Each figure is the average of 5 animals (Swiss male mice); results presented are the average of 3 successive experiments.

TABLE 3 Comparison of the Immunosuppressive Activity of 6-Mercaptopurine (6-MP) and Azathioprine (Imur) after Intravenous Injections of Equimolar Amounts in Mice

6-MP 50 mg/kg		Imur 81 mg/kg
Controls	6-MP	Imur
548 (353-850)	223 (209-396)	268 (130-550)
1102 (564-1552)	334 (228-491)	443 (350-560)
643 (468-947)	392 (240-643)	304 (173-535)
6-MP 100 mg/kg		**Imur 162 mg/kg**
Controls	6-MP	Imur
304 (194-475)	63.5 (23-170)	203.5 (120-413)
548 (353-850)	77 (32-186)	238 (178-318)
779 (449-1352)	138.5 (67-282)	486 (180-1308)

continued

Table 3 - continued

	6-MP 150 mg/kg	Imur 243 mg/kg
Controls	6-MP	Imur
729	47	208
(581-946)	(23-73)	(143-297)
548	64	245
(408-693)	(19-115)	(162-327)
806	51	284
(513-1228)	(34-80)	(190-381)

7 Swiss male mice/group. Treatment: $4x10^8$ SRBC i.p. on day 0 drugs were given i.v. on day 2, spleen plaque-forming cells were assayed on day 4. Results are expressed in PFC/$1x10^6$ nucleated spleen cells; geometric means of logarithmic transformation of the data are presented, in parenthesis the value of the mean plus or minus one standard deviation. By the analysis of variance no statistically significant difference was observed between animals given the low dosages of 6-MP or Imur; significant difference was observed using the intermediate and high dosages of 6-MP and Imur: $p<0.05$ - $p<0.01$ respectively.

TABLE 4 Comparison of the Immunosuppressive Activity of 6-Mercaptopurine (6-MP) and Azathioprine (Imur) after Intravenous Injections of Equimolar Dosages in Mice (results are expressed as percent reduction from control values)

6-MP 50 mg/kg	Imur 81 mg/kg	6-MP 100 mg/kg	Imur 162 mg/kg	6-MP 150 mg/kg	Imur 243 mg/kg
6-MP	Imur	6-MP	Imur	6-MP	Imur
41%	49%	20.9%	66.9%	6.5%	28.5%
30.3%	40.2%	13%	45.6%	8.6%	31%
61%	47.3%	14.2%	43.5%	12%	45%
46%	53.2%	13.4%	42.0%	6%	33%

7 Swiss male mice/group. Animals were given $4x10^8$ SRBC on day 0; 48 hr later they were given the drug i.v. and spleen hemolytic plaque-forming cells were counted on day 4. Results are expressed as reduction in percent of PFC values in treated animals from PFC levels of control, untreated mice equal to 100%.

TABLE 5 Comparison of the Immunosuppressive Activity of 6-Mercaptopurine (6-MP) and Azathioprine (Imur) after Oral Administration of Equimolar Dosages in Mice

6-MP 200 mg/kg		Imur 324 mg/kg	6-MP 400 mg/kg		Imur 648 mg/kg	6-MP 600 mg/kg		Imur 976 mg/kg
Contr.	6-MP	Imur	Contr.	6-MP	Imur	Contr.	6-MP	Imur
871±96	698±86	422±71°	715±146	287±69	110±61°	594±125	150±31	67±31°
675±113	425±38	281±54°	843± 77	250±61	87±63°	748± 86	107±29	41±18°

° $p < 0.05$

Treatment : 7 mice/group were treated with SRBC 4×10^8 i.p. on day 0; 6-MP or Imuran were given in carboxymethylcellulose (1%) on day 2 and the number of antibody-forming cells counted on day 4. Results are expressed as geometric means of numbers of PFC/1×10^6 nucleated spleen cells after logarithmic transformation of the data.

TABLE 6 Comparative Immunosuppressive Activity of 6-Mercaptopurine (6-MP) and Azathioprine (Imur) Given Intraperitoneally in Equimolar Doses

6-MP 50 mg/kg	Imur 81 mg/kg	6-MP 100 mg/kg	Imur 163 mg/kg	6-MP 150 mg/kg	Imur 244 mg/kg	6-MP 200 mg/kg	Imur 326 mg/kg
766±107 (87%)	724±71 (83%)	489±85 (56%)	635±64 (72%)°	168±49 (22%)	355±81 (46%)	113±36 (14%)	296±54 (38%)

° $p < 0.05$

Treatment: SRBC 0.5 ml of 10% suspension i.v. on day 0; drugs given i.p. on day 2; hemolytic cells evaluated on day 4. Values expressed are the geometric means of logarithmically transformed data plus or minus one standard error; averages of 3 experiments, 5 rats per group. In parenthesis percent reduction for control, untreated rats.

TABLE 7 Blood and Brain Pentobarbital Levels 7 Days after Cyclophosphamide

	BLOOD (µg/ml ±S.E.) AFTER MINUTES					$T_{\frac{1}{2}}$	BRAIN (µg/g ±S.E.) AFTER MINUTES					$T_{\frac{1}{2}}$
	1	20	40	60	90	(min)	1	20	40	60	90	(min)
Controls	26.6 ±0.4	14.4 ±0.3	11;2 ±0.4	8.4 ±0.5	3.7 ±0.3	35	24.4 ±1.8	19.7 ±0.9	14.6 ±0.6	10.4 ±0.6	4.5 ±1	31
Cyclo-phospha-mide	26.6 ±0.3	19.3° ±0.3	14.3° ±0.3	11.3° ±0.2	7.4 ±0.5	50	28.1 ±1.9	22.7+ ±0.7	18.4° ±0.7	14.6° ±0.3	7.4 ±0.7	42

Pentobarbital : 25 mg/kg i.v.
Cyclophosphamide: 120 mg/kg i.v.
+ $p < 0.05$
° $p < 0.01$

TABLE 8 Effect of Phenobarbital (PB) Pretreatment on the Immunosuppressive Activity of Various Drugs

TREATMENT	DRUG	DRUG+PB
6-MP	154 (126-171)	53 ° (38-65)
Cyclo-P	24 (9-59)	110 ° (75-163)
Imur	363 (298-428)	347 (271-415)
ALS	62 (31-88)	54 (27-86)
DIC	114 (78-143)	78 (56-131)
Dauno	498 (354-608)	334 (192-485)

° $p < 0.05$

Treatment : SRBC $4x10^8$ i.p. on day 0; phenobarbital 80 mg/kg i.p. on the two day immediately preceeding drug injection; number of antibody-forming cells was assayed on day 4. Results are expressed as geometric means after logarithmic transformation plus or minus one standard error of PFC/$1x10^6$ spleen cells; at least two experiments, 7 mice/group.

6-MP = 6-mercaptopurine 100 mg/kg i.v. on day 2 ;

ALS = antilymphocytic serum 0.25 ml i.p. on day -1;

Cyclo-P = cyclophosphamide 25 mg/kg i.v. on day 2;

DIC = 5-(3,3 dimethyl-1-triazeno)imidazole-4-carboxamide 200 mg/kg i.p. on day 2;

Imur = azathioprine 100 mg/kg i.v. on day 2;

Dauno = daunomycin 10 mg/kg i.v. on day 2.

TABLE 9 Effect of Phenobarbital (PB) Pretreatment on the Immunosuppressive Activity of Various Drugs

TREATMENT	DRUG	DRUG+PB
MTX	62 (28-94)	54 (19-131)
PCZ	17 (6-31)	15 (9-28)
Hydrocort.	339 (287-415)	839 * (709-921)
5-Fu	88 (36-139)	77 (51-116)
HN_2	273 (191-356)	550 * (479-625)
BCNU	430 (381-509)	191 * (88-236)
TEM	15 (3-29)	17 (5-34)
6-CH_3-MP	379 (254-548)	306 (186-467)

* $p < 0.05$

Treatment: SRBC $4x10^8$ i.p. on day 0; phenobarbital 80 mg/kg i.p. on the two days preceeding drug injection. Results are expressed as PFC/$1x10^6$ spleen cells assayed on day 4; geometric means of at least 2 experiments (7 mice/group), in parenthesis the mean value plus or minus one standard error.

MTX = methotrexate 1 mg/kg i.v. on day 2;

HN_2 = mechloroethamine 1 mg/kg i.v. on day 2;

continued

Table 9 - continued

PCZ = procarbazine 250 mg/kg i.v. on day 2;
BCNU = 1,3 bis(2 chloroethyl)-1-nitrosurea 5 mg/kg i.v. on day 2;
Hydrocort.= hydrocortisone hemisucc. 400 mg/kg s.c. 14 hr before SRBC;
TEM = triethylenemelamine 2 mg/kg i.v. on day 2;
5-Fu= 5-fluorouracil 100 mg/kg i.v. on day 2;
6-CH_3-MP = 6-methylmercaptopurine 100 mg/kg i.v. on day 2.

TABLE 10 Effect f Phenobarbital Pretreatment on the Immunosuppressive Activity of 6-Mercaptopurine 6-MP)

6-MP 180 mg/kg	6-MP+PB	6-MP 50 mg/kg	6-MP+PB	6-MP 25 mg/kg	6-MP+PB	
231.6	79.2 ° (34.2%)	540	97.6 ° (37%)	636	814	n.s.
106	35.2 ° (33%)	218	76 ° (35%)	855	1098	n.s.
126	46.5 ° (36.5%)	500.6	134 ° (27%)	738	761	n.s.
154±17	53±16 ° (34.4%)	414±55	97±21 ° (30.2%)	737±190	888±251	n.s.

° $p < 0.05$

8 Male Swiss mice/group; phenobarbital 80 mg/kg i.p. on day 0 and 1; SRBC 4×10^8 i.p. on day 0; 6-MP i.v. on day 2; anti-SRBC hemolytic plaque-forming cells in the spleen counted on day 4. Values are expressed as geometric means of PFC/1×10^6 spleen cells after logarithmic transformation; in parenthesis, percent PFC of phenobarbital treated animals in respect to animals given only 6-MP.

TABLE 11 Effect of Phenobarbital (PB) and SKF 525-A Treatment on the Immunosuppressive Activity of Cyclophosphamide (Cyclo-P)

Cyclo-P	Cyclo-P + PB	Cyclo-P	Cyclo-P + SKF 525-A	
19 (12.6-28)	117 (62-172)	24 (13-39)	44 (15-78)	
30 (10-69)	103 (80-134)	86 (44-141)	117 (49-162)	
24 (11-46)	110 ° (75-163)	36 (9-64)	41 (16-80)	
24 (9 - 59)	110 ° (75 - 163)	48.5 (21 - 84)	63 (22 - 128)	n.s.

° $p < 0.05$

8 Male Swiss mice/ group; phenobarbital 80 mg/kg i.p. on day 0 and 1; SRBC $4x10^8$ on day 0; cyclophosphamide 25 mg/kg i.v. on day 2; SKF 525-A (2-diethylaminoethyl-2,2-diphenylvalerate HCl) 50 mg/kg injected i.p. 1 hr before cyclophosphamide administration; results are expressed as hemolytic plaque-forming cells per $1x10^6$ spleen cells on the 4th day after SRBC stimulation. In parenthesis the values of the geometric means minus or plus one standard error calculated after logarithmic transformation of the data.

TABLE 12 Effect of a Single Injection of Phenobarbital (PB) Injected at Various Times before or after 6-Mercaptopurine (6-MP) Administration on the Immunosuppressive Activity of 6-MP in Mice

6-MP day +2°	PB day -2°	PB day -1	PB day 0	PB day +1	PB day +2	PB day +3
421±97	525±131	368±78	327±146	467±93	331±81	440±107
443±103	515±64	474±55	388±109	415±86	453±98	397±118

° relative to SRBC injection

SRBC $4x10^8$ i.p. on day 0; phenobarbital 80 mg/kg i.p.; 6-MP 50 mg/kg i.v. on day +2.

Values are geometric means of PFC/$1x10^6$ spleen cells counted on day +4. 7 mice/ group.

continued

Table 12 - continued

Effect of Phenobarbital Injection on the Number of Plaque-forming Cells in Normal Mice

CONTROLS		PHENOBARBITAL-TREATED	
PFC/1x10^6	PFC/SPLEEN	PFC/1x10^6	PFC/SPLEEN
611±104	159,100±46,800	559±87	134,210±47,200
431±153	117,900±37,550	462±94	108,580±51,800
801±146	184,300±61,080	962±156	198,730±58,490
924±181	217,850±75,430	1026±203	226,500±61,250

SRBC 4x10^8 i.p. on day 0; phenobarbital 80 mg/kg i.p. on day 0 and 1; Values expressed are geometric means of PFC/1x10^6 and PFC/spleen assayed on day +4. 8 mice/group.

TABLE 13 Effect of SKF 525-A on the Immunosuppressive Activity of 6-Mercaptopurine (6-MP) in Mice

CONTROLS	6-MP	6-MP+SKF 525-A
965 (751-1108)	138 (70-272)	129 (79-224)
695 (497-781)	143 (68-197)	151 (103-186)

SRBC $4x10^8$ i.p. on day 0; 6-MP 100 mg/kg i.v. on day 2; SKF 525-A 50 mg/kg i.p. one hour before 6-MP injection.
Values are geometric means plus or minus one standard error after logarithmic transformation of PFC/$1x10^6$ spleen cells assayed on day 4.

Effect of Phenobarbital (PB) Pretreatment on the Immunosuppressive Activity of 6-Mercaptopurine (6-MP) in Secondary Stimulated Mice

CONTROLS	6-MP	6-MP+PB
1899±438	992±273	512±107 °
2332±391	1034±315	470±256 °

° $p < 0.05$
SRBC $4x10^8$ on day 0 and 10; 6-MP 100 mg/kg i.v. on day 12; phenobarbital 80 mg/kg on day 10 and 11.
Values expressed are PFC/$1x10^6$ spleen cells assayed on day 14.

TABLE 14 Spleen Levels (µg/g) of 6-Mercaptopurine (6-MP) in Control and Phenobarbital-Pretreated Mice

DOSE OF 6-MP	TREATMENT	1'	5'	15'	30'
100 mg/kg	6-MP	54.7±4.2	88.1±5.7	41 ±2.8	21.1±1.9
	6-MP + PB	43.9±1.9	71.9±4.8	38.4±1.7	23.8±1.2
50 mg/kg	6-MP	34.2±1.8	18. ±3.1	2.8±1.1	< 1
	6-MP + PB	27.7±4	10.1±2.9	2.5±1.2	< 1

Treatment: phenobarbital (PB) 80 mg/kg i.p. on day 0 and 1; 6-MP i.v. on day 2.
Results tabulated are the average of 3 experiments, each including 5 animals/point.

TABLE 15 Serum Levels (μg/ml) of 6-Mercaptopurine (6-MP) in Control and Phenobarbital-Pretreated Mice

DOSE OF 6-MP	TREATMENT	1'	5'	15'	30'
100 mg/kg	6-MP	137 ±8.9	69.5±5.9	38.8±3.2	30 ±2.9
	6-MP + PB	158.6±6.2	88.7±4	48.5±1.8	32 ±2.6
50 mg/kg	6-MP	67.2±3.8	31.7±1.5	15.4±2.3	1
	6-MP + PB	57.1±2.9	28.9±0.8	18.9±0.8	2.3±1.1

Treatment: phenobarbital 80 mg/kg i.p. on day 0 and 1; 6-MP i.v. on day 2.
Results presented are the average of 3 experiments, each including 5 animals/point.

TABLE 16 Tissue Levels of Methylnitrosourea (20 mg/kg i.v.) in Mice Bearing Ehrlich Carcinoma

	BRAIN MNU (µg/g±S.E.)AFTER MIN.						TUMOR MNU (µg/g±S.E.)AFTER MIN.						LIVER MNU (µg/g±S.E.)AFTER MIN.					
	1'	5'	10'	20'	40'	60'	1'	5'	10'	20'	40'	60'	1'	5'	10'	20'	40'	60'
Controls	20.84 ±0.4	10.26 ±0.4	7.52 ±0.2	5.00 ±0.2	2.5 ±0.2	< 0.50												
Ehrlich carc. i.c.	26.7° ±0.8	10.7 ±0.4	7.9 ±0.2	5.6 ±0.2	3.3 ±0.2	< 0.5												
Controls	23.7 ±1.2	10.4 ±0.6	6.7 ±0.5	2.9 ±0.2	1.6 ±0.4	<0.5												
Ehrlich carc. s.c.	26.3° ±0.9	18.3 ±0.7	6.9 ±0.4	2.9 ±0.2	1.8 ±0.6	< 0.5	1.3 ±0.3	2.7 ±0.4	2.7 ±0.2	1.9 ±0.5	<0.5	<0.5						
Controls	18.2 ±1.5	10.6 ±0.4	6.8 ±0.5	3.6 ±0.2	2.0 ±0.0								12.3 ±0.6	9.1 ±0.7	4.0 ±0.4	0.8 ±0.0	<0.5	<0.5
Ehrlich carc. i.p.	27.6° ±0.7	15.5+ ±0.9	9.6 ±0.7	5.4 ±0.7	2.8 ±0.5		5.1 ±0.4	8.8 ±0.4	8.2 ±0.4	4.3 ±0.8	1.4 ±0.2							

The experiment was performed 12 days after i.c. tumor transplantation (100,000 cells/0.01 ml); 16 days after s.c. tumor transplantation (5.000,000 cells/0.1 ml) ; 13 days after i.p. tumor transplantation (1.000,000 cells/0.2 ml).
i.c. = intracerebral i.p. = intraperitoneal s.c. = subcutaneous

° $p < 0.05$ + $p < 0.01$

TABLE 17 Blood and Tissue Levels of Cyclophosphamide (85 mg/kg i.v.) in Mice Bearing Ehrlich Carcinoma

	BLOOD (μmoles equiv.HN_2/ml±S.E.)					BRAIN(μmoles equiv.HN_2/g±S.E.)					TUMOR (μmoles equiv.HN_2/g±S.E.)				
	5'	10'	20'	40'	60'	5'	10'	20'	40'	60'	5'	10'	20'	40'	60'
Controls	216.2 ±20.3	246.8 ±14.8	173.2 ±20.6	50.6 ±12.9	93.9 ±13.2										
Ehrlich carc. i.p.	227.9 ±25.8	240.9 ±14.6	155.7 ±10.2	121.0° ±22	109.1° ±16.4						21.9 ±2.6	34.9 ±1.9	60.4 ±1.7	57.7 ±3.1	49.9 ±5.9
Controls	239.4 ±24.4	258.8 ± 5.1	220.8 ±22.9	140 ± 6.3	53 ±7										
Ehrlich carc. s.c.	238 ±11.1	307.4 ±24.1	248 ±10	93.4° ±4.9	27° ±2						48.9 ±4.8	51.1 ±4.1	66.7 ±7.8	56.7 ±4.1	37.4 ±2.7
Controls	187.2 ± 9.1	274.4 ±19.4	309.6 ± 7.5	149.6 ±12.9	62 ±2	49.8 ±5.8	42.2 ±3.2	39.0 ±3.6	21.4 ±5.9	13.0 ±2					
Ehrlich carc. i.v.	206.7 ±36.8	261 ± 7	288 ± 9.6	138.4 ±10.3	69.6 ±6.5	57.8 ±8.8	43.6 ±5.8	75.0 ±9.2	32.6° ±4.7	24.6° ±3.9					

The experiment was performed 12 days after i.c. tumor transplantation (100,000 cells/0.01 ml); 16 days after s.c. tumor transplantation (5.000,000 cells/0.1 ml); 13 days after i.p. tumor transplantation (1.000,000 cells/0.2 ml).
i.p. = intraperitoneal s.c. = subcutaneous i.c. = intracerebral

° $p < 0.05$ • $p < 0.01$

TABLE 18 In Vitro Metabolism of Aniline (5 µmoles) and Cyclophosphamide (600 µg) with 9000xg Liver Fraction of Male Mice

		CONTROLS	EHRLICH i.p.
mumoles p-aminophenol/30 min ±S.E.			
	50 mg liver	63.8±8.9	
(°)	100 mg liver	130.7±10.4	
	200 mg liver	215.8±6.0	
mumoles equiv.HN_2 /45 min ±S.E.			
	200 mg liver	282.0+18	158.7±15 *

9000xg liver fraction equivalent to different amounts of liver were incubated in a total volume of 3 ml with cofactors as described under Methods.

(°) In the experimental conditions usually employed for testing the microsomal activity 100 mg liver of male mice metabolize 111.0±4.4 mumoles of p-aminophenol.

* $p < 0.01$

TABLE 19 Cytotoxic Effect of Cyclophosphamide in Vitro

KB CELLS AT To ($x10^3$ cells)	CELL GRWOTH AFTER 24 HR ($x10^3$ cells)			
	Controls	Cyclo-P	Microsomes°	Microsomes + Cyclo-P
101	293 ± 12	236 ± 21	309 ± 15	28*

* $p < 0.01$ relative to the other groups

° Mouse liver homogenate (9000xg) plus cofactors, incubated for 45 min. in presence of cyclophosphamide (Cyclo-P) 100 µg/ml. Inhibition: 89%.

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5.4. RADIOBIOLOGICAL INSTITUTE TNO, Rijswijk (ZH)

Prof. Dr. D.W. van BEKKUM

SCIENTIFIC REPORT 1971

Contract Euratom-EORTC nr. 079-69-1 BIAC

I. Histocompatibility research in Rhesus monkeys

The program of selecting phenotypically identical Rhesus monkeys was started in early 1971. A limited number of transplantations of bone marrow was performed between "incomplete phenotypically identical" M. rhesus monkeys, meaning that all the major identifiable leukocyte ag. antigens between the non-related host and donor were identical. In these cases, the severity of acute secondary disease was similar to that seen following the use of non-related marrow donors. Several groups of well-matched animals were selected for bone marrow transplantation, using ALS or stem cell separation as adjuvant therapy. From the first few experiments the impression was gained that the use of identical donor-marrow leads to a slightly milder form of delayed secondary disease in primates. These pilot experiments will be continued. Selection of donors will henceforth be done using groups of sera that were internationally accepted as defining antigens of the Rhesus monkey's major histocompatibility system. These results were exhaustively discussed and compared during an International Workshop and Symposium on Transplantation Genetics of Primates, organized in Rijswijk, September 1971. The Proceedings of this Symposium are in press and will be published in a separate issue of Transplantation Proceedings (1972).

In the course of this year, the mixed lymphocyte culture technique was introduced and adapted to monkey leukocytes and is being perfectionated

to further improve the selection of compatible individuals.
As a refinement in tissue typing of monkeys, the micro-complement fixation method is currently used in routine testing (in addition to cytotoxicity). Moreover, red cell typing has reached a stage whereby separate antigens of internationally accepted red cell systems can be identified. (These, as well as red cell enzymes can be used to determine chimerism in phenotypically identical host/donor combinations.)

Finally, the increased number of TNO raised Rhesus families will make it possible to begin bone marrow transplantation studies using full sibs (of various degrees of compatibility) as bone marrow donors.

II. Mitigation of secondary disease following grafting of allogeneic bone marrow

It was reported last year that concentrated stem cell preparations from monkey bone marrow which are deprived of PHA responsive cells, are capable of completely repopulating lethally irradiated allogeneic recipients, without concomitant development of acute secondary disease. The recipients do develop the delayed form of secondary disease, starting between 24 and 30 days following the grafting, similar to the syndrome which develops in lethally irradiated mice treated with allogeneic bone marrow. A comparable delay of secondary disease

can be obtained by pretreating the recipients with large doses of ALS shortly prior to the grafting of whole bone marrow from allogeneic donors. A combination of the two procedures namely pretreatment with ALS and grafting of concentrated stem cells did not further delay or diminish the secondary disease. This finding supports the hypothesis that the delayed secondary disease is initiated by lymphoid cells developing from precursor cells after grafting, while the acute form of secondary disease is initiated by immunocompetent cells which were present in the grafted material. The development of competent cells from precursors requires -at least in the mouse- the presence of a functional thymus. Therefore, these earlier findings in mice are being repeated and extended with the introduction of stem cell concentrates and the role of the thymus in the development of delayed secondary disease in primates is being investigated by performing concentrated stem cell grafts in thymectomized lethally irradiated monkeys.

Further studies were performed on the differences between ALS raised in rabbits and ALS raised in horses with regard to the killing effect of these sera on hemopoietic stem cells. From in vivo studies with grafted monkeys, it became evident that rabbit sera were outright toxic to stem cells so as to prevent the take of bone marrow grafts.

The problem was first studied more extensively with the mouse model for bone marrow grafting and with various anti-mouse lymphocyte sera. Here the difference between rabbit and horse sera with regard to toxicity for stem cells was only minimal when measured by the CFU assay following incubation of mouse bone marrow with the sera in the presence of complement. As during the past year the cloning technique for measuring hemopoietic stem cells of monkey bone marrow has become practicable, the in vitro toxicity of ALS preparations for monkey stem cells is now being studied quantitatively. This investigation should indicate whether a useful method can be developed for the testing of anti-human lymphocyte sera for their possible toxicity for hemopoietic stem cells and secondly whether in vitro incubation with ALS offers possibilities for the avoidance of acute secondary disease.

Preliminary experiments were performed both in rats and in monkeys with passive enhancement as a possible means of preventing acute secondary disease. In the rat model, the sera were specifically active against the recipient strain and were of proven effectiveness in cardiac and kidney grafting situations. Nonetheless no beneficial effect in the course of the secondary disease was achieved following treatment with these sera. In the monkey the sera were not host-specific and so far have shown no mitigating activity with regard to acute secondary disease. Further attempts can now employ specific donor-anti-host sera, because our technical possibilities have been greatly

extended by the effective preservation method (see below).

III. Stem cell identification and cloning of stem cells.

Following the tentative morphological description of the hemopoietic stem cell of the mouse, the identification of the monkey hemopoietic stem cell has been pursued employing similar methodology. Instead of pretreating the bone marrow donors with anti-mitotic agents, refractionation of gradient fractions has been pursued, which has resulted in roughly 40-fold concentration of stem cells. These preparations are being scanned by light- and electron microscopical methods for the presence of cells with similar morphological characteristics as the mouse stem cells.

Our in vitro cloning method of bone marrow cells has been subjected to a number of tests designed to establish the nature of the in vitro colony forming cells. All the evidence available points to the hemopoietic stem cell as being the colony former. These results have been extensively compared to those obtained by other groups with different tissue culture systems during a Workshop/ Symposium organized in the Radiobiological Institute TNO on the subject of In Vitro Culture of Hemopoietic Cells, September 29-October 5, 1971.

Similar in vitro systems have been developed from the cloning of monkey and human bone marrow stem cells and these systems are

currently employed in both experimental and clinical bone marrow transplantations. The self-replication of stem cells in such cultures is a matter of considerable interest because it may provide the opportunity to increase the number of stem cells available for grafting of patients, in those instances where large numbers of stem cells are required, and when only limited numbers of purified stem cells can be obtained from the donor. Various modifications of the culture conditions are being explored in attempts to increase the yield of stem cells following in vitro culturing.

IV. Preservation of bone marrow and stem cell preparations at low temperatures

Although several authors have reported takes of frozen and thawed bone marrow, a quantitative analysis of the results has only been performed in the mouse and the monkey. With mouse bone marrow the usual cryoprotective agents were found to provide excellent protection although considerable variation was seen between the results of different groups. With monkey bone marrow the loss of viable cells was about 50% when the preserved material was grafted into autologous hosts,and into allogeneic hosts preserved bone marrow grafts were never capable of restoring hemopoiesis, even when the number of grafted cells was 8 times that required with fresh bone marrow. These difficulties have now been analyzed by the use of in vitro cloning capacity of the cells as a parameter for "viability".

This approach has revealed that the damaging step in the preservation procedure was the rapid removal of the cryoprotective agent after thawing. By changing this step into a very gradual dilution procedure, nearly complete preservation of viability has been obtained, as confirmed by in vivo results with preserved marrow.

This dependable preservation method has provided a number of new possibilities, one of which has already been applied to clinical bone marrow transplantation. Purified stem cells are now being preserved until the results of the PHA response of the fractions -which takes 4-5 days- have become available. The technique also allows repeated transplantations from one batch of preserved cells and has numerous applications both for experimental and clinical aspects of bone marrow grafting.

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5.5.

Contrat n°: 079-69-1BIAC.

Organisme ou Institution, Lieu, Pays : Institut Jules Bordet, Brussels, Belgium.

Noms : P. Stryckmans, J. Manaster, G. Delalieux.

Thème, titre : DNA repair after ultraviolet irradiation and after alkylating agents.

Description générale succincte des travaux accomplis :

Corticosteroids are very effective in the treatment of human acute lymphoblastic leukaemia when used in association with chemotherapic agents. This observation introduces two questions :

a) Since corticosteroids were shown to decrease the flux of G_1 leukaemic cells entering DNA synthesis per unit of time, are these hormones capable also of inhibiting the capacity of repair DNA synthesis which follows ultraviolet irradiation?

b) Since many studies suggest that repair processes take place in the cells after their exposure to alkylating agents, is it possible to demonstrate a process of DNA excision similar to the one described after UV irradiation?
To answer this question, the DNA of the cells was labelled and the cells were thereafter exposed in vivo to cyclophosphamide.

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The Potentiality of Out-of-cycle Acute Leukemic Cells to Synthesize DNA. Blood 36 : 697-703, 1970.

G. Delalieux, P. Stryckmans, J. Manaster and R. Badjou.
DNA Excision Process in Blood Lymphocytes and Mature Granulocytes Exposed to Ultra-violet Light. Submitted to Blood.

5.5.

Projet n° : Contract n° 079-69-1 BIAC.

Titre : DNA repair after ultraviolet irradiation and after exposure to alkylating agents.

Noms des chercheurs : P. Stryckmans, J. Manaster, G. Delalieux.

Description des résultats :

1. The effect of prednisone on the process of post UV DNA repair.

a) In vivo study was performed on : 5 normal subjects, 2 patients with acute myeloblastic leukemia (AML), 1 patient with acute lymphoblastic leukemia (ALL), 1 patient with chronic lymphocytic leukemia (CLL), 2 patients with lymphosarcoma (LS) and 1 patient with chronic myeloid leukemia (CML). 40 mg/m^2 body surface of methyl prednisolone natrium succinate was injected IV. The capacity to incorporate ^{3}HTdR into the nucleus after UV irradiation was measured on autoradiographies from peripheral blood drawn prior to and 2 hours after the injection of hydrocortisone. The effect of prednisone on the DNA repair process was evaluated on lymphocytes in the normal, LLC and LMC patients and on leukemic cells in ALL, AML and LS patients. A considerable decrease of ^{3}HTdR uptake was observed in 4 normal patients out of 5, in 1 ALL out of 1, in 1 LLC out of 1, in 1 LMC out of 1.

b) In vitro study. The effect of hydrocortisone natrium succinate (5, 1 and 5 γ/ml) on the post-UV DNA repair process was investigated in vitro during a 2 hour incubation period. The study was performed on the blood of 4 CLL patients, 2 LMC patients, 2 ALL patients. A decrease of ^{3}HTdR uptake of 10 to 30% of the control samples (without hydrocortisone) was observed on ARG and by scintillation counting only in 2 patients with CLL.

The discrepancy between the in vivo and in vitro studies may have several explanations which are not elucidated yet. A) The decrease observed in vivo may result from a different distribution of the blood cells as a consequence of cortisone. In fact, the blood cells examined before and after cortisone may not be the same. B). on the other hand the in vivo experiment may be necessary for the action of prednisone which can not be observed in vitro.

2. In vivo effect of cyclophosphamide on prelabelled DNA

Two patients with an elevated number of leukemic cells in the peripheral blood were studied during the second relapse of their disease. Tritiated HTdR (0.1 mC/kg of body weight) was given IV. Two days later, at the expected maximum percentage of labelled blast cells in the blood, and 4 days later when the percentage of labelled blast cells was presumably still easily measurable, respectively 5 or 10 and 10 or 20 mg/kg of body weight of cyclophosphamide was injected IV. In order to detect eventual loss of DNA fragments, the mean grain count of the labelled cells was determined on autoradiographies several times just before and several times just after the injection of cyclophosphamide for a period of 4 hours. The results of patient (a) only are availably at present. A decrease in the radioactivity of the leukemic blood cells was observed during the hours following cyclophosphamide.

6. Administrative report

This Association Contract No. 079-69-1 BIAC is governed by a steering committee. The committee met twice in 1971 to execute the essential business. However, scientific coordination between the laboratories participating in this contract and other affiliated institutions has been executed at several meetings.

The heads of all contracting institutions belong to the Council of the European Organization for Research on Treatment of Cancer. This Council met in 1971 5 times in Brussels, Zurich, Paris, Rome, and Paris.in order to promote scientific cooperation in Europe. The Project Group on Clinical Gnotobiology has entered the stage of collaborative research and has founded a bacteriological typing centre and a virological and parasitological centre for antibody determination. The stem-cell club met 2 times during 1971 and is presently engaged in investigating the possibility of collaborative projects. The desire to actually coordinate stem-cell research was catalysed greatly by a stem-cell workshop in Rijswijk in October 1971 in which not only all groups of this contract met but a large number of overseas experts participated.

There is no doubt that the present Association Contract has been able to increase the coordination and cooperation of European research in a field relevant for the detection and treatment of radiation injury in man.

ASSOZIATION

Europäische Atomgemeinschaft - EURATOM
Gesellschaft für Strahlen- und Umweltforschung mbH-GSF

S t r a h l e n h ä m a t o l o g i e

Jahresbericht 1971

von

W. STICH und St. THIERFELDER

Bericht abgefaßt vom Institut für Hämatologie und der Abteilung Immunologie, Gesellschaft für Strahlen- und Umweltforschung-GSF, München, Deutschland, Assoziation Nr. 031-64-I BIAD

INSTITUT FÜR HÄMATOLOGIE
(Leiter: Prof.Dr.W.Stich)

Einleitung

Im Jahr 1971 wurden die wissenschaftlichen Untersuchungen entsprechend den im Anhang I des Assoziationsvertrages Strahlenhämatologie zwischen EURATOM und GSF niedergelegtem Forschungsprogramm und dem in der Zwischenzeit dem Stand unserer Kenntnisse angepaßten und ergänzten Forschungsprogramm 1971 durchgeführt.

Im Berichtsjahr galt das Forschungsprogramm im wesentlichen den drei Hauptproblemen Blutzellbildung und Blutstoffwechsel, Leukämie und Blutersatz.

Das Forschungsprogramm des Instituts wurde in klinischer und experimenteller Forschung durchgeführt.

Im Berichtsjahr gehörten dem Institut 59 Mitarbeiter, darunter 27 promovierte Wissenschaftler, 1 Gastwissenschaftler (Israel) und 4 Medizinalassistenten an. Außerdem waren 15 Doktoranden tätig.

ARBEITEN UND ERGEBNISSE

Das Forschungsprogramm 1971 wurde dem Stand der Kenntnisse und der neueren Entwicklung der Hämatologie und ihrer Grenzgebiete angepaßt. Für die Bearbeitung der drei Hauptprobleme Blutzellbildung, Leukämie und Blutersatz wurde entsprechend ihrer Größe und Bedeutung die Form der Projektforschung gewählt.

Blutzellbildung und Blutstoffwechsel

Auf dem Gebiet der Biosynthese des Hämoglobins und ihrer klinisch

bedeutsamen Störungen wurde die Bleivergiftung und ihre Wirkungen auf die Hämsynthese weiter untersucht. Zur Früherkennung der Bleivergiftung wurden zwei Schnellnachweise der Delta-Aminolävulinsäure - deren Ausscheidung im Urin den feinsten Indikator der Bleiaufnahme und der Bleivergiftung darstellt - entwickelt (D .SCHMIDT und W.STICH`). Die erste Methode stellt eine semiquantitative Schnellprobe und die zweite Methode eine einfache quantitative Bestimmung der Delta-Aminolävulinsäure im Urin dar. Beide Methoden sind zur Routineanwendung bei Blei-Exposition und Blei-Intoxikation geeignet. Eine spezielle Studie galt dem Krankheitsbild der erythropoetischen Protoporphyrie (D.SCHMIDT und W.STICH). Dabei konnte ein gegenüber der Norm um das 1300fache erhöhter Porphyringehalt und eine um das 15fache erhöhte Delta-Aminolävulinsynthethase-Aktivität in der Leber nachgewiesen werden. Diese Befunde sprechen entgegen der bisherigen Auffassung einer alleinigen Störung der erythropoetischen Hämsynthesestörung für eine gleichzeitige und beträchtliche hepatische Hämsynthesestörung (erythrohepatische Porphyrie !).

Die Untersuchungen über den Hämstoffwechsel unter Einsatz von synthetischem ^{14}C-Bilirubin wurden mit speziellen Studien über die ^{14}C-Bilirubinclearance bei Leberzirrhose (M.SCHMIDT, J.EISENBURG und W.STICH), die hepatische Aufnahme und Konjugation von ^{14}C-Bilirubin bei der chronischen lymphatischen Leukämie (M.SCHMIDT und W.STICH), die hepatische Bilirubinaufnahme und Bilirubinelimination bei unbestrahlten Kaninchen und nach Bestrahlung mit Cs-Gammastrahlen (M.SCHMIDT, F.SOUCHON, H.KRIEGEL und W.STICH) und die hepatische Aufnahme und Konjugation von ^{14}C-Bilirubin bei hochdosierter intravenöser 4-Stunden-Bilirubindauerbelastung von gesunden Menschen (M.SCHMIDT u. W.STICH)fortgesetzt In diesen Untersuchungen konnte erstmals mit einer hochleistungsfähigen Isotopenmethode nachgewiesen werden, daß für die hepatische Aufnahe und Konjugation von Bilirubin eine große Leistungsreserve

besteht. Bei einer normalen hepatischen Bilirubinelimination von ca. 250 mg pro Tag betrug die Bilirubinelimination bei Höchstdosisbelastung etwa das 60fache der Norm. Unter pathologischen Verhältnissen stellen die Halbwertszeiten der hepatischen Aufnahme und Elimination von ^{14}C-Bilirubin weitere Parameter der Hämstoffwechselstörung dar. Bei Ganzkörperbestrahlung nimmt die Bilirubinelimination erst bei Dosen von 1400 - 2100 R signifikant ab, bei 2100 R beträgt sie etwa 50 % der Norm.

Die Untersuchungen über die Hämopoese im menschlichen Knochenmark in vitro wurden intensiv fortgesetzt. Eine spezielle Studie über "The Kinetics of the Normal, the Megaloblastic and the Sideroachrestic Erythropoiesis estimated by Colchicine Blocking in vitro" wurde abgeschlossen. Der Mitosestopp durch Colchizin führt bei normaler Erythropoese zur verzögerten Reifung, denn die nächste Reifungsstufe kann nicht ohne reguläre N-2n-Teilung erreicht werden. Die gesteigerte Regeneration bei Megalopoese kann durch einen erhöhten Mitose- und Stathmakinese-Index, eine verkürzte Zellverdoppelungszeit und eine Verlagerung des Teilungs-Pool zu den oxyphilen Erythroblasten erklärt werden. In einer weiteren Studie wurde die Wirkung der Immunosuppressiva 6-Mercaptopurin und Azathioprin auf menschliches Knochenmark in vitro analysiert (I.BOLL, J.KLIMAS und B.WILLIGERODT). Schon 5 µg/ml beider Substanzen wirkten zytotoxisch. Bei 1 µg/ml zeigte sich eine Verminderung der Mitosehäufigkeit, 5 µg/ml beeinträchtigen die Regeneration des Knochenmarks nur vorübergehend und förderten die Reifung der Granulozytopoese signifikant. Weitere Studien galten der Bestimmung der absoluten Zellzahl menschlichen Knochenmarks unter Koagulum-Kultur-Bedingungen (H.P.RAMDOHR), den Erythroblasten- und Megaloblasten-Kerngrössen in Ausstrichen von Knochenmarkkulturen (Chr.GEBAUER) und der Wirkung von L-Asparaginase (U.RECKERS), 400 Rad schnellen Elektronen des 35 MeV Betatrons (J.CIARKOWSKI) sowie von Alkohol (J.WILDANGER) auf menschliches Knochenmark in vitro. Die Knochenmarkskultur hat sich dabei als

geeignete Methode für das Studium der Morphologie und Proliferationskinetik der Hämopoese unter der Einwirkung toxischer und therapeutischer Maßnahmen gezeigt.

Auf dem Gebiet der Zellphysiologie wurden bei den Untersuchungen über "Photometric Methods in quantitative Autoradiography" (P.DÖRMER) unter besonderer Berücksichtigung der eigenen methodischen Entwicklungen und Ergebnisse der gegenwärtige Stand dieses Gebietes dargestellt. Eine weitere Studie galt der quantitativen ^{14}C-Autoradiographie einzelner Zellen (P.DÖRMER und W.BRINKMANN).

Auf dem Gebiet der Knochenmarkhistologie wurden die Ergebnisse der Auswertung von 2003 eigenen Myelotomien in einer Arbeit über die diagnostische Aussage der Myelotomie (R.BURKHARDT und E.BEIL) zusammengestellt. Dabei ergab sich, daß 395 Fälle ausschließlich und weitere 847 Fälle mit wesentlicher Hilfe der Myelotomie geklärt werden konnten. Der Vergleich der histologischen und klinischen Ergebnisse führt bei 31 Krankheitsgruppen zu einem prospektiven Urteil über die diagnostische Aussage der Myelotomie. Eine weitere Arbeit galt der Knochenbiopsie (R.BURKHARDT). Dem bereits 1970 erschienenen Farbatlas der klinischen Histomorphologie von Knochenmark und Knochen (R.BURKHARDT) folgten inzwischen eine französische, italienische, spanische und japanische Ausgabe, eine englische befindet sich in Vorbereitung. Das Buch gilt bereits als führendes Standardwerk der normalen und pathologischen Histomorphologie des menschlichen Knochenmarks.

Leukämie

In Fortsetzung früherer Untersuchungen über die neue Enzymtherapie der Leukämien mit L-Asparaginase konnten die Studien über die unter Asparaginase-Therapie beobachtete Immunglobulinvermehrung und Antikörperbildung abgeschlossen werden (R.LAMERZ, A.FATEH-MOGHADAM, B.HORNUNG und H.EHRHART). Bei 12 Patienten mit akuter

und chronischer Leukämie und soliden Tumoren, darunter 4 Patienten mit symptomatischem Antikörpermangelsyndrom (AMS), konnte unter E.coli-Asparaginase-Therapie eine reversible Vermehrung der Immunglobuline IgG, IgA und IgM im Serum nachgewiesen werden. Dieser Effekt blieb dagegen bei einem idiopathischen und einem schweren symptomatischen AMS bei IgG-Paraproteinämie unter niedriger Asparaginase-Dosierung aus. Bei 11 der 14 Patienten ließen sich ab dem 7.Tag nach Beginn der Therapie präcipitierende und bei 8 Patienten komplementbindende Antikörper gegen Asparaginase nachweisen. Die unter Asparaginase-Therapie auftretende Immunglobulinvermehrung stellt eine unspezifische Reizwirkung der Asparaginase auf die Immunglobulin-bildenden Zellen dar. Eine weitere analytische Studie galt dem NAD-, ATP- und Lipidgehalt in normalen menschlichen Leukozyten, bei Leukämien und unter cytostatischer Therapie (H.EHRHART u.P.SCHWANDT). Beim 15.Kongreß der Deutschen Gesellschaft für Hämatologie berichtete H.EHRHART in einem Referat "Epidemiologie, Differentialdiagnose und Diagnose der chronischen myeloischen Leukämie" über die Erfahrungen unserer Münchner Gruppe. Auf dem Gebiet der akuten Leukämien wurden die Studien über die licht- und elektronenmikroskopische Morphologie und Zytochemie der Monozytenleukämie (D.HUHN, F.SCHMALZL u.K.DEMMLER) und über die Zytochemie, Elektronenmikroskopie und Zytogenetik der unreifzelligen myeloischen Leukämie (D.HUHN, F.SCHMALZL u.U.KRUG) in enger Zusammenarbeit mit der Medizinischen Universitätsklinik Innsbruck/Österreich (Vorstand: Prof.Dr.H. BRAUNSTEINER) abgeschlossen. Unsere Ergebnisse stützen die Annahme, daß die Monozytenleukämie eine Sonderform einer leukämischen Entartung der granulozytären Zellreihe darstellt. Die leukämischen Monozyten lassen sich gegenüber den Zellen anderer Leukämieformen abgrenzen. Es lassen sich außerdem promonocytäre, paramonocytäre Formen unterscheiden. Saure Phosphatase und Peroxidase ließen sich auch im feinstrukturellen Bereich (endoplasmatisches Reticulum, Golgi-Apparat, Granula) darstellen. Bei den unreifzelligen myeloischen Leukämien konnte gezeigt werden, daß die

leukämische Entartung der Zellen zu bestimmten morphologischen Besonderheiten führt, welche zum Teil generell bei Malignomzellen anzutreffen sind: Vergrösserung der Kernoberfläche durch Kerntaschen, locker angeordnetes Chromatin und große Nukleolen im Rahmen einer intensiven DNS- und Eiweiß-Synthese sowie großes Golgi-Feld, Anhäufung großer, pathologisch veränderter Mitochondrien, Fehlentwicklung azurophiler Granula zu Auer-Stäbchen.

Die Ergebnisse der vergleichenden klinischen, lichtmikroskopischen, elektronenoptischen, zytochemischen und zytogenetischen Studien bei allen Formen der unreifzelligen Leukämie wurden von D.HUHN in seiner Habilitationsschrift vorgelegt. In einer Studie über Gefäßveränderungen im Knochenmark bei Myelofibrose und Osteomyelosklerose (K.DEMMLER u.R.BURKHARDT) wurden 6 Patienten mit megakaryozytärer Myelose ohne Markfibrose, 10 mit megakaryozytärer Myelose mit starker Markfibrose und 19 mit Osteomyelosklerose qualitativ und quantitativ untersucht und einem Normalkollektiv gegenübergestellt. Die qualitativen Gefäßveränderungen waren in allen Gruppen ziemlich einheitlich. Dagegen wiesen die Gefäßmuster deutliche Unterschiede auf. Mit fortschreitendem myeloproliferativem Prozeß vermehren sich die arteriellen Gefäßabschnitte, vor allem im kapillären Bereich, während die venöse Kapillarisierung, repräsentiert durch die Sinus, nur bei gleichzeitig vermehrter Knochenbildung eine Vermehrung zeigte. Die umfassenden Untersuchungen über die Wirkung von heterologem Antilymphozytenglobulin bei chronischer lymphatischer Leukämie (H.PFISTERER, K.LANI, K.DEMMLER, S.THIERFELDER, A.FATEH-MOGHADAM, W.LAND, W.BRENDEL u.W.STICH) wurden abgeschlossen. Heterologes Antilymphozytenglobulin (ALG) wurde in hoher Dosierung bei 4 Patienten mit chronischer lymphatischer Leukämie geprüft. Die Agglutinationstiter des Serums - gewonnen durch Immunisierung von Pferden mit peripheren Lymphozyten lymphatischer Leukämien oder mit Lymphozyten aus Tonsillen gesunder Menschen - lagen zwischen 1:256 und 1:16000. Als Gesamtdosen wurden 50 - 390 ml, als Einzeldosen bis zu 40 ml intravenös appliziert. Außer einer peripheren

Lymphopenie konnten keine Zeichen einer Remission (Lymphknoten, Milz, Leber, Knochenmark) festgestellt werden. Allerdings mußte der Therapieversuch bei allen Patienten wegen allergischer Reaktionen, Venenreizung und Thrombopenie vorzeitig nach 5, 7, 20 und 34 Tagen abgebrochen werden. Erst wenn ALG in hohen Dosen über lange Zeit appliziert werden kann, läßt sich endgültig entscheiden, ob es bei chronischer lymphatischer Leukämie zur Therapie brauchbar ist.

Hämosubstitution

Die Untersuchungen auf dem Gebiet der Bluttransfusion und gezielten Hämotherapie wurden fortgesetzt. Besonderes Interesse galt der Entwicklung von geeigneten Isolierungsmethoden von Blutzellen mit dem elektronischen NCI-IBM Zellseparator und von kryobiologischen Methoden zur Tiefkühllagerung von Blutzellen sowie dem Studium der Transfusion von vitalen Granulozyten und Thrombozyten unter Berücksichtigung immunologischer Gesichtspunkte.

Bei der Leuko- und Thrombopoese bei Gesunden mit dem NCI-IBM-Blutzellseparator (K.LANI, H.PFISTERER, W.RUPPELT, H.BOLLAND und W.STICH) wurde die optimale Ausbeute an Thrombozyten, Lymphozyten und Granulozyten untersucht. Die Ausbeuten betrugen für Thrombozyten $4{,}8 \times 10^{11}$ (Entnahmedauer 2 Std.), für Lymphozyten $3{,}55 \times 10^{9}$ (Entnahmedauer 2 Std.) und für Granulozyten $1{,}5 \times 10^{9}$ (Entnahmedauer 1/2 Std.). Durch Verwendung eines ACD-Heparin-Gemisches an Stelle von ACD-Stabilisator konnte die Ausbeute bis zu 10^{10} Granulozyten gesteigert werden, so daß rein quantitativ die Voraussetzung für die Granulozytentransfusion gegeben ist. Die bereits auf dem XIII. International Congress of Hematology mitgeteilten "Studies on the Separation of Granulocytes for Transfusion from normal Persons by the IBM Cell Seperator (K.LANI, H.PFISTERER, W.RUPPELT, H.BOLLAND und W.STICH) wurden inzwischen ausführlich publiziert. Eine weitere Studie galt der Verkürzung der Lebensdauer $DF^{32}P$-markierter Granulozyten nach Isolierung mit Dextran

oder Ammoniumchlorid (H.BOLLAND, H.PFISTERER, W.RUPPELT und G.MICHLMAYR). Gegenüber einer HWZ von 6,6 Std. bei der Kontrollgruppe (ACD-Vollblut) zeigte sich bei Dextran eine Verkürzung auf eine HWZ von nur 1,6 Std. und bei Ammoniumchlorid waren nach 3 Minuten nur mehr 2 % der Granulozytenaktivität nachweisbar. Somit kann die Gewinnung von Granulozyten zu Transfusionszwecken durch Hämolyse der Erythrozyten mit Ammoniumchlorid wegen starker Zellschädigung als unbrauchbar, durch Sedimentation der Erythrozyten mit Dextran als nur bedingt angesehen werden. Das derzeit beste Verfahren der Granulozytenisolierung für Transfusionszwecke ist die Isolierung mit dem elektronischen NCI-IBM Blutzellseparator unter Verwendung von Heparinals Anticoagulans. In einer Studie "Increase of Platelets after Platelet Transfusion" (H.PFISTERER, W.STRECK, W.RUPPELT, K.LANI und W.STICH) konnte durch Markierung der Megakaryozyten im Knochenmark mit ^{75}Se-Methionin gezeigt werden, daß die Transfusion homologer Thrombozyten zu keiner am Ausstoß markierter Thrombozyten erkennbaren Stimulierung der Thrombopoese, sondern im Gegenteil zu einer Verminderung des Austritts von Thrombozyten aus dem Knochenmark führt.

Zusammenarbeit mit anderen wissenschaftlichen Institutionen

Die Zusammenarbeit des Instituts auf internationaler, europäischer, nationaler und regionaler Ebene wurde weiter intensiviert. Von den internationalen Kontakten seien insbesondere die Zusammenarbeit mit verschiedenen Institutionen in den USA (Clinical Center of the National Institute of Health in Bethesda, Blood Research Center in New York, Harvard University Medical Center in Boston) genannt. Mit einer Reihe von in- und ausländischen Kliniken und Instituten bestehen enge wissenschaftliche Verbindungen, insbesondere mit den Medizinischen Kliniken der Universitäten Innsbruck/Österreich und Genf/Schweiz. Das Institut ist an den Sonderforschungsbereichen "Restitution und Substitution innerer Organe" und "Medizinische Molekularbiologie und -biochemie" beteiligt. Eine Arbeitsgruppe

des Instituts ist in Berlin tätig. Regional besteht eine enge Zusammenarbeit mit dem Klinikum der Universität, insbesondere dem Lehrstuhl für Innere Medizin, spez. Hämatologie, dessen Vorstand in Personalunion der Institutsleiter ist. Auch zu den klinisch-theoretischen und theoretischen Instituten der Universitä den Max-Planck-Instituten, insbesondere dem MPI für Biochemie, dem Klinikum der Technischen Universität, dem Blut-Center und dem Südbayerischen Blutspendedienst bestehen enge wissenschaftliche und ärztliche Kontakte.

Vorlesungstätigkeit an Universitäten

Universität München:
Prof.Dr.Walther STICH
Innere Medizin, spez.Hämatologie
Kolloquium der Inneren Medizin (Blut- und Immunkrankheiten)
Klinische Hämatologie und Untersuchungskurs
Kurs für Klinische Chemie
Klinische Visite

Prof.Dr.Hans EHRHART
Innere Medizin, spez. Onkologie
Klinische Hämatologie mit Untersuchungskurs
Klinische Visite

Prof.Dr.Rolf BURKHARDT
Innere Medizin, spez. Osteologie
Klinische Hämatologie mit Untersuchungskurs
Klinische Demonstrationen und histologische Beurteilung hämatologischer und osteologischer Fälle
Poliklinische Visite

Freie Universität Berlin:
Prof.Dr.Irene BOLL
Wissenschaftliche Untersuchungen am menschlichen Knochenmark

Außerdem beteiligten sich Priv.Doz.Dr.Dieter HUHN, Dr.Brigitte WOLF-HORNUNG, Dr.Peter DÖRMER, Dr.Herbert PFISTERER, Dr.Dieter SCHMIDT und Dr.Manfred SCHMIDT von der Klinischen Abteilung des Instituts beim praktischen Unterricht am Krankenbett und im Laboratorium für Studenten am Lehrstuhl für Innere Medizin (Hämatologie) und der I.Medizinischen Klinik der Universität München.

Institut für Hämatologie
Abteilung Immunologie
(Leiter: Priv.Doz.Dr.S.Thierfelder)

Die Arbeitsgruppen der Abteilung konzentrierten sich auf die Analyse und Überwindung der immunologischen Komplikationen nach experimenteller Knochenmarktransplantation auf tödlich bestrahlte Säugetiere. Dieses Arbeitsgebiet geht von einer strahlenbiologischen Thematik ("Therapie des akuten und chronischen Strahlenunfalls") aus, hat jedoch in den letzten Jahren als Weg zur Heilung bestimmter Erkrankungen des Blutes und als einziger Weg zur lebenslänglichen Ausschaltung der Gewebsunverträglichkeit bei Organtransplantationen allgemeine medizinisch-biologische Bedeutung erlangt.

Das Forschungsziel wird im wesentlichen von drei Arbeitsgruppen verfolgt, die mit
1. immunchemisch-molekularbiologischen
2. immunbiologisch-tierexperimentellen Methoden und
3. mit den immunbiologischen in-vitro-Methoden der Zellkultur
arbeiten.

Die wissenschaftliche Tätigkeit der Abteilung erstreckte sich auf die Aufklärung der Struktur der Immunglobuline mittels Sequenzanalyse am Beispiel der leichten Ketten eines Bence-Jones-Proteins. Ferner wurde eine immunbiologische Methode zur Vorbereitung von Knochenmarkempfängern auf ihr Fremdtransplantat entwickelt, die weniger risikobelastet ist als die bisher verwendete Ganzkörperbestrahlung. Mit Hilfe von Knochenmarkskulturen wurde noch 36 Stunde nach dem Tod des Knochenmarkbildners ein 80%-iges Überleben teilungs fähiger Knochenmarkszellen festgestellt.

Der Abteilung gehörten im Berichtsjahr 6 wissenschaftliche Mitarbeiter, 5 technische Assistentinnen, 5 Doktoranden und 8 technische Kräfte an.

Arbeiten und Ergebnisse

Strukturuntersuchungen an Immunglobulinen

Die Arbeiten zur Sequenzanalyse des Bence-Jones-Proteins Scw wurde mit der Aufarbeitung der chymotryptischen Peptide des aminoäthylierten Gesamtproteins abgeschlossen. Nach der nun vorliegenden Primärstruktur gehört das Protein zur Subgruppe $\varkappa$/I. Mit vergleichbaren Proteinen (Roy, Ag, Au) stimmen etwa 80 % der Aminosäure im variablen Teil überein. Zunächst wurde das Protein der Untergruppe $\varkappa$ I/1 zugeordnet; ob darüberhinaus wegen mehrerer, bisher nicht beschriebener Aminosäureaustausche ins Protein Scw eine weitere Untergruppe charakterisiert wird, müssen weitere Sequenzuntersuchungen an Bence-Jones-Proteinen vom λ-Typ erbringen. Insgesamt enthält das Protein 214 Aminosäuren. Mit Leu in Position 191 ist es dem Allotyp Inv (a^+) zuzuordnen.

Knochenmarktransplantation auf bestrahlte Mäuse

Nach Transplantation gewebsunverträglichen Knochenmarks auf Tiere, deren Blutbildung durch Bestrahlung zerstört worden war, kommt es zu immunologischen Komplikationen (= Sekundärkrankheit). Eine Beeinflussung dieser Sekundärkrankheit mittels Immunsuppression gelang vor allem durch das biologische Immunsuppressivum Antilymphozytenserum (ALS). Es zeigte sich, daß bei Spender-Empfänger-Kombinationen, die sich in einem Haplotyp am Transplantationslokus (H_2) unterschieden, die zytologischen Veränderungen der Sekundärkrankheit durch ALS verhindert werden konnten. Während die transplantierten Tiere der unbehandelten Kontrollgruppe innerhalb von 3 Wochen an Sekundärkrankheit starben, waren die Empfänger von ALS-vorbehandelten Knochenmarktransplantaten auch noch nach 1 Jahr symptomfrei. Dabei hatte sich durch die nur 6 Tage mit ALS durchgeführte immunsuppressive Behandlung zunächst eine Gewebstoleranz gegenüber dem Knochenmarkstransplantat des Spenders eingestellt.

Darüberhinaus wurde eine Kombinationsbehandlung mit Teilkörperbestrahlung unter gleichzeitiger Immunsuppression durch Antilymphozytenserum entwickelt, die ein Einwachsen fremden Knochenmarks in die bestrahlte Körperhälfte ermöglicht. Da diese Behandlung einen Großteil des knochenmarkempfängereigenen blutbildenden Gewebes verschont, ist sie weniger risikobelastet als die bisher gebräuchliche Ganzkörperbestrahlung.

Zellkulturen

Im Rahmen der immunologischen Spenderauswahl konnten in Zusammenarbeit mit Dr.Albert und Dr.Burger (Univ-Kliniken) in mehreren Fällen 2 unverwandte Personen gefunden werden, die in allen serologisch erfaßbaren Transplantationsantigenen übereinstimmten. 15 solche Paare wurden mit Hilfe der gemischten Lymphozytenkultur auf ihr immunologisches Verhalten in vitro geprüft. Es zeigte sich nur in einer Kombination ein Verhalten wie zwischen Geschwistern mit gleichen Transplantationsantigenen. Außerdem wurde eine Familie gefunden, in welcher 2 serologisch identische Geschwister eine positive Reaktion zeigten, während serologisch unterschiedliche Geschwister nicht reagierten. Diese Ergebnisse deuten darauf hin, daß die gemischte Lymphozytenkultur und die serologisch erfaßbaren Transplantationsantigene (HL-A-Antigene) von 2 verschiedenen Genregionen gesteuert werden.

Mit Hilfe einer neu aufgebauten in vitro Technik wurde im Maus-System die Überlebensrate von Knochenmarksstammzellen (CFU_c) nach dem Tode verfolgt. Es zeigte sich, daß 36 Stunden nach dem Tode noch 80 % der CFU_c-Zellen überlebten.

Zusammenarbeit mit anderen wissenschaftlichen Institutionen

Dr.Thierfelder folgte Einladungen zu einem Vortrag bei der Ungarischen Gesellschaft für Hämatologie, Budapest, sowie des Bluttransfusionsdienstes Bremen zum 4.Bremer Bluttransfusionsgespräch, und hielt einen Vortrag im Johns Hopkins Hospital in Baltimore, USA. Außerdem nahm er an dem "Symposium on applied Immunology", Kitzbühel, teil.

Über die Fraunhofer-Gesellschaft bestand eine Zusammenarbeit mit der Akademie des Sanitäts- und Gesundheitswesens der Bundeswehr.

Die Abteilung Immunologie war außerdem an dem Sonderforschungsbereich 37 der Deutschen Forschungsgemeinschaft beteiligt.

Dr.Thierfelder hielt Vorlesungen an der Universität München über "Pathophysiologie immunologisch bedingter Blutkrankheiten".

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RELAZIONE DI ATTIVITA' 1971

Contratto No. 078-60-1 BIAC

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I M M U N O G E N E T I C S

SUMMARY

In the mouse immunized with SRBC specific antibodies are produced by bone marrow-derived (B) cells upon interaction with thymus-derived (T) cells and antigen. This interaction was shown to occur in vitro. As to whether cell receptors are involved, it was found that T cells, unlike B cells, were not prevented by rabbit antibodies against mouse whole serum from interacting with antigen and the cooperating cells. It was also found that cell-free medium of thymocyte cultures can enhance the in vitro immune response of B cells because it can replace T cells and display a similar helper effect.

A technique for measurement of antibody avidity at the level of single immunocytes was devised and applied to spleen cell populations immunized in vivo or in vitro.

RESULTS

The immune system protects the individual from invasion and pathogenic action of microorganisms. The efficiency of the immunologic surveillance relies on the integrity of populations of immunologically competent cells which partecipate in the processes of antigen recognition and antibody production. Recent studies have demonstrated that several immune responses result from the cooperation between thymus-derived (T) and bone marrow derived (B) lymphocytes. In mice immunized with sheep red blood cells (SRBC) it has been shown that antibody synthesis occurs in B but not in T cells and that T cells have to react specifically with antigen before the cooperation with B cells can take place. The nature of the T and B cell interaction is not known, although several mechanisms have been proposed. Results of in vivo experiments favor the possibility that the T cell binds antigen by receptor sites specific for some antigenic determinants and focusses other determinants of the same antigen molecule onto specific receptors of the B cell. Therefore, the former cell traps antigen so as to form a local concentration of it at some critical site on the latter cell which is then triggered to antibody synthesis. This mechanism requires the presence of antigen binding receptors on the lymphocyte membrane. The existence and the Ig nature of cell receptors are supported by several lines of experimental evidence.

An in vitro study was carried out on competent cells and their interactions involved in the immune response of mouse cells to SRBC. The aim of this work is the understanding of how mouse cells can develop a primary response in vitro, so that optimal conditions will be adapted to human cells for the detection of radiation induced immunologic deficiences.

Whether the interaction between T and B cells can occur in vitro was investigated by the Mishell and Dutton technique, whereby unprimed mouse spleen cells can be stimulated in vitro with SRBC to produce antibody-forming cells. The immune response was evaluated by the Jerne technique and expressed as number of hemolytic plaque-forming cells (PFC) per culture. Cooperation between T and B cells was shown to occur in vitro using thymocytes from normal mice and splenocytes from neonatally thymectomized mice or from thymectomized chimeras (mice thymectomized in adult life, lethally irradiated, and grafted with isogenic bone marrow cells). It was found that the addition of SRBC to mixed cell cultures of thymocytes from normal mice and splenocytes from thymectomized mice elicited an immune response comparable to that of normal spleen cells. When the two cell populations were stimulated in separate cultures no response of thymocytes

was ever observed, while the response of splenocytes from thymectomized mice was within the range of the PFC background in unstimulated control cultures. Appropriate controls ruled out that higher responses in mixed cultures were simply the result of greater cell density.

If receptor sites are involved in the cooperation between T and B cells, treatment of these cells with anti-receptor antibodies prior to culture may prevent cell interaction and the resulting immune response. Assuming that cell receptors are molecules shared by the serum, rabbits were immunized with normal mouse whole serum. Cells were incubated with complement-inactivated rabbit antiserum or normal serum and then washed prior to culture with SRBC. Treatment of normal spleen cells with the rabbit antiserum prior to culture drastically reduced the immune response to SRBC. This indicates that normal spleen cells possess receptor sites antigenically identical to or cross reactive with molecules of the normal serum. When thymocytes, splenocytes from neonatally thymectomized mice, or both were similarly treated with the rabbit antiserum or normal serum prior to culture, the immune response in mixed cultures could be inhibited only if the B cells were pretreated with the antiserum. Preincubation of T cells with the antiserum did not interfere with the cell cooperation events leading to antibody formation. Thus, T cells seem to lack demonstrable receptor molecules such as those detected by the same reagent on B cells and present in the normal mouse serum. This finding is in agreement with other results showing absence of Ig on thymocytes. Yet, it is not ruled out that cell-bound receptors for antigen exist also on the T cell, perhaps in lower density, but with higher turnover rate than receptors on the B cell. Therefore, the T cell receptors blocked by rabbit antibodies could be quickly secreted and replaced by new receptors. This possibility is compatible with the results of absorption experiments, in which the antiserum ability to prevent the immune response of normal spleen cells was lost after the antiserum had been absorbed with mouse spleen or thymus cells. Thus, T cells possess receptors which, as cell-bound or secreted molecules, can block those rabbit anti

bodies inhibiting the immune response of normal spleen cells. The findings that only B cells were the sensitive target for inhibitory antibodies in the cell cooperation experiment and that T cells could remove these antibodies in the absorption experiment suggest that T cell receptors are antigenically identical to or crossreactive with B cell receptors. However, T cell receptors differ from B cell receptors in that the former are not prevented by rabbit antibodies from partecipating in the process of cell cooperation. It should be pointed out that this conclusion is restricted to receptor molecules shared by T and B cells and secreted in the normal mouse serum, as T cells might display helper function by other cell-bound receptors for which the rabbit antiserum lacked specific antibodies. Furthermore, the present findings need not conflict with other results showing either thymocyte ability to bind antigen in a specific way or presence of Ig-light chains on T cells involved in hypersensitivity reactions of the delayed type or in rosette formation. It is, indeed, possible that the T cell population is heterogeneous in that the cells provided with helper function may be different from killer cells or antigen binding cells such as rosette-forming cells.

The helper function of the T cell might not be tied to a mechanism of cell to cell contact. The T cell may release a soluble substance which promotes triggering of the B cell by antigen. Accordingly, it was investigated whether cell-free medium from thymocyte cultures can enhance the in vitro immune response of spleen cells from neonatally thymectomized mice. It was found that such a cell-free medium can replace thymocytes and display a similar helper effect. When spleen cells from neonatally thymectomized mice were cultured with SRBC and cell-free medium from cultures of thymocytes maintained in vitro for 24 hrs a great enhancement of the immune response was consistently observed. The thymus specificity of the active factor is suggested by the finding that cell-free medium in which adult liver cells had been cultured for 24 hrs had no enhancing activity on the immune response of spleen cells from neonatally thymectomized mice. Presence of SRBC in the thymocyte culture for 24 hrs

did not change the enhancing activity of the cell-free medium. Thus, either T cells secrete a cell promoting factor that lacks antigen specificity or T cells release receptor molecules, some of which bind the test antigen and concentrate it onto specific B cells. Release of the promoting factor or of receptor molecules would be an antigen independent process. The hypothesis of a cell promoting factor that lacks antigen specificity is not supported by the results of in vivo experiments implying that T and B cell cooperation is antigen specific. However, experiments in progress may clarify whether the soluble factor acts directly on B cells or amplifies a small population of T cells that escaped neonatal thymectomy and that could specifically interact with antigen and B cells.

The effect of the helper function of T cells may be the activation of a larger B cell population, heterogeneous with respect to the receptor affinity for antigen, or the selection of B cells bearing high-affinity receptors. In vitro techniques for evaluation of defects of the immunologic potential should allow estimates of affinity of the antibodies produced, for it is well established that the protective ability of the immune system ultimately depends on the good fit of antibodies for antigens. Indeed, it has been shown that neutralization of toxins or viruses is a property of the antibody avidity, a function of affinity, which determines the stability of the antigen-antibody complexes.

A technique has been devised whereby antibody avidity can be assessed at the level of single immunocytes. 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 PFC. If spleen cells were plated with TNP-SRBC and TNP-BSA, PFC were inhibited. The percent of inhibition was a sigmoid function of the amount of TNP-BSA added. The reciprocal of the amount of inhibitor that suppresses 50% of PFC was taken as an estimate of antibody avidity, based on the fact that higher-avidity antibodies are blocked by lower amounts of antigen. Wide variations of avidity could be detected in spleen cell populations from mice immunized with different antigen doses and for different time intervals. Parallel estimates of antibody affinity at the

serum level are being carried out by equilibrium dialysis.

The culture technique was modified so that unprimed mouse spleen cells could be stimulated in vitro with TNP-HRBC to produce PFC anti-TNP. Changes of antibody avidity at the level of single immunocytes were detected also in this system, which can be adapted to study the role of T cells on the avidity of antibodies produced by B cells.

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INTRODUCTION

Le but général du projet est l'étude de mécanismes physiologiques et biochimiques dont l'altération détermine des effets à court et à long terme des radiations et de définir les méthodologies permettant d'étudier ces mécanismes. Les domaines investigués sont la régulation de l'erythropoïèse et le métabolisme de l'érythropoïétine, le leucocyte et sa capacité de phagocytose, les processus de réparation du DNA, le renouvellement des cellules des muqueuses, et l'établissement d'un modèle du métabolisme de l'iode dans la thyroïde.

I. HEMATOPOIESE ET RYTHROPOIETINE (J.P. NAETS, M. WITTEK)

Les effets de l'irradiation sur l'hématopoïèse sont de deux ordres : le premier, beaucoup étudié, est la dépression générale de l'hématopoïèse ; le second, que nous avons récemment démontré (Proc. Soc. Exper. Biol. Med., 100, 40, 1959), est une altération du métabolisme de l'hormone régulatrice, de l'hématopoïèse, de l'érythropoïétine. Chez le rat irradié, le catabolisme de l'érythropoïétine est en effet ralenti. Ceci pouvait suggérer que la réduction du catabolisme pouvait être due à l'aplasie médullaire qui suit l'irradiation. Cependant, la dégradation de l'érythropoïétine exogène est indépendante de la quantité d'érythroblastes disponibles dans la moelle et de la différentiation des cellules souches en erythroblastes (Am. J. Physiol., 217, 247, 1969), et de plus, le catabolisme de l'érythropoïétine endogène pourrait être moins affecté par l'irradiation que le catabolisme de l'érythropoïétine exogène. Notre hypothèse est que l'action des rayons X porte sur le rein qui serait le principal site de catabolisme de l'hormone. De fait, nous avons récemment montré que le rein joue un rôle dans le catabolisme de l'érythropoïétine (Experientia, sous presse). Cette année,

nous nous sommes attachés plus particulièrement à étudier le mécanisme de cette fonction. Nous avons utilisé à cet effet différents modèles expérimentaux. Nous avons comparé la décroissance de l'érythropoïétine exogène et endogène du plasma chez des rats normaux ou néphrectomisés bilatéralement.

A. Erythropoïétine exogène

a) Plasma de rat soumis à l'hypoxie, prélevé immédiatement après la sortie du caisson (E ine O h).

b) Plasma prélevé 2h après la sortie du caisson (E ine 2 h).

c) Eine de mouton Armour, grossièrement purifiée.

Nous avons observé que la décroissance plasmatique de l'Eine O h chez le rat néphrectomisé depuis 48h suit une double exponentielle (T/2 = 30 minutes et 10h30), alors qu'on n'observe qu'une seule exponentielle chez les rats témoins normaux (T/2 = 2h). Par contre, nous avons également observé une seule exponentielle, avec un T/2 très allongé (T/2 = 24h), si l'on injecte aux rats néphrectomisés de l'Eine 2h. Enfin, la décroissance de l'Eine de mouton suit une courbe comparable à celle de l'Eine 2h chez le rat néphrectomisé, alors qu'elle est identique à celle de l'Eine Oh chez les témoins.

B. Erythropoïétine endogène

Chez le rat néphrectomisé immédiatement après arrêt de la stimulation hypoxique, on observe aussi un T/2 très allongé (21h), et la disparition de l'activité érythropoïétique se fait également suivant une double exponentielle. Par contre, si la néphrectomie est pratiquée 2h après la sortie du caisson, on n'observe qu'une seule droite de décroissance dont le T/2 est de 14h (4h chez les témoins).

En conclusion, nous avons déduit de ces divers résultats que l'érythropoïétine se trouve sous 2 formes dans le plasma des rats qui ont été stimulés par l'hypoxie. Une forme disparaissant rapidement même en l'absence de rein (T/2 = 30') et une autre, beaucoup plus lentement métabolisée chez l'animal anéphrique (T/2 = 15 à 24h). Nous avons l'intention de poursuivre l'étude du mécanisme de dégradation de cette glycoprotéine qu'est l'Eine, et de l'effet de l'irradiation X sur ce catabolisme.

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- Markedly increased bone blood flow in myelofibrosis (en collaboration avec D.C. VAN DYKE, H.O. ANGER, H.G. PARKER, J. ME RAE, E.L. DOBSON, Y. YANO et J.A. LINFOOT) (J. Nucl. Med., 1971, 12, 506).

II. LEUCOCYTE ET PHAGOCYTOSE (E. SCHELL-FREDERICK, J. BERBEROF-VAN SANDE, J.E. DUMONT)

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 bactéricide et de phagocytose du leucocyte (Nature, 210, 158, 1966 ;

J. Reticuloendoth. Soc., 5, 538, 1968 et 7, 743, 1970). Le but du présent projet est détudier le mécanisme de la phagocytose par le leucocyte de l'homme et de l'animal et d'appliquer cette connaissance à l'animal irradié.

Nous avons démontré le rôle essentiel de la 3',5'-AMP cyclique (cAMP) dans le mécanisme de phagocytose de la cellule thyroïdienne (Vitamins and Hormones, 26, 287-412, 1971). Nous avons donc recherché un rôle éventuel de la 3',5'-AMP cyclique dans la phagocytose par le leucocyte. Nous nous sommes proposés, comme hypothèse de travail, que la cAMP pourrait agir comme médiateur intracellulaire de la phagocytose et de ses conséquences métaboliques d'une manière rappelant le mécanisme d'action de la thyrotropine sur le tissu thyroïdien. Dans ce modèle, le contact entre la particule phagocytée et la surface externe du leucocyte polynucléaire stimulerait l'enzyme adenyl cyclase et induirait ainsi une augmentation marquée de la concentration cellulaire en cAMP. Le cAMP serait responsable de la stimulation métabolique qui suit la phagocytose.

Méthodes

Les leucocytes polymorphonucléaires humains sont préparés par la méthode de Chodirker et al. (J. Lab. Clin. Med., 71, 9, 1968). Les leucocytes préparés étaient morphologiquement normaux au colorant de Wright et plus de 90% étaient polymorphonucléaires. La contamination en erythrocytes et plaquettes était très faible. Le cAMP cellulaire était mesuré par la méthode de Gilman (67, 305, 1970) ou de Berberof-Van Sande (sous presse). Les méthodes de mesure des autres paramètres métaboliques sont mentionnées dans le tableau résumé des résultats.

Résultats

Pour valider le modèle proposé, nous devons démontrer :

1): Une augmentation de la concentration intracellulaire de cAMP durant la phagocytose.
2) Que le cAMP et/ou un analogue peut reproduire les conséquences métaboliques de la phagocytose en l'absence de particules phagocytées.

Nous avons démontré que le taux intracellulaire de cAMP augmentait dans le leucocyte dès le début de la phagocytose (moins d'une minute après l'addition de latex). Des données moins complètes ont été présentées récemment par un autre groupe (Nature New Biology, 229, 27, 1971).

Le processus de phagocytose est suivi d'une série d'importantes modifications métaboliques : stimulation de la consommation d'oxygène, de l'oxydation du glucose par la voie des pentoses phosphates, de l'incorporation du phosphate ^{32}P dans les phospholipides, et de la glycogénolyse. Dans chaque expérience, un de ces paramètres a été testé comparant chaque fois des leucocytes au repos, des leucocytes incubés en présence de particules de latex et des leucocytes incubés en présence de nucléotide cyclique. La capacité de phagocytose des particules est testée par leur effet métabolique, par mesure du latex dans des extraits au dioxane des cellules ou par examen au microscope (Biochem. J., 89, 150, 1963). Le tableau suivant résume nos résultats.

(Voir Tableau 1)

Nous en concluons que le cAMP n'est pas le médiateur chimique de la plupart des conséquences métaboliques de la phagocytose. Le cAMP semble être responsable de l'activation de la glycogénolyse et peut donc jouer un rôle dans la mobilisation rapide nécessaire de substrat énergétique. Nous étudions actuellement d'autres rôles possible du cAMP et par exemple celui d'agent chémoattractant.

TABLEAU 1

PARAMETRE METABOLIQUE	METHODE	+ LATEX	+ cAMP	+DBcAMP
		% DU TEMOIN		
CAPTATION D'OXYGENE	ELECTRODE DE CLARKE	AUGMENTATION	PAS DIFFERENCE	PAS DIFFERENCE
Glycolyse	Glucose-6-$C^{14} \rightarrow C^{14}O_2$	200%		100%
Voie des pentoses phosphates	Glucose-1-$C^{14} \rightarrow C^{14}O_2$	400 - 1000%		100%
$^{32}P \rightarrow$ phospholipides	J. Biol. Chem. 236, 1895, 1961	200%	100%	100%
Glycogénolyse	Sur flycogène prémarqué	85%		77%

Publications

- Méthode de dosage de la 3',5'-AMP cyclique dans des tissus incubés in vitro (J. BERBEROF-VAN SANDE) (soumis à Biochimie).
- Role of cyclic 3',5'-AMP in phagocytosis by human leukocyte (E. SCHELL-FREDERICK, J. BERBEROF-VAN SANDE) (soumis à Blood).

III. LYMPHOCYTES AND DNA REPAIR (P. STRYCKMANS, G. DELALIEUX)

The recent findings in Xeroderma pigmentosum , a human hereditary disease characterized clinically by a high sensitivity to sunlight and 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 the DNA repair processes in relation to carcinogenesis.

The process of repair of U.V. induced DNA damage (the total uptake of ^{3}HTdR and the kinetics of this uptake) can be studied easily in human lymphocytes in several pre-malignant clinical situations : Down syndrome, Chediak Higashi syndrome, Fanconi syndrome, etc., and compared with normal control lymphocytes.

The second part of the report involves an investigation on the mode of chemotherapeutic agents on acute leukemia.

A. Search for DNA repair processes after use of antileukemic drugs

The process of DNA repair after UV irradiation starts by a first step of pyrimidine dimer excision. The process of pyrimidine dimer excision has been investigated on rat blood lymphocytes. The excision of thymidine dimer was objectivated on autoradiography after DNA labelling by

means of ^{3}HTdR followed by UV irradiation (Delalieux, Stryckmans et al., submitted to Blood).

In order to study a similar excision process after the administration of alkylating agents, two patients with acute leukemia were given ^{3}HTdR intravenously. Two days later, when a large fraction (30%) of the leukemic cells in the peripheral blood were labelled, cyclophosphamide was given at a dose of 20mg/m^{2}. Pheripheral blood smears were made of samples taken regularly during 4 hours before and 4 hours after the injection of cyclophosphamide.

Preliminary results in one patient seem to indicate a loss of ^{3}H radioactivity, from the leukemic blasts in the blood during the hours following the administration of cyclophosphamide.

B. Mode of action of chemotherapic agents on human acute leukemia (ALL) cells in vivo

The problem of the cell cycle phase-dependent action of vincristine was studied in 2 cases with acute lymphoblastic leukemia. Only one could be evaluated.

Method

500ml of blood were labelled in vitro by ^{3}H-cytidine and immediately thereafter autotransfused. The disappearance of labelled blasts was followed on autoradiographies of the blood prior to and after an injection of vincristine.

Results

Results are shown on Figures 1 and 2 -
Figure 1 shows on semilog paper the effect of VCR on one patient with ALL who achieved a complete remission. The population of labelled blasts expressed per mm^{3} decreased exponentially for 3 days with a T 1/2 of 60 hours. The decrease shown on this slide represents the natural exit of leukemic cells from the blood to the tissues. VCR

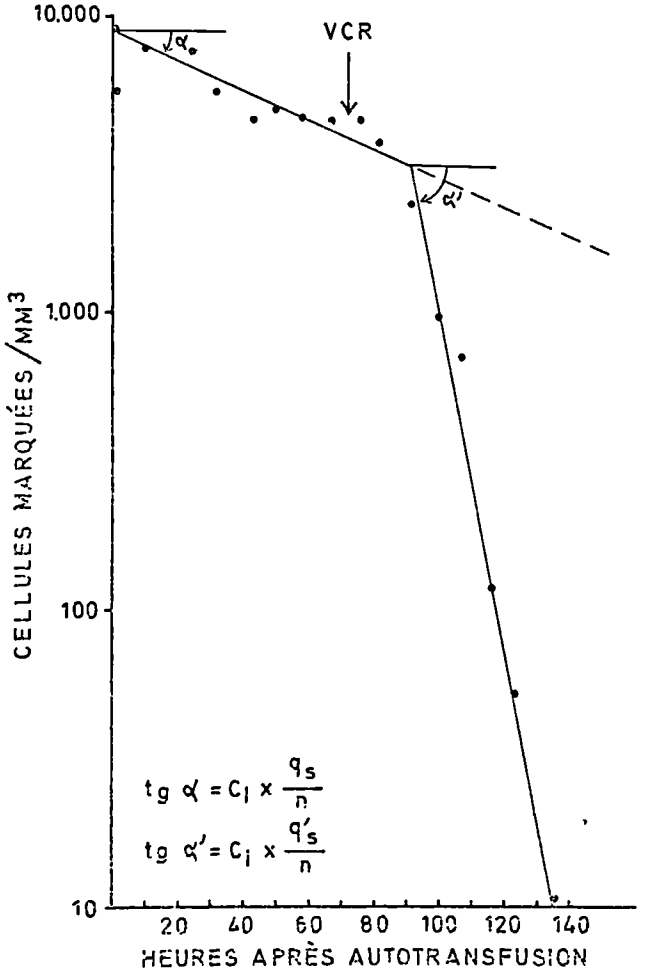

FIGURE 1

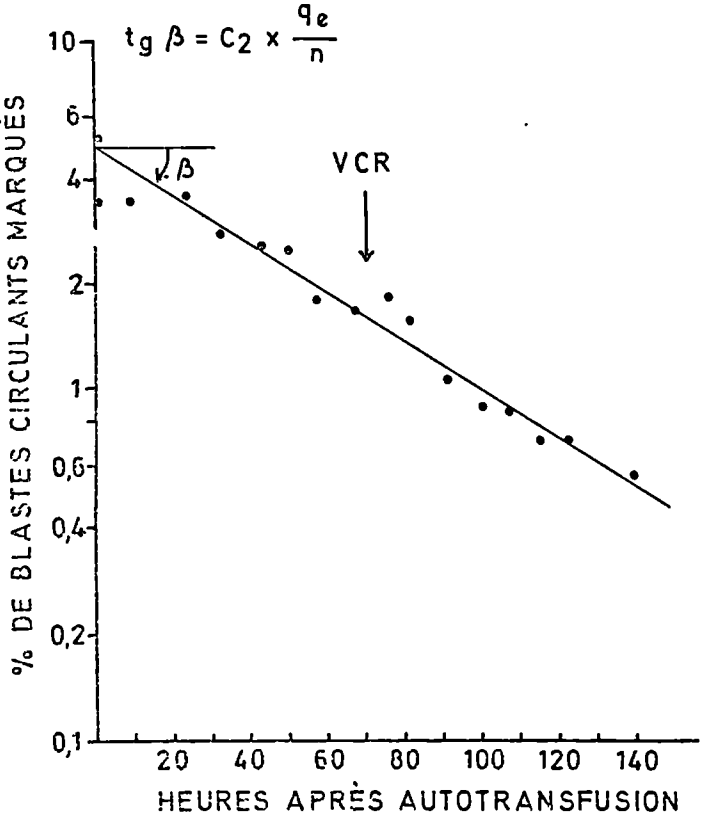

FIGURE 2

(1.5mg/m^2 of body surface) was given IV on day 4 and a few hours later the labelled cells decreased dramatically with an accelerated T 1/2 of 5 hours. This effect cannot be accounted for by an arrest of input of cells from the BM, since this could not affect the slope of the number of labelled cells from the BM. It is shown that the exponential decrease of labelled cells due to the exit of cells to the tissue before treatment is truncated as a consequence of the treatment by a more rapid exponential process which represents presumably a lethal effect on the circulating leukemic cells.

Figure 2 shows on semilog paper in the same patient the labelling index of the blood leukemic blasts as a function of time. The LI decreases exponentially and the slope is not affected by vincristine. The slope of this curve is equal to a constant (c) times the Kin (number of unlabelled cells entering the blood from the BM/unit of time over B, which is the number of cells in the blood at the same moment). The decrease of the LI after vincristine shows that the labelled cells continue to be diluted by new coming unlabelled cells from BM. The slope (tan β) is constant indicating that the ratio q_e/n is constant ; since n decreases dramatically as shown on the previous slide, q_e must decrease in the same proportion. Since q_e represents the input from the bone marrow, this would indicate a similar cytocidal effect on blood and marrow blasts which are, as shown in the beginning, different in terms of proliferation. The blood blasts being almost exclusively cells out-of-cycle it would indicate that vincristine may have at least in some cases a cytocidal effect on all the cells irrespective of their position in the cell cycle. This would indicate that in vivo vincristine may act on cell proliferation not only by blocking the cells in mitosis but also by destroying interphase cells. This work is now being prepared for publication.

Publications

- DNA excision process in blood lymphocytes and mature granulocytes exposed to ultra-violet light (G. DELALIEUX, P. STRYCKMANS, J. MANASTER, R. BADJOU) (submitted to Blood).
- The mode of action of chemotherapic agents in vivo on human acute leukaemia - I - Daunomycin (P. STRYCKMANS, J. MANASTER, F. LACHAPELLE, M. SOCQUET) (submitted to J. Clin. Invest.).
- Cell proliferation in chronic myeloid leukemia (P. STRYCKMANS, J. MANASTER, T. PELTZER, M. SOCQUET, G. VAMECQ) (submitted to British J. Haematol.).

IV. ETUDE DES PARAMETRES DU RENOUVELLEMENT CELLULAIRE DANS LES TISSUS HUMAINS : EMPLOI DE TISSUS BIOPSIES INCUBES IN VITRO (P. GALAND)

La mesure du renouvellement cellulaire peut servir de contrôle de l'action de divers agents ou traitements susceptibles d'interférer dans le déroulement normal du cycle mitotique. La sensibilité très grande de la muqueuse intestinale aux radiations ionisantes, connue depuis 1912 par les travaux Regaud, Nogier et Lacassagne, conduit à penser qu'il serait notamment intéressant de mesurer dans ce tissu l'apparition éventuelle d'anomalies dans la cinétique cellulaire, tant dans le cas d'exposition accidentelle aux rayonnements, que pour suivre les effets d'un traitement (simple ou non) par les rayons. Notre méthode in vitro permettant de telles études sans aucun risque pour le patient, constitue un outil de choix pour de telles investigations. De même, il serait intéressant de suivre à ce niveau, et aussi dans l'épiderme, l'action de traitements physiques ou chimiques (hormones, antimitotiques) utilisés dans certaines thérapeutiques.

A. Muqueuse digestive humaine (H. BLEIBERG, P. MAINGUET)

Nous avons poursuivi nos études sur le tissu rectal prélevé chez des sujets normaux dans diverses situations pathologiques. Deux cas de polype familiale ont été étudiés notamment. Les résultats montrent à la fois l'apparition d'une zone anormale de prolifération (jusqu'au sommet des cryptes) et l'existence d'un renouvellement cellulaire plus rapide dans cette pathologie, par rapport au tissu sain avoisant. Ces résultats déjà mentionnés ont été vérifiés. D'autre part des polypes adénomateux et des polypes villeux ont été étudiés. Ce matériel recueilli est en cours d'analyse.

Dans le cas de l'intestin grêle, nous mesurons un temps de renouvellement moyen de 33 heures (10 cas) et une phase S de 7 heures, chez les sujets normaux.

Des cas d'atrophie villositaire ont été également étudiés. Dans la maladie coelique, il ne semble y avoir aucune modification importante de ces paramètres. Une étude approfondie portant sur des patients à différents stades de l'évolution de la maladie est en cours. Elle porte sur des sujets considérés avant et après traitement.

B. Epiderme humain (M. HEENEN, ACHTEN)

Nous avons comparé le temps de renouvellement dans la couche basale de l'épiderme normal et de tumeurs de l'épiderme (épitheloma basocellulaire). Nous n'avons noté aucune différence significative, les résultats étant semblables à ceux obtenus antérieurement chez des sujets normaux. Grâce à l'injection locale (intradermique) de thymidine tritiée, nous avons également mesuré le pool proliférant (growth fraction) dans la tumeur. Les résultats montrent qu'il est proche de l'unité (0,80).

D'autre part, la phase S est significativement plus longue (19h au lieu de 10h) dans la tumeur que dans la

population basale normale. Une phase S de 19-20h est mesurée également dans un cas de tumeur de Pintens (considérée comme précancérose). Ces divers résultats font l'objet d'un article soumis à l'éditeur.

Publications

- Measurement of S phase duration in human epidermis by double labelling method (M. HEENEN, G. ACHTEN, P. GALAND) (Europ. Soc. Dermatol. Res., Noordwijk a/Zee, 1971).
- Mesure autoradiographique de la prolifération cellulaire à différents niveaux du tractus digestif normal et pathologique. Utilisation de biopsies incubées in vitro (H. BLEIBERG, P. MAINGUET, J. VANDENHENDE, P. GALAND) (Rev. Europ. Et. Clin. Biol., XVI, 233-239, 1971).
- Cell renewal in familial polyposis : comparison between polyps and adjacent healthy mucosa (H. BLEIBERG, P. MAINGUET, P. GALAND) (Gastroenterology, sous presse).
- Mesure de la durée de la phase S par les méthodes de double marquage (P. GALAND) (15 pages dans "Cinétique Cellulaire", Inserm, Ed. M. Tubiana).
- Etude de la prolifération cellulaire dans des tissus prélevés par biopsie et traités in vitro par la méthode de double marquage (P. GALAND) (Dans "Cinétique Cellulaire" Inserm, ed. M. Tubiana).

V. MODELE DU METABOLISME DE L'IODE DANS LA THYROIDE

(F. CANTRAINE, B. DEWANDRE, J.E. DUMONT)

La plupart des modèles mathématiques de la cinétique de l'iode chez l'homme sont des modèles descriptifs qui ne tiennent aucun compte des acquisitions récentes de la physiologie thyroïdienne. Ces modèles ne permettent donc

pas de calculer de manière adéquate la persistance et la localisation thyroïdiennes du radioiode ingéré dans la chaîne alimentaire. De plus, les modèles existants ne tiennent aucun compte de la répartition différentielle du radioiode dans les cellules et dans la lumière folliculaire et ne permettent donc pas le calcul de l'irradiation des cellules thyroïdiennes dans les traitements au radioiode d'affections thyroïdiennes (Basedow, etc.). Ceci explique la controverse actuelle sur les avantages réciproques et sur les dangers relatifs (hypothyroïdie, et surtout carcinogénèse) des traitements à I^{131} et I^{125} (New England J. Med., 285, 1099, 1971). Le follow up des patients traités ne permettrait de résoudre cette controverse que dans une vingtaine d'années. Il est donc d'un intérêt immédiat, pratique, de développer un modèle de la thyroïde permettant d'évaluer l'irradiation réelle des cellules thyroïdiennes après administration d'isotopes divers de l'iode.

Métabolisme de l'iodure intrathyroïdien (F. CANTRAINE)

Dans le but de construire un modèle physiologique du métabolisme de l'iode intrathyroïdien, nous proposons un modèle reprenant trois aspects aujourd'hui acceptés de ce métabolisme.

La compartimentation biochimique en MIT, DIT, T_3, T_4 a été introduite ainsi que des compartiments MIT', DIT', ceux-ci étant composés de MIT et DIT, mais se distinguant des précédents car ces thyronines ne sont iodables qu'une fois (MIT') ou ne se couplent pas pour former de la T_3 et de la T_4.

La compartimentation physique cellule, microvilli, colloïde a été introduite afin de permettre la description de la diffusion de la thyroglobuline au sein de la colloïde.

L'hétérogénéité fonctionnelle - La compartimentation physique permet l'introduction directe de la taille des unités

fonctionnelles (les follicules) et permet donc de décrire les différences de la cinétique entre des follicules de rayons différents. Comme toutes les étapes biochimiques aujourd'hui admises sont introduites dans ce modèle, les paramètres sont des grandeurs accessibles directement (pool biochimique, chromatographie, pool physique, mesure de section histologique) ; les paramètres de synthèse hormonale (cinétique chimique), les constantes de diffusion (autoradiographie), les paramètres de captation (expérience d'incubation de tranches thyroïdiennes - rapport 1970 - par exemple). Dans ce modèle, certains éléments ont déjà été étudiés : distribution des tailles des unités fonctionnelles (rapport 1968), transport de l'iodure (rapport 1969).

B. Etude de la diffusion intracolloïdale

Le modèle étudié présente de nombreux sous-systèmes similaires. En effet, les précurseurs réagissent à la membrane apicale, les produits résultants diffusent dans la colloïde, passent dans le compartiment microvial suivant ou passent dans la cellule après l'endocytose cellulaire.

Nous avons donc entrepris l'étude analytique de ce sous-système, afin de préciser l'importance de la diffusion de la thyroglobuline par rapport à la vitesse des réactions chimiques, ainsi que l'influence sur le renouvellement colloïdal du rayon de la lumière folliculaire.

Nous avons montré comment passer du modèle linéaire étudié aux cinétiques obtenues après l'injection de leucine tritiée (précurseur de la thyroglobuline) ou l'injection d'iodure marqué dans des tranches de thyroïde en incubation in vitro. Le modèle développé permet également, par la confrontation des autoradiographies avec les profils de radioactivité prédits par le modèle, d'estimer et de vérifier l'importance respective des paramètres du modèle. Les résultats obtenus ainsi que les implications expérimentales de l'étude seront soumis prochainement à l'éditeur.

Publications

- Solutions and modeling for linear differential systems with constant coefficients (V. DE MAERTELAER, F.R.L. CANTRAINE (Computers Programs in Biomed., sous presse).
- Modèles linéaires différentiels du premier ordre à coefficients constants (F.R.L. CANTRAINE, V. DE MAERTELAER, C. DELCROIX) (First European Biophy. Congress, Proceedings IV, Verlag der Wiener Medizinischen Academie, September 1971).
- Considérations sur l'élaboration de modèles biomathématiques. Un exemple : le transport de l'iode thyroïdien (F. CANTRAINE) (Bruxelles-Médical, 3, 193-197, 1971).
- Interpretation of surface aspects of cells sections (G. VASSART, J.E. DUMONT, F. CANTRAINE) (J. Cell Biol., 49, 210-212, 1971).

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GRUPPO COMMISSIONE - UNIVERSITA' DI PISA

Rapporto scientifico 1971

(Prof. L. Donato).

1. BIOFISICA E BIOMATEMATICA

Oggetto del programma: Automazione della scansione

E' continuato lo sviluppo del programma tecnologico sulla automazione delle apparecchiature di acquisizione dati nucleari in vivo.

A) Hardware

E' stata interamente progettata una interfaccia per il controllo degli 11 gradi di libertà dello whole body strip scanner da parte del calcolatore di processo HP2116B. Sono già state costruite varie parti di tale interfaccia. Si prevede la messa in opera dell'interfaccia HP2116B whole body strip entro i prossimi tre mesi.

B) Software

Tutto il software già funzionante su base conversativa per la acquisizione, elaborazione e display dei dati provenienti dalla gamma-camera è stato trasformato in modo da poter essere utilizzato in batch. Questo per consentire un migliore utilizzo del calcolatore e suoi periferici da parte dei molti utenti del sistema. La filosofia generale infatti è quella di usare i sistemi conversativi per lo sviluppo di nuove attività e di usare sistemi di elaborazione completamente automatica per la routine.

Il software suddetto è stato usato largamente per studi clinici in entrambe le versioni. Entro i prossimi tre mesi si prevede di realizzare anche il software di base per tale collegamento.

C) Elettromeccanica

Sono state interamente completate le costruzioni di parti relative alle modifiche del Whole Body Scanner che consentiranno di renderlo interamente gestibile dal calcolatore.

Nel corso del semestre prossimo si prevede il completamento del montaggio ed il collaudo.

2. RADIOCHIMICA

Oggetto del programma: Sviluppo di metodi radioimmunologici (RIA)

A) Definizione di un modello cinetico del RIA: scopo del programma è quello i misurare i parametri da cui dipendono specificità e sensibilità del dosaggio, RIA, utilizzando come modello l'angiotensina II; tra i 18 anticorpi a titolo variabile da 1/5000 a 1/400. 000 prodotti nel Laboratorio, ne sono stati selezionati quattro che mostrano diversa specificità nei confronti dell'ormone, del precursore Angiotensina I e dei cataboliti (1-7 eptatpeptide e 1-6 esapeptide). Applicando il modello di Sips e di Rodbard si è sviluppato un programma di calcolo che permette di misurare: il grado di eterogeneità di ogni singolo antisiero; la costante di reazione della classe di immunoglobuline a massima affinità presenti nell'antisiero; la costante di reazione media delle altre classi di immunoglobuline. In base a tali risultati un secondo programma permette di calcolare i valori ottimali dei parametri analitici: concentrazione dei reattivi; tempo di reazione ad ogni temperatura; tipo di adsorbente da utilizzare per la misura di B/F. Applicato al caso specifico dell'angiotensina II il modello ha permesso di definire le condizioni sperimentali per la misura separata dell'ormone e dei suoi cataboliti.

B) Standardizzazione del metodo di dosaggio della Angiotensina I

E' stato completato un programma di ricerca volto a definire le condizioni ottimali per il dosaggio diretto, senza estrazione, della angiotensina I (valutazione della attività reninica).

C) Sviluppo del dosaggio RIA dell'Aldosterone plasmatico

Sono stati ottenuti sette antisieri anti-aldosterone ad elevata specificità; di ogni antisiero è stata eseguita la caratterizzazione completa e la misura

dell'indice di reazione crociata rispetto agli steroidi interferenti. Tutti gli antisieri si prestano al dosaggio dell'aldosterone plasmatico, dopo purificazione mediante TLC monodimensionale, con elevata accuratezza e precisione.

E' stato inoltre sintetizzato il derivato Aldosterone-TME e sviluppato il metodo di marcatura con ^{125}I.

D) Sintesi dei coniugati con albumina di: Testosterone, Progesterone ed Estradiolo.

Sono stati sintetizzati i coniugati di ciascun steroide con l'albumina, utilizzando punti diversi di attacco all'anello ciclopentafenantrenico. Per ciascun steroide è stato inoltre sintetizzato il corrispondente TME-derivato e ne è stata studiata la marcatura con ^{125}I. I cicli di immunizzazione, in corso per ciascuno steroide, hanno dato esito positivo nel caso del testosterone e del progesterone. I relativi antisieri, ad elevato titolo, sono in corso di caratterizzazione.

E) Sintesi di "solid-phase antigens"

Scopo del programma è la preparazione di antigeni accoppiati a resine contenenti gruppi aminici, da utilizzare come fissatori selettivi di frazioni dei corrispondenti antisieri. Questi composti verranno utilizzati per il frazionamento degli antisieri nel quadro di un programma che avrà inizio nel gennaio 1972. Sono state studiate le condizioni ottimali di reazione nel caso dell'aldosterone emisuccinato e dell'angiotensina II seguendo due procedimenti di condensazione: reazione con carbodiimidi in soluzione acquosa e in vari solventi organici - reazione delle anidridi miste. I risultati hanno permesso di identificare come il più conveniente il metodo di condensazione che utilizza la dietil-carbodiimide in mezzo anidro (tetraidrofurano).

F) Marcatura di polipeptidi ormonali ad attività specifica superiore a ICurie/mg

Sono state isolate le due forme iodate della Angiotensina II, la monoiodoangiotensina e la diiodoangiotensina (MIA e DIA). Uno studio comparato delle proprietà antigeniche delle due forme ha dimostrato come solo la MIA possa essere utilizzata come tracciante nei dosaggi RIA. La generalizzazione di tali risultati ad una serie di polipeptidi ormonali (oxitocina, vasopressina e gastrina I) è stata dimostrata in collaborazione con altri laboratori (Saclay, Losanna).

G) Sviluppo di adsorbenti per la misura dell'ormone libero

Come programma derivato da quello di cui al punto A) sono state studiate le caratteristiche, come adsorbente degli ormoni steroidi, delle microsfere di albumina umana. I risultati hanno dimostrato come le microsfere di albumina, che mantengono la capacità di interagire con gli steroidi propri della albumina nativa, siano un vero adsorbente reversibile il cui impiego permette di effettuare la separazione dello steroide libero da quello legato in condizioni di vero equilibrio. Lo stesso tipo di studio è stato eseguito nei confronti del sistema T_3-TBC ed ha consentito la messa a punto di un metodo di misura del T_3-index che appare superiore, quanto ad accuratezza e precisione, ai metodi attualmente in uso.

3. PNEUMOLOGIA

Oggetto del programma: Distribuzione del flusso e del volume di sangue polmona

A) La distribuzione regionale del flusso di sangue polmonare studiata mediante scintigrafia

Lo studio sulla distribuzione regionale del flusso di sangue polmonare è stato esteso dalla proiezione antero-posteriore a quella laterale, specialmente destra, dei polmoni. Le proiezioni laterali, già ampiamente utilizzate nel nostro laboratorio nel corso di studi sulla diagnosi e sulla misura dell'estensione delle embolie polmonari e dei carcinomi broncogeni, consentono un esame molto più dettagliato delle alterazioni regionali della perfusione polmonare. E' stato così possibile indagare in maniera più diretta l'effetto della compressione esercitata dalle strutture cardio-vasali ingrandite sulla distribuzione regionale del flusso di sangue polmonare. In molti cardiopatici si osserva, nella proiezione laterale destra, ed anche in quella sinistra, che la distribuzione dei macroaggregati o delle microsfere è ridotta nelle regioni basilari solo in corrispondenza dell'impronta cardiaca, mentre è ben conservata nelle zone polmonari retrocardiache. E' evidente che in tali pazienti il metodo per la misura del flusso sanguigno regionale del polmone basato sull'iniezione endovenosa di ^{133}Xe e sulla rivelazione della radioattività mediante rivelatori toracici multipli fornirebbe una distribuzione di flusso sanguigno polmonare del tipo cosiddetto invertito o bilanciato, mentre, in realtà, si tratta di alterazioni principalmente focali, cioè da cause locali, della distribuzione del flusso di sangue.

Al contrario, in pazienti con aumento marcato delle resistenze vascolari polmonari conseguente ad elevazione della pressione atriale sinistra, le proiezioni laterali del polmone mostrano che la distribuzione dei macroaggregati o delle microsfere è ridotta nelle regioni basilari non solo in corrispondenza

dell'impronta cardiaca, ma anche di tutte le altre zone declivi del polmone. Risulta quindi evidente l'importanza delle proiezioni laterali al fine di distinguere gli effetti dovuti all'ingrandimento del cuore da quelli dovuti ad alterazione dei principali fattori, quali le resistenze vascolari polmonari e la pressione atriale sinistra, che regolano la distribuzione del flusso di sangue alle varie parti del polmone preso nel suo insieme.

Le osservazioni sopra riportate suggeriscono la considerazione che gli studi funzionali quantitativi di un dato organo sono molto più significativi e di interpretazione più aderente alla realtà quando vengono raccolti nel corso di uno studio che pure visualizza in qualche modo la forma e le dimensioni dell'organo allo studio.

Nel secondo semestre del 1971 è prevista l'estensione di questo tipo di studi e delle ricerche intese a realizzare l'analisi automatica degli scintigrammi polmonari la quale, nella forma richiesta per questi studi, non è stata ancora realizzata.

Gli studi sulla distribuzione del flusso di sangue polmonare sono già stati oggetto di alcune pubblicazioni, delle quali si allega un estratto, ed è in corso di preparazione un manoscritto più sistematico su queste ricerche.

B) Il volume di sangue regionale del polmone studiato mediante rivelatori multipli e gamma-camera

Sono proseguite le ricerche per l'acquisizione mediante gamma-camera dei dati sulla distribuzione regionale dei tempi di transito polmonare con ^{99m}Tc. Sono state ottenute le prime funzioni di frequenza dei tempi di transito su alcune zone polmonari selezionate ed analizzate mediante il calcolatore HP2116B.

Sono pure proseguiti i lavori per adattare lo Whole Body Scanner ad operare sia sul piano orizzontale che su quello verticale coi vari movimenti

di scansione comandati mediante il calcolatore.

Inoltre sono proseguiti gli studi in vitro ed in vivo per meglio caratterizzare il comportamento dell'Indio-113m come tracciante che si lega alla transferrina del plasma umano.

Infine, in vista dell'applicazione su larga scala dell'Indio-113m per la misura della portata cardiaca, dei volumi di sangue regionali, ecc., è stato sviluppato un metodo per la misura diretta, cioè senza ricorrere a nessuna diluizione in vitro od a pipettamento, dell'Indio-113m sia nel liquido da iniettare sia nel sangue prelevato ai pazienti.

Queste tecniche saranno ulteriormente testate ed anche impiegate nel secondo semestre 1971.

4. CIRCOLAZIONE CORONARICA

Oggetto del programma: Studio del flusso miocardico nei pazienti con insufficienza coronarica

A) Flusso miocardico regionale in relazione alla severità delle lesioni coronarie

Il flusso coronarico regionale è stato determinato in 11 pazienti a riposo ed in 8 anche durante tachicardia indotta da pacing, mediante l'iniezione intracoronarica di ^{133}Xenon e registrazione della sua curva di scomparsa mediante gamma-camera collegata al calcolatore (Maseri et al. J. Nucl. Biol. and Med. in stampa). Le zone distali ed una stenosi serrata sovente presentano flusso ridotto rispetto alle zone circostanti a riposo, sebbene il flusso miocardico medio sia sempre entro i limiti osservati in soggetti non ischemici. Nelle zone ischemiche il flusso può aumentare in risposta ad un aumento del carico, ma, se il carico diventa eccessivo e provoca l'insorgenza di angina pectoris il flusso diminuisce marcatamente mentre aumenta ulteriormente nelle zone ischemiche.

Questa osservazione suggerisce che la comparsa dell'angina è legata a meccanismi che riducono il flusso coronarico nella zona ischemica e non solo all'impossibilità dell'apporto ematico a questa zona di aumentare fino a soddisfare le richieste metaboliche.

E' in corso lo studio di pazienti con insufficienza coronarica grave con serrate stenosi o con occlusioni coronariche alla coronarografia per una valutazione funzionale in vista di un possibile intervento chirurgico di rivascolarizzazione e successivamente per valutare i risultati funzionali dell'intervento.

I risultati preliminari di questo studio sono stati selezionati per la presentazione al Congresso della American Federation for Clinical Research, Atlantic City Maggio 1971 (Maseri et al.) presentati al Congresso Internazio-

nale "Myocardial Blood flow in Man" Pisa Giugno 1971 (Maseri et al.).

B) Pazienti con infarto miocardico acuto

Sono state determinate le modificazioni del flusso coronarico nei pazienti con infarto acuto all'ingresso e durante la degenza nell'unità coronarica unitamente alle pressioni intracardiache e polmonari ed alla portata circolatoria e volume di sangue polmonare (Radiocardiografia) in 15 pazienti. E' stata usata la tecnica del ^{86}Rb modificata recentemente (Maseri ed al. J. Nucl. Biol. Med. in stampa).

E' stata osservata una riduzione del flusso coronarico durante la fase iniziale, indipendente dalla sede all'infarto. Successivamente il flusso coronarico tende a ritornare ai valori normali. Per contro in 4 soggetti che poi sono deceduti per cause diverse il flusso coronarico è rimasto a valori subnormali anche dopo la fase iniziale. La portata circolatoria è risultata ridotta inizialmente nella maggior parte dei soggetti ed è successivamente ritornata verso valori normali mentre l'aumento delle pressioni polmonari, osservato inizialmente nei pazienti con infarto anteriore e laterale, tende a persistere.

I risultati di questi studi sono stati presentati al Congresso della Società Italiana di Cardiologia ed al Congresso Internazionale "Myocardial Blood flow in Man" Pisa Giugno 1971 (Donato et al.).

NEFROLOGIA

Oggetto del programma: Sviluppo di metodi di diagnostica nefrologica

A) Studio della funzione renale separata per la diagnosi etiologica di ipertensione da nefropatia monolaterale.

i) Influenza dell'ipotensione indotta sulla portata plasmatica (PRP) di ciascun rene.

E' proseguito lo studio delle variazioni della portata plasmatica di ciascun rene in ipotensione medicamentosa (da clonidina endovena) per la diagnosi differenziale tra ipertensione renovascolare ed ipertensione da pielonefrite monolaterale. In 2 soggetti con pielonefrite monolaterale l'ipotensione non ha determinato variazioni della differenza di PRP tra i due lati. In un caso di ipertensione renovascolare tale differenza si è nettamente ridotta in ipotensione mentre in altri 2 casi i risultati sono apparsi di dubbia interpretazione.

ii) Misura della frazione di filtrazione (FF) di ciascun rene

In 4 soggetti con ipertensione da nefropatia monolaterale sono stati misurati mediante cateterismo ureterale il filtrato glomerulare (FG) (con hypaque-I^{131}) e la portata renale plasmatica (con hippuran-I^{125}). In 2 soggetti con pielonefrite monolaterale la FF del rene affetto e di quello sano è risultata uguale. Al contrario, in 2 casi di ipertensione renovascolare la FF del rene ischemico è risultata ridotta. Se questi risultati preliminari verranno confermati sarà possibile la diagnosi differenziale tra le due nefropatie monolaterali più frequentemente causa di ipertensione arteriosa mediante conteggio esterno, impiegando due traccianti a differente emissione gamma, quali l'hippuran-I^{131} ed il DTPA-La^{140} Sulle esperienze condotte

con quest'ultimo tracciante per la misura del FG è stato riferito in precedenti rapporti.

La determinazione della FF con conteggio esterno presenterebbe il vantaggio, rispetto ad altre metodiche utili per la diagnosi differenziale tra le nefropatie monolaterali ipertensive, di fornire la misura della funzione del rene affetto e di quello controlaterale.

B) Messa a punto e verifica di un nuovo test per la diagnosi di ipertensione renovascolare.

Il test consiste nell'iniezione endovenosa unica di hippuran-I^{131} e nella registrazione, mediante scanner veloce, di due scintigrafie renali, immediatamente dopo l'iniezione del tracciante e a distanza di 1 ora da questa. Esso si basa sulla iperconcentrazione del lato ischemico che subiscono le sostanze non riassorbite a livello tubulare (e quindi anche l'hippuran-I^{131}) per l'aumentato riassorbimento tubulare idrico conseguente all'ischemia. Nell'insieme sono stati finora esaminati (la maggior parte nell'ultimo semestre) 20 soggetti ipertesi, di cui 11 affetti da ipertensione renovascolare, gli altri 9 da pielonefrite monolaterale. In 9 casi di ipertensione renovascolare la scintigrafia con hippuran-I^{131} ha dimostrato una concentrazione del tracciante nettamente maggiore dal lato ischemico. Tale reperto si è normalizzato in 2 casi sottoposti a correzione chirurgica della stenosi dell'arteria renale. Negli altri 2 casi di ischemia renale ed in tutti quelli con pielonefrite monolaterale non si sono osservate differenze di concentrazione tra il rene affetto e quello controlaterale.

In base ai dati raccolti, questa semplice metodica, che richiede solo uno scanner convenzionale, appare utile per la diagnosi differenziale tra ipertensione renovascolare ed ipertensione da pielonefrite monolaterale.

C) Valutazione della ripetibilità di alcuni indici funzionali renali in soggetti con insufficienza renale.

In 10 soggetti con insufficienza renale, con valori di filtrato glomerulare compresi tra 5 e 30 ml/min, è stata ripetuta per 3 volte in giorni successivi la misura della clearance renale dell'urea, della creatinina endogena, dell'acido urico e del filtrato glomerulare (FG) determinato direttamente con il metodo del conteggio esterno sulla vescica, impiegando quale tracciante l'hypaque-I^{131}. Il FG e la clearance della creatinina hanno dimostrato una ripetibilità migliore della clearance ureica, che viene correntemente impiegata per la misura della funzione renale in questi pazienti.

6. ENDOCRINOLOGIA E METABOLISMO

Oggetto del programma: Studio della cinetica degli ormoni proteici.

A) Studio del catabolismo dell'insulina mediante doppio tracciante a partire dalla curva ematica e dalla curva di escrezione urinaria.

Con la sola ipotesi assai verosimile che il catabolismo dell'insulina avvenga in siti a rapido scambio con il plasma, la curva di escrezione urinaria dello iodio liberatosi nel catabolismo dell'ormone è data dalla convoluzione dell'integrale della funzione di distribuzione (Transit Time Distribution Function) del sistema dello iodio libero per l'attività plasmatica dell'ormone moltiplicata per il F. C. R. (Fractional Catabolic Rate). Tale relazione permette di calcolare il F. C. R. dalle curve di scomparsa plasmatica dell'ormone e dalle curve di escrezione urinaria dello iodio libero e ormonale.

Inoltre può essere ottenuta una stima della distribuzione dell'insulina fra pool di iniezione e rimanente spazio insulinico con il metodo del tempo di equilibrio o con metodi integrali.

Lo studio è stato condotto in :

a) soggetti normali (basalmente e a vari livelli glicemici ed insulinemici) n. 15

b) diabetici trattati con insulina da vari periodi di tempo n. 9

c) diabetici che non hanno mai ricevuto insulina n. 13

d) obesi normali n. 11

e) obesi diabetici n. 8

f) iperlipemici essenziali n. 6

B) Studio del catabolismo dell'ormone somatropo (HGII) mediante la tecnica del doppio tracciante.

La metodica impiegata è simile a quella descritta sopra per l'insulina; i presupposti teorici sono gli stessi.

La casistica studiata riguarda:

a) soggetti normali n. 8

b) diabetici insulino-dipendenti n. 3

c) diabetici insulino-indipendenti n. 3

d) acromegalici (prima e dopo intervento di ipofisectomia) n. 2

e) nanismo ipofisario e genetico n. 1

C) Studio del catabolismo dei proteormoni (insulina, HGH) nell'uremia cronica prima e durante trattamento emodialitico.

Lo studio condotto con le tecniche sopra descritte modificate, è stato condotto in pazienti uremici prima e durante il trattamento emodialitico per ambedue i proteormoni negli stessi soggetti per poter valutare le analogie e le differenze nella cinetica di questi due ormoni, che sono i maggiori responsabili della regolazione dell'anabolismo energetico e proteico, in una condizione metabolica profondamente ostacolata come quella dell'uremico cronico.

7. ONCOLOGIA

Oggetto del programma: diagnostica dei tumori.

In questo periodo sono proseguite le ricerche sull'impiego della scintifotografia con fibrinogeno-I^{131} nella diagnosi delle lesioni epatiche occupanti spazio e dei noduli freddi tiroidei.

Per quanto riguarda le affezioni epatiche è stata studiata in particolare la possibilità di differenziare le cosiddette "pseudomasse", di frequente riscontro in alcune epatopatie diffuse, dalle neoplasie epatiche. Il reperto di una o più lacune di radioattività nello scintigramma epatico ottenuto con radiocolloidi, in pazienti portatori di cirrosi epatica pone infatti il problema di stabilire se le immagini lacunari siano da riferire a zone di fibrosi o alla presenza di una neoplasia epatica, la cui incidenza nei cirrotici, come è noto, è superiore che nei soggetti non portatori di epatopatie.

Nel I semestre 1971 sono stati esaminati 16 pazienti nei quali lo scintigramma epatico con radiocolloidi aveva evidenziato una o più lacune di radioattività. Si trattava di 7 cirrosi o epatite croniche, 4 neoplasie primitive, 4 metastasi, 1 caso di linfogranuloma maligno.

L'esame scintifotografico con radiofibrinogeno ha fornito i seguenti risultati: nei sette casi di epatopatie diffuse si è ottenuto un risultato negativo; nei quattro pazienti con neoplasie epatiche primitive, tre hanno fornito un risultato positivo ed 1 un risultato incerto; nel caso di linfogranuloma il risultato è stato incerto.

Per quanto riguarda lo studio con radiofibrinogeno dei noduli freddi tiroidei sono stati esaminati 5 casi di noduli benigni (cisti od adenomi non funzionanti).

In tutti i casi l'esame scintifotografico con fibrinogeno-I^{131} eseguito in fase "precoce" ha mostrato, in corrispondenza della lesione una lacuna di radioattività che è rimasta immodificata nei rilievi della 24^ e 48^ ora (risultato negativo).

LANGZEITWIRKUNGEN UND TOXIKOLOGIE DER RADIOAKTIVEN ELEMENTE

EFFETS A LONG TERME ET TOXICOLOGIE DES ELEMENTS RADIOACTIFS

EFFETTI A LUNGA SCADENZA E TOSSICOLOGIA DEGLI ELEMENTI RADIOATTIVI

EFFECTEN OP LANGE TERMIJN EN TOXICOLOGIE VAN RADIOACTIEVE ELEMENTEN

LONG-TERM EFFECTS AND TOXICOLOGY OF RADIOACTIVE ELEMENTS

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

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

Altri lavori di ricerca al riguardo vengono descritti anche nelle seguenti relazioni annuali:

Verdere publikaties over dit thema zijn ook in de volgende jaarverslagen opgenomen:

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

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ETUDE DES RADIOLESIONS PRECOCES ET DE LEUR DETECTION

(Réactions d'Alarme)

Rapport Année 1971

1. Metabolism of 5 OH tryptophan in isolated perfused rat liver

(G. GERBER and J. DEROO)

It had been reported that the hepatic content of aromatic amino acid decarboxylase diminishes abruptly 12 days before mice die from a lethal radiation exposure. As less 5-OH-tryptamine (5HT) (serotonine) in tissues and more of its catabolites (5 OH indolacetate) (5 HIAA) in urine is found after irradiation, lack of 5 HT has been thought to play a certain role during the terminal phase of radiation disease.

In order to verify whether less 5 HT is formed under in vivo conditions we have studied metabolism of labelled 5-OH-tryptophan in isolated perfused liver from normal and irradiated rats (7-8 days after 1000 R). The results demonstrate that conversion of 5 HTP to 5 HT is much slower than catabolism and conjugation of 5 HT (formation of 5 HIAA, 5 OH tryptophol and various glucoronide and sulfate conjugates). After irradiation, conversion of 5 HTP to 5 HT and its catabolites is not depressed, rather an increased formation of 5 HIAA is noted, whereas conjugation pathways are slightly diminished.

2. Metabolism of nuclear proteins in isolated perfused rat liver

(G. GERBER, J. DEROO and J.P. DECOCK)

The relation between synthesis of nuclear proteins and DNA represents an interesting indicator for the effect of various agents on synthesis of the different components of the genetic structure. We have developed methods to isolate nuclei and to prepare two histone fractions (lysine rich and others), acid proteins, residual proteins, nuclear globulins as well as DNA from small (.5 g) tissue samples.
Lysine labelled with C14 or H3 and thymidine labelled with the corresponding other isotope were added to perfusions of normal and regenerating liver, and catabolism as well as incorporation

into different fractions was studied as a function of time. One study dealt with the effect of aflatoxins (potent cancerogenic substances which supposedly act on the genetic structure). As little as .1 mg of aflatoxin B-1 added to a perfusion of a 24 hours regenerating liver depresses immediately synthesis of DNA and to about the same extent that of histones and acid proteins. Synthesis of nuclear globulins and total proteins is much less susceptible to interference. It had been reported by others that synthesis of nuclear histones precedes that of DNA in regenerating liver by a few hours. Since in these studies in vivo no account could be taken of the effects of catabolism of the precursors, we have carried out similar studies in perfused liver. Indeed, there also takes place synthesis of certain histones prior to the onset of DNA synthesis. Studies on liver irradiated with up to 5 kR and perfused 3 hours later were carried out in order to learn whether a specific interference of radiation with the presynthetic phase exists (irradiation at 16 hours perfusion at 19 hours after hepatectomy). Surprisingly no effect of such higher doses on synthesis of DNA or nuclear proteins was found under these conditions.

3. Metabolism of deoxycytidine in mice and rats.

(G. GERBER, J. DEROO and J.P. DECOCK)

Deoxycytidine (dC) is excreted in excess after irradiation of rats. In mice (and probably in man), however, the amount of dC excreted under physiological conditions is much smaller than in rats and the changes after radiation are less consistent. This is due to the fact that the former species but not rat possesses an enzyme directly degrading dC to deoxyuridine (dU). In rat dC must pass to this end dCMP $\longrightarrow$dUMP, a reaction known to be active only in cells synthesizing DNA of a nucleoside precursor. We then studied the implications that this different handling may have on metabolism of

DNA and its precursors in intact rats and mice as well as in perfused rat and mouse liver and rat intestine. (a technique to perfuse mouse liver was developed for this purpose). Turnover-time of dC in mice is less than 1 hour whereas in rats it is more than 5 hours. Although much dC thus is catabolized directly to dU in mice, a considerable percentage can be reutilized for synthesis of DNA thymidine. On the other hand, rats, despite their low rate of conversion of dC to dCMP by excretion, loose part of their pyrimidine deoxyriboside precursors into urine. As a result of these divergent mechanism incorporation of dC into DNA is about the same for both species although the rate of synthesis of pyrimidine deoxyribosides is much greater in mouse than in rats.

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1. INFLUENCE DES RADIOPROTECTEURS SUR LA SURVIE A LONG TERME ET SUR L'INCIDENCE DES CANCERS INDUITS PAR UNE DOSE UNIQUE DE RADIATIONS IONISANTES.

(J.R. MAISIN, A. DECLEVE, G. MATTELIN, M. LAMBIET-COLLIER, C. BIESEMANS-VAN GENECHTEN et U. VAN GORP)

Des souris mâles BALB/c^+ âgées de 12 à 14 semaines (LD50 30 jours 576 R) sont, suivant les traitements administrés, irradiées par une dose de rayons X (50 $\longrightarrow$ 2000 R). Certaines de ces souris sont, avant d'être irradiées, traitées par de l'AET, de la 5-hydroxytryptamine ou par un mélange de 2-β-aminoéthylisothiourée-Br-HB de glutathion, de cystéine, de mercaptoéthylamine et de sérotonine. D'autres reçoivent après l'exposition aux rayons X une transplantation de 10 millions de cellules de cellules de moëlle osseuse isogénique.

Survie à long terme

L'analyse des résultats obtenus montre que les souris traitées par le mélange de 5 substances radioprotectrices ont une meilleure survie à long terme que les souris non traitées et irradiées et les souris traitées par l'AET. Cependant les différences observées entre les différents groupes varient fortement en fonction de la dose de radiations administrées.

Incidence des leucémies et des cancers

La différence de survie entre les groupes de souris traitées est due surtout à une variation de l'incidence des leucémies lymphoïdes et myéloïdes et secondairement à celle des autres types de cancers (fig. 1 et 2).
Après des doses de 350, 650 ou 1000 R de rayons X, les souris traitées par une association de substances radioprotectrices

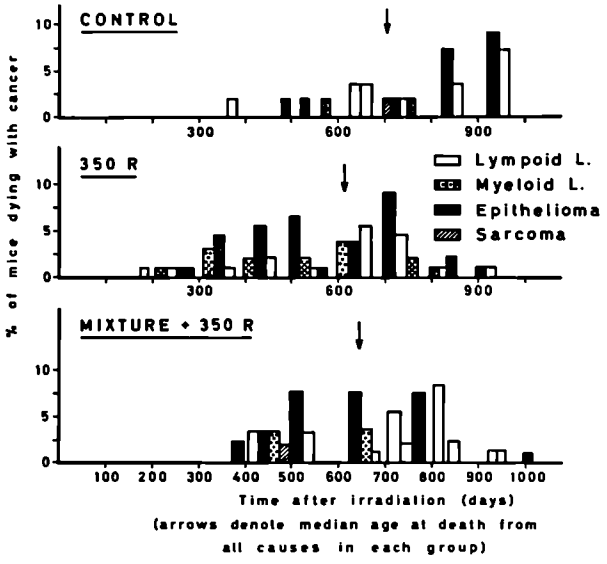


Fig. 1 : pourcentage de souris traitées ou non par un mélange de substances radioprotectrices et mourant de cancer à différentes moments après une dose de 350 R de rayons X.

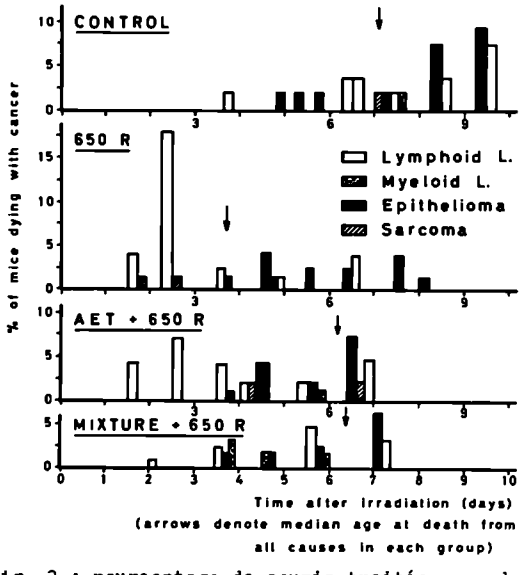


Fig. 2 : pourcentage de souris traitées par de l'AET ou par un mélange de substances radioprotectrices et mourant de cancer à différents moments après une dose de 650 R de rayons X.

présentent une incidence de leucémies lymphoïdes moins élevée que les souris irradiées sans protection ou que les souris protégées par de l'AET. Cette diminution du taux de leucémies lymphoïdes est due surtout à une moins forte incidence des leucémies thymiques. Quant aux leucémies myéloïdes, leur incidence est moins élevée chez les souris protégées par un mélange de substances radioprotectrices et irradiées avec 350 R de rayons X que chez les souris irradiées avec la même dose. Les leucémies lymphoïdes et myéloïdes surviennent également plus tard chez les souris protégées que chez les souris irradiées sans protection.

La protection obtenue par ce mélange de substances radioprotectrices contre l'incidence des leucémies thymiques s'observe également chez les souris C57Bl irradiées avec 4 doses de radiations à huit jours d'intervalle (125, 175 ou 250 R de rayons X). En effet, alors que chez les souris irradiées (250 R) sans protection, l'incidence des leucémies thymiques est d'environ 90 %, chez les souris protégées et irradiées avec la même dose, l'incidence des leucémies thymiques est voisine de 40 %.

Quant aux autres types de cancers, vu la faible incidence rencontrée dans les différents groupes de souris, il est encore trop tôt à l'heure actuelle pour pouvoir déterminer quelle est l'influence exacte des substances radioprotectrices sur ceux-ci. Ce qui est certain, c'est que les substances radioprotectrices augmentent la période de latence de la plupart des cancers.

Nous poursuivons à l'heure actuelle nos expériences pour confirmer les résultats déjà obtenus et pour élucider les mécanismes par lesquels les associations de substances radioprotectrices diminuent l'incidence des leucémies lymphoïdes et myéloïdes chez les souris irradiées.

2. ULTRASTRUCTURE DU POUMON DES SOURIS EXPOSEES A UNE DOSE SUPRALETALE DE RADIATIONS DONNEES SUR LE THORAX.

(J.R. MAISIN, A. DECLEVE, M. LAMBIET-COLLIER, U. VAN GORP et D. BAILLY)

Matériels et méthodes

Toutes nos expériences sont réalisées sur des souris BALB/c^+, irradiées sur le thorax ou sur l'hémithorax avec une dose de 2000 R de rayons X. Pour suivre le transport de la peroxydase à travers la paroi alvéolocapillaire, les souris sont injectées de 5 mg de peroxydase dans une veine de la queue à différents moments après l'irradiation. La peroxydase est mise en évidence par les techniques de Graham et Karnovsky.

Résultats

A. Modifications de l'ultrastructure des capillaires

Les modifications de l'ultrastructure du poumon de la souris après une dose de 2000 R de rayons X peuvent être subdivisées en trois phases :
- la phase aiguë ou phase exudative
- la phase intermédiaire ou phase prolifératrice
- la phase tardive

- Phase aiguë

Pendant la phase aiguë, les lésions sont localisées à certains capillaires. Trois heures déjà après l'irradiation, les cellules endothéliales présentent des modifications de leur ultrastructure (dilatation du réticulum endoplasmique, lysosomes plus abondants que normalement, espace périnucléaire des noyaux dilaté et ségrégation des constituants du nucléole). Six heures après l'irradiation, ces lésions s'accentuent. Notons entre autres dans le cytoplasme des cellules endothéliales

quelques inclusions lipidiques ainsi que des précipitations osmiophiles, rupture de la membrane plasmique, modification de la forme des noyaux, accolement de plaquettes à la membrane basale. Ces lésions s'accentuent dans les semaines qui suivent.

- Phase intermédiaire

Trois à quatre mois après l'irradiation, les lésions des capillaires se sont étendues et aggravées. Les lésions les plus importantes touchent la paroi des capillaires. La lumière de plusieurs capillaires est obstruée par des plaquettes, d'autres par des fibres collagènes ou des inclusions lipidiques. Les leucocytes et les plasmocytes sont nombreux.

- Phase tardive

Huit à quinze mois après l'irradiation, la paroi des alvéoles pulmonaires est fortement épaissie et sclérosée. La membrane basale de plusieurs capillaires est hypertrophiée et oedémateuse, la lumière d'un grand nombre d'entre eux est complètement remplacée par du collagène. Parfois on aperçoit de nouveaux capillaires qui se sont développés dans la lumière de capillaires plus anciens. Les cellules plasmiques et leucocytes sont rares.

B. Modifications de la perméabilité de la paroi alvéolo-capillaire à la peroxydase

- Phase aiguë

Chez les témoins, la peroxydase traverse l'endothélium par les espaces intercellulaires et par pinocytose. La membrane basale est fortement coloriée par la peroxydase et l'on aperçoit rarement de la peroxydase au niveau de la surface des alvéoles.

20 à 30 minutes après l'irradiation, le nombre de vésicules de pinocytose et de replis cellulaires, remplis de peroxydase, est augmenté chez les souris irradiées par rapport aux souris non irradiées.

Trois jours après l'irradiation dans les régions du poumon où les modifications de l'ultrastructure sont marquées, le cytoplasme des cellules endothéliales et des pneumocytes membraneux est entièrement imprégné de peroxydase qui semble libre dans le cytoplasme. Sept jours après l'irradiation, la majorité des cellules endothéliales et épithéliales ont leur cytoplasme infiltré de peroxydase. La distribution de la peroxydase dans le parenchyme pulmonaire est inégale. La membrane basale ne montre qu'une faible imprégnation ou l'absence complète de peroxydase.

Phase intermédiaire

Vers le 7ème mois après le traitement, la distribution de la peroxydase dans les capillaires apparaît irrégulière. Une grande partie des capillaires ont leur lumière altérée et obstruée. Il semble cependant que, dans des régions moins lésées, le transport de la peroxydase est accéléré par comparaison aux souris non irradiées du même âge. De nombreuses vésicules remplies de peroxydase s'ouvrent dans la lumière alvéolaire et une grande quantité de peroxydase se retrouve fréquemment à la surface des alvéoles.

Phase tardive

Chez les souris-témoins et âgées, le transport de la peroxydase semble plus lent que chez les souris jeunes (3 mois). Chez les jeunes souris, 90 secondes après l'administration de peroxydase, celle-ci se retrouve au niveau de la membrane basale et dans les cellules épithéliales ; or, chez les souris âgées, il faut attendre de 5 à 8 minutes après l'injection de peroxydase pour retrouver la même image. Chez les souris âgées irradiées, le transfert de la peroxydase semble encore plus lent.

Conclusions

Ces observations préliminaires semblent montrer que les lésions au niveau des capillaires jouent un rôle important dans le développement des lésions du parenchyme pulmonaire. Il est cependant difficile à l'heure actuelle de dissocier la part prise par l'action directe des radiations sur les différents constituants du parenchyme pulmonaire de la part indirecte due aux lésions des capillaires.

1. ETUDE DES TRANSLOCATIONS RADIOINDUITES DANS LES SPERMATOGONIES DE LA SOURIS.

(A. LEONARD, GH. DEKNUDT et G. LINDEN)

Les translocations réciproques qui impliquent l'échange de fragments terminaux entre chromosomes non homologues demandent 2 cassures voisines l'une de l'autre dans l'espace et dans le temps. On peut dès lors s'attendre à ce que le taux de translocations produites par irradiation des spermatogonies soit influencé par l'intervalle de temps s'écoulant entre les 2 fractions d'une même exposition.

Nous avons étudié ce problème en soumettant des souris mâles adultes à 2 expositions de 250 R séparées l'une de l'autre par des intervalles de temps de 1 à 24 heures.

Les animaux ont été sacrifiés de 120 à 150 jours après le traitement. Aucune anomalie n'a été observée chez les témoins tandis que les animaux exposés à une dose unique de 250 R ou de 500 R possédaient respectivement 4,25 et 8,05 % des spermatocytes en division porteurs de remaniements chromosomiques.

Le tableau I montre clairement que le taux de spermatocytes porteurs de translocation tombe de 8,05 % à un minimum de 5,73 % pour un intervalle de 2 heures ($t = 1,72$; $0,05 < P < 0,10$). Pour un intervalle de temps de 4 heures le taux remonte à ce qu'il était après une exposition unique à 500 R. Après 7 heures il passe par un minimum de 4,44 % pour remonter ensuite à 8,40 % après 16 heures.

Ces résultats concordent parfaitement avec ce qui a été observé avec des microspores de Tradescantia (Lane, 1951), des graines en germination de Viciafaba (Evans, 1967) ou des leucocytes humains en culture (Evans, 1967). Ils suggèrent que l'effet observé est lié directement au cycle cellulaire des spermatogonies irradiées.

T A B L E A U I

SPERMATOCYTES PORTEURS DE TRANSLOCATIONS EN FONCTION DU TRAITEMENT

Observations	Dose de R X			Intervalle de temps entre 2 expositions de 250 R (en heures)										
	0	250	500	1	2	3	4	5	6	7	8	10	16	24
Nombre de métaphases examinées	1.600	2.000	1.950	1.900	1.850	1.700	1.650	2.000	2.000	1.600	1.900	900	1.250	2.000
Nombre de métaphases anormales	0	85	157	131	106	111	145	144	102	71	91	55	105	135
Pourcentage de métaphases anormales	0	4,25 ±0,20	8,05 ±0,83	6,89 ±0,71	5,73 ±0,76	6,53 ±0,89	8,79 ±0,85	7,20 ±0,56	5,10 ±0,54	4,44 ±0,76	4,79 ±0,21	6,11 ±1,11	8,40 ±1,26	6,75 ±0,93

2. RADIOSENSIBILITE COMPAREE D'ANIMAUX APPARTENANT A DIFFERENTES RACES OU ESPECES.

(A. LEONARD, N. GILLIAVOD, GH. DEKNUDT, G. LINDEN)

Dans le cadre des recherches entreprises sur les possibilités d'extrapoler à l'homme les résultats des recherches expérimentales effectuées sur les petits mammifères, nous avons comparé la radiosensibilité génétique de diverses races de souris.
Dans ce but nous avons étudié le taux de léthaux dominants induits par irradiation des spermatozoïdes des souris de races RF, BALB/C, C3H, CBA, COI3, AKR/T1Ald, C57Bl et AKR. L'administration de 400 R aux souris mâles produit une légère diminution du taux de femelles gravides. Le taux de mutations induit par une dose de 400 R décroît de 0.43 pour la race C57Bl à 0.13 pour la race RF. Dans la plupart des races, l'augmentation de la mortalité prénatale est due à un accroissement simultané de la perte avant et après implantation.

La comparaison de nos résultats actuels avec ce qui a été observé sur des espèces telles que le rat, le cobaye et le lapin montre que les variations du taux de léthalité dominante observées entre les races sont plus grandes que celles qui sont notées entre les espèces (Tableau).
Seul le hamster apparaît quelque peu plus sensible.

T A B L E A U II

Taux de Mutation x 10^4 par R

Espèce	Race	Taux de Mutation	Référence
Souris	LP/J	1.68	Storer (1967)
	RF	4.63	*
	BALB/c	5.27 - 9.31	Gilliavod et Léonard (1971)
	CBA	5.61 - 9.39	* Frölen (1965)
	C3H	7.23	*
	AKR/T1Ald	7.92	*
	CO13	9.64	*
	DBA/2J	10.36	Storer (1967)
	C57B1	10.54	*
	Albino	11.43	Frölen (1965)
	SJL/J	12.55	Storer (1967)
	A/J	15.40	Storer (1967)
Cobaye		4.48	Lyon (1970)
Lapin		6.13	Lyon (1970)
Rat	Wistar	11.51	Gilliavod et Léonard (1971)
Hamster		22.55	Lyon (1970)

* Résultats actuels

3. ANOMALIES CHROMOSOMIQUES OBSERVEES DANS DES CULTURES DE SANG PERIPHERIQUE CHEZ LE PERSONNEL D'UN DEPARTEMENT DE RADIOTHERAPIE.

(A. LEONARD, GH. DEKNUDT, G. LINDEN)

Onze personnes appartenant à un département de radiothérapie ainsi que 6 témoins n'ayant reçu aucune dose de radiations ionisantes si ce n'est lors d'examens pulmonaires de routine ont été examinés afin de déceler la présence éventuelle d'anomalies chromosomiques dans les leucocytes du sang périphérique. Le tableau ci-dessous donne les caractéristiques de chaque individu.

Nos observations (Tableaux IV et V) ont montré que l'exposition chimique à des radiations de nature diverse peut entraîner une augmentation des anomalies que l'on rencontre d'ordinaire chez les témoins (lacunes chromatidiennes et chromosomiques, cassure des chromatides et des chromosomes) et l'apparition d'anomalies beaucoup plus spécifiques telles que des chromosomes polycentriques et des chromosomes en anneau. Etant donné que nous n'avons pu établir aucune relation entre le taux de cellules anormales et la dose moyenne annuelle enregistrée par les dosimètres personnels ou le temps passé dans le service, on doit en déduire que cette technique peut être tout au plus utilisée comme indicateur biologique, mais ne peut en aucun cas être employée pour faire de la dosimétrie biologique chez les personnes exposées aux radiations de par leur profession.

T A B L E A U III

Traitement	Sexe	Cas n°	Age	Nombre d'années passées dans le service	Nombre d'années avec dosi-métrie	Dose totale enre-gistrée	Dose moyenne par année	Nombre de surexpositions hebdomadaires
Travailleurs exposés	♂	I 1	33	7	7	5,002	714.6	3
		I 2	40	17	9	7,863	873.7	0
		I 3	40	19	9	13,845	1,538.3	9
		I 4	41	15	8	6,799	849.9	5
		I 5	46	20	5	1,748	349.6	0
		I 6	47	20	9	12,348	1,372.0	6
		I 7	55	30	9	20,217	2,246.3	20
	♀	I 8	30	14	8	9,565	1,195.6	4
		I 9	47	18	9	5,913	657.0	4
		I 10	50	16	8	9,522	1,190.3	5
		I 11	50	3	3	11,817	3,939.0	18
Témoins	♂	C 1	31					
		C 2	46					
		C 3	46					
		C 4	55					
	♀	C 5	30					
		C 6	31					

T A B L E A U IV

ANOMALIES NUMERIQUES

Cas	Nombre total de cellules analysées	Cellules euploïdes		Cellules aneuploïdes	
		Total	%	Hypoploïdes	Hyperploïdes
I 1	160	152	95.0	7	1
I 2	200	174	87.0	19	7
I 3	200	182	91.0	12	6
I 4	202	179	88.6	15	8
I 5	200	178	89.0	16	6
I 6	200	183	91.5	12	5
I 7	156	139	89.1	11	6
Total	1,318	1,187	90.1	92	39
I 8	200	170	85.0	28	2
I 9	202	178	88.1	19	5
I 10	207	180	87.0	23	4
I 11	151	125	82.8	22	4
Total	760	653	85.9	92	15
C 1	202	192	95.0	7	3
C 2	84	69	82.1	14	1
C 3	64	61	95.3	3	-
C 4	86	78	90.7	7	1
Total	436	400	91.7	31	5
C 5	200	185	92.5	14	1
C 6	66	60	90.9	5	1
Total	266	245	92.1	19	2

T A B L E A U V

ANOMALIES CHROMOSOMIQUES DE STRUCTURE

Sexe	Cas	Nombre total de cellules analysées	Cellules avec anomalies de structure										
			Total	%	Aberrations chromatidiennes			Aberrations chromosomiques					
					Lacunes	Cassures	Réarrangements	Lacunes	Fragments	Delétions	Translocations	Anneaux	Dicentriques
♂	1	160	17	10.6	9	3	-	1	4	-	-	1	-
	2	200	13	6.5	8	2	-	4	2	-	-	-	-
	3	200	9	4.5	10	1	-	-	3	-	-	1	-
	4	202	7	3.5	4	2	-	-	1	-	-	-	-
	5	200	20	10.0	5	4	-	3	9	-	-	2	-
	6	200	15	7.5	10	1	-	1	4	-	1	-	-
	7	156	30	19.2	18	3	1	2	5	2	5	-	2
Total		1,318	111	8.4	64	16	1	11	28	2	6	4	2
♀	8	200	7	3.5	3	-	-	-	3	-	-	-	1
	9	202	3	1.5	-	1	-	1	2	-	-	-	-
	10	207	15	7.2	12	1	-	1	2	-	-	-	-
	11	151	2	1.3	2	-	-	1	-	-	-	-	-
Total		760	27	3.6	17	2	-	3	7	-	-	-	1
♂	1	202	8	4.0	7	-	-	1	1	-	-	-	-
	2	84	3	3.6	2	1	-	-	-	-	-	-	-
	3	64	-	-	-	-	-	-	-	-	-	-	-
	4	86	1	1.2	2	-	-	-	-	-	-	-	-
Total		436	12	2.8	11	1	-	1	1	-	-	-	-
♀	5	200	8	4.0	6	-	-	-	4	-	-	-	-
	6	66	1	1.5	1	-	-	-	-	-	-	-	-
Total		266	9	3.4	7	-	-	-	4	-	-	-	-

1. Mécanismes de réparation du DNA mitochondrial

(L. BAUGNET-MAHIEU, R. GOUTIER et C. BAES)

L'étude des mécanismes de réparation du mt-DNA après irradiation "in vitro" de suspensions de mitochondries isolées, est en cours.

a) L'analyse en gradients de sucrose alcalin de suspensions de mitochondries ayant reçu une dose de 20 kR de rayons X indique, par rapport aux contrôles non irradiés, un abaissement et un étalement du pic, plutôt qu'un allègement réel du mt-DNA.
Le phénomène est plus accentué encore en présence d'EDTA, qui bloque l'activité de certains enzymes de réparation.
De plus, la radioactivité acido-insoluble des mitochondries irradiées ne représente que 60 à 80 % de celle des contrôles, ce qui suggère une perte de petites molécules acido-solubles (oligodeoxyribonucléotides). Ces premiers résultats indiquent une dégradation du mt-DNA après irradiation in vitro. Afin de voir si ces dommages sont dans une certaine mesure réparables, ce travail se poursuivra par une cinétique d'incubation des mitochondries irradiées, avec ou sans EDTA, précédant l'analyse en gradients alcalins.

b) Une stimulation de l'incorporation des précurseurs du DNA a pu être mise en évidence chez les mitochondries irradiées. Entre 5 et 20 kR, l'importance de cette stimulation semble indépendante de la dose d'irradiation. La dégradation du mt-DNA par l'irradiation peut s'accompagner d'une augmentation de la capacité primer du DNA et donc de l'activité de la DNA polymérase, due probablement à la libération de groupements 3-OH terminaux. (fig. 1)

c) Par analyse en gradients de chlorure de césium, il est

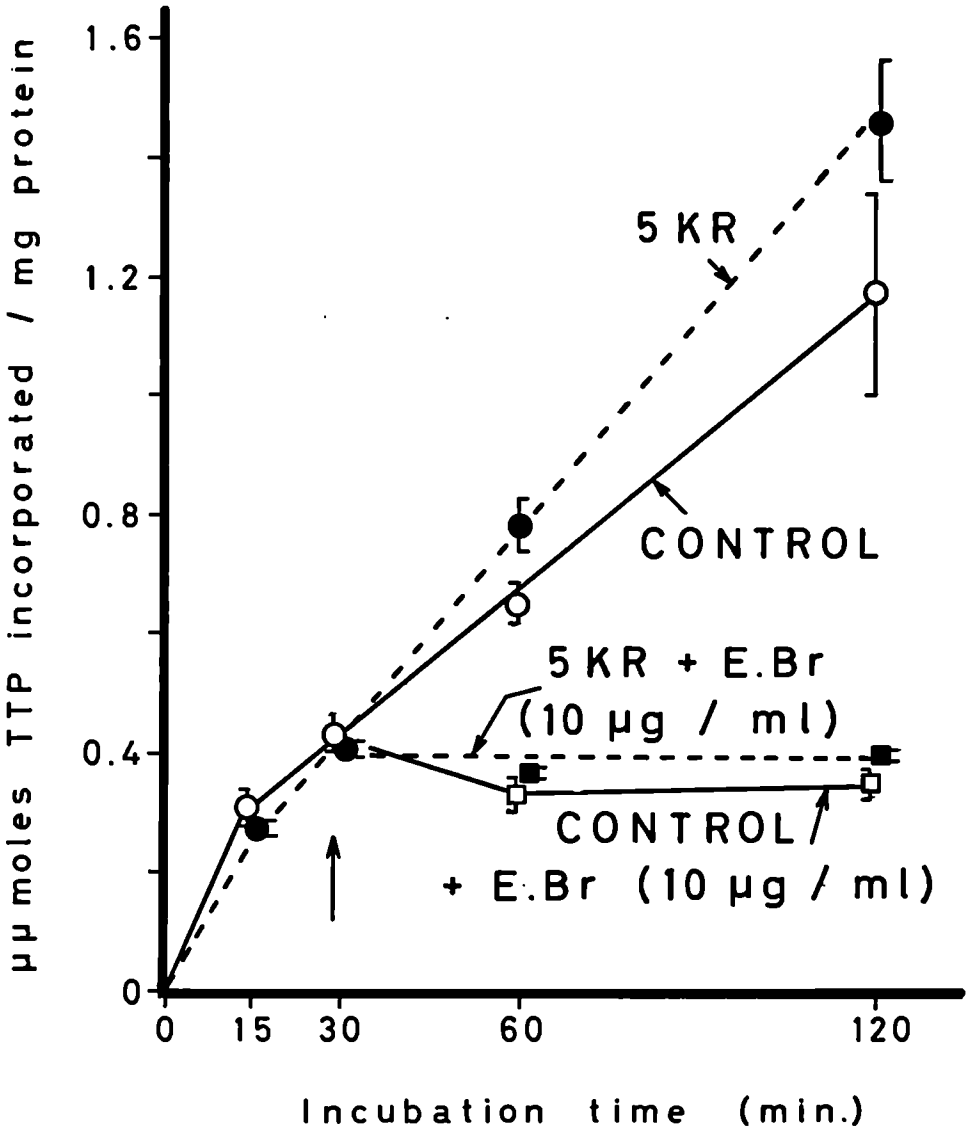


fig. 1. Mitochondries de foie de rat incubées à 37°C en présence de ^{3}H-TTP, immédiatement après une irradiation in vitro par 5 KR.

Lignes continues : suspensions non irradiées

Lignes interrompues ; suspensions irradiées

Après 30 min d'incubation (flèche) : addition de bromure d'éthidium (10 µg/ml).

possible de distinguer la réplication non-semi-conservative (repair replication) de la réplication normale, semi-conservative. Après avoir prémarqué in vivo le mt-DNA par des injections répétées ou une infusion continue de ^{14}C-thymidine, on isole les mitochondries et on les incube ensuite, irradiées in vitro ou non, en présence de ^{3}H-bromodeoxyuridine (^{3}H-BUdR).

La synthèse non-semi-conservative devrait se traduire par une hétérogénéité dans la densité du DNA isolé. Après avoir mis au point les techniques d'isolement et de purification du mt-DNA, nous effectuons les essais d'incorporation de BUdR et de localisation dans les gradients de CsCl du mt-DNA alourdi.

d) Certains inhibiteurs métaboliques sont largement utilisés pour distinguer la réplication semi-conservative de la "repair replication" (voir rapport annuel 1970).

Outre l'hydroxyurée, nous utilisons le Bromure d'Ethidium (EthBr), un inhibiteur de la réplication qui s'intercale dans la double hélice du DNA.

In vivo, nos premiers résultats indiquent que l'injection intrapéritonéale de 10 mg/kg d'EthBr à des rats normaux, exerce un effet inhibiteur sélectif (50 à 70 %) sur la synthèse du mt-DNA, sans affecter la synthèse du n-DNA. Une étude des effets différentiels du EthBr sur les synthèses du mt- et du n-DNA dans le foie de rat en régénération sera suivie d'une série d'expériences combinant l'irradiation et le traitement par EthBr.

In vitro, l'effet inhibiteur de l'EthBr (10 à 25 µg/ml) sur la synthèse du DNA (incorporation de ^{3}H-TTP), est également moins marqué dans les suspensions de noyaux de foie normal ou en régénération que dans les suspensions de mitochondries. On étudie actuellement l'effet de l'addition d'EthBr sur la stimulation de l'incorporation de précurseurs dans le mt-DNA chez les mitochondries irradiées.

2. Polyribosomes hépatiques

(W. BAEYENS, R. GOUTIER et V. VANGHEEL)

Nous avons établi que l'irradiation totale du rat produit une hausse précoce de radioactivité spécifique des polyribosomes périnucléaires. Contrairement à ce que suggéraient des études antérieures, il ne semble pas que les polyribosomes périnucléaires soient des particules intermédiaires entre les précurseurs nucléaires et les polysomes cytoplasmiques, mais bien des polysomes spécialisés qui synthétisent des protéines retournant immédiatement dans le noyau.

Peu de temps après irradiation, la sous-unité ribosomiale 40 S présente une radioactivité spécifique augmentée (après injection d'orotate ^{14}C). Cette sous-unité comprend le RNA messager, le RNA ribosomial et des protéines associées : seule la fixation au formol suivie de l'analyse en gradient de CsCl permet de séparer ces différents composants. Cette technique a été mise au point et appliquée à des extraits cytoplasmiques de foies de rat 6 h après irradiation totale par 2000 R et une injection d'orotate ^{14}C 50 minutes avant le sacrifice. On a pu ainsi mettre en évidence qu'au niveau de la fraction 40 S, l'irradiation totale augmentait la synthèse des deux types de RNA (messager et ribosomial). (fig. 2)

Des essais similaires seront entrepris dans le foie en régénération.

En collaboration avec Mademoiselle le Docteur Lemaire (Service de Radiothérapie, Université de Liège), on a étudié l'influence des hormones thyroïdiennes sur le comportement des polyribosomes hépatiques de rats irradiés ou non. L'injection de triiodothyronine provoque une hausse des polysomes lourds chez des rats thyroïdectomisés, mais, au contraire, une hausse légère des oligosomes chez des rats normaux. L'irradiation augmente la proportion de polysomes lourds chez les rats normaux comme chez les rats thyroïdectomisés.

Buoyant density profiles of 40 S particle fractions obtained from rat liver labeled with [^{14}C] orotate for 40 min. E.D.T.A. treatment: 30 min. at 0° C. Centrifugation of the preformed gradients was at 40.000 rev. / min for 16 h in a titanium S.W. 60 rotor (M. Christ)

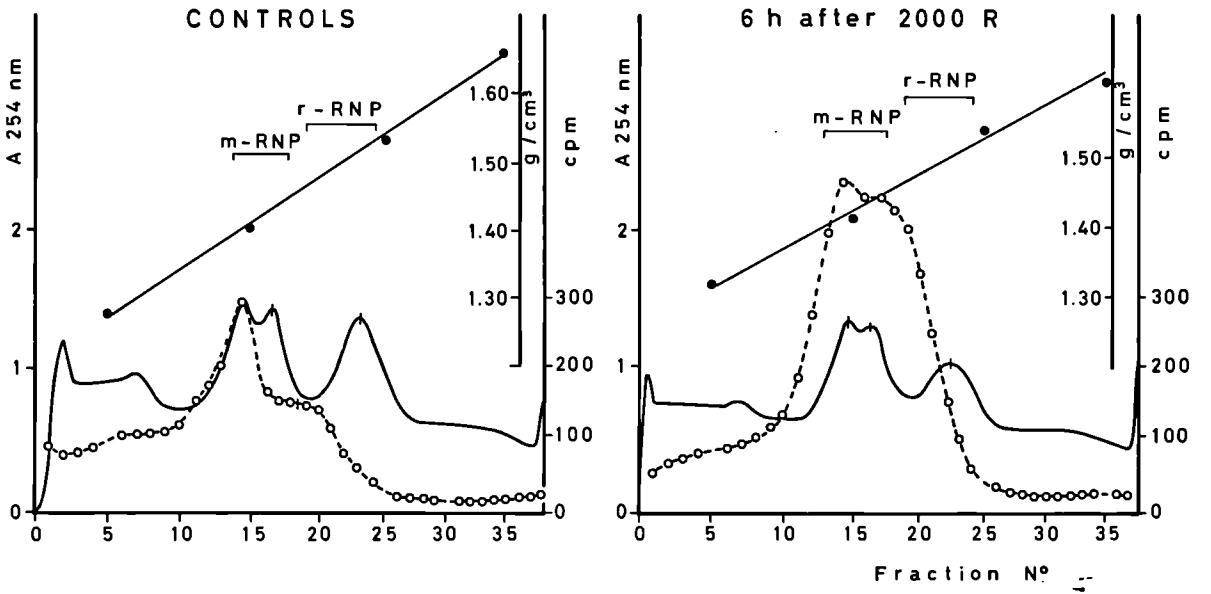


fig. 2. Profils de densité des fractions 40 S obtenues à partir de foie de rat, 40 min après injection d'orotate - ^{14}C. Les particules 40 S ont été traitées par de l'EDTA pendant 30 min à 0°C puis centrifugées sur gradients préformés de CsCl à 40.000 rpm pendant 16 h.

Ligne continue : OD à 254 nm

Ligne interrompue : radioactivité

Ces modifications de distribution des polysomes s'accompagnent de modifications parallèles d'incorporation d'acides aminés in vitro.

Des expériences récentes ont montré l'existence d'un antagonisme entre l'action de la triiodothyronine et l'action des hormones corticostéroïdes libérés à la suite du stress radio-induit.

Les modalités de cet antagonisme sont à l'étude.

Vertrag Nr. 045-65-1 BIAD

Institut für Biologie der Gesellschaft für Strahlen- und Umweltforschung, Neuherberg/München - Germany

O.Hug, W. Gössner, M. Bornhausen, A. Gerspach, T. Hanai, S. Holzberg, A. Luz, K.A. Marquart, J. Murken, F. Schales, J.H. Schröder, H. Spiess, W. Wiederholt, W.A. Winter.

Pathogenesis of genetic and somatic radiation damage.

Radiotoxicity

After incorporation of boneseeking radionuclides in rodents the nature and developement of radiation injuries, 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 isotopes of radium, thorium and cer differing by the radiation emitted (α- and β-emitter), their halflives and their mode of deposition in bone (volume- and surface seeker). In foregoing experiments the frequency of bone tumors after single and repeated injections of ^{224}Ra and its sex and age dependency has been investigated. Now analogous studies with ^{227}Th are in progress. Preliminary results indicate that ^{227}Th produces osteosarcoma with a higher overall incidence and after shorter intervals than 224 Ra. Comparative studies with the β-emitter 141 Ce have been started. Beside late effects initial radiation effects on cell structures and proliferation are studied.

Histogenesis, classification and nomenclature of radiation induced bone tumors are subject of study in contact with EULEP.

Two epidemiological studies are concerned with the late effects of ^{224}Ra after therapeutic application in juveniles and in patients with ankylosing spondylitis.

Radiation effects on the central nerves system

Otherwise undetectable alteration of brain function by relatively low doses of X-rays can be demonstrated after unilateral irradiation of the head in rats and cats by a correlation analysis of the electrocorticograms and of extracellularely recorded neuronal discharge patterns of both hemispheres. These correlations techniques has been achieved by means of a newly developed interphase to a central digital computer.

Genetic radiation effects in vertebrates

As a contribution to the evaluation of the genetic risk of ionising radiation extensive experiments with vertebrates may be considered. In an earlier study the dose dependency of dominant lethals in mice has been established. In a large group of mice no recessive sex-linked lethal mutations could be observed after doses up to 1200 R. Sex-chromosome losses occured after irradiation of spermatozoa and late spermatids only but not in dictyate stage oocytes, primordial oogonia or spermatogonia.
In fishes the mutability of polygenes and behavioral changes in the first postirradiation generation has been studied.

Project 1

Late effects after incorporation of short-lived bone seeking radionuclides

W. Gössner, B.Hindringer, O.Hug, A. Luz, W.A. Müller

Distribution studies and dosimetry were carried out with the short-lived radionuclides ^{47}Ca, ^{90}Y and ^{141}Ce.
The calculated mean skeleton dose for 141 Ce was 1.115 rad for one µCi/kg body weight.
Comparative studies with respect to the distribution of alkaline earths (^{47}Ca, ^{85}Sr, ^{131}Ba, ^{224}Ra) show an exceptional behaviour of ^{224}Ra in this series. Thus the Ra-concentration in the spleen of older animals exceed in the first days after injection even the Ra-concentration of the bone. These effects varied in the rats and two different strains of mice (Fig.1); they are in addition age and sex dependent.
Quantitative autoradiography on tibia diaphysis of mice demonstrates that after incorporation of ^{227}Th the relative number of α-tracks on bone surfaces - compared with the compacta - is about two times higher than after incorporation of ^{224}Ra (Fig.2). Additional γ-spectrometric measurements showed that ^{223}Ra-activity always occured combined with ^{227}Th-activity.
As ^{224}Ra the short-lived ^{227}Th produces bone tumors in mice. Female NMRI-mice 3-4 weeks old were divided in 8 dose groups each containing 100 animals and received between 0.1 and 100 µCi ^{227}Th/kg body weight. 12 months after injection there is a relatively steep increase of bone tumor bearing animals between 1 and 5µCi ^{227}Th/kg (corresponding to 200 and 1000 rads mean skeletal dose). The progressing cumulative osteosarcoma rate indicates at comparable mean skeletal dose ranges an earlier occurrence of osteosarcomas after application of ^{227}Th than after ^{224}Ra (see. Fig. 4). 20 months after incorporation of 5µCi ^{227}Th/kg (diagnosis in the recent cases only by X-ray pictures) the maximum osteosarcoma incidence reaches values of about 80%. Osteosarcomas in the head and in the other ske-

letal sites show a different dose dependence and in addition different morphological pictures (see Fig. 3).

Fig.1
Radium-224 in various organs of different rodents (48^{h} after injection)

Fig. 2
Relative distribution of α-tracks in the diaphysis of NMRI-mice (3-4 weeks old)

Fig. 3
Osteosarkoma incidence. (Corrected for the progressing decrease of the number of animals at risk by summing up the actual monthly tumor rate) versus mean skeletal dose. Female NMRI-mice 12 months after incorporation of ^{227}Th.

Fig. 4
Progressing cumulative osteosarcoma rate of ^{227}Th and ^{224}Ra.

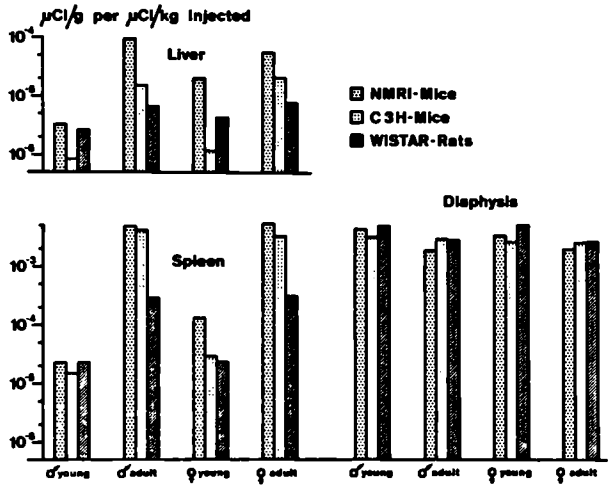


Figure 1

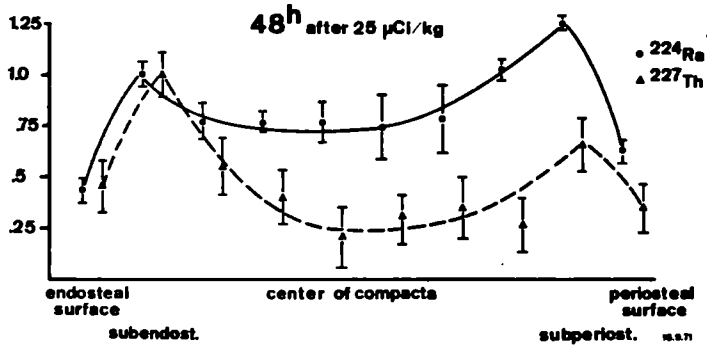


Figure 2

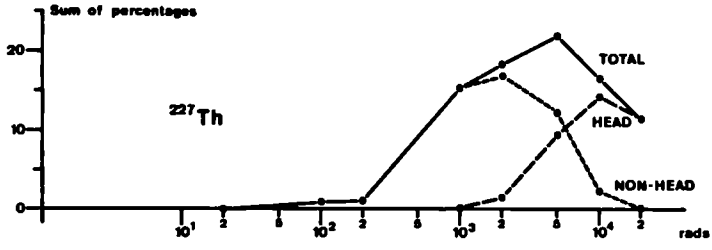


Figure 3

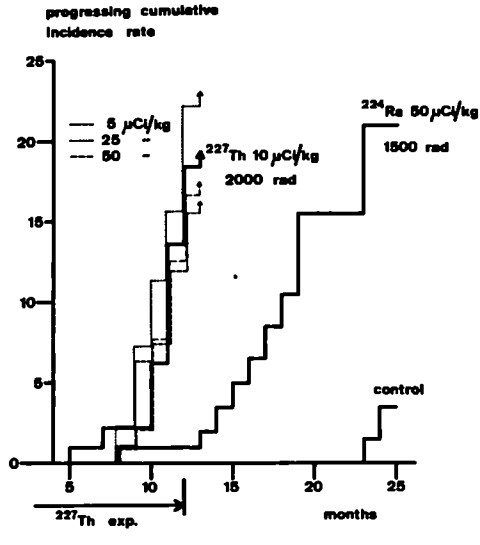


Figure 4

Project 2

Histogenesis, classification and nomenclature of radiation induced tumors.

W. Gössner, A. Lutz

Special interest is devoted to the evaluation of the histogenesis and to the classification of tumors observed in the long-time experiments (see Proj. 1). In the moment these studies concentrate on the morphological analysis of bone lesions ard tumors occuring in our ^{224}Radium and ^{227}Thorium experiments.

These histopathological studies are part of a cooperative research project within the European Late Effects Project Group (EULEP). In 1971 the reference center (committee on pathology of the EULEP) for the histological definition and classification of spontaneous and experimentally (radiation etc) induced tumors has been created in Munich.

A meeting of the committee on pathology has taken place in Munich on October 30-31, 1971, which was attended by members from

Abteilung für klinische Physiologie der Universität Ulm
Centre d'Etude de l'Energie Nucléaire in Mol
Centro di Studi Nucleari della Casaccia in Roma
Experimental Gerontology Unit TNO in Rijswijk
Gesellschaft für Strahlen- und Umweltforschung in Munich
International Agency for Research on Cancer in Lyon
Research Institute National Defence in Sundbyberg.

The main topic was a slide seminar on bone-tumors.
A set of 20 slides has been sent to the participating laboratories. Each case has been presented with the diagnoses and comments received from the different laboratories and discussed in details. After the final discussion the histological criteria for a tentative classification of bone tumors in mice and rats were presented.

Project 3

Pathogenesis of early effects

A. Electron microscopic study of osteoblasts of the tibia of mice after incorporation of ^{224}Ra

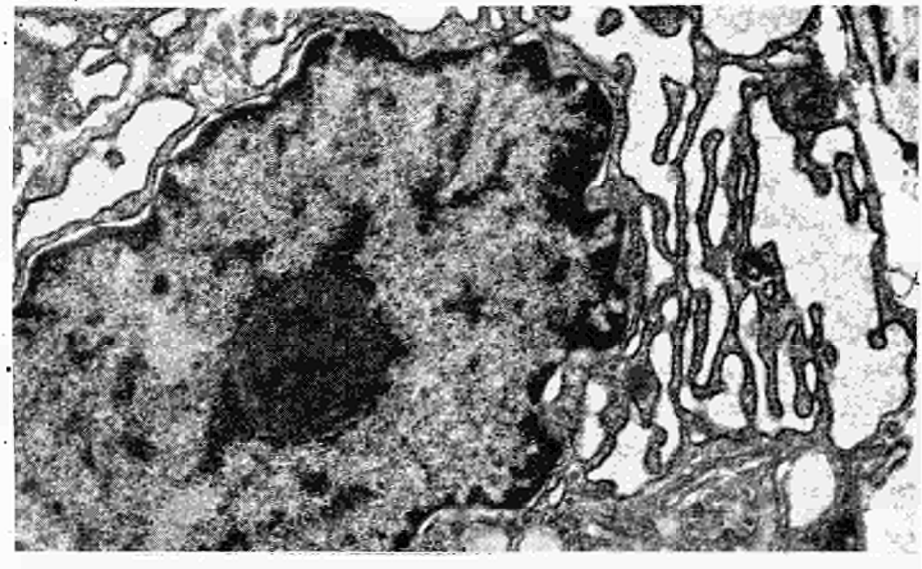
K.-H. Marquart

After incorporation of only 5 µCi ^{224}Ra/kg (NMRI mice, 3-4 weeks old) early ultrastructural changes of osteoblasts in the proximal tibia metaphysis can be demonstrated.

24 hours after incorporation the chromatin in the nuclei of the osteoblasts is condensed, especially in the marginal zone. The nucleoli show a condensed, and nearly homogeneous structure. The space between the two membranes of the nuclear envelop is enlarged. The endoplasmic reticulum is dilated and formes large cisternae (Fig.1). The mitochondria are swollen.

Fig. 1

Electron microscopic picture of an osteoblast of a mouse tibia 24 hours after incorporation of 5 µCi/kg body weight ^{224}Ra. X 20,000.



B. Irradiation of isoproterenol-stimulated salivary glands of mice

W.A. Winter

Changes of the morphological structure and of the cell-proliferation were studied in the parotid and submandibular gland of mice after isoproterenol (IPR)-injection and irradiation.

After IPR-stimulation alone the maximum of the DNA-synthesis occurres in the paratid gland 5 hours earlier than in the submandibular gland and reaches a higher value (72% higher).

After irradiation (1000 R) only in the parotid gland radiation induced cell death can be observed. Irradiation (1000 R) of IPR-pretreated salivary glands in the early and late G_1-phase and during the S-phase show no detectable histologic damage in both glands studied.

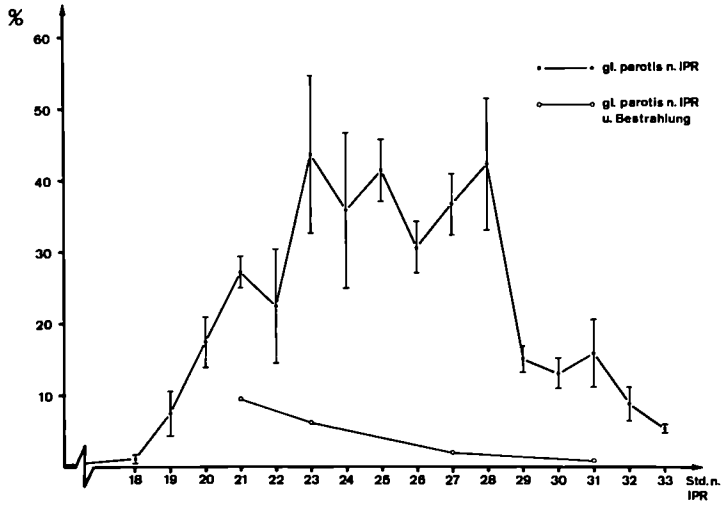
Therefore it is suggested that the IPR-treatment before irradiation may prevent cell-death.

Autoradiography studies with H3-thymidine have shown that irradiation (800R) during the late G_1-phase suppresses DNY-synthesis.

Fig. 2

Upper curve: H3-thymidine-labelling index in the paratid gland after IPR-injection (4mg).

Lower curve: The same after IPR-treatment plus irration (800R) in the late G_1-phase.



Project 4

Studies on late effects induced by ^{224}Ra in children and adults.

H. Spiess, A. Gerspach, J. Murken, W. Wiederholt

In 1971 the epidemiological study, started in 1969, was continued by questionnaires or by medical examinations of ^{224}Ra treated persons. The personal examinations were especially done in persons treated with ^{224}Ra as juveniles. They include the clinical, biochemical and X-ray examination of the skeleton. The pathological findings are summarized in table I.
In half of the checked persons we found an elevation of serum cholesterol above 250 mg% and no elevation of fibrinogen.

Chromosome studies were done in 31 persons treated with ^{224}Ra because of spondylitis ankylopoetica or bone tuberculosis. The total dose injected ranged from 280 to 3000 µCi ^{224}Ra. So far chromosome aberrations has been found in 5,3% of 1478 evaluated cells of 19 persons. The differentiation of the defects is being continued.

In 1971 a new study was started to evaluate the collected data. A special investigation concerns the growth retardation of persons treated as juveniles with ^{224}Ra.

Table I

Follow up of patients treated with ^{224}Ra

December 31, 1971

Number of ^{224}Ra-patients registered: 1814

no present adress: 236, doctors-report without name: 654, examination refused: 15, exact information available: 909

Age at time of 1.treatment:	1-20years	21years a.over	Total
Number providing information	219	690	909
Report by family doctor by questionnaire	40	369	409
Personally contacted Hospital examination	117	107	224
Death	62	214	276
Causes of death			
Basic disease	10	37	47
Not related to basic disease or malignancy	11	84	95
Unknown	3	13	16
Bone malignancy: osteogenic sarcoma	27 (+3)*	12 (+4)*	39 (+7)*
Bone malignancy: chondro-sarcoma	6	0	6
Other malignancy (mostly of lung and digestive tract in the elderly)	0 (+2)*	25 (+5)*	25 (+7)*
Liver disease	1	12 (+13)*	13 (+13)*
Renal disease	2	23 (+6)*	25 (+6)*
Panmyelophthisis, anaemia	2	5	7
Leukaemia	0	3	3
Other abnormalities			
Benign bone tumors: Osteochondroma solitary	17	0	23
Benign bone tumors: Osteochondroma multible	6	0	
Benign bone tumors: Chondroblastoma	1	0	1
Benign bone tumors: Osteofibroma	1	0	1
Teeth spontaneous fractures	13	6	19
Cataracts	7	12	19

*(The numbers in brackets concern living patients with malignant diseases).

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 clinic re-investigation.

In the first stage of the studies 3 hospitals are included. The case reports were scanned for patients with and without ^{224}Ra-treatment. In Frankfurt/Main 244 patients have been contacted. About 50 patients were re-investigated clinically 12 patients with and without ^{224}Ra have died; the causes of death have been recorded.
In Münster/Westphalen and in Hannover contacting of these patients and clinical re-investigation will start in 1972. The collection and evaluation of data (with the aid of electronic data processing) will be done in the same manner as in the Euratom-thorotrast study and parallel to project 4.

Project 6

Irradiation-induced changes of the CNS-function

M. Bornhausen, T. Hanai

Experiments on either prenatally or acutely irradiated cats and rats were performed in order to detect an alteration in the functional state of the brain after exposure to ionizing radiation. The detection of functional impairment in the CNS depends mainly on the comparison of statistically relevant quantitative data.
Three methods were applied and mutually associated:
- EEG-analysis by auto- and crosscorrelation
- serial correlograms of neural discharge pattern
- instrumental conditioning with intracranial selfstimulation in typical schedules of reinforcement.

The electrophysiological data are simultaneously obtained from the left and from the right hemisphere. Only one hemisphere is irradiated. Therefore, inter-individual and time-differences are avoided.

I. With appropriate correlation techniques it is possible to show marked left to right differences in the spectral characteristics of ECoG-pattern in animals with chronical implanted electrods. This correlation analysis - results of which have been reported earlier - can now be performed on-line in a special purpose computer (Intertechnique DIDAC 4000) which offers a ten times higher resolution than the former off-line method. A particular feature of correlation techniques i.e. the extraction of signals from noise is actually used to differntiate the mechnisms of cortical and subcortical death after lethal irradiation.

II. A series of investigations observe the reaction of EEG, evoked potentials and extracellular recorded neutral events to appropriate central or peripheral stimuli in acute unanesthetized preparations. More than hundred experiments so far showed an apparent irradiation-induced enhancement of the

excitatory effect of the stimulation. The evoked potentials also changed their form and/or amplitude in accordance with only this typs of synaptic reaction while other neurones which responded to the stimulation by an inhibition of their discharge rate did not show any alteration either in the duration or the strenght of that inhibitory effect after irradiation.(1000 rads of X-rays.)Corresponding selective irradiation effects upon the excitatory post-synaptic potential (EPSP) in the spinal motoneurone were found by Sato.

III.In order to find radiation or environmental effects upon the functional interactions of different neurones circumscriptidentical areas of both hemispheres were prepared for the simultaneous recording of several units using two pairs of steel-microelectrodes. The spontaneous activity of cells is stored on magnetic tape. Under conditions of experimental stationarity series of spike-intervals can be considered as stochastic point processes and therefore subjected to mathematical treatment. The computation of serial correlograms of single discharge pattern yielding an enormous amount of data has to be performed through computer processing before they can be related to the activity of other units in statistically adequate proportions.

With the help of collaborators from two other GSF-departments a special interface to the central digital computer of the GSF (ICL1902 A) was constructed which discriminates and converts the analog spike data for the computation of serial correlograms, which are displayed on an oscilloscope screen.

Project 7

A. Induction of recessive sex-linked lethal and detrimental mutations in germ cells of the house mouse

J.H. Schröder

A total of 5.775 replicas of irradiated or control X-chromosomes was examined for the occurence of recessive sex-linked lethals. Because no true recessive sex-linked lethal mutation could be found after doses up to 1200 R, the 99% confidence limits for the appearance of recessive sex-linked lethals was calculated to vary between 0.00 and 0.08%. However, if one assumes that at most 4 out of 5 spontaneous or induced mutations were lost by any exchange between the homologous X-chromosomes, the 99% confidence limits would vary between 0.0 and 0.4%. All the experiments performed hitherto for the purpose to induce recessive mutations of the murine X-chromosome favour the view that recessive sex-linked lethal mutations are substituted by detrimental mutations in mammals. Accordingly, the present experiments revealed a significant decrease of genically marked male which were suspected to be hemizygous carriers of radiation-induced detrimental mutations.

B. Aneuploidy of murine sex-chromosomes

No significant increase of sex-chromosome losses was found after X-ray irradiation of dictyate stage oocytes with 200 + 200 R (24 hours apart), of primordial oogonia or spermatogonia in unborn mice with 150 R and of spermatogonia in adult males with 600 R or 1200 R (300 kvp; ca. 53 R/min). However, a significant increase of sex-chromosome losses could be obtained after exposure of spermatozoa or late spermatids with 600 + 600 R of X-rays (24 hours apart) as compared to the controls. The frequency of sex-chromosome losses was either 16 or 23 times higher than that of the controls, corresponding to the already known differences of mutagenic radiation sensitivity between spermatozoa and late spermatids.

C. Radiation-induced mutations of teleosts

J.H. Schröder, S. Holzberg

The already reported experiments for the analysis of mutability of polygenes in teleosts were continued. Certain genotypes of the guppy (Lebistes reticulatus) affecting the synthesis of melanin were found to interact synergistically with recessive radiation-induced mutations expressed as reduced surviability of these homozygous genotypes. The vital vigour of wild-type fish originated from irradiated grandparents was rather improved than reduced.
The irradiation of juvenile cichlid fish (Cichlasoma nigrofasciatum) with 500 + 500 R of X-rays (24 hours apart, exposure of oogonia and spermatogonia) revealed a significant reduction of male agressiveness in the first postirradiation generation as compared to the control fish.

List of publications

W. Gössner, B. Hindringer, O.Hug, A. Luz, W.A. Müller

Early and late effects of incorporated ^{224}Ra in mice

Contamination by bone-seeking radionuclides and radioprotection, 528-548, Soc.Franc.de Radioprotection, Paris 1971

W. Gössner, M.Schwabe

Enzymhistochemie des Knochengewebes

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B. Hindringer, W. Gössner

Microscopic distribution of shortlived α-emitting bone seekers studied by quantitative autoradiography

Contamination by bone-seeking radionuclides and radioprotection, 340-359, Soc. Franc. de Radioprotection, Paris 1971

B. Hindringer, G. Büsing

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Histochemie, 26, 333-342 (1971)

S. Holzberg

Voraussetzungen und Probleme verhaltensgenetischer Analysen.

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K.H. Marquart

Frühe Ultrastrukturveränderungen an den Skeletmuskelcapillaren der Ratte nach lokaler Röntgenbestrahlung

Virchows Arch. Abt. B Zellpath. 10, 4 - 13 (1972)

W.A. Müller

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Contamination by bone-seeking radionuclides and radioprotection, 434-448, Soc. Franc. de Radioprotection, Paris 1971

W.A. Müller

Studies on short-lived internal α-emitters in mice and rats, Part I. ^{224}Ra

Int.J. Radiat..Biol., 20, 27-38, 1971

W.A. Müller

Studies on short-lived internal α-emitters in mice and rats, Part II. ^{227}Th

Int. J. Radiat. Biol., 20, 233-343, 1971

W.A. Müller

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Health Physics Vol. 21 No 6, Suppl. p. 63 (Abstract) 1971

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Dominant Letalmutationen in der Nachkommenschaft bestrahlter männlicher Mäuse. I. Untersuchung der Dosiswirkungsbeziehung und des Unterschiedes zwischen Ganz- und Teilkörperbestrahlung bei meiotischen und postmeiotischen Keimzellenstadien.

Mutation Res. 11: 215 - 245, 1971

J.H. Schröder

Wie mutieren quantitative Merkmale?

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Int. J.Radiat. Biol. 20: 195, 1971 (Abstract)

J.H. Schröder

A search for radiation-induced recessive sex-linked lethal and detrimental mutations in immature germ-cells of the house mouse (Mus musculus)

Genetics 68: 35 - 37, 1971

J.H. Schröder

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GSF-Bericht M 79, p.17, 1971 (Abstract)

J.H. Schröder, S. Holzberg

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Genetics (in the press)

H. Spiess, Ch.W. Mays

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Health Physics, 20, 543-545, 1971

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W.Abmayr, B. Hindringer, H. Platzer

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Seminartag im Institut für Radiochemie, Kernforschungszentrum Karlsruhe, 9.6.1971

W. Gössner, B.Hindringer, O,Hug, A.Luz, W.A. Müller

Tumor induction in mice after incorporation of ^{227}Thorium

VIIIthAnn.Meet. of the Europ.Soc. for Rad.Biol., Basko Polje, Yougosl.,Sept.20-23,1971(Abstract)

W. Gössner, B.Hindringer, A.Luz

Strahlenpathologie (Demonstration)

12.Jahrestagung der Deutschen Strahlenschutzärzte e.V. 8./9.Oktober 1971 München

B.Hindringer, K.Hermes, W.A. Müller, W. Poetsch

Topographic distribution of ^{224}radium and ^{227}thorium in long bones of mice

VIIIth Ann.Meet. of the Europ.Soc.for Rad.Biol., Basko Polje, Yougosl., Sept. 20-23, 1971 (Abstract)

A.Luz

Tumorinduktion nach ^{224}Ra-Inkorporation bei der Maus

Seminartag im Institut für Radiochemie, Kernforschungszentrum, Karlsruhe 9.6.1971

W.A. Müller

Dosimetrie und Verteilungsstudien nach Inkorporation von kurzlebigen α-Strahlern

Seminartag im Institut für Radiochemie, Kernforschungszentrum Karlsruhe, 9.6.1971

W.A. Müller

Dosimetry and late effects with ^{224}Ra in mice

Argonne National Laboratory, Argonne, Ill.(USA), 5.8.1971 Abstract: euro abstracts sect. I 9/923 1971

W.A. Müller

Studies on short-lived internal α-emitters in mice and rats

College of Medicine, University of Utah, Salt Lake City, Utah (USA) 22.7.1971. Abstract: euro abstracts sect. I 9/919 1971

W.A. Müller

Comparison of dose and radiotoxic effects by short-lived and long-lived internal α-emitters

University of California, Davis Laboratory, Calif. (USA)
27.7.71. Abstract: euro abstracts sect. I 9/920 1971

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Distribution and dosimetry of ^{224}Ra and ^{227}Th in mice

Battelle Pacific Northwest Laboratory, Richland, Wash. (USA)
30.7.71 .Abstract: euro abstracts sect. I 9/921 1971

W.A. Müller

Distribution, dosimetry and radiotoxic effects of ingested short-lived α-emitters

Lovelace Foundation for Medical Education and Research
Albuquerque, N.M. (USA), 3.8.1971
Abstract: euro abstracts sect. I 9/922 1971

A Vertrag Nr. 049-69-01 PST D

Institution: Institut für Nuklearmedizin des Deutschen Krebsforschungszentrums (DKFZ),Heidelberg.

Projektleitung: Prof. Dr. K.E. Scheer, Direktor des Instituts für Nuklearmedizin am DKFZ, Heidelberg,
Stellvertreter: Priv.Doz. Dr. W.J. Lorenz,
Institut für Nuklearmedizin am DKFZ, Heidelberg,
Koordinator: Dr. G. van Kaick

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 am Deutschen Krebsforschungszentrum, Heidelberg, und

Prof. Dr. A. Kaul, Klinikum Steglitz der Freien Universität Berlin, Nuklearmedizinische Abteilung,

durchgeführt.

Thema: Forschungsvorhaben "Thorotrast" - Untersuchungen zur Beurteilung der durch künstliche Bestrahlung bewirkten Spätschäden beim Menschen (Thorotrast-Patienten).

Allgemeine Darstellung der durchgeführten Arbeiten:

In dem vom Bundesministerium für Bildung und Wissenschaft sowie von EURATOM geförderten Thorotrast-Forschungsvorhaben werden biophysikalische Untersuchungen zur Abschätzung der durch die Thorotrastdepots bedingten Strahlenbelastung und eine klinische Untersuchung der Thorotrast-Patienten zur Ermittlung der durch das Kontrastmittel ausgelösten Schäden sowie eine statistische Auswertung der Untersuchungsergebnisse durchgeführt. Eine Bewertung der bei den Thorotrastträgern festgestellten Schäden ist nur möglich, wenn ein entsprechendes Kollektiv von Kontrollpatienten nach den gleichen Voraussetzungen untersucht wird. Außerdem ist die Aufklärung der Spätschicksale bei den bereits verstorbenen Thorotrastträgern sowie den Kontrollpatienten für die Ergebnisse des Forschungsvorhabens von Wichtigkeit.

Zur Beurteilung der thorotrast-induzierten degenerativen und neoplastischen Veränderungen der Leber wird eine begrenzte Anzahl der Patienten szintigraphisch und gegebenenfalls angiographisch untersucht.

Daneben hat sich der Einsatz der immunologischen Tumordiagnostik (Bestimmung von Alpha-1-Foetoprotein) als nützlich erwiesen.

Die bereits seit einigen Jahren vorgenommenen Bestimmungen der Chromosomenaberrationsrate bei Thorotrastträgern und Vergleichspersonen und ihre Abhängigkeit von der Strahlendosis werden nach der bisherigen konventionellen Methode weitergeführt. Darüberhinaus soll versucht werden, mit einer bereits erstellten Anlage zur Übertragung photographischer Informationen in rechnerkompatible Größen eine automatische Analyse von Chromosomenaberrationen durchzuführen.

Im Tierversuch muß die bedeutende Frage geklärt werden, ob ein Teil der schädigenden Wirkung des Thorotrast durch den Fremdkörperreiz bedingt ist und wie groß gegebenenfalls dieser Anteil ist.

B Projekt Nr. 1. Arbeitsgruppe Institut für Nuklearmedizin des Deutschen Krebsforschungszentrums (DKFZ), Heidelberg, in Zusammenarbeit mit dem Institut für Dokumentation, Information und Statistik am Deutschen Krebsforschungszentrum, Heidelberg.

Titel: a) Recherchen nach Thorotrast-Patienten; klinische und biophysikalische Untersuchungen; Aufklärung der Spätschicksale verstorbener Thorotrast-Patienten.

Namen der Forscher: Dr. H. Lohölter (bis 31.12.1971)
Dr. T. Oeftering (ab 1.12.1971)
Dr. G. van Kaick
Dipl.Phys. U. Herzfeld
Priv.Doz. Dr. W. J. Lorenz
Prof. Dr. K. E. Scheer

b) Recherchen; klinische und biophysikalische Untersuchungen; Aufklärung der Spätschicksale bei Patienten der Kontrollgruppe.

Namen der Forscher: Dr. D. Lorenz
Dipl.Phys. U. Herzfeld
Dr. P. Schmidlin
Prof. Dr. H. Immich

c) Diagnostik thorotrast-induzierter Lebertumoren.

Namen der Forscher: Dr. G. van Kaick
Dr. H. Kampmann / Dr. P. Georgi
Dr. H. Lohölter / Dr. T. Oeftering

Darstellung der Ergebnisse:

zu a):

Recherchen an folgenden Kliniken:
Düsseldorf - Chirurgische Universitätsklinik;
Gießen - Chirurgische und Neurochirurgische Universitätsklinik,
Tübingen - Versorgungskrankenhaus,
Frankfurt/M. - Psychiatrische und Neurologische Univ.Klinik,
Bamberg - Nervenklinik und ehemaliges Lazarett Bad Nauheim.
Ermittelt wurden insgesamt ca. 600 Patienten, bei denen zur Angiographie wahrscheinlich Thorotrast angewandt wurde.

Oesterreich: Durch Vermittlung von Herrn Prof.Dr. K.E.Scheer konnten die Anschriften von 120 Thorotrast-Patienten übernommen werden.

Arbeitsministerium Bonn: Wir erhielten eine Zusammenstellung von ca. 8000 Kriegsdienstbeschädigten, deren damalige

Erkrankung eine angiographische Diagnostik erforderlich gemacht haben könnte. Bei nachstehend aufgeführten Versorgungsämtern wurden die Akten "verdächtiger" Thorotrast - träger durchgesehen: Darmstadt (76), Frankfurt/M. (104), Gießen (64), Heidelberg (183), Marburg (62), Wiesbaden (61). Es wurde dabei kein Thorotrastträger gefunden, der uns bisher nicht bekannt war.

Aufgesucht wurden ferner das Städtische Krankenhaus Lüdenscheid, die Chirurgische Universitätsklinik Lübeck und das Landeskrankenhaus Bedburg-Hau. Hinweise auf Thorotrastanwendung in diesen Kliniken haben sich nicht bestätigt.

Die Anschriften von 105 in Westdeutschland lebenden Thorotrastträgern, die aus Gesundheits- und Altersgründen nicht reisefähig sind, wurden an die Arbeitsgruppe Homberg gegeben. Es ist vorgesehen, diese Patienten zu Hause mit dem Meßwagen des Homburger Instituts aufzusuchen (s. Projekt Nr. 2).

Klinische und biophysikalische Untersuchungen im Jahr 1971: Es wurden 170 Patienten aus allen Teilen der Bundesrepublik Deutschland nachuntersucht. Bei 120 Patienten konnten Thorotrastablagerungen nachgewiesen werden. Die übrigen 50 Patienten waren "thorotrast-negativ". Die Einbestellung und Untersuchung thorotrast-negativer Patienten läßt sich nicht vermeiden, da den alten Krankenblattunterlagen nur selten ein Vermerk über die Art des Kontrastmittels zu entnehmen ist. Bis zum Ende des Jahres 1971 wurden in Heidelberg insgesamt 690 Patienten nachuntersucht. Dabei fanden wir 481 Thorotrastträger; 209 Patienten hatten keine Thorotrastablagerungen.

Spätschicksale verstorbener Patienten: von den insgesamt ca. 5 800 erfaßten Patienten sind nach den bisherigen Ermittlungen

1 867 am Grundleiden verstorben (d.h. nach unserer Vereinbarung innerhalb von 3 Jahren nach Injektion des Kontrastmittels),

849 später als 3 Jahre post injectionem verstorben; die finale Erkrankung bzw. Todesursache konnte durch Obduktionsberichte und Arztbriefe geklärt werden,

192 später als 3 Jahre post injectionem verstorben; die Todesursache konnte bisher noch nicht geklärt werden.

Bei den 849 Verstorbenen mit bekannter Todesursache ergab die Obduktion 30 primäre maligne Geschwülste der Leber. In der Kontrollgruppe sind primäre Neoplasmen der Leber vergleichsweise noch nicht registriert worden.

Die statistische Auswertung dieser Untersuchungsergebnisse ist selbstverständlich erst nach Abschluß der gesamten Studie möglich.

Zu b):

Patienten der Kontrollgruppe wurden im Jahr 1971 aus folgenden Kliniken erhoben und angeschrieben:

Universitätsklinik Hamburg-Eppendorf (270), Neurologische Klinik der Universität Marburg (625), Chirurgische Klinik

der Universität Würzburg (353), Chirurgische Klinik der Universität Gießen (135), Chirurgische Klinik der Universität München (57), Psychiatrische und Neurologische Klinik der Universität Frankfurt/M. (114), Nervenklinik Bamberg(63).

1971 wurden 128 Patienten aus den oben genannten Kliniken in Heidelberg untersucht. Insgesamt beträgt die Zahl der untersuchten Vergleichspatienten 400.

Von den angeschriebenen Patienten der Kontrollgruppe waren ca. 800 Personen bereits verstorben. Die Todesursache konnte bisher bei 350 Verstorbenen geklärt werden.

Zu c):

Bei 50 Thorotrast-Patienten, die klinische Hinweise für eine Leberparenchymschädigung boten oder bei denen der Verdacht auf einen Lebertumor bestand, wurde ein Szintigramm der Leber mit ^{198}Au-Kolloid angefertigt. Bei der Mehrzahl der Patienten zeigten sich diffuse Rarefizierungen des Leberparenchyms, bei einigen Patienten sogar größere Aussparungen. Auf Grund dieser Ergebnisse sowie auf Grund von suspekten Veränderungen der Röntgenleeraufnahme des Abdomens (Thorotrastverschiebungen) wurde bei 6 Patienten eine Coeliacographie vorgenommen. Ein neoplastischer Prozeß konnte im Angiogramm jedoch nicht nachgewiesen werden. Es ist anzunehmen, daß die Speicherdefekte durch thorotrastbedingte degenerative Veränderungen hervorgerufen sind.

Die hepatozellulären und teilweise auch die cholangiozellulären Karzinome der Leber bilden einen pathologischen Eiweißkörper, der bei Erwachsenen normalerweise nicht vorhanden ist. Dieses sogenannte Alpha-1-Foetoprotein kann immunologisch im Serum nachgewiesen werden. Es ist uns gelungen, zum ersten Mal zu belegen, daß auch das thorotrast-induzierte hepatozelluläre Karzinom der Leber imstande ist, Alpha-1-Foetoprotein zu bilden: bei 1 Thorotrastträger konnte der Tumor vor der histologischen Bestätigung durch die immunologische Bestimmung erkannt werden. Insgesamt wurde 1971 bei 170 Seren die Untersuchung auf Alpha-1-Foetoprotein vorgenommen.

Veröffentlichungen:

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van KAICK, G., W. RAPP und H. LOHÖLTER: Die Bestimmung von Alpha-1-Foetoprotein als Screeningtest bei Thorotrastträgern. Vortrag,Deutscher Krebskongreß Hannover, 1971. Springer-Verlag, im Druck.

van KAICK, G., W. RAPP und H. BECKENBACH: Diagnostik thorotrast-induzierter Lebertumoren durch immunologischen Nachweis von Alpha-1-Foetoprotein. Vortrag auf dem 8. Kongreß der Südwestdeutschen Gesellschaft für Innere Medizin, Karlsruhe 1971.

B Projekt Nr.2. Arbeitsgruppe Institut für Biophysik der Universität des Saarlandes, 665 Homburg (Saar).

Titel: a) Klinische und biophysikalische Untersuchungen an Thorotrastpatienten.

Namen der Forscher: Dr. med. M. Austgen (bis 31.8.71)
Dr. med. B. Schatanek (ab 1.9.71)
Dipl.-Phys. P. Schneider
Prof. Dr. H. Muth
Prof. Dr. Dr. E. Oberhausen

Titel: b) Untersuchung von Chromosomenaberrationen bei Thorotrastpatienten.

Namen der Forscher: Dr. W. Kemmer (Biologe)
Prof. Dr. H. Muth

Darstellung der Ergebnisse:

zu a): Die Recherchierarbeiten zur Ermittlung weiterer Thorotrastpatienten sowie die biophysikalischen und klinischen Untersuchungen an Patienten wurden in enger Zusammenarbeit mit der Arbeitsgruppe Heidelberg (Projekt Nr.1) nach den gemeinsam entwickelten Methoden und Verfahren weitergeführt. Bis Ende 1971 wurden in Homburg (Saar) insgesamt etwa 180 Patienten untersucht. In der Berichtszeit wurden von Homburg weitere 105 Personen, bei denen mit hoher Wahrscheinlichkeit Thorotrastablagerungen angenommen werden müssen, die sich jedoch zunächst nicht bereit erklärt haben, zur Untersuchung nach Heidelberg oder Homburg zu kommen, angeschrieben. Es besteht die Absicht, diese Patienten im Rahmen einer Rundfahrt mit dem Meßwagen des Instituts aufzusuchen und an Ort und Stelle einmal durch Messung der Thoronkonzentration in der Atemluft die Thorotrastablagerung zu testen und zum anderen die notwendigen klinischen Untersuchungen zu veranlassen. Eine ähnliche Aktion war früher (1969) bereits sehr erfolgreich durchgeführt worden. Von den 105 angeschriebenen Patienten haben

sich inzwischen 35 zur Untersuchung mit dem Meßwagen bereit erklärt, 7 wurden bereits in Homburg bzw. Heidelberg untersucht, 13 haben abgelehnt, 16 sind verstorben und 34 Anfragen blieben bisher unbeantwortet. In Durchführung und Fortsetzung dieser Aktion hoffen wir im Jahre 1972 für Homburg die Zahl von 300 untersuchten Patienten zu erreichen. - Die bisherigen im Rahmen des Forschungsvorhabens erzielten Ergebnisse wurden in einem zur 4. Internationalen Konferenz über die friedliche Anwendung der Atomenergie, Genf, 6. - 16.9.1971 vorgelegten Bericht (AED-CONF-71-100-51 Germany May 1971) ausführlich dargelegt (1). Außerdem wurde über spezielle Ergebnisse der biophysikalischen Untersuchungen in einem Vortrag auf dem von der IAEA veranstalteten Symposium on the Assessment of radioactive organ and body burdens, Stockholm, 22. - 26. November 1971 berichtet (2). In einer weiteren Veröffentlichung wurde das Problem der Strahlenbelastung der Lunge von Thorotrastpatienten behandelt (3).

zu b): Untersuchungen von Chromosomenaberrationen wurden bisher an 51 Thorotrastpatienten durchgeführt und mit den bei einer entsprechenden Kontrollgruppe gewonnenen Ergebnissen verglichen. Die mittlere Expositionszeit des Thorotrast betrug 26 $\pm$ 2,8 Jahre. Die Lymphozytenkulturen wurden aus peripherem Blut nach den bekannten Methoden gewonnen. Als Maß für die bis zur Blutentnahme wirksam gewesene Strahlendosis wurde das Produkt aus dem sogen. ^{224}Ra-Äquivalentwert in 10^{-9}Ci und der Expositionszeit in Jahren (RA · Y) zu Grunde gelegt. Der ^{224}Ra-Äquivalentwert (R. Grillmaier, 1964) errechnet sich aus der Thoronkonzentration in der Atemluft, die zusätzlich zur Bestimmung der gesamten Thorotrastablagerung im Ganzkörperzähler bei jedem Thorotrastpatienten gemessen wurde. Dieser Wert ist direkt proportional der Thorotrast-Aktivität im RES, die für die Strahlenbelastung der Lymphozyten entscheidend ist. Bei allen Thorotrastpatienten wurden Chromosomenaberrationen gefunden, die statistisch signifikant höher lagen als die Ra-

ten der Kontrollgruppe (natürliche Rate: 2 Brüche pro 100 Zellen). Wie Abb.1 zeigt, ergab sich jedoch eine starke Streuung der Einzelwerte in Abhängigkeit von der Dosis. Eine statistische lineare Regressionsanalyse ergab eine Dosis-Wirkungsbeziehung der Form:

$$y_{B_1} = 19{,}4 + 0{,}15 \cdot D$$

(Korrelationskoeffizient 0,348; Irrtumswahrscheinlichkeit $< 1\%$). Hierbei ist yB_1 die Gesamtbruchrate und D die Strahlendosis. Sinnvoller erscheint allerdings eine Potenzfunktion der Form:

$$y_{B_2} = 7{,}0 \cdot D^{0{,}33}$$

(Korrelationskoeffizient 0,417. Irrtumswahrscheinlichkeit $< 1\%$). Die letzte Beziehung trägt der Tatsache Rechnung, daß die Kurve praktisch durch den Nullpunkt gehen muß, da bei Personen ohne Strahlenbelastung keine erhöhte Chromosomenaberrationsrate auftritt. Neben großen statistischen Schwankungen, die mit der Methode und Art der Auswertung der Metaphaseplatten in Zusammenhang stehen und auf die bereits im letzten Jahresbericht vom 15.3.71 (für 1970) hingewiesen wurde, sind für die starke Streuung der Einzelergebnisse offensichtlich auch biologische Faktoren maßgebend, wie die Fortführung der im letzten Bericht (für 1970) erwähnten Untersuchungen der Chromosomenaberrationsrate in Zeitabständen von 3 Wochen bis 2 Monaten beim gleichen Patienten (durchgeführt an mehreren Patienten) erkennen liessen. Hierbei zeigt sich eindeutig eine Schwankungsbreite der Ergebnisse, die nicht nur auf statistischen Abweichungen beruhen kann, sondern auch auf biologische Faktoren zurückgeht, wie z.B. die zeitliche Änderung des Lymphocytenpools im Blut bzw. im Körper. In einer Veröffentlichung wurden die bisher gewonnenen Ergebnisse dieser Untersuchungen ausführlich dargestellt (4). - Die bereits im Jahre 1969 begonnene Entwicklung einer Methode der automatischen Analyse von Chromosomenaberrationen wurde 1971 weitergeführt. Eine Apparatur, die nach dem flying spot-Verfahren arbeitet, wurde aufgebaut und vervollkommnet. Sie soll 1972

zur Auswertung eingesetzt werden (5). Dieser Entwicklung kommt im Hinblick auf die mit der sehr zeitraubenden bisherigen mehr oder weniger subjektiven Methode der individuellen Auswertung verbundenen Schwierigkeiten eine besondere Bedeutung zu. Ein automatisches Auswerteverfahren dürfte auch die unabdingbare Voraussetzung für die Weiterentwicklung der Methode als "biologisches Dosimeter" sein.

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(1) MUTH, H., HEYDER, J., van KAICK, G., KAUL, A., KEMMER, W., KOEPPE, P., LORENZ, D., OBERHAUSEN, E., ROEDLER, D., SCHMIDLIN, P.: Results of Biophysical and Clinical Examination of Thorotrast Patients and their Importance for Better Knowledge about Radiation Late Effects. Fourth United Nations International Conference on the Peaceful Uses of Atomic Energy, Geneva, 6-16 September 1971, AED-CONF-71-100-51 Germany May 1971

(2) MUTH, H., EDELMANN, L., GRILLMAIER, R., HERZFELD, U., KAUL, A., KOEPPE, P., KUNKEL, R., OBERHAUSEN, E., ROEDLER, D., WERNER, E.: Radiation Dose in Different Organs of Thorotrast Patients, paper read at the IAEA-Symposium on the Assessment of Radioactive Organ and Body Burdens, Stockholm, 22-26 November 1971 im Druck.

(3) GRILLMAIER, R., MUTH, H.: Radiation Dose Distribution in Lungs of Thorotrast Patients, Health Physics 20 (1971), 409

(4) KEMMER, W., MUTH, H., TRANEKJER, F. and EDELMANN, L.: Dose Dependence of the Chromosome Aberration Rate in Thorotrast Patients, Biophysik 7 (1971) 342.

(5) EICKEL, R.; Entwicklung und Aufbau einer Anlage zur Übertragung photographischer Informationen in rechnerkompatible Größen, insbesondere zur Analyse von Chromosomenaberrationen. Diplom-Arbeit, Mathematisch-Naturwissenschaftliche Fakultät der Universität des Saarlandes, Saarbrücken, 1971

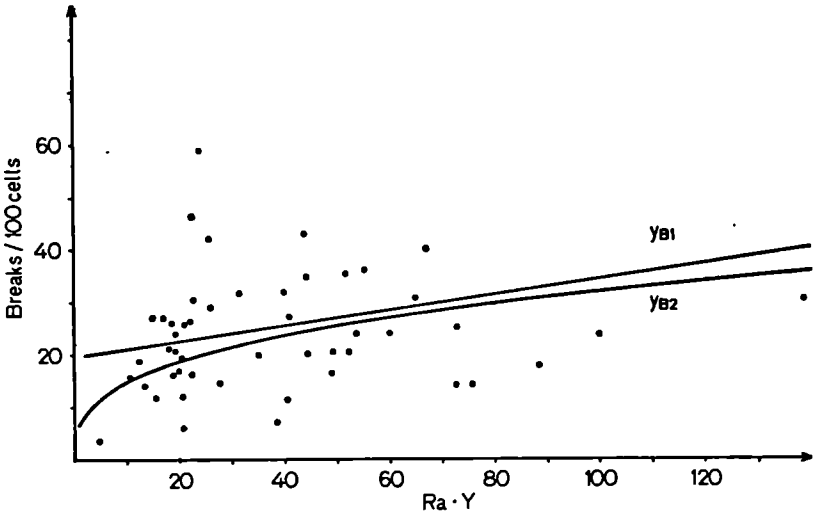

Abb.1: Chromosomenaberrationsrate in Abhängigkeit von der Strahlendosis. Lineare- und Potenz-Funktion der Dosis Effektbeziehung. Abszisse: Radium 224-Äquivalentwert x Jahre (RA · Y). Ordinate: Gesamtbruchrate pro 100 Zellen. y_{B_1}: lineare Beziehung. y_{B_2}: Potenzfunktion.

B Projekt Nr.3. Arbeitsgruppe Klinikum Steglitz der Freien Universität Berlin, z.T. in Zusammenarbeit mit der Gesellschaft für Strahlen- und Umweltforschung mbH München, Institut 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) Retention von kolloidalem ThO_2 und Thorium-Folgeprodukten in Ionen-Form aus dem Blut.

Namen der Forscher: Prof. Dr. A. Kaul
Dipl.-Phys. Dr. J. Heyder

b) Erfassung morphologischer Veränderungen von Thorotrast-Konglomeration durch elektronische Bildanalyse.

Namen der Forscher: B. Hindringer
W. Abmayer
Prof. Dr. A. Kaul

Darstellung der Ergebnisse:

zu a):

Problemstellung

Der Prozeß der Speicherung von kolloidalem ThO_2 läßt sich in drei Phasen beschreiben, die von der primären Retention des Kolloids aus dem Blut durch Phagocytose über eine zunehmende Konglomerierung der ThO_2-Teilchen zu größeren Aggregaten bis zur Speicherung des Kontrastmittels im Bindegewebe verlaufen. - Zum Verständnis der Kinetik der primären Speicherung von Thorotrast wurden Röntgenaufnahmen von Kaninchen nach Inkorporierung von 8 cm^3 einer Thorotrast-Suspension im Zeitraum zwischen 2 min und 180 Tagen nach Injektion des Kontrastmittels durchgeführt. Darüber hinaus wurde der zeitliche Verlauf der Retention der Thorium-Folgeprodukte Radium 228, Th 228, Ra 224 und Pb 212 mit der Teilchenfraktion und Ionenform untersucht

Methodik

Die Röntgenaufnahmen wurden bei einer Röhrenspannung von 60 kV und einem Fokus-Objekt-Abstand von 130 cm durchgeführt, die Expositionszeit betrug 10 Sekunden. Zur quantitativen Bestimmung der Retention der Thorium-Folgeprodukte wurde den Tieren im Zeitraum zwischen 5 Minuten und 75 Stunden nach Inkorporierung des Kolloids jeweils 2 cm^3 Vollblut entnommen.

Ergebnisse

1. Die erste Phase der primären Retention des Kontrastmittels durch Phagozytose gliedert sich in zwei Abschnitte, von denen der erste etwa 60 Minuten nach Injektion abgeschlossen ist. Während dieser Zeit findet eine nur vernachlässigbare Retention des Kolloids statt.
2. Im zweiten Abschnitt der ersten Phase der primären Retention erfolgt die Elimination des Kolloids aus dem Blut nach einer Exponentional-Funktion mit einer Halbwertzeit von etwa 90 Minuten.
3. Das gleiche gilt für die Ionenfraktion von Ra 228 und Ra 224, für deren Retention eine Halbwertzeit von etwa 75 Minuten errechnet wurde.

Schlußfolgerungen

Die quantitativen Ergebnisse über die Retention der Teilchenfraktion erklären den röntgenologisch erhobenen Befund, daß 2 Stunden nach Injektion des Kontrastmittels der Prozeß der Speicherung des Kolloids noch nicht abgeschlossen ist.

zu b):

Problemstellung

Nach Thorotrast-Applikation kommt es zur Ablagerung von Thorium-Dioxid-Konglomeraten in Leber, Milz und Knochenmark. Die Konglomerate erfahren während der Dauer der Speicherung des Thorium-Dioxid-Kolloids eine Größenänderung, woraus eine Änderung der Selbstabsorption der Alpha-Teilchen und damit der Alpha-Strahlendosis resultiert. Zur Berechnung der Alpha-Strahlendosis bei Thorotrast-Patienten ist es daher

erforderlich, die morphologischen Veränderungen der Thorotrast-Ablagerungen im Gewebe quantitativ zu erfassen. - Es wurden deshalb Mikro-Fotos von histologischen Schnitten von Leber- und Milzproben des Kaninchens hergestellt und mit Hilfe eines Bildanalysators im on line-Betrieb mit einer elektronischen Rechenanlage ausgewertet.

Methodik

Kaninchen wurden jeweils 2 cm^3 einer kolloidalen ThO_2-Suspension injiziert. Zu verschiedenen Zeiten nach Thorotrastinkorporation (0,125 bis 392 Tage) wurden die Tiere durch eine Überdosis eines Narkotikums getötet. Von den zu untersuchenden Organen Leber und Milz wurden histologische Schnitte angefertigt und Mikroaufnahmen von willkürlich ausgewählten Arealen einer Abmessung von 860 x 560 µm hergestellt. In einem Bildanalysator wurden die Thorotrast-Konglomerate nach Größe und Anzahl erfaßt. Zur Bilderfassung wurde das sogen. flying-spot-Abtasteverfahren angewandt.

Ergebnisse

Die Anzahl der Thorotrast-Konglomerate in den Milzschnitten lag zwischen 200 und 900, die in den Leberschnitten zwischen 100 und 500. Bereits ein Tag nach Inkorporierung des Kolloids (mittlerer Teilchendurchmesser zum Zeitpunkt der Injektion: 5 nm) haben sich die ThO_2-Teilchen zu Konglomeraten einer mittleren Fläche von etwa 500 μm^2 zusammengelagert. - Die Fläche der Konglomerate nimmt während der Dauer der Thorotrast-Speicherung nach einer monoton verlaufenden Funktion zu und beträgt 300 Tage nach Inkorporierung in der Milz etwa 2600, in der Leber etwa 1500 μm^2.

Diskussion

Obwohl die vorliegenden Ergebnisse noch als präliminar anzusehen sind, zeigt das Verfahren, wie durch Bildanalyse nach dem flying-spot-Verfahren die zeitliche Änderung der Größe der Thorium-Dioxid-Konglomerate quantitativ zu erfassen ist.

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(2) KAUL, A., HEYDER, J.: Retention of Th 232 and daughters from blood, Vortrag IAEA Symposium on Assessment of Radioactive Organ and Body Burdens, Stockholm, 1971, im Druck

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RAPPORT D'ACTIVITE 1971

Contrat n° 049-64-3 BIAF

Commissariat à l'Energie Atomique
Centre d'Etudes Nucléaires de Fontenay-aux-Roses-France

Dr. J. LAFUMA

MOUVEMENT DE CERTAINS ISOTOPES CHEZ LES ANIMAUX ET CHEZ L'HOMME

Cette étude expérimentale est destinée à obtenir des données métaboliques indispensables pour la fixation des limites de sécurité.

En 1971 les études métaboliques ont porté sur le Neptunium-237 dont on a précisé les formes de transport dans le plasma et la répartition dans l'organisme suivant les modalités d'administration.

Les recherches entreprises les années précédentes sur l'épuration alvéolaire ont été développées et les relations dose-effet ont été établies pour plusieurs éléments stables ou radioactifs.

Enfin en 1971 on a commencé l'étude des conséquences pathologiques entrainées par les différents comportements métaboliques des radioisotopes des séries lanthanides et actinides.

PROJET N°1

Métabolisme du Neptunium-237

Dr. J.C. NENOT
Melle MORIN

Les études ont été pratiquées sur des rats Sprague-Dawley SPF. Le neptunium a été administré à une dose pondérale égale au 1/40 de la dose létale. Deux formes chimiques ont été utilisées le nitrate et le complexe formé avec le DTPA et les injections ont été faites soit par voie intraveineuse soit par voie intramusculaire.

Les résultats sont résumés dans les tableaux suivants :

Voie intraveineuse - répartition à 1 jour
en % de la dose injectée

TISSUS	Nitrate pH 1.5	Nitrate pH 7.5	+ DTPA
Squelette	37.4	39.9	31.8
Foie	22.9	16.4	5.5
Muscles+Sang	6.5	7.2	11.4
Rein	3.5	3.	1.4
Urine	23.9	27.7	37.7
Fécès	3.2	4.3	4.3

Voie intramusculaire - répartition à 1 jour
en % de la fraction diffusée

TISSUE	NITRATE	+ DTPA
Squelette	40.3	34.6
Foie	5.7	2.7
Muscles + Sang	11.3	3.0
Rein	2.8	1.1
Urine	38.0	50.4
Fécès	2.0	2.8
Fraction diffusée en % de la quantité totale administrée	22.3	87.5

Ces résultats montrent que la charge hépatique constatée avec les nitrates injectés par voie intra-veineuse dépend de la fraction colloïdale formée, et que le complexe DTPA-Neptunium-237 n'est pas stable en milieu biologique.

Les études bio-chimiques pratiquées sur des échantillons de plasma par la méthode de l'électrophorèse en rideau ont permis de séparer 9 fractions protéinique ayant complexé le Neptunium-237.

PROJET N°2

Etude de l'épuration alvéolaire

W. SKUPINSKI
H. SCHORN
J. C. NENOT
M. MORIN

En 1971 les activités se sont orientées dans deux directions : l'intercomparaison des espèces et l'action toxique de polluants radioactifs ou stables.

Intercomparaison des espèces :

2 groupes ont été identifiés. L'un avec une période d'épuration alvéolaire de l'ordre du mois, l'autre avec une période de l'ordre de l'année.

Dans le premier groupe on trouve : le rat, le hamster, le chat, dans le second on trouve le singe, le chien et l'homme.

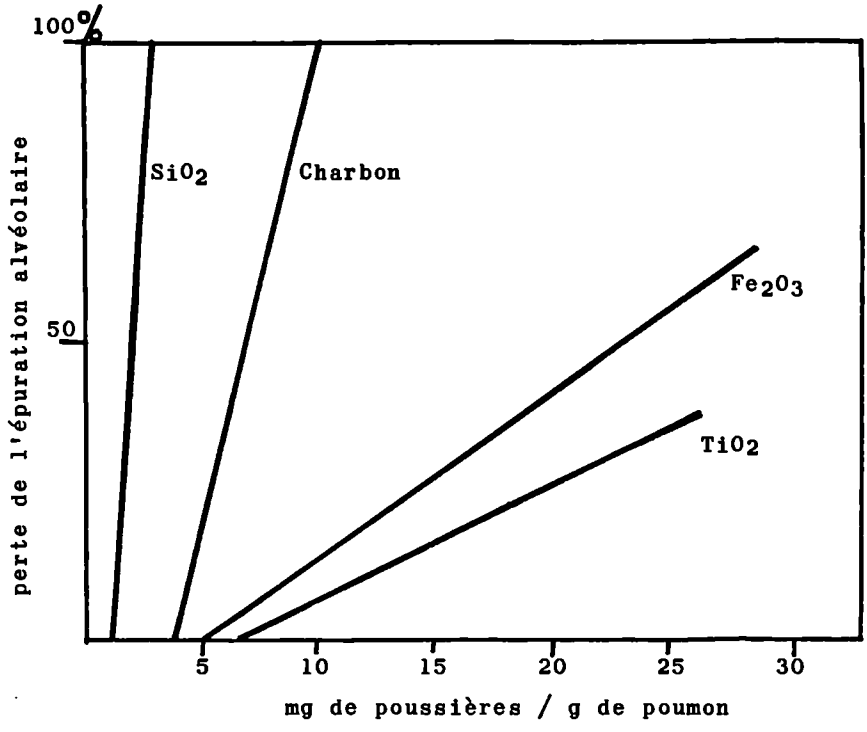
L'étude des causes de cette différence est en cours.

Action toxique des polluants

Les relations dose-effet sur l'allongement des périodes d'épuration alvéolaire ont été établies pour divers polluants radioactifs ou stables.

Polluants stables

Les résultats sont résumés dans la figure suivante :



Polluants actifs

Pour avoir la même perte de l'épuration alvéolaire, nous avons dû faire inhaler aux animaux :

- 15 µCi/g de cérium-144
- 1 µCi/g d'oxyde de plutonium-239
- 0.3 µCi/g de nitrate de plutonium-239

Ce résultat montre l'importance de la nature du rayonnement émis et de la répartition spatio-temporelle de l'irradiation sur cet effet toxique.

Accélération de l'épuration alvéolaire

Une augmentation de la fraction épurée en trois jours entrainant une diminution de 30% de la charge alvéolaire a été constatée après administration de metopyrone 1 heure avant ou après le test au fer 59.

PROJET N°3

Action toxique des lanthanides et actinides inhalés

J.C. NENOT
M. MORIN
W. SKUPINSKI
Avec la collaboration de R. MASSE pour l'analyse des effets pathologiques.

Depuis 1970 des animaux ayant inhalé des lanthanides et des actinides sous des formes physico-chimiques variées et à diverses activités sont conservés et suivis pour étudier les effets à long terme.

A la fin de 1971 nous avons des données permettant de tracer pour le rat ayant inhalé de l'oxyde de ^{239}Pu une courbe de survie comparable à celle tracée avec des chiens par W. BAIR

Une déposition initiale de 0.2 µCi par gramme est suffisante pour induire chez tous les animaux un cancer pulmonaire en 300 jours environ. Avec des chiens W. BAIR avait obtenu les mêmes cancers entre 1.000 et 3.000 jours pour des activités finales dix fois plus faibles.

Ceci montre l'importance de la durée de vie de l'espèce dans la genèse et la cinétique d'évolution de ces cancers pulmonaires.

Nous avons obtenu aussi des cancers avec du nitrate de ^{239}Pu, mais en utilisant des activités plus faibles qu'avec l'oxyde.

Dans ce domaine également, non seulement la nature de la particule mais la répartition spatio-temporelle de l'irradiation sont des paramètres fondamentaux.

En 1971 des animaux ont été contaminés avec du ^{239}Pu, du ^{238}Pu, de l'américium-241, du ^{144}Ce et de l'yttrium-90 afin d'étudier l'action pathologique de ces éléments.

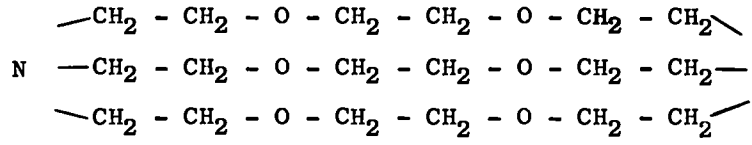
PROJET N°4

Décorporation du Strontium

W. MULLER

En 1971, les recherches sur la décorporation du Strontium se sont poursuivies en utilisant trois molécules appartenant à des classes différentes de composés chimiques.

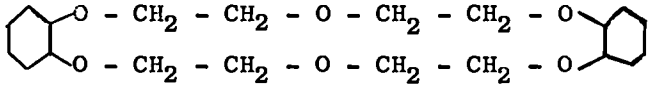
1/ Cryptateur $C_{18}\ H_{36}\ O_6\ N_2$



Chez le rat, la perte d'efficacité de cette substance semble suivre une loi exponentielle et le produit n'est efficace sur le Sr extracellulaire que pendant les premières heures après la contamination.

On a étudié également in vitro l'action du cryptateur sur les oligoéléments et mis au point une méthode d'extraction du cryptateur de l'urine.

2/ Polyethercyclique $C_{20}\ H_{36}\ O_6$



Ce produit a une constante de stabilité vis à vis du Strontium supérieure à celle vis à vis du calcium. Malheureusement sa toxicité le rend inutilisable pour la décorporation.

3/ amino polyetherpolyacétique $C_{12}\ H_{21}\ N_1\ O_9$

$$N\begin{cases} CH_2 - CH_2 - O - CH_2 - COOH \\ CH_2 - CH_2 - O - CH_2 - COOH \\ CH_2 - CH_2 - O - CH_2 - COOH \end{cases}$$

La synthèse de ce produit a été pratiquée selon le schéma suivant :

$$N(CH_2\text{-}CH_2\text{-}OH)_3 + 3 \text{ } \langle\bigcirc\rangle\text{-Li} \longrightarrow N(CH_2\text{-}CH_2\text{-}O\text{-}Li)_3 + 3 \text{ } \langle\bigcirc\rangle$$

$$N(CH_2\text{-}CH_2\text{-}O\text{-}Li)_3 + 3Cl\text{-}CH_2\text{-}COOC_2H_5 \longrightarrow N(CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}COOC_2H_5)3 + 3Cl\ Li$$

$$N(CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}COOC_2H_5)3 + 3KOH \longrightarrow N(CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}COOK)3 + 3\ C_2H_5OH$$

Les expériences effectuées in vitro et in vivo ont montré que ce composé ne permet pas la décorporation du Strontium.

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COMPOSITION DU COMITE DE GESTION

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RAPPORT D'ACTIVITE

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A Contrat n° 077 69 1 BIO C

Institut d'Embryologie et de Tératologie expérimentales du Centre National de la Recherche Scientifique et du Collège de France
49 bis, avenue de la Belle Gabrielle
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Professeur Etienne WOLFF

Effet des irradiations sur l'embryon et ses organes in vivo et in vitro.

Les recherches effectuées dans le laboratoire au cours de l'année 1971 ont porté sur les effets des rayons X sur les embryons de divers animaux, aux points de vue des inhibitions de développement, des malformations, des transformations génétiques d'une part, d'autre part des réparations des lésions et de la protection contre les rayons X.

Au nombre des effets d'inhibition et de transformations figurent :

1°) Les malformations produites par les rayons X dans les membres et leur explication.

2°) L'inhibition complète, la transdétermination et, dans certains cas, la stimulation des ébauches imaginales des Insectes au cours du développement larvaire.

3°) Les effets de doses uniques ou fractionnées des rayons X sur des tumeurs humaines cultivées in vitro, les modifications qu'elles entraînent au point de vue génétique et physiologique.

4°) L'effet des rayons X sur la régénération du pharynx de Planaires d'eau douce.

5°) La protection de l'activité enzymatique respiratoire des mitochondries isolées par le Tris.

6°) Des recherches de biologie moléculaire ont porté sur la dissociation, à l'aide de corps marqués, des différents ARN cellulaires de la peau d'embryons de poulet.

B

I - Production de malformations des membres de type ectromèle et hémimèle chez l'embryon de Poulet, à l'aide d'un agent radiomimétique, l'Ypérite azotée.

B. SALZGEBER

L'administration d'ypérite azotée (concentration 0,02 à 0,03 mg/ml) aux embryons âgés de 2 à 2 jours et demi, (10 à 23 paires de somites) détermine l'apparition de malformations des membres de type ectromèle et hémimèle avec réduction des parties distales. Ces malformations sont souvent associées à d'autres malformations telles que la célosomie ou la strophosomie (voir rapport 1970).

Afin d'étudier la genèse des malformations de membres, deux séries d'expériences ont été effectuées :

1°) L'évolution des ébauches de membres a été suivie dans l'oeuf pendant plusieurs jours après le traitement. Rappelons qu'au moment de l'intervention, ces ébauches ne sont pas visibles morphologiquement mais elles existent à l'état de territoires présomptifs. Les bourgeons apparaissent 20 à 24 heures plus tard chez les sujets témoins et traités. Au cours du développement ultérieur, la croissance des embryons opérés est plus faible que celle des sujets témoins. Les bourgeons prennent un aspect opaque ; leur contour est irrégulier. Ils s'allongent en pointe, à l'extrémité de laquelle, on reconnaît la cape apicale. Trois ou quatre jours après le traitement, certains bourgeons cessent de croître ; à l'autopsie, le membre est absent ou réduit à un petit moignon (ectromélie). D'autres bourgeons évoluent mais la palette distale n'apparaît pas ou ne se forme que partiellement. Seule, la partie proximale se développe ; à l'autopsie, le membre sera dépourvu de zones distales.

2°) L'observation de la structure histologique des membres pendant plusieurs jours après le traitement révèle la présence de 2 constituants du bourgeon de membre, 24 à 30 heures après l'administration de la substance tératogène à l'embryon. Le mésenchyme, à gros noyaux, à figures mitotiques aberrantes, est revêtu d'une cape ectodermique épaissie à sa partie distale. Cette crête apparaît dans tous les cas, quel que soit le degré de lésion du mésenchyme. Mais 48 heures après le

traitement, on observe des figures de dégénérescence : noyaux hypertrophiés, mitoses aberrantes, pycnoses. Selon son degré d'altération, la cape apicale s'effrite et dégénère, ou bien elle est maintenue et fait saillie à l'extrémité du bourgeon. Dans le premier cas, la croissance du bourgeon, est définitivement arrêtée, dans le 2ème cas, le bourgeon s'accroit mais la partie distale ne se forme pas. L'ectromélie, obtenue dans nos conditions expérimentales, n'est pas due à une agénésie des bourgeons. Ceux-ci apparaissent mais deux à trois jours plus tard, la croissance de certains d'entre eux est définitivement arrêtée. Des recherches en cours ont pour but d'élucider le rôle des 2 constituants du bourgeon de membre dans la genèse de ces malformations.

II - Etude autoradiographique de la synthèse des ADN.

B. SALZGEBER

Le précurseur radioactif (Thymidine tritiée) est administré à l'embryon de 3 jours, 3 à 48 heures après le traitement à l'ypérite azotée. Les membres sont fixés 2 à 4 heures après l'injection de substances radioactives.

Trois heures après le traitement à l'ypérite, on ne voit aucune différence entre les bourgeons témoins et les bourgeons traités. Une diminution d'incorporation est nettement visible à partir de 6 à 7 heures après le traitement. Après 24 heures le marquage est très faible dans les bourgeons de membres traités. Il y a donc un effet inhibiteur très net de la synthèse d'ADN. Malgré l'aspect altéré des bourgeons traités, on observe à 48 heures une augmentation très nette du marquage.

Au cours de ces expériences, nous avons également observé des différences d'incorporation entre les deux constituants du bourgeon de membre.

Protection de l'activité enzymatique respiratoire des mitochondries.

J.M. GASC et M. REINBOLD

L'essentiel des résultats obtenus a été publié dans deux notes à l'Académie des Sciences de Paris (Scéance du 17 Janvier 1972). Il ressort de ce travail que l'effet radioprotecteur du tampon Tris à pH 7,4 est important, bien que il apparaisse très différent selon les conditions de mesure de l'activité enzymatique. En effet, ce pouvoir radioprotecteur n'est probablement efficace que sur l'effet d'ionisation des rayons X sur le matériel irradié. Un effet secondaire, mais très important, de l'irradiation est la perméabilisation des membranes mitochondriales, qui entraîne une augmentation apparente de la mesure de l'activité enzymatique. Il n'a pas été possible d'éviter l'alternative suivante : soit mesurer l'activité enzymatique en présence d'un détergent dont l'effet varie avec l'état des mitochondries, donc avec la dose de rayons X reçue, soit mesurer l'activité enzymatique sans détergent, et dans ce cas mesurer aussi la perméabilisation des mitochondries par les rayons X. Dans le premier cas, l'effet radioprotecteur du Tris apparaît très nettement, mais il est discutable à cause de la présence du détergent ; dans le second cas, les conditions de mesure sont plus acceptables mais l'effet radioprotecteur n'est évident que si on le compare à celui de la cystéamine qui se manifeste alors de manière tout à fait analogue. Admettant que l'effet observé après addition de Cystéamine (0,64 mM) à la suspension mitochondriale irradiée est bien une radioprotection, il est possible de conclure à un effet radioprotecteur du même ordre de grandeur pour le Tris ajouté 3 heures avant l'irradiation. Ce délai de 3 heures est nécessaire pour assurer la pénétration du Tris à l'intérieur des mitochondries.

Effet des rayons X sur la régénération des planaires

C. ZILLER

On sait depuis longtemps que les rayons X inhibent la régénération des planaires en détruisant sélectivement les néoblastes (cellules de régénération). Ce phénomène a été étudié dans le cas de la régénération par épimorphose, qui est due à l'activité mitotique et à la migration des néoblastes (Stéphan-Dubois, 1949). Dans certains cas, la régénération se fait par morphallaxie, c'est-à-dire par transformation de cellules différenciées en d'autres types cellulaires. Par exemple, nous avons constaté que dans une queue isolée de la planaire Dugesia tigrina, un pharynx se forme au sein des tissus différenciés et que la morphogenèse du pharynx s'effectue aux dépens de l'appariel copulateur qui se dédifférencie et disparaît complètement. Nous avons pu montrer que les rayons X empêchent cette transformation : le pharynx ne se différencie pas dans une queue irradiée à 6 000 r. Nous nous proposons d'étudier l'effet de cette irradiation sur la morphallaxie dans la queue de D. tigrina au niveau histologique et cellulaire : mitoses, migrations, métaplasie.

Effets des rayons X sur les disques imaginaux de la Drosophile.

P. SIMPSON et C. ZILLER

Les expériences d'irradiation aux rayons X des disques imaginaux ont été poursuivies. D' une manière générale, les rayons X stimulent la croissance des disques et provoquent des transdéterminations non spécifiques. D'autre part, nous avons cherché à savoir si une même dose de rayonnement appliquée en plusieurs fois - 2 à 3 irradiations à 1 ou 2 heures d'intervalle - produisait le même effet qu'une irradiation unique. Les résultats de ces expériences semblent indiquer qu'il n'y a pas de phénomène de récupération entre deux irradiations successives.

Action des rayons X sur des nodules cancéreux humains maintenus en culture organotypique de longue durée.

R. BEAUPAIN

1°) Effets d'une dose unique sur l'incorporation de ^{3}H-U (Uridine tritiée) dans le nodule de la souche Z 200.

La synthèse d'ARN paraît moins sensible à l'action des rayons X que la synthèse d'ADN : l'incorporation de ^{3}H-U dans le noyau n'est pas affectée par les irradiations, même après de très fortes doses (5 000 r). Quarante huit heures après une dose de 5 000 r les nodules sont très nécrosés ce qui démontre l'action des rayons X. Les cellules qui sont encore vivantes incorporent activement la ^{3}H-U . La chute de l'incorporation qu'on remarque avec la ^{3}H -TdR 48 heures après une dose de 600 r, ne se produit pas avec la ^{3}H-U . Le passage du marquage du noyau au cytoplasme de la cellule n'est pas non plus affecté par les rayons X (une dose de 2 500 r).

2°) Effet d'une dose unique sur la caryotype.

Ce travail est effectué en collaboration avec le Dr. de Grouchy. Nous avons pu dénombrer le nombre de 66 chromosomes pour la souche non irradiée (témoin) et de 66/67 pour la souche irradiée (une dose de 600 r). Les 7 marqueurs sont dans la souche irradiée très variables en forme et en taille.

3°) Effets d'une dose unique sur l'incorporation de ^{3}H-TdR dans les nodules de la souche irradiée (Z 200X).

La lecture des lames, déjà prêtes, n'est pas encore assez avancée pour nous faire une idée des résultats de ces expériences et pour en tirer des conclusions.

Deux doses de 600 r à un intervalle de 2 heures et de 24 heures sont appliquées. Aucun des nodules n'a survécu : ils se comportent comme s'ils avaient reçu 1200 r en une seule fois. La réparation après la première dose est insuffisante pour permettre aux nodules de survivre après une deuxième dose.

4°) Effets d'une dose unique sur la survie des explants de la souche irradiée Z 200 X.

Les nodules ont subi une dose de 600 r et 1 200 r. Les chances de survie des nodules de la souche irradiée n'ont pas beaucoup changé par rapport à la souche non irradiée Z 200. 69 % des nodules survivent après une dose de 600 r ; aucun ne survit après 1 200 r.

Etude de la différenciation de la peau embryonnaire de Poulet à l'aide de l'Uridine tritiée.

L. SORIANO et J. DESVEAUX-CHABROL

Les populations de RNA ont une composition différente dans la peau des embryons de poulet aux différents stades de la différenciation (entre 7 et 11 jours). Ce résultat avait été apporté en 1970 grâce à des centrifugations en gradients de saccharose. La composition en bases des fractions ainsi séparées a été examinée en chromatographie en couche mince. Les ARN ont été extraits de peau embryonnaire de 9 et 11 jours marquée à l'uridine tritiée, et, dans certains cas, traitée à l'actinomycine. Le traitement à l'actinomycine à 9 jours fait apparaître deux fractions sédimentant entre 25 et 6 S (unités Svedberg) et dont la composition en bases ressemble à celle de l'ADN de l'embryon de poulet. A 11 jours, seule la fraction 6 S a conservé cette propriété. Dans les deux stades étudiés les expériences témoins sans actinomycine permettent d'isoler les fractions contenant des ARN ayant la composition en bases de type ribosomique.

Les fractions démasquées après blocage de la synthèse des rARN par l'actinomycine, et dont la composition en bases rappelle celle de l'ADN embryonnaire de Poulet, contiennent très probablement les molécules de mARN qui codent la synthèse des protéines de la kératohyaline dont l'apparition est spécifique à ce stade du développement.

RENDICONTO SCIENTIFICO ANNUALE

1° GENNAIO - 31 DICEMBRE 1971

DEL CONTRATTO DI RICERCA EURATOM-ENEL

N. 048 - 71 - 1 - PST I

Nel presente rapporto annuale si riferisce sui risultati degli esami citogenetici, autoradiografici e citologici effettuati su linfociti circolanti di lavoratori della centrale elettronucleare ENEL del Garigliano nel periodo 1-1/31-12-1971. Esso comprende pertanto i dati riportati nei rapporti trimestrali 1-1/31-3, 1-4/30-6, 1-7/30-9, nonchè quelli ottenuti nel corso del trimestre 1-10/31-12-1971.

Dal complesso dei 150 dipendenti dell'impianto, 62 hanno formato oggetto dell'esame cromosomiale mediante le modalità tecniche note; le dosi cumulate dall'inizio del lavoro con radiazioni al momento del prelievo variano da un minimo di 4.112 mrem ad un massimo di 19.386 mrem.

Il gruppo dei 15 soggetti esaminati nel trimestre 1-10/31-12 comprende i lavoratori che hanno cumulato le dosi relativamente più alte (da 13.302 a 19.386 mrem). Dalle colture a tempi lunghi (72 ore) si è avuto sviluppo di 930 piastre metafasiche, con una incidenza del 3,8% di aneuploidia e dell'8% di cellule con anomalie cromatidiche; nelle colture a tempi brevi (48 ore) le piastre metafasiche sono state complessivamente 415, le cellule aneuploidi 24 (5,7%) e le cellule con anomalie cromatidiche 32 (7,7%).

Nel corso del 1971 sono state allestite complessivamente 62 colture che hanno dato luogo allo sviluppo di 3.773 piastre metafasiche, 3.622 delle quali euploidi (96%) e 151 aneuploidi (4%); 237 cellule, pari al 6,2%, contengono anomalie di tipo cromatidico (gaps e delezioni). Dalle 62 colture a tempi brevi si sono ottenute 1.656 piastre metafasiche con una incidenza del 4,7% di aneuploidia e del 5,4% di cellule con anomalie cromatidiche.

Se si considera nel suo complesso il quadro cromosomiale dei lavoratori nucleari a circa 7 anni di distanza dall'inizio del lavoro con radiazioni, non si osservano significative modificazioni rispetto a soggetti di controllo non esposti, nonchè rispetto agli stessi lavoratori esaminati prima e durante l'attività lavorativa (con dose massima cumulata di circa 20 rem). Se si ripartiscono gli stessi lavoratori in gruppi sulla base delle dosi di radiazioni assorbite, si ha un'ulteriore conferma dell'aumento delle anomalie nei soggetti maggiormente esposti; sembra tuttavia che l'incidenza delle anomalie cromatidiche, dopo una fase di incremento da valori del 4% a valori dell'8%, si sia stabilizzata su quest'ultima cifra senza subire ulteriori sensibili modificazioni malgrado il persistente accumulo di dosi di radiazioni.

Tale reperto appare allo stato attuale di non facile interpretazione; è verosimile che esso rappresenti la manifestazione di un alterato metabolismo cellulare concretizzantesi in una certa fragilità dei cromosomi; la constatazione che il lento e costante accumulo delle dosi non determini, dopo una prima fase, un ulteriore aumento del numero di anomalie, fa postulare l'in-

tervento di meccanismi di compensazione o di riparazione.

Significativo anche il fatto che anche nel corso degli esami effettuati nel 1971 non si siano riscontrate aberrazioni di tipo cromosomiale.

Gli studi autoradiografici e citologici sono stati condotti su campioni di sangue prelevati da 5 soggetti di controllo e da 5 lavoratori nucleari che, al momento del prelievo, avevano cumulato rispettivamente dosi di 7.292, 9.818, 12.073, 15.947, 18.910 mrem. Per ciascun soggetto sono state eseguite 3 colture distinte; i risultati ottenuti, che non differiscono sostanzialmente nei controlli e negli esposti, mostravano una certa variabilità da coltura a coltura.

Le prime figure metafasiche si osservavano a 42 ore ed aumentavano di frequenza nei tempi successivi sino ad un picco tra 66 e 72 ore dalla somministrazione di PHA.

Impiegando ^{3}H-timidina, nessuna cellula marcata si osservava dopo 6 e 12 ore di coltura; cellule marcate iniziavano a comparire a 18 ore, aumentavano in seguito per numero ed intensità di marcatura sino ad un picco tra 36 e 42 ore, diminuendo quindi lentamente se il precursore era aggiunto a vari intervalli nel corso della coltura o mantenendosi a lungo elevate quando il precursore veniva immesso nel mezzo all'inizio della coltura.

Gli esperimenti di valutazione dell'indice mitotico e quelli autoradiografici dimostrano che i linfociti circolanti sono nel periodo G_1 del ciclo cellulare e che il trasferimento in coltura in presenza di PHA stimola l'attività proliferativa e la trasformazione blastica delle cellule. Il lungo intervallo tra la comparsa delle prime figure metafasiche e il picco di metafasi indica l'esistenza di una notevole variabilità da cellula a cellula; tale variabilità può dipendere essenzialmente da due fattori: diversa sensibilità di risposta all'azione stimolante della PHA, cioè diversa durata del periodo di latenza prima della mobilizzazione proliferativa e passaggio dallo stadio G_0 allo stadio G_1; oppure variabilità della durata degli stadi che precedono la divisione cellulare, G_1, S o G_2. I risultati delle ricerche autoradiografiche dimostrano che questo secondo fattore assume un ruolo fondamentale nella variabilità dell'attività proliferativa indotta dalla PHA nei linfociti; essi indicano che il periodo G_1 (fase presintetica) del ciclo cellulare è estremamente lungo, da 24 a 36 ore, e la somma dei periodi S (fase di sintesi del DNA) e G_2 (fase post-sintetica) molto variabile, da 18 a 30 ore.

La lunga durata del periodo G_1 dipende probabilmente dal fatto che i linfociti esposti alla PHA debbono subire profonde modificazioni chimiche e completare una serie di fenomeni metabolici per iniziare l'attività proliferativa in coltura. La variabilità della durata dei periodi S e G_2 è in rapporto verosimilmente alla inomogeneità della popolazione cellulare; è noto infatti che i linfociti circolanti, pur essendo morfologicamente simili, appartengono a classi cellulari funzionalmente diverse.

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ACTIVITY REPORT

BIOLOGY GROUP at ISPRA/Italy,
Biology Division, Commission of the European Communiti
Directorate General for Industrial, Technological and
Scientific Affairs

K.Gerbaulet

BIOLOGICAL RESEARCH IN THE FRAME OF THE COMMISSION'S PROGRAMME IN RADIOPROTECTION

GENERAL DESCRIPTION

The overall research work implemented during 1971 by the Biology Group at Ispra was conducted along the following lines:

- Radioactive contamination of the environment (project 1): Studies of the cycles of various radioisotopes in both the food chain and ecological systems under partially controlled conditions including studies in an aquatic system (transfer from water to sediments, seston and plankton, to molluscs and fish) as well as in a terrestrial one (direct contamination of plants by spray irrigation and indirect contamination from water through soils to plants in irrigated agriculture); influence of other environmental pollutants on these processes.

- Biochemical effects of ionizing radiations on nucleic acids (project 2): Isolation, purification and characterization of the nucleic acid enzymes from mammalian sources (DNA and RNA polymerases, terminal transferase and polynucleotide ligase) and preparation of DNA-like models used as significant substrates in assaying the former; analysis of the biochemical lesions caused by irradiation in DNA especially by examining their template properties for the nucleic acid enzymes.

- Effects of physical and chemical agents on biochemical systems (proj.3): Establishment of specific analytical tests for the characterization of interactions; qualitative and quantitative evaluation of the interactions

between Aflatoxin-B_1 and either serum albumin or calf thymus DNA, influence of UV-irradiation on the latter interactions; "in vivo" and "in vitro" studies of the metabolism of foreign compounds including some studies on pre-irradiation effects.

- Radiation biophysics and microdosimetry (project 4): Experimental and theoretical studies of the spectral energy deposition of fast neutrons of 0.5 - 6 MeV in small volumes of tissue and tissue equivalent matter; evaluation of the influence of the energy straggling of the recoil ions on these spectra and of their electronic and nuclear mass stopping power as well as their ranges; calculations of the energy distribution of ion produced delta-rays.

- Radiation sensitivity of insects (project 5): Studies of the effects of gamma-irradiation applied at different stages of life-cycle to some fruit fly species; examination mainly of changes in physiological functions; development of breeding procedures in laboratory conditions.

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PROJECT No. 1

Title: RADIOACTIVE CONTAMINATION OF THE ENVIRONMENT

Names of scientists: A.Berg, E.Levi, C.Myttenaere, M.Merlini, O.Ravera and P.Reiniger

RESULTS

The fate of various radioisotopes has been considered in both terrestrial and aquatic ecosystems. In the first case, direct (leaves) and indirect (roots) contamination of plants were studied in greenhouse experiments under partially controlled conditions; in the second one, investigations have dealt with seston, plankton, molluscs and fish.

Terrestrial ecosystem

- Foliar uptake studies of ^{85}Sr and ^{134}Cs by wheat plants treated by spray irrigation at various stages of life-cycle indicated a low retention for both elements. The radioactivity in the seeds was found to be dependent on the stage of growth at which plants were treated: post-flowering showed highest contamination due to a direct effect, whereas earlier contamination was only due to root uptake from spillage and run-off on the soil in the case of ^{85}Sr; in the case of ^{134}Cs, leaf to grain transport also occured. Further experiments were performed on rye grass to discriminate the simultaneous uptake by leaves of ^{89}Sr and by roots of ^{85}Sr; results confirmed both the separation of two transport paths and the lack of influence of root uptake on the foliar one.

- Soil studies considered the evaluation of the concentration factor of ^{137}Cs in bean plants as a function of its contamination level in different soils. The results so far available indicate a higher uptake by plants grown in a Podzol from Hannover compared with those grown in a Loess from Amiens and a Terra fusca from Bari; the radioactivity found in the material was proportional to that in the soils.

- Root absorption of ^{134}Cs by tomatoes plants grown in waterculture was determined as function of the stable Cs concentration in the nutrient medium. First results showed that an increase of the carrier concentration from 0 to 1 ppm was also accompanied by an increase of the total radioactivity taken up notwithstanding the decrease of the specific activity.

- Experiments with ^{51}Cr carried out in model rice paddies yielded the following results: although absorbed by the roots this element was found not to move appreciably to the rest of the plant, whereas an increase of the stable Cr concentration from 0.005 to 0.5 ppm in the irrigation water resulted in a higher root uptake as well as a translocation of the radioactivity to the shoot bases; no significant ^{51}Cr was detected in the grains; a further increase of the Cr concentration to 20 - 25 ppm became toxic.

Aquatic ecosystem

- In sediments the ^{137}Cs activity from fallout was measured in the different layers; its content in a definite layer was used to determine both the sedimentation rate as well as the chronological order of conventional pollution. Rates of 0.5 - 2 cm/year were found in the lakes surrounding Ispra. Analyses of C, H, N, P, of minerals and of metals in water and seston as well as determinations of the biomass of seston allowed important conclusions concerning the "health" conditions of these lakes.

- The accumulation of ^{134}Cs and ^{85}Sr by plankton was examined under controlled conditions: a preferential accumulation was observed for ^{134}Cs by the phytoplankton and for ^{85}Sr by the zooplankton. Determinations of the primary productivity of plankton by measuring the incorporation of ^{14}C or ^{32}P demonstrated that ^{32}P cannot substitute ^{14}C in such studies but may be used as an index of the metabolic activity of phytoplankton.

- Studies on the effects of chelating agents (EDTA and NTA) on the uptake of ^{58}Co and ^{65}Zn by molluscs and copepods revealed a strong reduction in their uptake; in the case of copepods EDTA was found to be more effective.

- The biological pathway of ^{65}Zn was determined in freshwater fish considering factors as: species, size, water quality, content of stable Zn in water and in both natural and synthetic food. The accumulation rate of ^{65}Zn, differentiating between direct gill or intestinal absorption, as well as its elimination and body distribution were measured. It was found that Zn is homeostatically controlled by fish and that low levels of Hg, Cd, ABS or EDTA did not affect the stable Zn content nor its body distribution but inhibited the uptake and concentration of ^{65}Zn. The exchange rate of Ca was reduced by low concentrations of Zn, Hg or ABS in the water.

- Addendum: The studies were partially performed with the EUR/ITAL Association and under a contract with the CEA.

PROJECT No. 2

Title: BIOCHEMICAL EFFECTS OF IONIZING RADIATIONS ON NUCLEIC ACIDS

Names of scientists: U.Bertazzoni, F.Campagnari and L.Clerici

RESULTS

- The purification and characterization of nucleic acid enzymes was pursued. A procedure was standardized for extracting DNA dependent nucleic acid polymerases from calf thymus nuclei. Large scale purification of a terminal transferase used for synthesizing polydeoxynucleotides yielded in a preparation of 40 mg of enzyme from 20 kg of thymus.
A replicative DNA polymerase was purified 75-fold over the crude nuclear extracts and found to be active on native and denaturated DNA templates; this deoxynucleotidyl transferase required Mn^{++} and Mg^{++} as metal cofactor and was different from the mammalian cytoplasmic DNA polymerase (complementative type synthesizing DNA only in the presence of single stranded DNA and Mg^{++}).
An unstable RNA polymerase could be partially purified (10 - 20 fold over the crude extract) and characterized as being of the type A inhibited by alpha-amantin.

- The synthesis of DNA-like models in radioactive form either as free polymers or as attached to cellulose particles proceeded at an extended scale: poly dA:(^{3}H)dT,dT-cellulose (substrate for assaying DNA ligase) and poly dA :(^{14}C)dT-cellulose and poly dA:(^{14}C)dT,(^{3}H)dC-cellulose (both used as differential substrates for endonucleases recognizing distortions of DNA) were synthesized. Most of these products were delivered to the laboratories of "Genetica Biochimica ed Evoluzionistica" of the C.N.R. at Pavia/Italy and of "Molecular Genetics" of the University of Leiden/Netherlands.

- Studies of the effects of ionizing radiations on nucleic acids were continued. The abnormal response of mildly irradiated DNA as a primer for the terminal deoxynucleotidyl transferase was standardized in conditions which minimize the unprimed synthesis of polynucleotides by the enzyme.
From poly(^{14}C)dT-cellulose, exposed in suspension to X-rays, the produced breakage of nucleotide chains was measured by the release of radioactive material into solution.

PROJECT No. 3

Title: EFFECTS OF PHYSICAL AND CHEMICAL AGENTS ON BIOCHEMICAL SYSTEMS

Names of scientists: H.Ott and P.Scoppa

RESULTS

- Under the above title a reorientation of the research activity has become necessary during 1971 by the nature of the study itself and by further needs of using the available scientific competences in a cooperative way. Therefore, metabolic studies "in vivo" as well as the complex studies on the effects of radiations on the metabolism of foreign compounds were dismissed. The results of the latter studies have shown that the quantitative metabolic changes observed are indirect effects mediated by hormones; sublethal doses of X-rays do not produce in the adult rat biochemical lesions on the enzyme systems involved in the metabolism of foreign compounds.

- Since exposure to radiations can modify the biological effects of various chemicals due to qualitative and quantitative changes of their binding with macromolecules, especially with DNA, the research activity was oriented to an "in vitro" evaluation of radiation induced modifications of such interactions. Aflatoxin-B_1, the most carcinogenic mycotoxin, has been chosen as adequate for pilot studies. Its interaction with bovine serum albumin has been determined quantitatively by spectrophotometric and chromatographic techniques and its interaction with calf thymus DNA by equilibrium methods in considering the influence of several parameters: like concentrations of ligand and DNA, temperature, ionic strength, magnesium ions. By irradiation with UV-light, a certain amount of Aflatoxin or its photodecomposition products became linked to DNA by irreversible binding.

- "In vitro" experiments on liver microsomal fractions of Aflatoxin-treated rats showed an inhibitory effect on the induction of cytochrome P-450, aminopyrine demethylase, benzpyrene hydroxylase and hexobarbital hydroxylase presumably as consequence of inhibition of RNA polymerase activity thus suppressing the "de novo" synthesis of specific enzyme proteins.

- Following specific analytical methods have been established in cooperation: fluorometric determination of chlorophyll in the presence of pheopigments, ATP determination in plactonic material and ß-glucosidase in zooplankton.

PROJECT No. 4

Title: RADIATION BIOPHYSICS AND MICRODOSIMETRY

Names of scientists: J.Booz and M.Coppola

RESULTS

The studies on the spectral energy deposition of ionizing radiations in small volumes of tissue and tissue equivalent matter have been continued looking mainly at fast neutrons of 0.5 to 6 MeV and the corresponding recoil ions. Further work was done on the radiation stucture of these recoil ions and their delta-rays.

The energy deposition in small volumes and in thin layers was evaluated by both experimental and theoretical approaches (Monte Carlo calculations). A good correspondence between theory and experiment served as a basis for calculations at very small volumes of high radiobiological interest where experimental measurements are not possible.

- The tissue equivalence of Shonka plastic and of several gases for fast neutrons of o.5 to 6 MeV has been evaluated. The spectral energy absorption in the various materials has been compared with the spectra in ICRU-tissue by using the Monte Carlo programme developped last year.

- The spectral energy deposition of fast neutrons of 5.8 MeV in small spheres of tissue equivalent matter was calculated for sphere diameters between 0.1 μ and 6.5 μ. The calculations were made at first with and then without taking into account the energy straggling of the recoil ions inside and outside of the sphere. Thus the influence of this effect on the energy deposition spectra could be studied.

- The electronic and nuclear mass stopping power and the ranges of most of the recoil ions (10 KeV - 7 MeV) in ICRU-tissue and in some tissue equivalent materials has been evaluated and tabulated.

- An additional test criterium for the energy distribution function of ion produced delta-rays has been developped. The test is based on the linear ion density of the ions and is as well a test for the energy dependence of the W-value of low energy electrons.

- A Monte Carlo programme "Ionplane" has been established which calculates the energy transfer of energetic ions and their delta-rays to an infinite plane of 100 Å to 10 µ. Making use of the delta-ray distribution described above, the energy transfer of monoenergetic alpha particles has been calculated and compared with the experimental results. The agreement between the experimental and the calculated energy distributions was found to be very good.
In addition the standard variation of the energy deposition of fast ions in thin layers of tissue equivalent matter was studied experimentally and with Monte Carlo calculations. It could be shown that the variation of the energy deposition is not identical to the variation of the energy lost by the ion within the studied layer. Although the two variations are nearly equal for thick layers the variation of the energy deposition becomes gradually smaller than the variation of the energy loss when the layer thickness is decreasing. It also was shown that for all ions the standard deviation can be described by an unique function.

- Some other problems connected with the W-value and the delta-rays emission of recoil ions were tackled experimentally. However, final results could as yet not be obtained. There is now sufficient information on fast neutrons and their recoil ions to initiate the studies on the relationship between energy deposition spectra and radiation damage for these radiations.

PROJECT No. 5

Title: RADIATION SENSITIVITY OF INSECTS

Name of scientist: R.Cavalloro

RESULTS

- Studies of the radiosensitivity of two fruit fly species (Dacus oleae and Ceratitis capitata) at different stages of their life-cycle were pursued.

The results showed that the radiosensitivity was fairly similar in both species and decreased with increasing age in each single stage of life-cycle as well as with the progression in metamorphosis. Thus, doses which reduced by 50% the coming out of eggs increased from 200 rad for 30 minutes old eggs to 30 krad for 36 hours old ones; the LD_{50} for larvae was found to increase from 1 to 2 krad; the hatching of adults from pupae was 50% inhibited by 2 krad at 3 days after pupation and by 45 krad at the last 3 days of pupal stage.

Shortening of 50% of the mean survival time of the developed insects required doses in the rage of 40 - 50 krad, here females were found to be more radiosensitive than males.

- A dose of 8 krad, delivered to pupae 3 days before adults hatch, was observed to be optimal for sterilization purposes. This dose produced vigorous males with competitive sterile spermatozoa and females which stopped laying of eggs. The longevity was not found to differ significantly from unirradiated controls.

- Further studies on Dacus oleae considered their bacterial symbiosis which influences the biological cycle of this species. Comparing natural and laboratory breeding conditions it was found that the absence of these microorganisms was associated with a reduced survival, fecundity and fertility.

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ANPASSUNGEN IN DER LANDWIRTSCHAFT

ADAPTATIONS AGRONOMIQUES

ADATTAMENTI AGRONOMICI

AGRARISCHE ADAPTATIES

ADAPTATIONS IN AGRICULTURE

1971 VERSLAG OVER ONDERZOEKWERKZAAMHEDEN

Institute of the Association EURATOM-ITAL
Wageningen, the Netherlands

Hoofd van de onderzoekgroep(en): Dr.Ir. D. de Zeeuw

Algemeen thema van het verslag:

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.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 9
Projectnummer	: 9	
Projecttitel	: Development and initial application of a semi-conductor assembly for biological research	
Onderzoeker(s)	: S.C. van de Geijn	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
„ „ -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	–	mandagen
Middelbaar personeel	–	mandagen
Lager personeel	–	mandagen

Beknopte weergave van achtereenvolgens

A. Verslag afgelopen jaar (1971) met vermelding van publicaties

B. Plan komende jaar.

A. Verslag over 1971

"In-vivo" localization of β-emitting isotopes with a semiconductor detector assembly.

Preliminary results have been obtained on the usefulness of the method for ^{32}P-localization in stems of Xanthium Pennsylvanicum after root uptake (see project 29, page).
Calibration measurements with ^{14}C show that the method can also be applied for this isotope. The results are in good agreement with theory for depths ranging from 0-200 μm (density 1g/cm^3), where a maximum accuracy of about 20 μm can be obtained.

A further study has been made of the extension of the in-depth localization method to the determination of the in-depth distribution of β-emitting isotopes. We have restricted ourselves in first instance to ^{45}Ca. The approach is based upon the following concept:
A labelled subject of a certain volume can be considered to consist of layers, oriented parallel to the surface of the properly collimated detector, each part of such a layer being at equal depth.
Each layer gives a contribution to the spectrum which depends upon:

A. activity in the layer.
B. thickness of the covering layer(s) between the considered source-layer and the detector.
C. geometrical factors.

In fact the spectrum of such a labelled subject can be described by a summation of the spectra of the separate layers. When introducing a set of spectra recorded in standard conditions from each of these layers we have the relation:

$$S(E_i) = \Sigma_j \alpha_j R_j(E_i) \text{ for all } E_i$$

where E_i = energy interval i.
α_j = proportionality factor (local activity in the j^{th} layer)
S = total spectrum of the subject under study
R_j = spectrum of the fererence source, covered by the same thickness as the j^{th} layer. Not only the total number of counts (fig. 1) but also the maximum energy (fig. 1, insert) is reduced by the introduction of absorbers of increasing thickness.

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Projectnummer : 9	

The problem of the in depth distribution is reduced in this way to the determination of the factors α_j, representing the activity in the j^{th} layer. To determine these α_j, the least squares criterion has been used:

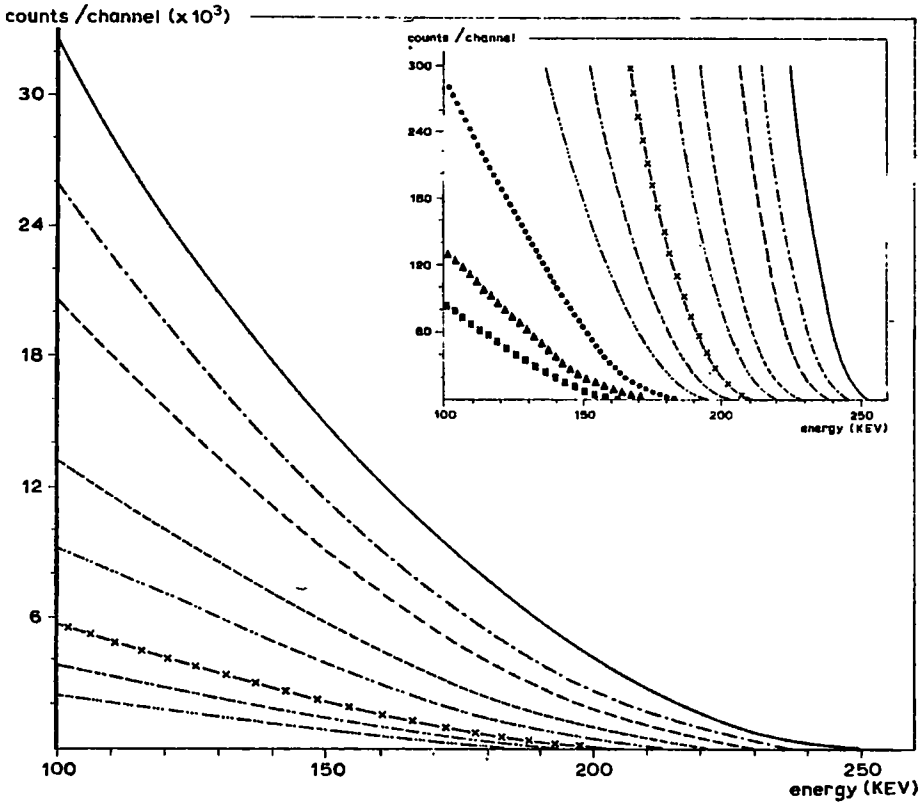
$$\sum_{i=1}^{M} (S(E_i) - \sum_{j=1}^{N} \alpha_j R_j(E_i))^2 \text{ minimum.}$$

Evaluation leads to a set of N equations which can be solved for the α_j by matrix inversion.
Preliminary results are promising (see table I) The activity measured at the depth of 315 and 350 μm is not a real one; it is probably due to geometrical factors slightly influencing the spectrum in the lower energy region.
Actually a program of testing (significance; resolution; sensitivity to geometrical factors) and refinement of the procedures is in progress.

Table I

Activity (a.u.) as a function of depth resulting from the analysis of the β-spectrum of a layered ^{45}Ca-source, with activity at depths of 35, 70 and 105 μm (density 1 g/cm^3) in the ratio 1.0 : 1.1 : 1.1.
Measurement repeated 9 times.

Measurement number	depth (μm)										
	0	35	70	105	140	175	210	245	280	315	350
T 44-1	0.40	1.27	3.52	1.61	-	-	-	-	-	-	5.40
2	0.06	2.75	2.04	1.32	0.84	-	-	-	-	-	-
3	-	2.91	1.68	2.19	-	-	-	-	-	-	2.81
4	-	2.63	2.11	1.80	0.31	-	-	-	-	-	-
5	-	3.00	1.17	2.80	-	-	-	-	-	-	-
6	0.19	1.94	2.63	1.99	-	-	-	-	-	-	8.03
7	0.51	0.77	3.53	2.04	-	-	-	-	-	-	6.30
8	0.05	2.47	2.13	2.07	-	-	-	-	-	2.94	-
9	0.18	2.17	2.12	2.38	-	-	-	-	-	-	3.00



Set of reference spectra, recorded per unit time interval.
From right to left increasing absorber-thicknesses
0, 35, 70, ..., 210 and 245 μm (density 1 g/cm³).
In the insert the maximum energy region is shown.

PROJECTVERSLAG

Onderzoekinstelling	:	
Projectnummer	:	
Projecttitel	:	ASSOCIATION EURATOM - ITAL 20 Organization and coordination of mutation breeding
Onderzoeker(s)	:	
Projectleider	:	
Afdelingshoofd	:	C. Broertjes

Registratie nr.: AA-009 / 20

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
„ „ -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	–	mandagen
Middelbaar personeel	–	mandagen
Lager personeel	–	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

During 1971 again many contacts between the Institute and (potential) users of our service (irradiation, literature, general information) have been realized. Five new irradiation projects were started, all with asexually propagated species (Achimenes, Cymbidium, Euphorbia, Hyacinth and Tulip) and all with private plant breeders. The total number of mutation projects, started since 1959 now amounts to 173, namely cross-pollinators 16, self-pollinators 23 and vegetatively propagated species (including apomicts) 134, the latter figure being an indirect proof of the potential importance for and the interest in mutation breeding in such crops.

In 1971 various mutants were commercialized, namely Dahlia cv. "Motive", a purple mutant from the orange flowering cv. "Arthur Godfrey" (Ballego and Sons, Leiden, The Netherlands), a white flowering mutant, called "White Wings", from the white-yellow flowering Alstroemeria cv. "Orchid flower" (Van Staaveren, Aalsmeer, The Netherlands) and three Achimenes mutants, obtained by using the adventitious bud technique. The mutants from the cv. "Paul Arnold", were two earlier flowering ones ("Springtime" and "Early Arnold") and a compact growing type ("Compact Arnold"). These results have demonstrated that the combined use of irradiation and the adventitious bud technique under certain conditions can lead to commercial results in an extremely short period (for further details see: Broertjes, C. Mutation breeding of Achimenes; Euphytica 21 (1), 1972 (in press)).

Promising results were reported in a number of other crops, such as onion, Poa pratensis, apple and pear, Lilium, to mention only a few.

PROJECTVERSLAG

Onderzoekinstelling	:	ASSOCIATION EURATOM - ITAL
Projectnummer	:	22
Projecttitel	:	Mutation breeding and radiation induction of self- and cross-compatibility in Pyrethrum.
Onderzoeker(s)	:	S. Roest, G.S. Bokelmann
Projectleider	:	
Afdelingshoofd	:	

Registratie nr.: AA-009 / 22

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
" " -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	—	mandagen
Middelbaar personeel	—	mandagen
Lager personeel	—	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

The scope of the project has actually been limited to the following aspect: vegetative propagation of Pyrethrum (Chrysanthemum cinerariaefolium Vis.) in vitro and in vivo.
Mutation breeding of Pyrethrum will be the object of further research in other specialized Institutes.
For experimental purposes different clones are maintained in the greenhouse and in a growth chamber. In order to keep these clones in an optimum condition it proved to be necessary to multiply old plants by cuttings 2 times a year.
Research concerning the adventitious root formation (initiation and subsequent elongation of adventitious roots) of flowerstem-explants in vitro and of shoot cuttings in vivo and ecological investigations with regard to vegetative and generative growth of plants at different temperatures are carried out by S. Roest.
Experiments regarding the adventitious bud formation and the outgrowth of axillary buds of flowerstem- and flowerhead-explants in vitro and anatomical research in relation to root and bud formation are performed by Miss G.S. Bokelmann.

1. Adventitious root formation of flowerstem-explants in vitro

Experiments are carried out with explants of the clones 1087, 4331 and MA 63/1889 grown in the greenhouse. Segments of about 1½ cm are cut out from the upper part of young flowerstalks, which are sterilized in a 5% sodium hypochlorite solution during 20 minutes. Explants are wounded by removing a bark strip over the whole length and placed with the wounded side on a sterilized medium containing: distilled water, agar 0,6%, Knop's macroelements and Heller's microelements (both at half strength), sucrose 2% (20 gr/l) and β-indolylbutyric acid 10^{-5} (10 mgr/l).
20 Explants per treatment are incubated at 20°C in continuous darkness during 4 weeks. The influence of plant, nutritional and environmental factors is investigated by varying one of these factors while keeping the other factors constant. Observations are made at least two times per week. Initiation of adventitious roots occurred during the first two weeks and elongation of root primordia during the last two weeks of incubation. At the end of the experiment only main roots had formed: speed of adventitious root formation, rooting percentage, average number of main roots and average dry root weight per rooted explant are measured.

The age of the plant material showed a very pronounced effect on adventitious root formation. Top explants of young flowerstalks yielded the best results. Short explants of 1 and 1½ cm showed a better rooting response than long explants of 2-2½-3 cm. Adventitious root formation is stimulated by wounding the explant.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 22	Registratie nr.:

The formation of adventitious roots is favoured by placing the explants horizontally with the wounded side on the medium instead of placing the explants vertically (upside up or upside down) in the medium.
For initiation of adventitious roots the addition of an auxin to the culture medium was required. The optimum concentration was about 10^{-5} of β-indolylacetic acid (IAA), β-indolylbutyric acid (IBA) or α-naphtylacetic acid (NAA).
If an auxin is absent during the first two weeks of incubation then very few roots are initiated during the last weeks.
For elongation of the initiated root primordia the presence of an auxin in the medium didn't appear to be essehtial; even the number of adventitious roots per rooted explant strongly increased in the absence of an auxin during the last 2 weeks of incubation.
Gibberellic acid showed a detrimental effect on adventitious root formation and similarly acted the cytokinins benzyl adenine and kinetin.
Both initiation and elongation of adventitious roots only occur if a sugar is present in the medium. The optimum concentration was about 2% glucose or sucrose. If sucrose is absent during the first two weeks then the explants are dying and roots aren't initiated. In the absence of sucrose during the last two weeks of incubation the initiated roots don't elongate.
The temperature showed a very marked effect on adventitious root formation. Initiation of root primordia is promoted by relative low temperatures of about 15°C; elongation of the adventitious roots is enhanced by relative high temperatures of about 25°C.
The best results were obtained with a combined temperature treatment: incubation during the first two weeks at a relative low temperature and during the last two weeks at a relative high temperature.
Light (Philips TL 33 RS) inhibited adventitious root formation of flowerstalk-explants. Even etiolation of flowerstalks of intact plants in darkness, from which later on the explants are cut out, enhanced adventitious root formation.

II. Adventiticus root formation of shoot cuttings in vivo.

Experiments are carried out with shoot cuttings of the clones 1087, 4331 and MA 63/1889 grown in the greenhouse.
Shoot cuttings of about 10 cm are cut from the base of the plant. Before planting the cuttings are dipped in a solution of 0,2% benlate to prevent fungal diseases and in a powder formulation of 1% IAA on talc. Shoot cuttings are planted in boxes containing a substrate of leaf mould + sharp sand and covered with thin polyethylene foil to create a high relative humidity. Shoot cuttings are incubated during 4 weeks at an air temperature of 15°C and a light intensity of about 25.000 lux (daylength 14 hrs, Philips TL 33 RS).
At the end of the experiment the cuttings are lifted and rooting percentage, average number of main and lateral roots per rooted cutting, average length of main and lateral roots, dry root weight and dry shoot weight are measured.

The root formation and the development of the upper vegetative part of the cutting are favoured by a treatment with powder formulations of the auxins IAA, IBA and NAA on talc; the most favourable results are obtained with IAA or IBA (1%).
Different soil temperatures in the range from 10 to 30°C were studied:
Initiation of main roots is promoted by relative low temperatures of about 15°C, elongation of main roots, initiation and elongation of lateral roots are enhanced by relative high temperatures of about 25°C. Again, as with the flowerstem-explants, the best quantitative and qualitative root formation were obtained by realizing during the first two weeks a relative low temperature and during the last two weeks a relative high temperature.
The substrates leaf mould + sand and perlite (fine and coarse grained) appeared to be suitable; vermiculite is unsuitable for root formation of shoot cuttings.

Onderzoekinstelling : Association Euratom-ITAL	Registratie nr.:
Projectnummer : 22	

III. Ecological research

Young, vegetative plants are incubated in the phytotron of the Department of Horticulture, Agricultural University, at different temperatures. In this way information is obtained with regard to temperature requirements for vegetative and generative (flower initiation and subsequent realization of flowering) growth. In the first experiment, 20 plants of clone RA 63/1889 were incubated at continuous temperatures of respectively 9, 13, 17, 21 and 25°C (daylength 16 hrs.) Five and a half months after incubation flower initiation and realization of flowering was observed at 9 and 13°C. The number of generative plants and the number of flowerbuds + flowers per generative plant were higher at 9° C; however realization of flowering proved to be better at 13°C. At 17, 21 and 25°C only vegetative growth occurred; the highest number of shoot cuttings per plant was realized at 17°C.

IV. Adventitious bud formation and the outgrowth of axillary buds in vitro.

In addition to adventitious root formation realization of the formation of adventitious buds or the outgrowth of axillary buds is essential in order to obtain whole plantlets in vitro.

Flowerstem-explants

So far adventitious bud formation didn't occur. The outgrowth of axillary buds of flowerstalk-explants of clone 4331 could be realized; this outgrowth of developing shootlets is stimulated by a treatment with gibberellic acid conc. 10^{-4}

Flowerhead-explants

Young flowerbuds of clone 4331 are sterilized in a 5% sodium hypochlorite solution during 30 minutes. The ray and disc florets are cut off from the flowerhead (receptacle) under a stereo-microscope. Receptacles are incubated on a sterilized medium containing distilled water, agar 0,6%, Knop's macro-elements and Heller's micro-elements (both at half strength), sucrose 2%, ß-indolylacetic acid 10^{-6}, benzyl adenine 10^{-5}, adenine 4.10^{-5} and several vitamins.
The explants are incubated at 14 hrs daylength (Philips TL 33 RS) and 18°C day-temperature, 14°C night temperature. Two to three weeks after incubation a high number of shootlets (1 to 100 with an average of about 50 per bud) had developed. The nature of the developing shootlets is uncertain; anatomical research can elucidate the origin: adventitious or axillary.
Summarizing the formation of adventitious buds and/or the outgrowth of axillary buds could be realized. Adventitious root formation of these shootlet-explants is now considered.

V. Anatomical research

Explants of the flowerstalk of clone 1007 are incubated in vitro at conditions suitable for adventitious root formation and these explants are fixed 0, 1, ... 9 and 10 days after incubation.
Microscopical research shows cell divisions in the interfascicular region of the pericycle 3 - 4 days after indubation. Division and elongation of cells of the root primordium take place rapidly during further development and about 10 days after incubation the first root primordia have penetrated the epidermis just between the ribs (the ribs consist of a tight, collenchymous tissue) of the explant. Growing from the interfascicular pericycle in radial direction the elongating root primordium brings about a collapse of endodermoid, cortical and epidermal cells. Between the ribs the cortex consists of a very loose, chlorenchymous tissue, easy to penetrate.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM-ITAL	Registratie nr.: AA-009 / 23
Projectnummer	: 23	
Projecttitel	: Kinetics of ion-uptake by intact plants	
Onderzoeker(s)	: G.R.M. Verfaillie	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
„ „ -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	—	mandagen
Middelbaar personeel	—	mandagen
Lager personeel	—	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971

Technical improvements.

The apparatus built at the Associations Institute for the study of the kinetics of ion-uptake by intact plants in a closed system and described in previous reports, has been modified. The improvements obtained by these modifications are:
- Larger space for the aerial parts, both in height and in diameter and better distribution of the air stream in the chamber.
- Constancy and homogeneity of the air temperature are notably increased.
- The maximum light intensity produced in the empty chamber has been increased from 30,000 lux up to 40,000 lux.
- Simplified air connections in the air chamber give more space for the rice plants, allowing the use of 24 plants instead of 20 for each experiment.
- Vibrational stirring of the nutrient solution in the culture vessel produces, at any point in this vessel, an average local stream velocity of 30 cm/sec, without harming the roots. Equivalent velocity obtained by the use of a circulation pump would require a flow-rate of 20 cubic meters per hour. It seems to be the best way to eliminate the film-diffusion problems in ion-uptake experiments.

The apparatus in its final version has, since October, been used in preliminary experiments, mainly to check its working and possibilities. Description and results of these experiments are given here.

The rate of phosphate-uptake as a function of the phosphate concentration and the chemical composition of the nutrient solution.

Material and methods.

For each experiment, a batch of 24, six weeks old, intact rice plants (Oryza sativa L., cv Maratelli) has been used. Using the ^{32}P tracing method, the uptake of phosphate ions has been followed continuously during the exhaustion of the phosphate from a closed constant volume of nutrient solution. With each batch of plants, two different treatments have been applied, namely, feeding first with a pure KH_2PO_4 solution (1 ppm P) and, later on, with a complete nutrient solution having the same phosphate concentration as in the first treatment. The whole

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series of observations has been repeated for each of five similar batches of plants. The results presented below represent average values.
During all experiments, the plants have been kept under strictly controlled conditions. These conditions are:

Temperature of the air	:	$25^{o}C \pm 0.5^{o}C$
Temperature of the solution	:	$25^{o}C \pm 0.2^{o}C$
Light intensity	:	20,000 lux ± 200 lux
Rate of transpiration	:	27.7 g water per hour and per batch. The variability was 1 g/h between batches and 0.2 g/h for a single batch in the course of one experiment.
Rate of photosynthesis	:	600 ml CO_2 (NPT) per hour and per batch. Reproducibility better than 1 percent.
Phosphate concentrations	:	Continuous scanning from 1 ppm P down to 25 ppb P.
pH	:	5.5 ± 0,02

Composition of the matrix nutrient solution (when used):

K - Ca - Mg	:	5 meq/l each.
Nitrate	:	12 meq/l
Sulphate	:	3 meq/l

Results and comments.

For both treatments, the variations of the uptake with the phosphate concentration are illustrated by the curves plotted in fig 1. A sudden change in the rate of uptake occurs in both treatments around 0.6 ppm P or 0.02 mM.
The enhancing effect of the matrix nutrient solution is obvious in fig 1 and it will be more precisely expressed by a factor defined as the ratio of the rate of uptake from a complete solution to that of the uptake from a pure KH_2PO_4 solution. This factor varies around a value of 2 depending on the concentration of the phosphate as it is shown in fig 2. According to these variations, bisedes 0.6 ppm, another threshold concentration might exist between 0.2 and 0.3 ppm. However, this preliminary information has to be confirmed by further experiments.

Rate of release of phosphate under natural conditions and after poisoning with DNP (2:4 - Dinitro-Phenol).

Material and methods

After the last uptake experiment described above, the root system of the radioactively tagged rice plants has been washed three times with demineralized water, and the release of ^{32}P has been followed over 46 hours. During a first period of 18 hours, the light intensity has been kept at 20,000 lux, as for the uptake experiments. The air circuit was open, what means that the photosynthesis proceeded in normal air containing about 0.04 percent CO_2. Afterwards, the air circuit has been closed, forcing the photosynthesis to proceed at compensation (photosynthesis and respiration cancelling each other) during 4 hours. Finally, from the 22nd to the 46th hour, switching out of the light suppressed all photosynthetic activity but allowed the respiration to go on.
After again washing the root system, a 3.10^{-4} M DNP solution has been fed to the plants, and the further release of ^{32}P has been followed in darkness during 43 hours.

Results and comments.

The results of this experiment have to be considered as an introduction to further research and the comments must be taken with some reserves.
The thin line drawn in fig 3 represents the cumulated release of ^{32}P plotted against the time, in absence of DNP. During the first phase of the experiment, when the photosynthetic conditions were normal, the efflux-rate of the radiophosphate was

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nearly constant, with the exception of the possible existence of several small steps. At the end of this phase, the release of radiophosphorus had reached a value of 0.3 percent of the total activity previously taken up by the plants. Later on, when the plants were submitted to compensation conditions, the activity of the solution remained steady, indicating that the release of phosphate was also compensated by an uptake still going on. Finally, in the last phase, when the respiration was proceeding alone, the activity of the solution decreased exponentially or, in other words, with a rate proportional to the phosphate concentration in the solution. The latter fact is in complete agreement with interpretations of the uptake from very dilute solutions, whatever the formalism used for the kinetics. At the end of the experiment, the residual phosphate concentration in the solution was below the detection limit (0.02 ppm P) of the colorimetric method with extraction.

The heavy curve in fig 3 represents the cumulative release of ^{32}P resulting from the poisoning with DNP. The efflux appeared in two waves, the later one overlapping the earlier one 56 minutes after poisoning and increasing suddenly the rate of release by a factor of 11.2. During the first wave, the efflux-rate was increasing with time and concentration, while, for the second wave, it was decreasing exponentially. These facts show that the phosphate ions have been released from two different pools. The pool producing the first wave immediately after poisoning must be very close to the solution, thus in the roots, and its capacity is much smaller than that of the second pool. The exponential decrease of the efflux-rate during the second wave implies a diffusion process, the rate of which is proportional to the difference between the inner and the outer concentrations. When the efflux stops, the phosphate concentration in the solution must be equal to that in the roots. By colorimetric analysis, 1 ppm P was found as equilibrium concentration and the fraction of the radiotracer recovered was 15.1 percent of the amount initially absorbed. Furthermore, if diffusion is the process of the second efflux wave, the distance between the second pool and the solution must be long enough to explain the time-interval between the poisoning and the start of the second efflux wave. The passage from the first to the second wave is illustrated by fig 4 representing a detail of the experimental record on chart paper at the original scale.

Finally, it is worthwhile to mention that, if the ^{32}P has been released after poisoning with the same specific activity as under natural conditions, the phosphate concentration we were not able to measure by colorimetry (see above) in the first part of this experiment, would be equal to 12.3 ppb P.

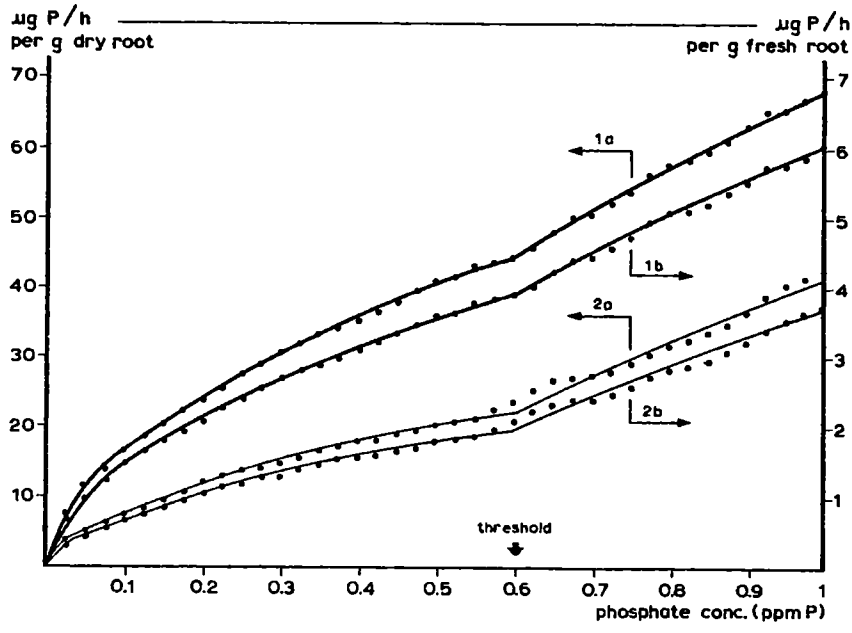

fig. 1

Rate of phosphate-uptake as a function of the phosphate concentration and of the chemical composition of the nutrient solution.

Numbering of the curves:

1 : uptake from complete nutrient solution

2 : uptake from a pure KH_2PO_4 solution

a : rate based on dry root weights

b : rate based on fresh root weights

dots : experimental points as calculated from continuous recordings.

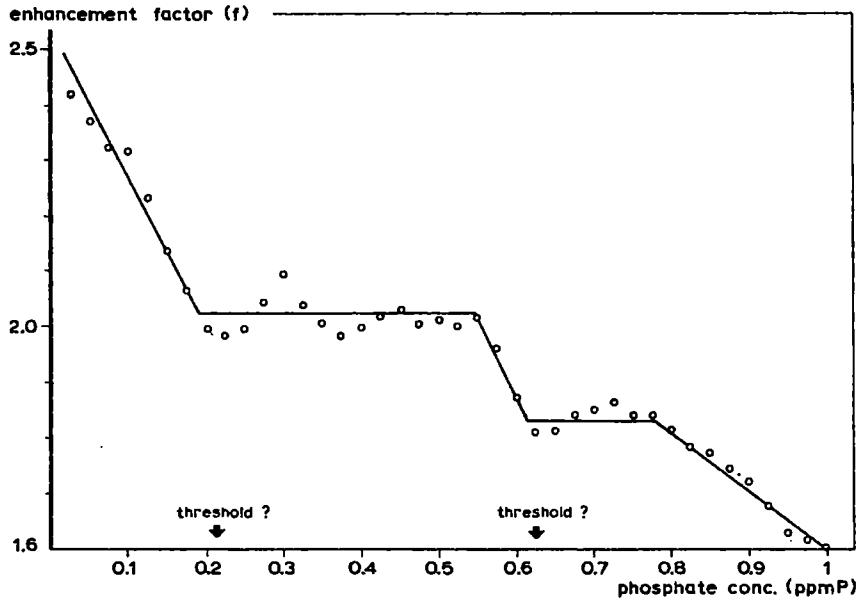

fig. 2

The enhancement factor (f) of the matrix nutrient solution as a function of the phosphate concentration

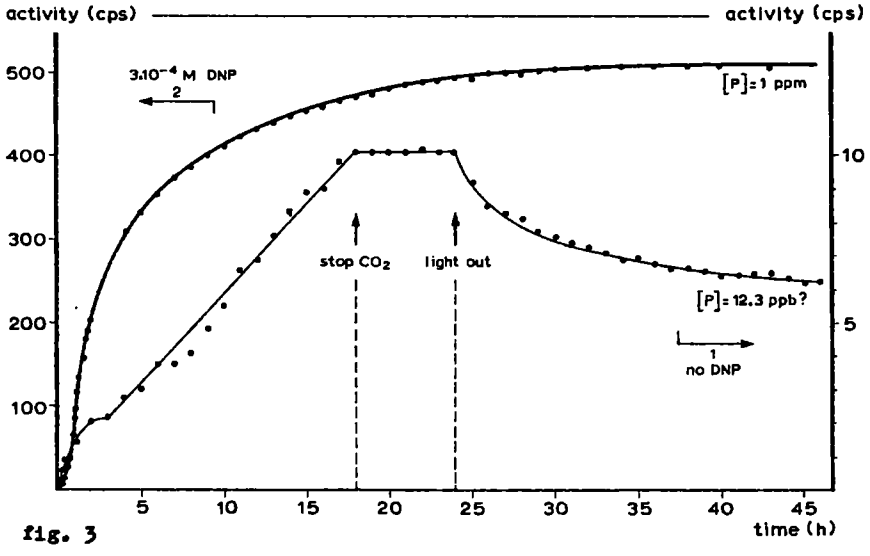

fig. 3

Activity of the nutrient solution due to the release of ^{32}P by intact rice plants.

Thin line (1) : release under natural conditions

Heavy line (2) : release after poisoning with 3.10^{-4} M DNP solution

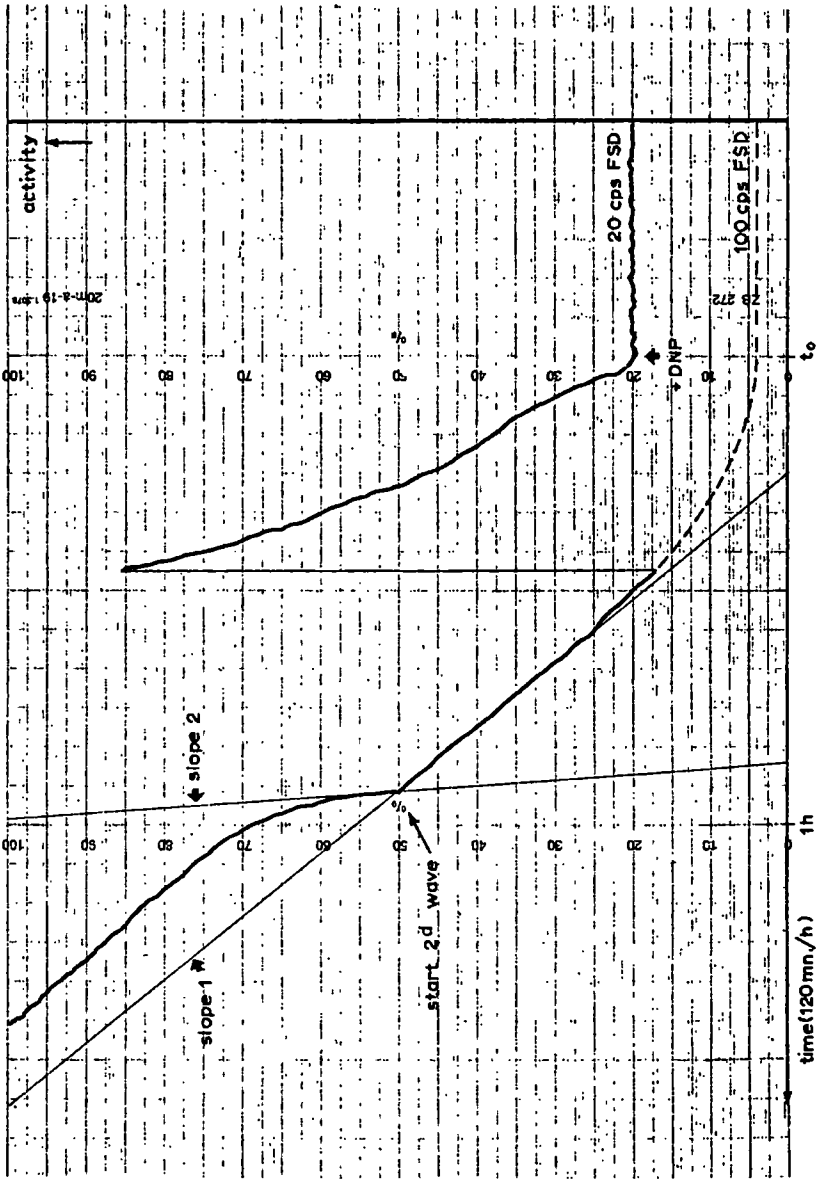

fig. 4

Details of the beginning of the phosphate release after poisoning with DNP as actually recorded on the chart paper

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 25
Projectnummer	: 25	
Projecttitel	: Microbiological research on irradiated food and radioresistance of micro-organisms.	
Onderzoeker(s)	: J.H. Becking, S.C.E. Romkes	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
„ „ -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	—	mandagen
Middelbaar personeel	—	mandagen
Lager personeel	—	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

A. Irradiated Food.

Irradiation of native henn's egg-white, and subsequent analysis by means of starch-gel electrophoresis, showed a gradual decline in intensity of the protein bands. This is mainly due to the denaturation of the protein. Some proteins, however, seem to be more sensitive to irradiation than others. The relative amounts of these proteins before and after irradiation are consequently indicative of the radiation dose. Conalbumin in particular is more sensitive to γ-radiation than albumin. The ratio of the sum of the three conalbumin bands to the sum of the three albumin bands, as determined by densitometry, can be used as a rough dosimeter. Below a dose of 200 krad the effects are too small to be measured in this way, while usually all conalbumin is denaturated above 1 Mrad. An attempt is made to improve the signal to noice ratio in the densitometry in order to extend this range.

The irradiated protein or egg-white was found to be inhibitory for the growth of micro-organisms. This inhibitory effect was investigated in more detail by using three bacteria strains, i.e. Staphylococcus epidermidis, Micrococcus flavus, and Micrococcus morrhuae. These micro-organisms were inoculated on synthetic medium containing irradiated pure gelatin as sole N source or in some instances irradiated gelatin both as N and C source. This inhibitory effect of the medium on bacterial growth persisted sometimes for periods up to several weeks at room temperature, although Staphylococcus epidermidis is rather insensitive to this post irradiation effect (Figure 1). The medium is inhibitory to growth as a consequence of certain radiation-induced changes in some of the constituents of the medium. The main influence arises from water, in which H_2O_2 is formed as most stable product, while irradiated glucose and irradiated gelatin contribute to a lesser degree to this effect. Catalase in the medium acts as a protector, and a radiomimetic effect can be obtained by adding reagent H_2O_2, although the concentrations needed are much higher than those induced by irradiation. The comparative small effects of irradiated glucose and irradiated gelatin can be separated by statistical methods.

B. Radioresistance of micro-organisms. (J.H. Becking and B. Harton).

The research on the radioresistance of micro-organisms was continued. In Micrococcus radiodurans no radiosensitizing effect was observed by application of N-ethylmaleimide (NEM). This effect could, however, be obtained with iodoacetamide (IAAM). NEM proved, however, to be a very active radiosensitizing agent in Escherichia coli. This NEM

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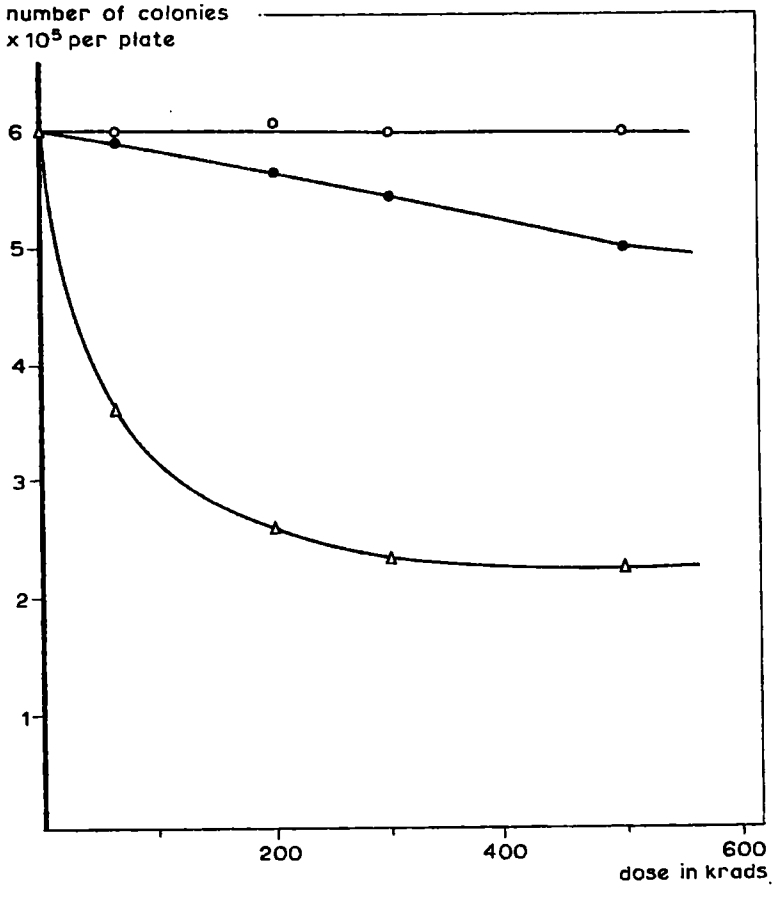
effect was influenced by environmental conditions. When tested in aerobic condition the radiosensitizing effect of NEM was rather low, but very pronounced under anaerobic (anoxic) conditions. In anaerobic conditions concentrations of 10^{-3}-10^{-4} NEM were already highly effective. Effects with NEM could, however, only be obtained if this radiosensitizing agent was in direct contact with the bacterial cell during irradiation.
Exposure to NEM and removal of this substance before irradiation (by washing the bacteria cells) induced no radiosensitizing effect in Escherichia coli cells.
Using C^{14}-labelled NEM and liquid scintillation techniques the problem was attacked, whether NEM actually penetrates the bacterial cell. Indeed a small fraction of the NEM applied could be detected within the bacterial cells. This NEM was irreversibly bound, since it could not be removed by washing the bacteria cells. Probably the irreversibly fixed NEM is bound to -SH groups of constituents of the bacterial cell. This problem is under further investigation.

These results of NEM fixation within the bacteria cell are rather contradictory to the observation mentioned above that exposure of bacteria to NEM and removal of this substance by washing the cells before irradiation resulted in no sensitizing effect of NEM after irradiation. No explanation of this phenomenon can be given at moment. Testing Micrococcus radiodurans, which is an unsensitive species for NEM as sensitizing agent, with C^{14}-labelled NEM also an irreversible fixation of NEM of about the same magnitude as found in Escherichia coli was observed. Thus the irreversible bound NEM in the bacterial cell is not directly correlated with a sensitizing effect.

A preliminary account of the results obtained is in preparation for publication (Hartog and Becking).


number of colonies
x 10⁵ per plate
6
5
4
3
2
1
200
400
600
dose in krads

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 29
Projectnummer	: 29	
Projecttitel	: Localization of ions in plant tissues following uptake	
Onderzoeker(s)	: G. Sauer, S.v.d.Geijn, A. Ringoet	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	–	mandagen
„ „ -materieel	f	Middelbaar personeel	–	mandagen
Semi-directe kosten	f	Lager personeel	–	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71.

Track-autoradiographic applications have been discontinued, pending further progress of the work on ion-uptake by chloroplasts.

Spectrometric localization by the semiconductor assembly has been limited to one biological application.

Localization of the phosphorus transport in Xanthium Pensylvanicum Wallr.

Based upon the results of calibration measurements with ^{32}P (Ann. rep. 1970), a first experiment was performed to test the maximum energy extrapolation technique for in vivo localization purposes. The choice of Xanthium was justified by its easiness to form rather abundant layers of parenchymous tissue inbetween the xylem and the phloem after decapitation and removal of the axial buds of seedlings (suggestion S. Shapiro).

It was expected that thus a discrimination of the transport through the xylem and the phloem should become possible. A very serious drawback of this method, however, is the very low phosphorus uptake, due to the absence of actively growing meristems and this phenomenon made a measurement in a short initial phase of the experiment impossible. After about 5 h. a significant amount of ^{32}P was located at a depth of about 700 µm (± 20%) (70 mg/cm^2 ∿ 100 kev energy loss). The results of an additional experiment with an intact plant show a transport during the first hours at a depth of 1300 µm (± 20%) and after 5 h. a significant amount of ^{32}P was again located at a depth of 700 µm (± 20%). The results of these experiments tends to the conclusion that the extrapolation technique for the determination of the depth at which ^{32}P is present can be used, provided that the uptake and specific activity are sufficiently high to measure a spectrum in a relatively short period.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM-ITAL
Projectnummer : 34
Projecttitel : Radiobiological and genetic studies on insects

Registratie nr.: AA-009 / 34

Onderzoeker(s) : C. Vijnands-Stäb.
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
" " -materieel f
Semi-directe kosten f
Omslag algemene kosten f
Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971

Study of genetical processes induced by irradiation in the onionfly, Hylemya antiqua (Meigen).

Selection of translocation heterozygotes.

Selection of translocation heterozygotes in the first generation after irradiation (dose range of 0.5 to 2.0 krad X-rays) was continued. The criterion is an evident reduction of fertility in single pair crosses. However, the fertility in the controls had declined beneath values normally expected as a result of translocation heterozygosis. Therefore in June 1971 fresh flies from the field in Flakkee have been introduced.
Their reproduction turned out to be much better: a reasonable quantity of eggs per female and good egg hatch-ability were obtained.

Radiation experiments.

In July 1971 radiation experiments have been started with the first generation obtained after introduction from the field into the laboratory. One day old flies, males and females have been irradiated with 1000 rad of X-rays and 1000 rad of fast neutrons. The irradiated flies have been outcrossed in cages containing 5 treated flies with 10 untreated partners. Twelve percent of the eggs, fertilised by males irradiated with 1000 rad of X-rays has hatched, corresponding to an average production of 103 eggs per female. Fifty-two percent of the eggs of females irradiated with 1000 rad X-rays has hatched. (Average production of 20 eggs per irradiated female). These data fit the dose-effect curve established by J.Ph.W. Noordink at the Institute of Phytopathological Research, Agric. University Wageningen.
In crosses of males irradiated with 1000 rad of fast neutrons only 3.4 % of the eggs has hatched, at an average produotion of 179 eggs per female. The females irradiated with 1000 rad fast neutrons have not produced any eggs.

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The egg-hatch of the control was 73 % when males were mated to 10 females, and 81 % in crosses of 5 females with 10 males.
(The number of eggs per female is higher when there is a smaller number of females than males).
The offspring of irradiated males has been outcrossed to 3 untreated partners to investigate the fertility of the F_1 individuals.
The observed F_1 after X-ray irradiation consisted of 18 males and 8 females. Among this progeny 10 had normal fertility, 3 a somewhat reduced fertility, 4 an extremely reduced fertility, 6 had no offspring at all and 4 were semi-sterile. Eight strains, suspected of carrying a translocation, have been maintained and in the F_2, for each strain, 10 single crosses have been made. The reduced fertility was a hereditary trait. In 3 strains Ir. C. van Heemert (Dept. of Genetics, Agric. Univ., Wageningen), has cytologically demonstrated a translocation and/or inversion and/or fragmentation. This research, which combines a preliminary selection with cytological analysis, is still in progress.
The fertility of 9 F_1-males and 4 F_1-females after irradiation with neutrons has also been determined. Five crosses had normal fertility, 2 were semi-sterile and 6 had no viable offspring. In the F_2 the semisterility of the 2 strains reappeared. No cytological data are yet available.
For a correct evaluation of the fertility in the F_1 after irradiation, the normal fertility in single crosses with 3 partners has been determined for 20 untreated males and 6 control females.
Fertility was normal (> 65 % egg hatch) in 15 crosses, in 3 cases fertility was reduced to a level of semisterility, and in 8 crosses no offspring has been produced.
The selection has to be done against a background of considerable variation occurring in the control groups and in the separated egg clutches. Nevertheless suspected strains mostly exhibit cytological aberrations, showing reciprocal translocations, as well as invorsions and fragmentation.

Simulation models.

Simulation models have been developed in collaboration with Dr. M.J. Frissel of the Association's Institute, to study the effect of translocation and other genetic manipulations on the density and growth of population. In this study it became obvious that a correct estimation of the growth capacity of the population during the year is essential. An 'educated guess' of the variation in growth capacity, dependent on good or bad years, is almost as important.
One single autosomal translocation, with only one third of the normal fertility left over for the heterozygote, is not capable of reducing the population density, even when released at the optimal ratio. Such translocations might delay the population growth and stabilise the population at lower densities than without control methods.
In cooperation with Prof.Dr. W. Scharlo, Institute of Genetics, Univ. of Utrecht, experiments with Drosophila melanogaster are in progress to investigate the effects of translocations on the growth of Drosophila populations in cages and to find out a way of verification of the assumptions made in the simulation for onionfly.

Onderzoekinstelling :	ASSOCIATION EURATOM-ITAL	Registratie nr.:
Projectnummer :	34	

Publications

Internal report

No X Wijnands-Stäb, K.J.A. and M.J. Frissel.
Joint FAO/IAEA Panel on Computer Models and Application of the Sterile Male Technique.
Viernna, Dec. 13-17, 1971.

In press:

Frissel, M.J. and K.J.A. Wijnands-Stäb.
Computer modelling of the Dynamics of Insect Populations.
Proceedings of a panel on 'Computer Models and Application of the Sterile Male Technique.'
Vienna, Dec. 1971.

Wijnands-Stäb, K.J.A. and M.J. Frissel.
Computer simulation for genetic control of onionfly Hylemya antiqua (Meigen)
Proceedings of a panel on 'Computer Models and Application of the Sterile Male Technique, Viernna, Dec. 1971.

List of participations at meetings.

1. Study Group on Genetical Methods of Pest Control (affiliated to OILB and ESNA), September 20-25, 1971.

   Langford House, Langford, Bristol

   Participant from Association Euratom-ITAL: K.J.A. Wijnands-Stäb.

2. Panel on Computer Models and Application of the Sterile Male Technique December 13-17, 1971.

   Headquarters Joint FAO/IAEA Division Vienna.

   Participants from the Association Euratom-ITAL: M.J. Frissel, K.J.A. Wijnands-Stäb.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM-ITAL	Registratie nr.: AA-009 / 37
Projectnummer	: 37	
Projecttitel	: Quantitative description of the behaviour of nitrogen in soils	
Onderzoeker(s)	: J. Beek, M.J. Frissel	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
„ „ -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	–	mandagen
Middelbaar personeel	–	mandagen
Lager personeel	–	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971 .

A few coworkers of C.T. de Wit worked this year on the extension of the simulation model of the behaviour of nitrogen in soil. The complexity of nitrogen behaviour in soils is reflected in the computerprogramme.
Therefore at the Association a new and complete description of all the models which in the last few years were built was prepared.
The purpose of this niew description was to specify the state of our actual knowledge as a basis for new developments and a source of information for other scientists. The publication contains about 75 pages, is edited by J. Beek and M.J. Frissel and is published as an internal report of the Association.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 39
Projecttitel : The propagation of nuclear methods in biology and agriculture

Registratie nr.: AA-009 / 39

Onderzoeker(s) : J.F. Stoutjesdijk
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
" " -materieel f
Semi-directe kosten f
Omslag algemene kosten f
Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

1. Courses

1.1 A general radioisotope course was organized from March 15 till April 2, 1971, for 20 participants, of whom 3 members of the Association's personnel.

1.2 Liquid scintillation Courses were organized from November 22 till December 3, and December 6 till 17, 1971, with 13 and 15 participants respectively. Among the participants was one member of the Association's personnell as well as three foreign questworkers at the Institute.

2. Cooperation with other institutes

2.1 Department of Food and Nutrition, Agricultural University, Wageningen.
The investigations of A.B. Cramwinckel on the Fe-metabolisms in rats with ^{59}Fe were continued. New plans are made for double-tracer-experiments with ^{55}Fe and ^{59}Fe,

2.2 Department of Food Science, Agricultural University, Wageningen.
The investigations of K. Slijkhuis on the irradiation of activated sludge were temporarily terminated with promising results.
The sludge was irradiated with electrons in doses from 50 to 1000 krad.
The specific resistance of the sludge-cake in vacuum-filtration decreased with 50% after an irradiation of 50 krad and with 75% after 1000 krad. The sedimentation was considerably accelerated by the irradiation and the Chemical Oxygen Demand of the filtrate or supernatant was increased by 50% (50 krad) and 250% (1000 krad). The viscosity of the sludge decreased with low doses, but increased with higher doses to twice the value of the untreated sludge.
All bacteria were killed with 700 krad.
Irradiation of the sludge after addition of $FeCl_3$ or $CaCl_2$ did not give a further improvement of the specific resistance in vacuum-filtration; the irradiation effect on the specific resistance was not influenced by adding H_2O_2.

3. Development of radiochemical methods. (J.F. Stoutjesdijk, S.K. Das).

In cooperation with a guest-worker from India, dr. S.K. Das, several destruction methods for plant material labelled with ^{3}H, ^{14}C and ^{35}S, in

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view of liquid scintillation counting, have been compared. For ^{35}S a wet destruction with HNO_3 and $HClO_4$ was found to be most suitable; for ^{14}C and ^{3}H total combustion proved to be better than digestion with $HClO_4 + H_2O_2$. With the acid digests of ^{35}S containing material emulsion counting using Triton X-100-mixtures or the commercially available Insta-Gel gave better results.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM-ITAL	Registratie nr.: AA-009 / 40
Projectnummer	: 40	
Projecttitel	: Behaviour of mercury in soils	
Onderzoeker(s)	: M.J. Frissel, P. Poelstra	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	—	mandagen
„ „ -materieel	f	Middelbaar personeel	—	mandagen
Semi-directe kosten	f	Lager personeel	—	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71 met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

In 1970 attention was focussed on the volatile product, dimethylmercury.
In 1971, the less volatile compounds, methyl mercury chloride and mercury dichloride, have mainly been considered. Both compounds can be converted to each other, so it was necessary to develop suitable methods for separating them.

A. Separation techniques.

A 100%-separation of both compounds can be obtained by binding to dithizonates, which are solved in carbon tetra chloride. This solution is added to an Al_2O_3 column and eluated with carbon tetra chloride. The anorganic mercury remains in the column, which is afterwards eluated with chloroform. A second separation method has been achieved by thin layer chromatography; here the components are also coupled to dithizonates.
Because of some retention by the column, determination of very small amounts of both mercury compounds is impossible.
Such small quantities (< 50 ng/g) can be determined by gaschromatography.
The electron capture detector, however, is not sensitive enough to detect the halogene part of the mercury (dithizon-chloride) molecule. Therefore we intend to use the atomic absorption spectrophotometer as specific mercury detector.
A separation at 130°C with gaschromatography was achieved but it appeared that the organic mercury compound was destroyed at that temperature. For checking this possibility we are now using a preparated column for a separate spectrometric analysis of the compounds.

B. Determination of Hg.

A determination technique of total Hg has been developed. This technique is based upon flameless atomic absorption spectrofotometry with a quartz cuvet.
The minimum detectable level is 10 nanogram or 0.002 ppm Hg in 5 g soil.

C. Extraction of mercury compounds from soil.

An extraction technique permitting the determination of the different Hg-components in soils under field conditions has been worked out. Extraction is done with a mixture of 4N HCl and some KCNS. Its yield is on the average 90-95% of the total amount of mercury present, but depends upon the type of soil. After filtration, KI, EDTA and citrate are added and the solution is brought to pH 7.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 40	Registratie nr.:

By liquid-liquid extraction the mercury compounds are transferred to the organic phase (carbon tetra chloride with dithizone), which extraction yields 95 to 99%. The compounds are then separated and determined.

D. Adsorption studies on Soils.

Adsorption of CH_3HgCl has been studied in two soils, a sandy soil from the bulb area (Hillegom) and a clay soil from the forelands of the river Rhine (Oosterhout). The duration of the adsorption experiments was 6 months. The pH of the soils and the concentration range of the methylmercurychloride were varied; the pH from 3 to 7; the concentrations were 0.1, 1.0 and 10.0 ppm. The influence of pH appears to be small for both soils but its effect might have been masked by the salts which were added for pH stabilisation. The effect of CH_3HgCl-concentration was negligible in the clay soil and small in the sandy soil. This small effect suggests that a relation exists between amounts adsorbed and in solution. When monomethyl concentrations in soils under field conditions are known, this effect will get further consideration. The effect of time is rather complicated; after one day 80 to 90% of the amount added has been adsorbed. The clay soil adsorbs somewhat more than the sandy soil and its adsorption level remains constant during about 2 months.
Measurements have shown that in the sandy soil part of the methyl mercury has been converted to anorganic mercury (Hg^{2+}) and probably to metallic Hg which has been evaporated from the system. Such conversions have not been noticed in the clay soil.

E. Sampling and storage techniques of soils.

Because almost no information is available on the distribution of mercury compounds over the soil profile under field conditions, a sampling technique had to be developed which enables sampling to a depth of 1.5 meter.
Perspex tubes of 2 meter length and 2.3 cm internal diameter, provided with a screw-thread at the lower end on which is fitted a sharp-edged hollow augerhead are driven into the soil to the desired depth. They are withdrawn from the soil with some type of jack. The soil can be removed from the tube by unscrewing the augerhead and cut into pieces. As mercury compounds are rather volatile precautions have to be taken during storage of soils before analysis. It has been shown that no volatilization occurs when soils are stored at -25°C.

F. Transport studies with CH_3HgCl.

For more than half a year the distribution of ^{203}Hg-labelled CH_3HgCl was followed after surface-application to two columns filled with soil from the bulbarea of Hillegom.
First some downward movement occurred, which later on, however, gradually diminished and finally stopped. It appeared, after 5 months, that all the organic mercury had been converted into $HgCl_2$, which is so strongly adsorbed to the soil that it does not move anymore. During the experiment a radioactive volatile compound continuously disappeared from the column. This may have been CH_3HgCl in the beginning of the experiment, but after conversion of the latter one, the volatile compound could be identified as metallic Hg. Another set of experiments is now in preparation to check the material-balance of the added mercury compound and to trap the disappearing volatile compound for analysis.

G. Measurements in the field.

Two sites were chosen for measurements in the field, one of the sites is on the grounds of the "Leidse Duinwatermaatschappij" at Katwijk, where water from the district Rijnland is infiltrated into sand dunes. The water passes through sandy layers of 50 to a few hundred meters and is then gathered in open channels. The water in Rijnland has been contaminated with mercury compounds from treatments in the bulb-fields during the last 20 years. To permit the sampling the infiltration was interrupted and the samples taken as soon as the soil became dry.

Onderzoekinstelling : Association Euratom-ITAL
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The other site is on the grounds of the "Gas- en Watervoorzieningsbedrijf voor de bloembollenteelt" at Hillegom. Its fields, except the control plots, were continuously used for bulb production during the last 20 years.
Soil samples from these two sites down to a depth of 2 meters have now been analysed for total mercury by activation analysis by Dr. Das, R.C.N., Petten.
The airdried samples have been irradiated for 2 days with a thermal flux of 5.10^{13} n cm^{-2} sec^{-1}.
The results of the analysis are shown in the table 1.
The first tentative, very preliminary, conclusion might be that there is no mercury-accumulation and that the mercury, which has reached these soils, has either been evaporated or transported beyond a depth of 2 meters.

Table 1 - The mercury content of some soils of drinking water companies in the Netherlands.

	Hg ppm, dry soil
Katwijk, control (no infiltration)	0.023*
Katwijk, infiltration area, mud, location 1	0.053
Katwijk, infiltration area, mud, location 2	0.052
Katwijk, infiltration area, depth 5 cm	0.020
Katwijk, infiltration area, depth 15 cm	0.023
Katwijk, infiltration area, depth 25 cm	0.023
Katwijk, infiltration area, depth 50 cm	0.023
Katwijk, infiltration area, depth 100 cm	0.016
Katwijk, infiltration area, depth 150 cm	0.017
Katwijk,iinfiltration area, depth 200 cm (small humic layer)	0.028
Hillegom, control(area which was never cultivated with bulbs)location 1	0.029
Hillegom, control(area which was never cultivated with bulbs)location 2	0.018
Hillegom, control(area which was never cultivated with bulbs)location 3	0.031
Hillegom, mixed profile, depth 5 cm	0.019
Hillegom, mixed profile, depth 15 cm	0.021
Hillegom, mixed profile, depth 25 cm	0.014
Hillegom, mixed profile, depth 50 cm	0.015
Hillegom, mixed profile, depth 100 cm	0.025
Hillegom, mixed profile, depth 150 cm	0.021
Hillegom, original profile, depth 5 cm	0.040
Hillegom, original profile, depth 15 cm	0.039
Hillegom, original profile, depth 25 cm	0.019
Hillegom, original profile, depth 50 cm	0.033
Hillegom, original profile, depth 100 cm	0.050
Hillegom, original profile, depth 200 cm	0.012
Hillegom, mud out of ditch between 2 profiles	0.020

*Standard deviation 0.005 ppm.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 41
Projecttitel : ileutron Activation Analysis

Registratie nr.: AA-009 / 41

Onderzoeker(s) : P. Poelstra and N. van der Klugt
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
" " -materieel f
Semi-directe kosten f
Omslag algemene kosten f

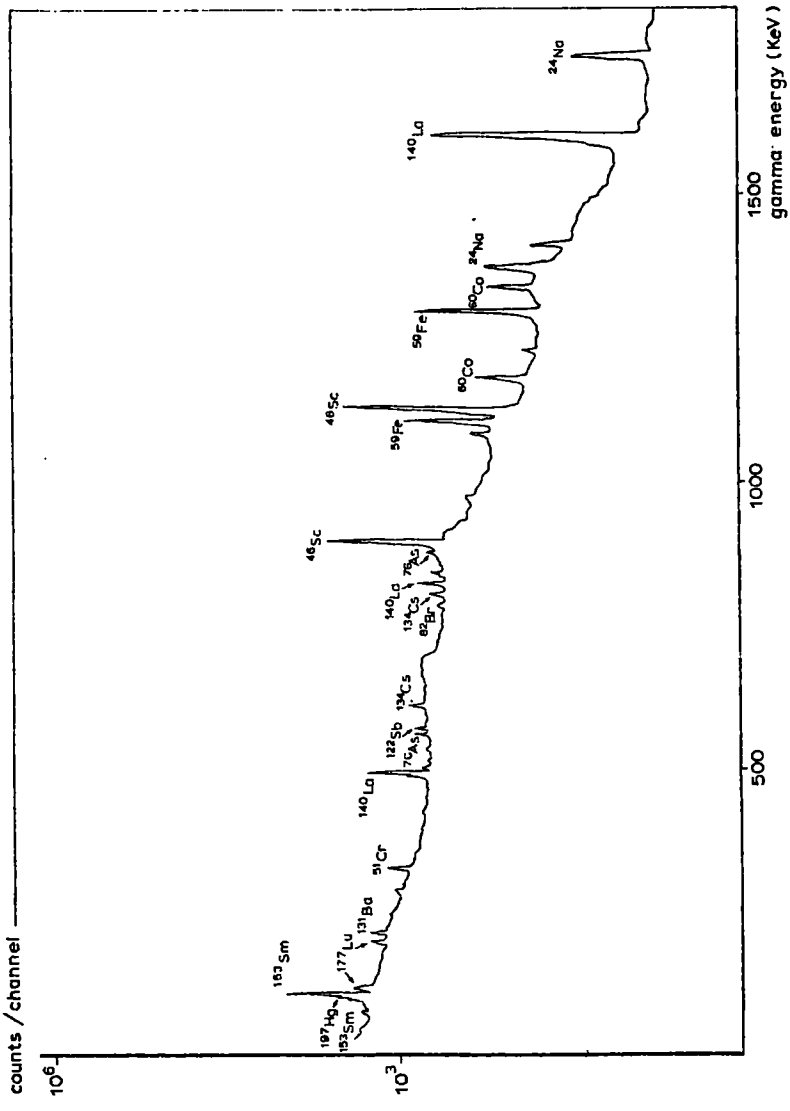
Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

As the punchtape reader of the IBM 360/50 has proved to be unreliable, punchtape recordings are now converted to punchcard recordings - using an IBM 1130 computer with a reliable punchtape reader - before being presented to the computer for treatment.
Two sub-programs have been developed and connected to the main computer program. The first program makes it possible to identify any arbitrary element in the sample and to calculate its concentration, with the only restriction that the element should be present in the reference standard. This program also calculates the Na-24 contribution to the 511 KeV peak of Cu-64. This implies that it is no longer necessary to remove the sodium from the irradiated sample in order to be able to determine the Cu-content accurately.
The second program developed takes care of the total administrative elaboration of the data obtained. This implies that the administration belonging to each irradiated sample is completely done by the computer and that the results are recorded on magnetic tape for later use.
Some of the topics up till now handled with the technique are summarized here.
On request of the Institute for Phytopathology, Wageningen a metal had to be chosen for labelling of onionflies before release and subsequent detection by neutron activation after capture. Preliminary experiments were done with dysprosium, copper, silver, bromium, gold and manganese.
In connection with investigations on heavy metals in fish products, (Netherlands Institute for Fisheries Research, IJmuiden) the amount of mercury has been determined in pike. Some further research has to be done in order to obtain a mercury reference standard with a albumine matrix.
The determination of some metals in soil, soil solution and rice from Dutch Guyana has started and will be finished in spring 1972.
The graph shows a γ-spectrum from a Dutch Guyana soil, irradiated during 48 hours and measured after 140 hours.
For some typical elements the following concentrations were found in this soil: cobalt: 50 ppm; copper: 470 ppm; manganese: 2300 ppm; iron: 32000 ppm.



Soil from Dutch Guyana

Irradiated for 48 hrs. Measured after 140 hrs.

PROJECTVERSLAG

Onderzoekinstelling	:ASSOCIATION EURATOM - ITAL
Projectnummer	:43
Projecttitel	:Mutation breeding via the adventitious bud technique

Registratie nr.: AA-009 / 43

Onderzoeker(s)	:C. Broertjes
Projectleider	:
Afdelingshoofd	:

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
" " -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	–	mandagen
Middelbaar personeel	–	mandagen
Lager personeel	–	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971

The experiments with Achimenes, to demonstrate that commercial results can be obtained within a short time, relatively speaking, if the adventitious bud technique is available, have resulted in the commercialization of three mutants. They were obtained within three years, from the very first irradiations of the leaves of Achimenes cv. "Paul Arnold", until the commercialization under the names "Springtime" and "Early Arnold" (two earlier flowering mutants) and "Compact Arnold" (a compact growing type). (See: Broertjes, C. Mutation breeding of Achimenes; Euphytica 21(1), 1972 (in press)).
The Kalanchoë adventitious plantlets have flowered and showed a fairly high number of mutants (20%), none showing the slightest sign of chimerism. It demonstrates again, that the apex of adventitious buds at the base of detached leaves, originate from a single (epidermal) cell.
Some of the mutants have been selected by a number of Kalanchoë breeders and growers; they will be propagated, together with a few marked mutants and compared on a clone basis amongst themselves and with the original cultivars "Josine" and "Annette". Especially the compact growing, free flowering mutants with good inflorescence characters were selected, but also an earlier flowering mutant and one with larger flowers. (see Broertjes, C. Mutation breeding of Kalanchoë; Euphytica 21 (1972) (in press)).
Of Ornithogalum an extremely low number of mutants have been observed.
A few mutants with larger flowers, probably tetraploids, were found after colchicine treatment of freshly cut leaves as well as a number of plants, slightly differing in size and form of flower or inflorescence. They will be propagated via adventitious bulbils to compare the aberrant types on a clone basis.
New experiments with leaves of Chrysanthemum have not been carried out.
Cooperation within the recently formed "Adventitious Bud Study Group" has started.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 44
Projecttitel : The behaviour of effluent phosphate in soils

Registratie nr.: AA-009 / 44

Onderzoeker(s) : J. Beek, M.J. Frissel, P. Poelstra
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
" " -materieel f
Semi-directe kosten f
Omslag algemene kosten f
Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

Due to the departure of Beek no experimental work on project 44 has been carried out. A few recommendations for the set up of the set up of the project have been prepared.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 45
Projectnummer	: 45	
Projecttitel	: Ion uptake by roots from diluted solutions	
Onderzoeker(s)	: A. Rinnoet, M.J. Frissel	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	–	mandagen
„ „ -materieel	f	Middelbaar personeel	–	mandagen
Semi-directe kosten	f	Lager personeel	–	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71.

1. Uptake and transport of phosphates in groundnut (Arachis hypogea L. cv. TM V2) plants-influence of light and temperature. (J. Chaudoir, G. Alagarswamy, H. Laudelout and A. Rinnoet).

Groundnut plants were grown in a climate-controlled chamber on complete mineral solutions, characterised by low phosphate concentrations (respectively 1.61, 16.1 and 32.2 $\mu M KH_2PO_4$. In one series of experiments uptake and translocation of phosphates in the plants were studied at different light intensities: 31.500; 23.000; 11.500 and 0 lux. In another group of experiments the temperature in the growth chamber was varied: 7, 13, 18, 23 and 28 °C. All experiments using ^{32}P-labelled solutions were done with 31 days old plants, which were generally pretreated at the selected light intensity or temperature for 48 h in a flowing culture solution of the same phosphate concentration. The rate of uptake was measured by the continuous flow method as described in previous reports. Transport of phosphates to the aerial parts was estimated either from measurements with a semiconductor detector assembly or by harvesting plants at different time intervals after the start of the experiment.
According to the available results in the present series of experiments uptake of phosphates is not directly affected by the light intensity, whereas their transport to the aerial parts increases at higher light intensities. This conclusion applies to all concentrations considered in these experiments.
The experimental data concerning the temperature effect show that the process, which governs the rate of phosphate uptake in the temperature range below 18 °C. has a high Q_{10} and a high apparent energy of activation (varying from 16.8 to 23.2 kcal mol^{-1} depending on the P-concentration). In the temperature range above 18 °C, the Q_{10} is 1.2 and the apparent energy of activation varies from 1.5 to 4.5 kcal mol^{-1}. These results suggest the intervention of a metabolic process in the lower temperature range and thus the possibility that the passage of the membranes of the rootcells constitute the rate determining step of the uptake. Above 18°C, film diffusion in the dilute solution around the roots may be the pace-making process of rate control.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 45	Registratie nr.:

II. Simulation of the phosphate uptake from a nutrient solution by a complex geometric root system. (M.J. Frissel, G. Alagarswamy, J. Sinnaeve, H. Laudelout).

Several models describing situations with one root or varying amounts of roots were worked out. Both mass flow and diffusion of phosphate ions towards the root surface were considered. The existence of a dual mechanism was used (see above), one mechanism operating at all concentrations but most efficiently in the lower concentration range, the other one being operative only at higher concentrations. The kinetic parameters used in the simulation models were of the same order of magnitude as those reported in the literature. Root efficiency and the threshold concentration, at which the second mechanism starts were taken into consideration. From the calculations, which were partly verified by experiments, it appeared that in the systems studied with only one or a few roots diffusion was generally the rate limiting step. With more complex root systems, the influence of diffusion in the root medium was reduced to a minimum. More information can be found in: Simulation of the uptake of ions from a nutrient solution by a complex geometric root system by M.J. Frissel, G. Alagarswamy, J. Sinnaeve and H. Laudelout.

Onderzoekinstelling : Projectnummer :	Registratie nr.:

III. Absorption of phosphate by rice (Oryza sativa L, cv IR 8) and groundnut (Arachis hypogea L, cv TMV2) plants grown under axenic and non-axenic conditions (G. Alagarswamy, H. Laudelout and A. Ringoet).

The absorption of phosphate from solutions, at concentrations of 0.32 µM, 1.61 µM and 3.22 µM, has been compared for rice and groundnut plants, respectively under axenic and non-axenic conditions. Results are presented in figure 1. At the three concentrations studied under non-axenic conditions, compared to axenic conditions, an increase of 57 to 119 percent in the root uptake of riceplants was observed. To the contrary the translocation to the shoots was more important under axenic conditions (see transport index in figure 1). Keeping groundnut plants for longer periods under sterile conditions proved to be impossible and therefore the information is limited to the rate of uptake by young plants for short periods: the rate of phosphate uptake by intact non-axenic groundnut plants was 1,5 times higher than by axenic plants.

Because of the similarity of the overall response of rice plants, at the different phosphate concentrations considered, to axenic and non-axenic conditions, there is, at least for this species, no indication that the low and high concentration mechanisms (see above) are due respectively to microbiological and plant activity.

Onderzoekinstelling : Association Euratom - ITAL
Projectnummer : 45
Registratie nr.:

IV. Analysis of uptake and growth response in intact rice (Oryza sativa L., cv IR 8) and groundnut (Arachis hypogea L., cv TMV 2) plants to increased phosphate concentration in the nutrient medium. (G. Alagarswamy, Tang vanHHai, M.J. Frissel, H. Laudelout and A. Ringoet).

In the present study, the uptake (absorption and transport) of phosphates, from solutions of different concentration, by intact (30 days old) groundnut and rice plants was considered. Also the relationship between the growth and the phosphate concentration of the solutions was studied for both species.
The rate of phosphate uptake was measured by the continuous flow method, which has been described in detail in previous reports. Climatic conditions and the composition of the nutrient medium were also similar to those in related experimental work over the past years.
As shown by fig. 2 and 4, there is a distinct discontinuity in the uptake response curves of both plants with respect to phosphate concentration. The two curves may be easily fitted to Michaelis-Menten kinetics (represented by the full lines in the figures). Respectively in the low and high concentration range the following equations were used:

$$V_{low} = \frac{V_{m_{low}} \cdot C}{K_{m_{low}} + C} \quad (1) \quad \text{and} \quad V_{high} = \frac{V_{m_{high}} \cdot (C-C_L)}{K_{m_{high}} + (C-C_L)} \quad (2)$$

Not too much weight may be attached to the fit thus obtained since it involves the arbitrary adjustment of five parameters, namely the Vm and Km values for both high and low concentration mechanisms and the threshold concentration, C_L, below which the high concentration mechanism is inoperative.
The results, pertaining to the relationship between uptake and phosphate concentration, have been replotted together with those for the relationship between growth and phosphate concentration in semi-logarithmic fashon, since phosphate concentration values were spread on about four orders of magnitude. Fig. 3 and 5 present these results for groundnut and rice respectively. The concentration range in which the maximum response to a concentration change occurs seems to vary with the process considered.
Because of the logarithmic scale the maximum responses can not be read directly from the figures 3 and 5. They have been calculated from Δ uptake/Δ concentration and Δ growth/Δ concentration ratios. In the case of groundnut, the maximum response change for growth appears to occur at a phosphate concentration of 20 μM while the corresponding value for the uptake-rate was about 80 μM. This result illustrates the well known phenomenon of luxury consumption of the substrate. No such difference was observed in the case of rice where both ratios showed similar but noticeably lower values than in the case of groundnut. Both for uptake and growth, a value around 3 μM was found.
Whatever the nature of the formalism chosen for explaining the kinetics of phosphate uptake -dual mechanism, irreversible thermo-dynamic considerations, or allosteric effects- it remains that a certain threshold concentration of phosphate does exist below which the response of the plant with respect either to growth or uptake remains constant, irrespective of the amounts of phosphate present in a fairly wide range of concentrations. This fact may have an important bearing on the possible economic return of phosphate manuring of soils with a low phosphate status.
The determination of the maximum response concentration range in laboratory experiments of the type described here, may be of direct interest to field fertilizer experiments with different species. The presented data show the considerable differences in maximum response, for both growth and uptake, between the monocotyledon rice and the dicotyledon groundnut. Further study of the growth and uptake responses of other species seems worthwhile.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 45	Registratie nr.:

V. Transport and accumulation of phosphates in the aerial part of soybean (Glycine Max L.(Merr.) cv. chippewa) plants after root uptake from diluted solutions. (H. Trabelsi, C. Petit, S.v.d.Geijn, G. Verfaillie, H. Laudelout, A. Rinnoet).

In relation to the studies on root uptake of phosphates reported elsewhere, its further transport and distribution in the aerial part of soybean plants has been considered. This research has mainly been done using a semiconductor-detector assembly, built at the Institute (see previous annual reports). This assembly, composed of 4 detectors, permits in vivo transport and accumulation measurements of elements, labelled with β-emitting isotopes.
The soybean plant has been chosen (see VI) because of the facility to apply the detectors correctly to the stem and leaf surface. The in vivo transport and accumulation measurements, using ^{32}P-labelled solutions, have frequently been controlled by sampling of different parts of other soybean plants treated in exactly the same way as the plants of the semi-conductor experiments. Counting of these samples has been performed according to the usual liquid scintillation techniques.
From the literature it is known that root-absorbed phosphates are mainly transported to the young metabolically active plant parts. Foliar-absorbed phosphates probably move through the phloem, either to the top of the plant or in a basipetal direction in the stem. There is experimental information showing that phosphates accumulate in or near the phloem tissue.
In the experiments related here, we have mainly been interested in the following points:

- Speed of the phosphates transport in groundnut (Arachis hypogaea L.) and soybean plants

The speed of transport has been determined using semiconductor detectors placed at known distances along the stem and highly labelled (up to 500 µCi) phosphate solutions of known concentration, applied to the root system.
Respectively in groundnut (35 days old) and in soybean (20 days old) plants, speeds of 1-3 cm/min and 5-7 cm/min were registered. The speed of transport is considerably reduced in darkness, at lower temperatures and by suppressing part of the foliage. This effect of changes in the environment again (see I) shows that phosphate transport is also influenced by the transpiration of the plants.
Although very sensitive semiconductor detectors as well as a considerable amount of ^{32}P-label were used, the precision of the speed measurements still depends upon the ^{32}P detection-efficiency in plant stems. It is also affected by a phosphate accumulation process in the tissue surrounding the xylem vessels, that almost immediately starts during acropetal transport (see below).

- Phosphate accumulation in soybean stems

Phosphor-32 labelled solutions of different (1ℓ.1 µM and 966 µM) concentration have been applied to the root system of soybean plants for various periods: 2 to 3 minutes, 1 to 2 hours, 2 to 3 days. After these periods the roots were washed and the plants again received a non-labelled solution at the same phosphate concentration.
The following observations were made:

- as long as the plant is in the radioactive solution, there is a continuous arrival of ^{32}P in the tissue in front of the detectors and after some time, this accumulation occurs at almost constant rate. Even at high phosphate concentration of the nutrient solution and over longer periods no saturation of the tissue is observed. (fig. 6).
- the rate of accumulation during the day is higher than during the night (fig. 6).
- when in older plants (35 days) the accumulation in front of 2 semiconductor detectors, one at the base and one at top of the stem is considered and radioactive solutions

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are applied over short periods, respectively at 10 a.m. and at 2 p.m., accumulation in the basal stempart is higher in the morning experiment. The opposite is true in the afternoon. (fig. 7).

- after replacement by the non-labelled solution, the rate of ^{32}P-accumulation is slowly reduced. After long ^{32}P-application periods several days are needed to stop all new accumulation. (fig. 8).

According to these results a tremendous phosphate accumulation capacity of the soybean stem has to be accepted. That accumulation is concerned is shown by the fact that, when the radioactive solution is replaced by a non-labelled one, the count rate in front of the detectors still increases, although at a slower rate, but eventually during several days. Apparently part of the ^{32}P root-pool is slowly replaced by ^{31}P-ions and the former are transported to the aerial part. In the stem tissue, however, exchange of ^{31}P-ions for ^{32}P, if it occurs, must be small as compared to the accumulation. The experiments with 2 semiconductor detectors at different moments of the day and the difference between day and night accumulation suggest that the availability of metabolites plays a role in the phosphate accumulation.
These results were at the origin of another series of experiments aiming at some more information concerning the accumulation process. For that purpose the effect of different treatments on redistribution of the accumulated ^{32}P was studied.

- Redistribution of the accumulated phosphates in soybean stems

As further experiments are in progress, only some preliminary results are related here.
These treatments were:
- After feeding the plants for 20 days on a 320 µM or more P solution they received the same solution, labelled with ^{32}P, during 3 days. Then they were transplanted, either to a solution without phosphates (treatment 1) or to a complete solution (without ^{32}P of course) containing the uncoupler of the oxydative phosphorylation 2-4 dinitrophenol (2-4 DNP) at a concentration of 10^{-4}M (treatment 2), or to a complete solution after cutting stem and roots below the cotyledons (treatment 3).
- Treatment 4 as treatment 3 mentioned above, but with plants which had grown in a 16 µM P solution and were labelled for 1 hr at this same phosphate concentration.

As mentioned before replacement of a ^{32}P-labelled solution by a non-labelled one never produces a measurable release from the sites of accumulation in front of the semiconductor detectors (fig. 8). In the treatments 1, 2 and 4 considered here, to the contrary, a decrease of the count-rate and therefore of the P-accumulation is observed by the detectors, respectively after a few days (fig. 9), a few hours (fig. 10) and a few minutes (fig. 11). In the third treatment the accumulation does not change.
The interest of these preliminary results is that they give some indications concerning the fate of root absorbed phosphates in the plant. The results of treatment 4 show that recently absorbed phosphates may easily be displaced (movement to younger parts?) by other ions directly absorbed by the stem. Phosphates absorbed over a longer period are not redistributed in those experimental conditions (treatment 3). The most probable explanation is that recently absorbed phosphates are still in the xylem. When absorbed over a longer period, phosphates have probably moved out of the conducting vessels or are accumulated in a form which is not exchangeable by recently absorbed ions. In experiments concerning labelled phosphate absorption over several days the fraction in the xylem, measured by the detectors would then be very small compared to the accumulated amount. (see before). Results in treatment 1 suggest that, when the phosphate source in the nutrient medium, is not longer available, the metabolically active younger plant parts probably first

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use the phosphates in the roots before starting to mobilize the reserves in the stem. The uncoupling reaction of DNP, however, by stopping the oxydative phosphorylation but not the respiration in the plant, makes phosphates in all plant parts immediately available for redistribution. From a kinetic point of view it is important to note that apparently rootabsorbed phosphates are directly mobilized from the conducting tissue according to the metabolic needs at different sites on their way upwards.

Finally, in relation to the experiments discussed here, a method has been worked out, using semiconductor detectors for the control of the phosphate concentration in solutions for the continuous flow experiments. Figure 12 schematically represents the experimental set-up. Due to the thickness of the solutionlayer, the semiconductor detector only observes concentration variations and not the adsorption to the plexi-glass bottom of the vessel. The technique is, of course, limited to experiments at rapid flow rates, but it can also be used for control of the uptake after changes in concentration or flowrate of the supplying solution.

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VI. The influence of urea on the uptake of phosphates from diluted solutions by soybean (Glycine Max L. (Merr.), cv. Chippewa) plants. (S. Stan and A.Ringoet).

In recent years phosphate uptake from diluted mineral solutions by different plant species has been studied at the Institute. In 1971 two studies concerning the influence of the interaction between different nutrients on uptake were started. In the first one phosphate/chloride ratios in tomatoplants (Lycopersicon esculentum L, cv. Mevette) were considered (see below). The present study is mainly concerned with the effect of the presence of urea in the mineral solution on phosphate uptake, the phosphate being present at various concentrations (3.22, 16.1, 32.2, 322, 644, 966 µM KH_2PO_4). Growth of the soybean plants, submitted to different treatments was also studied.
Plants were grown and experiments done in a climate controlled growth chamber (27000 lux, day t°: 28°C, night t°: 22°C, daylength: 13 hours, relative humidity: 75%). Bollard type nutrient solution was used as the standard one. For the urea solution, all nitrogen salts were replaced by urea. Where necessary the pH was adjusted to a value of 5.5 with small amounts of 0.1 N HCl. Micronutrients were added according to Hoagland. To avoid important development of microorganisms, mainly in the "urea" type solution, the solutions were changed every day. Twenty days old soybean plants were selected for the uptake experiments. These experiments were done with the continuous flow method in use at the Institute since several years; the solutions were labelled with 1 µCi ^{32}P per liter. Plants were always grown at the phosphate concentration used in the final uptake experiments; they were also pretreated for 24 hours under continuous flow conditions. After the uptake experiments (4-5 hrs) under steady-state conditions, plants were harvested, separated in roots and aerial parts, weighed for fresh and dry matter, analysed for total radioactivity absorbed during the experimental period.
All experimental data are not yet available. Some preliminary results concerning growth may be summarized as follows:

- the growth curves in relation to phosphate concentration in fig. 13 illustrate a sharp growth increase at the lower phosphate concentrations (3.22-32.2 µM) In the higher concentration range the increase is a more regular one. Even at the 966 µM concentration the maximum growth of the soybean plants is not reached.
- It is also interesting to note that at almost all phosphate concentrations growth and dry matter production is higher in "urea"-type solution than in the standard Bollard solution.
- the stimulating effect of urea mainly affected the growth of the roots and the leaves of the soybean plants.
- according to fig. 14 the rate of phosphate uptake by 20 days old plants also increases with the phosphate concentration in the solution. Again, the increase is most marked at the lower concentrations (3.22-32.2 µM).The maximum is not reached in the concentration range considered here.
- except at the 3.22 µM phosphate concentration, the rate of phosphate uptake is always higher in the "urea"-type solution.
- no dual uptake mechanism was observed in the present experiments on soybean plants. It remains possible that the threshold concentration between the high and low affinity mechanism for this species is situated in the concentration range below 3.22 µM. However, at concentrations in that range we did not succeed in growing healthy plants, even with frequent (several times a day) renewal of the nutrient solutions. The maximum responses of growth and uptake to varying phosphate concentrations (see above) both occur, according to the available results in the range from 3.22-32.2 µM. In this respect, the soybean plants, like the rice plants do not show the luxury consumption, which characterized the groundnut plants

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VII. Uptake of chlorine and phosphates by intact tomato (Lycopersicon esculentum L., cv. Mevette) plants from mainly dilute nutrient solutions. (P. Chhabra, A. Ringoet)

This study considers the uptake of chlorine from mainly dilute nutrient solutions, its interaction with phosphates, its accumulation and distribution in different plant parts and its effect on the growth of the tomato plants.

Method and material

For the uptake experiments the plants were grown on a complete aerated nutrient solution, at different generally low levels of chlorine and phosphates, under controlled climatic conditions (38000 lux, day temperature 20 °C (16 hours), night temperature 15 °C, relative humidity 70%). Different combinations of 11 chlorine levels (1.68 (control), 8.4, 14, 28, 42, 56, 84, 112, 140, 196 and 280 10^{-5} M Cl) and 4 levels of phosphorus (0.32, 1.6, 3.2 and 6.4 10^{-5} M P) were used. In some phosphate uptake experiments higher levels of P, up to 48 10^{-5} M P were also used. The nutrient solution was renewed every day. At the age of 30 days (start of the flowering stage), the plants were pretreated for 24 hours in the continuous flow system using unlabelled nutrient solution. The uptake experiment itself was done with the same solution labelled with ^{32}P (phosphoric acid in H_2O) and ^{36}Cl (0.1 N HCl). The rates of uptake of the elements were calculated as described by Tang Van Hai and Laudelout (1966).
For autoradiographic and long duration experiments a smaller number of plants per culture tank was used and the nutrient solution was changed every two days. For autoradiography, 30 days old plants were allowed to absorb either ^{36}Cl or ^{32}P for 24 hours. Freeze-drying and processing for exposure was as described by Levi (1969). The Cl-contents of the dry plant material were determined by potentiometric titration.

Results and discussion

1. Rates of chlorine and phosphate uptake by 30 days old intact plants and effect of their interaction on the uptake.

The results of the Cl-uptake experiments have been summarized in figure 15. There is a linear relationship between the rate of Cl-uptake and Cl-concentration in the nutrient solution up to at least 56 10^{-5}M Cl. At low levels (0.32-3.2 10^{-5}M P) of phosphates, in the nutrient medium , a sudden increase of the rate of Cl-uptake is observed at a solution concentration between 84 and 412 10^{-5} M Cl.
A dual mechanism thus seems responsible for the Cl-uptake at low phosphate concentrations. The first mechanism appears to reach its maximum rate between 56 and 84 10^{-5} M Cl. The threshold concentration between both mechanisms is near to 84 10^{-5} M Cl. The second mechanism tends to a maximum between 112 and 140 10^{-5} M Cl. At a phosphate concentration of 6.4 10^{-5} M P, to the contrary, no second mechanism is found: the rate of Cl-uptake increases linearly with the concentration up to 280 10^{-5} M Cl. This interesting effect of phosphates on the chlorine uptake mechanism needs further study.
The rate of phosphate uptake by intact tomato plants shows an almost linear increase up to a P-concentration of 6.4 10^{-5} M P (figure 16). At higher concentrations the rate of uptake decreases and tends to reach a maximum between 32 and 48 10^{-5} M P. Under the present experimental conditions no dual mechanism for phosphates uptake at concentrations up to 48 10^{-5} M P has been observed. There is no effect of the Cl-concentrations on the rate of phosphate uptake, except at the 1.68 10^{-5} M Cl level, where, for yet unknown reasons, the P-uptake is always higher.

2. Effect of chlorine on the growth of 30 and 60 days old plants

Sixty days old plants grown in climate-controlled conditions on mineral solutions at 1.68 10^{-5} M Cl, show deficiency symptoms and reduced leaf size, whereas no such observations are made in 30 days old plants. No toxic effect of chlorine up to

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concentrations of 280 10^{-5} M Cl are observed. There is an increase in the fresh (figure 17)as well as in the dry matter production of 30 and 60 days old tomato plants with increase in Cl-concentrations of the nutrient medium, at least in the low concentration range.
Chlorine has a marked effect on the fruit production of the tomato plant (table 1). Plants grown in 1.68 10^{-5} M Cl have fewer flowers than plants grown in nutrient solution containing higher levels of chlorine; they produce no fruits.
Plants of the 280 10^{-5} M Cl treatment produce flowers one week earlier than plants from the other treatments and apparently related to this effect, the fruit weight is much higher inspite of no difference in the average number of flowers. The fruits produced in the 280 10^{-5} M Cl treatment are, however, suffering from blossom-end-rot.

Table 1 - Influence of Cl- and P-concentrations in the nutrient medium on flowering and "fruit" production of 60 days old tomato plants.

Chlorine concentration ($\times 10^{-5}$ M)	Average number of flowers			Fresh weight of the reproductive parts (g)		
	1.6 x 10^{-5}M P	3.2 x 10^{-5}M P	6.4 x 10^{-5}M P	1.6 x 10^{-5}M P	3.2 x 10^{-5}M P	6.4 x 10^{-5}M P
1.68	3	2	4	4.3	1.7	3.3
14.0	19	42	59	60.6	20.1	174.0
28.0	17	49	59	51.6	36.9	187.9
112.0	18	47	62	81.7	37.3	(30.6)
280.0	21	45	59	92.6	121.2	174.0

3. Effect of phosphate on the plant growth

The phosphate concentration of the nutrient medium shows a great effect on the growth of 30 days old tomato plants up to concentrations of 3.2 10^{-5} M.
Further increase in phosphate concentration up to 48 10^{-5} M has no additional effect. But the older plants (60 days old) show that phosphate concentrations up to 6.4 10^{-5} M increase the fresh (figure 17)and the dry weight as well as the fruit production (with the exception of the 3.2 10^{-5} M treatment) of the tomato plants (Table 1).

4. Accumulation and distribution of chlorine

The accumulation of chlorine over 30 days of growth increases with the Cl-content of the nutrient solution but the rate of accumulation in the shoot is much slower than in the roots. As shown in figure 18,which applies to 60 days old plants, among the aerial parts the stem contains the highest amount of Cl(at the maximum 1.33% of dry matter), followed by the reproductive parts (0.90 %), the upper leaves (0.75%) and then by the lower leaves (0.72%). Phosphates have a pronounced effect on decreasing the Cl-content of the 30 and 60 (see fig.18) days old plants.
In 30 days old plants, this effect is, at the maximum, at 3.2 10^{-5} M P; in 60 days old plants, even at 6.4 10^{-5} M P, a further decrease of the Cl-content is found.
The total increase in the Cl-content of the dry plant material (both for 30 and 60 days old plants) with increase of the Cl-concentration in the nutrient medium, is smaller than the amounts which can be calculated from the observed rates of ^{36}Cl-uptake by roots (see before). This may be explained to a certain extent by the losses of chlorine from the aerial plant parts (see 5).

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5. Autoradiographic studies

The autoradiographs of ^{36}Cl-labelled 30 days old tomato plants show the maximum blackening in the root followed by stem, young leaves and the older leaves. The terminal parts of the fully grown leaves show minimum blackening. This confirms the results for 60 days old plants where Cl-contents decreased in the following order: root > stem > reproductive parts > upper leaves > older leaves.
The autoradiographs further suggest that in leaves chlorine is stored at the outer margin, especially at sharp ends and in some spots in the leaf blade. The rest of the leaf blade shows almost no blackening and therefore apparently no preference for ^{36}Cl accumulation. The spots on the leaves which correspond to the black spots on the autoradiograph and thus contain ^{36}Cl, appear only after drying. When examined under the microscope they show up as small cavities containing necrotic material and surrounded by many long hairs. The hairs taken from these spots and the leaf-margin tissue are found to contain hygroscopic columnar crystals of salts, especially in the basal cell of the hair, as well as parts of raptured cell walls. From these findings it is concluded that chlorine in the leaf is mainly stored at the leaf-margin and in some spots in the leaf-blade. These places are probable sites for hydathodes which are actively associated with the salt excretion from the plant. The chlorine might be present as one of the components of these salts and be eliminated from these sites by rapturing the cell wall. Such losses might explain why there is no proportional increase in the Cl-content of the aerial plant parts with increase in Cl-concentration of the nutrient solution inspite of higher rates of uptake by roots.
The qualitative results from the autoradiographs are also in this respect confirmed by the higher total Cl-content (0.27%, as compared to 0.18% for the inner leaf part) of the leaf margin of 60 days old plants.
The autoradiographs of the ^{32}P-labelled plants show the preference of phosphates for the apical young leaves. No effect of Cl-concentrations on the distribution pattern of phosphates in the plant is observed. Although the black spots (as observed after ^{36}Cl-labelling) are also found in ^{32}P-labelled plants no blackening of the X-ray films show any preferential accumulation of ^{32}P at such sites.

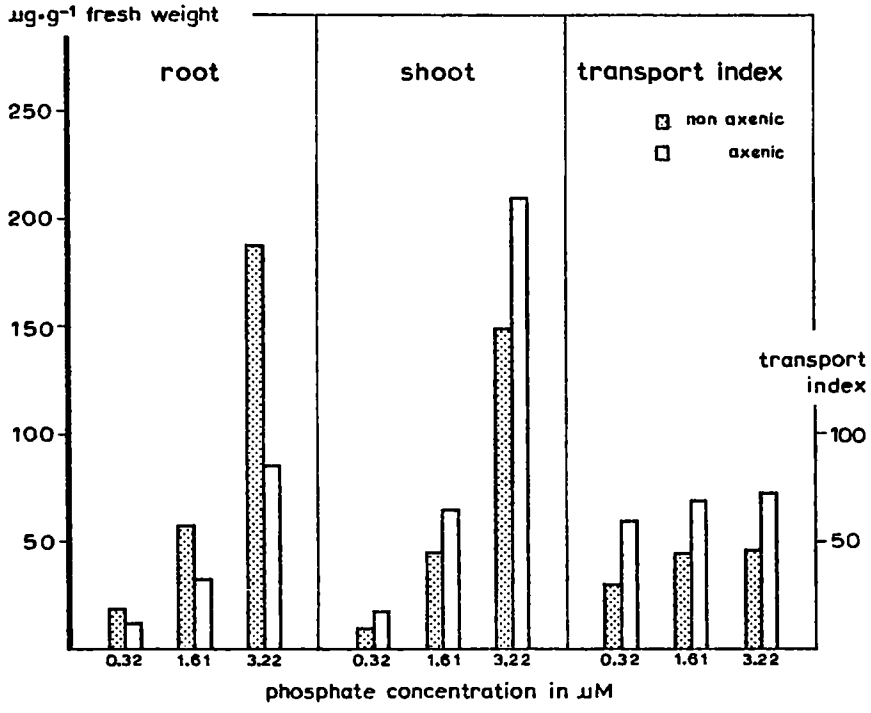

fig. 1

The distribution of phosphate after 24 h. in rice plants (21 days old) grown under axenic and non-axenic conditions

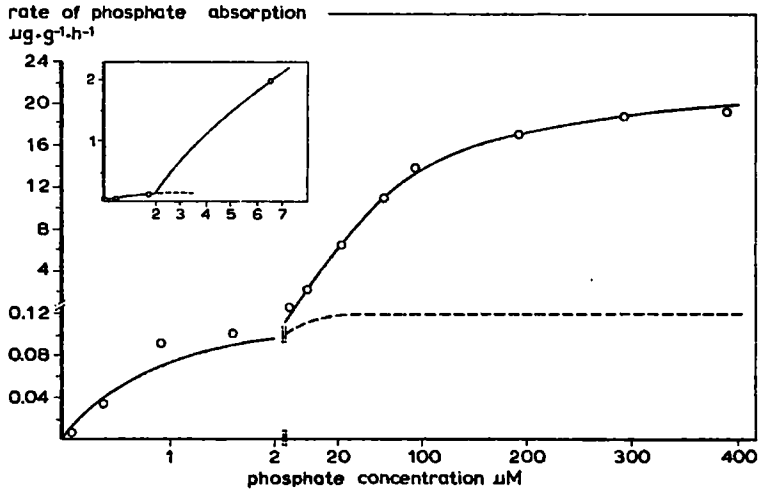

fig. 2

Rate of phosphate uptake by 30 days old groundnut plants, measured by the continuous flow method in relation to the phosphate concentration of the nutrient medium.

Insert: first part of the curve showing the transition from low to high concentration kinetics.

- experimental data: (•)
- theoretical curve calculated according the Michaelis-Menten kinetics: ————
- contribution of low concentration mechanism operating at maximal rate: --------

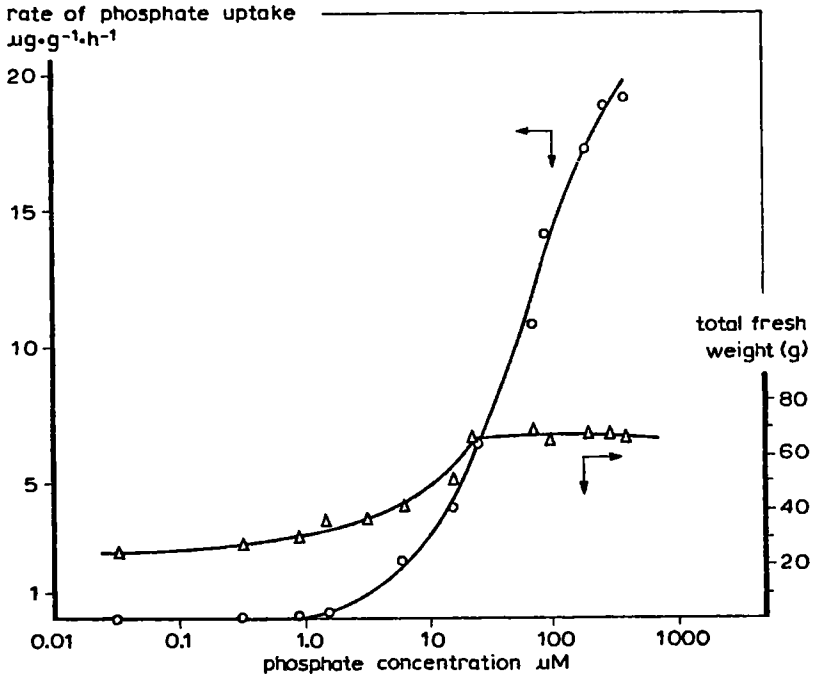

fig. 3

Yield and rate of phosphate uptake as influenced by phosphate concentration for groundnuts: uptake (o); growth (△)

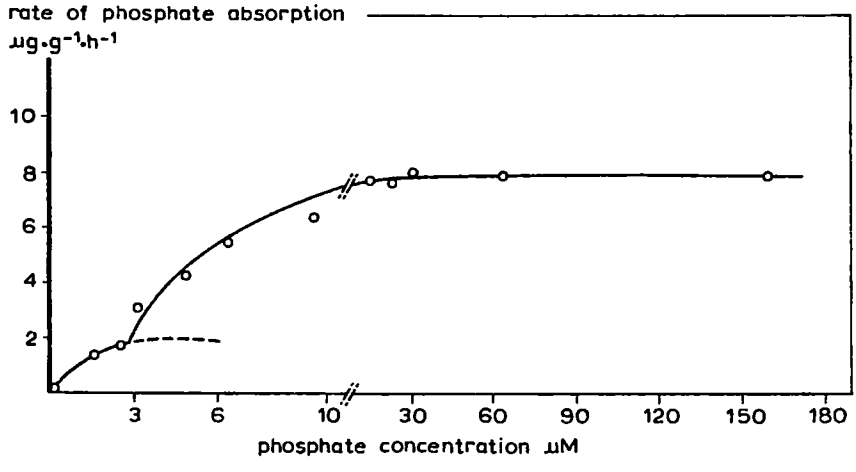

fig. 4

Influence of phosphate concentration on its uptake rate in rice plants

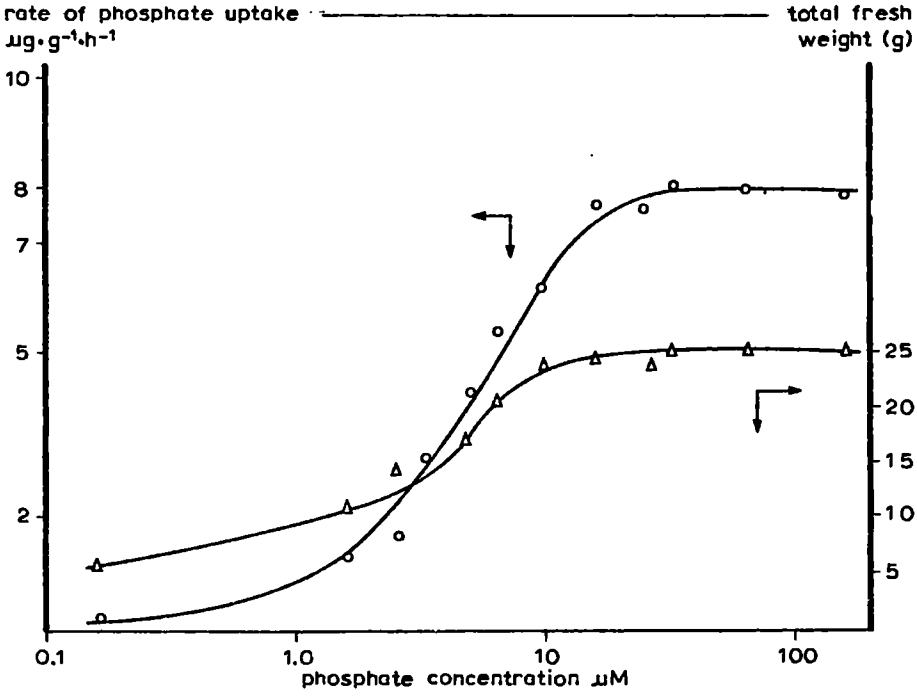

fig. 5

Yield and rate of phosphate uptake as influenced by phosphate concentration, for rice plants: uptake (o); growth (Δ)

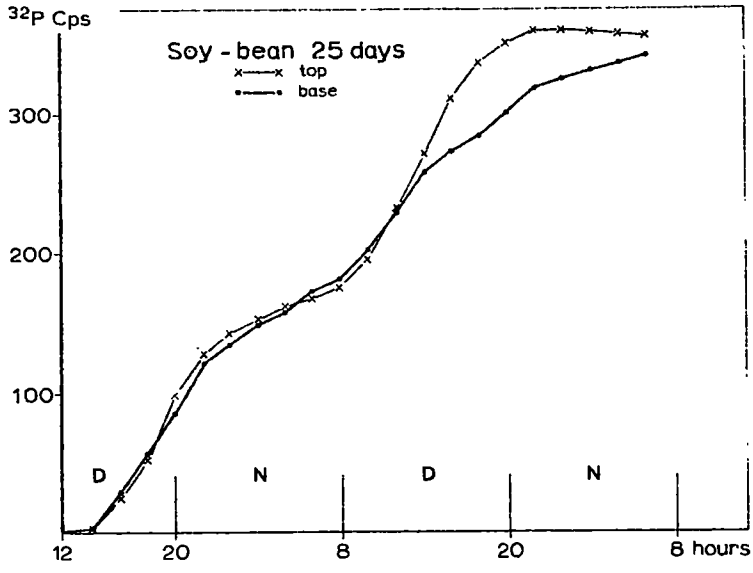

fig. 6

Phosphate accumulation at two sites in the soybean stem over longer periods, as measured by semiconductor detectors.

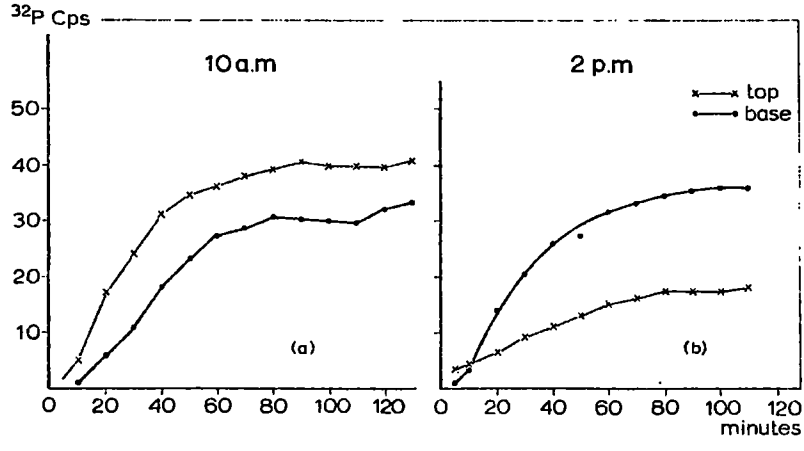

fig. 7

Phosphate accumulation at two sites in the soybean stem over short periods; (a) measurement during the morning

(b) measurement in the afternoon.

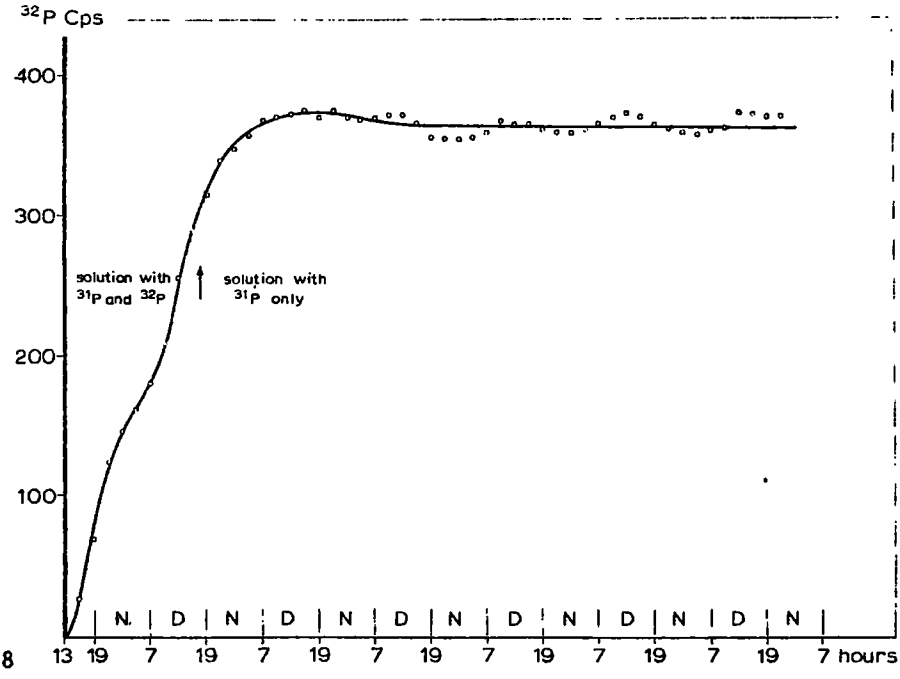

fig. 8

Phosphate accumulation in the soybean stem over a long period Effect on accumulation of the replacement of a ^{32}P-labelled solution by a non-radioactive solution.

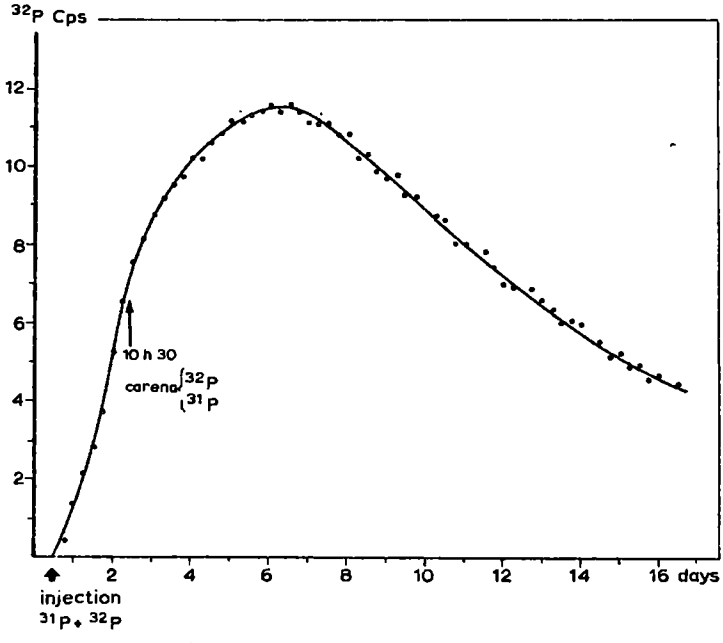

fig. 9

Phosphate accumulation in the soybean stem over a long period Effect on accumulation of the replacement of a P-labelled solution by a solution without phosphates

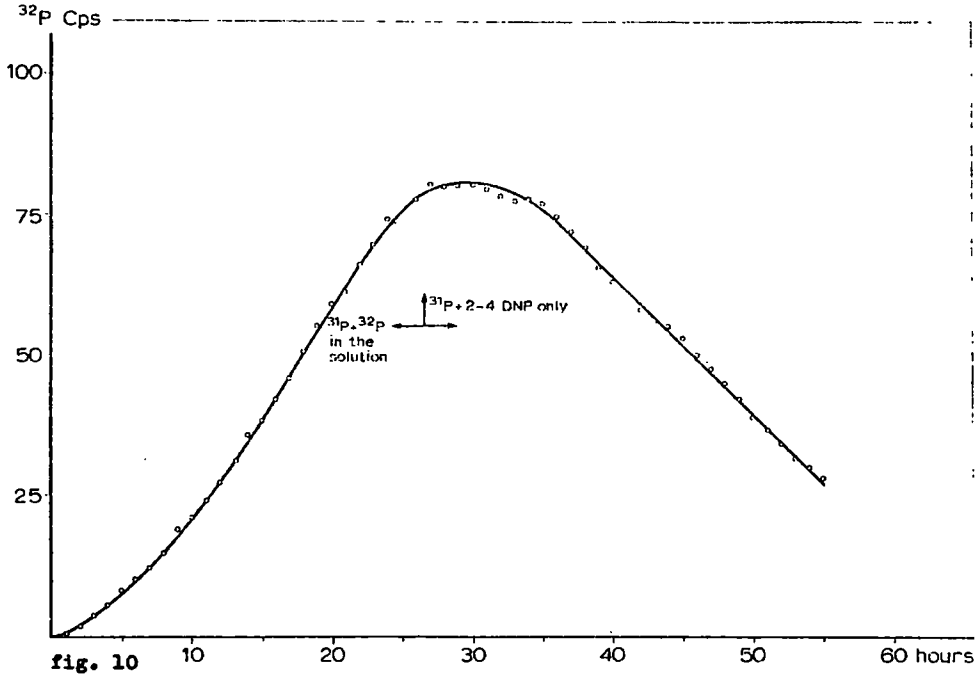

fig. 10 Phosphate accumulation in the soybean stem over a long period. Effect on accumulation of the replacement of a ^{32}P-labelled solution by non-radioactive one containing 2-4 DNP 10^{-4}M.

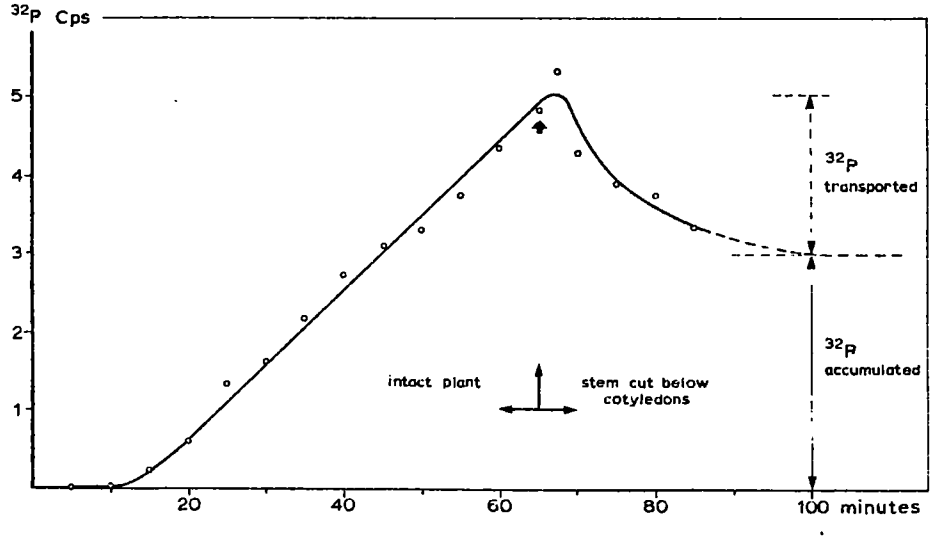

fig. 11

Phosphate accumulation in the soybean stem over a short period. Effect on accumulation of cutting the stem below the cotyledons and replacement of the ^{32}P-labelled solution by a non-radioactive one.

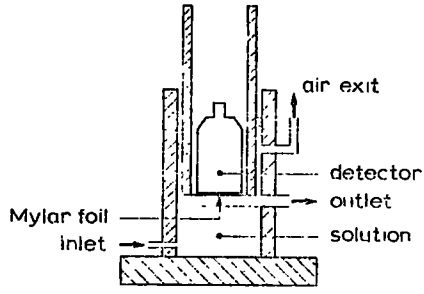

fig. 12
Experimental set-up with semiconductor detector for the control of concentration in a continuous flow system.

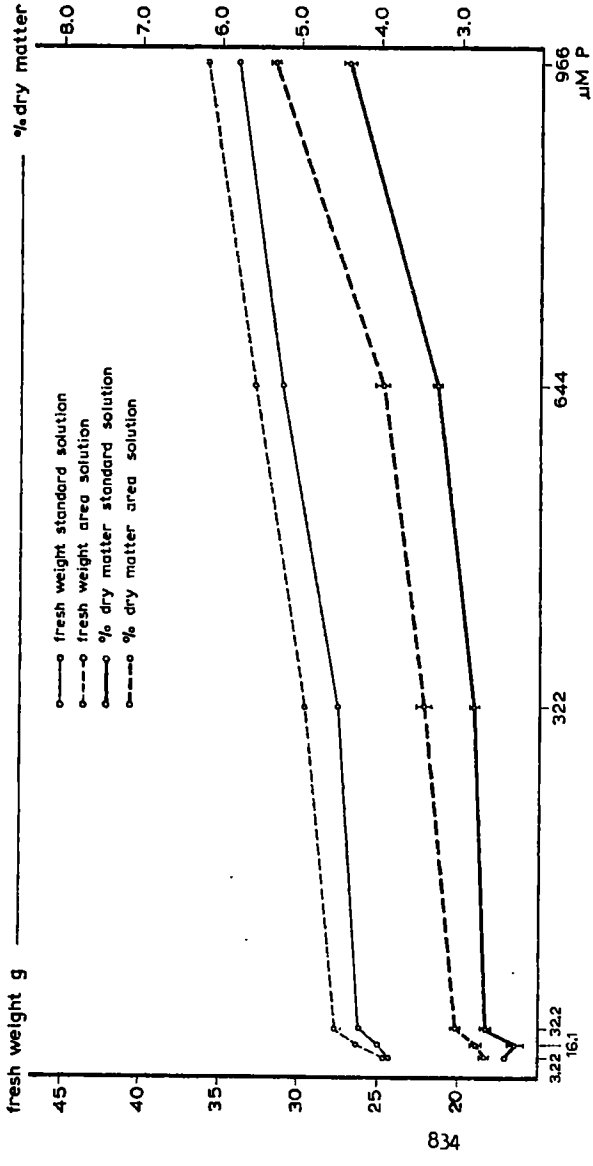

fig. 13
Increase of fresh weight and % dry matter in relation to the type of nitrogen source and phosphate concentration level in the nutrient solution.

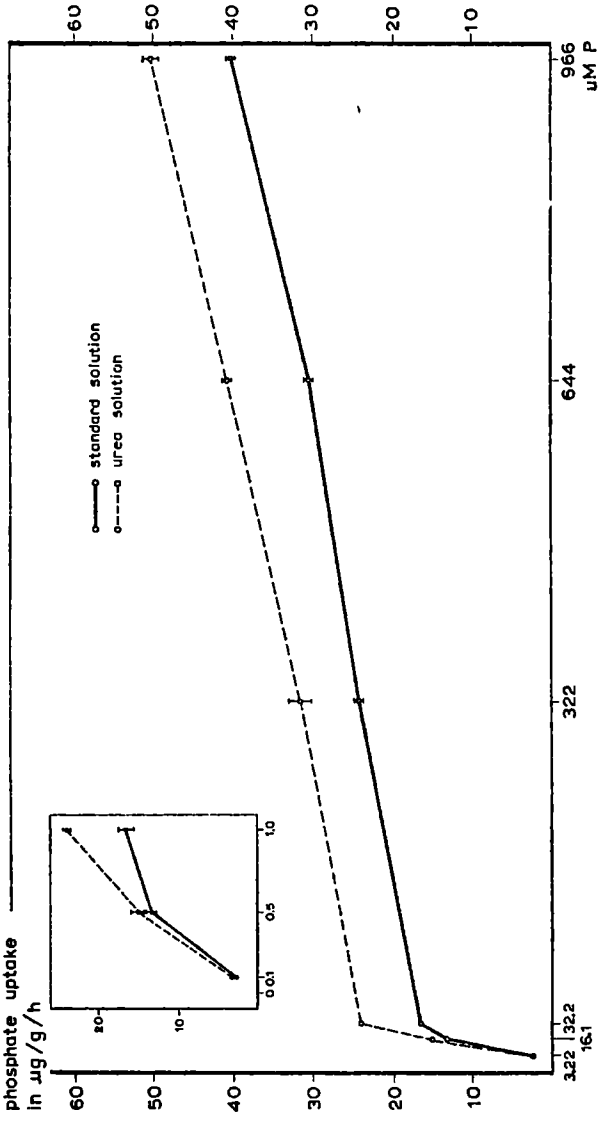

fig. 14

Rate of phosphate uptake by intact soybean plants in relation to the type of nitrogen source and phosphate concentration in the nutrient solution.

Insert: detail of the first part of the curves.

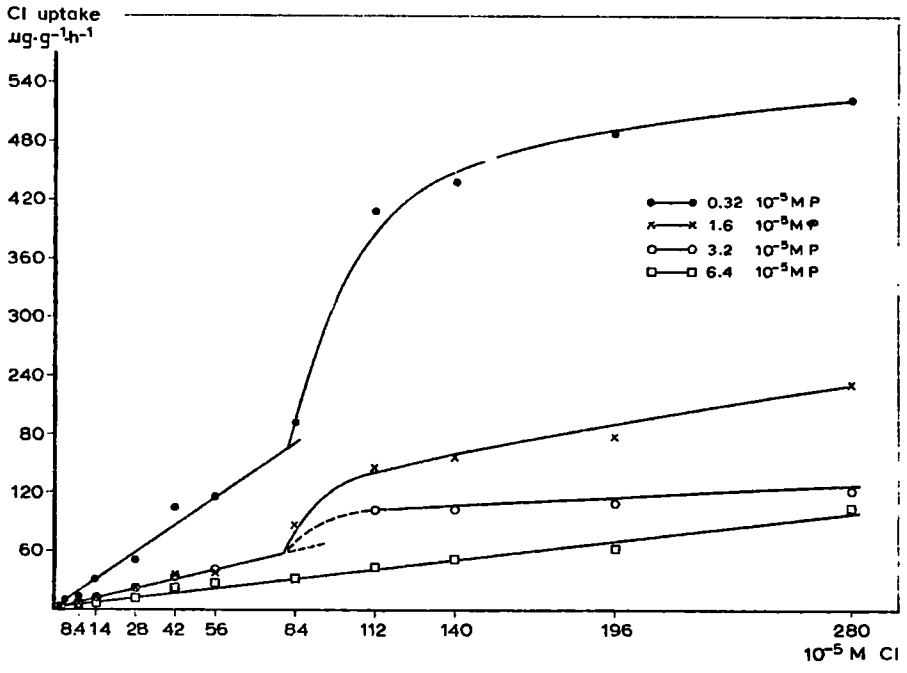

fig. 15
Influence of the chlorine and phosphate concentrations of the nutrient solution on the rate of Cl-uptake by 30 days old tomato plants

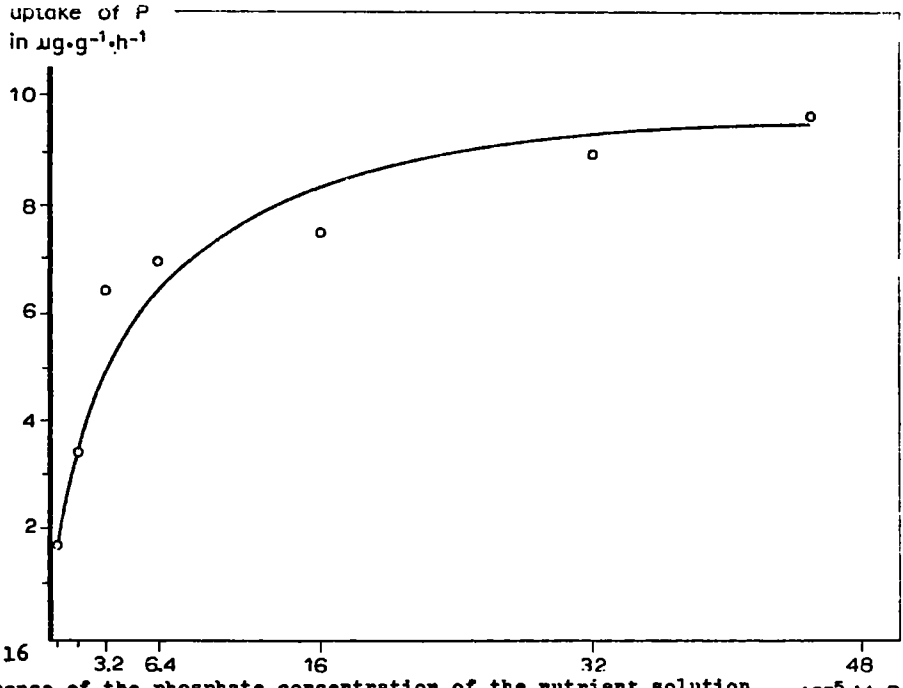

fig. 16 Influence of the phosphate concentration of the nutrient solution on the rate of P-uptake by 30 days old tomato plants.

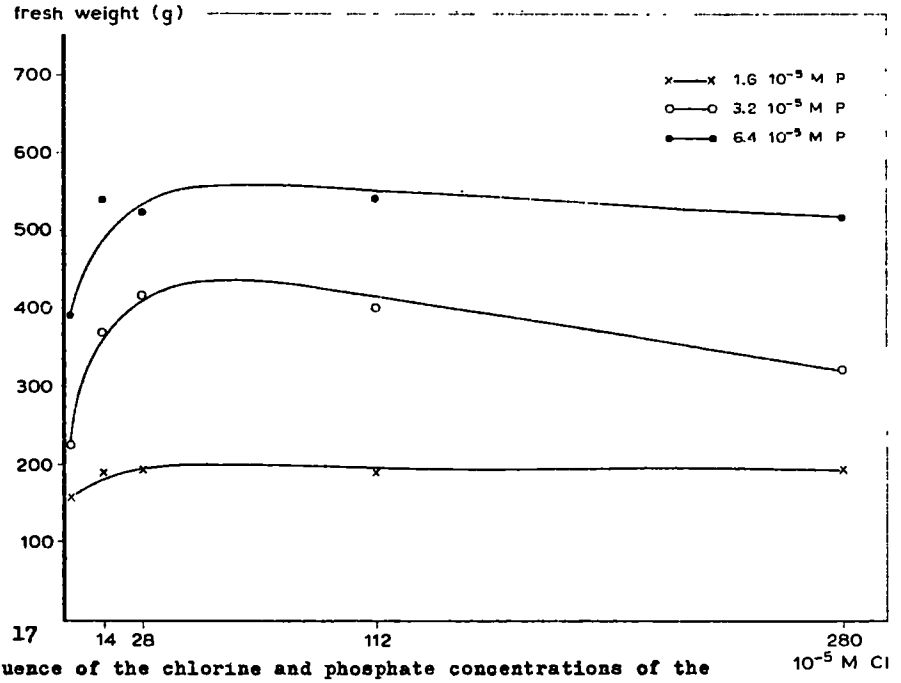

fig. 17 Influence of the chlorine and phosphate concentrations of the nutrient solution on the growth of tomato plants up to 60 days.

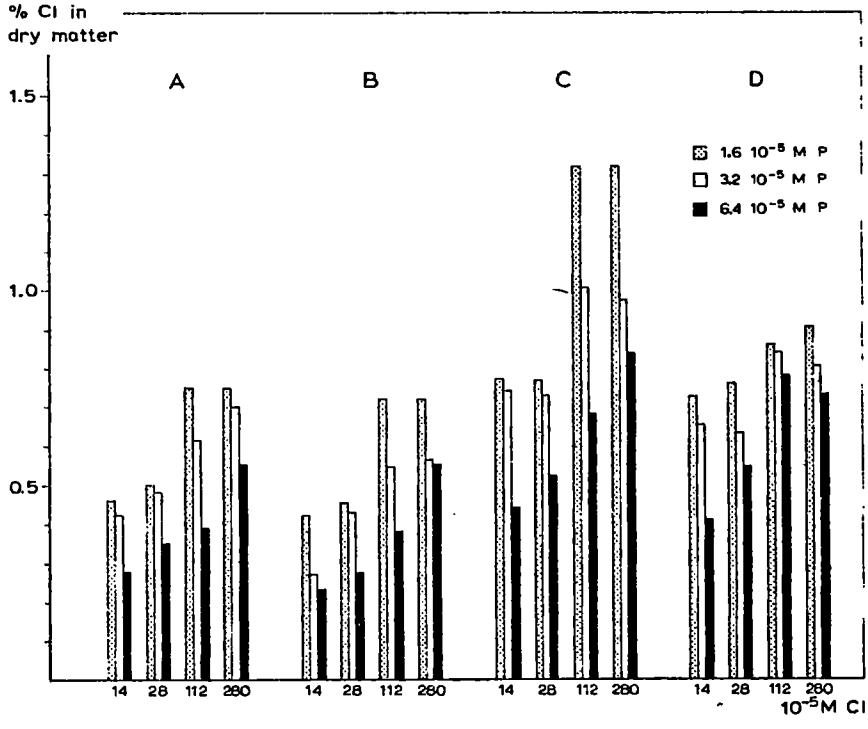

fig. 18

Influence of the chlorine and phosphate concentrations of the nutrient solution on the Cl-content of different plant parts of 60 days old tomato plants;

A: leaves, B:

A: younger leaves
B: older leaves
C: stem
D: reproductive parts

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 50
Projecttitel : Coordination of food research

Registratie nr.: AA-009 / 50

Onderzoeker(s) : J.G. van Kooij
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
" " -materieel f
Semi-directe kosten f
Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

Shelf life extension of precooked food commodities by irradiation; bacteriological aspects and sensory evaluation.

In cooperation with the Netherlands Institute for applied Home Economics Research, Wageningen, some preliminary experiments were carried out with carrots, endive and savoy cabbage. Only when irradiated at low doses (< 150 krad) these vegetables maintained an acceptable quality. Irradiation with such doses does not improve the sensoric value, but the shelflife of the vegetables, considered from a bacteriological viewpoint, was definitely increased.

Vitamin C assays in "ready to eat" vegetables.

These vegetables are normally subjected to the following treatments: precooking, storage at low temperature and finally reheating. In the present experiments the precooking vegetables were also irradiated before storage. As a first control of the effect of irradiation on the nutritive value of "ready to eat" vegetables, vitamin C was analysed in leek and brussels sprouts by the Food and Nutrition Dept. of the Agric. University, Wageningen.
By cooking alone and by cooking-irradiation respectively 33 and 50% of the available vitamin C is destroyed. Irradiation does not affect the further reduction of the vitamin C content of "ready to eat" vegetables during cold storage.

Irradiation for decontamination of egg products.

The Institute for Poultry Research "Het Spelderholt", Department Technology, Beekbergen, has investigated the radiation decontamination of crystallized eggwhite, as well as the effects of irradiation on foaminess, stability of the foam, viscosity of liquid eggwhite, colour and odour. Crystallized eggwhite (15% water) is not Enterobacteriaceae free after 600 krad. Similar observations were made the irradiation research on fishmeal. It is suggested, that the proteins of these products may act as radiation protection agents. However, it is also shown, that the heating time of these eggproducts can be reduced (50%), when the material is first irradiated with 100 krad. The viscosity and solubility are not changed by the irradiation doses applied, while foam-volume and stability increase with increasing dose. Irradiated crystallized eggwhite has an offensive odour (H_2S),

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but this odour may partly be avoided, when the irradiation is done under nitrogen. Irradiation has a bleaching effect; repair of colour occurs during storage in darkness.

Research on off-ocour in irradiated meat.

The Central Institute for Nutrition and Food Research, Zeist, has carried out a literature research on the nature of compounds, which may cause the objectionable "wet dog odour" in irradiated meat. As more than hundred different volatile compounds are known in meat; a good research plan has to be worked out to learn something concerning the compound responsible for off-odour in irradiated meat.

Sprout inhibition of onions by irradiation.

According to the general literature data doses of 4 to 10 krad are effective to prevent sprouting. One of the aims of the present experiments was to examine the possibility of a delayed irradiation (this in connection with a more efficient use of a gamma source for potato-irradiation). Five tons of non-irradiated onions and batches of onions irradiated at different time-intervals after harvest were stored at the ambient temperature from October till April, and then in a storage room at 1°C until June.
The results can be summarized as follows:
- after the dormancy period onions are not suitable for an irradiation treatment; sprouting is only retarded.
  The irradiation treatments have no effect on the sensoric value of the product. In cooperation with the Sprenger Institute, Wageningen, and the foundation of the Netherlands Onions federation, Alkmaar, further research on the effect of irradiation at different stages during dormancy of the onions and on sensoric parameters will be done.

Sprout inhibition of potatoes by irradiation.

In cooperation with the Institute for Research on Storage and Processing of Agricultural Produce, Wageningen and a commercial firm storage experiments were carried out with irradiated potatoes. The main aim of these experiments was to investigate on a large scale the effect of handling on the storage-quality of irradiated potatoes. A total of 150 tons of potatoes (variety Bintje) of the same origin has been used. Fifty tons were irradiated medio October with 10 krad (gamma source of the Pilot Plant for food irradiation), immediately after transfer of the potatoes to the small irradiation boxes; in this way healing of the transfer lesions was avoided.
After irradiation the boxes were emptied in a bulk storage room and the potatoes stored under commercial conditions (ventilation with outside air). Fifty tons were first kept for 3 weeks in the boxes, permitting wound-healing after transfer. Then the potatoes were irradiated with 10 krad early November, again stored for 3 weeks in the boxes and emptied in the bulk storage room. Fifty tons were chloroisopropylphenyl carbonat treated and stored under the same conditions as the irradiated potatoes. At intervals of one month the stored potatoes were inspected for sprouting and at the same time sampled for an evaluation of the sensoric quality after boiling. All batches of potatoes were examined on storage-loss and diseases. The Chloroisopropylphenyl carbonat-treated potatoes were discarded medio May because of total sprouting. The irradiated batches had an excellent appearance, did not sprout and were free from storage-disease. No difference was found between the potatoes, which were immediately irradiated after handling and those, irradiated after a healing period in the boxes.
The results show that tissue lesions, due to handling are not negatively affected by irradiation. However, generalization of this conclusion will always depend upon the level of Fusarium contamination. Weight losses in the irradiated batches were respectively 6 and 8,5%. After cooking irradiated potatoes were somewhat green shaded and this off-colour increased with the subsequent storage time at room

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 50	Registratie nr.:

temperature. This after-effect of irradiation in potatoes, which varies with the origin and variety of the material, is the main problem in the commercialization of the sprout-inhibiting irradiation process. In other aspects the reactions of the laboratory panels and groups of consumers were favourable to irradiated potatoes

Shelflife extension of packed fish fillets by irradiation.

The collaboration with the Institute for Fishery products is in the research planning stage.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 51
Projecttitel : Coordination of research in the field of wholesomeness of irradiated food.

Registratie nr.: AA-009 / 51

Onderzoeker(s) : J.G. van Kooij
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
„ „ -materieel f
Semi-directe kosten f
Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

Wholesomeness research with mini-pigs on an irradiated diet.

This research is carried out in collaboration with the Central Animal Testing Laboratory of the Medical Faculty, University at Nijmegen.
The mini-pig diet contains several human foodstuffs such as meat, fish, vegetables, cereals and has a caloric value of 1200.
The diets were irradiated with doses varying from 40 to 200 krad. There was no statistical difference in bodyweight increase over 5 months between animals receiving irradiated food and those fed with a normal diet.
Blood-tests, twice a year, did not show any difference between both groups.
Further analysis of the results is in progress.

Short-term toxicity study with rats on irradiated and autoclaved "Rostoch mixture" diet.

This research was done in cooperation between the National Institute of Public Health, Bilthoven, the Statens Serum Institute, Copenhagen and the Association's Institute. One hundred days old S.P.F. rats were fed either on a standard balanced diet, or on the same diet after sterilization by irradiation (5 Mrad) or by heat (110 °C during 10 min and 120 °C during 15 min). In the experiments of which the results are now available, each of the three groups was initially composed of 20 females and 10 males.

Feeding studies with fattening-pigs and reproduction tests with breeding-pigs.

In cooperation with the National Institute of Public Health, Bilthoven and the Vocational Training School for Poultry and Pigs, Barneveld, a wholesomeness test with pigs is in progress.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM-ITAL
Projectnummer : 52
Projecttitel : The heat and radiation resistance of bacterial spores.

Registratie nr.: AA-009 / 52

Onderzoeker(s) : H. Stegeman.
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
- Directe kosten-personeel f
- " " -materieel f
- Semi-directe kosten f
- Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
- Hoger personeel — mandagen
- Middelbaar personeel — mandagen
- Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971

In 1971 a detailed study, in collaboration with W. Pilnik (Department of of Food Science, Agric. Univ. Wageningen) and D.A.A. Mossel (Department of Food Preservation, Cath. Univ. Louvain) was started concerning the factors effecting the resistance of Bacillus stearothermophilus ATCC 7953 spores to irradiation and heat.

A first investigation concerned the sensitizing effect of irradiation to a subsequent heat treatment. In agreement with the observations on spores of Clostridium sporogenes PA 3679 by Morgan and Reed (Food Res., 19, 357 (1954)), spores of B. stearothermophilus ATCC 7953, suspended in water, were more easily destroyed by heat after a sub-lethal dose (100-200 krad) of X-rays.
Heating of spores before irradiation did not affect their radiation sensitivity.

Spores of B. stearothermophilus ATCC 7953, which were sensitized to heat by converting them to the 'hydrogen' form by acid treatment (Method - Alderton, Appl. Microbiol., 17, 745 (1969)), indeed lost their heat resistance to a certain extent, but the treatment had no effect on the radiation resistance.
The sensitizing effect of pre-irradiation to a subsequent heat treatment on spores in 'hydrogen' form was considerably increased. No synergistic effect was also observed when heat was applied before irradiation.
These results are in agreement with the idea that the biochemical mechanisms of inactivation by heat and radiation are different.

PROJECTVERSLAG

Onderzoekinstelling	:	ASSOCIATION EURATOM - ITAL
Projectnummer	:	53
Projecttitel	:	Studies on the metabolism of bacteria isolated from irradiated meat.

Registratie nr.: AA-009 / 53

Onderzoeker(s)	:	J.G. van Kooij.
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
„ „ -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

.Hoger personeel	—	mandagen
Middelbaar personeel	—	mandagen
Lager personeel	—	mandagen

Beknopte weergave van achtereenvolgens

A. Verslag afgelopen jaar (19 71) met vermelding van publicaties

B. Plan komende jaar.

A. Verslag over 19 71

A better knowledge of the effect of moderate doses of irradiation on the metabolism of the surviving flora in a natural environment, like meat, is desirable. Results from early work indicated, that Pseudomonas in non-irradiated, Achromobacter and Microbacterium thermosphactum in irradiated meat play an important role in the deterioration of fresh meat during cold storage. A few preliminary results concerning the metabolism of Microbacterium thermosphactum are now available. Colonies of Microbacterium thermosphactum from both, irradiated and non-irradiated minced meat react in the same manner to different biochemical tests.

Microbacterium thermosphactum survives and multiplies in a salt solution, irradiated with 30 krad; first a reduction in the number of bacteria in the order of magnitude of 1 log cycle was observed within 24 hrs, after this initial reduction the micro-organisms returned to its normal rate of growth, as found in a non-irradiated solution. When the inoculated solution is again irradiated with 30 krad, we observe not only a further reduction of the number of bacteria but also much slower recovery of the initial growth rate. It is remarkable that low doses of this order of magnitude create such stress conditions on a micro-organism that is known for its radiation resistance. Further research is in progress.

PROJECTVERSLAG

Onderzoekinstelling	:	ASSOCIATION EURATOM - ITAL
Projectnummer	:	54
Projecttitel	:	Uptake and release of ions by subcellular structures, mainly chloroplasts and mitochondria.

Registratie nr.: AA-009 / 54

Onderzoeker(s)	:	A. de Ruyter, A. Ringoet, G. Sauer.
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld
- Directe kosten-personeel f
- „ „ -materieel f
- Semi-directe kosten f
- Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
- Hoger personeel — mandagen
- Middelbaar personeel — mandagen
- Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71.

Ca uptake by isolated chloroplasts.

Methodology and Materials

Spinach (Spinacia oleracca L., cv. Noorman) plants, used in these experiments were initially bought at the local supermarket.
Oat (Avena sativa L., cv. "Marne") and spinach plants later on were grown under controlled (climate and nutrient medium) conditions.
Aqueous (Nobel 1969) but also so-called "dry" chloroplast isolation methods have been applied to spinach- and oat leaves. In the former method, on the average, 60% of the isolated chloroplasts are of the Class I type (inner and outer membrane probably intact). Separation of the chloroplasts from the isolation or incubation medium can be done either by centrifugation (once or repeatedly) or by filtration. Normal centrifugation (1000 x g) is used in experiments which are not time-bound. In all other cases, e.g. time related Ca-uptake, an improved filtration technique is applied. Millipore filters (0.8 µm pore size and 13 mm diameter), pretreated with a 1% $LaCl_3$ solution to avoid Ca-adsorption, on filter holders are connected to a plexi-glass box with a vial in which the filtrates (isolation or incubation media) are collected under vacuum.
The chlorofyll content of the isolated chloroplasts is determined by the well-known Arnon method (1952). Starting from 60 g fresh weight samples of the mesophyll part of spinach leaves, the average total chlorophyll content of isolated chloroplast suspensions amount to 3900 $\pm$ 257 µg.
The chlorophyll content of the spinach-chloroplast suspensions of increasing concentration in relation to the spectrophotometric extinctions proves the reproducibility of the chloroplast isolation technique. The chlorophyll a/chlorophyll b ratio, as measured in a certain number of isolated spinach chloroplast samples, is very constant: 2.06 $\pm$ 0.04.
Ca- and Mg-content of the suspensions, the isolated chloroplasts and the leaves is measured by Atomic Absorption Spectrophotometry (A.A.S.).
Preliminary potassium and sodium data are obtained by a flame-photometric method (Van Schouwenburg 1970). Almost all uptake experiments reported here for spinach chloroplasts are done in a 0.3 M saccharose incubation medium buffered at pH 7.9 by Tris.HCl. In recent experiments this buffer has been replaced by the MOPS buffer, which has a better buffer capacity below pH 7.5 (pH of the cell sap of the spinach

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leaves: 6.2) and which probably interferes less with the calcium binding process. The same medium is generally used for isolation of the chloroplasts. In all uptake experiments a ^{45}Ca label of 0,2 µCi ^{45}Ca/ml is applied. The experimental set-up has been improved by the use of a dark room and a better illumination device to avoid the variable day light and temperature interference with the calcium uptake process.

Results

1. Chlorophyll, dry matter and cation content of oat and spinach leaves.

Table 1 - Dry matter (% fresh weight), chlorophyll (µg/g fresh leaf) and cation content (mg/g dry leaf) of oat and spinach leaves.

	Chlorophyll	Dry matter	Ca	Mg	K	Na
Oats	1924	13.2	2.7-4.0	5.3	19.0	20.0
Spinach	1160	7.4	8.9-12.5	11.0	29.0	43.0

These preliminary results confirm some well known facts e.g. that the cation content is generally higher in dicotyledons (spinach) than in monocotyledons (oats). The Mg- content is not directly related to chlorophyll a and/or b content; Mg in oats is lower than in spinach, while the chlorophyll-content of oat leaves is higher than in spinach leaves. The total calcium content of the leaves shows much less variability than the Ca-content of the chloroplasts (see below).

2. Chlorophyll content of isolated chloroplast suspensions.

Chloroplast isolation being performed according to the same technique, starting from the same amount of leaf material, the chlorophyll contents of the chloroplast suspension are, respectively for spinach and oats, 355 and 240 µg/ml.
Variation of these data due to the age of the plants is negligible.
Correlating the number of chloroplasts (microscopic observation) with the chlorophyll content of the isolated chloroplast suspensions, it appeared that the chlorophyll content of oat-chloroplasts is higher than that of spinach chloroplasts. (See Table 1).

3. Cation content of isolated chloroplast suspensions.

Table 2 gives average values of the cation content of oat and spinach chloroplast suspensions (aqueous technique), as well as the relation of these data to the cation content of the whole leaf (see table 1).
The growth conditions of the plant material are also indicated.

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Registratie nr.:

Table 2 - Cation-content of oat- and spinach-chloroplast suspensions and its relation to the cation content of the whole leaf.

Cation	Growth conditions	µg Cation per mg chlorophyll	µg cation in chlorophyll of 1 mg dry leaf	cations in chloroplasts in % of cations in leaf
OATS				
Ca	growth chamber (see before)	39	0,51	12,7
Ca*		43	0,84	30,5
Mg		47	0,76	14,4
K		2	0,03	0,2
Na		203	3,29	16,4
SPINACH				
Ca	market	190	2,79	31,3
Ca*	market	190	2,03	16,8
Ca	growth chamber	38	0,60	4,8
Mg	market	64	0,81	7,4
K	market	150	1,91	6,5
Na	market	230	2,92	6,8

* average value at a different period.

Although the Mg, K and Na data are preliminary ones, some information about these cations can be drawn from table 2. Magnesium per mg chlorophyll is somewhat (25%) higher in the spinach chloroplast suspensions; the K-content of the oatchloroplast suspensions is negligible as compared to its concentration in the spinach suspension. The Na-content of both suspensions is highly chlorophyll-bound. Mg per mg dry leaf is very similar in both species and the higher Mg-fraction (%) in the oat chloroplasts is related to the lower Mg-content of this leaf (table 1).
Oat chloroplasts bind relatively much less K than Na; this is, however, not true in spinach chloroplasts. In view of what follows about the calcium data we insist once more on the preliminary nature of this information concerning Mg, K and Na.

4. Calcium content of isolated chloroplasts or their suspensions.

More data are available concerning the Ca-content of chloroplasts and their suspensions. The data concerning the Ca-content of chloroplast suspensions, show large variability (34-55 µg Ca/mg chlorophyll in oats; 19-619 µg Ca/mg chlorophyll in spinach). As shown before, neither variations in the chlorophyll content of the suspensions nor variations in the Ca-content of the leaves can be responsible for this variability. They may partly be explained by the origin of the spinach material: market spinach up to 619 µg Ca/mg chlorophyll, growth chamber spinach 19-199 µg Ca/mg chlorophyll. Variable contamination of the chloroplast suspension by Ca-oxalate crystals from the spinach cell-vacuole during isolation may be responsible to a certain extent (estimated at 6% of the measured Ca-content)
The variable Ca-content observed in the chloroplast suspensions has been the object of a great number of experiments, varying different factors:

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a. Neither the age of the plants, at least not within the growth stage selected for the experiments, nor the pH variation of the nutrient solution (4,8 - 6,3) and its calcium concentration (down to 1/10 of the normal concentration) did considerably affect the Ca-content of the isolated chloroplast suspensions.

b. NaCl, (0,175 M) versus saccharose (0,3 M) isolation medium either with or without added known amounts of $CaCl_2$. As already mentioned last year, the presence of Na in the isolation medium indeed reduced the Ca-content of the chloroplast suspension. When calcium is added to the medium the suspension itself shows - after substraction of this known amount - a higher Ca-content (see e. below).

c. The pH of the isolation medium:
The Ca-content of spinach chloroplast suspensions decreases at higher pH values of the isolation medium. The direct transfer of the chloroplasts from a medium at pH 6.2 (spinach leaf) to the isolation medium at pH 7.9 certainly reduces their Ca-content.

d. Temperature of the isolation medium.
Non clean-cut conclusions can be drawn from the experiments at different temperatures of the isolation medium. (0, 20, 30 °C).

e. Centrifugation and filtration of chloroplast suspensions.
Besides data concerning the Ca-content of the whole isolated suspension, by filtering on millipore or centrifugation information has also been obtained concerning calcium concentrations in chloroplasts and suspension liquid separately. On the average 70% of the total Ca in the suspension was found in the chloroplasts and 30% in the suspension liquid.
Therefore the number of ml to wash the chloroplasts after filtration, for removing the rest of the incubation or isolation medium, that might affect the measurement of the Ca-content, has been determined. One and a half ml is sufficient.
Repeated centrifugation of the suspension with renewal of the isolation medium leads to a progressive calcium depletion of mainly the chloroplasts but also of the suspension liquid. Desintegration microscopic observations of the chloroplasts) after the third centrifugation results in a considerable increase of the calcium depletion. Chloroplasts treated in this way only show an initial rapid uptake (adsorption?) not followed by any further (metabolic?) uptake (see below).

These results suggest that the variability of the calcium content of the isolated chloroplasts (suspensions) is due to the interaction of a certain number of factors. Reduction of the variability can be obtained by strict control of the growth and isolation techniques. Furthermore these experiments brought the important initial release of calcium from the chloroplasts to our attention. Both aspects, variability of the Ca-content and the initial release, have generally been neglected in all ion-uptake studies using chloroplasts.

5. Calcium uptake by isolated chloroplasts

a. Chlorophyll content of the suspensions and Ca-uptake.
The uptake being expressed with respect to the chlorophyll content of the suspension, it is important to know if the relation between Ca-uptake at a certain concentration of the incubation medium and increasing chlorophyll-contents of the suspension is a linear one. This appeared not to be the case, probably due to the interference light-amount of chloroplasts with the uptake process. For this reason uptake experiments will have to be done with suspensions of very similar chlorophyll concentration.

b. Ca-uptake at different Ca-concentrations in the incubation medium.
Most experiments were performed at 20°C, over periods from 15 sec up to 30 min in some cases. At each concentration, the uptake is characterized by a

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Projectnummer : 54	

rapid initial process (generally within 15 sec, see fig. 3) followed by a slower one, which reaches a maximum after 20 - 30 min. It is accepted that the first process is due to adsorption, the second one perhaps being of metabolic origin (see below). Assuming that the adsorption occurs within an infinite short time, its correct value can be determined by extrapolation of the first part of the metabolic uptake curve to the Y-axis. Figure 1 reproduces adsorption data (mg per mg chlorophyll) obtained in this way in relation to the initial Ca-concentration (log scale) of the incubation medium (0.3 M Saccharose, pH 7,9). Besides a small irregularity in the curve between 10^{-5} and 10^{-4} M Ca, adsorption increases with concentration up to 2.10^{-3} M, where it reaches a maximum.
From the first part of the metabolic uptake curves, starting at 15 sec, uptake rates at different Ca-concentrations have also been calculated. They are represented in fig. 2, with at the left the uptake rates (in % of the maximum rate observed) at low concentrations (log scale 10^{-6} - 10^{-4} M Ca) and at the right the rates at higher concentrations (10^{-4} - 10^{-2} M Ca).
The most characteristic result is the considerable increase of the uptake rate starting at approximately 3.10^{-4} M Ca. Again a small irregularity in the curve is found between 10^{-5} and 10^{-4} M Ca (see fig. 1). Another interesting fact is the drop in the uptake rate curve at concentrations higher than 10^{-3} M Ca. At this stage a physiological interpretation of these first data is difficult.

c. Influence of the Ca-concentration of the medium, and its temperature on Ca-uptake.
To learn more concerning the nature of the second process in Ca-uptake by isolated chloroplasts this uptake was studied at 20 and 0 °C and at 20 °C using heated (70°C) chloroplasts. Two different calcium concentrations in the external solution were considered: 10^{-6} and 10^{-3} M Ca. One can suppose that at 0 °C in normal chloroplasts the metabolic uptake is suppressed; the same assumption can be made for heated chloroplasts.
At 10^{-6} M Ca in the incubation medium and at 0 °C with normal chloroplasts or at 20 °C with heated chloroplasts there is indeed hardly any further uptake after the initial rapid adsorption (figure 3).
When the concentration in the external solution is 10^{-3} M Ca (fig. 4) the uptake at 0 °C by normal chloroplasts and at 20 °C by heated chloroplasts is even higher than the uptake by normal chloroplasts at 20 °C.

This preliminary information indeed shows that, after the initial adsorption, a metabolic process is probably involved in the uptake of calcium by isolated chloroplasts. However, according to figure 4, this metabolic process, which occurs in the lower range of external Ca-concentrations, is completely masked at higher concentrations by a third process, that again, as the initial adsorption, is more of physical nature. This latter process is responsible for the important increase of the Ca-uptake rate at higher concentrations (see figure 2). New experiments with an improved experimental set-up, which will permit a better estimation of the adsorption and continuous control of the Ca-concentration during the uptake process, are now in progress.

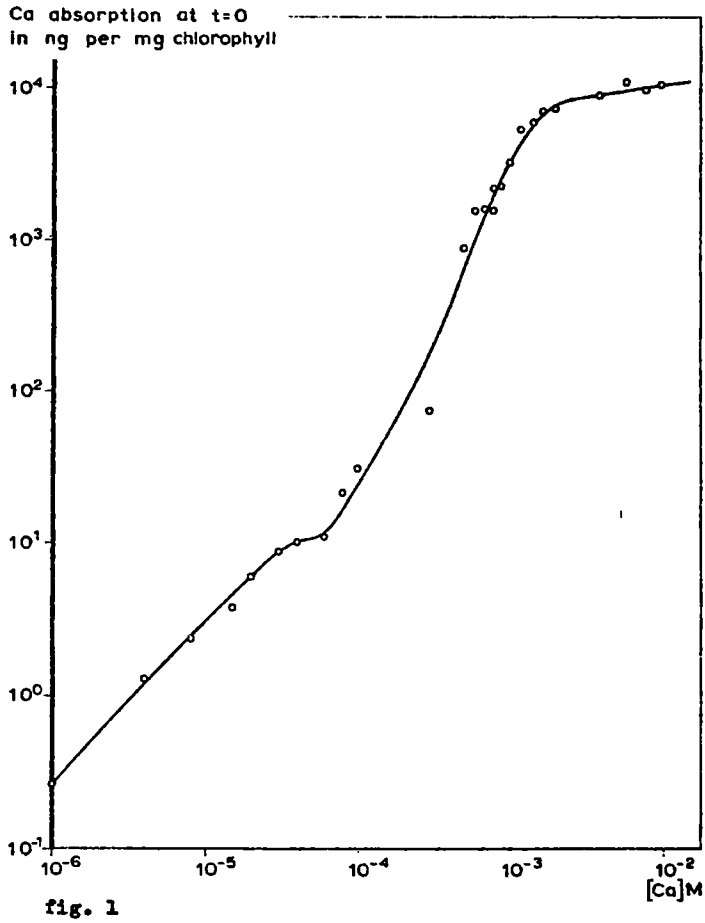

fig. 1

Calcium adsorption (ng/mg chlorophyll) by isolated spinach chloroplasts in relation to the Ca-concentration of the incubation medium.

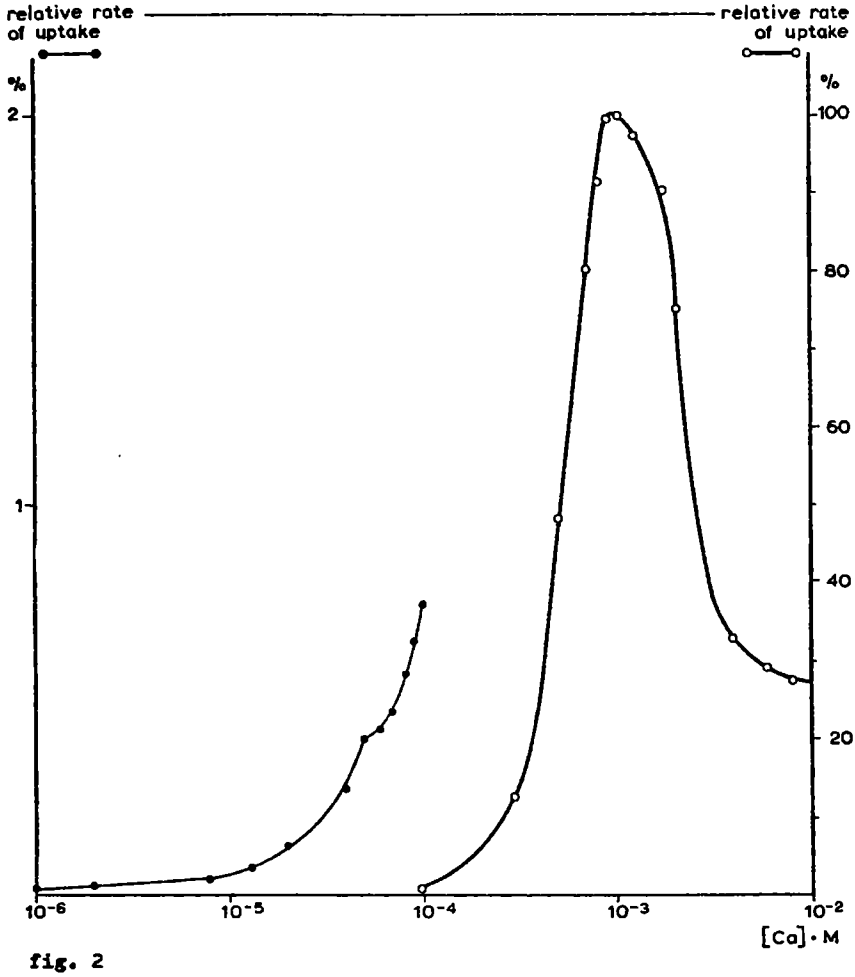

fig. 2

Rate of calcium uptake (in % of maximum rate of uptake) by isolated spinach chloroplasts in relation to the Ca-concentration of the incubation medium.

Different scale for low and high concentrations.

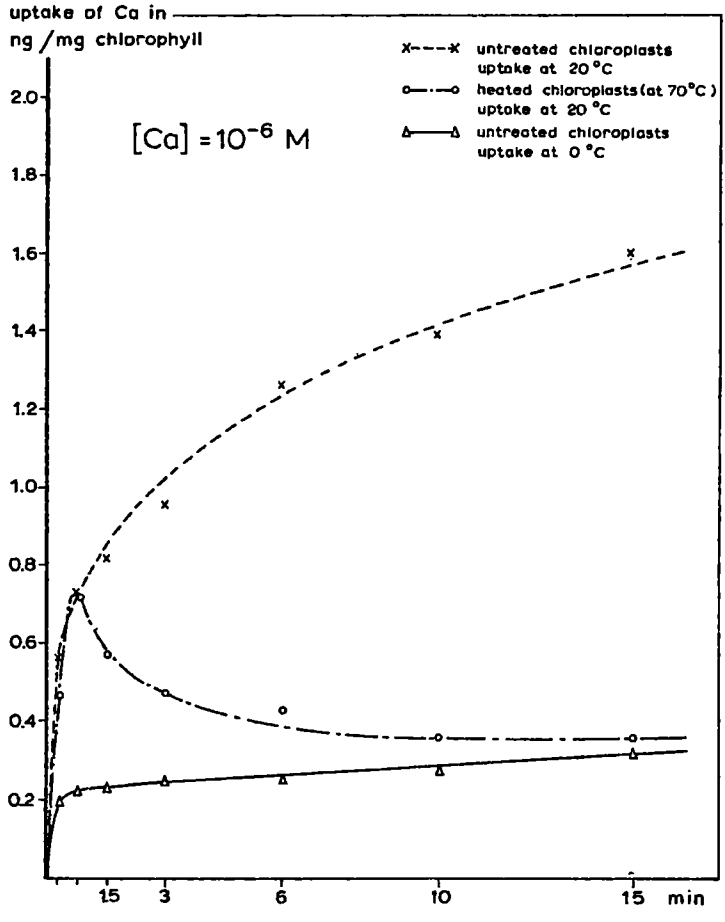

fig. 3

Total calcium uptake (ng/mg chlorophyll) by isolated untreated (0°C, 20°C) or heated (20°C) chloroplasts in relation to time and at 10^{-6}M Ca-concentration of the incubation medium.

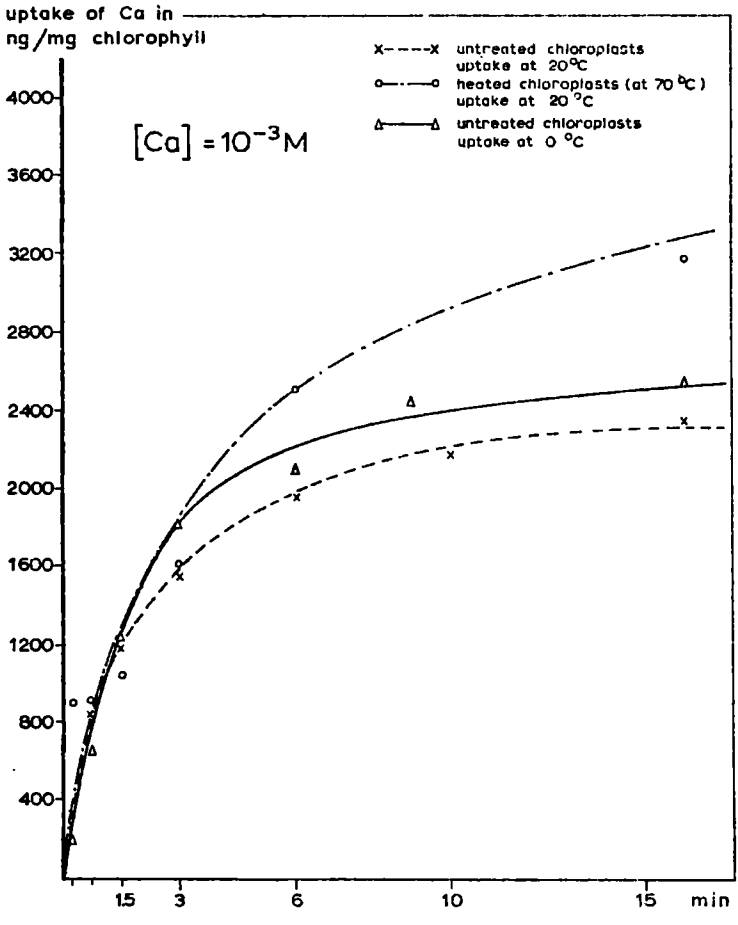

fig. 4

Total calcium uptake (ng/mg chlorophyll) by isolated untreated (0°C, 20°C) or heated (20°C) chloroplasts in relation to time and at 10^{-3}M Ca-concentration of the incubation medium.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 57
Projectnummer	: 57	
Projecttitel	: Genetical control of Adoxophyes orana Fr.	
Onderzoeker(s)	: D. Snieder	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	–	mandagen
" " -materieel	f	Middelbaar personeel	–	mandagen
Semi-directe kosten	f	Lager personeel	–	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

Genetical and radiobiological research on the summerfruit tortrix moth Adoxophyes orana FR.

Using preliminary information obtained by Dr. Ankersmit (Department of Entomology-Agricultural University, Wageningen) irradiations were carried out with X-rays (1,5 meV) and fast neutrons (1,1 x 10^8 n/sec/cm^2) to induce translocations in imagines of Adoxophyes orana Fr. (0-24 hrs old, not mated). The percentage egg-hatch has been tentatively used for isolation of translocation-strains. The percentage nitrogen (approximately 3,6%) of the moths was expected to be too low to discriminate, after irradiation with thermal neutrons, between the effect of the thermal neutrons (^{14}N-targets) and the effect of the contaminating gamma-rays in the BARN-reactor.

Almost 500 F1-descendants of the cross of an irradiated parent with an untreated parent were sampled and, as far as mating activities permitted, tested in single pair crosses for partial sterility. The obtained information lead to the following conclusions:
- the percentage egg-hatch of selected moths and their descendants was very variable. The possibility therefore exists that, either no translocations, but just fragrents and deletions are induced, or the percentage egg-hatch is no reliable criterion for isolation of translocation-strains;
- cytological research on L5-larvae, selected according to percentage egg-hatch, gave very few positive results. In one line, in the F3-generation, a chromosomal mutation (=translocation?) was discovered, but we did not succeed in maintaining this line. To the contrary, in the F1-generation of irradiated parents, chromosomal aberrations are very frequently found. Therefore more should be known concerning the heredity of these chromosomal aberrations.

Besides further research on this aspect of the programme, new experiments concerning dose-rates and dose-response curves for different radiation sources are in progress.

PROJECTVERSLAG

Onderzoekinstelling	:	ASSOCIATION EURATOM - ITAL
Projectnummer	:	58
Projecttitel	:	Genetical and radiobiological studies on the two-spotted spider-mite (Tetranychus urticae C.L. Koch)

Registratie nr.: AA-009 / 58

Onderzoeker(s)	:	A.M. Feldmann
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
„ „ -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	—	mandagen
Middelbaar personeel	—	mandagen
Lager personeel	—	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971 .

The project was started in May 1971. Since then breeding-facilities for the spider-mite and its host-plant (Phaseolus sp.) have been built and are now available.
A literature search on genetical insect-control and the possibilities of using translocations for this purpose was started in collaboration with the colleagues of the Insectgroup at the Institute.
A working-scheme, according to the programme of this project, has been prepared and the first experiments were started.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 59
Projecttitel : Transport of soil moisture.

Registratie nr.: AA-009 / 59 b

Onderzoeker(s) : M.J. Frissel, P. Poelstra, G.H. Bolt, L. Stroosnijder, F.van Dorn
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
- Directe kosten-personeel f
- " " -materieel f
- Semi-directe kosten f
- Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
- Hoger personeel — mandagen
- Middelbaar personeel — mandagen
- Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

It is expected that the soil moisture-column scanner will be operational in February 1972. Further no relevant news.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 61
Projecttitel : Ionic composition of the soil solution as a function of time and place

Registratie nr.: AA-009 / 61

Onderzoeker(s) : F. van Dorp, M.J. Frissel, P. Poelstra, J. Sinnaeve
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld.
Directe kosten-personeel f
„ „ -materieel f
Semi-directe kosten f
Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971 .

Literature study in progress.

Calcium concentration of the soil solution - P. Reiniger.

Ispra

Sampling of the soil solution in the Ispra lysimeters was interrupted from August 1970 until spring 1971 due to the departure of U. Marckwordt from Ispra and due to the winter weather. This interruption has made necessary a prolongation of the study and sampling was re-started the beginning of May 1971.
The heavy spring rainfall of more than 500 mm in May and June favoured the extraction of the soil solution and the collection of the effluent.
Preliminary results for three soils and three sampling dates are presented in table 1. (The soluble calcium has been calculated from conductivity data with the aid of a regression equation established for each soil).

Table 1 - Pasture soil lysimeters Ispra. Average soluble calcium concentration in the soil solution at three depths.

Depth cm	0 - 10			10 - 20			20 - 30		
Date	5.5	18.5	16.6	5.5	18.5	16.6	5.5	18.5	16.6
Soil	SOLUBLE CALCIUM, meq/L								
FEN	<1	<1	<1	1	2	1	5	5	5
RENDZINA	5	5	5	6	6	7	8	9	8
CLAY	6	5	5	6	7	6	8	9	8

Compared with the data from summer 1970 (Annual Report 1970) a decrease in the salt concentration in Fen and in the deeper layers of the clay soil may be noted. This decrease can be ascribed to the heavy spring rains. On the contrary, in the rendzina soil the calcium concentration stayed virtually constant due to the dissolution of solid calcium carbonate.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 61	Registratie nr.:

Measurements of the pH of some of the soil solutions, carried out the beginning of May, showed the following values:

Podzol 4.2, Fen 3.4 - 4.3, Loess 5.7 - 6.5, Rendzina 7.6 - 7.8.

No radioactivity could be detected in the effluent from the lysimeters.

Amiens

This spring, only one additional sampling of the soil solution could be carried out in one of the field installations at Amiens. Due to these scarce results this part of the project was abandoned.
The failure of the installations may partly be sought in the relatively long distance separating them from the laboratory, partly in the high suction values of the soils and the low rainfall, though the same soils render sufficient moisture for analysis under the conditions of Ispra.

No further relevant news.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 62
Projecttitel : Transport of insecticides, pesticides, fungicides in soils

Registratie nr.: AA-009 / 62

Onderzoeker(s) : J.P. Rolland, M.J. Frissel, P. Poelstra
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
- Directe kosten-personeel f
- „ „ -materieel f
- Semi-directe kosten f
- Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
- Hoger personeel — mandagen
- Middelbaar personeel — mandagen
- Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71.

No relevant news, no experiments have been carried out.

Literature orientation is in progress.

PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : XX 63
Projecttitel : Nitrogen fixation and nitrogen transformations in soil

Registratie nr.: AA-009 / 63

Onderzoeker(s) : J.H. Becking, J.P. Leyser* W.J. Gerritsen**
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
" " -materieel f
Semi-directe kosten f
Omslag algemene kosten f

Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

A study was made on the efficiency of urea dressing in rice soils, because urea is in practice the main nitrogen source for rice culture. Tropical, short-day rice varieties were used (Oryza sativa L., var. Temerin) in pot experiments with tropical (Surinam) soil. Light, temperature, sowing, dressing and water drainage conditions were as far as possible the actual conditions in the field. The results obtained showed that there was no significant difference between root plus shoot production in the pots with or without urea dressing. The high nitrogen content of the organic Surinam soil used was responsible for this effect. However, if urea was given, this nitrogen source was taken up in preference above another soil-nitrogen source. Inspite of about the same growth of the rice plants fertilized and not fertilized with urea, the plants with urea dressing showed a higher N-content per dry matter. Urea labelled with N^{15} was used and the N^{15} analyses showed that about 31.0-31.8% of the nitrogen applied as dressing was recovered in the shoot. Rice plants with the regime of water drainage during dressing showed a somewhat lower nitrogen recovery in the shoot. Although these differences were small, they were statistically significant. The lower urea efficiency under drainage conditions is probably due to an oxidation of urea-ammonium to nitrite and nitrate (nitrification) and subsequent volatilization (denitrification) under anaerobic conditions.

In connection with the results mentioned above, it was tried to estimate the N-losses to the atmosphere after N^{15}-urea dressing of tropical rice soils. Models, having a closed system, were used, and N^{15}-labelled urea and KNO_3 were applied to the soil. Volatile nitrogen products were screened by katharometric gas chromatography (especially N-oxides) and ammonia by absorption to boric acid. When the qualitative and quantitative composition of the atmosphere was known, the atmosphere was analysed for N^{15} enrichment by means of mass spectrometry. The analytical problems and procedures applicable to such a system were completely solved. However, there remained practical problems with regard to the growth of rice plants in a closed system having insufficient O_2 and CO_2 control (the rice plants showed lodging after a period of time). Moreover, it was impossible to connect the normal valves of such a system directly to the mass spectrometer, because these valves showed leakage when exposed to high vacuum. The application of special plastic valves will overcome this problem. In addition, a closed system for plant growth with O_2 and CO_2 regulation must be designed.

Onderzoekinstelling	: Association Euratom-ITAL	Registratie nr.:
Projectnummer	: 33	

Some preliminary experiments with Nude (Wageningen, the Netherlands) clay and the Surinam (Wageningen, Surinam) organic soil to which N^{15}-labelled KNO_3 or urea were added,were conducted. With Nude clay 16% of the potassium nitrate added was denitrified to N_2 and N_2O (other volatile compounds were not detected) in 10 days, whereas with urea application in 10 days only 3% of the urea-N was recovered in N_2 and no N_2O could be detected. With urea the Surinam soil showed no denitrification in 15 days, but in 20 days 4% of the urea-N added was recovered in the atmosphere as N_2. The ammonia determinations showed, in the alkaline Nude clay, a volatilization to ammonia of 12% of the applied ureum, but in the more acid Surinam soil no production of ammonia in the atmosphere could be detected.

With financial support of the W.O.T.R.O. (Wetenschappelijk Onderzoek Tropen, The Hague) a 2-month research was performed about the N-cycle in rice soils at Bogor, Java, Indonesia. The objective was to measure the N-increment (N_2-fixation) due to microbial processes in rice soils under natural conditions. Nitrogen fixation experiments were conducted with the irrigation water, surface soil and subsoil of ricefields and with the rhizosphere of rice plants. The survey moreover covered the whole hydrobiological system including Azolla. So far the detailed results are not yet available, because all samples have not yet been analyzed. The few results available indicate that the normal N_2-fixation in the heavily urea-dressed rice soils tested (no other soils were available) was rather low, but that Azolla when present, could contribute substantially to the N-increase of these soils.

* Student Agricultural University, Wageningen
Study Agricultural Science.

** Student chemistry, University of Utrecht.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 64
Projectnummer	: 64	
Projecttitel	: Genetical, Radiobiological and Biochemical research on the gamethophytic monofactorial system of self-incompatibility	
Onderzoeker(s)	: D. de Nettancourt, A.J.G. van Gastel, G. Bredemeijer and	
Projectleider	: Carluccio.	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	—	mandagen
„ „ -materieel	f	Middelbaar personeel	—	mandagen
Semi-directe kosten	f	Lager personeel	—	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

Analysis of the factors and mechanisms governing the generation of new alleles at the S-locus of Lycopersicon peruvianum Mill. (De Nettancourt, Van Gastel).

Testing of the hypothesis that new S-alleles are generated by equal crossing-over.

Pandey (Genetica, 1970; Nature, 1970) has expressed the opinion that intragenic crossing-over within the S locus may have generated the new allele (S_3) which we observed in our inbreeding experiments (De Nettancourt et al, 1969, 1971). In order to test Pandey's hypothesis, reversion tests were made to find out if the new allele (S_3), when in presence with one of the two original alleles, could generate the second oroginal allele. Screening for reversions was performed in both back-crossed and selfed progenies. At least six clear cases of reversion were observed in some back-cross populations whereas only one dubious reversion was detected after selfing S_1S_3 and S_2S_3 individuals. Not all results are yet available but it appears that:
- equal crossing-over is not involved in the generation of a new S-allele;
- the genetic factors which govern the generation of a new S-allele have sporophytic expression, are recessive and present in heterozygous condition in the mother clone.
- a number of these genetic factors are located on the S-bearing chromosome (new alleles are essentially generated in S-homozygotes) but other loci are involved which are situated elsewhere in the chromosome complement (only a fraction of S-homozygotes generate a new allele).

Testing of the hypothesis that the apparition of a new S-allele results from activation-inactivation switching processes on tandem segments.

The crosses and diallelic tests, necessary for testing Edström theory (Nature, 1963) that constructive mutations (in this case, changes in S-specificity) are due to the reactivation of allelic copies previously stored during outbreeding, are underway in both Wageningen and Roma.
The ancestry tests will all be carried out on S_1 and S_2 alleles. Ancestor-alleles are S_4 and S_5 in Wageningen and S_6, S_7 at the Casaccia.

Onderzoekinstelling : Association Euratom -ITAL Projectnummer : 35	Registratie nr.:

Nature of the relationship between spontaneous selfing and the generation of a new S-allele.

Plant I_2-53 is an inbred individual which is classified as S_1S_2 and regularly self-incompatible. On one occasion, however, the plant set a very large number of seeds after selfing which gave rise to a progeny segregating for three specificities (S_1S_2 and, the new specificity S_3). The problem was to find out if the generation of the new allele S_3 was the consequence of spontaneous inbreeding (S_3 would then result from the formation of a new genetic background in the I_3 generation) or its cause (S_3 is formed in the I_2 generation and is regularly transmitted, during the whole period covered by the formation process, to the I_3 generation).
For this purpose plant I_2 - 53 was subjected to inbreeding techniques which resulted into the production of a limited number of seeds after selfing. The results obtained to date are summarized in table I and indicate beyond doubt that the formation of S_3 is the cause and not the consequence of spontaneous inbreeding.

Table I: Segregation for S-alleles in the progeny of plant I_2-53 (S_1S_2) obtained after spontaneous selfing (A) and after obligate selfing (B).

Genetic segregation observed			
A) Spontaneous selfing		B) Obligate selfing	
No. plants	Genotypes	No. plants	Genotypes
25	S_1S_3	13	S_1S_2
8	S_2S_3	4	S_2S_2

Self-compatibility in Lycopersicon peruvianum Mill.

After obligate selfing in the progenies of S_1S_2 plants in the second generation of inbreeding, a self-compatible individual has been detected which is the mirror image of self-incompatibility: it rejects all pollen except its own!
The progeny of this plant segregates in approximatively 50% self-compatible individuals (which again, only accept self-pollen) and 50% self-incompatible offsprings. The phenomenum repeats itself at each generation of selfing.
The self-compatibility character detected is not only valuable from a practical point of view (possibility to apply the conventional selection method of self-pollinated crops to a cross-pollinated species) but provides an interesting material for analysing the processes which control function and expressivity at the S-locus.

Genetic mapping of the S-locus and identification of the S-bearing chromosome in Nicotiana alata, Link and Otto. (Van Gastel, De Nettancourt, Carluccio).

Two approaches have been made in 1971 for establishing the chromosomal localization of the S-locus and its eventual position within a linkage group.

Genetical approach

Using the adventitious method of propagation by isolated leaves, a number of morphological mutants have been obtained, after irradiation of excised leaves, in the same genetical background as the original S_2S_3 clone (De Nettancourt, Dijkhuis, Van Gastel and Broertjes, 1971). The fact that mutant individuals were also detected in the control series clearly shows that the leaf-propagation technique is, in itself, mutagenic.
All mutant plants have been submitted to progeny-testing and identity-crosses with S-homozygotes for ascertaining the hereditary nature of each induced mutation and an eventual linkage to the S-locus.

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In addition, 2 pollen-part and 2 stylar-part self-compatible mutants have been detected in the material screened so far. To our knowledge, this is the first time that self-compatibility mutations are induced and detected, in the complete absence of inbreeding effects, in the precise genetic background of the original material. In other words, mutants are now available which permit an accurate comparison of the genetical and biochemical properties of S.I. and S.C. (pollen part, style part) alleles in similar genetic backgrounds.

Cytological approach

Two interesting findings have been made so far. One concerns the discovery of an aberrant chromosome in a stylar-part mutant obtained from rooted-leaves. The aberration, which is not visible in all somatic metaphases, appears to involve the insertion of a translocation segment in one of the median chromosomes. All progenies of this self-compatible mutant examined to date (3) display an aberration which in one case resembles that of the mother-plant and, in the two others, appears as a tertiary constriction. Karyogrammes of control and aberrant plants are being made which will enable an exact classification of the cytological anomalies observed. The second finding is the detection, in the progeny of a self-compatible tetraploid individual, of a plant which is not self-compatible and displays 34 chromosomes instead of 36. The two missing chromosomes do not appear to be homologous. It is tempting to conclude that the plant should have been Sa Sa Sa Sb and that heterogenic pollen (self-compatible Sa Sb pollen) is not formed because Sb is located on one of the two missing chromosomes.

Mutation spectra analysis at the S-locus of Nicotiana alata Link and Otto. (Van Gastel, De Nettancourt).

The aim of the experiments is the production of self-compatible mutants by different radiation treatments. Analyses of the mutation spectra (type of mutation and ratio of fragment to non-fragment mutation) may produce information concerning the dose and radiation treatment inducing mutations like in nature. (spontaneous mutations for self-compatibility do not display a centric fragment).

After pollen mother cell (P.M.C.) irradiation with different dosages of X-rays several mutant seeds have been obtained. Dosages used are 300, 600 and 825 rad. The mutants obtained in this experiment will be test-crossed to find out the type of self-compatible mutation. All self-compatible mutants will be cytologically analysed for the presence or absence of a centric fragment.

Mutants obtained from preliminary crosses have already been test-crossed for that purpose. In one case a pollen-part mutation was induced whereas in two other cases a revertible mutation (probably pollen part) was obtained. Cytological analyses revealed that at least in one case a centric fragment was present.

In a chronic γ-irradiation experiment on self-incompatible plants rather great amounts of seeds (after selfing) have been obtained. The largest amount of selfed seed was found after irradiation at a dose-rate of 2.75 rad/h. The progenies will be tested to find out whether the seed set is due to a mutation at the S-locus or to a physiological effect. If the effect is indeed a genetic one, the results of this experiment shows that chronic irradiation is more effective for inducing self-compatibility than acute irradiation.

Induction of self-compatibility in dihaploid Solanum tuberosum L. (Van Gastel, De Nettancourt).

In an experiment using chronic γ-irradiation several mutant seeds have been obtained after selfing. These mutants will be tested for self-compatibility.

The effects of P.M.C.-irradiation with acute X-rays on the pollen sterility have been studied. The induction of selfcompatible mutants will be initiated next spring.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 35	Registratie nr.:

According to recent information the induction of self-compatibility in a dihaploid Solanum tuberosum is of limited interest to agriculture. Continuation of this project will therefore be reconsidered.

Biochemical aspects of self-incompatibility in Lycopersicon peruvianum Mill.and Nicotiana alata Link and Otto. (G. Bredemeijer).

Identification of S-genotypes in leaves of Lycopersicon peruvianum and Nicotiana alata.

Several investigators found a relation between the S-genotype (specificity for self-incompatibility) of a style and its protein composition (peroxidase, glutamate dehydrogenase and basic proteins).
To our knowledge, nothing has been published concerning a possible similar relation with the protein composition of vegetative plant parts (e.g. leaves). Such information, however, might be of considerable interest in detecting different S-genotypes at an early vegetative growth stage.
As revealed by starch gel electrophoresis the Lycopersicon peruvianum leaves contained 6 to 8 peroxidase isoenzymes and the Nicotiana alata leaves 8 to 10 peroxidase isoenzymes. In the case of Lycopersicon peruvianum leaves the various S-genotypes compared do show differences in the peroxidase isoenzyme patterns. The results showed that each S allele had its own specific isoenzymatic bands in homozygous plants. However, in plants which were heterozygous a number of the specific bands were absent. There are indications that the age of the leaves used also plays a role.

Studies on Nicotiana alata pollen germination in vitro.

In order to study the mechanism of the self-incompatibility reaction and its possible relation with peroxidase, pollen germination in vitro was examined. Preliminary experiments showed that the highest germination percentages were obtained by using fresh pollen in a medium with 10% sucrose and 0.01% boric acid. After four hours of incubation the peroxidase activity increased by approximately 25 per cent.

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 65
Projectnummer	: XII 65	
Projecttitel	: The use of ionizing radiation in food technology Collaboration with the Pilot Plant	
Onderzoeker(s)	: D.Is. Langerak	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	–	mandagen
" " -materieel	f	Middelbaar personeel	–	mandagen
Semi-directe kosten	f	Lager personeel	–	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

In 1971 the experiments with prepacked endive, asparagus and peeled potatoes have been continued.
Special attention has been paid to packaging, quality aspects and microbiological spoilage under commercial circumstances.
The experiments with asparagus have been carried out in cooperation with the Research Station for Outdoor Vegetables, Alkmaar; the Sprenger Institute, Wageningen; the Governmental Advisory Service for Horticulture, Roermond; the Pilot Plant foor Food Irradiation and the Auction Grubbenvorst, Venlo.

Prepacked endive:
The quality of this prepacked product is strongly affected by discoloration, desiccation and microbiological decay during storage, transport and marketing.
The discoloration is probably caused by:
(a) an oxidation of the phenole substances available in the cellsap, catalyzed by such enzymes as polyphenolase or tyrosinase;
(b) non-enzymatic discoloration through direct oxidation or polymerization of polyphenols. Free radicals or O_3 produced during irradiation, may affect this reaction.

Since cooling is generally insufficient during transport and sale the application of ionizing rays is studied to lengthen the keeping quality of prepacked endive. A number of experiments have been carried out to study the preservation of this product: the initial quality, the processing, the packaging, the irradiation dose and storage temperature. The changes in the product during storage were estimated by quality, organoleptic and microbiological tests. The following results were obtained:
- Careful processing extends the keeping quality. Addition of a mixture of citric-acid and ascorbic-acid, either with or without irradiation, increases the discoloration. This increase is probably due to the reaction: ascorbic-acid → dehydro ascorbic-acid → furfural.
- After an irradiation of 100 krad the colour-, organoleptic- and trade-value scores of the samples packaged in unperforated bags were higher than those of samples in perforated bags. This result suggests a synergetic effect of irradiation with low doses and the modified gas composition in the bag on the quality of the product. A possible explanation is the slowing down of the

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oxidation reactions in unperforated bags.
- The initial microbial count was within the range of 10^5 to 10^7 counts/g; for enteriobacteriaceae it was about 10^3 counts/g. Irradiation with 100 krad resulted in reducing the total bacteria count by a factor of 10^4 and in complete inactivation of the enterobacteriaceae.

The storage life of irradiated prepacked (polythene 0.05 mm, unperforated) cut endive was considerably prolonged at 10 and 20 °C (as compared to that of the control samples (see table 1).

Table 1 - Influence of irradiation on the keeping quality of cut endive at different storage temperatures.

storage temperature	0 krad	50 krad
0 - 2 °C	10 days	10 days
10 °C	3 days	7 days
20 °C	1 day	4 days

Comparative study on enzyme activities in prepacked cut endive. V. Kalinov, D.Is. Langerak.

The purpose of this study was to check the influence of irradiation at quality keeping doses on the overall activity of certain enzymes.
At first peroxidase which is considered as a key enzyme in the quality evaluation of preserved vegetables and catalase were chosen as objects of study. Later on polyphenoloxidase which enzyme is closely connected with the browning discoloration in the injured plant tissue, was also considered.
The activity of enzymes was measured in filtered and diluted extracts obtained by homoginizing the endive leaves in buffered media.
The peroxidase activity was measured with the peroxidase test as described by Chance and Machly but modified for the use with crude enzyme extracts. The oxidation of guaiacol to brown coloured substances catalyzed by peroxidase in presence of H_2O_2 was spectrophotometrically recorded.
The polyphenolase activity was measured similarly; however, the reaction mixture contained catechol instead of guaiacol and H_2O_2.
The following results were obtained:
- The catalase activity considerably decreased during the first 24 hours following processing and irradiation without difference between control and irradiated samples. Thereafter the activity slowly increased and, after 4 days storage at 10 °C, it was about 50% of the initial activity in the control and 30% in the irradiated samples was significantly lower than that of the controls.
  During storage at 2 °C, the activity in both the controls and the irradiated samples remained almost constant at the level reached 24 hours after the first treatment. After 3 days of storage the activity in irradiated material was significantly lower than that of the controls.
- The activity of peroxidase did not immediately change after irradiation with doses of 100 to 300 krad. The effect of storage on peroxidase activity was highly significant ($P < 6.01$). During storage at 10 °C the activity was increasing in both the control and the irradiated samples. At the end of the period (7-10 days) the activity in the samples irradiated with 100, 200 and 300 krad was respectively 70, 50 and 4% of the activity in the control. The same trend appeared in the storage experiments at 2°C.
- The polyphenolase-activity of the samples irradiated with doses of 100 to 300 krad was, immediately after irradiation, significantly ($P < 0.05$) higher than in the controls. The effect of storage was also very significant ($P < 0.01$). The activity linearly increased with time in the controls as well as in the irradiated samples. During storage at 10°C, the difference between both treatments was also significant.

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After 7 days of storage the activity in the controls was considerably increased in comparison with the initial one, where as in the irradiated samples, the activity showed a very slow rise (figure 1).
According to the observations during the present experiments the brown discoloration of endive is indeed associated with the activity of polyphenolase in this vegetable.

The irradiation of asparagus:
In order to get more information about the application possibility of a gamma irradiation for asparagus-preservation the experiments of the previous years were repeated and extended. Special attention has been paid to packaging and storage conditions. During storage the product was examined for quality (colour, desiccation, spoilage, "growth") trade value, pH value, titratable acidity, and organoleptic properties. In the packaging the CO_2 and O_2 contents were measured.

The following results were obtained:
During storage the non-irradiated asparagus soon discoloured (pink and borwn), while the irradiated asparagus better retained its white colour.
The cut surfaces of the stalks of the non-irradiated asparagus had a more desiccated (greyish) appearance than those of the irradiated ones. On the non-irradiated asparagus mould growth showed up after 2 days at 15°C; by irradiation this occured after 4 to 6 days. At the end of the storage time a few tops of the irradiated asparagus were soft (damage ?).
Microbiological observations showed that at a radiation dose of 100 krad, the viable total count (on Plate-Count-Agar (PCA)) was reduced by a factor of 10^4. The enterobacteriaceae were completely inactivated.

By irradiation the "growth" of asparagus was stopped and its trade value was improved. At 15°C the keeping quality was prolonged with 2 - 5 days. In the present experiments no clear connection was found between pH, titratable acidity and spoilage.
In the wrapped paper dishes, used for packaging, the CO_2 percentage rose to an average of 3%; in a few packages the O_2 percentage decreased till 1%. At this low O_2 percentage fermentation was observed. Perforation will be advised in the future.
The taste was not disadvantageously influenced by an irradiation dose < 200 krad.

Prepacked peeled potatoes
The keeping quality of peeled potatoes is limited by microbiological spoilage and enzymatic discoloration.
Previous research (1968, 1969) showed that the keeping quality of peeled potatoes treated with "sulphite" ($Na_2S_2O_5$) was considerably improved by irradiation. A disadvantage of the treatment, however, was that at 100 krad taste was affected; at 50 krad this only occured when the product was strongly (> 2%) sulphurized.
In connection with this research the influence of various radiation doses, anti-oxidants, pH and packaging on quality, shelf life and taste of peeled potatoes were again investigated. The following results were obtained:
- The shelf life at 15°C is prolonged by 1 or 2 days after irradiation (see table).
- The total viable count (Plate-Count-Agar-medium) and enterobacteriaceae (Violet-Red-Bile-glucose medium) are reduced by doses as low as 50 krad.
- Among the investigated anti-oxidants only $Na_2S_2O_5$ gives satisfactory results. Colour preservation is better after a combined treatment $Na_2S_2O_5$ (0.5%) - irradiation (50 krad) than after "sulphite" alone. At higher "sulphite" concentrations and/or a higher irradiation dosis off-flavour occurs more frequently.

There are indications that the quality of the product is improved by preservation in nm-perforated polythene bags.

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Projectnummer : 14
Registratie nr.:

Table 2 - Influence of sulphite and of a combined sulphite-irradiation treatment on the keeping quality of peeled potatoes at different storage temperatures.

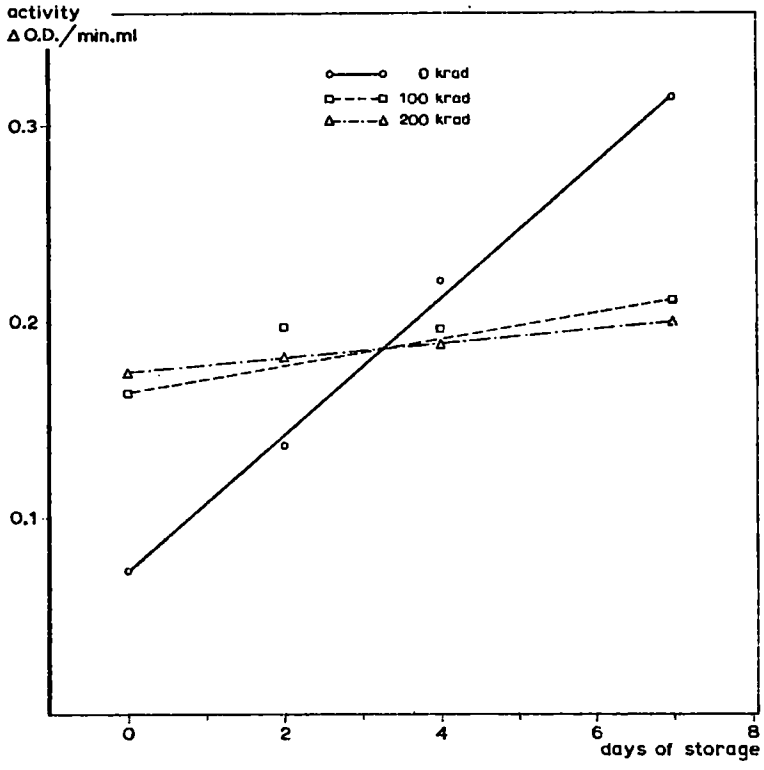
Storage temperature	0.5% $Na_2S_2O_5$	0.5% $Na_2S_2O_5$ + 50 krad
2°C	7 days	14 days
10°C	4 days	8 days
15°C	1,5 day	3 days

Experiments on the storability of strawberry yoghurt using irradiated strawberries.
I. Kiss, D.Is. Langerak.

The aim of the experiments was to find out whether the microbial flora of strawberries may be stabilized with ionizing radiations or combined treatments. The effect of heat, freezing, irradiation and various combinations of these treatments upon cell count and sensory quality was investigated. It was observed that the individual treatments were not satisfactory, within the permissible treatment limits. The combination of surfacial heat treatment at 55°C, freezing and irradiation with 0,4 - 0,6 Mrad substantially increased the storage life of strawberries or that of the yoghurt prepared with this fruit. When compared to yoghurt made with frozen strawberries by the dairy factory, the storage life increase was 2,5 fold at 15°C and 3,5 fold at 2°C.
The strawberries irradiated with doses above 0,2 Mrad showed aroma and flavour change immediately after treatment. This effect, however, was eliminated after some days.
The yoghurt made with strawberries, given a radiation treatment of 0,57 Mrad, did not differ organoleptically from the yoghurt made with untreated strawberries.
The above mentioned treatments (heating, freezing, irradiation) were only satisfactory, from the point of view of microbiological stability, when the injection level of the strawberries did not exceed 10^{-3} yeast cells per g.

Influence of irradiation on packaging materials.

A working group under the direction of Prof. H.A. Leniger (Dept. of Food Technology, Agric. Univ., Wageningen) has studied the influence of irradiation doses on the packaging material used for irradiated foods. Members of the working group came from the Institute for Packaging, Delft, the University Reactor Institute, Delft, the Packaging Industry (Ruys) the Association's Institute and the Pilot Plant for Food Irradiation, Wageningen.
The main conclusion was, that at the level of pasteurization doses the possibility of the formation of toxical compounds in the officially admitted plastic foils was very small (below the danger level of 1 - 10 ppm).



Influence of storage and irradiation on the polyphenolase activity

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 66
Projectnummer	: XII 66	
Projecttitel	: Electronic instrumentation development applied to biological research	
Onderzoeker(s)	: J.G. de Swart	
Projectleider	:	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	–	mandagen
„ „ -materieel	f	Middelbaar personeel	–	mandagen
Semi-directe kosten	f	Lager personeel	–	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (1971) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71.

The first part of this report is dealing with work directly related to the Association's programme (items 1, 2 and 3); the items 4, 5, 6, 7, 8 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 scanner is now in production and will be tested beginning 1972.

2. Punch tape reader/interface for the Association's table-computer.
A photocell-reader with interface has been realized for automatic handling of the teletype-tape and the Tally-tape, which makes it, for instance, possible, to transform liquid scintillation data of the Mark II directly into desintegrations/min without manual feeding of the figures to the computer-keyboard. The prototype was successfully brought into operation.

3. Because of the need for more modern nuclear counting systems, it has been decided to replace the old Philips units. The new instruments are partly custom built and are ready for operation. Much attention was paid to flexibility of the system by modular design in combination with automatic readout possibilities, using parallel printers and teletype interfacing.

4. Moisture content measurement in maize leaf - Kleinendorst, Institute for Biological and Chemical Research on Field Crops and Herbage (I.B.S.), Wageningen, The Netherlands.
The realisation of this system, using the absorption of the ^{45}Ca β's and G.M. detection, measuring in four channels with automatic registration, is finished. Production of special circuitry was carried out in the Association's Institute by

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technicians of I.B.S.
The system is working,under normal operating conditions, with satisfying results.
A report on the results by Kleinendorst, will be given separately by Kleinendorst.

5. Measurement of density variations of flowing granular material in elevator systems Van Zuilichem, Dept. for Food Technology, Agric. University, Wageningen.
A method is 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 by different granular materials flowing in a model of an elevator system is measured at different places in the model.
The γ-beam is obtained from a 50 mCi ^{137}Cs source and the measurement channel consists of a scintillation detector followed by a one-channel γ-spectrometer system.
The measurements are transformed by a computer programme in a density plot.
The first experiments showed that the elevatormodel needed modifications.
This has now been done and the measurements will start again in February 1972.

6. Velocity measurement of granular material in an elevator system - Van Zuilichem, Dept. for Food Technology, Agric. University, Wageningen.
The material is vertically transported in an air stream over more than 20 m with speeds of approximately 25 m/sec. The velocity of the solid material will depend on different factors, e.g. packing, tube diameter etc. However, a method for measuring this velocity at different heights in the transport tube is unknown at this moment. It seems to be attractive to bring a shot of activated material in the tube, during a normal transport process, and to measure the time the activity needs to reach a detector at a certain position along the tube. Because of the high speed of the material, the measurement time will be rather short (in order of m sec), c.q. the count rate needs to be high and the time resolution extremely good.
For this detection, the use of a multichannel analyzer in multi scaler mode seems possible. The address advance (time base) will be driven by a pulse train with a precisely known frequency, while the start of the pulse train has to be synchronized with the sample shot.
Preliminary experiments with motor driven rotating sources, for lining up the system, produced good results.
Necessary changes in the elevator system (pneumatic system to inject the activated material in the elevator tubing, for instance) are actually made by the laboratory for Food Technology.
Because of these mechanical improvements of the original installation, the first experiments with the final set-up had to be postponed until 1972.

7. Measurements of the residence time of maize in an extruder - Van Zuilichem, Dept. for Food Technology, Agric. University, Wageningen.
A $CuCl_2$ solution is brought into maize grit and after activation in the BARN reactor fed into the normal maize supply of the extruder. At the extruding head, a scintillation detector with collimator and a multi-channel analyzer in multi-scaler mode is used for registration of the signal.
The measurements have been completed with satisfactory results. More detailed information will be given separately by Van Zuilichem.

8. Investigation concerning the degree of uniformity in distribution of liquids by a spraying machine.- Institute of Agricultural Engineering and Rationalization, Wageningen.
A $CuCl_2$ solution is sprayed on 10 m long strips of filter paper in the field. After drying the strips are activated in the BARN reactor and the strips are scanned with a scintillation detector combined with a single-channel γ-spectrometer, set for ^{64}Cu.
According to the first experiments the method is reliable and especially suited for automatic interpretation via punch tape read-out and computer handling, which is important because of the large amount of experimental data (ca. 1000 figures per paper strip). Further experiments will be done in 1972.

Onderzoekinstelling	:Association Euratom-ITAL	Registratie nr.:
Projectnummer	:19	

APPENDIX I.

Measurements on growth and water content of maize plants following localized treatment with low temperature.
A. Kleinendorst, I.B.S. Project Nr. 435.

Methods

Changes in mass per unit leaf area due to changes in water content were measured by 4 beta-gauges. Fifteen µCi ^{45}Ca mounted in the top of a small perspex rod was placed under the leaf of an intact plant. A GM tube was mounted at the opposite side of the leaf. With help of a voltage supply, amplifier and count rate meters the output was continuously recorded on line recorders.
Leaf elongation of the youngest leaf is measured continuously by connecting the leaf via thin wire thread to a resistance meter. The output which is linearly related to the leaf elongation is recorded.

Results.

1. Low root temperature results in a reduction of the leaf elongation, which is caused by water deficit; cell elongation ceases or is inhibited. Subsequently, as a result of adaptation, the water deficit decreases and leaf elongation recovers.
2. Low meristem temperature results in a reduction of the leaf elongation caused by an influence on the biochemical processes in cell elongation and probably also to decreased cell division. No water deficit occurs in the plant and there is no shortage of carbohydrates in the meristem region.
3. Low temperature applied to a localized region on the stem above the meristem also results in a more or less important reduction of leaf elongation, caused by total or partial cessation of the transport of carbohydrates and/or growth substances (hormones) to the meristem.
   After some time the plant recovers from the low temperature inhibition.
   No water deficit occurs in these plants.

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APPENDIX II.

Measurements of residence time distribution in an extruder producing expanded corn pellets. (Agricultural University, Dept. of Food Science, Wageningen, The Netherlands, D.J. van Zuilichem, J.G. de Swart, G. Buisman.

1. Introduction

In the Food industry a well-known method of producing expanded products is given by extruding fine or coarse ground cereals through an orifice of certain restricted dimensions. The apparatus shown in fig. 1 was used in the present experiments. Finely ground corn is transported by means of the screw through a cylinder with hardened sleeve. The cylinder is preheated electrically.
The root diameter of the screw is steadily increasing towards the end-diameter in the high pressure part of the screw-cylinder combination.
Corn, transported by the worm through the compression section and high-pressure part is exposed to high shear forces and high temperatures (170 - 200 °C). The result is that corn looses its original structure and is transformed to a mass with more or less plastic properties under high pressure (200 - 600 kg/cm^2). Leaving the die through the narrow orifice (2.4 mm) the mass expands to barometric pressure and obtains a high porosity and a low filling weight.

2. Problems

When we consider the extruder as a process-apparatus some problems reveal themselves immediately. The high temperature causes destruction of amino-acids, vitamins and is responsible for a change in taste. The time during which the material is exposed to the high temperature determines the state to which destruction has proceeded. The length of the temperature section is fixed and the temperatures in this section are known, but the residence-time and its distribution had to be measured.
Factors which influence the residence-time distribution are the number of revolutions of the screw per unit time, the moisture content of the material, the compression-ratio of the screw and the diameter of the die. In these investigations we varied the speed of the screw, the moisture content of the ground corn and used different die-diameters.

3. Experiments

The experimental set up is shown in fig. 2. Copper 64, produced by neutron activated finely ground corn, which had been soaked in a saturated $CuCl_2$ solution was chosen as a tracer. A shot of 3.75 grams of this material with an activity of 1.8 mCi was added at each run to the normal supply of the working extruder, simulating a disturbance with the shape of an impulse in the feed part of the extruder. The response of this signal was detected by means of a scintillation counter, a 1 channel spectrometer and a 200 channel analyser (time mode). The result was read out by a parallel printer.
We obtained diagrams as shown in fig. 3 and 4, from which it is easy to determine the residence-time, using the methods, common in control-techniques. The residence-time distribution was read out from the diagrams by judging the shape of the curves.

4. Results

We carried out the investigations with 3 different moisture contents: (14.2%; 16.5%; 20.0%), 3 different speeds (80, 100 and 120 r.p.m.) and 2 different die-diameters (1.9 and 2.4 mm). The moisture content was measured before on a sample taken from the supply of the extruder. The production was measured in kg/hr. The average residence time τ is calculated in seconds.

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5. Results, conclusion and discussion

moisture content (%)	r.p.m.	die-diameter (mm)	production (kg/hr)	residence time (average) τ (sec)
14.2	80	2.4	-	42
14.2	100	2.4	-	33
14.2	120	2.4	-	32
16.5	80	2.4	26	47
16.5	100	2.4	30	39
16.5	120	2.4	32	37
20.0	80	2.4	26	46
20.0	100	2.4	30	38
20.0	120	2.4	34	37
19.8	80	1.9	24	50
19.8	100	1.9	28	44
19.8	120	1.9	31	40

The width of the response in time units amounts to approximately 10 seconds for all the runs we did. For this reason can be stated that the residence-time distribution in an extruder is small, when extruding finely ground cereals.
The influence of the moisture content on the residence-time decreases with increasing moisture content. This corresponds to the increasing values of the hour-production for the moisture contents 16.5% and 20.0%.

With increasing rotational speed the residence-time decreases; which seems to be plausible, as is the decreasing values of the hour-capacity, when the die-diameter decreases, with a corresponding increase in residence-time.

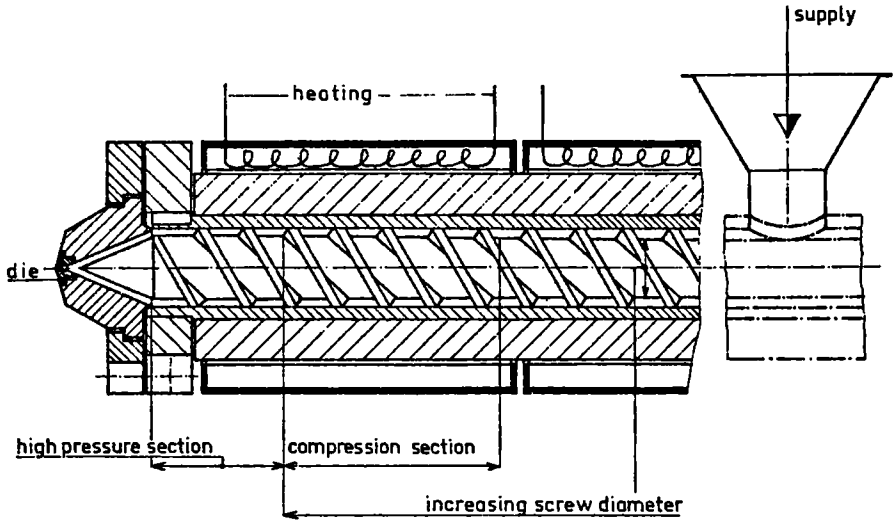


fig. 1 Section of screw-extruder

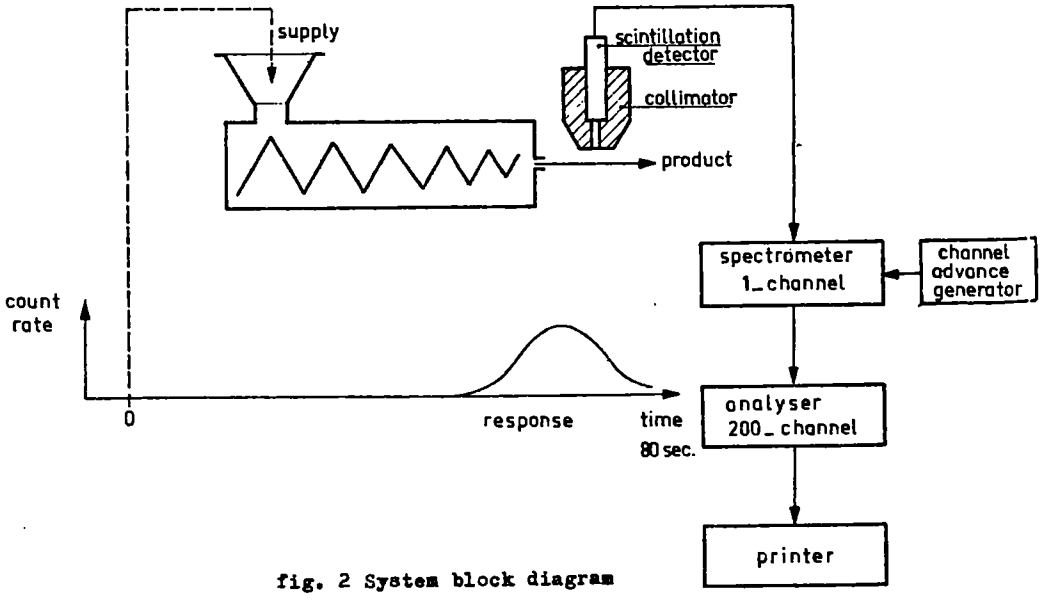
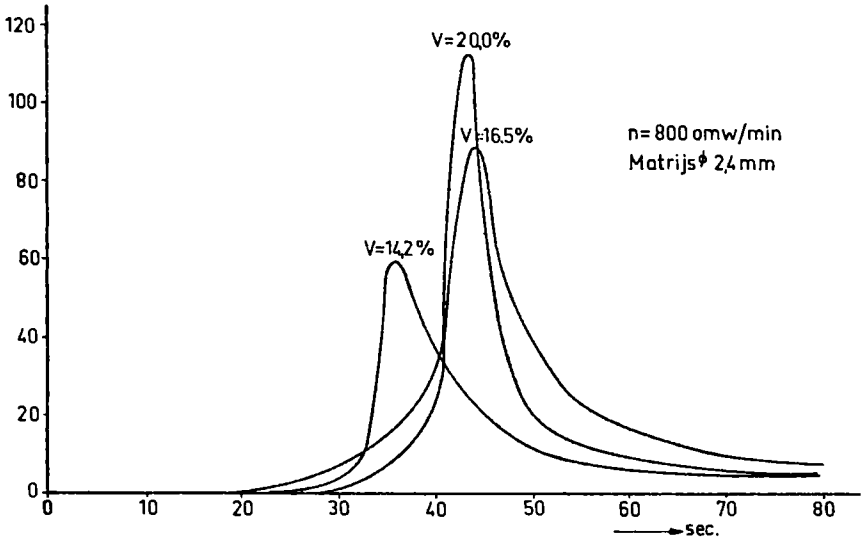


fig. 2 System block diagram


V=20,0%
V=16,5%
V=14,2%
n=800 omw/min
Matrijs ϕ 2,4 mm
120
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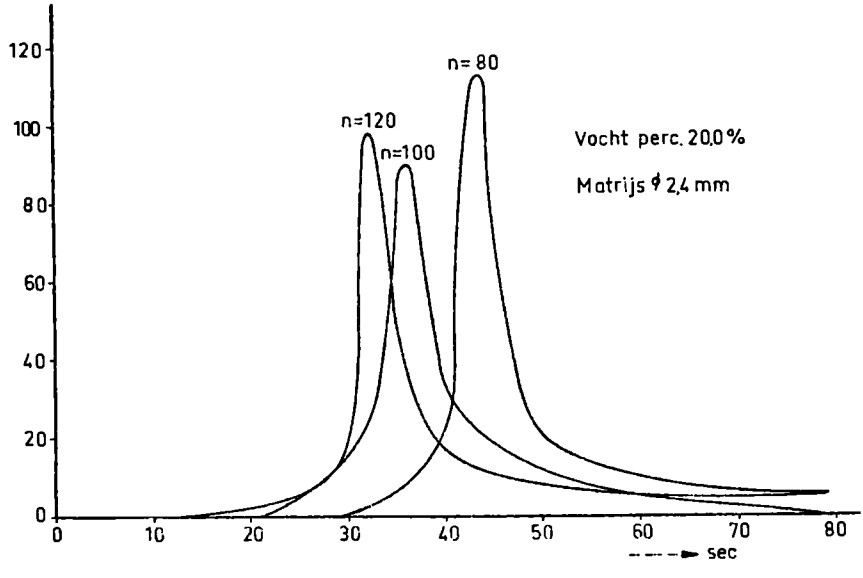
sec.

fig. 3


n=80
n=120
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120
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30
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50
60
70
80
sec

fig. 4

PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 67
Projectnummer	: ~~35~~ 67	
Projecttitel	: Genetical, Radiobiological and Biochemical research on the gamethophytic monofactorial system of self-incompatibility	
Onderzoeker(s)	: D. de Nettancourt, A.J.G. van Gastel, G. Bredemeijer and	
Projectleider	: Carluccio.	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld

Directe kosten-personeel	f
" " -materieel	f
Semi-directe kosten	f
Omslag algemene kosten	f
Totaal	f
Inkomsten	f

Tijdbesteding van direct bij het project betrokken personeel:

Hoger personeel	—	mandagen
Middelbaar personeel	—	mandagen
Lager personeel	—	mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

Analysis of the factors and mechanisms governing the generation of new alleles at the S-locus of Lycopersicon peruvianum Mill. (De Nettancourt, Van Gastel).

Testing of the hypothesis that new S-alleles are generated by equal crossing-over.

Pandey (Genetica, 1970; Nature, 1970) has expressed the opinion that intragenic crossing-over within the S locus may have generated the new allele (S_3) which we observed in our inbreeding experiments (De Nettancourt et al, 1969, 1971). In order to test Pandey's hypothesis, reversion tests were made to find out if the new allele (S_3), when in presence with one of the two original alleles, could generate the second oroginal allele. Screening for reversions was performed in both back-crossed and selfed progenies. At least six clear cases of reversion were observed in some back-cross populations whereas only one dubious reversion was detected after selfing S_1S_3 and S_2S_3 individuals. Not all results are yet available but it appears that:

- equal crossing-over is not involved in the generation of a new S-allele;
- the genetic factors which govern the generation of a new S-allele have sporophytic expression, are recessive and present in heterozygous condition in the mother clone.
- a number of these genetic factors are located on the S-bearing chromosome (new alleles are essentially generated in S-homozygotes) but other loci are involved which are situated elsewhere in the chromosome complement (only a fraction of S-homozygotes generate a new allele).

Testing of the hypothesis that the apparition of a new S-allele results from activation-inactivation switching processes on tandem segments.

The crosses and diallelic tests, necessary for testing Edström theory (Nature, 1963) that constructive mutations (in this case, changes in S-specificity) are due to the reactivation of allelic copies previously stored during outbreeding, are underway in both Wageningen and Roma.
The ancestry tests will all be carried out on S_1 and S_2 alleles. Ancestor-alleles are S_4 and S_5 in Wageningen and S_6, S_7 at the Casaccia.

Onderzoekinstelling : Association Euratom -ITAL	Registratie nr.:
Projectnummer : 35	

Nature of the relationship between spontaneous selfing and the generation of a new S-allele.

Plant I_2-53 is an inbred individual which is classified as S_1S_2 and regularly self-incompatible. On one occasion, however, the plant set a very large number of seeds after selfing which gave rise to a progeny segregating for three specificities (S_1S_2 and, the new specificity S_3). The problem was to find out if the generation of the new allele S_3 was the consequence of spontaneous inbreeding (S_3 would then result from the formation of a new genetic background in the I_3 generation) or its cause (S_3 is formed in the I_2 generation and is regularly transmitted, during the whole period covered by the formation process, to the I_3 generation).
For this purpose plant I_2 - 53 was subjected to inbreeding techniques which resulted into the production of a limited number of seeds after selfing. The results obtained to date are summarized in table I and indicate beyond doubt that the formation of S_3 is the cause and not the consequence of spontaneous inbreeding.

Table I: Segregation for S-alleles in the progeny of plant I_2-53 (S_1S_2) obtained after spontaneous selfing (A) and after obligate selfing (B).

Genetic segregation observed			
A) Spontaneous selfing		B) Obligate selfing	
No. plants	Genotypes	No. plants	Genotypes
25	S_1S_3	13	S_1S_2
8	S_2S_3	4	S_2S_2

Self-compatibility in Lycopersicon peruvianum Mill.

After obligate selfing in the progenies of S_1S_2 plants in the second generation of inbreeding, a self-compatible individual has been detected which is the mirror image of self-incompatibility: it rejects all pollen except its own!
The progeny of this plant segregates in approximatively 50% self-compatible individuals (which again, only accept self-pollen) and 50% self-incompatible offsprings. The phenomenum repeats itself at each generation of selfing.
The self-compatibility character detected is not only valuable from a practical point of view (possibility to apply the conventional selection method of self-pollinated crops to a cross-pollinated species) but provides an interesting material for analysing the processes which control function and expressivity at the S-locus.

Genetic mapping of the S-locus and identification of the S-bearing chromosome in Nicotiana alata, Link and Otto. (Van Gastel, De Nettancourt, Carluccio).

Two approaches have been made in 1971 for establishing the chromosomal localization of the S-locus and its eventual position within a linkage group.

Genetical approach

Using the adventitious method of propagation by isolated leaves, a number of morphological mutants have been obtained, after irradiation of excised leaves, in the same genetical background as the original S_2S_3 clone (De Nettancourt, Dijkhuis, Van Gastel and Broertjes, 1971). The fact that mutant individuals were also detected in the control series clearly shows that the leaf-propagation technique is, in itself, mutagenic.
All mutant plants have been submitted to progeny-testing and identity-crosses with S-homozygotes for ascertaining the hereditary nature of each induced mutation and an eventual linkage to the S-locus.

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In addition, 2 pollen-part and 2 stylar-part self-compatible mutants have been detected in the material screened so far. To our knowledge, this is the first time that self-compatibility mutations are induced and detected, in the complete absence of inbreeding effects, in the precise genetic background of the original material. In other words, mutants are now available which permit an accurate comparison of the genetical and biochemical properties of S.I. and S.C. (pollen part, style part) alleles in similar genetic backgrounds.

Cytological approach

Two interesting findings have been made so far. One concerns the discovery of an aberrant chromosome in a stylar-part mutant obtained from rooted-leaves. The aberration, which is not visible in all somatic metaphases, appears to involve the insertion of a translocation segment in one of the median chromosomes. All progenies of this self-compatible mutant examined to date (3) display an aberration which in one case resembles that of the mother-plant and, in the two others, appears as a tertiary constriction. Karyogrammes of control and aberrant plants are being made which will enable an exact classification of the cytological anomalies observed. The second finding is the detection, in the progeny of a self-compatible tetraploid individual, of a plant which is not self-compatible and displays 34 chromosomes instead of 36. The two missing chromosomes do not appear to be homologous. It is tempting to conclude that the plant should have been Sa Sa Sa Sb and that heterogenic pollen (self-compatible Sa Sb pollen) is not formed because Sb is located on one of the two missing chromosomes.

Mutation spectra analysis at the S-locus of Nicotiana alata Link and Otto. (Van Gastel, De Nettancourt).

The aim of the experiments is the production of self-compatible mutants by different radiation treatments. Analyses of the mutation spectra (type of mutation and ratio of fragment to non-fragment mutation) may produce information concerning the dose and radiation treatment inducing mutations like in nature. (spontaneous mutations for self-compatibility do not display a centric fragment).

After pollen mother cell (P.M.C.) irradiation with different dosages of X-rays several mutant seeds have been obtained. Dosages used are 300, 600 and 825 rad. The mutants obtained in this experiment will be test-crossed to find out the type of self-compatible mutation. All self-compatible mutants will be cytologically analysed for the presence or absence of a centric fragment.

Mutants obtained from preliminary crosses have already been test-crossed for that purpose. In one case a pollen-part mutation was induced whereas in two other cases a revertible mutation (probably pollen part) was obtained. Cytological analyses revealed that at least in one case a centric fragment was present.

In a chronic γ-irradiation experiment on self-incompatible plants rather great amounts of seeds (after selfing) have been obtained. The largest amount of selfed seed was found after irradiation at a dose-rate of 2.75 rad/h. The progenies will be tested to find out whether the seed set is due to a mutation at the S-locus or to a physiological effect. If the effect is indeed a genetic one, the results of this experiment shows that chronic irradiation is more effective for inducing self-compatibility than acute irradiation.

Induction of self-compatibility in dihaploid Solanum tuberosum L. (Van Gastel, De Nettancourt).

In an experiment using chronic γ-irradiation several mutant seeds have been obtained after selfing. These mutants will be tested for self-compatibility.

The effects of P.M.C.-irradiation with acute X-rays on the pollen sterility have been studied. The induction of selfcompatible mutants will be initiated next spring.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 35	Registratie nr.:

According to recent information the induction of self-compatibility in a dihaploid Solanum tuberosum is of limited interest to agriculture. Continuation of this project will therefore be reconsidered.

Biochemical aspects of self-incompatibility in Lycopersicon peruvianum Mill.and Nicotiana alata Link and Otto. (G. Bredemeijer).

Identification of S-genotypes in leaves of Lycopersicon peruvianum and Nicotiana alata.

Several investigators found a relation between the S-genotype (specificity for self-incompatibility) of a style and its protein composition (peroxidase, glutamate dehydrogenase and basic proteins).
To our knowledge, nothing has been published concerning a possible similar relation with the protein composition of vegetative plant parts (e.g. leaves). Such information, however, might be of considerable interest in detecting different S-genotypes at an early vegetative growth stage.
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PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 68
Projectnummer	: XX 68	
Projecttitel	: Genetical, Radiobiological and Biochemical research on the gamethophytic monofactorial system of self-incompatibility	
Onderzoeker(s)	: D. de Nettancourt, A.J.G. van Gastel, G. Bredemeijer and	
Projectleider	: Carluccio.	
Afdelingshoofd	:	

Besteding over het afgelopen jaar (19)

Besteding in geld		Tijdbesteding van direct bij het project betrokken personeel:		
Directe kosten-personeel	f	Hoger personeel	—	mandagen
" " -materieel	f	Middelbaar personeel	—	mandagen
Semi-directe kosten	f	Lager personeel	—	mandagen
Omslag algemene kosten	f			
Totaal	f			
Inkomsten	f			

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19$_{71}$) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 19 71

Analysis of the factors and mechanisms governing the generation of new alleles at the S-locus of Lycopersicon peruvianum Mill. (De Nettancourt, Van Gastel).

Testing of the hypothesis that new S-alleles are generated by equal crossing-over.

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The ancestry tests will all be carried out on S_1 and S_2 alleles. Ancestor-alleles are S_4 and S_5 in Wageningen and S_6, S_7 at the Casaccia.

Onderzoekinstelling : Association Euratom -ITAL Projectnummer : 35	Registratie nr.:

Nature of the relationship between spontaneous selfing and the generation of a new S-allele.

Plant I_2-53 is an inbred individual which is classified as S_1S_2 and regularly self-incompatible. On one occasion, however, the plant set a very large number of seeds after selfing which gave rise to a progeny segregating for three specificities (S_1S_2 and, the new specificity S_3). The problem was to find out if the generation of the new allele S_3 was the consequence of spontaneous inbreeding (S_3 would then result from the formation of a new genetic background in the I_3 generation) or its cause (S_3 is formed in the I_2 generation and is regularly transmitted, during the whole period covered by the formation process, to the I_3 generation).
For this purpose plant I_2 - 53 was subjected to inbreeding techniques which resulted into the production of a limited number of seeds after selfing. The results obtained to date are summarized in table I and indicate beyond doubt that the formation of S_3 is the cause and not the consequence of spontaneous inbreeding.

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The progeny of this plant segregates in approximatively 50% self-compatible individuals (which again, only accept self-pollen) and 50% self-incompatible offsprings. The phenomenum repeats itself at each generation of selfing.
The self-compatibility character detected is not only valuable from a practical point of view (possibility to apply the conventional selection method of self-pollinated crops to a cross-pollinated species) but provides an interesting material for analysing the processes which control function and expressivity at the S-locus.

Genetic mapping of the S-locus and identification of the S-bearing chromosome in Nicotiana alata, Link and Otto. (Van Gastel, De Nettancourt, Carluccio).

Two approaches have been made in 1971 for establishing the chromosomal localization of the S-locus and its eventual position within a linkage group.

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Using the adventitious method of propagation by isolated leaves, a number of morphological mutants have been obtained, after irradiation of excised leaves, in the same genetical background as the original S_2S_3 clone (De Nettancourt, Dijkhuis, Van Gastel and Broertjes, 1971). The fact that mutant individuals were also detected in the control series clearly shows that the leaf-propagation technique is, in itself, mutagenic.
All mutant plants have been submitted to progeny-testing and identity-crosses with S-homozygotes for ascertaining the hereditary nature of each induced mutation and an eventual linkage to the S-locus.

Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 35	Registratie nr.:

In addition, 2 pollen-part and 2 stylar-part self-compatible mutants have been detected in the material screened so far. To our knowledge, this is the first time that self-compatibility mutations are induced and detected, in the complete absence of inbreeding effects, in the precise genetic background of the original material. In other words, mutants are now available which permit an accurate comparison of the genetical and biochemical properties of S.I. and S.C. (pollen part, style part) alleles in similar genetic backgrounds.

Cytological approach

Two interesting findings have been made so far. One concerns the discovery of an aberrant chromosome in a stylar-part mutant obtained from rooted-leaves. The aberration, which is not visible in all somatic metaphases, appears to involve the insertion of a translocation segment in one of the median chromosomes. All progenies of this self-compatible mutant examined to date (3) display an aberration which in one case resembles that of the mother-plant and, in the two others, appears as a tertiary constriction. Karyogrammes of control and aberrant plants are being made which will enable an exact classification of the cytological anomalies observed. The second finding is the detection, in the progeny of a self-compatible tetraploid individual, of a plant which is not self-compatible and displays 34 chromosomes instead of 36. The two missing chromosomes do not appear to be homologous. It is tempting to conclude that the plant should have been Sa Sa Sa Sb and that heterogenic pollen (self-compatible Sa Sb pollen) is not formed because Sb is located on one of the two missing chromosomes.

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Onderzoekinstelling : Association Euratom-ITAL Projectnummer : 35	Registratie nr.:

According to recent information the induction of self-compatibility in a dihaploid Solanum tuberosum is of limited interest to agriculture. Continuation of this project will therefore be reconsidered.

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PROJECTVERSLAG

Onderzoekinstelling	: ASSOCIATION EURATOM - ITAL	Registratie nr.: AA-009 / 69
Projectnummer	: XX 69	
Projectitel	: Genetical, Radiobiological and Biochemical research on the gamethophytic monofactorial system of self-incompatibility	
Onderzoeker(s)	: D. de Nettancourt, A.J.G. van Gastel, G. Bredemeijer and	
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Besteding over het afgelopen jaar (19)

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Beknopte weergave van achtereenvolgens
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Projectnummer : 35
Registratie nr.:

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PROJECTVERSLAG

Onderzoekinstelling : ASSOCIATION EURATOM - ITAL
Projectnummer : 105
Projecttitel : Recycling of the waste

Registratie nr.: AA-009 / 105

Onderzoeker(s) : J.H. Becking, G. Ernst*
Projectleider :
Afdelingshoofd :

Besteding over het afgelopen jaar (19)

Besteding in geld
Directe kosten-personeel f
" " -materieel f
Semi-directe kosten f
Omslag algemene kosten f
Totaal f
Inkomsten f

Tijdbesteding van direct bij het project betrokken personeel:
Hoger personeel — mandagen
Middelbaar personeel — mandagen
Lager personeel — mandagen

Beknopte weergave van achtereenvolgens
A. Verslag afgelopen jaar (19 71) met vermelding van publicaties
B. Plan komende jaar.

A. Verslag over 1971.

Microbial degradation of plastics after a pretreatment of radiation.
(Preliminary study on one of the research aspects).

In view of the mounting threat of environmental pollution by waste products, a study was initiated on the degradation of plastics used as packaging material. The objective was to apply radiation to plastics in order to reduce the polymer length of the plastics and therefore making them more attackable by microbes. Apart from chain breakage smaller oxidized components are formed by radiation treatment.
Because commercial plastics have very different composition by the use of different plasticizers, in this study use was made of plastics of known composition. For this purpose plastics such as poly-ethylene (PE) and poly-vinylchloride (PVC) with known plasticizer, i.e.di-octyl-phtalate or di-octyl-adipate, were synthesized by the Kunststoffen en Rubber Instituut TNO, Delft, The Netherlands.
Plastic strips of this material irradiated with doses of 2-100 Mrads showed a darkening of the material. Irradiation caused a decrease (0.5 - 5%) of the dry weight of the material probably due to oxidation or volatization of certain constituents of the plastic such as chloride in the case of PVC.

Bacteria probably capable of degrading plastics were obtained by using microbiological enrichment techniques with a mineral medium containing poly-vinyl alcohol and poly-vinyl acetate as sole carbon source. These bacteria after isolation were tested for their capability to disintegrate unirradiated and irradiated plastic samples. Moreover, unirradiated and irradiated plastic samples were burried in soil (temp. 29°C, humidity 80-90%) for natural accumulation of micro-organisms on these plastics. In general these treatments showed a reduction of dry weight of these plastics, but the same reduction was also found in the unirradiated samples. In some cases the irradiated samples showed a higher degree of destruction compared to the unirradiated samples, but it is not yet known whether these differences are statistically significant. This study is further in progress.

*Student in biology University of Nijmegen.

ADDITIONAL AGREEMENT No. 1

to

CONTRACT SC 001/076-69-1 BIAN

between

EUROPEAN ATOMIC ENERGY COMMUNITY

and

UNIVERSITY OF PISA (INSTITUTE OF GENETICS)

"Cytology and Genetics of plant tissues and cells grown "in vitro"

Report on activities from January 1st to December 31st, 1971.

In agreement with the research lines proposed in the scientific program of the contract, the following investigations have been carried out:

1) Cell dedifferentiation in plant tissues grown "in vitro" and gene amplification.

Work has been continued on the cytochemistry and biochemistry of cell dedifferentiation (change from the differentiated to the proliferating state, induced by hormones) in two systems in vitro: the lettuce (Lactuca sativa) cotyledons and the pith tissue of Nicotiana glauca. Previous work on the lettuce cotyledons grown in vitro (see Report for the year 1970, research line 1), had provided cytochemical and autoradiographic evidences of extra DNA synthesis (gene amplification) in the first two days of in vitro culture, i.e. during the cell dedifferentiation process (NUTI RONCH 1971, Publ. no. 6). Some of techniques used on lettuce (Feulgen reaction; feeding with ^{3}H-actinomycin D for the autoradiographic detection of DNA; DNase digestion; acridine orange for the differential fluorescence of DNA and RNA; flash feeding with ^{3}H-thymidine) have been applied to the study of cell dedifferentiation in the Nicotiana glauca pith tissue (NUTI RONCHI, MARTINI and BENNICI). Starting from the second day of culture, cells of the prospective proliferating regions show extrusion of chromatin and whole

nucleoli from the nucleus into the cytoplasm: the amount of extruded chromatin varies from small portions to the dispersion of the whole nucleus. Flash treatments with ^{3}H-thymidine show that chromatin extrusion is not a degenerative phenomenon, but rather it concerns the extrusion of replicating DNA (extra DNA synthesis).

The phenomena of extra DNA synthesis observed in lettuce and Nicotiana glauca tissues during cell dedifferentiation might be indicative of gene amplification phenomena: among these, amplification of the cistrons coding for ribosomal RNA (rRNA cistrons or rDNA) can be demonstrated by rRNA-DNA hybridization on cytological preparations. This technique has been applied succesfully, for the first time, to a plant material by AVANZI, BUONGIORNO-NARDELLI, CIONINI and D'AMATO (1971, Publ. no. 1) using the embryo suspensor cells of Phaseolus coccineus. Therefore, cytological slides of lettuce cotyledons and N.glauca pith tissue have been subjected to hybridization with tritiated rRNA of Phaseolus coccineus with high specific activity (see below). The slides are now under exposure and will be developed during February-March 1972 (NUTI RONCHI, MARTINI and BENNICI).

Some biochemical aspects of cell dedifferentiation are now under study. First of all, a technique has been developed for the production, isolation and purification of tritiated rRNA with high specific acticity. Tritiated rRNA of Phaseolus coccineus, so obtained (180,000 d.p.m. per microgram) has been recently used for cytological rRNA-DNA hybridization in dedifferentiating cells of lettuce and N.glauca and in the polytene chromosomes of Phaseolus coccineus. Moreover, a technique has been developed for the analysis of DNAs by agarose-acrylamide gel diso- electrophoresis. By this method, the DNAs extracted from lettuce cotyledons and N. glauca pith at different times of in vitro culture are being analyzed (DURANTE, PARENTI, BUIATTI and GERI, Publ. no. 4).

The analyses on the cytolocalization of rRNA cistrons in the polytene chromosomes of Phaseolus are being carried out by AVANZI, CIONINI and D'AMATO.

2) Habituation in Nicotiana bigelovii.

Work on habituation in Nicotiana bigelovii (see Report for the year 1970, research line 4) has been continued. The exceptionally high capacity for transformation to autotrophy for hormones (habituation) in Nicotiana bigelovii has led to an investigation on the responses of two varieties (quadrivalvis and bigelovii) to different hormone treatments applied to their seeds sown in vitro. Hormones used were: kinetin (K: 0.8, 1.6 and 3.2 ppm), 2,4-dichlorophenoxyacetic acid (2,4-D: 0.1 and 0.4 ppm), indole acetic acid (IAA: 2 ppm) and naphtalene acetic acid (NAA: 1, 2 and 4 ppm). Callus formation and habituation were scored after transfer on minimal medium (without hormones) 3 months after sowing. The variety quadrivalvis showed a much higher ability to form callus, but the callus formed was autonomous for hormones in a lower percentage of cases when IAA or K treatment was considered. Analysis of auxin-like substances in leaves of the two varietis showed a higher content in N.bigelovii var. quadrivalvis. The observations made are of relevance to the tumorous phenomena in plants (BENNICI, BUIATTI, TOGNONI, ROSELLINI anf GIORGI, Publ. no. 2).

3) Studies on the RNase complement in normal and habituated tissues.

Electrophoretic analyses have been continued on normal and habituated (hormone autotrophic)tissues of Haplopappus gracilis and Nicotiana glauca and on habituated tissue of Nicotiana bigelovii, all grown in vitro (see Report for the year 1970, research line no.2).

Five bands were found at acid pH in both normal and habituated Nicotiana glauca tissues. Alkaline pH tests showed 5 and 4 bands respectively in habituated and normal material.

In Haplopappus normal callus 4 and 1 band were found at acid and alkaline pH. The corresponding values for hormone autotrophic tissues were 1 and 5.

N.bigelovii extracts showed 6 bands at acid pH and 4 at alkaline pH.

These results might have implications in the problems of tumorous transformation in plants, as discussed in previous papers by the same research workers (DURANTE, PARENTI, BUIATTI and GERI, Publ. no. 5).

4) Protoplasts from tissue grown "in vitro" of the tumorous hybrid Nicotiana glauca x N. langsdorffii (see Report 1970, res. line no. 5).

Protoplasts of higher plants can be obtained through enzymatical hydrolisis of the pectic-cellulose wall. They regenerate in a short time a new cell wall, are capable of nuclear and cellular division, can be fused.

Each protoplast can give origin to a callus in which differentiation of buds and roots that eventually develop into flowering plants can be induced.

Protoplasts culture may open up new possibilities in plant breeding and in cellular physiology studies.

To get good results it is necessary to isolate large numbers of protoplasts and to culture them in vitro in the best concentration for the growth.

A new technique is here reported of protoplast isolation from tumorous tissue of Nicotiana glauca x Nicotiana langsdorffii grown in vitro as a modification of that used by Chupeau and Morel for carrot tissue.

The enzymatic mixture used for protoplast production was dissolved in Linsmajer and Skoog medium and contained: 5% (w/v) Onozuka P1500 Cellulase, 5% (w/v) IBF Cellulase, 1% (w/v) Macerozyme and 20% (w/v) sucrose as plasmolysing agent; the pH was adjusted to 5.5 using KOH. The treatment was performed at 30°C for two hours. Protoplasts were released from tissues by mechanical breakage. To isolate,

concentrate and wash the protoplasts contained in the enzyme mediu they were centrifuged (700 g-2'). After this centrifugation we fou about 100% of protoplasts on the surface of supernatant while cell lar debris assembled on the bottom. Later on, a 30-40 mm high laye of Linsmajer and Skoog medium containing 15% (w/v) sucrose and 2.5 (w/v) sorbitol was added and the mixture centrifuged for a second time. The protoplasts floated on the surface in a very high concentration ($5x10^5$ protoplasts/ml); they were collected through a pipette and put into culture (BENNICI and PAGLIAI, Publ. no. 3).

5) "In vitro" regeneration of cauliflower plants.

Success has been obtained in the in vitro regeneration of cau flower (Brassica oleracea) plants (see Report for the year 1970, research line no. 6). Plants have been regenerated from the follow materials:

A) Callus from anthers grown "in vitro". Anthers of 7 inbred lines of Brassica oleracea in different developmental stages have been induced to form callus on Nitsch's medium: from the callus plantlets were differentiated whi are at present growing in pots. Determination of chromosome number in these plants is being carried out. Interesting differences have been detected between degree of inbreeding (2 and 4 years of inbreeding) on one side and callus production and frequency of plants differentiated on the other.

B) Callus from leaf petioles. Callus has been obtained from explan in vitro of leaf petioles; from the callus, plants have been regenerated. The explants have been taken from the same 7 inbred lines used in the experiment with anthers; clear differences between lines in callus production and frequency of differentiated plants have been observed. This interesting aspect is being closely investigated in a large scale experiment (BENNICI, BUIATTI and BARONCELLI).

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Pisa January 18th, 1972

Prof. Francesco D'Amato

A Vertrag Nr.: SC 006/076-69-BIA D

Organisation oder Institution, Ort, Land:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Thema, Titel:

Pflanzenmutation

Kurze allgemeine Darstellung der durchgeführten Arbeiten:

Under project I the possibility to use micro-mutations directly for creating an improved variety shall be investigated. The emphasis will be put on yield mutations. Under subject II it will be tried to remove the undesired pleiotropic effects of macro-mutations in a new genetic background. However, in the new genetic environment, the agronomically valuable character of the mutated locus shall be maintained. The aim is to breed highly productive lines which, in addition, possess the useful part of the original mutation. Under project III an attempt shall be made to breed vigorous and fertile tetraploid barley which behaves physiologically as an alloploid. This goal shall be achieved by (a) structural differentiation of the chromosome complements through accumulation of mutations from recurrent irradiation , (b) repeated intercrossing of a great number of different 4n varieties with such accumulated mutations and by (c) selection.

B Projekt Nr.: I

Titel: Significance of Micro-Mutations

Name der Forscher: Prof. Dr. H. Gaul, Dipl.-Landw. V. Lind

Darstellung der Ergebnisse:

The most advanced experiments in the program "Significance of micro-mutations" reached in 1971 the M_{11}-generation.
Investigations with micro-mutations are long term projects and we reported about results, aspects and problems during the last meetings of our Contact Group. Nevertheless we think, that now some general conclusions with regard to practical aspects can be drawn. (1) Mutagenically treated material of spring barley and winter wheat reaches, although no selection is carried out, about 100 % of the yield of the untreated control. This self improvement seems to be mainly a result of the action of a natural selection. The reason therefore is mainly a reduced vitality of "negative" mutants, resulting in low yielding and little seed production. (2) By selection within mutagenically treated material it is very easy to isolate families, having a yield potential of about 110 % as compared with the untreated but also selected control. (3) A reselection within high yielding mutant families is possible and leads to sub-families, being significantly different in kernel yield and other characters, as for example quality, maturity etc.
These results from our "pure" theoretical investigations encouraged us, to work out a maintenance-breeding program with micro-mutations. A breeder has to exert an intensive maintenance-breeding, to have a continuous increase in the productivity of his variety. Only by this he is able to keep a top place for a longer period, and a promising variety distribution. Micro-mutations are, per definition, "not" distinguishable from the mother variety. It is possible to select those, having only a higher yield, but being not distinguishable for morphological characters from the mother variety by using the standard methods of the official variety board.
There are no clear results up to now, whether chemical mutagens or ionizing radiations are superior for practical breeding purposes like the maintenance-breeding with micro-mutations. Our breeding program starts therefore with M_2-populations, derived from EMS- as well as from X-ray treatments. Out of these M_2-generations single plants are selected and the selection intensity is much stronger as in the conventional maintenance-breeding.

The selected M_2-plants are grown and tested in field trials and the first foundation-stock seed is available after five or six years. This means that an effective maintenance-breeding should set up early enough.

A second cycle of mutagenic treatment and selection can be started on a "higher" yield - or quality level after the improved variety has been stabilized.
Our maintenance-breeding concept with micro-mutations requires special knowledge and experience. Everybody who is going to start with this method should therefore have a connection with an expert. The technical utilization of our concept has been started in 1970 in cooperation with practical breeding firms. We hope to have first results in a few years.

Refering to our general conclusions mentioned above, a reselection within families for high yield is possible and can be used in addition to the selection of families. Besides the desired "true variety" micro-mutants another kind of micro-mutants appears using our maintenance-breeding program. These are easily distinguishable on the plot basis from the mother variety by visual inspection. Such strains can be regarded as a useful side product but should be tested in a completely separate column.

B Projekt-Nr.: II

Titel: Significance of Macro-Mutations

Name des Forschers: Dr. J. Grunewaldt

Darstellung der Ergebnisse:

In the past years the pleiotropic effect of macro-mutations and possibilities of obtaining mutant-character variation have been studied in this program. We showed that it is possible to modify individual characters of a pleiotropic complex independently from each other. So, for example, the short culm and the dense ear of a barley erectoides mutant could be changed by crossing the mutant with an other barley. The intensity of mutant-character variation was thought to depend on the genetic relationship of the cross parent used and the mutant mother line. Crossing experiments however between mutants of the same mother line, having therefore the closest possible genetic relationship, now showed, that mutant character variation occurs also in these combinations We got, for example, in the F_2-generation of an erectoides mutant and a lax mutant plants, having the short mutant culm but an ear as lax as the lax mutant parent.
Following these and earlier results out of our extensive crosses between mutants and barleys out of the world collection, we tend to assume, that the mutant-character variation depends on the difference between the means of individual features of the mutant and the cross parent used. To prove this hypothesis new crosses will be performed. If our hypothesis turns out to be correct, it would be possible to know from the difference of means the greatest possible mutant-character variation. This prediction possibility would further simplify the selection of suitable parents for cross-breeding programs with macro mutants and would make this method much more effective than we can handle it up to now.
In 1971 the analysis of an awn-length controlling suppressor was continued. We found this factor in a cross between an awnless African barley with a short awned mutant, which resulted in F_2-generation in awnless, awned (1 to 1o cm) and very long awned (14 to 17 cm) F_2-individuals. About 1oo single F_2-plant progenies were grown and nearly 14,ooo plants have been analyzed. The segregation ratios found indicate, that our hypothesis, advanced on the F_2-generation results, was right: A dominant suppressor inhibits in homozygous stage the awn development. The plants are awnless like the

African barley. In heterozygous stage there is an awn development possible, depending on awn length factors. The awns are between 1 and 10 cm long, and the individual awn lengths are controlled by one major factor. This is to be seen in the segregations we found in the F_2-plant progenies. In homozygous recessive stage the suppressor does "not" influence the awn length: Plants with very long awns, i. e. 14 up to 17 cm, appear and also such, having awns between 2 and 6 cm length. The last mentioned group includes the awn length promoting factor in homozygous stage like the mutant used. We can conclude that the African barley is awnless only if the suppressor is present in dominant stage but very long awned, if the suppressor is homozygous recessive.
In 1972 crosses for the localization of the suppressor will be started.

B Projekt-Nr.: III

Titel: Diploidization of Autotetraploid Barley

Name des Forschers: Dipl.-Landw. W. Friedt

Darstellung der Ergebnisse:

The aim of this long-term project is to obtain autotetraploid barley plants, which resemblediploids with regard to their meiotic behaviour, genetic segregation ratios and physiology of growth. To reach this "diploidization" our tetraploid material was handled in the same way as we reported already during the last years meeting, namely:
(a) Annually repeated mutagenic treatment of different varieties (and populations) to accumulate chromosome and gene mutations,
(b) multiple crosses of mutagenically treated varieties, to combine structural and genic divergency and to allow recombinations to occur and
(c) selection for seed setting, tillering capacity, earliness and other characters, according to the pedigree method.

The results of 1971, as far as they are already available show, that it is very difficult to overcome the lacking 10 to 15 % up to completely fertile tetraploid barleys, and a lot of work has to be done before the diploidization has been reached.
In a second cycle the meiotic behaviour of the best fertile strains will be analysed. Further more preliminary investigations will be started to study possibilities of influencing the fertility through changed environmental conditions.
Besides this work new varieties have been treated with colchicin to increase the number of rough tetraploids and by this have a broader genetic base for our diploidization program.

Contract EURATOM-ITAL-CNEN Laboratory for the Applications of Nuclear Energy to Agriculture

Annual report of activities for 1971

Genetical effects of irradiation on ontogenetic stages in higher plants.

1. After the researches performed at Casaccia on the biological and genetical effects of irradiation on different ontogenetic stages in higher plants (barley, durum wheat, tobacco, peas and tomato), a programme started in 1969 to study the effects of radiation treatments on genic recombination between groups of marker genes. In cross breeding experiments, the possibility of altering the recombination frequencies in the F_1 plants is of much value for the plant breeder and the programme aimed to see if acute or chronic irradiation of F_1 plants could be a technique to be applied.

1.1. In Pisum, chronic irradiation of F_1 plants heterozigous for ten genes has been performed in gamma field and the F_2 plants have been analyzed in greenhouses and in field according to the characters. The recombinations in the following groups of linked genes were studied: s-wo-k (chromosome II), b-st (chromosome III) and r-tl (chromosome VII). Some of the results are reported in table 1: after irradiation of F_1 plants, an increase of the recombination values has been found among genes of the chromosome II.

Increase in recombinations after chronic radiation treatment is in agreement with results in the literature obtained with acute treatments. A different heterochromatin content in the pea chromosomes can determine the inter-chromosomal differences in radiation responses.

1.2. In Tomato (Lycopersicum esculentum Mill.), the influence of irradiation on the recombination process has been studied through acute treatments of buds of different lenght in F_1 plants heterozygous for the marker genes localized in chromosome II. The F_1 plants were of the following constitution: $\frac{d\ suf\ Aw}{D\ Suf\ aw}$; three exposure rates were applied (100 R, 200 R and 400 R). Relations between the bud's lenght and the developmental stage of the flower were reported in the report of the last year: near all the buds shorter than 3.4 mm are in premeiotic stage, while 81% of the buds longer than 4.5 mm are from the second meiotic division to pollen grains; the leptotene or pachitene stages are mainly present in buds of 3.5 - 4.4 mm. Table 2 reports the recombination values found on near 100.000 F_2 plants coming from unirradiated and irradiated F_1 plants: premeiotic irradiation seems to increase the recombination values, while treatments of the meiotic and after meiosis stages decrease the recombination rate. It seems also that the recombination frequencies decrease with the increasing of the exposure rates.

Also in tomato, an analysis has started on F_2 seedlings coming from F_1 plants $\frac{d\ suf\ Aw\ Me}{D\ Suf\ aw\ me}$ and irradiated acutely at the zygote (4000 R) and seed stages (20 kR) - the recombination values among the four genes will be taken into account.

2. By gamete treatment, a very high frequencies of variegated M_1 plants was found in pea, tomato and durum wheat. Such M_1 plants were characterized by the presence of unstable chromosomes (dicentrics or rings), the behaviour of which has been studied and the results reported in previous reports. From such individuals, trisomic plants were originated. As no trisomics were known in the world collection of Pisum, genetic and cytological studies were carried out on the five trisomics so far isolated.

2.1. The biometrical analysis (table 3) shows that each of the five trisomics differs from the others and from the diploid mother line (cv. Parvus). The primary nature of the trisomy has been also ascertained by the cytological data. The transmission frequencies of the trisomy after selfing and after backcrossing are reported in table 4: an average of 24% of transmission was observed.
Only trisomic P251 shows to transmit itself via pollen and in fact two tetrasomic plants have been obtained in its progenies. Crosses of each trisomic with the mother line and with marker lines have been completed and the analysis of the F_2 material is in course in order to have genetic evidences for the identification of the extra-chromosomes.

2.2. Always to isolate other aneuploids, an X-ray treatment of pollen was performed in the canning cv. Sprinter. In 1971 the M_2 plants coming from the three M_1 variegated plants with unstable chromosomes were analyzed and the results are reported in table 5: M_2 plants with dicentric, telocentric and both abnormal chromosomes have been found. Plants with

$2n$ = 14 chromosomes will be carefully analyzed in order to find morphological differences due to a possible presence of duplicated chromosome segments coming from the breakage-fusion-brid cycle of the unstable chromosomes.

3. A preliminary agronomic trial has been performed at Casaccia to check the practical value of the mutant M86 obtained by gamete treatment on the cv. Moneymaker. Experimental data are reported on table 6: it seems that such brachytic mutant can be utilized for intensive cultivation in fields.
Brown seed mutants obtained in Moneymaker in previous radiogenetic experiment are under investigation to see their importance as markers of hybrid seeds.

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F_1 plants	RECOMBINATION				
	r-tl	s-wb	wb-k	s-k	b-st
irradiated with 25 R/d	5.59± 0.43	14.38± 1.05	18.60± 1.16	23.74± 1.38	28.07± 2.03
unirradiated	6.04± 0.41	9.49± 0.86	14.36± 1.04	20.97± 1.30	28.92± 1.49

Table 1 - Recombination values (%) among linked marker genes of Pisum estimated on F_2 plants from unirradiated and chronically gamma-irradiated F_1 plants. Marker genes in coupling phase.

Table 2 - Recombination values(%) among three marker genes located on chromosome II after gamma irradiation of buds of different lenght belonging to F_1 plants $\frac{\text{d suf Aw}}{\text{D Suf aw}}$.
(data from the analysis of 95840 F_2 plants)

Irradiated buds (mm)	d suf				suf aw				d aw			
	100R	200R	400R	Mean	100R	200R	400R	Mean	100R	200R	400R	Mean
1.8 - 2.4	6.74	-	4.64	5.99	13.13	-	12.03	12.59	8.44	-	15.35	11.01
2.5 - 3.4	5.11	4.71	3.47	4.26	11.63	8.14	6.33	8.69	11.22	11.83	12.03	11.22
3.5 - 4.4	4.78	4.41	3.75	4.10	8.99	7.62	4.40	6.41	9.43	12.21	8.41	9.81
4.5 - 5.5	4.33	4.01	3.84	4.01	7.49	8.12	5.72	6.89	6.81	12.22	9.71	10.12
Mean of irradiated material	4.98	4.26	3.75	4.18	10.12	7.97	5.76	7.56	8.66	12.03	10.12	10.35
Mean of control				4.64				7.09				10.35

Material	Leaflets lenght/width	Flower lenght of wings mm.	Flower width of vexillum mm.	Flower lenght of calyx mm.
Control	1.339±0.019 a	16.600±0.561 a	25.500±0.427 a	12.400±0.162 a
P.251	1.322±0.049 a	25.400±0.451 b	34.300±1.075 b	16.400±0.370 b
P. 17	1.187±0.031 b	21.100±0.481 c	27.100±1.026 a	12.200±0.249 a
P.133	1.342±0.001 a	25.777±0.146 b	33.444±0.579 b	15.333±0.333 b
P.111	1.612±0.033 c	15.666±0.421 a	18.666±0.210 c	9.666±0.210 c
P. 12	1.206±0.019 d	———	———	———

Table 3 - Some biometric data of five pea trisomic lines (data followed by the same letter are not significatly different from the others) (P= 0.01).

Material ♀ ♂	Plants analyzed n°	Trisomic plants %
P 251 - selfed	20	20.00
P 251 x Parvus	169	18.9
Parvus x P 251	38	2.63
P 17 - selfed	28	28.60
P 17 x Parvus	29	27.58
Parvus x P 17	33	0.00
P 133 - selfed	35	25.71
P 133 x Parvus	32	31.2
Parvus x P 133	-	-
P 111 selfed	25	28.00
P 111 x Parvus	28	28.57
Parvus x P 111	59	0.00

Table 4 - Rate of trasmission frequencies of the trisomy in four trisomic line of Pisum.

M_1 variegated plant	M_2 plants analyzed n°	Plants with 2n =			
		14	13 + 1 dicentric	13 + 1 dicentric + 1 telo.	14 + 1 telo.
A2	5	3	-	-	2
A31	7	3	2	1	1
A54	11	2	6	2	-

Table 5 - Segregation of M_1 variegated mutants characterised by genome with 2n= 12 + 1 dicentric + 1 telocentric chromosome.

Table 6 - Height, yeld per plant and characteristics of the fruits of the radioinduced mutant M86 and of the Moneymaker control(mean values with the same letter in the same column are not significantly different at level of P=0.05 according to Duncan test)

Material	no. plants for sq.mt.	no. of grown plants	height cm	Total yeld per plant (g)	Diameter of the fruit equatorial (cm)	Diameter of the fruit polar (cm)	fruit weight (g)
M 86	2,38	94	33,25 a	288,5 a	4,85 b	4,42 b	45,80 c
"	3,75	144	36,46 a	338,2 ab	4,72 ab	4,18 a	45,65 c
Moneymaker	2,38	96	95,42 b	378,5 b	4,68 ab	4,47 b	43,32 b
"	3,75	144	78,25 b	318,3 ab	4,54 a	4,21 a	40,00 a

CONTROL OF THE ONION FLY, Hylemya antiqua (Meig.), USING THE STERILE MALE TECHNIQUE

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General

In 1971 the activities of the onion fly team have been concentrated mainly on a first field trial, in which sterilized insects have been released in an isolated onion fly population in order to evaluate the influence of the released flies on the reproduction of the population. The field trial could only be carried out thanks to an intensive cooperation and a great enthousiasm. J. Theunissen was in charge of the coordination of the field experiment during the absence of J. Ticheler, who advised for six months for IAEA the Government of Madagascar on the possibilities of genetic control of insect pests for that country. An important part of the field observations has been done by W. Kelderman (student of Agric. University, Wageningen), who, besides, studie the induction of diapause. Through the common effort of every member of the team, an impressive amount of data has been collected, which will be used also for a simulation model that is being built in cooperation with C. Wijrands-Stäb and M.J. Frissel (ITAL). Thanks to the great cooperativeness of the ITAL, onion fly pupae could be sterilized every week by irradiation in total 550.000 pupae.

A specific attractant would be of great use for population studies and for following a release programme. P. Bennett and W. Wientjes (Centraal Laboratorium TNO, Delft) investigate the possibilities for such a substance.

The Federation of onion growers, SNUIF, Middelharnis, offered again hospitality and assistance which facilitated the study of population dynamics.

The field experiment

By the end of 1970 an onion fly population was built up on an isolated onion field of 1 ha, planted at the Schuilenburg, Lieńden. In 1971 onions have been sown on an adjacent 1 ha plot. On the basis of the estimation of the population size and of the available data from the phaenological studies, a curve has been constructed for the release of the sterilized flies.

The result of the release has been determined by means of a number of partly independent criteria a.o.:

1. the emergence of the sterilized flies
2. the numbers of flies actively flying in the field; of these flies we determined:
   a. the sex ratio
   b. the fertile/sterile ratio in the females
   c. the mating percentage of these females
3. the ratio fertile/sterile in the eggs laid by the fertile females
4. the number of infested plants
5. the number of pupae of the first and of the second generation; from these pupae:
   a. the percentage pupae in diapause.

Weekly sterilized pupae have been dug into the field. The emergence of the pupae was satisfactory: 80-90%. The expected 20-fold overdose of

sterile flies, however, has not been realized. In the field traps we found at the maximum a 5-fold excess. This might be attributed to an excessive mortality among the sterile flies in the early stages. This could be explained by the fact that the mass rearing in this period produced pupae with a rather low average weight.

The sex ratio among the trapped flies was roughly 1 : 1. The ratio fertile/sterile flies could, but for exceptions, namely when the sterile flies had been labeled with radioisotopes or dyes, be determined anatomically in the females. It is reasonable to accept that the ratio found in the females applies equally to the males. Both sterile and fertile females had mated for about 70%. The eggs laid in the laboratory by the trapped females come from the fertile females only. Sterilized females have undeveloped ovaria and are unable to lay eggs. The ratio fertile/sterile eggs in these batches reflects the mating activity of the fertile and of the sterile males. The ratio for the eggs is, during the first flight period, closely correlated with the ratio fertile/sterile flies as they had been trapped in the field. This shows that the active flying sterile males were competitive with the fertile males. The favourable picture of the first flight was not repeated during the second flight period. The fertility of the eggs was much higher than was to be expected on the ground of the fertile/sterile ratio among the trapped flies. We think that the released flies were affected much stronger by the parasitic fungus Entomophthora spec. The concentration of the young -and then most susceptible- sterilized flies on a few spots could have increased the chances of infection relative to the fertile flies, which emerge dispersed over the whole field.

As in the rest of the Netherlands, 1971 was a year unfavourable for the development of the onion fly. Notwithstanding the considerable numbers of flies emerging from the overwintering pupae, little damage to the crop occurred. The dry spring, unfavourable for eggs and young larvae, is probably responsible for this. The damage on the experimental field, compared with the check plots, can therefore not be used as a measure for the result. The damage did not exceed 5%.

From the hatching of the egg batches we could deduct that the majority of the fertile flies catched in the field had mated only once.

The experiment will be continued in 1972. Special attention will be given to the improvement of the quality of the reared insects, a.o. by starting the mass rearing of a strain freshly collected in the field. We will aim at a method to disperse the sterilized pupae more evenly over the field.

The maximum estimate for the population of hibernating pupae is somewhat smaller than last year's, notwithstanding a less intensive sampling. The conditions for a second field experiment are, therefore, favourable.

Mass rearing (J. Noorlander)

Paiting of cold room in october 1969 caused a very severe mortality among the stored pupae. 25% only of the pupae survived, and it is not known whether these suffered from lasting - genetic or other- damage. The offspring of these pupae have been used in 1971 fieldtrial, where their vitality appeared to be sub-normal. In order to get a better strain, infested onions have been collected in august 1971 in Flakkee, from which some 3000 pupae have been obtained. We intend to multiply this strain (E) for use in the 1972 field experiment.

Pupae which have been formed without an absorbent substrate, as was the practice in 1970, cannot be stored for a long period without considerable losses. The larvae, kept in glassjars in a humid atmosphere, made their surroudings and themselves filthy with excretion products and excrements. In such an environment they probably cannot form a good pupal skin, with the result that the pupae, during long storage, lose gradually too much water and dry out. Vermiculite as pupation substrate prevents this phenomenon. Addition of 8% (by volume) of water to the vermioulite creates an environment with a high relative humidity. The excretion products can easily be absorbed by the vermiculite.

For the preparation of the larval diet, we changed from lumps of dried carrot for livestock nutrition to slices of dried carrots for human consumption. This resulted in a more constant quality of the medium. Moreover the chance of contamination with insecticide residues decreased.

The comparison of flies reared for many generations in the laboratory with wild flies on vitality and behaviour could not yet be realized. These tests are laborious and require, when automated, expensive equipment.

An important step towards a week rhythm in the rearing is the possibility of storage of eggs up to seven days in a refrigerator, without undue mortality. This permits to reduce work in the weekends drastically.

We started rearing a fly strain with an easily distinguishable marker. To be useble this "green eye" strain should be tested on its vitality and behaviour. For reasons stated above, this has not yet been realized.

Induction of diapause (W. Kelderman)

In a series of experiments the influence of the temperature on the induction of diapause has been studied under long day conditions (16½ hrs.). During larval development and during the beginning of pupal formation, combinations of high (18°C) and low (12°C) temperatures have been given. Low temperature during the second and third larval stage promoted induction of diapause slightly; low temperature during the prepupal stage and the first three days of the pupal stage induced diapause very strongly. At temperatures above 18°C no diapause was induced. Lower temperatures induced up to 60% diapause.

At 16°C a photoperiod of 14 hours gave 85% diapause and 10 hours gave 95%.

The effect of temperature (3, 6, 10, 15 and 20°C) and time of storage (5, 10 and 15 weeks) on the breaking of diapause is being studied.

Histopathological studies (J. Theunissen)

The major part of the available time has been devoted to the planning, excution and analysis of the field experiment.

In cooperation with J.Ph.W. Noordink, the current studies on spermatogenesis and oogenesis in relation to the factor time were continued, using autoradiographic techniques.

Pupae have been irradiated, at the usual age, with 0 (check), 0.5, 1.0, 1.5 and 2.0 KR of hard X-rays. The emerging flies form the material for the study of histopathological effects on the cell-populations in testes and ovaries after irradiation with low doses. At the sterilizing dose of 3 KR, very characteristic changes occur in the cellpopulations which together form the testis. Moreover we investigate at which dose recovery of the cellpopulations occurs.

After irradiation with 3KR the development of the ovaries is strongly inhibited. This phenomenon should be less pronounced and might probably be better quantified and related to dosis after irradiation with low doses.

During spring, at the request of J. Noorlander, we checked regularly the females in the production cages of the mass rearing, in order to get information on development, activity and health of the flies. On the seventh and fourteenth day after the emergence of the first fly a rand random sample of ten females was taken. In the females we checked for the presence of sperm, the constitution and development of the ovaries, the number of ovarioles per ovary, the condition of the intestine and the appearance of the abdomen. These observations revealed that by the seventh day 6% only of the females had mated, by the fourteenth day this was 87%. The number of ovarioles per ovary was (average of 293 ovaries) 23.4 ± 3.9.

In order to obtain a quicker and more accurate estimate of the sterile/fertile ratio and, if possible, of the age distribution in the field population, it would be desirable to use not only the females but also the males. Therefor we studied the possibility of separating the males, using various criteria, into irradiated and normal males and of the age determination. Histologically, irradiation damage can be easily distinguished, but this is a procedure too time consuming and too intricate to be feasible for large numbers of flies. The criteria which could be used are: form, colour and dimensions of the testis and the appearance of the chromosomes in simple squash-preparations. Discrimination on the basis of each of these criteria was not precise enough for a clearly discriminating method, which would be practicable for the field experiment.

Aspects of the population dynamics M. Loosjes

The field studies suffered because of lack of assistance and because of the time devoted to the field experiment described above. On the other hadn the experiment yielded some interesting data relevant to this particular project.

Methods

The method for trapping the flies which we developed has been applied on a larger scale and gave satisfactory results. As was the case last year, with a similar activity dependent trapping method, the sex ratio appeared to be dependent on the age of the flies.

Sampling flies with a suction trap proved to be generally unpracticable. Time lacked to develop another sampling method.

Marking the flies at emergence has been further developed; if carefully applied, this method is satisfactory. Daily change of the dye layer, permitting the determination of the age of the recaptured flies to one day, has been applied succesfully in the field. Part of a group labeled with ^{65}Zn has also been provided with a dye-mark, in order to detect a possible deleterious effect of the colour label. The results of this experiment have not yet been analyzed.

Further study of labeling and catching methods has been continued on a limited scale.

Dispersal

Catches of onion flies in the field indicated a strong preference of the flies for verges and edges of onionfields. Preliminary results of an experiment in which 22,000 labeled flies emerged in two spots, show a relatively strong influence of the topography (ditches, dikes) on the dispersal of the flies.

The preliminary impression from a series of recordings of the behaviour of onion flies in an onion field is that their flights seldom surpass a distance of a few decimeters, and that there is a difference in the flight activity of the sexes. The presence of the observer does not seem of importance.

As in last year, the onion fields of a part of Flakkee have been mapped, in order to determine the density of onion fields and the distances between onion fields. These data permit also the choice of experimental sites for 1972.

Tracer methods J.Ph.W. Noordink

In order to recognize a labeled fly several months after the application of the isotope, ^{65}Zn has been tested. In january larvae have been reared on a diet containing 10, 40 and 90 μCi ^{65}Zn per 200 g. diet respectively. The pupae obtained were easily recognizable. After storage for eleven months at 3°C, these pupae gave rise, at a higher temperature, to flies which were still radioactive. This was shown by autoradiography. The flies reared on the highest isotope concentration, could also be distinguished from unlabeled flies using a Geiger-Müller tube.

For the sterile-release experiment several thousands of flies have been labeled with ^{32}P and ^{65}Zn. The first group, used for the study of life span and dispersal, was sterilized, while the second group has been released without sterilisation, in order to estimate the size of the natural population.

As onions are grown in a crop rotation, with the result that onions are never grown for two consecutive years on the same plot, it is important to know where the mating of the onion fly takes place, in last years onion field, where the flies hibernated as pupae, or in the new onionfield, where the flies come to deposit their eggs. In order to get better informed on this matter, we carried out two experiments, on the grounds of the IPO, in which ^{32}P labeled males were released together with not labeled females. Five flight interception traps have been installed at distances varying from 10 to 40 m from the release point. These traps have been checked daily. The captured flies were sexed, and the females were autoradiographed. Radioactivity meant mating. In the first experiment 1,472 radioactive males and 1,677 females were released, of which 42 males and 21 females were recaptured. Of these 21 females 20 had mated. In the second experiment 11,020 radioactive males and 11,850 females were released of which 73 and 60 were recaptured respectively. Of these females 35 had mated. One can conclude that a major part of the females mates before they start to search for an onion field. In 1972 a similar experiment will be held, but then close to an onion field.

The following experiments have been started in 1971, but the results will not be known before 1972:

1. Mating experiment, in which relative importance of the first and of the following inseminations on the fertilisation of the eggs is investigated.
2. The duration of spermatogenesis in the onion fly.
3. Neutron activation. As the determination of the sterile/fertile ratio in field releases is of prime importance, a method permitting the labeling of all sterile insects would be very useful. As both dyes and isotopes have drawbacks, experiments are under way to label flies with non radioactive substances. These substances become radioactive upon irradiation in the ITAL reactor. This permits distinction, in the catches, between reared and wild specimens.

Phenological observations L.E. van 't Sant

The results of the 1971 phenological observations carried out at Middelharnis and Wageningen show some similarity. At Wageningen the first fly was trapped on april 23rd, which is rather early (only once a fly emerged earlier, on april 20th). At Middelharnis where only few observations were carried out before may 5th, one was rather surprized by the considerable number of flies found in the depot on may 5th.

The peak of the emergence coincided for both localities on may 7th. At Middelharnis the emergence lasted longer than at Wageningen, till june 14th and may 25th respectively. At Middelharnis a second peak can be distinguished on may 26th. This peak, however, is due to the emergence from one depot only. Twelve depots have been installed at Middelharnis and ten at Wageningen. They had been installed in 1971 with pupae or larvae collected on different dates.

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TÄTIGKEITSBERICHT FÜR DAS JAHR 1971

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STRAHLENANALYSE IM LANDBAU

Die Anwendung von Radionukliden, radiometrischen Meßverfahren und Röntgenstrahlen in der Agronomie führt, wie in anderen Gebieten der Wissenschaft, zu vielseitigen Verbesserungen und Vervollkommnungen der herkömmlichen Methodik und eröffnet neue Möglichkeiten. Die Strahlenanalyse im Landbau geht davon aus, nukleare Meßverfahren bei der Lösung der sich dem Landbau künftig stellenden Aufgaben einzusetzen.
Der vorliegende Bericht betrifft insbesondere die Erarbeitung von kerntechnischen Meßmethoden, die bei der Beurteilung der Wachstumsbedingungen von Wild- und Kulturpflanzen hinsichtlich des Wasserhaushaltes des Bodens im Zuge einer besseren Nutzung der Anbaugebiete von großer Wichtigkeit sind. Daneben wird aber auch auf ein Verfahren eingegangen, das für die Landschaftspflege von erheblicher Bedeutung ist.

Im Berichtszeitraum wurden folgende Projekte bearbeitet:

1. Zur Evaporation im ariden Lößboden während der nächtlichen Temperaturinversionsphase.

2. Kontinuierliche Bestimmung des Wasserdampfes im Boden mit Hilfe einer Tritiummarkierung (Vom Grundwasserbereich ausgehende Wasserdampfbewegung).

3. Entwicklung einer Methode zur Bestimmung der Feuchtigkeit in dünnen Bodenschichten mit kapillar-porösen Körpern.

4. Untersuchungen zur Diagnose und Therapie von Holzfäule in lebenden Bäumen.

Projekt Nr. 1

Zur Evaporation im ariden Lößboden während der nächtlichen Temperaturinversionsphase.

W. Kühn, M. Elmdust

Es ist bekannt, daß in ariden Gebieten täglich Temperaturinversionen auftreten. Da diese auch die oberflächennahen Bodenschichten beeinflussen ist es wahrscheinlich, daß mit dieser regelmäßigen Umkehr der Temperatur durch Kondensation ein Feuchtigkeitstransport aus der Atmosphäre in den Boden verbunden ist. Dieser darf beim Wasserhaushalt des Bodens d.h. bei der Wasserversorgung von Irrigationskulturen nicht unberücksichtigt bleiben. Kontinuierliche Temperaturmessungen im Wüstenboden haben gezeigt, daß die Inversion noch 50 cm unterhalb der Bodenoberfläche nachweisbar ist. Vermutlich trägt daher die nächtliche Temperaturumkehr bei rel. tiefer Temperatur und hoher rel. Luftfeuchtigkeit zur Wasserversorgung des Bewuchses bei. Damit ergibt sich unter Anwendung von speziellen natürlichen Bewässerungssystemen die Möglichkeit, Pflanzen in Wüstengebieten trotz spärlicher Niederschläge zu kultivieren. Mit der nächtlichen Inversionsphase tritt auch ein vom Bodeninnern nach der Oberfläche hin gerichtetes Temperaturgefälle von ca 10-12°C auf, durch das u.U. auch eine gegenläufige Evaporation einsetzt. Ein Verlust an Feuchtigkeit in den oberen Bodenschichten wäre die Folge. Um zu prüfen wie bedeutsam dieser Verlust ist, bzw. bis in welche Tiefe er sich in der zur Verfügung stehenden Zeit der nächtlichen Temperaturumkehr auswirkt sind in Zusammenarbeit mit dem Department of Botany der Hebrew University of Jerusalem erste, orientierende Versuche ausgeführt worden, die über die Evaporation aus vorgegebenen Bodentiefen Auskunft geben. In einen Wüstenlößboden mit definierter Feuchtigkeit wurden zu diesem Zweck in vorgegebenen Tiefen, in getrennten Versuchszeiten, mit Tritium markierte Schichten gleicher Feuchtigkeit eingebracht. Zwischen 10 cm Tiefe und der Atmosphäre wurde eine

konstante Temperaturdifferenz aufrechterhalten. Dieser Temperaturgradient simuliert die nächtliche Inversionsphase. Wird nun die Atmosphäre über dem Boden mit trockenem Stickstoff durchströmt und führt man diese in kontinuierlichem Durchfluß durch ein geeignetes Proportionalzählrohr hindurch, dann kann sowohl der Zeitpunkt des Beginns der Evaporation aus der betreffenden Schicht als auch die verdampfende Wassermenge aus dieser Tiefe gemessen und von anderem verdampfendem Wasser unterschieden werden.
Da die nächtliche Inversionsphase etwa zu 8 Stunden auszusetzen ist, ist dieser Zeitraum im Vergleich zum ermittelten Evaporationsbeginn aus größerer Tiefe rel. kurz und es ergab sich, daß offensichtlich aus Schichten unterhalb 3 cm Tiefe keine nennenswerte Verdampfung während der Nachtzeit stattfindet.
Diese Untersuchungen werden unter besonderer Berücksichtigung der klimatischen Bedingungen, wie sie in der Negev-Wüste vorliegen weitergeführt.

Projekt Nr. 2

Kontinuierliche Bestimmung des Wasserdampfes im Boden mit Hilfe einer Tritiummarkierung.

M. Elmdust

Die Wasserdampfbewegungen im Boden sind auch heute noch nicht völlig geklärt. Es wurden daher die im vorausgegangenen Berichtszeitraum beschriebenen Untersuchungen über den aus der Atmosphäre in den Boden eindringenden Wasserdampf auf Versuche zur Messung des aus tieferen Schichten des Bodens, nämlich auf den aus dem Bereich des Grundwassers aufsteigenden Wasserdampf ausgedehnt. Dabei hat sich gezeigt, daß wahrscheinlich neben dem in früheren Versuchen festgestellten, aus der Atmosphäre eindringenden Wasserdampf, auch ein vom Kapillarsaum des

des Grundwassers aufsteigender geringer Anteil in der Wurzelzone der Pflanzen wirksam ist.

Das Vorkommen dieses bislang nur schwierig nachzuweisenden Teils des Wassers als Dampf in größeren Bodentiefen könnte durch Markierung des Grundwassers mit Tritium ermittelt werden.
In einer Bodensäule von 50 cm Durchmesser wurde während der Versuche ein konstantes Grundwasserniveau in einer Tiefe von 120 cm aufrechterhalten und oberhalb des Kapillarsaums wurden Dampfsammelröhren in den Boden eingebracht, so daß der aus dem Grundwasserbereich aufsteigende markierte Wasserdampf in verschiedenen Tiefen durch kontinuierlichen Durchfluß durch ein spezielles Proportionalzählrohr gemessen werden konnte. Um eine Wasserdampfbewegung innerhalb der Bodensäule zu erzeugen, waren der Natur entsprechend, verschiedene Temperaturgradienten zwischen Bodenoberfläche und Grundwasser hergestellt worden.
Die Ergebnisse haben gezeigt, daß sich das Wasser in Dampfform unter dem Einfluß des Temperaturgefälles zwar von unten nach oben bewegt, daß aber diese Bewegung im wesentlichen nur innerhalb des Kapillarsaumes stattfindet. Bis etwa 50 cm oberhalb des Grundwassers ist eine Wasserdampfbewegung nachweisbar, die nur noch in Spuren darüberhinaus reicht, aber immerhin noch von Bedeutung für bestimmte Pflanzen sein kann. Der mit dem Grundwasser gerade noch in Verbindung stehende Bereich des Bodens in 70 cm Tiefe (am Rande des Kapillarsaums) lieferte Werte von etwa $4 \cdot 10^{-4}$ mm H_2O/24 h bei einem Temperaturgradienten von 22°C, zwischen der Bodenoberfläche und 120 cm Tiefe während in 50 cm Tiefe nur noch etwa $1 \cdot 10^{-5}$ mm H_2O/24 h nachgewiesen werden konnten. Bei Temperaturgradienten um ca 18°C lassen sich in dieser Tiefe lediglich noch 10^{-6} mm H_2O/24 h feststellen. Daraus ist zu schließen, daß insbesondere der Kapillaraufstieg als solcher bei der Wasserdampfbewegung aus dem Grundwasser von Bedeutung ist. Insofern wird daher zumindest für die simulierten Temperaturgradienten zwischen

Δt = 22°C und Δt = 18°C, die Auffassung verschiedener Autoren, daß nämlich die Wassermenge unterhalb der Frostzone aus dem Grundwasser stammt, nicht bestätigt.

Auf dem landwirtschaftlichen Versuchsgelände der Technischen Universität Hannover wird aufgrund dieser in Laborversuchen erhaltenen Ergebnisse in Zusammenarbeit mit dem Institut für Meteorologie der Universität zur Zeit eine Freilandversuchsanlage installiert, die Aufschluß über den vertikalen Wasserdampftransport im Boden unter natürlichen Bedingungen erbringen soll.

Projekt Nr. 3

Entwicklung einer Methode zur Bestimmung der Feuchtigkeit in dünnen Bodenschichten mit kapillar-porösen Körpern.

H. Glubrecht, W. Kühn, H.P. Schätzler

Das Ziel dieser Entwicklung war der Bau einer Meßsonde zur Bestimmung der Bodenfeuchtigkeit mit hohem räumlichen Auflösungsvermögen und hoher Empfindlichkeit bei rel. geringen Feuchtigkeiten. Der Einsatz der bekannten radiometrischen Meßverfahren ist aufgrund dieser Forderungen kaum möglich; weil z. B. mit Neutronen wegen der erforderlichen Bremslänge bis zur Thermalisierung bei geringem Wassergehalt kein hohes räumliches Auflösungsvermögen erreicht werden kann. Ebenso ist die Messung des gesamten Flächengewichts durch Absorption von Gammastrahlung in diesem Falle problematisch, weil hohe Empfindlichkeiten mit langen Meßweglängen, die zusätzlich kolliniert werden mußten, zu erkaufen sind. Daher wurde die Kombination eines kerntechnischen und eines klassischen Verfahrens vorgezogen. Dieses, auf der Grundlage der Differenz des Kapillarpotentials des Bodens und eines kapillar-porösen Körpers arbeitende Meßverfahren, wird mit einer Absorptionsmessung mittels Gammastrahlung so kombiniert, daß die Geschwindigkeit des Wasserein- oder -austritts in den Meßkörper durch die Absorptionsmessung registriert werden kann.

Das Verfahren ist im Berichtszeitraum ausführlich beschrieben worden.

Ein Prototyp wurde im Jahre 1971 gebaut und bei der IV. Atomkonferenz in Genf (Sept. 1971) erstmals vorgeführt.

Die praktische Erprobung des Meßverfahrens unter Feldbedingungen ist Gegenstand weiterer Versuche.

Projekt Nr. 4

Untersuchungen zur Diagnose und Therapie von Holzfäule in lebenden Bäumen.

J. Handl, W. Kühn

Wie vorausgegangene Untersuchungen gezeigt haben, ist es möglich, mit Hilfe von Röntgenaufnahmen den Zustand im Innern eines lebenden Baumes zu erkennen. Der röntgenologischen Diagnose von Krankheiten in Pflanzen, insbesondere dem Erkennen von Holzfäule in Stämmen älterer, noch lebender Bäume kommt deshalb erhebliche Bedeutung zu. Bei diesen Aufnahmen werden die von Holzfäule befallenen Stammpartien im Vergleich zu gesundem Holz durch mehr oder weniger stark geschwärzte Streifen hervorgehoben, so daß sie recht gut gegenüber nicht befallenen Bereichen erkennbar sind.

Zum Zweck der Aufnahme von Belichtungsdiagrammen für unterschiedliche Baumdurchmesser und für Untersuchungen zur "Fehlerkennbarkeit" im Innern eines Baumes wurde ein Baumphantom angefertigt, dessen Durchmesser zwischen 1o und 5o cm variiert werden kann. Mit diesem Phantom können die in der Praxis auftretenden Parameter simuliert werden.

Gleichzeitig wurde geprüft, ob mit dem Holzabbau im kranken Material Mangelerscheinungen oder besondere Konzentrationen in bestimmten Elementen gegenüber gesundem Holz verbunden sind. Hierzu sind Fichtenholzproben von der Biologischen Bundesanstalt für Land- und Forstwirtschaft Hann. -Münden, die von bekannten Fäulniserregern befallen waren, aktivierungsanalytisch untersucht worden. Die Resultate weisen darauf hin, daß offensichtlich die kranken Bereiche einen höheren Mangangehalt haben als die gesunden. Andere Untersuchungen haben ebenfalls gezeigt, daß Mangan den Stoffwechsel der die Rotfäule hervorrufenden Erreger fördert. Um zu prüfen, in wie weit tatsächlich ein Zusam-

menhang besteht zwischen Holzfäule und Mangankonzentration, beziehen sich die Arbeiten nicht mehr nur auf das diagnostische Verfahren, sondern sie wurden auf Fragen ausgedehnt, die sich mit den Ursachen der Rotfäule beschäftigen, um gegebenenfalls ein therapeutisches Verfahren entwickeln zu können.

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ANNUAL REPORT OF ACTIVITIES FOR 1971

Contract Euratom - CNEN 083 BIOI

1. In vitro culture of higher plants for radiobiological and radiogenetical researches.

1.1. Anther cultures of Nicotiana tabacum in order to obtain haploid plant formation by androgenesis.

Anther cultures of Nicotiana tabacum have been carried out with success on different cultivars :

a) Virginia Bright Kusaka Mammuth : more than 30 haploid plants are actually in the greenhouse and part of them are already at the phase of chromosome doubling by in vitro culture of haploid stem disk.

b) Three cultivars of Burley, chosen among the most interesting for their agronomic value, also produced haploids by anther culture; 24 plants of each cultivar are in observation in the greenhouse.

c) In order to analyse the possibility to utilize the microspore of an isogenic line as test-material to detect a mutagenic agent, we have irradiated by X-rays, the microspores of an isogenic line of N. Tabacum cv. Xanthy-Yaka with low (5-10-20 R.) and high doses (500-1000-2000 R.). The anthers were cultured in vitro immediately after irradiation. This material, perfectly stable from a cytological point of view and perfectly homogeneous as far as the genetic constitution is concerned, would be the most interesting to use as biological test to detect a mutagen.

d) In order to observe the first steps of the embryoid formation, an analysis was performed in the anthers of an isogenic line of N. tabacum at both the cytological and the histological levels.

The conclusions of this research are :

1) The best stage for inducing embryogenesis of the microspore is confirmed as being the haploid mitosis which separate the generative and the vegetative cells in the gametophyte;

2) the degeneration of the tapetal cells in the anther takes place at the time of haploid mitosis. It is therefore difficult to find out whether it is this last event, the degeneration of the tapetum or the synchrony of the two events in the anther of tobacco which facilitates embryoid formation;

3) the divisions in the embryoids take place after the differentiation of the two nuclei (generative and vegetative) but, practically, the generative cell does not go through more that two mitoses while the vegetative one continues and gives rise to the embryoid. The generative nuclei are therefore practically eliminated;

4) the polarity fo the embryoid in vitro is very delayed as compared with the tobacco embryo "in vivo" where, already at the stage of bicellular proembryo, the polarity is well visible;

5) the embryoid in vitro has no suspensor;

6) the dispersion of the embryoids in the anther is absolutely casual;

7) embryogenesis is also very limited in the microspores from an isogenic plant, it therefore appears that the genotype of the haploid microspores does not play a role in difference between the embryogenic capacity of the male gametophytes. It is however clear that the diploid genotype of the sporophyte plays a role in the embryogenetic capacity of the male gametophyte.

e) Starting from an observation made by J. Nitsch with regard to the possibility to differentiate in vitro an embryoid from the tip of the pollen tube, we have tried to pollinate a stigma with a part

of the style attached to it, and transplanted in vitro. Different culture media were used in order to obtain the growth of the pollen tube in the medium and to try to differentiate the three haploid cells of the pollen tube in an embryoid. The penetration of the pollen tubes from the style to the medium gives very good results with N. tabacum, but all the attempts to obtain an embryoid differentiation have failed.

f) Isolations of nuclei from tobacco tissues were performed in order to measure the DNA content by Feulgen microdensitometry. The technique used is based on cellulase digestion of the cell wall and mechanical disruption of the cytoplasm which releases free nuclei without any appreciable cytoplasm contamination. The nuclear DNA content measurements were performed on nuclei isolated from piths of isogenic diploid tobacco stems from haploid and diploid callus derived from stem disks of different tobacco plants, and finally, from isolated cells in liquid medium. The results show that :

1) for the haploid tissue, free cells in liquid medium display a higher tendency to endoreduplication than the same callus tissue grown on solid medium.

2) in the diploid callus tissue, maintained in solid medium, the distribution of the DNA content in the nuclei reaches the same values as observed in the pith tissue in vivo;

3) in the haploid callus tissue, the endoreduplication tendendy remains very low as compared with the situation of the pith in the haploid plant "in vivo". In fact, the great majority of the nuclei shows values of DNA content ranging from 1 C and 2 C. An analysis of the variation of the DNA content in the nuclei isolated from a tissue culture of the diploid tobacco pith, coming from an isogenic line, is being carried out by means of autradiographic and cytophotometric techniques. The first stages of callus formation are studied.

1.2. Anther cultures of other species.

In order to obtain haploid plants in other species, anther cultures were carried out with Nicotiana sylvestris, Nicotiana alata, Brassica oleracea, Capsicum annum, Vicia faba, Lycopersicon peruvianum, Solanum melongena, Triticum durum and Panicum miliaceum. Haploid plants were obtained with the wild species Nicotiana sylvestris ($n = 12$); with the self-incompatible Nicotiana alata ($n = 9$) we have obtained many embryoids in the cultured anthers, but without any plantlet generation.
With Brassica oleracea, callus fromation was observed from the anther culture and, after transplantation on a differentiating medium, numerous roots have grown. By anther culture of Capsicum annum, numerous callus were obtained and are now on different types of medium in order to induce the differentiation of plantlets.
With other species, the results have so far been disappointing.

PUBLICATIONS

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2. M. DEVREUX et B. DONINI - Effets radiobiologiques de l'irradiation des gametophytes chez l'orge et le blé dur - Radiation Botany.(in pre

3. S. SACCARDO et M. DEVREUX - Effets des rayons gamma appliqués à différentes intensités en début et fin de période de repos sur le zygote de tabac. Radiation Botany 11 : 303-308, 1971

4. M. DEVREUX, F. SACCARDO et A. BRUNORI - Plantes haploides et lignes isogéniques de Nicotiana tabacum obtenues par cultures d'anthères et de tiges in vitro. Caryologia 24 : 141-148, 1971.

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6. M. DONINI and M. DEVREUX - Genetic effects of gametophyte irradiation in barley. Barley Genetics Newsletter 1 : 17-18, 1971.

7. M. DEVREUX, B. DONINI and G.T. SCARASCIA MUGNOZZA - Genetic effects of gametophyte irradiation in barley. II. Frequency and types of mutations. Radiation Botany (in press).

8. M. DEVREUX e A. BOZZINI - Piante in vitro geneticamente pure, la le prospettive a breve termine per il tabacco ed altre piante coltivate. L'Italia Agricola maggio 1971.

9. M. DEVREUX - Impiego delle colture in vitro in genetica e nel miglio amento delle piante coltivate. Atti della Associazione Genetica Italiana, S. Vincent 1971 (in press).

10. M. DEVREUX e F. SACCARDO - Ibridazione di linee isogeniche di tabacc ottenute da coltura in vitro di antere. Società Italiana Genetica Agraria, Bari 1971 (in press).

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Allevamento della mosca della frutta.

1.1 Sono continuate le ricerche intese ad ottenere una idonea razionalizzazione dell'allevamento massivo della Ceratitis.
A partire dal mese di ottobre del 1970, abbiamo condotto delle prove di produzione della mosca sia in singole strutture di allevamento, gabbie prismatiche (cm. 125 x cm.80 x cm.40), che in strutture multiple montate in serie di 9 gabbie rettangolari (35 x 10 x 80 cm
I dati sono riportati sulla Tabella N° 1.

Ulteriori miglioramenti sono stati apportati alle condizioni di allevamento per quanto riguarda gli aspetti fisici (temperatura, umidità, luce,scambi gassosi), la composizione delle diete. Nella Tabella N° 2 vengono riportati alcuni dati sulla densità di adulti di Ceratitis in rapporto alla produzione e fertilità delle uova.
I risultati complessivi ottenuti, ci permetto di aumentare in larga misura le nostre capacità di produzione. Inoltre, allo scopo di sincronizzare la produzione, sono stati determinati i periodi pupal della mosca della frutta a differenti temperature.

1.2 Sono proseguiti i cicli di selezione sia attraverso singoli imbreeding, che in piccole popolazioni di ceppi di Ceratitis selezionati opportunamente per longevità, vigore sessuale e fertilità.
Inoltre, si stanno effettuando selezioni genetiche su alcuni mutanti (maschio con setole non spatolate, pupa chiara, pupa scura), prc venienti da allevamenti di laboratorio dell'Università di Bologna.

Sterilizzazione della mosca della frutta.

1.3 Per quanto riguarda l'induzione della sterilità, nel 1971, sono incominciate delle prove di radio-sterilizzazione direttamente su adulti neo-sfarfallati. I dati preliminari indicano, rispetto agli irraggiamenti sulle pupe che, irraggiando gli adulti, la "qualità" delle mosche sterili subisce un vistoso miglioramento e che, per esempio, a parità di dose (7,5 Kr.), il vigore sessuale aumenta, irraggiando direttamente gli adulti, più del 50%. Esperienze di irraggiamento di Ceratitis neosfarfallate contenute nei sacchetti di lancio(5.000 adulti per sacchetto), confermano che é possibile effettuare tecnicamente, nella nostra sorgente gamma, degli irraggiamenti massivi. Le esperienze continuano. Idati preliminari sono indicati nella Tabella N°3, e nella Tabella N°4

Sono anche in corso delle prove di irraggiamento mediante neutroni termici e veloci. I primi risultati sono riportati nella Tabella N°5.

Cause biotiche ed abiotiche di mortalità delle pupe di Ceratitis.

1.4 Nell'ambito di un programma di ricerca istituito dall'IBP, sono state effettuate, in un'area della Casaccia, prove in pieno campo atte a raccogliere informazioni sulle cause biotiche ed abiotiche di mortalità delle pupe di Ceratitis ancora non completamente chiarite. I dati ottenuti, nel 1971, hanno dimostrato che i fattori biotici sono quelli che possono causare complessivamente la morte di oltre il 60% delle pupe esposte durante i mesi autunno-inverno e che in base alle caratteristiche del danno fisico riscontrato nei pupari,

i predatori e parassiti sono tra questi i nemici più importanti. Si ritiene che la precisa identificazione di questi nemici naturali sia di grande aiuto nell'eventuale impostazione di un programma di lotta biologica o integrata per il controllo di questa specie. Alcune informazioni si ricavano dalle Tabelle N° 6 e N° 7.

1.5 Sono proseguite le analisi sperimentali per ottenere conoscenze il più possibile dettagliate sul "behavior" della Ceratitis. Particolare attenzione é attualmente rivolta alla conoscenza degli stimoli e delle preferenze olfattive, visive, sessuali e nutrizionali della mosca della frutta. I dati sono in via di elaborazione.

Indagini sulla presenza e sulla dinamica di popolazione della Ceratitis lungo la costa laziale.

1.6 Nel 1970 sono incominciate le osservazioni e la raccolta di reperti sulla presenza della Ceratitis lungo la costa laziale. Le indagini ecologiche effettuate mediante controlli settimanali della cattura degli adulti e dell'infestazione hanno portato alle prime considerazioni:

a) Nelle varie località di ricerca, la presenza ed abbondanza della Ceratitis sono strettamente legate alle specie e quantità di frutta suscettibile all'attacco, presenti durante il succedersi delle varie generazioni che il Tripetide é obbligato a compiere in un anno. In linea generale si considerano i biotopi del pesco e dell'albicocco, con epoca di maturazione luglio-agosto, dei potenziali e precoci focolai di infestazione dai quali la specie può spostarsi nei vicini biotopi; quello dell'uva da tavola, specialmente della cultivar "Ca

dinal", un possibile ospite di cui non si conoscono, ancora, tutte le implicazioni ecologiche; quello del pereto sia un territorio prevalentemente di riproduzione per gli adulti sia, dove si svolgono le ultime generazioni, un biotopo di sopravvivenza per le pupe svernanti della specie.

b) In relazione all'abbondanza e continuità di catture, i biotopi maggiormente favoriti dalla Ceratitis sono stati quelli costituiti da intense masse verdi di piante di albicocco, di pesco e di vite allevata a tendone; decisamente sfavorevoli sono risultati quelli dell'oliveto e dei giovani frutteti allevati a palmetta(susino e pero). Si é osservato inoltre che la presenza o meno di frutta nei vari biotopi non influisce sull'andamento delle catture degli adulti, i quali probabilmente sono in rapporto dinamico con i biotopi tra loro vicini.

c) La Ceratitis manifesta sia un individuale movimento di "vagabonlaggio" presumibilmente effettuato per la ricerca di biotopi favorevoli alla sua riproduzione, sia un movimento "quantitativo" di popolazione (migrazione?) quale probabile risposta diretta degli adulti a condizioni ambientali sfavorevoli(scarsità di alimento,soprapopolazione, ecc.). Si osserva qumunque che questo ultimo aspetto non interessa grandi distanze e avviene solo verso la fine dell'anno.

d) Scarsamente efficaci sono risultati i trattamenti insetticidi nel controllo della Ceratitis la quale manifestando una netta preferenza a concentrarsi in determinati biotopi(con o senza frutta), una grande mobilità tra i vari frutteti ed una nuova adattabilità trofico-larvale (infestazione dell'uva da tavola), deve essere combattuta in maniera razionale e diversa di quanto fino adesso é stato fatto. Allo scopo si ritiene che, pur con le necessarie ed ulteriori ricerche ecologiche, l'applicazione di un sistema di lotta integrata, basata sull'utilizzazione di varie tecniche di lotta, quali quella con insetti radiosterilizzati, con esche avvelenate, con nemici naturali, possa vantaggiosamente essere attuata nel controllo di questa specie. Si allegano le tabelle del 1970-71 e si riportano nelle Tabelle N° 8 e N° 9 i dati preliminari in via di elaborazione per l'anno 1971-1972.

Tabella N° 1

Dati sull'allevamento permanente della Ceratitis capitata Wied.- (Fase di preparazione alla produzione massiva).

Periodo	uova raccolte	% uova schiuse	Produzione di pupe per mantenimento insettario	rendimento in pupe. % .
Ottobre 1970	3. 980.000	73,30	1.135.000	37,30
novembre 1970	11. 020.000	71,20	1.522.000	37,00
dicembre 1970	14. 490.000	74,00	774.000	33,30
Gennaio I971	6. 940.000	90,00	620.000	39,10
febbraio "	6. 220.000	90,80	1.390.000	45,16
marzo "	6. 340.000	92,00	1.107.500	38,33
aprile "	8. 980.000	84,20	462.000	29,23
maggio "	9. 380.000	81,00	1.390.000	34,17
giugno "	9. 560.000	86,80	782.000	31,09
luglio "	12. 660.000	83,50	935.000	24,50
agosto "	11. 920.000	85,00	1.067.000	27,39
settembre "	15. 100.000	82,00	1.037.000	29,00
ottobre "	14. 920.000	83,90	1.530.000	29,00
novembre "	46. 700.000	86,56	1.580.000	29,00
dicembre "	46. 320.000	89,00	1.500.000	32,16
gennaio 1972	48. 040.000	93,24	3.250.000	39,12
MEDIE	972 520 000	84,15	20.081500	3343

TABELLA N° 2

Densità di adulti di Ceratitis capitata Wied. in rapporto alla produzione e fertilità delle uova.

Dimensioni gabbia	Capacità	Densità degli adulti per gabbia	Quantità di uova prodotte per gabbia	% uova schiuse
125 x 80 x 40 cm.	400 dm^3	25. 000	2.400.000	95,8
" " " "	" "	30. 000	2.600.000	96,5
" " " "	" "	35. 000	2.760.000	92,5
" " " "	" "	40. 000	3.300.000	91,3
" " " "	" "	50. 000	3.440.000	87,8
" " " "	" "	75. 000	2.600.000	87,7
" " " "	" "	100. 000	3.100.000	73,3
80 x 35 x 10 cm.	280 "	10. 000	830.000	94,1
" " " "	" "	7. 500	770.000	95,2

TABELLA N° 3

Irraggiamento di adulti neosfarfallati di Ceratitis capitata Wied.

Dose	% schiusa uova			% sfarfall.	Intensità dose
	♂* x ♀*	♂* x ♀	♂ x ♀		
7,5 Kr. sorgente gamma	0,24	0,77	92,7	89,1	2,5 Kr. minuto
6,2 Kr. cella gamma	3,9	4,6	89,5	92,7	1,3 Kr. ora

T A B E L L A N° 4

Irraggiamento di pupe di Ceratitis capitata Wied. 24 ore prima dello sfarfallamento.

Dati preliminari.

Dose	% schiusa delle uova		Longevità (giorni)		Media uova per ♀
	♂* × ♀*	♂* × ♀	♂	♀	
0	81,9 ± 5,4		38,1 ± 3,5	41,6 ± 4,2	726 ± 114
500 rads	24,6 ± 4,8		32,6 ± 3,2	39,7 ± 4,2	778 ± 132
1.000 "	19,7 ± 2,2		34,6 ± 2,7	31,8 ± 5,4	689 ± 121
3.000"	0		41,2 ± 3,9	43,6 ± 4,4	0
4.000 "	0	0	35,5 ± 4,0	41,6 ± 5,1	0
7.500 "		1,9			
7.500 "		1,3			
7.500 "		1,4			
7.500 "		1,5			
7.500 "		1,4			
10.000 "		0,22			
10.000 "					
10.000 "					
10.000 "					
10.000 "					

Tabella N° 5

Ricerca dosi sterilizzanti.* Irraggiamento con neutroni termici e veloci.

Neutroni termici			Neutroni veloci		
Tempo	Dose	% uova schiuse	tempo	dose	% uova schiuse
0	0	93,09 ± 8,9	0	0	89,8
10minuti	250 r.	24,5 ± 2,7	20 sec.		66,2
50minuti	500 r.	29,5 ± 4,8	40 sec.	da determinare	42,4
			60 sec.		23,7
			80 sec		17,1
			100 sec.		4,5
			120 sec.		4,1

Potenza Reattore TRIGA 10 KW.

* pupe di 24 ore prima dello sfarfallamento.

TABELLE N° 6

C.capitata - Roma 70/71

Effect of Exposure Period on Pupal Mortality Components

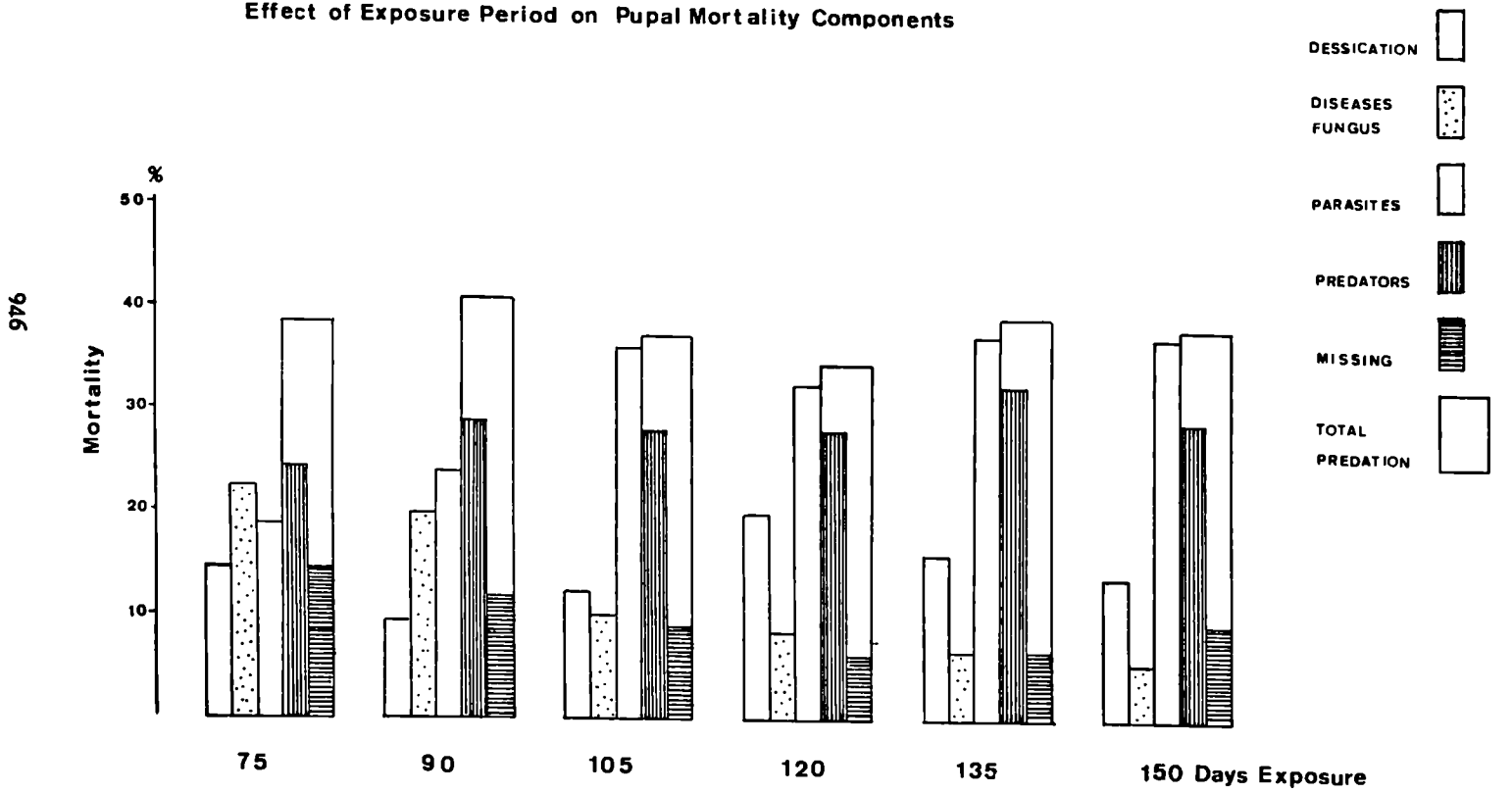
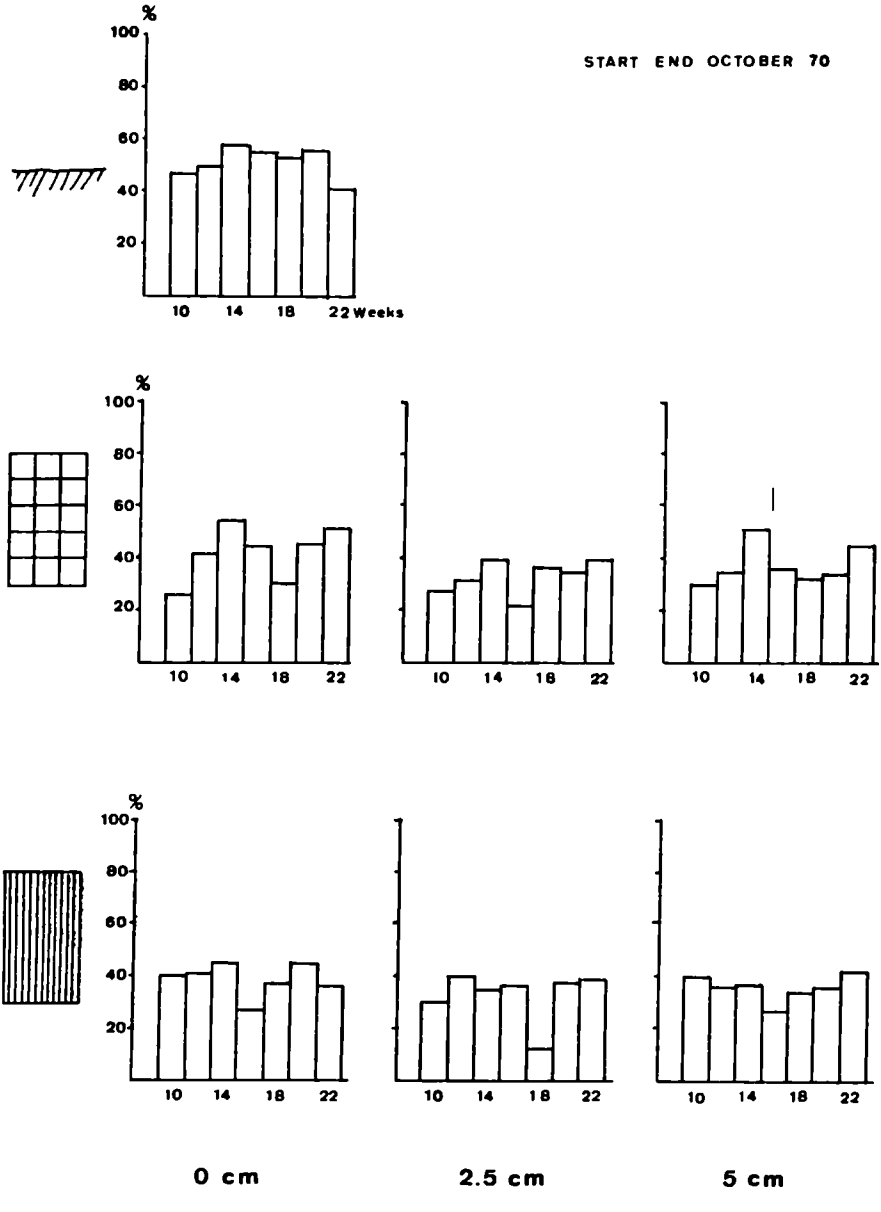


Tabella N° 7



Mortalità delle pupe di Ceratitis capitá Wied. Casaccia (ROMA).

TABELLA N° 8

Dinami-ca di popolazione di Ceratitis capitata Wied. lungo le coste del Lazio. Catture adulti con trappole.

I971

Località	Azienda ha.	catture di maschi di Ceratitis durante il 1971										
		Marz	Aprile	Maggio	Giug.	Lugl.	Agost	Sett.	Ott.	Novem.	Dicem.	
Tarquinia		==	==	00	00	00	00	54	45	225	58	382
Cerveteri		==	==	00	00	00	6	98	188	1125	38	1444
Maccarese		==	==	00	00	00	2	30	143	1047	3	1225
Ostia		==	==	00	00	1	70	16	105	724	0	916
Fiorano		==	==	00	00	1	205	3790	17716	7000	10	28722
Latina		==	==	00	00	00	38	385	557	2613	61	

Tabella N° 9

Cattura di maschi della mosca della frutta Ceratitis capitata Wied. lungo la costa laziale. 1971

DATA CONTROLLO	TARQUINIA			CERVETERI			MACCARESE			OSTIA			FIORANO			LATINA			LANUVIO		
	Maschi Catturati	Numero Trappole	Media per Trappola	Maschi Catturati	Numero Trappole	Media per Trappola	Maschi Catturati	Numero Trappole	Media per Trappola	Maschi Catturati	Numero Trappole	Media per Trappola	Maschi Catturati	Numero Trappole	Media per Trappola	Maschi Catturati	Numero Trappole	Media per Trappola	Maschi Catturati	Numero Trappole	Media per Trappola
31-V-71	–	24	–	–	27	–	–	20	–	–	20	–	–	30	–	/*	/	/	–	28	–
14-VI-71	–	24	–	–	27	–	–	20	–	–	20	–	–	30	–	/	/	/	–	28	–
28-VI-71	–	24	–	–	27	–	–	20	–	–	20	–	–	30	–	/	/	/	–	28	–
12-VII-71	–	24	–	–	27	–	–	20	–	–	20	–	–	30	–	/	/	/	–	28	–
26-VII-71	–	24	–	–	27	–	–	20	–	1	20	0,05	1	30	0,03	–	–	–	–	28	–
9-VIII-71	–	24	–	1	27	0,03	–	20	–	36	20	1,8	22	30	0,7	4	23	0,2	–	28	–
23-VIII-71	–	24	–	5	27	0,2	2	20	0,1	34	20	1,7	183	30	6,1	34	23	1,5	5	28	0,2
6-IX-71	35	24	1,4	26	27	0,9	7	20	0,3	7	20	0,3	433	30	14,4	159	23	6,9	3	28	0,1
20-IX-71	19	24	0,8	65	27	2,4	23	20	1,1	9	20	0,4	3357	30	111,9	226	23	9,8	7	28	0,3
4-X-71	15	24	0,6	117	27	4,3	68	20	3,4	9	20	0,4	7846	30	261,4	77	23	3,3	5	28	0,2
19-X-71	30	24	1,2	71	27	2,6	75	20	3,7	96	20	4,8	9870	30	329,0	480	23	20,8	1044	28	37,3
2-XI-71	134	24	5,6	620	27	22,9	764	20	38,2	407	20	20,3	3658	30	121,9	1361	23	59,2	1455	28	51,9
16-XI-71	91	24	3,8	505	27	18,7	643	20	32,1	317	20	15,8	3342	30	111,4	1252	23	54,4	1181	28	42,2
7-XII-71	57	24	2,4	37	27	1,4	2	20	0,1	–	20	–	8	30	0,2	61	23	2,6	4	28	0,1
15-XII-71	1	24	0,04	1	27	0,03	1	20	0,05	–	20	–	2	30	0,06	–	23	–	4	28	0,1

*/ Trappole non esposte

DATA CONTROLLO	TARQUINIA			CERVETERI			MACCARESE			OSTIA			FIORANO			LATINA		
	Maschi catturati	Numero Trappole	Media per trappola	Maschi catturati	Numero Trappole	Media per Trappola	Maschi catturati	Numero Trappole	Media per Trappola	Maschi catturati	Numero trappole	Media per trappola	Maschi catturati	Numero Trappole	Media per Trappola	Maschi catturati	Numero Trappole	Media per Trappola
29-VII	--	15	--	198	9	22,0	34	16	2,1	(*)	(*)	(*)	11	24	0,5	--	10	--
5-VIII	4	15	0,2	495	7	70,7	23	16	1,4	(*)	(*)	(*)	26	24	1,1	2	10	0,2
12-VIII	2	15	0,1	1480	7	211,4	12	16	0,7	(*)	(*)	(*)	52	24	2,2	4	10	0,4
19-VIII	85	15	5,7	2650	21	126,2	34	20	1,7	38	8	4,7	198	28	7,1	4	10	0,4
26-VIII	267	15	17,8	5024	23	218,4	133	20	6,6	274	8	34,2	1143	28	40,8	56	15	3,7
2-IX	412	15	27,5	2140	23	93,0	52	18	2,9	17	8	2,1	725	28	25,9	41	15	2,7
9-IX	661	15	44,1	3559	23	154,7	36	18	1,8	14	8	1,7	2204	28	78,7	59	14	4,2
16-IX	1330	15	88,1	1953	23	84,9	158	18	8,8	94	8	11,7	3137	26	122,0	118	14	8,4
23-IX	659	15	44,0	6710	23	291,7	211	20	10,5	319	8	39,9	4030	26	155,0	717	15	47,8
30-IX	2089	15	139,3	5811	22	264,1	340	20	17,0	316	8	39,5	3893	26	149,7	174	15	11,6
7-X	621	15	41,4	3426	23	148,9	419	20	20,9	504	8	63,0	2765	26	106,3	307	15	20,5
14-X	495	15	33,0	3038	23	132,1	515	20	25,7	375	8	46,9	1724	26	66,3	298	15	19,9
21-X	490	15	32,7	1537	23	66,8	261	20	13,1	165	8	20,6	932	26	35,8	99	15	6,6
28-X	256	14	18,3	453	23	19,7	1154	20	57,7	1274	8	159,2	1194	26	54,9	174	13	13,4
5-XI	167	14	11,9	355	23	15,4	953	14	68,1	1113	8	139,1	871	26	33,5	134	14	9,5
11-XI	336	14	24,0	456	23	19,8	391	14	27,9	337	8	42,1	168	25	6,7	24	14	1,7
18-XI	249	14	17,8	214	23	9,3	200	14	14,3	176	8	22,0	90	25	3,6	21	14	1,5
25-XI	5	15	0,3	17	23	0,7	13	14	0,9	15	8	1,9	16	25	0,6	1	14	0,1
2-XII	5	15	0,3	15	23	0,6	13	14	0,9	15	8	1,9	14	25	0,5	1	14	0,1
9-XII	5	15	0,3	15	23	0,6	--	14	--	--	8	--	--	25	--	1	14	0,1
16-XII	1	15	0,1	4	23	0,2	--	14	--	--	8	--	--	25	--	--	14	--
23-XII	-	15	--	3	23	0,1	--	14	--	--	8	--	--	25	--	--	14	--
Totale catture																		

(*) Trappole non esposte

TABELLA I - Tabella riassuntiva delle catture di ceratitis capitata Wied. effettuate nelle sei differenti località di osservazione . 1970

Data controllo	TARQUINIA Pesche		CERVETERI Pesche		CERVETERI Pere		MACCARESE Pere		OSTIA ANTICA Pere		FIORANO Pesche		FIORANO Pere		LATINA Pere	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
5-VIII	1348	0,0	1106	90,6	1489	0,0	680	0,0	1022	0,0	608	6,9	1887	1,0	--	--
12-VIII	1216	0,10	--	--	1022	2,1	1327	0,0	820	0,0	1401	6,0	1507*	40,9	690	0,0
19-VIII	1095	8,50	--	--	1933	34,9	910	0,0	1567	0,0	2022	31,2	6042	10,6	520	21,0
26-VIII	556	22,00	--	--	8450	67,5	1331	0,0	1550	0,0	419	42,0	432	15,7	660	43,6
2-IX	3068	96,50	--	--	3515	85,6	--	--	918	0,0	210	51,0	619	18,1	270	61,0
9-IX	400	100,00	--	--	--	--	--	--	1109	0,0	45	60,9	279	22,9	177	71,2
16-IX	--	--	--	--	--	--	--	--	601	0,0	17	76,5	155	23,9	512	92,0
23-IX	--	--	--	--	--	--	--	--	450	0,0	--	--	705	90,0	406	95,0

TABELLA II - ANDAMENTO DELL'ATTACCO NELLE VARIE LOCALITA' DI RILEVAMENTO 1970

A - Frutti esaminati

B - Percentuale di attacco

* - Controllo effettuato in un solo appezzamento

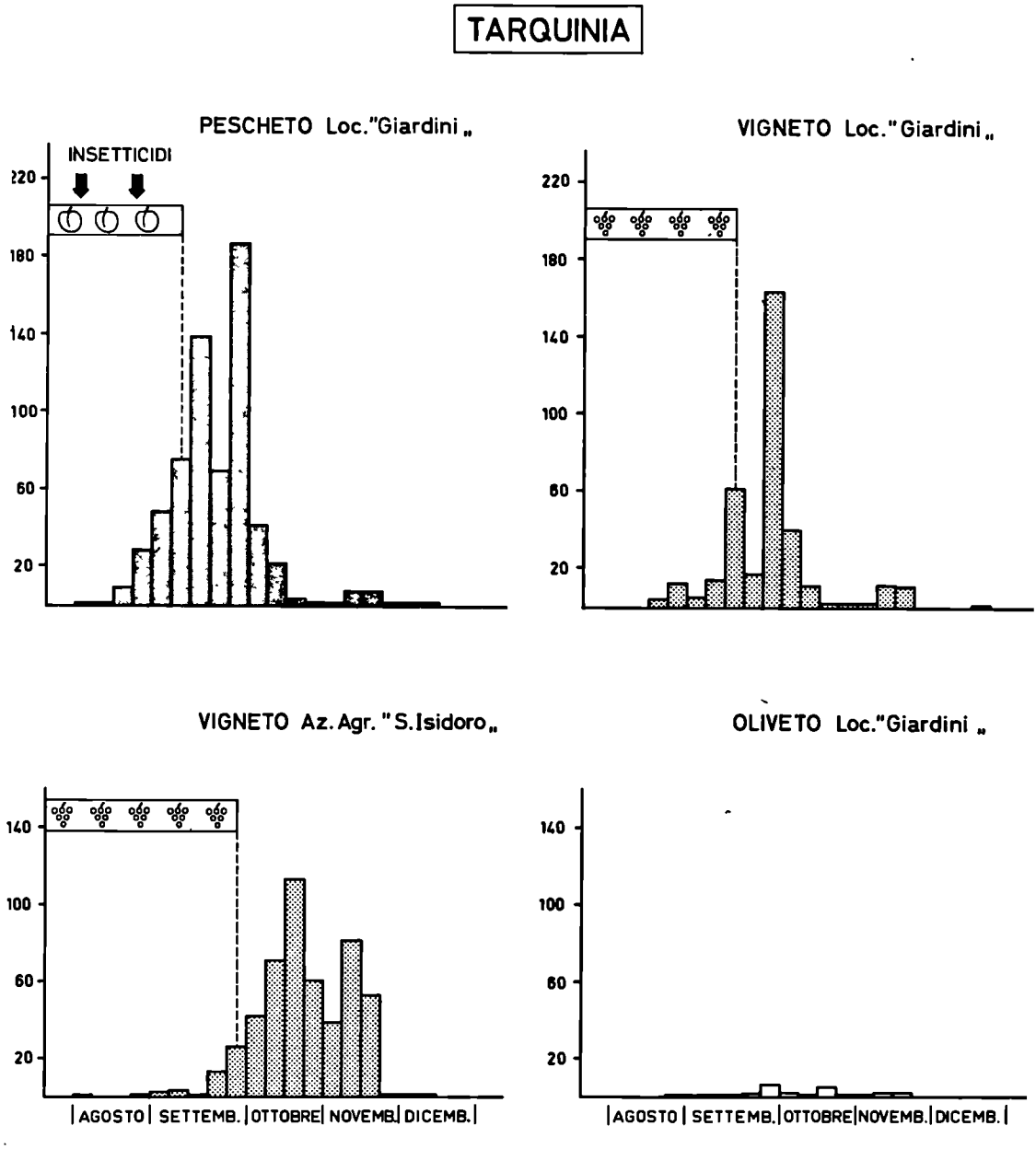


Fig. 2-Andamento delle catture in quattro biotopi diversi della località di Tarquini

- 1 9 7 0

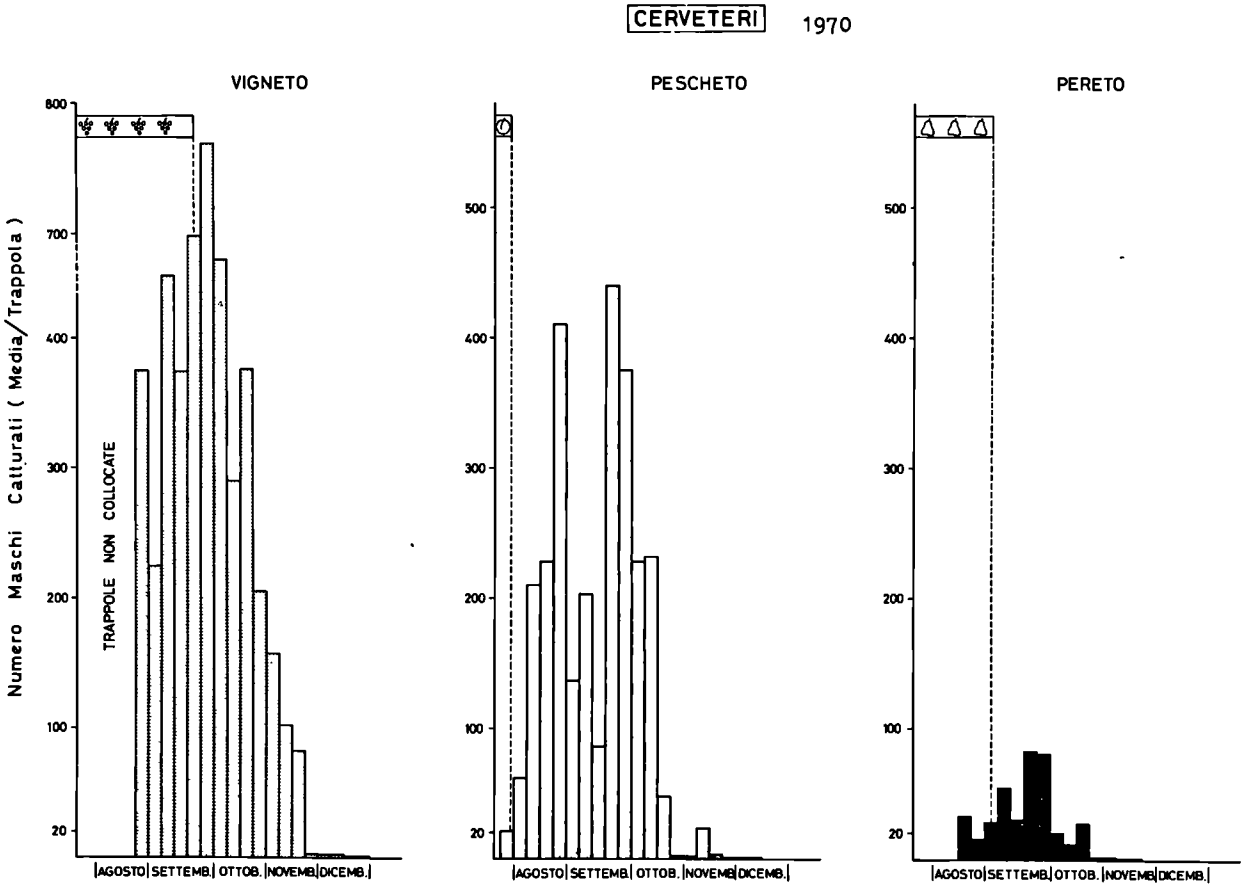


Fig.3-Andamento delle catture in tre biotopi diversi della località di Cerveteri .

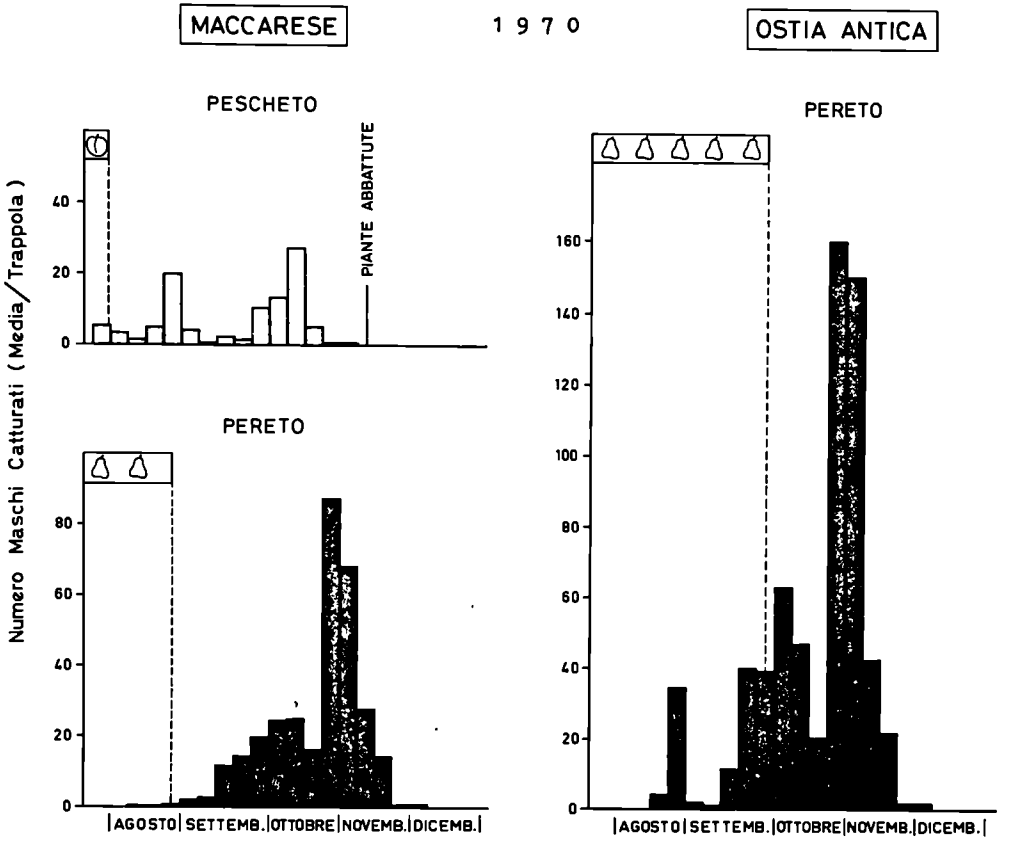


Fig. 4 - Andamento delle catture in tre biotopi diversi della località di Maccarese e di Ostia Antica.

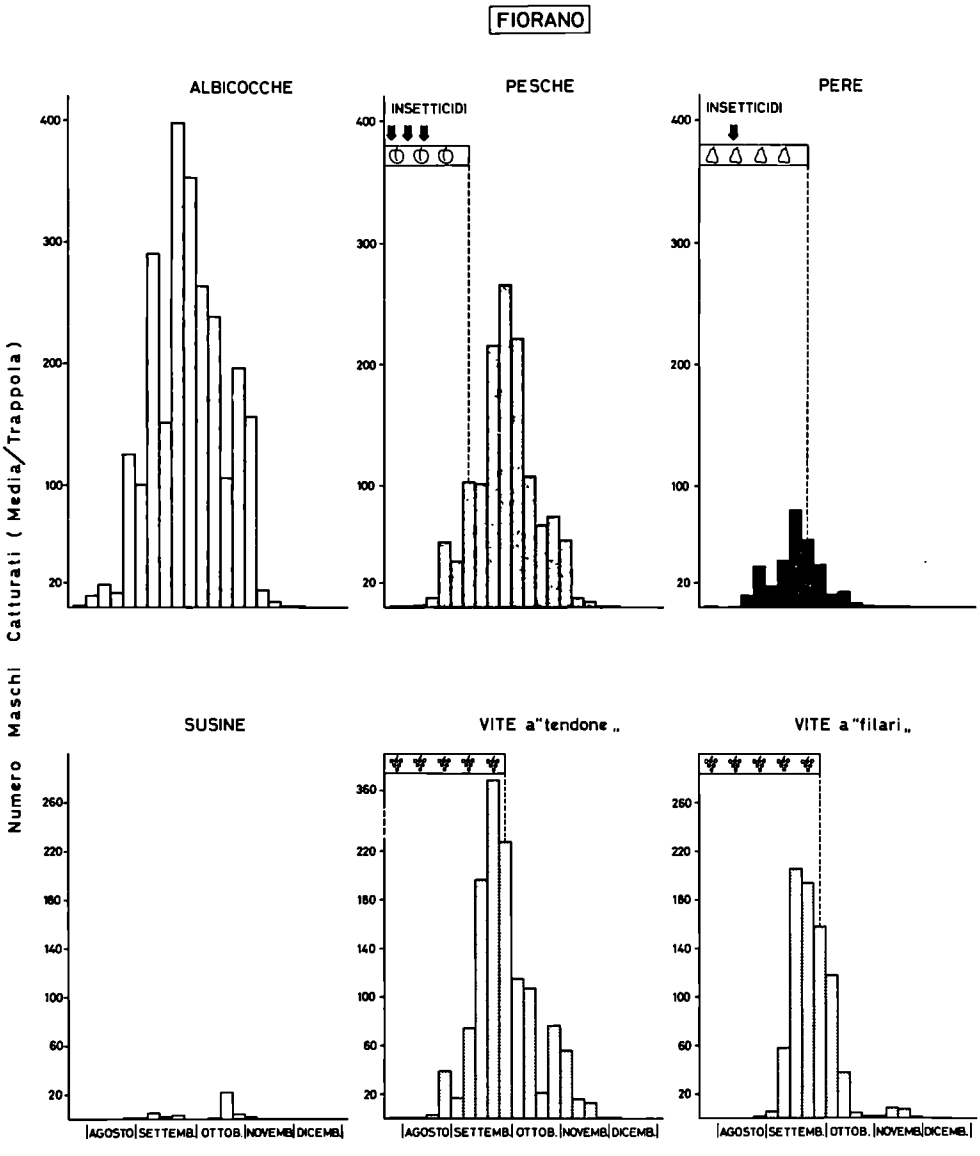


Fig.5 - Andamento delle catture in sei biotopi diversi della località di Fiorano.

LATINA

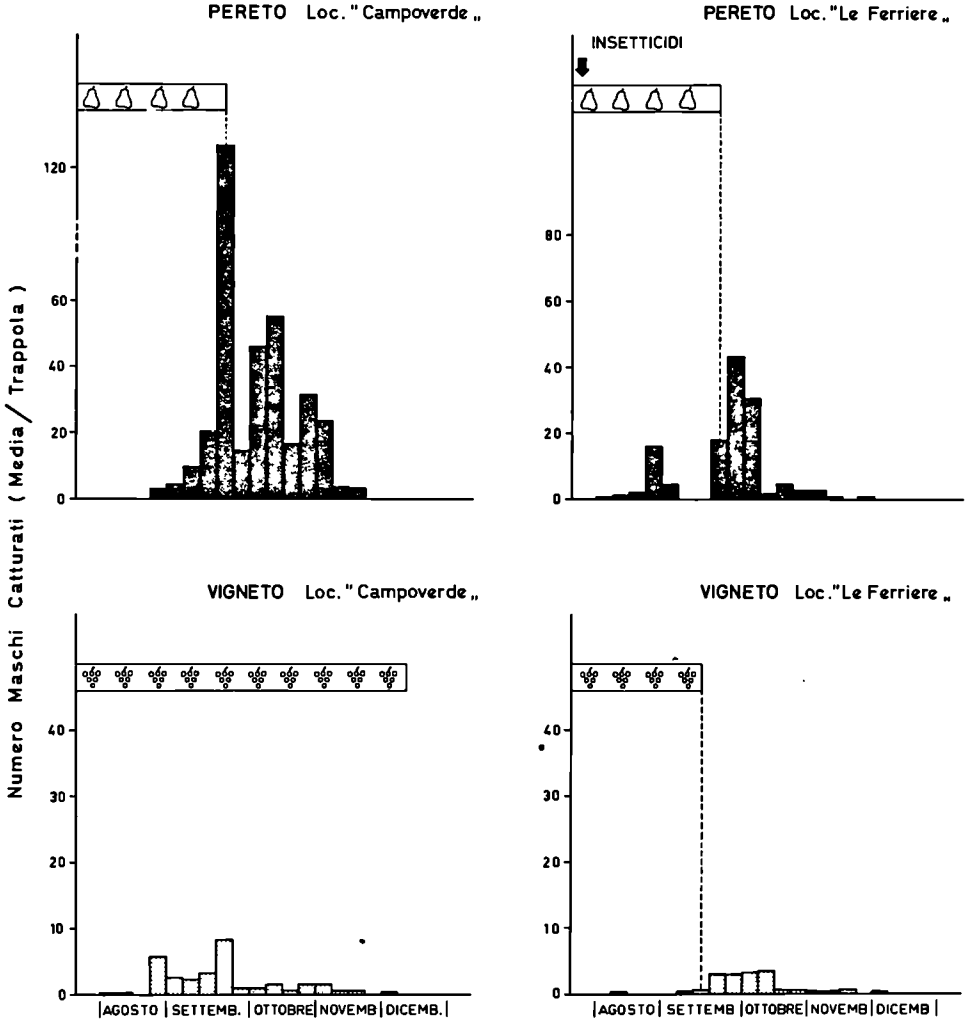


Fig. 6 - Andamento delle catture in quattro biotopi diversi della località di Latina.

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