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Triennial Report 1981-83

Institute for Genetics and for Toxicology

Kernforschungszentrum Karlsruhe

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Institut für Genetik und für Toxikologie

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Triennial Report 1981-83

Institute for Genetics and for Toxicology

(in association with the Institute of Genetics,
University of Karlsruhe, and the Institute of Radiotoxicology,
University of Heidelberg)

Editor: G. Hotz

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Abstract

The scientific activity of the Divisions of Genetics and of Toxicology of the Institute for Genetics and for Toxicology during the period January 1981 to December 1983 is described. In addition to reports on the various research topics this second "Report" also gives an overview of the external scientific and teaching activities of the staff members during the period. In the field of radiation genetics most of the interest has been focussed on the mechanisms of gene repair, gene regulation and the molecular biology of tumor viruses in human and other mammalian cells. The main emphasis of the long term toxicology program has been on studies of radiotoxicology of the actinides and other heavy metals, especially in relation to chelation therapy and to the development of biochemical and physical methods for investigation of their metabolic behaviour.

Tätigkeitsbericht 1981-83

Zusammenfassung

Es wird über die wissenschaftliche Tätigkeit des Teilinstituts Genetik und des Teilinstituts Toxikologie innerhalb des Instituts für Genetik und für Toxikologie von Spaltstoffen im Zeitraum Januar 1981 bis Dezember 1983 berichtet. Neben kurzen Berichten über wichtige Forschungsergebnisse gibt der Report auch einen Überblick über andere wissenschaftliche und akademische Aktivitäten der Mitarbeiter. Im Bereich der strahlengenetischen Forschung interessieren die Fragen nach den Mechanismen der Genreparatur, Genregulation und molekularen Biologie von Tumorviren in der Säugetierzelle. Schwerpunkte des Forschungsprogramms bilden Arbeiten über die Strahlentoxikologie von Actiniden und anderen Schwermetallen sowie ihre Therapie, die Entwicklung biochemischer und physikalischer Methoden und ihre Anwendung auf diesem Gebiet.

This report is the second of a series which will be issued multiennially by this institute to inform friends and colleagues, about what is going on in the IGT. This report covers the scientific activity during the years 1981 to 1983.

It should be stated that all experiments on recombinant DNA done in the IGT are carried out under biological safety regulations as defined by the Federal Ministry of Research and Technology of the Federal Republic of Germany, regulations which are comparable to those defined by the NIH Recombinant DNA Research Guidelines. Similarly all studies with living animals are performed in accordance with ethical and husbandry standards recommended both nationally and internationally.

Acknowledgments:

We are indebted to Mrs. Ulrike Baltzer, Mrs. Christel Heinold and Mrs. Gisela Kammerer for their patience and care in typing the manuscript.
We also thank Prof. Klose and our administration for their interest in this report and for the financial support.

REPORT 1981-83

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1. Introduction to the first issue

The Institute was first established in 1958 as the Institut für Strahlenbiologie (IStB) of the Kernforschungszentrum Karlsruhe. In those early days the aim was to start again in a field of biophysical research which had been studied prior to World War II in the Genetics Department of the Kaiser-Wilhelm-Institut in Berlin under the direction of such well known scientists as M. Delbrück, N.W. Timofeeff-Ressovsky and K.G. Zimmer. K.G. Zimmer started building the new Institute in Karlsruhe in 1957 and, together with the late Professor Alexander Catsch, directed the Institute for the next twenty years. Under the direction of Professors Zimmer and Catsch the Institute's scientists made many contributions to the analysis of radiation damage in phage DNA and to the problems of accelerating the removal of radionuclides from the body. Thus the tradition of carrying out both fundamental and applied research was firmly established.

Following the untimely death of Alexander Catsch in 1976 and with the retirement of K.G. Zimmer in sight, the Institute re organised into two new divisions, genetics and radiotoxicology. The Institute was renamed as the Institut für Genetik und für Toxikologie von Spaltstoffen on 1 July 1977. The Institute has close university affiliations and the two present directors also occupy respectively the Chairs of Genetics in the University of Karlsruhe (Peter Herrlich) and Radiotoxicology in the University of Heidelberg (David M. Taylor). These strong university affiliations are reflected in a steady stream of diploma and doctoral students and by the active programme of seminars, lectures, practical courses, workshops and journal clubs. Close links are maintained with other institutions having similar interest throughout the world and several collaborative studies are being carried out. During the report period, about 40 scientists and 40 non-scientific staff members worked in the Institute. The scientists coming from Germany, the United States of America, Belgium, Great Britain, Israel, Italy, Austria and Switzerland.
The research programmes of the Institute are assessed annually by the Board of Management of Kernforschungszentrum Karlsruhe, assisted by an independent Scientific Advisory Board, and the work is funded largely from the budget of Kernforschungszentrum but from time to time scientific posts are also financed by the University of Karlsruhe and other bodies.

Introduction to the second issue

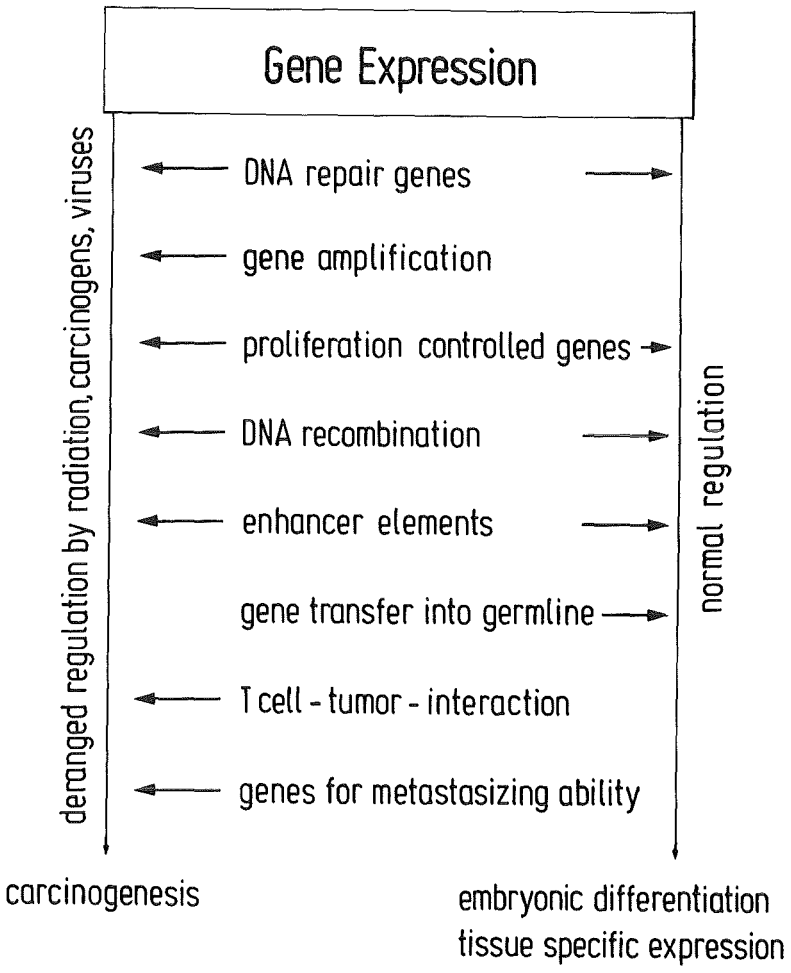
During the past 3 years interest in biological research increased within our research center. New activities

established an "R&D program" called TMU (Technik-Mensch-Umwelt) which covers areas like genetics, toxicology, waste disposal, water technology, climate research and systems analysis. Again many visitors from abroad have participated in our scientific life as visiting scientists, as guest-speakers in our seminars or students working on their doctoral thesis. In addition to these activities the number of staff members from European countries has increased quite considerably.

Since 1977 it was planned to improve the biological facilities by constructing a new building. However after calculating the costs it was decided to rebuild and to enlarge our old house. We are now in the first stages of this modernisation. We will get an annex for our directorate, secretariat, library and seminar room and another one for the service facilities, glassware wash-up, medium kitchen and workshops. The old Van-de-Graaf-building is being transformed into a modern animal house. A third annex will increase lab space for the biochemical toxicologists. This operation will probably last about 2 years and will absorb quite a bit of our time. But we certainly hope that all the effort will finally result in much better research facilities for the biology group of the centre.

Research in Genetics

The past few years since the start of the genetics division early in 1978, have been devoted to attempts to combine fruitfully modern genetic problems and technologies with the area of radiation biology. A large part of the efforts now goes into investigating the determinants of carcinogenesis and particularly radiation carcinogenesis. A scheme of these topics and their relationship to each other is given below:



The fate and biological consequence of a radiation induced DNA lesion is determined by the capacity of the cell to repair and reconstitute the original DNA sequence. The enzymatic repair functions in eucaryotic and particularly human cells are as yet largely unknown. Our efforts are concentrated onto isolating DNA repair gene since these will yield more reliable information on repair mechanisms than direct enzymatic tests. At the same time the isolated genes will reveal the patterns of their regulation and biological role. Human individuals genetically deficient in DNA repair are not only cancer-prone but have developmental defects. Thus some of these genes may help to study control functions of early development and the links between cancer and differentiation. The only enzymatic work on DNA repair done here attempts to define the limiting factors in mammalian mitotic recombination.
Although most carcinogenic agents are also mutagenic and mutations are part of the several step carcinogenic process, evidence for the existance of other carcinogen-induced mechanisms of higher efficiency than random mutation has accumulated. These are of particular interest for the question how low doses of radiation act. Gene activation that are mediated by the mutagen-induced change of proliferation, and radiation-induced gene amplifications are candidates for carcinogenic mechanisms of this kind. Among the proliferation controlled genes are cellular oncogenes. An extracellular factor was detected which transmits the induced genetic program onto non-treated cells. This factor could spread and magnify the effect of a carcinogen.
With the intention to elucidate how oncogenes are activated, the transcriptional control elements of the mouse mammary tumor virus have been delimited and isolated. The molecular details of action of hormone-dependent and independent enhancing sequences are intensively studied at the present time. Adjacent to these specific enhancer studies, the area of tissue specificity is interesting. How do cells ensure that only certain genes are expressed and that these are not expressed elsewhere. Mouse mammary tumor virus makes tumor of the mammary gland cells and serves as one system for this investigation. Quite recently, a new group joined us aiming at learning how certain liver-active Xenopus-genes are regulated.

Research in Toxicology

The long-term aim of the toxicology programme is to increase our understanding of the mechanisms by which radiation, especially that from α-emitting radionuclides, induce bone and other types of tumor. Such knowledge is of special importance in relation to the assessment of the risks to human health which may result from the entry of small amounts of plutonium, and or other components of the nuclear full cycle

into the human body. The evidence available from animal studies suggest that at very low doses, comparable to these to be expected following accidental exposure of human populations the incidence of tumors or other harmful effects will be very low, thus conventional toxicological methods may not yield adequate information and new investigational approaches are necessary. A primary requirement for such investigations is a detailed understanding of the mechanisms which control the deposition of the radioelements in cells and tissues and the induction of their harmful effects. The study of such mechanisms forms a major part of our research programme.

In the past three years our biochemical studies of the uptake and deposition of plutonium and related elements in cells and tissues have been expanded. Particular emphasis is being placed on the role of transferrin, and other iron-binding proteins, in the transfer of plutonium across the cell membrane and its subsequent transfer to lysosomes. The studies now involve the use of a variety of human and animal cells in vitro as well as in vivo studies in rat and hamster liver or rat lung. The possible role of a plutonium-transferrin-transferrin receptor mechanism in facilitating, or perhaps more probably limiting the uptake of plutonium by cells is under investigation, as are the chemical species involved in the intra- and extracellular transport of plutonium in vivo.

It is generally accepted that the risk of radiation-induced injury is related to the radiation dose received by the sensitive tissues. The distribution of plutonium and the actinides in bone is non-uniform and consequently the - radiation dose delivered to the cells at risk in bone is also non-uniform. Computer-controlled microdosimetrie methods for the study of the distribution of radiation dose from actinide elements deposited in bone have been in use in our studies for several years. These studies have been continued and extended and now include studies with Pu-239, Am-241, Np-239 and U-233 in rats, mice and dogs. An important development has been the active collaboration with the Radiobiology Laboratory of the University of Utah which has permitted the extension of this work to include studies on bones from dogs which received Pu-239, Am-241 or Ra-226.

New limited radiotoxicological studies with Np-237 in rats have been started and the long planned further studies with Pu-239 and possible Ra-226 are expected to begin as soon as our new animal facility becomes available in late 1984.

Reduction of the risk of late-effects following accidental intake of plutonium, or other actinides, by chelation therapy remains on important area of study. Studies in the period under review have shown the remarkable efficacy of orally administered ZnDTPA for the removal of plutonium and americium from bone and other tissues of rats.

More detailed accounts of these studies will be found in the

following pages or in the publications listed.
In both the genetics and toxicology programmes fundamental and applied studies are closely integrated. The combination of genetics and toxicology in one institution is quite unique and offers the chance to develop the programmes outlined which in fact cover interconnecting aspects of a joint problem. The combination has been designed to yield data which ultimately will assist in the evaluation of the effects of low doses of radiation in man.

P. Herrlich D.M. Taylor

Karlsruhe, Mai 1984

2. Staff of the Institute

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(Administration of the institute during the period (1980/81)

Prof. Peter Herrlich (Genetics)
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Berlin

4. Year-round Research (Short scientific reports)
a) Gene Repair

DNA REPAIR AND GENOME AMPLIFICATION DURING ALPHA RADIATION-INDUCED G2 ARREST

C.Lücke-Huhle, L.Hieber

With a few exceptions, most eucaryotic cells exhibit a G2 period in their mitotic cycle. This period separates the S phase from mitosis and represents the time necessary for preparing chromosome condensation, assembly of the mitotic spindle and cytokinesis. Ionizing radiation induces delay of cells in G2.
Our data provide evidence that radiation-induced G2 delay is caused by DNA damage preventing the condensation of chromosomes while the mitotic spindle and cytokinesis function normally. The lengthening of the G2 period is used for repair of the chromosomal lesions.
The extent of G2 arrest caused by alpha particles from an americium-241 source (1) depends on the age of the cell at the time of irradiation (2) and increases linearly with dose and LET (Linear Energy Transfer) of the radiation (3). A correlation between arrest in G2 and cell inactivation has been found for eight cell lines of human and animal origin. For all endpoints (chromosomal lesions, G2 delay and cell killing) the effectiveness of alpha particles is greater than that of gamma rays. The RBE values for G2 delay (4.6-9.2) are in general comparable to those for cell killing (4.2-7.4) if the latter are derived from the initial slopes of survival curves. An exception is made by cells from patients with Ataxia telangiectasia. Ataxia cells are highly sensitive to killing by gamma rays but show average G2 arrest per unit gamma dose. From the missing correlation between G2 delay and cell killing in Ataxia cells we deduce that the defect of these cells concerns repair of damage other than that leading to G2 delay. We believe that characteristic DNA double-strand breaks and their repair are the cause for radiation-induced G2 delay (3,5). This explanation is supported by results showing average sensitivity of Ataxia cells to killing by high LET alpha particles and normal repair of alpha ray-induced DNA double-strand breaks (4).

The chromatin of cells arrested in G2 by alpha irradiation is severely damaged. By means of the premature chromosome condensation technique the number of breaks, gaps and exchanges was scored. G2 arrested cells possess large numbers of breaks/cell: 6-20. In addition, cells with one or more chromosomes exhibiting long stretches of undercondensed chromatin are found. These cells increase in number as the number of breaks/cell increases with dose (5). This lead us to assume that the multiplicity of breaks/chromosome prevents exact condensation of chromatin in G2 cells and that

irradiated cells accumulate as a direct consequence of extensive unrepaired damage in the chromosomes.
Repair of these lesions is indicated by site-specific unscheduled DNA synthesis and by the observation that condensation of these regions improves during G2 arrest (6). Addition of 2 mM caffeine stops repair and releases cells prematurely from G2 arrest. Such cells exhibit a 3 times higher number of aberrations/metaphase as compared to cells which reach mitosis without the help of caffeine. The action of caffeine on DNA is far from being understood. We can nevertheless say that caffeine altered or interupted a process taking place on damaged DNA which then permitted cells to condens their chromatin and enter mitosis. Thus a cell blocked in G2 must have contained all macromolecules required for cell division including specific proteins, the spindle apparatus and membrane components.

A dose of 4.38 Gy of alpha particles arrests Chinese hamster V79 cells irreversibly in G2. In spite of extensive DNA lesions such cells initiate new rounds of replication (endoreduplication) without preceeding cell division. Flowcytometry, PCC analysis and DNA hybridization with radioactive labelled DNA probes reveal a fourfold genome amplification (figure). At 48 hr after exposure to alpha particles more than 50% of the cells have a DNA content higher 4C (7).
Observations related to the alpha radiation-induced genome amplification may be the mutagene induced gene amplification in mammalian cells (8) and the UV increased resistance to MTX by amplification of the DHFR gene (9). Experiments on the amplification of individual genes after alpha irradiation and their relevance for repair in mammalian cells are in progress.

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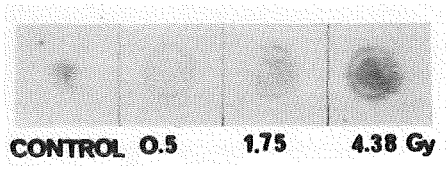
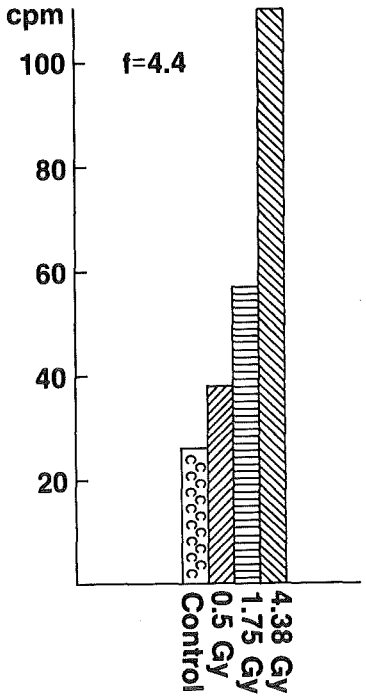
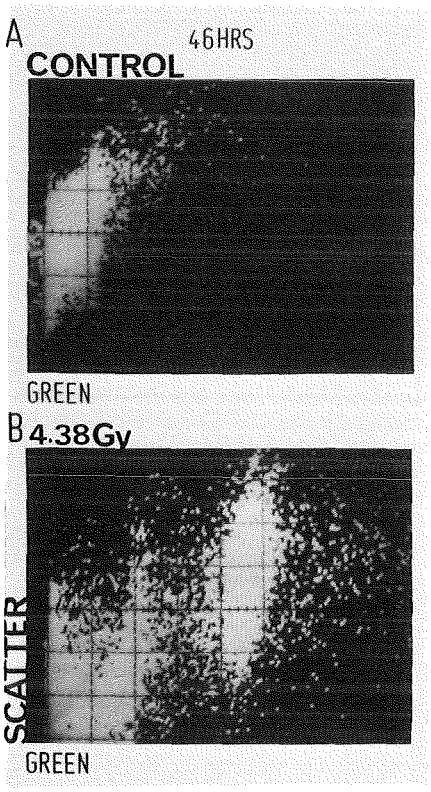
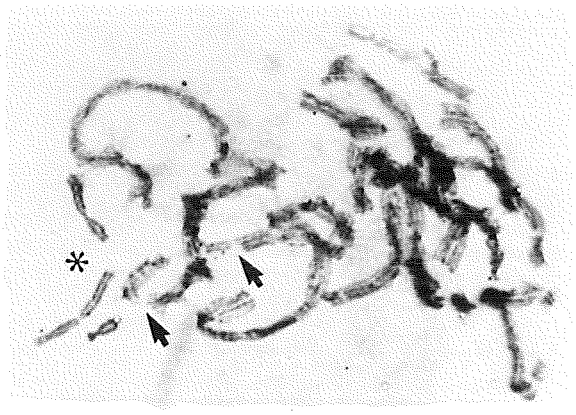


"In situ" hybridization of genomic DNA of 5 x 10^5 V79 cells to ^{32}P-labelled dhfr-gene shows a 4.4 fold amplification at 48 hr after 4.38 Gy of alpha particles.







G2 chromatin of an irradiated cell after fusion with a mitotic cell (PCC-technique) at 39 hr after exposure. Next to the star are 3 normal mitotic chromosomes. The arrows point to chromatin breaks.

Dot pattern display (flowcytometry) of V79 cells at 48 hr after irradiation. Green fluorescence and scatter signals are proportional to the DNA content and the size of the cell, respectively.

REJOINING OF DNA DOUBLE STRAND BREAKS IN MAMMALIAN CELLS

T. Coquerelle, K.F. Weibezahn

All organisms are able to rejoin radiation-induced DNA double strand breaks (DSB). In experiments using bacterial mutants we showed that DSB as measured by neutral filter elution are rejoined by two mechanisms with different velocities, a fast ligase-dependent process and a slow recombination process. To obtain information about the mechanisms of DSB repair in mammalian cells we investigated rejoining in different radiosensitive mutants, treated with chemicals and irradiated with radiation of different linear energy transfer (LET).

1. Action of caffeine and hypertonicity on DSB rejoining in V79 cells.
Caffeine and hypertonicity influence cell survival in different ways: caffeine alters the shoulder of the dose-effect curve, while hypertonic treatment affects mainly the final slope. Caffeine inhibits the late rejoining of DSB and part of the fast rejoined DNA is degraded (fig.1a), whereas hypertonic shock causes a temporary inhibition of rejoining but has no overall effect in the long term (fig.1b).
Irradiated cells are temporarly arrested in the G2 phase of the cell cycle. Cells treated with caffeine are not blocked in G2 after irradiation. There is evidence that unrepaired potential lethal damage after caffeine treatment consists of DNA DSB. The repair of these lesions appears to be responsible for the shoulder of the survival curve.

2. Impairment of DSB rejoining in CHO radiosensitive mutants.
To confirm that survival is influenced by repair of DSB and by the reversible arrest in the G2 phase we studied the rejoining of DSB and the G2 block in X-ray sensitive CHO cells kindly provided by P.Jeggo (1). These mutants are much more sensitive against ionizing radiation than the corresponding wild type CHO cells but exhibit the same resistance against UV radiation.
As illustrated in fig.2 the ability of the mutants to rejoin DSB during the late rejoining phase is impaired. Furthermore they are irreversibly blocked in the G2-phase.
We conclude that normally mammalian cells are able to rejoin a great amount of DSB and that during the rejoining period they are reversibly blocked in G2. Caffeine masks the DSB and the cells pursue their cycle unaware of lesions to be repaired. The CHO radiosensitive mutants are in part unable to repair the DSB and therefore cannot leave the G2 phase.

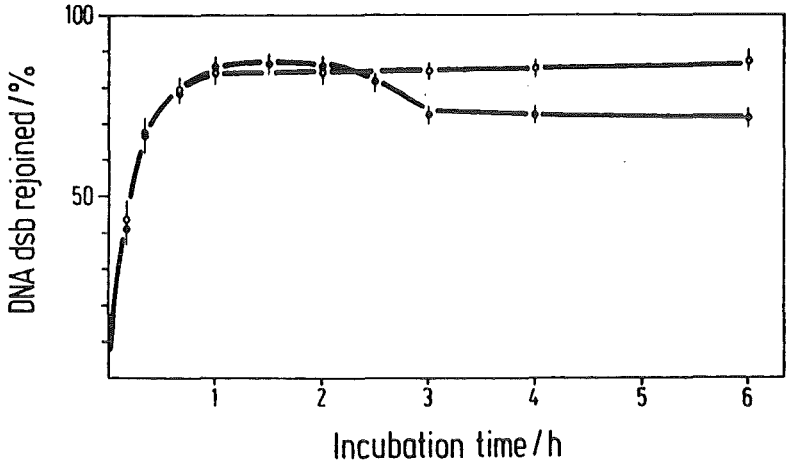


Fig.1a: Influence of caffeine on DSB rejoining: Percentage of V79 DNA DSB rejoined as a function of incubation time after irradiation with a dose of 100Gy γ-rays.
o: Irradiated cells incubated in medium at 37°C.
●: Irradiated cells incubated in medium with 2 mM caffeine at 37°C.
Standard errors of 6 independent experiments.

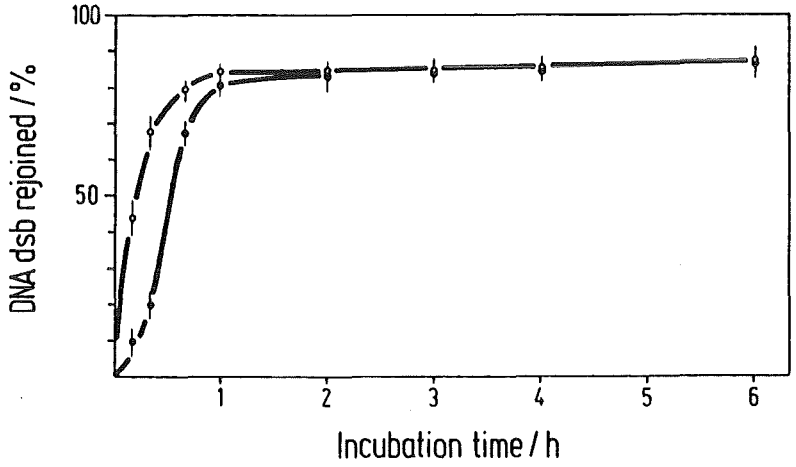


Fig.1b: Influence of hypertonicity on DSB rejoining: Percentage of V79 DNA DSB rejoined as a function of incubation time.
o: Irradiated cells are incubated in medium at 37°C.
●: During the first 20 min after irradiation the cells are incubated in hypertonic PBS.
Standard errors of 6 independent experiments.

3. DNA Rejoining in normal human fibroblasts and Ataxia Telangiectasia fibroblasts after exposure to γ-rays, Am-241 α-particles and bleomycin. (In cooperation with C.Lücke-Huhle).

Cells from A-T patients are sensitive to ionizing radiation. This has been shown by colony survival as well as by cytogenetic methods. The high frequency of spontaneous and induced chromosome aberrations in cells irradiated in early G1 or in the G2 phase of the cell cycle suggests that unrejoined DNA breaks are one type of damage responsible for the enhanced cell killing of irradiated A-T cells (2). We have demonstrated an impairment of DSB rejoining ability in AT2BE cells (3).

DNA DSB can be divided in different classes. They can result directly from a high energy particle track traversing the two opposite DNA strands or from neighbouring SSB in the opposite strands forming a DSB. Depending on the inducing agent the nature of the broken ends (blunt ends, sticky ends, or gaps) can vary. It is obvious that the configuration of the breaks influences the rejoining mechanisms involved. To investigate the genetic defect responsible for the impairment of rejoining in AT2BE cells we used agents inducing different kinds of DNA breaks.

Fig.3 illustrates the DSB rejoining kinetics of normal human fibroblasts and AT2BE fibroblasts after exposure to radiation with different LET. Normal human fibroblasts rejoin γ-ray induced DSB very fast: 50% of the DSB being repaired within 3 min. In the more sensitive AT2BE fibroblasts the rejoining occurs slowly and less completely.

In contrast to γ-rays, α-particles induce predominantly DSB with massive local destruction. The rejoining of this kind of DSB exhibits totally different kinetics: The percentage of rejoined DSB increases linearly with time during the first 60 min. and the number of residual breaks is 2-3 times higher than after γ-irradiation. The rejoining of α-induced DSB in AT2BE cells, however, is identical to the rejoining in normal fibroblasts. This is in agreement with the finding by Lücke-Huhle et al. (4) that AT2BE cells exhibit the same radiosensitivity with respect to cell killing after α-irradiation as the normal fibroblasts.

Reduced survival and increased chromosome breakage have been observed in A-T cells exposed to bleomycin (5). The radiomimetic action of bleomycin on DNA consisting in a release of bases, disruption of phosphodiester bonds and a destruction of the sugar moiety results in SSB and DSB. In normal human fibroblasts the rejoining kinetics of bleomycin-induced DSB is similar to that of γ-induced DSB (fig.4). In AT2BE cells the fast rejoining is still enhanced: about 55% of the breaks are rejoined immediately but very few additional breaks are rejoined during the following 3 hours. There is an evident correlation between the increased sensitivity of AT2BE cells against γ-rays and bleomycin and

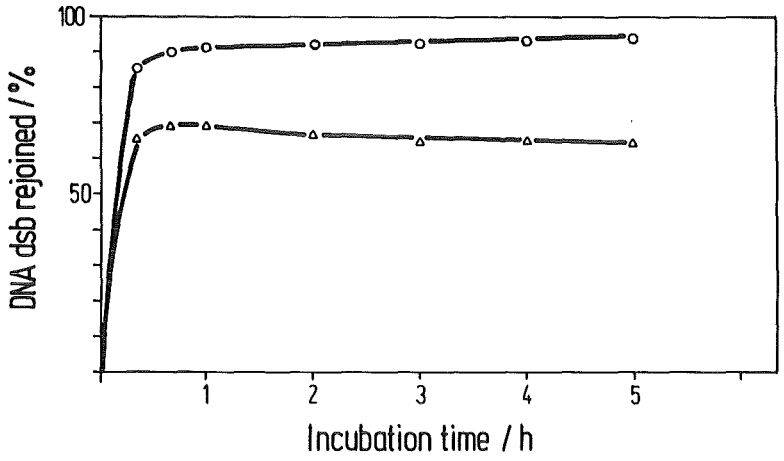


Fig.2: DSB rejoining in X-ray sensitive CHO cells: Percentage of CHO DNA DSB rejoined as a function of incubation time after irradiation with a dose of 100Gy γ-rays.
o: Wild type cell (K1) incubated in medium at 37°C.
Δ: X-ray sensitive mutant cell (xrs-2) incubated in medium at 37°C.
Mean values of 4 independent experiments.

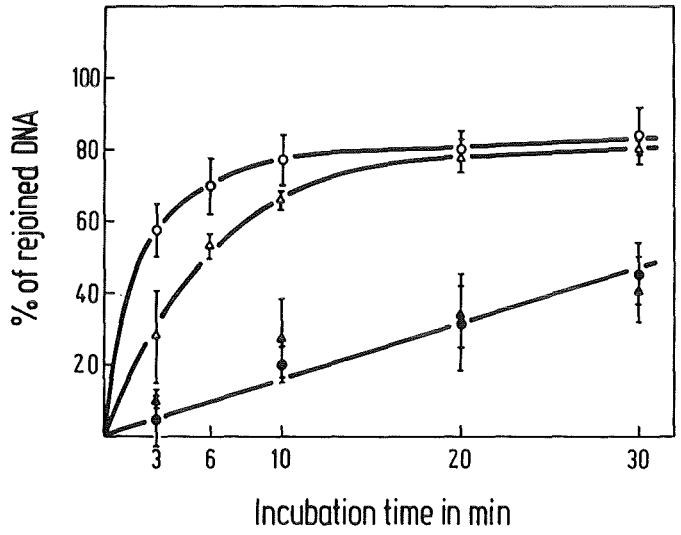


Fig.3: Rejoining kinetics of DSB in normal human fibroblasts exposed to a dose of 25 Gy of γ-rays (o) or α-particles (●) and in AT2BE fibroblasts exposed to the same dose of γ-rays (Δ) or α-particles (▲).

their reduced ability to rejoin DSB.

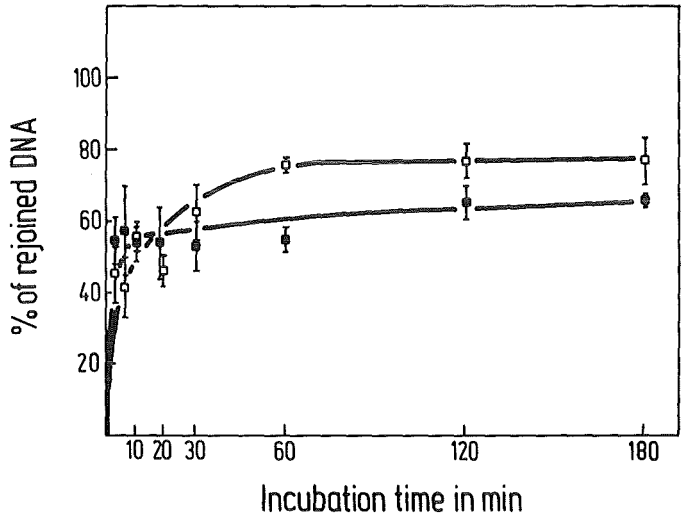


Fig.4: Rejoining kinetics of DSB in normal human fibroblasts (□) and AT2BE fibroblasts (■) treated with 400 µg/ml bleomycin for 1 hour.

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USE OF ADENOVIRUS TO STUDY THE RECOMBINATION MECHANISMS IN HUMAN CELLS.

T. Coquerelle

The exploration of recombination mechanisms in higher eukaryotic DNA is the subject of these investigations. In prokaryotic systems and lower eukaryotes the recombination mechanisms are well defined; in mammalian cells these reactions are yet poorly understood. The complexity of the mammalian genome is the principal cause of the lack of informations. An approach to the problem is the development of a model system based on viral recombination (e.g. human adenovirus). Owing to their homology the adenovirus 2 and 5 DNA molecules recombine after simultaneous infection with the two serotypes. Ad2 and Ad5 differ sufficiently in their restriction endonuclease patterns that cross-over regions in the recombinants can be mapped in progeny DNA (1).
With this experimental system we endeavour to determine host cells and viral functions involved in recombination. We will test whether radiation stimulates the recombination processes in human cells and whether cells from patients exhibiting a higher radiosensitivity or a proneness to develop cancer have a modified recombination capacity.
Investigations in vitro are planned to determine the influence of the DNA molecule conformation on the recombination mechanisms and the nature of proteins and factors taking part in these reactions.

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THREE-DIMENSIONAL CELL CONTACT, INTERCELLULAR COMMUNICATION AND RADIORESISTANCE

H. Dertinger, G. Hinz, A. Seiter

The phenomenon of "contact resistance" (CR) observed when electrically coupled cells are cultured and irradiated as cellular spheroids was investigated further. Work was focussed mainly on two problems:
(1) Is CR simply an artifact of the in vitro system (spheroids) or are we dealing with a general property of communicating cells when irradiated under conditions of their natural steric arrangement?
(2) What are the molecular mechanisms of CR and does its correlation with gap junctions reflect a functional requirement of intercellular communication?

1. Contact Resistance in Human Tumors

To answer the first question we demonstrated the occurrence of CR in an in vivo system. Several considerations concerning, for example, the direct methodological compatibility with the spheroid system, led us to utilize solid human tumors xenografted into athymic nude mice as a test system. We found that the tumor cells (oxic fraction) survived better when irradiated in the animals instead in Petri-dishes, provided that the tumor cells were electrically coupled by gap junctions (1,2). The latter was tested by micro-electrode measurements on small tumor cell aggregates (2). As an example, Fig. 1 shows the CR in the human melanoma BE 11. The survival curve termed "Euoxic Fraction" was obtained by subtracting the survival contribution of the hypoxic cells from the original data (1). Compared to the tumor cells irradiated in vitro ("Plates") the oxic tumor fraction is much more resistant. Thus, CR is also exhibited by xenografts of electrically coupled cells in vivo, regardless of their state of oxygenation and proliferation (1,2,3). In two cases we were able to grow spheroids from the tumor cells. They showed basically the same characteristics of radiation response as the corresponding tumors irradiated in vivo (2). As a practical consequence, these finding may possibly explain and, by micro-electrode measurements, predict the clinical radioresistance of certain human tumors. (This work was performed in collaboration with the Institut "Gustave Roussy", Villejuif).

2. Contact Resistance and DNA-Repair

Initial experimentation started from the working hypothesis that CR might be due to induced DNA-repair. However, we have not been able to obtain evidence for an induced error-prone repair function (4), nor was there any difference in DNA strand rejoining between contact-resistant spheroids and

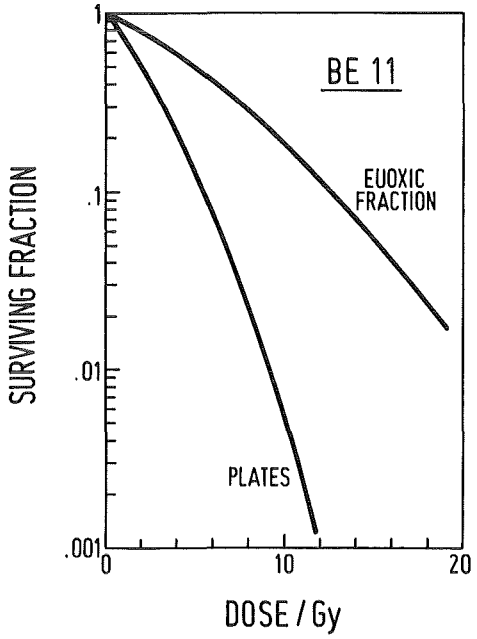

Fig. 1. Survival curves (colony forming capacity) after γ-irradiation of the human melanoma BE 11. "Plates": Tumor cells taken from the nude mice and irradiated as single cells on Petri dishes under aerobic conditions. "Euoxic Fraction": Response of the oxic tumor cell fraction when the tumor is irradiated on the animal and then plated for survival determination (see text for more details).

	UNTREATED	TPA-TREATED
SURVIVING FRACTION	0.20 ± 0.02	0.046 ± 0.004
ELECTRICAL SIGNAL TRANSFER		

Fig. 2. Irradiation of contact-resistant Chinese hamster B14 FAF28 spheroids with and without pre-treatment with the tumor promoter TPA (24 h; 20 ng/ml). As a result of this treatment, survival after a 10 Gy dose of Co-γ-radiation decreases from 20 to 4.6%, which is roughly the monolayer level. Micro-electrode measurements reveal that TPA inhibits intercellular electrical signal transfer. V_1: Hyperpolarization signal induced in a cell when a rectangular constant-current pulse (10 nA/70 Hz) is injected. V_2: Signal obtained in a neighbouring cell.

monolayers (5). In addition, two-dimensional protein gel electrophoresis did not reveal systematic and significant differences between contact-resistant spheroids and monolayers. Although these experiments do not rule out the induction of DNA repair genes by cell contact, we have given up this hypothesis, particularly in view of the data described below.

3. Biological function of gap junctions and relevance for induction of CR

Gap junctions are thought to induce tissue differentiation by supporting intercellular flow and equilibration of metabolites, as well as distribution of small "signal molecules" (6). This concept requires a three-dimensional network of coupled cells, as encountered in the spheroids, for establishing the gradients promoting such fluxes. When the spheroids grow in size, the volume to surface ratio increases thus changing channel-directed fluxes. As a consequence, this may give rise to a threshold in the spheroid cells, if the observed phenomenon were triggered by critical values of these fluxes and concentrations. The characteristics of CR observed by us can be explained on the basis of this biophysical concept of gap-junctional function:

a) Requirement of three-dimensional cell contact.
b) Requirement of a critical spheroid size (5).
c) All cells develop CR regardless of their location in the spheroid (3,5).

That the gap junctions are specifically involved in the induction of CR was tested by applying gap-junctional uncouplers. As an example, Fig. 2 shows the results obtained with the tumor promoter TPA. Pre-incubation of contact resistant spheroids (Chinese hamster B14 FAF28) with TPA inhibits intercellular electrical signal transfer and eliminates the CR: the survival of spheroid cells after a dose of 10 Gy is depressed towards the monolayer level. Monolayers do not change their radiosensitivity in response to a TPA pretreatment (not shown).

Intercellular communication by gap junctions is required to induce CR. However, once developed in spheroids, CR only slowly decays with time when the spheroids are trypsinized prior to irradiation (5). This apparent single cell character of CR is further stressed by our observation that the permeability of the gap junctions decreases after induction of CR (7).

4. Is CR associated with a certain stage of cellular "differentiation" as suggested by the biological function of the gap junctions?

Since cellular differentiation is frequently associated with changes in cyclic AMP production, we measured, as a first

approach to this problem, cyclic AMP synthesis in spheroids and monolayers of coupling and non-coupling cell lines (7). In contact-resistant spheroids, the activity of adenylate cyclase was found to be much lower than in monolayers and single cells. However, in non-coupling cells, cyclic AMP synthesis did not change noticeably between monolayers and spheroids. Thus, the presumptive differentiation of coupled spheroid cells is characterized by a low cellular cAMP level, and elucidation of the mechanisms of CR will ultimately require detailed biochemical analysis of this state of cell differentiation.

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4. Year-round Research (Short scientific reports)
b) Gene Regulation

REGULATION OF THE ALBUMIN AND VITELLOGENIN GENES IN XENOPUS

G.U.Ryffel, M.Kazmaier, M.Schorpp, U.Wagner.

We use the albumin and vitellogenin gene families of Xenopus to investigate the molecular mechanisms responsible for differential gene expression in eukaryotes. Both gene families are expressed tissue-specifically i.e. they are only active in hepatocytes (1,2). Whereas the activity of the vitellogenin genes depends on the presence of the steroid hormone estradiol, the albumin genes are constitutively expressed and their expression is reduced by estrogen (3).

1. Estrogen activates the vitellogenin genes on the transcriptional level whereas it represses albumin gene expression post-transcriptionally.
By in vitro transcription in isolated nuclei we could demonstrate that the silent vitellogenin genes are coordinately activated by estrogen. These results confirm and extend our previous finding that estrogen opens the chromatin containing the vitellogenin genes (4,5). Analysing the transcription of the albumin genes in isolated nuclei, we observed that estrogen does not alter the constitutive transcription of these genes. Furthermore quantitation of albumin precursor mRNA revealed that the amount of these RNA's remains unchanged after estrogen treatment (in collaboration with E.Brüning). These data indicate that estrogen does not decrease albumin gene transcription but rather reduces the half-life of the albumin mRNA. This contrasts to the well-known stabilization of the vitellogenin mRNA's by estrogen.

2. Structural analysis of the albumin genes of Xenopus
The exon intron structure of the 68kd and 74kd albumin gene of Xenopus is identical to the structure of the mammalian albumin genes (6). To define precisely the 5' and 3' end of the albumin genes we are subcloning and sequencing these gene regions. These sequence data are essential to construct albumin derivatives that can be used in transfection and injection experiments.

3. Transfection of albumin and vitellogenin genes into cell cultures.
Several Xenopus cell lines contain an estrogen receptor that might correspond to the middle affinity estrogen binding protein. These cell lines are used for transfection experiments to analyse whether the expression of transfected genes can be regulated by estrogen. First experiments have revealed that cotransfection with a DHFR gene cannot be used as selection system since Xenopus cells are highly resistant

to methotrexate. Therefore selection with neomycin using the plasmid pSV2-neo is used. We are attempting to select from these cell lines clones containing vitellogenin and albumin gene derivatives. Provided that hormonal regulation of the transfected genes is observed, we will be able to define the DNA sequences involved in regulation.
As an alternative approach we have transfected the albumin and vitellogenin genes of Xenopus into mammalian cell cultures using cotransfection with pSV2-neo (in collaboration with H.Ponta). Stable transformants will be analysed for expression and hormonal regulation of the Xenopus genes.

4. Persistence and expression of genes injected into fertilized eggs of Xenopus
(in collaboration with A.C.Andres and D.Muellener)
To define the regulatory DNA sequences involved in the expression of the albumin and vitellogenin genes, we inject gene variants into fertilized eggs of Xenopus and follow the fate of these DNA molecules through development and differentiation. In general a mosaic distribution of the injected DNA is observed between and within the various tissues of the developing frog, indicating that no integration occurred before the first cleavage stage. The persisting DNA may be partially integrated but can also be found in an episome - like form. The unintegrated form is not supercoiled and usually rearranged (7).
Using several genes, including a vitellogenin minigene, a chimaeric vitellogenin-gpt gene (constructed by P.Walker and W.Wahli) as well as the chicken conalbumin gene, we have detected transcriptional activity in the tailbud embryo resulting in the appearance of the corresponding polyadenylated RNA. At this early developmental stage 10 to 50 gene copies per cell are present and it seems that relatively high copy number of injected genes cannot be inactivated as the endogenous genes are at this developmental stage. At later stages i.e. in hepatocytes of frogs containing the injected genes in the liver, the persisting genes are silent and cannot be activated by estrogen so far.

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PROLIFERATION CONTROLLED GENES

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Introduction

A considerable portion of the genome must be devoted to controlling proliferation. This is concluded from the facts that there are mutations which stop cells in one or another phase of the cell cycle, that during ontogeny cells obey differentiation-specific signals for proliferation or non-proliferation, and that immortalization is achieved by genetic manipulation. To learn about this interesting portion of the genome many different approaches are being followed in many laboratories. Our approach has been to disturb the complicated process and to check what changes occur thereafter. We have chosen to screen for genes whose expression is altered by a change in proliferation: that is genes which are activated or inactivated in cells that go from G_0 to S, and others which are activated or inactivated in cells that are arrested from logarithmic growth.

What consequences could we expect, when we interfere with the logarithmic growth of mammalian cells by agents which damage DNA and consequently disturb cellular proliferation?

When cells no longer proliferate they will stop synthesizing proteins that are only required during proliferation. Obvious examples are the histones and genes involved in the replicative process.

It is harder to speculate how genes might behave which positively direct cells through the cell cycle. Some of the cellular and viral oncogenes are believed to function in this manner. They may be turned off because a cycle-derived signal or because an externally triggered function is now missing, or vice versa. Such genes could be overexpressed if the progress through the cell cycle delivered a negative signal which is now lacking.

Work on bacteria suggests a totally different additional response. The cell could provide for functions directed towards removing the metabolic or replicative block. Mammalian cells may just like bacteria induce DNA repair and recombination enzymes.

Physiologically, periods of proliferation and non proliferation occur during differentiation. It would be a "reasonable" mechanism of economy if the change of proliferation would trigger the appropriate differentiation program. Various steps of terminal differentiation have indeed been induced or inhibited in vitro by agents which alter proliferation.

Results
We have shown that in cells treated with various mutagens the synthesis of new proteins is induced; the proteins were differentiation specific, in B-lymphocytes proteins were synthesized different from those in fibroblasts. In vitro translation experiments suggested, that for most of the proteins the induction was at the level of translatable mRNA. The fibroblast specific proteins induced by carcinogens and cocarcinogens were produced spontaneously in a cancer prone human mutant, Bloom's syndrome (1,2,3,4,5).
In the last three years we concentrated on three topics. i) We charaterized the properties of some of the induced proteins and of the induction system in detail. ii) We discovered the functional context for two of the proteins. iii) We isolated cDNA clones derived from genes which are regulated by mutagens or tumor promoters. In the following we will first discuss our knowledge of protein induction and function in lymphoid cells, then describe what we know about mutagen-regulated proteins in fibroblasts and finally present the state of isolation of inducible (and repressible) DNA sequences.

1. Mutagens induce the synthesis of the MHC class II associated invariant chain in B-lymphocytes
The major protein induced in mouse myeloma cells or pre-B-cells is a basic 35Kd glycosylated protein with high turn over. It is synthesized in resting B-lymphocytes or in derived cell lines at a high rate without mutagenic treatment, but is not inducible in T-cells or in fibroblasts (4). Because of its B-cell specificity we investigated its relationship to known B-lymphocyte markers. Immuno-precipitation experiments with antibodies directed against class II proteins (polymorphic proteins on the surface of B-cells and macrophages which regulate the immune response) showed, that the inducible protein is the MHC class II associated invariant chain. In the cytoplasm it is associated non covalently with the class II proteins and might have some function for the expression of these proteins. We found that the invariant chain is a specific marker for B cell development and that its synthesis is regulated in parallel to the class II proteins (6). Pre-B-cell lines (characterized by cytoplasmic immunoglobulin chains) do not synthesize the protein and we assume that they mirror the situation in primary pre-B-cells, which cannot yet be isolated in sufficient amount to allow a biochemical characterization. When a pre-B-cell, after rearrangment of both immunoglobulin genes and expression of IgM on the cell surface, transforms into a non-dividing small B-lymphocyte, the synthesis of the invariant chain protein is induced. After polyclonal stimulation it falls again, not on a per cell basis, but in relation to total protein synthesis (7). At least some of the immunoglobulin secreting cells discontinue the synthesis of

the invariant chain completely and in these cells it can be reinduced by stopping proliferation.

2. In human fibroblasts UV-light and the tumor promoter TPA induce the same set of proteins. One of the induced protein communicates this genetic program to non mutagen hit cells.
Two hours after treatment of human fibroblasts with TPA or with UV light a set of at least 8 proteins (XHF1, a-g) is induced. The synthesis of 1 protein is diminished. The proteins a-g are cytoplasmic proteins, XHF1 and another major protein are secreted into the culture medium. The proteins a, b and c share peptides after limited proteolysis. None of the proteins migrates to the position of heat shock proteins. The UV-induced response is mediated by DNA damage. This was deduced from experiments with fibroblasts derived from patients with deficiencies in DNA repair. Fibroblasts from patients with Xeroderma pigmentosum, which are unable to excise UV induced thymidine-dimers, are fully induced for protein synthesis at 2 J/m^2, in contrast to repair proficient fibroblasts which need 30 J/m^2 (5,8).
To investigate a possible role of the induced proteins in the early steps of oncogenic transformation, we tested the action of a variety of inhibitors of TPA. An early inhibitor of tumor promotion, the glucocorticoid fluocinolone acetonide, also inhibited the induction of proteins. Other inhibitors of tumor promotion, like retinoic acid or protease inhibitors, did not interfere with TPA mediated protein induction. We think therefore that we are studying one of the early effects of TPA.
While testing for the function of the induced secreted proteins, we discovered in the medium of UV irradiated cells a cellular factor which induces, in non mutagenized cells, the same genetic program as UV-light or TPA. We called this factor EPIF (Extracellular proteinsynthesis inducing factor). We conclude from it's properties, that EPIF is a protein: It is not digested by nucleases, it is heat labile, not dialysable nor sedimentable at 100 000 g and it can be precipitated with ammonium sulfate. EPIF can be harvested from UV-treated cells maintained in low serum and it can be deep frozen (8,9). This will be important for its purification. The factor is not identical with plasminogen activator (10) or a low molecular weight DNA breakage factor (clastogenic factor) (11). Since we do not yet have antibodies against the TPA-induced secreted proteins, we do not know whether EPIF is identical to one of the secreted proteins which show up in electrophoretic separations.

3. Isolation of inducible cDNA clones
Since the induction of proteins was at the level of translatable mRNA, the isolation of cDNA clones coding for these proteins was straightforward. We constructed a cDNA

library from cells which synthesized the proteins and screened it by differential colony hybridization with ^{32}P cDNA's from uninduced and induced cells. From about 1200 recombinant cDNA clones constructed from mRNA from TPA treated cells, 5 cDNA clones were isolated containing nucleic acid sequences which are induced by TPA and UV and one which is repressed (12). First expression studies with these clones support our protein data and show that the induction system is regulated on the level of mRNA. We find fast induction kinetics after TPA treatment, the accumulation of mRNA is partly inhibited in the presence of cycloheximide. This might suggest that a protein of high turnover which is necessary for the induction is lost during cycloheximide treatment or that the induced proteins stimulate their own synthesis. The induction of the mRNA's seems to be specific for fibroblasts, we do not find it in human lymphoblastoid cells (B cells), in a human hepatoma cell or in the tumor cell line HL 60. In UV sensitive human cell lines the mRNA is induced to maximal level by 10 fold lower UV doses than in repair proficient cells. EPIF also exerts its action through the elevation of specific mRNS's (13).

We did not find experimental evidence that the TPA regulated cDNA clones which we isolated are identical with known oncgene sequences. However when we tested whether the expression of onc genes is regulated by UV or TPA, we found that treated cells contain a slightly elevated level of fos mRNA. The induction factor for fos could be enhanced dramatically, when the treatment was performed in the presence of cycloheximide. Cycloheximide alone induces some fos and myc transcription. We are currently testing the hypothesis that both m-RNA's are normally controlled by an unstable repressor which disappears in the presence of cycloheximide. The accumulation of the c-fos mRNA can then be increased dramatically by UV-light or by TPA (13). A summary of some of the expression data is given in the table.

We screened a human cDNA library from B-cells for the invariant chain protein by differential colony hybridization between cDNA's from B-cells and from fibroblasts. Inbetween 3500 cDNA clones from human B-cells we found about 60 clones which were expressed preferentially in B cells. 20 of those contain information for the invariant chain, as shown by hybrid selected translation and cross hybridization. The human cDNA clone for the invariant chain did not give a signal with mRNA from mouse B-cells. To get a probe for the mouse invariant chain, we subcloned a cosmid which we obtained from M. Steinmetz (Basel Institute of Immunology). With these molecular probes we reached the same conclusion as with the in vitro translation studies: The regulation of the invariant chain during B-cell differentiation and after mutagenic treatment is at the level of mRNA.

Conclusions and perspectives

Low doses of mutagens induce in higher cells a set of differentiation specific proteins, probably by interfering with cellular proliferation. The mutagen does not reach the gene directly, but a signal is formed in the mutagen-treated cell which induces the genes (14). We discovered the functional context for two of the induced proteins: from the available information the invariant chain protein plays a role in MHC class II protein expression. But we suppose that this protein has also other functions.

EPIF belongs to a new class of communication molecules, and transfers a genetic program from mutagen-hit cells to cells which have not been reached by a mutagen, thus amplifying the action of low doses of mutagens.

With the cDNA and genomic clones for the induced proteins we propose i) to study the regulation of synthesis ii) to narrow down signals and signal sequences on the DNA which mediate the induction iii) to determine the proteins which the clones code for and iv) try to get better insight into the function of the induced genetic program.

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Table

Gene probes	Size of RNA	Response** to Cycloheximide(Cx)	UV	UV+Cx	TPA	TPA+Cx	TPA+FA	EPIF***
cDNA clone 1	2.3 kb	n.d.	++	n.d.	+++	n.d.	+	++
2	2.65; 2.3*	n.d.	++	n.d.	+++	n.d.	n.d.	(+)
3	1.0	n.d.	++	n.d.	+	n.d.	++	+
4	2.7	o	+++	n.d.	+++	+	+	++
5	2.55	o	+++	n.d.	+++	+	+	+
6	2.5	n.d.	-	n.d.	-	n.d.	-	-
7	2.1	o	o	o	o	o	o	o
v-fos	2.2	+	(+)	++	+	+++	+	o
v-myc		++	n.d.	n.d.	o to +	++	++	+
v-ras^{ki}		o	n.d.	n.d.	o	o	(+)	n.d.

*) The smaller RNA is constitutive, the larger strongly induced.

**) The response is scaled semiquantitatively as unchanged (o), increased RNA levels (+, ++ or +++), decreased RNA level (-) as compared to untreated cells. RNA samples were prepared 4 and 8 hrs, in some examples 48 hrs after treatment.

***) EPIF = Extracellular Proteinsynthesis Inducing Factor (8,9)
n.d. = not determined

4. Year-round Research (Short scientific reports)
c) Biological Cancerogenesis

MOUSE MAMMARY TUMOR VIRUS: A SYSTEM FOR STUDIES OF TRANSCRIPTIONAL AND TRANSLATIONAL CONTROL MECHANISMS, OF TISSUE SPECIFIC GENE EXPRESSION AND OF TUMORIGENESIS

H.Ponta, B.Salmons, W.Günzburg, G.Knedlitschek, B.Groner, U.Rahmsdorf, P.Butkeraitis, P.Skroch and P.Herrlich

Multiple copies of MMTV proviral DNA are present in the germ line and the somatic tissues of most inbred strains of mice. The inherited (=endogenous) MMTV copies present in different mouse strains vary in number, chromosomal location and biological properties. Additionally, some mouse strains contain MMTV copies arising from exogenous infection. Effective virus production is found, almost exclusively, in the mammary glands of lactating mice, suggesting the involvement of steroid hormones in the control of virus expression. Although several proviral copies are present in the mouse genome, only some are transcriptionally active.
We have investigated the differential control of transcriptional activity of the various proviral elements, and the proviral sequences involved in the regulation of expression by steroid hormones. Virus production in the mammary gland ultimately results in mammary carcinoma. Since MMTV appears not to carry an oncogene, mechanisms involving the insertional activation of a cellular oncogene have also been investigated.

The role of methylation in the control of MMTV expression
The methylation of MMTV proviruses present in different organs of various inbred strains of mice was characterized using the restriction endonucleases Hpa II and Msp I. Although both endonucleases recognize and cleave the recognition sequence CCGG, Hpa II is selectively inhibited by methylation of the internal cytosine residue.
Previous studies of the methylation patterns of endogenous MMTV proviral copies have been limited by the inability to identify changes in the methylation of individual proviruses (9). Using double-digestions with Eco RI in conjunction with the methyl sensitive enzyme Hpa II, we have determined the methylation of the individual MMTV proviruses simultaneously, and have discovered discrete tissue and locus specific differences in the methylation of the endogenous MMTV proviruses present in five different mouse strains (8). Since the proviral structures are very similar (8), the difference in methylation cannot be determined by the proviral DNA sequence but rather by the chromosomal location into which the provirus has integrated.
The methylation of mouse cellular DNA flanking the proviral

integration site shows an identical pattern to that of the provirus. The methylation of "pre-integration" sites was investigated in strains not carrying integrated MMTV DNA at loci occupied in other lines. The methylation of the mouse DNA at these "pre-integration" sites was found to mirror that of the provirus integrated at that locus in strains carrying the occupied locus. These data suggest that the methylation of MMTV proviral DNA is imposed on the provirus by the site at which the provirus integrates (8). These findings are corroborated by studies on the DNase I sensitivity of MMTV proviruses and their "pre-integration" sites. Integrated MMTV DNA appears to be packaged into chromatin in a configuration similar to the surrounding genomic DNA. The thus imposed chromatin configuration then determines the transcriptional activity of the provirus (4).

Hypomethylation has been correlated with gene expression in many different eukaryotic systems (for review see ref. 2). In the adult GR mouse at least two endogenous loci, Mtv-2 and Mtv-3, are transcriptionally active. Mtv-2 is implicated in virus production and in mammary tumour formation (14) whilst Mtv-3 is correlated with the production of group specific (gag) or core antigens in the mammary glands, which can be detected in the milk (15). Both of these loci are hypomethylated in tissues in which they are expressed. Infection with exogenous MMTV results in additional proviral information present in the mammary tumours of many mouse strains (1,5). These exogenously acquired proviruses are hypomethylated and transcriptionally active.

The tight control over the methylation pattern of MMTV proviral DNA appears to be limited to differentiated tissues in vivo. There is a loss of methylation specificity both in mammary carcinomas and in SV40 transformed GR cell lines. The loss of methylation specificity in transformed tissues is accompanied by MMTV expression both in vivo and in vitro. Upon transformation of normal non-expressing primary liver cells with SV40, a low level of MMTV specific RNA can be observed. RNA production is further increased after demethylation using the drug 5 aza-cytidine. Further, normal transcriptional enhancement is attained by administration of the steroid hormone dexamethasone (Fig.1). A similar demethylation accompanied by MMTV specific RNA production can be artificially induced in vivo. Partial hepatectomy is followed by rapid regeneration of liver tissue. This regeneration is accompanied by a general demethylation of MMTV proviral information, and MMTV specific RNA expression (Fig.2)

The expression of MMTV specific RNA after demethylation via (1) transformation (2) 5 aza-cytidine treatment and (3) rapid cell proliferation suggests that demethylation may be directly involved in gene control, though it is unlikely to be the sole mechanism. In no case was MMTV expression observed in tissues not containing hypomethylated MMTV

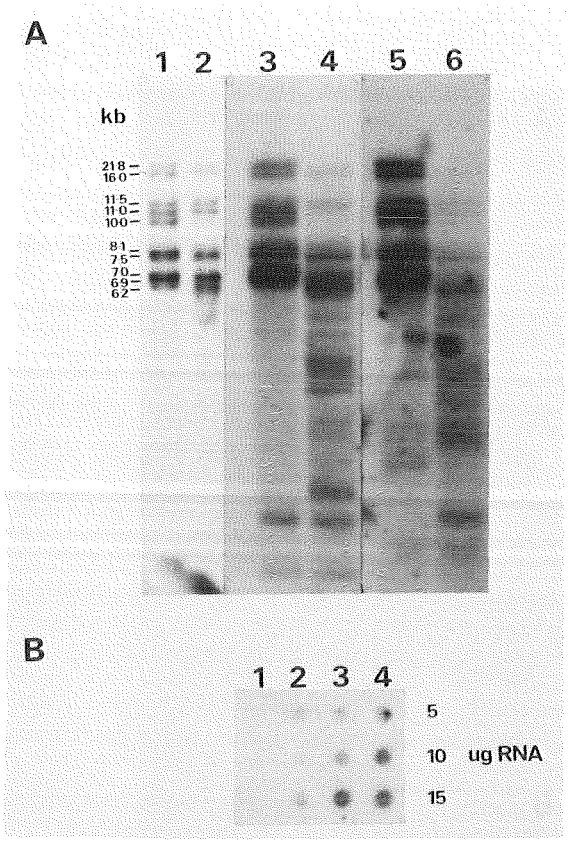

Figure 1

A. Analysis of the Extent of Methylation of MMTV Proviral Genes by Southern Blotting Visualisation of Restriction Fragments

DNA was digested with the restriction enzymes Eco RI (lanes 1,3,5) or Eco RI and Hpa II (lanes 2,4,6). After size separation by gel electrophoresis, the DNA fragments were transferred to a nitrocellulose filter and hybridized to a p-32 labeled, nick-translated MMTV probe.
Lanes 1 and 2: DNA from livers of GR mice
Lanes 3 and 4: DNA from GR liver cells immortalized by transfection with SV40 DNA
Lanes 5 and 6: DNA from SV40 immortalized GR liver cells treated with 5-azacytidine

B. Quantitation of MMTV-specific Transcription by Dot Blot Analysis

RNA prepared from various cells was dotted onto a nitrocellulose filter, and MMTV-specific transcripts visualized by hybridization to the p-32 labeled MMTV probe.
Lane 1: RNA from livers of GR mice
Lane 2: RNA from liver cells immortalized by transfection of SV40 DNA
Lane 3: RNA from SV40 immortalized GR liver cells treated with 5-azacytidine
Lane 4: As in lane 3, after treatment with glucocorticoid hormone

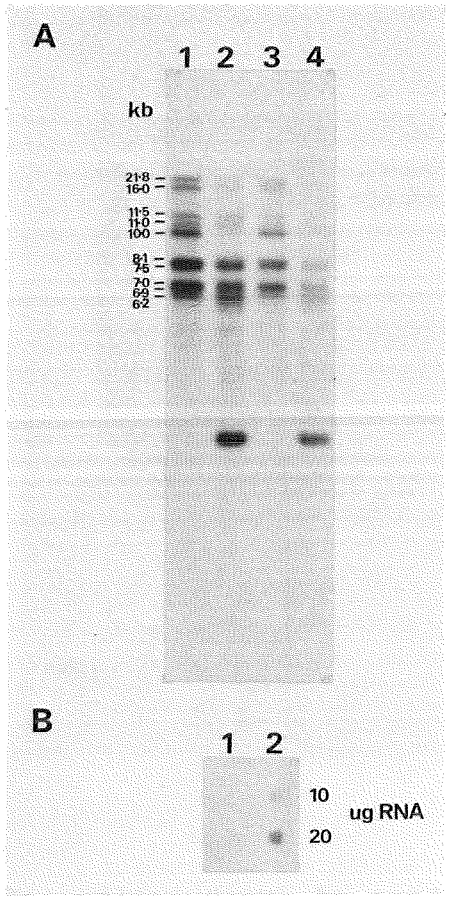

Figure 2

A. Analysis of the Extent of Methylation of MMTV Proviral Genes by Southern Blotting Visualization of Restriction Fragments

DNA was digested with the restriction enzymes Eco RI (lanes 1 and 3), or Eco RI and Hpa II (lanes 2 and 4). MMTV-specific fragments were visualized as described in Figure 3A.
Lanes 1 and 2: DNA from livers of GR mice
Lanes 3 and 4: DNA from a 5-day regenerating GR mouse liver

B. Quantitation of MMTV-specific Transcripts by Dot Blot Analysis

RNA prepared from GR livers (lane 1), or 5-day regenerating GR liver (lane 2) was dotted onto a nitrocellulose filter, and the MMTV-specific transcripts visualized by hybridization to the p-32 labeled MMTV probe.

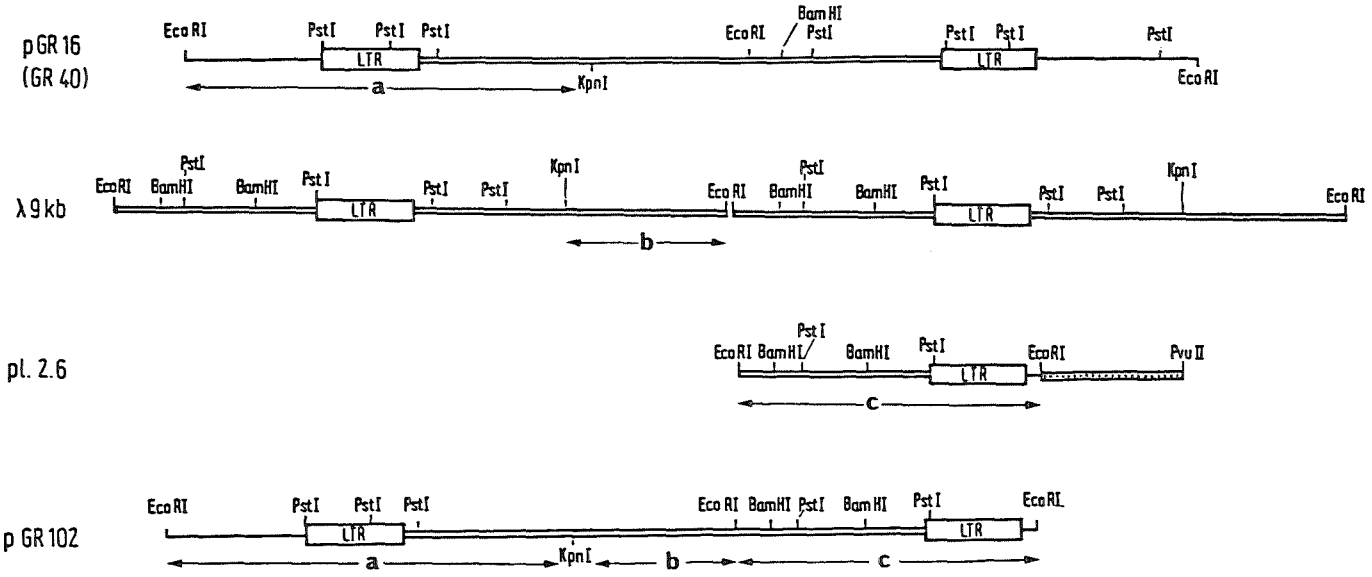

Figure 3

Schematic Representation of MMTV Proviral DNAs and Recombinant DNAs used in Transfection Experiments

Inserts cloned into the Eco RI site, or the Eco RI and PvuII sites of pBR322 (prefix p), or lambda (prefix λ) vectors, are shown. Mouse genomic DNA is indicated by a single line, MMTV sequences by a double line and thymidine kinase sequences by a double line with dots. The MMTV long terminal repeat sequence is marked LTR.
The hybrid provirus, pGR102, consists of the gag region (a), of the endogenous provirus GR40, and the pol and env regions (b and c, respectively) of exogenous type MMTV DNA.
The molecular clones shown have been described before: pGR16 (GR40) (10,20); λ9kb (27); pl 2.6 (11).

information. However, one MMTV locus is demethylated in liver tissue where no detectable MMTV RNA production occurs. Thus in accordance with the findings of others (18), hypomethylation per se, appears not to be a sufficient prerequisite for gene expression.

Production of MMTV virions upon transfection of a proviral variant

The Mtv-8 associated provirus is present in most inbred mouse strains (7,8,12). This endogenous provirus is methylated in all tissues of all mouse strains examined (22) and is apparently transcriptionally inactive (1). Although most MMTV proviruses have proved difficult to clone, the Mtv-8 associated provirus, GR 40, has been cloned in its entirety (10) (Fig.3). Transfection of the cloned, and thus demethylated, proviral DNA into both homologous and heterologous cultured cell lines results in the synthesis of apparently normal MMTV transcripts. Further, the transcription of the MMTV specific RNA is enhanced when transfected cells are grown in the presence of the synthetic glucocorticoid dexamethasone (10,20).

Mature virions, however, were not detected in the medium of transfected cells, even though at least one of the cell lines used, has been reported as fully permissive for MMTV. According to immunopreciptation of labelled cell extracts prepared from GR 40 transfected cells, the gag related proteins were indistinguishable from those found in the virus producer cell line, GR. However, only one env related protein is detected in the transfected cell lysates. This protein does not co-migrate with either the env precursor protein $gPr73^{env}$ or the mature env protein gp52 found in the GR cell line, and has an apparent molecular weight of around 66-68 kD (Fig.4). These results suggested that the Mtv-8 associated provirus carries a smaller or defective env gene resulting in the synthesis of a env precursor protein which, apparently, cannot be cleaved. Since MMTV is poorly infective in vitro and shows a high degree of tissue specificity in vivo, the lack of virus production upon transfection of GR 40 may alternatively reflect an inability of cultured cells to correctly process the viral structural proteins.

Recently, the env gene of the endogenous GR 40 as well as that of the biologically active exogenous provirus have been sequenced. A comparison of the nucleotide and predicted amino acid sequences of the GR 40 env gene with that of the exogenous MMTV (21) shows that although the predicted amino acid sequence around the cleavage site is conserved, the GR 40 env gene contains stop codons in all three reading frames just to the left of the 3' LTR, predicting a premature termination of the env protein. Thus, the carboxy terminus would lack 68 amino acids, equivalent to a reduction of 6-7kD in molecular weight. This is in good agreement with the smaller env precursor found in GR 40 transfected cells. The

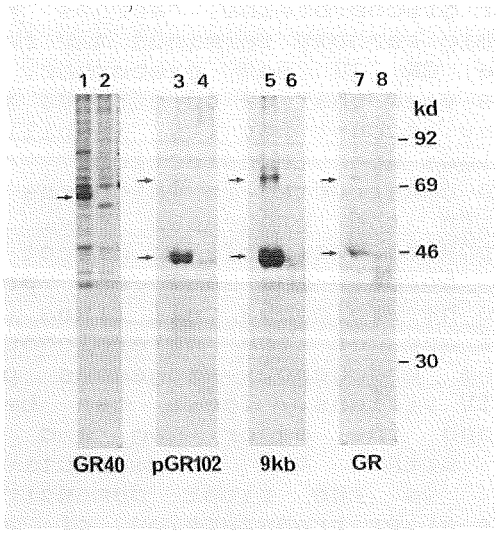

Figure 4

Immunoprecipitation of MMTV Specific Env Proteins with Anti-gp52 Serum

Transfected cat kidney cells were labeled overnight in S-35 methionine containing medium. Cell extracts were prepared and immunoprecipitated with anti-gp52 serum (lanes 1,3,5,7) which recognizes $Pr73^{env}$ and gp52 (indicated by arrows in lanes 3,5,7) and $Pr68^{env}$ (arrowed lane 1). The immunoprecipitates were recovered using Protein A sepharose and analysed by SDS-polyacrylamide electrophoresis. As a control, the same cell extracts were immunoprecipitated with normal rabbit serum (lanes 2,4,6,8).

Lanes 1 and 2: Cells transfected with the endogenous provirus GR40 (Mtv-8)

Lanes 3 and 4: Cells transfected with the hybrid construct pGR102 (Fig.1)

Lanes 5 and 6: Cells transfected with concatenates of exogenous (Mtv-2) DNA (9kb, Fig.1).

Env-specific MMTV proteins were also immunoprecipitated from a labeled cell lysate prepared from virus-producing GR cells (lanes 7 and 8).

hydrophobic carboxy terminus is probably involved in membrane interaction (13,21). The env precursor is thought to be processed, like other glycoproteins, across membranes (3). Lack of the hydrophobic carboxy terminus may severely affect the processing of the GR 40 env precursor. Thus, recent evidence supports the view that GR 40 carries a defect in the env gene, and this may account for its inability to direct the synthesis of virions upon transfection.
A hybrid molecule was constructed in which the env gene of GR 40 was replaced with the exogenous env. (Fig.3). The resultant molecule, pGR102, was able to direct the synthesis of both apparently normal gag and env proteins after transfection into two heterologous cell lines (Fig.4).
The culture medium of one of the heterologous cell lines transfected with the endogenous-exogenous pGR102 hybrid molecule contains MMTV virions. These can be purified by isopycnic banding and the major gag and env proteins immunoprecipitated with the respective antisera (unpublished). This is the first demonstration of the production of virions after transfection of a cloned MMTV molecule into cultured cells.
The availablity of a defined cloned molecule that gives rise to virus production upon transfection allows in vitro mutagenesis studies in which site specific mutations may be used to functionally define regions of the MMTV viral genome. This in turn may yield an insight into the mechanism of tumorigenesis by MMTV.

The hormone responsive element of MMTV
The expression of cloned MMTV proviral DNA, transfected into cultured cells, can be regulated by the addition of glucocorticoid hormones (10,20). In vitro recombination of the long terminal repeat (LTR) of the proviral DNA with several indicator genes such as the thymidine kinase (tk) gene, the alpha-globin gene and the bacterial neomycin resistance gene, followed by transfection of the chimeric DNAs into recipient cells has demonstrated that the DNA sequence responsible for hormonal regulation is located within the LTR. Glucocorticoid regulated transcripts were found to be correctly initiated in the LTR, but also at the initiation site of the adjacent indicator gene. Thus the hormonal regulation was conferred upon genes adjacent to the LTR.
A MMTV-tk chimeric gene was used to define the hormone responsive element (HRE) contained within the LTR (Fig.5). After progressive deletion of nucleotide sequences from either the 5' or 3' ends of the LTR, the resultant molecules were transfected into cultured cells. Correctly initiating LTR or tk transcripts from cells grown in the presence or absence of dexamethasone were quantitated by the S_1 mapping technique. Progressive deletions from the 5' end showed that 950 nucleotides of the 1328 nucleotide LTR could

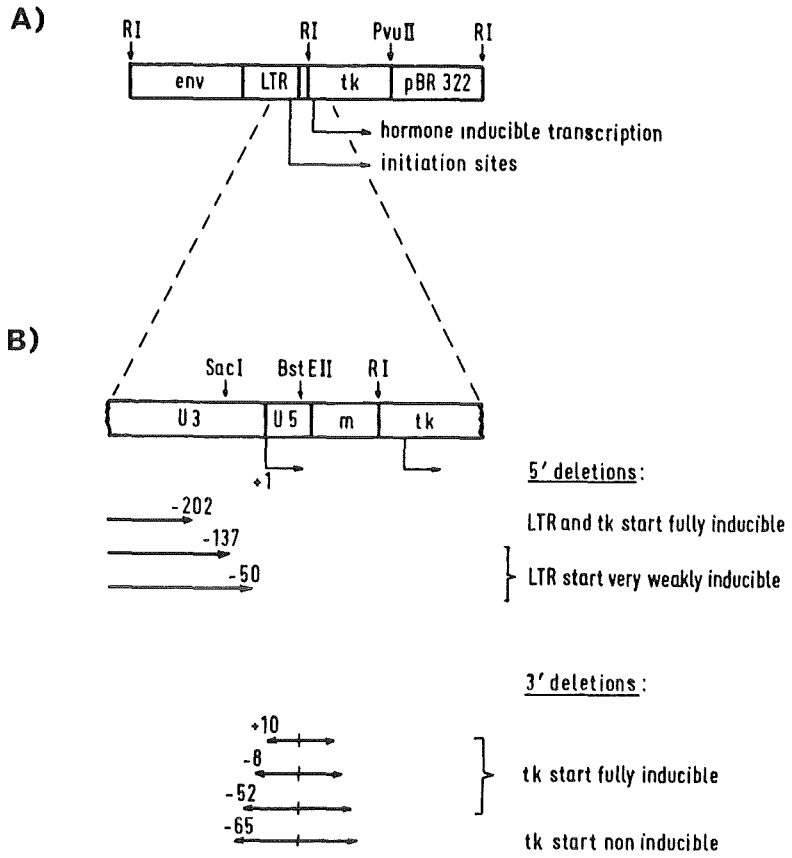

Figure 5

Schematic representation of deletion in MMTV-tk chimeric gene

The chimeric MMTV LTR-tk plasmid shown has been described (11). This plasmid contains two initiation sites for transcription by RNA polymerase II, one located in the MMTV LTR and the other in the adjacent tk gene. Both starts are regulated by glucocorticoid hormones (11). The plasmid was manipulated in vitro and the sequences which have been deleted are indicated by arrows beneath the enlarged portion of the LTR-tk fragment in B. Hormonal inducibility of the deletion mutants was tested by the S1 nuclease protection assay upon transfer of the plasmid DNA into mouse L-cells and the results are summarized in B.

be deleted without affecting hormone inducibility, both from the LTR (at nucleotide 1194) and the tk initiation sites (11). Progressive deletions from the 3' end of the LTR showed that both the proviral initiation site and the proviral promotor (TATA box), are not part of the hormonal regulation sequence. The removal of 3' sequences up to a position 59 nucleotides 5' to the LTR initiation site does not affect the hormonal induction of correctly initiating tk transcripts (6).
We have isolated an Eco RI fragment containing 184 nucleotides of the MMTV LTR (-236 nucleotides to -52 nucleotides 5' to the initiation site) which should encompass the HRE. This fragment was independantly combined with two indicator genes, the tk gene and the mouse alpha-globin gene. When the HRE was placed upstream and in the same orientation as the indicator gene, the expression of the respective gene was enhanced 3 to 5 fold by glucocorticoid treatment. This demonstrates that the area of the LTR defined as the HRE by deletion mapping contains all the sequences necessary for hormone regulation. Furthermore, since the HRE is positioned 460 bp upstream of the tk initiation site and 1100 bp upstream of the alpha-globin initiation site, the positioning of this element with respect to a promoter appears to be quite flexible.

Position and orientation dependent enhancement of the alpha-globin and the tk gene by MMTV proviral sequences
Insertional activation of a cellular oncogene is very likely a central step in MMTV mediated tumorigenesis (17). In various MMTV induced mammary carcinomas, either of two cellular genes, Int-1 and Int-2, were found to be activated by integration of MMTV proviral DNA in a region spanning about 10kb 5' or 3' to these genes (16,19). In nearly all cases, the transcriptional orientation of the integrated proviral DNA was directed away from the Int genes (schematic representation in figure 7). This is inconsistent with a promoter insertion model of oncogene activation and suggests the existance of a different mechanism of activation: the insertion of enhancing sequences. To define the MMTV sequences required for enhancement of an unrelated promoter, two series of constructs were made. We independently combined the tk or the mouse alpha-globin indicator genes with the HRE fragment in various positions and orientations (Fig.6). In addition the indicator genes were also inserted 5' and 3' to a complete provirus in both orientations (Fig.7). The rationale of this set of constructs is to mimic the tumour situation where the indicator gene represents the Int gene and allows the effect of the provirus on Int transcription to be monitored. After transfection of the different constructions into recipient cells, the expression of the indicator genes was examined, both in the presence and absence of hormone. The term "expression" is used synonymous

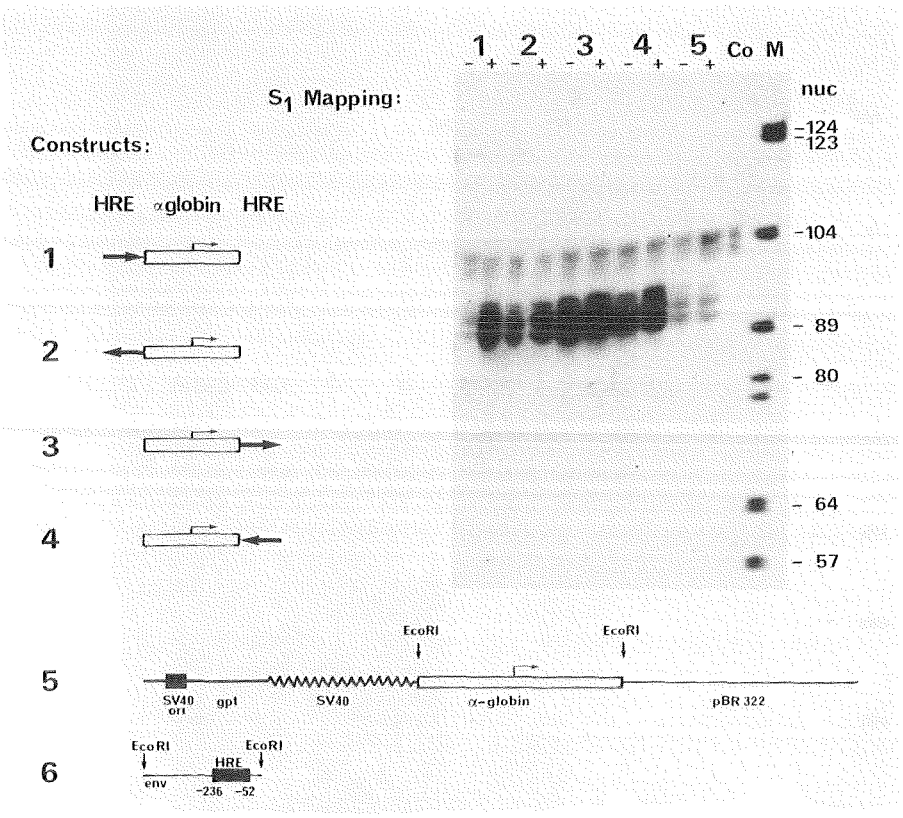

Figure 6
Position and Orientation Effects of the MMTV HRE on the Transcription of the Mouse α-globin Gene

The mouse α-globin gene flanked by Eco RI sites (25) was inserted into the pSV2 gpt vector (26). This plasmid was kindly provided by E.Wagner. A 774 bpEco RI fragment containing 184 bp of LTR sequences (-236 to -52, construct 6) was inserted into Eco RI site located 1.1 kb 5' of the globin cap site in both of the possible orientations (constructs 1 and 2) or into an Eco RI site located 1.1 kb 3' of the globin site (constructs 3 and 4). Mouse L cells were transfected with the constructs 1 to 5 and stable transfected clones were grown in mass culture in the presence or absence of hormone. Cellular RNA was hybridized to a 104 nucleotide, Hae III, PstI fragment, in which the Hae III site was 5' end labeled. This fragment spans the transcription start of the globin gene. Accurately initiated globin mRNA protects a 98 nucleotide fragment. A slight heterogeneity in the initiating nucleotide can be observed resulting in the bands seen on the autoradiograph. The numbers at the top of the autoradiograph refer to the construct used for transfection shown on the left. In the control lane (co) yeast RNA was introduced into the S1 mapping reaction.

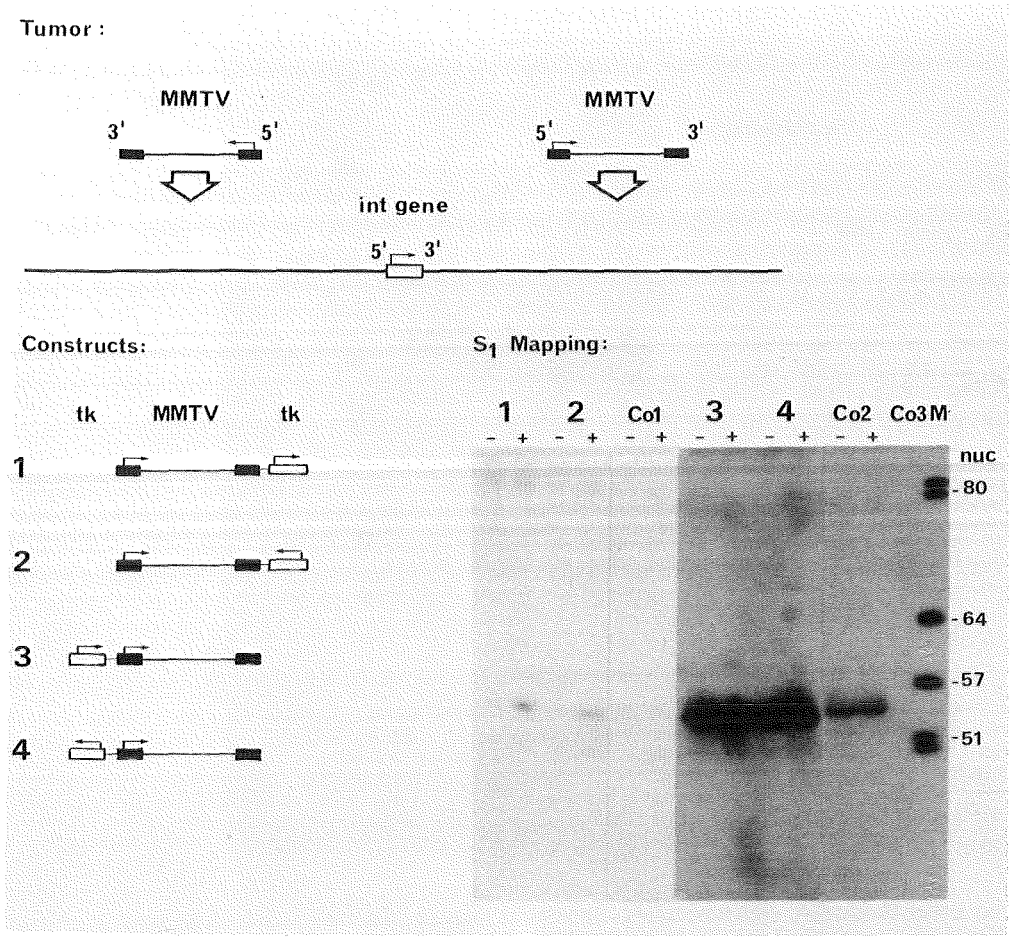

Figure 7

In Vitro Recombination of MMTV Proviral DNA with the Thymidine Kinase (tk) Gene

A molecular clone of MMTV proviral DNA (pGR21), similar to pGR102 (Fig.3) was used for in vitro recombination. A 2.4 kb Eco RI fragment of the tk gene (24) was inserted into the Eco RI site located 222 bp 3' of the right LTR in both of the possible orientations (constructs 1 and 2). A 3.6 kb Bam HI fragment of the tk gene was inserted into the Bam HI site located 1 kb 5' of the left LTR in both of the possible orientations (constructs 3 and 4). The constructs were transfected into L cells and grown in the absence or presence of glucocorticoid hormone. Transcripts originating from the tk start were quantitated by S1 mapping analysis as described in figure 5. The number at the top of the autoradiograph refer to the constructs shown on the left. The controls refer to transfections with the cloned tk Eco RI fragment (co 1) and to the cloned tk Bam HI fragment (co 2) devoid of proviral DNA. No signal was observed when yeast RNA was introduced into the S1 mapping reaction (co 3).
The upper part of the figure shows schematically the integration of proviral DNA in the vicinity of the cellular int-gene in tumors.

for RNA levels determined by S1 analysis. A summary of results obtained using the alpha-globin gene coupled to the HRE is shown in Figure 6. Hormone induction was convincingly found only when the HRE was 5' of the indicator gene, and had the same orientation with respect to the direction of transcription as it had in the original LTR. In the three other constructions the hormone effect was much less pronounced. Thus the HRE functions in an orientation and position dependent way, the functional orientation being exactly opposite to the one found in most tumors. Also in the experiment using the intact provirus coupled to indicator genes (Fig.7) hormone regulation was conferred to the indicator gene when the provirus was placed 5' to the indicator gene in the same transcriptional orientation. In contrast to the HRE recombinants ,the indicator gene could be turned around without loss of hormone inducibility. We remember that both these situations do not occur in the tumors. The relevant positioning of the indicator gene 5' to the intact provirus, in either orientation, did not result in hormonal enhancement of transcription.
The comparison of expression of the chimeric constructs with the transcription of transfected constructs containing the indicator genes alone, led to the discovery of a new enhancing activity. In all the constructions containing the MMTV provirus or the HRE linked to an indicator gene, where hormonal regulation of the indicator gene was not observed, the hormone independent transcription was greater by a factor of more than 5 as compared to the transcription from the indicator gene alone. Thus it seems that we have discovered an MMTV enhancer element, which activates adjacent genes in a hormone independent way. These situations correspond to those found in the tumor cells, in the neighborhood of both int 1 or int 2. Integrated MMTV provirus most likely exerts its activating effect on adjacent genes by this hormone independent enhancing sequence.

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4. Year-round Research (Short scientific reports)
d) Radiationtoxicology of Actinides

AMERICIUM-241 IN THE BEAGLE SKELETON: MICRODISTRIBUTION AND LOCAL DOSIMETRY

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Americium-241 if entering the blood by one of the usual routes of intake, inhalation, ingestion or wounds, deposits preferentially in the skeleton. Its initial skeletal distribution is highly non-uniform on internal and external skeletal surfaces. The purpose of this study is to quantitate the distribution of Am-241 with respect to definite anatomical regions and determine localized radiation doses.

Methods

Two groups of young adult beagle dogs, 461-485 days old and comprising 3 animals each, received 104.3 kBq/kg (level 5) or 33.3 kBq/kg (level 4) Am-241 by i.v. injection, respectively. Level 5 dogs were sacrificed between 7 and 20 days, level 4 between 1300 and 1569 days after injection. The proximal humerus (PH), distal humerus (DH), proximal femur (PF), distal femur (DF), proximal ulna (PU) and a lumbar vertebral body (LVB) were embedded and sectioned into slices by a rotary saw. The sections were then stained with Alizarin red and mounted in contact with CR 39 alpha-track detectors. After finishing the exposure and etching (6.25 NaOH, 60^{o}C, 1 hr) the plastic track detectors and stained bone sections were analyzed by means of a scanning microscope photometer controlled by a PDP 11/02 minicomputer. Evaluation of scan data was performed by a series of FORTRAN programs on the same minicomputer.

Results and Discussion

The average concentration of radioactivity was found to be highest in the proximal and distal femur and lowest in the humerus in level 5 animals. For level 4 animals the concentrations in the distal humerus and the proximal ulna are distinctly lower than the values in the other bones. The average skeletal concentration for level 5 was estimated to be 592 Bq/g which is in good agreement with autoradiographic data (table 1). The mean spec. surface activity was also highest in both parts of the femur in level 5 (table 1). Considering the standard errors of the means (not given in the table) there does not seem to be much variation between individual bones. It is possible to obtain a global estimate of the specific surface activity for the whole skeleton based on known values for trabecular and cortical surface/volume ratios (170/cm and 30/cm). Thus a value of

14.3 Bq/cm^2 results which is also comparable to autoradiographic measurements in individual bones (table 1). Fig. 1 shows as an example the probability density of spec. surface activities in the humerus. The curves illustrate the non-uniformity of the surface deposition of Am-241. The relative variation of the spec. surface activity defined as the ratio standard deviation/mean ranges between 0.31 and 0.49 and does not reveal any conclusive trend between level 4 and level 5 samples. The average ratio between spec. surface activities of level 5 and level 4 samples is 2.61. This figure is close to the global skeletal ratio of total activities (2.29) which can be derived from skeletal retention curves. With the exception of the distal humerus the dose rates in 'deep' marrow of level 5 samples are comparable. In level 4 samples this parameter shows more variation (table 1). No obvious tendency to clustering of tracks with time in the marrow could be seen.

Local dose rates to the 10 microns wide band of marrow adjacent to bone surfaces have been calculated in three ways:

1. Burial of surface deposits is ignored.
2. The locally determined burial depth has been taken to calculate the dose rate.
3. The mean burial depth and mean spec. surface activity over the area scanned was used to calculate the dose rate.

The results are listed in table 1. Procedure 2 tends to underestimate the dose rates, procedure 1 is a conservative estimation of this quantity. To give the dose rate figures an intuitive meaning it may be noted that a surface activity of 10 Bq/cm^2 of Am-241 results, for a spherical nucleus of 5 μm diameter at a distance of 5 μm from the surface, in 1.25 alpha-particle traversals per week.

For the case of cells lining resting bone surfaces it is possible to estimate accumulated radiation doses. Such an estimation has to be based on specific assumption concerning the residence time of surface labels. It one assumes that this residence time is distributed exponentially (random remodelling) then the mean residence time is 1/r (r = bone turnover rate). Using turnover rates as determined in an earlier experiment by Kimmel et al. one obtains the cumulative radiation doses as listed in table 1. The distal humerus and proximal ulna have the lowest specific surface activity in level 5 samples but nevertheless show the highest cumulative doses due to the low turnover rates in these bones.

True burial depths of Am-241 labels at individual surface sites cannot be determined directly with sufficient accuracy with the photometric scanning procedure employed. This is due to confounding factors such as section thickness, alpha-particle range, inclinination of bone surfaces and the finite size of the probing frame. Consequently even if all labels are on surfaces a distribution of burial depths extending into marrow and bone will always be measured. The true

Table 1

Parameter	LVL	PH	DH	PU	LVB	PF	DF	
Average concentration over scan area (Bq/g)	5	445.1	213.8	362.3		558.2	538.6	
	4	190.3	122.1	99.1	196.3	217.1	194.1	
Mean specific surface activity (Bq/cm**2)	5	17.67	13.90	16.27		24.85	21.25	
	4	7.90	5.56	5.37	7.94	9.76	7.66	
Dose rate to 0-10 µm band (mGy/d)	5	388.8	305.8	357.9		473.4	467.6	1)
	5	168.4	139.2	169.9		212.6	186.3	2)
	4	145.5	122.3	118.2	174.8	214.8	164.5	1)
	4	49.8	37.4	37.4	53.2	66.6	42.8	2)
	4	65.8	58.1	43.9	68.2	104.7	66.5	3)
Dose rate in deep marrow (mGy/d)	5	15.10	2.75	26.6		16.55	19.19	
	4	9.53	0.46	4.0	7.5	10.11	13.62	
Accumulated rad. dose (Gy) from initial deposits	5	87.7	209.4	251.6		155.4	150.3	
Surface/ volume ratio (1/cm)	5	213.4	134.1	122.8		173.0	200.7	
	4	164.8	104.6	84.5	127.9	132.9	145.8	

1) Burial ignored 2) Local burial depth 3) Average burial depth

LVL- injection level, PH- proximal humerus, DH- distal humerus, PU- proximal ulna, LVB- lumbar vertebra, PF- proximal femur, DF- distal femur.

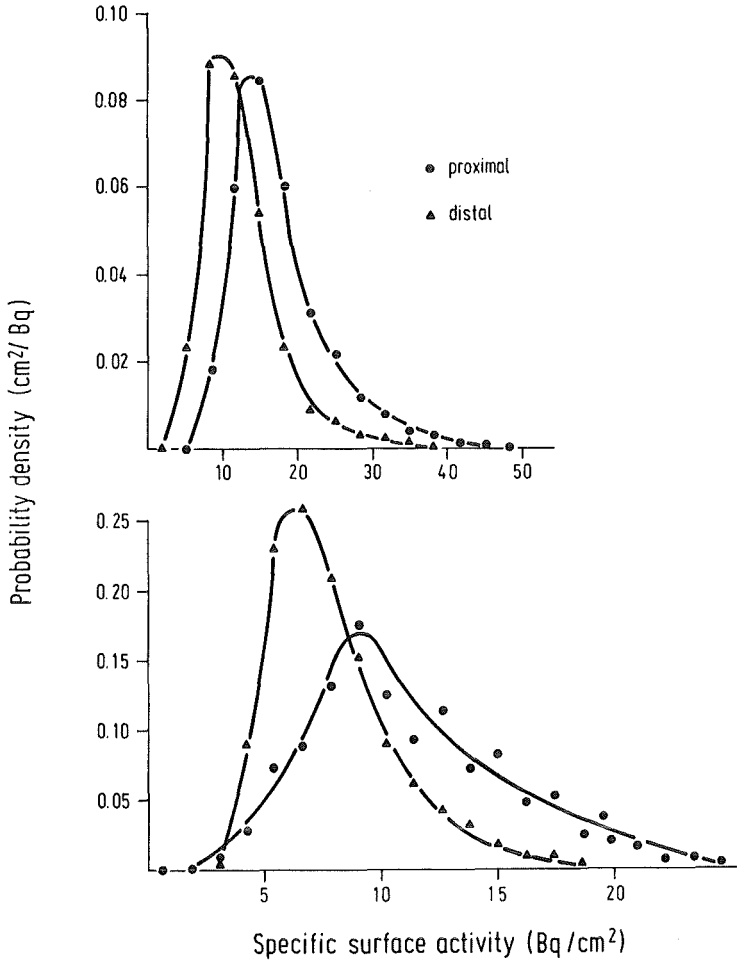

Fig.1: Probability density of specific surface activity of Am 241 in trabecular portion of proximal and distal humerus. Top: Level 5 (104.3 Bq/kg). Bottom: Level 4 (33.3 Bq/kg). Horizontal axis is scaled according to ratio of injected activities.

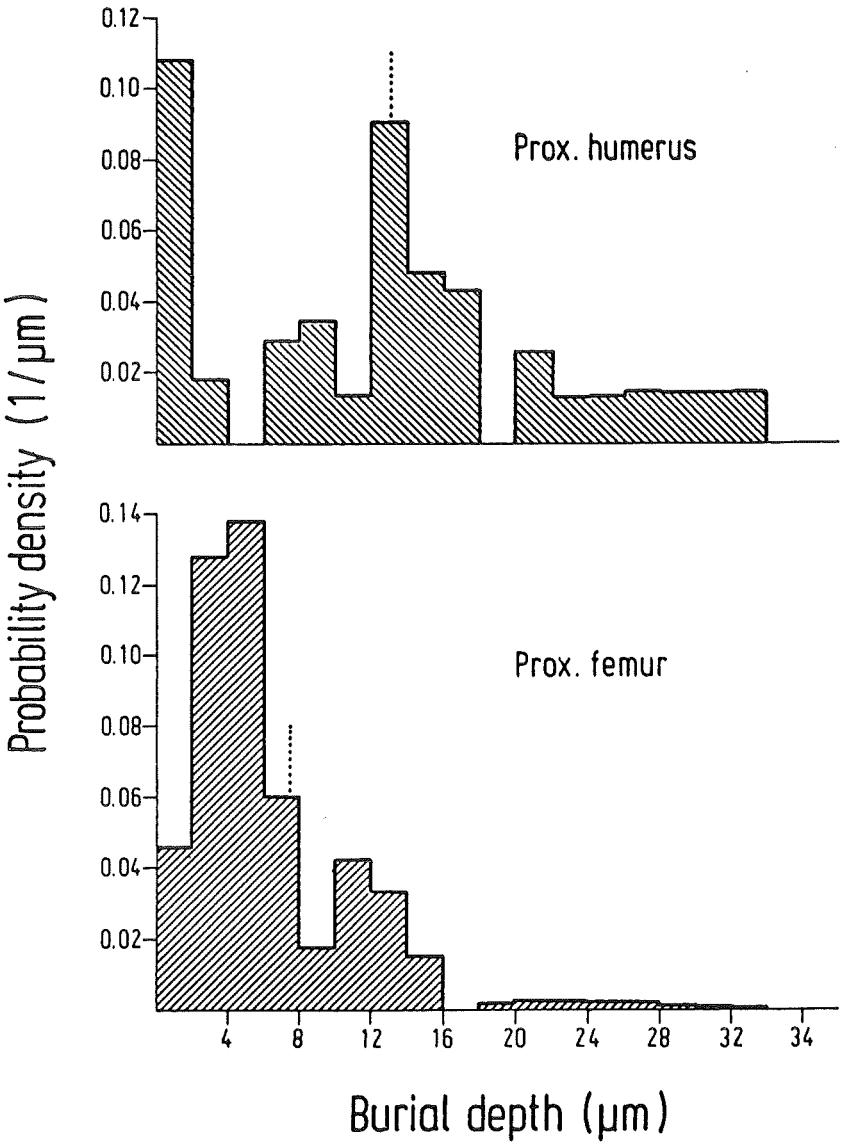

Fig.2: Density of burial depths obtained by deconvolution for level 4 sections of a prox. humerus and prox. femur.

distribution of burial depths was therefore determined by deconvolution from a reference distribution obtained from samples in which Am-241 labels are known to be coincident with bone surfaces. The reference distribution was calculated from selected samples of level 5 animals, and the densities of burial depths calculated for level 4 samples using deconvolution combined with a numerical optimization technique. Fig. 2 shows as examples the densitites found in a particular sample of the proximal humerus and proximal femur. The diagrams reveal that the average burial depth in the proximal humerus sample is 13 µm, that 38.2% of all surface labels have a burial depth between 0-10 µm and that 9.1% of the surface labels are located deeper than 28 µm, the range of Am-241 alpha-particles in bone. The corresponding figures for the proximal femur are 6.7 µm, 78.2% and 1.3%, respectively. The mean burial depths are marked as dotted lines.

To check whether a similar correlation as existing between initial uptake of Pu-239 and bone turnover rates could be established for Am-241 too, the correlation coefficients between each spec. surface activity, average concentration over scan area, concentration in deep marrow on one hand, and bone turnover rate on the other hand, was calculated. They were found to be 0.59, 0.72 and 0.74, respectively, and significantly different from zero (95% level). This positive correlation thus implies that the affinity of Am-241 to bone surfaces, like Pu-239, increases with increasing bone turnover.

Some morphometric parameters of trabecular bone are obtained as a by-product of the bone structure scanning process. The most appealing differences between level 5 and level 4 samples were observed with respect to the surface/volume ratio S_V (table 1). Level 4 animals show distinctly reduced values of S_V compared to level 5 animals. In control dogs S_V can be expected to be increasing with age. Thus it must be assumed that radiation damage caused alterations of bone structure in a similar way as observed in rats in a previous experiment.

Conclucions

Specific surface activities and dose rates to the 0-10 µm band are not very different in the types of bones investigated but there is a positive correlation between these parameters and bone turnover. Dose rates in deep marrow differ widely between individual types of bone but no tendency towards 'star formation' could be detected in autoradiographs. Average burial depths for surface labels are comparable in the various types of bone (5.3-8.6 µm). Morphometric parameters indicate that radiation damage caused alterations of bone structure.

COMPARISON OF BONE TURNOVER RATES CALCULATED BY TWO DIFFERENT METHODS: THE VITAL STAINING TECHNIQUE AND THE ANALYSIS OF AUTORADIOGRAPHICS FROM BONE SURFACE SEEKERS.

W. Sontag

1. Introduction

To understand the dynamic alteration of the skeleton in normal and pathological conditions many investigations have been made in recent years using the vital staining technique. After application of one or more injections of substances such as tetracycline or calcein, which form fluorescent calcium-complexes on sites of bone mineralisation, these coloured bands can be detected using a fluorescence microscope (Fig. 1A and 1B). This was the basis of qualitative and quantitative measurements of bone formation and indirect bone resorption, because the distance between the fluorescence bands, which are only a few micrometers thick, or the distance between fluorescence band and bone surface can be measured with a high accuracy. From the known distance and the length of the bands the appositional rate and the bone formation rate can be calculated easily.

Some radionuclides, such as plutonium and americium, are initially bound in different concentration on bone surfaces. They form a layer less than one micrometer thick, which appear in autoradiographs as a zone of high track density, called a "hot line" (Fig. 1C). The breadth of the hot line is dependent of the range of the emitting alpha-particle and varies between 50 and 70 micrometers. If the radionuclides are on or near bone surfaces, the radiation dose from the emitting alpha-particles may damage the cells lying near bone surfaces, causing a disturbance of the bone turnover rate.

The aim of the present investigation is to use the hot-line distribution for calculating bone turnover rate. From earlier investigations it is known that within bone matrix the buried calcein and plutonium lies in the same position (1), on the other hand the behaviour of calcein and the radionuclides differs, dependent on the state of the bone surfaces. Bone surfaces can be divided into three classes (Fig. 2): a) Forming surfaces: New bone is formed by osteoblasts, the calcein or radionuclide lying initially on this surface is buried under newly forming bone. b) Resorbing surfaces: Existing bone matrix is resorbed by osteoclasts, the calcein or radionuclide lying initially on this surface is resorbing, also. c) Resting surfaces: Over the whole observation time no remodeling takes place, the initially bound radionuclide layer does not change, and no calcein fluorescence is observed.

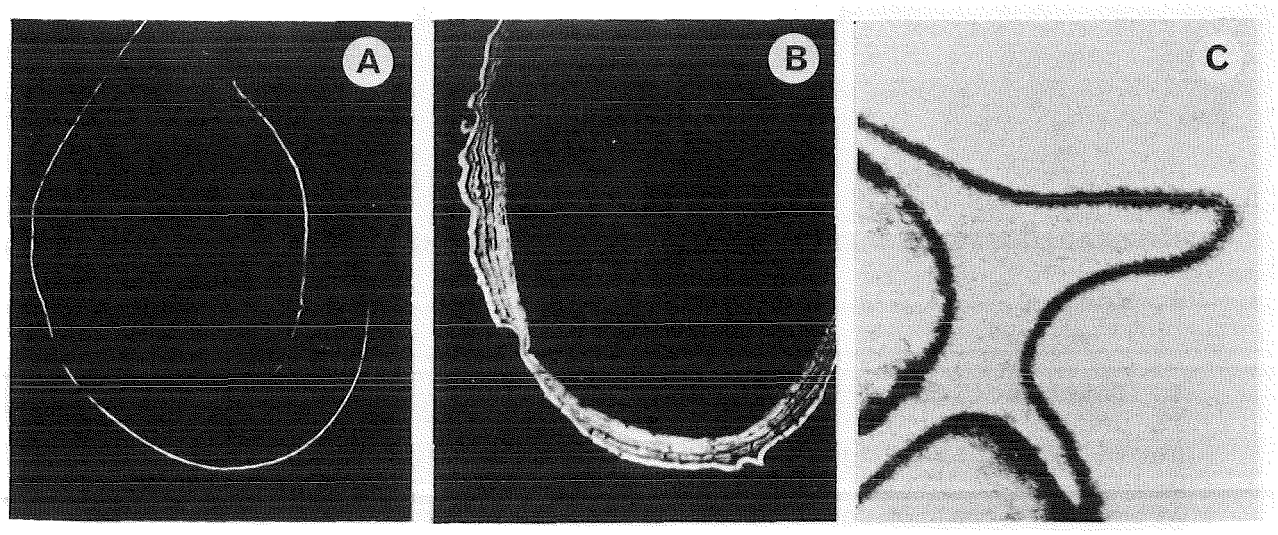

Fig. 1: Photomicrographs of single (A) and multible (B) injections of calcein and in rat femur, a neutron-induced autoradiograph (C), showing a hot-line distribution, following injection of Pu-239.

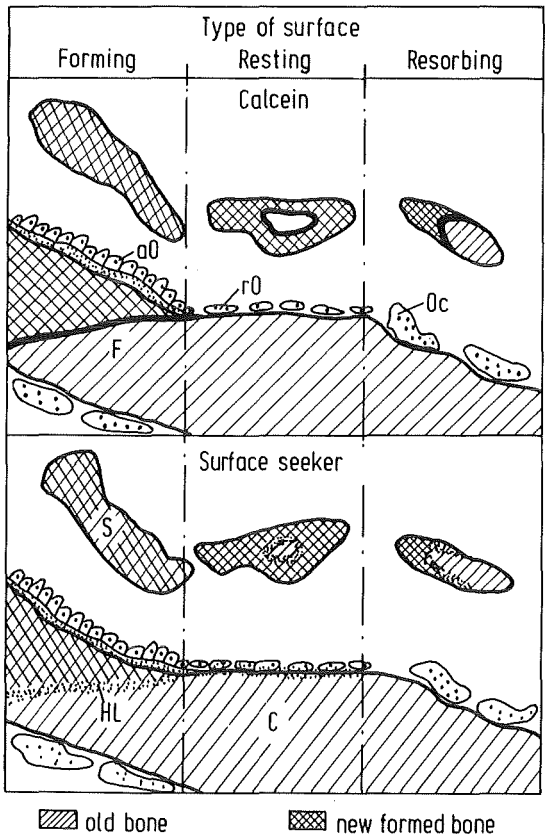

Fig. 2: Schematic description of the behaviour of calcein (F) and a surface seeker (HL) in cortical (C) and cancellous (S) bone with active (aO) and resting (rO) osteoblasts, and osteoclasts (Oc).

2. Material and Methods

For the present examination three groups of 60 day old female rats of the Heiligenberg strain were used: Group 1 received an intravenous injection of plutonium-239 (11 kBq/kg) and few hours later the first subcutaneous injection of calcein. The calcein-doses were repeated twice weekly until death. Group 2 received only one injection of plutonium-239 (11 kBq/kg) and group 3 received only the multiple injections of calcein. The rats were killed between 1 day and 32 days after injection; selected bones were removed, embedded in methyl-metacrylate and cut, on a Leitz sawing microtome, into about 60 micrometer thick sections. From the same bone sample in group 1 both neutron-induced autoradiographic as well as fluorescence-analyses were made. Detailed descriptions of the fluorescence- and autoradiographic analysis have been published (2,3), so that only the calculation of bone turnover from autoradiographics is described briefly here (Fig. 2): After detection of the hot-line, the distance between the middle of the hot-line and the bone surface is calculated. If the hot-line is lying on bone surfaces, that means in a layer 5 micrometers into bone and 10 micrometers into marrow, the distance is set equal to zero and the surface is quantified as resting; if the hot-line is more than 5 micrometers into bone then the surface is quantified as growing; the rest of the surface must be, per definition, of the resorbing type.
In each measuring field the distance between the endosteal and periosteal surfaces and the label has been calculated from the autoradiographs as the shortest distance between the middle of the hot-line and the surface; the same criterion was used after single injections of calcein. In the case of multiple injections of calcein the shortest distance is calculated from the calcein labeled bone border to the bone surfaces, that means if the whole bone is labeled (left trabeculae in Fig. 2) no border exists. Calcein labeled bone surfaces describe these fields which contain surface as well as a calcein label, that means the whole surfaces in the left and the middle trabeculae in Fig. 2 would be labeled.

3. Results and Discussion

Hard tissue can be divided into cortical and cancellous bone. In man between 70 and 80% of the total bone is assumed to be cortical bone, the rest is cancellous or spongy bone (4). Therefore the comparison between calcein and plutonium has been made in the diaphysis as an example of cortical bone and in the metaphysis as an example of predominantly spongy bone. The calculation of the morphological parameters such as bone and marrow area and the length of the endosteal and periosteal surfaces is made in the same way in both parts of examination, so that the results obtained are comparable within the experimental error.

Diaphysis

To compare the different methods of measurement the diaphysis is an ideal object for examination, because the wall thickness is relatively great, no resting surfaces exist and sites of resorption and formation are separate over a long observation periods (5). Fig. 3 shows the behaviour of the endosteal surface after treatment of plutonium and calcein. In the calcein treated rats the labeled surface and the ratio of label to surface show a similar behaviour, both increase with increasing time after injection from 40% to a plateau of about 55% which agrees with the fraction of forming surface. At one day after injection of plutonium more than 80% of the surface is designated as resting, because most of the hot-line is still lying near the surface, but the fraction of resting surface decreases rapidly with increasing time after injection to less than 5%, whereas the forming surface increases to the expected value of about 60%.
The distances calculated for the two different indicators (Fig. 4) is similar, whereas the fraction of formed bone (Fig. 5) calculated from the distance fluorescence label to surface and the distance hot-line to surface are comparable within the first 20 days, but differ at 32 days after injection.

Metaphysis

The metaphysis is more complicated than the diaphysis, because the thickness of the trabeculae is very thin (about 80 micrometer), and due to the growth in length new trabeculae are formed near the epiphyseal plate and resorbed again near the diaphysis, so that in the whole metaphysis the processes of formation and resorption run simultaneously and cannot be separated in some morphological regions.
In Fig. 3 the behaviour of the endosteal surface is shown, the calcein labeled surface is 2-fold higher than the ratio label to surface, whereas the former increases with increasing time after injection, the ratio label to surface increases up to 12 days and then decreases after this time point and thereby shows a similar behaviour as the growing surface in the plutonium treated animals. A similar approach shows fig. 4, that in both cases the distance between surface and label increases to a maximum and then decreases at longer times.
This can be explained by consideration of the bone turnover dynamics in this region of bone. After injection of plutonium the whole surface is contaminated, but a short time after injection this label on sites of resorption is removed again, in contrast after injection of calcein only forming surfaces are labeled. With increasing time after treatment new bone is forming, so that the calcein- and plutonium labels become buried and the distances surface to label increase in both cases, simultaneously near the epiphyseal plate new unlabeled trabeculae are formed (Fig. 2). If the labeled trabeculae

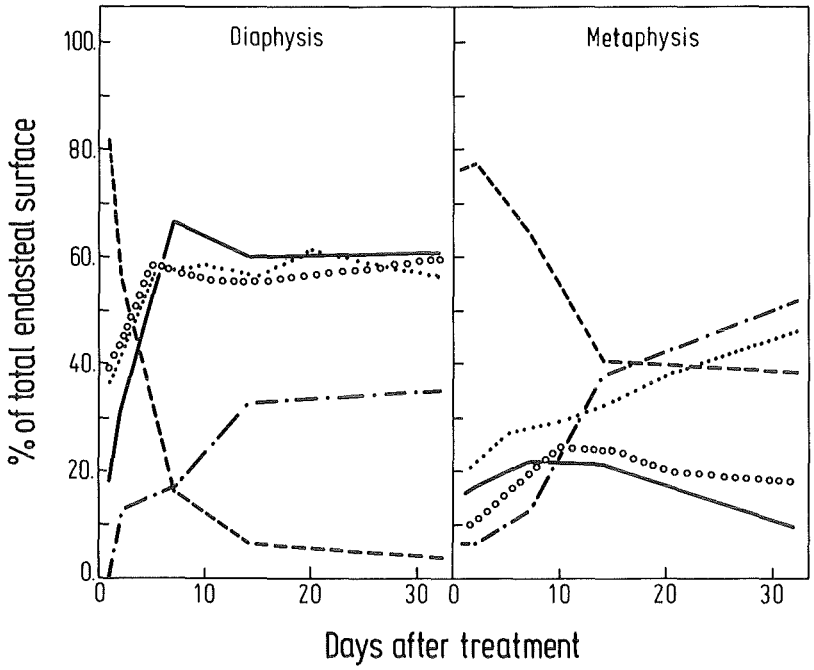

Fig. 3: Classification of the endosteal surfaces in the diaphysis and metaphysis of the femur as a function of time after injection. After treatment with calcein the labeled bone surfaces (...) and the ratio inner label to bone surfaces (ooo) were calculated, whereas after injection of plutonium the surfaces were divided into growing (——), resting (- -) and resorbing (-.-).

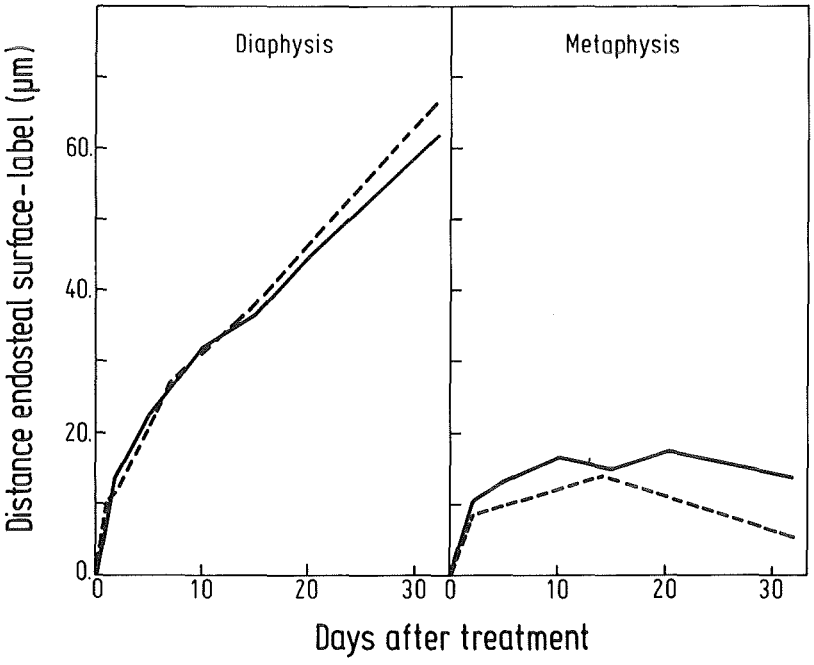

Fig. 4: Distances between endosteal bone surfaces and label in diaphysis and metaphysis of the femur as a function of time after injection calculated from the calcein label (——) and the plutonium hot-line (- -).

Table 1 Remodeling parameters in diaphysis and metaphysis 2 days and 32 days after injection, data are obtained from calcein (C)and from plutonium (P) analysis. Arithmetic means ± S.E.; 4 animals per group (Group 1).

Parameter	Typ	Diaphyse		Metaphyse	
		2 days	32 days	2 days	32 days
Endosteal growing surface (%)	C	42 ± 6	59 ± 5	11 ± 2	18 ± 3
	P	27 ± 7	35 ± 6	17 ± 4	10 ± 3
Distance endosteum to label (µm)	C	13 ± 3	66 ± 11	10 ± 2	14 ± 4
	P	11 ± 3	61 ± 18	8 ± 3	5 ± 3
Total of new formed bone (%)* real	C	3.2 ± .5	33 ± 4	14 ± 2	59 ± 6
label	C	4.0 ± .3	29 ± 3	12 ± 3	18 ± 5
label	P	2.8 ± 1.1	24 ± 7	11 ± 4	14 ± 6

*The per cent of new formed bone is calculated both from the number of calcein labeled measuring fields (real) as well as from the distance label to surface (label).

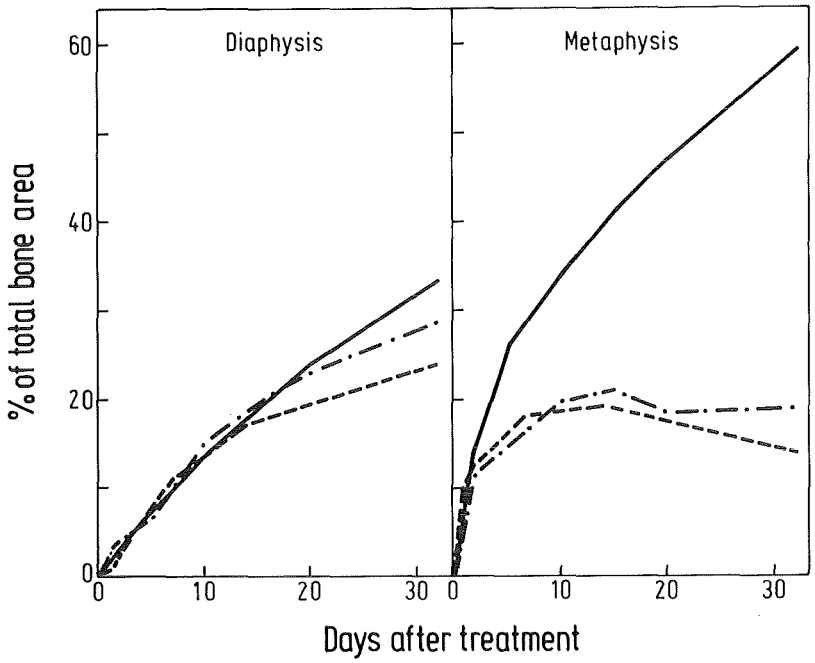

Fig. 5: Newly forming bone in diaphysis and metaphysis as a function of time after injection calculated from the calcein-labeled bone (——), from the distance calcein label to surface (-.-) and from the distance plutonium hot-line to surface (- -). In this presentation the endosteal and periosteal parts of the growing bone have been summed.

reach zones of resorption, the whole trabeculae become smaller (right trabeculae in Fig. 2) and the distance label to surface decreases.
Figure 5 shows the new forming bone area calculated by three different ways. Whereas the number of labeled fields indicate that the real total amount of new bone diminished in proportion to the resorbed fraction in the meantime, both calculations from the distance label to bone surface yield values which are too small. Only in the first two days are the results obtained from the three different ways of calculation comparable.
From the preceding discussion it is clear that in principle it is possible to calculate the remodeling parameters both from calcein labeled bone as well as from plutonium labeled bone. However, the values obtained from autoradiographs must be interpreted carefully, because they may be underestimates at the earlier, or later time points, when the hot-line is lying near the surface, especially in cancellous bone. Further the values are dependent on the accurate determination of the hot-line and the distance between the middle of the hot-line and the bone surface, this can be measured more exactly in neutron-induced autoradiographs, where hot-lines are smaller, than in alpha-autoradiographs. Normally the error is considerably greater for data obtained from autoradiographs (Table 1).

4. Conclusions

In young growing female rats bone remodeling has been studied in the diaphysis and the metaphysis of the femur in two different ways: From calcein labeled bones and from plutonium labeled bones. Whereas in the diaphysis both methods yield to the same results over a long period of observation, in the metaphysis the autoradiographic data lead to an underestimation for both early and later time points.

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THE GASTROINTESTINAL ABSORPTION AND LONG-TERM RETENTION OF NEPTUNIUM IN RATS

R. Wirth, V. Volf

1. Gastrointestinal Absorption

The aim of the present study was to estimate the intestinal absorption of neptunium as influenced by the mass and acidity of the administered solution as well as by the age, sex and nutrititional status of the animals. Furthermore, the kinetics of neptunium following intravenous and oral administration were compared with those of simultaneously administered plutonium. The experiments were carried out with young and adult Sprague-Dawley rats.

Nutrititional status seems to be a dominant factor for the absorption of neptunium from the gut. In our experiments there was a 6-fold higher absorption in fasted than in fed male rats, irrespective of their age (Table 1). Under the same conditions our fasted adult male rats absorbed 0.12% Np-239 which is approximately twice the amount absorbed by the fasted adult females (0.05% of administered dose).

The gavage of 1 and 10 mg Np-237/kg body weight resulted in an absorption of 0.23% and 0.26%, respectively. These values correspond to those reported by Harrison et al. (1) for about 2.5 mg Np-237/kg (0.26%) and by Sullivan et al. (2) for 5 and 10 mg Np-237/kg (0.1% and 0.2%). On the other hand, the absorbed fractions reported after gavage of 22 mg and 43 mg of Np-237/kg were 1.5% and 2.7%, respectively (2). Thus it appears that a high absorption of neptunium in adult animals is to be expected only if a large mass is ingested, more than 10 mg Np-237/kg in the rat.

The total average absorption of neptunium in adult male rats was independent of the nitric acid concentration in the gavaged solution (over the wide range from 10E-1 to 10E-7 mol/l).

The comparison of the biological behaviour of Pu-238 and Np-239 shows, that the fractions of both radionuclides that are absorbed through the gut wall and are retained in skeleton, liver and kidneys are statistically equal. However, the total retention of intravenously injected Pu-238 in the organs examined was significantly lower than that of Np-239, so that we can draw the conclusion, that some more of the gavaged Pu was absorbed than Np.

2. Long-term Retention

This is a summary of observations made during the first year after a single intravenous injection of Np-237 nitrate (0.2 or 1.0 mg Np-237/kg body weight) into adult female Sprague-Dawley rats; further preliminary data were also obtained with young animals. The retention of Np-237 was followed up by

Table 1: Absorption of Neptunium from the Gastrointestinal Tract of Rats Administered the Nitrate (pH 1) by Gavage

Sex	Age	Nutritional status	Number of animals	Isotope	Mass administered (per kg)	Absorption [a] (% of dose)
Male	Adult	fasted	14	Np-239	110 pg	0.12 ± 0.02
		fed	10	Np-239	110 pg	0.02 ± 0.01
	Young	fasted	10	Np-239	220 pg	0.18 ± 0.06
		fed	9	Np-239	220 pg	0.03 ± 0.01
Female	Adult	fasted	11	Np-239	110 pg	0.05 ± 0.02
		fasted	10	Np-237	1 mg	0.23 ± 0.10
		fasted	8	Np-237	10 mg	0.26 ± 0.07

[a] Arithmetic means ± SEM ; absorption = retention (without skin, paws, tail and gastrointestinal tract) plus excretion (estimated as 50 % of retention).

whole body counting and serial sacrifice of groups of animals.
The data illustrated in Figure 1 show that within the first month there is a rapid loss of Np-237 from the body, about 35% in young rats and about 55% in adults. By the end of this time virtually all the remaining radionuclide is found in the skeleton. The whole body retention data for the adult animals can be fitted to three component exponential equations which show little significant difference between the two Np-237 dose levels used in these studies. The half-times and the extrapolated initial fractions calculated from the first two exponential terms indicate that about 40% and 15% of injected Np-237 was excreted within the first 5 days and 5 months, respectively, while the rest was excreted with a half-time of about 3.5 years. The final long term component is assumed to indicate the rate of loss of Np-237 from the skeletal compartment.
Our studies do show that Np-237 injected in a dose up to 1 mg/kg body weight shows a similar retention time in bone to that reported recently for four other highly radiotoxic bone seekers Ra-226, Pu-239, Am-241 and Cm-244 in rats (3).

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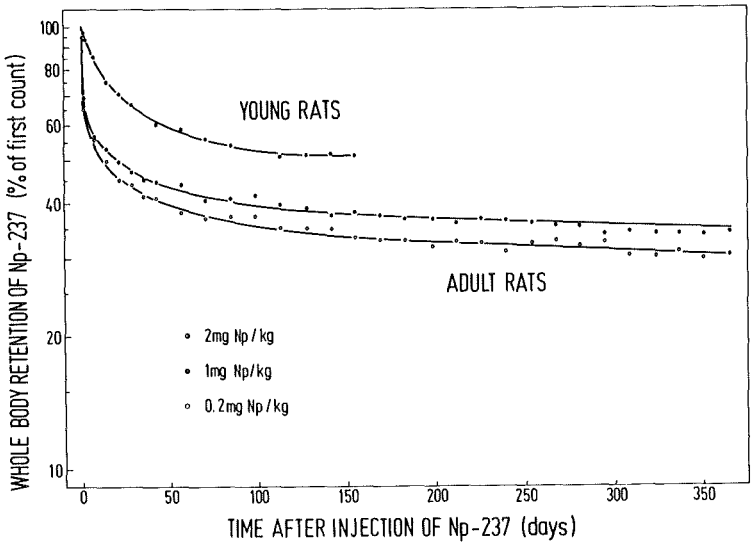


Fig. 1: Whole body retention of Np-237 injected as nitrate into rats (1 mg Np-237 = 26 kBq). Each point indicates a mean of 40 and 25 adult and young rats, respectively, at the beginning of experiment; the last points are means of 30 - 32 adult and 5 young animals.

DECORPORATION OF Pu-238, Pu-239, Am-241 BY DTPA IN DRINKING WATER

V. Volf

The aim of the present investigation was to collect further experimental data to support the hypothesis that prolonged administration of quite small amounts of DTPA represents a true alternative to the hitherto accepted schedule of repeated DTPA injections.
In the first experiment on female Heiligenberg rats (Tab. 1) the effects of various concentrations of Zn-DTPA in drinking water on the retention of Pu-238 and Pu-239 were compared with the effect of Ca-DTPA injected in human equivalent doses (1 g Ca-DTPA in a 70 kg man). After Ca-DTPA injections the contents of both isotopes in the bone and liver were reduced by about 40% and 70%, respectively. A similar effect was achieved with Pu-238 and Pu-239 by drinking 0.0001 and 0.001 molar Zn-DTPA, respectively. Thus, the effect of oral Zn-DTPA was substantially more pronounced with Pu-238 than with Pu-239, both in the bone and liver. This suggests that with low chelate concentrations in the tissues following oral administration of DTPA the mass of Pu becomes a critical factor: For equal activities the mass of Pu-238 is about 280 times less than that of Pu-239.
The total DTPA intake in rats drinking Zn-DTPA was between 1 and 30 times higher than in those injected with Ca-DTPA yet it proved equally effective in reducing the contents of Pu-238 in the bone and liver, respectively. The effect in the liver of oral DTPA can be easily understood assuming that only about 3% of ingested DTPA is absorbed from the intestine. In the bone, however, it is obviously important that a low level of DTPA is maintained for longer periods of time, thus preventing the redeposition of the small quantities of Pu-238 released. The higher mass of released Pu 239 can be bound only if about 30 times higher local DTPA concentrations are achieved.
In man, chelation therapy is indicated after incorporation of substantially lower amounts of Pu-239 than are those usually administered in animal experiments (e.g., the activity injected in the above experiment was 7 µCi per 70 kg body weight). Thus, animal studies with the low mass Pu-238 seem to be a suitable model for human accidental incorporation of Pu-239.
In the second experiment Zn-DTPA was added to drinking water so that the intake equalled approximately 300 µmoles per kg body weight per day, i.e. 10 x the human equivalent dose. Male Sprague-Dawley rats were injected with Pu-238 and Am-241 citrate, 4 d or 30 d before the beginning of treatment which was continued to 105 d, when the animals were sacrificed. The two actinides were administered simultaneously in order to

TABLE 1. RETENTION OF ACTINIDES IN FEMALE HEILIGENBERG RATS AS INFLUENCED BY DTPA

Treatment			Actinide content (% of injected amount; arithm. means + S.E.)			
			Skeleton		Liver	
Substance	Concn. or amount	Total amount (mmol / kg)	Pu-238	Pu-239	Pu-238	Pu-239
Controls 4d	–	–	63.9 ± 4.1	65.8 ± 2.3	19.2 ± 1.7	20.9 ± 1.0
Controls 28d	–	–	54.1 ± 1.3	61.7 ± 1.9	4.1 ± 0.2	4.6 ± 0.4
Zn-DTPA (Drinking-water)	1×10^{-4} M.	0.09	40.8* ± 1.3	59.5 ± 2.6	2.9* ± 0.2	4.5 ± 0.6
	3×10^{-4} M	0.27	35.2* ± 1.3	56.6 ± 2.9	2.2* ± 0.1	3.7 ± 0.3
	1×10^{-3} M	0.90	32.6* ± 0.9	54.2* ± 3.0	1.9* ± 0.2	3.4* ± 0.3
	3×10^{-3} M	2.70	30.5* ± 1.5	49.5* ± 2.8	1.8* ± 0.1	1.9* ± 0.1
	1×10^{-2} M	9.00	30.9* ± 1.8	39.4* ± 2.9	1.2* ± 0.1	1.1* ± 0.1
	3×10^{-2} M	27.00	31.4* ± 1.4	27.7* ± 2.6	1.0* ± 0.1	0.6 ± 0.1
Ca-DTPA (s.c.in-jection)	30 µmol/kg	0.27	34.2* ± 1.2	37.7* ± 2.9	1.4* ± 0.1	1.2* ± 0.1

Treatment started 4d after i.v.injection of Pu-citrate (0.1µCi/kg) and continued 3 x per week for 3 weeks. Rats (5-10 per group) sacrificed 4 weeks post Pu.

* Statistically significant difference between control and treated group ($p < 0.05$).

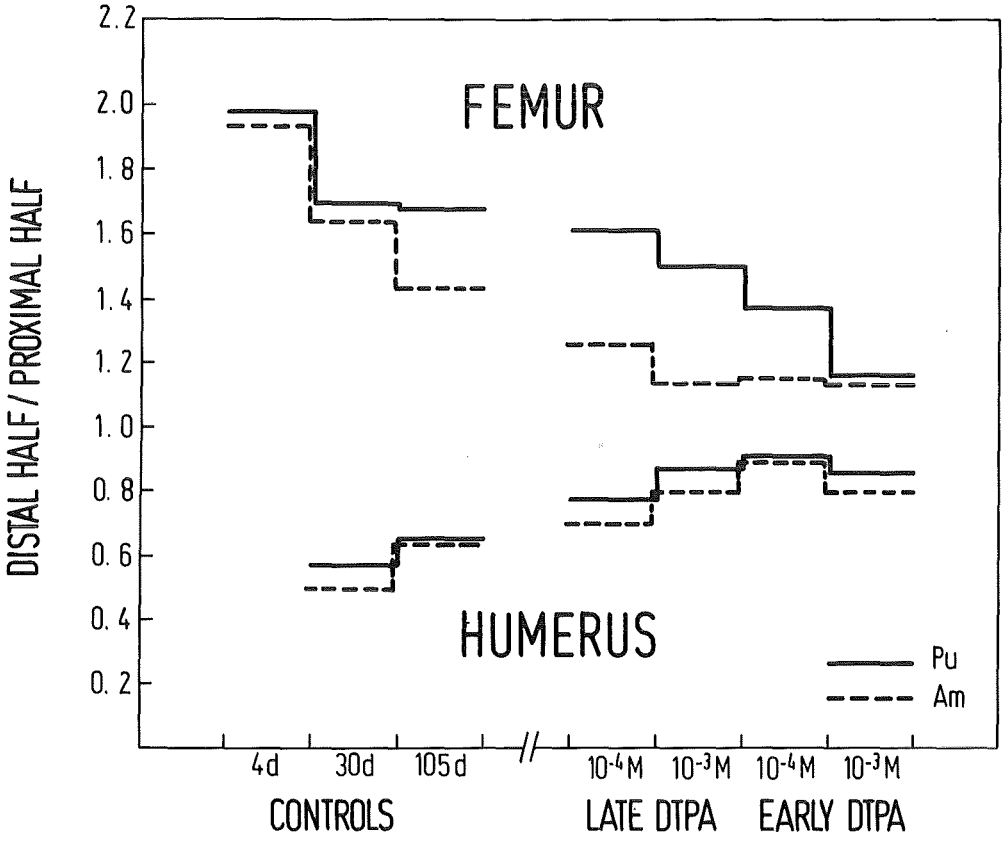


Fig. 1. Ratios of ^{238}Pu and ^{241}Am in distal to proximal halves of rat femur and humerus.

compare their response to treatment under identical conditions. The changes in the overall retention of Am-241 in vivo were followed by repeated whole body counting.
At 15 weeks postinjection the whole body retention of Am-241 as well as the content of Pu-238 and Am-241 in the skeleton were reduced to approximately 60% and 40% by the treatment with Zn-DTPA beginning late or early after injection of the actinides, respectively. In the soft tissues, both Zn-DTPA treatments reduced the Pu-238 content to below the detection limit, and that of Am-241 to about 10% and 50% in the liver and kidneys, respectively.
The latter results indicate that substantial fractions of Pu-238 and Am-241 can be removed even by delayed administration of DTPA in drinking water, except for a small fraction of Am-241 which remains fixed in the kidney, even if DTPA treatment begins only 4 d after Am-241 injection.
We were further interested to see if Zn-DTPA is equally effective in different bones as well as in various parts of the same bone. There was no marked difference in the effectiveness of the four different treatment schedules in the whole femur and humerus. Initial uptake of Pu-238 and Am-241 in the distal half of the femur and in the proximal part of the humerus was about twice as high as that in the other halves of these bones. The removal of Pu-238 and Am-241 from the sites of high initial uptake was more pronounced than that from the sites of lower uptake, both in untreated controls and in rats drinking Zn-DTPA, the distal: proximal content ratio for Pu-238 or Am-241 in the femur and humerus changed with time and after administration of Zn-DTPA approached 1 (Fig. 1).
There was a good correlation between Pu-238 and Am-241 retained in the halves of the two long bones taken from control animals at 4, 30 and 105 d post-injection as well as from treated animals at 105 d. This indicates that there was neither a preferential uptake nor a preferential removal of either of the two actinides studied. The data from untreated and Zn-DTPA treated rats could be fitted by single regression curves, which suggests that removal of Pu-238 and Am-241 was governed by essentially the same mechanism. It is well known that bone growth and remodelling rates in distal and proximal parts of the long bones are different and these obviously determine the degree of homogeneity of radionuclide deposition and removal. Thus it appears that prolonged administration of DTPA reduces the retention of Pu-238 and Am-241 mainly by preventing their redeposition during bone remodelling. Since only small amounts of the actinides are set free, small amounts of DTPA may be as effective, or nearly as effective, as larger ones.

4. Year-round Research (Short scientific reports)
e) Biochemistry of Heavy Metals

BIOCHEMICAL STUDIES ON THE SPECIES DIFFERENCES IN LIVER RETENTION OF TRANSURANIUM ELEMENTS WITH RAT AND CHINESE HAMSTER

A.Seidel, U.Sütterlin, M.Balani, W.-G.Thies, G.Hotz

One of the most striking aspects of the comparative behaviour of transuranium elements is the distinct species variation in their retention times in mammalian liver (1,2). For several years the aim of our studies has been to explain these species variations in order to provide a better basis for the understanding of the comparative behaviour of transuranium elements in man and animals. Of primary importance is a knowledge of the subcellular component, or components, to which the nuclides are initially bound and the question whether this is the same for all animal species. For this purpose we have compared the subcellular distribution in the whole liver of rats and Chinese hamsters as examples of species with rapid and slow elimination transuranium elements from liver, respectively.
The animals were injected with Pu-239- or Am-241-citrate, for some experiments also with Fe-59-citrate, and sacrificed at time intervals ranging from one hour to about 300 days after nuclide injection. From the liver homogenates the postnuclear supernatant (E) was prepared, which was used for gel chromatography on Sephacryl S-1000 (3). Mitochondrial-lysosome fractions (ML) were analyzed by sucrose-, Metrizamide-, or Percoll-gradients or by free flow electrophoresis (4,5). All other experimental details have been described elsewhere (4) together with the conditions for the assay of the marker enzymes (lysosomes: acid phosphatase, AP; arylsulphatase, AS; N-acetyl-glucosaminidase, N-Ac; and cathepsin D, Cath; mitochondria: glutamate dehydrogenase, GDH; endoplasmic reticulum:glucose-6-phosphatase, G-6-P; Pericellular membranes: alkaline phosphodiesterase, AlPD). Data from density gradients are presented as frequency histograms, where Q=fractional amount of constituent in each fraction and =density increment from fraction to fraction.
The retention of H-3 Triton WR 1339 in rat and hamster organs was studied with Triton labelled by the Wilzbach method with Tritium gas or by catalytic exchange in tritiated water. The fraction with a molecular weight of 110 000 (obtained by dialysis against distilled water) was used for injection. The product obtained by the Wilzbach method was injected as carrier-free, intravenously and that produced by catalytic exchange, intraperitoneally, with 750 mg/kg body weight of inactive Triton carrier ($2\text{-}8 \times 10^5$ Bq/kg).

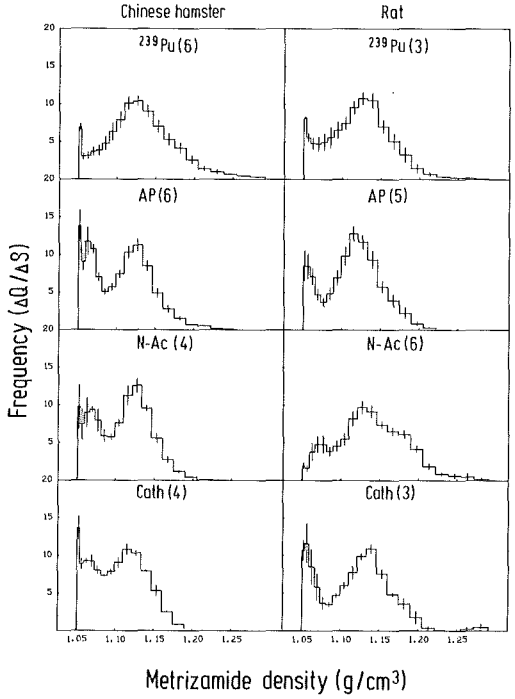


Fig. 1: Density gradient profiles of Pu-239 (4-10 days after injection) and lysosomal marker enzymes after centrifugation of the liver ML-fraction in a linear metrizamide gradient. Arithmetic means + S.E., number of experiments in brackets (4).

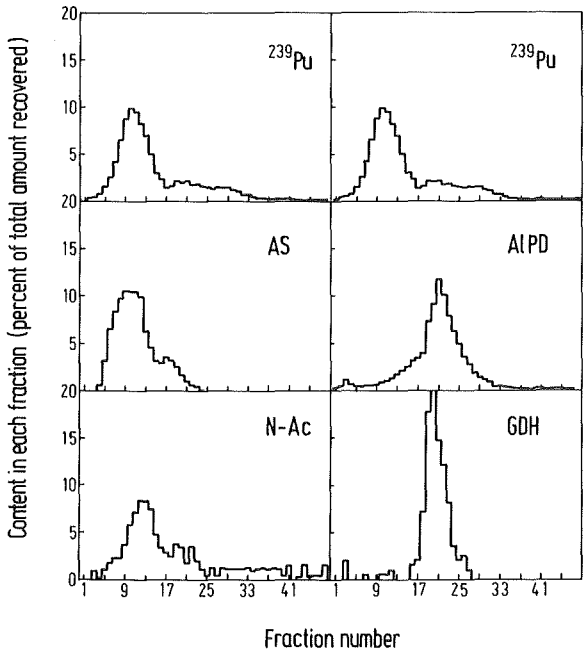


Fig. 2: Profiles of Pu-239 and marker enzymes after free flow electrophoresis of the ML-fraction of Chinese hamster liver at day five after Pu-239-injection. Representative for three experiments (5).

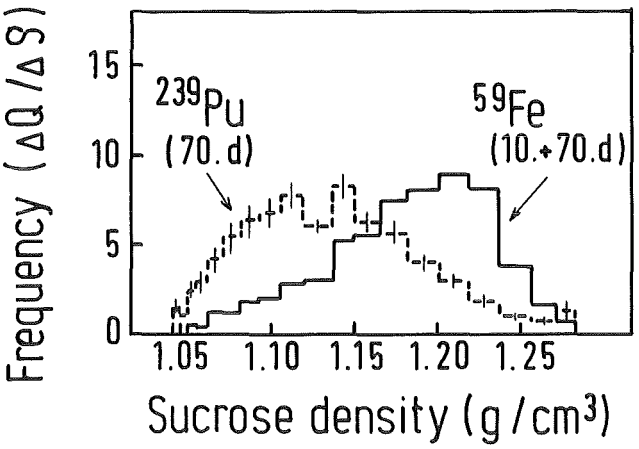


Fig. 3: Density gradient profiles of Fe-59 and Pu-239 after centrifugation (16 h, 88 000 g) of the liver ML-fraction in a linear sucrose density gradient. Seven experiments for Pu-239, two for Fe-59 (4).

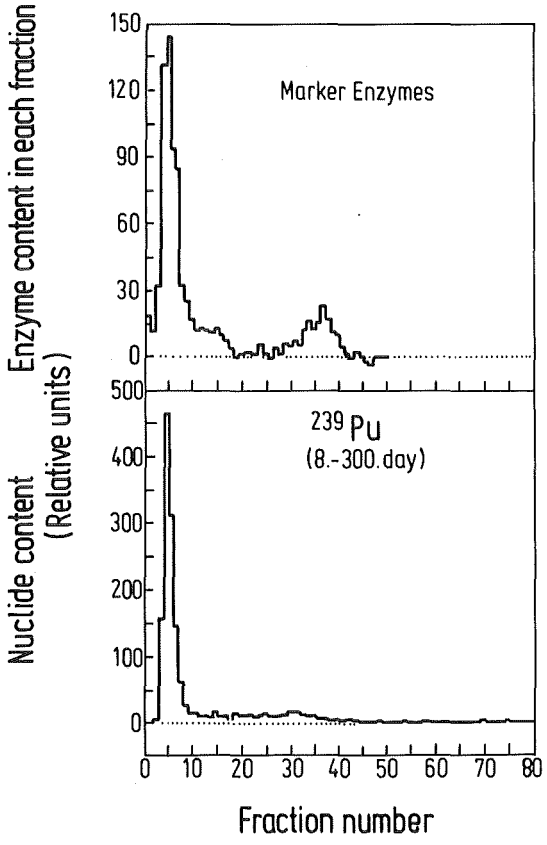


Fig. 4: Behaviour of marker enzymes and Pu-239 after chromatography of the E-fraction (postnuclear supernatant) from Chinese hamster liver on a Sephacryl S-1000 column (3). The profile for marker enzymes is representative for the marker enzymes mentioned in the text. The results shown are representative for five experiments.

Results and Discussion

In both animal species the relative nuclide content in the ML fraction increases at a similar net rate to 80-90% of the total liver burden within the first 6 to 10 days (Table 1). In rat as well as in Chinese hamster liver a close correlation of Pu-239 and Am-241 (not shown, see 6) and the lysosomal enzyme profiles exists. For the sake of clarity the profiles of the other marker enzymes are not shown. These results are shown elsewhere (6) together with those from sucrose gradients. They permit a clear distinction between the profiles of the nuclides and those of either mitochondria or endoplasmic reticulum. Figure 1 illustrates the profiles for Pu-239 and for the lysosomal enzymes observed in Metrizamide gradients for the time period day 4 to 10 after injection. These results have been confirmed by using a quite different method, i.e. free flow electrophoresis. Due to their higher surface charge the lysosomes migrate more rapidly to the cathode (Fig. 2, left side of the diagrams) than all other cell organelles and the Pu-239 profile again correlates well with the lysosomal enzyme profiles in both animal species (only Chinese hamster is shown). With this method the clear separation of the Pu-239 and AlPD peaks permits the exclusion of the pericellular membranes as binding sites for Pu-239; this is difficult with sucrose and metrizamide gradients alone (4,6). Some separation of the Pu-239-profile from pericellular membranes was previously observed with Percoll gradients (6). From the results presented so far we conclude, that secondary lysosomes are the primary target organelle for Pu-239 and Am-241 in rat as well as in Chinese hamster liver. This implies that the species differences in nuclide elimination are not due to binding to different cell organelles with a different biological fate.

Our main interest was then focussed on the question of the ultimate fate of Pu-239 in Chinese hamster liver. The first question was whether the nuclide remains bound to a cell organelle or whether it escapes from the lysosomes and becomes bound to cytosolic proteins,for example to ferritin. As shown in Table 1, more than 90% of the nuclide remain in the ML-fraction at day 70 after injection, which is in sharp contrast to the behaviour of stable and radioactive iron. Also within the ML-fraction, the behaviour of Pu-239 and stable and Fe-59 in sucrose gradients is quite different (Fig. 3). By gel-chromatography of the postnuclear supernatant E on a Sephacryl S-1000 column particles larger than 400 nm can be separated from smaller cell constitutents (3). From day six until day 300, Pu-239 in Chinese hamster liver was found exclusively with particles larger than 400 nm (Fig. 4).

Electrophoretically these Pu-239-binding particles consist at late time intervals (several months after injection) of two groups (Fig. 5); a slowly migrating group, which is not

Table 1

Relative distribution of stable and radioactive iron, ^{239}Pu and ^{241}Am in subcellular fractions of rat and Chinese hamster liver cells (% of E)[a]

Day of sacrifice	Metal	Rat ML	Rat P	Rat S	Rat n	Chinese hamster ML	Chinese hamster P	Chinese hamster S	Chinese hamster n
-	Stable iron	11	23	51	1	38	14	53	1
10	^{59}Fe	16	31	51 ± 2[b]	1	27 ± 3	24 ± 1	47 ± 1	3
70	^{59}Fe	20	35	47	1	32	21	45	2
10	^{239}Pu	78 ± 1	10 ± 1	9 ± 1	6	93 ± 2	3 ± 0	5 ± 1	3
70	^{239}Pu	75 ± 2	9 ± 1	16 ± 1	5	96 ± 2	2 ± 1	3 ± 1	5
10	^{241}Am	83 ± 2	6 ± 2	8 ± 1	3	91	3	4	2
70	^{241}Am	70 ± 2	10 ± 1	15 ± 1	3	97	2	2	2

Arithmetic means ± S.E., n = number of experiments

[a]Nuclide content in postnuclear supernatant fraction is taken as 100%.

[b]n = 5

Table 2

Influence of an intraperitoneal injection of 750 mg/kg Triton WR 1339 on the median densities (g/cm^3) of lysosomal marker enzymes in sucrose density gradients

Time of sacrifice[a]	Chinese Hamster						Rat					
	Acid phosphatase	n	N-acetyl-glucosaminidase	n	Aryl sulfatase	n	Acid phosphatase	n	N-acetyl-glucosaminidase	n	Aryl sulfatase	n
Control	1.161±0.003	23	1.184±0.002	8	1.185[b]	2	1.183±0.001	8	1.192±0.002	7	1.187[b]	2
4	1.108±0.006	9	1.123±0.004	6	1.126[b]	2	1.112±0.002	12	1.121±0.002	6	1.134[b]	2
24	1.115±0.004	3	1.123±0.003	3	1.122	2	1.137	2	1.147	2	1.148	2
70	1.126±0.001	3	1.132±0.002	3	1.130±0.001	3	1.160±0.002	4	1.170±0.004	4	1.169±0.002	4

Arithmetic means ± S.E., n = number of experiments per value

[a] Days after Triton WR 1339 injection

[b] Data from Winter et al., obtained with an MLP-fraction

Table 3

Concentration of ^{3}H-activity after intraperitoneal injection of ^{3}H Triton WR 1339 (with 750 mg/kg Triton WR 1339 as carrier) into rat and Chinese hamster. Data represent percent of injected dose per gram fresh weight

Day of sacrifice		4		24		60	
Liver	Rat	3.6 ± 0.1	(5)	1.5 ± 0.2	(6)	1.3 ± 0.2	(6)
	Chinese hamster	15 ± 1	(5)	10 ± 1.2	(5)	12 ± 1.1	(5)
Kidneys	Rat	0.5 ± 0.02	(5)	0.2 ± 0.2	(6)	0.04± 0.01	(6)
	Chinese hamster	2 ± 0.6	(5)	0.5 ± 0.1	(6)	0.1 ± 0.09	(6)
Lung	Rat	0.6 ± 0.03	(5)	0.2 ± 0.02	(6)	0.1 ± 0.02	(6)
	Chinese hamster	6 ± 2.4	(5)	0.8 ± 0.1	(5)	0.3 ± 0.1	(6)
Spleen	Rat	1.6 ± 0.2	(5)	1.9 ± 0.1	(6)	1.6 ± 0.2	(6)
	Chinese hamster	11 ± 0.7	(5)	6.2 ± 0.8	(5)	0.3 ± 0.2	(5)

Arithmetic means ± S.E., number of animals per group in brackets

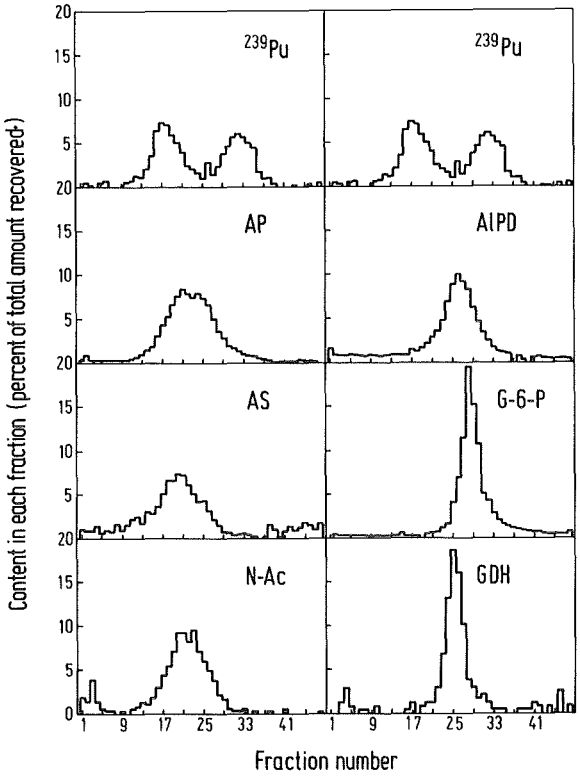
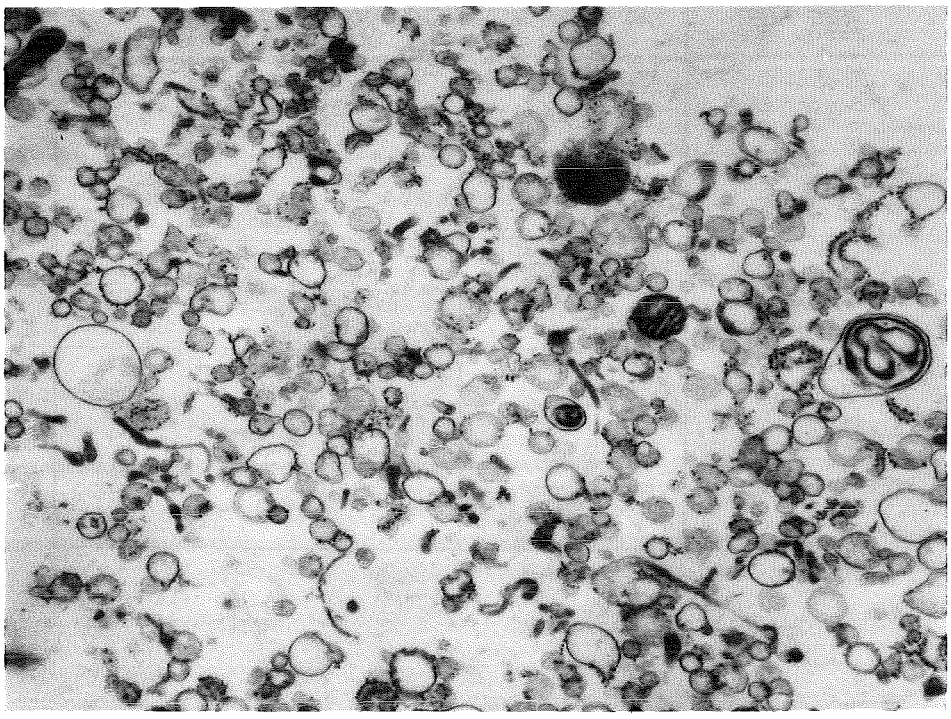


Fig. 5: Profiles for Pu-239 and marker enzymes after free flow electrophoresis of the ML-fraction of Chinese hamster liver. Representative for five experiments between day 200 and 300 after injection (5).

Fig. 6: Electron microscopic appearance of the constituents of fraction with $\rho = 1.125$ g/cm^3 from a sucrose density gradient (Chinese hamster). This fraction contains the highest Pu-239 content at intervals ≥ 70 days after nuclide injection. Uranylacetate/lead citrate staining, magnification 24 000 x.



correlated to any of the marker enzymes tested so far, becomes apparent at day 12 and is responsible for about 50% of the Pu-239 at later time intervals after injection. In sucrose gradients, these particles are less dense than typical lysosomes and their Pu-239-content can no longer be released by addition of Triton X-100 (6). Electron microscopic examination of the gradient fraction with the highest nuclide concentration shows a prevalence of not yet identified vesicular elements (Fig. 6) and some constituents with more electron dense material. Electron microscopic studies with Pu-241 are under way in order to identify the nuclide binding constituents also morphologically.

A main difference between rat and hamster is the considerably higher relative nuclide content in the rat liver cytosol (Table 1) at late time intervals, which possibly correlates with the higher nuclide mobility in rat liver. During the phase of rapid elimination from rat liver, Pu-239 remains bound to typical lysosomes (4,6).

The presently available data suggest, that the species differences in the biological half-life of transuranium in liver probably reflect differences in the composition, properties or biological fate of liver lysosomes or in the mechanisms of biliary metal transport. It seems possible, that transuranium elements are eliminated into the rat bile by a normal, relatively rapid biliary excretion of lysosomal material for which there is some evidence in this animal species (6). In Chinese hamster liver, at least those lysosome to which the nuclides are bound may be transformed gradually into inert residual bodies. A further indication for species differences between rat and Chinese hamster liver lysosomes was found by comparing the time dependence of the effect of an injection of the lysosomotropic substance Triton WR 1339. The decrease of lysosomal density produced by this substance is reversible in rats but not in Chinese hamsters (Table 2).

The retention of H-3 labelled Triton WR 1339 (catalytic exchange method) in various organs of rat and Chinese hamster is shown in Table 3. In rat liver, the H-3 concentration decreases to 1/3 of the 4-day burden until day 60, whereas only 1/4 are eliminated from hamster liver. With respect to the elimination rate the results with the "Wilzbach-labelled" H-3 Triton WR 1339 are in general agreement with these data. The H-3 liver burden of rats decreased from 20±2 at day 4 to 9±1% of 3-H-dose at day 25, whereas that in Chinese hamster liver remained virtually constant (17±1% at day 4 and 20±2% at day 25, 6 animals in most groups). On the other hand the decrease of H-3 concentration in hamster lung and spleen is very rapid, which is in sharp contrast to rat organs. The differences in H-3 Triton retention in liver between rat and Chinese hamster are parallel to the differences in lysosomal density and support the view of species differences in composition and/or turnover of liver lysosomes. However, the

differences in lung and spleen indicate that species differences in the response towards lysosomotropic agents may also exist in other organs than liver. The H-3 concentration in these other organs is relatively high and prompted us to analyse the rat lung after intraperitoneal injection of 750 mg/kg Triton WR 1339 by electron microscopy (Fig.7).

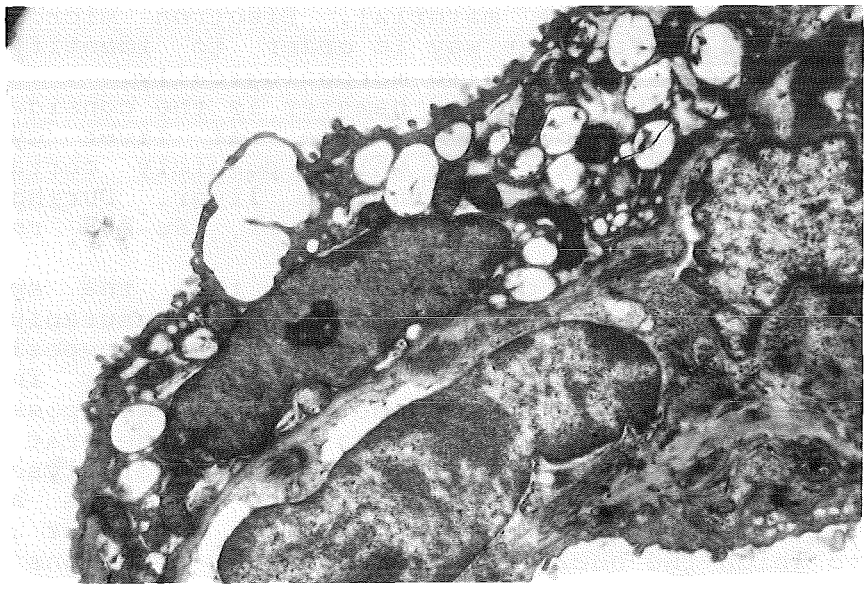


Fig.7: Electron microscopic appearance of rat lung after intraperitoneal injection of 750 mg/kg Triton WR 1339. Magnification 12 000 x.

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DOES THE BINDING OF METALS TO TRANSFERRIN PLAY A ROLE IN METAL UPTAKE INTO CELLS?

J.R.Duffield, F.Planas-Bohne

The iron transport protein transferrin (Tf) appears also to mediate the uptake of iron into cells. Since Tf also transports a number of non-physiological metals in the blood, the question arose as to whether it plays a similar role in the uptake of these metals. A better knowledge of the mechanism of metal uptake might lead to an understanding of why metals are concentrated in some tissues while they are excluded from others.
We, therefore, started a comparative investigation into the binding characteristics of human Tf with iron and some exogenous metals,and into the in vitro uptake of metal-Tf complexes by human lymphoblasts (WI-L2 - cells.)

1. Chemical investigations:
The stoichiometry and strength of metal binding to Tf has been studied by visible and ultraviolet spectroscopy, iso-electric focusing and gel-filtration chromatography. To prevent hydrolysis and aid metal binding to Tf nitrilotriacetate (NTA) metal complexes have been used. All determinations have been carried out at $20^{o}C$ with an ionic strenght of 150 mmol per l NaCl.
As Tf binds a metal ion its absorbance changes at a characteristic wavelength in either the visible or U.V regions of the electromagnetic spectrum. For Fe^{3+} this change occurs at 465 nm and for Hf^{4+} and Pu^{4+} at 244 nm. The absorbances reach a maximum when each Tf molecule has bound its maximum complement of metal ion. Thus if a plot is made of absorbance versus the ratio moles metal: moles Tf a plateau is reached when Tf is fully saturated.(Figure 1). In this way it is found that each Tf molecule has two specific binding sites for Fe^{3+}, Hf^{4+} and Pu^{4+}.
It has proved possible to separate the different metal Tf species formed at different percentage metal saturations using iso-electric focusing (IEF). Four major species can be isolated for Fe^{3+} and Pu^{4+} - Tf complexes, corresponding to:

(I) Apo-Tf, the metal free protein
(II) M_NTf, monometallic Tf with metal at the N-terminal site
(III) TfM_C, monometallic Tf with metal at the C-terminal site
(IV) M_2Tf, dimetallic Tf.

By scanning the intensities of the bands for a given percentage saturation (Fig.2a) a species concentration profile can be constructed (Fig.2b). Thus under the

FIGURE 1: SPECTROPHOTOMETRIC STUDY OF THE SATURATION OF TF WITH HF^{4+}.

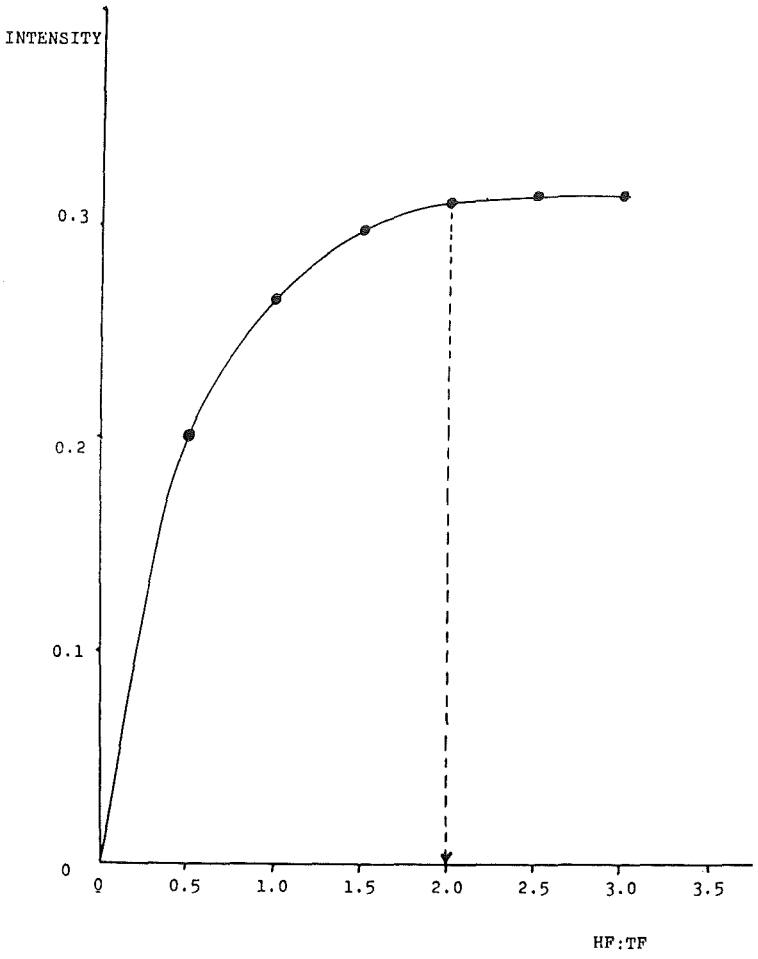


FIGURE 2a: DENSITOMETER SCAN OF TF PROFILE AT 30 % FE^{3+} SATURATION.

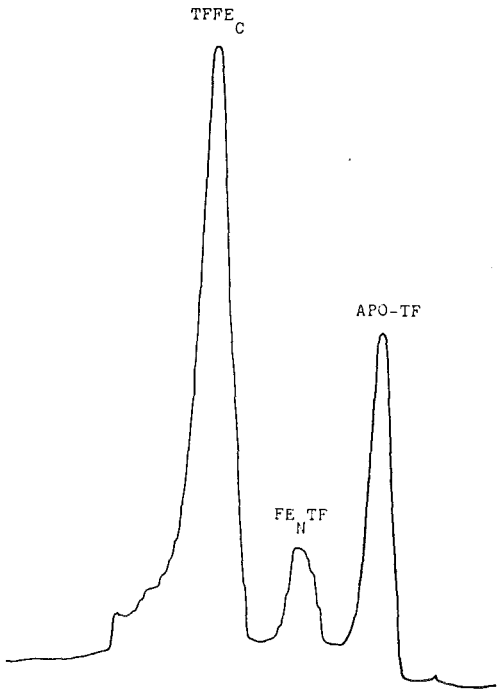
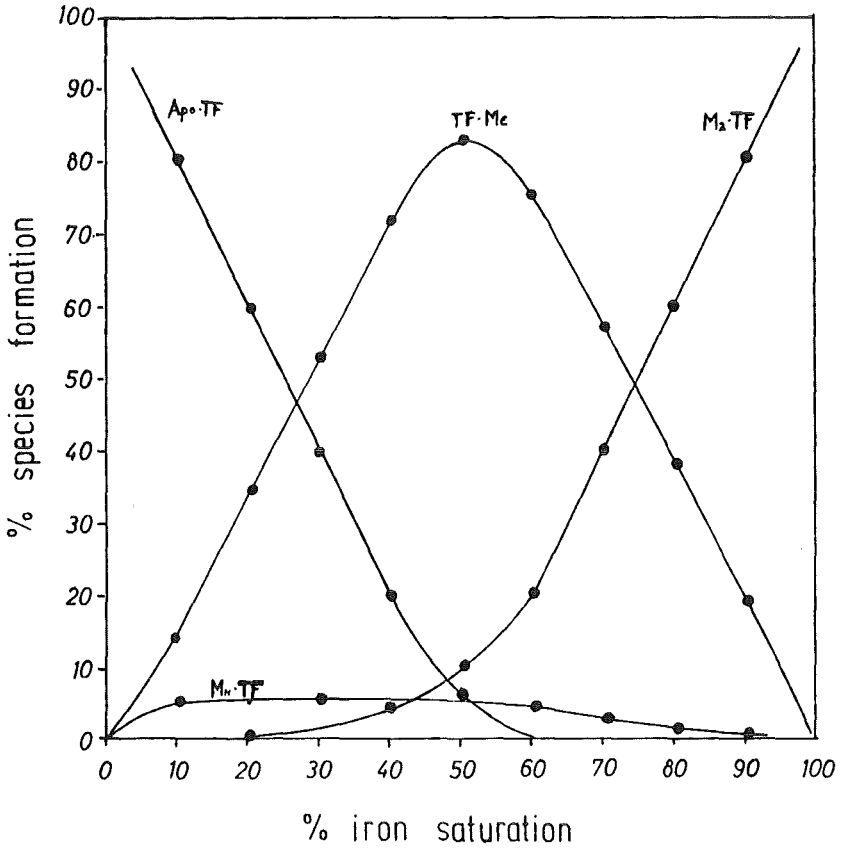


FIGURE 2b: TF SPECIATION AS A FUNCTION OF FE^{3+} SATURATION.



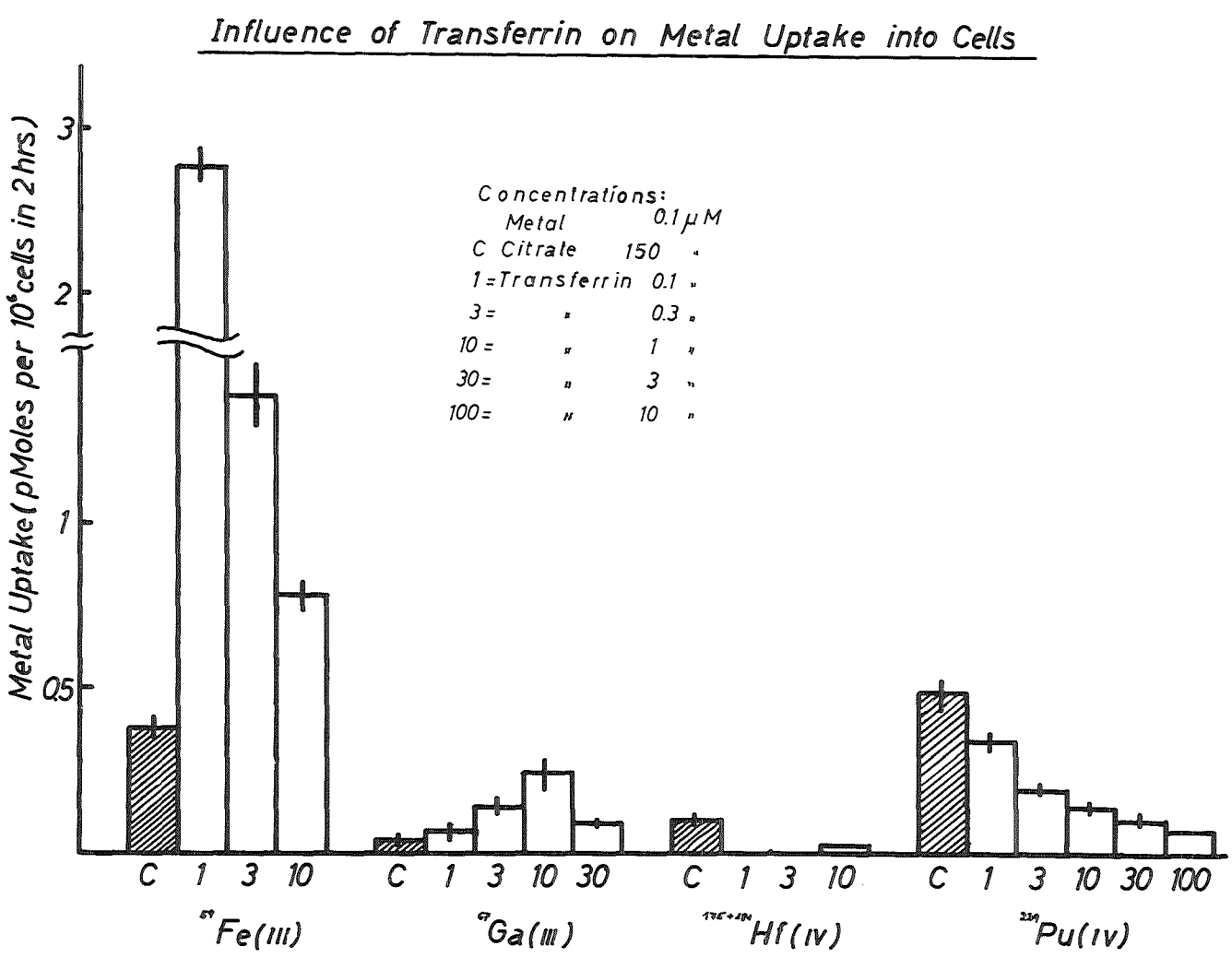


Fig. 3. Metal uptake as pmole per 10^6 viable cells after 2 hr incubation with metal citrate or metal transferrin solution.

conditions used it can be seen that metals occupy the C-terminal binding site in preference to the N-terminal.
Investigations of the strength of binding of metals to Tf using special gel-filtration techniques which are not shown here indicate that it increases in the order GaIII < HfIV < PuIV < FeIII.

2. Cell Experiments:
Metal uptake into cells was studied in human lymphoblasts of splenic origin (WI-L2). Cells were washed in serum free medium and then incubated with radioactive metal-citrate or -Tf solutions. The final cell concentration was 10E6 cells per ml. After two hours cells were washed twice with serum free medium and once with trypsin (0.05% w/v trypsin in 0.15 M citrate buffer). The radioactivity (metal content) was measured in the cells and the combined washings.

The uptake of metal citrate - and metal Tf - complexes is shown in fig.3. Five different metal to Tf concentration ratios 1:1, 1:3, 1:10, 1:30 and 1:100 were used. Each of the metals studied shows a different pattern of uptake from the transferrin solutions. With a 1:1 Fe-59:Tf ratio the uptake is about seven times greater than from citrate, but the stimulatory effect decreases with increasing Tf concentration. Increasing Tf concentrations stimulate Ga-67 uptake up to a ratio of 1:10. In contrast, increasing Tf concentrations progressively reduce Pu-239 or Hf-175+181 uptake as compared to that from citrate.

3. Discussion
The results of the chemical experiments show that Hf and Pu resemble Fe closely as they also bind to two specific binding sites in the Tf molecule. Moreover, we could show that these exogenous metals like iron occupy the C-terminal binding site of Tf in preference to the N - terminal. For Ga it was not possible to perform these experiments because its binding to Tf is not strong enough. This was also seen in the gel filtration experiments.
The low stability of the Ga (4) complexes may also be the reason why this metal is taken up into cells from citrate an order of magnitude less than Fe or Pu. Dissociation of Ga-citrate may lead to the formation of charged hydroxides which cannot penetrate the cell wall. The tenfold higher Fe-59 uptake as well as the difference in behaviour with varying metal to Tf ratios was also observed by other authors (5) who also attributed it to the lower stability of the Ga-Tf complex leading to dissociation especially at low Ga:Tf ratios.
The reason why the uptake of Pu and Hf, which like iron and gallium are transported by Tf in the blood of mammals, into WI-L2 cells should be inhibited by Tf remains an open question. More detailed studies designed to elucidate this

problem are in progress. However, these results may indicate differences in the binding of the different metals to transferrin or of the metal protein complex to the transferrin receptors which are known to be present on the surface of WI-L2 cells (6).

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LOW LEVEL ALPHA-SPECTROSCOPY

W.-G.Thies, A.Seidel

To study the subcellular distribution of actinides and their biochemical binding, very low alpha-activities must be detected. This is especially important in long - term experiments, where radiation damage due to high alpha-doses should be avoided. Therefore a very sensitive method is needed to measure picocurie and femtocurie quantities of alpha-emitting radionuclides.

A Low-Level Alpha-Spectrometer was developed, which can be used to analyse up to 16 samples simultaneously. The precise evaluation of the data is done on a PDP 11 Computer, for which a complete system of programs has been written. The Low Level System is based on eight ORTEC 476 Dual Alpha-Spectrometers. The detectors (silicon surface-barrier detectors) have a linear energy response, an excellent energy resolution (25 keV), and they are insensitive to Gamma-radiation. Calibration constants and efficiencies were determined for all 16 detectors and stored in a system library file. These data can be accessed by all the programs to perform a fully automatic data evaluation. Figure 1 shows one of the calibration spectra, measured with a standard sample. The mean efficiency of the alpha-counters is about 20%.
The chemical preparation of the samples is carried out according to the method described in (1). The radioelements are then electrodeposited onto thin stainless steel disks. As it is nearly impossible to avoid losses of activity during the sample preparation, a small amount of a calibration-standard (e.g. Pu-236) is added to the probe prior to the complete procedure. The activity of the other isotopes may then be determined by comparison with this known tracer-activity. The analysis-program determines the energy-windows for the measured alpha-isotopes using an alpha-energy table. The settings of the windows and the consistency of the data-evaluation may be checked visually on a TEK 4010 graphic terminal, or on a BENSON 1202 Plotter. Figure 2 shows a typical energy spectrum from a measurement of a Pu-sample. The different isotopes can be easily identified and their activity measured separately.
The sensitivity of the Low-Level-System depends mainly on the background, which has been investigated carefully. Due to the small energy windows the background of the detectors is very low, e.g. 2 counts/day for Pu-239. A more serious problem is slight contamination from the sample preparation, which may increase this value by a factor of ten. The lower limit for the precise determination of Pu-239 in biological samples was

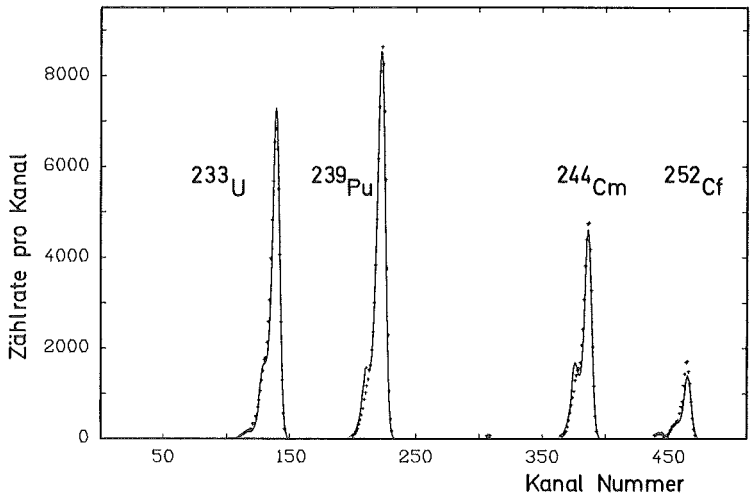

Fig. 1: Energy-calibration spectrum with four different alpha isotopes. From the fit of the theoretical lineshape to the data the calibration parameters are determined.

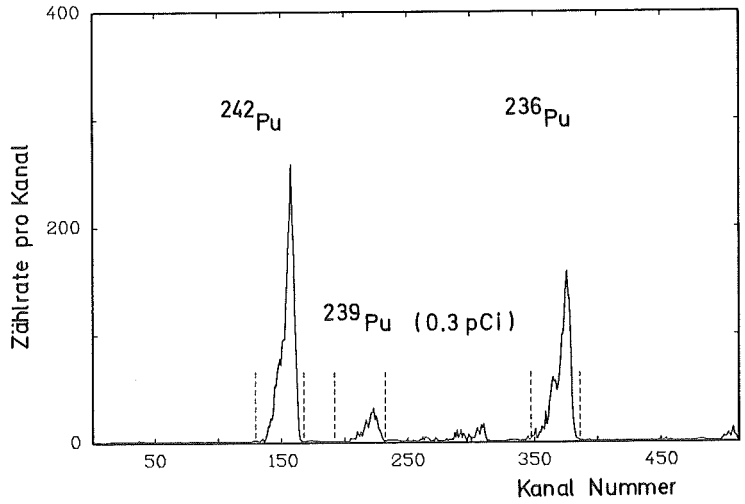

Fig. 2: Measurement of several Pu-isotopes with activities from 0.3 to 1.9 picocuries.

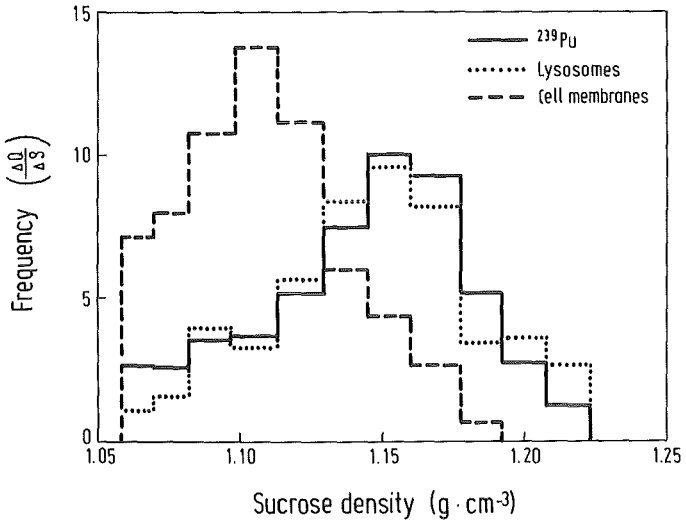

Fig. 3: Profiles of Pu-239 (7 days after injection) and subcellular organelles in a linear sucrose density gradient. Material: Mitochondria-lysosome fraction from Tupaia liver.

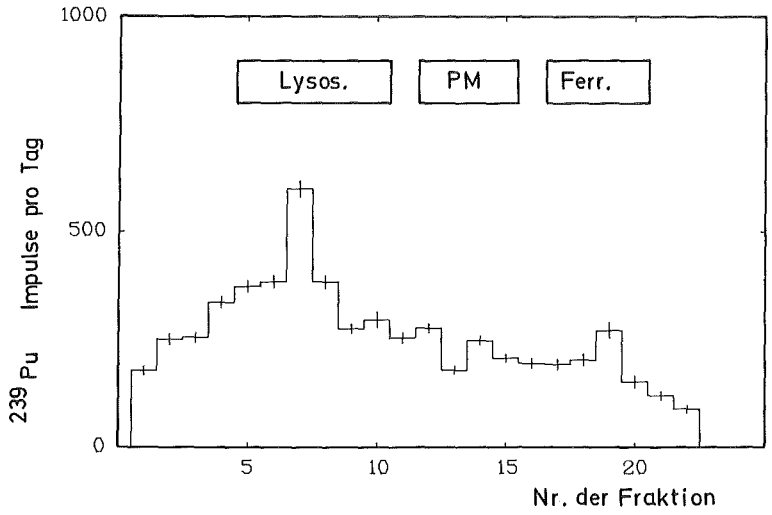

Fig. 4: Subcellular organelles and ferritin (schematic) and Pu-239 in sucrose density gradient, 3 years after Pu-injection (Mitochondria-lysosome fraction from Tupaia liver).

estimated to 50 fCi.
The Low-Level-System is currently being used for long-term studies of the metabolism and the toxicity of Pu-239 in Tupaias (2). The investigation of the distribution and the biochemical binding of Pu-239 in the Tupaia liver is one of the aims of this research. The different cell fractions of the liver contain very small quantities of Pu-239, and these measurements are only possible with the Low-Level Method. Figures 3 and 4 show some results of these experiments.

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THE SUBCELLULAR DISTRIBUTION AND BINDING OF RADIONUCLIDES IN MARINE ORGANISMS

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In 1980 a collaborative study was started, under the auspices of the Indo-German Agreement on the peaceful uses of atomic energy, with scientists from the Bhabha Atomic Research Centre in Bombay. The aim of this project was to attempt to apply the biochemical techniques developed for the study of the sub-cellular distribution of actinide elements in rat liver and other tissues to the investigation of the intracellular localisation and binding of radionuclides in marine organisms, especially in those species which were known to concentrate radionuclides released into the sea from coastal nuclear power stations.
Studies in a number of marine species collected in the vicinity of the discharge from the Tarapur Nuclear Power Station about 100 km north of Bombay, India, were made in order to determine the extent to which the methods developed for rat liver could be applied directly to marine species. The initial investigations established that the lysosomal enzymes acid phosphatase and aryl sulphatase were present in hepatic tissue from four different marine species from this region. Table 1 compares the data obtained with those for rat liver. Except in the crab (Scylla serata) the acid phosphatase activity, expressed either as units per g wet tissue or units per mg protein, were similar in the rat and the other three marine species. However, the activity of aryl sulphatase in rat liver was found to be between 4 and more than 30 times less than that in the marine species examined; this markedly higher activity of aryl sulphatase in the marine species probably reflects the greater occurrence of organic sulphates as utilisable components of the diet of these organisms. In marked contrast to the rat which shows a very high degree of structure related latency for these two lysosomal enzymes, the latency of the enzymes in the marine species was low. This latter observation suggests that in the marine species the lysosomes are more fragile than in the rat and, thus, are more easily ruptured by the gentle homogenisation procedures used; alternatively in these marine creatures only a relatively small fraction of the total activity of these enzymes is normally present in lysosomal structures. Because of this apparent lability of the marine lysosomes no satisfactory distribution profiles for any radionuclide have been obtained for any of the species examined. Electron microscopic techniques, which have now

Table 1 Comparison of the activities of the lysosomal enzymes acid phosphatase and aryl sulphatase in hepatic tissue of marin organisms and rats

Species	Acid phosphatase		Aryl sulphatase	
	U/g	U/mg protein	U/g	U/mg protein
Clam - Anadara granosa (hepato pancreas)	3.5	0.04	14.1	0.17
Sea hare - Aplysia benedicti (hepato pancreas)	4.8	0.09	1.7	0.03
Crab - Scylla serata (hepato pancreas)	0.4	0.01	3.0	0.04
Mudskipper fish - Boleophthalamus boddaerti (liver)	4.0	0.05	1.4	0.02
Wistar rat (liver)	4.8	0.03	0.4	0.002

been introduced into this work, may yield more reliable information on the subcellular location of radionuclides than any of the biochemical techniques so far studied.
Further studies by our Indian collaborators have shown that in the arcid blood clam, Anadara granosa, the activities of acid phosphatase and aryl sulphatase in the digestive gland change with the salinity, temperature and other environmental factors and suggest that the activity of these enzymes may provide useful indicators of the effects of various types of pollutants, including radionuclides and heavy metals (Patel and Patel, 1984 - to be published).

Certain species of sponge have been shown to have a remarkable capacity to concentrate radionuclides from sea water. For example the species Spirastrella cuspidifera concentrates ^{60}Co from the surrounding water by factors of up to 10^5. The chemical form of the ^{60}Co in this species was investigated using gel- and ion-exchange chromatography. About 85% of the ^{60}Co concentrated by S. cuspidifera was found in the aqueous phase when samples were disintegrated in a high speed homogeniser. Gel chromatographic analysis on Sephadex G-50 showed that more than 80% of the soluble ^{60}Co was present as a complex with a molecular weight of less than 1000 which appeared to be of low stability. The nature of the complexing ligand had not yet been identified. The relatively weak complexing of the ^{60}Co in this sponge is in contrast to the binding of this radionuclide to protein which appears to be predominant in other marine species such as prawn, abalone or sea hare. A more detailed account of this work will be published shortly (3).
These exploratory studies in the field of radioecological biochemistry suggest that further work in this field could provide valuable information not only about the fundamental mechanisms involved in the uptake of radionuclides and heavy metals by marine organisms but also about the chemical form and the bioavailability of radionuclides entering the human food chain from marine products.

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4. Year-round Research (Short scientific reports)
f) Biophysics of Metallorganic Compounds

BIOPHYSICS OF METALLO-ORGANIC COMPOUNDS

The transport and deposition phenomena of metal ions in mammals have been of particular interest in recent years. Apart from general studies of metal uptake and decorporation special consideration of actinide ion binding has been encouraged.
It is well established that transferrin is the essential metal binding and transport protein in the serum protein of man. Although it has been studied for more than forty years, comparatively little is established about the details of the making and breaking of the metal ion bond. There are two binding sites per molecule (molecular weight of about 79550), situated in the C- and N-terminals of the protein, respectively.
Disagreement still exists in the literature about whether these sites are chemically and/or functionally equivalent or not and whether the answer to this question might be dependent on the type of the metal ion.
Recently it has been demonstrated in an in vivo experiment (1), that also the group IV metal hafnium, which as a plutonium analogue is of great interest with respect to its metabolism in mammals, binds to transferrin. Using time differential perturbed angular correlation (TDPAC)-technique binding parameters for Hf were now determined after an in vivo uptake of Hf-181 in rat plasma.
As a starting chelate for the metal transfer to transferrin the metalnitrilotriacetate (NTA)-complex, which permits short incubation times, is widely used. Aiming at a fundamental understanding of the metal delivery to the protein the binding and complex formation of iron and hafnium in NTA has been studied employing TDPAC- and Mössbauer techniques.
In addition, as a first example of the actinide series several organic compounds of Np-237 has been studied.

It has been convincingly demonstrated that the parameters isomeric shift, electric quadrupole- and magnetic dipole splitting observed by Moessbauer- and TDPAC-techniques, respectively, are extremely sensitive and specific with respect to chemical bonds and details of complex formation between metal ions and biomolecules.
The relaxation constants that can be extracted from the spectra provide further helpful information on the dynamic properties of the molecule.

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THE BINDING OF HAFNIUM TO NITRILOTRIACETATE

G.Then, I.Zell, H.Appel, J.H.Raudies, W.-G.Thies, J.Duffield, D.M.Taylor

The $1/2^+(133\ keV) \rightarrow 5/2^+(482) \rightarrow 7/2^+$ cascade in Ta-181, that succeeds the β^--decay of Hf-181, was used for TDPAC studies of the Hf-binding parameters in NTA and of the chemical structure of the complex.
The samples were prepared from $HfCl_4$ which was dissolved in 2 - 11 molar HCl. The Hf^{4+} ions then form hydro-complexes. Solutions of different pH were prepared by titration with NaOH to the required pH value. The samples were measured predominantly in the dried state.
The data were collected with a conventional 4-detector arrangement. The usual R(t) ratio was formed which is plotted versus time in all the TDPAC spectra shown.

$$R(t) = 2\,\frac{W(\pi,t) - W(\pi/2,t)}{W(\pi,t) + 2W(\pi/2,t)} \approx A_{22}^{eff} \cdot G_{22}(t)$$

The perturbation factor G_{22} can be written as

$$G_{22}(t) = \sum_{k=0}^{3} S_{2k}(\eta)\cdot e^{-\frac{1}{2}(n_k(\eta)\omega_0\delta t)^2} \cos(n_k(\eta)\omega_0 t)\, e^{-\frac{1}{2}(n_k(\eta)\omega_0\sigma)^2/8\ln 2}$$

with δ, the frequency distribution coefficient and η a (possibly existing) asymmetry parameter. The time resolution, measured with the cascade in question, was δ = 2.5 ns. For spin I = 5/2 in the intermediate state the modulation frequency ω_0 and the quadrupole coupling constant ν_Q are related by

$$\nu_Q = \frac{10}{3\pi} \cdot \omega_0 \quad .$$

The findings can be summarized as follows:
For a molar ratio of 1:2 or smaller a constant fraction of the Hf-ions was subject to a quadrupole coupling with ν_{Q1} = (646 ± 12) MHz and an asymmetry parameter η_1 = 0.22 ± 0.04. This frequency may be attributed to a specific Hf-NTA binding in an 1:2 complex. The small frequency distribution factor δ < 10% observed for Hf: NTA = 1:2 increases with the NTA excess due probably to the changing steriochemical conditions. The spectra (see fig. 1 as an example) allow identification of a second fraction characterized by ν_{Q2}

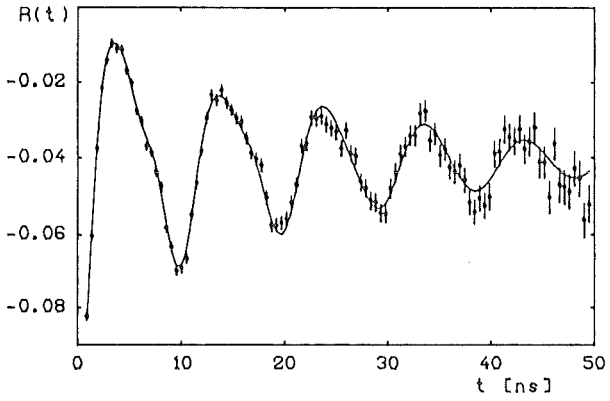


Fig. 1: R(t)-spectrum for a Hf-NTA ratio of 1:2 at pH = 5.2

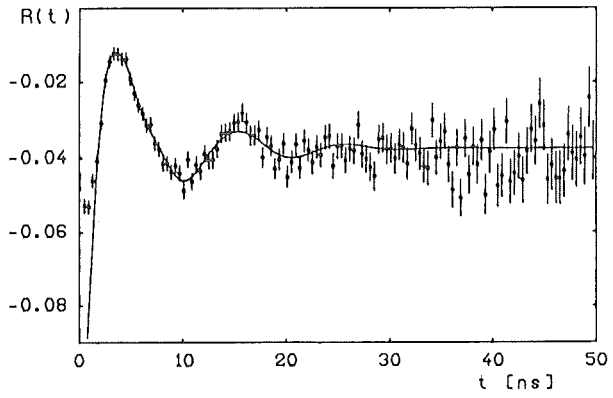
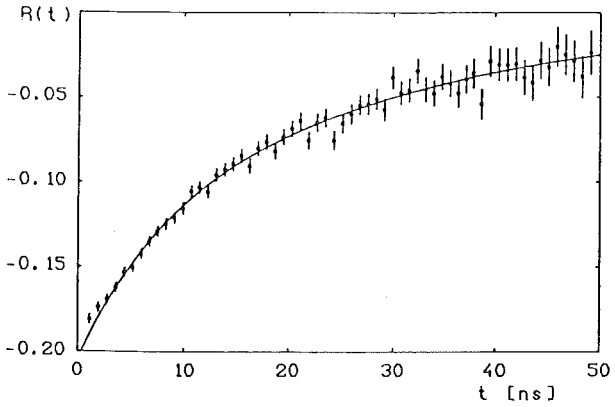


Fig. 2: R(t)-spectrum of a frozen Hf-NTA solution (pH = 5.0) at 77 K



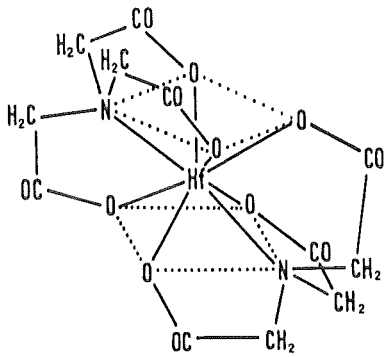


Fig. 3: R(t)-spectrum of a Hf-NTA solution (pH = 5.0) taken at room temperature

Fig. 4: Spatial structure of the $(Hf(NTA)_2)^2$-complex

= (771 ± 33) MHz with an $\eta = 0.29 \pm 0.09$ and a fairly broad distribution: $\delta = 25\%$. This frequency may be ascribed to complexes where one or more carboxyl bonds of Hf-NTA are substituted by hydroxyl ions and for the formation of Hf-hydroxo species. This binding will be called non-specific.

From pH = 3 up to pH = 6.5 a constant fraction of the specific Hf-NTA frequency was observed. For higher pH-values this fraction decreases because very probably Hf-NTA-hydroxo-complexes are formed.

Thus Hf-NTA seems to be stable only within this pH-range. This is an important result with respect to the preparation of Hf-protein complexes using Hf-NTA as an agent for metal delivery.
The bulk of the samples was studied in the dried state. The two observed frequencies and their relevant fractions do not change significantly when liquid samples are studied in the frozen state at 77 K (see fig. 2), but δ_1 becomes considerably larger and approaches the value of δ_2. For liquid samples the spectra can be described by two pure time dependent interactions with relaxation constants $\lambda_1 \approx 90$ MHz and $\lambda_2 \approx 26$ MHz (see fig. 3).
Hafnium, atomic number 72, belongs to the group IVb metals. Its physical and chemical properties are very similar to those of Zr. From the titration curve of Zr-NTA it was derived (1) that a $(Zr(NTA)_2)^{2-}$ complex is formed between pH=3 and pH=6.6. Since the pH dependence of the TDPAC data can be interpreted in a similar way to a titration curve, the results for Hf can be attributed to the formation of a $(Hf(NTA)_2)^{2-}$ species of similar structure to $(Zr(NTA)_2)^{2-}$. The eightfold coordinated Hf^{4+} ion can be envisaged as being surrounded by two $(NTA)^{3-}$ ions in a dodecahedral structure (see fig. 4).

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HAFNIUM BINDING TO RAT SERUM TRANSFERRIN

G.Then, I.Zell, H.Appel, W.-G.Thies, J.Duffield, D.M.Taylor

Sprague-Dawley rats were injected intravenously with 1 ml of a solution containing 1 mCi Hf-181 (equivalent to 0.029 µmoles Hf) plus 0.12 µmoles nitrilotriacetic acid (NTA), adjusted to physiological pH with $(HCO_3)^-$. The retention of Hf-181 in the plasma, collected two hours after injection, was 15%. Gel-chromatographic analysis indicated that 98.5% of the Hf was bound to transferrin. The TDPAC-measurements were made with liquid samples (at room temperature) and in the frozen state. It was verified that the metalprotein binding is not altered when the plasma samples are cooled to 77 K and then warmed up to room temperature again.
The data were taken with a conventional 4-detector arrangement using the $1/2^+$(133 keV) → $5/2^+$(482 keV) →$7/2^+$-cascade in Ta-181. The usual ratio

$$R(t) = 2\,\frac{W(\pi,t) - W(\pi/2,t)}{W(\pi,t) + 2W(\pi/2,t)} \approx A_{22}^{eff}\, G_{22}(t)$$

was formed. The effective anisotropy coefficient (including solid angle and decay branching corrections) was determined to be $A_{22}^{eff} = -0.219$.
Fig. 1 shows the data taken from rat plasma at room temperature. The R(t)-spectrum can be approximated by the perturbation function (see for example (1)):

$$G_{22}(t) = f_1 \exp(-\lambda t)\, G_{22}^{(1)stat}(t) + f_2\, G_{22}^{(2)stat}(t)$$

The main fraction ($f_1 \approx 78\%$) of the Hf-181 nuclei exhibits a well defined electric field gradient (EFG): $\nu_{Q1} = (1516 \pm 15)$ MHz with a frequency distribution $\delta_1 = (5.3 \pm 0.8)\%$. Superimposed on this is a time dependent EFG that leads to a relaxation-damping with $\lambda = (46 \pm 8)$ MHz. A smaller fraction ($f_2 \approx 22\%$) is subject to a broad distribution of EFGs with $\nu_{Q2} = (1014 \pm 37)$ MHz and $\delta_2 = (16 \pm 3)\%$. Because of the limited experimental time resolution of 2.5 ns and the comparatively high observed frequencies, harmonics can not be resolved. Thus it is not possible to decide whether the EFG associated with the

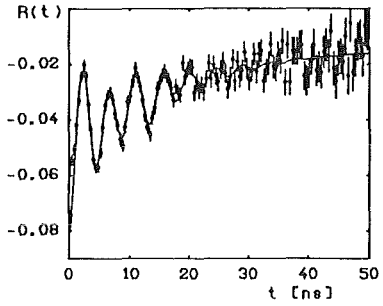

Fig. 1: R(t)-spectrum of in vivo applied Hf-181 to rat plasma taken at room temperature

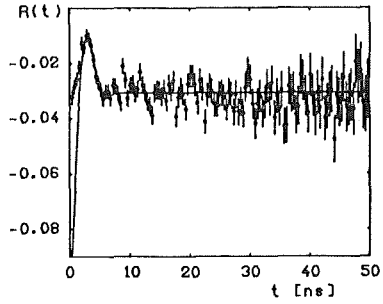

Fig. 2: R(t)-spectrum of in vivo applied Hf-181 to rat plasma taken at 77 K

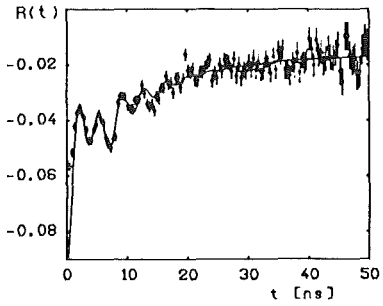

Fig. 3: R(t)-spectrum of in vitro prepared Hf-181-transferrin taken at room temperature

quadrupole constant ν_{Q1} is axially symmetric or whether a finite asymmetry parameter η is involved.
The R(t)-spectra change considerably when the sample temperature is lowered to 77 K (see fig. 2). A broad distribution of a reduced EFG is then observed: $\nu_{Q1} \approx 600$ MHz with no relaxation effects to be seen. Since it has to be assumed that the specific Hf-transferrin binding is not altered at this low temperature two possible mechanisms have to be discussed in interpreting the finding: The perturbation might be due to a superposition of the static EFG(1) and a 'frozen' time-dependent EFG that gave rise to the relaxation at room temperature. Alternatively many different conformations of the protein in the frozen plasma could have contributed to a markedly reduced asymmetry parameter η.
The rat plasma results may be compared with those taken from in vitro labeled Hf-181-human-transferrin. For the in vitro studies crystalline human transferrin was dissolved in $(HCO_3)^-$ buffer at pH = 8. Then the Hf-181 NTA solution, as characterized above, was added. The pH was again adjusted to the physiological value of 7.4.

The spectrum of this sample (see fig. 3) looks rather similar to the in vivo results with respect to the relaxation behaviour. The dominating frequency ($\nu_{Q1} \approx 1800$ MHz) however, appears to be significantly higher than in the in vivo case. This indicates different EFGs or asymmetry parameters η at the binding sites of the two transferrin species studied.
A definite assignment of the observed frequencies to specific binding properties of Hf in transferrin cannot be given yet. It seems likely that the ν_{Q1} can be attributed to a specific binding of the Hf ions while non-specific binding in native plasma may give rise to ν_{Q2}. It is interesting to speculate on the significant difference between the dominating frequencies observed in the in vivo and the in vitro measurements. This finding may be interpreted in terms of a preferred binding of metal ions to the C-terminal site of in vitro prepared transferrin whereas there is preferential occupation of the N-terminal site in serum, as pointed out in ref. (2). If any cooperativity phenomena between the two binding sites in transferrin exist it has also to be taken into consideration that in plasma about 30% of the binding sites were already taken up by ferric ions. Finally, in serum the simultaneous anion binding requirement, a characteristic requirement for metal binding to transferrin, may have been fulfilled in a different manner to that which occured in the in vitro preparations.

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TEMPERATURE- AND pH-DEPENDENCE OF THE Fe-BINDING TO NTA

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Iron (III)-nitrilotriacetate (Fe-NTA) is a favourite starting chelate for delivering iron to proteins in in vitro studies. The details of the iron binding to NTA are not yet known. They are basic, however, for an understanding of the metal transfer to proteins which seems to be a rather involved process.
Fe-57 NTA was studied with the pH-range 3 to 9 and at different temperatures using the Moessbauer method. The probes were prepared from pure $FeCl_2$ solutions. For Fe: NTA ratios of 1:4 equilibrium was achieved within minutes at room temperature. The pH was adjusted by adding NaOH.
Two characteristically different bond conditions were observed for the iron ion in NTA. They exist in complementary fractions depending on the pH range and can be described as follows:
- At low pH (typically pH = 3) as a six-line pattern due to paramagnetic interaction together with a small quadrupole splitting. The relaxation behaviour becomes more pronounced with rising temperature (see figures 1a through 1c).

The following parameters were evaluated from the data using the Hamiltonian

$$\hat{H} = A_z \hat{S}' \hat{I}_z + \frac{eQV_{zz}}{4I(2I-1)} \quad (3\hat{I}_z^2 - I^2)$$

For T = 70 K they are
A_z = (-13.02 ± 0.07) mm/s for the magnetic hyperfine parameter of the ground state,
ΔQ = (-0.05 ± 0.02) mm/s for the electric quadrupole splitting,
δ = (0.39 ± 0.02) mm/s for the isomeric shift versus Co-57 in Rh, and
τ = (55 ± 9) ns for the relaxation time.

The spectra can be fitted reasonable well with the cited finite value for A_z and $A_x = A_y = 0$, i.e. under uniaxial symmetry conditions. The experimental value for A_z is consistent with $5a_o$ = -13 mm/s (with the free iron ground state hyperfine constant a_o taken as -2.60 mm/s). The shapes of the spectra observed depend quite critically on the rate of the electronic relaxation processes which determine the hyperfine fields in the nucleus. They can be described in a simple approximation by using the Scherer-Blume model (1,2) with an effective spin $S'_z = \pm 1/2$ and

the Hamiltonian quoted above. The procedure applied to the data permits the conclusion that the lowest $|S = 5/2, S_z = \pm 5/2>$ Kramers doublet is occupied. It is then sufficient to parametrize the process by a characteristic time τ. Simulated spectra have been produced for different relaxation times τ and with other parameters kept constant as taken from the experimentally observed spectra. The temperature dependence of τ is different from that expected for superparamagnetism (3), thus a dependence on the particle size, i.e. collective phenomena like polymer formation can be excluded here.
The appearance of Kramers doublets necessarily requires an odd number of electrons: Hence the iron ion in NTA must be in the 3^+ state. This finding is supported by the value for the isomeric shift which is also characteristic for this 3^+ valence state.
It should be noted that the small quadrupole contribution causes the spectrum, which appears essentially like an effective hyperfine spectrum, to become slightly asymmetric. The smallness of ΔQ points towards a high symmetry environment with only a weak distortion. The field is very likely to be of tetrahedral or octahedral symmetry (4).
The part of the data that deviated from the fitted spectrum close to the center of the six-line pattern, is attributed to a chemical compound, possibly other than Fe-NTA which is not yet identified.
- For high pH (typically pH = 8) as a pronounced quadrupole doublet and a weak six-line relaxation spectrum (see figure 2). The parameters extracted from the data at 70 K are
$\Delta Q = (1.53 \pm 0.05)$ mm/s
$\delta = (0.36 \pm 0.01)$ mm/s and
$\Gamma = (0.29 \pm 0.02)$ mm/s for the line width.

The comparatively large quadrupole splitting indicated that the iron environment is strongly distorted. Possibly dimers (5,6) are formed at high pH values via two OH^- bridges.
Polymer formation is very unlikely. The isomeric shift turns out to be constant within the temperature range chosen. The value is again typical for Fe^{3+}.
The results can be interpreted as follows: At low pH Fe(III) NTA exists as a monomer complex. The iron atom is probably fourfold coordinated and arranged in an essentially tetrahedral configuration. A slight axially symmetric distortion is obviously introduced by the Fe-N coordination in comparison to three Fe-O-coordinations and leads to a small quadrupole splitting. In general the complex shows paramagnetic behaviour whereas the spectrum shape is characteristically modified at different temperatures by relaxation phenomena. The monomer complex gradually changes into a dimer configuration at higher pH values via OH^- bridges. The iron atoms are then sixfold coordinated. The comparatively high field gradient and consequently the large

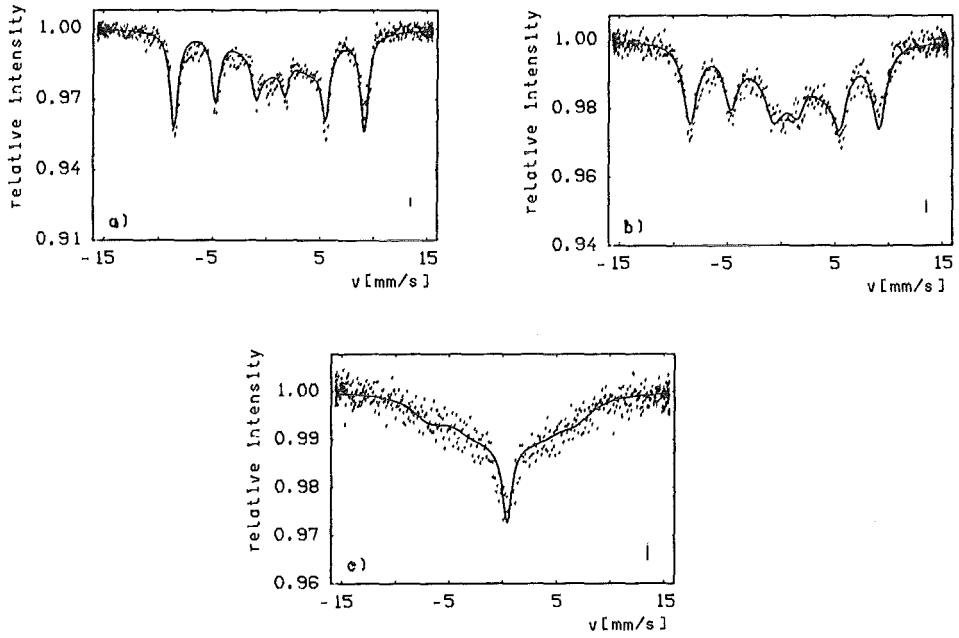

Fig. 1: Fe-NTA, pH = 3
a) T = 11 K, b) T = 70 K, c) T = 200 K

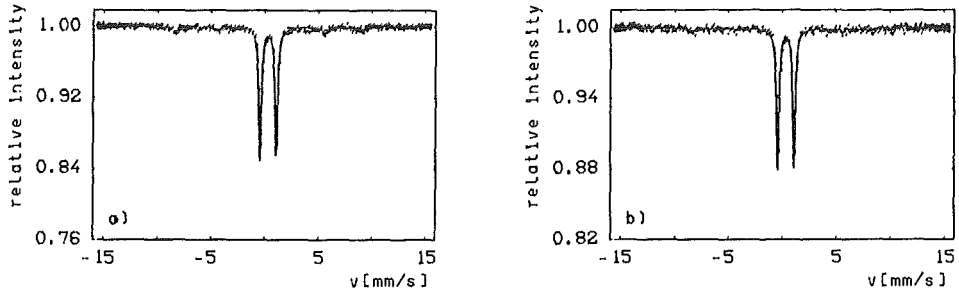

Fig. 2: Fe-NTA, pH = 8
a) T = 12 K, b) T = 70 K

quadrupole splitting is then very likely to result from the distortion of the octahedral symmetry due to the two neighbouring Fe atoms.

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MÖSSBAUER STUDIES OF Np-237-ORGANOMETALLIC COMPOUNDS

Th.Krüger, H.Appel, H. Haffner, B.Kanellakopulos

Np-organometallic compounds show rather small resonance effects of the order 10^{-3}. The probes studied were therefore kept at 4.2 K during the measurements. With a 140 mCi metallic Am-241 source and a lead loaded plastic scintillator a 60 keV count rate of about 2×10^8 per channel and week was registered. The chosen narrow geometry and the necessary high Doppler-velocities led to a marked deformation of the base line, thus making the separation of the small resonance effects difficult.
The interaction of the nuclear spin with an effective electron spin S = 1/2 results - in the absence of external fields - in a splitting into two sub-states each for the excited and the groundstate, respectively. Two out of the four occuring transitions remained unobserved because of comparatively small intensity.
The following compounds have been studied (1):

1) $Np(C_5H_5)_4 = NpCp_4$
Figure 1 shows the spectrum of the paramagnetic, quasi-tetrahedral $NpCp_4$ (2) in the Γ_6 groundstate (S = 1/2). Both lines are considerably broadened by relaxation phenomena. An interpretation of the data is readily possibly using the Scherer-Blume model for paramagnetic relaxation in cubic environment and S = 1/2 (3,4). A simulation program indicated a relaxation frequency of about 600 MHz for the observed pattern. The hyperfine interaction parameter A and the isomeric shift (versus $NpAl_2$) were determined to be

$$A = 3392 \pm 68 \text{ MHz}$$
$$\delta = 16.7 \pm 0.5 \text{ mm s}^{-1}.$$

Mössbauer spectra taken at 4.2 K with an Am-241 metal source

2) $(C_5H_5)_3NpCl = Cp_3 NpCl$
As can be observed from figure 2, the resonance effect for Cp_3NpCl is considerably smaller than 10^{-3} even at 4.2 K. The probe material is of axial C_{3v} symmetry (5), the lowest electron state again being Γ_6. The crystal structure is, however, not cubic, thus the Hamilton operator is not isotropic anymore. The isomeric shift was determined to be

$$\delta = 18.8 \pm 1.00 \text{ mm/s}$$

3) Np-Nitrilotriacetate = Np-NTA
The spectrum taken can be approximated by a quadrupole splitting and isomeric shift, respectively, of

$$\frac{1}{4} eQV_{zz} = 15.13 \pm 0.90 \text{ mm s}^{-1} \text{ and}$$
$$\delta = 6.8 \pm 0.3 \text{ mm s}^{-1}.$$

The value for δ supports the assumption of a strong ionic character of the metal ion bond. Np-NTA may also be used as a starting complex in studies where Np(IV) is supposed to bind to polypeptides.

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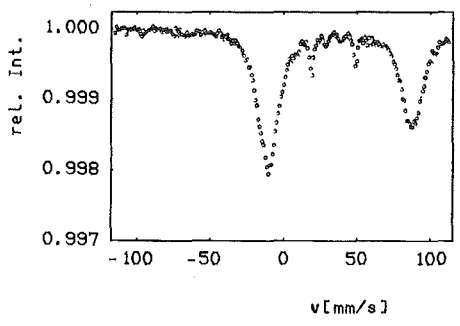

Fig. 1: $Np(C_5H_5)_4$

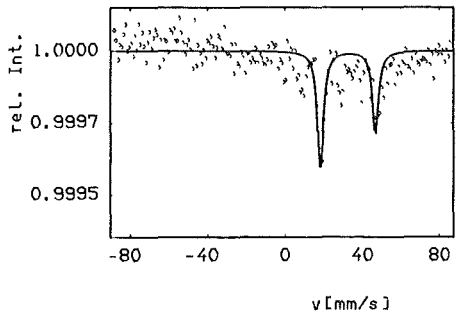

Fig. 2: $(C_5H_5)_3$ NpCl

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F.Planas-Bohne, M.Lehmann
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W.Sontag
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R.Winter, U.Sütterlin, M.Glück, A.Seidel
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F.Planas-Bohne, D.M.Taylor, V.Volf
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A.Seidel, W.Sontag
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A.Seidel
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D.M.Taylor
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D.M.Taylor
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D.M.Taylor
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V.Volf
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W.Sontag
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D.M.Taylor
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V.Volf
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R.W.Heidinger
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S.Adelski
Mössbauer-Untersuchungen an Transferrin
Diplomarbeit, Universität Karlsruhe 1982

M.Glück
Untersuchungen des Verhaltens von intratracheal verabreichten polymeren Transuranen in der Rattenlunge
Diplomarbeit, Universität Karlsruhe 1982

Th.Krüger
Untersuchungen von Np-237-Verbindungen mit Hilfe des Mössbauereffekts
Dissertation, Universität Karlsruhe 1982

E.Peter
Untersuchungen zum biologischen Verhalten und zur Dekorporation von Th-234 bei der Ratte
Dissertation, Universität Karlsruhe 1982

U.Sütterlin
Der Einfluss der Zeit auf die subzelluläre Verteilung von Transuranen und Fe-59 in der Leber von Ratte und Chinesischem Hamster
Dissertation, Universität Karlsruhe 1982

G.M.Then
Untersuchung von Bindungsverhältnissen in Hafnium-Komplexen mit Hilfe der zeitdifferentiellen Beobachtung von gestörten Winkelkorrelationen
Diplomarbeit, Universität Karlsruhe 1982

J.H.Raudies
Untersuchungen zur Bestimmung elektrischer Feldgradienten in dotierten Diamanteinkristallen
Dissertation, Universität Karlsruhe 1983

R.Wirth
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6. In-House-Seminars

1981

January

H.Kleinkauf, TU Berlin
"Die Biosynthese von Antibiotika-Peptiden"

H.Lehrach, EMBL Heidelberg
"Klonierung und Sequenzierung von Semliki Forest Virus 26s-RNA"

E.Jacob, MPI Tübingen
"Analyse der frühen Embryonalentwicklung von Xenopus laevis mit Hilfe von klonierten c-DNA Seequenzen materneller RNA"

U.Sütterlin, IGT
Weitere Untersuchungen zur subzellulären Verteilung von Pu-239"

February

E.Peter, IGT
"Dekorporation von Thorium bei der Ratte"

J.Horst, Univ. Ulm
"Über prokaryote Genexpression in animalen Zellen"

O.Pongs, Inst. f. Biochemie, Univ. Bochum
"Ecdyson-Rezeptoren und Chromosomenpuffs"

K.N.Raymond, Univ. Berkeley
"New Chelating Agents for Plutonium"

March

B.Dobberstein, EMBL Heidelberg
"Molekularbiologische Analyse von Histokompatibilitätsantigenen der Maus (H-2)"

April

R.Friis, Institut for Virologie,Univ. Gießen
"Rous Sarkom Virus Src-Genprodukt und der transformierte Genotyp"

T.Wurtz, ISREC Lausanne
"Characterization of a chromatin fraction enriched for active transcribing sequences"

May

M.Delius, EMBL Heidelberg

"Elektronenmikroskopie von Nukleinsäuren"

U.Bernard, DKFZ Heidelberg
"Die Verwendung von DNA vermitteltem Gentransfer zur Isolation eukaryotischer Gene"

P.Philipson, Biozentrum Basel
"Ty, a mobile element in yeast"

A.Pillai, Bombay
"Pu-239 at environmental levels"

V.Volf, IGT
"Wechselwirkungen von Plutonium mit stabilen Metallen"

June

G.Vossius, Inst.f.Biokybernetik, Univ.Karlsruhe
"Funktionelle Stimulation von Querschnittsgelähmten"

D.M. Prescott, Univ. of Colorado at Boulder, USA
Arrangements of genes in chromosomes and isolation of genes in protozoa

S.Kozma, Univ.Lüttich
"Analysis of MMTV proteins in mouse organs"

B.Groner, IGT
"Hormonregulation des MMTV-Gens"

G.Darai, Inst.f.Virol., Univ.Heidelberg
"Virologische Aspekte der Tupaias: Isolierung und Charakterisierung von Herpes- und Adenoviren"

E.Fanning, Fachbereich Biologie, Univ.Konstanz
"Multiple forms of Simian Virus 40 large tumor antigen"

July

I.Diehl-Marshall, IK II
"Die Wirkung kleiner Dosen verschiedener Strahlenqualitäten auf Vicia faba"

K.Mölling, MPI Berlin
"Genprodukte von RNA-Tumorviren"

S.Nelson, Inst.f.Angew.Mathem. Univ.Heidelberg
"Models for DNA damage formation and repair"

G.B.Schofield, British Nuclear Fuels Ltd.
"Plutonium in Man"

F.Vogel, Inst.f.Anthrop.u.Humangenetik, Heidelberg
"Mutationsbelastung menschlicher Populationen"

R.Winter, A.Seidel, IGT
"Biochemische Studien mit Schwermetallen in marinen Organismen"

M.Moos, Physiol.-Chem.Inst., Univ.Marburg
"Untersuchungen zur Struktur des Aktin-Gens"

September

A.Nordheim, MIT
"Left-handed Z DNA: A search for its natural occurrence and biological function"

R.Moses, Baylor College, Houston, z.Zt.Heidelberg
"Cloning of the uvr-C gene in E.coli"

H.Blüthmann, Univ. Genf
"Teratocarcinome als Differenzierungsmodell"

D.Grunberger, Columbia Univ. New York
"Molecular Mechanisms in Chemical Carcinogenesis"

I.Mattaj, Friedr.Miescher-Inst. Basel
"Studies of estrogen inducible and constitutively expressed genes in chicken liver"

October

J.Stoye, Friedr.Miescher-Inst. Basel
"Retrovirus expression in murine lymphocytes"

U.Gehring, Univ. Heidelberg
"Biochemische und genetische Untersuchungen über Steroidrezeptoren"

November

F.W.Bonner, Univ. of Surrey
"Toxicology of Cadmium"

R.Friedrich, Univ. Freiburg
"Replikation der RNA-Tumorviren"

W.S.S.Jee, Univ. of Utah
"Pu-239 induced bone sarcomas in beagles"

K.Holmes, MPI f.Med.Forsch.,Heidelberg
"Mechanismus der Muskelkontraktion"

A.Pöting, IGT
"Ecdyson-induzierte Proteine in Drosophila melanogaster"

R.Heiermann

"RNS-Polymerase und 'in vitro' Transkription"

December

H.J.Ache, IRCH
"Anwendung nuklearer Techniken für die Lösung biologischer Probleme"

P.Möller, Patholog. Institut, Univ. Heidelberg
"Pathologisch-histologische Untersuchungen an Tupaias"

M.Steinmetz, Calif.Inst.of Technol.,Pasadena
"Organisation of the genes encoding mouse transplantation antigens"

N.Harth, Inst.f.Mol.Gen., Univ. Heidelberg
"Regulation auf Translationsebene beim Phagen T7"

W.Doerfler, Inst. f. Genetik, Univ. Köln
"DNA-Methylierung: Ein Signal für die Genregulation bei Eukaryonten"

1982

January

W.Keller, DKFZ Heidelberg
"In vitro-Transkription von viralen Genen"

February

W.Sontag, IGT
"Plutonium im Tupaia-Skelett"

G.Bornkamm, Inst.f.Virologie, Univ. Freiburg
"Das Genom des Epstein-Barr-Virus und sein Nachweis in menschlichen Tumoren"

G.Schütz, DKFZ Heidelberg
"Das Lysozymgen: Transkription in vitro und Expression nach Transfer in eukaryotische Zellen"

H.Weber, Univ. Zürich
"Modification of the rabbit Betaglobin gene by restructuring and site directed mutagenesis"

F.Planas, IGT
"Aufnahme von Actiniden und Schwermetallen in Abscessen"

March

B.Wasylyk, CNRS Strasbourg
"Promoter sequences of eukaryotic protein coding genes"

J.Jacobsen, Univ. Essen
"Der tödliche Elektrounfall im Gefährdungsmodell"

S.Buntenkötter, Hochschule Hannover
"Einfluß von Pharmaka auf die elektrische Herkammerflimmerschwelle"

M.Lehmann, IGT
"Der Metabolismus von Hafnium"

C.E.Johnson, Univ. Liverpool
"Mößbauer-effect studies of ironsulphurproteins"

M.Govindan, DKFZ Heidelberg
"Purified corticoid receptor binds to LTR-sequences of MMTV in vitro"

April

A.Iyengar, Kalpakham, Indien
"Radioecological preoperational survey at the Kalpakham Reactor Power Station"

D.Ganten, Univ. Heidelberg
"Neue Aspekte der Blutdruckregulation"

D.Jähner, Pette-Institut Hamburg
"De novo Methylierung von Maloney-Retrovirus-genomen in vivo"

E.-L.Winnacker, Univ. München
"Rekombinations- und Replikationsprozesse in Säugerzellen"

May

G. Smith, Univ. Cardiff
"Studies on Cadmium-binding in biological systems"

M. Ciriacy, Inst. Mikrobiol., TH Darmstadt
"Insertionselemente in Saccharomyces cerevisiae"

A. Gräßmann, Inst.f. Molekularbiol., FU Berlin
"Kartierung von SV-40 spezifischen Funktionen"

P.H. Hofschneider, MPI München
"Hepatitisviren und primäre Lebertumoren"

J. Sussenbach, Univ. Utrecht
"Adenovirus DNS-Replikation"

H.-P. Zimmermann, DKFZ Heidelberg
"Colchicin-Derivate, ihre biologische Aktivität und intrazelluläre Verteilung"

June

U. Sütterlin, IGT
"Subzelluläre Verteilung von Transuranen in der Leber in Abhängigkeit von der Zeit"

E. Harms, Univ. Heidelberg
"Biochemische Untersuchungen zu den lysosomalen Speicherkrankheiten"

D. Drahovsky, Univ. Frankfurt
"Enzymatische Methylierung der eukaryotischen DNS"

M.H. Weisenseel, Inst.f.Botanik, Univ. Karlsruhe
"Ionenströme als Kontrollmechanismus bei Differenzierung und Wachstum"

J. Beck, CNRS Strasbourg
"Cytolytic and antitumor agents from

traditional Chinese and other oriental drugs"

M.C. Balani, BHABHA, Indien
"Distribution of radionuclides in marine organs"

B.A. Rahn, Lab. Exp. Chirurgie, Davos
"Zirkulation und Knochenumbau"

July

P. Starlinger, Inst.f.Genetik, Univ. Köln
"Transponierbare Elemente bei Zea mays"

M. Glück, IGT
"Untersuchungen zur biochem. Bindung von Transuranen in der Rattenlunge"

September

J.A. Martial, Univ. Liege
"Structure and regulation of the Prolactin gene"

A. Lehmann, Univ. Sussex
"The Cockayne Syndrome"

R. Müller, Salk Institute, San Diego
"Expression of cellular oncogenes"

W. Bodmer, Univ. Erlangen
"Transkription und Translation in Herpesvirus Saimiri und Ateles infizierten Zellen"

October

I. Grummt, Inst.f.Biochemie, Univ. Würzburg
"Signalsequenzen und regulatorische Faktoren bei der Transkription ribosomaler Gene der Maus

R. Dofuku, Nederlands Kankerinstituut, Amsterdam
"A possible selective endomitotic origin of trisomy of chromosome 13: implications for mammary tumorigenesis of mice"

T. Tslty, Stanford University
"Enhanced frequency of generation of methotrexate resistance and gene amplification in cultured mouse cell lines"

L. Thilo, MPI f. Biol., Tübingen
"Das Verhalten von internalisierter Membran während der Pinozytose bei Makrophagen"

W. Lubitz, Univ. Kaiserslautern
"Klonierung von OX-174 Lysefunktion"

R. Ball, Ciba Geigy, Basel
"Analysis of SV 40 tumor antigens with monoclonal antibodies"

H. Kohler, Roswell Park Memorial Inst.,Buffalo
"The dextran-specific B-cell repertoire in Balb/c"

B. Brown, DKFZ Heidelberg
"Isolation of transforming genes by genomic DNA transfection - Problems and Pitfalls"

November

M. Wiener, Univ. Karlsruhe
"Wirkung von Schwermetallen auf Pflanzen"

V. Braun, Univ. Tübingen
"Rolle der Eisenversorgung für die Pathogenität von Bakterien"

W.A. Müller, GSF München
"Verteilung, Dosimetrie und Spätschäden verschiedener Radionuklide nach Inkorporation bei Mäusen"

G. Ryffel, Inst.f.Zoologie, Univ. Bern
"Regulation of Vitellogenin Genes"

H. Gronemeyer, LGME Strasbourg
"Photoaffinity labeling of steroid hormone receptor"

R. Gebhardt, Univ. Tübingen
"Aspekte der Struktur und der Funktion des biliären Pols in kultivierten Hepatozyten"

U. Rüther, Univ. Köln
"Neueste Klonierungssysteme für Eukaryontengene"

K.H. Klempnauer, Univ. of California, San Francisco
"Structural analysis of an oncogene: The leukemia gene myb"

G. Metzger, Strasbourg
"Effects of the binding of the carcinogen acetylaminofluorene to chromatin

H.J. Lipps, Univ. Tübingen
"DNA-Organisation im Makronukleus des Ciliaten Stylonychia mytilus"

C.M. Calberg-Bacq, Univ. Liege
"The MMTV envelope: structure, composition and eventual role during natural infection"

D. von Wettstein, Carlsberg Lab.
"Zur molekularen Biologie des Chloroplasten"

V. Herzog, Univ. München
"Die Rolle der Plasmamembran beim sekretorischen Prozess"

December

P. Czernilofsky, Österr.Akad.d.Wiss.Salzburg,
"Die Struktur des Src-Gens und seine flankierenden regulatorischen Regionen"

M. Wabl, Friedr.Miescher Lab. der MPG, Tübingen
"One lymphocyte - two immunoglobulin classes"

A. Sergeant, DKFZ Heidelberg
"In vitro transcription of RNA-polymerase II transcribed genes"

W. Zumft, Inst.f.Mikrobiol., Univ. Karlsruhe
"Regulation der Stickstoff-Fixierung bei phototrophen Bakterien"

J. Döhmer, MPI f.Biochemie, Martinsried
"Molony Sarkomavirus und Rinder-Papilloma-Virus als eukaryotische Vektoren"

S. Weiß, Friedrich-Miescher-Labor MPG, Tübingen
"Kappa supprimierte Mäuse als Modellsystem zum Aufbau des immunologischen Repertoires"

H. Debuch, Univ. Köln
"Lysosomaler Phospholipidstoffwechsel"

F. Anders, Univ. Gießen
"Biologie eines Onkogens und eines Differenzierungsgens bei Xiphophorus"

V. Ullrich, Univ. Homburg
"Mechanismen des Fremdstoffabbaus"

1983

January

R.O. Williams, ILRAD, Nairobi, Kenia
"Relationship between multiple copies of a parasite Trypanosoma brucei membrane antigen gene whose expression is not controlled by duplication"

W. Schaffner, Inst. Mol. Biol., Univ. Zürich
"Stimulation der Transkription durch virale DNA-Segmente"

U. Schäfer, Uni. Düsseldorf
"Isolierung und Analyse männchenspezifischer Gene von Drosophila melanogaster"

E. Amann, Behringwerke Marburg
"Neue Methoden zur Expression eukaryotischer Gene in E.coli"

O.G. Issinger, Univ. Homburg/Saar
"Vergleichende Untersuchungen der nukleären und cytoplasmatischen Phosphoproteine von menschlichen Meningeomen"

M.C. Balani, IGT
"Pu-239 in Chinese hamster liver. Studies on the size of the nuclide binding cell organelle"

J.R. Duffield, IGT
"The chemistry of metal ion binding by transferrin"

February

L. Henninghausen, Inst.f.Genetik, Univ. Köln
"Structure and hormonal regulation of milk protein gene"

March

M. Schwab, Univ. of California, San Francisco
"Amplifikation zellulärer Onkogene: Molekularbiologische Basis für Krebsbildung?"

R. Seiff, CNRS Gif-sur Yvette (France)
"Malignant transformation induced by polyoma virus or SV40"

April

E. van Rensburg, Univ. of Pretoria
"Nucleoid sedimentation as a method for detecting DNA damage and repair"

M. Geiser, Friedrich Miescher-Institut, Basel
"Structure and sequence of the promoter region of the estrogen-regulated chicken vitellogenin II gene"

E.A. Nigg, ETH Zürich
"Cellular localization of retroviral transforming proteins and of substrates for tyrosine protein kinases"

L.König, KfK, HS
"Tritiumbelastung der Umgebung des Kernforschungszentrums Karlsruhe"

May

G. Röderer, Univ. Hohenheim
"Toxische Wirkung von organischen und anorganischen Bleiverbindungen in einzelligen Algen"

R. Renkawitz, DKFZ Heidelberg
"Hormonregulation transferierter Gene"

G. van Kaick, DKFZ Heidelberg
"Die Deutsche Thorotrast-Studie"

A. Belayew, Univ. of Liege
"Genetic analysis of -fetoprotein synthesis in mice"

W. Ostertag, Pette-Institut Hamburg
"Myeloproliferatives Sarkomvirus: Struktur und Funktion"

J. Rosmanith, Univ. Aachen
"Interaktionen zwischen Metallen"

D. Heck, KfK, IAK II
"Elementaranalyse einzelner Zellen mit protoneninduzierter Röntgenstrahlung"

June

T. Wurtz, ISREC Lausanne
"Characterization of a transcriptional active chromatin fraction"

E.K.F. Bautz, Inst.f.Mol.Gen., Univ. Heidelberg
"Genregulation in Drosophila"

C. Kellershon, Med. Fakultät Paris
"Mössbauer study of rare earth bone uptake

and of iron in hepatic and splenic tissue"

M. Breindl, Pette-Institut Hamburg
"Transkription klonierter Retrovirusgenome nach Injektion in Froschoocyten"

J. Burckhardt, EMBL Heidelberg
"Reaction mechanism of the restriction and methylation enzyme EcoK"

C. Stuart, Pette-Institut Hamburg
"Teratocarcinomas and gene expression"

V. Eybl, Univ. Pilsen
"The interaction of chelating agents with Cadmium"

J.R.J. Sorensen, Univ. Arkansas, USA
"Copper Complexes for the Treatment of Inflammatory Diseases and Cancer"

July

P.W. Durbin, Berkeley
"Licams for the removal of plutonium"

H. Land, MIT
"Two complementary oncogenes are required to transform primary rat embryo fibroblasts"

B. Wittig, Inst.f.Molekularbiol., FU Berlin
"Genprogrammierung durch Nucleosomenpositionen"

H.E. Schmidt, TU Karlsruhe
"Vorkommen und Bedeutung von Kernbrennstoff-Aerosolen"

N. Spoerel, Harvard Biological Laboratories
"Chorion genes of Bombix and Drosophila"

T. Igo-Kemenes, Inst. f.Physiol.Chemie, Phys. Biochemie und Zellbiologie der Univ. München
"Die Chromatinstruktur aktiver und inaktiver Genbereiche

N. Cohen, Institute of Environmental Medicine
New York University, Medical Center
"The need for future research in decorporation therapy"

C. Streffer, Inst.f.Med.Strahlenphys., Univ. Essen
"Strahlenbiologische Untersuchunngen am Prä-

implantationsembryo der Maus"

M. Pfahl, The Salk Institute, San Diego
"Characterisation of the glucocorticoid receptor binding site on the MMTV-LTR"

August

M.E. Wrenn, Radiobiology Div. Dept. of Pharmacology, Univ. of Utah, Schoool of Medicine
"Dosimetry, pathology and dose response for bone sarcomas in beagles injected with Ra-226"

F. Bosch, Inst.f.Virologie, Univ. Gießen
"Molekulare Grundlagen der Pathogenität von aviären Influenza-Viren"

V. Bosch, Univ. Gießen
"Structure and Assembly of Rous sarcoma virus"

September

P.G. Strauss, NIH Bethesda
"Induktion von Thymus-Tumoren in der Ratte durch MoMuLV. Mechanismen der Entstehung von Leukämie"

K.W. Kohn, NIH Bethesda
"Protein-associated DNA strand breaks: A cellular response to DNA intercalation"

October

L.I. Wiebe, Univ. Alberta, Edmonton, Canada (z.Zt. DKFZ, Heidelberg)
"Synthesis and biochemical properties of some radiohalogenated nucleosides"

H. Lehrach, EMBL, Heidelberg
"Cloning of chromosomal regions containing developmental mutations: the t-complex of the mouse"

K.H. Scheidtmann, Univ. Freiburg
"Beziehung zwischen Phosphorilierung, DNA-Bindung und Oligomerisierung des SV40 T-Antigens"

November

F.W. Bruenger, Univ. of Utah, USA
"Plutonium translocation from a deposit of soluble particles in liver and spleen, its elimination and toxicity"

F. Grummt, Univ. Würzburg

"Zink und Adenosintetraphosphat als Signale der Proliferationskontrolle und Trigger der Replikation"

H. Schüttelkopf, HS
"Der Transfer von Actiniden aus dem Boden in Pflanzen"

E. Wagner, EMBL, Heidelberg
"Studies on gene transfer into mice"

R.O. Williams, ILRAD Nairobi
"A model of antigen gene expression in trypanosomes"

D. Paul, Inst.f.Pharmakol., Univ. Hamburg
"Regulation des Wachstums von normalen und transformierten Hepatozyten in Kultur durch Wachstumsfaktoren"

December

K. Stanley, EMBL, Heidelberg
"Cloning of rare mRNA molecules by the immunological detection of hybrid protein in situ"

K. Kloppstech, Univ. Hannover
"Regulation der Genexpression während der frühen Phase der Ergrünung bei höheren Pflanzen"

W. Hunziker, Veterinärpharmakol. Institut der Univ. Bern
"1.25-Dihydroxi-Vitamine D as a steroid hormone: receptors and regulated gene products"

7. Teaching Activities at the Universities of Karlsruhe and Heidelberg

Einführung in die Genetik	P.Herrlich, U.Mallick
Spezielle Genetik (Immmungenetik)	P.Herrlich
Einführung in die molekulare Genetik	P.Herrlich
Molekulare Mechanismen der Reparatur von Strahlenschäden an der DNS	T.Coquerelle
Einführung in die molekulare Strahlenbiologie	C.Lücke-Huhle
Einführung in die zelluläre Strahlenbiologie	C.Lücke-Huhle
Analytische Methoden in der Zellbiologie	A.Seidel
Biochemie umweltrelevanter Schwermetalle	H.Appel F.Planas-Bohne A.Seidel D.M.Taylor
Verhalten und biochem. Wirkungs-mechanismen von Schwermetallen	A.Seidel
Allgemeine Toxikologie der Radionuklide	V.Volf
Biologisches Risiko der Kerntechnik I	A.Seidel
Molekulare Genetik	B.Groner,N.Hynes N.Kennedy,H.Ponta H.J.Rahmsdorf
Vererbung geistiger Eigenschaften	P.Herrlich
Physik der Atomkerne und Elementarteilchen Kursvorlesung Physik IV (Atomphysik) Spektroskopie an biologischen Systemen Anwendung physikalischer Methoden auf biologische Systeme Kursvorlesung Physik VI (Kernphysik) Hyperfeinwechselwirkung I: Kernspektroskopische Methoden	H.Appel
Genetisches und Toxikologisches Seminar	P.Herrlich D.M.Taylor
Seminar: Genetik für Anfänger	N.Kennedy

Literaturseminar für Fortgeschrittene	P.Herrlich
Vorbereitungsseminar zu den Genet.Kursen	P.Herrlich H.Ponta H.J.Rahmsdorf
Strahlenbiologisches Seminar	T.Coquerelle G.Hotz,A.Seidel V.Volf
Biochemie für Lehramtskandidaten (Seminar)	H.Ponta
Genetisches Seminar	P.Herrlich
Molekulare Genetik für Fortgeschrittene (Seminar)	B.Groner H.Ponta H.J.Rahmsdorf
Seminar Vererbung und Intelligenz	P.Herrlich
Fortgeschrittenen Seminar	B.Groner P.Herrlich N.Hynes N.Kennedy H.Ponta H.J.Rahmsdorf R.Renkawitz
Seminar: Grundlagen der Mössbauer-Spektroskopie	H.Appel
Seminar: Elektronenstruktur und chemische Bindung Seminar zur Hyperfeinwechselwirkung Seminar Biophysik der Aktiniden Seminar über spektroskopische Methoden Forschungsseminar zu laufenden Arbeiten	H.Appel,H.Haffner W.-G.Thies
Seminar Genstruktur: DNS und Chromatin	R.Renkawitz
Gen-Transfer: Neuer Weg zur funktionellen Analyse von Genen	R.Renkawitz
Großes Genetisches Praktikum	P.Herrlich, S.Matzku H.Ponta, H.J.Rahmsdorf R.Renkawitz
Genetikkurs für Fortgeschrittene	P.Herrlich

Einführung in die immunologische Technik	S.Matzku
Strahlenbiologisch-mikrobiologisches Praktikum	G.Hotz
Zellbiologisches Praktikum	A.Seidel
Gentechnologisches Praktikum (für Fortgeschrittene)	B.Groner N.Hynes
Anleitung zu wissenschaftlichen Arbeiten (im Rahmen von Dissertations-, Diplom- und Zulassungsarbeiten)	H.Appel P.Herrlich G.Hotz H.Ponta H.J.Rahmsdorf A.Seidel D.M.Taylor V.Volf

8. Extramural Activities of Institute Members

a) Genetics

1981

T.Coquerelle, K.F.Weibezahn
Rejoining of Radiation Induced Double Strand Breaks in Different Cellular Systems
Annual Meeting of the Radiation Research Society, Minneapolis, 3.6.1981

T.Coquerelle, K.F.Weibezahn
Rejoining of DNA Double Strand Breaks in Ataxia Telangiectasia and Fanconi Fibroblasts
International Workshop on Experimental Oncology, Wisconsin, 29.5.81

B.Groner, N.Kennedy, P.Herrlich, G.Knedlitschek, L.Fabiani, U.Rahmsdorf, N.Hynes
Stability state of modification and expression of a cloned mouse mammary tumor virus gene in transfected cells
IX. Congress of the International Society of Developmental Biologists, Basel, 28.8.-2.9.1981

B.Groner, U.Rahmsdorf, N.E.Hynes
Hormone responsive expression of transfected mouse mammary tumor virus DNA and chimeric MMTV-thymidine kinase genes
Joint Meeting of the French, German and Swiss Biochem. Society, Strasbourg, 21-23 Sept. 1981

P.Herrlich
Regulation eines Tumorgens
Festkolloquium aus Anlaß des 25-jährigen Bestehens des Kernforschungszentrums Karlsruhe, Kernforschungszentrum Karlsruhe, 11. Juni 1981

U.Mallick, H.J.Rahmsdorf, N.Yamamoto, H.Ponta, P.Herrlich
TPA-inducible proteins are synthesized at elevated rate in Bloom's Syndrome
IX. Congress of the International Society of Developmental Biologists, Basel, 28.8.-2.9.1981

1982

B.Groner, H.Ponta, N.Kennedy, U.Rahmsdorf, P.Herrlich, N.E.Hynes
The long terminal repeat (LTR) of mouse mammary tumor virus (MMTV) confers hormone inducibility to the expression of the proviral gene and a chimeric MMTV-thymidine kinase gene
Tagung der DGHM, Sektion IV Virologie, Heidelberg, 25.-27.3.1982 und Special FEBS-Meeting, Athens, 25.-29.4.1982

P.Herrlich, H.J.Rahmsdorf, H.Ponta, U.Mallick
B lymphocyte specific nuclear proteins are induced by

mutagenic treatment
Special FEBS-Meeting, Athens,25.-29.4.1982

P.Herrlich, H.J.Rahmsdorf, L.Hieber, C.Lücke-Huhle, H.Ponta, U.Mallick
Ionisierende Strahlung aktiviert Gene
Wiss. Vortragsveranstaltung der AGF, Bonn, Wissenschaftszentrum, 4./5.11.1982

H.J.Rahmsdorf, U.Mallick, P.Herrlich, N.Koch
Arresting replication induces Ia associated invariant chain synthesis in proliferating B cells
Joint Meeting of the Nordic Biochemical Society and the Ges. für Biol. Chemie, Sept. 27-29, 1982, Damp/Kiel

1983

B.Groner, H.Ponta, A.J.J.van Ooyen, N.Kennedy, M.Beato, M.-L.Carrozza, P.Herrlich, N.E.Hynes
Transcriptional control signals contained in the long terminal repeat sequences of mouse mammary tumor virus
Jahrestagung Zellbiologie, Hamburg, 23.-25.3.1983

B.Groner, A.J.J.van Ooyen, N.Kennedy, P.Herrlich, H.Ponta, N.E.Hynes
Fifty nucleotides preceeding the MMTV LTR RNA cap site can cause hormone inducible transcription
CETUS-UCLA Symposium in Gene Expression, Park City, Utah, USA 26.3.-1.4.1983

W.H.Günzburg, N.E.Hynes, B.Groner
The methylation pattern or proviral genes of mouse mammary tumor virus (MMTV) in normal tissue DNA and in transformed cells
Sowjetisch-deutsches Symposium 1983

P.Herrlich, L.Hieber, C.Lücke-Huhle, U.Mallick
Alpha radiation induces changes of gene expression
IAEA Internat. Konferenz über die biologischen Auswirkungen schwacher Strahlung, April 11-15, 1983, Venedig

L.Hieber, C.Lücke-Huhle
DNA repair and genome amplification in mammalian cells during alpha-radiation induced G2 arrest
Workshop on Heavy Particles in Biology and Medicine, Darmstadt, 27.-29.6.1983

S.Kozma, N.E.Hynes, B.Groner
Transformation by gene transfer of mammary carcinoma oncogenes
International Symposium on Oncovirology, May 23-27, 1983, CSSR

C.Lücke-Huhle, L.Hieber, N.Kennedy, P.Herrlich
Gene and genome amplification in mammalian cells following exposure to 3.4 MeV alpha particles
UCLA-Symposium, April 10-15, 1983, Keystone, Colorado

H.Ponta, N.E.Hynes, N.Kennedy, M.L.Carrozza, A.van Ooyen, M.Beato, P.Herrlich, W.Günzburg, B.Salmons, G.Knedlitschek, B.Groner
Transcriptional control signals contained in the long terminal repeat sequences of mouse mammary tumor virus
Frühjahrstagung der Sektion Virologie, Würzburg, 17.-19.3.1983

H.Ponta, B.Groner, N.Kennedy, P.Herrlich, A.van Ooyen, C.Scheidereit, M.Beato, N.E.Hynes
Transcriptional control signals contained in the long terminal repeat sequences of mouse mammary tumor virus
Cold Spring Harbor Meeting on RNA Tumor Viruses, Cold Spring Harbor, May 25-29, 1983

K.F.Weibezahn, Th.Coquerelle
Impaired double strand break repair by caffeine in mammalian cells
Eleventh L.H. Gray Conference, Glasgow, 18.-22.7.83

b) Toxicology

1981

S.Adelski, H.Appel, H.Haffner, T.Krüger, D.M.Taylor
Mössbauer Spectra of Fe-57 in Human Transferrin
Intern.Conf. on the Application of the Mössbauer Effect
JAIPUR-Conference, Indien, Dec. 14-19, 1981

H.Haffner, H.Appel
Demonstration of a Stable Sulfenyl Iodide Bond for Cysteine in Tobacco Mosaic Virus (TMV)
Intern. Conf. on the Application of the Mössbauer Effect
JAIPUR-Conference, Indien, Dec.14-19, 1981

E.Holzschuh, W.Kündig, P.F.Meier, B.D.Patterson, J.P.F.Sellschop, M.E.Stemmet, H.Appel
Muonium in Diamond, Conference, Reading, GB, 8.-11.7.1981

F.Planas-Bohne
Probleme der Dekorporationstherapie von Schwermetallen und Radionukliden
Deutsche Gesellschaft für Wehrmedizin und Wehrpharmazie, München, 3.6.1981

1982

H.Appel
Application of Moessbauer-Methods to Biomolecules

Vortrag Andhra University Visakhapatnam, AP, India, 1982

H.Appel
Beta-Decay Shape Factor Measurements and Test of the Conserved Vector Current Hypothesis
Vortrag Andhra University, Visakhapatnam, AP, India, 1982

J.Duffield
Metal Binding Properties of Human Transferrin
Symposium "Toxicology of Metals and Metal Deficiency", London, Dec. 20-21,1982

F.Planas-Bohne, J.R.Duffield, D.M.Taylor, G.Darai
The role of transferrin in the uptake of metals by human lymphoblasts (Wil-2) in vitro
Symposium "Toxicology of Metals and Metal Deficiency", London, Dec. 20-21, 1982

D.M.Taylor
Chelation Therapy: Retrospect and Prospect
Tagung der Inorganic Biochem. Discussion Group (Royal Society of Chemistry) and Association of Clinical Biochemists: "Toxikologie der Metalle und Metallmangelkrankheiten", London 20.-21.12.82

V.Volf
Reduction of risk after incorporation of radionuclides
Colloquium on the Toxicity of Radionuclides, Nov. 19-20, 1982, Liege

1983

G.Adrian, H.Appel, J.Duffield, H.Haffner, D.M.Taylor
Temperature- and pH-dependence of the Fe-binding to NTA
Intern. Conference on the Applications of Mössbauer Effect, Alma-Ata/USSR, 26.9.-1.10.83

M.Balani, A.Seidel
Binding of Pu-239 to lysosomes in Chinese hamster liver: studies with gel chromatography and free flow electrophoresis
Jahrestagung der Deutschen Gesellschaft für Zellbiologie, Hamburg, 23.-25.3.1983

R.Heidinger, P.Peretto, S.Choulet
TDPAC measurements on phase transition and hydrogen up-take in C15 Laves phases
Intern. Conference on Hyperfine Interactions, Groningen, 4.-8.7.83

W.Jung, R.Gebhardt
Biliary Transport of Organic Anions in Primarry Cultures of Rat Hepatocytes
Hepatologie-Symposium, Wien, 13.-14.10.1983

T.Krüger, H.Appel, H.Haffner, B.Kanellakopulos
Mössbauer Studies of Np-237-organometallic Compounds
Second Seeheim Workshop on Mössbauer Spectroscopy, Seeheim, 23.-26.5.83

T.Krüger, H.Appel, H.Haffner, B.Kanellakopulos
Structure and magnetic ordering in BaNp-oxide (03)
Intern. Conference on the Applications of Mössbauer Effect, Alma-Ata/USSR, 26.9.-1.10.83

E.Polig, D.Kimmel
Dimensions of bone cell nuclei and hit probabilities from surface-seeking alpha emitters
IAEA Internat. Konferenz über die biologischen Auswirkungen schwacher Strahlung, April 11-15, 1983, Venedig

J.Raudies, H.Appel, G.M.Then, W.-G.Thies, K.Freitag
TDPAC studies on Hf-181 implanted into diamond
Intern. Conference on Hyperfine Interactions, Groningen, 4.-8.7.83

G.M.Then, H.Appel, J.Raudies, W.-G.Thies, J.Duffield, D.M.Taylor
The binding of Hafnium to nitrilotriacetate
Intern. Conference on Hyperfine Interactions, Groningen, 4.-8.7.83

G.M.Then, I.Zell, H.Appel, W.-G.Thies, J.Duffield, D.M.Taylor
Hafnium binding to rat serum transferrin
Intern. Conference on Hyperfine Interactions, Groningen, 4.-8.7.83

V.Volf
Carcinogenesis by Incorporated Isotopes
Seventh International Congress of Radiation Research, Amsterdam, 3.-8.7.83

Additional Year-round-activities:

Th. Coquerelle: Member of the "Study group on primary effects of radiation on nucleic acids", Commission of the European Communities, Brussels.

P. Herrlich: Beiratsmitglied der Deutschen Forschungsgemeinschaft (DFG) für das Zentralinstitut für Mutagenitätsforschung, Freiburg.

G. Hotz: Member of the "Study group on biochemistry and genetics of DNA repair", Commission of the European Communities, Brussels.

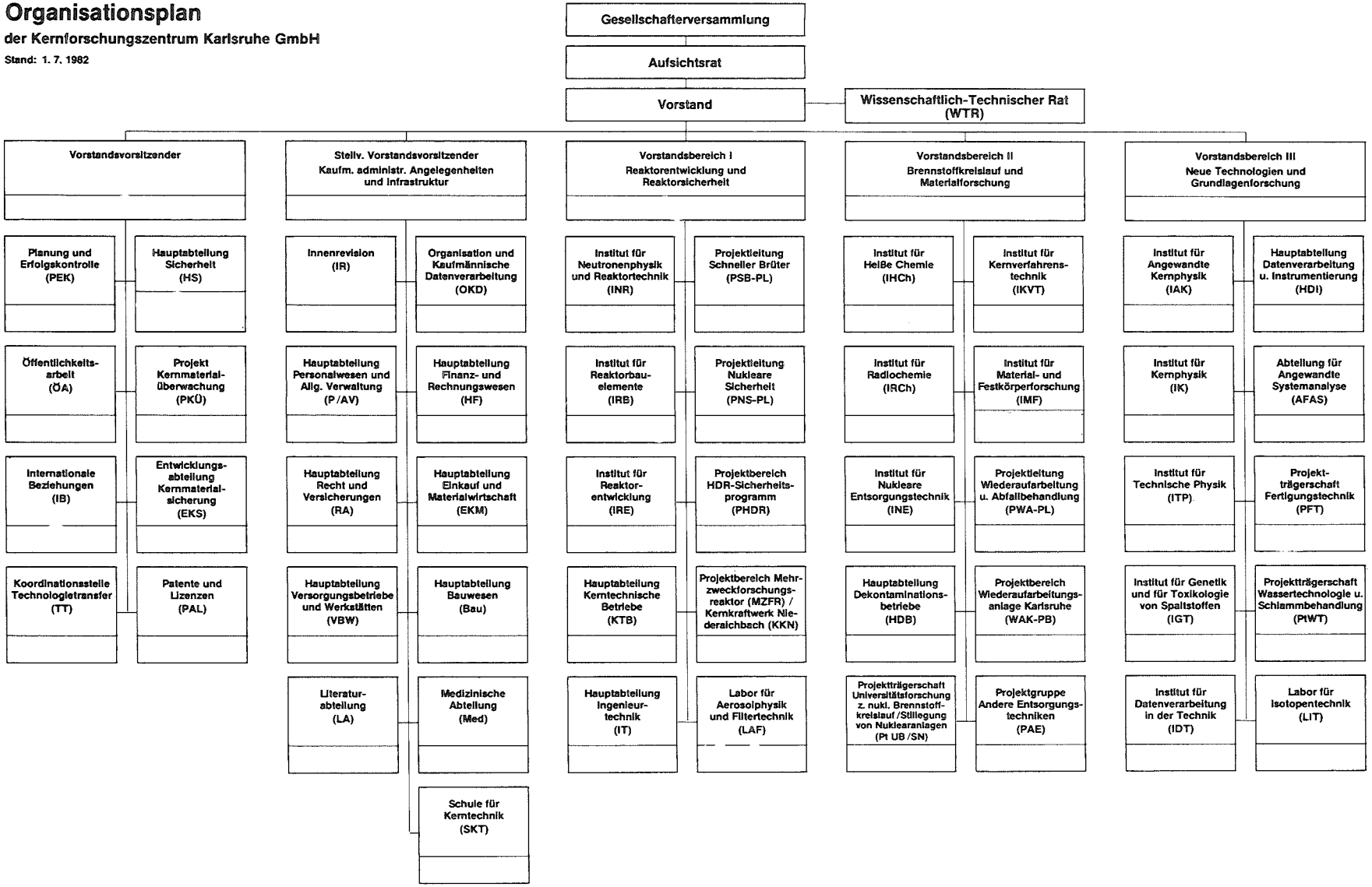
E. Polig, A. Seidel, W. Sontag, D.M. Taylor, V. Volf: Members of the "European Late Effects Project Group (EULEP)", Brussels.

V. Volf: Mitglied des Arbeitskreises "Arztliche Hilfe bei Kernenergie-Katastrophen" des Wissenschaftlichen Beirats der Bundesärztekammer, Köln. Mitglied des Ausschusses "Strahlenmedizin" des Hauptverbandes der Gewerblichen Berufsgenossenschaften, Köln. Mitglied des Arbeitskreises "Inkorporationsüberwachung" beim Fachverband für Strahlenschutz.

Organisationsplan

der Kernforschungszentrum Karlsruhe GmbH

Stand: 1. 7. 1982



10. Address of the Institute

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V. Volf	823209
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