RADIATION EFFECTS IN PHYSICS, CHEMISTRY AND BIOLOGY

PROCEEDINGS OF THE SECOND INTERNATIONAL CONGRESS OF RADIATION RESEARCH

HARROGATE, GREAT BRITAIN, AUGUST 5–11, 1962

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1963

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FOREWORD

The problem of scientific communication at large congresses presents a challenge, in response to which the organizers of the Second International Congress of Radiation Research employed the rapporteur method for the presentation of about half the proffered papers. Each Rapporteur reviewed a group of related papers. This volume contains the Rapporteurs' reports on some 300 papers, and summaries of the discussions to which the reports gave rise. In a unique way, therefore, it presents the thought of several hundred leading scientists, drawn from over forty countries, on twenty-five topics broadly representative of the advancing frontiers of radiation research.

It is a fine tribute to the quality of both the reports and the discussions that some nine hundred copies of this volume were sold in advance to Members at the Congress. It is no less a tribute to the original research which formed the basis of the reports, and I should like to offer my personal thanks to the authors for the self-effacing manner in which they entered into the spirit of the rapporteur experiment.

I think this volume is remarkable not only for its contents but for the speed with which it has been published. This has only been achieved by the very close cooperation between the Contributors, the Editors, and the North-Holland Publishing Company, to all of whom we offer our congratulations. The Editors deserve our special thanks for their part in the preparation of this volume.

During the five days of the Congress, which began with messages from two of our Honorary Presidents, Dr. Bugher and Professor Kuzin, and the Opening Address by Lord Adrian – a characteristic blend of wit and wisdom – and which ended with the remarks of Dr. Hollaender at the Closing Ceremony, much was said that was memorable but cannot be recorded in this volume. Happily, however, it has been possible to include an historical sketch of the development of radiation research which was given by Dr. Hollaender in reply to the Chancellor of the University of Leeds on behalf of the four Congress Members upon whom Her Royal Highness The Princess Royal had conferred the Degree, Doctor of Science, *honoris causa.*

At the outset we were greatly disappointed when ill health robbed us of the presence of our senior Honorary President, Professor George de Hevesy, to whom the Officers and Editors respectfully dedicate this volume, as a token of our affection and of our esteem for his outstanding contributions to radiation research.

L. H. GRAY
(PRESIDENT)

British Empire Cancer Campaign
Research Unit in Radiobiology
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January 1963

ACCEPTANCE SPEECH

by ALEXANDER HOLLAENDER

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. (U.S.A.)
(Operated by Union Carbide Corporation for the
U.S. Atomic Energy Commission)

(On occasion of Conferment of Honorary Degrees, Doctor of Science honoris causa *to Louis Harold Gray, Alexander Hollaender, Alexander Mikhajlovich Kuzin and Raymond Latarjet)*

Your Royal Highness and Chancellor, Members of the Faculty, Ladies and Gentlemen:

We feel highly honored to be given Honorary Degrees on this great occasion.

As you probably know, several of us have approached the problem of radiation research from a physical or physicochemical point of view, though not all of us have received Physics or Physical Chemistry Degrees. We therefore feel especially honored to receive the Degree from a University which for many years has been and now is so outstanding in physics and physical chemistry and, especially in the last decade or so, in radiation chemistry. These fields form the foundation stones of radiation biology.

It is interesting to follow the path that radiation research has taken during the past 30 years. With the increasing understanding of basic phenomena in the nuclear science field, interest in radiation biology was reawakened. Physical approaches have been and are being used extensively with great succes in the interpretation of effects in radiation biology. However, it has become more and more obvious, especially during the past 10 or 15 years, that radiobiology will require also a broad interpretation from the chemical point of view. This attitude has, as you know, taken hold of us very strongly during the last few years and changed our outlook on the mechanism of radiation effects. Modern developments in biochemistry and molecular biology have given us a much broader point of view in looking at living cells, seeing how they function, how they maintain their status, how they protect themselves against

environmental factors, such as radiation, and how they repair damage. These are points of view which have emerged very strongly during the past 20 years. Even in the late Thirties and early Forties, the mention of repair mechanism in living cells was regarded as rather unusual and not considered essential enough to be mentioned in important reviews. It is only with the new developments in these fields, which are being brought out so strikingly in so many papers at this Second International Congress of Radiation Research, that we feel we are getting closer to an understanding of how radiation affects living cells.

What are the fields necessary for the radiation biologist to have at his finger tips to be able to follow these developments? He has to be well-versed in physics and in chemistry. He has to be in contact with the newest developments, especially in biochemistry, which have made possible so many strikingly new interpretations of the function and structure of the nucleic acids, the physical and chemical structure of chromosomes and genes, as well as of a variety of cytoplasmic particles. The new developments in our knowledge of the transfer of information from the nucleus to the cytoplasm which is involved in nucleic acid and protein synthesis have opened entirely new fields and ways of looking at biology. These developments have not only influenced radiation research, but radiation research has often opened the way for these advances. Radio- and stable isotopes have made possible investigations which were formerly impossible. What would our status be in these fields without advances in modern chemical genetics which were initiated by mutations produced and analyzed by radiation? The development in our understanding of genetic coding, which has become so important in the last few months, would have been impossible without the progress made in radiation biology. Developments in biology have often to wait for new advances in physics and chemistry. The new and very elegant biochemical tools, like the special mutations in microorganisms and viruses with highly specific properties, have helped extensively the new advances in molecular biology.

Radiation research also has given the immunologist, the radiologist, the cancer investigator many modern tools that have helped in interpreting medical problems, as well as in developing therapeutic methods. Let me follow this thread in a different direction.

Today we know more about the effects of radiation on living cells than of almost any other agent. And not only the effects on cells, but on living organisms in general. This became obvious to me when a survey was made on chemical mutagenesis. It turned out that radiation mutagenesis actually leads the way in the development of this important field, which is becoming more and more important with the wide use of insecticides, of food additives, and certain types of medicine. Here the new advances lie in directions pointed out by radiation research, and it looks again as if the systematic and thorough investigation of a fairly narrow field like radiation research has produced tools and approaches and ways of following things which will be most useful in the field of cancer, toxicology and in developmental biology.

Several of these areas have been approached in a routine manner by screening compounds for their effectiveness, whereas they should have been developed by studies on the mechanism of the function of such compounds. Here radiation studies, as exemplified by many papers at this Congress, can point the way. The new developments in genetics, cytology, biochemistry, and physiology – the most important research in radiation chemistry, the new thrilling approach to radiation absorption and measurement, and isotope developments – all these different fields brought together have helped to advance science in many ways, and not the least in the peaceful application of atomic energy. A survey of the abstracts of the more than 700 papers given at this congress is most impressive. They illustrate the productive cooperation between physicists, chemists, biologists, and medical investigators. This congress is indeed an excellent demonstration of peaceful cooperation of the scientists from the 40 nations represented at this meeting.

Development in sciences that border on radiation biology, which is beautifully illustrated in the development of basic science at the University of Leeds, has given us the interpretation and approaches which are so very important to modern biology. The discovery of a new method of crystal analysis, without which the new concept of the structure of nucleic acid would not be possible, was made here at the University of Leeds by Professor William Bragg many years ago. The physical chemistry necessary for modern approaches to biology has been developed most impressively here

at the University of Leeds. Again, I want to say how much we feel honored to receive Honorary Degrees from a University which has played such an important role in these fields that border on, and strongly support, research in modern biology.

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PREFACE

This book contains the twenty-five reports given by Rapporteurs at the Second International Congress of Radiation Research at Harrogate, Yorkshire, August 5–11, 1962. Following each Rapporteur's report is an account, written by a Discussion Secretary, of the discussion which took place after the report.

The Association for Radiation Research proposed in 1959 that the Second International Congress of Radiation Research should be held in Great Britain. At the same time it was decided to devote a large part of the Congress time to the Rapporteur method as an experiment in scientific communication. The subjects for the Rapporteur sessions were chosen to represent the growing points of radiation research as defined by the scientific material submitted to the Congress. The Rapporteurs were selected from workers in this country and were given a number of papers to summarize and present in a way which would be comprehensible to a non-specialist audience.

Over three hundred papers are included in these reports. It is our duty to point out to the reader that the scientific content of this book is almost entirely based on these papers, and that the Rapporteurs acted as reviewers of current research in progress.

We would like to express our thanks to all the Rapporteurs and Discussion Secretaries, whose promptness in completing their manuscripts has made possible the early publication of this book, and to acknowledge the cooperation and help of the publishers towards the same purpose.

MICHAEL EBERT
ALMA HOWARD

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Physical Parameters in Radiobiology

LINEAR ENERGY TRANSFER

L. F. LAMERTON

Physics Department, Institute of Cancer Research, The Royal Cancer Hospital, Clifton Ave, Belmont, Sutton, Surrey, Great Britain

a) P. ALEXANDER and Z. B. MIKULSKI,
Qualitative differences between the action of α and X rays on lymphoma cells in tissue culture.
Chester Beatty Research Institute, London SW 3, Great Britain

b) TIKVAH ALPER, D. K. BEWLEY and J. F. FOWLER,
Alpha-particle irradiation of bacterial spores in dormant and germinated forms.
Medical Research Council, Hammersmith Hospital, Ducane Road, London, Great Britain

c) D. A. BARBER and M. EBERT,
The effects of neutrons and X rays on extension growth and survival of barley roots.
D.A.B.: *Agricultural Research Council, Letcombe Regis, Wantage, Berks., Great Britain*
M.E. : *Medical Research Council, Hammersmith Hospital, Ducane Road, London, Great Britain*

d) G. W. BARENDSEN and H. M. D. WALTER,
Differences between densely and sparsely ionizing radiations with regard to modification of radiation damage in human tissue culture cells.
Radiobiological Institute, TNO, Rijswijk (ZH), Netherlands

e) R. J. BERRY and J. R. ANDREWS,
Some observations of relationships between ionization density (LET) and lethal efficiency studied by irradiation and assay of surviving mammalian tumour cells *in vivo*.
Radiation Branch, National Cancer Institute, National Institute of Health, Bethesda, Maryland, USA

f) J. F. FOWLER, D. K. BEWLEY, G. W. BARENDSEN and H. M. D. WALTER,
Technique and dosimetry for irradiation of mammalian cells in tissue culture with cyclotron accelerated particles of various LET.
J. F. F. and D. K. B.: *Medical Research Council, Radiotherapeutic Research Unit, Hammersmith Hospital, Ducane Road, London, Great Britain*
G. W. B. and H. M. D. W.: *Radiobiological Institute, TNO, Rijswijk (ZH), Netherlands*

g) J. F. FOWLER, S. HORNSEY, D. K. BEWLEY, R. L. MORGAN, J. A. SILVESTER, B. A. TURNER and G. SILINI,
Differences in macroscopic damage to pig skin not predictable from cell population studies as exemplified by ascites cells in mice after X ray and cyclotron neutron irradiations.
Medical Research Council, Hammersmith Hospital, Ducane Road, London, Great Britain

h) G. J. NEARY, J. R. K. SAVAGE, H. J. EVANS and J. WHITTLE,
Limiting values of the RBE of fast neutrons and gamma rays at low dose rates.
Medical Research Council, Harwell, Didcot, Berks., Great Britain

i) L. D. SKARSGARD,
A comparison of some of the biological effects of heavy ions and X rays on mammalian cells.
Biophysics Department, Yale University, New Haven, Connecticut, USA

j) G. F. WHITMORE and D. O. SCHNEIDER,
Comparative effects of neutrons and X rays on mammalian cells.
Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada

1. INTRODUCTION

The subject of this, the first of the rapporteur sessions, is the significance of radiation quality in radiobiological studies, radiation quality here being defined by Linear Energy Transfer, or LET, as the mean rate of energy loss along the paths of the ionizing particles. One reason why LET and the related subject of RBE were chosen for the first session was clearly because Linear Energy Transfer and Relative Biological Effectiveness are both basic concepts in radiobiology. Another reason was, I suspect, because the terms LET and RBE can themselves express very well the philosophy behind the rapporteur session:

Let Everybody Talk?

No!

Rapporteurs for Bare Essentials.

That, at least, is the theory, but only the rapporteur who has had to summarize papers so full of information and thought as the present ones will recognize just how bare the essentials have to be.

Over the last few years there have been many developments, experimental and theoretical, in our understanding of the biological significance of LET and its exploitation in the study of biological mechanisms. One reason is the much wider range of radiations now available. In the present series of papers the LET values used range from 0.2 KeV/μ, using high energy electrons, to 2000 KeV/μ

using accelerated argon ions. Another very important reason has been the improvement in techniques and precision of dosimetry with the particulate radiations, so that much of the previous uncertainty in dose values has been removed.

A major factor, however, has been the development, initially by Puck[1]) and his colleagues, of the culture techniques which allow quantitative studies on mammalian cell survival. This has greatly extended the range of biological materials for which dose-response curves can be determined over a wide range of dose levels. If full advantage is to be taken of the range of radiation qualities available it is not sufficient to make single determinations of RBE. The whole dose-response curves have to be compared over a wide range of radiation conditions.

Ten of the twelve papers given in the programme for this session have been made available in full for this report. The main areas of work covered are:

(i) comparison of dose-response curves for a range of LET values and with a variety of biological materials, including a number of types of mammalian cells in culture, plant material and bacteria.

(ii) the modification of these curves by various factors, including fractionation and protraction of the radiation dose, degree of oxygenation and culture conditions.

2. LET DEPENDENCE OF DOSE-RESPONSE CURVES

Work already published on plant and mammalian material has established the general principle that, when there is a difference in shape of the dose-response curves for radiations of different LET, the curve for the high LET radiation is more nearly exponential than that for the low LET radiation. However, there is little published work on the detail of how the shape of the curve varies with LET.

In the present series the paper of Fowler, Bewley, Barendsen and Walter[f]), gives dose-response curves for a range of LET values from 5 to 140 KeV/μ, for survival of a culture of T-1 cells, kidney cells of human origin. These are shown in Fig. 1. The curves for the three highest LET values, 140, 85 and 60 KeV/μ, obtained with α radiation, are exponential within experimental error. Thereafter, with reduction in LET, a shoulder begins to develop on the curve. One consequence of such a change in shape of the curves is that, for any

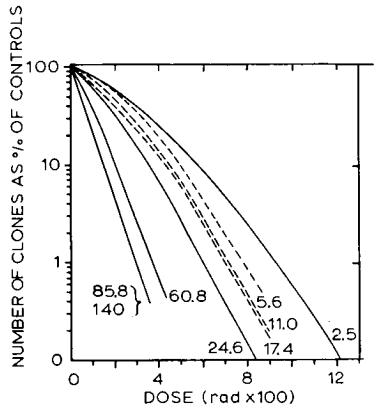


Fig. 1. Dose response curves for T1 cells. LET values in KeV/μ given on curves. (Fowler, Bewley, Barendsen and Walter.)

two radiations, the RBE will not have a constant value but will increase as the dose is reduced, except when both curves are exponential. Fig. 2, taken from the same paper, shows how the RBE value relative to that for 250 KVp X rays varies with LET for different levels of radiation damage (corresponding to 80%, 20%, 5% and 0.5% survival).

The LET value for maximum RBE is an important parameter for estimation of "target size". In these experiments the LET value for maximum RBE would appear to be about 100 KeV/μ, but is somewhat uncertain because it is at the extreme end of the LET range used.

Studies up to much higher values of LET are reported by

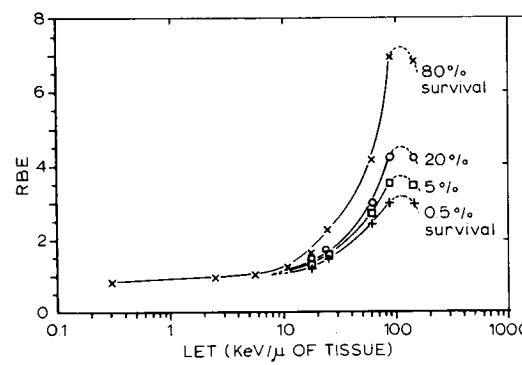


Fig. 2. RBE values for T1 cells for different levels of radiation damage, as indicated on the curves. (Fowler, Bewley, Barendsen and Walter.)

Skarsgard[1]), who has studied the response of Chinese hamster cells in culture to irradiation from X rays (LET 2 KeV/μ) and to accelerated heavy ions up to a maximum LET of 1950 KeV/μ, for argon ions. The RBE-LET relationship found for cell survival and for mitotic delay is shown in Fig. 3. For cell survival the peak RBE occurs at an LET of between 100 and 200 KeV/μ. It will be seen that both RBE values and LET values for peak RBE are different when mitotic delay is the end-point. I will return to this result again later.

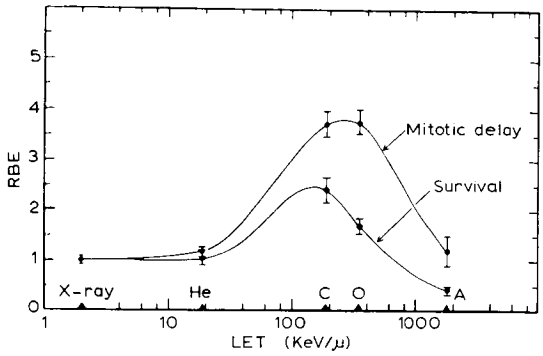


Fig. 3. Relationship of RBE and LET for mitotic delay and survival in Chinese hamster cells $CH2B_2$. (Skarsgard.)

A basic question, in any review of radiation quality, is how far radiation quality can be adequately characterized by the mean LET, and whether two different types of radiations will necessarily have the same biological effect because they have the same LET. This, and the question of what precisely is meant by "mean LET", are not specifically the subject of any of the papers contributed. However, Berry and Andrews[e]), in their studies of the response of mouse lymphatic leukaemia cells, irradiated and assayed *in vivo*, have employed two very different types of radiation but having the same mean LET of about 0.5 KeV/μ. One was ^{60}Co γ radiation and the other accelerated protons of 340 MeV energy in the plateau region. They found similar values, not only of RBE, but also of the oxygen effect. A further demonstration of the significance of LET was their finding, *in vivo*, of different RBE's for the same accelerated α beam, depending on whether the plateau region or the Bragg peak was used.

3. LIMITING VALUE OF RBE

When the dose-response curves vary with LET in the way shown in Fig. 1, one may ask whether the RBE of high LET to low LET radiation will increase indefinitely as the dose is reduced, or will reach a limiting value. This is a matter of considerable theoretical interest, as well as of practical importance in radiation protection.

On a target theory the interpretation of the change in shape of dose-response curve with LET is that the passage of a single ionizing particle through the target is sufficient to produce the effect for high LET radiation, whereas more than one passage may be required for low LET radiation. If, for the low LET radiation, more than one passage is always required, the RBE of high to low LET radiation will increase indefinitely as dose is reduced. However, if there is a finite, though perhaps only small, probability that the effect can be produced by a single passage with low LET radiation, there will be a limiting value of RBE, given by the ratio of the coefficients of the linear dose terms for the two radiations. On a simple target theory one would expect this limiting value of RBE to apply to very low doses at any dose rate and, if repair mechanisms operate between successive passages, for low dose rates at any total dose level. As a number of the present contributors point out, experiments to test this involve many difficulties, because of the low doses or long exposure times needed.

However, Neary, Savage, Evans and Whittle[h]) present, in their paper, a study of this problem based on chromosomal aberrations in Tradescantia microspores, using ^{60}Co γ rays and 3 MeV fast neutrons as the radiation sources. They have analysed the dose-response data for short exposures and for exposures up to 48 hr and have computed the coefficients of the linear dose terms and square dose terms. The conclusion is that there is a linear term for the low LET radiation, but much smaller than that for the high LET radiation, so that the limiting values of RBE are high, as shown Table 1. These RBE values are much higher than those that have been previously reported for Tradescantia.

Several of the contributors working on survival of mammalian cells in culture conclude that here also the RBE has a limiting value. Barendsen and Walter[d]), in their paper on the response of T-1 cells, use their data on X ray fractionation to infer the limiting

TABLE 1

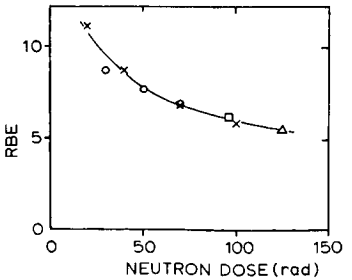
Extrapolated RBE values for combined aberrations in Tradescantia

		RBE	95 per cent Confidence Limits
Long Exposure	Neutrons, 0.7 MeV mean (24 and 48 hr) and gamma rays (48 hr)	87	46 to 570
Long Exposure	Neutrons, 3 MeV mean (7 hr) and gamma rays (48 hr)	52	29 to 220
Short Exposure	Neutrons, 3 MeV mean (20 min) and gamma rays (10 min)	24	11 to 1000

(Neary, Savage, Evans and Whittle.)

curve for low doses and dose rates. For α rays of 3.4 MeV energy to X rays, their results would indicate a limiting RBE for low dose rates of about 12, under anoxic conditions. Under oxygenated conditions the limiting RBE might be much less than this. There is much uncertainty in these values but, nevertheless, they would appear to be much lower than those for Tradescantia.

In general, and not only for low doses, the RBE values found for mammalian cells seem to be considerably less than those found for plant material, as has been pointed out by Alper[2]). This suggestion is supported by the results of Barber and Ebert[c]) on plant material, who in their paper give RBE values based on growth and survival of the seminal roots of barley. The radiations used were



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□ Expt. 5/61
△ Expt. 34/60

Fig. 4. RBE of fast neutrons compared with 1.2 MeV X rays for the growth rate and survival of seminal roots of barley. Aerobic irradiation. (Barber and Ebert.)

1.2 MeV X rays and 2 MeV fast neutrons. Fig. 4 shows some of their data. The RBE values (for oxygenated conditions) are higher than those reported for mammalian cell systems and a higher value than 11 might well be obtained at dose levels lower than those used. For anoxic conditions one would expect these values to be further increased.

4. DOSE-RESPONSE CURVE FOR ANTHRAX BACILLUS

Compared with the mammalian and plant data the studies of Alper, Bewley and Fowler[b]) on bacteria (using *B. anthrax*) show a very different form of LET response. In fact, they show no LET effect

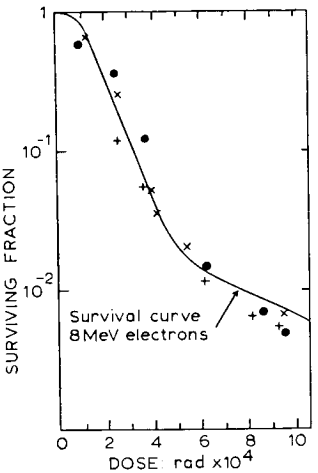

Fig. 5. Survival of germinated spores of *B. anthrax* after irradiation. (Alper, Bewley and Fowler.)

+---×-----Separate runs with 27 MeV α-particles.
● -----5.2 MeV α particles.

at all, as illustrated in Fig. 5, for survival of germinated spores, where points corresponding to LET's of 0.2, 24.6 and 85.8 KeV/μ all fall on the same curve. For dormant spores it was found that each of the radiations used was eight times less effective than for germinated spores.

These results are very interesting not only from the point of view of interpretation; they are also a tribute to the precision of the dosimetry. An RBE of unity for all dose levels would imply, on a target theory, that the radiation sensitive structure in the organism was very thin, less than the ion cluster spacing of the most densely ionizing radiation used. A thickness of less than 10 Ångstrom units is indicated and this, together with inferences from the magnitude

of the inactivation dose for the low LET radiation, would suggest that the lethal damage occurs at an interface.

5. POSSIBLE QUALITATIVE DIFFERENCES IN RESPONSE AND EFFECT OF MODIFYING FACTORS

Several of the contributors ask whether one should regard the effect of high and low LET radiation as being different in nature as well as in degree. The work of Alexander and Mikulski[a]) shows some interesting differences in the response of a mouse lymphoma cell culture to α radiation (from a radium source) and 200 KV X rays. The dose-response curves followed the general pattern, being exponential for the α radiation and having an initial shoulder for the X rays. However, after X rays the damaged cells appeared to divide once before death, whereas after α radiation there was no evidence of further division of the damaged cells. Another example of difference concerned a mutant strain of cell which was isolated from the culture. For X rays the mutant was found to be $2\frac{1}{2}$ times as sensitive as the original strain, but for α rays the dose-response curves were identical.

Such effects can perhaps be explained on a difference in magnitude of the initial effect rather than on a difference in nature. In other words, the passage of an α particle track does considerably more than the minimum damage required for cell death. For less densely ionizing radiation, however, the LET range available spans the range of energy requirements for various end-points.

The results of Alexander and Mikulski are a good example of the very general principle discussed by Quastler[3]) that high LET radiation is always less, or at best equally, selective than low LET radiation, implying that when factors such as dose rate, dose fractionation, oxygen tension and other physiological conditions have an effect, it will be less with high than with low LET radiation. This is clearly an important generalization for the therapeutic applications of high LET radiations, and it is very necessary to obtain quantitative information.

So far as dose fractionation is concerned, the principle is confirmed by Fowler, Bewley, Barendsen and Walter, who found no effect of dose fractionation with α radiation on their cultures of T-1 cells. The fractionation effect found with X rays on mammalian cell cultures, and studied so comprehensively by Elkind and his

colleagues[4]), can be explained, on target theory, by repair processes between successive "hits". One very important question is whether a fractionation effect can be observed whenever there is an initial shoulder on the dose-response curve, even when this occurs with relatively high LET radiation. If no fractionation effect were found with the higher LET radiations it would suggest a difference in the nature of the initial cell injury.

Whitmore and Schneider[j]) report that the fast neutron dose response curves for survival of H1 (Chinese hamster) cells show an initial shoulder, but fractionation studies gave no indication of repair, at least over a period of 2 hr. This differs from the result obtained by Hornsey and Silini[5]) in previously published work. In studies with mouse Ehrlich ascites tumour cells, using X rays and fast neutrons of LET about 20 KeV/μ, they found no difference in shape of survival curves, and the same rate of recovery in fractionation experiments. Hornsey and Silini are joint authors with Fowler, Morgan, Bewley, Silvester and Turner[g]) of a paper presented in this session, where they irradiated the skin of pigs and used, as criterion of effect, the severity of the second phase of the erythema. They found a higher RBE for fast neutrons relative to X rays for fractionated doses than for single doses (3.3 against 2.5), indicating a difference in recovery capacity between the effects of high and low LET radiations perhaps associated with a difference in shape of response curve. The authors discuss various reasons for the difference between response of the ascites tumour cells and the skin of the pig. It is evident that there are a number of unanswered questions on the relationship between fractionation effect and shape of dose-response curves for radiations of different LET.

With regard to the modification of response by change in oxygenation of the system there is substantial agreement between all the workers in the present group who have studied the problem. The oxygen enhancement ratio, that is, the increase in effectiveness of a given dose of radiation from anoxic to oxygenated conditions, is found to be greater for low LET than for high LET radiation, and Fig. 6 summarises the data from Barber and Ebert; Berry and Andrews; Barendsen and Walter; and Whitmore and Schneider[j]). For some of these points I have had to make a rough estimate of the LET value.

Barendsen and Walter suggest that the effect of anoxia might be

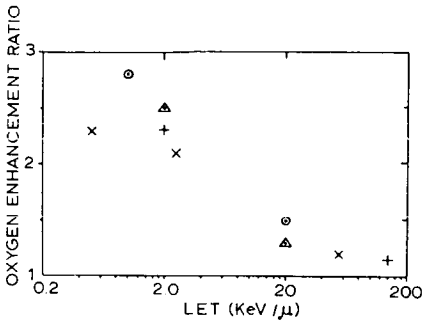

Fig. 6. Change of oxygen enhancement ratio.

◎ ---- Barber and Ebert
× ---- Berry and Andrews
+ ---- Barendsen and Walter
△ ---- Whitmore and Schneider

to render ineffective some 20 to 30% of the ionizations produced. This will reduce the effective LET of the radiation, and the biological significance of such a reduction would be much more serious for low than for high LET radiation, which could explain the observed difference in response to anoxia. A similar mechanism could hold for chemical protectors.

The results on dose fractionation and on the oxygen effect represent, in the main, two different aspects of the lower selectivity of high LET radiation, the dose fractionation effect concerned with repair and the oxygen effect mainly, at least, with the magnitude and perhaps nature of the initial damage. A more general demonstration of the principle is given by Barendsen and Walter, who find that the culture conditions, including time between plating and irradiation and the temperature, and also the action of cysteinamine, modify the shape of the X ray curve much more than that of the α ray curve.

If the LET data are to be used for the elucidation of radiation mechanisms and for estimates of "target" size, reproducibility of the dose-response curves has to be adequate. Whitmore and Schneider mention that, in cell culture studies, the addition of 10^4 heavily irradiated "feeder" cells per ml of medium greatly improves the reproducibility from experiment to experiment, particularly with regard to the final slope of the curve.

6. ELUCIDATION OF MECHANISMS

"Target" size calculations for mammalian cells present many complications. In this series of papers, such a calculation has been attempted by Fowler, Bewley, Barendsen and Walter but, as they

show, it needs a detailed knowledge of the change in shape of the dose-response curve over a wide range of LET values, and also involves assumptions concerning the definition of LET and, in particular, the extent to which delta rays shall be included in the estimate of the LET value.

Such complication is not required if one's interest is the comparison of the underlying mechanisms of different biological effects. Here a straight comparison of RBE values will give useful information; a difference in RBE will indicate a difference in mechanism, while the same RBE may be a strong indication of similar basic mechanism. This is the approach of Whitmore and Schneider in their paper.

These authors, however, make use of a new definition of RBE, which is the ratio of the slopes of the dose-response curves at a given level of response. From the point of view of interpretation of mechanisms they feel that this has advantages over the definition normally used. It implies that the RBE of two radiations will have a constant value in the region where the shapes of the dose-response curves are the same, which for mammalian cell survival corresponds to that of effective linearity at the higher dose levels.

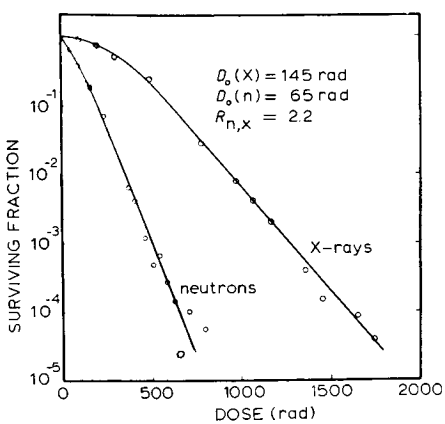

Fig. 7. Survival of hamster fibroblast cells (cell line H1). (Whitmore and Schneider.)

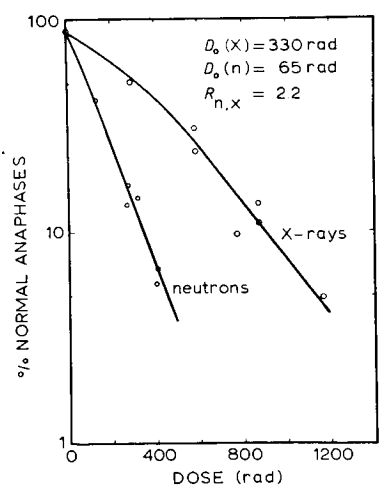

Fig. 8. Survival of hamster fibroblast cells (cell line H1). (Whitmore and Schneider.)

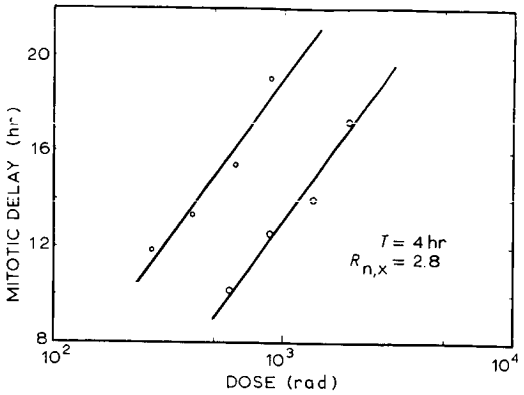

Fig. 9. Mitotic delay in hamster fibroblast cells (cell line H1). (Whitmore and Schneider.)

They have studied several species of mammalian cell and have found RBE values, under their definition, for cell survival, for the production of abnormal anaphase figures and for mitotic inhibition. Their results for H1 cells are shown in Figs. 7, 8 and 9. The authors conclude that the mechanism which produced the loss of proliferative capacity is chromosomal in nature. They suggest that the radiation damage which causes mitotic inhibition may not be the same as the damage which leads to the loss of the ability of a cell to proliferate indefinitely. This is also the conclusion from the work of Skarsgard discussed earlier, who showed that the curves of RBE against LET had a different shape for cell survival and for mitotic delay.

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DISCUSSION

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Powers (*Argonne Nat. Lab., Ill., USA*) pointed out that for dry bacterial spores the killing efficiency of radiation is the same with increasing LET up to 200 KeV/μ for the oxygen dependent part of the effect, but it increases for the oxygen independent part of the effect. It is this increase which gives rise to the decrease in the oxygen enhancement ratio. He also observed that up to 200 KeV/μ the free radical production is constant but then falls with increasing LET up to that produced by neon ions.

Since the rapporteur's remarks might be interpreted to indicate that the influence of modifying factors was always reduced with increase in LET, Tobias (*Donner Lab., Univ. Cal., USA*) quoted two exceptions. The induction of certain specific mutations remains oxygen dependent over the range of LET studied, although cell survival is only slightly oxygen dependent[1]). Also glycerol shows a dose modification factor of about 2 in haploid yeast cells, which is maintained over the whole range of LET studied[2]). It appeared as if certain types of damage associated with chromosome breaks were dependent on LET in contrast to certain mutations which were LET independent. Barendsen[d]) felt that the explanation for the different effectiveness of glycerol in either yeast cells or mammalian cells may be found in the different shape of the survival curves. The yeast cells quoted showed exponential survival curves whereas mammalian cells had an initial shoulder.

Tobias further pointed out that the delta rays associated with the densely ionizing tracks give a relatively low LET. These delta rays could induce mutations in the surviving cells and this would be oxygen dependent.

In connection with the problem of the initial slope of dose response curves, Alper[b]) stressed the importance of using homogeneous cell populations in which all cells had the same sensitivity. Heterogeneity could account for a variety of initial slopes. Elkind (*Nat. Cancer Inst., Bethesda, Md., USA*) indicated that the question of the initial slope was of importance in determining the extent of

recovery at very low dose rates, and asked Barendsen whether he had any experimental data concerning this point. Barendsen replied that he had given fractionated irradiation in 3 to 4 fractions at 3 to 4 hr intervals and had obtained a survival curve which was parallel to the single exposure curve up to a dose of about 300r. This suggested a linear component of the survival curve at low doses.

Wolff (*Oak Ridge Nat. Lab., Tenn., USA*) suggested that the increase in RBE at low doses occurred because the contribution of "two hit" events to the total damage produced by X rays is small for low doses. In addition there is recovery between hits at low dose rates leaving predominantly the "one hit" effect which is dose rate independent and which predominates in high LET radiations. This would also reduce the oxygen effect.

Neary[h]) said that at low doses it is probably not adequate to consider X or gamma radiation simply as low LET radiation. This was relevant to rapporteur Lamerton's comment on the RBE at low doses for anaerobic conditions where he suggested that under aerobic conditions the RBE would be less because of the oxygen modification factor. Neary said that he and Savage had looked at the dose modification factor of oxygen for chromosomal damage at low doses in Tradescantia microspores. They found that the oxygen factor was very much smaller than in the normal range of doses. This probably had to be attributed to delta rays or to the high LET portions of the spectrum with X or gamma radiation, as the fraction of the total dose associated with high LET was of the right order to account for the extrapolated RBE found in this system. It thus seemed that there was little qualitative difference at low doses or dose rates between the effects of low and fairly high LET radiations and the large values of extrapolated RBE observed were probably the ultimate maximum values.

Burch (*Univ. Leeds, Great Britain*) entered a plea that whenever average values for the LET of a particular radiation are quoted, the method of determining the average should be stated. The average could be calculated in several ways and under extreme conditions different methods could vary by a factor of 50. Quoting average LET values tended to obscure the fact that any particular radiation produced a range of LET's.

Szybalski (*McArdle Lab., Univ. Wis., USA*) asked why Alexander

and Mikulski[a]) had interpreted their data on the basis of two different mechanisms and why DNA was not considered to be the target. Could one not have two different mechanisms operating on the same molecule; for instance, with low LET the breaking of one strand of DNA and with high LET the breaking of both strands, the target in each case being the same?

Alexander gave two reasons for thinking that damage to DNA was not the main cause leading to cell death. Using a model system, DNA *in vitro*, he had found that X rays did rather more damage to DNA than did alpha rays. Miss Alper added that this was also true of bacteriophage which is predominantly DNA and in a more biological form than DNA in an *in vitro* chemical system.

The second reason was that, with the mutant strain of lymphoma cell which he had isolated, the sensitivity to X rays was $2\frac{1}{2}$ times greater in one strain than in the other, in spite of both cell types being diploid and having the same DNA content per cell; also DNA extracted from both types of cell after irradiation showed the same kind of damage. However, it is possible to explain this difference by means of a repair mechanism.

Miss Alper stressed that one should not regard chemical protection as similar to the oxygen effect since, with bacteria, the oxygen enhancement ratio decreased with increase in LET and in her experiments this did not happen with chemical protection.

Munro (*Strangeways Res. Lab., Cambridge, Great Britain*) asked Whitmore[j]) whether he had any explanation as to why Ehrlich ascites tumour cells (Hornsey and Silini)[4]) recover from fast neutron irradiation in contrast to his H1 Chinese hamster cells. Whitmore had no explanation to give. Barendsen suggested that the 2 hr interval used by Whitmore was too short to allow for recovery since the cells were irradiated at room temperature. Whitmore said that this was unlikely since these cells show recovery at room temperature after X irradiation within this time. Scott (RRU, *Mt. Vernon Hosp. Northwood, Middx., Great Britain*), elicited from Whitmore that the method of determining the mitotic delay was simply to extrapolate the percentage of cells in mitosis as a function of time to zero effect.

Boag (RRU, *Mt. Vernon Hosp., Northwood, Middx., Great Britain*) asked how the Bragg peak of the proton and alpha particle beams were used to irradiate mice. Berry[e]) replied that this was done

by inserting perspex absorbers into the beam so that the Bragg peak could be displaced and the mouse irradiated in four steps with the peak at different positions within the mouse.

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Physical Parameters in Radiobiology

DOSE RATE AND TIME FACTOR

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Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berks., Great Britain

a) IRENE U. BOONE,
The effect of dose rate on the incidence of leukaemia in several strains of mice.
Los Alamos Scientific Laboratory, Los Alamos, New Mexico, USA

b) V. DOREEN COURTENAY,
Studies on the protective effect of isologous and homologous marrow grafts in rats given lethal doses of radiation at various dose rates.
Institute of Cancer Research, Belmont, Sutton, Surrey, Great Britain

c) I. JOHANSEN,
The effect of fractionated irradiation under various oxygen concentrations on the radiosensitivity of the Drosophila embryo.
Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway

d) R. F. KIMBALL,
Fractionation of dose and radiation intensity in the induction of recessive lethal mutations by X-rays in *Paramecium aurelia.*
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

e) H. A. KÜNKEL and F. OBERHEUSER,
Ultrafractionation and biological efficiency of fast electrons, investigations on "eggs" of *Drosophila melanogaster* of different stages of development.
Universitäts-Frauenklinik, Hamburg-Eppendorf, Germany

f) P. J. LINDOP and J. ROTBLAT,
The effect of anaesthesia on radiation sensitivity to whole-body exposure of mice.
Medical School, St. Bartholomew's Hospital, London, EC 1, Great Britain

g) S. P. STEARNER and S. A. TYLER,
Comparative aspects of the acute radiation response.
Argonne National Laboratory, Argonne, Illinois, USA

h) Y. TAZIMA and S. KONDO,
Further studies on two types of dose-rate dependence of radiation-induced mutation rates in spermatogonia and oogonia of the silkworm.
National Institute of Genetics, Misima, Japan

i) S. A. TYLER and S. P. STEARNER,
A model of the kinetics of injury processes associated with acute lethality in the mouse following ^{60}Co gamma radiation.
Argonne National Laboratory, Argonne, Illinois, USA

1. INTRODUCTION

"Dose rate" is an ambiguous term. When it is used to mean the rate of absorption of energy per unit of time nothing is said about how the absorption is fractionated within that unit of time. It is as well to distinguish instantaneous dose rate, the rate of absorption during an uninterrupted exposure, and the average dose rate, the total absorption divided by the overall exposure time. A further ambiguity arises when particulate radiation is considered. An increase in the number of particles arising in a given mass of matter per unit of time leads to an increase in the overall rate of energy absorption but, unless the concentration is so high that particle tracks interact, does not lead to an increase in dose rate, as the term is usually understood. The instantaneous dose rate around a particle track is determined by that particle and is independent of how many other tracks there may be. Fortunately, perhaps, particulate radiation need not be mentioned again since all the papers allocated to this session deal with X or gamma radiation. With these radiations an increase in rate of energy absorption entails a greater density of ionizations and an increased probability of interaction of separate ionizations at all levels physicochemical and biological.

The simplest kind of experiment on dose rate is to compare the effects of giving the same total dose at two different instantaneous dose rates as illustrated by A and B in Fig. 1. This entails two different overall exposure times and, if there is a difference in effect, it is not possible to decide whether this is due to the difference in dose rate or to the difference in time of exposure without further experiments using fractionated exposures. In fact dose rate cannot be investigated using single uninterrupted exposures only; multiple exposures are essential. Fig. 1 (F_1–F_4) illustrates some possibilities for distinguishing the relative importance of instantaneous dose rate and of the two orders of time, the relatively long overall duration of exposure and the relatively short combined duration of a single fraction and its subsequent radiation-free interval.

It is always possible, however short the duration of an exposure to radiation, to divide the exposure up as in Fig. 1, conceptually at least. Thus instantaneous dose rate has a pragmatic meaning only and implies that the time of delivery of the dose is short in

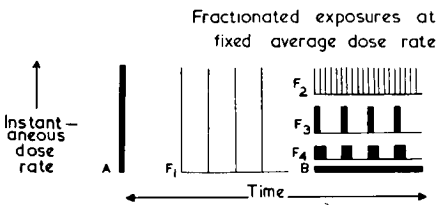

Fig. 1. Methods of fractionation as a means for distinguishing the effects of instantaneous dose rate, average dose rate and time. The dose (instantaneous dose rate multiplied by total duration of "radiation-on" time) is the same for each method of exposure. The overall exposure time for B, F_1, F_2, ... is the same and therefore the average dose rate is too. The plus sign indicates the factor of over-riding importance in determining the experimental result.

Experimental Results		Instantaneous dose rate	Time
$A = B$	$(= F_1 = F_2 = \ldots\ldots)$	—	—
$A \neq B$	$A = F_1 \neq F_2 \ldots\ldots$	+	—
	$B = F_1 = F_2$	—	+ overall
	$B = F_2 \neq F_1$ or F_3	—	+ between fractions

comparison with the characteristic time constant of the process of interest. It is convenient to distinguish several different scales of time.

Category	Characteristic time scale	Process of interest
Physical time	fractions of seconds	diffusion of ions and radicals and of oxygen
Cellular time	minutes to hours	repair of injury by active metabolic processes
Somatic time	days to years	cellular division, movement and organization
Evolutionary time	generations	dispersal and combination of genes in a population

Within each category of time it is possible to use the fractionation patterns of Fig. 1 to distinguish the relative importance of time and of dose rate.

In addition a secular change in radiosensitivity during an ex-

posure is a possibility which the biologist at any rate should not forget, especially when working in cellular or somatic time.

The major problem in surveying the proferred papers has not been their mere bulk but the fact that they deal with a number of quite different biological systems. The details of these are highly relevant to the planning and interpretation of the experiments which concern them, and the serious scientist will expect to be told about them as well as about the dosimetry and the experimental arrangement before being invited to consider results. However, in order to make this survey intelligible to the non-specialist, all technical detail has been omitted except when unavoidable and also all observations which seemed to me inconclusive.

2. PHYSICAL TIME AND MODIFICATION OF BIOLOGICAL EFFECT

Drosophila "eggs" have been a favourite test-object for many years. The "eggs" are really developing embryos and the measured end-point is the proportion which fail to hatch into adult larvae. Unfortunately they are not very stable, speaking radiobiologically, since synchronous nuclear division is occurring every 10 min or so. Künkel and Oberheuser[e]) compared the effect of 2, 25 and 50 pulses per sec of 15 MeV electrons, each pulse lasting one μsec, generated in a betatron with special equipment for regulating the emission of pulses. As can be seen (Fig. 2) the proportion of "eggs" killed by a given dose was slightly but consistently modified according to the number of pulses per sec whether the overall expo-

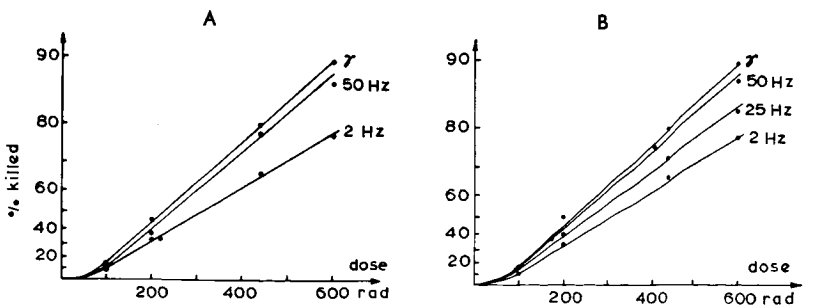

Fig. 2. Dose-response curves for killing of 1.75 hr Drosophila "eggs" by pulsed 15 MeV electrons. 2 Hz, 25 Hz, 50 Hz are 2, 25, and 50 pulses per sec respectively. A: Constant average dose rate (17 rad/min) so that pulse dose varied inversely with pulse frequency. B: Constant pulse dose (140 mrad) so that overall exposure time varied inversely with the pulse frequency. (Künkel and Oberheuser.)

sure time and the average dose rate were kept constant or the instantaneous dose rate was kept constant at $\sim 10^5$ r per sec and the exposure time varied 25 fold. The overall exposure time in the experiments illustrated ranged from $\frac{1}{4}$ to 35 min. Continuous gamma radiation was also used but the minor difference from 50 pulses per sec of 15 MeV electrons cannot be interpreted without a knowledge of the relative biological efficiency of the two kinds of radiation. The shortest radiation-free time between pulses was 20 msec in Künkel and Oberheuser's work but, when this was reduced to 40 μsec, Johansen[c]) found that a pulsed exposure of X rays was indistinguishable from a continuous one.

Drosophila "eggs" give a more or less "exponential" survival curve against dose, which may vary with the experimental conditions. Künkel and Oberheuser deprecate the interpretation of such curves in terms of target or hit theory but Johansen felt that his experiments on the effect of dose rate gave an interesting empirical result when analysed according to the formal "hit" formulae. There was little change in the 50 per cent killing dose with change in average dose rate for a continuous exposure but a striking inverse correlation between dose rate and extrapolation number (Table 1).

TABLE 1

Dose rate of continous exposure and killing of Drosophila "eggs" aged 60 $\pm$ 5 min (Johansen)

X ray dose rate (r/min)	50% killing dose		extrapolation number (best fit)
	mean	SE	
25	427	12	14
50	385	18	12
75	370	6	9
100	385	3	7
200	370	6	4
470	348	14	3

Of course this must entail a corresponding correlation with D 37, the slope of the straight part of the exponential survival curve, which could not be determined directly since the dose range did not extend much beyond the 10 per cent killing dose.

For the dose rates of 50 r/min and higher there was a linear relation between duration of the 50% killing dose and (extrapolation number —2).

Pulsed radiation was also used by Lindop and Rotblat[f]), 400 pulses of electrons per sec, each 1.3 μsec long. The dose per pulse and therefore the instantaneous dose rate could be varied within wide limits. They found a less than 10 per cent change in the 50 per cent killing dose for unanaesthetized mice when the average dose rate ranged from 1.3 to 2600 rad/sec as shown in Fig. 3. This end-point,

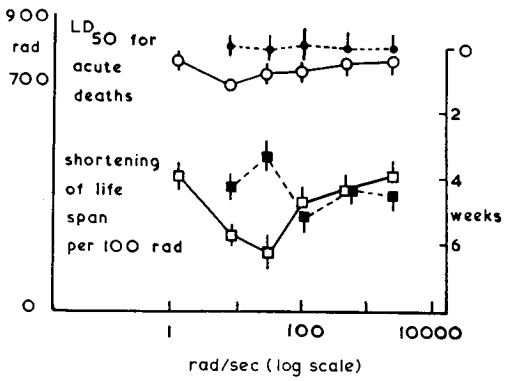

Fig. 3. Effect of dose rate on the acute and delayed killing of mice by whole body irradiation. (Lindop and Rotblat.)
Anaesthetized ● ■
Conscious ○ □

the killing of the whole animal, is probably the direct consequence of the killing of particular kinds of cell, the stem cells of haematopoietic tissue, and the basic phenomenon examined is therefore quite comparable with the killing of Drosophila "eggs". The broad, and important, conclusion therefore seems to be that for dose rates of 1 rad/sec and higher there is quantitatively little effect of instantaneous or average dose rate on cell killing.

Lindop and Rotblat also determined the ultimate shortening of life in the survivors of the acute experiments, a phenomenon which has no generally accepted cellular basis but is certainly not due merely to cell death. They feel that the results in Fig. 3 suggest a systematic change of effect with average dose rate. One of the difficulties of investigating mechanisms in complex organisms like mammals is that technical procedures, like anaesthesia, which are employed merely to facilitate investigation, may by themselves modify the radiation effect appreciably as demonstrated in Fig. 3.

3. DECAY OF CELLULAR RADIATION DAMAGE WITH TIME

Recovery from radiation or repair of radiation damage is a well-attested phenomenon and can occur in a variety of ways. In the

scale of somatic time, cells, lost from a multicellular organism because they have been killed by radiation, may be replaced if surviving cells divide. In cellular time at least three different processes seem to occur. In a single cell breaks in chromosomes may heal by rejoining. Second, Elkind and Sutton[1]) have produced convincing evidence that another kind of intracellular repair can occur associated with an increase in extrapolation number from one to the value characteristic of the unirradiated cell. A third kind of process is described by Kimball in this series of papers.

The commonest way of following the time course of recovery, whatever its mechanism, is to use a second dose of radiation at progressively increasing time intervals after the first dose. The decay of radiation damage thus observed may be monotonic or may be phasic. Healing of chromosome breaks is thought to occur monotonically (exponentially) with time. The Elkind type of recovery is demonstrably phasic in tissue culture. Recovery of the whole organism due to synchronized cell division may appear to be phasic with time when tested by a second dose of radiation because radiosensitivity may vary during the cell cycle from division to division.

Stearner and Tyler's[g]) experiments on recovery concern three species of bird and one species of mammal, which were all found to behave similarly. The distribution of deaths with time after exposure was very similar in mouse, parakeet and pigeon (Stearner and Tyler[2]). The chick a few days old may die at earlier times for other reasons but only deaths between 7 to 30 days were considered in the present analysis. When the overall duration of a continuous uninterrupted exposure to γ radiation was increased from 10 minutes to six hours there was an increase in the LD 50, the 50 per cent killing dose, in all four species. As the overall exposure time was still further increased from 6 to 24–25 hours there was little further increase in LD 50. There is a 2 to 3 fold range in absolute values for the LD 50's of the different species and when these are expressed as multiples of the LD 50 for an instantaneous exposure (derived by extrapolation), it becomes clear (Fig. 4) that the three species of bird all form one group with a faster proportional rate of change of LD 50 with overall exposure time than the mouse. In both groups however the LD 50 appeared to increase linearly with exposure time up to the limit of six hours or so and to a level some 30 to 50 per cent above the instantaneous value. There is supporting

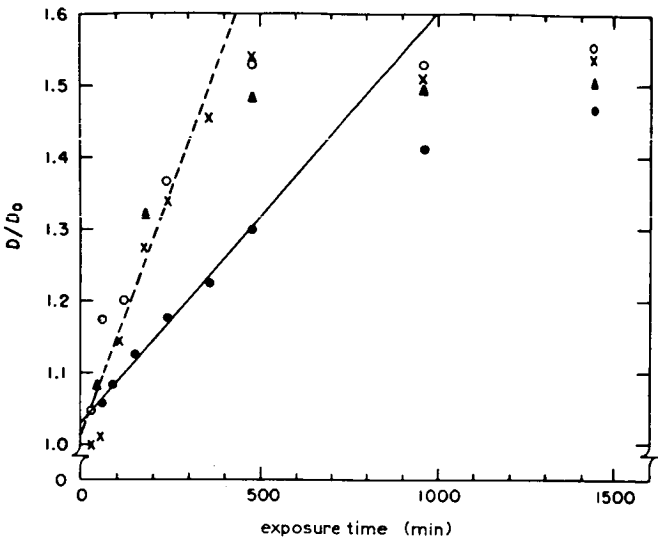

Fig. 4. Total (unpartitioned) mortality. LD 50/30 for protracted γ irradiation of pigeon, parakeet, chick and mouse expressed as a multiple of the derived LD 50/30 for an instantaneous exposure. (Stearner and Tyler.)

	Calculated LD 50/30 for instantaneous exposure	
	mean	SE
Pigeon ▲	2050	550
Parakeet O	1500	450
Chick ×	1110	340
Mouse ●	985	84

evidence from published work on the mouse from six different laboratories that this rapid but time-limited increase in LD 50 is a general phenomenon.

Tyler and Stearner[1]) propose two main kinds of explanation:

(i) that there are two types of injury process, the first producing reversible injury (upon which the instantaneous LD 50 depends) and the second irreversible injury (which determines the LD 50 for prolonged exposures of 12 to 24 hours). This is the model the authors themselves chose to use for their analysis; Stearner and Tyler[2]).

(ii) that there is some form of recovery process which is limited in time. Such a time-limited decay of radiation damage has indeed been observed in the mouse (Brown, Corp and Mole)[3]). The parti-

cular kind of cellular recovery demonstrated by Elkind and Sutton[1]) is also completed in a similar period of time and there are now several examples in widely differing biological objects to suggest that what may be called an Elkind type of recovery is a basic radiobiological phenomenon.

When a radiation exposure of a warmblooded animal is spread over several days it is to be expected that cellular division will occur during the exposure and so add a further order of complexity to interpretation. Courtenay[b]) compared exposures of about 30 min, about 20 hr and about 6 days at radiation levels all of which were 100 per cent lethal. By grafting bone marrow into the irradiated rats varying degrees of survival were obtained. Fig. 5 shows

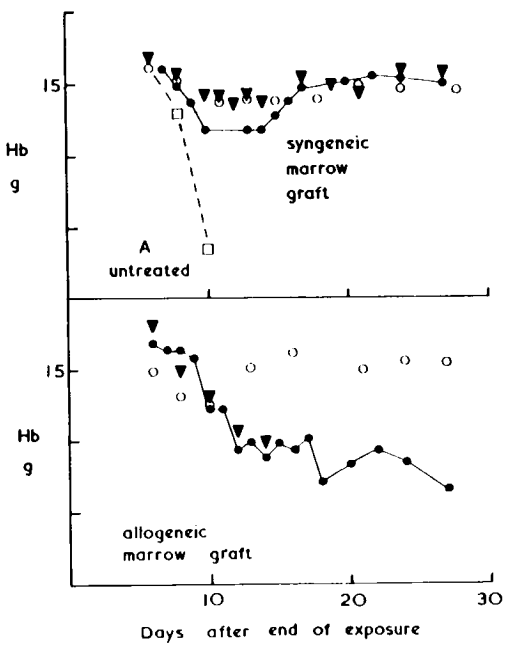

Fig. 5. Haemoglobin levels and survival after supralethal radiation exposures at different dose rates of August rats given marrow grafts after exposure. Dose of whole body irradiation ~ 1.2 × LD 100. (Courtenay.)

A 800 r	29 r/min	○
B 1600 r	1.4 r/min	▼
C 2460 r	0.3 r/min	●

	30 day deaths	mean survival
A	nil	+ +
B	nil	+ +
C	nil	91 days
A	nil	43 days
B	9/10	
C	15/16	

the fall in haemoglobin concentration in untreated rats given a 20 min exposure and the absence of the fall after grafting with allogeneic marrow, that is bone marrow from a genetically different stock of rats. The success of allogeneic grafts was due to the effect of the radiation in depressing the natural ability to recognize genetically foreign cells and so get rid of them.

After larger doses of lower dose rate radiation this ability seems to be less impaired and allogeneic marrow was less successful than syngeneic as illustrated in Fig. 5. Such a result is not easy to interpret without much additional quantitative information on the relation between success-rate and numbers of cells grafted, but extended experiments of this sort are necessary if bone marrow is to be grafted between non-identical human subjects.

4. MUTATION AND THE SURVIVAL OF VIABLE BUT PERMANENTLY ALTERED CELLS

Practically all the effects discussed so far can plausibly be attributed to the direct cell-killing effects of radiation but consequences such as leukaemia or other kinds of cancer clearly depend on the production, directly or indirectly, of cells with a permanently altered genetic make-up. Boone[a]) has scored leukaemia incidence and shortening of lifespan in several strains of mice given various fractionated or continuous exposures lasting overall from 2 min to 6 weeks. Her work illustrates some of the practical problems of designing and carrying out such experiments. Scoring of cancer incidence, for instance, which depends on the autopsy, therefore depends on an experimental design which ensures that practically every mouse is properly autopsied, and not too decomposed or eaten for this to

TABLE 2
Life shortening of female RF mice aged 8 to 16 weeks at start of irradiation (Boone)

Kind of exposure	Duration of exposure	Dose rate (rad/min)	Total dose (rads)	Mean life span (days)		Life-shortening per cent per 100 rad
				Exposed	Control	
Daily X rays	Six weeks	50	1500	374	567	2.3
Continuous γ	21 days	0.05	1500	395	608	2.3
	14 days	0.05	1000	443		2.7
	7 days	0.05	500	513		3.2
Fractionated γ	3 exposures at monthly intervals	13	800	466	679	3.9

be possible. Lifespan shortening is often expressed as per cent of the control lifespan but this, even if it should be constant, can unfortunately vary from experiment to experiment. Lifespan shortening was not linearly dependent on dose for protracted exposures (Table 2) so that if comparisons are to be made between differently fractionated and protracted exposures several different total doses are needed for each different kind of exposure.

Macroscopic phenomena like cancer and lifespan may perhaps not be linearly dependent on dose even if based on molecular phenomena, like mutation, which are thought to be linearly dependent. Nevertheless recent work has shown that gene mutation by radiation is not the time-independent phenomenon which it was once believed to be.

Kimball[d]), working with *Paramœcium aurelia*, has found two situations in which the frequency of mutations can be changed by altering the time of exposure (Table 3).

TABLE 3
Mutation in *Paramœcium aurelia* (R.F. Kimball)

	Dose rate (Kr/min)	Mutation index M per 10^4r phase of growth: log phase (usually G 1)	Mutation index M per 10^4r phase of growth: stationary phase
Single exposure			
	0.03	2.7	1.4
		sig	not sig
		3.5	1.5
	1.0–1.7	3.6	
		3.7	2.1
			sig
Fractionated exposures			
Two fractions within one generation	1.4	3.6	
Four fractions within two days	1.6		1.7
Two fractions four generations apart	1.6	3.5	

Sig = statistically significant difference in M.

In the stationary growth phase a change in the duration of a continuous exposure had no effect on mutation frequency whereas dividing a high dose-rate exposure into several fractions given over

two days led to a significant reduction in mutation frequency. This last is the classical result to be expected from the action of some time-dependent repair process, such as rejoining of chromosome breaks, and clearly in the metabolically inactive stationary phase this rejoining is a slow process. In the metabolically active log phase of growth, however, the difference in M with fractionation of the high dose rate exposure was small, showing that this form of repair by rejoining is either quantitatively small or fast or both. In either case the fact that in log phase a significant change in M resulted from a change in dose rate of a continuous exposure indicates that a second kind of time-dependent recovery process occurs. This Kimball interprets, in agreement with earlier work (Kimball, Gaither and Perdue[4]), as a relatively slow repair of one-hit events. A one-hit genetic event used to be considered synonymous with irreversible point mutation but these observations on Paramoecium show that a form of reversible premutational damage can occur.

Kimball's work with Paramoecium deals with the integrated effects of all genes behaving as recessive lethals. Tazima, Kondo and Sado[5]) examined the effects of dose rate on mutation rate in a simpler manner by making use of specific loci in the silkworm which determine egg colour. During the first 10 days after hatching, the silkworm larva undergoes two successive moults about 5 days apart. Doses of 1000 r at about 0.1 r min take about 5 days to give but whatever the particular 5 days over which such a continuous dose was given to silkworm larvae, the mutation rate for a particular specific locus in a particular sex was constant within the limit of experimental error. However, when the dose was given in 3 min at 320 r/min the mutation rate varied cyclically according to the particular day of the 10 day larval development period on which the dose was given. In the first half of the period the mutation rate was higher and in the second half lower than for the low dose rate exposures. A variety of interpretations are possible and in their latest experiments Tazima and Kondo[h]) have not only confirmed these differences between acute and protracted irradiation but have shown that there is probably a linear relation between dose and mutation frequency at 320 r/min in larvae six days old. This result makes untenable the assumption that highly mutable cells are more easily killed by radiation and so disposes of one possible explanation for the remarkable finding that fewer mutations were produced

by high dose rate exposure than low dose rate exposure. Another new experiment shows that the change in mutagenic efficiency occurs somewhere between 1 and 18 r/min.

5. CONCLUSION

Not all the papers surveyed make the sort of comparisons which allow a clear distinction between the effects of dose rate and of time. However it seems that changes in instantaneous dose rate from 1 rad per sec up to 10^5 rad per sec produce surprisingly little change in effect, surprisingly, that is, if such instantaneous dose rates are large enough to modify radical production and reactions in an aqueous medium. On the other hand the demonstration of even a relatively small change in mutation rate with variation in exposure time (Kimball, Tazima and Kondo) confirms current ideas that genetic mutation is not the instantaneous, permanent and invariant response which classical theory postulated.

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DISCUSSION

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Several questions were directed to Johansen[c]) about the results from his experiments on Drosophila embryos which were presented in Table 1. Robev (*Dept. of Radiobiol. Sofia 56, Bulgaria*), asked if chemical protective agents might have modified the shape of the survival curve from which the data in Table 1 were taken. In reply Johansen said that the irradiations were carried out in air or nitrogen and that effects of chemical protective agents had not been investigated. Wolff (*Oak Ridge Nat. Lab., Tenn., USA*) drew Johan-

sen's attention to errors which might be involved in obtaining extrapolation numbers from survival curves that were only carried down to the 5% survival level. Biophysicists have often pointed out that in order to make such extrapolations, the survival curve should be taken down to at least 10^{-3} to 10^{-4} because only at these low levels of survival does the curve become truly exponential. Johansen said that he was aware of this problem and he had overcome it by making a study of theoretical multi-hit curves which he compared with his experimental survival curves. The values for the extrapolation number given in Table 1 were obtained from the theoretical curves which best fitted the experimentally obtained survival curves. Charlesby (*RMCS, Shrivenham, Great Britain*) enquired which theoretical formula was used in calculating these theoretical curves and Johansen replied that they were obtained from the standard multi-hit formula. More comment on Johansen's work came from Kaplan (*Stanford Univ., Palo Alto, Cal., USA*) who commented that the LD 50's were the same for several different dose rates but the extrapolation numbers were not the same, which suggests that the LD 50's were estimated from the non-linear portion of the curve, and this might be an ambiguous and confusing way to interpret the data. He suggested that it might have been better to interpret the data from the D 37 dose taken from the truly linear part of the survival curve rather than from the LD 50 which is derived from the non-linear portion. A general warning was given by Elkind (*Nat. Cancer Inst., Bethesda, Md., USA*) who pointed out that one must be careful in associating survival responses with changes in particular parameters which in turn require the assumption of a particular formal model the applicability of which is generally not proven. Johansen stated that he thought his experimental data were correct and that he was not drawing conclusions which went beyond the data.

Burch (*Dept. Med. Physics, Univ. Leeds, Great Britain*) said he understood the general idea behind the selective killing hypothesis, referred to in the interpretation of the results of Tazima and Kondo[h]), was that there were cells which were highly mutable and at the same time very prone to the lethal effects of radiation. Burch suggested that at high doses and high dose-rates a potentially *mutant* lesion might get converted by cooperative phenomena to lethal damage and so reduce the mutation frequency. Furthermore he thought

it might not be necessary to postulate that cells at a given stage differ in their mutability or sensitivity to lethal effects. In reply Tazima explained that his data showed two types of dose rate dependent phenomenon. In the first dose rate phenomenon, chronic irradiation was found to be less effective than acute irradiation and the other type of phenomenon showed the reverse effect. The first type may be explained by assuming a repair mechanism, but the other type cannot be explained in this way. However, it may be accounted for by invoking the selective cell killing hypothesis, but he and his colleagues were not certain of this interpretation. Purdom (*Radiobiol. Res. Unit, Harwell, Didcot, Berks., Great Britain*) wondered whether it was possible to explain Tazima and Kondo's data on the grounds of differential sensitivity of germ cells rather than on intensity effects. It is known that rapid changes of sensitivity could occur during the period of irradiation. Therefore, it might not be logical to compare, on an intensity effect basis, a radiation exposure of 3 min at a particular time with a continuous exposure of several days covering many stages of development which may have varying radiation sensitivity. In reply Tazima said it was unfortunately not possible to carry out a chronic irradiation which could be compared with an acute irradiation in the early stages of egg development. In later stages, a chronic irradiation could be compared with an acute one given in the middle of the chronic time period and the effects of changing sensitivity might be averaged out.

Barendsen (*Radiobiol. Inst. TNO, Rijswijk, The Netherlands*) asked Rotblat[f]) if he thought that a dose of about 1000 rad was sufficient to deplete the oxygen content of the body fluids in a normal animal to such an extent that it modified the radiation response. It was generally assumed that about 20 000 rad was required to use up the oxygen in a solution. Rotblat replied that it would depend on the amount of oxygen present in the cells and body fluids at the beginning of the radiation. This point was again brought out in a question by Mewissen (*Dept. Radiophysics and Radiobiol. Inst., Bordet, Brussels, Belgium*) who asked Rotblat whether he had any explanation for the phenomenon, which he and Lindop had reported, that anaesthesia decreases the lifespan shortening of mice following irradiation. In reply Rotblat said that there were two protective effects observed in their animal experiments. If a dose

is given to unanaesthetized animals in a shorter and shorter time, as the dose rate was increased, then the latter part of the dose may be delivered to a somewhat anoxic animal. This is becausc oxygen might not be able to diffuse fast enough into the cells to replace that used up by the radiation induced chemical processes. Therefore, some protective effect might come from this slight anoxia which could explain the observed changes in LD 50 and life shortening. In the case of the anaesthetized animals the anaesthetic itself may act as a chemical protective agent. However, this could not explain the observed dose rate effect with anaesthetized animals, and Rotblat could not suggest any mechanism to explain this effect.

Lamerton (*Inst. Cancer Res., Belmont, Surrey, Great Britain*) asked about the difference in dose rate response between birds and mammals which he found extremely interesting, and wondered if the authors had any information on the possible physiological basis for this. In reply Stearner[g,i]) said that she did not have any explanation for this difference and the only difference which might have any significance was in the metabolic rates of these animals.

Radiation Chemistry of Water

PRIMARY SPECIES IN WATER

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School of Chemistry, University of Leeds, Leeds, Great Britain

a) H. AEBI and J. P. HEINIGER,
Effect of gas phase (O_2, N_2, N_2O) on radiation induced formate oxidation.
Medizinisch-Chemisches Institut, University of Berne, Switzerland

b) A. APPLEBY, J. HOLIAN, G. SCHOLES, M. SIMIC and J. J. WEISS,
Radiation induced carboxylation and carbonylation.
Department of Chemistry, King's College, Newcastle-upon-Tyne, Great Britain

c) J. H. BAXENDALE,
Transient species in the flash photolysis of water and aqueous solutions.
Argonne National Laboratory, Argonne, Illinois, USA and *Manchester University, Great Britain*

d) J. W. BOAG, R. E. STEELE and E. J. HART,
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Research Unit in Radiobiology, Mount Vernon Hospital, Northwood, Middx., Great Britain and *Argonne National Laboratory, Argonne, Illinois, USA*

e) F. S. DAINTON and F. T. JONES,
The irradiation of aqueous solutions of mineral acids containing N_2O and/or Fe^{2+} ions between 77 and 350 °K.
School of Chemistry, University of Leeds, Great Britain

f) L. M. DORFMAN, R. E. BUEHLER and I. A. TAUB,
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Argonne National Laboratory, Argonne, Illinois, USA

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Physiologisch-Chemisches Institut der Universität, Göttingen, Germany

h) N. GETOFF,
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Institut für Radiumforschung und Kernphysik der Österreichischen Akademie der Wissenschaften, Vienna, Austria and *Department of Chemistry, King's College, Newcastle-upon-Tyne, Great Britain*

i) J. JORTNER, M. OTTOLENGHI, J. RABANI and G. STEIN,
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Department of Physical Chemistry, Hebrew University, Jerusalem, Israel

j) J. P. KEENE,
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Research Physics Department, Christie Hospital and Holt Radium Institute, Manchester 20, Great Britain

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Physics Department, Guy's Hospital Medical School, London, Great Britain
l) J. Kroh and Z. Czerwik,
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Politechnika, Lódz, Poland
m) J. Marquissée and E. W. Abrahamson,
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Department of Chemistry, Case Institute of Technology, Cleveland, Ohio, USA
n) Y. Raef and A. J. Swallow,
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Nuclear Technical Laboratories, Chemical Engineering Department, Imperial College, London SW 7, Great Britain
o) J. K. Thomas and E. J. Hart,
Radiolysis of aqueous solutions at high radiation intensities.
Argonne National Laboratory, Argonne, Illinois, USA

1. INTRODUCTION

About six years ago the author overheard a fellow participant at a conference remark that the radiation chemistry of water was finished. At that date it was generally accepted that the state of affairs in the track of a fast charged particle about 10^{-8} sec after its passage could be represented by equations (1) to (4).

Low LET

$$G_{-H_2O}H_2O \xrightarrow{10^{-8}\text{ sec}} G_H H + G_{OH}OH + G_{H_2}H_2 + G_{H_2O_2}H_2O_2 \quad (1)$$

$$G_{-H_2O} = G_H + 2\,G_{H_2} = G_{OH} + 2\,G_{H_2O_2} \quad (2)$$

High LET ($> \sim 1$ eV/Å)

$$G_{-H_2O}H_2O \rightsquigarrow G_H H + G_{HO_2}HO_2 + G_{OH}OH + G_{H_2}H_2 + G_{H_2O_2}H_2O_2 \quad (3)$$

$$G_{-H_2O} = G_H - G_{HO_2} + 2\,G_{H_2} = G_{OH} + 2\,G_{HO_2} + 2\,G_{H_2O_2} \quad (4)$$

In these equations G_H, G_{OH} and G_{HO_2} are the numbers of hydrogen atoms, hydroxyl radicals and perhydroxyl radicals per 100 eV absorbed energy, which are available to react with solutes present in concentrations insufficient to interfere with the *combination reactions* ($2H \rightarrow H_2$ and $2OH \rightarrow H_2O_2$), the *reconstitution reaction* ($H + OH \rightarrow H_2O$) and the *intra-track reactions* ($HO + H_2O_2 \rightarrow H_2O + HO_2$) which, in the period 10^{-13} to 10^{-8} sec, are in competition with the

diffusive escape of H and OH from spherical spurs (low LET systems) or from cylindrical tracks (high LET systems). The comment implied that the primary species were definitely a hydrogen atom and a hydroxyl radical, that the diffusion model was essentially correct and would only be modified in detail as a result of further work which should be directed along the lines of (i) measurement of the radical and molecular yields and exploration of the dependence of these on such experimental variables as temperature, LET (linear energy transfer), *p*H etc. and (ii) determination of the absolute rate constants of all the elementary reactions of H, OH and HO_2.

In the intervening years experimental studies have proceeded along these lines and have demonstrated that the remark referred to earlier (which epitomised a widely held opinion) could hardly have been further removed from the truth. We now know that some, if not all, of the supposed hydrogen atoms are solvated electrons or polarons and may either react with solutes in this form *or* be converted to hydrogen atoms by reaction with a proton-donor, XH^+, according to equation (5)

$$XH^+ + e^-_{aq} \rightarrow X + H \tag{5}$$

and then react with solutes. Many of the papers presented in this session reflect this change of emphasis or are concerned with the application of recently developed techniques to investigate the properties of e^-_{aq}, H and OH and, to make these points more evident, I shall present the papers, *not* in the order given in the abstract book, but in groups, the nature of each group being indicated by the sub-headings.

2. PULSE TECHNIQUES

Controlled delivery of a large pulse of energy of short duration compared with the kinetic lifetime of the intermediates offers the possibility of (i) measurement of their spectra and (ii) spectrophotometric observation of the decay in their concentration. Most investigations made hitherto have involved pulsed electron beams of between 1.5 and 15 MeV energy. Marquisée and Abrahamson[m]) are developing an X ray source giving ~ 500 joule pulses of 50 to 100 keV X rays of ~ 10 μsec duration, which promises to have considerable utility. The apparatus (shown in Fig. 1) comprises 6 razor-edged tungsten ribbons acting as field emission cathodes align-

ed parallel to a thin, cylindrical, beryllium anode target (OD 1″) within which the material to be irradiated can be placed. A similar shaped X ray source operating at 1.2 MeV is currently being designed by W. P. Dyke.

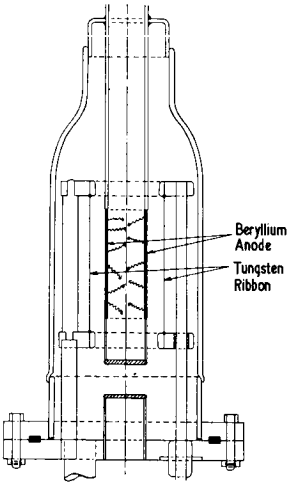

Fig. 1. Pulsed X ray source for irradiating cylindrical samples. (Marquisée and Abrahamson.)

Three papers are concerned with data obtained by single electron pulse radiolysis. The first by Boag, Steele and Hart[d]) describes the use of a linear accelerator giving 2 μsec pulses of 1.8 MeV electrons of 0.5 A maximum beam current, which has already been described[1]). Very narrow, ribbon-like, conical beams can be directed on to an open-ended or thin-windowed quartz cell containing the solution to be irradiated. Absorption bands attributed to I_3^- and I_2^- were detected in irradiated aqueous solutions of potassium iodide and iodine. Irradiated oxygenated water gave an unidentified absorption band with a maximum at $\sim$ 2400 Å (see Fig. 2) which persists for less than 10 μsec and is therefore thought not to be due to the perhydroxyl radical. In the Fricke solution there is a very rapid development of the 2400 Å absorption and an approximately 1000-fold slower production of the ferric absorption at 3050 Å. In ceric sulphate solutions, absorption due to ceric species also disappears slowly. Just before the Congress Boag informed me of his recent, beautiful experiments which indicate that a short-lived ($t_{\frac{1}{2}} = \sim 10$ μsec) species is formed on irradiation of deaerated aqueous systems. This species has broad absorption ($\lambda_{max} \simeq 7000$ Å)

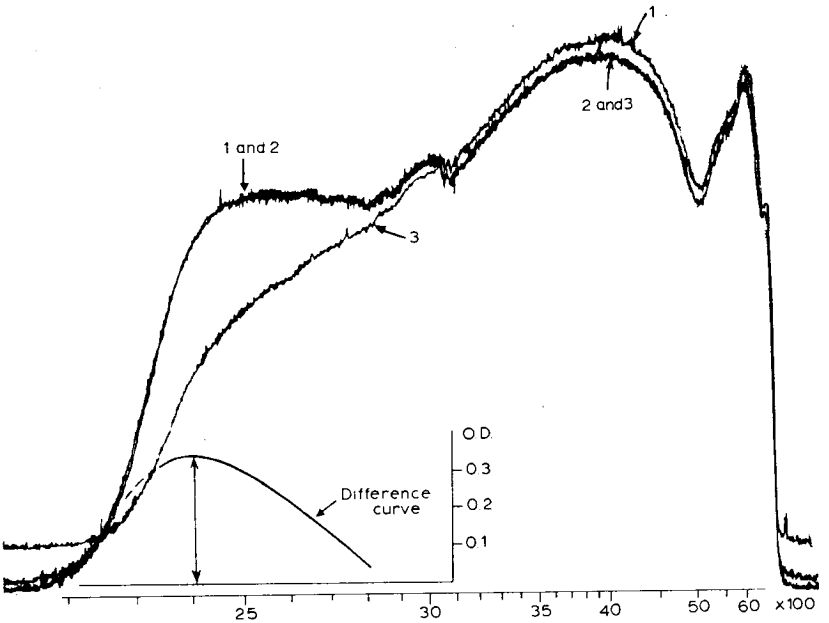

Fig. 2. Transient (< 10 μsec) absorption in irradiated aerated water. Abscissa: Wavelength in 100 Å. Ordinate for difference curve: Optical density. Curve 1) before irradiation. Curve 2) 30 sec after irradiation. Curve 3) simultaneous with irradiation. (Boag *et al.*)

in the region expected for the aquated electron. The yield is larger in Na_2CO_3 solution, and in 12 *M* NH_3 aq. the band is displaced to longer wavelengths. Keene[j]) has used broader beams of 4 MeV electrons of similar pulse length to that of Boag *et al.* but smaller doses (~ 10^4 rad) per pulse and care was taken to minimise effects due to shot noise and Cherenkov radiation. His results for potassium iodide solutions and water are similar to those of Boag *et al.*, but he also observed an absorption in the near UV during the pulse which is larger in deaerated than in oxygenated water. He analysed the absorption at 3040 Å caused by irradiation of the Fricke solution into four components having mean lives of < 1 μsec, 200 μsec, 20 msec and 2 sec, the last being due to the Fe^{2+}-H_2O_2 reaction. The μsec component is tentatively assigned to the Fe^{2+}-OH reaction, the 200 μsec to the Fe^{2+}-HO_2 reaction and the 20 msec to ligand substitution.

The paper by Dorfman, Buehler and Taub[f]) provides a good ex-

ample of the power of the pulse radiolysis method when allied to kinetic spectroscopy in a suitable system. Using pulses of 15 MeV electrons of up to 5 μsec duration to irradiate aqueous solutions of benzene derivatives in high purity quartz cells and a four-fold pass, analysing beam from a xenon spectroflash lamp or monochromatic radiation from a mercury lamp, the authors were able to identify the absorption spectra of many transient intermediates including C_6H_6OH, $CH_3C_6H_5OH$, $HOC_6H_6O_2$ and $C_6H_5CH_2$.

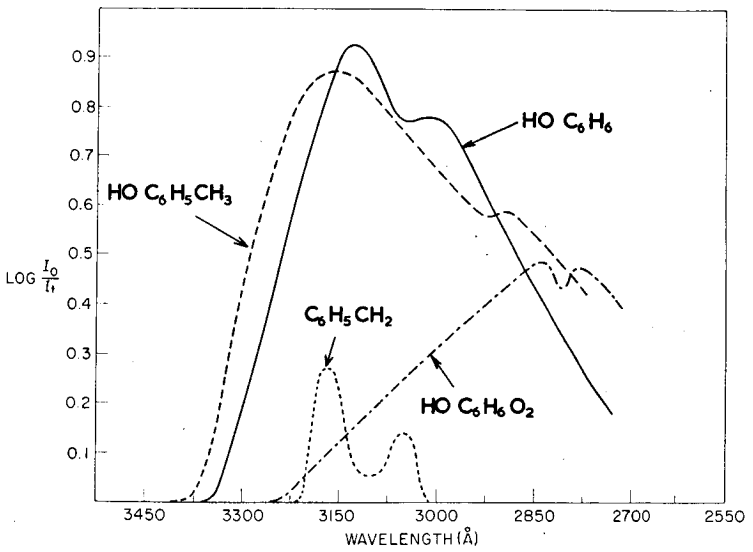

Fig. 3. Absorption spectra of aromatic transients. (Dorfman *et al.*)

These are shown in Fig. 3. The 1st or 2nd order decay curves for bimolecular destruction of the radical by reaction with oxygen or itself are of very high quality (see e.g. Fig. 4) and permitted the determination of (i) the following rate constants:
$k(OH + C_6H_6 \rightarrow HOC_6H_6) = 4.9 \times 10^9$; $k(OH + C_6D_6 \rightarrow HOC_6D_6) = 4.7 \times 10^9$, and $k(HOC_6H_6 + O_2 \rightarrow HOC_6H_6O_2) = 5 \times 10^8$ (l mole^{-1} sec^{-1}) and (ii) the molar decadic extinction coefficient of the hydroxybenzene radical at 3130 Å as 3500 $\pm$ 800 M^{-1} cm^{-1}.

Irradiation of liquid water with light of wavelength 1850 Å

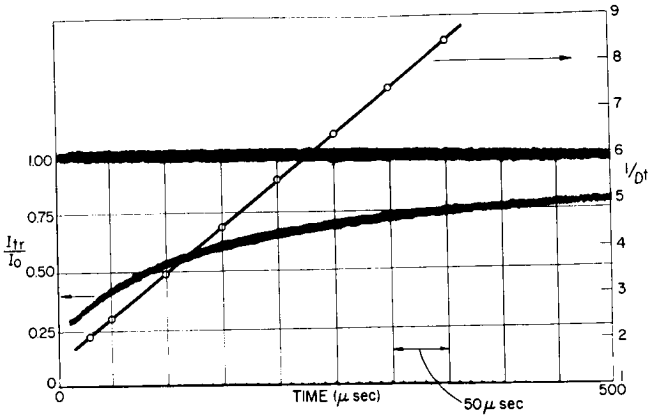

Fig. 4. Bimolecular decay of the HOC_6H_6 radical. (Dorfman *et al.*)

causes the dissociation (6)

$$H_2O + h\nu \xrightarrow{1850\ \text{Å}} H(^2S) + OH(^2\pi) \tag{6}$$

so that flash photolysis of aqueous solutions offers an alternative means of studying reactions important in the radiolysis. The analogy with radiolysis is emphasized by the fact that pure water gives H_2 and H_2O_2 on irradiation. In his paper, Baxendale[c]) describes (i) the yields of H_2O_2 formed on "flashing" aqueous solutions containing O_2, CH_3OH, H_2O_2, and OH^- (ii) the transient spectra and the kinetics of their growth and decay for aerated solutions of alcohols and formic acid. He concludes that the perhydroxyl radical has a molar decadic extinction coefficient at 2537 Å in the region 350 to 380 $M^{-1}\,cm^{-1}$, and that the rate constants of reactions (7) and (8)

$$2HO_2 \rightarrow H_2O_2 + O_2 \tag{7}$$

$$HO_2 + Cu^{2+} \rightarrow H^+ + O_2 + Cu^+ \tag{8}$$

are 2.5×10^6 and 1.5×10^7 $M^{-1}sec^{-1}$ at pH 2.3. Measurements at pH 7 and pH 11 suggest that k_7 passes through a maximum in neutral solution and that ε^{2537} increases with increasing pH.

3. HIGH INTENSITY EFFECTS

It was first established by Sutton and Rotblat[2]) that, at high dose rates, bimolecular combination and reconstitution reactions involving H and OH radicals drawn from adjacent tracks cause an apparent increase in molecular yields and a diminution in radical

yields. Thomas and Hart[9]) have extended this work using 13 MeV electron pulses. Assuming $G(Fe^{3+}) = 15.6$ at dose rates below 10^{25} eV $l^{-1}sec^{-1}$, because the oxidation yield is here insensitive to changes in $[Fe^{2+}]$ and $[O_2]$, they were able to measure higher intensities from the magnitude of the total charge collected in a massive lead block. Their results on this system show that the fall-off in $G(Fe^{3+})$ occurs at lower dose-rates the lower the oxygen or ferrous ion concentrations and, somewhat surprisingly, the larger the chloride ion concentration. Irradiation of deaerated 0.8 N H_2SO_4 at dose rates below 10^{26} eV $l^{-1}sec^{-1}$ gave molecular yields $G(H_2) = (H_2O_2) = 1.23$, but when H_2O_2 was also present initially $G(H_2)$ was smaller. The diminution of $G(H_2)$ with increasing added H_2O_2 is illustrated in Fig. 5, from which it is seen that a higher initial H_2O_2

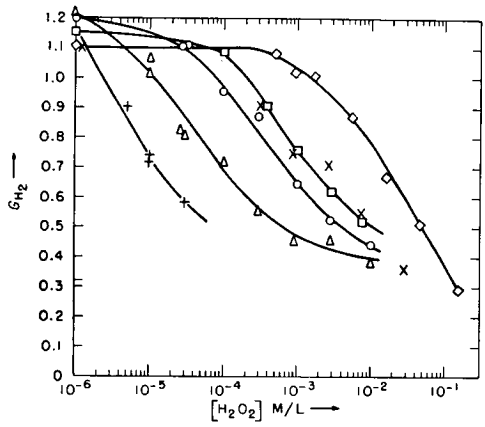

Fig. 5. Suppression of intertrack combination of H atoms by added H_2O_2 at different dose rates. (Thomas and Hart.)

Relative dose-rate per pulse:

◇	×	□	○	△	+
3200	1000	216	12	2.5	0.24

concentration is required to produce the same decrease in $G(H_2)$ at higher dose-rates. These and other results are interpreted on the basis that there is no reaction during the pulse and that reactions (9) and (10)

$$H + H \rightarrow H_2 \tag{9}$$

$$H + H_2O_2 \rightarrow H_2O + OH \tag{10}$$

are the sole competitors. Repeated pulsing,

so that H atoms which survived from a preceding pulse augmented those formed in the next, gave data which were also consistent with this simple one-radical model and $k_9 = 5 \times 10^9$ and $k_{10} = 4 \times 10^7\ M^{-1}\text{sec}^{-1}$. At very high intensities this model is inadequate and, notwithstanding the last sentence of the summary, no alternative explanation is presented in the paper.

4. LOW TEMPERATURE STUDIES

At a sufficiently low temperature any massive intermediates produced by irradiation of solid aqueous systems will be immobile and can be preserved for post-irradiation investigations. A commonly used tool for detection of non-zero spin intermediates is that of electron-spin resonance (ESR) spectroscopy and this has been applied by Kilgour, Smith and Wyard[k]) to glassy mixtures of H_2O_2 in H_2O irradiated at —196 °C. These workers find that the relative yields of a radical giving a doublet ESR signal with 13 gauss splitting and g-value anisotropy for irradiation by 250 kV X rays, 15 MeV deuterons and 5.7 MeV α particles are approximately the same as those found for G_H and G_{OH} in liquid water. Although there is no perceptible deuterium isotope effect at —196 °C comparable with that observed in liquid water, the ESR doublet referred to above is much less abundant in D_2O_2–D_2O glass irradiated with ultra violet light than in H_2O_2–H_2O glass. This result is attributed to the probable greater rate of reaction (11)

$$HO + H_2O_2 \rightarrow H_2O + HO_2 \qquad (11)$$

as compared with that of its deuterium counterpart at the same low temperature. Since (a) the line width of an ESR signal increases with the concentration and (b) a new spectrum appears at half the magnetic field strength when the radicals are very close together, two methods are available for estimating local concentrations of radicals. The results indicate a much wider spread of radicals for both X and α rays than is assumed in the diffusion model and, after allowance has been made for diffusion before trapping, it is concluded that the ESR spectra of irradiated peroxide glasses demonstrate that the ionization electron is *not* recaptured by positive ions.

This conclusion is identical with that of Dainton and Jones[3]) who investigated the ESR spectra and yields of Fe^{3+}, N_2 and H_2 when acid glasses containing N_2O and Fe^{2+} are irradiated at —196 °C and

higher temperatures with either γ rays or 2537 Å light. The effects of temperature, composition and state of aggregation on the signal strengths and product yields indicate, (a) that the H-atom-like species is mobile and can travel distances of 50 to 70 Å from its point of origin to a solute, (b) that this migration is more facile in the glassy state and is presumed to occur preferentially along the polymer chains which are also responsible for the glassy state, (c) that the photochemically generated species is identical with that produced by γ rays and, (d) that both react equally rapidly with N_2O and Fe^{2+}. The mobile species is considered to be an electron, produced according to reactions (12) and (13).

$$H_2O_{gl} \rightarrow H_2O^+_{gl} + e^-_m \tag{12}$$

$$Fe^{2+}_{gl} + h\nu \xrightarrow{2537} Fe^{3+}_{gl} + e^-_m \tag{13}$$

and capable of being trapped by Fe^{2+}, N_2O or $\sim\sim SO_4H$ groups (equations (14), (15) (16)).

$$e^-_m + Fe^{2+}_{gl} \xrightarrow{77^\circ K} Fe^+_{gl} \; (\rightarrow \overbrace{Fe^{3+} + H_2 \text{ or } S\text{-}S}^{\text{warming}}) \tag{14}$$

$$e^-_m + N_2O \xrightarrow{77^\circ K} N_2O^- \; (\rightarrow N_2 + OH \rightarrow N_2 + Fe^{3+}) \tag{15}$$

$$e^-_m + \sim\sim SO_4H \xrightarrow{\sim 77^\circ K} \sim\sim SO_4 + H_T \; (\xrightarrow{\sim 90^\circ K} \sim\sim SO_4H + e^-_m). \tag{16}$$

H_T is here a trapped hydrogen atom and reaction (16) is reversed at about —180 °C.

Kroh and Czerwik[1]) have measured $G(Fe^{3+})$ for frozen Fricke solutions irradiated at different temperatures in the range —20 to —180 °C. For ^{90}Sr-Y β particles they find a linear dependence of $G(Fe^{3+})$ on temperature ($G(Fe^{3+}) = 0.016\ T°K - 0.46$), whereas for γ rays the relation is more complex. It must be emphazised that these systems are crystalline and yield $G(Fe^{3+})^{O_2}_{90°K} \simeq 1$ whereas Dainton and Jones[e]) find that irradiation at —196° of a deaerated, acidified, glassy solution of ferrous sulphate gives $G(Fe^{3+})_{90°K} = \sim 8$.

5. THE NATURE AND REACTIVITY OF THE PRIMARY SPECIES IN LIQUID AQUEOUS SYSTEMS

Recognition of two types of hydrogen atom H_a and H_b related as acid and conjugate base according to equation (17)

$$H_a + H^+ \rightarrow H_b \tag{17}$$

depends upon H_a and H_b either reacting with the same reagent to give different products (e.g. with $ClCH_2COOH$ giving Cl^- or H_2) or reacting at grossly different rates with a given pair of reagents (e.g. with O_2 and H_2O_2). Evidence for the fact that the most basic form of hydrogen atom formed by the action of ionizing radiation on water has unit negative charge has recently been provided by investigation of primary salt effects on certain rate constant ratios[4]. The results are illustrated in Fig. 6. The paper by Appleby, Holian,

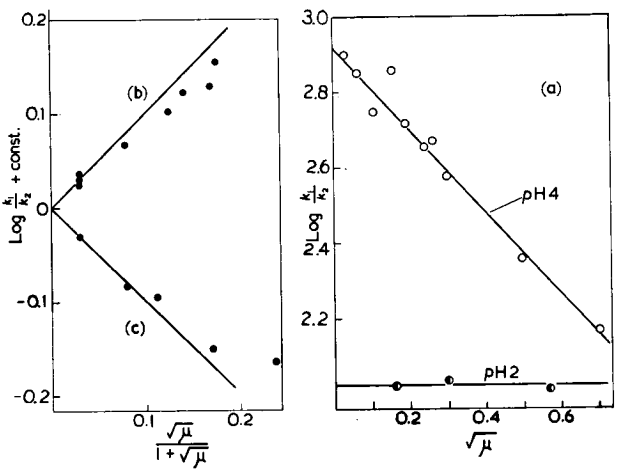

Fig. 6. Ionic strength effects on rate constant ratios, for two reagents competing for "H"[4]. (a) Ag^+ and $CH_2CH.CONH_2$ at pH 4 and pH 2; (b) NO_2^- and H_2O_2[4]; (c) H^+ and H_2O_2[4].

Scholes, Simic and Weiss[b]) is concerned with the reaction of H_a (called by the authors a "polaron" and represented as $(H_2O)^-$) with carbon dioxide and carbon monoxide. In deaerated solutions of an organic substrate RH containing CO or CO_2 the important reactions are (18), (19) and (20)

$$OH + RH \rightarrow R\cdot + H_2O \tag{18}$$

$$H_a(=(H_2O)^-) + CO_2 \text{ (or CO)} \rightarrow CO_2^- \text{ (or CO}^-) \tag{19}$$

$$R\cdot + CO_2^- \text{ (or CO}^-) \rightarrow RCO_2^- \text{ (or RCO}^-) \tag{20}$$

so that radiation-induced carboxylation or carbonylation of organic solutes can be achieved. The carboxylation is markedly pH dependent as seen in Fig. 7 but the fact that the yield of carboxylic acid is less

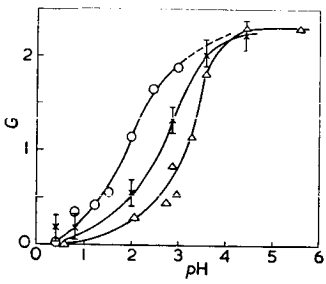

Fig. 7. Effect of pH on the yields of H_2, glycol and glycollic acid from the γ radiolysis of 10^{-1} M aqueous methanol equilibrated with 1 atm of carbon dioxide. (Appleby *et al.*)
△ Yield of glycollic acid; ○ Decrease in hydrogen yield, $G(-H_2)$, on adding carbon dioxide. × Decrease in glycol yield, G (-glycol), on adding carbon dioxide.

than the decrease in hydrogen yield is provisionally attributed to the ionic dissociation(21)

$$COOH \rightleftharpoons CO_2^- + H^+, \tag{21}$$

the evident poorer carboxylating power of COOH as compared with CO_2^- and the tendency of the former radicals to undergo disproportionation to CO_2 + H·CO·OH. In marked contrast to CO_2, carbon monoxide can accept H_b as well as H_a and therefore the carbonylation reaction occurs as readily in acid solution as it does in neutral solutions. An interesting aspect of this work is the conclusion that the conventional hydrogen yield $G_{\text{«H»}}$ = 2.9 comprises 0.6 true hydrogen atoms and 2.3 polarons. Other authors have reached different conclusions.

Getoff[h]) has made a detailed analysis of the variation with dose at different values of pH within the range 2 to 11 of the yields of the many reduction products of carbon dioxide. The primary products appear to be CO^-, CO, CO_2^-, HCO and ·COOH (or H·COO·) and again a major rôle is allotted to the solvated electron. Most of the products in this system are derived from bimolecular reactions between radicals, some of which may be interfered with by selective reaction of a product (e.g. H_2) or an added reagent (e.g. $FeSO_4$) which can react with both primary and secondary radicals.

Nitrous oxide is an extremely efficient electron scavenger in the gaseous, polar liquid (H_2O and NH_3) and glassy (aqueous and organic) phases and has the merit that one product of this reaction, namely nitrogen, is so inert chemically as to be immune from attack by radicals. Furthermore the stoichiometrically equivalent reaction of H atoms with N_2O has an energy of activation in the

range 10 to 16 Kcal/mole. Consequently the decrease in $G(N)_2$ when the pH is diminished can be used to obtain the rate constant ratio $k(e^-_{aq} + N_2O)/k(e^-_{aq} + H^+)$ and if ferrous ions are present to scavenge H atoms according to equation (22)

$$H + Fe^{2+}_{aq} \rightarrow H_2 + Fe^{3+} \tag{22}$$

the variation of $G(H_2)$ and $G(Fe^{3+})$ with pH can be used to confirm this ratio and to investigate the variation of $G_{\text{"H"}}$ with pH. The results are illustrated in Fig. 8. It appears that the increase in

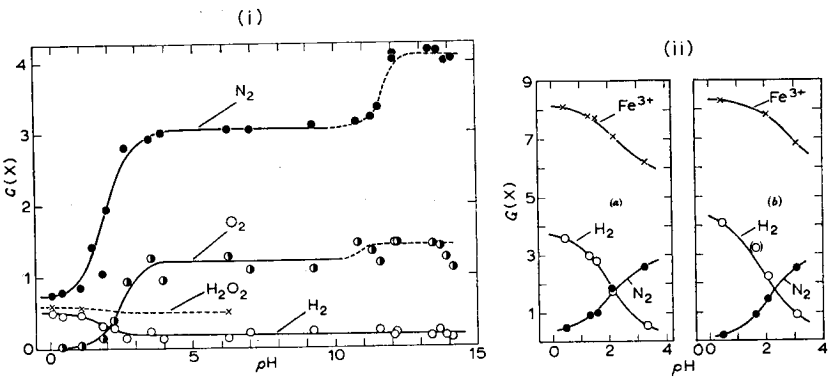

Fig. 8. Effect of pH on the radiolysis of solutions of N_2O in the presence and absence of ferrous sulphate. (Dainton and Peterson.) (i) absence of ferrous sulphate $[N_2O] = 0.014\ M$. (ii) presence of ferrous sulphate. $[N_2O] = 0.013\ M$, $[FeSO_4] = 1.93\ mM$ (a) and $10\ mM$ (b).

$G_{\text{H'}}$ and G_{OH} at pH < 3 and the fact that $G(N_2)$ does not fall to zero even at pH 0 are due to the reaction of H^+ with either an excited water molecule (H_2O^*) or with a radical pair (H + OH) forming H_2^+ which reacts rapidly with N_2O or oxidizes Fe^{2+}. Recent work by Mr. Watt[5]) on the ferrocyanide-ferricyanide-N_2O system has shown that these reactions have their basic analogues which are, incidentally, responsible for the step in $G(N_2)$ at pH 11 shown in Figure 8; so that we now have additional "H" and OH yields available by virtue of reactions (23) and (24).

$$H_2O^* \text{ or } (H + OH) + H^+ \rightarrow H_2^+ + OH \tag{23}$$

$$H_2O^* \text{ or } (H + OH) + OH^- \rightarrow e^-_{aq} + OH \tag{24}$$

Jortner, *et al.*[i]) have concluded that the so called hydrogen atom produced by photolysis of potassium iodide solutions is a solvated electron. This is a reversal of the carefully argued conclusions of Jortner, Ottolenghi, Levine and Stein[6]) and contrary to the conclusions of several other authors. They have studied the effect of $H_2PO_4^-$ and NH_4^+ ions on this reaction and on the radiation chemistry of deaerated aqueous solutions of acetone and sodium formate and in the presence of ferricyanide or nitrite or nitrate ions. From their results they have derived the rate constant ratios shown in the Table 1.

TABLE 1

Rate constant ratios for e

	a	b	a	b	a	b	a	b
Substrates	$(CH_3)_2CO$	$H_2PO_4^-$	$Fe(CN)_6^{3-}$	$H_2PO_4^-$	NO_2^-	$H_2PO_4^-$	NO_3^-	$H_2PO_4^-$
k_a/k_b	570		1000		740		1300	
Substrates	$(CH_3)_2CO$	NH_4^+	H^+	$(CH_3)_2CO$				
k_a/k_b	$\sim 10^4$		2.3					

Carbon monoxide shares with carbon dioxide the power to scavenge e_{aq}^- and also combines with H and OH. Raef and Swallow's[n]) data on the irradiation of deaerated, acidified aqueous solutions of carbon monoxide can be understood in terms of the bimolecular reaction of CHO and COOH radicals with one another and with H, OH, H_2O_2 and HCHO, provided it is assumed that the COOH radical persists as such and does not add to carbon monoxide. In alkaline solutions, formic acid is produced via a chain reaction in which the propagation reactions are thought to be:

$$H + OH^- \rightarrow e_{aq}^- \tag{25}$$

$$e_{aq}^- + CO \rightarrow CO_{aq}^- \text{ or CHO or } HCOOH^- \tag{26}$$

$$CO_{aq}^- \text{ or CHO or } HCOOH^- \rightarrow HCOO^- + H \text{ or } HCOOH + e_{aq}^-. \tag{27}$$

When ferrous ions are present they compete with CO for OH and H radicals and the resultant ferric ions are reduced by CHO and COOH radicals. The rate constant ratios $k_{OH+CO}/k_{OH+Fe^{2+}}$ and

$k_{H+CO}/k_{H+Fe^{2+}}$ can therefore be evaluated and the results are discussed.

Aebi and Heiniger[a]) studied the X ray induced oxidation at *p*H 6.8 of ^{14}C-labelled formate in the presence and absence of beef liver catalase. The enzyme causes the H_2O_2 to oxidize more formate with liberation of additional $^{14}CO_2$. In the presence of oxygen $G(CO_2)$ is increased from 3.3 to 5.2 by addition of the enzyme whereas in a nitrogen atmosphere there is no increase in $G(CO_2)$ when the enzyme is present. Nitrous oxide produces a larger non-enzymatic yield of CO_2 than oxygen, a result which is interpreted in terms of a short chain oxidation of formate and by the reaction sequence (28) and (29)

$$\cdot COOH + N_2O \rightarrow CO_2 + N_2 + OH \tag{28}$$

$$HCOOH + OH \rightarrow COOH + H_2O. \tag{29}$$

This greater effect of N_2O as compared with O_2 is also observed in the X ray induced oxidation of hemolysate to methaemoglobin.

Engelhard, Prchal and Froben[g]) have carried out a detailed paper chromatographic and electrophoretic study of the products of 50 KVp X ray irradiation of a 0.02 *M* aerated aqueous solution of L-lysine hydrochloride at *p*H values in the range 0.8 to 11.8. They find the products to be very similar in nature and relative abundance to those obtained when the gas from an electrical discharge through water vapour is passed through the same solutions. Earlier experiments have led them to believe that the major components of the gas discharge are hydroxyl radicals. They therefore regard the major influence of X rays on aqueous systems as ascribable to hydroxyl radicals. This inference clearly depends on the validity of their conclusions about the gas discharge and these have been rigorously tested. The very marked effects of change of *p*H on the product distribution suggest that perhaps other species besides OH may be important.

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DISCUSSION

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The discussion was opened by Platzman, (*Argonne Nat. Lab., Ill., USA*), who commented that Professor Dainton had differentiated between solvated electrons and polarons. He asked for a definition of these species. In reply, Weiss[b]) stated that the differences could not be distinguished chemically but that the polaron could be defined in physical terms according to the concepts of Landau[1]), Mott and Gurney[2]) and Pekar[3]). The polaron was not an electron with a number of water molecules orientated around it as in a hydrated ion. Since the de Broglie wavelength of the electron is greater than the diameter of the water molecule, the polaron should be considered rather as an electron spread out over several water molecules. In this manner the medium becomes polarized and stabilizes the electron. The work of Dainton and others had given adequate proof for the existence of a radiation produced electron in water. Recent work of Emmerson, Schneider and Weiss[4]) has given definite ESR spectrographic evidence for the presence of both positive and negative polarons in ice. There should be no difficulty in extrapolating these results to liquid water.

Platzman considered that ideas on the nature of the solvated electron were unnecessarily confused. In his opinion radiation chemists had never considered the solvated electron to resemble a solvated ion.

In view of the fact that it was believed that the polaron only concerned trapping in respect of energy lowering and that the polaron was not necessarily held rigidly in space, Matheson (*Argonne Nat. Lab., Ill., USA*) asked Weiss if he could clarify how a sufficiently great concentration of polarons was obtained to enable electron spin resonance spectra to be studied. Weiss replied that

the results were obtained in aqueous systems frozen in liquid nitrogen. The spectrum consisted of a very narrow line of maximum width 0.5 gauss with a g factor of 2.0044, which there was reason to ascribe to the solvated electron, and a second resonance with a g factor of 2.016 which had been tentatively assigned to the positive polaron. It should be remembered that there would be a positive hole left by the electron. Further work would be carried out in liquid helium. Jortner[1]) commented that the work of Pekar[3]) was concerned with electron binding in ionic crystals. A recent analysis has shown that in polar solvents the treatment should be somewhat modified.

Weiss did not think this called for much modification of the theory since the medium is characterized only by its optical and static dielectric constants and a great distinction could not be drawn between ionic crystals and polar liquids. Grossweiner and Matheson[5]) have measured the trap depth in the thermoluminescence of irradiated ice and their value of 0.3 eV is in good agreement with the theory of Pekar. The maximum absorption of the polaron should be greater by a factor of 3, and based on the continuum theory the maximum absorption should appear at about 10000 Å. Jortner pointed out that, according to the theory of Pekar in a polar medium the additive electron is bound in the polarization field produced by the electron itself. The Landau-Pekar model is based on the idea that the binding energy and hence the kinetic energy of the additive electron is low compared with the energy of the electrons of the medium. The approximation involved is equivalent to the adiabatic approximation in molecular physics. The medium electrons are affected by the field of the additive electron located at a certain position, while the latter is affected by the mean charge distribution of the medium electrons. These assumptions are applicable for electrons having a binding energy of about 0.1 eV (as calculated for ionic crystals). In the case of polar solvents the binding energy is higher and the application of the electronic adiabatic approximations appear to be unjustified. An alternative way to treat electron binding in polar solvents is to assume that the additive electron and the medium electrons may be treated independently by the Hartree[6])-Fock[7]) approximation. In this type of calculation the quantum mechanical interaction energies are replaced by the electrostatic polarization energy of the dielectric medium. This

treatment differs for the Landau-Pekar model as it introduces the contribution of the electronic polarization of the medium to the binding energy of the electron, and thus will yield a higher binding energy and a more confined charge distribution for the additive electron. Calculations carried out for electron binding in water lead to the thermal dissociation energy of 1.2 eV and predict that the first 1s $\rightarrow$ 2p electronic transition of the solvated electron will lie at 0.9 eV.

Schiller (*Cent. Res. Inst. Phys., Budapest, Hungary*) commented that Dainton had defined three types of hydrogen atoms. He did not think that this definition was necessary. The initial local concentrations of hydrogen atoms in acid solution is lower, not because of a lower value of the rate constant, but because the initial spatial distribution of hydrogen atoms is greater in acid than in neutral solutions. Similarly, the increased radical yield in acid solution follows from the greater distribution of hydrogen radicals and scavenger reactions are enhanced.

Concerning the presence of solvated electrons in neutral aqueous solutions, Kuppermann (*Dept. Chem., Univ. Ill., USA*) stated that diffusion kinetic calculations using a three radical model had been compared with experimental yields in neutral aqueous solutions. In the model, OH and H_3O^+ were assumed to have initial Gaussian spherical distributions of 10 Å diameter, and e^-_{aq} an initial distribution of 20 Å diameter. The average number of these species was assumed to be 9 in each spur. The reaction $e^-_{aq} + H_3O^+ \rightarrow H + nH_2O$ was included in the mechanism as well as the usual radical-radical and radical-solute reactions. All diffusion coefficients were taken to be 2×10^{-5} cm^2/sec, all rate constants, 6×10^9 l/mole/sec and the solute concentrations 10^{-3} *M*. The calculated yields were $G_{H_2O_2} = 0.7$, $G_{H_2} = 0.27$, $G_{OH} = 2.22$ $G_{e^-_{aq}} = 2.21$ and $G_H = 0.9$. These results were in reasonable agreement with some of the experimental measurements and this agreement could easily be improved by relatively small changes in the parameters used in the calculation. On this basis it can be said that a model of the gamma-radiolysis, or 1 MeV electron radiolysis of dilute neutral aqueous solutions in which there are assumed to be isolated spherical spurs containing initially OH, H_3O^+, and e^-_{aq} is consistent with the observed experimental results. Jortner stated that Rabani, Ottolenghi, Stein and himself had recently examined the general mechan-

ism of the conversion of solvated electrons to hydrogen atoms. The conversion in solutions containing H_3O^+, $H_2PO_4^-$, HF and NH_4^+ was investigated by competitive kinetics, in aqueous solutions irradiated by 200 KV X rays. The results of an investigation of the nature of the reducing radicals produced by the photolysis of the iodide ion in aqueous solution were interpreted in terms of dissociation of the spectroscopic excited state, leading to the formation of an iodine atom and a solvated electron in a solvent cage. The solvated electron may be scavenged by the above general acids, leading to the production of a hydrogen atom. These experimental results were treated using scavenging equations to account for competition between the secondary cage recombinations and scavenging. The agreement between the rate constants ratios $ks + e^-/kH_3O^+ + e^-$ derived from radiation and photochemistry is satisfactory. These results show that the conversion of e^-_{aq} by the four acids investigated correlates with the pK values of the acids. The results fit quite well with the Brönsted general acid catalysis law, yielding evidence that the conversion $e^-_{aq} \rightarrow H$ involves a proton transfer from an acid. Considering the role of water as a Brönsted catalyst these results provide an explanation for the fact that the reaction $e^-_{aq} + H_2O \rightarrow H + OH^-_{aq}$ is a surprisingly slow process. From the pK values of H_2O and H_3O^+ it was estimated that $\frac{kH_2O + e^-}{kH_3O^+ + e^-} \leq 2 \times 10^{-7}$. Hence the efficiency of the scavenging of e^-_{aq} by H_2O can be determined within a narrow range.

Uri (*Min. Aviation, Waltham Abbey, Great Britain*) asked Baxendale[c]) if the pK value of the HO_2 radical, previously assumed to be about 2, should be revised. Did Baxendale's result support a pK of 7? Since some of the work has been carried out in the presence of alcoholic scavengers, was it not likely that the RO_2 radicals were also present? Could one derive any data from the present investigations about the RO_2 radicals?

Baxendale agreed that in the generally held opinion, the pK value was about 2. This result was originally estimated by Evans, Hush and Uri[8]) but has so far not been confirmed experimentally. Since the estimate was made from thermochemical data there was considerable room for uncertainty. An error of a few kilocalories would make differences of several orders of magnitude in the estimated pK value. Although it could not be proved that

the species concerned was definitely the HO_2 radical, this was believed to be the case and that the appropriate *pK* value was rather greater than that first calculated by other workers.

It was stated by Czapski (*Dept. Phys. Chem., Hebrew Univ., Jerusalem, Israel*) that combination values for the HO_2 radical had been measured at different *p*H values. At *p*H 2 the figure was found to be in agreement with Baxendale's value, but at *p*H 7 the value was in agreement with that of Schmidt, but in disagreement with Baxendale. *pK* for HO_2 was found to be 4.4 ± 0.4.

Uri asked Boag whether his technique would make it possible to determine if the ion-pair complex $Fe^{3+}OH^-$ is formed as the first intermediate in the electron transfer reaction: $Fe^{2+} + OH \rightarrow Fe^{3+} + OH^-$ in acid solution. In general it appeared that more information concerning the intimate mechanism of electron transfer reactions would be of great importance, both in radiation chemistry and the study of metallic catalysis. In reply, Boag[d]) pointed out that the technique would be helpful if the intermediate complex had an absorption spectrum clearly distinguishable from that of the final products, and if the resolution of the method, which was at the moment of the order of a few microseconds, was adequate to distinguish the rate of change with time.

Keene[j]) added that his colleagues and himself had observed several optical absorptions in pure deaerated water. The largest of these occurred with a 2 μsec pulse. Its spectrum increased steadily from 3000 to 6000 Å and had a life time of 1 to 2 μsec. In aerated water, its amplitude was reduced about five fold and in oxygenated water the amplitude fell by a further factor of three. After the pulse much smaller absorptions occurred having spectra in the far ultraviolet. The life time of these absorptions appeared to have two components, one lasting for a few tens of microseconds and the other very much longer. These absorptions ultimately return to zero.

Dorfman[f]) added to Keene's remarks. He had found that in oxygenated water a second transient appeared at shorter wavelengths. Buehler and he had found that this transient at 2967 Å, decayed following a first rather than a second order relationship, and that it had a half life of several hundred microseconds.

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Radiation Chemistry of Water

SOLUTE KINETICS

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1. INTRODUCTION

Most of our present ideas concerning the radiation chemistry of aqueous solutions begin with the so called radical diffusion model. The basis of this model is that the H and OH radicals formed during irradiation of water are produced either in localized clusters or in tracks, so that initially they tend to combine in pairs to form the molecular products hydrogen, hydrogen peroxide, and water. The probability of such combination reactions diminishes rapidly as the radicals diffuse away, so that we have a fairly sharp distinction between molecular products on the one hand, and on the other the free radicals which escape this combination by diffusion, or by reaction with added solutes. It follows from this model that the total yield of water decomposition should be nearly independent of solute concentration, while the yield of molecular products should diminish slowly as the concentration of scavenging solutes is increased.

In general the experimental facts conform with this view, and the studies reported in this session are concerned either with particular exceptions to this general pattern, or with detailed investigations of the nature and rates of the secondary reactions initiated by the free radicals.

2. EFFECT OF SOLUTE CONCENTRATION

The first paper, by Anderson and Knight[b]) deals with the γ radiolysis of concentrated potassium iodide solutions, in which the products are (a) molecular hydrogen and (b) oxygen and iodine, formed in amounts which together are equivalent to (a). Their observations are shown in Fig. 1, and demonstrate that the yield of hydrogen per unit of total energy absorbed by the system increases

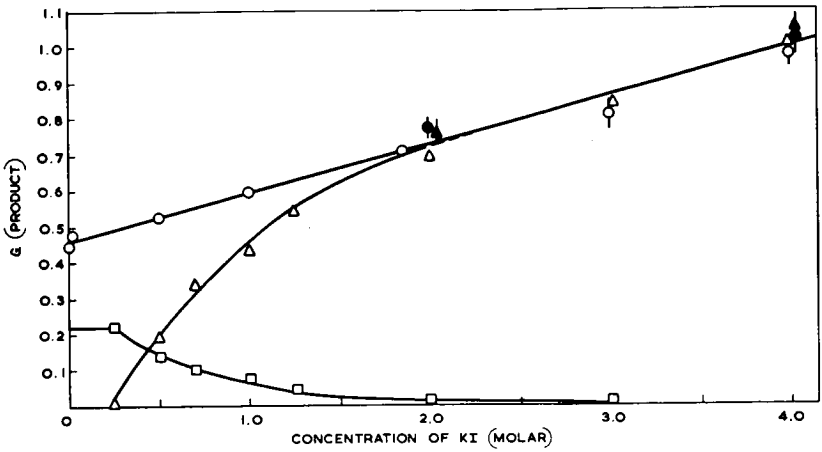

Fig. 1. Product yields in the $^{60}Co\,\gamma$ radiolysis of neutral, air free aqueous potassium iodide solutions. (Anderson and Knight.)

Unshielded { ○ H_2 ● △ I_2 ▲ □ O_2 } Shielded by 2 mm of lead

linearly with the iodide concentration. Iodide ions do not react with the precursors of molecular hydrogen, so we would expect the yield to remain nearly constant. This exceptional behaviour could conceivably be due to the energy directly absorbed by iodide ions, but the authors argue from photochemical evidence that this is unlikely. Instead, they attribute the effect to so called "sub-excitation" electrons having energies too low to excite water molecules, i.e. less than 6.8 eV, but sufficiently high to excite solvated iodide ions and cause their decomposition. This view is supported by the fact that iodide ions absorb light strongly in the region of 2200 Å, and are therefore likely to become activated by electrons of about 5.7 eV energy. However, other mechanisms are not excluded.

Returning now to the more general situation, we consider various systems in which the yield of hydrogen peroxide varies in the expected manner with increasing concentration of solute. The first concerns the formation of hydrogen peroxide from aerated water by the α rays of polonium. This has been studied by Carmo Anta[c]) and collaborators, and some of their results are shown in Fig. 2. Various solutes have been used to react with the precursors of the

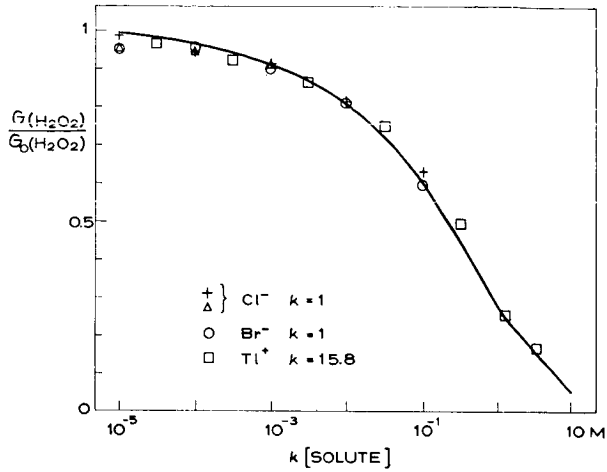

Fig. 2. The effect of solutions on hydrogen peroxide yields in the γ radiolysis of aerated water. (Anta *et al.*)

hydrogen peroxide and hence reduce the yield. Evidently the effects of all these may be expressed by a single curve if we multiply the solute concentration by an appropriate factor, k. If this precursor is the OH radical, as we suppose, then k must be proportional to the rate constant of its reaction with the solute. In this way the relative rates of such reactions have been obtained, and we shall refer to these values later. In the case of chloride, bromide, and thallous ions the effect follows the predictions of the radical diffusion model, but quite a different curve is obtained when nitrate ions are added to the system. This is attributed to the production of nitrite by energy directly absorbed in the nitrate ion.

The second paper in which this method is employed is by Madame Koulkes-Pujo and Ziemeckie[1]). These authors have studied the reduction of solutions of sodium chloraurate to metallic gold induced by γ radiation. They have succeeded in showing that the precipitated gold does not redissolve, and they find that the mechanism of the reaction is similar to that of the reduction of ceric sulphate, so that the yield may be written as follows:

$$AuCl_4^- \rightsquigarrow Au\downarrow$$

$$G_{(-\mathrm{Au\ III})} = G_{H_2O_2} + G_H - G_{OH} = 2.3 \text{ at } pH\ 2.8.$$

If chloride ions are added to suppress formation of hydrogen peroxide, then the gold reduction yield decreases in accordance with the radical diffusion theory. A similar, but larger, effect of chloride ions is observed in the acid ceric sulphate system since the reaction

$$OH\cdot + Cl^- + H^+ \rightarrow OH^- + Cl + H^+$$

proceeds more rapidly in acid solution.

3. COMPETITION BETWEEN SOLUTES

A more general method of comparing reaction rates is to study the competition between two solutes for the same free radical. Ferradini and Madame Koulkes-Pujo[f]) have irradiated air free solutions of potassium bromide under high pressures of hydrogen, so that these two solutes both react with OH radicals. The reaction sequence is then as follows:

$$H_2O \rightarrow H_2, H_2O_2, H, OH\cdot$$

$$OH\cdot + H_2 \rightarrow H_2O + H \qquad (1)$$

$$OH\cdot + Br^- \rightarrow Br + OH^- \qquad (2)$$

$$H + H_2O_2 \rightarrow OH + H_2O$$

$$H + Br \rightarrow HBr.$$

Hence

$$G(H_2O_2) = G(H_2) - \left(\frac{G_H + G_{OH}}{2}\right)\frac{k_1[H_2]}{k_2[Br^-]}.$$

This relation has been verified experimentally as shown in Fig. 3, from which one obtains $k_2/k_1 = 830$. This result may be regarded as confirmation of previous independent determinations of k_2 (1.6

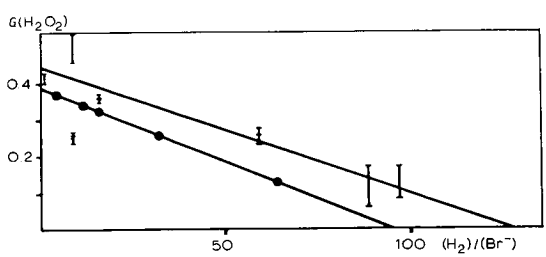

Fig. 3. Hydrogen peroxide yields in the γ radiolysis of bromide solutions containing added hydrogen. (Ferradini and Koulkes-Pujo.)
● --- 0.8 N H_2SO_4; I -- pH 2.15

$\times 10^{10}$ l mole^{-1}sec^{-1}) by Burton and Kurien[1]) and of k_1 (4.4×10^7 l mole^{-1}sec^{-1}) by Schwarz[2]).

Reactions of OH radicals have also been studied by M. Anbar and Miriam Bobtelski[a]). These authors have irradiated solutions of iodo-benzoic acid, and also of iodo-phenetole. In each case the substance is de-iodinated with a G value of approximately 3, which is independent of the presence or absence of oxygen. From comparison of the products formed by γ radiations with those obtained with the Fenton reagent it is concluded that the de-iodination reaction is due to the OH radical. If a second solute is added to compete for OH radicals then the de-iodination is lowered, and the rates of such competing reactions have been compared in this manner. Some of the results obtained are shown in Table 1, in which the rate of the chloride ion–OH· reaction in acid medium is arbitrarily taken as unity. Results obtained by other methods are also included for comparison.

The results of these methods agree in order of magnitude, though there are some discrepancies. In particular the small value obtained for the bromide ion by the last two methods is surprising, since we have just seen that the rate constant for this reaction is so high that it must proceed on nearly every collision.

TABLE 1

Reactions rates of OH radicals

	Suppression of $G_{H_2O_2}$		Suppression of $G_{(-I)}$
	γ-rays[3])	α rays (Anta)	(Anbar)
Cl^- (acid)	1	1	1
Br^-	6	1	1.4
I^-	4		11
Tl^+	14	15.8	31
Cystine			32
Iodophenol			46
Benzene			1

Somewhat similar conclusions have been reached by Zagorski and Minc[n]) during studies of a reaction initiated by γ rays in aerated solutions of alkaline sodium sulphite. This is a chain reaction in which oxygen is consumed in very high yield, and the OH

radical appears to be the chain carrier. Added solutes suppress this chain, and their efficiency increases in the order chloride < hydrocarbons < proteins < hydrogen peroxide. The peculiarly high efficiency of hydrogen peroxide has been examined more closely.

In many systems the H atom, or its precursor (the solvated electron) may enter into competing reactions in addition to the OH radical, and the kinetic situation then becomes extremely complicated. Such a situation is encountered in the radiolysis of iron salts and Shubin and Dolin[m]) have sought to simplify it by using high pressures of hydrogen to convert the OH radical to an H atom. This initiates a short chain reaction and we shall shortly consider one aspect of their conclusions.

Cercek[d]) also reports a study of ferric sulphate solutions, to which vinylpyridine sulphate has been added. The latter substance is transformed into a reducing species by the action of H and OH radicals, so that under appropriate conditions the following sequence of reactions takes place:

$$\begin{aligned} \text{H (or OH}\cdot) + \text{Vinylpyridine (VP)} &\rightarrow \text{R}^{\cdot} \\ \text{R}^{\cdot}_{k-1} + \text{VP} &\rightarrow \text{R}^{\cdot}_{k} \\ \text{R}_k + \text{Fe}^{3+} + \text{H}_2\text{O} &\rightarrow \text{R}_k\text{OH} + \text{Fe}^{2+} + \text{H}^+ \\ \text{H} + \text{Fe}^{3+} &\rightarrow \text{H}^+ + \text{Fe}^{2+} \\ \text{H}_2\text{O}_2 + \text{Fe}^{2+} &\rightarrow \text{OH}\cdot + \text{OH}^- + \text{Fe}^{3+}. \end{aligned} \tag{3}$$

Hence $G(\text{Fe}^{2+}) = G_{\text{H}} + G_{\text{OH}}$, and this relation has been experimentally verified. A common feature observed in all iron solutions is the reduction of ferric ions by H atoms or their precursors, represented formally as reaction (3) above. In aerated solutions of ferrous sulphate this reaction occurs only with high concentrations of ferric ion and leads to a reduction in $G(\text{Fe}^{3+})$. Shubin and Dolin interpret this as a competition for H atoms between ferric ions and oxygen ($\text{H} + \text{O}_2 \rightarrow \text{HO}_2\cdot$) while Dobson and Hughes[3]) in a somewhat similar study regard the process as a competition for solvated electrons, as follows:

$$\begin{aligned} \text{Fe}^{3+} + \text{H}_2\text{O}^- &\rightarrow \text{Fe}^{2+} + \text{H}_2\text{O} \\ \text{H}_3\text{O}^+ + \text{H}_2\text{O}^- &\rightarrow \text{H} + 2\text{H}_2\text{O} \end{aligned}$$

Such solvated electrons play a comparatively minor part in Shubin and Dolin's experiments, since most of the reducing species produced in their case are H atoms formed from reaction of OH· with

hydrogen. Cercek also observes an increasing influence of ferric ions at high concentrations and at high pH, which causes a reduction in the yield of molecular hydrogen in his system. He attributes the pH effect to a change in the nature of the ferric ion complex from $Fe_2(OH)_2^{4+}$ at pH 2 to $FeSO_4^+$ at pH 0, with a consequent decrease in the rate of reaction with H atoms.

A system somewhat similar to ferrous sulphate has been investigated by Seddon[1]). This concerns the γ radiolysis of nitric oxide solutions, in which the following mechanism is suggested:

$$\begin{aligned} H + NO &\rightarrow HNO \\ HNO + NO &\rightarrow N_2O + OH\cdot \\ OH\cdot + NO &\rightarrow HNO_2. \end{aligned} \tag{4}$$

Hence $G(N_2O) = G_H = 3$; $G(NO_2^-) = G_H + G_{OH} = 5.9$; and $G(\text{-}NO) = 3\,G_H + G_{OH} = 12$. The values reported were observed at pH 5.

If sufficient nitrite is added to the solution then the reaction

$$H + NO_2^- \rightarrow OH^- + NO \tag{5}$$

competes with reaction (4), and one obtains

$$G'(NO_2^-) = G_H + G_{OH} - \frac{2G_H}{1 + k_4\,[NO]/k_5\,[NO_2^-]}.$$

The experimental observations conform with this relation, from which one obtains $k_4/k_5 = 4.5$.

Our studies of irradiated water have been limited in the past by a lack of suitable systems for use in alkaline solution. Haissinsky and Patigny[g]) suggest that tellurium salts may be useful for this purpose, and they describe the radiolysis of sodium tellurite. This is oxidized to tellurate with a G value of approximately 6 in aerated solution. The evidence suggests the following mechanism:

$$\begin{aligned} HO_2\cdot &\rightarrow H^+ + O_2^- \\ HTeO_3 + O_2^- &\rightarrow HO_2^- + TeO_3^-(\rightarrow H_2TeO_4^-) \\ H_2TeO_4^- + OH\cdot &\rightarrow HTeO_4^- + H_2O \\ 2\,Te^{V} &\rightarrow Te^{VI} + Te^{IV} \\ HO_2^- + Te^{VI} &\rightarrow Te^{VI} + OH^-. \end{aligned}$$

Hence

$$G(H_2O_2) + G(Te^{VI}) = G_{H_2O_2} + \tfrac{1}{2}\,G_{OH} + \tfrac{3}{2}\,G_H.$$

In airfree solution, a very small yield of oxygen is observed and is attributed to decomposition of hydrogen peroxide, which is also formed in small yield. In this case the H atom acts as a reducing agent, so that

$$G(\mathrm{Te^{VI}}) = \tfrac{1}{2}\,(G_{\mathrm{OH}} - G_{\mathrm{H}}) - G(\mathrm{O_2}) = 0.18$$

and

$$G_{\mathrm{H_2O_2}} = G(\mathrm{H_2O_2}) + G(\mathrm{O_2}) = 0.09.$$

In this way all the yield values have been determined, with results which we shall consider in a moment.

Another study of alkaline solutions deals with the reduction of potassium ferricyanide, investigated by Hughes and Willis[h]). It is found that the ferricyanide ion is rapidly reduced by hydrogen peroxide and also by the alkaline form of the HO_2 radical, so that in aerated solutions the reduction yield becomes

$$G\ (\text{ferrocyanide}) = 2\ G_{\mathrm{H_2O_2}} + G_{\mathrm{H}} - G_{\mathrm{OH}} = 2.0.$$

When methanol is added to the solution both H and OH radicals become converted to reducing species and the yield becomes

$$G\ (\text{ferrocyanide})\ \text{methanol} = 2G_{\mathrm{H_2O_2}} + G_{\mathrm{H}} + G_{\mathrm{OH}} = 7.8\,.$$

Results obtained by the two methods are compared in Table 2.

TABLE 2

Yields at *p*H 13

	$HTeO_3^-$ (Haissinsky and Patigny)	$Fe(CN)_6^{3-}$ (Hughes and Willis)
$2G_{\mathrm{H_2}}$	0.6	—
G_{H}	2.75	4.85
$2G_{\mathrm{H_2O_2}}$	0.2	
G_{OH}	3.15	2.95

Dainton informs me that he and Watt[4]) have also investigated alkaline ferricyanide solutions with results similar to those of Hughes and Willis. There is evidently satisfactory agreement between the two methods with regard to G_{OH}, but the hydrogen peroxide yields differ considerably.

The variation of radical and molecular yields with *p*H has also been studied by Schiller, Kósa-Somogyi and Kiss[k]), using aerated

solutions of potassium dichromate. In this system the reduction yield decreases progressively to zero as the *p*H is increased from 0 to 5. The authors interpret these observations in terms of (a) variation in distribution of primary radicals with *p*H, and (b) variations with *p*H of the reactivity of the HO_2 radical and of hydrogen peroxide. They make the interesting suggestion that (a) may be due to capture of electrons by solvated hydrogen ions.

4. CHEMICAL DOSIMETRY

Finally, we consider two papers which deal with radiation chemical methods of dosimetry. In the first, by Dale, Davies, Keene and Law[e]) it is shown that the yield of the ferrous sulphate dosemeter varies somewhat with the purity of the sulphuric acid used in preparing the solutions. Variations of up to 20% are reported in one instance, and these are attributed to the presence of small amounts of sulphur dioxide in the acid. The second paper is by Moroson and Laughlin[j]). This concerns the induction of fluorescence in solutions of hydroxy benzoic acid, which may be used to monitor doses in the range 10 to 10000 rads. The optimum conditions for this purpose have been determined, and they find that the greatest sensitivity is obtained when light of 295 mμ wavelength is used to excite the fluorescence.

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DISCUSSION

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Dorfman (*Argonne, Nat. Lab., Ill., USA*) commenting upon the work of Anbar and Bobtelski[a]), suggested that the mechanism put forward by these authors for the reaction of aromatic systems with the hydroxyl radical was ingenious but incorrect. His arguments were threefold.

(i) Spectroscopic evidence from studies with pulsed radiation[1])

indicates that the iodine remains in the initially formed complex between hydroxyl radical and iodo-aromatic compound, and is only lost after a relatively long time.

(ii) The relative rate constants for reactions of the OH radical with different substances, as obtained by Anbar and Bobtelski, taken together with Dorfman's absolute rate constant of 4.3×10^8 mole $l^{-1}sec^{-1}$ for the reaction $OH\cdot + C_6H_6 \rightarrow C_6H_6OH$ leads to a value of approximately 5×10^{11} mole $l^{-1}sec^{-1}$ for the rate constant of the reaction $OH\cdot + IC_6H_4\ COOH \rightarrow$ products. This value was stated by Dorfman to be almost two orders of magnitude too high for purely diffusive encounters of molecular species of the dimension concerned in liquid water, and hence led the entire interpretation of Anbar and Bobtelski into serious difficulties.

(iii) In aromatic systems of this kind a general characteristic is the occurrence of a large number of post-irradiation reactions arising from the formation of cyclohexadienyl dimers. Dorfman suggested that as a consequence of this, measurements made at "infinite" time, such as those of Anbar and Bobtelski, reflected the lability of the iodine atom in various cyclohexadienyl dimers and was not a measure of the relative rates of the primary processes.

Replying to Dorfman, Anbar suggested that his value for the relative rate constant of the reaction $OH\cdot + C_6H_6$, compared with reactions of OH· with other aromatic compounds more soluble in water, may be low owing to the low solubility of benzene in water and the consequent importance of interference by adsorption. He supported this assertion by noting that of some forty water-soluble aromatic compounds studied, only three gave relative rate constants for reaction with OH· radicals which were 100 times less than that for iodobenzoic acid. These were the least water-soluble compounds, benzene, toluene and chlorobenzene. All the more water-soluble compounds showed rate constants which were within a factor of ten of that for iodobenzoic acid. If an absolute rate constant for the OH· + iodobenzoic acid reaction were deduced from the relative value with respect to certain inorganic ions and the absolute values for these ions as quoted by Schwarz[2]), the rate constant obtained was not unreasonable. Hence Dorfman's second point created no real difficulty and the reaction of OH· with iodobenzoic acid probably occurs at a rate close to the diffusion limit. Anbar did not want categorically to assert that reactions of OH·

with aromatic compounds occurred by electron transfer processes rather than via an addition complex. However, he thought it significant that the reaction of OH· with iodobenzoic acid was 3 to 10 times faster than other reactions with OH· known to proceed by electron transfer, e.g. OH· + nitrite, arsenite, thallous, cupric ions etc. Anbar also drew attention to a change in the relative rate of the reaction for OH· + Br^-; the figure of 1.4 quoted in Table 1 of the rapporteur's report should now be replaced by 4.0.

Loeff (*Imperial College, London, Great Britain*) said his work indicated that addition of OH· to benzene does take place. The evidence for this hinged on the dependence on dose rate of the major products, phenol and mucondialdehyde. He thought that the electron transfer mechanism proposed would almost certainly imply a dependence on *p*H in some cases at least. No such dependence had in fact been found. With regard to the post-irradiation effects to which Dorfman had referred, Loeff thought it possible that in the very high dose rate region in which Dorfman worked, such effects might arise through the formation of unstable molecules resulting from the addition of water to excited benzene molecules. Anbar maintained that even if a complex of the type $\cdot C_6H_6OH$ were formed it could not be the direct precursor of phenol, because when iodobenzoic acid was irradiated in the presence of isopropyl alcohol the yield of deiodination was undiminished but the main product was benzoic acid. The same result was obtained photochemically.

Kuppermann (*Univ. Ill. Urbana, Ill., USA*), referring to the work of Anderson and Knight[b]), suggested that the increasing yield at high concentrations was unlikely to be due to sub-excitation electrons. Kuppermann and Raff had recently measured singlet-triplet excitation energies of isolated molecules by a technique involving the scattering of a beam of electrons in the energy range 25 to 100 eV by those molecules. Preliminary results for water indicated a state with a vertical excitation energy of 4.2 $\pm$ 0.2 eV above the ground electronic state. If this result were confirmed, and if this value were also appropriate for the liquid state, then sub-excitation electrons in dilute aqueous solutions would have an energy less than 4.2 $\pm$ 0.2 eV. This was inconsistent with the 5 eV required to give photochemical excitation of iodide ions in water. Anderson (*AERE, Harwell, Berks., Gr. Britain*) replying, said that the participation of sub-excitation electrons was one of five explanations which they

had considered, none of which was wholly satisfactory, to explain the increase in yields of molecular products. He was grateful to learn that one of these possibilities could be eliminated. Another possibility was that OH radicals could be scavenged within expanding spurs by iodide ions, since the rate constant for the reaction $OH\cdot + I^- \rightarrow OH^- + I$ is high, 2×10^{-11} cm^3 molecule^{-1}sec^{-1} as shown by Senvar and Hart[3]). The consequent replacement of OH radicals by I atoms could change the observed yields of molecular products if the rate constants for the intra-spur reactions involving I atoms differed from those for the corresponding reactions involving OH radicals. In particular, if $k_{(e^-_{aq} + OH)} > k_{(e^-_{aq} + I)}$ the present observations are qualitatively explicable. Anderson asked Kuppermann whether, on the basis of his experience of calculations on a two radical model, he thought this explanation a feasible one. Kuppermann said he intuitively thought it a better explanation. Thomas (*Argonne Nat. Lab., Ill., USA*) made the point that any increase in G_{H_2} arising from this effect tends to be offset by the slower rate of the reaction $I + I \rightarrow I_2$ than that of reaction $OH\cdot + OH\cdot \rightarrow H_2O_2$. This subsequently has the effect of increasing the importance of the reaction $H + I \rightarrow HI$ compared with $H + H \rightarrow H_2$. Machine calculations, and the pulsed radiation techniques referred to in the previous session, indicate that G_{H_2} in the system containing I^- ions should be, and is, lower than in the absence of I^-.

Matheson (*Argonne Nat. Lab., Ill., USA*) asked Kuppermann over what range of energy could electrons effectively produce water molecules in the triplet state, and how did the cross section for such excitation compare with that for excitation (in the sub-excitation energy range) of solutes such as iodide ion? Can one say that sub-excitation electrons will only be important in water below 4.2 eV, which is the threshold for the production of triplet state water molecules? Kuppermann maintained that by analogy with the case of ethylene he would expect the cross section for excitation of the triplet state of water to remain high (of the order of optical cross sections) up to about 50 V above the onset of formation at 4.2 eV. Thus energy would be transferred preferentially to water until this energy was reached.

Zagorski[n]) stated that from measurements on sulphites in alkaline solution (at *p*H 12; 1 *N* NaOH) the molecular yields of hydrogen

lay between 0.45 and 0.50. This was to be compared with the value of 0.6 quoted by Haissinsky and Patigny[g]).

Dainton (*Univ. Leeds, Gr. Britain*) said that he and Watt[4]) had recently shown that the ferrocyanide-ferricyanide-nitrous oxide system can be used to explore the variation with pH of the radical and molecular yields in alkaline solution. At $p\mathrm{H} > 11$ the yields G_{H} and G_{OH} increase with pH whilst $G_{\mathrm{H_2}}$ and $G_{\mathrm{H_2O_2}}$ undergo but a slight diminution. A similar increase in the yields of H and OH· at $p\mathrm{H} < 3$ is already well established. These data suggest that two entities, stoichiometrically equivalent to "H" and OH·, are made available to the solute by the interaction of $\mathrm{H^+}$ or $\mathrm{OH^-}$ with some intermediate which would otherwise revert to water. Dainton and Watt had suggested that this intermediate might be either an excited water molecule $\mathrm{H_2O^*}$ and Kuppermann's reference to a triplet state at ~4 eV was, therefore, most interesting, or a radical pair (H + OH·). (He stressed the point that a considerable proportion of the energy absorbed in water is used to form isolated radical pairs.) The reactions with $\mathrm{H^+}$ and $\mathrm{OH^-}$ might then be

$$\mathrm{H_2O^* \text{ or } (H + OH\cdot)} + \mathrm{H^+} \rightarrow \mathrm{H_2^+} + \mathrm{OH\cdot}$$
$$+ \mathrm{OH^-} \rightarrow \mathrm{e_{aq}^-} + \mathrm{OH\cdot}.$$

In an earlier session a speaker had suggested that the increase of G_{H} and G_{OH} in acid solutions is due to the scavenging of "H" by $\mathrm{H^+}$ in the spurs. Though this may be true, it is difficult to envisage a mechanism by which the addition of $\mathrm{OH^-}$ would augment the yield of $\mathrm{e_{aq}^-}$ (in which form the extra "H" is known to react).

Farhataziz (*Pakistan Atomic Energy Comm. Karachi, Pakistan*) said that he and Anderson had studied the γ radiolysis of acid dichromate solutions and had found that the presence of $\mathrm{H_2}$ increases the yield for reduction. Their proposed interpretation was the occurrence of the reaction $\mathrm{OH\cdot} + \mathrm{H_2} \rightarrow \mathrm{H} + \mathrm{H_2O}$. In sulphuric acid, however, the OH radical is replaced to some extent by the $\mathrm{HSO_4}$ radical which does not react with $\mathrm{H_2}$. Farhataziz wondered whether Shubin and Dolin[m]) had observed a similar effect. In the absence of these authors the same question was put to Schiller[k]) who said they had no evidence for two types of oxidizing radical in acid solutions in the pH range of 0 to 5. He said that in his view OH radicals could not reverse the reduction of $\mathrm{Cr_2O_7^{--}}$ as they had found no evidence of such reversibility. He agreed that in the presence of

hydrogen the yield of reduction might be expected to increase but he did not know the fate of the OH radicals in the absence of hydrogen.

Schiller also suggested (in a written comment) that it was unnecessary to postulate three forms of reducing species to interpret *p*H effects in the radiation chemistry of water. In his view all such effects would be interpreted on the basis of competition for electrons by the ions H_2O^+ and H_3O^+. The initial spatial distribution of H atoms is thus broader in acid solution than in neutral solution and this accounts for higher yields of reducing radical species in acid than in neutral solution[5]).

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Radiation Chemistry of Macromolecules in vivo and in vitro

RADIATION CHEMISTRY OF MACROMOLECULES

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j) H. WILSKI,
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1. INTRODUCTION

In addition to its direct value to polymer scientists, the study of radiation effects in polymer molecules is of considerable importance to other branches of radiation research. The radiation chemist concerned with low molecular weight compounds will be interested in the effects of physical state and molecular weight. The radiobiologist will wish to separate out the purely physical and chemical effects also present in simple macromolecules from the more specific biological ones in his more complex structures. The physicist will be attracted by the problems of energy transfer and radiation protection, and by such phenomena as radiation-induced conductivity and thermoluminescence.

Many of these basic reactions occurring in irradiated macromolecules appear to be deceptively simple, and a large number of the changes produced can be ascribed to the phenomena of cross-linking (dimerization) and degradation (main chain scission). These changes are readily revealed by changes in viscosity and solubility. However these basic reactions are themselves frequently modified by the radiation conditions, and many of the papers discussed here deal in one way or another with such modifications. Because of the variety of materials irradiated, and the different techniques used for their study, it is impossible to present a generalized outline of the main conclusions. I shall, therefore, attempt to indicate for each paper, how far the results are influenced by radiation conditions. Individual authors may then wish to apply or amend my remarks, or draw attention to aspects which I have not considered. Unfortunately not all the contributors are present, and I note with regret the continued absence of one who has had far more experience of radiation work than any of us — I refer of course to the sun.

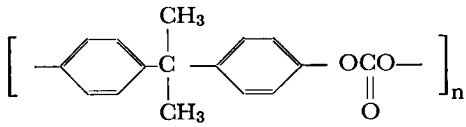
One may consider the papers contributed to cover the following aspects:

1. Irradiation of polymer macromolecules in the solid state, leading to changes in physical properties.
2. Influence of radiation conditions, in particular dose rate, oxygen.
3. Energy transfer and radiation resistance.
4. Effect of solvent.

5. Related biological studies, in which changes in hydrogen bonding, crystallinity and molecular flexibility may be important.

2. RADIATION-INDUCED CHANGES IN PHYSICAL PROPERTIES

Most of the published work on the irradiation of polymers deals with vinyl systems, having a carbon main chain. Golden and Hazell[e]) have carried out a comprehensive study on the effect of radiation on a polycarbonate, including G values (number of molecules changed per 100 eV absorbed), changes in mechanical properties, optical changes and gas evolution. The result is a paper of value to polymer scientists who are concerned with mechanical properties and to radiation chemists concerned with protection and radiation reactions.



polycarbonate.

The polycarbonate degrades under radiation (i.e. suffers main chain scission) with G values for main chain scission of 0.09 *in*

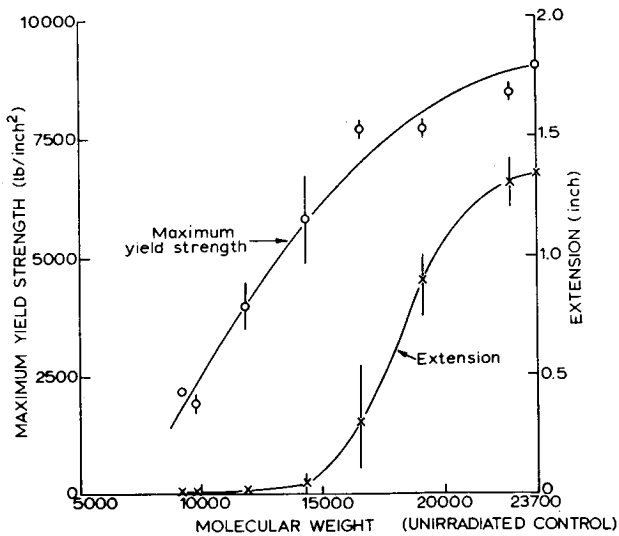


Fig. 1. Yield strength and extension in terms of molecular weight (as modified by radiation). (Golden and Hazell.)

vacuo, and 0.14 in oxygen. These low values are indicative of protection offered by the aromatic group. The gases evolved (mainly CO and CO_2) are due to destruction of the carbonate linkage; there is little evidence of breakdown products from the isopropylidene grouping. The weak point in the macromolecule is therefore clearly pinpointed. The effect of oxygen is to increase the amount of CO_2, with only a minor increase in CO.

The UV spectrum at 305 mμ shows a linear increase in absorption with dose. Colour changes in the visible part of the spectrum are typical of those found in many hydrocarbons, and may be due to conjugated unsaturation or trapped radicals. Infra-red data are also given.

Since radiation causes a reduction in average molecular weight, proportional to dose, the effect of this reduced molecular weight on the mechanical properties can be readily followed. Thus both yield strength and extension fall with reduced molecular weight, though in different ways (Fig. 1). An interesting result is the curve relating fracture energy to molecular weight (Fig. 2). Notable is the mole-

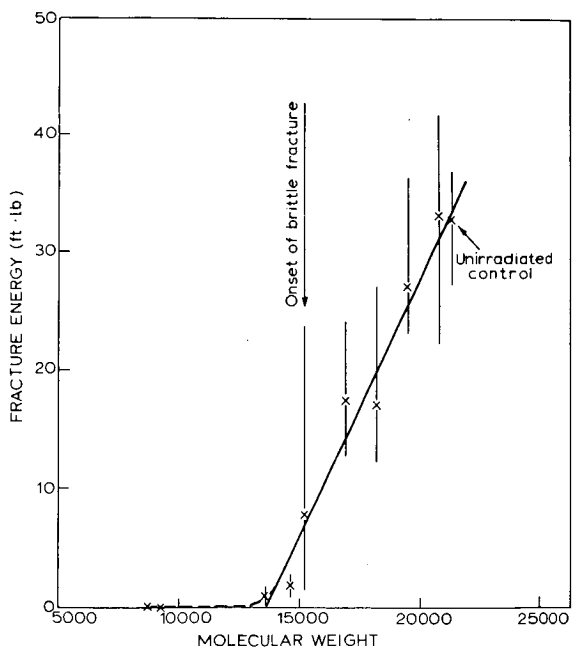


Fig. 2. Fracture energy in terms of molecular weight. (Golden and Hazell.)

cular weight at which brittle fracture begins to appear. One would be interested to hear whether the same relationships are found at different rates of stressing. This paper may be taken as an excellent example of the use of radiation to study physical properties in polymers.

3. ESR DATA ON TRAPPED RADICALS

In many irradiated macromolecules it is difficult to follow the individual steps occurring between the initial ionization and excitation, and the final chemical changes. This difficulty arises because in measuring the chemical change, the specimen is treated in some way (e.g it is heated or dissolved). One does not know whether the major chemical changes have occurred immediately following irradiation, or during subsequent treatment. This problem can be solved in part by the use of electron spin resonance (ESR) to determine the nature of any trapped intermediate radicals. Polymethylmethacrylate (PMM) is a promising system to study because of its rigid structure at room temperature. Unfortunately the ESR pattern is found to change on standing; it is affected by the presence of trace monomer, and is apparently inconsistent with the reactions we expect to occur. The paper by Sliemers, Ungar, Gulbaran, Gager, Kircher and Leininger[1]) follows their previous work in attempting to elucidate the problem. It thereby provides information on the reactions which can occur in a solid matrix.

A number of methacrylate polymers were studied; the gases evolved give an indication of the likely radical sites. These may then be related to the ESR patterns as shown in Table 1.

Although the number of chain scissions increases uniformly with dose, the overall number of radicals measured by ESR does not increase in the same way, but tends to a maximum value at about 10 to 30 Mrad (Fig. 3). The disappearance of these radicals may be attributed to their interaction in the solid state. This is also shown by the fact that their concentration is lower than that expected from the amount of gas evolved. The maximum concentration corresponds to an average distance between radicals of about 50 Å. This limiting spacing has been attributed by the authors to the mobility of the chains. I would like to suggest that since distances of the order of 100 Å have frequently been observed in ESR patterns of irradiated polymers and in protection effects, a

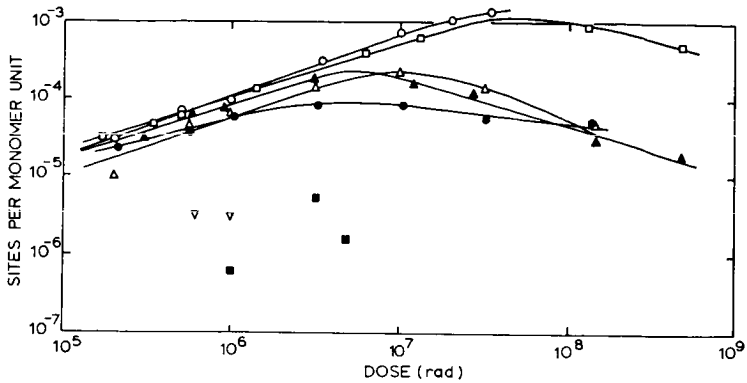
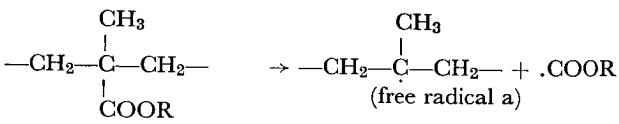


Fig. 3. Sites per monomer unit versus dose for polymethacrylic acid and several poly-n-alkylmethacrylates. (Sliemers, Ungar, Gulbaran, Gager, Kircher and Leininger.)

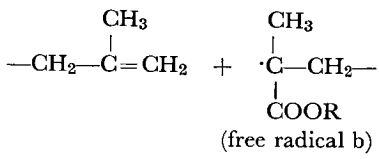
- ○ Polymethacrylic acid
- □ Polymethylmethacrylate
- △ Polyethylmethacrylate
- ▲ Polypropylmethacrylate
- ● Polybutylmethacrylate
- ▽ Polyamylmethacrylate
- ■ Polyhexylmethacrylate

more basic property is involved, possibly the range of energy transfer prior to the formation of radicals, or the range at which H atoms react with radicals rather than with each other.

The reactions suggested by the authors are as follows.



Scission then occurs with double bond formation giving

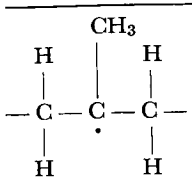
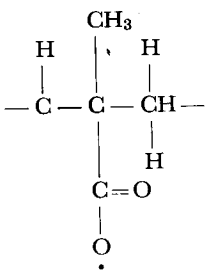
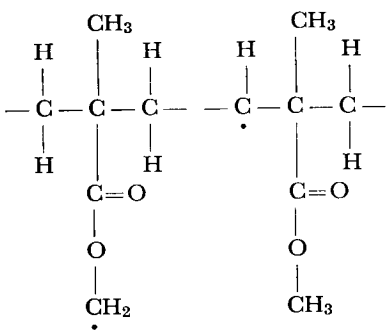


Free radical a can give a 4, 5 or 6 line spectrum, depending on orientation of the methyl. Free radical b gives the characteristic 5–4 spectrum. H and CH_3 radicals are also produced, but are assumed to be shortlived. To account for the observed ESR pattern, the authors assume that it is due to the superposition of spectra from a and b, giving rise to the initial broad spectrum, which subsequently sharpens as a converts to b. Any monomer present will

TABLE 1

Estimated concentrations of various free radicals on the polymer chain in irradiated polymethylmethacrylate(A)

(*Sliemers, Ungar, Gulbaran, Gager, Kircher, Leininger*)

Type of free radical		Concentration in PMMA, sites per monomer unit × 10^3
	(B)	2.52
	(C)	0.42
	(D)	2.70
total		5.64
total by ESR		0.8

(A) Based on results obtained from gas chromatography and mass spectrometry.
(B) Calculated from sum of concentrations of CO, CO_2, and esters.
(C) Calculated from sum of concentrations of hydrocarbon, CH_3OCH_3, and CH_3COOCH_3 minus CO_2.
(D) Calculated from sum of concentrations of H, CH_3OH, $HCOOCH_3$, and CH_4.

also react with a to give a branched radical structure, showing the same ESR spectrum as b.

One point that might deserve elucidation is the contribution of other radicals which, according to the gas evolution data, must be produced. What happens to the ESR patterns of the radicals C and D of Table 1 which, according to the data in the paper, represent 55% of the total radicals in PMM?

4. PULSED RADIATION

As part of a programme on factor-dependency in radiation effects, the paper by Degering, Calderella and Evans[b]) investigates the many parameters other than radiation dose, which influence the degree of polymerization of styrene[1]). Whereas most of the published work in this field deals with low intensity continuous radiation, so that steady state conditions may be assumed, Degering uses high intensity pulsed electron radiation with dose rate effects which may involve not only the propagation chain, but the initiation and the lifetime of the growing chains. Furthermore, some contribution from ionic polymerization may also be present since this is favoured at high intensities.

A number of other variables are also investigated including the presence of various gases (oxygen, nitrogen, helium), temperature, moisture, additives such as acrylic acid.

The overall yield falls off with increasing dose rate, though not as rapidly as might be expected from the radical reactions studied at low gamma intensities. The temperature coefficient corresponds to an activation energy in the range 5 to 8 Kcal/M for pure styrene, but much less in a 1/1 solution in benzene.

5. OXYGEN EFFECT AND PHYSICAL PROPERTIES

The paper by Wilski[j]) deals with the effect of dose rate on the tensile strength and viscosity of various polymers irradiated in air. When irradiation is carried out *in vacuo*, the resultant changes in molecular weight and consequent mechanical properties are independent of dose rate, and depend only on the total dose[2]). On the other hand, marked dose rate effects appear when irradiation occurs in the presence of oxygen. In this paper this dose effect has been measured in terms of the changes in viscosity and tensile strength at the same overall dose. Polyethylene and Caprolactam for example show a

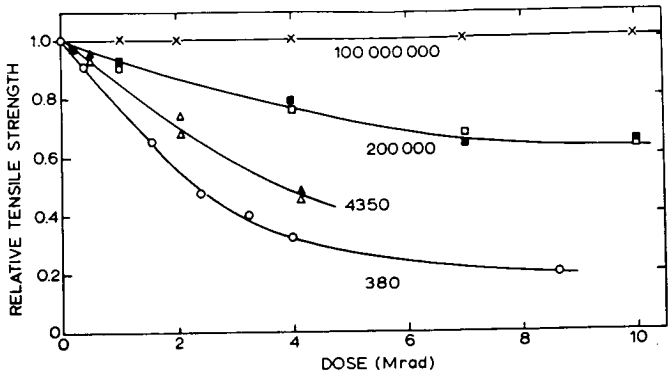


Fig. 4. Tensile strength of low pressure polyethylene, irradiated in the presence of air. (Figures give dose rate in rad/hr.) (Wilski.)

reduced strength at dose rates of 380 rad/hr, but little if any at 10^8 rad/hr (Fig. 4). Polypropylene shows an even more marked effect at low doses, but is in any case very sensitive to radiation even in the absence of oxygen. Polyvinyl chloride shows only a small dose rate effect and polyethylene terephthalate none at all. This is in agreement with the known resistance of these polymers, and is clearly determined by oxygen solubility and diffusion. It would be helpful if these changes in physical properties could be correlated with the oxygen reacting, e.g. by measurement of carbonyl.

We may perhaps lead on from this analysis to consider two basic problems.

(i) Oxygen presumably causes a weakening of the polymer by reducing its average molecular weight. Does it do this by stabilizing broken main chain links, produced by radiation, which might otherwise recombine to eliminate the initial damage? Or does it induce some further chemical reaction which eventually produces a main chain break?

(ii) The reaction of oxygen with radicals trapped in the crystalline region of these polymers can occur long after radiation has ceased. Does this occur by diffusion of oxygen into these crystallites, with the radicals completely immobile, or by the movement of radicals out from the crystallites into the amorphous regions where there is plenty of oxygen? In fact, are these radicals mobile along the chain (e.g. by a H transfer process), and if so, is this the major cause of post-irradiation effects?

6. INTER- AND INTRAMOLECULAR ENERGY TRANSFER

The paper by Gardner and Smith[d]) attempts to analyse the rather ill-defined process of energy transfer which occurs immediately after the incident radiation deposits energy in a system, and before any chemical reaction has occurred. Some such transfer is needed to account for the fact that, while energy absorption is usually taken as substantially random, the ensuing chemical effects are specific. The presence of certain groups, and particularly aromatics, is known to reduce the net effect of exposure by an amount which is greatly in excess of its direct energy absorption (assumed random[3])).

Various mechanisms have been suggested for the process; quantum mechanical resonance processes acting over large distances; stepwise processes somewhat akin to diffusion; direct transfer via photons. A recent approach considers the initial energy absorbed to be essentially delocalised at first (in accordance with the uncertainty principle), and only subsequently is it tagged onto one group. These various alternatives are not necessarily exclusive.

Gardner and Smith investigate the problem by studying the degree of "protection" offered to polymethylmethacrylate (PMM) against degradation by UV or high energy electron radiation. Two systems are chosen: one in which PMM is intimately mixed with n-propylbenzene, although the molecules are separate; the other in which PMM is copolymerized with α methylstyrene. Thus in the first system any protection offered by the aromatic group is necessarily intermolecular; in the second system it can be intramolecular. Closer coupling may be expected in the second system, giving a higher degree of protection against degradation. Radiation damage can result either from ionization, or from excitation, and the authors compare the effect of UV light with electron radiation; the former produces only excitation, the latter excitation and ionization. Fig. 5 shows the reduction in degradation (per unit additive) in terms of concentration, position of aromatic, and type of radiation.

The results show that protection against excitation is somewhat greater (1.47 ± 0.5) when the protecting aromatic structure is present in the chain. For ionization, protection is greater by a factor of 2 when the aromatic structure is present in the PMM molecule.

Thus to reduce the effect of excitation by one half, 0.7% of aromatics is needed in the chain, or 1% if adjacent to it. The same

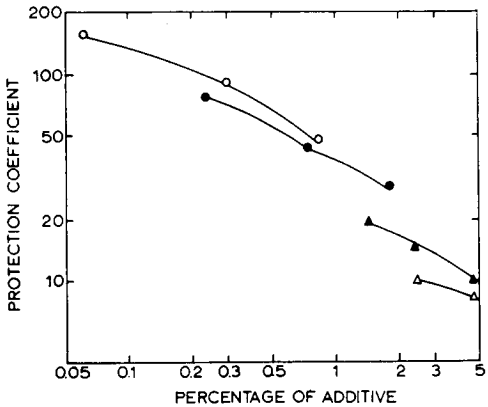


Fig. 5. Protection against degradation of polymethylmethacrylate by UV or electron irradiation. (Gardner and Smith.)

Abscissa: Per cent of additive.

Ordinate: Protection coefficient which is the ratio of the fraction of energy removed by the additive over the concentration of the additive.

—▲— electrons

—○— UV, PMM copolymerized with methylstyrene.

—△— electrons

—●— UV, PMM mixed with n-propylbenzene.

degree of protection against ionization requires 6% aromatics in the chain, or 12.5% adjacent to it. Thus energy transfer within a molecule is more effective than between molecules. Then it may be concluded that the absorbed energy is not delocalized over a large volume, comprising a number of molecules, since it would not then distinguish between inter- and intramolecular protection effects.

One may compare this work with earlier results on aromatic groups attached to alkyl chains, in which protection falls off at distances of about five carbon atoms; similarly aromatic groups in a styrene-isobutylene copolymer protect over about three monomer units.

The protection against radiation effects due to the presence of aromatics is often taken for granted. It does however require closer study. For example a question may well be asked concerning the irradiation of PMM in dilute benzene solutions. Why is the degradation of PMM by UV and by ionizing radiation so little affected by the presence of a very high concentration of aromatics in its immediate neighbourhood?

7. RADIATION DAMAGE AND NUCLEAR QUADRUPOLAR RESONANCE

The paper by Read and Depireux[h]) deals with a new technique for determining overall radiation damage in polymers, and in particular in measuring their radiation resistance. Since it is based on the use of nuclear quadrupolar resonance, it is primarily confined to nuclei such as Cl, Br, I, Cu. However by the use of additives containing such nuclei, as probes in a polymer matrix, damage in the latter may be evaluated.

For a pure crystalline lattice with quadrupolar nuclei, a sharp resonance line is found in the nuclear magnetic spectrum. If impurities are present they reduce the intensity of the line, due to (i) their electric fields, and (ii) the distortion of the electronic shells. These displaced resonances are shifted and lost in the background. Thus the intensity of the resonance line falls off exponentially with concentration of impurity.

If the damage is caused by irradiation, an exponential relation is also found between the intensity I of the resonance line and the radiation dose D.

$$I = I_0 \exp(-Kv_r D)$$

where v_r is the perturbed volume, and K represents the radiation sensitivity of the material. v_r varies little with the type of impurity. Thus a smaller value of K represents a more radiation resistant material. This agrees with experiments using aromatic or aliphatic crystalline solids. The method differs from the usual radiation chemical techniques in that it integrates the damage from all the radiation defects.

The authors then extend this technique to the use of polymer systems with no quadrupolar nuclei. This is achieved by adding about 5% of a probe molecule which does give nuclear magnetic resonances. Experiments proved that with different probe materials in a matrix, consistent results were obtained.

The technique was used for matrices of polyethylene glycol (PEG) of varying molecular weights, with 5% p-dibromobenzene as probe. In addition to the resonance from the probe, a new sharp resonance doublet is also observed, possibly due to some molecular complex. Fig. 6 shows the reduction in the resonance signal for the probe resonance, and for the new doublet. The curves are linear with dose (although they do not extrapolate to the same point). Since the

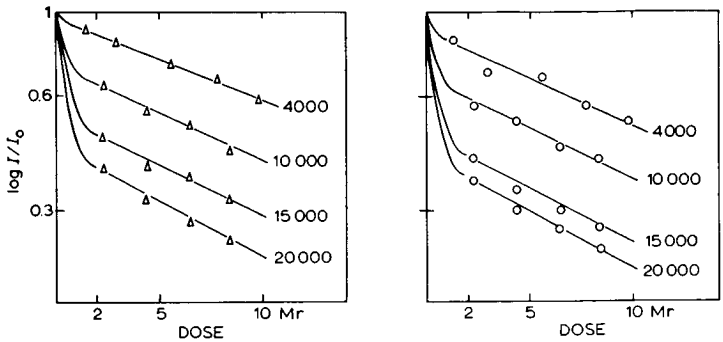


Fig. 6. Reduction in resonance amplitude due to radiation damage in polyethylene glycol. (Read and Depireux.)

curves have different slopes, depending on molecular weight, the authors conclude that the higher molecular weight PEG have lower radiation resistance. This conclusion could presumably be verified by other means, and may perhaps be compared with the influence of molecular weight on the behaviour of linear paraffins.

This use of quadrupole resonance appears of interest as a new technique for studying radiation effects, but it obviously requires further testing before its uses and limitations are clearly understood. The concentrations of probe material appear somewhat high, perhaps higher than are customary in radiation protection work for example.

8. INFLUENCE OF SOLVENT

We can now turn to the effect of solvents on the behaviour of polymers irradiated in solution. Here we have both direct and indirect effects, and also other possible reactions. The paper by Dieu and Desreux[c]) is concerned with this problem and also the abnormal behaviour of polyvinyl chloride (PVC) under irradiation.

One would expect PVC to crosslink readily under radiation, particularly as it has Cl atoms which are very radiation sensitive. In fact doses of about 50 Mrad are needed to render the polymer partly insoluble. Certain anomalies also appear in its behaviour when irradiated in solution. The authors have therefore studied the polymer in 0.5 and 2% solutions in five solvents: cyclohexanone, o-dichlorobenzene, 1–4 dioxane, tetrahydrofurane and dimethyl formamide.

The importance of polymer concentration[4, 5]) has been shown in previous experiments, e.g. on polyvinyl alcohol, which crosslinks readily in aqueous solutions at 1% concentration or above, but not at all at 0.5% or less. This distinction is usually ascribed to competition between internal and external linking. Each crosslink requires the combination of two radicals, and at sufficiently low concentrations one radical is more likely to react with another on the same polymer chain, rather than with a radical on a more distant polymer. This leads to cyclization and microgel rather than full gel formation.

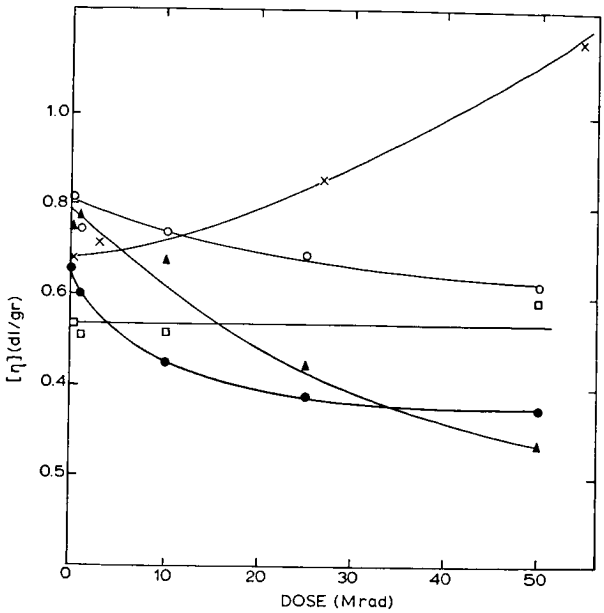


Fig. 7. Change in viscosity of PVC (2%) solutions irradiated in various solvents. (Dieu and Desreux.)

○ cyclohexanone, ● dioxane, ▲ tetrahydrofurane
■ dichlorobenzene, × dimethyl formamide

In the solvents studied, the behaviour of the dissolved PVC has been followed by viscosity measurements, sedimentation and in one case, infra red spectroscopy. Three different patterns of behaviour are observed: (see Fig. 7)

(i) In two solvents (cyclohexanone and o-dichlorobenzene) there

is little change up to doses of 50 Mrad. This appears to be due to the nature of the radicals formed in the solvent, which, according to mass spectrometer data may be expected to be Cl· and C_3H_6·.

(ii) In two solvents (1–4 dioxane and tetrahydrofurane) there is a reduction in intrinsic viscosity and an increase in the sedimentation distribution. The corresponding solvent radicals are expected to be CH_2O·.

(iii) With dimethylformamide (major radical $H\dot{C} = 0$) the same effects are observed as in (ii), but only at low concentration (0.5%). At higher concentration (2%) crosslinking occurs, with a rise in viscosity and of the sedimentation constant. These results show the considerable importance of both concentration and type of solvent.

In the second series of solvents the authors conclude from the reduction in viscosity that the polymer degrades. Since this same behaviour is observed in 0.5% solutions in dimethylformamide, we might deduce that this solution also degrades, whereas the 2% solution certainly crosslinks. It would therefore help us to know whether the same results cannot be explained primarily in terms of crosslinking; in the second series of solvents (0.5% and 2%) due to the shape of the polymer molecules in solution, such links are predominantly internal, whereas in dimethyl formamide internal crosslinks only predominate at the lower concentration of 0.5% .If this is true, we would expect crosslinking and gel formation to occur in the second series of solvent at higher concentration.

To account for the high gelation dose found in PVC may we consider the possibility of both crosslinking and degradation occurring to nearly equal extents? In polypropylene for example, which has the same —CH_2—CHR— structure, this is certainly the case, resulting in a high gel dose. End linking may also be a possible reaction, as suggested by the authors.

9. DOSE RATE EFFECTS IN POLYMER SOLUTIONS

Odours and flavours are produced in radiation processing of food and it has been claimed that these are smaller at very high dose rates. The milk protein β lactoglobulin was chosen by Hansen, Glew and Ley[f]) since it is a food protein readily prepared in pure form, and contains cysteine, cystine and methionine, the breakdown products being possibly responsible for the flavour changes. In the first paper by Hansen and colleagues attention was directed

to the effect of concentration, *p*H of solution, and temperature, while in a second contribution, the effect of oxygen was investigated. The third paper in the series, and the one contributed to this meeting, deals primarily with dose rate effects. Apart from their relevance to the specific system under study, the results obtained are of considerable interest in the study of radiation induced reactions in solutions of macromolecules.

Three aqueous concentrations (0.1, 1 and 10%) of β lactoglobulin were studied. Irradiation was carried out either with very high intensity electrons, pulsed at various rates, or with cobalt gammas at a much lower uniform radiation intensity. The effect of these radiation intensities was followed by four parameters; UV absorption, viscosity changes, concentration of sulphydryl groups and solubility. Any differences in the effects due to this extremely wide range of dose rates is capable of providing information on the lifetime of the active species. Unfortunately measurements on the effect at the three concentrations used are not usually measured in terms of the same parameter, so that far less information can be gained on the importance of concentration.

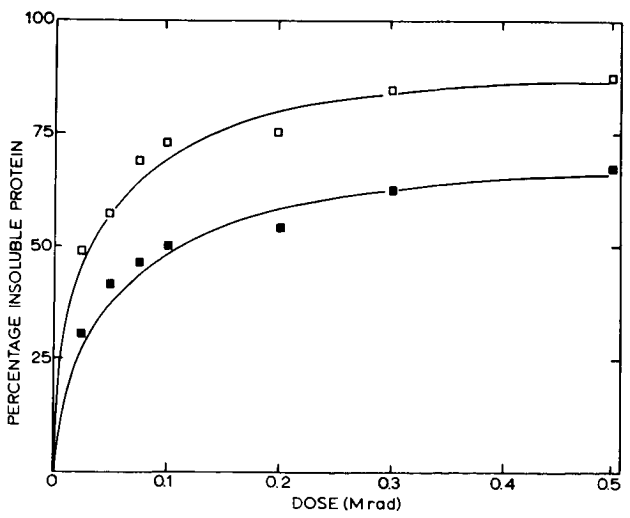


Fig. 8. Effect of electron dose rate on change in solubility of 0.1% solutions in 0.004 *M* $NaHCO_3$. (Hansen, Glew and Ley.) The samples were made up in a nitrogen atmosphere and then sealed.

— □ — 1.1×10^6 rad/sec.

— ■ — 1.1×10^8 rad/sec.

The main conclusions are as follows:

(i) At high concentrations (20%) there is no significant dose rate effect in the ranges 2.49×10^9 to 2.66×10^7 peak rad/sec. The viscosity increases steadily in this range showing crosslinking as a function of dose only.

(ii) At very low concentrations (0.1%) a dose rate effect appears on the viscosity and on solubility, (Fig. 8) which is however small compared to the wide range of dose rates studied (1.11×10^8 to 2.22×10^6 peak rad/sec). At first the viscosity rises, then falls, and this is ascribed by the authors to initial unfolding or crosslinking, followed by degradation at higher doses. On the basis of the data presented in the paper, this can be disputed, and there is still considerable doubt about the behaviour of very dilute macromolecular solutions under irradiation. One alternative is some form of competition between inter- and intramolecular crosslinking, with the formation of globular microgel. A further factor is the influence of oxygen solubility and diffusion.

(iii) Changes in UV absorption, depending on peak dose rate, are also observed in 0.1% solution, although in view of the very great intensity differences (a factor of hundred in peak dose rate), these changes are not very large.

(iv) The solubility of oxygen-free 1% solutions is also affected; a hundred fold increase in peak dose rate (1.1×10^6 rad/sec to 1.1×10^8 rad/sec) merely reduces the effect of a dose by a factor of 3.

(v) At the same peak dose rate, but at different pulse frequencies, there was no difference in SH formation nor in solubility of 0.1% solutions. On the other hand SH formation at low dose rates from ^{60}Co gammas does show such an effect.

Various interesting features arise from this investigation. One concerns the concept of initial protein unfolding, followed by crosslinking or degradation. The second relates to the lifetime of the active groups; if, as the authors state, all reactions in the protein system are complete in less than 10^{-3} sec, then the dose rate effect for γ rays is difficult to explain since two separate ionization tracks will not cover the same volume within this period. Yet such an overlap is necessary to account for an intensity dependence.

Another question arises. Are the various effects observed always directly related to one another? For example is SH decay affected

to the same extent by dose rate as viscosity or solubility? Much of the information to answer these points is referred to in passing by the authors, and it is hoped that they will publish their valuable data in the fullest detail.

10. HYDROGEN BONDS

The effects of irradiation on soluble proteins has been extensively studied. The paper by Bailey and Rhodes[a]) deals with the corresponding problem for the insoluble fibrous protein collagen, the fibrous protein of connective tissues. The changes produced are studied both in terms of the temperature and extent of hydrothermal shrinkage, and also of changes in solubility.

Collagen has an oriented, almost crystalline structure, which can be modified by radiation. Radiation primarily destroys the hydrogen bonds binding the triple helix, and the helices to each other. As a result the thermal stability of the molecule is reduced (Fig. 9).

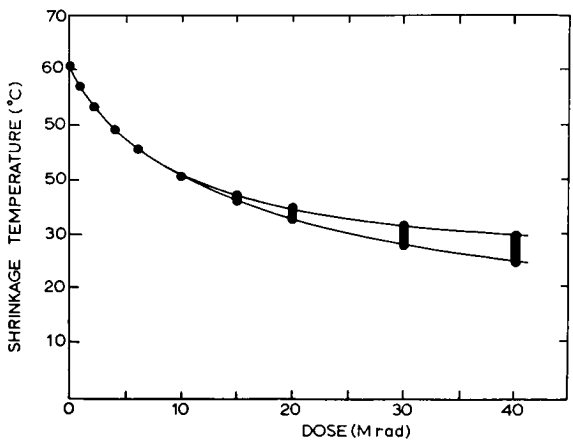


Fig. 9. Effect of irradiation on the shrinkage temperature of rat tail collagen. The two lines indicate the increasing temperature interval between which shrinkage occurs after higher doses. (Bailey and Rhodes.)

Simultaneously the shortening under a small load rose from a few percent with no radiation, to a maximum of 70% at 6 Mrad (Fig. 10). At doses below 6 Mrad, a further shrinkage occurred at the usual (unirradiated) shrinkage temperature, indicating only partial dam-

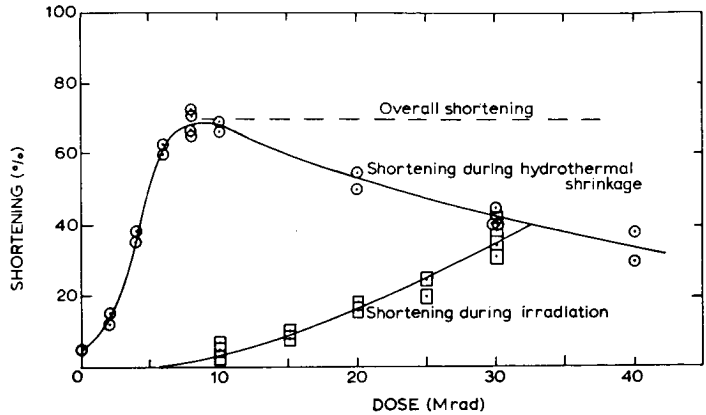


Fig. 10. Shortening of rat tail fibres during irradiation and subsequent hydrothermal shrinkage. (Bailey and Rhodes.)

age. Thus a dose of 6 Mrad is required to completely collapse the original structure.

With macromolecular structures it is difficult to account for such marked changes with relatively modest doses. The explanation is due to the hydrogen bonding. According to theoretical work by Platzman and Franck, 15 to 20 H bonds are ruptured per ionizing event. There are about 300 H bonds for each of the three helix chains, and there are 5 ionizing events per collagen triple helix for a dose of 6 Mrad. Thus about 10% of the hydrogen bonds are broken, and it is this which accounts for the collapse of the structure.

Reduction in solubility (due to crosslinking) was observed when the fibres were irradiated in the presence of water (Fig. 11). Although this might be explained as due to the indirect effect of the water producing H and OH radicals, futher evidence contradicts this view. A more plausible explanation is the increased flexibility of the protein chains in the presence of water; it is known that macromolecules such as polyvinyl alcohol (crystalline) can only be crosslinked in the presence of trace water, which allows some motion of the chains. This is further shown by the fact that the heat shrunk and water swollen fibres are more readily crosslinked, due to their disordered structure. The density of crosslinking quoted by the authors corresponds to a G value for crosslinked units of 3.5, in

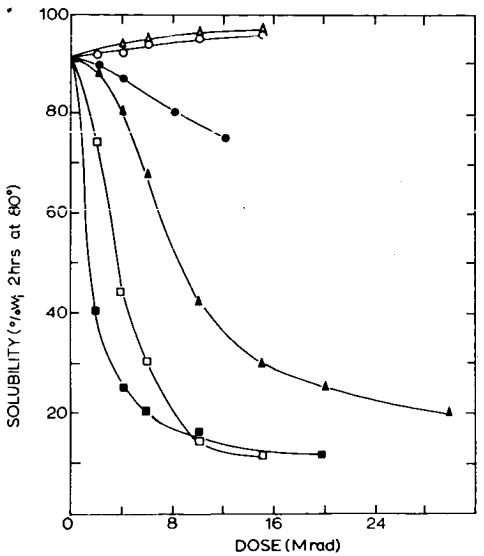


Fig. 11. Effect of ionizing radiation on the solubility of collagen (rat tail tendon) (Bailey and Rhodes.)

—■— water swollen, 0° C
—□— heat shrunk, 0° C
—▲— 0° C saline
—●— 0° C, oxygenated saline
—○— — 20° C saline
—△— dry.

good agreement with many other macromolecules, but of course far lower than the loss of hydrogen bonds. The nature of these crosslinks is as yet unspecified. A suggestion that such bonds occur via —S—S— links or tyrosine groups is not applicable in view of their virtual absence in collagen. As there is some evidence for the radiation protection effect of sulphur, one might expect the *G* value for crosslinking in other proteins to be lower.

11. UV AND IONIZING RADIATION

The importance of environment in simple polymer systems is also revealed in the more complex biological system studied in the paper by Moroson and Alexander[g]). In previous work the direct effect of ionizing radiation on DNA was found to promote crosslinking and consequent insolubility. Now the corresponding reactions are studied *in vivo* for both UV and ionizing radiations. With UV irradiation DNA could be crosslinked in sperm heads, though not in

E. coli or lymphoma cells. With ionizing radiation crosslinking between DNA molecules is marked in sperm heads, but is only slight in *E. coli* and lymphoma cells. Crosslinking between DNA and protein in the cell occurs with UV radiation, which in this case is more effective than ionizing radiation.

The most general conclusion to be drawn from this series of papers is that they show, for a wide range of macromolecules, the considerable influence which can be exercised on the basic reactions by such factors as dose rate, concentration, and type of solvent, oxygen diffusion, crystallinity, hydrogen bonding, and molecular flexibility. Thus in studying such basic reactions considerable care must be exercised in controlling these conditions.

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DISCUSSION

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At the invitation of the Chairman, two of the authors replied to questions raised by the rapporteur. Dieu[c]) said that he had previously given a clear-cut demonstration of the internal crosslinking of polyvinyl alcohol in aqueous solution at low concentration. This was shown by a strong decrease in viscosity and a strong increase in sedimentation constants. Such important changes were not observed for polyvinyl chloride irradiated at 0.5% concentration in the three active solvents (including dimethylformamide). The sedi-

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mentation constants of the peaks did not change very much, but there was an important spreading of the distribution of the sedimentation constants towards lower and higher values. Hence although at such doses, internal linking in these three solvents is possible, degradation and probably endlinking are certainly very important. At high doses, in all cases, there is an important change in the chemical composition of the system, so that we do not really know whether it is possible to use the physico-chemical relations which are valid for the non-irradiated polymer.

Leininger[i]) said that work was still proceeding on the non-appearance in the electron spin resonance spectrum of the radicals formed by abstraction of hydrogen from the chain backbone and from the methyl ester. These radicals were postulated to explain the formation of hydrogen, alcohol and ester as radiolysis products. They are perhaps short-lived and disappear through interchain reactions (crosslinking) or by migration along the chain to form double bonds by an intra-chain reaction of two radicals.

Leininger's explanation of the appearance of maxima in the number of sites made use of the influence of increasing molecular mobility. This was based on the known degradation of polymethacrylates during irradiation and on observations of different maxima starting with polymers of varying molecular weight.

Leininger also made a comment on the paper by Gardner and Smith[d]). Leininger and his colleagues had studied polyphenylmethacrylate and found that it is very comparable to polymethylmethacrylate in stability, i.e. the phenyl group, when separated from the main chain by an ester linkage, afforded no protection in contrast to its action when attached directly to the chain as in poly-α-methylstyrene.

Referring to Charlesby's report on Degering's paper[b]), Roberts (*UKAEA, Wantage, Berks., Great Britain*) said that he and his colleagues had not found any change in the mechanism of styrene polymerization at dose rates up to 10^8 r/hr. The rate was still proportional to $\sqrt{I}$. At room temperature there is no evidence for anything other than free radical processes.

Uri (*Min. Aviation, Waltham Abbey, Essex, Great Britain*) directed some questions to Wilski[j]).

(1) Wilski interpreted the observed dose-rate effect in the radiation-induced oxidation of polyethylene as entirely due to the rate

of oxygen diffusion. In view of the importance of this assumption, Uri wondered whether Wilski had attempted a quantitative correlation of the dose-rate effect with oxygen diffusion data.

(2) Could Wilski offer any explanation for the extraordinary stability of polyethylene terephthalate, and had he found an upper limit for the radiation tolerance of this polymer in the presence of oxygen?

(3) Had he measured the effect of added antioxidants in the radiation-induced oxidation of polyethylene?

(1) Wilski replied that polymer degradation was a result of oxidation. (This could be shown, for example, for the polyolefines, by the increasing absorption of the C=O group in the infra red.) This oxidation is oxygen-diffusion controlled and, therefore, dependent on the dose rate. A quantitative treatment is in progress but not yet finished.

(2) Perhaps polyethylene terephthalate is stable because of its benzene rings. It should be noticed that it is also very stable against photo- and thermal oxidation. As regards the upper limit for radiation stability, the experiments were stopped after about 3 yr when the sample had had a dose of about 10 Mrad. The upper limit for short time irradiation can be found in the papers of Bopp and Sisman[1]).

(3) The commonly used antioxidants and stabilizers (the ones effective against thermal and photodegradation) are ineffective against γ irradiation. However there are some quite effective substances.

Hunter (*41 St. Bernards Rd., Solihull, Great Britain*) had carried out experiments on the polymerization of vinylchloride with conversions of up to 90%. There was little change in the molecular weight at different stages of conversion in these experiments. There was no evidence of any serious degradation as determined by evolution of hydrogen chloride, nor was there any obvious evidence of any substantial crosslinking. Bawn of Liverpool University had spoken privately of evidence for crosslinking in the gamma irradiation initiated polymerization of vinylchoride, but Hunter had not obtained evidence of this.

Okamura (*Kyoto Univ., Japan*) asked some questions about Gardner's[d]) paper. Had Gardner done any experiments to compare the rigidity or softening point of his two systems, and had he done any

work on the change in properties of poly-α-methyl styrene? Regarding the first question, the rigidity of polymer systems has a large influence upon the reactions occurring under irradiation, because differences in the solid state may induce changes in the tendency of polymer radicals to recombine. Poly-α-methylstyrene is interesting since according to experimental results by Okamura and colleagues, poly-α-methyl-styrene undergoes some degradative type reactions associated with the methyl side chain, like those undergone by polymethylmethacrylate. Also this polymer crosslinks at high doses. Hence some of the α-methyl styrene units in the copolymer molecules may undergo both degradation and crosslinking.

In answer to the first part of this question, Gardner had not studied the effect of radiation on any of the mechanical properties of the polymer systems. Work on the pure α-methyl styrene polymer was not yet complete but Gardner was interested in the comment that the α-methyl styrene polymer crosslinks under irradiation.

Ormerod (*RMCS, Swindon, Great Britain*) made some comments on Leininger's paper. Ormerod and colleagues had used electron spin resonance to study polymethacrylates and acrylates at 77°K. After irradiation at 77°K a single line was observed, which was due to trapped electrons as indicated by studies with additives. This ESR spectrum was very sensitive to the presence of impurities owing to the electrons being trapped in different environments. This may explain why the presence of monomer affected the room-temperature spectra of Leininger *et al.* Secondly, on keeping low-temperature-irradiated samples at 77°K, the trapped electrons decayed giving rise to radical intermediates. These intermediates disappeared immediately on warming to room temperature, leaving the well-known stable room temperature spectrum. These radical intermediates are probably those postulated by Leininger *et al.* Their failure to observe them is due to the instability of the radicals, but the intermediates can be observed if the correct radiation conditions are used.

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Radiation Chemistry of Macromolecules in vivo and in vitro

DNA AND THE EFFECTS OF RADIATION

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Medical Research Council
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a) B. DJORDJEVIC,
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Institute of Nuclear Sciences "Boris Kidrich", Beograd, Yugoslavia

b) M. DRAKULIC and E. KOS,
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Department of Chemistry, King's College, Newcastle-on-Tyne, Great Britain

d) F. HUTCHINSON,
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Yale University, New Haven, Connecticut, USA

e) R. F. ITZHAKI,
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Department of Radiotherapeutics, University of Cambridge, Great Britain

f) B. MILETIC, Z. KUCAN, S. STAVRIC, DJ. NOVAK and LJ. ZAJEC,
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H.M.: Sloan-Kettering Institute, New York, USA. P.A.: Chester Beatty Research Institute, London SW 3, Great Britain

h) S. OKADA and L. H. HEMPELMANN,
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Division of Experimental Radiology, University of Rochester, Rochester, New York, USA

i) K. C. SMITH,
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Department of Radiology, Stanford University School of Medicine, Palo Alto, California, USA

j) N. B. STRAZHEVSKAYA and V. A. STRUCHKOV,
Molecular structure of DNA and radiation damage to cells.
Institute of Biological Physics, Academy of Sciences, Moscow, USSR

1. THE IMPORTANCE OF DAMAGE TO DNA

The very arrangement of the three plenary sessions in this group exposes at once one of the attitudes widely adopted in present thinking in radiobiology, one that can be expressed most simply as: radiation damages a vital macromolecule; that vital macromolecule is DNA; this damage sets in train a series of biochemical events which lead to the visible effects, cell death, chromosome abnormalities, mutation and so on, which are the basic matters of our subject. This attitude has a respectable history and I can only briefly justify it in general terms before describing the papers in my charge.

The first step in the argument is that the initial biochemical lesion is a single radiation event which can affect only a few atoms. In order that this single event can be so important it is likely that these few atoms must be part of a bigger unit, the most likely candidates being the high polymer structures, the proteins and the nucleic acids. Charlesby has in the previous report discussed some of the profound changes in the structure and properties of synthetic polymers which radiation can produce. Indeed more than twenty-five years ago the effect of X rays on the physico-chemical properties of proteins and DNA were already being studied, a study still intensively pursued.

For transforming principle and the simpler viruses, there is abundant evidence for the second step in the argument that it is DNA which constitutes the important macromolecule, and one would like to extrapolate to the cells of bacteria and higher organisms. (In the case of ultra-violet light, the biological efficiency is greatest for the wavelengths most strongly absorbed by nucleic acid. The initial events due to ionizing radiation are distributed at random.) The central rôle which DNA plays in molecular biology makes this hypothesis attractive, especially for mutagenesis when the final effect is certainly a change in the hereditary material. It is our lack of knowledge about the third step which prevents us from holding this hypothesis as anything more than a working model. Alper will take up this point and indicate that it is by no means the only mechanism for radiobiological effects. However, our current prejudices predispose many of us towards it and one line of research has recently lent a great deal of strength to this argument. Zahmenhof, De

Giovanni and Greer[1]) showed that bacteria which have incorporated 5-bromouracil (5-BUDR) in place of thymine are much more sensitive to lethal effects of UV-irradiation than are bacteria which contain normal DNA. This increased sensitivity is shown even by biologically active DNA, transforming principle, which contains 5-BUDR (Szybalski)[2]). So far this sensitization has only been observed with analogues which enter DNA.

If we make the assumption that damage to DNA is the primary radiation event, we can ask questions about what kind of damage is important.

The complexity of the structure of the twin helix and our ignorance of the metabolic processes which involve DNA make this a difficult question, even if we were to ignore the protein part of the nucleoprotein complex in which DNA exists in the cell. As far as DNA itself is concerned we can envisage some seven kinds of damage, those listed in Table 1.

TABLE 1

1. Change of a base (for example deamination)
2. Loss of a base
3. Single chain fracture (one phosphodiester backbone)
4. Double chain fracture (both chains simultaneously)
5. Cross linking within the helix (for example thymine dimerization)
6. Cross linking to other DNA molecules
7. Cross linking to protein

All seven kinds of damage have been studied and all seven demonstrated to occur. Which of these effects is important and to what extent there are mechanisms in the cell to repair or bypass this damage[3–5]), and what rôle oxygen and other agents play in enhancing or reducing the damage, is now one of the most interesting studies: the papers I have now to review bear on all these points and in their different ways exemplify the difficulties in this field.

2. RADIATION AND TRANSFORMING PRINCIPLE

The kinds of damage listed in Table 1 which have been observed in DNA after irradiation have been based on physical and chemical methods; using transforming principle it is possible to correlate these observations, to some extent at least, with a biological property which is easily measured. Hutchinson[d]) has made an analysis

TABLE 2

Treatment	Size	Authors
X irradiation of dry TP		Marmur and Fluke (1955)[6]
		Guild and DeFillipes (1957)[7]
X irradiation of frozen solutions	190000	Ephrussi-Taylor and Latarjet (1955)[8]
X irradiation of dry cells		Hutchinson and Arena (1960)[9]
X irradiation of dilute solutions	210000*	DeFillipes and Guild (1959)[10]
Heating in solution	260000*	Ginoza and Zimm (1961)[11]
Sonication	1100000	Litt, Marmur, Ephrussi-Taylor and Doty (1959)[12]

* Calculated by Hutchinson from the experimental data given in the references cited.

of a number of investigations of transformation which have used the streptomycin resistance character in Pneumococcus. Bringing together these data he has shown that with modest assumptions several methods of inactivation agree in defining a unit with a molecular weight about 200000 (Table 2). Hutchinson suggests that the common feature of these modes of inactivation is that they cause breaks in only one of two chains in the double helix. This is especially likely for heating at temperatures below those which dissociate the twin helical structure when the main cause of inactivation is likely to be depurination. Marmur and Lane[13]) have shown that one intact chain is sufficient to effect the transformation process. Sonication, however, gave a much larger size for the sensitive unit (1000000) but physical chemical studies show that in this case the principal form of damage is double-chain scission. Quite naively, one would anticipate that double-chain scission would be more likely to occur under bombardment with the densely ionizing atomic nuclei, if sparsely ionizing radiations break one strand. The author reports in his paper the results of irradiating Pneumococcus DNA (in the dry state) with α rays and the ions of carbon and oxygen (Table 3). The D 37 dose can be translated into a cross-sectional area for the target molecule, but for densely ionizing particles inactivation is produced by two processes, the first by direct impact of the heavy ion and the second by δ rays outside the core of high density of ionizations. The cross section due to direct impact, σ_{scission}, is calculated on the assumption the target is a 1.1 million daltons

TABLE 3

The experimental and calculated cross-section for cutting DNA strands by fast heavy ions

ion	D37		No. of events passing through DNA helix	σ experimental	σ scission	σ δ rays	σ calculated
	$\frac{\text{ions}}{\text{cm}^2}$	megarad					
	$\times 10^{11}$			$10^{-12}\,\text{cm}^2$	$10^{-12}\,\text{cm}^2$	10^{-12} cm^2	$10^{-12}\,\text{cm}^2$
He	4.88	1.5	1.1	2.1	1.8	0.295	2.1
C	0.85	2.2	10	11.8	8.85	3.33	12.2
O	0.69	3.3	18	14.5	8.85	5.92	14.8
X rays		3					

The calculated cross-section for scission for the helium ion corresponds to an efficiency of 20% for breakage in the passage of a helium ion through a DNA double coil.

double helix, and the second cross section $\sigma_{\delta\text{-rays}}$, is calculated from the fraction of the total energy dissipated as δ rays assuming that they are as efficient as X rays. In Table 3 one can see there is very good agreement between the experimental and calculated values of these two component cross-sections, a result which strongly supports the analogy between sonication and heavy ion bombardment as a cause of double chain scission.

Hutchinson suggests that these two rather different values for the apparent size of the marker can be reconciled. The first, the million unit, is probably the minimum size of the double stranded molecule which can be absorbed successfully by the bacterium; the second, the 200000, the minimum unit of single-stranded DNA which can readily enter the process of recombination: the actual size of the gene may well be much smaller. This interpretation, of course, depends very much on the detailed mechanism of the transformation process[14]), still largely unknown, and may therefore be limited only to this process. However, the author feels that these observations may be generalized with two interesting suggestions. It may be that only single chain breakage is increased by oxygen so that one might expect a decrease in the enhancement of radiation damage by oxygen on going to more densely ionizing radiations. (It should be pointed out that transforming principle only shows an oxygen effect when the intact bacteria are irradiated.) The second

is that in higher organisms it may be that the fracture of a unit bigger than a double helix is required to produce a biological effect. This would be easier for densely ionizing particles, so again one might expect them to produce more damage, rad for rad, than the sparsely ionizing.

Although the combination of chemical purity and biological activity which transforming principle affords is an attractive one, the mechanics of the transformation process are so little understood that it is still difficult to assess what meaning these results might have for interpreting what goes on in the cell. Attention must therefore still be paid to physical and chemical observations of isolated nucleic acid and nucleoprotein.

3. RADIATION EFFECTS ON DNA IN THE CELL

The length of the DNA thread as it exists inside the cell is still very much a matter of speculation but recent emphasis on very gentle techniques of isolation has yielded preparations of very high molecular weight. Attention to this was first drawn by Davison[15]) and his suggestion that the DNA in the T even bacterio-phage particle constitutes one molecule of 120 million molecular weight units is now widely accepted[16]).

Strazhevskaya and Struchkov[1]) have applied similar techniques to the isolation of DNA from a variety of tissues and obtained

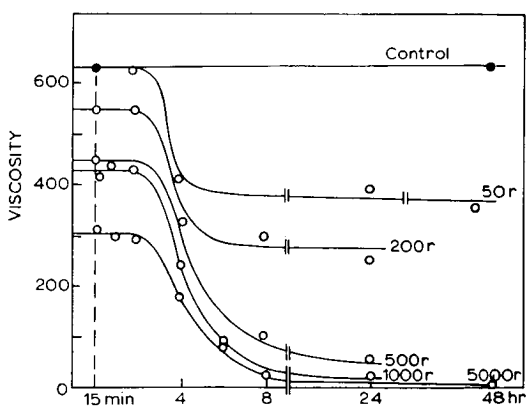

Fig. 1. The change of the viscosity of DNA extracted from rat thymus glands with time of isolation after irradiation of the whole animal, with the doses shown. (Strazhevskaya and Struchkov.)

preparations of such high viscosity that they do not commit themselves as to whether these contain single molecules or complexes; they call them supermolecular[17]). However, the viscosity of such preparations is changed in a most interesting way when the animal is irradiated prior to the isolation. Sacrificing 70 to 90 g rats at various times, the first only 15 min after irradiation, they have isolated the DNA from the thymus glands and the livers. In Fig. 1 one can see even with a dose as small as 50 r that there is already a perceptible decrease in viscosity of the thymus DNA 15 min later. At higher doses this initial drop is followed 2 hr later by a persistent decline. This is a direct effect on the thymus tissue: the DNA from an animal irradiated with the thymus shielded does not show these changes.

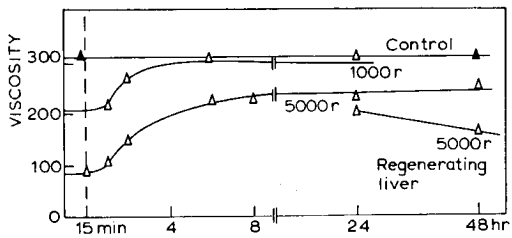

Fig. 2. The change of the viscosity of DNA extracted from the livers of the same animals as used for the previous figure. (Strazhevskaya and Struchkov.)

By contrast the results shown in Fig. 2 of similar preparations from the livers of the same animals manifest a much smaller effect and, moreover, one which is apparently reversed after some hours. This difference in sensitivity between the two tissues is emphasised by an experiment in which 5000 rad were given to animals which had a 2/3 partial hepactectomy 17 hr earlier. Again there is an apparently irreversible change in the viscosity of the isolated DNA (Fig. 2).

These authors suggest that despite the apparent absence of DNA synthesis in the liver there are processes of regeneration which restore the viscosity of the DNA. However, this difference may be more apparent than real, for the typical radiation induced chromosomal abnormalities do occur in liver if cell division is stimulated by partial hepactectomy even if this stimulus is not given for some months after the irradiation[18]). Apart from the observations on

nuclear phosphorylation by Creasey and Stocken[19]) there have been few indications of biochemical differences to help to elucidate the apparent distinction often made between the radiation-sensitive tissues of which the thymus is typical and the insensitive such as the liver. These results are extremely interesting but one must make one reservation; the authors in their paper give no indication of the percentage recovery of DNA from the irradiated tissues. Several authors have noticed that it is more difficult to recover DNA from irradiated cells and I shall now refer to papers dealing specifically with this point.

4. CROSS-LINKING OF DNA BY UV

Some years ago I noticed that the recovery of DNA from fish sperm fell dramatically after irradiation with electrons when using the detergent (sodium dodecyl sulphate) method of purification[20]). At the time we were inclined to the view that this loss was due to cross-links between DNA molecules. But subsequent observations showed that treatment of the irradiated fish sperm with trypsin abolished the difference in the amount of DNA recovered, although the amount of protein involved must have been very small[21]). The possibility that a similar loss of nucleic acid by cross-linking with protein can be induced by UV has been brought to light and is reported in two papers. In the first Smith[1]) has found that if cultures of *E. coli* are irradiated and the cells extracted with a detergent, sodium

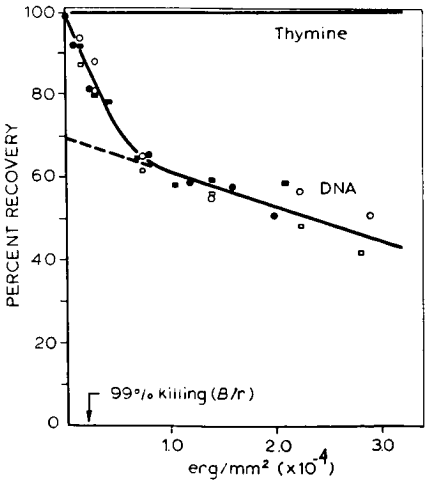

Fig. 3. The extractability of DNA from *E. coli* following irradiation with ultra-violet light. The thymine of the bacteria was labelled with C^{14} and the recovery of the radio-isotope was a measure of the free DNA[22]). The data of Smith[32]) on the loss of extractable thymine under the same circumstances is plotted for comparison. The strains of *E. coli* compared were: ● B/r; ■ B; ○ B; □ T^-. (Smith.)

dodecyl sulphate, the amount of DNA recovered falls markedly with increasing dose of UV (Fig. 3)[22]. The author checked that this was not a failure to lyse. It can be seen that this effect is much more striking than the formation of the thymine dimer to which I will refer again later. Smith does point out it may not have any biological significance, for this effect appears not to be related to the radiosensitivity of the organism: the same curve of DNA recovery is obtained for *E. coli* B and its radioresistant mutant B/r. Nor is there any difference produced after a photoreactivation treatment which raised the survival 20 fold (to 20% survival). Doses of X rays up to 1 Krad produce only a small (5%) loss and further irradiation to 40 Krad does not increase it. But irradiation with visible light in the presence of the photodynamic dye acridine orange, which complexes strongly with DNA, results in a great loss of extractable DNA, much more than does UV for the same percentage killing. The cross-link seems, as in the case of fish sperm, to be observed only when detergent is used to lyse the cell; if first the bacteria are ground with alumina before the addition of the detergent there is no loss of DNA.

Smith, impressed by the sensitivity and ease of detection of this effect, is inclined to believe that protein cross-links with DNA must be significant in the killing of bacteria by UV, but I feel that his own demonstration of the poor correlation between loss of DNA and killing points to the opposite conclusion. The effect of X rays in the case of fish sperm too seems not to correlate with biological phenomena; it is less in the presence of oxygen and for particles of high LET[20].

However, he is supported by Moroson and Alexander[g] who have found similar losses of extractable DNA after the UV irradiation of fish sperm, *E. coli* and a murine lymphoma maintained in tissue culture. They distinguish between DNA-DNA cross-linking which they found only in the fish sperm and DNA-protein cross-links which were formed in the other two. Digestion with trypsin had only a limited effect on the loss of DNA from fish sperm but abolished the effect in the bacteria and lymphoma. They further show that the viscosity of the extracted DNA begins to fall after a dose only 2 to 5 times that required to reduce the bacterial survival to 10^{-3}. A dose some 25 to 40 times greater must be delivered to the isolated DNA to produce a comparable effect.

5. RADIATION EFFECTS ON NUCLEOPROTEINS

If the problems of dealing with DNA are formidable, those of investigating nucleoproteins are daunting. It is not surprising that only recently have a few workers ventured studies of this kind. One of the difficulties is that of finding means to characterize the material. Ruth Itzhaki[e]) has used electric birefringence methods for a preparation of soluble nucleoprotein from rat thymus. This appears to be an aggregate which is broken up to some extent by irradiation, but it is not at present possible to say in what way and to what extent.

Emmerson, Scholes, Thomson, Wheeler and Weiss[c]) have, over many years, investigated in detail the chemical effects of radical attack on DNA, in particular base destruction and breakage of the phosphodiester backbone. They have now applied their methods to a soluble nucleoprotein prepared from calf thymus by the method first described by Doty and Zubay[23]). Measurements of phosphate release and base destruction reveal that the protein has substantially protected the DNA against radical attack, probably by as much as 90%. The G value for base destruction is only about 0.1 as compared with 1.09 for pure DNA[24]). Part of this protection is by simple competition but a large fraction must be due to steric hindrance. In synthetic mixtures of DNA and histone there is more attack on the DNA than when the two have never been dissociated.

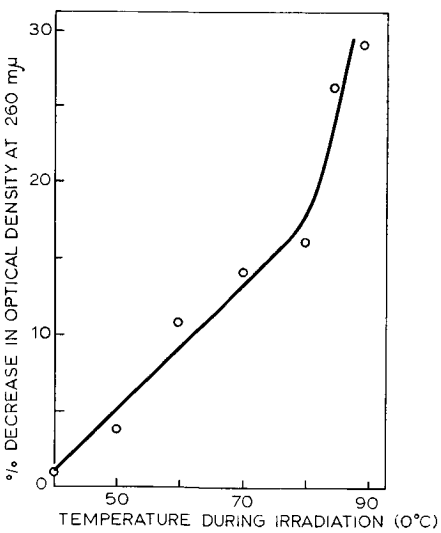

Fig. 4. The destruction of the nitrogen bases of DNA in 0.01% solutions of calf thymus nucleoprotein by 1.1×10^5 rad of Co γ rays as a function of the temperature at which the irradiation is performed. (Emmerson, Scholes, Thomson, Wheeler and Weiss.)

Fig. 4 shows that the protection decreases if the irradiation is performed at elevated temperatures.

They have followed up these observations with an attempt to establish the sites of radical attack in intact nuclei. This method is based on the reaction of the $CO\cdot_2^-$ radical ion, itself produced by radical attack on the bicarbonate ion, with the radicals (R·) fixed in macromolecules, to produce a carboxyl group[25]).

$$HCO_3^- + (H_2O)^- \rightarrow CO\cdot_2^- + OH^- + H_2O$$
$$RH + OH\cdot \rightarrow R\cdot + H_2O$$
$$R\cdot + CO\cdot_2^- \rightarrow R\cdot COO^-.$$

If the bicarbonate is labelled with ^{14}C, the incorporation of radioactivity is then a measure of the radiation induced carboxylation. After irradiation the proteins, nucleic acids and lipids in rat liver nuclei were separated and the ^{14}C in each fraction measured. Only 3 to 4% of the total radioactivity is associated with DNA while the lipid fraction, only 2% by weight of the nucleus, contained 30% of the total counts. This again suggests that the DNA is substantially protected from radical attack, but this must at the moment be qualified by uncertainties about the efficiency of this method. Certain carboxyl groups may be labile and therefore not measured after isolation and the amount of radioactivity in the lipid fraction may simply be due to the large surface the outer membrane presents to the surrounding medium. However, this is a method of great promise for locating the sites of radicals formed in biological materials.

6. POST-IRRADIATION METABOLISM OF DNA

Thus far I have dealt, in the main, with the damage DNA suffers under irradiation with little reference to the part played by the subsequent metabolism of the cell. This is the subject of the next two papers, both of which follow up an observation of Stuy[26]) who found that the DNA in bacteria is rapidly degraded after X irradiation. Miletic, Kucan, Stavric, Novak and Zajec[f]) have studied further this process of degradation. First a practical point: it appears that the method of measurement of the amount of DNA modifies the result obtained; different methods of DNA analysis give somewhat different results. Fig. 5 shows a typical pattern of behaviour for *E. coli* B. The immediate degradation is followed

after some 30 min by new synthesis. This immediate inhibition of synthesis has of course often been observed to be a consequence of irradiation. These authors have attempted to correlate the amount of degradation and the period of inhibition with cell death.

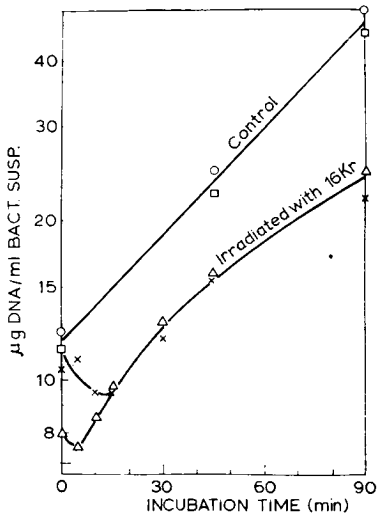

Fig. 5. The synthesis of DNA in *E. coli* B irradiated with 16 Krad X rays (210 KV). Aliquots of the two cultures were analysed by both the Schmidt Thanhauser ○△ and the Burton methods □ ×. (Miletic, Kucan, Stavric, Novak and Zajec.)

This has not so far yielded any clear-cut indication of the part the degradation plays. Although the extent varies with the bacterial strain, it clearly depends on a number of variables relating to the cell's metabolism. For instance, in the presence of chloramphenicol the extent of breakdown and in particular the loss of nucleic acid precursors is much greater for cells that have received 16000 r. Curiously this degradation does not occur if a second dose of X rays of 10 Kr is given after an initial dose of 1 Kr.

Drakulic and Kos[b]) have studied other aspects of the same phenomenon. Stuy[26]) had shown that high concentrations of citrate inhibited this post-irradiation degradation, an observation which seemed to implicate an enzyme which degrades DNA, DNAase I, since citrate is a known inhibitor of this enzyme. Drakulic and Kos[27]) had been unable to find any activation of DNAase in irradiated bacteria and they have, therefore, pursued the hypothesis that the addition of citrate affects the normal metabolic processes within the bacterial cell. They irradiated log phase cultures of *E. coli* B with 16000 rad of X rays and incubated them, with the

appropriate controls, in media which contained certain selected metabolites and antimetabolites, measuring at various times the DNA content and the viability. They tested succinate, glutamate, versene, Mg, ATP, ADP, and AMP (usually about 10^{-3} M). In the main those agents, notably ATP, which inhibited breakdown inhibited DNA synthesis in the unirradiated controls. Again it is by no means clear what part the degradation plays but the authors make the very interesting suggestion that the breakdown is the result of an unsuccessful attempt at replication.

The influence of post-irradiation conditions on the survival and mutation rate of bacteria is a field of radiobiology rapidly growing in importance and is touched on by Thornley and Lajtha in their reports.

7. RADIATION EFFECTS ON NUCLEASES

The part which activated nucleases play in post-irradiation events, despite much work, is by no means clear[28]). The release of nucleases offers another and subtle way in which the cell's machinery could be damaged by radiation. Okada and Hempelmann[h]) have studied in detail the rise in the activity of the enzyme, DNAase II, which is particularly marked in the spleen following whole body irradiation. Their results now show that this is probably the result of new synthesis in the surviving radio-resistant cells, and it seems very likely that it is an adaptive response to the liberation of nucleic acid from the dead and lysing cells. This does not rule out, but it does render unlikely the idea that this enzyme is involved in the damage which leads to cell death.

8. SENSITIZATION BY BROMOURACIL (5 BUDR)

The last topic I have to mention concerns the sensitization which follows on the incorporation of the halogen containing analogues of thymine (see also the report of Alper). Djordevic and Szybalski, had shown 5 BUDR to be an effective sensitizer of the Detroit 98 cell-line[29]). Djordevic[a]) has now found that he can partially synchronize cultures of these cells and that synchrony is only slightly affected by the addition of BUDR and FUDR (Fig. 6). Irradiating samples of these cultures with UV at various times through the first division cycle he has observed a progressive change in sensitivity (Fig. 7), the most marked effect being the steady reduction in the size of

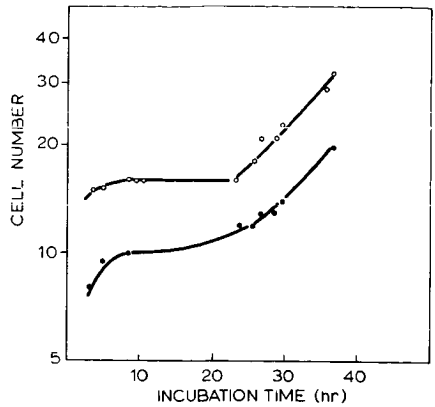

Fig. 6. The increase in cell number with time of cultures of Detroit 98 cell-line, synchronized by the method of Terasima and Tolmach[30]). The division time is slightly lengthened and the degree of synchrony less when the endogenous supply of thymidylic acid is blocked by 5-fluorodeoxyuridine (5 FUDR) and the cells are forced to utilize the analogue, 5 bromodeoxyuridine (5BUDR) to make DNA. Control O; with added 10 μg/ml 5 BUDR and 4 mμg/ml FUDR ● (Djordevic)

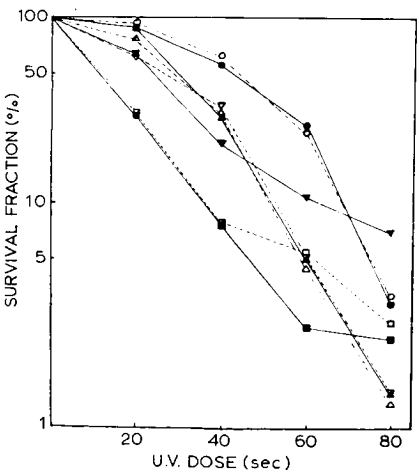

Fig. 7. The UV survival curves for samples taken from the cultures shown in the previous diagram. It may be seen that as the cells go through the division cycle they become increasingly sensitive. The effect of the 5 BUDR is only apparent at 22 hours, and this may partly be due to the lengthening of the division cycle (see Fig. 6). The cultures were irradiated at the following times, the untreated being shown by the closed symbols and those treated with 5 BUDR, by open symbols: 3hr ●—●, O—O; 7 hr ▲—▲ △—△; 19 hr ■—■, □—□; 22 hr ▼—▼, ▽—▽. (Djordevic.)

the shoulder. The sensitization by the analogue appears only late, as one might expect if one of the effects is to slow down cell-division (see Fig. 6). However, this preliminary result indicates that this is a most promising method of correlating the appearance of the analogue in the DNA with the increase in sensitivity.

Smith[1]) has been investigating the effect of ultraviolet light on some of the nitrogen bases and their analogues in an effort to find a mechanism for this sensitization by bromouracil. His method

stems from the striking observation of Beukers and Berends[31]) that thymine, hitherto thought rather resistant to UV, forms a dimer when irradiated in frozen solution. Irradiating with UV or X rays frozen solutions of the various purine and pyrimidines, singly and in various mixtures, using a dose which would produce 90% killing of *E. coli* (BT), Smith has found a bewildering number and variety of photoproducts. The most striking of his many observations is that bromouracil alone or with thymine is not changed, but if mixed with uracil or cytosine it undergoes a number of reactions. Cytosine itself is not changed but in its presence 3 photoproducts of 5-BU appear, one possibly the dimer.

It is clear from these results, confirming the original observation on thymine dimerization, that the conditions of irradiation greatly influence the effect of UV, and only by studying the effect of UV on intact DNA can one hope to establish the biologically important reactions. This Smith has attempted, irradiating bacteria with thymine or bromouracil labelled with ^{14}C [32]). The whole cells were hydrolysed and the bases located by paper chromatography. When recovery is plotted as a function of dose, it can be seen in Fig. 8 that within the DNA molecule, 5 BU is in fact more sensitive to photo destruction than is thymine by a factor of 1.9. And further work has shown that bromouracil gives rise to a great number of photoproducts.

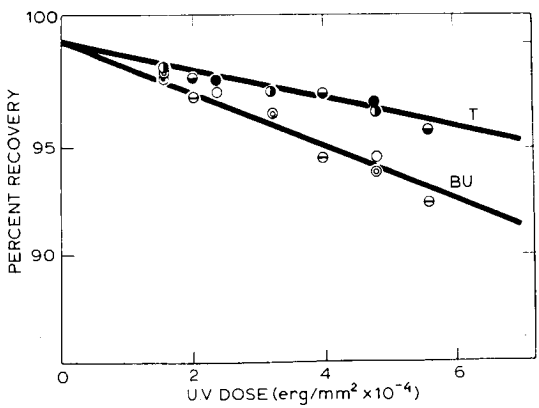

Fig. 8. The recovery of thymine (T) and bromouracil (BU) as a function of the UV dose delivered to the intact cells. Aliquots of *E. coli* B/r, after irradiation, were hydrolysed and these bases located by paper-chromatography. (For details of procedure see ref.[32]).) (Smith.)

9. CONCLUSIONS

The papers I have so briefly described illustrate some of the lines of progress towards an understanding of how radiation damages DNA. But it must be apparent that how and even whether this damage leads to the cellular injuries studied in radiobiology is still largely unexplained. My own feeling is that we have to deal with a spectrum of damages, many reparable, some not, which, in the main, have their effect when the DNA is replicated. Recently, Hudnik-Plevnik and Stocken[33]) have shown that DNA made in UV-irradiated cells is not identical with that made in undamaged cells. Experiments using irradiated DNA as "primer" for DNA polymerase show that this is potentially a method for locating the lesions which prevent replication[34]).

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DISCUSSION

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Marcovich (*Lab. Pasteur, Paris, France*) asked if anything was known of the nature of the bond formed between DNA and protein as a result of UV irradiation and whether the protein was bonded to a sugar moiety or to a base. Smith[1]) pointed out that the production of DNA-protein cross-links is not a direct effect of the irradiation; indeed, if DNA is subsequently isolated by dispersion of the cell homogenate in strong salt solution there is no evidence for any DNA-protein linkage. The bonds only appear to be formed during the isolation of DNA by the detergent method thus suggesting that the detergent molecule may itself be involved in the bond. Moroson[g]) pointed out that the extent of the bonding depended upon cell-type. Far fewer DNA-protein bonds were formed in UV irradiated sperm heads than in lymphoma cells or in *E. coli* B. Only in the case of the sperm heads did irradiation produce any intramolecular cross-linking of the DNA molecules themselves.

Smith did not agree with the rapporteur's suggestion that the cross-linking and protein was not important biologically. Although the data for UV irradiated *E. coli* B and B/r were similar (in contrast to their different resistance to X irradiation) it had been found that this lesion did not exhibit photoreactivation. The irreversibility of the process certainly suggested that it was of importance in relation to cell death. Analytically, the cross-linking of DNA and protein is a much more sensitive parameter of the effects of UV irradiation than is the dimerization of thymine. At the 1% survival dose

only 2% of thymine dimers can be detected whereas the cross-linking reaction accounts for 11% of the DNA. It remains to be determined to what extent the cross-linking reaction contributes to the total effect of UV light on the biological activity of the cells.

Kuzin (*Acad. Sciences, Moscow, USSR*) and Szybalski (*Univ. Wisconsin, Madison, Wis., USA*) questioned whether the loss of DNA from bacteria following UV irradiation could be simply interpreted in terms of a degradation of DNA. Kuzin suggested that the variable response observed with different strains of bacteria might be due not merely to the direct effect of X rays but in part to variations in metabolism, in particular the exchange reactions, between the different strains under normal conditions. Szybalski pointed out the parallelism with the effects of thymineless death where degradation of DNA has been found to occur only during the process of extracting the DNA. Drakulic[b]), replying in the absence of Miletic[f]), stated that there was no suggestion that the degradation of DNA was a direct result of the irradiation; it was quite clear in the full paper that the effects observed are the result of metabolic processes occurring after the end of the irradiation. Her own studies and those of Miletic and Djordevic[a]) suggested that the effects observed were made evident during the DNA replication stage of the cell cycle, perhaps indicating that the X irradiation damaged the information system thus leading to abortive replication of DNA.

In response to a request from Alexander[g]), Strazhevskaya[j]) discussed in more detail the nature of the high molecular weight DNA preparations described by herself and Struchkov. She pointed out that the preparations are made by repeated partition between phenol and physiological saline. The final product contains less than 1% protein. Preliminary analyses of residual protein showed that it contained at least ten amino-acids and that its composition depended upon the tissue used. Previously reported work had shown that the physical properties of this "super-molecular" DNA vary from one tissue to another. Electron microscope studies suggest that the preparations contain bundles of DNA molecules oriented in parallel with an approximate diameter of 400 Å. The response to X irradiation suggested that the function as well as the structure of these preparations was different in thymus and in liver. It seems likely that damage to these structures may be a very important aspect of radiation injury.

Radiation Chemistry of Macromolecules in vivo and in vitro

CELL DEATH AND MUTATION

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Laboratoire Curie de l'Institute du Radium, 11, rue Pierre Curie, Paris 5, France

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Isotope Research Division, Wantage Research Laboratory (AERE), Wantage, Berks. Great Britain

c) M. M. ELKIND and A. HAN,
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National Cancer Institute, National Institute of Health, Bethesda, Maryland, USA

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Committee on Biophysics, University of Chicago, Chicago, Illinois, USA

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Radiological Research Laboratory, Columbia University, New York, New York, USA

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Laboratoire Pasteur, Institut du Radium, Paris 5, France

g) H. S. KAPLAN,
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Department of Radiology, Stanford University School of Medicine, Palo Alto, California, USA

h) G. E. MAGNI,
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Institute of Genetics, University of Pavia, Italy

i) ETHEL MOUSTACCHI,
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Laboratoire Pasteur, Institut du Radium, Paris 5, France

j) K. C. SMITH,
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Department of Radiology, Stanford University School of Medicine, Palo Alto, California, USA

[k]) W. SZYBALSKI,
Radiosensitization of cells by halogen labelling of DNA, the principal target of radiation effects.
McArdle Memorial Laboratory, University of Wisconsin, Madison, Wisconsin, USA

1. INTRODUCTION

In his report, Stacey[1]) indicated that the links between chemical studies on macromolecules and radiobiological effects are still elusive. Speculations as to the nature of the target for a particular type of damage in the cell must be based on inference, and the contributors to this session have dealt with two important consequences of ionizing radiation: mutagenesis, and cell death — which will be defined for our purposes as the loss of the cell's ability to originate a clone of daughter cells. The importance of radiation mutagenesis requires no emphasis. The killing of cells by radiation is now believed to account for many effects seen in the irradiated animal, and this exciting development will be dealt with in a later report[2]).

It must be borne in mind that uniqueness, rather than sensitivity to radiation, is a necessary condition that any particular molecule or group of molecules should constitute a target. It is unlikely that the destruction of one out of a large number of like molecules would bring about cell death; but the destruction of a unique molecule, or the change of one molecule in a unique structure, might very well do so. Stacey has already mentioned the line of thought which leads to the concept that this unique structure is the cellular DNA.

2. INVESTIGATIONS WITH INCORPORATED BASE ANALOGUES

This concept has indeed been the starting point for the studies carried out by Szybalski[k]) and Kaplan[g]), who, with their co-workers, have examined the radiobiological consequences of inducing cells to incorporate unnatural base analogues in the DNA. Cells grown in this way become more sensitive to radiation, a result which is interpreted as demonstrating that DNA is a principal target for cell killing. Such an interpretation is supported by Szybalski's evidence on the similarity in the degree of sensitization imposed by the incorporation of the base analogue BUDR (5-bromo-deoxyuridine) on cells as a whole and on transforming principle, which is effectively pure DNA.

In my opinion, however, some of the data demonstrate features which complicate this picture. For example, the increased sensitivity to UV is much more dramatic than to X rays, as shown by Fig. 1, and it is relevant that UV, at the wave length used, is specifically

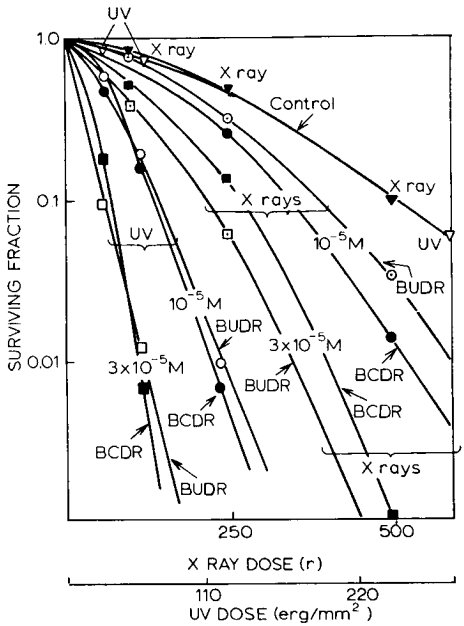


Fig. 1. Sensitivity to radiation of cells incorporating thymine analogues compared with that of controls. Fraction of original population still able to form colonies (surviving fractions) plotted on a logarithmic scale as a function of X ray or UV dose. (Szybalski.)

absorbed by nucleic acids and bases, while no such specific absorption occurs with ionizing radiation. Another example arises from Erikson and Szybalski's report[3]) that glycerol protects both normal and sensitized mammalian cells. Szybalski regards this as an indication that the primary site of damage is the same, the glycerol, presumably, interacting at that site. As shown by Fig. 2, however, the sensitized cells are protected to a somewhat greater extent, and this difference could perhaps be as significant for deducing the nature of the damage done to the two types of cell as the fact that, qualitatively, glycerol is protective for both.

Kaplan, Zavarine and Earle[4]) have compared the enhancing action of oxygen on cells with natural and unnatural bases, using bacteria as test organisms. They found that thymine substituted cells were subject to the same oxygen enhancement ratio as normal ones; but, when purine analogues had been incorporated, the picture was quite different; the cells irradiated in anoxic conditions de-

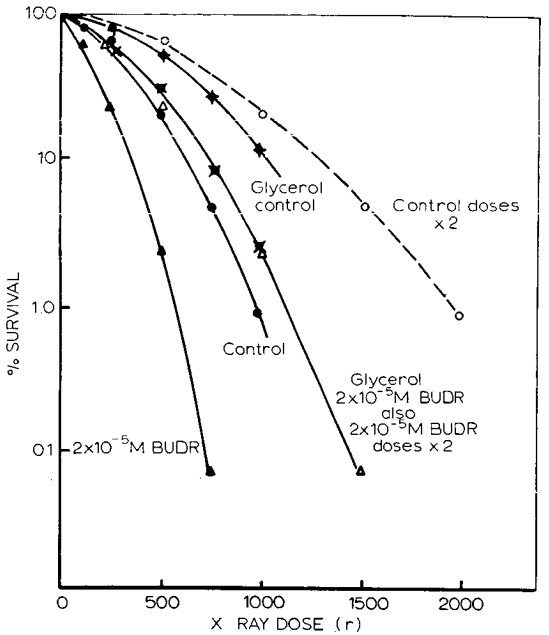


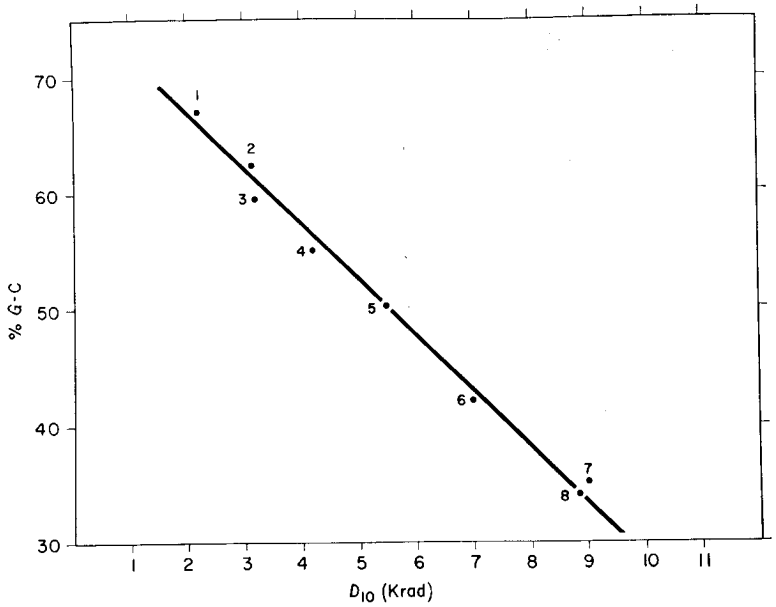
Fig. 2. Protection of sensitized and normal mammalian cells by glycerol against X ray induced loss of colony forming ability (Erikson and Szybalski[3]).

▲ 2×10^{-5}M BUDR, unprotected.
△ 2×10^{-5}M BUDR, unprotected, plotted against double the doses given.
× 2×10^{-5}M BUDR, protected by glycerol.
● Control, unprotected.
O Control, unprotected, plotted against double the doses given.
+ Control, protected by glycerol.

monstrated identical survival curves, the treated cells being sensitive only when irradiated in the presence of oxygen. This intriguing result demonstrates that, if effects on DNA are heavily implicated in cell killing, the phenomena involved are likely to be more subtle than gross effects such as main chain breakage.

3. RADIOSENSITIVITY AND DNA BASE COMPOSITION

Kaplan, Zavarine and Earle have followed a different line of evidence from which they have inferred that cytosine may be a particularly "sensitive" target for the killing of cells by X rays. This is based on a striking correlation between the radiosensitivity of bacteria and the proportion of bases represented by the guanine-cytosine moiety as shown in Fig. 3.



1 - Pseudomonas aeruginosa
2 - Pseudomonas fluorescens
3 - Serratia marcescens
4 - Azotobacter agile
5 - E. coli B.
6 - B. subtilis
7 - B. cereus
8 - M. pyogenes, var. aureus

Fig. 3. Correlation between base composition and sensitivity to X rays: Ordinate: guanine + cytosine content of each strain used, expressed as the percentage of total DNA bases. Abscissa: dose of X rays to give 10% survival (based on the final slope of the survival curve). (Kaplan, Zavarine and Earle[4].)

A dramatic contrast between the actions of UV and ionizing radiations is demonstrated by Haynes's[5]) recent finding of a correlation in exactly the opposite sense for killing by UV, a result confirmed by Kaplan for some of the strains used.

4. THE INFERENTIAL APPROACH

Haynes, Inch and Uretz[d]) have used an inferential approach in attempting to obtain an insight into the primary sites of radiation action. They used as their starting point the evidence that nitrogen mustard (HN_2) effects chemical changes in DNA which lead to cell death, and examined the combined effects of radiation and HN_2 treatment.

It was necessary first to develop a technique for assessing dose-effect relationships, and concentration of HN_2 was used as the variable; the end-point for any given concentration was established by observing the final survival, which reached an equilibrium value after sufficiently long exposure. The extent of interaction of this preliminary treatment with UV or X irradiation was judged by the extent to which survival curves resembled either those for cells irradiated without HN_2 treatment (no additivity), or those in which the initial killing by HN_2 might have been caused by the radia-

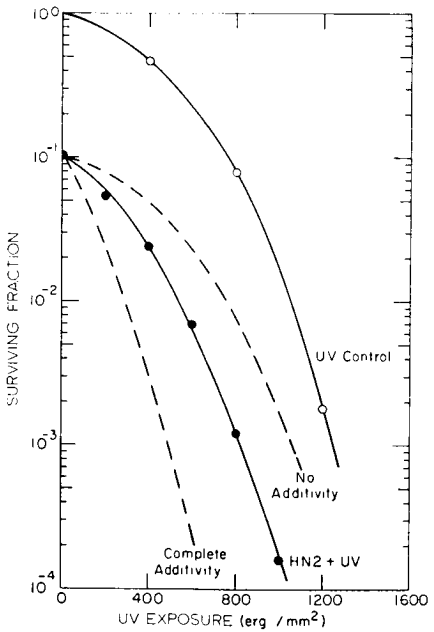


Fig. 4. Survival of *E. coli* B/r after UV (○) or after UV following treatment by nitrogen mustard (HN_2) (●). Dotted lines show expected survival curves for no additivity or complete additivity of the treatments. (Haynes, Inch and Uretz.)

tion itself (complete additivity) as shown in Fig. 4. Examination of these effects with haploid yeast and *E. coli* B/r led them to conclude that there was considerably more interaction on HN_2 treatment with UV than with X rays. They inferred that structural or physiological factors in the cell are more important for determining response to X rays than the "mere presence of DNA molecules".

So far we have distinguished only surviving and non-surviving cells, without considering at which stage failure occurs. It is known that mammalian cells rendered inviable by radiation may divide one

or more times before they die. Elkind and Han[e]) have made precise measurements of growth and division in total irradiated populations as well as in the survivors. The results shown in Figs. 5 and 6 may be summarized as follows:

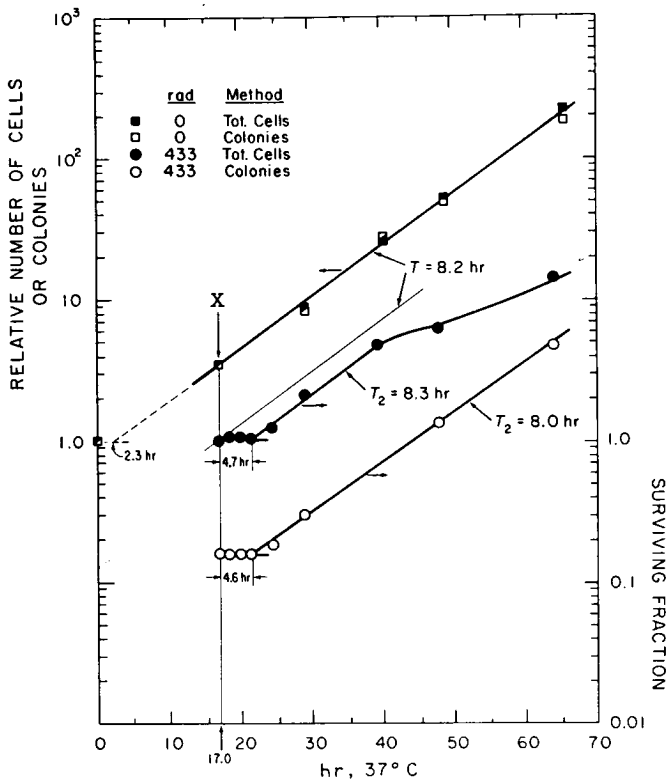


Fig. 5. Growth of unirradiated Chinese hamster cells and of the total population and the surviving cells after 433 rad of X rays delivered to a population in the logarithmic phase of growth (17 hr after plating). T_2 = average time required for population to double. (Elkind and Han.)

(i) Division delay is the same for surviving cells and total population (and therefore for non-survivors), and is linearly related to dose. The delay is just over one-tenth of the generation time per 100 rad.

(ii) After the initial division delay, generation times of surviving

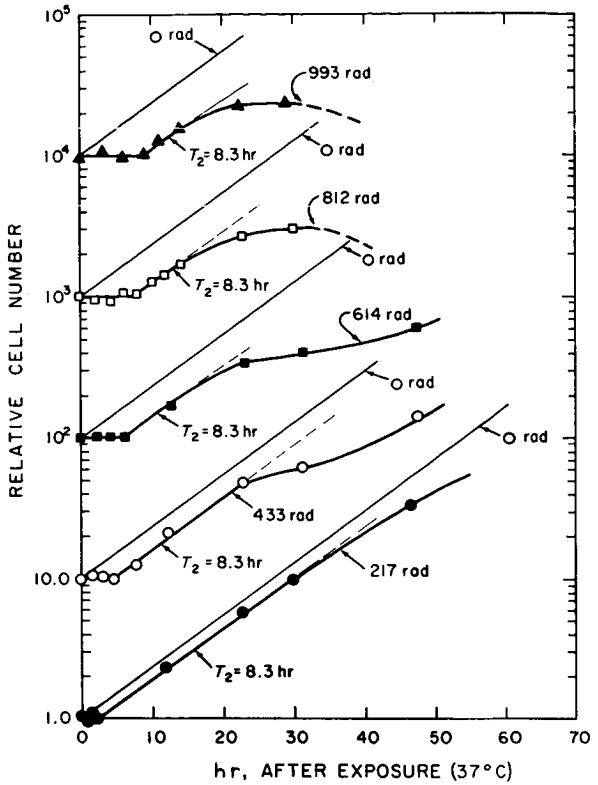


Fig. 6. Growth curves for total irradiated populations of Chinese hamster cells after various doses of X rays. (Elkind and Han.)

and non-surviving cells are equal to each other and to that of a non-irradiated population.

(iii) The total number of divisions undergone by non-surviving cells depends on dose, as shown by Fig. 7. A dose which has killed 90% of the cells leaves the non-survivors able to go through an average of two divisions.

In the light of these results, the authors are unable to agree with the postulate that cell death, division delay and the appearance of chromosomal aberrations all stem from an initiating damaging event involving DNA chain breakage, since division delay is imposed to the same extent on survivors and non-survivors alike. The authors conclude that there are substances other than DNA which

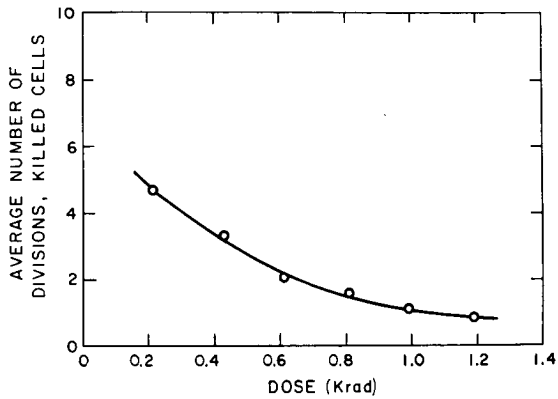


Fig. 7. Average number of divisions, as a function of X ray dose, of Chinese hamster cells no longer able to form colonies (i.e. killed cells). (Elkind and Han.)

are required for replication: in normal, unirradiated cells the synthesis of these substances and of DNA (and indeed all syntheses) proceed in step; but in the irradiated cell these synthetic processes are uncoupled, and the inhibition which leads to division delay occurs independently of the loss of ability to synthesize the substances needed for replication. Elkind and Han suggest that the latter loss is responsible for cell death, and that the killed cells are able to go through just so many divisions as are required to dilute these substances below a required level. The postulated damage of the ability to synthesize some one particular cell constituent is in accord with the classical concept of a unique target, but it is an important part of the argument that it is different from the target for delay in division. The dependence on dose of the number of divisions a killed cell may undergo is more difficult to interpret in terms of target theory. It would seem plausible that, if a given concentration of this material in the cell is postulated, sufficient to support several divisions, the dose dependence is a result of the destruction of molecules of the postulated substance itself.

5. CHEMICAL TESTS ON DNA IRRADIATED *in vivo* AND *in vitro*

The differing conclusions arrived at by all these authors in regard to DNA as a principal target for cell killing by radiation may be examined also in the light of studies by Smith[1]) of the effects of radiation on DNA in cells. Of all those he was able to observe, the

one most sensitive to detection by chemical methods was the cross-linking of DNA to protein. Like Moroson and Alexander[1]), Smith found that this effect was observable only to a very small degree after X irradiation, but was more marked after UV. Smith found the effect to be most closely associated with cell death when this was brought about by the action of visible light on cells treated with acridine orange, a compound thought to form a complex with cellular DNA.

6. EFFECTS OF RADIO-ELEMENTS INCORPORATED IN NUCLEIC ACID

Yet another technique for assessing the importance of interactions of radiation with cellular DNA has been used by Apelgot[a]) and Moustacchi[i]). They caused radio-elements to be built into the molecules of the cells by growing these in media containing one or other cell constituent in radioactive form, at very high specific activities. The cells are stored, and, during storage, the disintegration of an atom may cause cell death. Usually this so-called "suicide" is due to transmutation rather than to ionizations caused by the emitted particle. Apelgot studied the suicide phenomenon in the strain *E. coli* B/r, used by Haynes, Inch and Uretz[d]). In her experimental conditions, as in theirs, exponential survival curves were observed after X rays, but they were sigmoid after UV.

Apelgot examined the suicide effect of ^{32}P, of ^{14}C labeled thymine and of ^{3}H labeled thymidine. The two latter radio-elements were presumably incorporated exclusively into DNA, whereas the ^{32}P was incorporated into both RNA and DNA. Cell deaths following decay of ^{32}P or of ^{14}C atoms were due overwhelmingly to transmutation of atoms, rather than to ionizations, whereas ionizations caused by the very soft ^{3}H β-particles contributed a large part of the effects due to ^{3}H decay. Suicide curves for ^{14}C and ^{32}P decay were respectively of exponential and sigmoid form, i.e. were respectively reminiscent of killing by X rays and by UV. The results are difficult to interpret, since the differences may arise from the different chemical nature of the events following a disruption of the phosphate linkage, and a change in the thymine or its bonding when the ^{14}C atom disintegrates.

Moustacchi has compared dose-effect relationships for the killing of haploid yeast by X rays, ^{32}P and UV. When yeast cells are freshly harvested from a suitable medium, a proportion of cells

are in the resting stage, while the remainder are dividing or budding. The budding cells are comparatively much more resistant to X rays[6]), particularly when these are delivered in aerobic conditions[7]): the difference in sensitivity is far more than can be accounted for by the doubling of any cell constituent. Moustacchi's results are paradoxical: suicide by ^{32}P yields an exponential form of curve, such that the whole population appears uniformly sensitive; on the other hand, the longer the cells are stored, the greater the proportion of survivors which demonstrate that sensitivity to X rays which is characteristic of budding cells (Fig. 8). It seems as if the ^{32}P

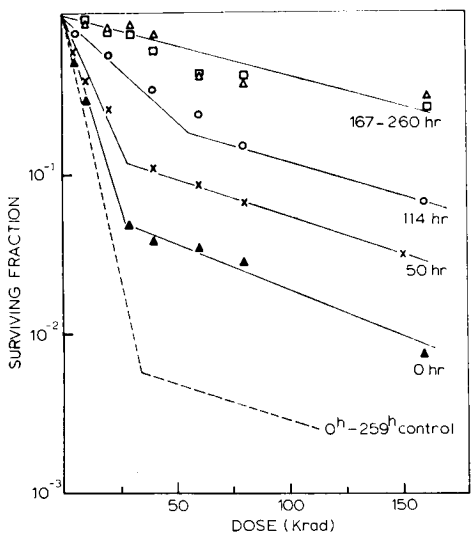


Fig. 8. Survival of irradiated haploid yeast cells, grown in ^{32}P (30 mC/mg), after various times of storage at 0° C. (Moustacchi.)

decay killed the resting cells selectively, although no sign of this selection is apparent in the suicide curve. The situation is complicated by Moustacchi's unexpected discovery that the survivors of ^{32}P decay mutate at high frequency in respect of resistance to X irradiation. These genetically radioresistant strains are not diploid strains, as she showed by genetic analysis, and they contain the same quantity of DNA as the parent strain, and have about the same sensitivity to UV. Furthermore, the resistances of the budding fractions are the same in the original and resistant strains (Fig. 9).

Moustacchi has therefore examined two different kinds of radio-resistance, as compared with the sensitive vegetative cells of the original wild type. (i) The budding cells, whether of the original strain or resistant strain, have the same sensitivity to ^{32}P as the

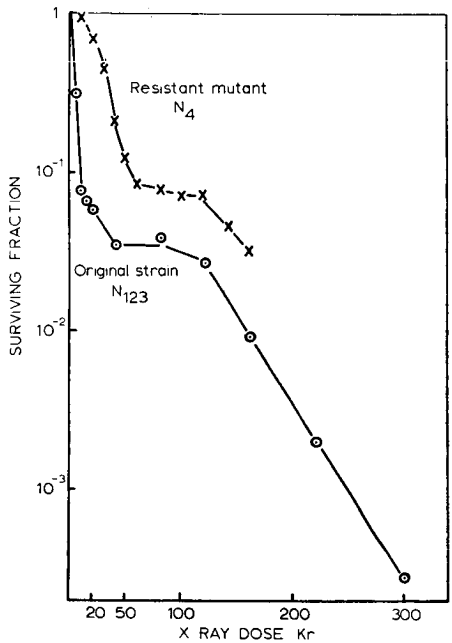


Fig. 9. Survival of haploid yeast cells exposed to X rays. ◉: Wild type strain, ×: Resistant mutant (resistance induced by ^{32}P incorporation). (Moustacchi.)

vegetative cells; deaths appear to be due to ^{32}P incorporated into DNA, rather than RNA; and they occur at the rate of about one death for 100 disintegrations in the DNA. (ii) The genetically X ray resistant strains, like the physiologically X ray resistant fractions of each strain, have about the same sensitivity to UV and to ^{32}P as the sensitive strains.

I believe that Moustacchi's results might be interpreted by postulating that some substance required for replication forms a target for the killing of vegetative cells by X rays and so renders them more sensitive than the budding cells which are capable of synthesizing it anew. This agrees with other data[7]), and the increased sensitivity to X rays, but not to ^{32}P decay or UV, is accounted for by postulating that this target is not nucleic acid.

7. RADIATION INDUCED MUTATIONS IN MICROORGANISMS

A disruption which does occur primarily in the genetic material of the cell, and consequently kills it, is regarded by many workers as a lethal mutation. In yeast, it is possible to make crosses, and therefore to determine whether a lethal event is a mutation in the classical sense, and whether it is dominant or recessive. By using this technique Magni previously concluded that a large proportion of deaths caused by X rays in haploid yeast are non-genetic[8]). In the present study[h]), however, he has called all these deaths recessive lethal damage, which he assesses by subtracting the observable dominant lethal damage. The dose-effect relationship for the so-called recessive lethal damage in a haploid population may then be used to calculate the survival curve for a diploid strain derived from the haploid, given any postulated number of sites for recessive lethal damage (Fig. 10). Magni's own data give a good fit to a

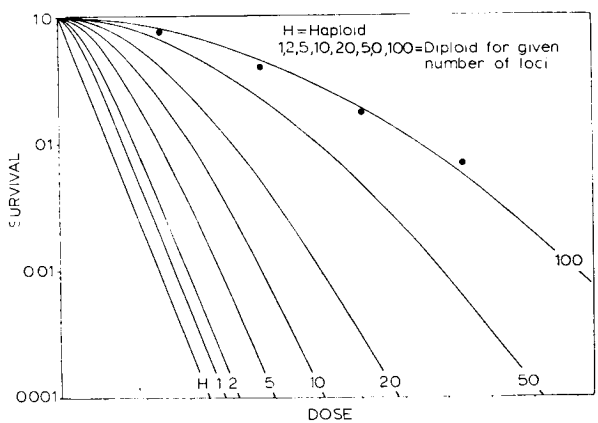


Fig. 10. Expected survival curves of diploid strains, with numbers of sites of recessive lethal damage as shown. ● Experimental points of Magni. (Magni.)

curve for a postulated number of about 100 loci. The strain used by Magni was "prototrophic", i.e. the cells could synthesize all their requirements from organic salts. Such cells may suffer mutations to "auxotrophy" (inability to utilize one or other chemical pathway), and Magni studied the induction by X rays of mutational events of this nature. The number of loci at which such mutations can occur in yeast is estimated to be 70 to 100, i.e. of the same

order as the number of sites at which lethal mutations can occur. Provided the induction of biochemical mutations is linearly related to dose, Magni's experiments show that an average of one such biochemical mutational event per cell would occur if 2000 Kroentgen were delivered, whereas an average of one lethal event per haploid cell occurs if 2.5 Kroentgen are delivered. Magni concludes, therefore, that the average sensitivity to lethal mutations is several hundred times greater than to biochemical mutations. In the language of target theory, the target for a lethal event is very much greater than for a biochemical mutation, which Magni inclines to regard as a "point" mutation, while lethal events, he thinks, are likely to be associated with chromosome breaks.

The study of the mutational step from auxotroph to prototroph is technically easier than the reverse, because large populations of the auxotrophic parent can be seeded on minimal medium, but only the prototrophs will grow out into colonies. Witkin[9]) observed that mutations of this type, when induced by UV, occur in considerably higher yield when amino-acids are available to the bacteria immediately after irradiation than when they are plated on minimal medium. Kada[f]) has gained further insight into this phenomenon by using a strain of *E. coli* requiring tyrosine, methionine, uracil and thymine for growth. He examined the induction of mutation to the ability to synthesize tyrosine, and assessed the relative importance of active protein, RNA and DNA synthesis after irradiation by withholding or supplying respectively methionine, uracil and thymine. There was a striking difference in the mutational effects of UV and X rays: with UV, the yield after a given dose was considerably reduced if either protein or RNA synthesis were inhibited; whereas the yield of X ray induced mutants was unaffected by the presence or absence of any of the substances which the parent auxotrophic strain required for growth. Kada suggests that the difference might be explained by assuming that the X rays act directly on the DNA, and the UV on some other component to produce the mutation. This is a surprising conclusion, since ionizations can occur at random in any cellular constituent, whereas UV is specifically absorbed in nucleic acid. Kada offers in support of his suggestion evidence from some previous results[10]) obtained with *E. coli* K12, a strain which demonstrates sexuality, in that genetic characters are transferred from donor to recipient cells. A donor

parent, auxotrophic for threonine, gave rise to prototrophic mutants when irradiated; when these donor cells were mated immediately after irradiation with similarly auxotrophic recipient cells, prototrophs were also recovered from the offspring of the mated cells. The ratio of prototrophic zygotes to prototrophic mutants in the donor cells was considerably greater after X than UV irradiation, which, in the author's opinion, points to a greater likelihood of the mutational event having occurred in the DNA when X rays were used.

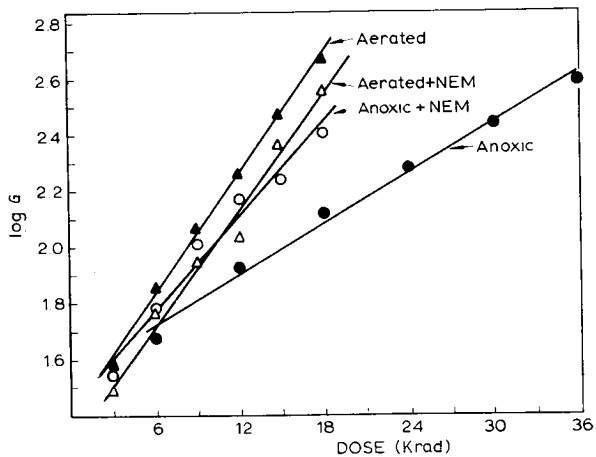


Fig. 11. Reversion to prototrophy of tryptophan-requiring mutant of *E. coli* B/r. Effect of oxygen and n-ethyl-maleimide on induction of prototrophs by γ radiation. (G = number of induced mutants per 10^8 survivors.) (Bridges.)

A different possibility for the site of an X ray induced mutational event in bacteria is postulated by Bridges[b]), who used a strain of *E. coli* requiring tryptophan for growth, and examined the effect of oxygen and of n-ethymaleimide (NEM), a chemical which he had previously found to enhance radiation killing[11]).

From previous studies Bridges had proposed that this chemical might act in a manner analogous with oxygen; furthermore, that it is likely to react with —SH groups or —S free radicals, which are contained in certain amino-acids or in protein molecules. It seems likely, therefore, that NEM modifies radiation damage to protein.

Bridges has plotted his results as logarithm of number of mutants per 10^8 survivors ("G"), as shown in Fig. 11. Enhancement ratios

TABLE 1
Enhancement of radiation effects on *E. coli* WP 2 by oxygen and N-ethylmaleimide

	Enhancement ratio		
	Lethality	Mutation	Induction
	Based on slopes	Based on slopes	Based on dose for which log $G=2.5$
Oxygen	3.16	2.44	2.10
NEM	2.09	1.83	1.70
Oxygen + NEM	3.12	2.36	1.85

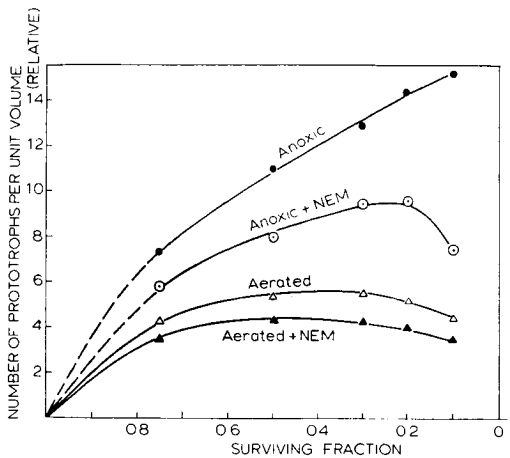


Fig. 12. Number of prototrophs per unit volume, induced by X rays in different conditions, as a function of surviving fraction in those conditions. (Bridges.)

are given in Table 1. This shows an enhancement by NEM which is nearly as high for mutation induction as for killing, and he suggests that this may be evidence for mutation induction via damage to protein with which NEM is complexed, or, alternatively, that damage to protein might at a later stage express itself in DNA.

In Fig. 12 Bridges' data have been plotted as absolute yield of mutants against surviving fraction. This shows clearly the markedly smaller oxygen enhancement ratio for mutation induction (M_I) than for killing (M_K). Indeed, we see from Fig. 11 that M_I

would be very low indeed if it were based on doses to give a much lower value of "G". The change in M_1 with dose might be evidence that mutation induction is not a completely random event in the population as a whole, which is a basic assumption in assessing mutation yield as mutants per survivor. That there is not complete randomness is the conclusion Hille[e]) comes to, in examining the same mutation after UV. She deduces that the physiological state of some cells in the population may predetermine their greater probability of becoming mutants. Further, from her studies of the prototrophs themselves, and their progeny, she has suggested a mechanism which has a bearing on all the papers dealing with biochemical mutants. Her studies of the action of UV in inducing tryptophan-requiring cells of *E. coli* B/r to revert to prototrophy, and of the characteristics of the induced prototrophs and their progeny, have led her to conclude that there is a "suppressor" locus in the genome, i.e. the ability to carry out the synthetic step in question may be suppressed. This, it is suggested, may happen when a particle called an episome[12]) attaches to the locus, and the cell with the attached episome is auxotrophic. Detachment changes the cell genetically to prototrophy, but, as her studies show, this form of prototrophy is unstable, i.e. the episome can readily be re-attached, and can remain re-attached in the progeny. There is good evidence that episomes exist, i.e. particles which subsist in the cell, but which may or may not form part of the genome; and there is some evidence that they are DNA particles[13]). If there is generality in the phenomenon of auxotrophy via the attachment of an episome at a suppressor locus (and Hill quotes evidence which shows that there may be), then we might envisage the induction of prototrophs from auxotrophs by radiation as being due either to destruction of the episome, or to disruption of the bond holding the episome in place. In the light of such suggestions perhaps the evidence offered by Kada that DNA is the seat of the primary X ray mutational event, but not the UV one, should be re-examined.

Bridges' suggestion that DNA linkage to protein may be involved in reversion from auxotrophy to prototrophy could fall into place here. However, I should like to mention one aspect of Hill's results, namely the effect of visible light after UV (Fig. 13). If the number of prototrophs per unit volume is plotted as a function of surviving fraction, it can be seen that, at every level of survival, fewer mutants

are observed in suspensions which have been exposed to visible light. This indicates that the "photo-restoration" phenomenon is more effective in reversing mutational than killing events; if we take into account also the relatively low oxygen effect, observed

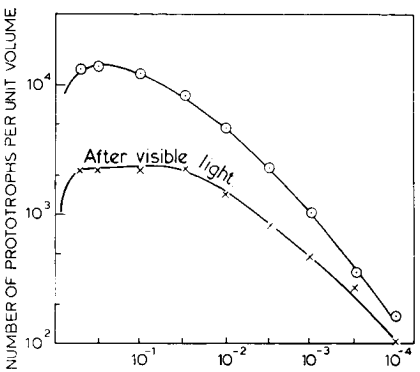


Fig. 13. Reversion to prototrophy of tryptophan-requiring mutant of *E. coli* B/r. Number of prototrophs per unit volume induced by UV. **O**: Kept in dark after irradiation. ×: Exposed to visible light after irradiation. (Hill.)

by Bridges, for mutations induced by X rays, it is tempting to regard the target for the induction of biochemical mutants as nucleic acid, whereas killing may be due to a large extent to a target of a different chemical nature. At any rate, these observations strongly support Magni's conclusion that biochemical mutations and cell death are due to primary events of a very different nature.

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DISCUSSION

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The rapporteur's superimposition of Haynes'[d]) data in which he shows an increase in UV sensitivity as the DNA thymine content increases, upon that of Kaplan[g]), who finds an increase in X ray sensitivity as the guanine-cytosine (G-C) base pair composition of DNA increases, brought comment from both audience and authors. Howard-Flanders *(Yale Univ. New Haven, Conn., USA)* remarked that it is unlikely that an effect of base ratio would be evident, unless DNA is a principal target of UV and X irradiations; furthermore, he noted that the findings of these workers support the view that the G-C base pairs may be involved in the cellular inactivation by X rays and the adenine-thymine (A-T) pairs in the cellular inactivation by UV. This latter point was taken still further by Haynes, who remarked that thymine dimerization may well be the chemical reaction induced by this type of photoexcitation. This author described current experiments in which sensitivity to nitrogen mustard is being investigated as a function of base pair composition of DNA in bacteria. On the basis of recent evidence of Brookes and Lawley[1]) that nitrogen mustard alkylates guanine in DNA, Haynes predicted that the results from these new experiments would parallel Kaplan's results with X rays.

Kaplan agreed with the remarks of Howard-Flanders summarized above, and offered supporting evidence for his interpretation of the radiosensitization of bacteria containing halogenated purine and pyrimidine analogues in their DNA. According to the author, his results are consistent with those obtained by other workers who carried out *in vitro* irradiations of aqueous solutions of purines and pyrimidines; the radiation-induced chemical changes show a dependence on oxygen with purines and an independence with pyrimidines. In this connection, Kaplan called attention to a difference in hydrogen bonding between the two strands of the DNA helix

containing different substituted analogues of purine and pyrimidine bases.

In reply to a request for his interpretation of the mechanisms of radiosensitization by base analogue substitution, Szybalski[k]) emphasized the need to consider two aspects of sensitization: (1) the intrinsic sensitization of DNA molecules, and (2) the effect of substitution on the repair of DNA molecules. Szybalski quoted evidence for a repair mechanism based on work performed with the radiosensitive bacterial strain *E. coli* B_s [2]) which seems to be devoid of any repair mechanisms. With substitution of 5-bromodeoxyuridine (BUDR) in this organism most of the analogue radiosensitization does not occur. Further support for his concept of intrinsic sensitization was drawn from his recent experiments involving *in vitro* UV irradiations of DNA with and without BUDR substitution. In contrast to normal DNA, substituted DNA shows fragmentation and increased reactivity.

Magni[h]) elaborated on the rapporteur's presentation of his work by stating that his most recent findings suggest that the number of sites sensitive to recessive lethal damage in his yeast strain is 25 to 30, rather than 70 reported in his paper. He drew attention to the fact that this smaller number is similar to the number of chromosome arms and suggested that recessive lethal damage is due to large chromosome rearrangement or large chromosome deletions.

Moustacchi[i]) reiterated the explanation for ^{32}P decay induced X ray resistance given in her paper, an explanation opposed by the rapporteur. The author stated that the appearance of X ray resistant cells in populations of yeast cells containing incorporated ^{32}P could not be explained by the selective killing of resting cells, since this radioresistance remained unchanged upon isolation and subculture of these cells. Further, she showed that this characteristic is genetically transmissible and she feels that these findings point to a genetic basis for induced X ray resistance rather than the explanation proposed by the rapporteur.

In a final statement to this session, Kada[f]) re-emphasized his position on the observed difference in the transmission of X ray and UV induced mutants in *E. coli* K12 Hfr. From the high level of incorporation of X ray induced mutants, he concludes that the site responsible for X ray induced mutation is on the chromosome. However, he does not draw the corresponding conclusion that the

principal site for UV action is in the cytoplasm from his observation of a low level of incorporation with this type of radiation. His suggestion was that this result may well stem from repair or other mechanisms in the female bacteria.

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Factors Influencing the Sensitivity of Cells

CELLULAR AND MACROSCOPIC DAMAGE IN RELATION TO CYTOLOGICAL OBSERVATIONS

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1. INTRODUCTION

It is a commonly held view in radiation biology that cell killing by ionizing radiations is primarily a consequence of the deposition of energy in the cell nucleus. The first of the papers in this session has a bearing on this general concept, for Bowen's[b]) paper is an extension of the studies of Sparrow and his colleagues[1]) on the relation between radiation-induced growth inhibition and nuclear volume or DNA content in higher plant species. Bowen has studied the relation between the LD 50 values obtained from irradiated seed of forty plant species and the DNA content per cell in these

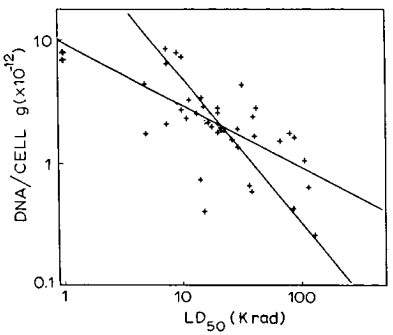

Fig. 1. The relationship between LD 50 and DNA content for 40 plant species. The lines represent regressions of log DNA on log LD 50 and *vice versa*. (Bowen.)

forms. The results are shown in Fig. 1, the two lines representing regression of log DNA content on log LD 50 and *vice versa*; the difference in the slopes of the lines being a measure of the scatter of the data. Although the LD 50 values extend from about 1000 rad for seeds of sensitive plants, such as pine trees, to about 100000 rad for the more resistant mustard seeds, the correlation is highly significant ($p = 0.003$). This result thus supports the contention that the vital target in the cell is the nucleus and that the amount of DNA is an important variable.

2. CHROMOSOME DAMAGE AS A CAUSE OF CELL DEATH

If we accept the hypothesis that the nucleus is all important, then it is logical to suspect that a large proportion of the lethal effects of radiation are simply consequences of the genetic loss which inevitably follows upon breakage and rearrangement of chromosome structures. Read and Wakonig[e]) have attempted to test this hypo-

thesis in experiments using *Vicia faba* roots. Comparisons were drawn between the chromosome breaking and growth inhibiting capabilities of a variety of chemicals, such as triethylenemelamine and nitrogen mustard, and also various kinds of ionizing radiation. Their results show that although different concentrations or doses of the various radiations or chemicals were required to produce a given level of growth inhibition, at this level the frequency of chromosome fragments, or micronuclei, is similar between treatments. In other words a strong correlation exists between the degree of growth inhibition and the amount of chromosome damage, regardless of the type of radiation or chemical employed. Furthermore, it appears that the time of expression of growth inhibition is correlated with the time of appearance of chromosome damage.

If genetic loss is the important factor in cell killing then certain aberration types, such as those which yield bridges and fragments at anaphase, should have a greater damaging effect than others, such as symmetrical translocations, which result in little or no genetic loss. This problem is considered by Revell[f]) who presents data on frequency at metaphase of the various aberration types induced in *Vicia faba* roots exposed to 50 r X rays. The data are analysed in an attempt to determine what proportion of the cell population,

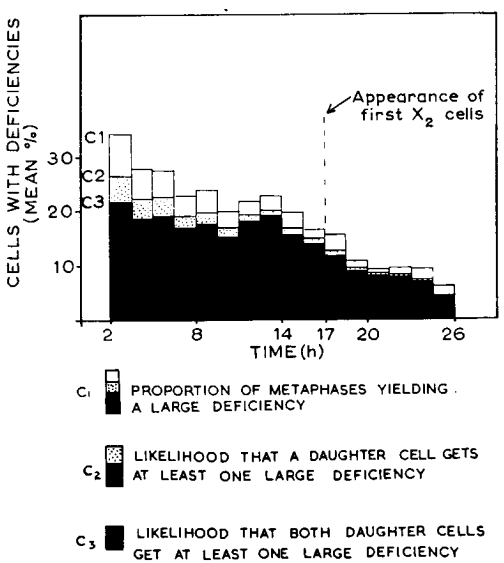

Fig. 2. Frequency of deficient cells induced throughout one mitotic cycle in Vicia root tip cells exposed to 50 r of X rays. (Revell.)

and their descendants, suffer large chromosome deficiencies. The data and conclusions are summarised in Fig. 2, from which it may be seen that the average probability of a cell being so damaged that both its daughter nuclei contain large deficiencies is about 0.15.

Since Revell's paper was received for this report, Hall, Lajtha and Oliver[2]) have published a paper giving survival curves for Vicia root cells as deduced from root growth inhibition studies. From these curves, at a dose of 50 r of X rays, the fraction of cells which lose their reproductive integrity is about 0.2 to 0.25. This estimate is clearly similar to that arrived at by Revell, despite the fact that in the cytological study small fragments, described as minutes, were deliberately excluded in classifying deficient cells.

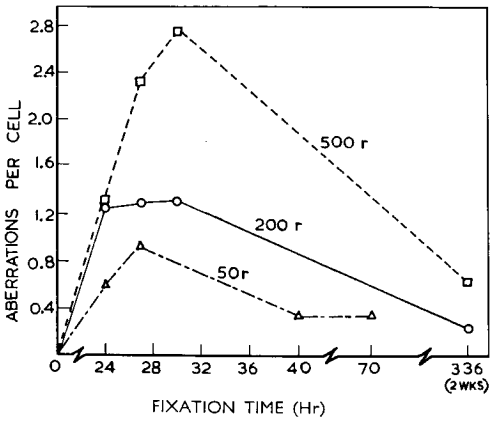

Fig. 3. Aberration frequencies in X irradiated pig kidney cells. (Stroud, Brues and Svoboda.)

Another approach in the study of the importance of chromosome aberrations is to seek information on the fate of cells carrying chromosome damage, by serial examinations following irradiation. We have to remember, however, that aberration frequencies determined in the first hours or days after irradiation may be influenced by mitotic delay. For instance, in Painter's[d]) experiments with HeLa cells, which will be reported on in detail by Lajtha in a later session, it has been shown that a dose of 300 r of X rays gives a mitotic delay of about 17 hr, whereas with 500 r cells may be delayed in reaching mitosis for about 28 hours. Mitotic delay is evident in the results of early fixation times in the data of Stroud, Brues and

Svoboda[1]) on aberration frequencies in X irradiated pig kidney cells (Fig. 3). These authors show that the aberration frequency is highest in the first division cycle following irradiation, and then undergoes a progressive decline, damaged cells either losing their capacity for proliferation, or competing unfavourably with cytologically undamaged cells. Dicentric aberrations were found to be rapidly eliminated, but, as has been shown in other materials, reciprocal translocations were maintained within the populations.

How and why some of the aberrations are eliminated and others perpetuated is the question to which Bora, Singh, Patil and Mouli[a]) address themselves. Their paper describes an attempt to see whether or not there is a consistent pattern in the elimination of various aberration types in X irradiated seed of tetraploid (*T. dicoccum* $2n = 28$) and hexaploid (*T. aestivum* $2n = 42$) wheat. Cells were sampled from root tips up to 18 days after irradiation and were classified into five categories:

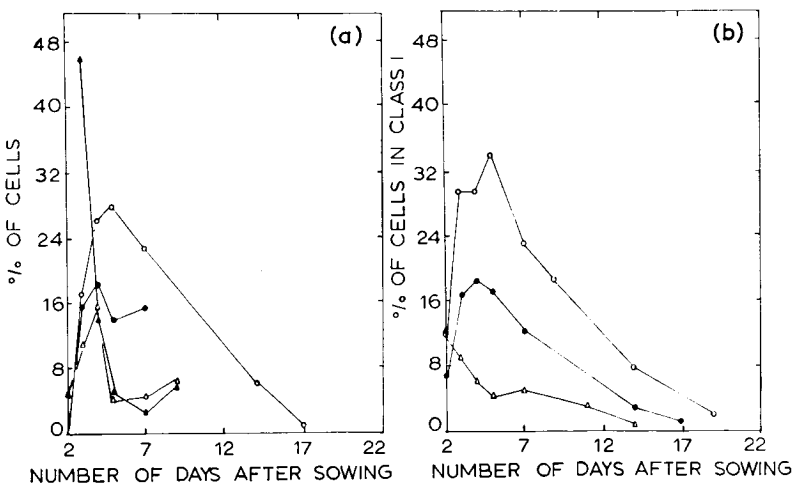

Fig. 4. Relative frequencies and rates of elimination of the various aberrant cell types in Triticum (Bora, Singh, Patil and Mouli.) For classes of cells see text.

a. Elimination of Class 1–4 cells in *T. aestivum* var. Kenphod ($6x = 42$) after 30000 r X rays

O—O Class 1 △—△ Class 3
●—● Class 2 ▲—▲ Class 4

b. Elimination of Class 1 cells in *T. dicoccum* var. Jaya 808 ($4x = 28$)

O—O 30000 r △—△ 7500 r
●—● 15000 r

class 0 – cells without any apparent chromosome abnormalities,
class 1 – cells with either a single centric ring or a single dicentric chromosome, but no fragments,
class 2 – cells with two aberrations, either dicentrics or centric rings,
class 3 – cells of class 1 or 2 but accompanied by fragments,
class 4 – cells with gross changes such as polycentrics and cells containing only acentric fragments.

A typical result illustrating the relative frequencies of the various classes is shown in Fig. 4, from which it may be seen that the class 1 cells are not only predominant over the other aberrant classes, but also persist for the longest period. An analysis of the data is complicated by the fact that a cell could shift from one class to another, e.g. by loss of a fragment. In other words, the aberrant chromosome structure could be eliminated rather than the cell carrying it. Kinetic studies on the rates of elimination lead to the conclusion that the probability of undergoing further division decreases with increasing class number, within both tetraploid and hexaploid species. Furthermore, the actual probabilities for mitosis in a given class of cells varies with dose and may differ between the two species.

3. RADIOSENSITIVITY AND PLOIDY

The data discussed thus far strongly implicate chromosome damage as a cause of loss of reproductive capacity, so that it is reasonable to expect that polyploid cells should be more resistant than diploid cells, since they may require inactivation or loss of a larger number of homologous chromosome regions. This possibility is explored by Révész and Norman[g]) and by Hornsey and Silini[c]).

Révész and Norman X irradiated cell suspensions of a hyperdiploid (ELD) and hypertetraploid (ELT) line of Ehrlich ascites tumour cells *in vitro*, either in the presence or complete absence of oxygen. Following irradiation, the cells were introduced into diffusion chambers and placed in the peritoneal cavities of mice. The rates of increase in cell population size were then determined at various times, and the results are shown in Fig. 5.

Following irradiation in nitrogen, the slower growing ELT line was found to be more resistant to the depressant effect of radiation than the ELD line. The doses required to prevent any increase in

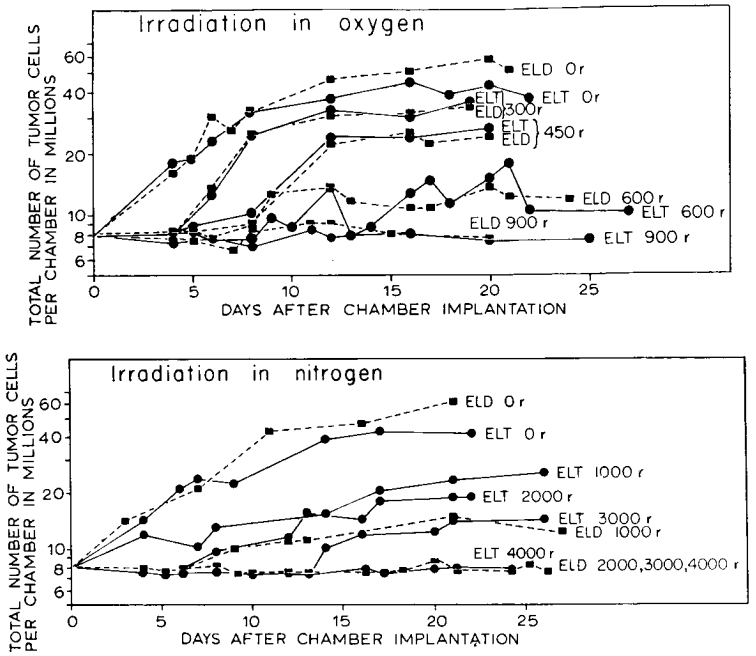

Fig. 5. Cell population size following irradiation in oxygen (a) or nitrogen (b) of hyperdiploid (ELD) and hypertetraploid (ELT) Ehrlich cells. (Révész and Norman.)

cell number over and above the initial inoculum size were about 4000 r and 2000 r respectively. However, irradiation in oxygen gives quite a different picture, in that the radiosensitivity differences observed between the diploid and tetraploid lines irradiated in nitrogen were abolished. The growth curves obtained at a given dose in oxygen for both cell lines were similar, and in both lines no increase in cell number occurred after a dose of 900 r. Expressing the results in a somewhat different way we may say that the oxygen enhancement factor for the ELD line is roughly about 2.2, but for the ELT cells is about 4.4.

Révész and Norman suggest that the correlation between cell ploidy and radiosensitivity under anoxic irradiation implies that genetic damage is a major cause of cell death. The results for the irradiation in oxygen, however, are taken to imply that factors other than genetic ones are mainly involved in cell death under aerobic conditions of irradiation.

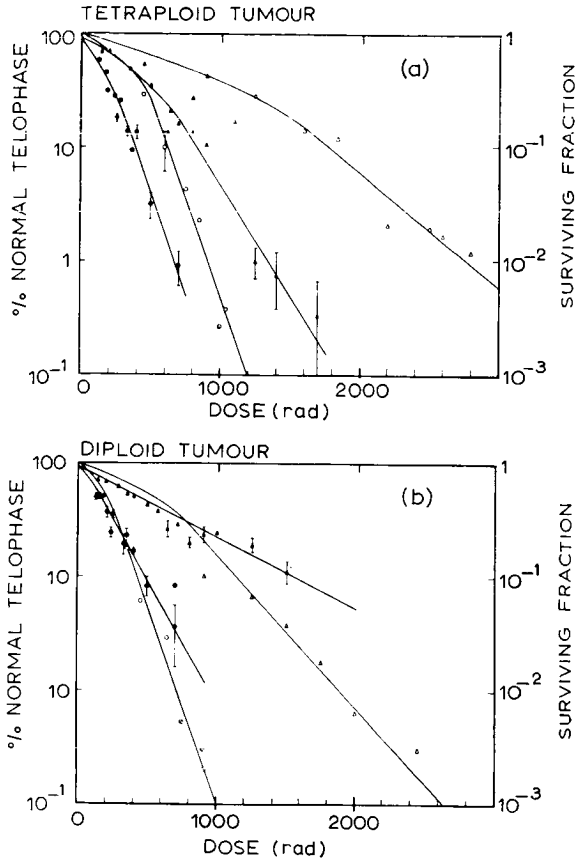

Fig. 6. Cell survival and telophase abnormalities in diploid and tetraploid Ehrlich ascites tumour cells (D_0 is the slope of the exponential part of the curve). (Hornsey and Silini.)

a. Tetraploid tumour

Oxygenated cells: ● Normal telophase (15 hr) $D_0 = 110$ r, ○ Surviving cells

Anoxic cells: ▲ Normal telophase (15 hr) $D_0 = 220$ r, △ Surviving cells

$\frac{D_0N_2}{D_0O_2}$: for abnormal telophase: 2.0
for cell killing: 3.1

b. Diploid tumour

Oxygenated cells: ● Normal telophase (18 hr) $D_0 = 220$ r, ○ Surviving cells

Anoxic cells: ▲ Normal telophase (18 hr) $D_0 = 600$ r, △ Surviving cells

$\frac{D_0N_2}{D_0O_2}$: for abnormal telophase: 2.7
for cell killing : 2.7

Hornsey and Silini also used a hyperdiploid and near tetraploid line of Ehrlich ascites tumour cells, with modal chromosome numbers of 46 and 96, in their studies on the relationship between telophase abnormalities and cell survival. As with the materials used by Révész and Norman, the tetraploid strain had twice the nuclear volume and DNA content of the diploid, and the cells were irradiated as suspensions *in vitro*, at ice temperature, and under controlled gas conditions. Cell survival was determined from the ratio of tumour takes from irradiated and unirradiated cells injected subcutaneously into young mice[3]). Telophase abnormalities were scored from cells inoculated intraperitoneally and fixed at 15 or 18 hr after irradiation, and the results are summarized in Fig. 6.

If the radiation target volume for chromosome aberrations is the chromosome volume, then the ratios of the slopes, or D_0 values, for aberrations in the $2n$ and $4n$ cells should be in the ratio of 1:2. This is observed if the irradiation is given in O_2, but in N_2 the ratio is higher, approximating to 1:3. In other words the oxygen enhancement ratio for aberrations in tetraploid cells is less (2.0) than in diploid cells (2.7). However, as Révész and Norman also found, the oxygen enhancement ratio for cell killing is the other way around: $4n = 3.1$ and $2n = 2.7$. Hornsey and Silini conclude that the $4n$ cells present some additional target for chromosome damage which is relatively insensitive to the presence of oxygen at the time of irradiation. Furthermore, as the oxygen enhancement ratios for cell killing (3.1) and telophase abnormalities (2.0) in the tetraploid line are different, then those aberrations which are little affected by oxygen appear to play little part in cell killing.

4. RADIOSENSITIVITY AND PROLIFERATIVE CAPACITY

In the work of Stroud *et al.*, to which we have already referred, and in the paper by Sinclair[h]) attention is focussed on the cytological structure and behaviour of mammalian cells which survive high doses of X rays. Sinclair shows that following exposure to a single dose of X rays of predominantly diploid Chinese hamster cells (clone V79-1) cultured *in vitro*, the surviving single cells give rise to clones which vary in size and growth rate. So-called large colonies and small colonies are produced, and the relative frequency of small colonies increases with increasing dose (Fig. 7).

By subculturing individual colonies, or parts of colonies, informa-

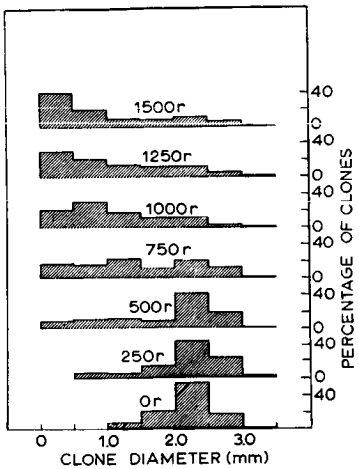

Fig. 7. Distribution of hamster cell clone sizes with X ray dose. (Sinclair.)

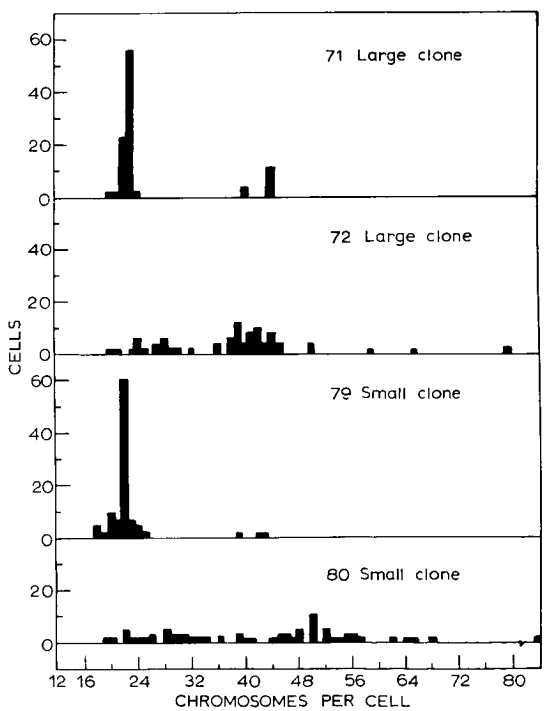

Fig. 8. Distribution of chromosomes per cell in hamster cells from small and large clones. (Sinclair.)

tion was obtained on growth rate, chromosome number, cell size, plating efficiency, aberration frequency, and radiosensitivity of cells from colonies of the two types. The results are summarized by reference to four clones

clone 71 – large colony, normal cell size distribution.
clone 72 – large colony, increased frequency of large cell types.
clone 79 – small colony, normal cell size distribution.
clone 80 – small colony, increased frequency of large cell types.

Cell size and chromosome number in these clones were found to be closely correlated, but no correlation existed between these parameters and clone size (Fig. 8). The differences in clone sizes were therefore a function of growth rate (Fig. 9). From this figure it may be seen that the average cell doubling times for large clones is the same as in unirradiated cells, about 11 hr, whereas the population number in small clones increases at less than half the normal rate.

Cells from large and small clones were exposed to a second dose

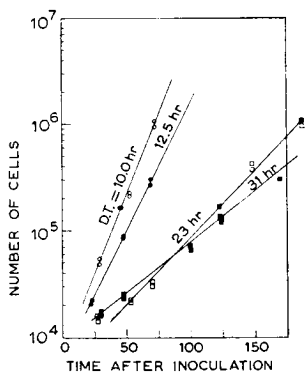

Fig. 9. Growth of large and small clones of hamster cells after transplantation. (Sinclair.)

○ Large clone 71
● Large clone 72
□ Small clone 79
■ Small clone 80
DT = doubling time.

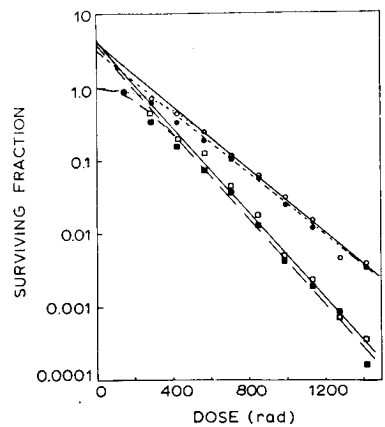

Fig. 10. Survival curves for large and small clones of hamster cells. (Sinclair.)

	Extrapolation number	D_0
○ Large clone 71	~ 4.1	200 rad
● Large clone 72	~ 3.2	205 rad
□ Small clone 79	~ 4.3	148 rad
■ Small clone 80	~ 3.8	145 rad

of X rays and the survival curves obtained are shown in Fig. 10. Cells from large clones appear not to be influenced by their previous radiation history, and yield a normal survival curve with an extrapolation number of 3 to 4 and a D_0 value similar to the D_0 value of 203 rad obtained from cells exposed to radiation for the first time. Cells from small colonies also show little change in extrapolation number, but are more radiosensitive, having a D_0 value of about 145 rad.

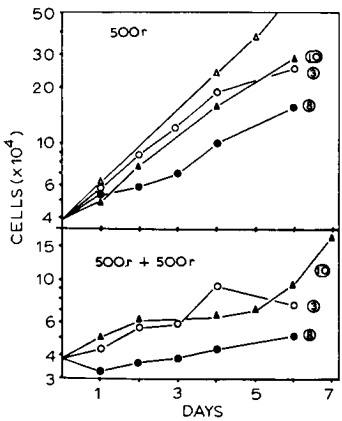

Fig. 11. Growth curves of pig kidney cells after X irradiation. Upper curves: growth rates of control and three surviving clones following a single X ray dose. Lower curves: growth rates of the three clones after a second dose of radiation given 9 months after the first. Note the higher sensitivity of the slower growing clones. (Stroud, Brues and Svoboda.)

Doubling time after the single dose:

control (B_2): 1.5 days
(10): 2.04 days
(3): 1.75 days
(8): 3.3 days.

From these results Sinclair suggests that the damage resulting in small colony formation occurs in sites other than those directly involved in cell survival. Furthermore, neither the radiation-induced changes in chromosome number nor, apparently, the aberration frequency appear to be correlated with what might be called the proliferative capacity, as measured by growth rate and plating efficiency. Radioresistance is, however, positively correlated with proliferative capacity, a finding which is supported by the results of Stroud *et al.* as shown in Fig. 11.

5. CONCLUDING COMMENTS

The proliferative capacity of a cell is clearly a function of the whole cell, although in the last analysis the nucleus, as the cell governor, must be of supreme importance. The results of the papers discussed are in accord with this concept, in that nuclear damage, or more

specifically chromosome fragment loss, appears to be a primary cause for loss of proliferative capacity. However, it is not always a cause, for cells may carry considerable chromosome damage without being inviable.

Again, under differing conditions, the relative importance of various intra- and extra-cellular factors may alter. This is well illustrated by the fact that in Sinclair's experiments (and see also Till[4])) tetraploidy *per se* did not appear to influence radiosensitivity, whereas with Ehrlich cells irradiated under anoxia, appreciable differences between the radiation response of diploid and tetraploid cells became apparent. At the moment it seems that we are in no position to draw any simplified general conclusions relating chromosome damage and cell viability, but recent advances, particularly in single cell mammalian biology, offer high hopes for the future.

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DISCUSSION

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In response to a request from the Chairman Tobias (*Univ. of Calif., San Francisco, USA*), Révész[g]) pointed out that the stem line of tumour cells was often aneuploid. His ELD line had a diploid number of 46, against the normal number of 40 for the mouse. The two distinct marker chromosomes in this line were monosomic, and it was possible that the genome might be monosomic for certain chromosomes which were critical in determining radiosensitivity. In the ELT hypertetraploid line, on the other hand, the stem line appeared to be an exact doubling of that of ELD.

Hornsey[c]) mentioned that she had also measured survival by

inoculating cells into the brains of adult mice. These survival curves differed from those obtained in baby mice; they were steeper, and the extrapolation numbers were greater. Immunity developing in the adult hosts did not seem to account for the whole of this difference; other post-irradiation culture factors seemed to be of importance, and they affected cells irradiated in nitrogen more than those irradiated in oxygen. As a result the oxygen enhancement ratio obtained with inoculation into the brains of adult animals was 2.4 compared with 3.1 when baby mice were employed. She emphasized that the telophase damage had been studied in cells grown under conditions different from those in which survival was measured, and urged caution in comparing the two sets of results. She then expressed surprise at the smallness of the doses which Révész had found sufficient to prevent proliferation. Révész replied that his cells were grown in diffusion chambers bounded by membranes with pores 0.5μ wide, which permitted peritoneal fluid to diffuse through but protected the cells from cell-mediated host reactions. In comparison with direct intraperitoneal inoculation, these were adverse conditions so far as cell multiplication was concerned.

Sinclair[h]) said that, in addition to small irradiated colonies, he had isolated a small slow-growing clone from a population of normal unirradiated hamster cells. Its performance and increased radiosensitivity were very similar to those of irradiated small clones. This and other evidence ruled out the possibility of the difference in sensitivity being due to residual radiation damage. The evidence he had accumulated since the Colorado Springs meeting showed clearly that ploidy *per se* did not influence the radiosensitivity, but that cells of low proliferative capacity were always more radiosensitive.

Tobias asked Sinclair why he believed that the damage which resulted in small colony formation occurred at sites other than those which determined cell survival. Sinclair replied that according to simple multitarget theory, at doses beyond the shoulder of the curve all surviving cells would have received $(n - 1)$ hits. Further increase in dose should affect the number of survivors but not their character, unless additional damage which was still dose dependent was being recorded at other sites. Since the spectrum of colony sizes continued to decrease even beyond the shoulder, the increase in intermitotic time did seem to be due to damage at sites other than those determining survival. Other explanations were possible, but less likely,

and Sinclair emphasized that the sites involved might well be nuclear.

In reply to a question by von Borstel (*Oak Ridge Nat. Lab., USA*) Bowen[b]) said that his LD 50's were scored at maturity, or after one year in the case of gymnosperms. Von Borstel, quoting work by Sicard[1]) said that in timothy seeds a number of cells entered into the germination process, but only a fraction of these needed to be killed in order to inhibit germination. This complicated the situation and he urged caution in attempts to relate sensitivity with the DNA content per cell.

Asked by the Chairman to justify his postulated correlation between DNA content and LD 50, when one of the points was out by a factor 10 so far as DNA content was concerned, Bowen said that he had no explanation for the scatter in his points, and had tried to emphasize that it was not possible to predict the LD 50's of seeds by measuring their DNA content. Evans mentioned that in Bowen's work some 40% of the species were polyploid. Work by Sparrow[2]) and his colleagues had shown that polyploidy *per se* was quite an important factor in sensitivity tested in this way in higher plants; some of the scatter might thus be due to the inclusion of tetraploid and hexaploid plants. Sparrow (*Brookhaven Nat. Lab., USA*) said that, when different diploid species were compared, his group had found that the average amount of DNA per chromosome gave a much better correlation with radiosensitivity than the amount of DNA per nucleus. They concluded that chromosome number or, more specifically, chromosome arm number, was also a factor which must be considered. Alper (*Hammersmith Hospital, London, Great Britain*) asked Sparrow to comment on the fact that in mammalian cells, where there was a wide range of chromosome numbers, the sensitivity appeared to vary by a factor of at most two. Sparrow assumed that this might be ascribed to the greater complexity of mammalian systems.

Barendsen (*Radiobiological Inst., Rijswijk, Netherlands*) asked Hornsey whether there was a difference in the oxygen enhancement ratios at high and low dose levels, and whether the difference between the figures of 2.7 and 3.1 was significant over the whole dose range. She replied that the ratios given referred to doses which gave the same survival in the middle of the exponential part of the curve. The actual ratios would then increase slightly, but not significantly, as the dose was increased. The "shoulder" of the

survival curves was not sufficiently accurately defined for an enhancement ratio referring to this region to be measured.

Lindop (*St. Bartholomew's Hospital, London, Great Britain*) said that the work of Hornsey and Révész could be interpreted as meaning that the presence of oxygen masked a difference in sensitivity, which could be demonstrated under anoxic conditions. She mentioned that she had found a variation in LD 50 of only 10% with age when mice were irradiated in oxygen. In nitrogen, the variation over the same age range was by a factor of nearly two.

Cohen (*General Hospital, Johannesburg, South Africa*) mentioned that he had found that attempts to induce tumour resistance in mice with irradiated isogenic tumours always failed when the inoculum was irradiated under fully anoxic conditions, but occasionally succeeded when the irradiated tissue was oxygenated. He suggested that irradiation under anoxic and oxygenated conditions produced qualitatively distinct antigenic changes, and asked Hornsey to comment. She replied that in her system cells given 12600 r anoxically increased the TD 50 of untreated tumour cells to a greater extent than cells given 1720 r in oxygen, so that the effect of oxygen seemed to be in the reverse direction. On the other hand, cells given very high doses (36000 r) under anoxia produced little if any immunizing effect on the host. She emphasized that her system was quite different from Cohen's, and that the tumour-host relationship was in no way isogenic.

Goldfeder (*Cancer and Radiobiol. Res. Lab., New York 32, USA*) focussed attention on cytoplasmic damage. She had made electron microscopical observations on mouse lymph nodes and tumour cells grown *in vivo*. At 100 min after 200 r, dilation of vesicles in the endoplasmic reticulum was observed, leading to breakage of the plasma membranes. At 10 min after 3000 r, the plasma membrane collapsed and intracellular constituents like mitochondria and microsomes poured out. Damage was particularly impressive in plasma cells.

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Factors influencing the Sensitivity of Cells

STAGES IN THE CELL CYCLE AND GERM CELL DEVELOPMENT

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a) E. BALLARDIN and P. METALLI,
X ray effects in relation to the stages of oogenesis in parthenogenetic *Artemia salina* of different ploidy.
Istituto di Genetica, Università di Milano, Italy

b) A. J. BATEMAN,
Induction of mutations in mouse spermatocytes.
Cytogenetics Laboratory, Christie Hospital, Manchester 20, Great Britain

c) K. B. DAWSON and E. O. FIELD,
Cell radiosensitivity studied by a double isotope autoradiographic technique.
Chester Beatty Research Institute, Fulham Road, London SW 3, Great Britain

d) W. C. DEWEY and R. M. HUMPHREY,
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Physics Department, M.D. Anderson Hospital, Texas Medical Centre, Houston 25, Texas, USA

e) ALOHA HANNAH-ALAVA,
Rates of visible mutations as related to the maturity of the male germ cells of *Drosophila melanogaster* at the time of radiation.
Department of Genetics, University of Turku, Finland

f) V. MONESI,
Variation of the X ray sensitivity during the cell cycle in spermatogonia of adult mouse.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA and *Research Group for Embryology, Biology Division, CNEN, Frascati, Italy*

g) J. A. PURO,
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Department of Genetics, University of Turku, Finland

h) M. SINGH,
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Agricultural Bio-Sciences Division, Puerto Rico Nuclear Centre, Mayaguez, Puerto Rico

i) Y. TAZIMA and S. KONDO,
Further studies on two types of dose-rate dependence of radiation-induced mutation rates in spermatogonia and oogonia of the silkworm.
National Institute of Genetics, Misima, Japan

[j]) T. TERASIMA and L. J. TOLMACH,
Variations in the response of HeLa cells to X rays during the division cycle.
Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri, USA

[k]) ANNA R. WHITING,
A comparison of visible mutation rates in Habrobracon eggs X rayed in first meiotic metaphase or prophase.
University of Pennsylvania, Philadelphia, USA

[l]) D. E. WIMBER,
DNA synthesis and cell proliferation in chronically irradiated Tradescantia roots and mouse gut studied with a ^{14}C- and ^{3}H-thymidine double labelling technique.
Brookhaven National Laboratory, Biology Dept., Upton, NY, USA and *Royal Marsden Hospital, Belmont, Surrey, Great Britain*

1. INTRODUCTION

The papers to be discussed in this session can be arranged in the following scheme.

A first group deals with changes in sensitivity during oogenesis, that is with sensitivity differences between stages of meiosis in the female. The material for one investigation is the wasp Habrobracon. For the other it is parthenogenetic lines of the crustacean Artemia.

The second group deals with sensitivity changes during spermatogenesis, that is with sensitivity differences between the germ cell types from early spermatogonia to mature spermatozoa. Bateman[b]), working with mice, scores effects that presumably are due to translocations, while the two Drosophila workers scored mutations as the criterion of damage. The work on silkworms is mainly concerned with mutations but considers also cell death. This leads to the paper by Monesi[f]) on mice, which I have put into a group of its own because it is concerned only with death of irradiated germ cells, in relation to the temporal pattern of DNA synthesis. This forms a link with:

The third group of papers, which deal with sensitivity changes during the mitotic cycle. The cells are treated either in the living organs (mouse gut, plant roots) or in culture (HeLa cells, Chinese hamster cells). Two papers describe results obtained by a double-labelling technique. All but one use cell death and mitotic delay as criteria of damage. The paper by Dewey and Humphrey[d]) measures chromosome damage and is concerned with the effects of dose fractionation and dose rate. This forms a link with the work

by Tazima and Kondo[i]), who compare dose rate responses in different stages of spermatogenesis.

2. SENSITIVITY DURING OOGENESIS

Let us consider the papers in group I. Whiting[k]) compares the frequencies of visible mutations induced by X rays in prophase and metaphase in oocytes of Habrobracon. This investigation rounds off a series of studies in which similar comparisons have been made for lethal mutations. Fig. 1 summarizes the results for dominant and

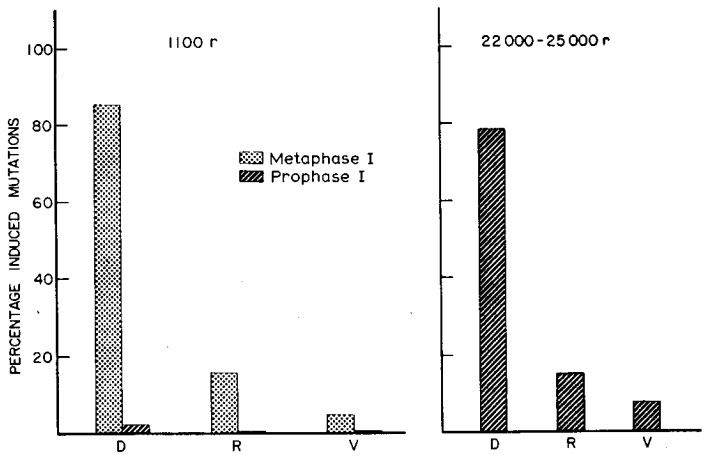

Fig. 1. The frequencies of Dominant (D) and Recessive (R) lethals and of Visible mutations (V) in irradiated oocytes of Habrobracon. (Whiting.)

recessive lethals and for visibles. A given dose of X rays produces all these effects much more frequently in metaphase than in prophase. In order to obtain quantitatively comparable results in prophase, one has to use a 20 times higher dose. For dominant lethals, cytological studies have suggested an explanation of the difference. Breaks produced in prophase give rise to bridges at the first meiotic anaphase. In Habrobracon, as in Drosophila, such bridges tie together the two inner nuclei of the tetrad and do not harm the zygote nucleus, which is one of the outer ones. On the contrary, breaks produced in metaphase give rise to bridges at the second meiotic anaphase, and half of these are lethal to the zygote nucleus. This still

leaves unexplained the refractoriness of prophase nuclei to the production of recessive lethals and visible mutations.

Ballardin and Metalli[a]) have used Artemia, an organism in which the timing of meiosis is facilitated by synchronous development of the oocytes, each stage having its characteristic position within the genital tract. The criterion of damage was hatchability inhibition, interpreted as dominant lethality. Their main results are shown in Fig. 2. Hatchability is lowest in the earliest prophase stage scored

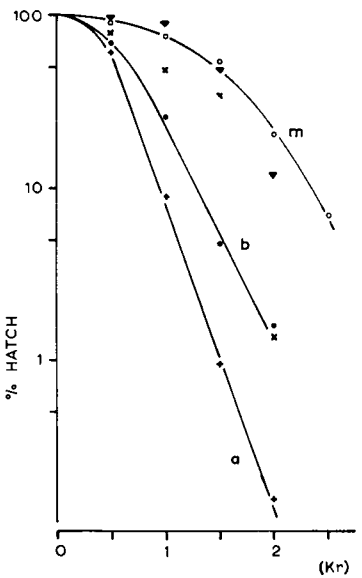

Fig. 2. The frequency of dominant lethals in irradiated oocytes of *Artemia salina*. a – Early prophase; b – Middle prophase; m – Metaphase. (Ballardin and Metalli.)

and increases throughout prophase to a maximum at metaphase. This is in direct contrast to the data on Habrobracon, and the authors discuss several tentative interpretations of the discrepancy. I should like to mention an additional factor which might be considered: the absence of the cytological mechanism which in Habrobracon protects the zygote nucleus against most of the damage due to chromosome breakage in prophase. It should also be realized that the Italian workers and Mrs. Whiting use the term prophase in a different sense. Prophase in Habrobracon is the stage preceding diakinesis while in Artemia even the earliest scored oocytes had already passed diakinesis.

One more paper belonging in this group arrived too late for

inclusion in the scheme. Singh[h]) studied chromosome fragmentation after irradiation of premeiotic inflorescence buds of tomatoes. He too found highest sensitivity in early prophase; a second peak of sensitivity occurred in metaphase II.

3. SENSITIVITY DURING SPERMATOGENESIS

We now come to the second group of papers, those dealing with spermatogenesis. Bateman[b]) compared the sensitivity of spermatids and spermatocytes of mice by determining the incidence of sterility and semi-sterility among sons and daughters conceived at different times after irradiation. Semi-sterility is presumed to be mainly due to translocations. A summary of the data is shown in Table 1.

TABLE 1

Sterile and semi-sterile progeny of males given 200 rad

Treated cell stage	Weeks after irradiation		
	3 spermatids	4 and 5 spermatocytes	Control
F_1 males			
Nr. effectively mated	65	54	34
% sterile	17	5.6	2.9
% semi-sterile	13.8	2.0	0
F_1 females			
Nr. effectively mated	52	49	26
% sterile	11.5	8.2	11.5
% semi-sterile	30.4	8.2	0

(Bateman).

Among sons, the frequency of semi-sterility was almost 7 times as high in the third week, representing irradiated spermatids, as in the fourth and fifth, representing irradiated spermatocytes. This agrees with previous findings of the author on dominant lethality. The incidence of sterility paralleled that of semi-sterility, contrary to what had been concluded in the abstract from preliminary data. Among the daughters, sterility was not increased by irradiation of the father. Semi-sterility showed the same trend as among the sons but at a considerably higher level, a finding for which Bateman offers various tentative explanations. It can be seen that the sum of sterile and semi-sterile sons equals that of semi-sterile daughters

in both periods. This supports a suggestion made to me by Cattanach, who thinks that many translocations (not necessarily involving the X or Y) cause complete sterility in males but only semi-sterility in females.

Hannah-Alava[e]) has determined the sensitivity pattern for visible mutations at 9 specific loci on the third chromosome of Drosophila. She also scored Minutes and other dominant visible mutations. The results of 7 replicate experiments are summarized in Fig. 3. They

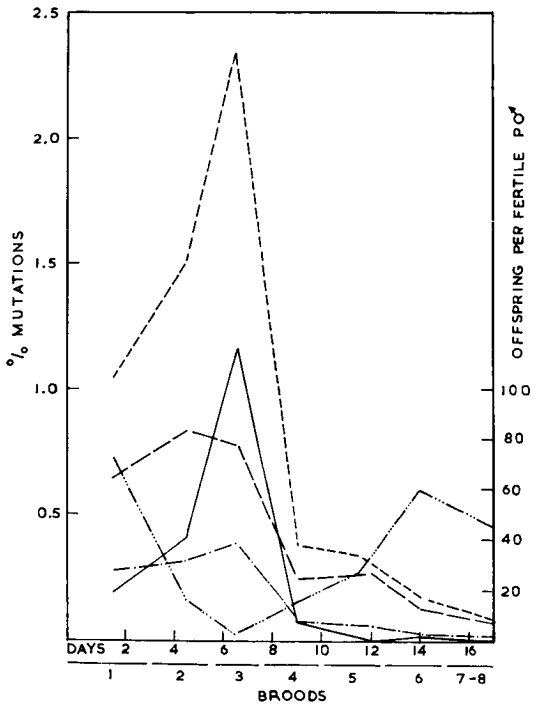

Fig. 3. Brood pattern of mutation rates in irradiated males of *Drosophila melanogaster*. (Hannah–Alava.)

———— Recessives
—·—·— Dominants
– – – – – – – Minutes
- - - - - - - - Per chromosome
—··—··— Offspring per fertile male.

show the familiar pattern of a rise of mutation frequency in an intermediate brood (here the third) followed by a steep drop in late broods. The fact that maximal mutation frequency coincided with minimal fertility suggests that the peak of mutation frequency oc-

curred in spermatocytes. However, so few offspring were obtained in the third brood (less than 300 flies carrying 6 mutations) that the confidence limits of mutation frequency in this brood must have been very wide. Mutation frequency in spermatogonia was probably underestimated because clusters of identical mutations (that is of mutations induced in a spermatogonium and subsequently multiplied by replication) were counted as single mutations, apparently without taking account of the fact that the non-mutant flies in these broods must have occurred in similar clusters.

While cluster formation does not affect the calculation of mutation frequencies, it raises a statistical problem for the calculation of the standard error, which has to be based not on the known number of tested spermatozoa but on a smaller unknown one of spermatogonia. Puro[g]) has tried to determine this number by an analysis of mutant clusters in late broods, after the sterile periods. He subjected flies from Hannah-Alava's experiments to tests for recessive lethal and visible mutations on the third chromosome. Lethals from the same male were cross-tested for identity. Table 2

TABLE 2

	Frequencies of different cluster sizes in successive broods				
	Brood days	4 8 to 10	5 11 to 12	6 13 to 14	7 15 to 16
Number of clusters of size	1	27	13	1 →	← 1;1
	2		2	2;1 →	← 1
	3		4;1 →	1	
	4			1	
	5 or 6		1	1 →	← 1
	8			← 1 →	
	11 or 12			2	← 1

(Puro).

shows how the mutants in successive broods are distributed over clusters of various sizes. Several clusters extended over two or more broods. This has been indicated by arrows next to the numbers. Such continuing clusters are attributed to mutations in the primary or predefinitive spermatogonia, which act as stem cells for the secondary, cyst-forming ones. From the pattern of clusters, their absolute sizes and their sizes relative to the total progeny of which they formed a part, Puro has drawn the following conclusions: (i) broods

6 and 7 are derived from treated primary spermatogonia. (ii) Brood 5 traces back to a mixture of treated primary and secondary spermatogonia. (iii) Brood 4 is also derived from secondary spermatogonia, but the clusters were too small to be discovered in his progeny samples. (iv) The number of primary spermatogonia is estimated as 5 per male. This provides a basis for calculating the standard error of the observed mutation frequency. (v) The ratio of mutation frequencies in mature spermatozoa and spermatogonia is 2.9 with fiducial limits 4.0 and 2.3.

The last paper in this group, by Tazima and Kondo[i]) has been discussed already in a plenary session. Its relevance to our present session rests on the evidence it provides for a difference between germ cell stages in response to dose rate. In both male and female silkworm larvae only the earliest germ cells show the same dose rate response as the spermatogonia of the mouse, i.e. they yield higher mutation frequencies after acute than after chronic irradiation. Late spermatogonia and oogonia show exactly the reverse response, that is: lower mutation frequencies after acute irradiation. Cytological evidence suggests that, at least in the testis, this difference is due to the more extensive killing of late spermatogonia by acute radiation. Repopulation of the depleted testis seems to occur from dormant germ cells which are highly resistant to killing as well as to mutation. This will reduce overall mutation frequency. After chronic irradiation, there is less cell death among late spermatogonia and, consequently, less dilution of mutation-sensitive by mutation resistant cells.

Cell death at different stages of the spermatogenic cycle is the subject of Monesi's[f]) investigation. Spermatogonia in the mouse go through the following stages: 4 generations type A, 1 generation intermediate, 1 generation type B. It is known from Oakberg's work[1]) that type B and intermediate spermatogonia are much more radiosensitive than type A ones; moreover, type A spermatogonia form a heterogeneous population, including some highly resistant cells. Monesi has formed an attractive hypothesis to account for these observations. In a previous study he had determined the lengths of the periods of DNA synthesis (S) and of those preceding and following it (G 1 and G 2) in different types of spermatogonia[2]). The results are shown in Table 3. It will be seen that the average generation time and G 1 are constant throughout all stages, while S

TABLE 3

Average duration (hr)	Spermatogonia					
	A I	A II	A III	A IV	Int.	B
Generation time	—	28–30	26–28	30–31	26–28	29–30
S	7–7.5	7.5	8	13	12.5	14.5
G 2	14	14	11	8	6	4.5
G 1	—	7.5	8	9.5	8.5	10.5

(Monesi).

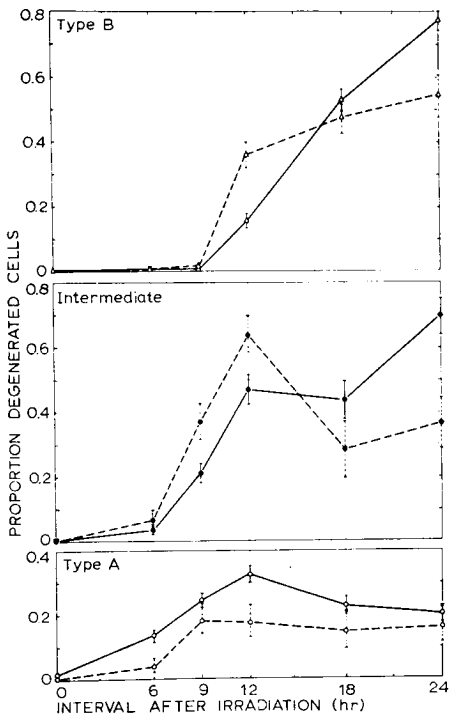

Fig. 4. The proportion of degenerated cells in the irradiated mouse testis at various times after irradiation. (Monesi.)

——— Number of degenerated unlabelled cells/Total number of unlabelled cells

– – – – Number of degenerated labelled cells/Total number of labelled cells

95% confidence interval.

increases and G 2 decreases from the earliest to the latest. This suggested to Monesi that DNA synthesis is the most sensitive period and that differences in sensitivity between spermatogonial types reflect differences in the length of this period. He has now tested this hypothesis by irradiating mice in which spermatogonia in DNA synthesis had been labelled with ^{3}H thymidine. The animals were killed at various periods up to 24 hours after irradiation, and their testes were examined for degenerating spermatogonia. According to the hypothesis, these should contain a higher proportion of labelled than of unlabelled cells. The results, shown in Fig. 4, did not fulfill this expectation. Among degenerating type A cells, unlabelled cells were more frequent than labelled ones at all times, whereas among degenerating intermediate and type B spermatogonia the relative proportion of labelled and unlabelled cells was reversed midway through the period of observation. Monesi believes that these findings do not invalidate his hypothesis, if account is taken of the stage at which damaged spermatogonia die. According to Oakberg,[3]) now confirmed by Monesi, type A spermatogonia die at late interphase or early prophase, and for those labelled towards the end of the S period death might not be observed in the cell cycle following irradiation. Type B spermatogonia, on the other hand, are shown to die during anaphase-telophase. Monesi concludes that G 2 is relatively resistant in type B spermatogonia, and may be so also in the other types. If this is true, differences in the duration of G 2 may contribute to differences in sensitivity.

4. SENSITIVITY DURING THE MITOTIC CYCLE

Lastly, we come to the group of papers dealing with sensitivity changes during the mitotic cycle. Here, labelling is the only reliable method for non-synchronized cell populations. In two investigations, double labelling with ^{3}H thymidine and ^{14}C thymidine has been used. When the second label is applied a short time after the first, a proportion of cells will have passed from S into G 2 during the interval; these will be labelled only with ^{3}H. A corresponding proportion will have entered S from G 1. These will be labelled only with ^{14}C. Where the double label cannot be distinguished from ^{14}C, it still is possible to calculate the period of DNA synthesis from the ratio of cells labelled with ^{3}H alone to cells labelled with ^{14}C alone or in conjunction with ^{3}H (Fig. 5). By extending the interval

between the two labels so as to allow some of the first labelled cells to go through mitosis, rough estimates can be made of G 1, G 2 and the total generation time. Wimber[1]) has applied this technique to cells in mouse gut and in Tradescantia root tips which had previously been exposed to chronic gamma irradiation. In both materials,

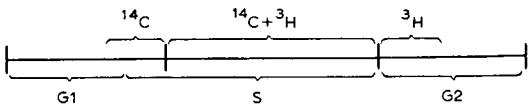

Fig. 5. Representation of the cell cycle with the principle of double labelling. (Wimber.)

the duration of S was unaffected by radiation. Taken at their face value, the data showed a prolongation of G 1 and G 2 in both materials. According to the author, however, this describes the true situation only for Tradescantia. For the mouse, he concludes that G 2 is unaffected and G 1 slightly shortened. This conclusion is based on a comparison of the data from double labelling with labelled mitosis curves.

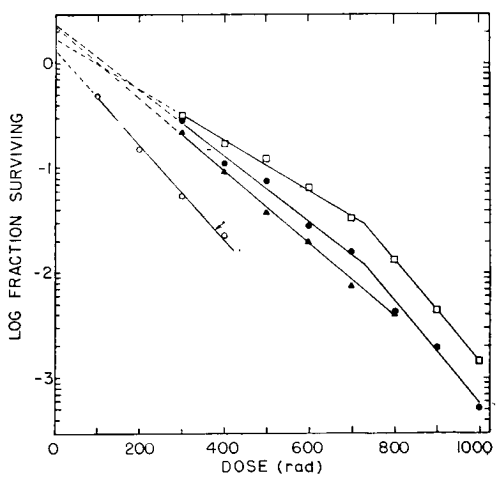

Fig. 6. Survival of HeLa cells after irradiation at different stages of the mitotic cycle. (Terasima and Tolmach.)

○ — ○ M 0 hr ● — ● G 1 6 hr
▲ — ▲ S 14 hr □ — □ G 2 18 hr

Dawson and Field[c]) have improved the double labelling technique by using two layers of autoradiographic stripping emulsion, the first of which records predominantly the distribution of tritium while the second layer is reached only by beta particles of higher energy. The two layers are separated by celloidin, and the silver grains in either or both may be dyed by a differential dye-coupling procedure. Using either this technique or a single label, the authors found that exposure of HeLa cells to 300 or 500 r increases the total generation time without affecting the duration of DNA synthesis; the main prolongation takes place in G 1.

Terasima and Tolmach[j]) used a synchronized population of HeLa cells for studying radiosensitivity throughout the cell cycle. As their paper will be reported in to-morrow's plenary session, I want to show only two of their figures, which are relevant to our discussion. Fig. 6 shows survival curves after irradiation in mitosis (0 hr), early G 1 (6 hr), late G 1 or early S (14 hr) and G 2 (18 hr). It will be seen that at lower doses survival is highest in early G 1 and G 2, lower in S, and lowest in mitosis. At higher doses, however, the

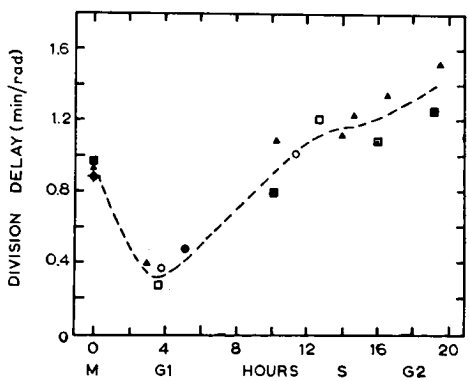

Fig. 7. Division delay in HeLa cells irradiated at different stages of the cell cycle. (Terasima and Tolmach.)

slopes of the survival curves become similar to each other. Fig. 7 shows that division delay was minimal after irradiation in G 1 and rose progressively to a maximum after irradiation in mitosis.

The last paper, by Dewey and Humphrey[d]) forms a link with the genetical papers. Dewey and Humphrey measured not so much

radiation damage as what they call repair of radiation damage, namely the speed with which broken chromosome ends rejoin at different stages in the cell cycle. It should, however, be pointed out that rejoining of broken chromosome ends leads to true repair only when the old order is restored. Reunion to form new arrangements is a special kind of damage and often lethal. The method used in this work is familiar to geneticists. It is based on the fact that chromosome exchanges are formed from two independently produced breaks, and that their frequency therefore increases as the square of the dose. If a given dose of X rays is applied in two fractions, and if the breaks produced by the first exposure are still open at the time of the second, the square law remains valid exactly as if the whole dose had been given in one exposure. If, however, the breaks produced by the first exposure have rejoined before the second, the overall frequency of exchanges will simply be the sum of the exchanges after the two single treatments. Dewey and Humphrey scored exchanges in Chinese hamster cells in which the synthetic stage had been labelled with ^{3}H thymidine. The time of fixation was chosen in such a way that the unlabelled cells had been in G 1 at the time of irradiation. The results are shown in Table 4.

TABLE 4

Exchange frequency (%) per cell
(Control frequency 0.04)

Cells	300 r	600 r in 1.1′	600 r in 4.7′	300 r + 300 r
labelled	0.29	1.03	1.04	1.07
unlabelled	0.29	1.03	0.58	0.56

Expected value for fractionated dose
without reunion 1.04
with reunion 0.54.
(Dewey and Humphrey.)

The interval between the two fractions of X rays varied from 10 min to 1 hr, but the result was the same for all intervals. The difference between cells treated in S and G 1 is striking and clear. While in S all breaks had remained open for at least 1 hr, in G 1 all had rejoined within 10 min. Perhaps the most interesting result from our present point of view is the effect of dose rate at the two

stages. Exchange frequency in S followed the square law independently of whether the dose was given in 1.1 min or 4.7 min. In G 1, breaks rejoined so fast that lowering the dose rate to one quarter resulted in a very material reduction of exchange frequency.

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DISCUSSION

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Mandl (*Dept. of Anatomy, Univ. Birmingham, Great Britain*) asked Metalli[a]) for further details of the chromosomal configuration in Artemia oocytes and the relationship of the condensed phases to "prophase". Metalli explained that, following diakinesis, the chromosomes became very diffuse during the most sensitive stage (which has been described as "early prophase"). Later, they condense again into very small dense metaphase chromosomes.

La Chance (*Dept. Agric., Kerrville, Texas*, USA) commented on the suggestion of Whiting[k]) that the large difference in radiosensitivity between metaphase I and prophase I could to some extent be accounted for by the formation of bridges at anaphase I. This would explain the effects observed with dominant lethals, but not those with recessive lethals and visibles. In reply, Whiting stated that she had no theory which would explain the different response of prophase I and metaphase I to X rays as measured by recessive lethals and visible mutations. There was, however, an indication that permanent bridges, which withheld incomplete chromatin from the egg pronucleus, might account for the reduced ratio of dominant lethals to visibles when prophase I stages are irradiated, compared with metaphase I. Whiting further suggested that a possible factor which would interfere with restitution and perhaps increase radiosensitivity at metaphase is the "stretched" condition of the chromosomes when viewed at metaphase. The region between the centromere and the single chiasma at each end appears to be under tension.

Hannah-Alava[e]) was asked by Sobels (*Dept. of Rad. Genetics, Univ. Leiden, The Netherlands*) whether in her experiments the mutation frequencies for visibles were higher in spermatids or spermatocytes than in mature sperm. An increase in radiosensitivity from mature sperm to spermatids should give an increase in point mutations. Hannah-Alava explained that her statistical analyses did not show conclusively a significant difference between broods one and two. Her view was that the data showed an increase in recessive mutations in brood two (spermatids) over brood one (mature sperm), which was probably also true for dominants and Minutes. There was currently no evidence that the increase in recessives was due to point mutations, since in each brood the number of homozygous viable mutants was about the same. In answer to Auerbach's comments on the small sample size for brood three, Hannah-Alava explained that the same males which produced 21 000 offspring in brood one yielded 256 flies with six mutations in brood three. She thought that it would be a long-term project to obtain reliable statistics for this brood. In answer to a question from Mossige, (*Norsk-Hydro Inst., Oslo, Norway*), Hannah-Alava said that the dose used was 3 000 rad hard X rays and agreed that, with lower doses, maximum sensitivity might be earlier than maximum sterility.

Bateman[b]) welcomed the hypothesis of Cattanach (*Mutagenesis Res. Unit, Inst. Animal Genetics, Edinburgh, Great Britian*) to explain the sex difference in incidence of translocations induced in the male. He quoted some data from his own paper which showed that semisterile females give two peaks for the proportion of lethal embryos, and favoured the interpretation that the high peak corresponds to those translocations which produce complete sterility in males. The group of semisterile males with a low lethal incidence would be due to translocations which would go undetected in females.

Savage (*MRC, Harwell, Berks., Great Britain*) asked Dewey[d]) what criterion of damage was used in assessing the interaction between breaks. Two types of aberrations will be involved, and with a given dose the yield of chromosome-type (in G 1) will be much less than the yield of chromatid-type (in S). Dewey replied that the criterion of damage was the total number of changes per cell. For G 1 cells, 80% were chromosome-type, and for S cells about 95% were chromatid-type.

Evans (*MRC, Harwell, Berks., Great Britain*) raised a query concerning

the danger of radiation damage arising from the use of ^{3}H-thymidine affecting results of radiation studies. In particular he asked whether the relatively greater duration of S in the more radiosensitive type B spermatogonia discussed in Monesi's[f]) paper might be partly explained by this effect, which might also affect Wimber's[l]) results. Both of these authors were of the opinion that these effects, if they existed, were insignificant. Monesi said that with a dose of 30 μC per mouse, he had not detected any mitotic delay or cell death.

In a discussion of various aspects of the paper by Terasima and Tolmach[j]), Pelc (*King's College, London, Great Britain*) enquired if there was any evidence that the S period in the synchronized population of HeLa cells was, in fact, synchronous. He had found that it was not. Tolmach showed a graph of his results demonstrating a high degree of synchrony in the S period commencing 7 to 8 hr after mitosis. Evans made a comment referring to the observed high radiosensitivity of the mitotic period. He referred to evidence indicating that when a cell enters mitosis, oxidative metabolism is reduced and there may therefore be an increased intracellular oxygen tension which would raise the radiosensitivity above that of interphase cells in which active respiration occurs. Tolmach thought that this was an interesting possibility but he had no data on oxygen effect. Neary (*MRC, Harwell, Berks., Great Britain*) stated that the observations of Terasima and Tolmach on sensitivity to mitotic delay in HeLa cells are opposite to his own in the bean root meristem. In HeLa cells, delay was greater the later the cell was in interphase when irradiated: in the bean root, delay was greater the earlier the cell was in interphase.

In further discussion of the effects of radiation on delay of these various phases, Painter (*NASA, California, USA*) said that he and his collaborators had found a dose-dependent delay in S, but no effect on G 1. Dawson and Field[c]) find a delay in G 1 but none in S. Field pointed out that their findings related, not to the immediate post-irradiation period, but to cell kinetics during succeeding cycles when the growth rate is retarded. This reduction in growth rate was due to prolongation mainly of G 1 and to a small extent of G 2. Tolmach added that according to their results the duration of G 1 and S was not significantly increased in the second generation following irradiation of the cells with 300 rad, though they had

not followed the cells through as many generations as Dawson and Field. Till (*Ontario Cancer Inst., Toronto, Canada*) pointed out that, in general, G 1 is lengthened in mammalian cell cultures if the generation time increases.

The session was concluded with a short discussion following a comment by the chairman that although in much of this work it was evident that radiation produced a delay in mitosis, there appeared to be no evidence for this in spermatogenesis in Drosophila. Auerbach agreed, and thought that ^{3}H-thymidine labelling might reveal such an effect. Bateman said that the time scale was different and that only a few hours delay was involved which could be overlooked when assessing broods. Monesi said that he had observed delay in mouse spermatogonia to the extent of 3 to 4 hr after 100 r, and 6 hr after 300 r.

Sparrow (*Brookhaven Nat. Lab., USA*) reported that in Trillium the time required for microsporocytes to pass through meiosis is related to the dose and to the relative radiosensitivity of meiotic prophase stages.

Factors Influencing the Sensitivity of Cells

METABOLIC CONDITIONS BEFORE AND AFTER IRRADIATION

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a) A. V. BEATTY and JEANNE W. BEATTY,
Modification of X ray effects.
Emory University, Atlanta 22, Georgia, USA

b) HEDI FRITZ-NIGGLI,
The decisive importance of chromosomal activity for the origin of radiation-induced back-mutations (point mutations).
Strahlenbiologisches Laboratorium, Roentgeninstitut, Kantonsspital, Zürich, Switzerland

c) N. E. GILLIES,
Restoration of X irradiated *Escherichia coli* strain B.
Medical Research Council, Hammersmith Hospital, Ducane Road, London W 12, Great Britain

d) BRENDA HODGKINS and TIKVAH ALPER,
Anaerobiosis as a metabolic factor influencing radiosensitivity.
Medical Research Council, Hammersmith Hospital, Ducane Road, London W 12, Great Britain

e) M. N. MEISSEL, T. S. REMEZOVA, R. D. GALZOVA, G. A. MEDVEDEVA, N. A. POMOSHCHNIKOVA, E. N. SOKUROVA, L. A. SELIVERASTOVA, M. N. POGLASOVA and A. T. NOVICHKOVA,
Cytophysiological and biochemical investigation of microorganisms in the process of post-radiation reactivation.
Institute of Microbiology, Academy of Sciences of the USSR, Moscow

f) D. K. MYERS,
Factors affecting the radiosensitivity of rat thymocytes *in vitro*.
Biology Branch, Atomic Energy of Canada Ltd., Chalk River, Ontario, Canada

g) D. PETROVIĆ, B. MILETIĆ and B. BRDAR,
Restoration of irradiated L-strain cells by subcellular fractions.
Department of Radiobiology, Institute Rudjer Boskovic, Zagreb, Yugoslavia

h) F. H. SOBELS,
Modification of the mutagenic effect of X irradiation in Drosophila males by chloramphenicol and ribonuclease.
Department of Radiation Genetics of the State University, Leiden, Wassenaarseweg 62, Netherlands

i) G. E. STAPLETON, M. S. ENGEL and L. V. ORCE,
Physiological aspects of radiosensitivity in microorganisms.
Biology Division, Oak Ridge National Laboratory, Tenn., USA and *Department of Biology, CNEA, Buenos Aires, Argentina*

1. INTRODUCTION

Metabolic conditions after irradiation act on the chain of events leading from the initial radiation damage to its eventual expression, a process whose earlier stages have been dealt with in previous sessions; here we are concerned with the later phases and means by which they can be modified. This subject of research has been popular in recent years, and from a large and confusing mass of results, one trend is apparent. This is an increasing emphasis on the importance of metabolic processes, both for the expression of the initial radiation damage, and for the contrary process of repair.

The collection of papers in this session illustrates both the magnitude of possible modifications, and the variety of agents which can produce them. The subjects are so diverse that I shall deal very briefly with individual papers in turn, beginning with chromosome effects and going on to inactivation work, and then will attempt to summarize factors common to a number of the papers.

2. EFFECTS ON CHROMOSOMES

Beatty and Beatty's[a]) work concerns effects on Tradescantia anthers, the aberrations studied being of the ring and dicentric types.

Table 1 shows a small selection of their results. An oxygen effect

TABLE 1

Aberration yields in Tradescantia anthers with 400 r of X radiation

	Outer area		Inner area		Total	
	No. of cells	Average interchanges	No. of cells	Average interchanges	No. of cells	Average interchanges
Helium	300	0.28 ± 0.03	149	0.26 ± 0.04	499	0.27 ± 0.03
100% oxygen	329	0.96 ± 0.05	190	0.86 ± 0.07	519	0.92 ± 0.04
5% O_2 in He	280	0.74 ± 0.05	179	0.44 ± 0.05	459	0.61 ± 0.04
5% O_2 in He, Post-treatment with CO (15 min)	377	0.64 ± 0.04	230	0.66 ± 0.05	607	0.65 ± 0.03

(Beatty and Beatty)

of the usual type was found by comparing irradiation in pure helium and pure oxygen, but when 5% oxygen was present in the gas mixture, many more aberrations per cell took place in the outer region

of the anther than in the inner region. This aberration gradient was present, though less marked, with 20% oxygen. Results of this type could be explained on the basis of an oxygen gradient, caused by metabolic consumption of oxygen within the tissue while the supply was limited by diffusion from the outside; but these authors' results with carbon monoxide do not seem to fit this interpretation. With carbon monoxide post-treatment for 15 min, the gradient of damage was completely abolished. It therefore seems most unlikely that the gradient was caused by differential damage, since it is hard to see how differences might be evened out by a post-treatment, and the authors explain it on the basis of a recovery process, which for some reason was more effective in the inner region of the anther. They suppose the recovery to be inhibited by CO, which prevents the supply of energy from respiration.

Sobels[h]) paper concerns experiments with Drosophila males, which carried a ring-shaped X-chromosome, and this ensured that the effects observed were confined to lethals originating as point mutations and possibly small deletions. The aim was to study effects of various modifying agents, and as an example of his results,

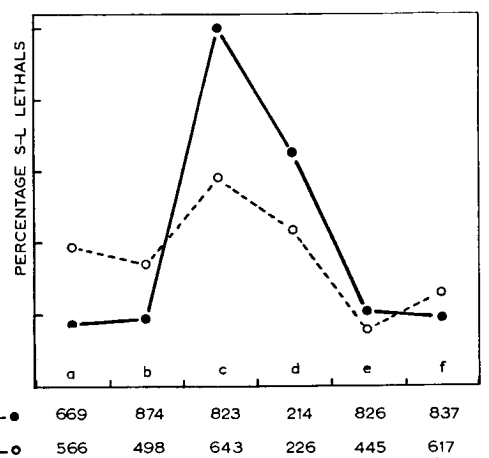

Fig. 1. Effect of pre-treatment with chloramphenicol (0.04 mg/ml, 2 injections separated by 1.5 hr) on the radiation-induced mutation frequencies in successive stages of spermatogenesis (a–f) in Drosophila. [●—●: 1200 r; ○- - - ○: chloramphlenicol + 1200 r. The numbers of tested gametes are shown at the bottom. Chloramphenicol causes an increase ($p < 0.01$) in mutation frequency in stages a-b, and a decrease ($p = 0.001$) in stages c-d. (Sobels.)

Fig. 1 shows the results of pretreatment with chloramphenicol. The proportion of lethals was highest in the progeny from gametes which, at the time of irradiation, were in the most sensitive stages, probably spermatids and late spermatocytes. Chloramphenicol treatment decreased the proportion of mutants in these stages while a small but significant increase was found in mutants in the mature sperm. A very similar picture to this was obtained with ribonuclease pretreatment.

Canavanine sulphate was tried because it is an arginine analogue and arginine is presumably incorporated in the protein in the later stages of maturation of sperm. It gave an increase of mutants in the sensitive stages, in contrast to the effect of chloramphenicol.

The effects of post-treatment with chloramphenicol were rather variable, both increase and decrease in mutation frequency being observed at the spermatid stage in different experiments. Sobels explains these results in terms of a repair system involving two processes, as suggested by Kimball[1]). One process is the loss of premutational damage, while the second is the process terminating the first, possibly the first cell division. The differing results obtained with chloramphenicol post-treatment might be explained if it affected both processes, since an altered balance could result in either an increase or decrease in eventual damage.

As for the pretreatments described, the author thinks they may change sensitivity by acting on the composition of proteins during the transition from spermatids to mature sperm, or possibly they may be acting on subsequent repair processes.

Fritz-Niggli[b]) studied genetic changes in the fission-yeast *Schizosaccharomyces pombe*. The material studied consisted of adenine-requiring mutants, originally induced by UV, X rays and nitrite. She examined the effect of X rays on reversions in these mutants, and found that the number was about the same for the three different types. Other factors, however, influenced the result, notably the age of the culture at the time of irradiation (Fig. 2). Twelve and 20 hr cultures containing actively dividing cells gave 2 to 3 times the number of mutants that were induced in 28 hr cultures, consisting of resting cells, and this difference was observed with all three types of mutant. Fritz-Niggli attributes this effect to the difference between chromosomes in mitosis and in resting stages, whether in oxygen content, water content, or inherent susceptibility,

but this explanation is difficult to reconcile with her results on starvation of cells in physiological saline for a two hour period before, during and after irradiation. This was said to inhibit mitotic activity, but not to alter the mutation rate. It would be interesting

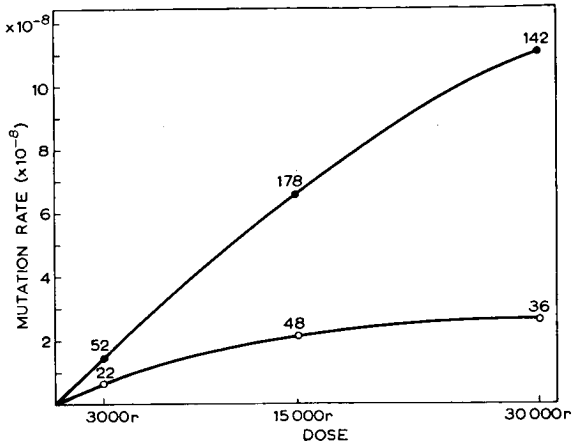

Fig. 2. Numbers of reversions induced in the X ray mutant M_2 of *Schizosaccharomyces pombe*. ●: 20 hr cells (dividing). ○: 28 hr cells (not dividing). The numbers of reversions which occurred are indicated above the points on the curves. (Fritz-Niggli.)

to know, also, whether the variation in mutability she observed was correlated with variation in inactivation, since this has been shown by Stapleton with *E. coli* to be at a maximum at the late logarithmic phase[2]).

The effect of metabolic inhibitors present before and during irradiation on reversion in this yeast was surprisingly small. Neither dinitrophenol nor chloramphenicol made any difference to mutability, and with chloramphenicol applied after irradiation, a change was observed only with the nitrite mutant, where the rate was reduced to 68% of the control value.

3. EFFECTS ON SURVIVAL

Mammalian Cells

Myers[f]) describes a post-treatment effect on a really large scale with rat thymocytes, in which survival after irradiation was measured by the number of cells not stainable by erythrosin B. Post-

irradiation incubation temperature altered the 50% lethal dose by a factor of 600 (see Table 2). With post-irradiation incubation

TABLE 2

Effects of incubation temperature and various additives on % survival (by a staining method) of X irradiated thymocytes

	% survival after	
	Incubation at 25° for 19 hr	
Dose (r)	Normal medium*	Normal medium +0.01M nicotinamide
20000	78	99
40000	39	87
100000	2	71
200000	0	59

	Incubation at 37° for 19 hr		
Dose (r)	Normal medium* *p*H 7.4 1.2 mM $CaCl_2$	Low *p*H: *p*H 6.1 1.2 mM $CaCl_2$	Excess calcium *p*H 7.4 11.2 mM $CaCl_2$
70	20–34	84	71
140	12–22	71	60
280	0–10	60	54
560	0–6	55	50
1000	0–5	50	46
2000	0–2	43	40

* A modified Krebs-Ringer's solution containing 5.5 mM glucose. (Myers.)

at 37° for 19 hr the cells were extremely sensitive, only 50 r being required to kill 50%, while with 25° incubation approximately 30000 r was required. It was clear from his results that death occurred by two quite different processes at the two temperatures, since cells incubated at 25° could be protected by nicotinamide at 0.01 M, although it made little difference to cells at 37°, whereas cells at 37° could be protected by alteration of *p*H from 7.4 to 6.1 or addition of an excess of calcium to the medium.

In an attempt to find the mechanism for this differential effect of temperature, various inhibitors were studied. Dinitrophenol gave a similar differential effect with temperature, and it was suggested that leakage of ATP from the cells caused this. However, the quantitative aspects of ATP loss were very different for irradiation and dinitrophenol, and further evidence would be needed to establish similarity of the two processes. Loss of nucleotides from irradiated cells at 37° had also been studied, and was mentioned as a possible contributory cause of death at this temperature.

Petrović, Miletić and Brdar[g]) experimented with L strain mouse fibroblasts, which after irradiation were plated and incubated to form colonies. The object was to show the effect on survival of sub-cellular fractions of the L strain cells, used as additives after irradiation. Homogenates were made and separated as far as possible into the different fractions, and portions of these were added to each plate containing L strain cells after irradiation. The results (Table 3) were not very clear-cut, since they varied from one ex-

TABLE 3

Effects of isologous subcellular fractions on the irradiated L-cells

Experimental number	Number of colonies after 500 r X rays*					
	Non-treated	Treated with nuclei	Treated with mitochondria	Treated with microsomes	Treated with supernatant	Growth medium+sucrose
32/I	100	304	141	125	125	67
34/I	100	164	136	136	127	100
35/I	100	—	160	150	90	90
38/I	100	166	141	133	150	56
39/I	100	109	85	118	54	83
40/I	100	95	93	88	56	53
42/I	100	105	67	74	—	—
42/I	100	100	85	55	—	—
1/II	100	141	131	93	86	32

* The numbers of colonies are relative. The numbers of colonies in irradiated and treated samples are corrected to 100 colonies in irradiated nontreated samples. (Petrović, Miletić and Brdar.)

periment to the other, and the enhancing effect on survival was not always present, differences probably being due to the long and

complicated preparation involved. Where the numbers were increased, the largest effect was found with the nuclear fractions and the next largest with the mitochondria; and these also caused marked increases in colony size. The entire homogenates also gave increased colony counts. The incomplete separation of the fractions made it difficult to say which component was causing the effect observed, but subsequent experiments where isologous DNA gave an increased number of survivors made it seem likely that this factor was responsible.

Yeasts

Meissel and his collaborators[e]) have produced a most comprehensive paper, dealing with the process of reactivation in *Saccharomyces vini* and other yeasts, a process originally studied by Korogodin[3]).

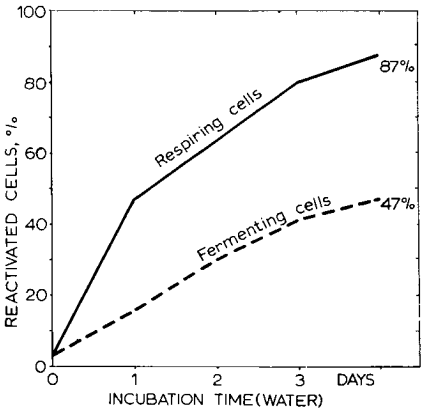

Fig. 3. Post-irradiation reactivation in *Saccharomyces vini* depending on the type of precultivation (aerobic or anaerobic). Irradiation dose 100 Kr. (Meissel, Remezova, Galzova, Medvedeva, Pomoshchnikova, Sokurova, Seliverastova, Poglasova and Novichkova.)

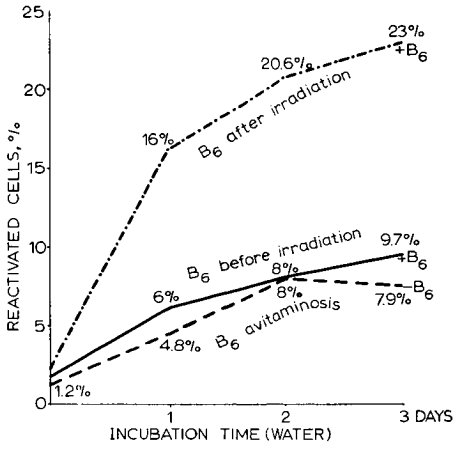

Fig. 4. Effect of short-term treatment with pyridoxine on the post-irradiation reactivation of B_6-exhausted yeast cells (*Saccharomyces carlsbergensis* 4228). Irradiation dose 100 Kr. (Meissel *et al.*)

The procedure consists of a post-irradiation incubation in tap-water at 28° to 30° for several days, during which time no budding takes place. The number of cells able to form colonies when transferred to wort agar substantially increases (Fig. 3). Cytological

studies showed abnormalities developing with immediate incubation on wort agar which were largely avoided by the preliminary tap water incubation.

Physiological work indicated the aerobic nature of the reactivation, which failed to take place under anaerobic conditions. Endogenous respiration was necessary, and was 50% higher in irradiated cells. First carbohydrate reserves and then lipids were respired, while the ATP content remained constant in irradiated cells, though increasing slightly in controls.

The free amino acid pool increased, in both control and irradiated cells, though less in the irradiated. A pyridoxine deficient strain of *Saccharomyces carlsbergensis*, cultivated without pyridoxine, was more sensitive to radiation and showed less reactivation than when supplied with this vitamin (Fig. 4), and its amino acid pool was almost twice as large, presumably indicating decreased incorporation of these compounds into protein. A supply of pyridoxine for one hour after irradiation followed by washing gave increased reactivation, although the same treatment before irradiation had no effect.

The reactivation was greater in cells previously grown in aerobic conditions than anaerobically (Fig. 3). It was also greater in cells with a full supply of nutrients than in starved cells, and in cells with a high lipid content than in those with reduced lipid.

When the results were compared for different species of yeast, it was found that no reactivation took place in haploid yeasts. Yeasts showing this phenomenon were found to have both respiratory activity, that is, a full cytochrome system, and the capacity for aerobic glycolysis.

Meissel concludes that the development of the initial radiation damage into cell injury is due to an imbalance of metabolism, and that a decreased level of metabolism favours repair processes. These are themselves metabolic, requiring an energy supply and involving amino acid metabolism.

Bacteria

The next two papers, by Stapleton *et al.*, and Hodgkins and Alper, are developments from the results of Hollaender, Stapleton and Martin[4]).

Stapleton, Engel and Orce[1]) showed the effects of growth conditions before irradiation on subsequent survival. Cells of *E. coli* B/r

CSH produced alkaline conditions in a peptone medium, while a peptone-glucose medium containing no buffer became acid towards the end of the logarithmic period (Fig. 5), and at about this time the cell size and DNA content increased. The radiation resistance gradually developed from a minimum after 4 hr to a maximum

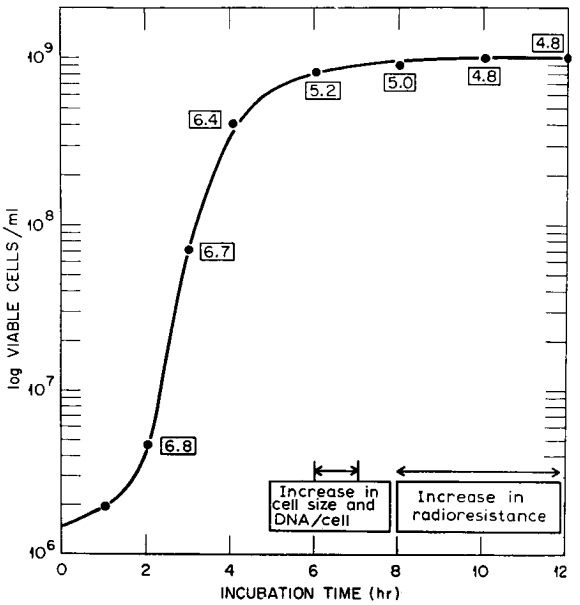

Fig. 5. Time scale for growth, change in *p*H, cell size, DNA content/cell and change in radioresistance. The curve shows the rate of growth in 1% peptone + 0.4% glucose + 0.0066 M phosphate buffer. The numbers in boxes along growth curve are the measured *p*H of the culture at the various times. The times for increase in cell size and DNA content and increase in radioresistance are indicated along the time axis. (Stapleton, Engel and Orce.)

after 18 hr incubation. The authors tend to correlate the increased resistance with the higher DNA content, but they realize that the difference in time scale is a serious drawback to this theory.

The expression of the higher resistance depends on the plating medium (Fig. 6). The addition of 1% peptone to the minimal medium has only a small effect on the peptone-grown cells, while it gives a large increase in survival of 18 hr glucose-grown cells, mainly shown by an increased extrapolation number. The fact that this only appears with appropriate post-irradiation conditions im-

plies that it is not due to multiple targets, as was also indicated by the work of Shekhtman[5]), who found a sigmoid curve even with α rays in a very similar system.

Reversions induced by irradiation at three different loci were

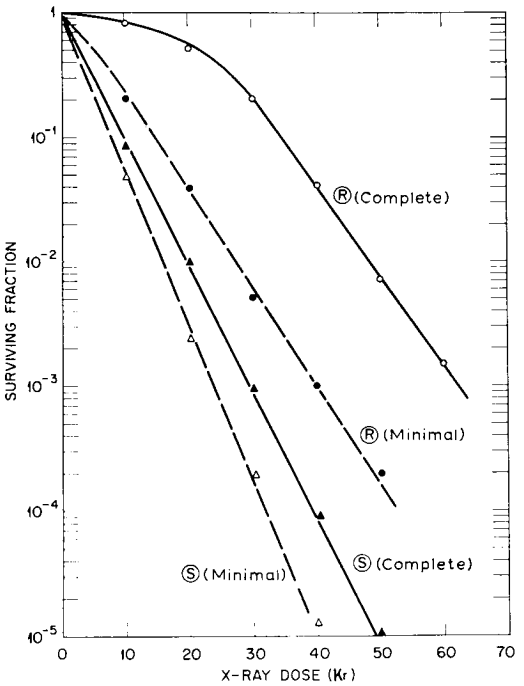

Fig. 6. The effect of post-irradiation plating media on resistant (R) and sensitive (S) populations of *E. coli* B/r CSH. The irradiated cells were plated immediately on solid minimal (M–9) medium (Anderson) or M–9 + 1% peptone, and incubated for 48 hr at 37° C. (Stapleton Engel and Orce.)

also studied, in cells grown by the two methods, and these varied in the same manner as inactivation, with the more resistant cells showing fewer mutations.

Hodgkins and Alper[d]) studied the effect of anaerobic compared to aerobic growth of *E. coli* B and B/r both before and after irradiation with UV, X rays, and 8 MeV electrons, and a selection of their results is shown in Fig. 7. Anaerobic growth before irradiation always gave a higher survival than aerobic growth, and in general the curves became more sigmoid. In strain B/r the extrapolation

number showed an increase up to eight. The anaerobic growth of cells after irradiation gave no difference at all in survival of B/r, which was shown to have the same growth rate in aerobic and anaerobic conditions. *E. coli* B had a longer lag and a slower growth

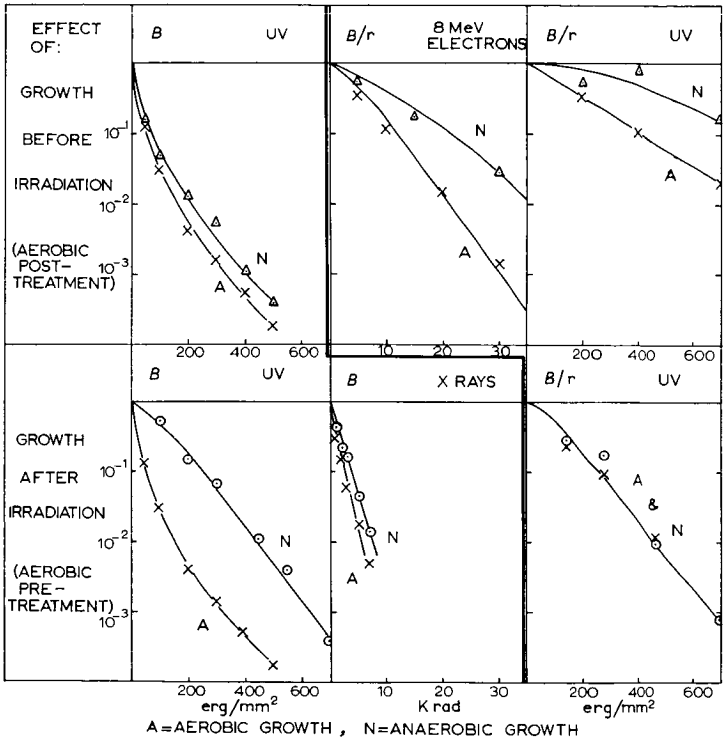

Fig. 7. Effects of anaerobic compared with aerobic incubation before and after irradiation in air on survival of *E. coli* B and B/r. (Hodgkins and Alper.)

rate in anaerobic conditions, and also gave higher survival. These differences occurred after both UV and X rays, and the UV curves also showed changes in shape from concave to sigmoid. With X rays, bigger differences were found after anoxic rather than aerobic irradiation. As shown by the UV curves, under favourable conditions for survival of strain B, similar inactivation of both B and B/r took place, thus confirming earlier results[6]).

The combination of chloramphenicol treatment with either aero-

bic or anaerobic incubation gave lower survival than anaerobic conditions alone.

The time scale of the recovery process was studied by transferring from aerobic to anaerobic conditions and vice versa at different times during incubation. The results indicated that the recovery process was complete before the first cell division took place (Fig. 8).

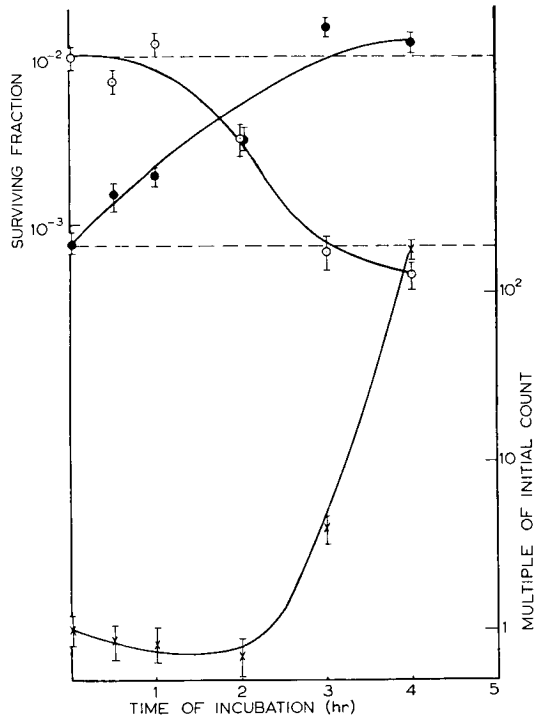

Fig. 8. Survival and growth of *E. coli* B (log plase cells grown on Oxoid broth) after exposure to 31.5 Krad X rays in anoxic buffer. (Hodgkins and Alper.)
O Transferred from aerobic to anaerobic conditions at time shown on abscissa.
● Transferred from anaerobic to aerobic conditions at time shown on abscissa.
× Viable counts on irradiated cells growing aerobically.

Gillies[c]) followed up his earlier work on amino acid deficient mutants[7]) by studying a proline deficient mutant of *E. coli* B. After X irradiation under anoxic conditions, survival curves on different media were obtained (Fig. 9). As in previous work[8]), initial chloramphenicol treatment gave a much higher count than plating

on a complete nutrient medium. Omission of proline for an initial period from the complete medium also gave a higher count, but not as high as that with chloramphenicol. A simple medium containing mineral salts, glucose and proline also gave higher counts

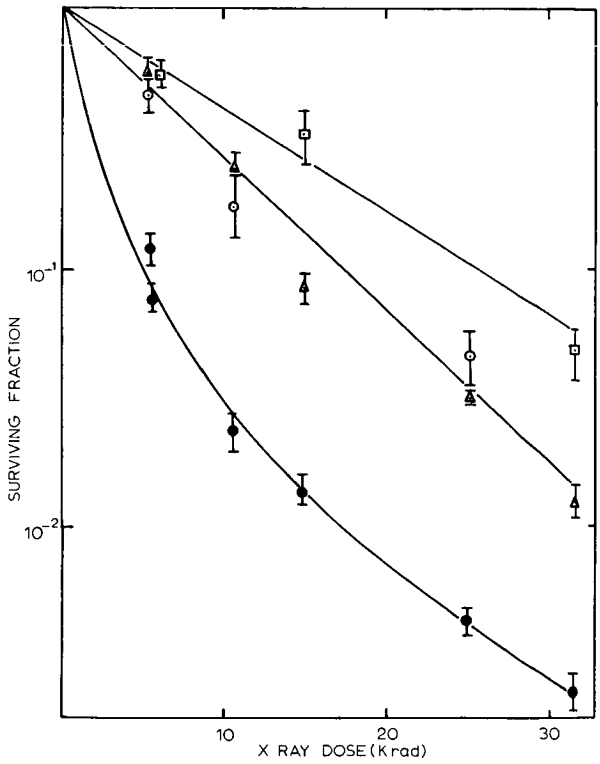

Fig. 9. Effect of plating medium on survival of proline-deficient *E. coli* B33 (stationary-phase cells) after exposure to X rays under anoxia. 2 media used: Simple, containing inorganic salts, glucose and proline; and N, containing in addition other amino acids, purines, and pyrimidines. (Gillies.)

Incubation on: □ Chloramphenicol 5 μg/ml in N initially; later N. △ Simple medium throughout. ○ N lacking proline initially: later N. ● N throughout.

than the complete medium, and was in fact indistinguishable from the complete medium without proline initially. The increase in count obtained by preliminary incubation without proline was followed over the first few hours, and the maximum increase was found to take place within about 5 hr after irradiation.

TABLE 4

Summary of conditions used by the various authors, and their effect on eventual radiation damage

Authors	Material	Effect observed	Conditions, and influence on damage:		
			Before irradiation	During irradiation	After irradiation
Beatty and Beatty	Tradescantia anthers	Chromosome aberrations		X rays Various gas mixtures	+CO Gradient abolished
Sobels	Drosophila males	Lethals	RNase ↓ Chl. ↓ Canav. ↑ on sensitive stages	X rays	Chl ↓↑
Fritz-Niggli	*Schizosacc. pombe* (Adenine-requiring)	Reversions	Active division (i.e. young culture) ↑ Chl– DNP–	X rays Air N_2	Chl: nitrite mutant↓ 2 radiation mutants -
Myers	Rat thymocytes	Death (staining reaction)		X rays	37° inc. ↑ *p*H 7.4→6.1 ↓ +Ca^{++} ↓ 25° inc. + nicotinamide↓
Petrović *et al.*	Mouse L-strain cells	Inactivation		X rays	+ subcellular fractions Nuclei ↓ Mitochondria ↓ Homogenates ↓

Meissel *at al.*	*Sacc. vini* and other yeasts	Inactivation	Aerobic growth ↓ Reserve foods ↓ $n \geqslant 2$, cytochrome system and capacity for aerobic glycolysis necessary for reactivation	X rays	Aerobic incubation in tap water, at 28 to 30°. ↓
Stapleton *et al.*	*E. coli* B/r CSH	Inactivation Reversions	Growth in: peptone medium glucose medium (18 hr) ↓↓	X rays	Synthetic medium Same + 1% peptone ↓
Hodgkins and Alper	*E. coli* B and B/r	Inactivation	Anaerobic growth B↓ B/r ↓	UV, X rays electrons Air N_2	Anaerobic growth B↓ B/r –
Gillies	*E. coli* B33 (proline-)	Inactivation		X rays N_2	Medium N Medium N – proline ↓ Simple medium Med. N + Chl. ↓

Radiation damage, with conditions indicated: ↑ = increased
↓ = decreased
– = unchanged

Chl = chloramphenicol. Canav. = canavanine sulphate.

4. DISCUSSION AND CONCLUSIONS

Table 4 shows the conditions used by the various authors, and the arrows indicate the effect each condition had on the eventual expression of the radiation effect, as compared with the converse treatment. Where two conditions are shown, an arrow pointing upwards indicates that the upper condition enhances radiation damage as compared with the lower one.

Pre-irradiation treatments

Pre-irradiation treatments can sometimes be separated into effects taking place during and after irradiation, as with some of Meissel's treatments, but in general it is very difficult to sort out the two processes. Further complications are introduced by varying growth conditions, since these may result in different cell size, DNA content, reserve food materials and enzymic composition, to mention a few possibilities, and a great deal of information is needed to trace such effects to their cause. Interesting comparisons can be made between pretreatments shown in Table 4, but I have not time to elaborate this point.

Post-irradiation treatments

Post-irradiation treatments used here can be regarded in three main groups: first of all, those concerned with energy supply.

The need for energy in repair processes has been well established in genetic work, particularly that concerning chromosome breaks. Beatty and Beatty included some information on this in their abstract, not dealt with in their paper, and Sobels[9]), in previous work, has shown that respiratory inhibitors interfere with repair in Drosophila.

Fritz-Niggli found a lack of effect with dinitrophenol, but she states that this does not inhibit the formation of energy rich phosphate in yeast, so this observation is not contradictory.

In the inactivation field, Meissel's work has stressed the importance of energy supply from metabolism, and in this case only an aerobic system would function. This was in contrast with Alper's observation of a decrease of radiation effects with anaerobiosis, but besides the different organisms used, the experiments differed in the supply of external nutrients by Hodgkins and Alper.

The second set of observations deals with metabolic inhibitors (other than respiratory ones) and with inhibitory conditions in general.

Of the inhibitors, we are mainly concerned here with chloramphenicol, known from work on bacteria to inhibit protein synthesis, while allowing nucleic acids, mainly RNA, to accumulate. Its effects after irradiation are usually attributed to the protein effect but sometimes to increased nucleic acid.

The work on mutations stems from that on bacteria and protozoa, which have been very thoroughly studied, and here chloramphenicol decreased the mutations observed. Interpretations differ, since Witkin[10]), and Doudney and Haas[11]), think that chloramphenicol interrupts the chain of metabolic events leading from premutational damage to irreversible fixation of mutations, whereas Kimball[1]) thinks that it acts on his dual system, consisting of the repair process, and the time limiting process for repair. Whatever the interpretation, chloramphenicol certainly seems to have some effect in Drosophila, probably depressing mutation in the sensitive stages of sperm formation. In yeast, it had surprisingly little effect, acting on only one out of the three mutants studied, and then only to a relatively small extent.

It is particularly interesting to see similar effects in inactivation studies. With *E. coli* B, inactivation is also depressed by chloramphenicol and by other agents which slow down growth or protein synthesis, as shown in the last two papers. Alper and Gillies tend to use Barner and Cohen's experiments on death due to unbalanced growth[12]) as an explanation for these observations.

Meissel's work is another example in which conditions unfavourable to growth led to an increase in eventual survival, given the right properties in the cells before irradiation. He too considers unbalanced growth responsible for damage, but stresses the importance of metabolic repair processes for reactivation. His reactivation system did not decrease the number of mutations expressed.

Having just mentioned Kimball's theory, concerning the time limitations for repair processes, it is worth pointing out that the time scale for the increase in counts observed in the last two papers was quite short, and corresponded to the lag phase in Alper's experiments. It seems that an intensive study of this period, like that made in some mutation experiments, might be useful also in inactivation work.

The third type of result is that in which complex media after irradiation decrease the damage. This does not apply to any muta-

tion studies, but is seen in the inactivation work of Petrović *et al.* and Stapleton *et al.* Stapleton's earlier work with *E. coli* B/r[13]) is the best known example of this type of result, which is thought possibly due to the necessity for adaptive enzyme synthesis to repair radiation damage.

These are the three main classes of post-irradiation effect studied. Results for mutation work seem to be in general agreement, although only an oversimplified outline has been possible here.

The picture is more confused in inactivation work, and further studies of both mutation and inactivation in the same system might be useful for clarifying it. The second type of effect has been shown to occur in a wide range of yeasts and *E. coli* B, while the third type takes place in *E. coli* B/r and some other vegetative bacteria. There is a tendency to generalize about bacteria from the *E. coli* results, but information on other genera is very scanty, and when obtained[14]), often shows no post-irradiation medium effects. It would therefore be extremely useful to know whether the various *E. coli* effects are, in fact, exceptional, or of widespread occurrence.

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DISCUSSION

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Sobels[h]) said that the findings of Hodgkins and Alper[d]) and the earlier work of Gillies and Alper[1]) show that *E. coli* B responds in greater measure to variations of culture conditions after radiation exposure under anoxic conditions than after irradiation in O_2. Alper, in her rapporteur's report (page 114) had likewise suggested that after radiation exposure in O_2 not only the chromosomal DNA but other cellular systems may be deranged.

Recent findings observed in cooperation with Barry Leigh in Sobels's department suggest a similar situation in Drosophila spermatids. The data show that, if a dose of 3000 r is given under strictly anoxic conditions, post-treatment with O_2 results in a considerable repair of the premutational damage in spermatids, as compared to that in flies which were post-treated in N_2. After exposure to 1000 r in O_2, on the other hand, the effect of post-treatment with O_2 exactly equalizes that of post-treatment with N_2. The same mutation frequency is then observed as after exposure to 3000 r in N_2 followed by post-treatment in N_2. Thus depending on the gaseous atmosphere during post-treatment, the O_2/N_2 enhancement ratio in Drosophila spermatids is profoundly changed. The results are interpreted to mean that the biochemical system responsible for repair of premutational damage has been damaged to a greater extent by exposure to irradiation in O_2 than in N_2.

Fritz-Niggli[b]) said that she would first like to ask Sobels whether the different reactions after post-treatment with chloramphenicol which he had found might not be due to the fact that his material is a mixture of point mutations and small deletions. In her work on pure (as she hoped) point mutations she had always found that post-treatment with chloramphenicol reduced them, both in the very active phase and in resting cells.

Turning to the problems of the different sensitivity of cells in mitosis and the resting stage and the effect of post-treatment with chloramphenicol, Fritz-Niggli said that metabolic conditions, ATP content and perhaps oxygen content are important factors in differential sensitivity, but DNA synthesis is less important. The

action of chloramphenicol after irradiation could be due to depression of DNA synthesis, thereby affecting mutation fixation, interactions of long lived free radicals, selection of mutationally deranged cells or (and this was her opinion) a depression of some metabolic product important for mutation fixation.

Sobels' reply to Fritz-Niggli was that it is impossible to distinguish which portion of the lethals induced in a ring X-chromosome might possibly be due to small deletions. On the basis of other evidence obtained in the same experiments on the frequency of loss of the ring X-chromosome, it is however interesting that recovery due to post-treatment with O_2, after irradiation in N_2, only happens with small deletions.

Meissel[e]) said that he would like to make a few comments on the excellent Rapporteur's report. As already noted (and pointed out by Thornley as well) reactivation of yeast cells requires a normal cytochrome system along with the capacity of aerobic glycolysis.

It is a well known fact that yeasts cultivated under fermentation conditions differ from aerobic ones in some respects, the cytochrome system included. It seemed worthwhile, therefore, to test the reactivation-capacity of a great number of yeast species differing in the prevalence of one or another type of energy metabolism, viz. respiration and fermentation. Concurrently, cytochrome spectra of these yeast species were taken. The following were the results obtained. All well-reactivated species exhibited distinct respiration and fermentation. Yeasts with a reduced respiratory activity were not reactivated. Nor was reactivation possible in yeast varieties with a deficient cytochrome system, whether they were naturally occurring (strains belonging to the *Saccharomyces carlsbergensis* group) or artificially produced mutants deprived of cytochrome oxidase.

Meissel had followed up in more detail, by means of a differential spectrophotometer, the rate of reduction of the oxidized cytochromes in 11 different cultures of yeast both capable and incapable of post-radiation reactivation. In all reactivated yeast the time of reduction of the cytochromes varies within the range of 3 to 7 min. If this is less than 3 min or more than 7 min the yeasts are not reactivated.

This investigation is now in progress. Much remains to be clarified with regard to complex relationships between the state of the

cytochrome system and post-radiation reactivation capacity.

Laser (*Molteno Inst., Cambridge, Gr. Britain*) asked Meissel whether his cytochrome-deficient yeasts have an oxygen effect of irradiation, but Meissel had no data on this point.

Alper[d]) noted with interest Sobels's report of a differential modification of mutational damage, by metabolic conditions, depending on whether or not irradiation had been in the presence or absence of oxygen. In her work she had not seen evidence of damage occurring only in the presence of oxygen. The data suggest rather that there are at least two sites of damage, one of which is much more influenced by the presence of oxygen during irradiation than the other. This is brought to light by differential modification, which is not necessarily always greater after anoxic irradiations; for example, the rescue of *E. coli* B/r by subsequent incubation at 18° is more efficient after irradiation in oxygen.

Rescue of *E. coli* B by chloramphenicol treatment and by anaerobic growth are not only not additive; in fact they counteract each other. Evidence from the kinetics of the rescuing process suggest that quite different mechanisms are involved in rescue by chloramphenicol and by other means, such as poor media or anaerobiosis. Examples of other microorganisms whose radiation response is affected by metabolic conditions are *Salmonella typhimurium* which respond in the same sense as *E. coli* B. Many strains of *E. coli*, like B/r, show increased response to radiation if they are treated with chloramphenicol. The relationship between *E. coli* K 12 X and K 12 S seems rather like that between *E. coli* B and B/r.

Haynes (*Committee on Biophysics, Univ. of Chicago, Ill., USA*) said he wished to enlarge briefly on the work of Meissel and his collaborators. He had made studies of the same post-irradiation phenomenon at Chicago in collaboration with Patrick and Uretz. These confirmed all Meissel's essential results for X rays using *Saccharomyces cerevisiae* haploid through hexaploid strains.

They had also shown that

(i) cells exhibit a dose modifying factor in recovery and this factor is the same for irradiations in the presence and absence of oxygen i.e. about 2 in each case.

(ii) populations of diploid yeast which have recovered are more sensitive to subsequent X ray inactivation than they were originally.

(iii) these populations will recover yet again.

Recovery of UV irradiated cells by storage in distilled water has been studied. In contrast with X rays, haploid yeasts recover from UV inactivation as well as the higher ploidies. The storage recovery from UV inactivation is independent of and additive to photoreactivation, e.g. inactivation with UV to 10^{-5} or 10^{-6} survival can be reversed in two stages, first by photoreactivation to 10^{-1} survival and then by storage to give a final recovery of practically 100% for UV doses of less than 4000 erg/mm^2. With higher doses a combination of photoreactivation and storage does not give 100% recovery. No evidence of a similar recovery following treatment with acridine orange and visible light or with nitrogen mustard had been found, although a long term recovery of *E. coli* B and B/r was observed after nitrogen mustard treatment.

Haynes expressed surprise that Meissel had found reactivation using tap water. Haynes had found no recovery unless double distilled water or glass distilled water carefully freed from trace metals was used. Hollaender had suggested that trace metals might poison recovery enzymes.

Meissel, expressing his appreciation to Haynes for his question, said that the water which he used in his experiments was sterile tap water. This water could not have had any toxic effects and reactivation of the yeast after radiation had always been obtained and the results were excellent.

Meissel added that he could obtain reactivation with yeast in the following way. After irradiation and after maintenance for 24 hr under anaerobic conditions, if the yeast was transported then under aerobic conditions, reactivation was found to be perfectly normal. Lastly, reactivation of yeast by this method of allowing it to rest, could be obtained also after exposure to chloramphenicol.

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The Linking of Disciplines in Radiation Research

ELECTRON SPIN RESONANCE IN BIOLOGY

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Free radicals produced by X ray irradiation of organic acids and their peroxides.
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Direct and indirect action of ionizing radiation as studied by electron spin resonance spectroscopy.
Norsk Hydro's Institute for Cancer Research, Oslo, Norway

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Comparative ESR measurements of the absolute concentration of radiation-produced free radicals.
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Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA
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Electro-Chemistry Institute, Academy of Sciences of the USSR, Moscow, USSR
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Biology and Nuclear Physics Divisions, Atomic Energy Establishment, Trombay, Bombay, India
[l]) K. SOMMERMEYER and O. SCHIRMER,
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Radiologisches Institut der Universität, Freiburg i.Br., Germany

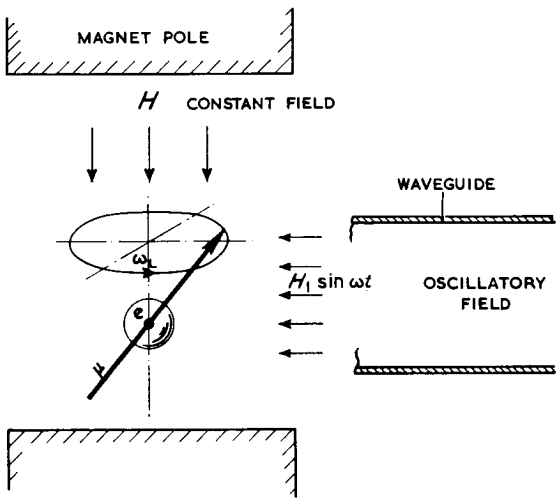
1. INTRODUCTION

In the biological or chemical action of ionizing radiation, there is in most instances a step which involves the formation of free radicals and their subsequent reaction with one another and with the surrounding molecules. It is, therefore, of great interest to discover what radicals are formed in different systems, how long they persist, and how their reactions can be modified by changes in the physical or chemical environment during or after irradiation.

2. PRINCIPLES OF THE METHOD

Electron spin resonance (ESR) offers a remarkably direct way of studying these questions. It does this by exploiting the characteristic feature of any free radical — the unpaired valency electron. Such an electron has both angular momentum and a magnetic moment, and the latter is no longer masked, as it is in most complete molecules, by the equal and opposite moment of a partner in the same orbital. When a radical is placed in a constant magnetic field the

unpaired electron behaves like a magnetic top, as demonstrated in Fig. 1 and precesses with a frequency proportional to the field strength H. If an oscillatory magnetic field acts at right angles to the steady fields, its perturbing effect on the precessing electron will average to zero, unless its frequency corresponds with that of the precession, when resonance will occur, and some of the electrons



ELECTRON SPIN RESONANCE OCCURS IF $\omega = \omega_L = \gamma H$

Fig. 1. Electron spin resonance.

will have their spin direction "flipped over," accompanied by a net absorption of energy from the oscillatory field.

A free electron should give a single sharp absorption line, as the magnetic field strength causing precession is varied through the resonance value (see Fig. 2). In radicals the unpaired electron will not be entirely free[1]; it will be associated with one or more nuclei, and the local magnetic field in which it moves will be modified by contributions from the magnetic fields of these nuclei. If the electron is bound, for instance, to a single proton which has a spin $\frac{1}{2}$, the proton magnetic moment will align itself in either of two ways — along the applied field or opposed to it — and about half the protons in any sample will have the one orientation and half the other. Thus about half the number of unpaired electrons will be in a field which is slightly higher, and half in a field which is an equal

amount lower than the applied field. The observed effect is thus a splitting of the single resonance line for a free electron into a doublet centred about the original position. If the unpaired electron is associated with two protons, there are four possible combinations of spin orientation, in two of which the proton magnetic fields cancel out. The ESR spectrum of an electron coupled equally to two

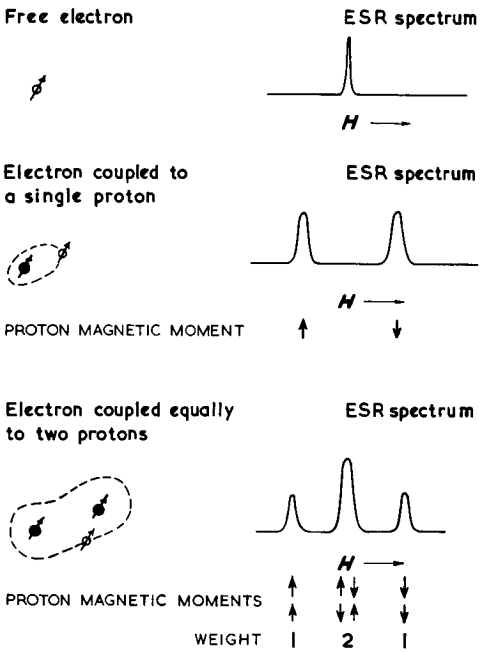


Fig. 2. Electron spin resonance spectra.

protons is thus a triplet with the central peak at the free electron position, and twice the height of the side peaks.

If the unpaired electron is associated with a nucleus of higher spin than the proton, a more complex spectrum is obtained, and the width and spacing of the peaks may be influenced by many subsidiary factors. It is seldom possible to deduce the nature of a radical from its ESR spectrum alone, but the latter can often provide strong clues to distinguish between different species which are thought to be possible on other grounds.

I should like to show in Fig. 3 two ESR spectra of organic salts

irradiated in the presence of oxygen; potassium palmitate by Revina and Bach[1]), and sodium succinate by Fränz and Randolph[a]). The close similarity between these is obvious, and points to the unpaired electron being associated with the similar end-groups. In fact, the suggested radical end-group is as shown in Fig. 3. A weak interaction of the electron with the two protons on the

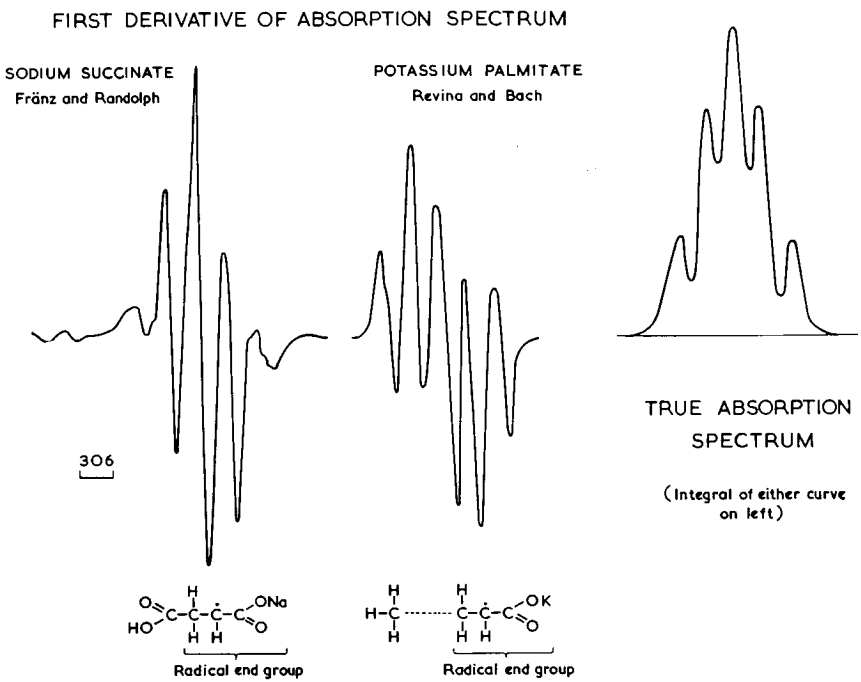


Fig. 3. ESR spectra of sodium succinate (Fränz and Randolph) and of potassium palmitate. (Revina and Bach.)

neighbouring carbon atom gives a narrow triplet splitting, and a stronger interaction with the single proton on the same carbon atom gives a wider doublet splitting, so the result is a double triplet in which the two central peaks happen to coincide.

3. APPLICATION TO RADIATION STUDIES

In radiation chemistry and radiobiology the problems which have been attacked by ESR methods fall principally into four groups:

(i) identification of the nature of the radicals formed in simple chemical systems

(ii) measurement of radical yields

(iii) kinetics of radical disappearance

(iv) correlation of radical spectra in irradiated materials with the biological damage observed.

The effective use of a new technique of this kind demands a fresh approach to the chemical and biological problems. New questions can now be asked with some hope of an answer. One can irradiate at low temperature when most of the radicals formed will be "frozen-in", and then study their change in number and in nature as the temperature is raised. One can have oxygen present during irradiation or introduce it afterwards at low temperature, or still later at room temperature, and so on. Several of the papers in this session make use of such methods.

4. ORGANIC ACIDS AND RELATED SUBSTANCES

Revina and Bach selected a group of three related substances, palmitic acid itself, one of its salts, potassium palmitate, and an ester, tripalmitin. These substances have long chain molecules which differ from one another only in the end-group. The different ESR spectra obtained after irradiation *in vacuo* at − 196 °C are shown in

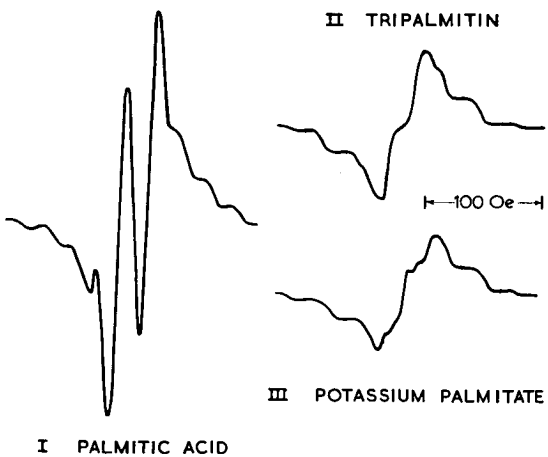


Fig. 4. ESR spectra of palmitic acid (I) tripalmitin (II) and potassium palmitate (III) after irradiation in vacuo at −196 °C. (Revina and Bach.)

Fig. 4 and indicate that the radiation damage must occur preferentially in or near the carboxylic group. The highest yield of radicals occurs in the acid, in agreement with previously reported gas yields. In this, as in most experiments involving radiation damage, one must expect to find at least two types of radicals in the sample, and often many more than two. It is never easy to separate a complex spectrum into its components, but progressive changes in the form of the spectrum may be taken to indicate a change from one type of radical to another type. If a sample of tripalmitin irradiated at -196 °C *in vacuo* is warmed to room temperature, irreversible changes in the ESR spectrum occur as shown in Fig. 5, which point

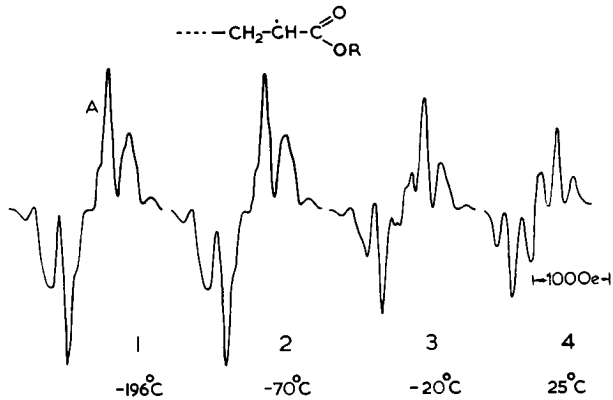


Fig. 5. Changes in the ESR spectrum of tripalmitin irradiated at low temperature on warming to room temperature. (Revina and Bach.)

to the transformation of those original radicals which do not disappear by recombination into new ones. No changes are observed up to about -70 °C, but they are becoming evident at -20 °C and the new double triplet structure characteristic of the radical shown in Fig. 5 is complete at $+25$ °C. The intermediate pattern (3) at -20 °C can be fitted very closely by an appropriate admixture of (1) and (4); the residual radicals amount to some 50% of the number originally formed, and are stable *in vacuo* at room temperature for months.

All the foregoing results were obtained from samples irradiated and kept *in vacuo*. If oxygen was present during irradiation at

−196 °C, this hardly changed the yield or type of radicals formed in palmitic acid, but considerably reduced the yields and changed the type in the salt and in the ester (Table 2). No radicals of characteristic peroxide type were found in any of the three compounds at −196 °C.

When oxygen was admitted after irradiation at −196 °C, no effect was observed on any of the three substances until the sample was warmed. At −70 °C asymmetric spectra characteristic of peroxide radicals were obtained in the ester and especially in the salt, but not in the acid (though this could be due to differences in the diffusion of oxygen into the crystalline samples, rather than to difference in reactivity). On further warming, the peroxide radicals still exist at −20 °C, and indeed at 0 °C, but had all vanished at +25 °C. They decayed much more rapidly than did the initial radicals *in vacuo*. Even in the acid, where the original radicals did not show a change to the peroxide type, their intensity diminished much more rapidly at room temperature when oxygen was present

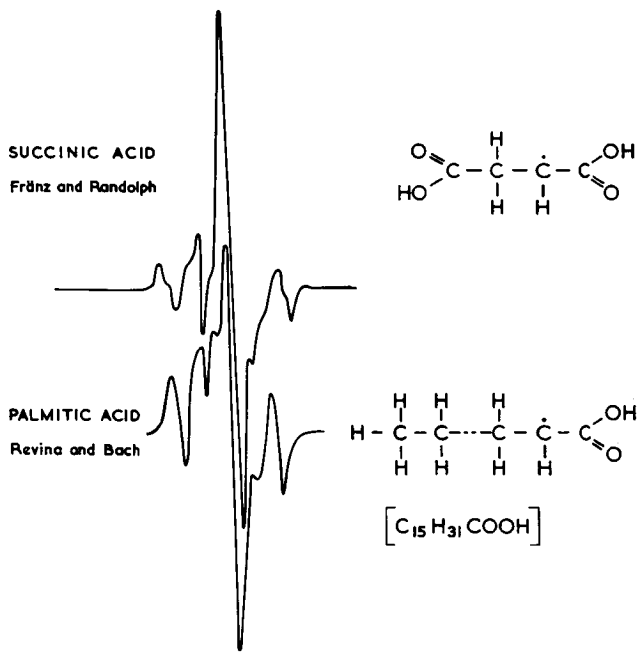


Fig. 6. Comparison of ESR spectra of irradiated succinic acid and palmitic acid.

than in its absence. Some part of the quenching of radicals by O_2 is reversible even at room temperature, and one must assume that in this reaction the O_2 retains its molecular structure since it can be pumped off again. Irreversible reaction with O_2 occurs if the sample is warmed very slowly, or if it is irradiated to much higher dose levels.

Fränz and Randolph report studies on succinic acid and a number of related substances. They irradiated the materials in air at room temperature, and it is again interesting to see in Fig. 6 that the spectrum they obtained for succinic acid is almost identical with that which Revina and Bach obtained for palmitic acid irradiated in air and brought up to room temperature. I have already referred earlier to the similarities in the spectra of the salts, and in the acids also the radicals must be situated on the similar end-groups. Fränz and Randolph found that the radical concentration decayed to half its initial value in about three days, and was unaffected by irradiation with UV. The form of the spectrum did not change during decay: this agrees with the findings of Revina and Bach that the oxidized form of the radical was stable.

The yield curve obtained by Fränz and Randolph at room temperature for succinic acid was similar in form to that of Revina and Bach for palmitic acid at low temperature as shown in Fig. 7.

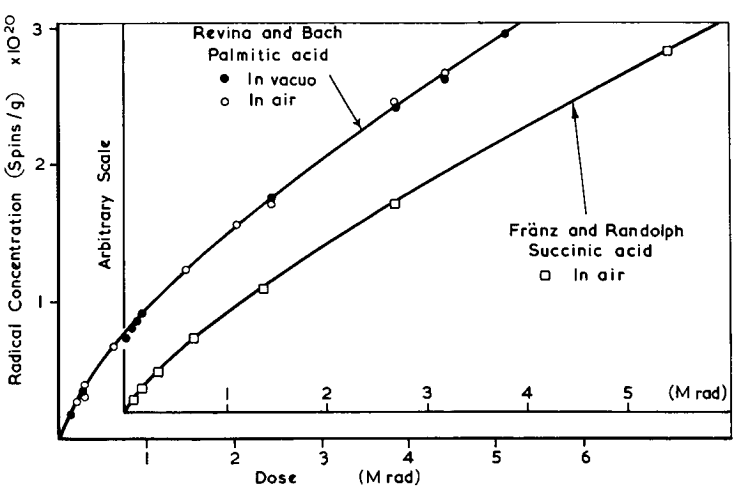


Fig. 7. Comparison of dose-effect curves of succinic acid and palmitic acid.

However, their absolute yield was lower by a large factor, presumably due to the rapid decay before measurement of a large proportion of the radicals originally formed. Fränz and Randolph point out that their findings lend weight to Latarjet's hypothesis[2]) that peroxides, such as disuccinoyl peroxide, may be formed in irradiated biological material in biologically significant amounts, even at moderate doses.

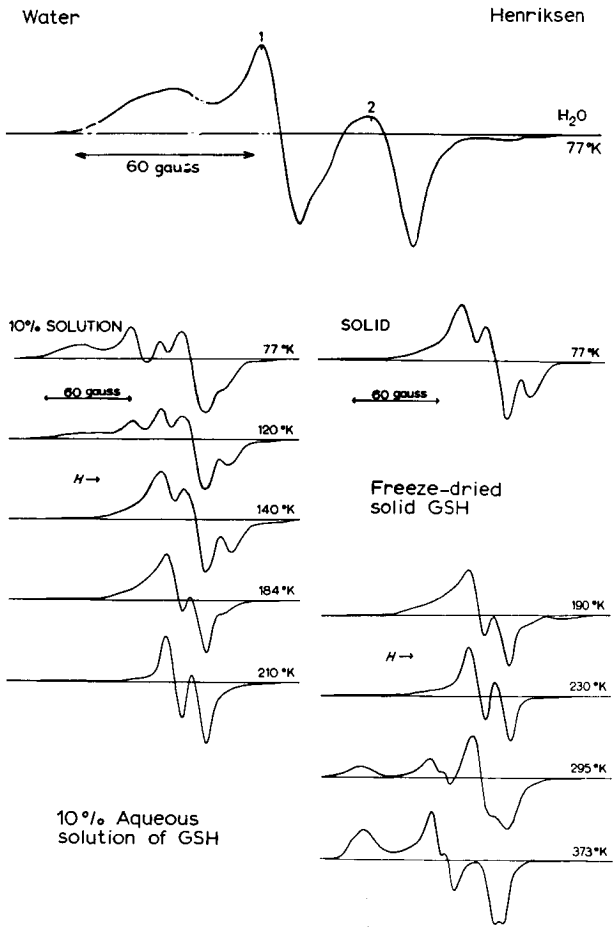


Fig. 8. ESR spectra of irradiated water and aqueous solutions of glutathion at low temperatures and of freeze-dried solid glutathion. (Henriksen.)

5. DIRECT AND INDIRECT ACTION

Henriksen[b]) reports a series of experiments designed to distinguish between the direct and the indirect action of radiation on aqueous solutions of sulphur-containing compounds such as glutathione, penicillamine, and others. Recognising that the resonances observed at room temperature are usually the result of secondary processes, he irradiated frozen aqueous solutions of the substances in question at 77 °K, and studied the changes in ESR spectra as a function of time, when the sample was held at a fixed temperature, and also as a function of annealing temperature when annealing was carried out for a fixed time (3 min).

The radicals found in pure water (Fig. 8, top) are the least stable, and as the temperature is increased they disappear, partly by recombination with one another and partly by reaction with the solute to form new solute radicals.

Henriksen irradiated and examined the spectra at 77 °K. At this temperature the H-atoms are not immobilized, and so they had already disappeared in reactions before the first observations were made. The initial ESR spectrum of the solution exhibited a composite pattern, due to the remaining water radicals, together with those solute radicals formed by the direct action of radiation, and any which had been formed by subsequent reactions between H-atoms and solute molecules.

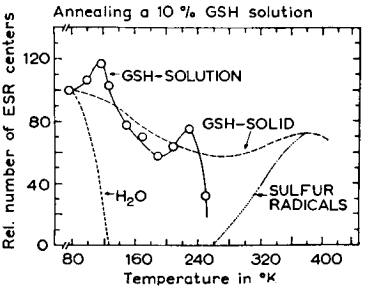


Fig. 9. Changes in the number of radicals in a frozen aqueous solution of reduced glutathion. (Henriksen.)

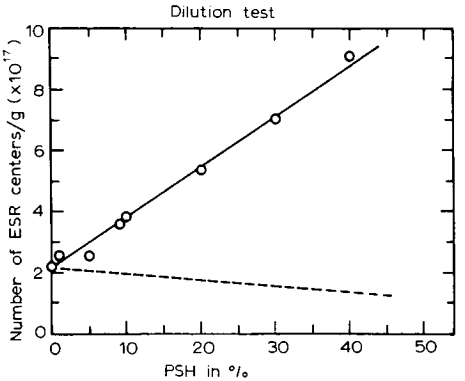


Fig. 10. Dilution test at 77 °K for penicillamine solutions (Henriksen).

In the lower part of Fig. 8 the ESR spectra found in a frozen 10% solution of glutathion (on the left) are compared with those found in the freeze-dried solid material at the same temperatures (on the right). The water radicals disappear rapidly between 100 °K and 130 °K. Above 140 °K the remaining resonances in the solution are exactly similar to those in the solid. The changes in the number of solute radicals in a frozen 10% solution of reduced glutathione as the temperature is raised are shown graphically in Fig. 9. The initial rise is presumed to be due to attack by water radicals, but no explanation of the rise between 190 °K and 230 °K is offered.

Henriksen tested for the effect of the H-atoms on the yield of solute radicals by a "dilution test" at 77 °K, (Fig. 10). The total number of radicals of all types in a penicillamine (PSH) solution at 77 °K was found to vary linearly with the penicillamine concentration up to high values of the concentration, and the straight line extrapolated back exactly to the value for pure water. In water the H-atoms disappear without trace, but if they had been effective in producing solute radicals in the solution this should have led to some curvature in the yield versus concentration, and thus to an extrapolated value somewhat higher than that for pure water.

6. INTERCOMPARISON OF STANDARD SAMPLES

For quantitative measurements an ESR spectrometer is calibrated by introducing known quantities of some stable free radical such as DPPH (diphenyl picryl hydrazyl), and the spin concentration in the unknown sample is deduced by a double integration of the derivative absorption curves of sample and standard. These processes introduce numerous possibilities of error, and values of radical yields reported in the literature from different laboratories have varied by large factors. An attempt to standardise quantitative measurements among a number of laboratories is, therefore, a step to be welcomed, and this is the subject of a paper by ten authors[d]).

As a result of a suggestion made some years ago by Zimmer, a number of workers interested in ESR studies in radiobiology met in Oslo two years ago with the support of the Norsk Hydro Institute of Cancer Research, and drew up a standardization programme. They chose as standard substance irradiated sucrose which Ehrenberg and Löfroth had found to be stable over long periods. Sam-

TABLE 1

Centre	Names	Standard of comparison	3×10^{-5} MnS in ZnS	Sugar samples (spins/g) Nominal values		
				2×10^{18}	10^{18}	0.5×10^{18}
				measured values		
Karlsruhe	Müller Köhnlein	DPPH $CuSO_45H_2O$ LiF optical	—	1.2	0.66	0.36
Stockholm	Ehrenberg Ehrenberg Löfroth	Titration with NO	0.104	1.22	0.64	0.36
Oslo	Henriksen	DPPH	—	2.02	0.98	0.55
Utrecht Geleen Eindhoven	ten Bosch Braams Smidt v. Wieringen	absolute DPPH Ti^{+++} chelate MnS in ZnS	0.18	2.0	1.0	0.6
Frankfurt	Redhardt Pohlit	DPPH di-phe-anisil	—	2.0	1.0	0.56
Oak Ridge	Randolph	DPPH pitch	—	1.44 0.68	0.74 0.39	0.40 0.22

ples of this sugar irradiated to three dose levels were circulated by the Swedish centre to each of the participating groups, who measured the spin concentration on their own apparatus, and returned a portion of the sample for subsequent check measurement by Ehrenberg *et al.*, who found no significant changes. In the early work five groups were involved, and these were recently joined by two others (Redhardt and Pohlit in Frankfurt, and Randolph in Oak Ridge).

Two calibrations have now been made, and the present status of agreement among the different groups is represented in Table 1. It can be seen that the values fall into two classes, differing by a factor of 1.6, and there is at least a suggestion that a systematic error of some kind may account for the difference between these two classes, but its nature has not yet been determined. Reproducibility and relative measurements within any one centre are as a rule considerably better than the accuracy of comparison between groups, but in one centre two different methods of standardization gave results

differing by a factor of 2. Of course, the same systematic error could have been present there, too.

Although it would have been pleasant to record more satisfactory agreement at this stage, the most important matter is perhaps the programme of continuing intercomparisons which one hopes may be extended to include other centres far and near, – for the International Postal Union provides a very satisfactory collection and delivery service for small samples, provided one can prevent Customs officers from X raying them too heavily. The participants in the present intercomparison would be interested to meet informally with other workers who may be present here, with a view to extending this intercalibration. The work involved, particularly for the issuing and collecting centre, is quite considerable, but in the long run this could properly be done by one of the national or international standardizing laboratories.

7. IMPROVEMENTS IN TECHNIQUE

Reitböck and Redhardt[h]) report on an elegant and skilful improvement in ESR apparatus. They show that a molecular amplifier (or maser) can be expected to increase the absolute sensitivity of an ESR spectrometer, especially in work at low temperature, provided the frequency response of the spectrometer is a very flat one, and provided certain other conditions are also satisfied. They have devised a twin cavity microwave circuit which greatly reduces spurious signals arising from frequency variations, and have built for use in conjunction with this circuit a ruby maser operating at the conveniently high temperature of 90 °K, (i.e. liquid air instead of liquid helium,) which gave a stable amplification of 32 dB with a band width of 540 Kc/s. These improvements in measuring technique are important, and will open up new experimental possibilities.

8. AMINO ACIDS AND PROTEINS

Turning to studies on the amino acids, which must by now be the most heavily irradiated of all biological substances, Sommermeyer and Schirmer[l]) report work on the yield and diffusion distances of radicals in glycin, alanin, leucin, serine and valine. The ratio of initial radical yield for alpha radiation to that for X radiation ranges from 0.4 for glycine to 0.8 for alanine and leu-

cine. The yields are reduced at doses above 1 Mrad, and in the case of glycine this falling-off in yield occurs at a lower dose level in powdered material than in single crystals, which contradicts the hypothesis that a saturation of superficial sites is involved. The authors attribute this falling-off in yield at the higher dose levels, and also the lower yield from α particle irradiation, to an initial recombination of radical fragments such as protons, $NH_2\cdot$, NH_3^+ and $\cdot COOH$ which, they postulate, will find one another by simple diffusion through the crystal. They start from the negative exponential "random-walk" formula for the probability that two entities initially at a given distance apart shall diffuse to within an assumed "capture distance," and they apply this formula to their experimental data. On the assumption that the capture distance lies between 0.5 and 3 Å, and making further assumptions about the initial separation of radicals in an α track, they deduce that the radical fragments diffuse over distances of the order of 150 to 300 Å before recombining.

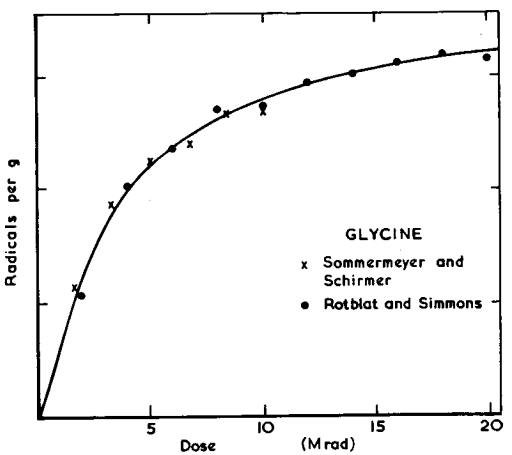


Fig. 11. Dose-effect curve for glycine. (Sommermeyer and Schirmer; Rotblat and Simmons.)

Rotblat and Simmons[1]) have studied the change in radical yield with total dose given to the sample in five amino acids. They find the same general shape of the dose-response curve in glycine as do Sommermeyer and Schirmer (see Fig. 11), and they determined an

initial yield corresponding to 27 eV/spin. The signal from glycine reached a "plateau" at about 20 Mrad, while in alanin the plateau was only reached at some 200 Mrad, and in valine, aspartic acid and glutamic acid between 100 and 200 Mrad.

Amino acids and proteins have been irradiated by Randolph[g]) with UV, and by Koch and Fränz[c]) with both UV and X rays. Randolph found a low *G* value for radical formation by UV, and in the case of glycyl glycine a different shape of ESR spectrum from that characteristic of the X irradiated material. Koch and Fränz found that the materials they tested fell into three groups: – (i) Those in which UV produced the same type of signal as X rays, and the two radiations together produced merely an enhanced signal. Cystine and silk fibrin belonged to this group; (ii) Those in which UV produced a different type of signal from X rays; UV given after X rays then caused a transformation of the ESR signal from the X ray into the UV form. All the sulphur-containing amino acids were in this group; (iii) Those in which UV produced no signal at all. Alanin and tryptophane were of this kind, and tryptophane showed no signal after 10^5 rad of X radiation. All the substances tested in which UV produced an ESR signal contained sulphur, excepting only silk fibrin, and some workers have reported a fraction of one per cent cystine in the latter; enough, in fact, to give a measurable signal.

9. COMPLEX BIOLOGICAL MATERIALS

Turning finally to more complex biological materials, Singh, Venkateraman, Notani, Chandra Mouli and Bora[k]) have irradiated seeds belonging to four different strains of maize. They found differences in the yield and decay rate between high oil and low oil seeds, but they state that no clear correlation between radiosensitivity and radical content could be observed. In the seeds of *Ornithogallum* virens they found that the narrow ESR line observed resided entirely in the seed coat. Rajewsky and Redhardt[f]) have studied the gradual change in ESR spectrum which takes place in dry keratin after irradiation, – using feather quills as experimental material. The ESR spectrum immediately after irradiation is as shown in Fig. 12. The line A at the low frequency end of the keratin spectrum is taken as indicating the formation of radicals containing sulphur, and in carefully dried material this grows within one or

two hours of irradiation to an equilibrium value which is some 130 to 150% of its initial value. The shape of this line as measured by the ratio A to B does not change significantly in this time, but a transient line near the free spin position decays as the line A increases.

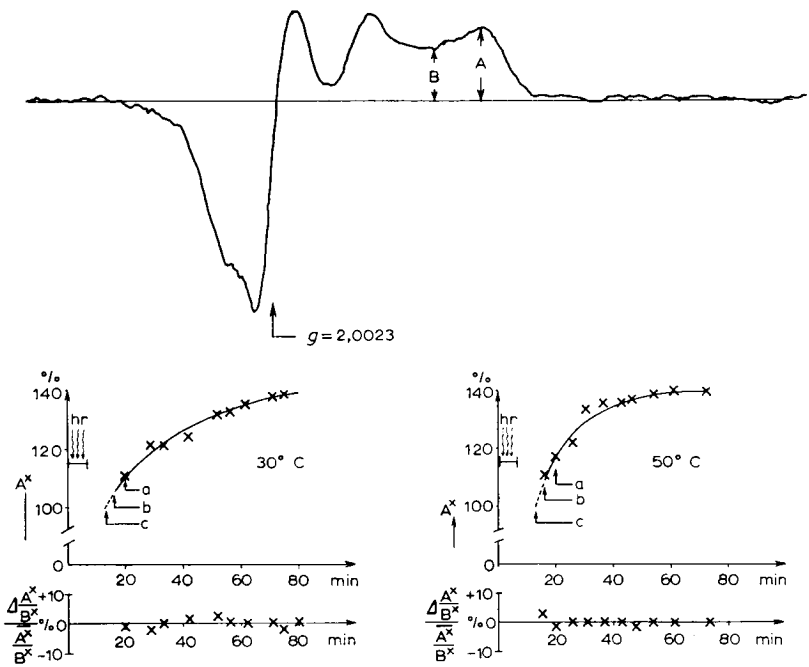


Fig. 12. ESR signal from irradiated keratin and changes after irradiation. (Rajewsky and Redhardt.)

The growth of this line cannot, however, account quantitatively for the absence of any ESR spectra characteristic of the radicals which one would expect to find in the sulphur-free amino acids contained in keratin. For short time intervals after irradiation the rate of change of A depended on temperature in a manner from which an activation energy of 0.65 eV could be calculated. The authors draw an analogy between the enhancement of the sulphur-containing radicals in solid keratin and the indirect effect on solutes in aqueous solution.

Müller[e]) has studied the ESR signals induced in T2 phage, and also in DNA extracted from T2 phage by irradiation either at 77 °K or at room temperature. These radiation-induced signals are easily distinguishable from the very broad paramagnetic absorption spectrum previously reported by Müller in unirradiated phage. He has now made the very important observation that the ESR signal in irradiated DNA is no longer linear with dose above about 100 Krad, as seen in Fig. 13. In the case of the amino acids a

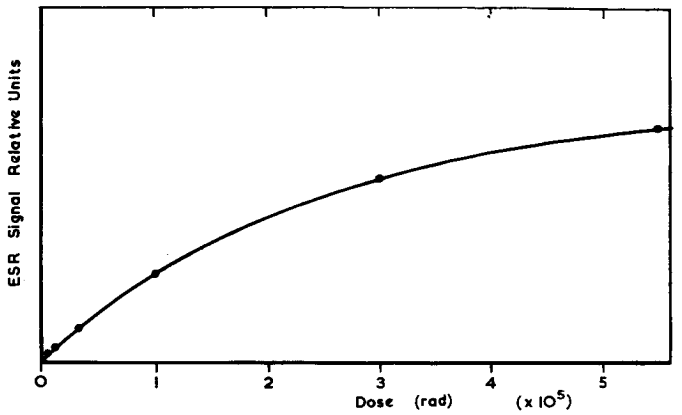


Fig. 13. Dose-effect curve for dry DNA from T2 phage. (Müller.)

similar decrease in the slope of the dose-effect curve begins in the megarad or multi-megarad range, as reported by Rotblat and Simmons. At very high doses the number of radicals in irradiated DNA reaches a constant value corresponding to about 10 radicals per whole DNA molecule. Earlier ESR studies on DNA had used high doses, and had, therefore, given an altogether false idea of the efficiency of radical formation. Müller finds that the initial slope of his dose effect curve for DNA corresponds to an energy expenditure of only 15 eV per radical. In radiation-chemical terms, the G value is 6.6. This means that radical formation in DNA must be given very close attention instead of being dismissed as a rather inefficient process. The ESR signal from whole T2 phage was intermediate between that for DNA and that found for proteins. It departed from linearity above 200 Krad, but did not show a plateau as did the extracted DNA. In the whole phage, but not in the DNA, some

radicals giving narrow ESR lines were present in the unirradiated material.

It may be useful to conclude this review with a composite table (Table 2) bringing together the various values of radical yields which are reported in the papers I have been reviewing.

TABLE 2

Substance	Authors	Yield radicals/100 eV	
		In vacuo	In air
Palmitic acid	Revina	20	20
Tripalmitin	and	12	7
Potassium palmitate	Bach	9	7
Succinic acid	Fränz and		1
Disuccinoyl peroxide	Randolph		1
		Dry	Frozen sol.
Glutathione	Henriksen	5	6
Oxidised glutathione		8	6
Penicillamine		7	8
Cysteamine		11	7
Cystamine		13	14
			In air
Glycine	Rotblat		4
Alanine	and		2
Valine	Simmons		2
Aspartic acid			1.3
Glutamic acid			1.5
		In vacuo	
T_2 phage DNA	Müller	6	

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2) R. Latarjet, Les péroxydes organiques en radiobiologie (Masson et Cie., Librairies de l'Académie de Médicine, Saint Germain, Paris, 1958)

DISCUSSION

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Müller's[e]) observation of a radical saturation in DNA irradiated at low doses at room temperature aroused considerable interest. Hutchinson (*Yale Univ., New Haven, Conn., USA*) wanted more details of this saturation, while Alexander (*Chester Beatty Res. Inst., London, Gr. Britain*) asked specifically about the effect of temperature on it and Bertinchamps (*Euratom, Brussels, Belgium*) asked how stable the saturated radicals were after the cessation of radiation. Müller replied that at liquid nitrogen temperatures radical build-up was linear up to a dose of a few megarads and that saturation had only been observed at room temperature. The level of saturation had been found to differ from one sample to another. The room temperature spectra showed marked hyperfine structure, but there was much less structure in the spectra taken at 77 °K after irradiation at that temperature. This could be due either to different protein contents in the samples or to the proposed ability of one DNA molecule to contain only one radical; damage during preparation might give a variation of molecular weight between samples which would then give a variation of saturation levels. After saturation had been reached, if radiation was stopped it was found that the radicals were comparatively stable. Decays of about 10% in 24 hours were normal.

Wyard (*Guy's Hospital Medical School, London, Great Britain*) wanted to know why irradiated sugar was used as a standard in the absolute calibration comparison made between several laboratories. He pointed out that several laboratories used a carbon sample which gives a much simpler spectrum and this might lead to more consistent results. Müller said that the line shape of the sugar spectrum is nearly Gaussian while that from a coal sample is Lorentzian. A Lorentzian line has a longer tail to it which leads to greater errors during integration.

Fowler (*Hammersmith Hospital, London, Great Britain*) asked whether the curves of radical concentration versus dose in glycine owed their saturation to any effect of the high dose rates used, for example in heating of the sample. Rotblat[j]) said that in this work a range of

dose rates had been used and that the curves shown were not due to dose rate effects.

Kuzin (*Academy of Science, Moscow, USSR*) said that in similar experiments to those by Fränz and Randolph[a]) he had obtained different results, in particular in succinic acid, and asked if this could be due to the presence of more than one radical species. Fränz said that he thought that this might be so and asked if a more detailed answer could be given by Whiffen (*National Physical Laboratory, Middlesex, Great Britain*), who pointed out that the observed radical concentrations and species depended on the thermal history of the sample after irradiation. The single crystals studied at the NPL were usually thermally annealed to give a single radical species.

Read (*Wakari Public Hospital, Dunedin, New Zealand*) said that the first effects of irradiation are the production of a positive molecular ion and a free electron. The latter travels some distance while losing its energy and in the crystalline or glassy materials at low temperatures may be held in an electron trap. He asked if the positive molecular ions and the trapped electrons would give ESR signals. Ormerod said that during studies of irradiated polymers at 77 °K it was found that most polymers gave spectra due to neutral free radicals. These spectra had been identified by studying oriented specimens. However, in polymethacrylic acid and its esters trapped electrons had been observed and identified by the use of particular additives. Unfortunately there was no ESR evidence for the positive ions.

Hart (*Argonne Nat. Lab., Ill., USA*) found two features of the communication by Revina and Bach[1]) of considerable interest. They were the extreme simplicity of the free radical $RCH_2\dot{C}HCOOH$ formed at room temperature in a complex fatty acid and the high *G* (radical) reported. He wanted to know if Bach could suggest mechanisms that could account for these observations. Bach replied that the high *G* (radical) was due to the measurements being made at low temperatures and at low radiation dose rates, so that all the radicals were trapped. In most early published work only a few of the radicals formed were observed owing to the measurements being made at too high a temperature. It was not possible to say why a particular radical is observed at room temperature since too little is known about radical reactions in these systems. More data on the kinetics of radical decay would have to be obtained before this question could be discussed more fully.

The Linking of Disciplines in Radiation Research

INHIBITION OF DNA SYNTHESIS IN RELATION TO CELL DEATH

L. G. LAJTHA

Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester 20, Great Britain

a) P. ALEXANDER and Z. B. MIKULSKI,
Qualitative differences between the action of α and X rays on lymphoma cells in tissue culture.
Chester Beatty Research Institute, London SW3, Great Britain

b) P. BIANCHI, J. A. V. BUTLER, A. R. CRATHORN and K. V. SHOOTER,
Effects of γ radiation on thymidine kinases and DNA biosynthesis in leukaemic mice.
Chester Beatty Research Institute, London SW3, Great Btiain

c) J. H. CURTIS and CATHRYN CROWLEY,
Chromosomal aberrations in neutron and chronic gamma irradiation.
Biology Department, Brookhaven National Laboratory, Upton, NY, USA

d) K. B. DAWSON and E. O. FIELD,
Cell radiosensitivity studied by a double isotope autoradiographic technique.
Chester Beatty Research Institute, London SW3, Great Britain

e) P. P. DENDY,
Studies on nucleic acid synthesis following the irradiation with an ultraviolet microbeam of parts of single cells grown in tissue culture.
Department of Radiotherapeutics, University of Cambridge, Tennis Court Road, Cambridge, Great Britain

f) W. C. DEWEY and R. M. HUMPHREY,
Recovery of radiation induced chromosomal damage in Chinese hamster cells related to the phase of the cell cycle.
Physics Department, M. D. Anderson Hospital, Texas Medical Centre, Houston 25, Texas, USA

g) M. DRAKULIC and E. KOS,
On the relation between the stability of DNA and colony-forming ability of gamma-irradiated *Escherichia coli* B.
Department of Radiobiology, Institute "Rudjer Boskovic" Zagreb, Yugoslavia

h) R. M. HUMPHREY, W. C. DEWEY and A. CORK,
Sensitivity of Chinese hamster cells to ultraviolet and ionizing radiation in relation to the cell cycle.
Physics Department, M. D. Anderson Hospital, Texas Medical Centre, Houston 25, Texas, USA

i) O. F. NYGAARD,
Insensitivity to ionizing radiation of DNA-polymerase in a slime mould.
Division of Radiation Biology, Department of Radiology, Western Reserve University, Cleveland, Ohio, USA

[j]) R. B. PAINTER,
The sequence of division of HeLa S3 cells irradiated during DNA synthesis and in the G 2 phase.
Batelle Memorial Institute, Columbus, Ohio and *Ames Research Center, Moffett Field, Mountain View, California, USA*

[k]) H. QUASTLER,
Autoradiographic studies of DNA synthesis in irradiated mice.
Biology Department, Brookhaven National Laboratory, Upton, NY, USA

[l]) C. L. SMITH,
Effect on nucleic acid synthesis of irradiation of parts of single cells in tissue culture with a microbeam of α particles.
Department of Radiotherapeutics, University of Cambridge, Tennis Court Road, Cambridge, Great Britain

[m]) T. TERASIMA and L. J. TOLMACH,
Variations in the response of HeLa cells to X rays during the division cycle.
Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri, USA

1. INTRODUCTION

That ionising radiations inhibit DNA synthesis has been known since the classical experiments of Hevesy twenty years ago. The two questions which emerge from his observation are, first, what is the mechanism of effect of radiation on the DNA synthesis, and second, is this mechanism connected in any way with the radiation induced "genetic death" of dividing cells. Although twenty years have gone by since these questions were posed, they are still not fully answered. The thirteen papers in this session bring some interesting information on this problem, and the material presented in these papers can be put under three headings: effects on DNA and DNA synthesis, effects on cell cycle, and effects on the chromosomes and reproductive integrity of the cells.

2. THE EFFECT OF DNA AND DNA SYNTHESIS

Drakulic and Kos[g]) have irradiated *E. coli* with 16000 r and find that in the irradiated samples there is a loss of DNA per cell as well as a loss of reproductive integrity. They have tested a number of compounds which prevent the breakdown of DNA after irradiation, but they find that most of them inhibit DNA synthesis as well, e.g. sodium citrate, succinic acid, glutamic acid, ATP, AMP, DNP, salicylate, and sodium azide. The effect is less clear with EDTA and ADP, which did not significantly inhibit DNA synthesis, but pro-

duced only moderate protection against DNA breakdown. The authors state that they could not observe any positive correlation between DNA content and viability during the post-radiation incubation period. They advance the interesting hypothesis that the radiation-induced breakdown of DNA in *E. coli* is but an unsuccessful attempt at replication, and hence the same enzymes may be involved in DNA breakdown as in DNA synthesis, but in a disorganised manner. Nygaard[1]) attempted to analyse the enzymes involved in DNA synthesis using the plasmodial slime mould *Physarum polycephalum*. This is a beautiful biological system in which synchronous division can be produced at will. Nygaard isolated the enzyme polymerase from these cells to test whether irradiation affects the polymerase activity in the organism. The polymerase content of the organism does not change very much during its cell cycle, apart from a small but perhaps significant drop about two hours after mitosis. Nygaard has irradiated this organism with 100000 r X rays, but failed to detect any effect on the polymerase activity, irrespective of the stage in the cell cycle at which the organism was irradiated. This observation is significant in view of the fact that smaller doses of radiation (21000 r gamma rays) lead to complete sterilisation of the nuclei, although even that dose did not affect DNA synthesis when delivered during the period of synthesis. The author came to the conclusion that in the slime mould the enzyme DNA polymerase is not a target of primary importance with respect to ionizing radiation. From his data it also appears that cells can be completely sterilized without any measurable effect on DNA synthesis.

Nygaard's observations are somewhat at variance with the results of Bianchi, Butler, Crathorn and Shooter[b]). They were studying the pathway of DNA synthesis in mouse leukaemia, and in particular they followed the various steps involved in the incorporation of thymidine into high polymer DNA. As their results in Table 1 show, the activity in high polymer DNA is more depressed by irradiation than the step leading to monophosphate or triphosphate formation. They come to the conclusion that in this mammalian system the polymerase reaction giving rise to polymerized DNA appears to be affected more by irradiation than either of the two previous steps in which the kinases are involved.

There are obvious differences in the two systems, the mouse pre-

TABLE 1

Fraction of the total ^3H thymidine present in tissue which has been converted to TMP, TTP and DNA half an hour after injection of $7\mu C$ ^3H thymidine *in vivo*. Each result is the mean of 3 experiments.

	Spleen			Lymph nodes		
	DNA	TTP	TMP	DNA	TTP	TMP
Control	0.69	0.78	0.90	0.69	0.75	0.84
½ hr after irradiation	0.53	0.68	0.82	0.42	0.53	0.72
1 hr after irradiation	0.52	0.63	0.78	0.52	0.68	0.80
2 hr after irradiation	0.20	0.45	0.65	0.14	0.30	0.55
3 hr after irradiation	0.15	0.25	0.57	0.10	0.17	0.38
4 hr after irradiation	0.13	0.29	0.55	0.17	0.25	0.51

(Bianchi, Butler, Crathorn and Shooter.)

senting a marked depression of the final DNA synthesis by as little as 600 r gamma rays, while this process is not affected in the slime mould by as much as 20000 r gamma rays. The question arises whether the polymerase content of the two systems are similar or vastly dissimilar. If the polymerase content of the slime mould is relatively high, while that in the mammalian system is very low, a mild degree of inactivation may not be detectable in the first, although showing a significant loss of activity in the second cell type. What is perhaps even more likely is that the slime mould contains a store of the enzyme polymerase, while the mammalian system depends on the continued production of the enzyme, and perhaps it is not the enzyme molecule itself but its production which is affected by irradiation. This, however, is a subject for further study.

That there are bewildering differences even between mammalian cells, is demonstrated by a paper from Alexander and Mikulski[a]) who gave 300 r X rays to mouse lymphoma cells L 5178 Y *in vitro*. They found that this dose of radiation and similar doses of alpha particles do not affect the DNA content of the cells until the cells actually begin to disintegrate. During the short post-radiation period the DNA content of their irradiated cultures does not differ from that of the normal. The cells either perform one or two post-radiation mitoses or simply form giant cells, but DNA synthesis is not apparently inhibited. In both these last two papers mouse lymphoid malignant cells are being studied, the difference being

that in the case of Alexander and Mikulski the system is *in vitro*, while in the case of Bianchi and his collaborators it is *in vivo*. It is conceivable also that in Alexander and Mikulski's system a mild temporary depression of the rate of DNA synthesis may not show up as well as in the more direct approach of Bianchi and his colleagues. It would be highly interesting to compare the two systems using the same parameter for the measurement of the DNA synthesis.

Using a different strain of cells, (HeLa cells *in vitro*), Terasima and Tolmach[m]) found that 300 r X rays do affect the process of DNA synthesis (as measured with ^{3}H thymidine incorporation on autoradiographs) but that the effect depends on the stage of the cell cycle at which the radiation is delivered. They find, for example, that if radiation is given during the process of DNA synthesis they can detect a depression of the order of 30 per cent. As the DNA synthetic period occupies a considerable part of the interphase in these cells, their system should again be comparable to that of Alexander and Mikulski, if similar parameters for the measurement of DNA synthesis are used. Again a comparison between the two systems would be very interesting indeed.

Two papers from Cambridge, by Smith[1]) and Dendy[e]), report on microbeam effects by alpha particles and UV radiation. These authors use massive doses of radiation, and they find an immediate effect on DNA synthesis in mouse cells *in vitro* (L cells and embryo-fibroblasts) using ^{3}H thymidine autoradiographs. The effects of UV and alpha particles are fairly similar. It is not necessary to irradiate the nuclear volume only: irradiation of the cytoplasm, and indeed of the medium in close proximity to the cell wall, also produces depression of DNA synthesis. In the case of cytoplasmic irradiation only about 2 per cent of the cytoplasm is exposed. For this reason the authors come to the conclusion that, with these massive doses, some inhibitory mechanism may be released which is eventually responsible for the depression of the rate of DNA synthesis. It may be noted that in an earlier work Smith[1]) has reported that the HeLa cell shows very little effect in respect of DNA synthesis following large doses of radiation, his dose-response curve showing 90000 rad to give a 63 per cent depression. This is in contrast to Terasima and Tolmach's observation on the same cell type, also *in vitro*, where 300 r given in the synthetic period produces about a 30 per cent depression.

Quastler[k]) in a summary paper, gives a very important correlation of factors which are involved in the incorporation of a labelled precursor into DNA. His conclusions, based on fast growing mouse tissues *in vivo*, are presented in Fig. 1. He points out that in various cell types and in various systems, even with the same cell type, different parts of this scheme may be differently affected by radiation,

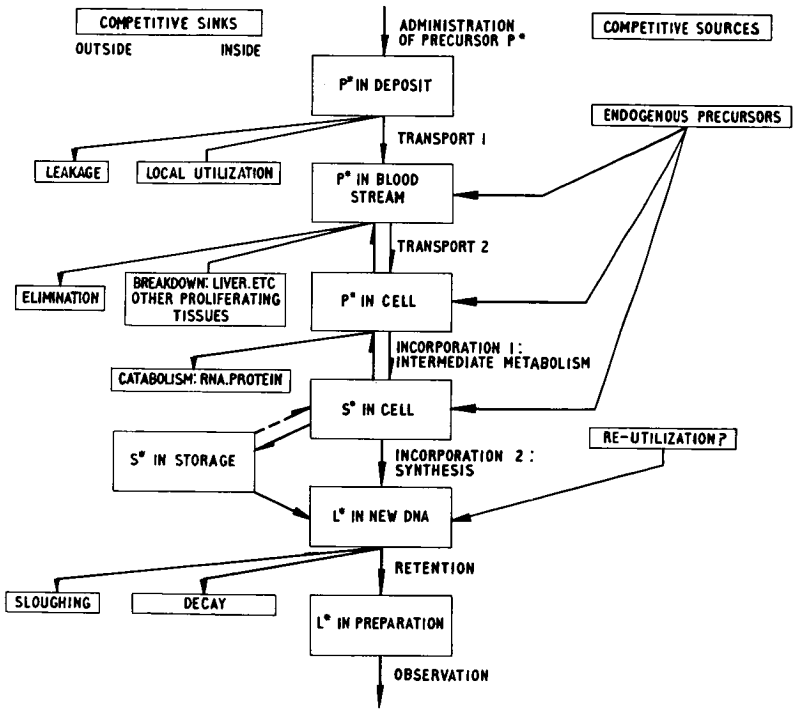

Fig. 1. Factors involved in the incorporation of a labelled precursor in DNA. (Quastler.) P = precursor, S = store, L = label.

and a more accurate analysis of all these factors is required before a full understanding of the radiation effect on DNA synthesis can be achieved. To this scheme I should like to add Fig. 2, which is based on some of our recent observations which compare the shape of the dose-response curve of X rays on DNA synthesis in different systems. It is clear that systems with a pronounced S_0 component will show extreme sensitivity, while a similar system which has lost the S_0 component will behave like one with the S_1 and S_2 components

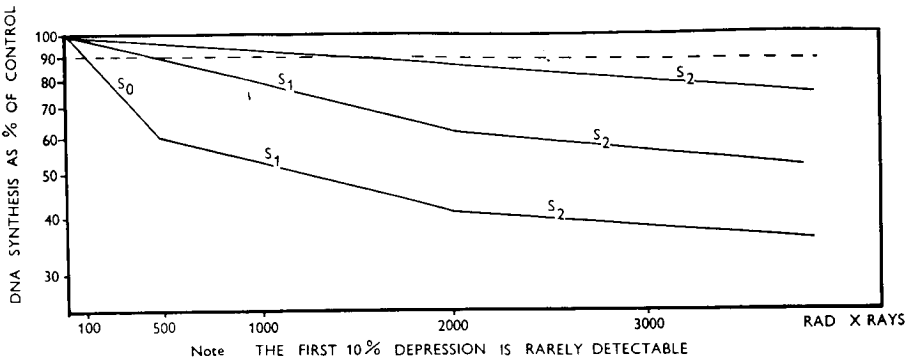

Fig. 2. X ray dose response relationships for the process of DNA synthesis. The S_2 slope is frequently observed in anoxic or damaged tumour cell systems and it may represent the integrity of the DNA template. The S_1S_2 curve applies for most oxygenated mammalian cell systems *in vitro*, in which S_1 represents the sensitivity of a rate limiting factor. The $S_0S_1S_2$ curve represents response of lymphatic tissue *in vivo*, where both S_0 and S_1 represent rate limiting factors. (Lajtha, to be published.)

only, and show a greatly diminished response. Lymphoid tissues *in vivo* show the S_0, S_1 and S_2 response; *in vitro*, they show only S_1 and S_2. It is suggested, therefore, that in further studies on the effects of radiation on DNA synthesis, as many as possible of the factors listed by Quastler and the full analysis of the dose-response relationship ought to be investigated.

3. EFFECTS ON CELL CYCLE

There are three papers dealing with effects on the cell cycle, all three using the HeLa cells, and the same S_3 clone — as far as it is possible to call three clones in three different laboratories the same. In an earlier paper, Painter[2]) has reported on a cell cycle delay, in particular of the G 2 period of the cell cycle, produced by 500r X rays. In his present paper[1]) he confirms his earlier observations (Fig. 3), but proposes the interesting theory that the delay produced may not be a specific G 2 delay but a particular damage which requires a certain time for repair. If the cells are irradiated in the G 2 period, which is very close to the subsequent mitosis, this delay will show up, while cells early in their cell cycle (G 1 or early S period) may have sufficient time to repair the lesion, so that when they come

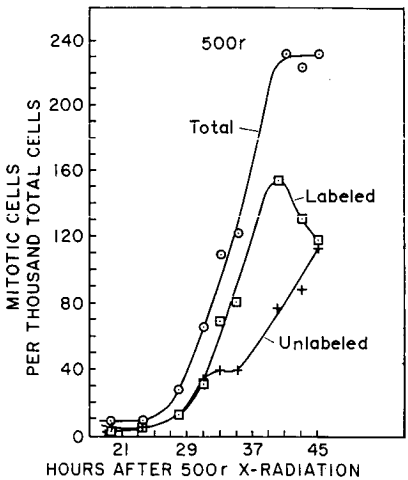

Fig. 3. G 2 delay in HeLa cells *in vitro*. (Painter.)

to their G 2 period, the damage will not show up so markedly. Terasima and Tolmach find essentially the same thing as Painter, and they graph the delay in entering mitosis in terms of minutes of delay per rad radiation (Fig. 4). They find that the delay increases

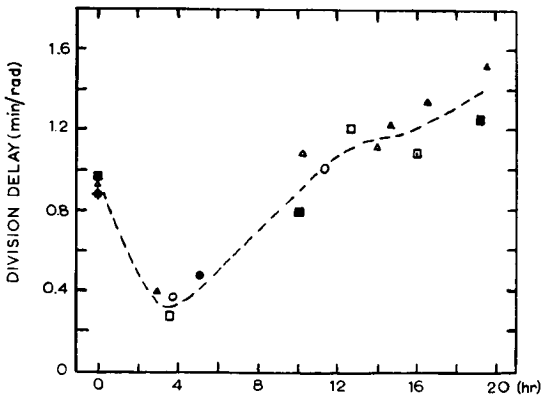

Fig. 4. The correlation of mitotic delay with stages in the cell cycle (hours after mitosis) at the time of irradiation of a synchronized population of HeLa cells. (Terasima and Tolmach.)

throughout the cell cycle: it is about 0.3 min per rad in early G 1 and 1.4 min per rad in G 2. On the face of it, their curve would not suggest a standard damage such as Painter suggests, but it is not certain whether their system is sensitive enough to make a clear

distinction between Painter's scheme and that of a general but unexplained interrelationship between delay and the cell cycle state.

Both Painter and Terasima and Tolmach report on immediate effects, that is, delay within the first cycle after irradiation. The next paper, however, that of Dawson and Field[d]), investigates the cell cycle delay apparent 7 days after irradiation of HeLa cells with doses of radiation similar to those of Terasima and Tolmach, and of Painter. Dawson and Field found earlier[3]) that the overall cell generation time is slowed down for several generations following irradiation, and that this delay is dose-dependent. With a most ingenious double isotope technique they analysed the cell cycle of their cells on the 7th day post-irradiation, and came to the conclusion that at that time it is only the G 1 period which is significantly elongated (6 to 18 hr depending on the dose), while the G 2 period shows a mild elongation of one to two hours. At that time the rate of DNA synthesis in the cells is not affected. The interesting point of these three papers is that the cell cycle delays observed cannot be correlated in either of the cases with any direct interference with the processes of DNA synthesis, and furthermore, that the cell cycle changes may vary in a way which depends on how soon after irradiation they are investigated. It might be pointed out, however, that the results of Alexander and Mikulski do not indicate either an immediate cell cycle delay or delay appearing after several cell generations, as shown by Dawson and Field. Alexander and Mikulski make it clear in their paper that an initial temporary delay lasting for only a few hours would not be detectable in their system. However, the growth curves after the fourth day certainly exclude a late elongation of the generation time. This is an interesting difference between the lymphoma cells they use, and the HeLa cells used by Dawson and Field.

4. CHROMOSOMES AND REPRODUCTIVE INTEGRITY

Alexander and Mikulski have studied the reproductive integrity of their mouse lymphoma cells *in vitro* by extrapolating from growth curves and using the limiting dilution method. In this way they were able to construct dose-response curves similar in nature to those found with mammalian cells by Puck[4]) and Hewitt[5]). Presumably because of the motility of these cells a clonal culture method is not suitable. The cells have a cell cycle of about 11 to 14 hours, and after moder-

ate doses of X rays, they are able to perform one or two post-irradiation divisions before the onset of cell death. As has been mentioned before, those cells which cannot divide still continue DNA synthesis and grow into giants. The authors have developed a radioresistant strain of cells which requires about 2½ times the X ray dose to produce the same sterilization rate as the sensitive strain. Using alpha irradiation, however, they find that there is no post-irradiation division at all, although the sterilized cells still can grow into giant cells. Furthermore, with alpha irradiation, there is no shoulder to the dose-response curve; it follows a straight exponential relationship, and there is no difference in the response between the X ray sensitive and X ray resistant strains of cells to alpha particles. This finding compares well with a paper by Curtis and Crowley[c]), who studied chromosome aberrations produced by gamma rays, X rays and fast neutrons in regenerating liver. These authors find that 7½ r/day chronic gamma exposure produced only a small increase of aberrations above that found in control animals, and call attention to the fact that even in the control animals the frequency of aberration increases with the age of the animal. A single dose of 234 rad X rays produces a significant number of aberrations but these decrease with time, that is, there is evidence that the aberrations produced by X rays can "heal". Furthermore, the authors find no good agreement with a theoretical extrapolation from their chronic irradiation to the single exposure data, which indicates that the X ray produced chromosome aberrations follow a "multihit" type of curve as a function of the dose. It is significant that while the aberrations produced by X rays can heal, the aberrations produced by a single exposure of 323 rad fast neutrons do not show any healing, in fact, they possibly increase further with time. The authors state that this fits in well with other observations on the lack of recovery from neutron irradiation, as far as life shortening is concerned, and they suggest that the acceleration of ageing produced by irradiation may be due to induced mutations.

Alexander and Mikulski claim that alpha particles may have a different mechanism of radiation sterilization than X rays, a statement well in keeping with the results and conclusions of Curtis and Crowley.

The phenomenon of post-irradiation division is further discussed by Dawson and Field, who report that in their HeLa cultures one or

two divisions occur during the first four days, even under a fractionated regime of daily 200 to 300 r (Fig. 5). The numbers of cells decrease only after the fourth day, that is, at about the same time as Alexander and Mikulski's lymphoma cells following 500 rad.

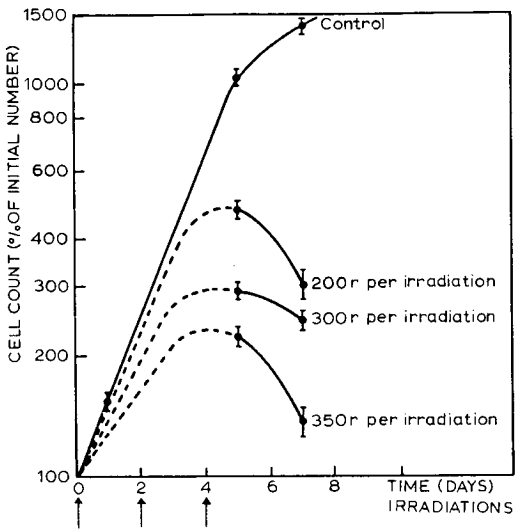

Fig. 5. Post-irradiation division of HeLa cells under a regime of repeated irradiation. (Dawson and Field.)

Terasima and Tolmach also find this post-irradiation division, but they claim that the number of post-irradiation divisions depends to a great extent on the phase of the cell cycle in which the cells were irradiated (Fig. 6). They also constructed X ray dose-response curves for their synchronous HeLa cell population using the Puck plating method[6]). They confirm that differences in dose sensitivity in respect to reproductive integrity are found, depending on the phase of the cell cycle which is irradiated. Greater sensitivity is found in mitosis and in the early S period. The minimum sensitivity is in the G 1 and G 2 periods. They have constructed, for a mixed cell population, a theoretical composite dose-response relationship predicted from the cell cycle of their HeLa cells. This curve shows a typical "multihit" shape with a straight exponential path down to at least the sixth decade. Although they do not state whether this curve fits their cells in a non-synchronised condition, it is parti-

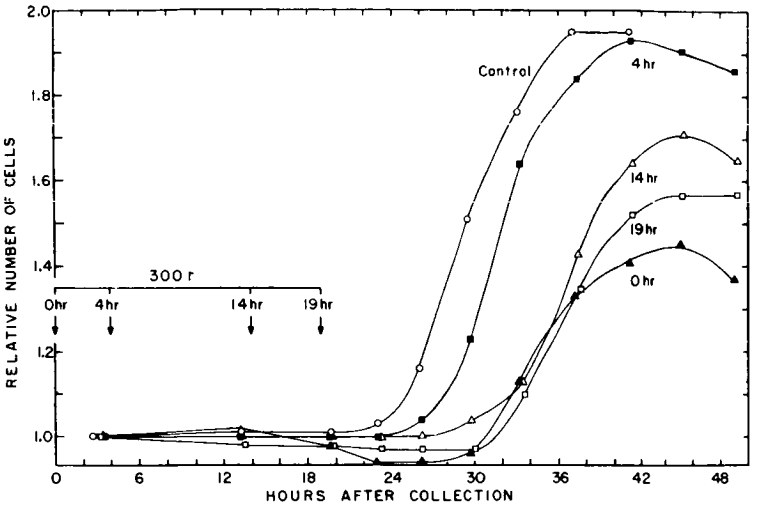

Fig. 6. The relation between the number of post-irradiation divisions in synchronized HeLa cells and the phase of cell cycle during irradiation. (Terasima and Tolmach.) (0 hr: cells in mitosis. 4 hr: G 1 stage. 14 hr: S stage. 19 hr G 2 stage. Control: non-synchronous cell population.)

cularly important as it indicates that a single dose-response curve, even if it has a straight portion reaching down to six decades, is not a sufficient indication of homogeneity of the cell population in respect of radiosensitivity. These results tie up well with the two papers by Dewey and Humphrey[f]), and Humphrey, Dewey and Cork[h]). These authors perform a careful analysis of the types of chromosome damage produced by 300 r X rays and UV irradiation of their Chinese hamster cells *in vitro*. Their results have been discussed in respect of chromosome morphology by Auerbach in a previous session. What is relevant here is their statement that chromosome restitution is complete in about 5 to 10 min if the irradiation is delivered in the G 1 period, whereas the breaks remain open for about an hour if they are produced during the S period. They also state that dose-rate is particularly important because the difference found between G 1 and S period is much less at a rate of 518 rad/min than at 128 rad/min. Their results show some interesting differences between the effects of UV and of ionizing radiation, as summarized in Fig. 7. UV produces most breaks when given in the early S period, whereas gamma rays are most effective in the

G 2 period. With gamma rays, 70 per cent of the damage produced in G 1 is of the chromosome type: with UV the chromosome type of damage is rare, and mostly chromatid type is produced. The authors state that their finding is in support of the two stranded structure of the chromosome proposed by Taylor[7]) and that in this

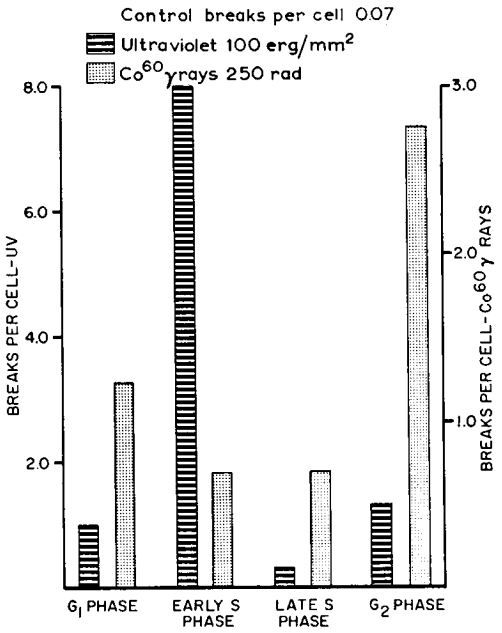

Fig. 7. Comparison of the effect of ultraviolet and gamma irradiation in producing mitotic abnormalities by exposure at different stages of cell cycle. (Humphrey, Dewey and Cork.)

way some information about the physico-chemical state of the interphase chromosome in G 1 and S period may be obtained. The Texan authors did not investigate dose-response relationships in respect of reproductive integrity, but their results would not easily extrapolate to those found by Terasima and Tolmach. Humphrey and Dewey found about four times as much damage from gamma rays when cells were irradiated in G 2 compared with those irradiated in S, whereas Terasima and Tolmach found a greater sensitivity in early S than in G 2. The cell types used by the two groups of workers are not the same, and this may explain the different results. It

would, however, be very important indeed to correlate these two types of investigations using the same cell strain in the same culture condition, to determine whether or not visible chromosome damage of any kind is directly related to damage to reproductive integrity. The above two papers indicate no good correlation, but, as has been said, the cell types used were different.

5. CONCLUDING REMARKS

To summarize, the results of these 13 papers indicate the following (see also Table 2).

TABLE 2

Immediate effects of X or gamma irradiation related to cell cycle state

State of cell cycle	Rate of DNA synthesis	Total delay of cycle	Chromosome breaks	Sensitivity (reproductive integrity)
G 1	slight	minimal	moderate	minimal
S	moderate	long	slight	maximal
G 2		maximal	maximal	minimal
M	slight	maximal		maximal

(Terasima and Tolmach; Humphrey, Dewey and Cork.)

(a) DNA synthesis may be affected by radiation in different ways, depending on the cell type used, on the milieu in which the cells are kept, on the cell cycle state and on the quality and quantity of radiation.

(b) Cell cycle delay may vary with the state of cell cycle during irradiation, on the time after irradiation when the phenomenon is investigated, and possibly on the cell type studied. The nature of the mechanism of cell cycle delay is not understood. It is likely that several different and perhaps unrelated mechanisms are involved.

(c) Although some correlation between immediate cell cycle delay and chromosome damage can be established, this will vary with the quality and dose rate of radiation used. Neither of these parameters, nor inhibition of the rate of DNA synthesis, can be correlated with loss of reproductive integrity.

This apparent lack of easy correlation between data from different approaches should not be discouraging. On the contrary, these papers make it clear that, for a proper understanding of the

mechanism of radiation effects on cells, a concerted effort of these various fields of study is essential. It is also clear that the time has come when all the measurable parameters should be investigated in the same experiment with the same cells, in the same milieu and cycle state, with the same quality of radiation, in one laboratory. There may not be many teams of workers who could perform such manysided experiments, but it would be a most profitable effort if a number of groups of workers could agree to form a temporary team for the purpose to work together in one laboratory for the required few months. I would venture to predict a great future indeed for such temporary working parties.

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DISCUSSION

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The discussion was opened by Nygaard[i]), who emphasized that the apparent discrepancy between his results on the insensitivity of polymerase in slime mould and its sensitivity in the leukaemic cells used by Bianchi *et al.*[b]) might be due to the difference in controlling factors in these different cell types, especially in physical factors not yet adequately understood.

Alexander[a]) thought that in the murine lymphoma cell system, metabolic factors might be particularly important in governing the post-irradiation behaviour of cells. The number of divisions through which cells might pass before death was reduced in starved cells. Dewey[f]) pointed out that in mouse L cells there was a good correlation between chromosome damage and decreased cell survival

in agreement with the results of Terasima and Tolmach[m]). In hamster cells dose rate was important, 100 rad per min in G 1 allowing greater restitution of breaks than 500 rad per min. Conditions at the time of irradiation were very critical.

Savage (*MRC, Harwell, Berks., Great Britain*) asked Dewey for further details about the type and amount of chromosome damage induced in S and G 1 cells. In reply, Dewey said that, when a high dose rate (518 rad per min) was used, cells irradiated in S showed 1.05 exchanges per cell, 95% of them of the chromatid type. G 1 cells showed the same exchange rate but only 48% were chromatid. With fractionation of the dose into two equal parts separated by 5 min to 1 hr, the exchange rate was unaltered in the S cells, but in G 1 cells it dropped to 0.56 per cell and only 35% were chromatid. In the S cells the breaks remained open for at least an hour, whereas in cells irradiated before synthesis starts they remained open for less than 5 min.

In answer to a question from Muldal, (*Paterson Lab., Christie Hospital, Manchester, Great Britain*) Curtis[c]) explained that he had scored only gross chromosome damage in liver cells, recording bridges and fragments.

Barendsen (*Radiobiol. Inst., TNO, Rijswijk, Netherlands*), Burch (*Dept. Med. Physics, Univ. of Leeds, Great Britain*) and Rajewsky (*Max Planck Inst., Frankfurt, Germany*) had questions about the papers of Smith[1]) and Dendy[e]). In amplification, Smith said that about 50 α particles were delivered per μ^2. The distance between the microbeam and the cell membrane was 8 to 12 microns. Irradiation of the nucleolus and the nuclear sap were about equally effective in delaying DNA synthesis; irradiation of the cell sap was only about half as effective. These effects on DNA synthesis took time to develop. Earlier work had shown that irradiation of the whole nucleus produced an immediate effect. Irradiation of the nutrient medium produced an effect on DNA synthesis at 2 hr but this was no longer apparent 6 hr after exposure. No investigations had been made of the effects of temperature on the system. Goldfeder drew attention to the fact that the irradiated medium affects the response of cells in culture. Cells transferred to a fresh medium immediately after exposure were less affected than if allowed to remain in medium which had been exposed. The importance of cytoplasmic irradiation on DNA synthesis was also stressed by Kuzin. Philpot mentioned that

he and Horgan[1]) had suggested that the production of single free radicals at a membrane surface might produce localized chain autoxidation which would be physically equivalent to puncturing a hole in the membrane, thus leading to a metabolic disturbance in the adjacent region. With electron microscopy of cells fixed a few minutes after X irradiation, Goldfeder had seen rupture of the plasma membrane.

Neary (*MRC, Harwell, Berks., Great Britain*) mentioned his results with bean root meristems[2]) which were in marked contrast to those of Painter and of Terasima and Tolmach for mammalian cells. In the asynchronous bean root cells, delay in reaching the next mitosis is greater in cells exposed in G 1, and progressively less as interphase advances. Cells exposed in G 2 are not much delayed.

Again in the bean root meristem, Hornsey (*MRC, Hammersmith Hospital, London W12, Great Britain*) quoted her own results[3]) which differed from those of Dawson and Field. Hornsey had found that in cells dividing 3 or 10 days after X irradiation the cell cycle was not slower than in normal cells. She emphasized that the changes in population size could be accounted for by cell death rather than by any lengthening of the division cycle, and pointed out that if dying cells were included in an analysis carried out by a labelling technique, the apparent cell cycle would be artificially lengthened. Dawson replied that in his material some of the cells were going to die and that these non-viable cells would affect the estimated values for the average lengths of the phases of the cycle.

Barendsen (*Radiobiol. Inst., Rijswijk, The Netherlands*), with reference to the paper of Alexander and Mikulski, questioned whether the different effects of α and X radiation should be considered to be fundamental. Such effects as failure to complete one division after irradiation, compared with failure only at the second division, may be produced with different efficiencies by different radiations with a continuous change of these efficiencies with LET. In the same context, Neary said that in the bean root meristem, and by using Hall's kinetic model[4]), he had found that after both X, gamma and fast neutron irradiation, non-surviving cells can make several divisions, the number being inversely proportional to dose. For doses giving equal root growth inhibition there was no difference in the mean number of divisions after gamma rays compared with fast neutrons.

In answer to a question from Hewitt (*Westminster Hospital, London, SW1, Great Britain*), Tolmach said that in his synchronized HeLa cell cultures, 85 to 90% of cells are simultaneously in mitosis soon after the synchronizing procedure. There is a loss of synchrony at later times, so that when DNA synthesis begins 7 or 8 hr after mitosis, cells enter S over a 6 hr period, and at the next mitosis at 18 hr, the population divides over an 8 to 10 hr period.

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The Linking of Disciplines in Radiation Research

EFFECTS ON ORGANIZED SYSTEMS IN THE LIGHT OF CELLULAR RADIOBIOLOGY

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Department of Materia Medica and Therapeutics, University of Aberdeen, Great Britain; J.A.R.M.: Department of Medical Physics, University of Aberdeen, Great Britain

c) LUCILLE A. DU SAULT,
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Henry Ford Hospital, Detroit 2, Michigan, USA

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f) A. D. GLINOS and H. H. NORTH,
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E.J.H. and L.G.L: Physics and Radiobiology Laboratories, Radiotherapy Department, Churchill Hospital, Oxford, Great Britain. F.A.L.C: Department of Botany, University of Oxford, Great Britain

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Radiobiological Research Unit, Cancer Institute Board, Melbourne, Australia

1. INTRODUCTION

A concise statement of the theme of this session appears in one of the papers which it is my very great privilege to report on this morning. Corp and Mole[a]) wrote: "It is one of the aims of radiobiology to interpret macroscopic phenomena in cellular terms".

It is my impression that all our authors concede that certain basic principles of cellular radiobiology, established in very simple

systems, are likely to retain their validity for cells incorporated in an organized system.

I suggest that these generally acceptable principles are summarized in the typical features of a tissue cell survival curve established with an assay technique for counting reproductively intact surviving cells. According to Morkovin and Feldman[1]), the parameters which fit the widest range of data, at least for well-oxygenated mouse and human cells, are a D 37 (or D_0) value of 140 rads and an extrapolation number of about 2.0 (Fig. 1). The initial shoulder

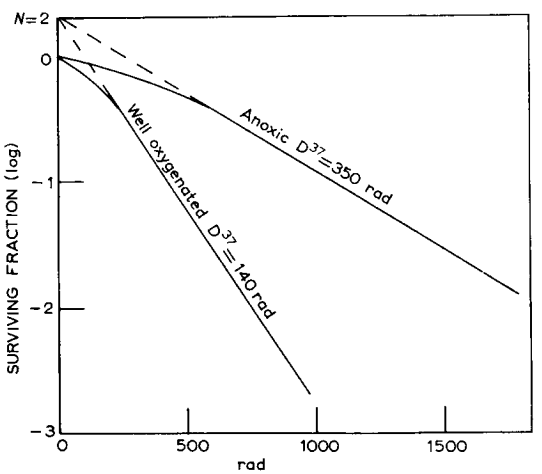

Fig. 1. Typical survival curves for well-oxygenated and anoxic mammalian tissue cells.

of the curve is understood, from Elkind's brilliant studies, to indicate the capacity of cells to accumulate sublethal damage, from which they can completely recover within 12 hr. The sparing effect of fractionation is completely accounted for by the Elkind form of recovery provided that a contribution from cellular proliferation can be excluded. For X rays and γ rays, a dose enhancement factor of about 2.5 is required for tissue cells irradiated under anoxic conditions. Moreover, from the oxygen effect curve determined by Deschner and Gray[2]), it is apparent that this relative radioresistance becomes significant only at levels of hypoxia which are distinctly low by physiological standards.

Two further principles of wide validity are these: firstly, that the

reproductive integrity of a tissue cell is its most radiosensitive function; secondly, that damage to this function is not usually manifested by dissolution of the cell until it has undergone one or more post-irradiation divisions. Indeed, Glinos and North[f]) have demonstrated this last principle by tissue culture experiments with L strain cells. I shall not describe their experiments, but their work sanctions the inference that the greater macroscopic damage seen in rapidly proliferating tissues does not imply a greater intrinsic radiosensitivity of the cells of such tissues.

To satisfy authors who seek to orientate the inevitable bias of their rapporteur I must describe the point of view from which I have studied their papers. In interpreting phenomena due to the operation of multiple factors, many of which cannot be adequately controlled, one must start with some assumptions. For my part I have assumed that all the basic principles I reviewed, established in simple systems, are fully valid for cells irradiated within an organized tissue; I have assumed also that the only extrinsic physiological variable affecting the radiosensitivity of cells is the oxygen tension in their environment. With these assumptions, I am inclined to suspect that the deduction of atypical cell survival curves from experiments with complex systems betrays the complicity of incidental physiological influences affecting the statement of the "surviving fraction".

2. RADIOSENSITIVITY OF MOUSE CELLS

Conflicting evidence about the radiosensitivity of a given cell strain, as signified by the D 37 value, can be obtained even with simple systems. Hornsey[i]) determined survival curves for Ehrlich ascites cells irradiated *in vitro*, surviving fractions being determined by transplantation assay. The D 37 value was grossly modified when the assay site was changed from new-born mice to the brains of adult mice (Fig. 2). It is important to realize that a difference of intrinsic radiosensitivity between two cell strains might be inferred if one strain were to be examined by one technique and the other strain by the other.

The haematopoietic system of mice presents the radiobiologist with several technical advantages: firstly, it displays radiation damage promptly, on account of the high rate of proliferation of its cells; secondly, the separateness of the constituent cells facilitates

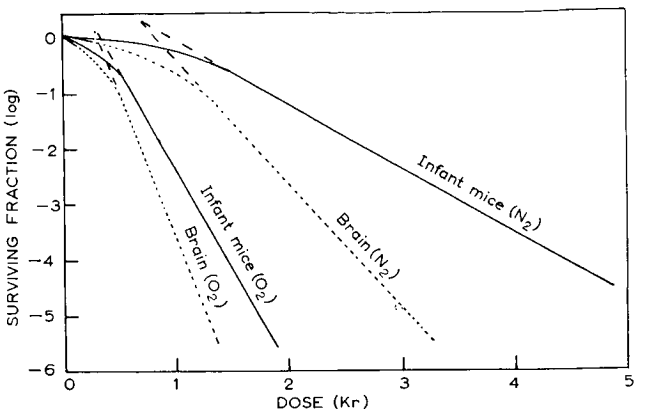

Fig. 2. Modification of radiation survival curves for Ehrlich ascites cells associated with change in the method of assaying surviving cells.
O_2: irradiation of cells in oxygen.
N_2: irradiation of cells in nitrogen. (Hornsey.)

quantitative grafting; thirdly, the LD 50/30 days dose of whole-body irradiation for initially healthy mice can be attributed exclusively to damage sustained by this tissue.

Corp and Mole[a]) used this last feature in dose-splitting experiments which enabled them to give an exceptionally good demonstration of the Elkind recovery phenomenon. The LD 50/30 dose of radiation for a single dose to their F_1 hybrid mice was 800 r; for two equal doses with a 6 hr interval, it was 970 r. With a 6-hr interval, this saving of effect by fractionation is unlikely to be contributed to by cellular proliferation and can reasonably be attributed exclusively to the Elkind type of recovery. When the primary dose given was 300 r, 500 r, or 700 r, the intermediate dose of 500 r enabled the largest total dose to be given for attainment of the LD 50. The illustration from their paper (Fig. 3) adequately describes an explanation of their results. It is assumed that in all cases the LD 50 effect requires reduction of the stem cells to the same surviving fraction. The shoulder representing the initial part of each dose fraction indicates a relatively inefficient contribution to the total dose. With a first fraction of 500 r, the dose-effect curve would include the whole of both shoulders. With 300 r, part of the first shoulder is lost; and with 700 r, part of the second shoulder. It is indeed remarkable that so neat a display of fundamental phenomena

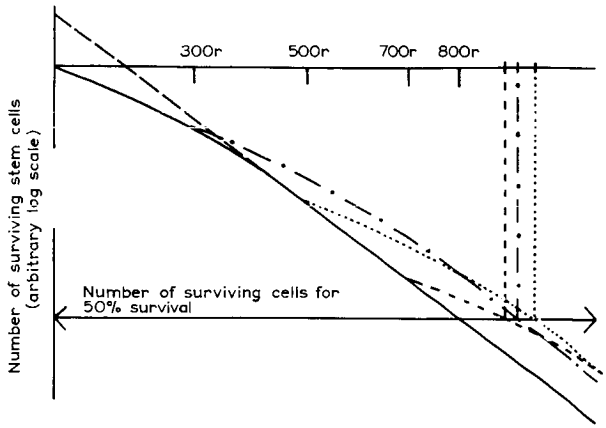

Fig. 3. Effect of size of first dose on the LD 5 0/30 split dose of whole-body irradiation for F_1 hybrid mice (theoretical interpretation of results). (Corp and Mole.)

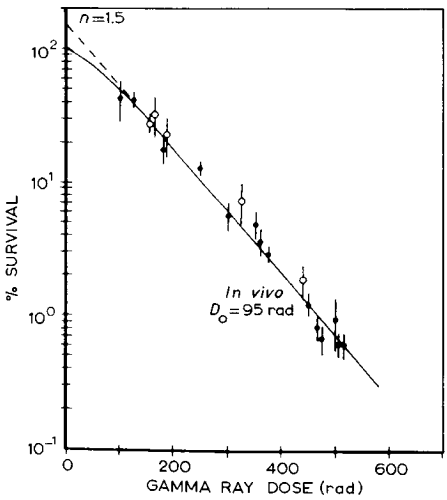

Fig. 4. Survival curve for mouse marrow cells irradiated *in vivo*. (Till and McCulloch.)

can be got from a system using the grossest radiobiological endpoint of all — death of the animal.

The outstandingly ingenious technique of Till and McCollucht[n]), which defies useful description in the time available to me, has already given us the first survival curve for mouse marrow cells irradiated *in vivo* (Fig. 4). These authors have now used their technique to demonstrate occurrence of the Elkind phenomenon in their system: a second shoulder appears on their curve when irradiation

is discontinued after 200 r and resumed after 3–4 hr (Fig. 5). The saving of effect achieved by dose splitting, with a primary dose of 200 r, varied with the time interval between fractions, being

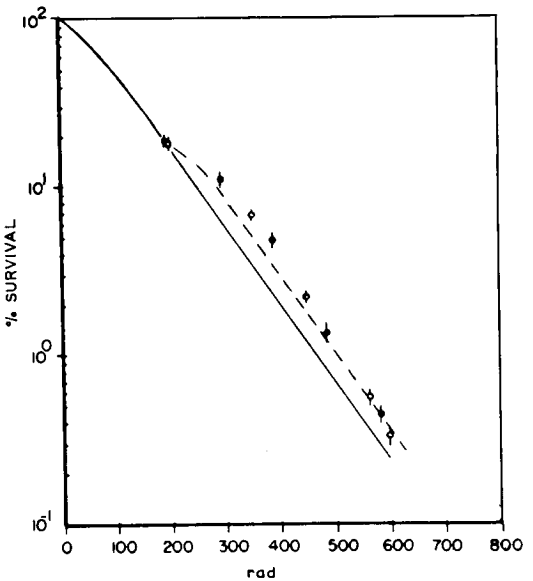

Fig. 5. Survival curve for mouse marrow cells irradiated *in vivo*. Effect of dose-splitting. (Till and McCulloch.)

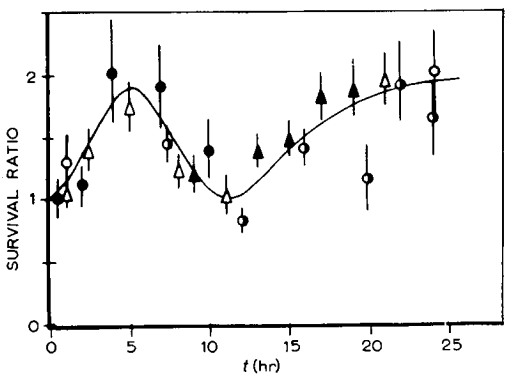

Fig. 6. Irradiation of mouse marrow cells *in vivo*. Effect of time between fractions on relative saving of effect achieved by fractionation. (Till and McCulloch.)

maximal at 5 hr and minimal at 11 hr (Fig. 6). The interpretation of their minimal value at 11 hr appears to invite discussion.

The paper by Gurney[8]) also describes an ingenious technique

for measuring radiation-induced depression of mouse marrow function. Erythropoiesis ceases in hypertransfused mice, but the stem cells retain their integrity and will respond to erythropoietin. This response can be measured by determining radio-iron incorporation in newly-formed red cells. Irradiation preceding the erythropoietin stimulus reduces the response exponentially with dose (Fig. 7).

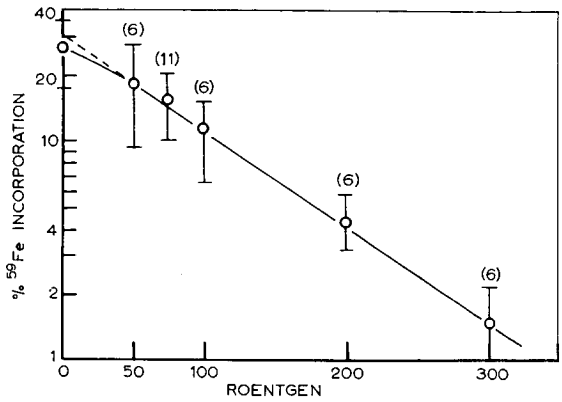

Fig. 7. Effect of radiation on response of marrow stem cells to erythropoetin in hypertransfused mice. (Gurney.)

To discuss Gurney's results in more detail would involve a consideration of complex haematological problems. However, it is important to our present theme to observe that modification of the assay system changed the character of the dose-response curve. A study of Gurney's paper teaches an important lesson in our present context: this is, that full interpretation of radiation response in an organized system may be held up by deficiencies in our knowledge of the relevant physiology. For example, Gurney refers to the possible pluripotentiality of the stem cells, and discusses the complication of his data which could result from a deviation of stem cell destinies from erythropoiesis to leucopoiesis.

3. BEAN ROOT CELLS

As our understanding of the radiobiology of organized systems improves, we shall use radiation increasingly to elucidate strictly biological problems, especially in respect of the cell kinetics of regeneration. Hall, Lajtha and Oliver[3]) published previously a dose-re-

sponse curve for the cells of *Vicia faba* meristems. In their present paper Hall, Lajtha and Clowes[h]) have used this information together with the results of further experiments to show that the quiescent centres of root tips need play no special role in root regeneration, as had been suggested by others. Their failure to find evidence to support a contention that the cells of the quiescent centre are atypically radioresistant saves us from a radiobiological dilemma: we need not entertain the disturbing notion that bean roots have built-in mechanisms for repairing damage done by radiobiologists.

4. RAT THYROID

It is apparent that we are relatively deficient in quantitative techniques for studying radiation damage to epithelial organs with a negligible rate of cell division. In such tissues, radiation damage can be revealed only by applying a stimulus to proliferation. The contribution of Crooks, Greig, Macgregor and McIntosh[b]) to this session is, therefore, of exceptional value. They studied the response to radiation of the adult rat thyroid. When rats receive continuous administration of the goitrogen methylthiouracil, hormonal control of the thyroid is disturbed and the gland undergoes a 4- to 5-fold enlargement during the first 4 weeks of treatment. The authors have evidence that enlargement during the first two weeks is largely due to hypertrophy or enlargement of the individual cells, whereas that during the 3rd and 4th weeks is largely due to hyperplasia or cellular proliferation. Irradiation of the thyroid before administration of goitrogen barely affected the early hypertrophic phase of enlargement. Hyperplastic enlargement during the 3rd and 4th weeks was, however, depressed. The D 37 value of their dose-effect curve (Fig. 8) was about 1000 rad, but I find that this is reduced to a more familiar level if their data is restated in a way that eliminates the contribution due to hypertrophy. Their results were not affected by increasing the interval between irradiation and the start of goitrogen treatment from 1 day to 2 weeks, showing that the radiation damage was stored until the stimulus to cellular proliferation was applied. It is this phenomenon which Glinos and North[f]) demonstrated so admirably with their simple tissue culture system. I should add that this thyroid system provides the important facility of enabling a distinction to be made between the effect of radiation

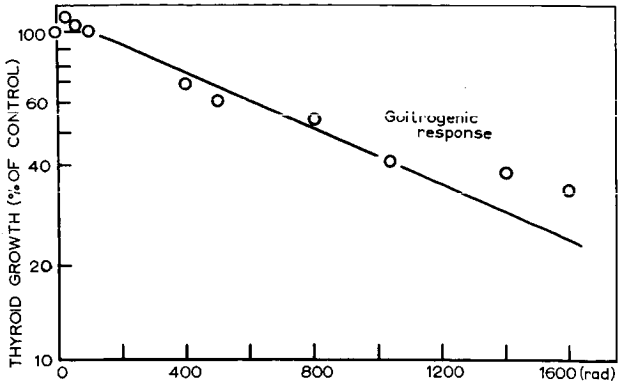

Fig. 8. Effect of preliminary irradiation on the hyperplastic response of rat thyroid glands to methylthiouracil. (Crooks *et al.*)

on the reproductive capacity of the cells and the effect on the cells' other functional capacities.

5. TUMOURS

More than half of the fifteen papers allocated to this session relate, directly or indirectly, to the effect of radiation on tumours.

In an animal tumour-host system, the cancerologist and radiotherapist seek a model of the human disease by means of which the empiricism of the clinical situation may be elucidated. The pure radiobiologist perceives the autonomous tumour as a discrete population of cells of estimable size; eradication of the growth potential of this population, or "cure" of the tumour, presents itself as a deceptively precise radiobiological end-point. The ideal analysis is conceived in which the number of reproductively intact cells in the tumour is estimated with fair accuracy, a relevant cell survival curve can be consulted, and the dose of radiation which must be administered for a reasonable chance of cure can be simply calculated. Naturally, embarrassments are encountered by those who make this immaculate approach. We are reminded that a tumour is a disorganized system, that the host differs from a tissue culture vessel in being itself radiosensitive and in being improvident, and sometimes aggressive, towards the tumour cells it harbours. Stromal inadequacies and architectural peculiarities result in foci of anoxic cells; transplanted tumours commonly evoke cyto-

lethal influences from the host; with fractionation, earlier fractions may induce a change in the extent of anoxic foci or in the level of host immunity, so that later fractions act on cells in a transformed environment.

The experiments of Suit[1]) provide the best circumstances for checking experimental radiotheraphy results against survival curve data. He removed uncertainties associated with immunity, the oxygen effect and fractionation by finding the single dose required for control of 50% of totally anoxic spontaneous mouse mammary tumours. This 50% tumour control dose was calculated as 5940 rad. Fig. 9 shows the excellent correlation of Suit's results with the

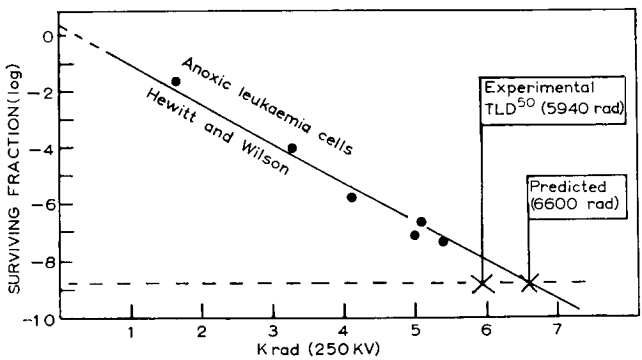

Fig. 9. 50 per cent control dose of radiation for anoxic spontaneous mouse mammary tumours (Suit) seen in relation to the prediction of survival curve for anoxic mouse leukaemia cells irradiated *in vivo* (Hewitt and Wilson[4]). The experimental points represent data additional to that published previously.

prediction from the survival curve for anoxic leukaemia cells *in vivo* by Wilson and myself[4]), assuming that one or more surviving cells are sufficient to cause failure of tumour control. The interrupted horizontal line, indicating the surviving fraction required, is based on Suit's estimate of the number of cells in his tumours. The solid points are our experimental points for the leukaemia cells. The departure of Suit's 50% tumour control dose from our predicted dose could result from a 10% error in the alignment of our dosimetry, and it is to be noted that Suit's dose is described as the minimum tumour dose $\pm$ 5%. The significance of persistent quiescent nodules of histologically normal-looking tumour tissue in tumours which received 6600 rad up to 90 days previously, and

which were probably controlled, is a suitable topic for discussion.

Kallman and Tapley[j]) found that 4230 rad of 140 KV X rays in a single dose gave 100-day control of 50% of unclamped spontaneous mammary tumours irradiated in mice breathing air. Calculations similar to those made by Suit show that this value, too, conforms to the implications of a typical cell survival curve, if it is assumed that the tumour contains a proportion of anoxic cells. These authors also showed that, following a conditioning dose of 1120 rad, the subsequent dose required for 50% "cures" varied with the time interval between fractions (Fig. 10). They have ex-

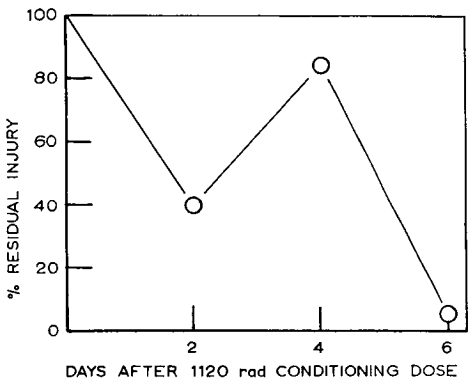

Fig. 10. Per cent residual injury in spontaneous mouse mammary tumours at intervals after a conditioning dose of 1120 rad. (Kallmann and Tapley.)

pressed their results in terms of "residual injury" following the first dose. It is seen that residual injury is less after 2 days than after 4. Sequential changes in the oxygen status of the cells surviving the first dose might explain their results. The authors suggest that the conditioning dose may cause the surviving cells to become temporarily blocked, after 2 days, at a hyperresistant phase of their division cycles.

6. TUMOUR RESPONSE, FRACTIONATION, AND OXYGEN EFFECT

The papers by Du Sault[c]), by Van den Brenk and his collaborators[o]), and by Thomlinson[m]) are all concerned with the oxygen effect and with fractionation as they affect the response of tumours.

Du Sault, irradiating spontaneous mammary tumours with 6000 r in 6 fractions, found that the number of tumours "cured"

rose from 13% for tumours irradiated in air to 38% for tumours irradiated in 3 atm oxygen. One atm oxygen gave no advantage over air (Fig. 11). It is probable that the author's "cure" rates would have been lower had her mean follow-up period been longer than 57 days. There is no doubt that the validity of tumour control or "cure", as a radiobiological end-point, increases with the length of the follow-up period.

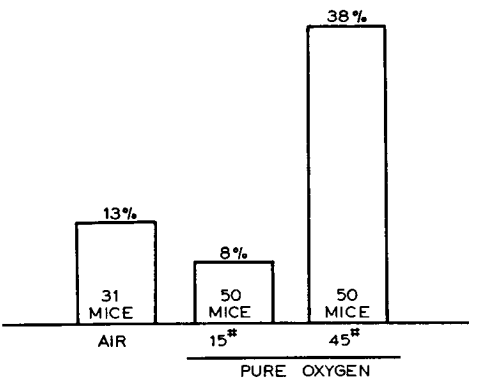

Fig. 11. Effect of oxygen tension in the respired atmosphere on the cure rate of spontaneous mouse mammary tumours. (Du Sault.)

Thomlinson[m]) measures the effect of radiation on his transplanted rat sarcomas, not in terms of cure rate, but in terms of displacement of the growth curves of his tumours. He finds that single doses of radiation become progressively more effective over the range of conditions presented by anoxia, irradiation in air, and irradiation in 3 atm oxygen. He also made observations which may enable the proportion of anoxic cells in the tumours to be roughly assessed: the advantage conferred by irradiation in high pressure oxygen instead of in air was found to be altered in tumours which had received a conditioning dose of 2000 rad in air 1 or 5 days previously. However, the complexity of the situation is apparent from Thomlinson's conclusion that the therapeutically optimum time interval between fractions may be influenced by the growth rate of a tumour and by the species.

Van den Brenk and his collaborators[o]) compared the effects of a great variety of oxygen conditions and fractionation schedules for the irradiation of Ehrlich tumours in mice. For a given total dose of radiation, the effectiveness was increased over the range from anoxia to high pressure oxygen; under all conditions of oxygena-

tion, for a given total dose, cure rates were less for a fractionated than for a single dose. Measurements of skin reaction were made coincidently with the determination of cure rates, and these authors were able to distinguish schedules with a favourable therapeutic ratio. They reduced immunological influences in their system by preliminary exposure of their mice to total body irradiation. They did, however, find that a substantial degree of immunity had returned within the period of their experiments. This may be reflected in the fact that the 50% curative single dose for totally anoxic tumours (4620 rad) was significantly less than that obtained by Suit for spontaneous tumours (about 6000 rad). It is of great interest that skin reaction was reduced by anoxia and increased by high pressure oxygen, indicating that the cells of this tissue in air are not fully oxygenated, at least in anaesthetized mice.

7. SKIN AND SKIN TUMOURS

I come finally to three papers which have this in common: the authors present quantitative data for radiation effects in very complex situations, from which they conceive inferences having far reaching implications at a fundamental level. It is desirable to scrutinize these inferences.

Fowler and his collaborators[d]) have determined the effectiveness of fast neutrons, relative to 8 MeV X rays, using the skin of the pig as the target tissue. For single doses of radiation the relative biological effectiveness (RBE) was 2.5, but for 6 fractions in 18 days it was 3.3. This difference was not predictable from previous work of two of the authors (Hornsey and Silini[5]), using mouse ascites cells irradiated *in vitro*. They found that neutrons and X rays gave survival curves of similar shapes and that the RBE of neutrons relative to X rays was 2.1 for irradiation of the cells in oxygen and 3.6 for irradiation in nitrogen. The present authors seek to explain the apparent discrepancy at a rather fundamental level, and this, I believe, requires caution. We are more ignorant of the physiology and pathology of the skin than of almost any organ in the body. The reactivity of the skin is influenced by hormonal, neural and emotional factors, and many skin lesions resembling radiodermatitis occur which are of unknown aetiology. I should have thought that the profusion of uncontrolled factors in this system makes it unreliable for the elucidation of fundamental issues. I

should like the authors to comment on my suggestion that the rise of RBE associated with fractionation could result from induction of anoxic conditions by the earlier fractions. My remarks should not detract from the immense practical importance of these observations for the clinical application of neutron irradiation.

Fowler and Stern[e]) have set down iso-effect curves for 14 sets of published data for the irradiation of human tumours or skin, showing the rise in total dose required with increasing numbers of fractions. Reasoning that the sparing effect of fractionation is largely an expression of the Elkind phenomenon, they have deduced that the relevant survival curve has an extrapolation number greater than 10 or is convex upwards.

Munro[k]) finds that the curative dose level for human skin tumours, as observed from general radiotherapy experience, is significantly lower than we should expect from the predictions of mammalian cell survival curves relevant to populations of cells containing moderate proportions of anoxic cells. He suggests that the discrepancy may disclose a regular and sizeable contribution to cure from host immunity factors active against the autologous tumours.

As a cancer research worker I feel bound to comment broadly on these approaches, which take us into uncharted seas. In attempting to bridge the gap between radiotherapy experience and cellular radiobiology we need to realize the limited precision of much of the clinical data: tumour dose is subject to quite wide ranges between maximum and minimum values; use of prolonged fractionation almost certainly involves some selection of patients with large or ill-defined tumours requiring large fields; failure of therapy may involve recurrence at the boundary of the treated area; and such is the slow growth rate of many clinical tumours that clinical cure is far from being synonymous with radiobiological eradication. Immunity to autologous tumours is a most debatable issue, even amongst tissue immunologists; the postulation of host immunity has always been a notorious device for conveniently accommodating theory to practice in all fields of cancer research. For us, I feel that it should be the very last resort.

The splendid experimental work with complex systems, which I have reported here, has tended to confirm me in the bias to which I confessed earlier. In our fundamental concepts of cellular radio-

biology, as established in simple systems, I believe that we have forged a sharp and sturdy instrument for measuring some of the many ill-defined physiological influences which distort the shape and parameters of our typical survival curve.

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DISCUSSION

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Fowler[d]) explained that it was the close similarity between the relative biological effectiveness (RBE) for fast neutrons relative to X radiation which they obtained for pig skin and that obtained by Hornsey *et al.*[1]) using ascites tumour cells (when due allowance was made for the different qualities of X radiation used in these two investigations) that led them to try to explain these results in terms of each other. Their dose fractionation experiments for skin were not undertaken as a fundamental investigation, but in connection with the practical study of neutron therapy. However, it was of interest to seek an explanation in terms of the results of radiobiology, rather than leave them as empirical findings. They had used the second phase of reaction at 40 to 100 days as this appeared to be less variable than the early wave of erythema, and likely to be related to structural breakdown of the skin – that is to cell depopulation. Little was known about the variation of oxygenation of the skin during a series of irradiations, and the effect might be opposite to that suggested by the rapporteur. Therefore, at present, after considering the possible effect of oxygenation, they preferred to explain the loss of effectiveness of dose due to fractionation in terms of the smaller doses falling on the less efficient shoulder of the dose response curve (as discussed in the rapporteur's report), the shape of this curve varying with the radiation used.

Hornsey[1]) stated that recent work by Vatistas (*Athens, Greece*) and herself had given similar results to those of Corp and Mole[a]) reported here. They used the death of the animal at 4 days, which effect was related to damage to the dividing cells of the intestinal villi. The LD 50 for a single exposure was 1300 rad compared with a total dose of 1760 rad for two exposures separated by 6 hr. This increase in the total dose was approximately the same for first fraction doses between 600 and 1000 rad, but rather smaller when a larger first dose was used. This result again suggests that doses of less than 600 rad do not include the whole of the shoulder of the cell survival curve.

Suit[1]) emphasised that the D 37 dose quoted by the rapporteur was not measured, but only estimated from the tumour cure dose. This calculation was complicated by uncertainty in the number of viable cells in the tumour, in the extrapolation number for their dose response curve, and in the relationship between the tumour cure dose at any one time (e.g. 120 days) and the dose giving a survival of less than one cell. Small nodules of about 1 mm diameter had persisted up to 225 days, and on examination at 47 to 99 days after a dose of 6600 rad these had appeared to contain foci of histologically intact tumour cells. The D 37 dose given at present must, therefore, be regarded as a minimum value which may have to be increased as the animals are observed for longer periods of time.

Glinos[f]) explained that in their experiments the dose response was studied for mouse L cells originating in both the lag and stationary phases of growth in two ways – by observing the post-irradiation population growth in replicate cultures, and by evaluating the ability of single cells to form clones. With the former technique, as observation was made at increasing times after irradiation, the dose for 37% survival decreased linearly up to the fifth post-irradiation division. After this, the D 37 remained constant at about 250 rad, equal to that obtained with the clonal technique. These results applied for both cell types, and are in agreement with the inverse relationship between dose and the number of divisions made by non-surviving cells reported by Elkind at another session of the Congress.

As Radotic (*Inst. Boris Kidrich, Belgrade, Yugoslavia*) had found elevated erythropoeitin levels in dogs after irradiation, he was con-

cerned whether the doses given in Gurney's experiments might have affected the erythropoeitin turnover. However, Gurney[g]) explained that the hyper-transfused animal is presumed to be able to deliver oxygen in excess of any demand, and that as a result, the mechanism of endogenous erythropoeisis is considered to be eliminated. In these circumstances the response to a standard dose of erythropoeitin is taken to provide a direct estimation of the integrity of the stem cell compartment.

A number of discussants were interested in the time variation of recovery (first demonstrated by Elkind *et al.*[2]) as shown by changes in the effectiveness of two split doses as the interval is varied. Cater (*Dept. Radiother. Univ. Cambridge, Great Britain*) referred to diurnal variation in many systems of cell proliferation of the mouse or rat, and felt that the possible effect of partial synchrony of cell division in the marrow should be excluded from the experiments such as those of Till[n]) reported by the rapporteur, where a marked minimum in the recovery cure was found with an 11 hour interval. In reply Till agreed that some synchronization within the cell cycle could exist and this could possibly be investigated. However, he pointed out that there was no cell multiplication between the two doses in the conditions of his experiments. Further, Berry (*Churchill Hospital, Oxford, Great Britain*) reported that in experiments with mouse transplantable leukaemia, he had obtained similar discontinuous recovery curves of this type for irradiations carried out both during the day and the night, providing no suggestion of an diurnal variation.

Alper (*MRC, Hammersmith Hospital, London, Great Britain*) pointed out that in the tissue culture experiments recovery was minimal at about the end of the lag period and according to Elkind the delay in division which was produced was proportional to dose, corresponding to about one tenth of a generation time per 100 rad. In Kallman's experiments[j]) a dose of 1100 rad was used so the delay might be about equal to the generation time. Kallman agreed with this suggestion, which fitted their data as their peak resistance (minimum recovery) occurred at about 2 days, with a generation time for their spontaneous mouse tumour cells of between 1 and 4 days. He believed that the same type of reasoning could explain Till's results referred to above.

Read (*Wakari Hosp., Dunedin, New Zealand*) drew attention to

the fact that the shapes of the survival curves for mammalian cells irradiated in tissue culture are very similar to those which describe the induction of dominant lethals in Drosophila or wasp eggs or in mouse sperm. A theory for this induction was proposed by Catcheside and Lea[3]) some 20 years ago, and elaborated later by Haldane and Lea. They supposed that isochromatid breaks with rejoining of sister chromatid ends were produced in proportion to the dose and were lethal, this effect determining the initial shape of the curve. All other chromosome breaks were assumed to rejoin – eucentric arrangements being viable whilst di- and meta-centric arrangements proved lethal. This second effect then determined the ultimate shape of the curve. It seemed that this theory should be considered as a possible explanation for the shape of the mammalian cell survival curves also.

Elkind (*Nat. Cancer Inst., Bethesda, USA*), asked to reply on this subject, said that he was familiar with this work and would like to think that it could offer a general explanation of the type of survival curve obtained in tissue culture. But he pointed out that there appeared to be no clear relationship between chromosomal damage and survival. The experiments of Till[4]) for instance had shown essentially the same survival curve for lines of mouse L-cells with average chromosome numbers varying by a factor of two. Read agreed that the implications of this theory for various chromosome numbers should be considered further but it must be remembered that many of the cells concerned are aneuploid.

This relationship between chromosome damage and cell survival data is obviously of great importance in radiobiology and should always be born in mind by workers in both fields.

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CHEMICAL PROTECTION IN CHEMICAL SYSTEMS

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Research Chemistry Branch, Atomic Energy of Canada Ltd., Chalk River, Canada

1. INTRODUCTION

From a strictly chemical view point an added chemical substance can only be said to protect if it reduces the total amount of chemical change that occurs as the result of irradiation; i.e. transfers energy from a radiosensitive to a radioresistant molecule. In its relation to radiobiology, however, the term "chemical protection" is applied if an added substance reduces the extent of the radiation induced alteration of a particular material under investigation, and in model systems of the type to be discussed in this session protection is therefore synonymous with the behaviour of mixtures.

Protection of living systems against ionizing radiations was first demonstrated with mice by Patt and by Bacq and subsequently the phenomenon has been demonstrated at all levels of biological organisation. The biological data is consistent with the hypothesis that chemical protection occurs at the stage of the primary chemical lesion; that is, the protector increases the dose needed to attain the degree of radiochemical damage necessary to initiate the long chain of events which lead to the observed biological end effects. In wet systems such as vegetative cells, the primary chemical lesion is established within a fraction of a second of the uptake of energy. In relatively dry systems (e.g. seeds, and sperm heads of fish) steric factors prevent the rapid interaction of free radicals and the chemical reaction which constitutes the primary lesion may not be established for some time and this makes relatively dry systems convenient for studying mechanisms of protection. The group of papers presented in this session are concerned with the large variety of mechanisms by which protection can occur in model systems. For simplicity, these mechanisms may be divided into two broad categories (i) prevention of chemical reaction from occurring in the target molecule; this can be achieved by competitive removal of radicals formed in water before they have had an opportunity to react, or by energy transfer. (ii) Reaction of the protector with the target molecule after this has been converted into a radical, but before it has undergone the further chemical changes necessary for irreversible damage: this we call repair. Some of the papers provide us with

a guide for determining which, if any, of these reactions apply when protection occurs in living systems.

2. PROTECTION IN DILUTE AQUEOUS SOLUTIONS

The pioneer investigations of Dale demonstrated that added materials, in particular those containing sulphur, increased the dose of radiation needed to inactivate enzymes in dilute solution and he suggested that this occurred by competitive removal of the free radicals such as OH· which were responsible for the inactivation. In many cases however, Dale[1]) and others[2]) found that the extent of protection was not related to the concentration of the protector in the expected manner and suggested that other reactions might participate. The papers in this session provide ample support for this view.

The paper by Kalkwarf[f]) supplies essential basic information about the relative rates of reaction of amino acids and sugars with radiation produced OH· radicals. The values shown in Table 1

TABLE 1
Relative rate constants for reactions with OH· radicals

Compound	Relative rate constant	Compound	Relative rate constant
Tryptophane	$(6.3 \pm 0.3) \times 10^{-1}$	Methionine	$(5.7 \pm 0.6) \times 10^{-1}$
Cystine	$(5.2 \pm 0.6) \times 10^{-1}$	Tyrosine	$(4.7 \pm 0.4) \times 10^{-1}$
Phenylalanine	$(4.6 \pm 0.4) \times 10^{-1}$	Histidine	$(3.3 \pm 0.4) \times 10^{-1}$
Cysteine	$(2.1 \pm 0.4) \times 10^{-1}$	Leucine	$(1.33 \pm 0.06) \times 10^{-1}$
Valine	$(1.1 \pm 0.1) \times 10^{-1}$	Arginine	$(1.1 \pm 0.8) \times 10^{-1}$
Glutamic acid	$(8.6 \pm 0.2) \times 10^{-2}$	Lysine	$(5.0 \pm 0.7) \times 10^{-2}$
Threonine	$(3.4 \pm 0.5) \times 10^{-2}$	Serine	$(2.5 \pm 0.2) \times 10^{-2}$
Proline	$(2.4 \pm 0.2) \times 10^{-2}$	Alanine	$(4.8 \pm 0.2) \times 10^{-3}$
Aspartic acid	$(3.0 \pm 0.9) \times 10^{-3}$	Glycine	$(8.3 \pm 0.2) \times 10^{-4}$
Glucose	$(1.1 \pm 0.1) \times 10^{-1}$	Fructose	$(1.5 \pm 0.2) \times 10^{-1}$
Galactose	$(1.8 \pm 0.2) \times 10^{-1}$		

were obtained by comparing the rate of bleaching of an aqueous solution of a triphenyl methane dye by OH· radicals produced by Co^{60} γ rays. The rate of reaction of different organic substances with OH· radicals varies very greatly. Amongst the amino acids there is a factor of 1000 and the sugars react as rapidly as the most reactive amino acids. The rate constant for macromolecules is very

TABLE 2

Relative rate constants for reactions with OH· radicals

	Relative rate constants	
	Estimated	Observed
Bovine serum albumin	87	6.0 ± 2.0
Bovine haemoglobin	57	6.2 ± 0.2
Polysaccharide lagarose	1.8×10^{-1}	2.1×10^{-2}

much less than that to be expected from its constituents, see Table 2, and Kalkwarf attributes this to the inaccessibility of many groups due to folding and other steric factors.

Knowing these relative rate constants, it can now be decided whether in a particular system protection occurs by competitive removal of OH· radicals or by some other mechanism. Kalkwarf, see Fig. 1, has shown that the ability of different substances to

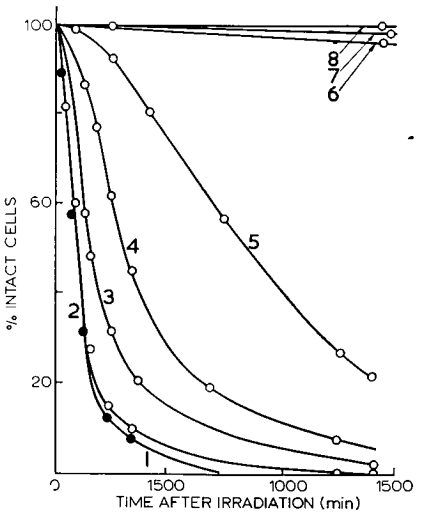


Fig. 1. Haemolysis of red blood cells at 37°C plotted against time in minutes after irradiation in the presence of protective substances. (Kalkwarf.) 1. Irradiated control. 2. Valine ($K_p/K = 0.11$). 3. Histidine ($K_p/K = 0.33$). 4. Tryptophane ($K_p/K = 0.63$). 5. Erioglaucine ($K_p/K = 1.00$). Curves 6, 7 and 8 represent unirradiated controls in which the red cells were treated with histidine, erioglaucine and valine respectively.

protect red blood cells against haemolysis runs quite parallel with their relative rate of reaction, i.e. K_p/K with OH· radicals and the conclusion seems warranted that in this case protection takes place by preferential reaction of the protector with OH· radicals.

On the other hand, for the system studied by Charlesby and Kopp[b]) we can conclude that protection does not occur by removal of OH· radicals. These authors have studied the influence of a large

number of additives at low concentrations of the order of 10^{-4} M on the conversion of solutions (usually 5%) of the water soluble polymers, polyvinyl pyrrolidone and polyethylene oxide into a gel. This reaction is known to be due to crosslinking[3]; presumably the OH· radicals formed in water abstract hydrogen from the polymer molecules, RH, to give polymer radicals

$$RH + OH\cdot \rightarrow R\cdot + H_2O$$

$$R\cdot + R\cdot \rightarrow R{-}R$$

which interact to form crosslinks. The solution sets to a gel when, on average, one crosslink has been formed for every molecule of polymer present. Alexander and Charlesby[3]) observed powerful protection by low concentration of thiourea and cysteamine and concluded that this occurred by a repair mechanism[4]), to which I will refer again later, and not by reaction with OH· radicals. In Table 3, is a selection of part of the data by Charlesby and Kopp which shows the amount of protection offered by those compounds for which Kalkwarf had determined relative reaction rates with OH· radicals. It is immediately apparent that protection in this polymer system is not correlated with reactivity with OH· radicals.

TABLE 3

Comparison of protection of 5% polyvinyl pyrrolidine against crosslinking with relative rate constant for OH· radicals

Protector	Dose reduction factor for crosslinking at 10^{-4} M concentration	Relative rate constant for reaction with OH· radicals
Cystine	2.0	5.2×10^{-1}
Cysteine	1.9	2.1×10^{-1}
Methionine	1.15	5.7×10^{-1}
Histidine	1.15	3.3×10^{-1}
Tyrosine	1.0	4.7×10^{-1}
Tryptophane	1.1	6.3×10^{-1}
Glycine	1.1	8.3×10^{-4}
Valine	1.2	1.1×10^{-1}
Serine	1.1	2.5×10^{-2}
Glucose	1.0	1.1×10^{-1}
Colloidal sulphur	2.9	—

The paper by Hills, Johnson and Restall[e]) also illustrates the complexity of protection against the indirect action of radiation.

These authors made a detailed study of the effect of high doses (2.5×10^6 and 10^7 rad) of γ rays on aqueous solutions of glucose (either 5% or 10%) their object being to prepare sterile solutions for medical use. They followed the alteration of glucose by measuring its optical activity in a polarimeter and by appearance of acidity. The apparent G value (number of molecules changed for every 100 eV absorbed) for destruction of glucose based on loss of optical activity was 3.6 and that for acid production (i.e. appearance of hydrogen ions) was 1.3. Some added substances protect very effectively, but the surprising feature is that the relative order of effectiveness of the various compounds is quite different whether acid production or loss of optical activity were chosen as the criterion for damage, see Table 4. The authors put forward no chemical mechanisms for their results. It seems to me that removal of OH· radicals by the agent shown in the table is unlikely to be responsible for protection since (i) protective capacity does not parallel rate of reaction with OH· and (ii) glucose has a high rate of reaction with OH· so that additives at much lower concentration (e.g. 1/50th) are most unlikely to compete effectively for OH· radicals. The protection found with relatively high concentrations of sodium iodide

TABLE 4

Protection of 5% glucose by additive

Protective agent	Dose reduction factor based on	
	Change in optical rotation	Acidity formation
Diethyl dithio carbamate	1.0	14
Sodium citrate	1.1	enhanced
Sodium dihydrogen phosphate	1.4	enhanced
Urea	1.6	1.2
Ascorbic acid	2.1	5.2
Sodium lactate	2.1	2.1
Thiourea	∞	25
Cysteamine	∞	—

and sodium chloride can probably be ascribed to reaction of these anions with OH· radicals.

Flemming[c]) reports a reaction in which the addition of cysteine potentiates. He finds on irradiation of dilute (5×10^{-3} M) solutions of the amino acids histidine, phenylalanine, tyrosine and trypto-

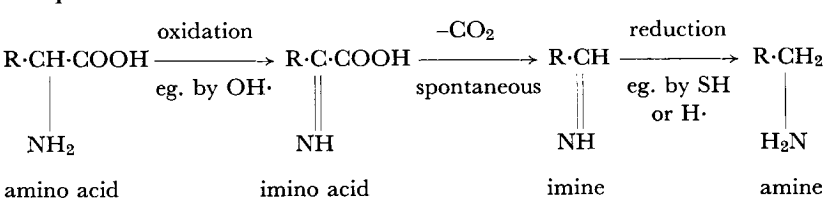
phane with 6×10^5 r of X rays that very small quantities of the corresponding amines (i.e. histamine, etc.) are formed. Table 5

TABLE 5

Formation of histamine by irradiation of 0.05% histidine with 6×10^5r of X rays

Irradiation condition	μg/ml histamine	Apparent *G* (histamine)
Solution saturated with O_2	0.6	0.01
Solution saturated with air	1.4	0.02
Solution saturated with nitrogen	2.5	0.04
Solution saturated with air + 3×10^{-3} M cysteine	7.8	0.13
Solution saturated with nitrogen + 3×10^{-3} M cysteine	28.0	0.45

shows the amount of histamine that is formed and I have calculated an apparent *G* value, but it must be emphasised that no information is available about the dose or concentration dependence of this reaction which represents only a very small fraction of the total amount of the amino acid changed by radiation. This decarboxylation reaction is greatly enhanced by a combination of anoxia with added SH compounds, and Flemming postulates that the conversion of the amino acid to the amine proceeds via the imino acid. The first oxidation step may be brought about by OH· radicals while the second step — the reduction — is brought about by SH compounds.



The paper by Nakken[1]) on the effect of X rays on several members of the B-vitamins in dilute solution has important implications both for radiation chemistry of organic compounds and for protection. Unfortunately I can deal only with the latter aspect in the time available. The degradation of all the vitamins studied was shown to be due to OH· radicals. From the protection offered by

these vitamins to pyridoxal-5-phosphate, the relative rates of reaction of these compounds with OH· radicals could be calculated: see Table 6. A value for the powerful biological protective agent

TABLE 6

Rate of reaction of test substance with OH·*

	Rate of reaction of pyridoxal-5-phosphate with OH·
Nicotinic acid	0.4
Pyridoxal-5-phosphate	1.0 (by definition)
AET	1.1
Pyridoxine	1.3
p-amino benzoic acid	3.1

* For reaction in solution containing dissolved oxygen.

AET (δ-β-aminoethylthiouronium BrHBr), was also obtained and its position in this table has a direct bearing on the mechanism of protection of cells to which I will refer again later.

Of particular interest is the observation that the destruction of all the vitamins except nicotinic acid is greatly enhanced by the presence of oxygen during irradiation, see Table 7. Yet O_2^- or HO_2·

TABLE 7

Influence of oxygen in destruction of vitamins

	Rate of destruction in air / Rate of destruction in nitrogen	in air $G_{(-\text{vitamine})}$
Nicotinic acid	1.1	1.2
Pyridoxal-5-phosphate	2.2	2.4
Pyridoxine	2.2	2.6
p-amino benzoic acid	6.0	0.9
Folic acid	2.2	1.1

radicals cannot be involved because the yield of hydrogen peroxide is that obtained from irradiation of aerated water without any other solutes. Nakken believes that the destruction of the vitamins (HB) is solely due to OH· radicals and that oxygen intervenes as follows:

$$HB_6 + OH\cdot \rightarrow B_6\cdot + H_2O\ ;$$

$B_6\cdot$ can either break up or react with H· radicals, which are formed radiolytically from water in the absence of oxygen.

$$B_6\cdot + H\cdot \rightarrow HB_6 \quad \text{(H· radical induced back reaction).}$$

In the presence of oxygen the back reaction is prevented by

$$B_6\cdot + O_2 \rightarrow B_6O_2\cdot \rightarrow \text{breaks up.}$$

This reaction scheme is very interesting as it has some similarities with the process referred to as protection by repair[4]) to which reference will be made later.

3. ENERGY TRANSFER

A method of protecting against the direct action of radiation is by processes which occur extremely rapidly after the passage of the ionizing particle and which funnel energy away before there has been time for a chemical reaction such as radical formation to occur. Reactions of this type are known as energy transfer; they have been shown to occur in the solid state[5]) both at room and at very low temperatures[6]), under conditions where the recipient of the energy (i.e. protective substance) must be, on a molecular scale, far away from the site of energy deposition.

I now have to review three papers dealing with energy transfer in mixtures of organic liquids where the "protecting" molecule can come into collision with the molecule that has been ionized or excited. Stone and Dyne[k]) have reinvestigated the effect of the addition of benzene on the radiolysis by Co^{60} γ rays of cyclohexane. Manion and Burton[7]), who first studied this system, observed that the radiolysis products of cyclohexane were reduced in the presence of small amounts of benzene which they concluded protected by energy transfer. Recently, several groups of investigators have questioned this interpretation and have suggested that the energy from the cyclohexane is not transferred to the benzene where it is dissipated without reaction, but that the benzene interacts with radical intermediates (e.g. scavenges hydrogen atoms). Stone and Dyne have determined quantitatively both the products formed and the rate of disappearance of cyclohexane and of benzene. In addition they have tested for "chemical" interaction by irradiating deuterated benzene (C_6D_6) with ordinary cyclohexane and analysed for C_6H_5D.

The results from this most thorough study seem unambiguous.

The G value of all the principal radiation induced reactions of cyclohexane is reduced equally by the addition of benzene and the magnitude of this effect increases regularly as the amount of benzene present is increased. The amount of benzene destroyed is quite insufficient to explain the protection by chemical interactions, such as scavenging of radicals, unless a series of reactions of the type occurs:

$$\underset{\text{(cyclohexane)}}{C_6H_{12}} \rightsquigarrow \underset{\text{(initial radicals formed on irradiation)}}{C_6H_{11}\cdot + H\cdot}$$

$$C_6H_6 + H\cdot \rightarrow C_6H_7\cdot$$

$$\underset{\text{(radical from cyclohexane)}}{C_6H_{11}\cdot} + C_6H_7\cdot \rightarrow C_6H_6 + C_6H_{12}$$

However the possibility that benzene successively reacts with both primary radicals has been decisively eliminated by the experiments with deuterium.

The physical mechanism of the energy transfer processes involved here remains unresolved. The first step will be to determine whether ionic processes such as charge or electron transfer are involved.

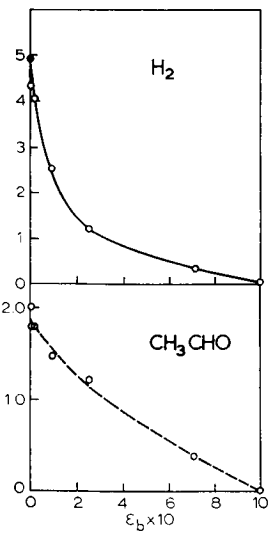


Fig. 2. Effect of benzene on the yield of H_2 and CH_3CHO from irradiated liquid ethanol. (Myron and Freeman.) E_b is the electron fraction of benzene. The filled point on the H_2 curve refers to the initial yield of H_2 in pure ethanol and the point at $G = 4.33$ represents the yield of H_2 in pure ethanol at the doses used in this experiment.

Generalizations cannot, however, be made in the complex situation of the irradiation of mixtures, as is shown by Myron and Freeman[h]) in their investigation of the influence of small amounts

of benzene on the radiolysis of ethanol. Fig. 2 shows that benzene is much more effective in reducing the yield of hydrogen than of acetaldehyde. The yields of methane and ethane are reduced to about the same extent as acetaldehyde, but the amount of ethylene is hardly affected at all. To interpret these data, the mechanism for the decomposition of ethanol by ^{60}Co γ rays has to be known and Myron and Freeman provide an exhaustive analysis of the products formed. There is no time to deal with their discussion of the many reactions which occur except to indicate their conclusion that the sequence of reactions below make an important contribution.

$$CH_3CH_2OH \rightsquigarrow CH_3CH_2OH^+ + e \quad \text{(primary act)}$$
$$CH_3CH_2OH^+ + CH_3CH_2OH \rightarrow CH_3CH_2OH_2^+ + CH_3CH_2O$$
$$CH_3CH_2OH_2^+ + e \rightarrow CH_3CH_2OH + H$$
$$H + CH_3CH_2O \rightarrow H_2 + CH_3CHO$$

In this system benzene protection would not seem to be confined to energy transfer at the level of the primary act, but that capture of hydrogen atoms contributes.

Goodman and Steigman[d]) have obtained convincing evidence for energy transfer via excited states (i.e. not ionic or electronic) in a three component system involving polymerization. Benzene did not have any influence on the rate of polymerization of styrene (i.e. neither sensitized not protected) but a number of scintillators (e.g. anthracene and p-terphenyl) protected. Since thermally induced polymerization was not protected against by these compounds, an energy

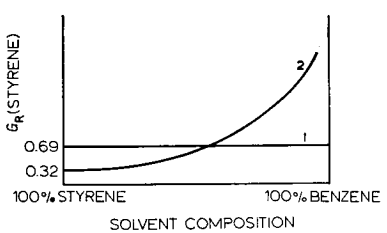


Fig. 3. Predicted dependence of G (R styrene) on solvent composition in the p-ter-phenyl-benzene-styrene system. (Goodman and Steigman.) Curve 1: system with no p-terphenyl. Curve 2: system containing 1.0 g/l p-terphenyl.

transfer process and not reaction with styrene radicals must be involved in the protection against radiation. In luminescence experiments, it had been shown that energy absorbed by benzene could be transferred to styrene in the presence of terphenyl. Con-

sequently one would expect first protection, and then sensitization in a three component system made up of a constant but low amount of p-terphenyl and variable amounts of styrene and benzene. Fig. 3 shows the predicted values for the yield of styrene radicals (derived from polymerization). The *G* value should be reduced (i.e. protected by terphenyl) if there is little benzene because energy transfer from styrene to p-terphenyl predominates. The *G* value should be enhanced if there is much benzene because of energy transfer from benzene to styrene via p-terphenyl which is an essential intermediate. Considerations of the energy levels of the excited states explain why the sensitization phenomenon is not seen with anthracene which, however, protects.

4. REACTION OF "PROTECTOR" WITH AN INTERMEDIATE RADICAL

In many radiochemical reactions whether initiated by direct or by indirect action, loss of a hydrogen atom to give a free radical is an essential first step (i.e. $RH \rightarrow R\cdot$) in a series of successive reactions which lead to chemical change necessary for loss of biological activity. Alexander *et al.*[3,4]) concluded from kinetic data that prevention of radiation induced crosslinking in aqueous solutions of polymers by sulphydryl compounds occurred by this reaction, and Ormerod[1]) has now obtained direct proof for this mechanism in the protection by cysteamine of DNA when irradiated as the nucleoprotein complex of salmon sperm heads, which consist of 65% DNA and 35% protamine. Under conditions where direct action predominates, irradiation of DNA or sperm heads leads to crosslinking and this is prevented by the addition of cysteamine.

On irradiation DNA gives a characteristic ESR signal and exactly the same spectrum is obtained from sperm heads when presumably energy transfer from the protein to the DNA takes place[6]). Ormerod examined the influence of cysteamine on the ESR signal of irradiated sperm heads. At the temperature of liquid nitrogen the ESR spectrum of sperm heads is the same in the presence and absence of cysteamine, but on standing at room temperature characteristic differences appear[8]). Firstly, the DNA signal decays more rapidly in the presence of cysteamine. In the absence of cysteamine the disappearance of $R\cdot$ can be correlated with the appearance of crosslinks (i.e. $R\cdot + R\cdot \rightarrow R—R$), while in the presence of cysteamine this is no longer the case. Secondly, on standing, a new spectrum

appears never seen without cysteamine, situated to the left of the R· signal. Fig. 4 shows the rate of appearance of this new radical expressed as *G* values in sperm heads containing different amounts of cysteamine. The work of Kurita and Gordy[9]) has established that the ESR spectrum of a —S· radical corresponds to the new spectrum seen in sperm heads with cysteamine.

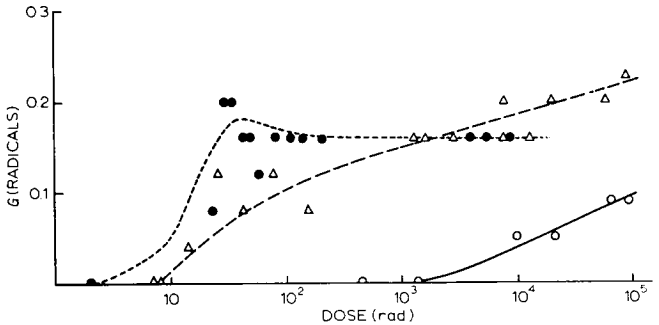


Fig. 4. The build-up of P· type free radicals at room temperature in sperm head irradiated at −196°C. (Ormerod.)
— O — spermheads plus 1% cysteamine
-- △ -- spermheads plus 5% cysteamine
-- ● -- spermheads plus 10% cysteamine.

Quantitative considerations rule out the possibility that the new signal in the sperm heads is merely due to the irradiation of the 1 to 10% of cysteamine present. The fact that this new radical which is identified as being —S· appears slowly shows that it is formed in a post-irradiation reaction and cannot therefore be due to irradiation of the cysteamine. The ESR data coupled together with the observed prevention of crosslinking by cysteamine indicates that

$$R\cdot + NH_2C_2H_4SH \rightarrow \underset{\text{repaired DNA}}{RH} + NH_2C_2H_4S\cdot$$

A repair reaction comparable to that observed with DNA also occurs in proteins. When dry salmine is irradiated, a typical radical is obtained which persists unchanged for many days at room temperature in the absence of oxygen. When salmine is mixed with cysteamine then the typical irradiated protein spectrum slowly dis-

appears on standing and a new S· type spectrum appears (Fig. 5). The reaction R· + PSH → RH + PS· appears to occur in proteins (P) in the same way as in DNA, except that in proteins the repair is not in competition with a crosslinking reaction, i.e. the protein radical is much more stable than that for DNA at least if oxygen is excluded.

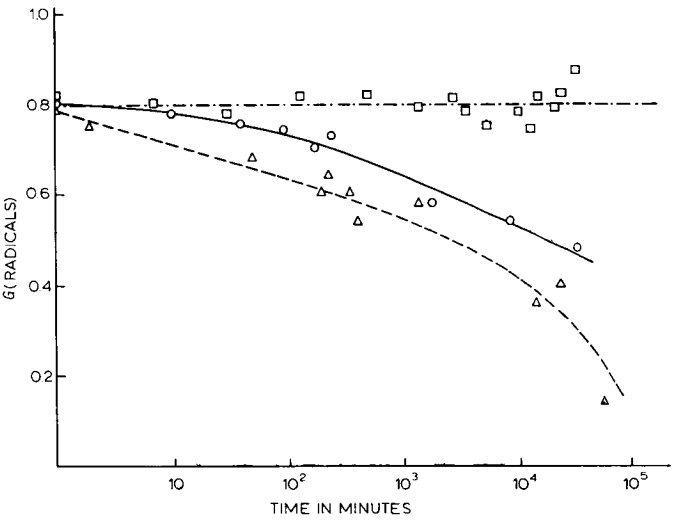
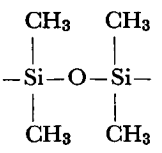


Fig. 5. The decay of R· type free radicals at room temperature in salmine irradiated at −196°C. (Ormerod.)
•—• □—•— salmine only.
—O— salmine plus 1% cysteamine
--△-- salmine plus 5% cysteamine.

Reaction with the intermediate radical may appear as protection even though it is not repair when a particular end effect is prevented without however restoring the molecule to its original state. This is well illustrated by Charlesby and Garratt[a]) who have investigated the mechanisms by which several added substances prevent the crosslinking (i.e. conversion into a gel) of the polymer dimethylsiloxane;



This material is a thick oil of 26000 molecular weight. The gel point, when there is, on average, one crosslink per molecule, is reached at 7.8 Mrad and from this it is concluded that one crosslinking centre (e.g. an R· radical) is formed for every 21 eV (i.e. $G = 4.6$). The crosslinking reaction is accompanied by gas formation, principally methane ($G = 1.15$) and hydrogen ($G = 2.0$).

Benzophenone at a concentration of 0.1 moles/l of polymer doubles the dose of radiation needed to crosslink and also cuts down the evolution of hydrogen, but does not affect the evolution of methane. Iodine is a much more efficient protector and at 0.005 moles/l doubles the dose needed to produce a gel and with higher doses the protection is still more marked. But iodine does not alter the amount of gas produced.

Anthracene surprisingly does not influence the dose needed to reach the gel point, but disappears as such from the system with a G value of 2.5 based on the total energy absorbed. If the polymer did not interact with anthracene then a G value of less than 10^{-4} would be expected with the concentration of anthracene used.

The authors conclude that energy transfer is not involved in the interactions between any of these three substances and the polymer. They interpret the observed protection as follows:

$$\mathrm{RH} \rightsquigarrow \mathrm{R}\cdot + \mathrm{H}\cdot$$
$$\mathrm{H}\cdot + \mathrm{RH} \rightarrow \mathrm{R}\cdot + \mathrm{H_2}$$
$$\mathrm{R}\cdot + \mathrm{R}\cdot \rightarrow \mathrm{R{-}R}\ \text{(crosslink)}.$$

Benzophenone captures H· and thus reduces evolution of hydrogen and cuts crosslinking efficiency to one half. Iodine reacts with R· and the product formed can no longer crosslink; it has to be postulated that iodine does not react with H· because H_2 evolution is unaffected.

Anthracene(A) reacts with R· and the radical formed RA· is still capable of forming crosslinks of the type RAR or RAAR. Consequently, there is no reduction in the rate of crosslink formation and the dose needed to gel is unaffected by the presence of anthracene.

A difficulty of all these suggested mechanisms is that both with and without additives the dose needed to gel the polymer is independent of dose rate in the range of 9.2×10^4 to 6.2×10^5 rad/min. This imposes severe restrictions on the possible mobilities and life times of the radicals involved. At the present, none of these mechanisms

can be considered as established and there is clearly much to learn about the reactions involved.

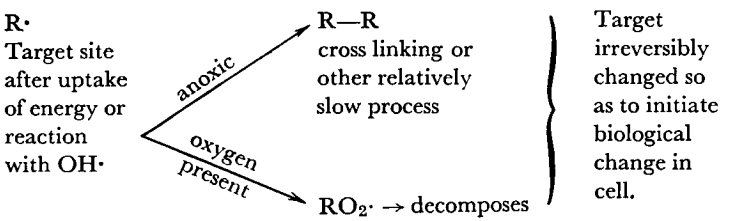
Perhaps the most remarkable aspect of the investigations by Charlesby and Garrett with dimethyl-siloxane and by Charlesby and Kopp in the polymers in aqueous solutions is that like Dale[1]), they find colloidal sulphur extremely effective as a protective agent. A concentration of 8×10^{-5} g atoms per liter in the form of a colloidal suspension doubled the dose needed to gel a 5% solution of polyvinyl pyrrolidone and the comparable figure for dimethyl-siloxane is 10^{-4} g atoms. From such figures it can be readily computed that each sulphur atom prevents approximately ten cross-links from being formed; and it must be remembered that the sulphur is present in colloidal aggregates containing hundreds, if not thousands, of atoms many of which are sterically inaccessible. This high efficiency of sulphur points to the occurrence of some chain reaction, but the authors have not suggested a mechanism.

5. IMPLICATIONS OF THESE RESULTS FOR MECHANISMS OF BIOLOGICAL PROTECTION

Nakken points out that the OH· radical scavenging ability of the B-vitamins is greater than that of AET, yet these vitamins are unable to protect *E. coli*. While Kalkwarf has found that the rate of reaction with OH· radicals parallels protective ability against haemolysis of red cells, many of the substances he has found most reactive are known not to protect dividing cells against death by X rays even though compounds like tryptophane would be expected to penetrate into cells. Polycyclic conjugated systems are the most efficient energy transfer agents, but none of them have protected cells *in vivo* or *in vitro*. Considerations such as these suggest that combination of the protective agent with a target molecule after it has been converted into a radical is the most likely mechanism for protection by SH compounds and for the role of oxygen in radiobiology (cf. Bacq and Alexander[10]). Both Nakken and Ormerod in their papers state that their data support this deduction.

The direct experimental evidence for repair in a nucleoprotein by low concentrations of SH compounds greatly strengthens the hypothesis first proposed some years ago by Bacq and Alexander, but based at the time only on experiments in model systems (ref. [4])). The hypothesis was that in vegetative cells, repair by SH and peroxi-

dation by dissolved oxygen compete for reaction with the "target" radical. The oxygen effect at the cellular level is then seen as a consequence of the prevention of repair by peroxidation. Added SH compounds protect cells in the presence of oxygen because the the SH concentration is now sufficient to compete with the oxygen for R· whatever this may turn out to be, protein, nucleic acid, phospholipid. This reaction scheme may be written:



Cellular SH compounds repair the majority of anoxic damage because reaction

$$R\cdot + —SH \rightarrow RH + —S\cdot$$

occurs more rapidly than does $R\cdot + R\cdot \rightarrow R—R$ or other inactivition reactions. But in the presence of oxygen, $R\cdot + O_2 \rightarrow RO\cdot_2$ occurs more rapidly than repair and only if SH compounds (i.e. protective agents) are added at high concentration can "oxygen" damage be prevented.

The existence of repair by physiological agents can explain the sensitizing action of oxygen and the much greater degree of protection provided by SH substances to oxygenated than to anoxic systems.

This interpretation has been adopted by Howard-Flanders to explain his data and this is discussed in the next session.

In conclusion I must deal inadequately with a paper of great practical importance which concerns the reverse of protection. Malhotra and Reber[g]) have studied the nutritional value of meat which had been sterilized with 5.5×10^6 rad. Weanling rats fed a diet of which this meat formed the major part died within 8 to 14 weeks from a haemorrhagic disease due to vitamin K deficiency. This was prevented by the addition of 3 μg vitamin K three times a week to the diet. When methionine was also added then 1 to 2 μg

of vitamin K was sufficient. If there had been no interaction with the other constituents of the meat, then a dose of 5.5×10^6 rad would have destroyed only about 1% of the vitamin K and methionine content. These feeding experiments show that the destruction must have been much greater and these substances must therefore have taken part in the kind of reactions considered earlier in this report under the heading of protection. Energy deposited in constituents of the meat, other than the vitamin, must have caused destruction of the vitamin K and methionine, though at the present time the mechanism by which this has occurred is not known. Indeed, one man's meat is another man's poison!

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DISCUSSION

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In reviewing the paper by Malhotra and Reber[g]), the rapporteur had brought out the similarity of the destruction of vitamins in irradiated food to the process of protection. However, the results also have considerable importance in experimental nutritional or other work involving feeding and irradiation. In order to draw attention

to this aspect, Reber contributed additional information on three points.

First, it had been suggested in their paper that the male rat was protected against haemorrhages when vitamin K was added to the diet in sufficient quantities to overcome a 50% loss following irradiation and that methionine must be added to compensate for a 27% loss following irradiation. The losses of vitamin K on irradiation varied from 1% in the system described by the rapporteur to 50 to 85% in beef as reported by other workers, and are still being investigated. It was reported in 1955 that, during the process of mixing rations, more vitamin E was destroyed when irradiated beef was mixed with the other ration components in comparison with non-irradiated beef. This indicates the presence of substances, possibly autoxidized lipids, which destroy vitamin E. Second, this haemorrhagic disease occurs only in male rats. It was not possible to produce it in female rats or male cats and dogs. Third, in feeding irradiated beef to rats the practice of coprophagy, which normally supplies vitamin K, no longer takes place. This suggests that one should consider the possibility, when substances produced by radiation are taken into the body, that vitamin requirements may increase and, if these are not present in sufficient quantity in the diet, physiological aberrations might occur.

Goodman[d]) reported that the prediction in his paper that increasing benzene content in a mixture containing styrene and a small constant proportion of p-terphenyl would first cause protection and then sensitization had not been fully confirmed by recent experiments. The sensitization does not occur. Instead, the increase in styrene radical yield which occurs up to 2½% benzene, appears to be due to inhibition by benzene of the protective effect of terphenyl that normally occurs in pure styrene monomer. Possibly styrene can quench luminescence due to a benzene-to-terphenyl energy transfer by a preferential benzene-to-styrene transfer which does not result in formation of styryl radicals. However, the question why benzene inhibits the protection due to terphenyl but not that due to anthracene remains unanswered.

Glubrecht, (*Inst. Strahlenbiol. T.H. Hannover, Germany*) suggested that radioactive labelling would be a valuable technique to use in the study of radiation protection and asked whether any such work had been done with S^{35} labelled materials. Kopp[b]), reported

some preliminary protection experiments using C^{14}– or S^{35}-labelled thiourea in aqueous polymer systems made with the object of determining the kinetics of the protection reaction and gaining information on the fate of the additive. It was found that both C and S parts of the thiourea molecule were incorporated into the polymer. The amount incorporated was proportional to dose until the thiourea was consumed. Thereafter, crosslinking started and proceeded as for the unprotected polymer. More detailed studies are in progress using labelled thiourea, and colloidal S^{35} in aqueous polymer solutions, in pure liquid organic polymers and in solid polymers.

Hutchinson, (*Biophys. Dept. Yale Univ. Conn., USA*) asked Kalkwarf to discuss the possibility that his results did not necessarily give the relative reactivities of the substances to OH· radicals because the observed products might result from a series of reactions with several intermediates. Kalkwarf[1]) agreed that the radiation chemistry of compounds in aqueous solution was complex but maintained that the relative rates quoted were those of the very first steps in a fairly complicated mechanism. The results related to a competition between the dye, which was the reference compound, and the various amino-acids in the initial stages of reaction where there was little destruction of any reactant.

Bach (*Acad. Sciences, Moscow, USSR,*) commented on the question of energy transfer from cyclohexane to benzene. Voevodsky[1]) and his collaborators have irradiated a synthetic hydrocarbon containing both cyclohexyl and phenyl groups. They have shown by the ESR method that when a phenyl group is present in the molecule, the *G* value for cyclohexyl free radicals decreases so markedly that in practice only radicals from the benzene appear. This seems to be rather direct experimental evidence that there is energy transfer.

A warning and a plea for work on hydrocarbon systems other than benzene-cyclohexane was made by Miss Nosworthy (*Phys. Dept. Guy's Hosp. London, Great Britain*). She suggested that, while the study of energy transfer in benzene-cyclohexane was valuable, other hydrocarbon systems should also be studied. In some work on scintillator solutions with which she was associated at the University of Notre Dame it was found that the observed luminescence intensities could be explained by energy transfer from a non-aromatic to an aromatic solvent. Simple kinetics gave quantitative agreement in most cases, including the n-hexane-benzene system.

However, with benzene-cyclohexane and some other mixtures containing rather flat, ring molecules, if a simple energy transfer were responsible for the observed intensities it would be necessary to assume a higher rate of transfer when the aromatic solvent was present in concentrations of a few per cent. This suggested that special molecular interactions occurred in benzene-cyclohexane solutions.

Flemming[c]), said that in his paper, in addition to explaining the mechanism of amine formation in a scheme involving several steps, he had also discussed another reaction. He found, when working with UV light that addition of H_2O_2 to the water caused a considerable enhancement of amine formation which, therefore, resulted from OH· or through OH· radicals. When cysteine was present in the irradiated solutions, up to 100-fold enhancement of amine production occurred. In his opinion, this was due to increased OH· radical formation and the effect of cysteine was related to the presence of its —SH group; none of the other compounds examined contained —SH groups. He was happy to find agreement with Kalkwarf in his haemolysis results, namely that aliphatic amino-acids have a weak protective action and the aromatic amino acids strong protection; this ran parallel with the formation of OH· radicals. However, he also found a similar difference in effect during UV haemolysis, where OH· radicals could not be effective.

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CHEMICAL PROTECTION AT THE CELLULAR LEVEL

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1. INTRODUCTION

Some of the possible mechanisms of protection from radiation in chemical systems have been dealt with in the previous report by Alexander. It is clear that there are still many uncertainties even where the conditions can be most closely controlled. In the whole animal the added complexities of varying cell types and different physiological conditions make interpretation of the results of chem-

A. Protective Agents

TABLE 1

Protective agents

(1) −SH	(2) N_2	(3) Glyc	(4) Others
Cysteamine	Anoxia	Glycerol	37°C to 2°C
Cysteine		Ethylene glycol	Freezing −190°C
Hydrogen sulphide		Ethanol	Drying
Mercaptoethanol(a)		Dimethyl sulphoxide (b)	Amidines
MEG			Thiourea (c)
(AET)			etc.

(a) $SH.CH_2.CH_2.OH$ (b) $CH_3—\underset{\underset{O}{\|}}{S}—CH_3$ (c) = S potential sulphydryl.

ical protection exceedingly difficult. One advantage of using isolated cells is that physiological complications can be avoided. However, to quote Miss Alper[a]), one of the authors in this session, "The mechanism of true chemical protection remains elusive."

The first three tables show the groups of substances used by the thirteen contributing authors.

(1) Sulphydryl compounds (—SH). Cysteamine and cysteine are the most frequently used compounds. Aminoethylisothiouronium bromide (AET) is included in brackets in this list because, although it is not a sulphydryl compound itself, in certain circumstances it is converted to mercaptoethylguanidine (MEG) which possesses the active —SH group.

(2) Anoxia (N_2). Anoxia is usually produced by flushing the system with specially purified nitrogen but other inert gases can be used. Of the materials reported on here, only phage in the absence of sulphydryl compounds fails to show protection by means of anoxia.

(3) Glycerol (Glyc.). A number of substances, typified by glycerol, will protect cells from damage by freezing and also from the effects of radiation. Relatively high concentrations of the order of two molar must be used. In this connection compare the 10 – 100 mM concentrations of sulphydryl compounds required for full protection.

(4) Other agents. Cooling from 37 to 2°C, freezing to — 190°C and drying also protect. A group of amidines and related compounds and =S compounds such as thiourea, methylthiourea and thioacetamide have also been found to protect.

B. Sensitizing Agents

TABLE 2

Sensitizing agents

(1) O_2	(2) NEM
Oxygen	N-ethyl maleimide (a)
Nitric oxide	

(a) combines with – SH compounds.

(1) Oxygen (O_2). Nitric oxide behaves similarly to oxygen in sensitizing cells but there are some quantitative differences.

(2) N-ethyl maleimide (NEM). This compound combines with sulphydryl compounds and its action is consistent with this.

C. Inactive Compounds

TABLE 3
Inactive compounds

Disulphides —S—S—	Pharmacologically active agents
Cystamine (a)	5-hydroxytryptamine (serotonin)
Cystine	Tryptamine
	Adrenalin
	Nor-adrenalin

(a) Betz found protection *in vitro* with thymocytes.

(1) Disulphides (—S—S—). Cystamine and cystine are the disulphide counterparts of cysteamine and cysteine and are usually inactive *in vitro* although cystamine is active *in vivo* possibly due to its conversion in the body to cysteamine.
(2) Pharmacologically active amines. These substances which may act by lowering oxygen tension in whole animals do not protect cells *in vitro.*

D. Radiation Types and Doses

TABLE 4
Types of radiation used

Type of radiation	Energy or source	Authors
X rays	50–250 kV	All except Biebl
Electrons	8 MeV	Alper
α	5 MeV	Alper
α	27 MeV	Alper
α	Polonium	Biebl

TABLE 5
Doses used (rad)

	Order of dose for 10% survival
Phage and bacterial spores	10^5
Bacteria (vegetative)	10^4
Mammalian cells	5×10^2

The use of α particles with their known slight "oxygen effect" is of particular interest.

E. Cell Types

TABLE 6

Types of cells used.

	Phage
Hotz	T1, 2, 3, 4, 6, 7, T4 Bo^r, P22, P22—VM
Howard-Flanders	T2. Free in suspension and also inside host bacterium but not replicating.
	Bacteria and yeast
Alper	*E. coli* B/r, *Salmonella typhimurium*, *Saccharomyces cerevisiae* (haploid)
Bridges	Pseudomonas sp.
Dewey	*Serratia marcescens*
Howard-Flanders	*Shigella sonnei*
Nikolov, Robev	*E. coli* 823
Webb	*Staphylococcus aureus* Spores of *Bacillus megatherium.*
	Mammalian and plant cells
Betz	Rat thymocytes (not dividing)
Eker	Mouse fibroblasts strain *L*
Vergroesen, Vos	Human kidney cells
Biebl	Outer epidermata of red onion scales (*Allium cepa*)

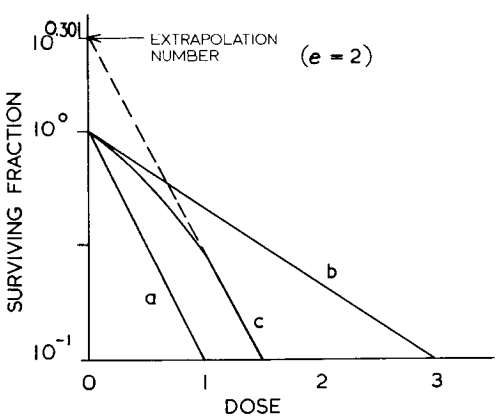

Fig. 1. Typical survival curves in which log surviving fraction is plotted against radiation dose.

Most authors have depicted their results by plotting log surviving fraction (vertical scale) against dose (horizontal scale). The survi-

vors are defined by their ability to reproduce and form colonies, plaques or infective centres. The slopes of these curves (Fig. 1, a, b) therefore represent the radiosensitivity and can be compared for different treatments, giving dose reduction factors (DRF), dose modifying factors or dose reduction coefficients. Frequently cells are more resistant for small doses and the curve has an initial "shoulder" before becoming straight (Fig. 1, c). The straight part can then be extrapolated back to zero dose and the point at which it strikes the vertical axis is known as the extrapolation number or sometimes as the "hit number".

2. MAIN PROBLEMS AND RESULTS

The chief objects have been to find new compounds and throw light on the mechanisms of chemical protection.

A. New compounds

Robev[j]) and Nikolov[i]) have protected *E. coli* by a series of amidines and related compounds. All these contain the chemical grouping shown in Fig. 2 and do not contain sulphydryl. Maximum

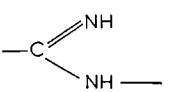

Fig. 2. Chemical grouping contained in amidines and related compounds. (Robev; Nicolov.)

DRF's between 2 and 3 were recorded, although compound twelve (Table 7) sensitized under certain circumstances. The mechanism of

TABLE 7

Robev	Nikolov
1 N-phenyl-4-toluylamidine	1 2-aminopyridine
2 N-phenyl-4-methoxybenzamidine	2 benzamidoxine
3 N-(4-iodophenyl)-benzamidine	
4 N-(2-tolyl)-benzamidine	
5 N-phenyl-4-dimethylaminobenzamidine	
6 N-phenyl-3:4-methylenedihydroxybenzamidine	
7 N-phenyl-3:4-dimethoxybenzamidine	
8 N-(4-iodophenyl)-4-methoxybenzamidine	
9 N-(4-tolyl)-3:4-methylenedihydroxybenzamidine	
10 N-(-4-iodophenyl)-3:4 methylenedihydroxy-benzamidine	
11 N-phenyl-2-naphthamidine	
12 N-phenylbenzylideneacetamidine	

action of these compounds is quite unknown and they warrant further investigation.

B. The problems of mechanisms of action

(1) Do sulphydryl compounds act only by lowering the oxygen tension of the the medium surrounding the cells? This problem has sorely taxed many radiobiologists, particularly in this country. Sulphydryl compounds, such as cysteine and cysteamine, undoubtedly do consume oxygen in solution, but the evidence presented here shows that they can protect in the complete absence of oxygen, and also that they do, in fact, protect when oxygen is undoubtedly present (this is interpreted as meaning that there is probably competition between oxygen and sulphydryl groups for damaged sites).

Dewey and Liddon[e]) have tested the radiosensitivity of the bacterium *Serratia marcescens* over a very wide range of oxygen pressures from 1 part per million to 100 atm (Fig. 3). They found that reduc-

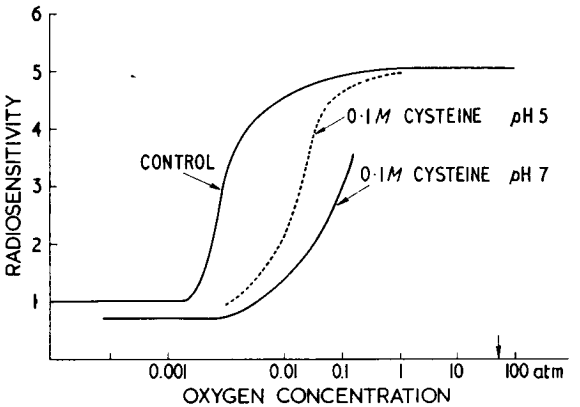

Fig. 3. Radiosensitivity of *Serratia marcescens* in relation to oxygen concentration. (Dewey and Liddon.)

ing the oxygen content of the carrier gas below 200 ppm did not further protect the bacteria, but the addition of 0.1 molar cysteine gave significant further protection. Using 100% oxygen at 1 atm, cysteine protection was lost. At *p*H 5 little autoxidation was thought to occur.

Vergroesen[k]) passed pure nitrogen above and below human kidney cells resting on 20 μ thick melinex membranes and treated

them with cysteamine. He found added cysteamine protection even when the gas leaving the irradiation chamber gave zero reading on his Hersch cell (less than one part per million).

Bridges[d]) obtained dose reduction factors of up to 1.8 with cysteine in complete anoxia.

Alper[a]) provides the following arguments against the idea that sulphydryl compounds act only through lowering oxygen tension.
— Cysteine protects most effectively when bacteria are irradiated as a monolayer open to the air.
— The oxygen effect would be expected to be small for 5 and 27 MeV alpha particles which have high LETs. Cysteine however protected against these radiations. Note also Biebl's[c]) finding of chemical protection of mature onion cells from polonium alpha particles.
— Cysteine in concentrations adequate to give full protection caused little change in the oxygen tension of the medium. The oxygen tension was measured with platinum electrodes.
— If cysteine altered the oxygen tension of the medium surrounding cells then the fall in oxygen tension should be the same for all strains and species. However, the different strains of bacteria behaved differently to similar concentrations of cysteine and, further, yeast was not protected at all by cysteine.
— *E. coli* pre-incubated with cysteine retained the protective effect even when no cysteine was present in the medium at the time of irradiation.

The compelling conclusions are firstly that sulphydryl compounds can afford protection in complete anoxia and secondly that they can give chemical protection in the presence of oxygen.

(2) Concerning the problem of the protection by pre-

TABLE 8 (Hotz)

	Phage type	
	Osmotic shock Sensitive T 2, 4, 6	Osmotic shock Resistant T 1, T 5, T 7 T 4 Bo^r, P 22
Retention of —SH protection	Present	Absent

incubation with sulphydryl compounds. Hotz[g]) found that only some phage types retained protection after pre-irradiation incubation with sulphydryl compounds. They were subsequently irradiated in negligible concentrations of sulphydryl compound in the medium (Table 8). It is suggested that the membrane that causes the osmotic shock sensitivity also traps the sulphydryl compound inside the phage.

(3) Why are free phage not protected by anoxia unless sulphydryl compounds are present? The answer given by Howard-Flanders *et al.*[h]) is that sulphydryl compounds are normally present inside bacterial cells but not in a free phage suspension. A diagram of his experimental results is shown in Fig. 4. The addition

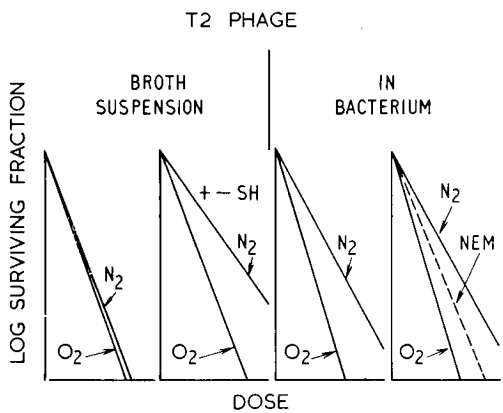

Fig. 4. Survival of T_2 phage irradiated in broth suspension or inside bacterial cells. + —SH: in the presence of mercaptoethanol. NEM: after pre-incubation of bacteria with N-ethyl maleimide. (Howard-Flanders, Levin and Lynch.)

of mercaptoethanol to a free phage suspension mimicked the results of phage irradiated inside bacteria. Pre-incubation of the infected bacteria with N-ethyl maleimide partially depleted the cell of sulphydryl compound. A scheme for DNA inactivation is shown in Table 9.

(4) Competiton between sulphydryl and oxygen. We have already seen the results of Dewey and Liddon showing this competition using bacteria. Howard-Flanders *et al.*, using phage, keep the oxygen concentration at 2 mM and vary the sulphydryl concentration. The radiosensitivity of phage in 2 mM oxygen is

TABLE 9

Reactions of DNA radicals* (Howard-Flanders, Levin and Lynch)

Biologically active DNA is denoted by RH

Formation of DNA radicals

(1) $$RH + \left\{\begin{matrix}\text{two radicals,}\\ \text{multiple ionization, etc.}\end{matrix}\right\} \rightarrow \text{inactive product}$$

(2) $$RH + \left\{\begin{matrix}H\\ OH\\ h\nu\\ \text{etc.}\end{matrix}\right\} \rightarrow \left\{\begin{matrix}H_2\\ H_2O\\ e^- + H^+\end{matrix}\right\} + R\cdot$$

where R· denotes a DNA radical which may then react as follows

		Product	*Reaction speed at 4° C*
(3)	$R\cdot + O_2 \rightarrow ROO\cdot$ etc.	inactive	fast (diffusion limited)
(4)	$R\cdot + NO \rightarrow RNO$ etc.	inactive	fast (diffusion limited)
(5)	$R\cdot + -SH \rightarrow RH + -S.$	active	slower by factor of 30
(6)	$R\cdot \rightarrow ?$	inactive	complete in less than 0.01 sec

* Compare Alexander and Charlesby[1]).

greatest when no sulphydryl compound is present and least when 200 mM sulphydryl compound is used. Assuming that sulphydryl and oxygen penetrate into the phage without difficulty, Howard-Flanders *et al.*, argue that the reaction rate of the DNA radical with oxygen is 10 to 100 times faster than the reaction rate with sulphydryl.

(5) The action of glycerol, sulphydryl compounds and freezing. Bridges has studied glycerol and dimethyl sulphoxide in combination with cysteine under both aerobic and anoxic conditions. Using anoxic conditions only, he obtained nothing but straight curves and therefore his results can be completely described by single dose reduction factors (Table 10). He argues that combining protective agents would lead to a final DRF that is a product of each if the agents act on different components of damage. If they act on the same component then their combined DRF's will not only not be a multiple of each other but will not exceed the theoretical maximum of the best. His results showed dependence (Table 10).

Dewey and Liddon's protection curves are complex, and they conclude that for certain types of cellular damage sulphydryl compounds and glycerol compounds protect through common mecha-

TABLE 10
Protection of Pseudomonas (Bridges)

	Observed dose reduction factor for given conc.	Calculated maximum dose reduction factor for infinite conc.
Glycerol 2 M	2.0	2.6
Dimethylsulphoxide 1 M	1.6	2.0
Cysteine 0.1 M	1.8	2.0
Glyc + DMS	2.3	
Glyc + —SH	2.5	

(N.B. $2.0 \times 1.6 = 3.2$
$2.0 \times 1.8 = 3.6$).

nisms but that each group also affords protection to different types of damage as well.

Webb and Powers[m]) have produced a series of very elegant curves for their various protective procedures (Fig. 5). These are put in numerical form in Table 11. From these results one sees that the

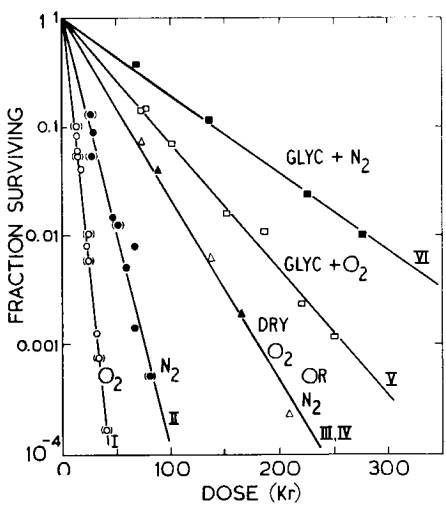

Fig. 5. Survival of *Staphylococcus aureus* irradiated under various conditions. (Webb and Powers.)

○: Buffer, air ●: Buffer, N_2
△: Lyophilized, air ▲: Lyophilized, N_2
□: Glycerol, air ■: Glycerol, N_2.
() : Bubbled

TABLE 11
Protection of *Staphylococcus aureus* and spores of *Bacillus megatherium* (Webb and Powers)

Condition	Dose reduction factors	
	Staph. aureus	*B. mega.* spores
O_2	1	1
N_2	2.3	2
Freeze dried* O_2 or N_2	5	–
O_2 + Glyc. 8.8 M	8	2.2
O_2 + Glyc. 11.6 M	9.2	2.5
N_2 + Glyc.	13	2.8

* Frozen and dried at reduced pressure for 20 hr.

effect of oxygen is reduced in glycerol and abolished by drying and that glycerol is more effective than drying even when oxygen is present. The authors also show that the changes in radiosensitivity that occurred with increasing concentrations of glycerol can be fitted to the Langmuir adsorption equation, and for this and other reasons they propose (and I here quote) that "the action of certain protective agents against ionizing radiations can be considered in terms of physical absorption on molecules and surfaces within cells."

Vos[1]) has protected human kidney cells in tissue culture with glycerol, dimethyl sulphoxide, cysteamine and freezing to −190° C. All his curves have a marked shoulder and the author has calculated all his dose reduction factors on the relative doses that give 1% survival. He has continued to draw his curves with increasing steepness down to lower levels of survival, but if the author's points are re-plotted after proportional reduction by the stated DRF at the 1% level it seems to me that a straight part of the curves could be found. A summary of his results is shown in Table 12.

TABLE 12
Protection of human kidney cells in tissue culture (Vos)

	DRF (O_2 = 1)
Glycerol 2 M	1.9
Cysteamine 4 mM	1.9
Freezing −190°C	1.9 to 2.8*
Glycerol + cysteamine	2.8
Glycerol + cysteamine + −190°C	4.5 to 6.4*

* Final slope would give even higher figures but note 2.8 × 2.8 = 7.8.

(6) The slopes of curves. How should one interpret the slopes and extrapolation numbers of the curves relating log surviving fraction to dose? Howard-Flanders *et al.* and Hotz using phage nearly always obtained straight lines.

Bridges, and Webb and Powers, using bacteria and spores, also obtain straight curves for all conditions. Alper, and Dewey and Liddon, on the other hand, obtain straight or "shouldered "curves depending on conditions. Alper, using *Salmonella typhimurium*, and Howard-Flanders *et al.*, using *Shigella sonnei*, obtain curves

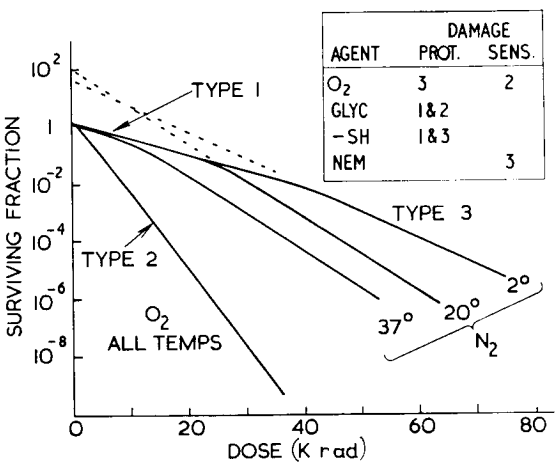

Fig. 6. Survival of *Serratia marcescens*. Three types of damage are deduced. Type 1: damage caused by small doses in N_2. Type 2: the additional damage in O_2. Type 3: the excess damage at high doses in N_2. The insert shows the effect of various agents in sensitizing to or protecting against the 3 types. (Dewey and Liddon.)

TABLE 13

Classification of radiation damage (Dewey and Liddon)

Type of damage	Temp. effect	Due to
1 N_2 low dose	0	H, OH radicals etc.
2 O_2 extra damage	0	HO_2, R-peroxide radicals, rejoining inhibition
3 N_2 high dose	+	Damage to proteins (multihit)
3a N_2 high dose	0	

with continuously decreasing slope, that is, "concave," if one looks from above. Alper's curve for yeast is at first concave and at higher doses convex. Dewey and Liddon relate different types of damage to different parts and slopes of their curves (Fig. 6 and Table 13).

3. SOME APPARENT INCONSISTENCIES AND POINTS OF INTEREST

Vos observed a peculiar toxicity of cysteamine for his mammalian cells at concentrations of 0.25 to 2 mM but not, surprisingly, at higher concentrations.

Vos and Vergroesen found that cysteine, cysteamine and AET protected their tissue culture cells but Eker and Pihl[f]), using mouse fibroblasts in tissue culture, failed to obtain protection with cysteamine and only AET "probably" protected. However, they were using only 2 mM cysteamine and they did experiments to show that up to half the free —SH disappeared during the time of irradiation so that the concentration may have been insufficient. Further, they were measuring changes in growth curves of cells which are usually very difficult to interpret.

Vos found no protection with the disulphide cystamine, but Betz[b]) was able to protect rat thymocytes from pyknosis. Here the cell type and end effect are quite different and this may explain the difference in results.

In conclusion one may say that, firstly, we have a wealth of new evidence that shows that sulphydryl compounds protect in the presence and absence of oxygen. Secondly, we can conclude that the diverse substances (sulphydryl compounds and glycerol-like compounds) protect, at least partly, against the same type of damage.

Finally, I would like to say that I am acutely conscious that I have omitted many very important points, but this has been due partly to the enormous amount of work these authors have produced and partly to the complexity of the subject.

REFERENCE

1) P. Alexander and A. Charlesby, in Radiobiology Symposium 1954 (Butterworths, London, 1955) p. 49.

DISCUSSION

JENNIFER SHEWELL
Department of Pathology, St. Mary's Hospital Medical School, London, Great Britain

The Discussion was opened by Powers[m]) who felt that it was better to describe the magnitude of the oxygen effect in term of absolute probabilities of cell death rather than by comparing ratios of dose reduction factors as the rapporteur had done. He referred to his previously published work on the effect of X irradiation on bacterial spores, which had shown that damage was here due in part to the production of free radicals. The presence of free radicals had been demonstrated by classical radical scavenging techniques, later verified by ESR techniques. Many compounds, among them NO, H_2S, various other mercaptans, and some amines, protected by removing free radicals which become toxic if they are allowed to react with oxygen. Glycerol, in addition to removing free radicals, also protected against damage from mechanisms that are oxygen independent. A very good fit had been obtained to the Langmuir isotherm, and using this fit it can be predicted that at infinitely high glycerol concentrations only 0 to 10% of the radiation effect would remain in these bacterial cells. Theoretically then in the spore, and perhaps in the cell, all energy absorbed can be dissipated harmlessly.

Alexander (*Chester Beatty Res. Inst., London, Great Britain*) pointed out that the normal curve relating radiosensitivity to oxygen also paralleled the Langmuir absorption isotherm.

Alper[a]) felt that the point that had emerged clearly was, both with bacterial cells and spores, although not with bacteriophage, that whatever protective compounds were used the extent of protection was less in the absence of oxygen. The extent of the variation of effect varied with the cell used and the compound used. Bridges[d]) had shown that different chemical compounds (e.g. sulphydryl compounds, dimethyl sulphoxide and glycerol) probably protected the same site of damage. As Alexander and Howard-Flanders[h]) had put forward the same mechanism for protection by sulphydryl compounds, Alper wondered whether this mechanism could be extended to include protection by the alcohols and dimethyl sulphoxide. (This point was not answered in discussion.)

Rajewsky (*Max Planck Institute, Frankfurt am Main, Germany*) asked

Dewey[e]) what was meant by high and low doses (i.e. rate or total) and following from that, whether there was any dose rate dependence. Dewey explained that at low doses, (i.e. doses up to 20 to 30 Krad) the shape of the survival curve in nitrogen was the same at all temperatures. At high doses, where there was a visible temperature effect, there was also a dose rate effect. The effect of dose rate changes was too complex to discuss fully; a drastic change in rate would affect the phenomenon described only slightly.

Philpot (*MRC, Harwell, Berks., Great Britain*), commenting on Robev's[j]) demonstration of the protective action of amidines, stated that he had once considered that malonic dialdehyde (a decomposition product of the hydroperoxide of linolenic acid) might be the substance responsible for radiation damage. Amidines should react with malonic dialdehyde to give pyrrole derivatives. Protection studies in mice with formamidine acetate and acetamide hydrochloride were unsatisfactory.

Robev stressed that not all amidines were protective and that the compounds he had investigated were n-phenyl substituted amidines. Protection was dependent on the structural variation of the molecule. Increased survival in mice, and in albino rats, had been demonstrated with the n-phenylamidine derivative of thiophene-2-carboxylic acid. Only 3 or 4 of the substituted amidines shown by the rapporteur in Table 7 had radioprotective action, although their pharmacological effects were the same.

Hutchinson (*Yale Univ., New Haven, USA*) emphasized that the inconsistencies reported in this field should not obscure the regularities. Dilute solutions of enzymes and transforming principle resembled bacteriophage in exhibiting only a slight oxygen effect, unless —SH were added, when an oxygen effect was obtained.

Howard-Flanders (*Yale Univ., New Haven, USA*) agreed that there were marked regularities in the data presented. Competition between —SH and oxygen obeyed simple kinetic considerations. There was a demonstration of unity in behaviour ranging from the inactivation of different enzymes, transforming principle and bacteriophage to organisms such as bacterial and other cells. Sulphydryl was normally necessary to demonstrate the oxygen effect on systems in aqueous suspensions, but this is ordinarily present in the cell. He felt that one could look forward to a speedy rationalisation of the mechanism of sulphydryl protection at least.

Charlesby (*Royal Military College, Shrivenham, Great Britain*) arguing from the assumption that the biological effects are due to radical–radical interactions, thought that at high intensities of radiation a high concentration of additive would be needed to protect because a high concentration of biological radicals would be formed, and at lower intensities with a lower concentration of radicals greater protection would be evident. Was there any dose rate dependent effect?

Alper thought that the range of dose rates necessary to discuss this point would have to be very wide. Would Charlesby accept that evidence from radiation of different LET would provide an equivalent situation? Sparsely ionizing radiations have ionizations far apart, while the closely spaced ionizations in an α particle track would simulate high intensity radiations. Both she and Biebl[c]) had demonstrated chemical protection to radiation of high LET, and more chemical protection was obtained with 27 MeV α particles than with 8 MeV electrons. Protection against 5 MeV α particles and 8 MeV electrons was much the same.

Powers referred to the published work of his group as having established beyond doubt that in the bacterial spore and vegetative cell free radicals are indeed formed by radiation; that they react with oxygen, and that they can be removed by chemical scavengers.

Meissel (*Institute of Microbiology, Academy of Sciences, Moscow, USSR*) considered the cytological mechanism of the protective effect. In 1950, he, Pomoshcknikova and Shavlovsky demonstrated that the vital blocking of mitochondria in yeast with berberin sulphate resulted in radioprotection. Later work showed that ethyl ether and some other surface active agents could replace berberin sulphate at the mitochondrial surface, when protection was still observed.

The final contribution was from Butler (*Chester Beatty Res. Inst., London, Great Britain*) who felt that a rewarding approach to the problem might be, not the study of the modification of the oxygen effect by protective substances, but rather the variations in protective action brought about in the presence of oyxgen. There was evidence that with pure substances in the solid state the oxygen effect was small or negligible, while a marked oxygen effect appeared when protective substances were added. In the cell, protective substances are invariably present. It was noteworthy that no oxygen effect was demonstrable in isolated bacteriophage.

CHEMICAL PROTECTION IN MAMMALS

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Defense Research Kingston Laboratory, Kingston, Ontario, Canada

1. INTRODUCTION

Chemical protection in mammals has generally been investigated by observing the effect of drugs on the mortality of total body irradiated mice during the 30 days following irradiation. Fig. 1

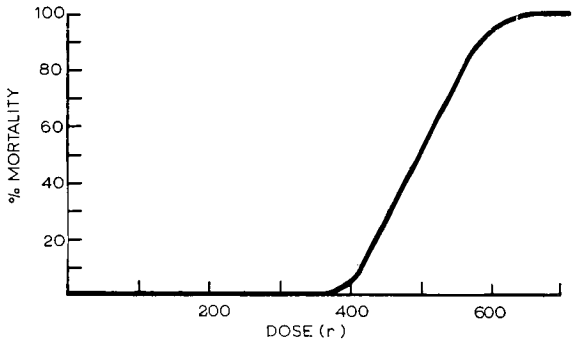

Fig. 1. Mortality curve for mice after total body irradiation. (After Catsch[1].)

shows a typical mortality curve taken from Catsch[1]). The curve rises very steeply between 400 and 700 r. An important consequence of this steep rise is that a small dose-reduction factor (DRF) produced by a protector may have a great effect on the percentage mortality in this critical range of dose. Fig. 2 demonstrates that a

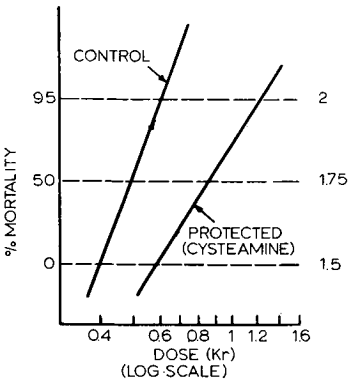

Fig. 2. Mortality curves for control and protected mice. (After Catsch[1].) Ordinate: working probit scale.

true DRF is not always obtained; in this case the apparent DRF increases with increasing dose[1]).

Mortality curves of this type show considerable variations according to the sex, age, and strain of mice used. It is essential that the

animals should be healthy, as irradiation increases susceptibility to disease. The spread of infection may explain the non-random distribution of deaths per box, or so-called cage effect, observed by Raventos[2]).

An extremely important factor in mammalian protection is the toxicity of the drugs themselves. All the protectors so far discovered are really effective only at levels of dosage near the limit which the animal can tolerate. It is therefore essential to examine the pharmacological action of these agents.

Since the last International Congress, work by van der Meer in Holland[3,4]) and Graevski[c]) in Russia, and van den Brenk in Australia[5]) has shown, with a high degree of probability, that a whole class of protectors, including the pharmacologically active amines, such as adrenalin, histamine, and serotonin, and also cyanide, all act by producing tissue anoxia. As a result of this work, the unsolved problems of mammalian protection have been limited to the mode of action of the cysteine-cysteamine group (Fig. 3) and a few others.

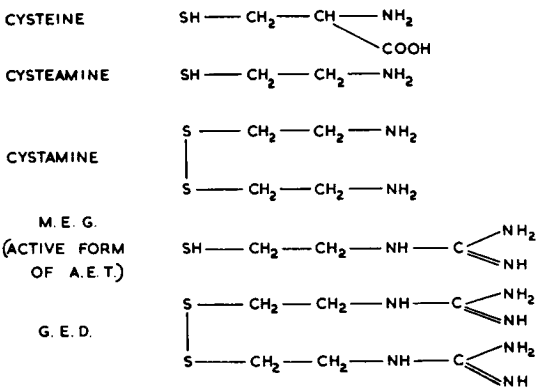

Fig. 3. Structure of agents of the cysteine-cysteamine group. MEG: mercaptoethylguanidine; AET: aminoethylthiouronium. GED: guanidoethyldisulphide.

Although cysteine and cysteamine have to be given in nearly toxic doses to obtain effective protection, direct measurement of the oxygen tension in the spleen has not revealed any evidence of anoxia[6]). Recent reviews on cellular protection have indicated that protection is not necessarily related to anoxia. It is still not quite clear whether the effects observed *in vitro*, using rather high concen-

trations of sulphydryl compounds, are in fact responsible for the protection of the whole animal.

2. EFFECTS OF MIXED DISULPHIDES

Bitny-Szlachto[a]) has carried out an experiment to test the validity of Eldjarn and Pihl's hypothesis[7]) that the formation of mixed disulphides is related to protection. Bitny-Szlachto tested in mice the protective activity of a mixed disulphide of mercaptoethylguanidine (MEG) and cysteine, and compared it with that of guanidoethyldisulphide (GED). The initial reactions of these two compounds with a sulphydryl-containing target molecule are shown in Table 1. In each case one product is MEG linked to a protein by

TABLE 1

Initial reactions of a mixed disulphide of mercaptoethylguanidine (MEG) and cysteine, and of guanidoethyldisulphide (GED), the disulphide of MEG, with an —SH target molecule

A.	M—S—S—Cy Mixed disulphide, MEG + cysteine	+	R.SH "Target molecule"	=	R—S—S—M + cysteine
B.	M—S—S—M Disulphide of M.E.G. (=G.E.D.)		+ R.SH. "Target molecule"	=	R—S—S—M + MEG

a disulphide bond, although in case A, the MEG-cysteine disulphide molecule contains only half as much MEG as is present in GED, which is composed of 2 MEG molecules. In terms of MEG content, therefore, A may be more efficient than B for the production of mixed disulphides. On the other hand, free MEG is released in case B.

Mice were given the cysteine-MEG disulphide at 0.5 mM/Kg, and

TABLE 2

Effect of GED and mixed disulphide on survival in mice (Bitny-Szlachto)

Dose of X-rays	Survivors at 30 days		
	Controls	GED	M—S—S—Cy
660	4/20	11/20	15/20
800	0/10	3/10	1/10

GED at 0.25 mM/Kg, followed by total body irradiation with 660 or 800 r. No obvious difference in the protective action was observed with the comparatively small number of mice used (Table 2), but further work based on this new approach would be welcome.

3. TRACER STUDIES WITH MEG AND GED

Shapiro, Schwartz and Kollmann[g]) point out that it is essential to know in what form protective drugs exist at the time of irradiation. They have administered ^{35}S-labelled MEG and GED (Fig. 3) to mice, both intraperitoneally and orally, and determined the products in the different organs. They identified protein-bound ^{35}S, GED, guanidoethanesulphinic acid, taurocyamine, and sulphate. When MEG was compared with GED given in equal dose (140 mg/Kg) the total ^{35}S (expressed as AET ^{35}S) was lower in serum and bone marrow 20 min after MEG injection than after GED injection. The values for the spleen were the same, however, and this may account for the equal radiation protection observed.

The various products were compared 20 and 120 min after intraperitoneal injection of GED, i.e. at times when radiation protection is observed, and when it is absent. The only metabolic products whose concentration could be correlated with the presence of protection were protein-bound sulphur and free GED. These results would appear to be compatible with different theories of protection.

3. ANAESTHETICS

Evans and Orkin[b]) have investigated the effect of mixtures of nitrous oxide and oxygen on the response of 8 week old mice to total body irradiation. A mixture of 83% nitrous oxide and 17% oxygen was without effect at 1 atm pressure. There was a slight protective effect with 90% nitrous oxide and 10% oxygen, but this was shown to be due to partial anoxia.

When experiments were carried out at 2 atm pressure, it was evident that nitrous oxide and nitrogen had different effects (Fig. 4). Nitrous oxide prevented most of the increase in mortality which was observed in air at 2 atm, but it was not protective if radiosensitivity in air at normal pressure is taken as the baseline. The authors attempted to rule out the possibility that this relative protection was due to nitrous oxide narcosis by anaesthetizing mice with pen-

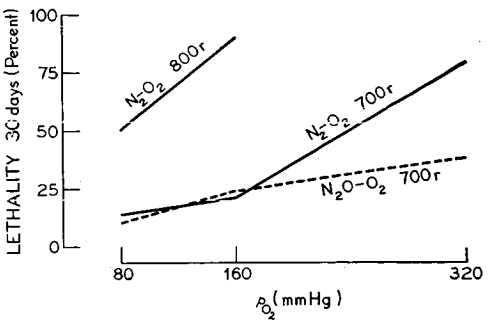

Fig. 4. Lethal action of X rays in mice in air (N_2—O_2) or a mixture of N_2O and oxygen having the same partial pressure of oxygen as air. The oxygen pressure was varied by varying the total pressure of the gas mixture. (Evans and Orkin.)

tobarbital (50 mg/Kg). Pentobarbital was protective in air at 1 atm, but not at 2 atm. The validity of this control depends on the assumption that this dose of pentobarbital produces the same pharmacological effects as nitrous oxide. The possibility that the protective effect is secondary to tissue anoxia has not, therefore, been eliminated.

Lindop and Rotblat[e]) have also studied the effect of pentobarbital (Nembutal) at a slightly higher dose than Evans and Orkin, i.e. 60 mg/Kg (Table 3). They measured the protective effect at different dose rates, and found that the dose reduction factor decreased with increasing dose rate. They consider that anoxia is the most likely mechanism of the protective effect, but the reason why the dose rate affects the control and not the anaesthetized animals is not clear. A separate experiment, in which the oxygen tension was measured in the marrow, revealed that ether anaesthesia was accom-

TABLE 3

Effect of Nembutal in protecting mice aginst lethal radiation damage at various dose rates (Lindop and Rotblat)

Dose rate (Rad/min)	LD 50		
	Control	Anaesthetized	DRF
~ 480	686	800	1.17
~ 2000	715	796	1.11
~ 6000	726	805	1.11
~ 31000	748	795	1.06
~160000	752	791	1.05

panied by a drop in oxygen tension. Lindop and Rotblat considered two mechanisms by which anaesthesia could lead to anoxia.

(i) Vasoconstriction secondary to cooling of the animal

(ii) Diminished rate and depth of respiration.

Oxygen was administered to a group of anaesthetized mice, which were then irradiated at 180000 rad/min. The LD50 was 738 rad in this group, suggesting that the very small protective effect of anaesthesia observed at high dose rates had been abolished by oxygen.

4. CYSTEAMINE AND ANOXIA

Shewell and Wright[h]) point out that comparatively few experiments with mammals have been carried out in which chemical protection is combined with hypoxia.

Mice, anaesthetized with 65 mg/Kg of Nembutal, were rendered anoxic by administration of pure nitrogen for 50 or 55 sec. Irradiation with 8 MeV electrons was carried out during the last 5 sec of nitrogen breathing. Another group received cysteamine in addition to anoxia, a third group cysteamine alone, and a fourth was irradiated in air. The results of 5 experiments combined showed that cysteamine and anoxia together gave a higher DRF (~2.8 compared with irradiation in air) than either alone. (~1.5 for cysteamine and ~2.3 for anoxia.) The surprising observation was made that cysteamine rendered the mice less susceptible to the hazards of the anoxic treatment. It is possible that cysteamine had shifted the haemoglobin dissociation curve. Whatever the explanation, this is another example of the pharmacological effects of protective drugs.

Both Nembutal and cysteamine lowered the body temperature. Given together, the temperature drop lasted longer. At least two interpretations of these results of the combination of cysteamine and anoxia are possible, namely that cysteamine renders partially anoxic mice completely anoxic, and that cysteamine and anoxia are operating through different pathways.

5. PHYSIOLOGICAL MECHANISMS OF CHEMICAL PROTECTION

Grigoresco, Tranco, Popp, Haulica, Vandrovschi, Lecca, and Nedelesco[d]) have carried out a very extensive investigation of many different protective agents. At the outset they had considered four classes of these drugs.

(i) Inhibitors of the central nervous system, such as chlorpromazine.
(ii) Those which release adrenal hormones, such as ACTH or adrenalin itself.
(iii) Inhibitors of the reticulo-endothelial system (trypan blue).
(iv) Competitors for free radicals (cysteamine).

They found that representatives of all these classes of drugs produced a drop in the body temperature of rats, which was most pronounced at 1 hr. They observed many other physiological responses, such as delay in stomach emptying time, lowering of blood pressure, inhibition of conditioned reflexes, etc. The authors conclude that the agents tested may be acting through a common pathway, and they refer to Mole's[8]) hypothesis that pharmacologically induced anoxia may be such a pathway. They suggest that two stages in mammalian protection must be considered, first, physiological response of the animal to the drug before irradiation, and second, specific effects, such as radical capture, during irradiation.

Graevsky, Barakina, Constantinova, and Smirnova[c]) have investigated many protective agents in mice, to clarify the relationship between the mechanism of protection and the oxygen effect. The criteria of radiation damage studied were LD 100/30 days, the number of cells in intestinal crypts and bone marrow, and chromosome aberrations. Irradiation of mice in oxygen, as compared with air, did not increase the mortality in controls. Oxygen reduced, but did not abolish, the protective effect of adrenalin and sodium

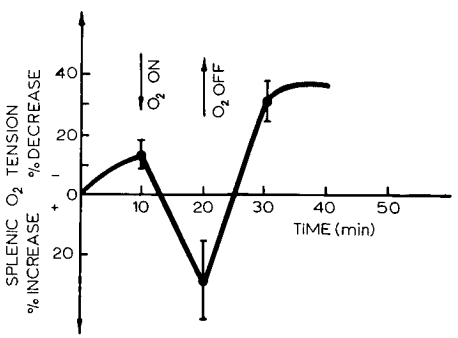

Fig. 5. Changes in oxygen tension in the spleen of mice previously treated with adrenalin, and the influence of oxygen administration. (Graevsky, Barakina, Constantinova and Smirnova.)

nitrite. Measurement of oxygen tension in the spleen by polarography showed that adrenalin lowered oxygen tension. This reduction could be abolished, or even reversed, by giving oxygen. Thus the polarographic results are not quite in agreement with the protection observed; possibly the electrode does not detect small islands of anoxic tissue (Fig. 5). Breathing oxygen did not alter the protection by cysteamine, cystamine, or AET, and polarography showed that on the administration of oxygen, the splenic oxygen tension rose to the same extent as in controls. Combining AET with hypoxia, (6.5% or 8.2% oxygen) gave more protection than hypoxia alone. Examination of tissues showed that AET protected marrow effectively, and crypt cells to a lesser extent.

Graevsky *et al.* distinguish two groups of protective agents: those, like the amines, which reduce tissue oxygen tension, and the monothiols which (as judged by polarography) do not.

Robev[f]) tested on rats the series of amidines which were discussed in the report by Wright (page 281). The compounds were given intraperitoneally 0 to 5 min before irradiation. He considered that some of these compounds may release adrenalin in the animal. A simple pharmacological explanation of their effect was not apparent, since some amidines with similar pharmacological properties show different degrees of protective ability. The protective effect of one of the amidines was abolished by its pharmacological antagonist, hexamethonium. I have taken some of Robev's data and rearranged them in Table 4 to illustrate the point that the more

TABLE 4

Radioprotective and toxic doses of drugs (Robev)

Drugs giving 30 to 40% survival at 720 r	Protective dose (mg/Kg)	Toxicity (LD 50) (mg/Kg)	Ratio of toxic to protective dose
N—phenyl-4 chloro-benzamidine	50	106	2.1
N-(4-nitrophenyl) benzamidine	200	445	2.2
N-(4-nitrophenyl) 3-nitro 4-methyl-benzamidine	200	380	1.9

effective protective agents must be give at near-toxic doses. On the other hand, a drug may be toxic without being protective.

6. RADIOACTIVE CHROMIUM UPTAKE: SEROTONIN

Vittorio, Wight, and Sinnott[1]) have investigated a new technique for measuring radiation effects, using radioactive chromium. Chromium plays a vital rôle in many biochemical systems, and the tissues of the newborn are particularly rich in it. In this study four groups of mice were examined

(i) Controls receiving saline.
(ii) Given serotonin.
(iii) Saline and X ray (600 r).
(iv) Serotonin followed by X ray.

All four groups received 25 μC sodium radiochromate. The ^{51}Cr content of various tissues was followed at frequent intervals up to 24 hr; organ weights and chromium content were also measured up to 40 days.

During the first 24 hr, serotonin alone had more effect on ^{51}Cr levels than X rays alone. In the irradiated tissues, the ^{51}Cr content (as counts/mg/min) was increased, and serotonin protected against this effect. The increase in chromium content was interpreted as an indication of accelerated repair processes. The serotonin group were less damaged, and therefore less repair was necessary.

7. SUMMARY

Five of the nine papers reviewed are concerned with the interrelationship between mammalian protection and tissue anoxia. There appears to be fairly general agreement that the majority of pharmacologically active compounds, such as the amines, or anaesthetics such as pentobarbital, act by inducing tissue anoxia. Evans suggests that nitrous oxide may protect by an independent mechanism.

The cysteine-cysteamine group, on the other hand, do not appear to induce tissue anoxia. The most direct evidence bearing on this point are the polarographic measurements of splenic oxygen tension carried out by Graevsky *et al.*

The work of Bitny-Szlachto, and of Shapiro *et al.* is concerned with testing the validity of the Eldjarn–Pihl hypothesis of mixed disulphide formation, but the results do not yet point to a conclusion.

The toxicity studies of Robev, and the biochemical approach of Vittorio, both emphasize the importance of the pharmacological effects of protective drugs in mammals.

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DISCUSSION

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Bitny-Szlachto[a]) commented that the low rate of survival of his animals treated with mixed disulphides of cysteine and MEG may well have been due to the toxicity of the disulphides. In fact, a number of animals receiving this treatment died at an earlier time than did some of the controls; in contrast, the substances were apparently harmless in unirradiated animals. It is clear that the pharmacological properties of GED and its mixed disulphides, as compared with those of cysteine, are very different: for example, the toxic effect of mixed disulphides is more lasting than that of GED. Moreover, irradiation may impede recovery following poisoning by the mixed disulphides of MEG and cysteine, an observation which may at least partly explain why a proportion of animals receiving these substances died earlier than did some of the controls. Bitny-Szlachto finally reminded the audience that two compounds were used in these studies, namely GED and mixed disulphides of MEG and cysteine, in the form of pure hydrobromides.

Shapiro[g]) wished to add that, since submission of the abstract, his group have been studying the nature of protein binding of the protective substance in the tissue. At 20 min, when the animals are protected, there are two main types of protein binding: a mixed disulphide bond, and a thiolester bond. There was also a trace of a third type of binding, of which the nature is obscure. At 2 hr, when the animals are no longer protected, both mixed disulphide and thiolester concentrations decrease, while the concentration of the third, as yet unidentified, type of protein binding increases. One may therefore conclude that the latter effect is not correlated with protection.

Evans[b]) then raised the question as to whether mice breathing nitrous oxide with 20% oxygen at 2 atm pressure may be protected from radiation death by anoxia due to narcosis. Polarographic measurements of oxygen in the spleen, originally undertaken as a result of Scott's suggestion, showed that oxygen increases as compared with air at normal pressure. A small increase was also found as compared with air at 2 atm pressure. Since the spleen may not be the critical organ for this protection, Evans and his colleagues have also measured total oxygen consumption under these conditions; they found no change from air at equal pressure.

Lindop[e]) stated that the very small protective effect reported could be reduced by giving anaesthesia combined with oxygen. There is some indication that anaesthesia may exert its protective effect by hypoxia, but at another site. When the animals breathe nitrogen, the DRF, as judged by acute effects, is 2.8; when protection is assessed by long term effects, the DRF is decreased to 1.4. For anaesthesia, the DRF for acute effects is 1.2, but for long term effects is it increased to 1.4. This would suggest that hypoxia and anaesthesia are protecting at a similar site for long term effects, in contrast to their mode of protection against acute effects.

In response to the Chairman's invitation for discussion from the audience, Mewissen (*Bordet Inst., Brussels, Belgium*) said that a few years ago, while investigating the relationship between dose and DRF, he and his colleagues observed that the latter was positively correlated with the dose of whole-body irradiation following pretreatment with either cysteamine or cystamine. These observations were fully confirmed by Catsch[1]) using a larger number of animals. He wondered whether Lindop may have observed the same pheno-

menon in her large series of irradiated and unirradiated mice.

Mewissen's second question related to late effects of irradiation in mice. In his experience, irradiated mice pre-treated with cysteamine or cystamine showed the same shortening of life-span, as well as the same incidence of radiation-induced tumours, as did control irradiated mice. He again enquired whether Lindop had made similar observations.

Lindop replied that the observation that long term effects are reduced by severe hypoxia during irradiation has been confirmed not only by herself and her collaborators, but also by Lamson *et al.*[2]). She suggested that protection against acute effects may not necessarily be mediated by a mechanism which also affects recovery.

Cohen (*Rad. Therapy Dept., General Hosp., Johannesburg, South Africa*), addressing himself to the rapporteur, commented that the latter's statement that pharmacologically non-toxic compounds are inert as chemical radiation protectors may not be universally applicable. There are many pharmacologically inert substances, like the sugars, which effectively influence the response not only to whole-body radiation, but also to the development of tumours (e.g. flavanoids).

In this discussion of Lindop's observations, Cohen enquired whether there may be a change in the exposure geometry of the animals following induction of anaesthesia. Unanaesthetized animals can walk about during the exposure, while those that are anaesthetized are immobile. All the animals may not therefore receive precisely the same dosage of equal homogeneity. He therefore urged that studies of this nature be accompanied by details of exposure geometry.

In reply to Cohen's enquiry, Lindop said that the physical distribution of doses in her various experiments were uniform within 2%, and that "hot-spotting" did not occur because of the high energy of the X rays used, 15 MeV. Those animals which were not anaesthetized were restrained from free movement, during irradiation, by being placed in a perspex box. Every effort was made to ensure that anaesthetized animals were consistently treated in a strictly identical manner to that of unanaesthetized controls.

In reply to Cohen's comments, Scott wished to emphasize that his previous remarks about toxicity were only applicable to such

substances as exert a protective effect equivalent to a DRF of at least 1.5.

Howard (*Res. Unit. in Radiobiol., Mount Vernon Hosp., Northwood, Middx., Great Britain*) asked Evans whether he had also tested xenon at different pressures, as well as other inert gases, for their effect upon oxygen consumption. Evans replied that so far only Nembutal (pentobarbital) and nitrous oxide had been tested. Pentobarbital decreased oxygen consumption; its protective effect was about the same as that of nitrous oxide, i.e. it gives a protection of about 20% in the LD 50 range, equal to that of N_2O at an atmospheric pressure of about 1.5 times normal.

Wright[h]) raised a more general point by comparing the whole animal to a complex dynamic mosaic of oxygen tensions, a system which is liable to be disturbed by almost any procedure. He pointed out that changes in such a complex mosaic would hardly be expected to be accurately detectable by means of only a few electrodes measuring oxygen tension, placed here and there. In his experiments, the acute and chronic protective effects of anoxia were similar with regard both to tumour induction and shortening of life-span. In contrast, there was little protective effect against the induction of cataracts, possibly because, during the administration of nitrogen, the oxygen tension in the lens of the eye does not fall to the same extent as that of other tissues.

In commenting on Scott's classification of protective compounds, van Bekkum (*Radiobiol. Inst. TNO, Rijswijk, The Netherlands*) said that he and van der Meer had studied the effects of dithiocarbamates upon intact mice; the results suggested that the action of these compounds could be explained completely in terms of a depression of the oxygen content of the spleen. For these reasons, he considered that dithiocarbamates should be classed in the group of substances which cause haemopoietic tissue anoxia.

Philpot (*MRC, Harwell, Berks., Gr. Britain*) said that some time ago he performed an experiment which might be described as a test of the Eldjarn–Pihl hypothesis, though actually it was done a year or two before the hypothesis was first postulated. Thinking it would be beneficial to block sulphydryl groups temporarily during irradiation, he gave mice a lethal dose of sodium arsenite, quickly irradiated them, and then restored them with BAL. The mice recovered from the arsenite but later died as a result of the irradia-

tion. One snag in this experiment, as a test of the Eldjarn–Pihl hypothesis, arose from the fact that arsenite is known to react preferentially with paired, as against single, sulphydryl groups. Philpot suggested that it might therefore be of interest to repeat the experiment with some reversible agent which is guaranteed to react with all single sulphydryl groups, as well as paired ones.

Before closing the discussion, the Chairman (*Eldjarn, Inst. of Clinical Biochem., Rikshospitalet, Univ. of Oslo, Norway*) pointed out that the essential feature of the Eldjarn–Pihl mixed disulphide theory is that the S-containing protectors act by a particular sort of repair process. The theory implies (i) the formation of the mixed disulphide, i.e. a particular form of protector concentration on susceptible loci and (ii) that the protector may eventually "repair" damage to the loci. No doubt arsenite does concentrate at the loci of paired disulphide groups, but the Chairman doubted very much if it would effect any repair.

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Radiopathology of Mammals

THE EFFECT OF AGE ON RADIOSENSITIVITY

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1. INTRODUCTION

Experiments with mice and rats by Quastler[1]), Abrams[2]), Hursh and Casarett[3]), and Sacher[4]) indicated an age-dependency of the sensitivity to ionizing radiation. Newer investigations by Kohn and Kallman[5]), Gengozian[6]), and Rotblat and his collaborators [7,8]) have shown that the dose causing 50% lethality at 30 days (LD 50/30) is a function of age of the animals at the time of irradiation. Fig. 1 shows

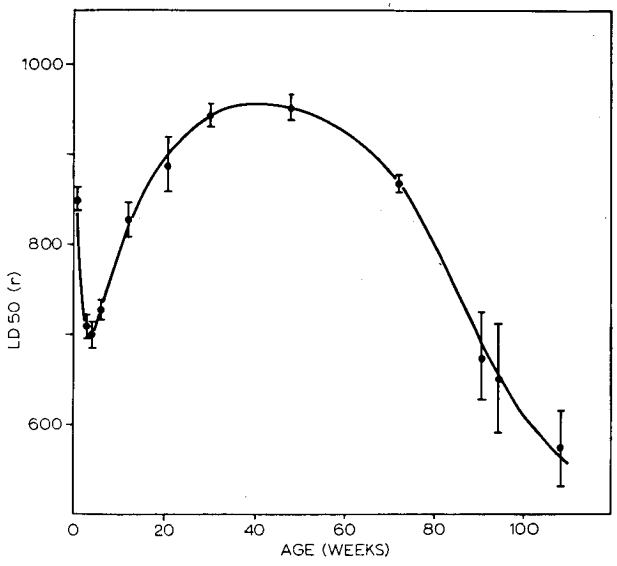

Fig. 1. LD 50/30 for SAS/4 mice as a function of age at irradiation. The radiosensitivity passes through a maximum value at 3 to 4 weeks of age. (Crosfill *et al.*[1]).)

the well-known curve of Crosfill, Lindop and Rotblat[7]), with two new points at older ages (private communication). It is of the greatest interest that the long-term life shortening caused by radiation also follows a very similar age variation, as shown both for acute exposures[16]) and for doses accumulated in small daily incre-

ments[4,4a]). Lindop and Rotblat[16]) gave a full discussion of results following acute exposures, and their implications, at the Annual Congress of the British Institute of Radiology in 1961. It is also relevant that radiotherapists have found empirically that X ray doses to very young and very old patients must be lower, by some 20% (Tudway)[17]), than those given to middle-aged patients.

Is there a single specific physiological function, an "intrinsic radiosensitivity factor" which varies with age and which is common to several mechanisms of injury? Or does the radiosensitivity of different physiological functions vary with age in different ways? Why is sensitivity so great at 3 to 4 weeks of age in mice? Is such a peak of radiosensitivity also found in other mammals?

The twelve papers in this session give some new results which are relevant to these questions, and also report many detailed results which cannot be given here in the time available. The main trends of the new investigations have been to examine the radiosensitivity of a number of functions other than LD 50/30 in the relatively unexplored range from birth to a few weeks of age, and where possible to investigate the effects of much smaller doses than hitherto.

2. EFFECTS IN MICE WHICH SHOW AN AGE DEPENDENCY OF RADIOSENSITIVITY SIMILAR TO THAT SHOWN BY LD 50

Table 1 summarizes the results on mice from seven of the papers for effects other than LD 50/30 days. The left-hand side of the table

TABLE 1

Mice

Effects in which radiosensitivity was found to vary:

Similarly to LD 50/30 (ref.[7]))	Differently from LD 50/30
Shortening of lifespan [16,4a]	
GI death[c,k])	Immune response[k])
Granulocyte and lymphocyte recovery rate[k])	Lens weight (8 weeks youngest)[l])
Sterility (females)[g,h])	Intercapillary glomerular sclerosis[a])
Total reproductive capacity (females)[h])	

lists those effects which showed a greater radiosensitivity at about 3 to 4 weeks of age than at earlier and later ages; this is the restricted meaning of "similarly to" in the table.

A. Gastro-intestinal death

K. Heuss[c]) gave 1000 r (X rays) to mice of ages between 2 and 67 weeks, and plotted the mean survival time (MST) against age, as shown in Fig. 2. The good correlation with Fig. 1 is obvious.

Smith and Alderman[k]) measured the radiosensitivity of gastro-intestinal death in terms of the LD 50 for death between 3 and 6

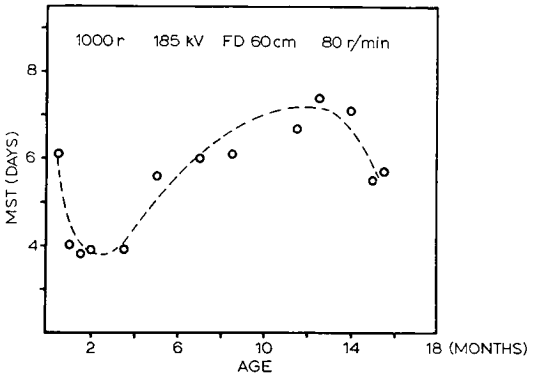

Fig. 2. Mean survival time (MST) after 1000 r whole body dose as a function of age in mice. (Heuss.)

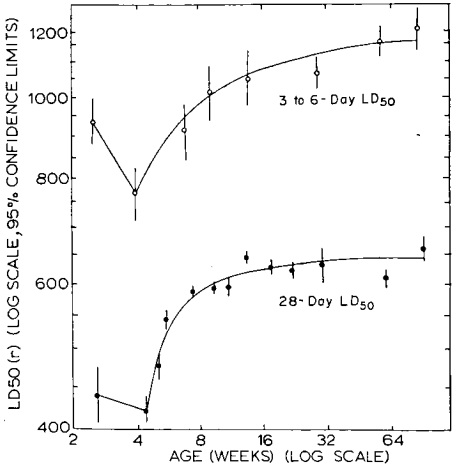

Fig. 3. LD 50 as a function of age in mice. (Smith and Alderman.)

days. Fig. 3 shows that their results followed very closely the pattern they obtained for LD 50/30. This is a very satisfactory agreement with the previously reported paper and with Fig. 1, and also provides a good justification for the use of mean survival time as a measure of radiosensitivity in this effect.

B. Granulocyte and lymphocyte concentration

Smith and Alderman also investigated femoral marrow cellularity (estimated 7 to 12 days after irradiation) and found it to be more depressed in 7½ week old mice than in those aged 32 weeks, so the younger mice were more radiosensitive. They went on to investigate the recovery times of peripheral lymphocyte and granulocyte concentrations after a dose of 420 r. Fig. 4 shows the results,

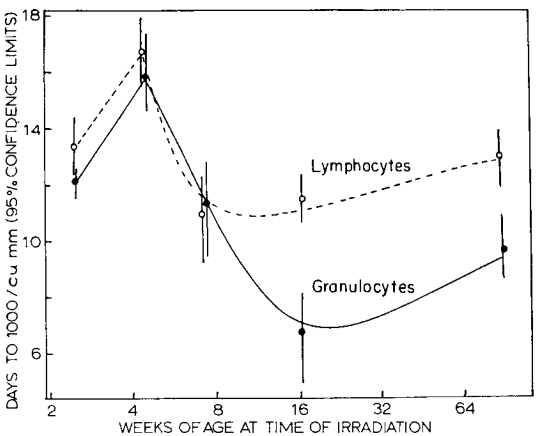

Fig. 4. Recovery time of lymphocyte and granulocyte concentrations as a function of age in CDBA mice after 420 r. (Smith and Alderman.)

plotted as time required to recover to 1000 cells/mm^3 against age. The shape corresponds well with the pattern of LD 50 *vs* age (Figs. 1, 2 and 3), showing a maximum sensitivity at 3 to 4 weeks. The correlation is best for granulocytes. The authors pointed out that "the times observed for granulocyte recovery (7 to 16 days) are consistent with the view that granulocyte recovery is causally related to the ability to survive the period of greatest danger from septicaemia," and may thus account for the low LD 50/30 at 3 to 4 weeks of age.

C. Sterility and total reproductive capacity

Earlier work[8–13]) has been done with doses exceeding 150 r given

within a few days of birth, or with doses down to 20 to 25 r given at ages greater than 5 weeks. Peters and Levy[h]), after tabulating these earlier results, reported experiments giving doses of 20 r to female mice at various ages between 0 and 35 days. The doses were to the whole body except that the head was shielded. Two functions were studied: the total number of offspring produced during the life span (total reproductive capacity) as shown in Fig. 5; and the

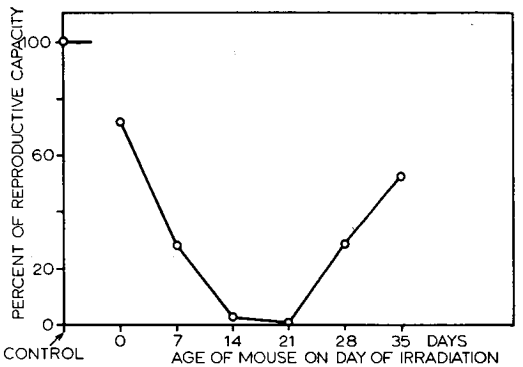

Fig. 5. Total reproductive capacity of female mice as a function of age when irradiated with 20 r. (Peters and Levy.)

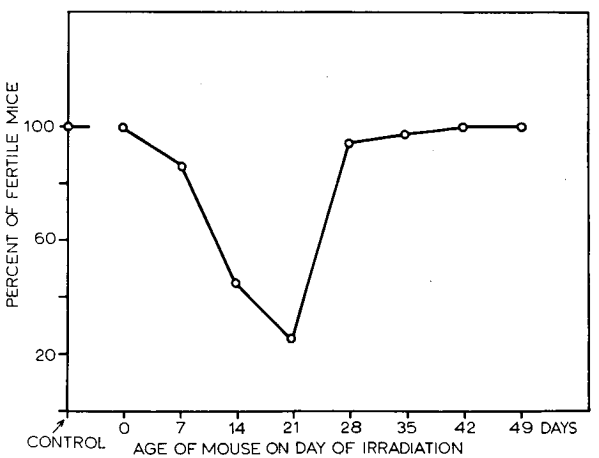

Fig. 6. Proportion of female mice not rendered sterile as a function of age when irradiated with 20 r X rays. (Peters and Levy.)

proportion of mice made completely sterile (Fig. 6). These curves agreed in showing very serious damage after 20 r given at 14 to

21 days. Irradiation at these ages also caused the reproductive life-span to be shorter. There was, however, an important difference between the radiosensitivity of sterility and that of total reproductive capacity. Sterility is very small for irradiation at birth or after 4 weeks of age, but the reduction in total number of offspring is substantial after 20 r given at all the ages tested. Some of this difference was due to cannibalism of litters at birth, which varied markedly with age at irradiation in the same way. Further, Peters and Levy presented curves of rate of production of offspring versus age, and found no improvement in the damaged reproductive capacity even after a latent period. The total number of offspring is thus shown to be a very sensitive index indeed of radiation damage even at ages outside the most dangerous 2 to 4 week period in mice.

Lindop, Morgan and Rotblat[g]) obtained results on fertility in female mice in general agreement with Peters and Levy's conclusions. They found that 1 day old mice were less sensitive than at 7 or 21 days of age; and further, were also less sensitive than at 15 or 35 weeks of age. They went on to study the effect on fertility of radioprotection by hypoxia.

3. PHYSIOLOGICAL PARAMETERS WHICH MIGHT CAUSE RADIOSENSITIVITY TO VARY WITH AGE

The effects described thus far all show a maximum radiosensitivity in mice at about 3 to 4 weeks of age, so that there is some substance in the question: can there be some general sensitivity to ionizing radiation which is common to several mechanisms of injury?

Possible candidates for an "intrinsic factor" which may determine the age variation of radiosensitivity include (i) oxygen tension in certain vital tissues such as bone marrow, (ii) the rate of change of weight of mice, i.e. growth rate and (iii) hormonal effects.

A. Oxygen tension in vital organs

Lindop and Rotblat[16]) have previously excluded the age variation of oxygen tension as a main factor in the age dependence of radiosensitivity, with the possible exception of the neonatal period. Lindop, Morgan and Rotblat have carried out experiments to study the effect of hypoxic protection upon fertility in young mice, but the results are not yet sufficient to establish a pattern of age variation. Their finding of a "failure to protect" in 7 day old

mice does not by itself rule out the hypothesis, because the result is probably simply due to the extremely high sensitivity of reproductive capacity at that age, which meant that the doses were too high to leave any mice fertile, whether they were breathing nitrogen or air.

B. The rate of increase of weight

It has previously been pointed out[16]) that the period of maximum sensitivity to radiation in mice corresponds to the peak in the rate of change of body weight (g/day) occurring at 3 to 4 weeks of age (Fig. 7). Hazzeldine and Lindop[b]) have attempted to investi-

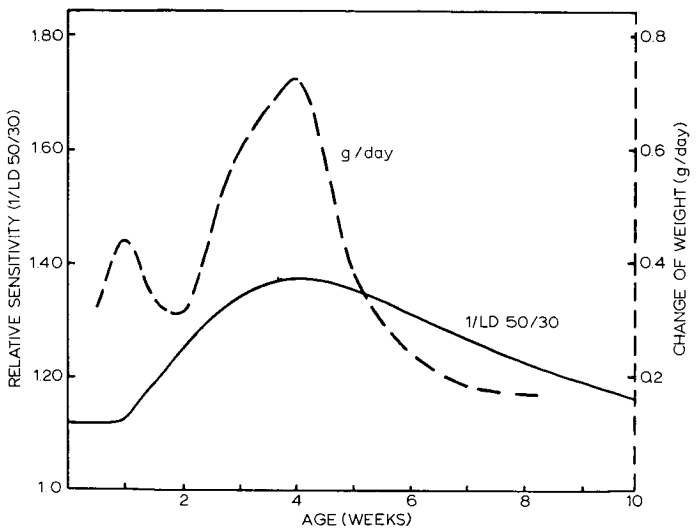

Fig. 7. Rate of increase of weight in mice *vs* age (dotted line), and relative radiosensitivity *vs* age (full line). (Hazzeldine and Lindop.)

gate the interdependence of age, weight, and radiation sensitivity by irradiating groups of mice of the same chronological age but different body weight, i.e. different physiological age. Some groups were fasted to reduce their weight and rate of growth. Two ages were chosen: 27 weeks when the radiosensitivity varies slowly with age, and 34 days when it is at its maximum value. Experiments with the 34 day old mice gave a clear result. Any change in the "physiological age" induced by fasting should give rise to a decreased radiosensitivity. The results showed, on the contrary, that the

sensitivity was increased by 20%. Therefore dietary-induced growth retardation certainly increased the radiation sensitivity of mice, as shown by other workers; but the retardation did not produce simply a change in physiological age. The desired separation of the parameters could not, in fact, be achieved in this way. It is still possible that the many changes which must be taking place at the time of maximum growth rate are connected in some way with the high radiosensitivity of a number of physiological effects in mice.

C. Hormonal factors

Sacher and Grahn[1]) have investigated the radiosensitivity of mice by means of continuous exposures starting at a certain age and continuing until death, which took place 20–160 days later depending upon the dose per day. They concluded that the effects of gonadectomy, and the correlation of radiosensitivity with the course of growth, suggested that the actions of oestrogens and androgens on the pituitary secretions, together with the suppression of oestrogens by radiation exposure, might be important factors in the variation of radiosensitivity with age. Their conclusions referred to mice aged 100 to 600 days; but the possibility obviously arises of some effect of radiation, either direct or abscopal, on pituitary function in infant mice.

4. EFFECTS IN MICE WHICH VARY WITH AGE DIFFERENTLY FROM LD 50/30

The right-hand side of Table 1 summarizes results which showed a

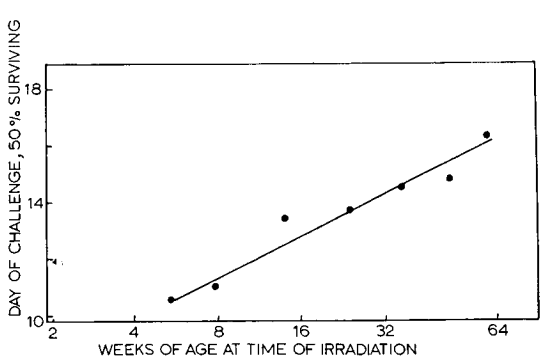

Fig. 8. Time to recovery of ability to reject homologous (DBA) ascites cells in LAF_1 mice after 420 r. (Smith and Alderman.)

different pattern of radiosensitivity at various ages from that just described for LD 50 and the other effects in Table 1.

A. Immunological response

Smith and Alderman[k]) measured the rate at which mice recovered their immune response after a whole body dose of 420 r by finding the time after irradiation at which 50% of the mice could survive an injection of 10^6 homologous lymphosarcoma ascites cells. Young mice recovered this immunity faster than did older mice (Fig. 8). The increase in apparent "sensitivity" with age is in marked contrast to the decreasing sensitivity with age after 5 weeks for the other effects so far discussed, and shows that immune response cannot be a major factor in the age variation, at least for mice older than 5½ weeks. (This was the youngest age tested.) It is likely, however, that this experiment measures the rate of recovery of immune response at various ages to a greater extent than it measures the radiation damage to the system.

This brings up the point that two main factors are always involved in measurements of "radiosensitivity". One is the amount of damage actually produced; the other is its modification during the time taken for it to become apparent.

B. Lens weight

R. L. Straube[l]) found that the weight of the lens of the mouse eye depended rather precisely upon age, but was unaffected by whole body doses of 400 to 500 r given at 8 to 13 weeks of age, even though the lenses were still actively growing in 8 week old mice. In a second experiment, Straube found that regeneration of liver was affected both by radiation and age in a similar way. A third system did not vary with age or with irradiation: this was the colonisation of the spleen of lethally irradiated mice by marrow from rats irradiated with 500 r at 1 to 5 or 18 to 24 months of age.

It is no surprise to find a process which is affected neither by radiation nor by age; and if a process varies with age but not with radiation, then possibly the sensitive time to irradiate has not been discovered. This brings up again the point that the time required for any effect to appear must be taken into account, and this will depend upon rate of physiological repair or of organ development, or cellular repopulation, as well as upon the degree of immediate radiation damage.

C. Intercapillary glomerular sclerosis

Guttman and Kohn[a]) have previously described the lesion of intercapillary glomerular sclerosis (IGS) in mice. Cellular and ground-substance changes in the spaces between capillaries in the kidney can be seen histologically, and graded on a scale of four. The changes are progressive with age in normal mice, and they are accelerated by irradiation, as already shown for large doses (several hundred rad) and mice over 58 days old. The present experiments were carried out mainly to determine the radiosensitivity of the effect in the unexplored range from late gestation to 54 days of age. The resulting grades of IGS, sampled either at 100 or 195 days, are shown in Fig. 9. The grading was doubled after 250 rad in 1 day

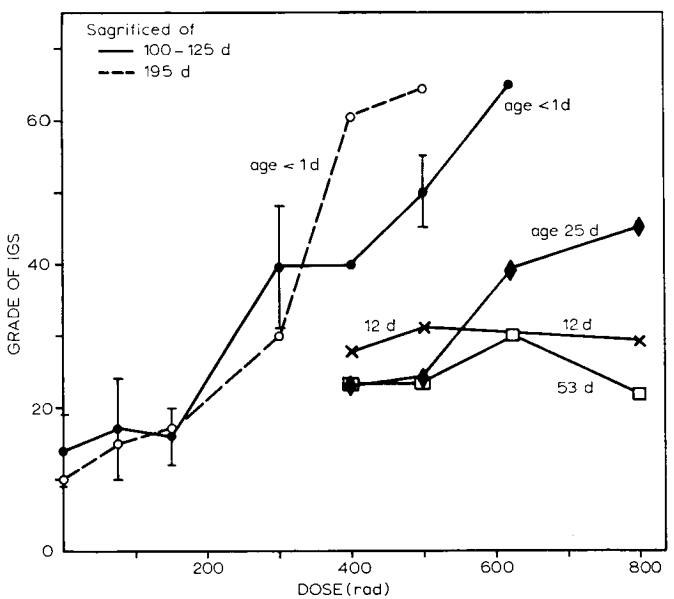

Fig. 9. Grade of intercapillary glomerular sclerosis (IGS) *vs* dose for mice of various ages; one curve for each age group. (Guttman and Kohn.)

old mice, 400–500 rad in 12 day old mice, and 500–600 rad in 53 day old mice. The conclusions were, first, that all these young mice showed much greater damage than one year old mice, and second, that the changes produced in one day old mice were greater than in the 12 day old animals. The latter is the point of difference

from the LD 50 age variation. There appears from Fig. 9, however, to be a greater effect in 25 day old mice given doses higher than 600 rad, than in 12 or 53 day old mice, suggesting a possible second peak of greater sensitivity at 25 days. The differences were, however, barely significant in this experiment.

Two possible mechanisms for the greater sensitivity at 1 than at 12 days of age were discussed by the authors. (i) there is a change of glomerular sensitivity with age, and (ii) the sensitivity to X rays remains constant, but the rate of the processes necessary for the development of visible damage may be slower after 12 days of age than before. Experiments giving equal doses to 1 day and 12 day old animals will help to distinguish between these mechanisms. If (i) is correct, mice irradiated when 1 day old will, at all subsequent times, show increasingly greater gradings of IGS than those irradiated at 12 days. If (ii) is correct, the difference will change with time, being greater at, say 200 days when the damage in the older animals will be only half developed, and decreasing by 300 days when it is fully developed in both age groups; experiments are in progress to determine this difference. The authors suggest the possibility that a delay between irradiation and the appearance of IGS might be due to the activation of an auto-immunity response, assuming that X rays produce changes which are antigenic. The host reactions would be of greater magnitude at birth than later in

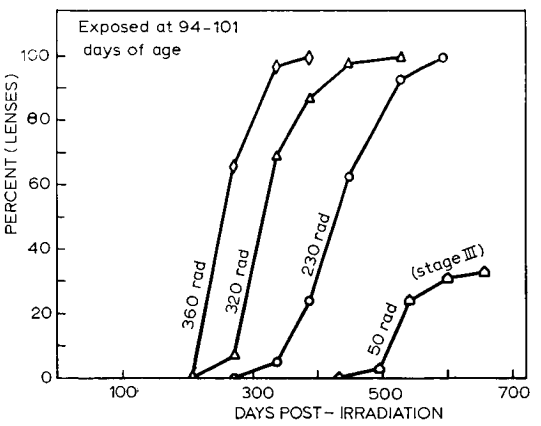

Fig. 10. Proportion of rat lenses showing complete (Stage IV) opacity as a function of time after the irradiation of one age group by 360, 320 or 230 rad fast neutrons, and stage III after 50 rad. (Kimeldorf.)

life, and would be consistent with the findings, both of this experiment[a]) and those of paper[k]) reported in Section 4.A above.

5. EXPERIMENTS WITH RATS: CATARACT INDUCTION

D. J. Kimeldorf[d]) used cyclotron-produced fast neutrons (p on Be) with a spectrum similar to that of fission neutrons. One experiment showed that doses exceeding 230 rad induced complete lens opacity, but that 50 rad never did so. The higher doses caused the opacification to begin sooner (Fig. 10). A second experiment showed that in groups of different ages given the same dose, the onset of opacification appeared at the same interval after irradiation for

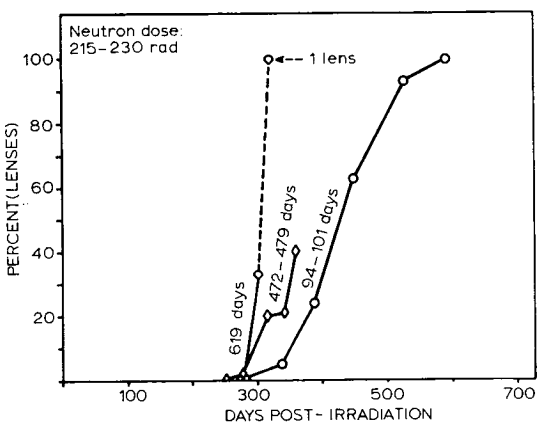

Fig. 11. Proportion of rat lenses showing complete opacity as a function of time after the irradiation with 215 to 230 rad fast neutrons of three different age groups. (Kimeldorf.)

all ages, but that it progressed more rapidly in the older rats (Fig. 11).

This finding is difficult to explain, and further discussion is necessary.

6. EXPERIMENTS WITH RATS: IS THE VARIATION OF RADIOSENSITIVITY WITH AGE SIMILAR TO THAT IN MICE?

Two of the papers compared fertility in rats aged 8 and 17 days, and both papers contained a number of other interesting results. The question arises as to whether other mammals than mice show

a peak of radiosensitivity within the first weeks of life. The summary of results concerning this question is given in Table 2.

TABLE 2

Rats

Effects in which radiosensitivity was found to be:

Greater at 17 days than 8 days	Greater at 8 days than 17 days
	LD 100/30 (ref.[f]))
	LD 50/30 (ref.[3a]))
	Reduced litter size
Retardation in weight[f])	(males – slight effect)[f])
	(females – definite[f])
Delay in producing first offspring (males)[f])	Sterility (males)[f&J])
	(females)[f])
	Histological reduction in number of spermatogonia[f])

A. Lethal dose

Leonard, Lambiet and Maisin[f]) showed that the LD 100/30 days in rats increased from about 350 r at 8 days of age through 450 r

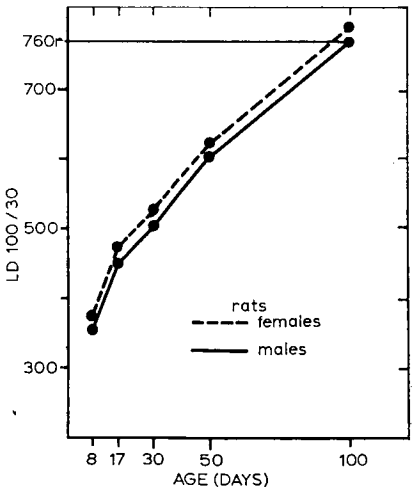

Fig. 12. LD 100/30 as a function of age and sex in rats. There is no maximum of radiosensitivity at 3 to 4 weeks, as seen in mice (cf. Figs. 1 and 3). (Léonard, Lambiet and Maisin.)

at 17 days to 760 r at 100 days (Fig. 12). This result, being based on LD 100, cannot be considered to be of high accuracy, but it is in agreement with some LD 50/30 results on rats obtained in 1955 by

Sikov and Noonan[3a]), quoted in reference[3]). It appears that rats may show a different pattern from mice, in not having a peak of radiosensitivity at 3 to 4 weeks of age; their sensitivity immediately after birth is greater than that of mice.

B. Retardation in growth rate

Leonard, Lambiet and Maisin[f]) went on to show that the reduction in weight compared with controls produced by a dose of 250 r was negligible for 8 day old rats, but was 25% for 17 day old animals. The difference was greater in rats protected with 200 mg/Kg AET and given 500 to 600 r. These results suggest that a peak in radiosensitivity for this effect may after all be present in rats at or after 17 days. After five months the irradiated rats had regained the weight of control animals.

C. Fertility of female rats

Female rats showed much greater disturbances of reproductive function than males.

17 days old: After 200–250 r to unprotected, or 500–600 r to AET-protected, females some delay between the first mating and the birth of progeny was found, and also slightly reduced numbers of offspring per litter[f]).

8 days old: All unprotected females given 200–250 r were sterile, as were most of those given 500 r with AET. Unprotected females given 50–100 r showed some delay in producing offspring, and produced less than half the normal number of offspring per litter[f]).

This result shows clearly a greater radiosensitivity of 8 day old compared with 17 day old rats for damage leading to reduced number of offspring. The sensitivity of these rats was less than that of mice aged 14–21 days. (Figs. 5 and 6).

D. Fertility of male rats

Leonard, Lambiet and Maisin[f]) found that the radiation-induced delay in the birth of progeny was greater in male rats irradiated at age 17 days than 8 days. This was true with and without 200 mg/Kg AET protection (Table 2). The rats were mated with unirradiated females 100 days after irradiation. The numbers of offspring produced in the first litter were normal for the 17 day, but slightly reduced for the 8 day old rats. These two effects therefore show opposing trends in age *vs* radiosensitivity.

Histological examinations showed, however, that fewer spermatogonia were present in the tubules (70 days after the irradiation)

of 8 day than 17 day old rats: this latter effect was more sensitive at the earlier than the later age.

Savkovic, Radivojevic and Hajdukovic[j]) confirmed the latter trend, showing quite definitely that fertility was reduced much more by 600 r (whole body) to 8 day old than to 17 day old male rats (to 14% compared with 25%). Cysteamine gave better protection to the younger group than to the 17 day old rats, as did AET and also homologous DNA administered immediately after irradiation. Local irradiation of the testes gave a broadly similar result, except that the reduction in fertility was not so great, and cysteamine provided no protection.

With both head and testes locally irradiated, this difference between 8 day and 17 day old animals was also clearly shown (26% and 70% fertile respectively). When only the head was irradiated, no significant reduction in fertility was caused, even by 600 r which reduced the pituitary gland weight to half normal.

The differences in sensitivity appear, on balance, to favour an opposite trend in rats to that in mice at 8 and 17 days of age (Table 2), although further results would be valuable.

The progeny of the irradiated males were studied by Savkovic and her colleagues[j]) with some curious results. First, the surviving progeny showed no reduction in growth rate after 600 r local irradiation of the testes, but they did show a reduced growth rate by 25% after 600 r local irradiation of the head only, or after local irradiation of the head and testes, or after 600 r whole body irradiation. Second, some abnormal offspring (e.g. with cataracts) were observed among the progeny of animals given 600 r local irradiation of the head only. Could these be due to a genetic effect produced in some way, possibly as a consequence of pituitary disturbance? Dr. Savkovic has informed me that the second generation offspring also show a marked weight reduction, so the effect must be a genetic one, from irradiation of the head only.

7. RADIOSENSITIVITY OF THE MOUSE FOETUS

The final paper concerns radiosensitivity of the mouse foetus at various ages during gestation, with respect to different kinds of abnormality. Doses of 50–400 r were given, most often about 200 r. Langendorff, Kriegel and Shibata[e]) have obtained a detailed picture of the variation of radiosensitivity with day of gestation for

abnormalities of the tail and limbs (left side of Fig. 13), and also for the lethal abnormalities of brain, eyes, nose and ventral closure (right side of Fig. 13). The very short period of high sensitivity for a given abnormality is demonstrated particularly well for limb de-

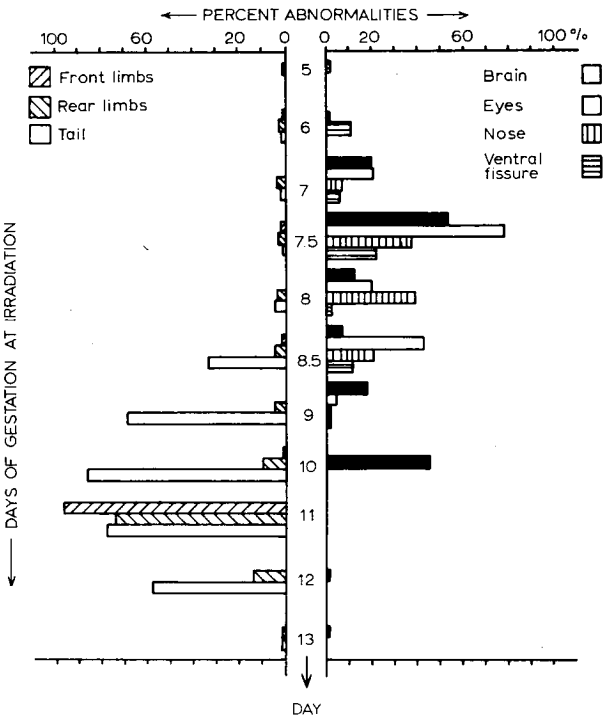

Fig. 13. Prenatal macroscopically recognizable deformities in the mouse after X irradiation during pregnancy, in percentage of abnormal relative to normal foetuses. (Langendorff, Kriegel and Shibata.) *Left*: Abnormalities consistent with life. *Right*: Serious, usually fatal, abnormalities.

formities at the eleventh day. The effect of different doses was also investigated (Fig. 14). The increase in proportion of abnormal foetuses with dose shown in Fig. 14a is accompanied by more foetal deaths (Fig. 14b), thus giving rise to the optimum dose of 175 to 200 r for the production of viable deformities in mice (Fig. 14c). These results are very similar to those reported previously by Russell[14]) and Russell and Russell[15]).

Such results are examples of the type of information which is

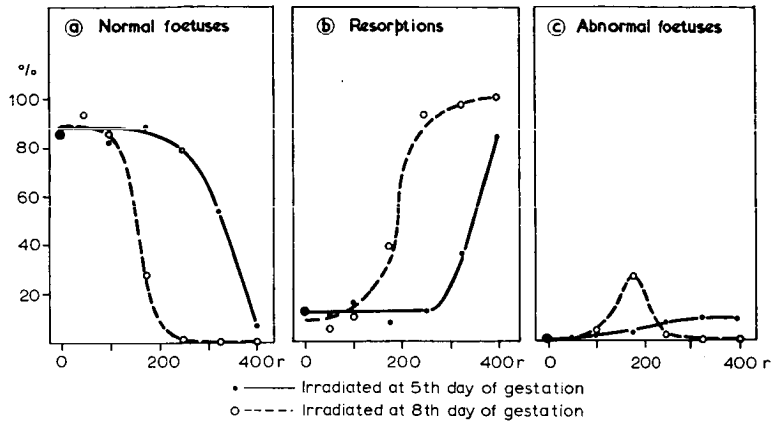

Fig. 14. Effect of varying doses, given on 5th or 8th day of gestation, on embryonic development in mice. (Langendorff, Kriegel and Shibata.) (a) Proportion of normal foetuses found at operation on 18th day. (b) Proportion of dead foetuses. (c) Proportion of deformed foetuses.

required for other kinds of radiation-induced damage, i.e. a specific time is known when the process is at its most sensitive to damage, and the relevant dose-effect curves have been explored. It is, of course, in principle easier to determine these things in the foetus than in older animals because of the grossness of the effects studied, and because their development takes place quickly so that problems of time taken for the damage to develop until it is visible do not arise.

8. CONCLUSION

To attempt to sum up what is already a very attenuated summary of the relevant results of twelve papers, I shall say:

(a) Several important physiological effects in mice in addition to LD 50/30 have been well established as showing a peak of radiosensitivity at 3 to 4 weeks of age.

(b) In other effects which show different variations of radiosensitivity with age, considerations of the time taken for the damage to appear, and of other processes which might mask or repair the damage, i.e. population kinetics, are probably predominant.

(c) The age variation of radiosensitivity in rats seems to differ from that in mice, over the first few weeks of life.

The following points of interest also arose:

(d) The total reproductive capacity of female mice is an ex-

tremely sensitive measure of radiosensitivity, more so than sterility except at 14 to 21 days of age, and is affected by a few rad given to infant mice.

(e) Irradiation of the head alone of rats was found in one investigation to cause the offspring to show a retardation in weight of some 25%, and some foetal abnormalities. These experiments must be repeated.

It is well recognized that problems of *in vivo* "radiosensitivity" must be studied with the appropriate population kinetics in mind, and the question of radiosensitivity at various ages of irradiation is no exception.

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DISCUSSION

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Smith[k]) agreed with the rapporteur's suggestion of the possible rôle of the age dependence of the ability to develop an immune

response, in contrast to the degree of damage, in her experiments on the failure to reject homologous tumour cells in irradiated mice of different ages. A similar process might also be operative in granulocyte and lymphocyte recovery after irradiation, and even in the LD 50/30 experiments, since it is not logically necessary that recovery processes should be solely dependent upon initial damage as such in animals of different ages.

Kimeldorf[d]) referred to his observations on lens opacification. A speculative explanation for the more intense rate of opacification following irradiation in later life might lie in the lens being a self-contained system whose radiosensitivity is largely due to those cells which are in mitosis. Since the mitotic index is known to decrease with age in some systems, the same radiation dose might be more effective in an older animal with fewer surviving mitotic cells.

Sacher[i]) suggested that the apparent simplicity of the age dependence of radiosensitivity at later ages is deceptive. Although most data indicate a plateau region from 200 to 500 days followed by an increase in radiosensitivity, there is also a greater increase in variability of response with age. Some of the oldest animals exhibit as great a resistance to radiation as the younger animals, which suggests that there are perhaps two processes involved — an increasing morbidity with age as well as a real systemic ageing process. At later ages the increased sensitivity would be mostly due to increased morbidity.

Rogers (*Dept. of Anatomy, Univ. of Birmingham, Great Britain*) had the impression that there was a peak in the mortality curve for mice at about three weeks, near the time of weaning, which might be relevant to the radiosensitivity data. Rotblat[g]) confirmed that there was a slight peak at 2 to 3 weeks, but he was not sure of its relevance in view of other factors, such as growth rate, which also changed at this time. Curtis (*Brookhaven Nat. Lab., Upton, NY, USA*) added that at the other end of the age range the drop in LD 50/30 was again paralleled by the mortality curve, an observation which had given rise to speculation in the past.

Cole (*US Naval Radiol. Defense Lab., San Francisco, Cal., USA*) referred to some observations made with Rosen (*Chester Beatty, London SW7, Great Britain*) on LAF_1 mice irradiated with 690 r of X rays on the day of birth. Three effects were noted:

(1) Intercapillary glomerulosclerosis was much greater after 6 to

9 months than in mice irradiated at 3 months with the same dose, in agreement with the results of Guttman and Kohn[a]).

(2) Severe growth reduction occurred during the first three months, and the animals subsequently remained small for the duration of their lives.

(3) The hair turned grey at 3 to 5 weeks, but the normal dark brown colour reappeared during the second month and persisted until the animals were several months old. This resistance to greying in new born mice seems analogous to the effect on fertility observed by several of the authors.

Lindop[g]) suggested that the resistance of the new-born mouse ovary to radiation might lie in the existence in the ovary at this stage of cells with a greater developmental potential than at later ages. Although the number of cells surviving irradiation at different ages might be the same, the damage would appear to be less in the new-born animal. A similar situation existing with cells at the base of the hair follicle might account for Cole's results.

Mole (*M.R.C., Harwell, Berks., Great Britain*) drew attention to the absence of hair in new-born rats and mice, and their inability to regulate body temperature compared with 30-day old animals. In view of the effect of body temperature on radiosensitivity he wondered whether Peters and Levy[h]) and Lindop and her colleagues had taken precautions to avoid this complication, and whether it might account for the difference between young rats and mice due to differing experimental techniques. Peters agreed with the pertinence of Mole's remarks, but said that her mice had only been away from the mother for one minute during irradiation. Whether this was sufficient to cause appreciable cooling was not known. (She added in answer to a question by Fowler that in her experiments, the heads of the mice had been shielded to avoid pituitary complications.) Lindop also agreed with Mole and cited some of her earlier work with unheated gases passed over mice, which might have affected the experimental results. In the present experiments all the gases had been heated to body temperature. It was observed, however, that the sensitivity of one day old mice could be increased by breathing oxygen, whereas that of adult animals was hardly affected. Thus the possibility of increasing the sensitivity in the new-born mouse by oxygen persisted despite temperature control.

Kohn[a]) said that he had measured the LD 50 for 1-day old rats

and mice after allowing them to cool to room temperature for 20 to 30 min. The rat was more sensitive than the mouse. At later ages the rat became appreciably more resistant. At the other end of the life span, 2-year old female mice given a single dose of 260 r or two doses each of 260 r one week apart showed either increased or unaffected survival, despite the rapid decline in LD 50 at this age.

Shellabarger (*Univ. of Michigan, Ann Arbor, Mich., USA*) pointed out that the age dependence of radiosensitivity seemed to parallel that of thyroid function, which in turn is related to body temperature and many other physiological processes.

Upton (*Oak Ridge Nat. Lab., Tenn., USA*) asked Lindop whether the effect of underfeeding mice applied equally well to life-shortening as to acute lethality, since underfed animals might not stand the acute stress of the LD 50 exposure so well. Lindop replied that only acute effects had been studied. In reply to a suggestion by Thiemann (*Jülich, Germany*) that food differences between young and adult mice might result in different secondary processes and physical spreading of energy dissipation, Lindop answered that differences in food certainly led to different intestinal flora, and information on this might be important for lethality in the age range from 1 day to 3 or 4 weeks. A reason for the high resistance of the new-born mouse might lie in the absence of intestinal flora.

Murphree (*Univ. Tennessee Agr. Res. Lab., Oak Ridge, Tenn., USA*) said that 15 to 18 month old cattle were 20% more sensitive to radiation than 4 to 5 year old animals, and presumably temperature differences in such a species were not important. Also pigs fed different nutritional levels and exposed to chronic irradiation showed no difference in sensitivity.

Referring to the period before birth, Schwarz (*Philadelphia Gen. Hosp., Philadelphia, USA*) said that his work with Riggs and Vross on brain lesions following irradiation *in utero* depended on the time of gestation. Mistakes could arise by trying to evaluate the effect of irradiation on a metabolic or enzymatic system without reference to its degree of development.

Rajewsky (*Max Planck Inst. Biophys., Frankfurt am Main, Germany*) asked how the authors had ensured homogeneity of irradiation, what degree had been achieved, and whether it was the same for all ages. Smith[k]) replied that for each irradiation they had used vessels containing 10 or 20 animals belonging to two or three age

groups. Sacher[i]) suggested that the important heterogeneity was biological rather than physical, e.g. disease, and that the physiological state of each animal at irradiation needs to be known. Cohen (*Radiother. Dept. Gen. Hosp. Johannesburg, South Africa*) thought that physical heterogeneity could not be so easily dismissed, since it was known that the proportion of high atomic number elements changed with age. For example, the increase in calcium during skeletal development could influence the dose to the haemopoietic system. Rotblat[g]) agreed with the importance of avoiding dose "hot spots" due to high atomic number material, and for that reason in their experiments high energy radiation was used. The dose variation with depth and over the surface of the animal had been less than 2%. As regards biological variation, the most essential factor in age dependence was surely age itself. Rajewsky agreed that high energy radiation afforded some improvement, but it was not ideal and the animals should be rotated. The effect was not simply physical, but was an important biological factor since the degree of homogeneity in different organs might vary with age. Woodard (*Sloan Kettering Inst., New York, USA*) pointed out that the amount of energy absorbed by the mineral in the skeletons of rats and mice is known, and amounts to an increase in dose of about 5% to adult mouse bone marrow in whole body exposure to 250 KV X rays. She added that, by correcting the marrow dose, Sinclair (*Argonne Nat. Lab., Ill., USA*) has been able to bring the RBE figures for lethality in rats and mice into harmony with those for uncalcified organisms.

Radiopathology of Mammals

RADIATION HAEMATOLOGY

E. V. HULSE

Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berks., Great Britain

a) S. DAVYDOFF-ALIBERT and B. BROUILLET,
Measurement of the mitotic index in individuals subjected to therapeutic irradiations.
Centre d'Etudes Nucléaires, Fontenay-aux-Roses, France

b) J.-F. DUPLAN,
Effect of X ray irradiation on the haemopoietic function of the liver of a mouse foetus.
Laboratoire Pasteur, L'Institut du Radium, Paris, France

c) A. FORSSBERG, B. TRIBUKAIT and C. ÖSTERDAHL,
Radiation effects on permeability of membranes and white cell mobility in the animal body.
Department of Radiobiology, Karolinska Institutet and Department of Physiology, Karolinska Sjukhuset, Stockholm, Sweden

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The leukocyte response following upper body X irradiation of beagles.
Armed Forces Radiobiology Institute, National Naval Medical Center, Bethesda 14, Maryland, and Atomic Energy Project, University of Rochester School of Medicine and Dentistry, Rochester 20, New York, USA

e) F. C. LUDWIG and H. I. KOHN,
Marrow response to whole-body X irradiation in rodents of different LD 50.
AEC Radiological Laboratory, University of California Medical Center, San Francisco, California, USA

f) M. A. MALONEY, H. M. PATT and C. L. WEBER,
Comparative analysis of neutrophil kinetics in X irradiated dog and monkey.
Argonne National Laboratory, Argonne, Illinois, USA

g) H.-J. MAUSS,
The effect of ionizing radiation on iron metabolism.
Universitätsfrauenklinik, Hamburg 20, Germany

h) W. K. METCALF,
The effects of whole-body irradiation on the nitrite sensitivity of intracorpuscular haemoglobin in rats.
Department of Anatomy, the University of Bristol, Great Britain

i) D. G. OSMOND,
Effects of gamma irradiation on lymphocyte production in the guinea-pig.
University of Washington, USA

[j]) J. N. SHIVELY, A. R. WARNER, JR., H. P. MILLER, H. J. KURTZ, H. L. ANDREWS and K. T. WOODWARD,
Study of swine surviving exposure to the gamma-neutron flux of a nuclear detonation.
Walter Reed Army Institute of Research and the National Cancer Institute, Bethesda, Maryland, USA

[k]) R. O.SPERTZEL, T. J. BUCCI and M. INGRAM,
Platelet size distribution following X irradiation.
University of Rochester School of Medicine and Dentistry, Atomic Energy Project, Rochester, New York, USA

[l]) L. SZTANYIK, O. GESZTI AND E. MANDI,
Effects of whole-body X irradiation on iron metabolism of rabbits and mice.
Institute for Radiobiological Research, Budapest XXII, Hungary

1. INTRODUCTION

As early as 1903 it was discovered that X rays could produce changes in the haemopoietic tissues (Senn,[1,2]) and some excellent radiation haematology was done during the next few years. Heineke's[3,4,5]) pioneer work is very well known but other very early workers (e.g. Milchner and Mosse[6]); Aubertin and Beaujard[7]); Morris[8]); Benjamin, v. Reuss, Sluka and Schwarz[9]); Krause and Ziegler[10]), and Warthin)[11]), also had a remarkable insight into the changes which followed irradiation and the problems which they posed. That the subject still has its fascination and, indeed, its problems is clearly demonstrated by the present series of papers. They also demonstrate how many facets there are to radiation haematology and how many techniques may be used.

2. CHANGES IN PERIPHERAL BLOOD

Three of the papers in this session are based on the simplest of all haematological investigations, namely, examination of the peripheral blood. The increase in neutrophil granulocytes which occurs shortly after irradiation is not as well recognized as it ought to be and it is certainly not easy to explain. However Forssberg, Tribukait and Österdahl[c]) present data which suggest that irradiation in the region of the liver is important in producing the increase. Whole-body irradiation of guinea-pigs with 300 r of X rays gave the expected increase in neutrophils 4 hours after exposure, and irradiation of the right half of the body or a small field (2.5 × 3.0 cm) over the liver also produced a neutrophilia. Irradiation of the left half of the body or the same sized field over the left part of the in-

testine did not produce a neutrophilia. Maloney and Patt[12]), who are also presenting a paper in this session, considered that the early neutrophilia after irradiation was due to mobilization from vascular reservoirs. It is interesting to speculate whether Forssberg *et al.* have identified an important area of that nature or whether other factors, e.g. hormonal, are at work. They also report a statistically significant increase in the numbers of blood lymphocytes after irradiation of the small field on the left side of the abdomen i.e. the side which did not initiate a neutrophilia.

Leucocyte changes after half-body irradiation are also reported by Hansen, Michaelson and Howland[d]). They were concerned mainly with recovery and their dogs were irradiated over the cranial half only. With their lower doses i.e. 800 r and 1000 r, the shielded marrow was capable of producing enough cells to prevent death. One might imagine that the shielded marrow would be able to colonize the damaged marrow, but in spite of this possibility the granulocytes and lymphocytes were still below normal at 60 days after irradiation, in one instance being 55 to 60 per cent of normal. Animals given higher doses, i.e. 1500 r and 1750 r, developed infective lesions of the mouth with ulceration and haemorrhage. Those which survived produced a sharp rise in granulocytes at that time, up to 133 per cent of the initial count. It is interesting to note that, although recovery was by no means complete at 60 days, these animals could produce sufficient granulocytes to cope with a severe infection as early as 21 days after irradiation.

Shively, Warner, Miller, Kurtz, Andrews and Woodward[j]) watched the recovery of swine which had been exposed to gamma rays and neutrons from a nuclear detonation. During the first 2 years the chief clinical difference from the control group was that the survivors of a mid-lethal dose gained less weight. The erythrocyte sedimentation rate, haematocrit and haemoglobin of the irradiated animals did not differ from those of the controls, and the reticulocytes and platelets returned to normal at 2 months after irradiation. The white cells took longer to recover, those of the mid-lethal group returning to normal at 10 months. The authors recall that the white cell count in burros took 2 years to recover, and that of the Marshall Islanders even longer. White cell recovery in the swine was, therefore, much quicker than in other species. Such results should warn us of the danger of using experimental data from one species to

judge the time when recovery would be expected in another species (e.g. man). Some of Shively's swine were given a dose of X rays (380 r to 650 r) 4 months after the original experiment. In these animals a low white count persisted for 2 years.

3. COMPARISON OF DIFFERENT SPECIES

We have two other papers which compare haematological changes in different species. Maloney, Patt and Weber[f]) compared neutrophils in the dog and monkey. They first followed the appearance of tritium-labelled neutrophils in the blood of normal dogs and monkeys and concluded that the chief difference was that the monkeys had a larger reservoir of mature neutrophils in the marrow. After 1000 r of X rays the neutropaenia developed very similarly in both species except that it began about a day later in the monkey, presumably because the monkey has a larger reservoir of neutrophils in its marrow. It would be interesting to know whether there is any indication of the different size of these reservoirs in the myelograms of the two species. The authors state that they have also studied data from mice, rats, hamsters and rabbits, and conclude that the initial injury to the granulocyte series is similar in all species but that differences exist in the kinetics of recovery.

Ludwig and Kohn[e]) examined the bone marrow to see whether differences in radiation response could be correlated with differen-

TABLE 1

LD 50/28 days and total nucleated cells in the marrow of irradiated and unirradiated animals (Ludwig and Kohn).

	LD 50 (rad)	Total nucleated cells in marrow: Controls (cells per mm of shaft) ($\times 10^6$)	Irradiated as % of control: 4 days after 250 rad	10 days after 250 rad	18 days after 250 rad
BABL/c mice	540	1.8–2.3	34–29	77–52	66
C57Bl mice	620	2.0	61	96	106
BALB/c x C57Bl mice	645	1.7	39	97	89
A mice	630	0.78	38	63	–
Chinese hamster	825	0.5	54	70	119

ces in mean lethal dose (LD 50). Table 1 shows the 28 day mid-lethal doses of the animals they used viz.: 3 inbred strains of mice, a hybrid between two of the mouse strains and Chinese hamsters. A dose of 250 r, well below the LD 50, was used. The mouse with the lowest LD 50 (BALB/c) had the highest control marrow count, but the percentage of nucleated cells remaining in the marrow at 4 days was the lowest of the group. The 18 day percentage suggests that the strain took longer than the others to regain the control level, a finding which emphasizes Maloney *et al.*'s remarks on the importance of the kinetics of recovery. The LD 50 of the hybrid strain was higher than either of the parent strains, but the percentage of the nucleated cells at 4 and 18 days indicated an intermediate response between the two strains. The Chinese hamster had the highest LD 50 and the lowest control marrow count, but Ludwig and Kohn do not consider that the total marrow counts indicate a more rapid recovery. However the hamster's most immature undifferentiated cells were not decreased in numbers at times when the same cells were definitely low in the mice, and the authors think that this may be the reason for the hamsters' high LD 50.

It must not be presumed that a return to normal cellularity (Table 1) necessarily indicates that the marrow has permanently recovered. Hansen *et al.* have emphasized how long haemopoiesis takes to recover completely and a temporary return to normal values in haemopoietic tissues has frequently been reported. Indeed in this session Osmond's paper on lymphopoiesis demonstrates such a temporary recovery.

4. LYMPHOCYTES

Osmond[1]) irradiated anaesthetized guinea-pigs with 150 r of gamma rays and examined lymphocyte changes in a number of situations by means of tritiated thymidine labelling and, where possible, by total cell counts. He chose a relatively long post-thymidine interval in order to obtain near-maximal labelling of small lymphocytes. After irradiation there was a rapid reduction in lymphocytes of all sizes (Fig. 1). Shortly afterwards an increase in numbers occurred, and at 3 and 4 days after irradiation the ratio of labelled medium and large lymphocytes (i.e. those synthesizing DNA) to labelled small lymphocytes was much greater than usual. As a long post-thymidine interval of about 25 hr was used, Osmond considers that

this difference in the proportions of the two groups of cells was not merely due to the less mature cells being labelled first, and he concludes that the mitotic cycle was prolonged at that time. The temporary nature of the first increase became apparent at 11 days when

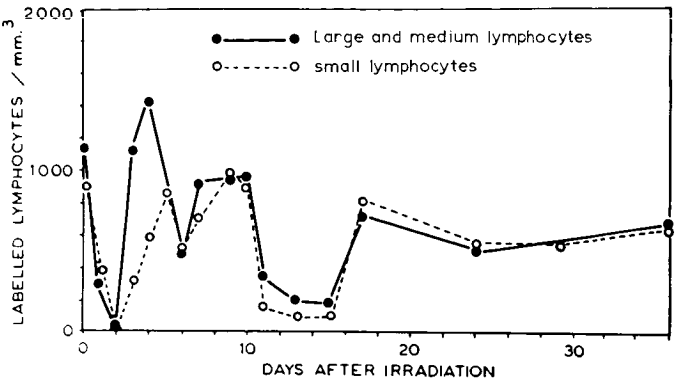

Fig. 1. Effect of whole-body irradiation (150 r) on the numbers of labelled small lymphocytes and labelled large and medium lymphocytes in thoracic duct lymph 24 to 36 hr after administration of tritiated thymidine (1 μC/g) (Osmond).

a second reduction occurred. Osmond suggests that this pattern of recovery may indicate that the initial increase in cell numbers is due to the proliferation of surviving but nevertheless damaged cells which die after a limited number of divisions. He thinks that the low number of labelled cells seen at 11 to 15 days may denote either changes in a dying population of cells or the appearance of new cell lines which at first have a longer generation time. Similar responses occurred in other situations but in the thymus the timing was different and this has made Osmond consider the possibility that there may be a prolonged retention of lymphocytes in that organ.

5. BONE MARROW RESPONSE IN MAN

In radiobiology we sometimes try to relate our observations on animals to possible human exposure. Davydoff-Alibert and Brouillet[a]) have done more than speculate. They have studied hospital patients directly, determining the mitotic index of bone marrow after therapeutic whole-body irradiation. Unfortunately none of

their patients had a normal mitotic index before irradiation and, of necessity, their observations were rather infrequent. Consequently they do not reach any general conclusions but nevertheless their findings are full of interest. Three patients were given a single dose of gamma rays and two patients received two doses with a four day interval between. After single doses of 100 rad or 250 rad the erythropoietic mitotic index was soon reduced (Fig. 2). Recovery

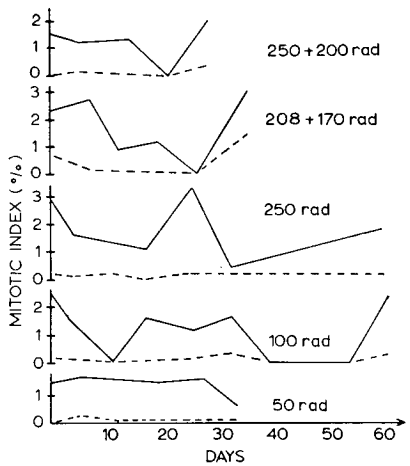

Fig. 2. Changes in the mitotic index of the erythropoietic and granulocyte series in the bone marrow of five patients given whole-body irradiation. Where two doses of radiation are quoted there was an interval of 4 days between the two irradiations (Davydoff-Alibert and Brouillet). ——— Erythropoiesis; - - - - - Myelopoiesis.

ensued but at about 30 days a second reduction occurred, and at the same time a similar reduction was seen in the patient who received 50 rad. The lack of an early fall of erythropoietic mitotic index in the patients given two doses may, as the authors suggest, be due to their first examinations in these patients being rather late. They think that the fall in this index which occurred between 20 and 30 days after two doses may be an earlier manifestation of the fall which occurred at 30 days in the patients who received a single dose. The patient given 208 + 170 rad (Fig. 2) was the only one with a normal granulopoietic mitotic index before irradiation and was the only one showing a convincing fall in that index.

6. FOETAL HAEMOPOIESIS

The effects of radiation on foetal haemopoiesis is only rarely studied, but Duplan[b]) has assembled some interesting data on radiation changes in the livers of foetal mice. The uterus of the mother was exteriorized and each foetus irradiated separately through the uterine wall, the doses being 240, 580 or 960 r of X rays. Irradiations were performed on the 13th, 14th or 15th day of intra-uterine life and the liver was examined daily for the next 3 or 4 days. The weight of the liver measures the effect of radiation on both the

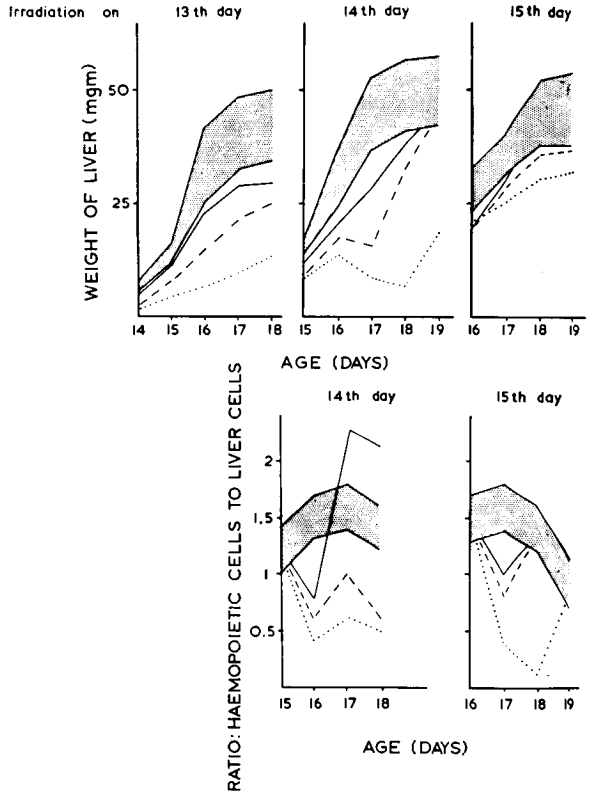

Fig. 3. *Upper 3 graphs*: changes in weight of foetal liver after irradiation on the 13th, 14th or 15th day of intrauterine life. *Lower 2 graphs*: changes in the ratio of haemopoietic cells to liver cells in the foetal liver after irradiation on the 14th or 15th day of intra-uterine life. (Duplan.)

Controls — — 580 r

——— 240 r ····· 960 r

haemopoietic tissue and the liver cells (Fig. 3, upper part). The changes observed depended on the age at which the foetus was irradiated. After irradiation of 13 day and 14 day foetuses the normal rate of increase in liver weight was retarded but after irradiation on the 15th day of foetal life the much less pronounced slowing down of growth rate occurred only after the highest dose.

The amount of change due to haemopoietic damage was assessed from the ratio between haemopoietic cells and liver cells (Fig. 3, lower part). Irradiation of the 13 day foetus did not alter this ratio, indicating that liver cells at that age were as sensitive as haemopoietic cells. Severe changes were noted when irradiation was on the 14th or 15th day. After irradiation on the 15th day signs of recovery were seen on the third day after irradiation but when a dose of 580 r or 960 r was given on the 14th day of intra-uterine life there were no signs of a return to the normal ratio during the experimental period. From previous experience with adult mice of the same strain Duplan thinks that the haemopoietic tissue of the 13 to 15 day foetus may be less sensitive than that of the adult mouse.

7. PLATELET SIZE

Spertzel, Bucci and Ingram[k]) studied an unusual parameter, namely

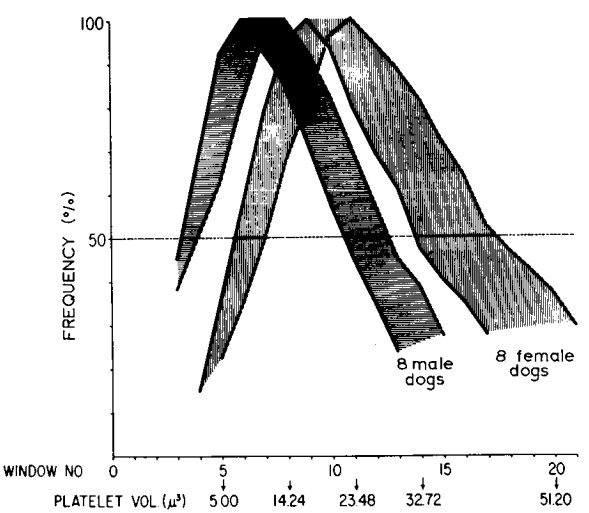

Fig. 4. Platelet volume distribution in male and female dogs. The volumes were measured with a Coulter Counter Model B; "window no." refers to the way in which particle sizes were recorded. (Spertzel, Bucci and Ingram.)

that of platelet size. By means of an automatic counter they measured the frequency in which platelets of different volumes were found in the blood of male and female dogs. Consecutive weekly samples taken from the same unirradiated animal were very consistent but there was a remarkable sex difference, platelets of females being larger and having greater range in size (Fig. 4). After establishing the normal size-pattern a group of animals was given 450 r of X rays. Changes after irradiation in one of the dogs are shown in Fig. 5, the size and size distribution being summarized by plotting the range of sizes into which 50 percent of the platelets fell. During the first seven days there was a slight decrease in the number of larger platelets, but from 11 to 21 days the thrombocytopaenia was too severe for measurements to be made. At 28 days the range of sizes was greater than normal and there was a shift towards larger sizes. At later times these increases subsided and pre-irradiation levels were regained at times ranging from 46 to 113 days after irradiation (Fig. 5).

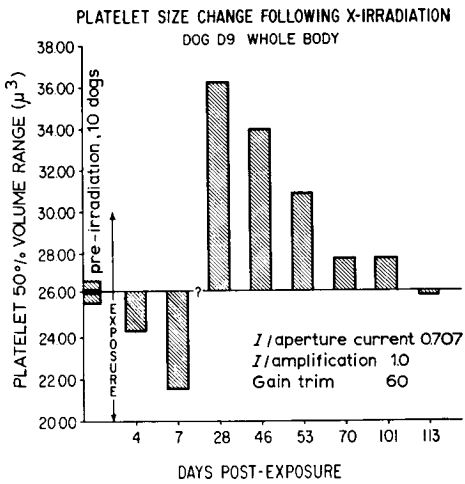

Fig. 5. Changes in platelet size in a dog given 450 r of whole-body X irradiation. (Spertzel, Bucci and Ingram.)

The authors have not as yet suggested any reason for these changes. The haematological literature contains occasional references to variations in platelet size (Wintrobe[13])) so presumably the changes they describe are not confined to radiation damage. It

may be worth considering whether the contractile vacuoles which have been described in platelets (Bessis[14])) play any part in the changes in size after irradiation.

8. IRON METABOLISM

Three of our papers are concerned with the red cell series, two of them with iron metabolism. Sztanyik, Geszti and Mandi[1]), using ^{59}Fe, studied five different factors in whole-body irradiated rabbits and mice. They found that plasma iron clearance times were increased at one day after irradiation; the higher the dose the longer the injected ^{59}Fe took to leave the circulation (Fig. 6, upper graph).

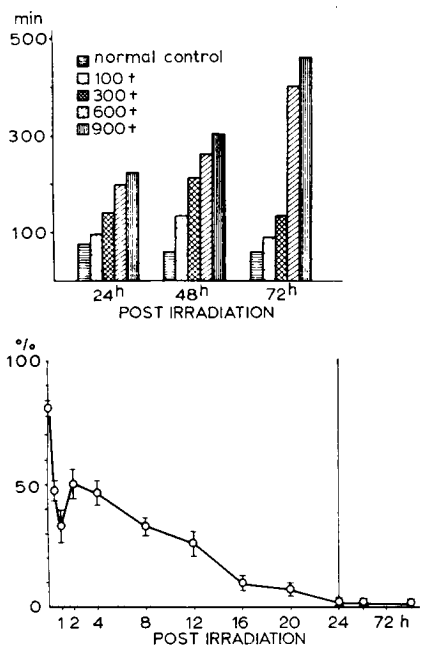

Fig. 6. *Upper graph*: Mean values of plasma iron clearance half-time of rabbits at various times after irradiation. *Lower graph*: Incorporation of ^{59}Fe into the erythrocytes of mice given 600 r of X rays. The abscissa gives the time after irradiation when the ^{59}Fe injection was given. The ordinate shows the activity of the peripheral blood 72 hr after ^{59}Fe injection expressed as a percentage of the injected dose. (Sztanyik, Geszti and Mandi.)

At 2 days the clearance times were even greater but at 3 days animals given 100 r and 300 r showed signs of recovery. The plasma iron concentration was increased after irradiation and remained high for 3 to 5 days, the maximal values being linearly related to the logarithm of the dose. The plasma iron turnover was decreased at one day but was normal on the second and third day. The amount of iron incorporated into the mature red cells in the blood (Fig. 6,

lower graph) was markedly reduced very soon after irradiation and was almost zero from 24 to 96 hr after 600 r. The authors also noted that after irradiation the proportion of iron in the liver and kidneys increased, but in the spleen it decreased.

Mauss[g]), who used rats for his experiments with ^{59}Fe, is also interested in the fate of the unused iron. He confined his attention to plasma iron clearance and plasma iron turnover and used part-body as well as whole-body irradiation. The two measurements agreed well, and the plasma iron turnover can be used to demonstrate his findings (Fig. 7). There was a marked reduction immediately

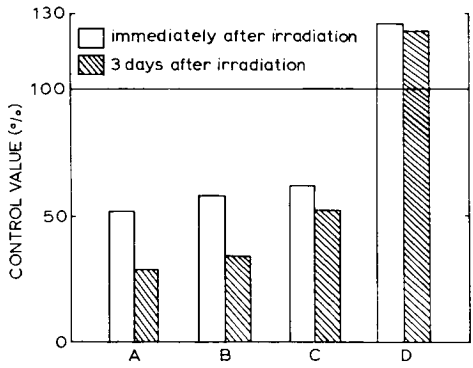

Fig. 7. Plasma iron turnover in rats after 1600 r. (Mauss.)
A Whole body irradiation
B Liver shielded
C Bone marrow only irradiated
D Liver only irradiated

after 1600 r of whole-body irradiation and an even greater reduction at 3 days after exposure. If the region of the liver was shielded, or if, by a series of shieldings, the irradiation was confined mainly to the bone marrow, the effect of the radiation was reduced. Similar but less pronounced changes occurred after 800 r. When an animal is given an intravenous dose of ^{59}Fe the iron may go to wherever erythropoiesis is taking place, and to those places where iron is normally stored. The liver is one of these stores, and Mauss found that when erythropoietic tissues were irradiated but the liver was not irradiated then the effect of the radiation was less (Fig. 7). He concludes that irradiation interferes with the storage of iron in the liver. He also irradiated the liver region by itself and found that the plasma iron turnover was increased (Fig. 7) and the plasma iron

concentration decreased. He thinks that in this case the irradiation may be causing iron to leave the liver. Other workers who have used similar techniques will no doubt wish to discuss, both statistically and theoretically, the significance of these findings. It is interesting, however, that out of these twelve varied haematological papers four are concerned in one way or another with the liver.

9. REACTION TIME OF OXYHAEMOGLOBIN WITH NITRITES

Finally, we turn to the paper by Metcalf[h]). He reports that 1/30 rad of whole-body gamma-irradiation can cause a measurable change in the blood of rats. His rather unusual test involves treating a suspension of erythrocytes from the irradiated animal with an isotonic solution containing small amounts of nitrites. The red oxyhaemoglobin then changes into brown methaemoglobin and the speed of the reaction can be measured; Metcalf calls it the "reac-

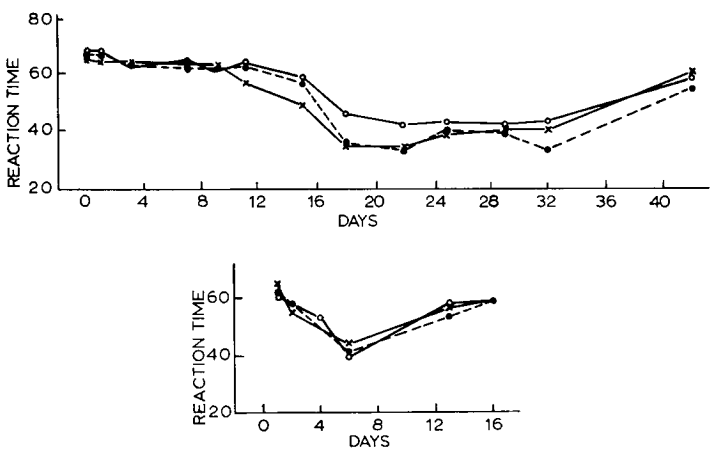

Fig. 8. Reaction time* in seconds at various times after whole-body irradiation with 2 equal doses of gamma rays. Groups of 4 to 5 adult rats were used. The standard deviation is approximately ± 4 sec. (Metcalf.)

Upper graph: after first dose *Lower graph*: after second dose

O — O each dose $^1/_{30}$ rad
● — ● each dose $^1/_{10}$ rad
× — × each dose $^1/_3$ rad.

* Reaction time is the interval between mixing red cell suspensions with nitrite solution and the first visually detectable colour change from the red of oxyhaemoglobin to the brown of methaemoglobin.

tion time". After 500 rad the reaction time was reduced very soon after irradiation and the alteration lasted 3 months. After small doses, e.g. fractions of a rad (Fig. 8), it is several days before the full effect is obtained. Eventually the reaction time returns to normal but if the rat is then irradiated a second time the changes in reaction time occur very much sooner. The mechanisms involved are as yet completely unknown. It may not be possible to use the test to detect minute exposure in man because other insults and various pathological conditions produce the same sort of change. However it is of great interest that the effect of such low doses can be demonstrated by means of changes in the haemopoietic system.

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DISCUSSION

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Shively[1]) wished to emphasise that in their studies on swine, it seemed likely that recovery from a second irradiation was slower than from a single irradiation. The experiments were however not strictly

comparable as the swine recovering from a second irradiation were twice the age of the other animals. This age difference might be an important factor.

Geszti[1]) noted that she had found diminished iron uptake 5 to 10 min post-irradiation with 600 r in mice. As the number of iron incorporating cells in the bone marrow was normal at this time it was possible these cells had suffered considerable damage. Geszti mentioned that Metcalf[h]) had also observed the peripheral red blood cells were damaged. By treating red cells or haemoglobin solutions with hydrogen peroxide Geszti had overcome the extinguishing effect of haemoglobin on the fluorescence of certain dyes. This hydrogen peroxide action is much reduced when the blood is from tumour-bearing patients or irradiated animals, a measurable difference in intensity of fluorescence induced by fluorescent dyes being detectable in a few hours following irradiation with a small dose such as 25 r. She thought the underlying mechanism might be due to a change in methaemoglobin formation, which she had since confirmed by spectrophotometry. As a result of Metcalf's work she had tried nitrites instead of hydrogen peroxide and found the effect more marked. In congenital methaemoglobinaemia there was a marked diaphorase deficiency, and a similar enzyme deficiency in the red cells might be responsible for the phenomena found by herself and Metcalf.

In reply Metcalf said that in 48 hours the same increased haemoglobin sensitivity could be induced in rats by starvation alone. Riboflavine and riboflavine alone given subcutaneously seemed able to restore the reaction time to normal. He felt this supported Geszti's impression that a flavoprotein enzyme may be involved. Following a query from Patt (*Argonne Nat. Lab., Argonne, Ill., USA*) the author stated that it was of little value to irradiate cells *in vitro* and then test them, as under these conditions the reaction can be easily upset.

Upton (*Biol. Div., Oak Ridge Nat. Lab., Oak Ridge, Tenn., USA*) asked if Osmond[i]) had observed any evidence of direct labelling of the small lymphocyte. This possibility was suggested by the similar pattern of labelling with tritiated thymidine of the large and small lymphocyte, as a function of time.

Osmond replied that the small lymphocyte in the thoracic duct is not a DNA-synthesizing cell, and labelled small lymphocytes do not appear in thoracic duct lymph until 2 to 3 hr after the ad-

ministration of tritiated thymidine. Thereafter there is an increase in the proportion of labelled small lymphocytes to labelled large and medium lymphocytes. He said that in all the present studies a constant post-thymidine interval of 24 to 26 hr was employed. At this time approximately 40% of the labelled cells in the thoracic duct lymph were normally of the small lymphocyte variety.

Upton noted that the size variations in platelets observed by Ingram and her colleagues[k]) might be consistent with the concept that platelets decrease in size with age, as observed by Odell *et al.* in rats. He then requested fuller details on the method of platelet size determinations.

Ingram answered first that they studied the platelets after sedimenting blood with polyvinylpyrolidone. She felt that it was reasonable to conclude, from a histogram of platelet volume against frequency of occurrence, that their results were both accurate and reproducible. From clinical studies, there was evidence that large platelets appear when platelet production is resuming after injury, and in disease, such as idiopathic thrombocytopaenia purpura, or following thrombotoxic agents. It was presumed these were immature platelets. However they needed more information as there may be an osmotic factor involved which could produce similar results.

Foissac (*Dept. Biol., Commis. à l'Énergie Atomique, Gif sur Yvette, S & O, France*) asked what was the method used by Ludwig and Kohn[e]) to find the total and individual absolute numbers of bone marrow cell types. Ludwig explained they suspend a known volume of marrow in a known volume of medium which contains spores in a precisely known concentration. Smears are then made and it was assumed that both bone marrow cells and the standard particles were distributed randomly. With a light microscope the ratio of cells to spores is counted. This will then give the total cell concentration, and that of any particular cell type as necessary. The advantage of the method over other methods of counting is that once the smear is made the count can be carried out at any convenient time afterwards.

Nagy (*Inst. for Med. Physics, Budapest, Hungary*) stated that he had given whole body irradiation to rats at doses from 25 r to 500 r, followed by intraperitoneal methionine-^{35}S. 24 hr later the animals were bled and the blood centrifuged into red cell, white cell and platelet fractions and tested for methionine-^{35}S activity. He found

the activity of the platelet fraction increased with increased doses of irradiation whereas the activity of the other two fractions decreased. These changes and differences are marked up to a dose of 100 r but above this the differences were less obvious.

Sacher (*Argonne Nat. Lab., Argonne, Ill., USA*) said he had studied donor labelled mouse red cells injected into a normal mouse. If these cells were irradiated before injection they died sooner, and at the same time there was a decreased reutilization of iron. There was perhaps abnormal metabolism of iron after it had been liberated from the cell in a possibly abnormal death process.

Mauss[g]) emphasized that it was important, in iron studies on the liver, to distinguish between experiments in which the liver only had been irradiated and those in which whole body irradiation was given.

If the whole body is irradiated then the iron content of the liver will increase because the irradiated bone marrow will have a reduced iron incorporation. Any decreased ability of the liver cells to take up iron will be masked by this effect.

Pietrantoni (*Dept. of Radiol., Ospedale Galliere, Genova, Italy*) asked if Mauss had studied the effect of irradiation on the incorporation of ^{59}Fe into developing red cells. Mauss thought the results of irradiation on iron incorporation were similar to the effects of irradiation on serum iron levels. After whole body irradiation iron incorporation in red cells is reduced to almost zero, but if the liver is isolated and irradiated there is a slight increase in iron incorporation which on the tenth day is of the order of 8 to 10%. In order to study early changes in iron turnover following irradiation, he no longer was using ^{59}Fe incorporation in bone marrow, which necessitated a 24 hr delay before making observations, and was now studying serum iron levels which enabled him to obtain data from 16 minutes after irradiation.

Kowalski (*Inst. of Nuclear Res., Warsaw 9, Poland*) noticed that his results on iron metabolism in irradiated animals were similar to those of Geszti and Mauss. He found that the plasma iron level increased at the same time as the iron in the organs increased, particularly in the spleen, and wondered if the authors could explain this. Mauss said he had made no study of the spleen. He had found a definite increase in the serum iron level following whole body irradiation, and possibly as the bone marrow had been irradiated at the same time the available iron may go to other organs.

Manoilov (*Inst. of Pharm. Chem., Leningrad, USSR*) added that they had been studying alterations in the mechanism of iron incorporation and found that any decrease in the synthesis of iron-incorporating compounds is associated with a decrease of general cellular biological activity. He felt that radiation acts specifically on iron-incorporating substances taking part in the tissue respiration processes. This might result in a disturbance of electron-transference and therefore a reduction in the general level of bio-energy in the cell.

Radiopathology of Mammals

RADIATION CHIMAERAS

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a) J. S. BURKLE, R. B. GRESHAM, T. WHEELER, D. CRANMORE, C. E. BRODINE and A. E. ALBRIGHT,
Survival in lethally irradiated dogs following autologous frozen bone marrow administration.
Radiation Exposure Evaluation Laboratory, US Naval Hospital, Bethesda 14, Maryland; Tissue Bank, National Naval Medical School, Bethesda 14, Maryland, and *Naval Medical Research Institute, Bethesda, Maryland, USA*

b) G. CUDKOWICZ,
Suppression of the foreign marrow reaction in mouse chimaeras by pre-irradiation of donor cells.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

c) A. J. S. DAVIES and A. M. CROSS,
Peripheral blood studies upon isogenic radiation chimaeras.
Chester Beatty Research Institute, London SW3, Great Britain

d) SHEILA M. A. DOAK,
Radiation chimaeras and transplantation immunity
Chester Beatty Research Institute, London SW3, Great Britain

e) G. DORIA,
Direct evidence for graft-anti-host immune reaction in mouse radiation chimaeras.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

f) JOAN WRIGHT GOODMAN,
Transplantation of white blood cells in irradiated mice.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

g) A. LENGEROVÁ, H. S. MICKLEM and C. M. CLARKE,
Immunological tolerance as a factor in transplantation therapy of lethally irradiated mice.
Institute of Experimental Biology and Genetics, Prague, Czechoslavakia

h) R. A. POPP,
Haemoglobin as an erythrocyte marker.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

i) D. W. VAN BEKKUM,
Foreign bone marrow transplantation following fractionated whole-body irradiation in mice.
Radiobiological Institute TNO, 151, Lange Kleiweg, Rijswijk (Z.H.), The Netherlands

j) O. Vos,
A specific immunological tolerance in homologous radiation chimaeras.
Medical Biological Laboratory RVO-TNO, 139, Lange Kleiweg, Rijswijk (Z.H.), The Netherlands

k) W. R. Wooles and N. R. Di Luzio,
Reticulo-endothelial participation in bone marrow transplantation following lethal X irradiation.
Department of Physiology, University of Tennessee Medical Units, Memphis, Tennessee, USA

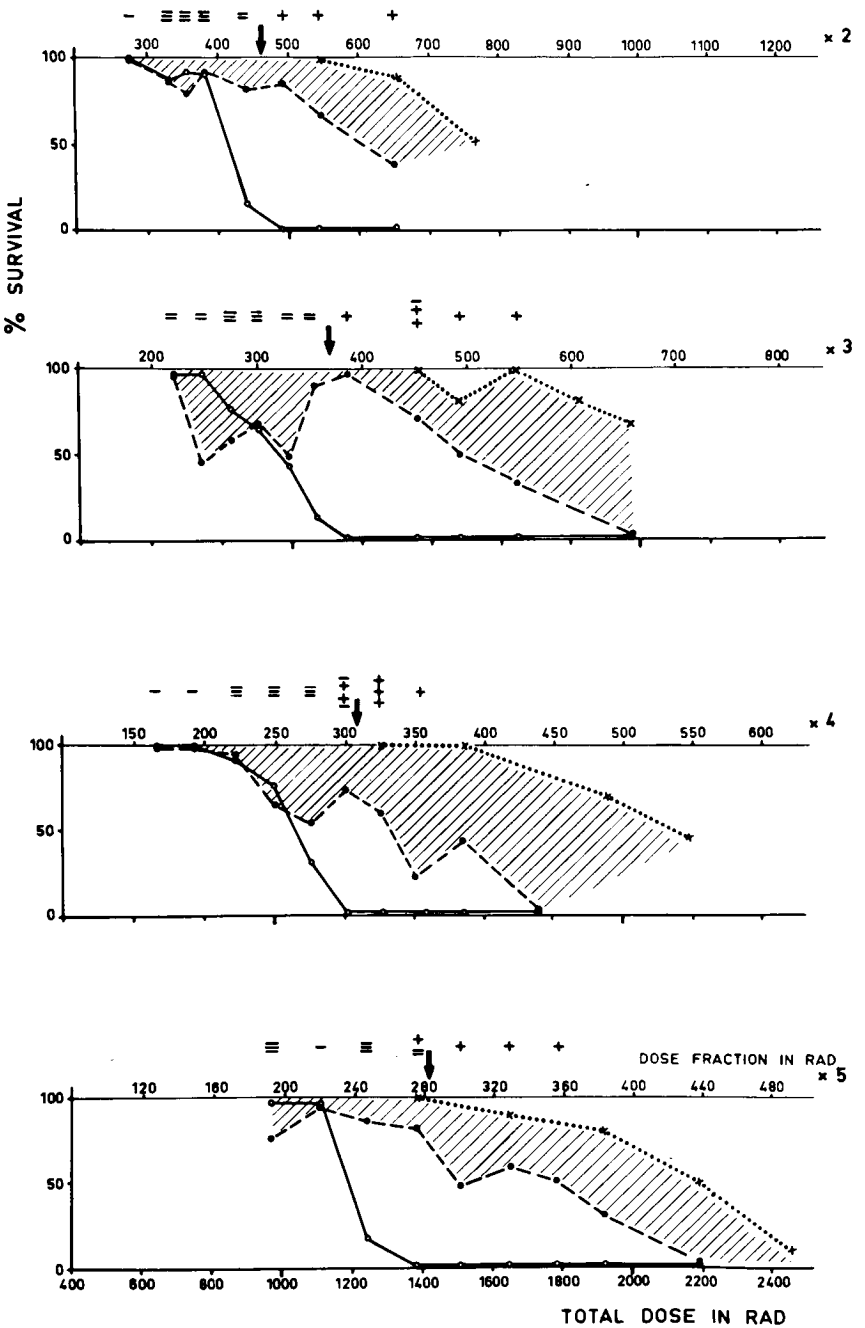
1. INTRODUCTION

By way of introduction, I must explain for those of you not engaged in this field of radiation research what is meant by the archaic term radiation chimaera. It has been known for twelve years[1]) that mice which received a large whole-body dose of radiation could be saved from death by treatment after the event. At first the treatment consisted of the intraperitoneal implantation by surgery of portions of spleen from young mice, but with time more sophisticated techniques of intravenous injection of bone marrow, spleen, or liver from embryonic animals have been developed. The fact that haematopoietic tissue from a species different from that irradiated was effective was at first thought to imply a humoral mechanism of action. However, proof of the establishment of a haematopoietic graft was soon obtained by a variety of techniques[2-4]). This coexistence of tissues derived from different individuals in the one organism constitutes a chimaera which was classically part goat, lion and serpent.

2. RADIATION TREATMENT AND BONE MARROW TAKES IN MICE

With the passage of time, the laboratory mouse has shown itself for a number of reasons to be the animal most amenable to research in this field. To an animal of this size the administration of a sufficiently uniform dose of whole-body irradiation is easily performed and is usually given in a single exposure lasting 10 to 20 min. With larger animals and man[5]), fractionation of the dose is often necessary and van Bekkum[i]) reports the effect on repopulation and survival of mice with syngeneic* bone marrow or rat bone marrow

*) I am using the terminology where syngeneic means antigenically identical, allogeneic means of the same species but different genetically, and xenogeneic of different species.


% SURVIVAL
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120 160 200 240 280 320 360 400 440 480
× 5
400 600 800 1000 1200 1400 1600 1800 2000 2200 2400
TOTAL DOSE IN RAD

following doses of radiation divided into 2, 3, 4 or 5 fractions. The results which are summarized in Fig. 1 show (i) that rat bone marrow is less effective than syngeneic bone marrow and that fractionation accentuates this effect; (ii) with three fractions rat bone marrow increases the mortality over irradiated but otherwise untreated animals at the mid-lethal dose level and (iii) that chimaerism with a permanent take of rat bone marrow only results from radiation doses in the 90 to 100% lethal range.

3. IDENTIFICATION OF HAEMATOPOIETIC GRAFT

Identification of the foreign cells within the irradiated animal was a problem which was solved in a number of different ways. In these experiments reported by van Bekkum, circulating rat erythrocytes were identified by agglutination with specific antiserum, and granulocytes of rat and mouse can be distinguished by the alkaline phosphatase reaction[6,7]). Other immunological methods[8]) and cytological methods[4]) involve the sacrifice of the animal to be typed. Another method of identification is by the haemoglobin specificity of the red cells. Techniques recently developed by Popp[h]) have increased the sensitivity of this method and thereby reduced the size of sample required. By his electrophoretic method[9]) the relative quantities of mixtures could be determined within 10 to 20%. Determination of the solubility of carbonmonoxyhaemoglobin in high concentration of phosphate buffers has advantages on this method. A large number of separate estimations, each requiring only 0.1 ml of blood, can be done in one day, and two or more haemoglobins in a mixture can be distinguished within ±5%. Further, breakdown of the haemoglobin molecule by tryptic hydrolysis yields characteristic peptide patterns on paper chromatograms. Some thirty peptides can be dis-

Fig. 1. Therapeutic effect of bone marrow transplantation following fractionated irradiation. From top to bottom: results for 2 to 5 daily irradiations
——— untreated controls.
– – – – rat bone marrow.
. . . . . syngeneic bone marrow.
Minus and plus signs at the top of each graph represent the findings in groups of surviving rat bone marrow treated mice subjected to the radiation doses indicated: — : reversals. + : mouse/rat chimaeras. Arrows indicate the minimal dose level at which permanent chimaeras were produced in all experiments (Van Bekkum.)

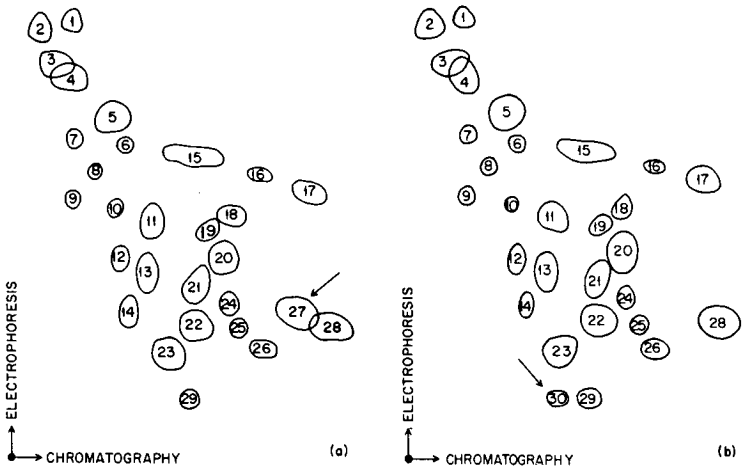

Fig. 2. Paper chromatograms showing ninhydrin-positive tryptic peptides of (a) C57BL and (b) 101 globin. The peptides have been numbered for reference. Differences are indicated by arrows: i.e. peptide number 27 is present on C57BL chromatograms, but absent on the 101 one. (Popp.)

tinguished (Fig. 2). Analyses in this detail fail to show any difference between haemoglobin from erythrocytes of chimaeras and from erythrocytes of normal animals of the donor strain.

4. EFFECTIVE NUMBER OF CELLS AND THEIR ORIGIN

It has been known for some time[10]) that the quantity of myeloid tissue required to save the irradiated mouse must be increased progressively with increasing genetic diversity; thus the largest amount of bone marrow is required from the rat. Davies and Cross[c]) have been following survival and the recovery of the peripheral blood picture in chimaeras after varying quantities of syngeneic bone marrow. Only their largest dose (10^7 cells) gave 100% survival, and doses from 2.5×10^4 to 1 million cells all gave about 80% survival. The peripheral blood leucocyte count was restored to normal within ten days by the largest dose of bone marrow, but progressively smaller doses of marrow did not give shorter survival. In a further experiment, doses of 10^7 syngeneic or allogeneic bone marrow cells were found not to differ in their effect on the peripheral leucocytes within 48 hours after irradiation. It is concluded that the granulo-

cyte is the critical cell required in the peripheral blood for survival of the mouse after irradiation and a granulocytopaenia lasting for more than 12 days was always associated with subsequent death.

Qualitatively also, the cell-type of the myeloid tissue which leads to repopulation after irradiation has intrigued workers in this field. Goodman and Hodgson[11]) have recently found evidence of stem cells in the peripheral blood of mice following the use of a white cell suspension to treat lethally irradiated mice. A total of 20 to 70 million cells had to be administered in up to three doses and the problem is to maintain the viability of the white cells while separating as many as possible of the large mass of red cells. Recent results relate to the use of lymph node cell suspensions and cells harvested from peritoneal exudates. Of 20 recipients which received between 50 and 200 million lymph node cells, none was found histologically to have any significant cellular proliferation in bone marrow or spleen. ^{59}Fe incorporation values for these mice were higher than in controls which had received irradiation only, but did not approach those of other irradiated mice which received 50000 syngeneic bone marrow cells. Peritoneal exudate cells were harvested from untreated donors and donors pretreated with glycogen intraperitoneally 1 to 2 days before. They consisted largely of small lymphocytes and reticulum cells. Histological examination of irradiated recipients of these cells killed within the first 2 weeks did not show significant marrow regeneration. However, cytotoxicity studies of cell suspensions from spleen, lymph node and hypocellular marrow showed the presence of donor cells.

5. RETICULOENDOTHELIAL STIMULATION

Previous sensitization of the host to donor antigens before irradiation[12]), or administration of iso-immune serum in very small quantities from host strain animals sensitized to the donor-strain[13]), each prevents the survival of the marrow cells and the animals all die. The importance of the reticuloendothelial system (RES) in the survival of injected foreign bone marrow is shown by Wooles and Di Luzio[k]). RES hyperplasia can be stimulated by the injection of derivatives of yeast cell walls. The authors used glucan, a polysaccharide of molecular weight about 6500, or trypsinized zymosan, a cell wall preparation, to stimulate the reticuloendothelial system in mice which were then irradiated and treated with saline or with

syngeneic, allogeneic or xenogeneic (rat) bone marrow. The results are given in Table 1 and show no effect of reticuloendothelial stimulation in comparison with normal animals with syngeneic marrow treatment, but a complete failure to survive in the RE hyperfunc-

TABLE 1

Influence of the functional state of the RES on transplantation of bone marrow in X irradiated mice

Recipient	Marrow treatment	30 day survival*	Percentage
Control	—	0/38	0
RE hyperfunctional	—	0/32	0
Control	Syngeneic	23/26	88
RE hyperfunctional	Syngeneic	24/30	80
Control	Allogeneic	13/15	92
RE hyperfunctional	Allogeneic	0/15	0
Control	Xenogeneic	10/40	25
RE hyperfunctional	Xenogeneic	0/37	0

* Values denote the number of mice that survived 30 days/the number which was initially in the group. The mean data are derived from a series of 6 experiments in the syngeneic, 3 in the allogeneic, and 7 in the xenogeneic groups. (Wooles and Di Luzio.)

tional group with foreign marrow. I understand that studies are under way with isotopically-labelled bone marrow suspensions to determine whether the mechanism of action with foreign bone marrow is increased phagocytosis or enhanced antibody function or a combination of these by the hyperfunctional RES.

6. AUTOGRAFTS

In larger laboratory animals and man we do not have the advantage of the inbred strains of mice. For this reason, immunological reaction by the host due to insufficient radiation causing a shedding of the graft, and reaction by the graft against the host if chimaerism is achieved, can usually be avoided only by the use of marrow removed before irradiation and stored at low temperature until required[14]). It has proved difficult to produce chimaerism in dogs by radiation and grafts of allogeneic marrow[15,16]). Burkle and his coworkers[a]) report success with autografts of stored bone marrow after lethal irradiation of dogs. They have found that at least 2500 million

cells must be aspirated for the marrow to be successful. The marrow was stored either in glycerol or the new agent dimethylsulphoxide which is protective to cells during freezing[17]) .They confirm that dimethylsulphoxide is an improvement on glycerol since it does not have to be removed after thawing and gives better cell counts and cell staining of thawed specimens.

7. SECONDARY DISEASE

When foreign bone marrow was used in irradiated mice, it was soon observed that long term survival was less good than when bone marrow from the same inbred strain was used. After three weeks or so, the mice fail to gain weight, pass fluid faeces, waste progressively and often die sporadically over the next one or two months. This delayed morbidity and mortality has been called "secondary disease". Histological examination of a mouse in secondary disease reveals that although the bone marrow is still cellular and active in erythro- and granulopoiesis, the lymphatic tissue of nodes and spleen is aplastic. This condition, with other evidence, leads to the conclusion that secondary disease results from a graft-versus-host reaction with self-destruction of the graft-derived immunologically competent tissue. Doria[e]) has direct evidence of this immune reaction following transfer into radiation chimaeras of spleen cells from mice sensitized to rat red blood cells. Secondary disease may result, of course, if bone marrow from the parent strain is used to treat irradiated F_1 hybrid

TABLE 2

Secondary agglutinin response in irradiated (800 r) recipients by transferred spleen cells presensitized against rat red blood cells. (Doria.)

Spleen cell donors		Irradiated recipients		
		F_1	P	P anti F_1
F_1 anti R	x	11.79	11.14	0
	s_x	0.20	0.30	0
	n_x	14	14	12
P anti R	x	11.00	11.33	10.93
	s_x	0.16	0.29	0.28
	n_x	16	12	14

P = C57BL/6 F_1 = (C57BL/6 X DBA/2)F_1 R = rat red blood cells
x = mean $\log_2$ titre. s_x = standard error. n_x = number of observations.

mice, although it does not arise in the reverse combination – parent mice treated with F_1 bone marrow[18]). Equally, mice of the parent strain can be sensitized to F_1 tissues before irradiation with the consequence that F_1 bone marrow will no longer take as a graft and save the mouse from death. After irradiation, spleen cells from donors sensitized to rat red cells were injected intravenously, followed immediately by rat red blood cells intraperitoneally. Reproducible results were obtained (Table 2) of secondary agglutinin response in all groups except where the parent strain mice presensitized to F_1 antigens were injected with spleen cells from F_1 donors. Similarly the test gave uniform results (Table 3) in syngeneic chimaeras formed 7, 15 and 30 days previously. However where graft-versus-host reaction was to be expected, the secondary agglutinin response was slightly reduced at 7 days and considerably so at 15 and 30 days.

Other evidence[19]) of early graft-versus-host reaction in allogeneic chimaeras has been adduced from the failure of host-type skin grafts

TABLE 3

Secondary agglutinin response in irradiated (800 r) recipients by transferred spleen cells presensitized against rat red blood cells. (Doria.)

Spleen cell donors		Irradiated recipients			Chimaeras age (days)
		F_1/F_1	P/P	F_1/P	
	x	7.75	8.06	5.28	
	s_x	0.22	0.33	0.55	7
	n_x	16	16	14	
	x	7.50	7.39	1.28	
F_1 anti R	s_x	0.14	0.17	0.49	15
	n_x	22	18	18	
	x	8.00	7.91	1.40	
	s_x	0.14	0.28	0.84	30
	n_x	10	11	6	
	x	10.40	10.17	10.11	
P anti R	s_x	0.39	0.33	0.26	15
	n_x	5	6	9	

P = C57BL/6 x = mean $\log_2$ titre
F_1 = (C57BL/6 X DBA/2)F_1 s_x = standard error
R = rat RBC n_x = number of observations
Chimaeras = irradiated host/bone marrow donor.

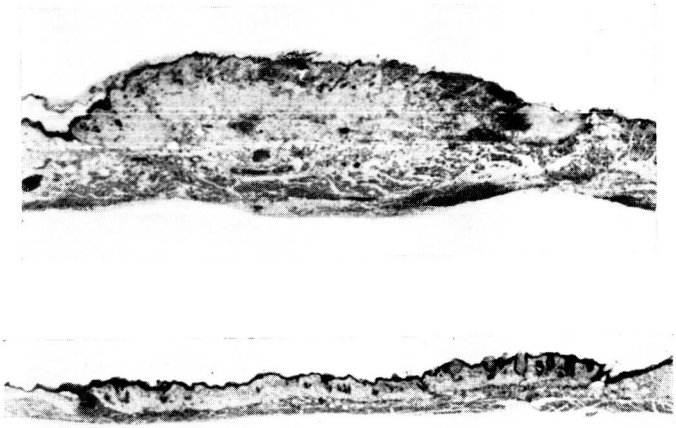

Fig. 3. Appearance of skin grafts on Balb/c → CBA chimaeras 12 days after grafting. Upper, graft of host-type skin. Lower, graft of donor-type skin. (Doak.)

(syngeneic or autografts) to be accepted when applied to chimaeras 24 hr after formation, although skin grafts from mice of the bone marrow donor strains were accepted. Factors in this rejection of skin grafts have been investigated by Doak[d]. Technique of grafting is the first point; if the graft is fitted tightly to the bed it survives better than if it is placed centrally in a much larger bed referred to as "open - style" grafting. The thickness of grafts of host-type skin are found to be 3 or 4 times greater than those of donor-type skin (Fig. 3). The oedema of the graft has led Doak to investigate the effect of removing the "ghost" surface layers at deplastering which leads to drying and reduction of graft acceptance (Table 4). A further distinction noticed between open and fitted grafts was the degree of epidermal hyperplasia. Irradiation of the skin donor was performed to stimulate epithelial activity and this resulted in a reduction of the number of accepted grafts by either technique in comparison with non-irradiated grafts.

TABLE 4

Survival of host-type grafts of Balb/c (BM) → CBA radiation chimaeras. (Doak.)

	State of grafts at 20 days		
	Accepted	Vestigial	Rejected
Ghost intact	10/11	1/11	0/11
Ghost removed	6/17	9/17	2/17
Ghost removed			
Fitted grafts	3/8	5/8	0/8
Open-style grafts	3/27	4/27	20/27
Ghost intact			
Non-irrad. open-style grafts	5/5	0/5	0/5
Irrad. open-style grafts	4/13	6/13	3/13
Ghost intact			
Non-irrad. fitted grafts	5/6	1/6	0/6
Irrad. fitted grafts	3/8	4/8	1/8

8. AVOIDANCE OF SECONDARY DISEASE

Clinical applications of the principle of the radiation chimaera would involve the problem of the avoidance of secondary disease. It was noticed that secondary disease was reduced in mice if foetal

haematopoietic tissue was used in place of bone marrow[20,21]). This was attributed to a maturation of the immunological capacity of the injected tissue within the new host and thereby specific actively acquired tolerance[22]) of the new environment.

Another approach to the problem is the removal of the immunologically active cells from the marrow suspension before injection into the irradiated recipient. Cudkowicz[23]) has subjected mice destined to be donors of bone marrow to 400 to 500 r of X rays, and found a reduction in the incidence of secondary disease in allogeneic recipients. Pretreatment of the irradiated donor mouse with the radioprotective drug s,β-aminoethylisothiouronium. Br.HBr. (AET) improved survival when the marrow was used in lethally irradiated recipients. It was suggested that AET showed a greater protective action for the haematopoietic cells than for the immunologically competent cells during the pre-irradiation of the donor. His latest results[b]) follow pre-irradiation of donor cells *in vitro*, both bone marrow and spleen cell suspensions. The constitution of the surviving animals has been determined by the haemoglobin characteristics using Popp's technique.

The results in one experiment are shown in Table 5. It is worth noting that with more radiation pretreatment there was more rever-

TABLE 5

Survival of lethally irradiated (C3H × 101)F_1 mice given (C57BL/6 X DBA/2) F_1 marrow cells pre-exposed *in vitro* to X rays. (Cudkowicz.)

Donor cells			Recipients						
Pretreatment	No. infused per recipient ($\times 10^7$)	No. mice	Surviving				Haemoglobin 90 days after transplanation		
			40 day		90 day		Donor	Mixed	Host
			(no.)	(%)	(no.)	(%)	(no.)		
None	0.5–2	40	6	(15)	0	(0)	0	0	5*
None	4.0–8	40	24	(60)	10	(25)	10	0	0
300 r	18	40	32	(80)	23	(57)	23	0	0
AET + 300 r	15	60	54	(90)	45	(75)	45	0	0
400 r	20	40	20	(50)	10	(25)	7	0	3
400 r	30	50	41	(82)	34	(68)	20	8	6
AET + 400 r	20	40	27	(68)	20	(50)	10	0	10
AET + 500 r	30	40	28	(70)	28	(70)	0	0	28

* 60 days after transplantation.

sion to host-type haemoglobin, suggesting that there might have been a "tiding-over" by the large white cell infusion in a manner similar to that suggested by Goodman's[f]) results with peritoneal exudates. The experiment with irradiated spleen tested concurrently the haematopoietic and immunological activity of the suspensions using lethally irradiated syngeneic recipients for the former and sub-lethally irradiated F_1 for the latter, as admirably displayed in Fig. 4.

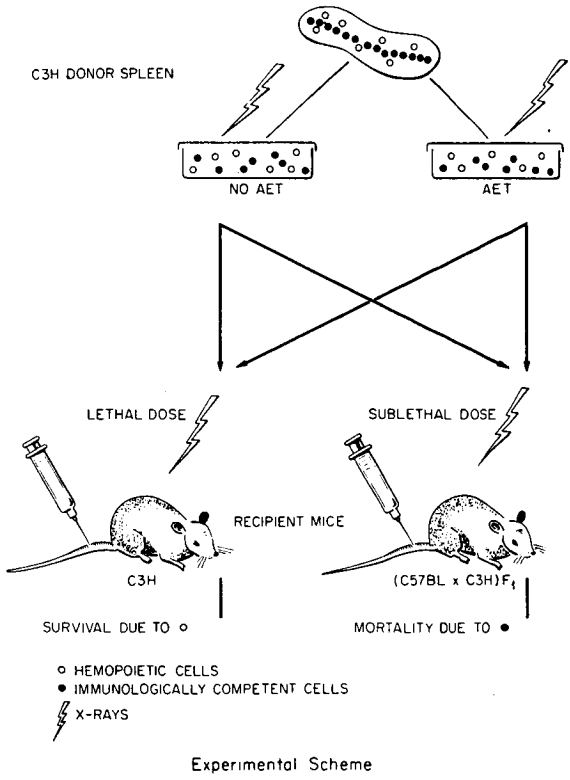

Fig. 4. Design of experiment with spleen cells exposed to X rays *in vitro*, with and without AET, and then transplanted into two types of recipient. On left, lethally irradiated syngeneic recipients to test haematopoietic activity. On right, sub-lethally irradiated F_1 hybrids to test immunological capacity to induce lethal wasting disease in the recipients. (Cudkowicz.)

The ability of AET to protect the haematopoietic cells, with very little effect on the immunologically competent cells, is illustrated in Fig. 5.

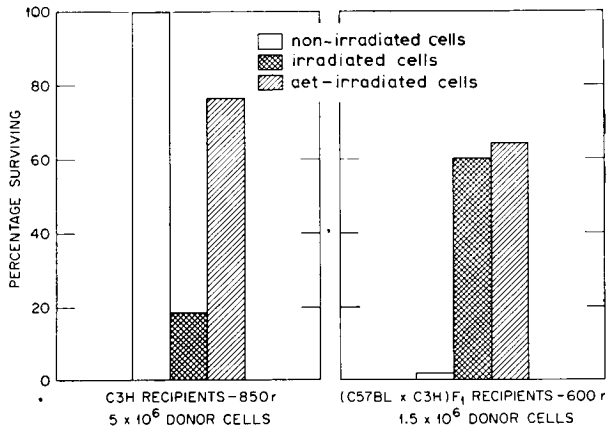

Fig. 5. Survival of irradiated recipients of C3H spleen cells preexposed *in vitro* to 200 r X rays. (Cudkowicz.)

TABLE 6

Immunological interactions of two cellular grafts in the environment of lethally irradiated CBA mice. (Lengerová, Micklem and Clarke.)

Experiment	Origin of the grafts		Relative frequency of dividing cells in bone marrow, spleen and lymph nodes scored 5 to 22 weeks after inoculation.		Interpretation
	(CBA x A) F_1 (T6−)	(C57BL x T6T6), or (A x T6T6)F_1 (T6+)	T6−	T6+	
1	embryonic	embryonic	755*	198*	Mutual tolerance between embryonic T6− and T6+ cells
2	adult	embryonic	787	1+1?	Immunity of adult T6− to embryonic T6+ cells

* T6+ cells were administered 1 to 10 days after T6− cells.

9. ACQUIRED AND SPECIFIC TOLERANCE

Lengerová[24,25]) has previously shown that embryonic grafts can acquire tolerance in the chimaera. The method adopted was to prepare chimaeras with a mixture of embryonic liver cells from two different strains. If they did not show mutual tolerance then the cells of one strain would be expected to outgrow those of the other. Extending these observations to a mixture composed of embryonic liver from one strain and adult bone marrow cells from another, Lengerová, Micklem and Clarke[g]) have evidence that adult cells do not show tolerance. The two types of cell were distinguishable cytologically and the adult cells outgrew the embryonic cells (Table 6). It could be argued that this preponderance was due to an advantage in proliferation rather than for immunological reasons. This, however, is not borne out by another experiment designed to test their relative capacities for proliferation where no immunological incompatibility existed (Table 7).

TABLE 7

Comparison of the proliferative activity of adult bone marrow and embryonic liver cell grafts without the interference of immunity. (Lengerová, Micklem and Clarke.)
Hosts: CBA males irradiated 1000 rad.
Donors: bone marrow CBA-T6T6 ♂ (3 to 6 $\times 10^6$ cells/mouse)
embryonic liver
(CBA x CBA-T6T6)F_1 (3×10^6 cells/mouse)

Interval between adult and embryonic cell inoculum	Dividing cells in chimaeras' bone marrow 2 months postirradiation		
	T6++ (adult graft)	T6+ (embryonic graft)	T6− (host)
4 hr (2 mice)	8	137	—
1 day (2 mice)	34	201	1
5 days (4 mice)	142	45	—

A proportion of animals do survive secondary disease and they come to resemble chimaeras which have never shown this condition. Vos[j]) has investigated whether their survival is due to a diminished sensitivity of the host to the graft-versus-host reaction, or whether it is due to specific tolerance of the graft for the host. At Rijswijk, bone marrow from their CBA mice gives very little secondary dis-

ease in their C57BL and (CBA × C57BL) F_1 mice but a high incidence of chimaerism. The test employed the "killing effect" of small numbers of CBA lymph node cells in addition to bone marrow following lethal irradiation. Normal mice and chimaeras of C57BL and F_1 strains showed a similar sensitivity to graded numbers of lymph node cells by this test, indicating that there was no difference in sensitivity of graft-versus-host activity. On the other hand, lymph node cells from chimaeras, shown to be of donor type by cytotoxicity tests, gave an equivalent killing effect to normal CBA lymph node cells in irradiated mice of third-party (RF) constitution, but not in irradiated mice of C57BL or F_1 strains.

10. CONCLUSION

The importance of the cells which repopulate the lymphatic tissue of the chimaera is only now being appreciated. If incompatible cells are added to the haematopoietic tissue, an early fatal result ensues. If the cells are derived from foreign haematopoietic tissue, they may become sensitized to the host and give rise to severe secondary disease; only if clones of immunologically competent cells specifically tolerant of the host arise is there hope of long-term survival. The provision of additional cells compatible with both host and donor may only be possible where inbred strains are available.

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DISCUSSION

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In response to the Chairman's invitation, some of the authors made additional comments. Van Bekkum[i]) opened the discussion by commenting that daily fractionation of the radiation dose in F_1 hybrid mice given rat bone marrow did not result in the MLD phenomenon when the mice were subsequently given a single dose of radiation, in contrast to CBA and C57BL mice. The results also showed that the MLD phenomenon was increased with up to four daily fractions.

Goodman[f]) added that she now had chimaeras of donor type, produced by the injection of peritoneal cells, living as long as 60 to 90 days after injection, with haemopoietic and lymphatic elements all being present. Di Luzio[k]) emphasised the importance of the host reticulo-endothelial system (RES) in the acceptance or rejection of

allogeneic bone marrow. It could be shown that the mortality of C57BL mice exposed to 800 r after induced RES hyperfunction was directly related to the degree of hyperfunction. Graft rejection and death of allogeneic chimaeras 1 to 3 months after marrow transplantation could be brought about within 5 days of the start of RES stimulation.

Dunjic (*Lab. de Radiobiologie, Institut du Cancer, Louvain, Belgium*) drew attention to the fact that in non-inbred rats given bone marrow of the same strain after irradiation, no homologous disease supervened. Homologous disease was also absent in similar non-inbred rats given lethal doses of Myleran when observed for 1 to 3 months.

Cole (*Naval Radiol. Defence Lab., San Francisco, Calif., USA*) emphasized that the principles which obtain for mice also apply in the case of dogs, apart from differences in radiosensitivity of the haemopoietic system and the system involved in the homograft reaction in the dog. Supralethal radiation doses in the dog do not suppress the homograft reaction, but single doses of radiation together with chemical agents such as 6-mercaptopurine or urethane permit homologous transplants to be accepted, and in support of this he quoted experiments with 15 dogs with excellent haemopoietic recovery in 8 to 9 days but with characteristic secondary disease in 3 months. Turning now to the use of peritoneal cells, Cole referred to experiments in which the injection of 10 to 15 $\times$ 10^6 peritoneal cells from normal donors into lethally irradiated mice resulted in survival for 3 months or more. He considered that peritoneal cells were 30 to 50 times less effective than normal bone marrow cells.

Goodman commented further that peritoneal exudate cells resulting from the stimulation with glycogen, though giving rise to lymphatic regeneration, plasma cell formation and some granulopoiesis, did not give rise to cells of the erythroid series, and in consequence, did not promote survival. She now used fresh peritoneal cells together with an F_1 to parent combination, so that antigenic markers were available to detect donor type red and white cells in the surviving chimaeras. In response to a question by Roylance (*Inst. of Cancer Res., Belmont, Sutton, Surrey, Great Britain*) regarding the true identity of the peritoneal cells, Goodman replied that she was not really able to say which cell or cells were responsible for lymphopoiesis or haemopoiesis. Smears and differential counts had not thrown any light on the problem. As to the cell dose necessary, she

had found peritoneal cells to be 500 to 1000 times less effective than bone marrow cells in terms of ^{59}Fe uptake at 8 days after transplantation. If survival is taken as an end-point, however, they may be regarded as equally effective.

Micklem[g]) underlined the importance of the results of Vos[j]) in demonstrating that immunologically competent cells from the donor can show specific tolerances towards the host in allogeneic chimaeras, i.e. that this capacity to develop tolerance is not exclusive to cells from young animals and that some chimaeras formed with allogeneic cells may acquire competent lymphoid systems. Questioned by Micklem as to the incidence of secondary disease in his system and whether any of the animals recovered from secondary disease, Vos quoted an incidence of 30 to 40% but said that it was difficult to be sure about the recovery of animals from secondary disease in the CBA to C57BL combination, though in the CBA to F_1 combination secondary disease was never found.

Arghittu (*Centro Applicacioni Militari Energia Nucleare, Pisa, Italy*) was interested to know the length of time the bone marrow was kept before injection and whether the irradiated dogs might have recovered with conventional supportive therapy alone. Burkle[a]) replied that the marrow was stored for periods not longer than 10 days, and that the control dogs treated conventionally but without bone marrow all died.

In returning to the subject of the amelioration of secondary disease, Cole pointed out that it had been assumed that this comes about by the removal of immunologically competent cells by prior irradiation and that a discrepancy existed between this state of affairs and that described by Barnes, Loutit and Micklem[1]), where secondary disease was observed in irradiated mice which were given syngeneic cells and which subsequently developed lymphoid aplasia. In replying to this, Cudkowicz[b]) emphasised that in bone marrow, immunologically competent cells were numerically fewer than cells of the myeloid or erythroid series. It was not really correct to assume selective removal of the immunologically competent cells by X rays alone, and it was rather with chemicals such as AET that these cells were selectively reduced in number below a critical level. He also referred to his own experiments in which a large proportion of the recipients of irradiated bone marrow showed a syndrome indistinguishable from secondary disease but which were not in fact chim-

aeras since the haemoglobin was of host type. Cole referred to experiments in which marrow, after irradiation with 400 to 500 r was then given 12 days later with much reduction in the incidence of secondary disease. At the time of giving the irradiated marrow the haemopoietic elements but not the lymphoid elements will have recovered, and he asked if the amelioration of secondary disease followed the reduction in the number of lymphoid cells in the inoculum. Cudkowicz reiterated his view that the total number of marrow cells injected was important.

Van Bekkum took up this point and said that as long as mixtures of lymphoid and haemopoietic cells were used it would be difficult to demonstrate a selective action. He referred to Fig. 5 in the Rapporteur's report and asked if the one fixed dose of cells which was employed was a critical dose. Cudkowicz replied that titrations of spleen cell mixtures were carried out and the cell dose was in fact selected on a basis of the titration curves.

Davies[c]) in reply to a question by Roylance as to whether the white cells were necessary for marrow repopulation or to counter systemic infection, said that he considered that the white cells were essential for marrow repopulation. In answer to a question by Goodman as to whether he had studied the platelets in his mice, Davies indicated that thrombocytopaenia could be tolerated for longer periods than granulocytopaenia.

The majority of authors to whom the rapporteur made reference based their experiments on either the rat or mouse. However, Shaw (*Dept. of Radiology, University of Wisconsin, Madison, Wis., USA*) presented some interesting material based on chimaeras formed between the pigeon and dove. In contrast to the experiments of those who work with rodents, he reminded the audience that far higher mean lethal doses (LD 50) obtained in these birds e.g. the LD 50 for the dove was 2600 r and for the pigeon, 2200 r. Normally in these pigeon-dove chimaeras it was possible to detect haemagglutinins produced against the host by the donor cells. In order to eliminate this haemagglutinin production in the dove, doses of 1400 r were required. He expressed the hope that it might be possible to separate the haematological and immunological activities in these chimaeras, but his own experimental findings were not complete as yet.

The discussion was wound up by Van Bekkum, who pointed out

that the conclusions of Barnes, Loutit and Micklem[1]) might lead to the idea that the signs and symptoms of secondary disease could be attributed to lymphoid aplasia alone. In the case of primates, specific tissue lesions in the intestinal mucosa could be seen in the face of very active lymphoid regeneration by cells presumably of donor origin.

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Late Effects in Mammals

CARCINOGENESIS

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1. INTRODUCTION

We are in plenary session with our colleagues of the physical sciences. Thus in talking about carcinogenesis, the production of cancer, we biologists must expose our ignorance. We cannot yet satisfactorily define cancer. We may recognise that the constituent cell of new growths, or tumours, may be abnormal in structure, or function, or physical and chemical constituents, or in all these re-

spects. This description is, however, incomplete. Cancer cells are in some way free of the disciplines which normally limit the growth of tissues. It is still unknown whether this is a specific characteristic, such as gene-mutation at a particular locus might be, or whether it is general imbalance of extremely variable origin. What is certain is that, in mammals including man, the probability of cancer developing spontaneously increases in geometric progression with age, that inherited factors affect susceptibility and that physical, chemical and biological agents in the environment play a part. Chemical carcinogens, of which coal-tar derivatives are the best documented examples, are now well known. Viruses, which may be considered chemical or biological agents according to one's point of view, are being increasingly incriminated. And tissues which either undergo cyclical changes in response to physiological hormonal influences, e.g. breast, or are affected by periodic injury and repair, e.g. the uterine cervix, are likely sites. Furthermore we have always difficulty in separating the simple or benign tumours from the malignant: it is only the latter which are completely undisciplined.

Carcinogenic physical agents include radiations. Ordinary sunlight with its ultraviolet component is a recognised cause of skin cancer. Ionizing radiations of all sorts are, per unit of energy delivered, still more effective. The radiation may be delivered from an external source — and most interest has been taken in the penetrating radiations, gamma rays and fast neutrons — or from within the tissues due to deposition there of radioactive materials. With conventional experimental animals external radiation commonly increases the yield or advances the onset of naturally occurring malignancies, especially of the blood-forming tissues (leukaemia) and of the breast; internal irradiation affects particularly the tissues where the respective radioactive nucleides concentrate, e.g. bone with bone-seeking elements.

Amongst the 16 papers on the subject of carcinogenesis which I am reviewing, eight are concerned with internal sources of radiation. The dosimetry here is much less straightforward than with external radiation. In the former case excretion and redistribution within the tissues tend to confuse the calculations. If the total dose delivered to the region of interest were the only relevant parameter, the uncertainty about dose would not be very serious; but we are now very well aware that intensity of irradiation, that is dose rate,

and fractionation of the total dose markedly influence the induction of cancer. *Prima facie* this makes it very difficult to invoke the simplest form of the somatic mutation hypothesis — mutation at a specific locus.

2. EXTERNAL IRRADIATION

Leukaemia in mice. I shall use the term "leukaemia" in the broadest sense to include several pathological states. Dr. Irene Boone's[c]) paper is entitled: "The effect of dose rate on the incidence of leukaemia in several strains of mice"; but in fact only one strain (RF) was submitted to irradiation at different dose-rates and her experiment may be regarded as exploratory only. X irradiation, 50 rad daily at 50 rad/min. to a total of 1500 rad, was too much for 2 strains, C57Bl and Swiss, since only one-third were alive to develop leukaemia after 2 months, but about half her RF mice were alive after 6 months. The mortality-pattern of these mice from "leukaemia", which may involve either the granular or lymphoid cells in RF mice, was compared with that of other RF mice given either 800 rad of γ rays at 13 rad/min in 3 fractions spaced at 4-week intervals, or continuous dosage for 1, 2 and 3 weeks at 70 rad/day.

TABLE 1

Incidence of "leukaemia" in RF mice. (Boone.)

Total dose (rad)	1500	800	494	987	1480
Dose rate (r/min)	50	13	0.05		
Total time	5 weeks	8 weeks	1 week	2 weeks	3 weeks
Female RF mice, number scored	12	19	33	28	25
Leukaemia, %	67	68	44	71	72
Control, % (Number of mice)	59(17)	47(15)	38(98)		

Boone concludes from her observations that total dose and dose rate are both important. I concur; but with their high natural incidence of "leukaemia" RF mice are poor indicators of the dose rate effect. Boone doubts too if the mice were homogeneous (see mortality data of controls in 3 separate experiments – Fig. 1).

Cole and Nowell[e]) have compared the induction of "leukaemia" in LAF_1 mice by a dose of 260 rad of X rays, either as weekly frac-

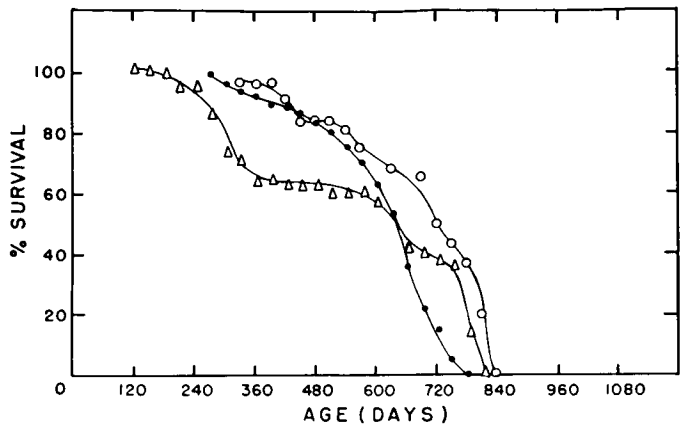

Fig. 1. Survival of mice plotted against time. The three sets of data refer to three separate control experiments using RF mice. (Boone.)

tions of 5 rad over a year or as a single dose at the beginning or end of the year. The numbers of animals available for scoring were only 13 to 19 per group. These were sufficient to show a significantly higher incidence of "leukaemia" from fractionation. This is in accord with current theory. A further interesting result was that shielding of one hindleg did not diminish the incidence of the generalized form of lymphomatosis, whereas shielding has been shown to affect the so-called thymic lymphoma[1]).

TABLE 2

"Leukaemia" induced by fractionated and single doses. (Cole and Nowell.)

Group	Age at death (months)	No. of mice	No. with leukaemia
I (5 rads × 52)	20–36	19	9
II (5 rads × 52: thigh shielded)	26–36	13	7
III (260 rad)	16–33	19	3
V (260 rad at 14 mos.)	24–34	13	3

In the thymic type of lymphoma the malignant change apparently starts in, and may be confined to, the thymus. Perhaps not unexpectedly surgical excision of the thymus before exposure of the

animal to radiation or chemical carcinogen precludes this form of leukaemia. In intact animals the leukaemogenic action of radiation is reinforced by urethane, provided it is given after the irradiation. Trainin and Berenblum[n]) consider radiation as initiator and urethane as promotor in a two-stage process. They were unable to promote "leukaemia" by urethane in thymectomized mice. However, they deduce that tissue (brain) from a thymectomized irradiated mouse contains a "transmissable factor": when injected into intact recipients, which were then submitted to urethane, it has caused so far 10 per cent to develop "leukaemia" within 9 months (Table 3).

TABLE 3

The "transmissable factor" in thymectomised mice. (Trainin and Berenblum.)

Group	Treatment of donor	Treatment of recipient	No. of mice with "leukaemia"
1	Thymectomy: irradiation	Donor brain-tissue: urethane	9/90
2	Irradiation	Thymectomy: donor brain-tissue: urethane	0/100

Mammary tumours. Mammary tumours, like murine leukaemias, can be of several histological types. Some are benign, some malignant. Shellabarger and co-workers[2]) have already noted that the natural susceptibility of the Sprague-Dawley rat to develop mammary tumours increases to nearly 100 per cent after sub-lethal total-body irradiation of young females. The presence of ovaries is necessary to produce the maximal effect, that is, the tumours are often hormone-dependent. In their present communication[k]) they have studied another strain (Long Evans rats) with comparable if slightly less dramatic results.

Male rats developed malignant fibrosarcomata, females variants of fibro-adenomata, usually more benign (Fig. 2). Evidence of disturbed humoral relations was adduced from changes in weight of ductless glands and frequent finding of tumours in these tissues also.

For many of these mammary tumours radiation might well have been the inducing agent, and hormone, for example ovarian oes-

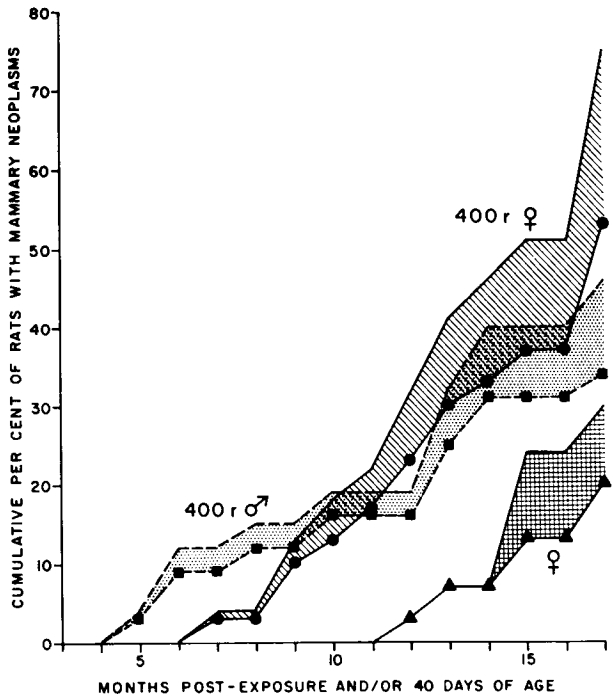

Fig. 2. Incidence of neoplasms in sublethally irradiated Long Evans rats as a function of age and time after exposure. (Shellabarger *et al.*)

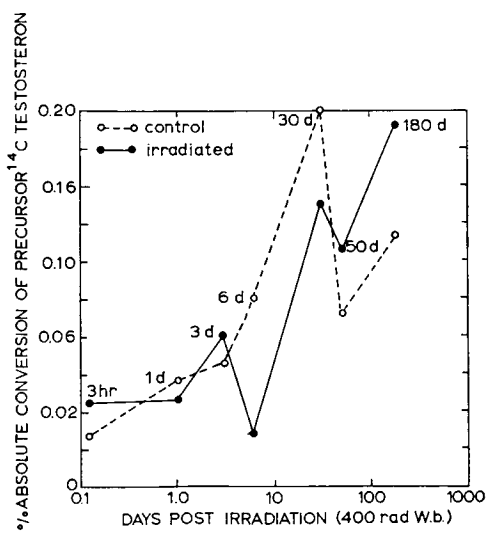

Fig. 3. Effect of 400 rad whole body irradiation on the *in vitro* capacity of ovarian tissue to synthesize oestradiol-17β. (Stitch.)

trogen, the promoting agent. Thus the biochemical assays of Stitch[m]) are especially relevant, though carried out in yet another strain (Harwell albino rats). The synthesis of ^{14}C-oestradiol-17β from ^{14}C-testosterone *in vitro* by minced tissue of ovaries excised at various times after 0 and 400 r is shown in Fig. 3. After an initial lag the ovaries from the irradiated animals show a capacity for oestrogen biosynthesis at least as great as normal controls. The author notes that the biosynthesis of only one of the many oestrogens has been measured and that histological examination showed some follicles still to be present.

In the experiments just reported adolescent animals were irradiated. Sikov and Lofstrom[1]) irradiated their subjects (rats of Sprague-Dawley strain) at the 10th or 15th day of intra-uterine life. Fig. 4 gives their results for subsequent mammary tumours. In three of

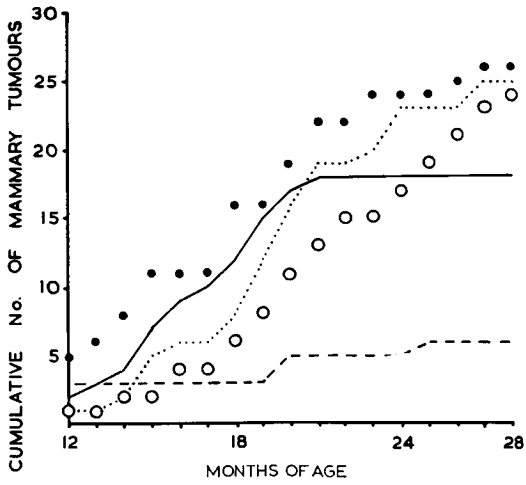

Fig. 4. Incidence of mammary tumours in Sprague-Dawley rats irradiated at the 10th or 15th day of intra-uterine life as a function of age. The X ray doses were 20 or 100 r on the 10th day, and 50 or 185 r on the 15th day. (Sikov *et al.*)

——— Control ● 15^{50}
○ 10^{20} – – – 15^{185}.
..... 10^{100}

the irradiated groups there is a suggestive increase of mammary tumours, but their development is late in life. This contrasts with the early development in Shellabarger's animals irradiated as adolescents. In the 4th group there was a decided paucity of tumours,

associated with failure of development of ovarian follicles. Only in the group given 100 r at the 10th day of embryonic life were histologically malignant tumours diagnosed.

So far we have considered only X and γ radiation. Vogel and Jordan[p]) have compared the incidence of mammary tumours in Sprague-Dawley rats, from a pathogen-free colony, irradiated with 4 weekly fractions of either 200 rad of ^{60}Co γ rays or 100 rad of fission neutrons. Almost all the experimental animals died with mammary tumours (Fig. 5), but unhappily no histological examina-

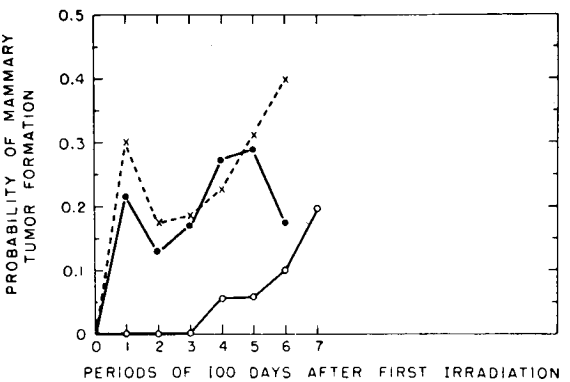

Fig. 5. Probability of mammary tumour formation in a pathogen-free colony of Sprague-Dawley rats after 200 rad ^{60}Co γ radiation or 100 rad fission neutrons. (Vogel *et al.*)

× Irradiated with fission neutrons (57)
● Irradiated with ^{60}Co γ rays (55)
o Unirradiated controls (35)

tion is available. One can deduce that the relative biological effectiveness of these neutrons relative to γ rays was 2, or perhaps more, for induction of these unspecified tumours. There was no observable effect in this experiment in altering the dose rate of both radiations by a relatively small factor of 35 (1 rad/min to 35 rad/min).

Miscellaneous tumours. Lieut. Col. Berdjis[b]) for comparison with his previously published work, adopted a more complex schedule of irradiation. Adolescent Sprague-Dawley rats of both sexes were given at monthly intervals 3 doses of 350 r to the whole body, 1000 r to the chest, 900 r to the right kidney, 900 r to the left kidney and 500 r to the whole body. Fifteen out of 52 irradiated rats failed to survive the course, leaving 37 to be compared with 36 contempora-

ry survivors of 40 controls. An increased incidence of tumours occurred in irradiated rats, an average of 4.6 tumours per rat, many malignant, in 100 per cent of the animals, compared with 1.3 mostly benign tumours, in 55 per cent of controls sacrificed at 27 months of age. Another feature of the irradiated rats was the finding of multiple adenomata of endocrine glands associated with lesions of gut and kidney.

3. INTERNAL IRRADIATION

Two of the papers concern that most interesting substance, tritiated thymidine (^{3}HT), so much used for marking cells synthesizing deoxyribosenucleic acid (DNA).

Lisco and his colleagues[3]) have already shown that one injection of 1 μC/g of body weight induces malignant tumours in mice. In the present communication (Baserga *et al.*[a]) they extend their data and also report on ^{14}C-thymidine. Mice at ages varying from 2 to 12 months were all susceptible especially the youngest. Newborn mice injected with 1 or 10μ C/g of ^{3}HT or with 0.2 or 2μC/g of ^{14}CT have an increased incidence of tumours after 22 and 18 months respectively: and the offspring of female mice injected with 250 μC of ^{3}HT in the terminal week of pregnancy have a higher than normal incidence of tumours after 18 months. The malignant tumours of the experimental animals were widespread and not confined to lung tumours and non-thymic lymphomas as in the controls. The authors conclude that malignancy may result from radiation confined within multiple single cells.

Duhig and Rifaat[g]) have also used ^{3}HT. They say: "AKR leukemia lymphocytes labeled with small doses of titrated thymidine appeared to be capable of inducing lymphoid leukemia in young AKR mice earlier than could unlabeled leukemic lymphocytes". According to their most recent data, mice receiving labelled cells have all developed leukaemia within 24 days, compared with 68 per cent of those receiving unlabelled cells.

4. EFFECTS OF BONE-SEEKING RADIONUCLEIDES

Five papers concern bone-seeking radionucleides. Finkel's group[h]) noted for their work with mice, now report on ^{90}Sr in doses of 150 μC/Kg to dogs. Four of 6 survivors of a single dose to adults developed 7 osteosarcomas (in spine or near the ends of long bones). Many

pups injected daily over a year to the same total died from a haemorrhagic syndrome, but survivors developed numerous osteosarcomas mainly in the shafts of long bones. Adolescents and adults given the dose spread over a year are with one exception now, 1 to 3 years later, still without diagnosed tumours.

Also using ^{90}Sr, van Putten[o]) has scored the effect of treating mice for 6 weeks with a diet low in phosphorus. When the diet was started 48 hr after the injection of ^{90}Sr (Group B), the median life span was increased to 392 days from the 304 days of the injected controls on normal diet (Group A). There was also a lower incidence of bone tumours (Fig. 6) particularly in the spine. When the phosphate-deficient diet was instituted 13 weeks after the injection (Group C), there was no effect on life span or incidence of tumours.

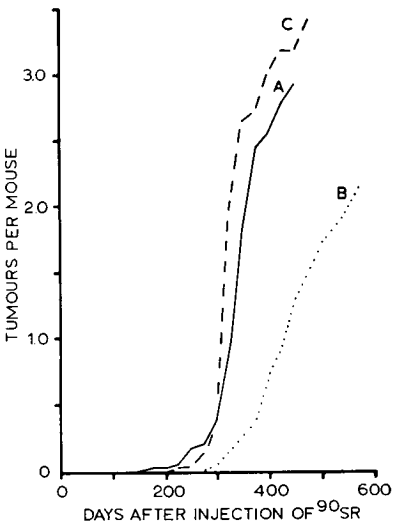

Fig. 6. Tumour frequency in mice after ^{90}Sr injection. Curve A: control animals on a normal diet. Curve B: animals kept on a low phosphorus diet for six weeks after ^{90}Sr administration starting 48 hours after injection. Curve C: low phosphorus diet started 13 weeks after ^{90}Sr administration. (Van Putten.)

Treatment is also the subject of Marcia Rosenthal and her colleagues[j]). ^{239}Pu was the nucleide considered, 2.6 μC/Kg being given intravenously to mice. This dose induces tumours in 65 per cent of untreated mice. 3 days later the treated mice received 1 to 12 daily injections of DTPA (diethylene-triamine-pentacetic acid). The groups having 8 or 12 injections survived on average 8 weeks longer and their incidence of bone tumours was significantly reduced (Fig. 7).

Two papers stem from the comparative radiotoxicity experiment

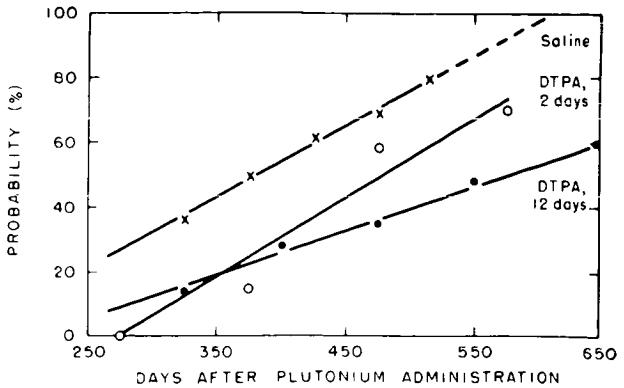

Fig. 7. Effect of DTPA on bone tumour expectancy at death in plutonium injected mice. (Rosenthal *et al.*)

in Utah. Dougherty[f]) gives current data on the 5 different bone seekers: ^{226}Ra, ^{228}Ra, ^{239}Pu, ^{228}Th and ^{90}Sr. He considers "the most meaningful way to make a comparison is to pick a common time of cancer formation for two radionuclides and compare their dose rates." In this way, as in Table 4, he derives provisional values for

TABLE 4

Effectiveness of other nucleides relative to radium in producing osteosarcoma. (Dougherty.)

Nucleide	Days to death	Nucleide rads/day	Radium rads/day	Eff. rel. to radium
^{239}Pu	1500	0.7	3.7	5
^{228}Ra	1140	3.7	10.0	3
^{228}Th	1140	0.7	10.0	14
^{90}Sr	1140	8.5	10.0	1

toxicity relative to radium varying from 1 for ^{90}Sr to 14 for ^{228}Th. He also concludes that "if the present trend continues, osteosarcoma may appear at maximum permissible concentration (MPC) levels towards the end of the beagle life span of about 20 years."

Jee and Stover[i]) concentrate their very substantial effort on ^{239}Pu, given as (+4) citrate to beagles in doses ranging from 2.8 down to 0.096 μC/Kg. Their studies are based on roentgenograms and histo-

logical sections of bone *post mortem*. All doses were highly carcinogenic, but concentration of plutonium in certain bones was only one of the factors which must determine the elective sites of tumours (Fig. 8). Most of the affected bones were of the spongy type (spine

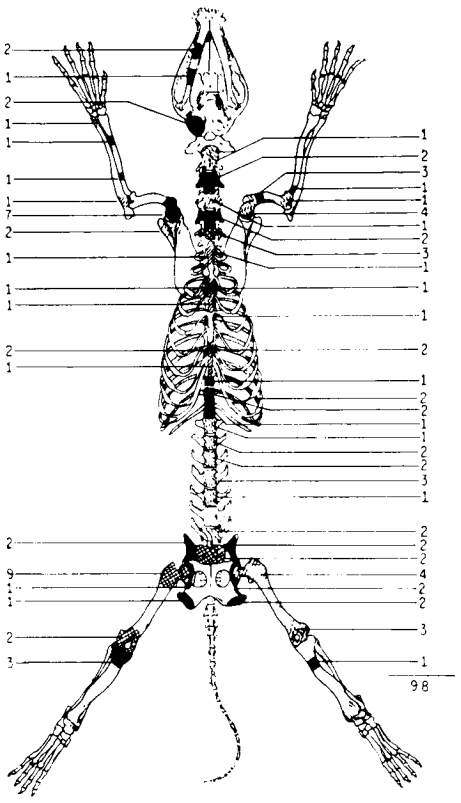

Fig. 8. Anatomical distribution of all osteogenic sarcomas induced by plutonium in beagles. (Jee and Stover.)

and ends of long bones) but the microscopic appearances here varied widely with dose, and gross tissue damage seemed not to be an essential prerequisite.

5. LUNG CANCER

Finally, in view of our need for a better understanding of lung cancer, we can welcome an experiment by Cember and Stemmer[d]) on the administration of radioactive material via the air passages, unhappily by the rather unphysiological method of intratracheal injection. Four groups of rats were injected with 21.3 μg of cerium as

0.15 ml of cerous chloride solution containing 0, 10, 15 or 30 μC of $^{144}CeCl_3$. A high early mortality is attributed to the radiation, but the method of administration may have been a contributory cause in a strain with obviously endemic pathological lungs. Late mortality in those given the radioactive material was due in large measure to malignant neoplasms (Table 5).

TABLE 5

Summary of $^{144}CeCl_3$-induced lung cancer. (Cember and Stemmer.)

Treatment (μC)	No. of rats observed	No. of primary lung tumours	Days till observation of first tumour	Absorbed dose (rad) for first tumour
0	21	0	—	—
10	61	9	306	14000
15	52	31	197	19600
30	37	26	70	25500

These data indicate a higher incidence of lung cancer from $^{144}CeCl_3$ than from insoluble $^{144}CeF_3$ given in a previous experiment. This is attributed to local fixation of cerous ions by protein.

6. CONCLUSION

These papers constitute a useful addition to our general knowledge. No revolutionary new concept has been put forward, but they do affirm current theory. In particular I welcome the results obtained with internal emitters of radiation. In a new field one requires a background of data obtained the hard way by careful and unremitting observation. We are fortunate that our author-investigators are providing this baseline for future thinkers.

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DISCUSSION

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Studitsky[q]) was asked to give a summary of his paper which had been submitted too late for inclusion in the rapporteur's report.

In work carried out with Bereskina on exposure of rats to sublethal doses of whole body irradiation, he had shown, in agreement with Shellabarger *et al.*, that the first site for malignant change was the mammary gland. Why was this so? The facts needing consideration were not limited to the immediate action of physical, chemical, viral and other factors, but also concerned more complicated mechanisms. These included the induction of cancer by chemically inert polymers of the cellophane type, by simple metals, for example cobalt and nickel, by vital staining by trypan blue, or even by simple disturbances of tissue relationships as by explantation and transplantation.

Studitsky *et al.* had demonstrated the induction of malignant change in muscle by cellophane film wrapped round the muscle. He postulated some disturbance in the control mechanism of these tissues produced by a discontinuity of their connection with adjacent tissues.

In the light of this work, they had examined radiation induced tumours, in relation to the relative sensitivity of the connective tissue to radiation, compared with that of the tissues which it controlled. They showed that local γ irradiation with a dose of 2000 r promoted regeneration of muscle tissue, whilst inhibiting connective tissue activity. A similar radiation response in the endocrine controlling system was also demonstrated, and exemplified further the complex results of both tissue and endocrine imbalance on the mammary carcinogenic properties of whole body exposure.

Finkel[h]) drew attention to van Putten's[o]) work, which showed that the yield of bone tumours produced by ^{90}Sr was modified by a phosphorus deficient diet, in a time relationship which indicated that the significant tumourogenic dose was accumulated from the 2nd to the 91st day only. Calculations of accumulated rads to produce tumours should take these data into account. Van Putten was not

sure that the whole significant dose was delivered by the 13th week; and raised the problem of the co-carcinogenic effect of the diet *per se*. He could not state whether the dose to produce tumours was received between the 2nd to 13th week, or during the whole time for which they were irradiated.

Dougherty[f]) pointed out that Furth *et al.*[1]) had shown that a single dose of X rays was leukaemogenic, and repeated doses were not necessary. He also said that although the amount of radiation decreased to zero with time, Ra Th was still the most carcinogenic of internal emitters.

Finkel queried the assumptions on which Dougherty based his statement that doses of the order of the present MPC (maximum permissible concentration) of plutonium could produce tumours within the lifetime of the beagle (20 years). Dougherty replied that with further investigation, increasingly smaller doses of plutonium were found to be producing tumours. At the time the paper was submitted in April 1962, no beagles at the lowest levels had developed tumours. One death from bone tumour had now however occurred at the lowest level. At this rate of production, extrapolation to 20 years leads to an expectation of tumours caused by doses yielded by the present maximal permissible levels.

Cole[e]) asked Loutit to explain the current theory for the induction of leukaemia following fractionated radiation, as referred to in the report. This question was seconded by Kaplan (*Stanford Univ., Palo Alto, Cal., USA*). In reply, Loutit explained that the current theory to which he referred was not a theory of the mechanism of induction of leukaemia, but of the repair of radiation damage in cell systems at low doses as based on the Elkind type data. These showed a shoulder to the cell survival curves indicating recovery from damage which would have killed the cell. Accumulation of residual damage after fractionated doses may however result in a carcinogenic transformation.

Vogel[p]) asked whether Shellabarger[k]) considered the process of mammary carcinogenesis to be progressive in the female rat, and also about the question of multiple tumour formation. He had found an average of 1.5 tumours per rat in animals irradiated with both fission neutrons and Co γ for which he reported an RBE of more than 2 for mammary tumour incidence and longevity. Shellabarger discussed the mechanism in terms of radiation as an insult super-

imposed on *normal* hormonal factors. Studitsky's discussion of the role of tissue interrelations had been of interest. Bond *et al.*[2]) had conclusively shown that local irradiation of the breast alone, without exposure of the endocrine glands, could produce mammary tumours. About the multiple tumours, 1.5 to 2 or more per rat, depending on the time after irradiation, up to 10 had been found.

Boone[c]), asked for details of Duhig's[g]) technique for leukaemia work in the AK mice, since she had found the induction time for leukaemia to be less than 10 days, regardless of technique. Duhig had not found the induced leukaemia to occur before the 12 to 14th day after injection of thymidine-labelled lymphocytes. The cells harvested from spleen and thymus of leukaemic animals were ground up with Tyrode's solution and these suspensions were adjusted by counts to 10^7 cells/ml. Portions of these suspensions were injected intraperitoneally into animals which were examined for leukaemia at death.

Kaplan called for the reassessment of Boone's result after separating the incidences for myeloid and lymphatic leukaemia. Different types of dose dependence may be masked by grouping all leukaemias together. He asked Cole how he distinguished between thymic and non-thymic lymphoma in mice which were not thymectomized, since when the disease is disseminated it is not possible to determine the site of origin. He stressed the importance of the findings of Trainin and Berenblum[n]) and referred to his work with Leberman on transmissible leukaemia, and Gross's confirmation of this in C_3H mice and Latarjet's in C57/Bl. Details of the time relationships and preparation of the material used by Trainin would be useful.

Cole said that as in their previous studies with LAF_1 mice, the reticular tumours observed were nearly all generalized non-thymic lymphomata. Although no tumour limited to the mediastinum was found, the possibility that some of the generalized lymphomata had originated in the thymus could not be excluded. The majority of these reticular tumours were classified histologically as lymphosarcoma and a few were reticulum cell sarcomas. Trainin discussed a two stage mechanism for the induction of leukaemia, with urethane as the promotion factor. Urethane did not produce leukaemia if given before irradiation. Tissues from mice given whole body radiation 24 hours previously induced leukaemia in mice given urethane. The transmissible agent seemed to be subcellular in nature and ex-

periments were in progress to elucidate these points. Thymectomy did not stop the production of leukaemia, when done one week before irradiation, followed by urethane 24 hr later. Normal animals without the thymus did not produce leukaemia.

Berdjis[b]) drew attention to the extreme care needed in labelling different types of leukaemia too hastily, because even in so called thymectomized mice, there are remnants of thymic tissue, which can develop into generalized tumours, which have the morphological criteria of a thymoma, lymphoid leukaemia or lymphosarcoma.

Hug (*Univ. München, Germany*) asked a question of Baserga[a]) in order to distinguish between only intracellular effects or more widespread tissue damage from high doses of tritiated thymidine. Might it be that with the doses used, even in this case, chronic tissue damage was precarcinogenic? The data presented indicated that isolated cell damage could produce tumours. Baserga had found, from histological examination of the animal, no gross or microscopic damage to the tissues. This indicated an intracellular mechanism.

Kaplan, although accepting Trainin's work as a valuable contribution, pointed out that its interpretation depended on the lack of induction of leukaemia by urethane alone. In fact, 100% leukaemia had been induced by much larger doses of urethane alone. Some leukaemia had been induced by intermediate doses of urethane (Doull[3])).

Brues (*Argonne Nat. Lab., Ill., USA*) said that the discussion of mammary tumours in rats has revealed a disagreement more apparent than real. External β-radiation locally applied to the skin produced mammary tumours. The rat is, however, a peculiar substrate for mammary tumour development. One sees various anomalies of the endocrine organs, including adenomas, in these rats. The impression exists that these anomalies are induced, or are favoured, by total-body irradiation. Have the authors any data on this question?

Rajewsky (*Max Planck Inst. Frankfurt am Main, Germany*) closed the session with a warning that although we had accumulated much data, there had been little progress made on the mechanisms of carcinogenesis, especially in relation to dose size effects. In assessment of the hazards of accidental exposure, long term effects would result from a chronic exposure rather than from a short term single exposure. We could be misled if we interpreted the results from high doses in terms of mechanisms which may apply only to low doses.

It is also important, that in investigations involving the incorporation of radiochemicals into the cell, only very small quantities should be used lest a killing effect of a larger dose masks non-lethal changes which may result from smaller doses.

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Late Effects in Mammals

IRREPARABLE DAMAGE OTHER THAN MALIGNANT DISEASE

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Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

1. INTRODUCTION

In the short time at our disposal, I will try to present those aspects of the 15 selected papers which appeared to me to be of particular interest at a plenary session.

The main themes developed in the papers before us may be summarized as follows:

(i) the mechanisms of the initial radiation damage leading to the observed late effects, which are of many types, including shortening of life span;

(ii) effects of age at the time of irradiation;

(iii) repairable and non-repairable injuries;

(iv) the response and specific role of different organs and tissues especially the spleen, blood vessels, kidney, liver and the lens of the eye;

(v) the part played by the production of genetic effects and of chromosome abnormalities in relation to late effects on both physiological and pathological processes;

(vi) comparison of the effects of fast neutrons with those of γ and X rays in the production of late somatic injury including cataract formation, and evidence concerning the relevant values of the RBE, especially for low dose rates: and finally

(vii) the effects of alpha-radiations on the bones, as shown by the evidence of animal experiments and long-term clinical studies.

2. EARLY X RAY EFFECTS ON THE AORTAL INTIMAL MATRIX

It is well known that a single aortic irradiation in dogs with 1500 to 2500 r will cause atherosclerosis in 30 to 40 weeks, not to be distinguished from the "natural" process but strictly localized to the irradiation field. The paper by Brinkman and Lamberts[b]) gives direct evidence of an immediate initial radiation effect. The authors measured the fall in the injection pressure in the aortic wall during irradiation, in the same way as they had done in their earlier experiments on skin[1]). In this exciting work, a salt solution is injected slowly at a constant rate and the pressure set up by the infusion is recorded. Irradiation, with doses of 80 r or even much less, of the spot around the needle tip causes a striking fall in pressure with a lag of not more than one second. Impairment of the function of the aortic basement membrane or "gel filtration layer" by irradiation is shown by dye diffusion experiments both *in vitro* and *in vivo*. In the experiments reported here the doses used are in the region of 1000 to 3000 r. An interesting point made is that in all the radiation effects on the mucopolysaccharide systems studied, including vascular effects, sodium thiosulphate ($Na_2S_2O_3$) acts as an efficient protector appearing to be an extracellular protector, although intracellular effects of radiation are not influenced by thiosulphate.

3. AGE AND WEIGHT EFFECTS IN MICE

Hazzledine and Lindop[g]), in common with other workers, have shown that dietary-induced growth retardation increases radiation sensitivity. A measured hypocaloric but balanced diet administered from birth produced mice with a large reduction in weight but without reduced viability. The irradiation was by means of the X rays produced by a 15 MeV linear acceleration; this gave physical

homogeneity of dose; "sham" irradiation experiments were also carried out to determine the mortality due to causes other than radiation. Two age groups were chosen which differ considerably with respect to radiation sensitivity. The 34 day old group coincides with the period of maximum sensitivity, as well as maximum rate of change of body weight. Any change in physiological age should have resulted, therefore, in an increase of the mean lethal dose (LD 50). The body weight of the starved mice was equal to that of the control mice 21 days of age, when the LD 50 is slightly higher than at 34 days. Actually a 20% reduction in the LD 50 was observed. From the experiments reported it is concluded that the increase in sensitivity to radiation is not due to a possible change in the physiological age.

A report on the effect of age at X ray exposure on the acceleration of intercapillary glomerulosclerosis (IGS) in mice has been presented by Guttmann and Kohn[e]). A striking acceleration of progressive IGS was observed when mice were irradiated either during the three days before birth or during the two days after birth with X ray doses mainly between 75 and 600 rad. This effect was diminished when the animals were irradiated at 12 to 53 days of age. However, in all the present experiments the renal changes were more marked and occurred earlier than those previously observed in similarly treated adult mice irradiated at 1 year of age or later. It is shown that the degree of severity of the IGS increases with increasing dose of radiation.

Attention is drawn to the similarity of IGS to the experimentally induced Masugi nephritis. Accordingly, the possibility may be suggested that the activation and functioning of an auto-immunity mechanism is the cause of delay between irradiation and the appearance of IGS. As the authors say "This hypothesis is predicated on the assumption that X rays produce cellular or intercellular changes that are antigenic". Further investigations are needed.

4. EXPOSURE OF FOUR DAY OLD RATS

The experiments reported by Billings, Yamazaki, Bennett and Lamson[a]) deal with observations of the growth, the incidence of various diseases, the time of onset of the characteristic features of the ageing process and the date of natural death of a group of rats after they had received either a control sham irradiation or total body X irradiation with doses of 5 r, 25 r or 125 r on the fourth day of their lives.

In the females, which in all the groups have a longer mean life than the males, life shortening has apparently occurred in the 125 r group. The mean longevity for this group is 21.7 months, while the control value is 24.2 months. Other differences in longevity are of doubtful significance. External tumours appeared earlier in the female rats in the 25 r and 125 r groups. The radiation doses employed were below the threshold for radiation induced greying of hair. When the eight experimental groups were sub-divided into 48 groups based on sex, radiation dose and paternal origin, a striking genetic effect on longevity became apparent. The influence of the male parent on the length of lifespan of its progeny was found to be of greater magnitude than that of irradiation.

5. PATERNAL EXPOSURE AND LIFESPAN IN MICE

The paper by Kohn and Bailey[1]) is basically one of genetics, but nevertheless is of great interest for the present session. Male CAF_1 mice were irradiated with filtered 250 KVp X rays both to the whole body and to the caudal third of the body, and after an interval of not less than 6 months, i.e., after the germinal epithelium had recovered, were mated with unirradiated female BALB/c mice. Any residual genetic injury would therefore be transmitted only by the spermatogonia or stem cells. Kohn had reported previously[2]) that neither the sex ratio nor the litter size of the progeny was influenced by paternal doses as high as 720 rad. The present paper reports on the lifespan of the progeny for controls and for paternal doses between 248 and 720 rad. There were two main findings. The effects of paternal irradiation were small. The largest difference observed between the control and treated groups was about 10% and occurred in the progeny of the paternal group most heavily irradiated with a single dose of 720 rad. Further, the sexes were affected differently. There was a tendency for the male progeny to show life-shortening and the female progeny to show life-lengthening.

6. REPARABLE INJURY AND RECOVERY IN DOGS

The complexity of the physiological and pathological processes following total body irradiation is so great that it is desirable to devote a major effort to try to analyse the factors involved in quantitative experiments. A detailed study of the mechanisms of injury and recovery from whole and partial body exposure to ionizing radiation is

presented by Michaelson, Odland and Howland[l]). These experiments were carried out with dogs.

It has been assumed that total body irradiation produces injury, one component of which is reparable exponentially, while another is permanent or residual and cumulative in relation to the total dose received. As an index of reparable injury, the recovery half-time has been utilized. If experimental animals are irradiated and after a suitable period of time are re-exposed to a second dose sufficient to produce lethality, it is found that the LD 50 for death within 60 days (LD 50/60) is considerably lower than the single dose LD 50/60. The reduction of the magnitude of the second dose of radiation required to produce lethality is taken as evidence that there is a latent injury remaining after the initial exposure. For the dog, the recovery half-time was found to be 8.6 days. Residual injury is expressed as a 15 to 20% reduction in the acute LD 50/60 in dogs tested 3 to 9 months after a single near lethal dose of radiation. Although lethality is the indicator of injury commonly used, it is shown that haematologic changes may provide a more sensitive method of assessing the processes of injury and recovery. Finally, it may be estimated that the recovery half-time for man after whole-body irradiation is between 15 and 22 days.

7. REMOVAL OF SPLEEN AND APPENDIX

The interactions of different tissues and organs in the course of events leading to the late results of damage produced by irradiation are considered from a more detailed point of view, with especial reference to the importance of the role of the spleen, in the paper submitted by Messerschmidt, Shibata and Melching[k]). White mice of an inbred strain were splenectomized 14 days before total body X irradiation. The survival rate was studied and the dose effect curves compared for the treated and control animals. Further evidence was gained by examining the daily urinary excretion of taurine. In addition, similar series of experiments were carried out to compare the effects of appendicectomy with those of splenectomy. It is pointed out that in the mouse the appendix is not only a relatively large lymphatic organ but it has also a particular functional importance. In the experiments reported in this paper, it was found that splenectomy of the white mice 14 days before total body irradiation resulted in an increase of the survival rate in the dose region

below the LD 100/30. For example, after 690 r, the survival rate was 18.0% (18/100) for the controls but 67.1% (47/70) for the splenectomized animals. In contrast to these findings, appendicectomy 14 days before irradiation produced no significant increase in survival. Similarly, the increased urinary excretion of taurine, which occurs during the first 3 days after irradiation, is reduced by splenectomy before irradiation but not by appendicectomy.

It is important to note that the protective action of splenectomy is only found in the region of lower doses.

8. X-RAY EFFECTS ON THE LUNG

The effects of radiation on the lung have been studied as a matter of importance since the early days of the development of radiotherapy. In the present session we have a detailed paper by Smith and Lavik[m]). This describes an experiment in which in groups of rats the right hemithorax was irradiated with a single dose of 3000 r of filtered 220 KV X radiation and the surviving animals were subsequently sacrificed at intervals. Of the 120 rats irradiated, 43 died before the scheduled time of sacrifice.

The pathological changes observed were generally progressive. In the rat, the first change is a focal and microscopic accumulation of macrophages within alveoli that is accompanied by a depopulation of the lining surfaces of the air sacs. Cells rendered incapable of further division by irradiation cease to contribute to the cell population of the tissue so that atrophy gradually ensues. Hyalinization occurs and, as is well known, is attributable to an alteration of the connective tissue of the alveolar walls. With partial collapse of the air sacs, the tissue displays progressive shrinkage and contraction. Since the radiation reaction is atrophic rather than inflammatory it is suggested that the process be termed "pulmonary radiatrophy" rather than radiation pneumonitis, although this latter term is, as the author says, "blessed with the sanction of established usage".

9. OXYGEN EFFECT AND RENAL FUNCTION

The next paper, by Concannon, Brewer, Summers, Teal and Hayeslip[c]), is a preliminary report of a carefully designed experiment to determine whether a normal well-vascularized mammalian organ, of which the kidney is taken as an example, is more radiosensitive

and shows increased damage to renal function after irradiation under conditions of increased oxygen tension. Dogs were irradiated at three different levels of tissue dose (1900 r, 2500 r and 3100 r in 13 days) at normal atmospheric pressure and at 3 atm absolute pressure of oxygen. In all, 36 dogs were used. Measurements of glomerular filtration rate, effective renal plasma flow and maximum rate of tubular excretion were made pre- and post-irradiation for times up to 15 months after exposure. The mean effects of dose and time are highly significant ($P < 0.0001$).

The mean effects of O_2 pressure-dose interaction were insignificant for changes in glomerular filtration rate and effective renal plasma flow. These effects, however, were significant for maximum rate of tubular excretion ($P = 0.05$ to 0.01).

The preliminary observations would seem to offer supportive evidence that irradiation of well-vascularized tissues under 3 atm pressure of O_2 does not produce a major increase in radiosensitivity.

10. CHROMOSOME ABERRATIONS IN REGENERATING LIVER

In studies of the mechanisms of production of the late effects of irradiation, it is important to try to assess the part played by induced chromosomal aberrations. Leong, Pessotti and Krebs[j]) describe experiments, the purpose of which is to relate the incidence of abnormal mitoses in the regenerating liver after partial hepatectomy to the functional competence of the organ which has been irradiated at one day after birth. The irradiated and also control rats were subjected to partial hepatectomy at the age of 90 days.

A most important and interesting finding is that in the animals exposed to 200 rad at one day after birth, the incidence of abnormal mitoses was approximately 50% of the total anaphases and telophases, the corresponding value for the controls being approximately 10 to 15%. However, the presence of these abnormal mitoses apparently does not impair the functional ability of the liver to produce bile or to remove ^{35}S-labelled bromosulphophthalein from the peripheral blood and excrete in into the bile. As the authors comment: "It is only possible to say at this time that the functional tests that we have used to describe the physiology of the irradiated livers are not sensitive enough to characterize the damage which has been produced within the cells".

Curtis and Crowley[d]) have studied the frequency of chromosomal

aberrations in regenerating liver cells of mice, particularly from the point of view of the somatic mutation theory of ageing.

The regenerating liver cells of the mouse were examined at 72 hours after subcutaneous injection of CCl_4, at the peak of mitotic activity. Squash preparations were used. Abnormal mitotic figures were scored as either bridges or fragments or both. The experiments were started when the mice were 8 weeks old. The percentage of chromosomal aberrations present in the regenerating liver cells was scored as a function of (i) age in normal mice, (ii) time following irradiation with a single LD 50 dose of 323 rad of fast neutrons produced by the Brookhaven reactor (iii) age of animals subjected to chronic ^{60}Co gamma irradiation at the rate of 7.5 rad per day and (iv) time after irradiation with a single dose of 234 rad of filtered 250 KVp X rays.

It was found that the frequency of aberrations is very high in the neutron treated animals and even increases to nearly 90% in succeeding months. This finding is consistent with the fact that there is little or no recovery from fast neutron irradiation as far as life-shortening is concerned. Comparison between the effects of chronic gamma irradiation and acute X irradiation shows that recovery occurs and that chromosome healing must take place. These observations support the tentative hypothesis that acceleration of ageing by radiation is due to the induction of mutations in somatic cells. However, it is considered that there must be other factors also involved in ageing.

11. RBE OF FAST NEUTRONS

This brings us to the problem of the relative biological effectiveness (RBE) of fast neutrons. The paper by Upton, Randolph and Conklin[9]) deals with studies of the influence of the dose rate on the RBE of fast neutrons of different energies in relation to ^{60}Co γ rays for late somatic effects in mice. Fast neutrons in three energy ranges were used, with mean energies of about 1, 5, and 14 MeV. The female RF mice, starting at 10 weeks of age, received total body irradiation at different dose rates, ranging from about 100 rad/min to less than 7×10^{-5} rad/min until a predetermined dose had been accumulated or until 50% of the animals had died.

The results confirm and extend observations by other workers that the effectiveness of gamma-rays may vary inversely with inten-

sity by a factor of at least 3 to 5 whereas that of fast neutrons is generally less, if at all, intensity dependent. There was no detectable change of the effectiveness of fast neutrons with dose rate for shortening of life, but a substantial rate dependent variation in gamma-ray effectiveness for this process. The low RBE of fast neutrons for induction of ovarian tumours in mice is particularly puzzling. The energy of the neutrons is of quantitative importance, e.g., the 1 MeV neutrons appeared to be most highly cataractogenic, possibly because of the high values of LET. The RBE exceeded 10 only for cataract induction.

12. LENS OPACIFICATION

Cataract formation, i.e., opacification of the lens, is one of the most constant findings in the study of the late effects of exposure to fast neutrons. The paper submitted by Kimeldorf[h]) describes work designed to provide information on two aspects of the problem (i) the lens opacification response to fast neutrons used in young adults with respect to dose, and (ii) the effect of age at exposure upon the development of opacification for the same dose. The fast neutrons had an energy distribution similar to that of fission. All doses were administered as single total body exposure. The degree of opacification was graded in severity on a four grade scale. The incidence of non-irradiation cataracts was less than 4% in the eyes examined.

The three higher doses used (230, 320 and 360 rad) were found to be capable of inducing complete opacification. The proportion of animals with complete opacification at a given time increases with dose. The data suggest that both latency and incidence of complete opacification are dose dependent.

The threshold dose for complete opacification in rats exposed as young adults is above 50 rad and less than 230 rad of the fast neutrons studied. The dose of about 230 rad was capable of inducing complete opacification, i.e., Stage IV cataract, regardless of age at exposure, in all cases with a latency to complete opacification of 36 to 40 weeks.

The proportion of lenses which did not show any opacification increased with the age of exposure from 3% for irradiation of young adults to 60% for irradiation during old age. There are still many problems to be elucidated in this field.

13. BONE

Finally, we come to the problem of the late effects of incorporated alpha-emitters on bone. In a detailed experimental study Stover and Jee[n]) describe selected skeletal samples obtained from healthy control beagles, and from beagles injected with a single intravenous dose of about 3 μC of ^{239}Pu per Kg in young adulthood at 14 to 23 months of age. These exposed dogs lived for 3 to 5 years after injection and at death their skeletons and livers had been severely damaged. Various areas of control and irradiated humeri and lumbar vertebrae have been analysed for water, organic and mineral contents, and similar samples have been studied by histological and microradiographic techniques.

The level of dosage of ^{239}Pu used is known to result in severe skeletal degeneration, with spontaneous fractures and osteoporosis, as well as the development in some cases of osteogenic sarcoma. However, the ratios of the average bone weights are essentially the same as the ratios of the average body weights, which is a remarkable finding.

A number of significant differences between average control and average irradiated composition values were observed. The decrease in fractional water content of the irradiated vertebrae correlate well with the histological and microradiographic findings. The loss of marrow and the hypermineralization of the existing bone in the vertebral body are consistent with decrease in water and increase in mineral contents. In the irradiated humeri, hypermineralization is observed in the proximal epiphyseal region.

The results discussed illustrate some aspects of the complex problem of evaluating radiation dose rates and doses from ^{239}Pu deposited in bone, for bone changes with time and with irradiation.

The last paper to be summarized is the work of Hasterlik, Finkel and Miller[t]). In the environs of Chicago there are approximately 400 women who worked as dial painters at some time in the past. However, this study has concerned itself primarily with women whose occupational history antedated 1925. Of the 264 subjects whose body radium content has been determined at the present time, it has been possible to complete detailed radiographic studies of the entire skeleton in 236. Gamma ray spectroscopy showed evidence of mesothorium in addition to ^{226}Ra in only 2 cases. The criteria for coding

the radiographic changes in the bones have been mutually agreed and discussed with the other two research groups carrying out similar studies in the USA. The regions of radium burden above and below the present occupational maximum permissible level of one-tenth of a microcurie are of extreme importance.

In the range of one-hundredth up to one-tenth of a microcurie, 90 subjects were radiographed. Of these, 80 showed no bone lesions, 8 had minimal changes, one had mild changes and one had moderate changes. None had advanced lesions or malignancies.

In the range of one-tenth up to 1 μC, 61 patients were radiographed. Of these, 25 showed no bone changes, 13 showed minimal changes, 9 mild and 9 moderate and 5 showed advanced changes; three patients had malignant neoplasms. It is to be noted that the lesions classified as "advanced" are for the most part crippling and of clinical significance.

There were 41 patients with more than 1 μC of radium; 40 of these were radiographed. 28 had advanced bone lesions and of these 14 had malignant neoplasms. There have been no cases of myeloid leukaemia.

It is clear that a correlation exists between the terminal radium content of the body and the number and severity of bone lesions. The evaluation of the initial radium burden is obviously a very difficult problem. It is of great interest that no advanced bone changes or malignant neoplasms were seen when the terminal radium content was below 0.1 μC of ^{226}Ra.

14. CONCLUSION

So far I have tried to be objective. The Chairman suggested that it would be useful for me to add some personal comments. It is clear that there is a need to continue these investigations during the next four years. Further, I would like to suggest that there is a particular need for two main types of study: first, the biochemical basis of the initial lesions, including macromolecular lesions, leading to the late effects, and second, clinical investigations of the effects of irradiation, especially at relatively low dose rates in patients and at low dose rates in exposed populations.

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DISCUSSION

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Leong[j]) said that the irradiated animals in his experiments were as well able to replace liver mass as the controls. Moreover, the cells which made up the regenerated mass after partial hepatectomy were of the same size in irradiated and control animals.

Hasterlik[f]) said that in his opinion it was important to study the excretion rates for radium in man experimentally. He thought it could be done safely. It was not possible to extrapolate with certainty from results obtained with small animals.

Kohn[e,i]) referred to the estimate of Michaelson and his colleagues[1]) that the recovery half-time after whole-body radiation in man is between 15 and 22 days. He asked how this estimate was made. In reply Michaelson said that in animals a linear relationship existed between the recovery half-time in a given species and the basal metabolic rate. Davidson[1]) had also shown that there was a relationship between recovery half-time and the time required for maximum depression of white blood cells. On the basis of basal metabolic rate, it was, therefore, possible to estimate the recovery half-time (and likewise the time of maximum leukocyte depression after a single exposure) in man.

Alexander (*Chester Beatty Res. Inst., London SW7, Great Britain*) wanted to know for what reasons Guttmann and Kohn ascribed radiation-induced intercapillary glomerulosclerosis to an auto-immune process. Guttmann[e]) replied that this view was only hypothetical, but that there were three reasons for putting it forward. Firstly, the glomerular lesions were very similar to those seen in nephrotoxic nephrosis produced experimentally by anti-kidney immune sera. Secondly, one had to explain the presence of a latent period between X irradiation and the development of pathological changes. Thirdly, it had recently been demonstrated in his labora-

tory that gamma-globulin appeared in the glomeruli 45 to 65 days after irradiation; this was not direct evidence of an antigen-antibody reaction, but in conjunction with the other findings it seemed to warrant further investigation of the rôle of auto-immune processes in the pathogenesis of intercapillary glomerulosclerosis.

Alexander also asked whether Hasterlik and his colleagues thought that their new data made it necessary to reconsider the currently accepted levels for maximum permissible body burdens of radium. Hasterlik replied in the negative. He pointed out that his examinations had been carried out long after exposure to radium, and that earlier body burdens had undoubtedly been much higher. Also it was possible to detect radiographically lesions which were far too small to have a clinical effect; and it was on a basis of clinical effect that permissible body-burdens were generally determined.

Rajewsky (*Max Planck Inst., Frankfurt am Main, Germany*) asked whether Hasterlik had found tumours other than sarcomata in his studies. Hasterlik replied that they had seen 8 epithelial tumours of the mastoid and para-nasal sinuses. Such tumours were normally very rare. He did not think that this implied a specially high radiosensitivity for these epithelia; they were very closely applied to bone and therefore susceptible to irradiation from it.

Mewissen (*Inst. Bordet, Brussels, Belgium*) said that in an experiment carried out in Chicago with Rust and others[2]), he had demonstrated a significant reduction of lifespan in the progeny of irradiated male C57BL mice mated with non-irradiated CBA females. No difference was however observed when the female was the irradiated parent. Did Kohn have any data concerning the progeny of irradiated females mated to non-irradiated males? Kohn replied that the ovary was exceptionally radiosensitive, 50 to 100 rad being sufficient to sterilize. In contrast, up to 750 rad could be given to the testis, with recovery after about 6 months. For this reason, no experiments had been performed on the effect of irradiating the female parent.

Hulse (*MRC, Harwell, Great Britain*) wanted to know whether Smith[m]) had any theories as to how irradiation initiated the accumulation of macrophages in the alveoli. Presumably the macrophages were attempting to remove some substance. What might the substance be? Smith replied that Bertalanffy and Leblond[3]) had shown the alveoli to be populated by vacuolated and non-vacuolated cells, which had generation times of about 8 and 30 days.

The suppression of mitosis of these cells, together with obstruction of the lymphatic channels through which they might leave the lung, could account for their accumulation within the alveoli.

Kaplan (*Dept. Radiol., Univ., Palo Alto, Cal., USA*) wondered whether the results of Messerschmidt[k]) and his colleagues could be interpreted in terms of a delayed compensatory hypertrophy of the bone marrow 14 days after splenectomy. By analogy with the events following oestrogen injection, 14 days was the interval at which compensatory hypertrophy might be expected to develop, and it might make the animal less radiosensitive. In reply Melching[k]) said that a protective effect could be seen even in animals splenectomized 6 hr after irradiation.

Arghittu (*Centro Appl. Militari Energia Nucleari, Pisa, Italy*) recalled the classic experiments of Jacobson, Lorenz, Ellinger and others, which showed that the spleen possessed a factor which combatted the effects of radiation. He asked how Messerschmidt and his colleagues explained the apparent paradox that splenectomy in their experiments had a protective rather than an aggravating effect. Melching replied that the phenomenon could best be explained in terms of biochemical changes which they had shown to occur in the spleen after irradiation. These might well have an adverse effect on survival. Smith pointed out that massive cytolysis of lymphoid tissues occurred within 6 hours of whole-body irradiation, and that this might be the reason why removal of the spleen had a favourable effect.

The Chairman (*Bugher, Agr. Bio-Sci. Div., Univ. of Puerto Rico, Mayaguez, Puerto Rico*) concluded the discussion with the observation that participants in it had tended to avoid the difficult and practically important question of life-shortening after irradiation.

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IRREPARABLE DAMAGE TO THE REPRODUCTIVE ORGANS

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a) S. O. BROWN, G. M. KRISE and H. B. PACE,
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Radiation Biology Laboratory, Texas A and M College, College Station, Texas, USA

b) J. DE BOER,
The effects of chronic whole-body irradiation on the reproduction of C57 black mice.
Air Force Special Weapon Centre, Kirtland Air Force Base, New Mexico, USA

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Department of Anatomy, University of California, San Francisco, California, USA

d) PATRICIA J. LINDOP, B. W. G. MORGAN and J. ROTBLAT,
The effect of whole-body irradiation on the fertility of female mice.
St. Bartholomew's Hospital Medical College, London EC1, Great Britain

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St. Bartholomew's Hospital Medical College, London EC1, Great Britain

f) K. G. LÜNING,
The breeding capacity in offspring of a mouse population given spermatogonial irradiation.
Laboratory of Radiation Genetics, Stockholm, Sweden

g) ANITA M. MANDL,
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Department of Anatomy, University of Birmingham, Birmingham 15, Great Britain

h) HANNAH PETERS and EMELIA LEVY,
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Finsen Laboratory, Finsen Institute and Radiumstation, Copenhagen, Denmark

i) N. V. SAVKOVIC, D. V. RADIVOJEVIC and S. I. HAJDUKOVIC,
Effect of whole body and local X irradiation on the reproductive ability of infantile male rats and changes in the first generation.
Institute of Nuclear Sciences "Boris Kidrich", Belgrade, Yugoslavia

j) A. G. SEARLE,
The productivity of daughters of X irradiated mice and the sex-ratio in their offspring.
Medical Research Council, Radiobiological Research Unit, Harwell, Berkshire, Great Britain

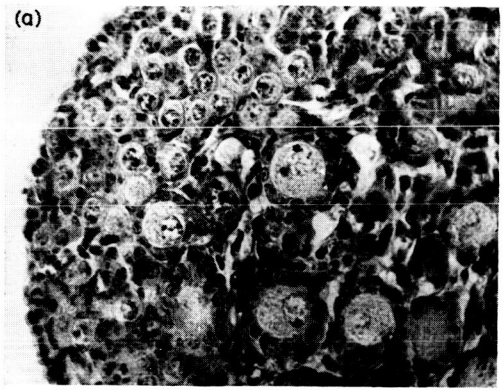

Fig. 1a. Photomicrograph of a 3-day control ovary showing primary follicles (Stage 1) and many primary oocytes.

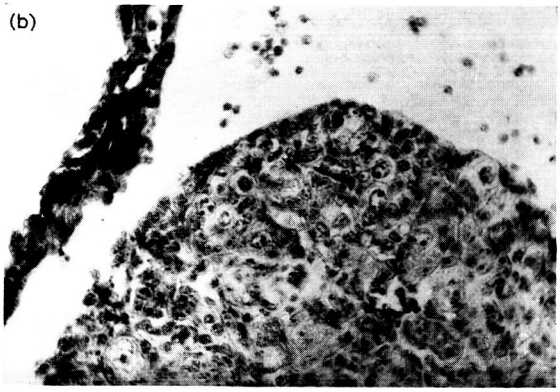

Fig. 1b. Photomicrograph of the ovary 3 days after irradiation. Most of the oocytes show degenerative changes, and the number of normal primary oocytes is greatly reduced. The activity of the germinal epithelium is suppressed.

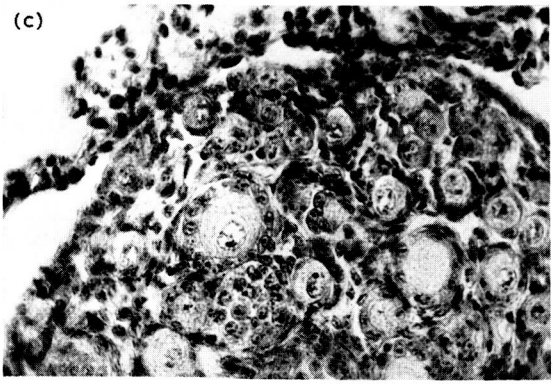

Fig. 1c. Ovary 6 days after irradiation, showing the resumption of oogenesis in the germinal epithelium and the increase in the number of healthy primary follicles. (Sundaram and Desai.)

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Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico, USA

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Medical Division, Atomic Energy Establishment, Trombay, Bombay, India

[m]) NIEL WALD, C. E. MILLER, W. H. BORGES and JIP KIM,
A cytogenetic study of some radium dial-painters and their progeny.
University of Pittsburgh, Graduate School of Public Health, Pittsburgh 13, Pennsylvania, USA

1. INTRODUCTION

It is now well recognized that the gonads are highly sensitive to the effects of ionizing radiation. These post-irradiation changes vary in intensity and depend not only on the age of the animals at the time of exposure, but also on the dose rate, a single acute dose of radiation being much more effective in producing damaging effects than the same dose applied chronically. The methods of evaluating the resulting damage have included studies of histological and cytological changes in the gonads themselves and of impairment of subsequent reproductive capacity. Much can also be learned from a quantitative study of the dose-response interrelationships, but great caution must be exercised in their interpretation. Contributions along these lines are well represented in this session and will be considered under the following sub-headings:

Structural and functional responses to radiation (section 2).
Genetic hazards of ionizing radiation (section 3).
Modification of the biological response of mammals to radiation (section 4).

2. STRUCTURAL AND FUNCTIONAL RESPONSES TO RADIATION

In studying the histological effects of exposure of the female mouse to a single dose of 20 r of deep X rays given within 24 hours of birth, Sundaram and Desai[l]) observed extreme sensitivity, manifested by suppression of oogenesis from the germinal epithelium, a pronounced reduction in the number of primary oocytes, and a marked increase in degenerating oocytes (Fig. 1). These radiation effects were, however, temporary and reversible, ovarian histology

returning to normal after 14 days without showing an influence on the subsequent maturation of follicles. Even though morphological recovery in the ovary appeared complete, the reproductive performance of the animals was, nevertheless, greatly reduced.

These data, together with those available on the effects of a single exposure of 27 r (Murray[1])) on sexually mature animals, led to the conclusion that there is a critical phase in the development of the ovary when marked sensitivity to irradiation is exhibited at a dose level that, in the adult animal, is ineffective in causing histological damage.

Peters and Levy[h]), employing other biological criteria, adduced physiological evidence to corroborate the known differences in histological sensitivity to the effects of radiation at different stages of postnatal gonadal development. With the same dose of radiation used by Sundaram and Desai, namely 20 r, they showed (Figs. 2 and 3) that the reproductive capacity of their mice was depressed

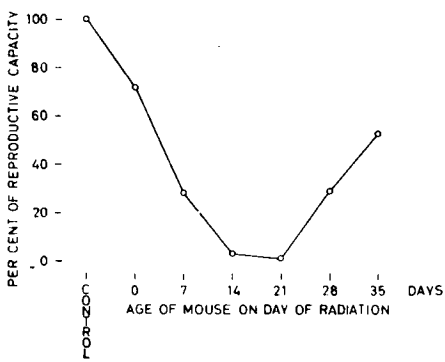

Fig. 2. Reproductive capacity of female mice as a function of their age when exposed to 20 r. (Peters and Levy.)

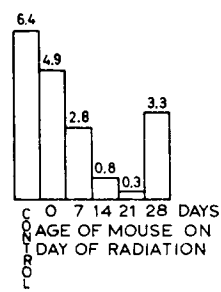

Fig. 3. Average number of litters per mouse in females irradiated with 20 r, at different ages. (Peters and Levy.)

to its lowest value of 1.4% of normal when the animals were irradiated at 21 days of age; irradiation before and after this critical period effected a less striking reduction in fertility. In contrast, however, to the findings of Sundaram and Desai, the radiation-induced impairment of reproductive functions was progressive. No improvement in the reduced fertility was observed even after a latent period, suggesting that damaged oocytes neither recover nor are replaced.

In these two studies, the differences in radiosensitivity, as judged by fertility, could have resulted from differences in chromosomal configuration in the two strains of mice at the time of birth, dictyate on the one hand and pachytene on the other, since it is known that oocytes in the rat are more radiosensitive in the dictyate than in the pachytene phase.

Utilizing a wide dose range of X rays from 15 r to 2200 r, Mandl[g]) studied the effects of radiation on the fertility of the female rat

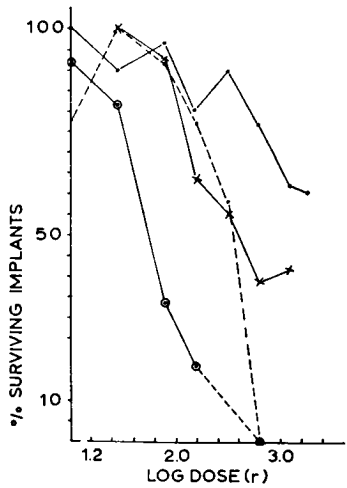

Fig. 4. Survival of implants after irradiation of female rats at different times before mating. (Mandl.)
. —— . 4.5 to 35 days before first mating.
× —— × Noon of pro-oestrus.
× - - - × Early afternoon of pro-oestrus.
⊙ —— ⊙ Early evening of pro-oestrus.

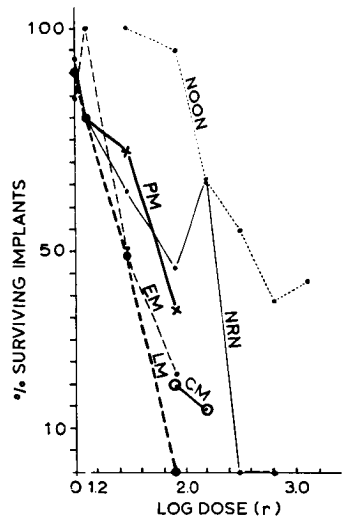

Fig. 5. Survival of implants after irradiation at different stages of the maturation division. (Mandl.)
. —— . Resting nucleus.
⊙ —— ⊙ Chromatin mass.
× —— × Premetaphase.
. - - - - . Early metaphase.
● - - - - ● Late metaphase.

emphasizing the extreme sensitivity of the germ cell. With the knowledge that there is some degree of synchronization of maturation division and the diurnal light cycle, and that under normal laboratory conditions of lighting ovulation takes place at about 2.0 a.m. and early diakinesis at about 5.0 p.m., the ovary was irradiated at various times that could be closely correlated with meiosis.

Judging by the proportion of viable implants, as shown in Figs. 4 to 6, radiosensitivity of the pre-ovulatory follicle rose between noon and early evening preceding ovulation; irradiation at noon on the day of pro-oestrus was followed by a higher mortality than that performed 5 to 35 days before fertilization. Furthermore, the shorter the interval between irradiation and ovulation, the greater was the number of eggs which failed to implant and, to an even greater extent, the incidence of embryonic mortality. It was clear, nevertheless, (Fig. 7) that the total number of implants was not significantly decreased except by high doses of radiation.

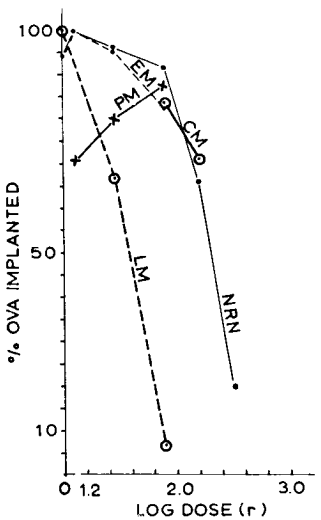

Fig. 6. Number of ova implanted after irradiation at different stages of the maturation division. (Mandl.)
Key as in Fig. 5.

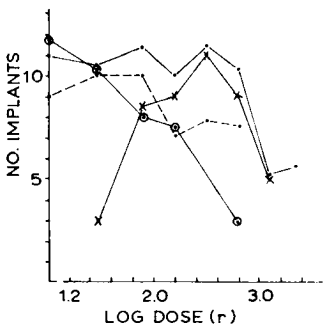

Fig. 7. Number of ova implanted after irradiation at different times before mating. (Mandl.)
Key as in Fig. 4.

In unilaterally spayed rats, in which the excised ovary served as an index of the maturation stage at which the animal was irradiated, histological examination confirmed that radiosensitivity increased as maturation division approached and proceeded. In fact, there was some evidence suggesting that radiosensitivity began to increase even before any unequivocal morphological changes associated with impending division could be discerned in the oocyte nucleus. Prior

to fertilization, the oocyte in small and medium-sized follicles (4 days or more before mating), was shown to be three times as radioresistant as that some 12 hr before fertilization, whereas after fertilization the radiosensitivity of the zygote again declined. Indeed, as early as 12 hr after fertilization the zygote exhibited considerably less radiosensitivity than the unfertilized oocyte during meiotic metaphase.

Mandl has recorded an additional observation of some interest regarding the corpora lutea of pregnancy; the number of ova shed was not drastically reduced by even high doses of radiation. On the contrary, Mandl has provided proof for the suggestion of Brown and his co-workers[a]) that doses in the range of 150 r to 600 r frequently resulted in superovulation of "under-sized follicles," particularly in animals exposed a few days before mating. Relatively few of these ova implant, however, particularly following irradiation during the evening of pro-oestrus.

Whereas the three preceding studies were concerned with the effects of acute doses of ionizing radiation, de Boer's[b]) experiments involved the exposure of C57 black mice to chronic whole body gamma radiation from a ^{60}Co source for 23 hr daily, so that the animals received daily doses of chronic radiation amounting to 2 r, 5 r, 10 r and 20 r before conception. In this investigation radiation damage was assessed by combining histological criteria with fertility studies that included the time of production of the first litter, a valid parameter when one takes into account the adverse effect of ageing *per se*.

De Boer's experiments revealed a greater sensitivity of the male to the deleterious effects of radiation. There were no indications, however, of a decrease in the reproductive life span of the animals, corroborating histological evidence suggesting that the organism as a whole is capable of making adjustments which lessen the effects of chronic low dose irradiation.

The histological criteria comprised the quantitative estimation of Sertoli cells, which did not vary significantly at any radiation level, and of the following four cell types which decreased significantly: type A spermatogonia, spermatocytes in leptotene and pachytene stages of development, and developing spermatozoa. Grossly, there was a significant decrease in the body weight and in the weight of the testes. In the case of the ovary, however, such

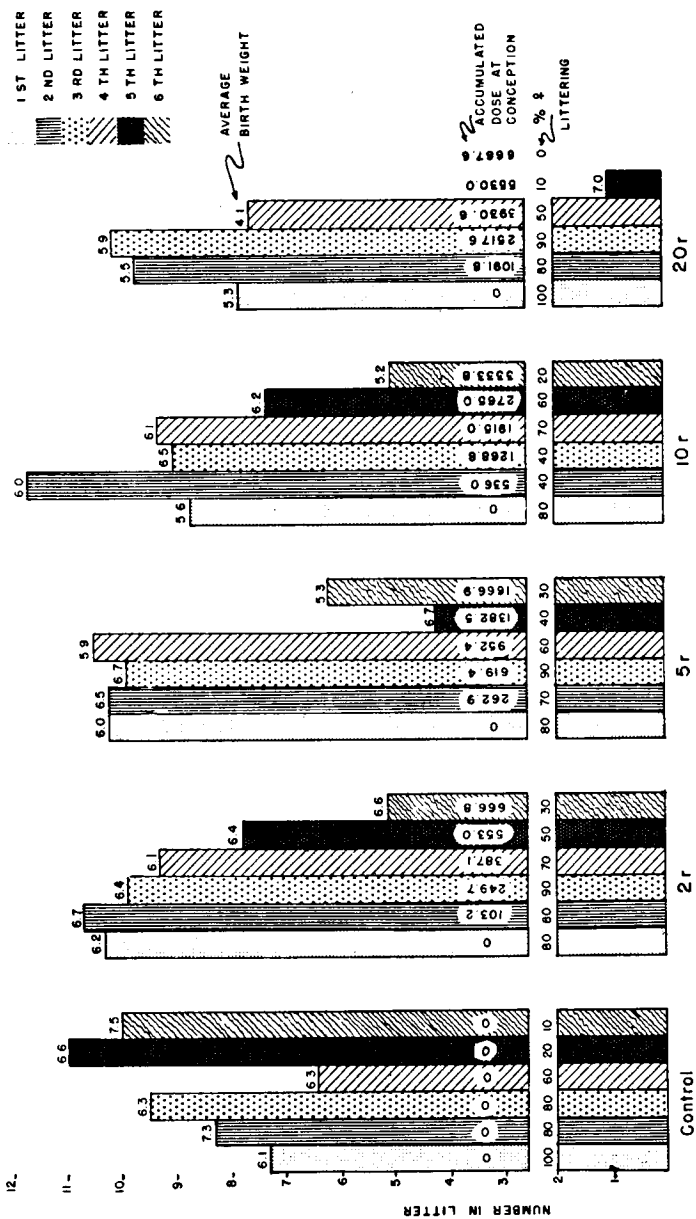

Fig. 8. Litter size of albino rats as influenced by chronic γ-radiation of the mothers from 2 r to 20 r/day from first conception throughout reproductive life. (Brown, Krise and Pace.)

specific correlations of gross and histological structure with radiation damage were not possible, although there was a strong su-

spicion of a relationship between gonadotrophic hormone levels and damage due to radiation, which might account for the less pronounced radiation effects.

Utilizing albino rats rather than mice, as in de Boer's studies, Brown, Krise and Pace[a]) evaluated the effect of chronic low-dose gamma radiation upon the fertility and reproductive capacity of the female and her offspring. The test animals, in groups of 10, were exposed to chronic gamma-radiation from a ^{60}Co source for 23 hr daily at levels of 0 r, 2 r, 5 r, 10 r and 20 r immediately following conception, and were kept in the radiation field for the duration of their reproductive lives.

Analysis of the results (Fig. 8) showed that for six successive litters at the 2 r, 5 r and 10 r daily radiation levels, neither the fertility nor the reproductive capacity differed significantly from the controls. On the other hand, at 20 r daily only one offspring was born in the fifth litter to the five surviving females and none was born in the sixth; all the offspring appeared normal, except for a slight decrease in average weight of the newborn. Age would thus appear to be an important factor influencing the size of litters produced near the termination of the reproductive life span of the albino rat.

Whereas, however, offspring from the third litter reproduced when irradiated males were mated with irradiated females on the 2 r and 5 r levels of daily radiation, males that received 10 r and 20 r from the time of conception were sterile. These results are in agreement with data regarding the differing radiosensitivity of the germ cells in the two sexes. They indicate, moreover, that during chronic radiation the ability to repair the radiation-induced damage within the animal is sufficient to maintain the functional integrity of the oocytes during the normal reproductive life span of the albino rat subjected to 2 r and 5 r daily levels of a whole-body chronic irradiation.

3. GENETIC HAZARDS OF IONIZING RADIATION

Turning to the genetic hazards of ionizing radiation, there is increasing evidence that many mutations may have slight dominant deleterious effects that may culminate in a greater population damage than that caused by the lethal effects of homozygous mutations (Russell, Russell and Oakberg[2])). In any attempt, therefore, to esti-

mate the genetic effect of ionizing radiation on fitness in general, it is very important to obtain data on the immediate offspring of those exposed in order to ascertain the nature of the dominant deleterious factors being induced. Equally important is the necessity to employ sibling, as well as random matings, since the expression of both recessive and dominant genes can be detected in brother × sister matings, whereas random mating reflects mainly dominant mutations.

In studying the production of both dominant and recessive mutations that adversely affect fertility in mice, Lüning[f]) utilizing both sibling and random matings, tested the effect of irradiation of spermatogonia with 276 r of X rays for four generations. Impairment of fertility due to dominant genes, manifesting itself by a reduction in the number of litters per female surviving the test period, was not observed, nor was complete sterility. Furthermore, a significant difference in post-natal lethality between the control and the irradiated group was not detected. There was, however, the suggestion of a slightly higher intra-uterine death rate among the brother-sister matings in the irradiated group, similar to that reported by Searle and due possibly to the action of recessive lethals. Such differences did not, however, reach a significant level. This relative absence of radiation effect was similar to that found by Carter and Lyon[3]) who used much higher doses (600 r). On the other hand, the only frankly significant finding was a decrease in the number of corpora lutea in females from the irradiated group (Tables 1 and 2). This suggests some general deleterious dominant genetic effect of the radiation, since the females were the progeny of an outcross, and probably polygenic. It calls attention also to the necessity of considering factors other than foetal loss in evaluating the effects of radiation in the reduction of litter size at birth.

Spalding and Strang[k]) have studied the comparative resistance of two lines of mice with different parental histories of exposure to chronic gamma and fractionated X irradiation.

The grand offspring of a line in which the sires received 200 rad of X rays at weaning for 15 consecutive generations were significantly less resistant to both continuous low intensity X rays and to fractionated acute X ray exposure than were control line mice. This reduced radioresistance was attributed to radiation-induced genetic damage, but could conceivably have been due to changed genetic selection at the time the separate lines were established.

TABLE 1

Effect of irradiation on fertility in mice (Lüning)

A. Brother × sister matings between offspring from the 4th generation of the irradiated and the control populations. Dissection of the 1st, 2nd or 4th litter.

Series	Litter	Alive	Dead or Males	Im-planted	% Dead	Corpora Lutea	Fe-males	Corpora Lutea/ female
Irradiated	1	711	97	808	12.00	894	133	6.72
Irradiated	2	886	122	1008	12.19	1080	141	7.66
Irradiated	4	171	43	214	20.1	232	28	8.29
Total		1768	262	2030	12.91	2206	302	7.30
Control	1	898	83	981	8.46	1091	151	7.23
Control	2	864	111	975	11.38	1060	138	7.68
Control	4	163	39	202	19.3	203	26	7.81
Total		1925	233	2158	10.80	2354	315	7.47
B. Non-brother × sister matings.								
Irradiated	1	1370	169	1539	10.98	1720	245	7.02
Irradiated	4	219	44	263	16.7	277	35	7.91
Control	1	1639	216	1855	11.64	2118	289	7.33
Control	4	135	31	166	18.7	174	21	8.28

Statistical analysis of the figures given in this table reveal that at the first pregnancy the number of corpora lutea is significantly lower in the irradiated than in the control group ($P<0.001$). At the second and fourth pregnancy this was not the case.

TABLE 2

The number of corpora lutea at the first litter in females from the fourth generation of the irradiated and the control populations of mice (Lüning)

Series	Corpora Lutea	Females	Corpora Lutea/female
Irradiated	2614	378	6.92
Control	3209	440	7.29

In an attempt to verify Russell's[4]) observation that the offspring of irradiated male mice have a life span shorter than that of the offspring of non-irradiated fathers, Lindop and Rotblat[6]) compared the life span of unirradiated mice from unirradiated fathers with that of mice from one or more generations of irradiated male ancestors. For this purpose the mice were given a single whole-

body dose of 350 r at four weeks of age. After statistical analysis, the authors conclude that there was evidence neither of decreased longevity in the offspring of irradiated fathers nor of a cumulative effect of three generations of parental exposure either on life span or sensitivity to radiation. In the first generation, however, a significant difference in fertility between irradiated and non-irradiated fathers was observed (52 $\pm$ 5% compared with 30 $\pm$ 5%), although by the second breeding complete recovery had taken place.

An incidental, and as yet unexplained, observation was the gradual decrease in the loss of weight between irradiated and non-irradiated groups in successive generations. By the third generation in fact, the difference in weight between the two groups was virtually abolished.

Searle[1]), in a study methodologically similar to the three just described, investigated the effect of X radiation on fertility in mice, with particular reference to the sex-ratio of the offspring. Three generations of female mice, the fathers of which had received 2 doses of 600 r high intensity X ray to the gonads at 6 and 14 weeks of age respectively, were observed. The daughters of the irradiated males were outcrossed, allowed to produce four litters and autopsied during their fifth gestation. The two most striking effects of irradiation of the grandfathers were decrease in litter size and in the proportion of males in the F_2 generation.

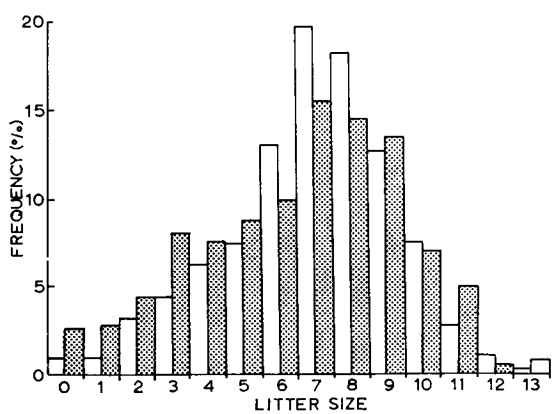

Fig. 9. A comparison of litter size frequency distributions in the offspring of daughters of control (open columns) and irradiated (solid columns) male mice. (Searle.)

Study of litter size frequency distributions (Fig. 9) shows that in the irradiated series there is a distinct flattening of the histogram, with a lower proportion of litters containing 6 to 8 young and a higher proportion with 0 to 5 young. The decrease in litter size and proportion of males was interpreted as reflecting induced sex-linked recessive lethals carried by the daughters of irradiated males and killing about half the sons of females carrying them. The irradiated males might, of course, also have produced a higher proportion of XO daughters. As Russell[5]) has shown, these females would be normally fertile, except that half their sons would die because of lack of an X chromosome (YO condition). Both these conditions would tend to decrease the sex-ratio in the second post-irradiation generation, and together would contribute to a decrease in litter size. Searle believes, however, that the decrease in litter size (approximately 6%) is due in part to the induction of dominant semi-sterility, attributable to chromosomal translocations. In this connection the results of studies by Lyon, Phillips and Searle, presented elsewhere at this Congress[6]), confirm the finding of induced semi-sterility following spermatogonial irradiation.

Turning briefly to the human species, one must appreciate that information concerning the biological effects of radiation in man is of immediate importance, not only because man is man, but also because during the last 50 years of his existence, many important artificial sources of radiation have been released by him and are used extensively. In human beings, genetic effects are, however, very difficult to evaluate. No experiments can be undertaken and a pure strain of *Homo sapiens* does not exist. Many intelligent approaches have nevertheless been made, such as the family study by Wald and co-workers[m]) of women who, as radium dial painters, were exposed to long term internal radiation.

These investigators were especially concerned with ascertaining firstly, whether any cytogenetic abnormalities were present in the blood cells of the women undergoing continuous internal radiation and secondly, whether any cytogenetic evidence could be found of a relationship between a significant maternal body-burden exposure to radium and anomalies in the progeny. Preliminary analyses in four of the dial painters revealed two instances of increased aneuploidy and three of increased chromosomal aberrations. It was difficult, however, to quantitate the radiation exposure that may have

produced the observed results, and even a crude estimation of the gonadal dose was not possible.

Although certain genetic anomalies were observed in the progeny of these dial painters, the authors properly stressed that selection had been exercised, in that the families studied were those in which frequent miscarriages and congenital anomalies were recorded. It is interesting, however, to consider that the three instances of definite anomalies in the offspring were discovered in association with increased aneuploidy in the peripheral blood cells. The issue in question is whether the cells that divide in peripheral blood culture are derived from the bone marrow. If they are produced in the lymph nodes, as recent work by MacKinney and his co-workers[7]) would seem to suggest, this method may be an inappropriate way to

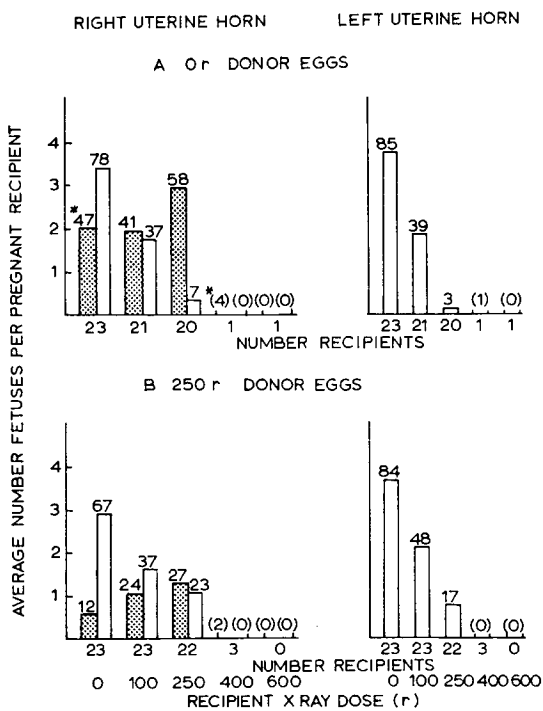

Fig. 10. Effect of *in vivo* and *in vitro* X irradiation on foetal development. (Glass and Lin.) Hatched columns: living donor foetuses. Open columns: living native foetuses. Figures at the tops of columns show the number of living foetuses observed.

study bone marrow radiation effects in the dial painters themselves, although it appears quite suitable for the study of their progeny.

4. MODIFICATION OF THE BIOLOGICAL RESPONSE OF MAMMALS TO RADIATION

Attempts to elucidate factors capable of modifying the histological and functional responses to radiation comprise the substance of the remaining three papers.

In their experiments Glass and Lin[c]) tried to distinguish maternal from oocyte or embryonic response to irradiation. For this purpose a comparison was made of the effect of X radiation on unfertilized donor oocytes *in vitro* (250 r) and on the native egg *in vivo* after irradiation of the mother with increasing doses from zero to 600 r. The data (Figs. 10 and 11) revealed that as whole body radiation

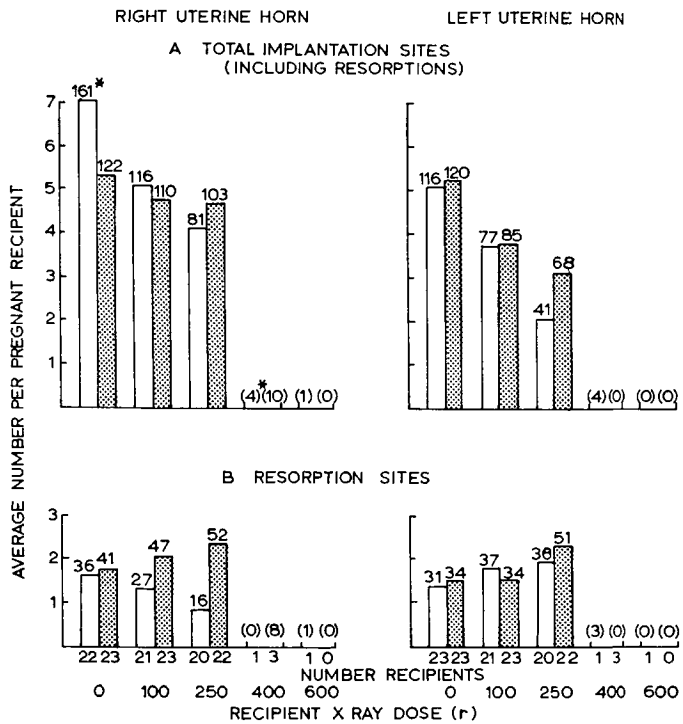

Fig. 11. Effect of whole body X irradiation on implantation and resorption. (Glass and Lin.) Hatched columns: recipients of unirradiated donor eggs. Open columns: recipients of 250 r donor eggs. Figures at the tops of columns show the number of sites.

of the recipient mother increased, the proportion of native to total eggs that developed decreased, although the number of resorption sites did not change significantly. Moreover, the number of animals the uteri of which contained only native eggs, or both native and recipient eggs, decreased as the X irradiation to the mother increased, whereas those containing only donor embryos increased. It may therefore be inferred that the effect of radiation on the oocytes *in vivo* is greater than the effect of similar doses *in vitro* and that the destructive effect is directly on the egg, rather than on the ability of the uterus to maintain the pregnancy. Whether, however, maternal injury was additive to oocyte injury *in vivo*, or the whether physiological conditions of oocytes *in vitro* was radioprotective remains uncertain.

The radioprotective effects of hypoxia were shown in the experiments of Lindop, Morgan and Rotblat[d]) to be age-dependent, as were the effects on fertility itself in female mice. The authors had previously indicated that irradiation of the female with 100 rad at 4 weeks of age resulted in complete sterility. In the present experiments mice irradiated at 1 day of age remained partially fertile after doses as high as 400 rad. That the protective effect of hypoxia on the ovary is also age-dependent was evidenced by the complete protection of ovarian function when the one-day old animal was exposed to 400 rad while breathing nitrogen. Some protection is afforded even after 800 rad, but no such protection was apparent in the one week old animals, in which complete sterility results from the same amounts of radiation. In other experiments with 21 week old animals, using lower doses of radiation, a reduction in fertility followed exposure to 50 rad while breathing air, whereas very little decrease in fertility followed irradiation with 150 rad while breathing nitrogen.

A final observation requiring further investigation concerned the cytology of the 1-day old ovary, which is composed of over 50% oocytes in the pachytene or other pre-stationary phase. These are the cells which Peters and Borum[8]) considered highly radiosensitive. Lindop *et al.* suggest, however, that the surviving cells retain their developmental potential and so populate the ovary, with the result that the gonad appears to have been less seriously damaged, although the actual number of cells destroyed may be large. Breeding experiments bearing on this problem have in fact previously reveal-

ed shortening of the reproductive life span, but such observations could not be made in the present studies because an infection in the colony precluded extended breeding.

Whereas Lindop and co-workers were interested in the effects of hypoxia, protective action by chemical and biological agents was demonstrated by Savkovic and collaborators,[1]) who utilized 8- and 17- day old rats, stating that previous work had not been performed on the infantile animal. These immature animals were subjected to 600 r of X rays and the protective effects of two chemical compounds

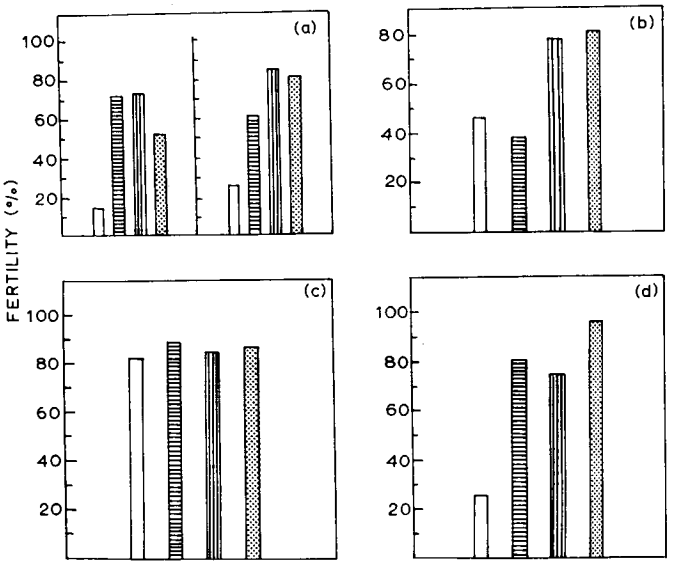

Fig. 12. Effect of protective agents on radiation-induced loss of fertility in rats. (Savkovic, Radivojevic and Hajdukovic.) Ordinate scales: fertility in per cent.
(a) Effect of 600 r whole body irradiation.
Left side: animals exposed at 8 days.
Right side: animals exposed at 17 days.
□ Controls. ▥ AET
▤ Cysteamine ▩ DNA
(b) Effect of 600 r local irradiation of the testes.
□ Controls, 8 days ▥ Controls, 17 days.
▤ Cysteamine, 8 days. ▩ Cysteamine, 17 days.
(c) Effect of 600 r local X irradiation of the head. Key as in (b).
(d) Effect of 600 r simultaneous local X irradiation of the head and testes. Key as in (b).

and of DNA were assessed by administering them to the test animals. Whole body irradiation reduced fertility markedly, an effect which was diminished by administering cysteamine and AET prior to radiation, and DNA immediately after exposure (Figure 12a).

When radiation was directed locally to the testis, fertility was less adversely affected than in the case of whole-body irradiation. In this locally irradiated group, the 8-day old animals suffered twice as great a loss of fertility as the 17-day animals. Cysteamine had no protective effect in the testis irradiated group (Fig. 12b). When only the head was irradiated, fertility was 80 per cent of normal, and again cysteamine exerted no protective action (Fig. 12c). When both head and testis were irradiated in the same animals, fertility was markedly reduced in the 8-day old, but much less so in the 17-day old (Fig. 12d). The authors concluded that cysteamine was protective only in the 8-day old animals. It must be pointed out, however, that the fertility of the control irradiated 17-day old group is over 75%; and therefore a striking protective effect could not be expected. A further point of interest is that despite 50 per cent reduction in the weight of the pituitary, no profound alterations in the reproductive organs were observed. Hence it was assumed that impaired fertility may have resulted from damage to the central nervous system rather than to the gland itself.

In the progeny of the irradiated animals, reduction in growth was most marked in the progeny of animals subjected to whole-body irradiation, less so in those with local irradiation to head or head and testis, and absent in those with testicular irradiation only; 50% of the animals died and here again cysteamine exerted no protective action.

In addition to the usual histological changes observed following testicular irradiation, the appearance of giant cells was striking in the animals subjected to both whole-body and local irradiation. These cells are in all probability spermatids within macrophages, but this like so much else remains to be proved.

From the foregoing it must by now have become apparent that much remains to be determined concerning the precise sequence of events that occur in living cells between irradiation and the production of a detectable change. The coming years will doubtless bring new observations and insights, be it in the directions indicated above or along other lines. In some years hence we may again be

wondering where we stand and what further progress we have made: let us hope that we may meet again with greater control over man-produced radiation sources and with more precise knowledge of the methods capable of modifying the responsive capacity of the living organism to radiation.

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DISCUSSION

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The Chairman having invited comments from the authors, Brown[a]) began the discussion by mentioning the work of his colleague Coppenger. Normal litters were born to female rats placed in a radiation field of 50 r daily at the time of conception, the total embryonic dose being about 1000 r gamma rays. The young animals were viable and apparently normal after three months of post-natal growth out of the field. In the author's own work albino rats had been born in the 10th generation of constant irradiation in a field of 2 r per 23 hr day. There were no apparent differences between control and irradiated animals in general appearance; possibly there were genetic effects which had not been determined.

In presenting the paper of Glass and Lin[c]) the rapporteur had stated that the *in vivo* irradiation of eggs was more damaging than *in vitro* irradiation. Glass pointed out that this conclusion could not strictly be drawn since the two types of eggs differed genetically. Donor eggs irradiated *in vitro* were hybrid agouti C3H eggs fertilized

by albino Cal A sperm; native eggs irradiated *in vivo* were Cal A eggs fertilized by Cal A sperm. Irradiated donor eggs did better than native irradiated eggs, which suggested that *in vivo* irradiation was more deleterious than *in vitro* irradiation, but did not demonstrate it conclusively. Glass also wished to underline the point that recipient females whose own eggs had been killed were still able to carry donor embryos successfully. This suggests strongly that the primary cause of embryonic death was damage to the egg, rather than damage to the maternal organism. Concerning the number of resorption sites, the data showed a trend, though not a statistically significant one, towards an increase with radiation dose. This was particularly true in females to whom irradiated donor eggs had been transplanted. In earlier work, where irradiated donor eggs were transferred to unirradiated recipient females, there was an increase in pre-implantation death as irradiation dose to eggs increased. The resorption data could be interpreted as due to increased pre-implantation death of native eggs with increasing whole-body dose to the recipient females. Finally, there was the point that those interested in egg transplantation had interpreted failure of donor eggs as due partly to competition for uterine implantation sites. The present data showed that as dose of maternal whole-body irradiation increased, the number of successful native embryos decreased. However, the increased success of donor embryos was not nearly equivalent to this decrease. While the data supported the hypothesis of competitive interaction for implantation sites between donor and native eggs, they suggested also that additional factors were involved in donor egg success or failure.

De Boer[b]) was asked by Peters[h]) to enlarge on his remark that histological observations suggested that the organism as a whole could make adjustments which would lessen the effects of radiation on the reproductive organs. He replied by considering his data from irradiation at 2, 5 and 10 r/day and the time needed to accumulate a certain total dose, e.g. at 2 r/day a dose of 600 r requires 300 days, and at 20 r only 30 days. Comparison of the data suggested an adjustment in the animals getting a low dose for a long time when compared with those receiving a high dose for a short period.

Spalding[k]) elicited the information from Rotblat that the radiation used in the work of Lindop and Rotblat[e]) on life span had been 15 MeV X rays, and commented that he himself had carried out

experiments using both gamma rays and fission neutrons and agreed with Lindop and Rotblat in finding no correlation between exposure dose to the sire or grand-sire and life span.

In his studies on the decreased productivity of the daughters of irradiated males Searle[j]) wished to point out that one of the two possible causes was much better established than the other. The evidence for the induction of heritable semi-sterility was highly significant statistically, whereas the decrease in the sex-ratio was only just significant and certain other data suggested that the effect was not really as great as it appeared. The induction of semi-sterility was of interest because it had previously been thought that translocations were not induced by spermatogonial irradiation, either in the mouse or in Drosophila.

The first questioner from the floor, Sobels (*Dept. of Radiation Genetics, Leiden, The Netherlands*) asked Searle what proportion of the reduction in sex-ratio among the progeny of irradiated grandfathers was due to genuine sex-linked lethals, and what to the induction of XO females. Searle was unable to answer this point since his experiment was on too limited a scale. Crosses with suitable sex-linked markers had been made but too few individuals were recovered to give an accurate estimate.

Gowen (*Genetics Dept., Iowa State Univ., Ames, Iowa, USA*) gave data in extension of Brown's work. Investigations on 8 long-inbred strains of mice irradiated continuously with ^{60}Co gamma rays over 22 hr/day at 0.06 to 0.10 r/hr were now in the 16th generation for 3 of the 8 lines. These progeny were and had been over the generations good mice of comparable first litter progeny to those of the controls. Success in reaching the 16th generation early was largely dependent on the biological characteristics fixed in the particular inbred strains. It was useful to think of the radiation as having been delivered in three ways: ancestral, embryonic, and somatic irradiation. In normal animals maturation of the germ cells in each generation eliminated many conditions of the ancestral plasm which were detrimental to the parents. These experiments supported the view that similar conditions held for changes brought about through ancestral irradiation, leaving the progeny relatively free to complete their normal lives.

Fowler (*MRC, Hammersmith Hosp., London, Great Britain*) asked for a comment on Lindop, Morgan and Rotblat's[d]) finding

that breathing nitrogen during irradiation gave no protective effect on fertility in mice aged 7 days. Lindop replied that it was probable that, at the ages of highest radiosensitivity, the radiation dose used was too high, so that even if nitrogen had given a protection factor of 3, the dose would still have been a sterilizing one. More investigations were needed.

Savkovic, Radivojevic and Hajdukovic[1]) after irradiating the head only in adult males had found the progeny to be of small adult size. Leonard (*Dept. Radiobiol., Centre d'Etudes de l'Energie Nucléaire, Mol, Belgium*) had made experiments of the same type and obtained normal young. He asked Savkovic how she explained an effect on the descendants from irradiation of the head, what she thought was the factor producing this effect, and whether other workers had obtained similar results with mice. Savkovic thought that perhaps the pituitary was involved. When pituitaries were examined at sacrifice of these rats which received radiation to the head they were smaller than normal. Perhaps this explained the effects on the progeny.

Kohn (*Radiol. Lab., Univ. Cal. Med. Center, San Francisco, Cal., USA*) wished to comment on life-shortening in the progeny of irradiated male mice. In the two pertinent reports in this session, no effect was found, whereas Kohn had found a small decrement in male progeny and a trend suggesting an increment in female progeny. He suggested that some of the variation between experiments might be attributable to points of experimental design, including the strain of mice used, the radiation doses, and the statistical techniques. His own results were obtained by fitting a regression line to the results for seven dose groups (0 to 720 rad).

Curtis (*Biol. Div., Brookhaven Nat. Lab., Upton, NY, USA*) pointed out that, from the paper by Lindop and Rotblat, one might infer that there was selection against deleterious genes in succeeding generations in mice. He asked if the authors would care to speculate on the effect of this conclusion on the maximum permissible dose for industrial exposure, since this was based largely on genetical considerations. Lindop replied that the authors had certainly been surprised that a life span shortening was not found after irradiating the ancestors, and that selection against deleterious genes could be important. This point seemed even more relevant when one considered the decrease in the loss of weight produced by a given radiation dose in succeeding generations. However, these findings did not

justify extrapolating from mouse to man in terms of the maximum permissible dose.

Sobels asked for Lüning's[f]) comments on the relatively small number of recessive lethals recovered in his experiments. Could these results be due to a smaller number of loci which could give rise to recessive lethal mutations in the mouse than in Drosophila? Lüning replied that data from offspring of the first generation indicated a slightly higher intra-uterine death rate in brother × sister matings from the irradiated series, the difference from control being statistically insignificant although over 300 pairs had been tested. Now data from the 5th generation of irradiation were available. Pooling the data from the 4th and 5th generations there was a significant difference in the rate of uterine death. The figures were 11.6% and 9.8%, respectively, which was in very good agreement with the data presented by Lyon in another session. Concerning the sex-ratio, when offspring of daughters of the first 5 generations were pooled, there were more than 32000 animals examined in each of the irradiated and control series. The proportion of males was 50.74% in the irradiated and 50.36% in the control series. Similar results were obtained when irradiating spermatozoa or oocytes in females. Thus three independent studies gave the same type of results, so that it can be concluded that there was no indication of changes in the sex-ratio in the direction expected if recessive lethals were present.

Biochemical and Physiological Effects

EFFECTS ON ENZYMES

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The kinetics of enzyme inactivation in solution by ionizing radiation.
Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital Montebello, Norway

[m]) H. P. SCHWARZ, L. DREISBACH, M. BARRIONUEVO, A. KLESCHICK and I. KOSTYK,
The effect of X irradiation on the lipids of the liver mitochondria.
Division of Biochemistry, Philadelphia General Hospital, Philadelphia, Pennsylvania, USA

[n]) B. M. TOLBERT, MARIE G. KRINKS and C. O. STEVENS,
Radiation stability of amino acids, peptides and proteins.
Department of Chemistry, University of Colorado, Boulder, Colorado, USA

1. INTRODUCTION

The key rôle which enzymes play in every function of every single cell justifies the interest of the radiobiologist and enzymologist in the question whether enzymes, in fact, are not only the initial but the crucial point of attack of ionizing radiation, leading to inactivation of cellular functions and to degradation and destruction of cells, organs and organisms.

The papers included in this report belong to this category. In reviewing these, it is the aim of the rapporteur to lay stress on those points considered most essential by the authors. In this respect I ask the authors to bear with me if, in their opinion, I have not succeeded in doing so.

The papers to be reviewed fall into three groups, dealing with the effects of irradiation on enzymes

(i) as dry material
(ii) in cells, organs or whole body
(iii) as pure or purified enzymes in solution.

2. IRRADIATION OF DRY MATERIAL

The first two papers deal with the effect of irradiation on dried material. Thus Braams[c]) irradiated yeast invertase with both protons (2 MeV) and electrons (1 MeV). He established two important points:

1. A certain fraction of enzyme molecules is absolutely inactivated.

2. Another fraction of molecules, although catalytically still active, has become heat labile, that is, it is destroyed by exposure to about 60 °C for 15′ to a greater extent than non-irradiated enzyme.

The D 37 (the dose which leaves 37% of the original material intact) for the totally altered fraction, i.e. inactive and labile molecules, is less than half the dose required for inactivation by radiation without subsequent exposure to heat. The author suggests that heat lability, that is, lowering of the threshold for denaturation, is caused by the breakage of a number of H-bonds concomitant with a small change of configuration which prevents reforming of the bonds.

Tolbert, Krinks and Stevens[n]), also using dry material, report extensive work on irradiation produced changes in amino acids, peptides and proteins (0.66 MeV gamma rays from a ^{137}Cs source). One striking fact was the great variability in radiosensitivity of different amino acids, the $G(-M)$-values (number of molecules M affected per 100 eV) varying by a factor of 15, i.e. $G = 2$ for L-glutamic acid and 30 for L-isoleucine. The initial stability of dicarboxylic acids was especially pronounced, while in all amino acids roughly 1/5th of decomposition was caused by decarboxylation. An example is given in Fig. 1.

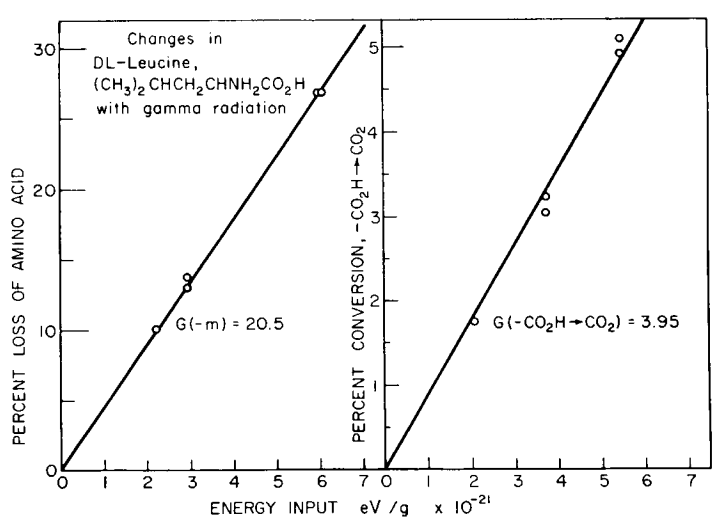

Fig. 1. Decomposition and decarboxylation of DL-leucine as function of energy input. (Tolbert, Krinks and Stevens.)

The radiation stability of amino acid residues in peptides, however, is not comparable to that of equivalent free amino acids, nor are different amino acid residues in a peptide equally sensitive to radiation. Lastly, but not least, the authors conclude from work with the enzyme lysozyme, where at 50 eV/molecule almost none of the original protein is left, yet 60 to 80% of the enzyme activity remains, that enzymic inactivation requires very extensive chemical changes.

I feel bound to add that the radiation doses applied were very large in terms of biological usage, up to 500 Mrad in the experiments of Tolbert and co-workers, and that the absence of water, i.e. exclusion of indirect effects, does not easily lend support to equating the results concerning enzyme inactivation with potential happenings in the animal body or in a bacterium. It is only fair to say that the authors have never claimed so. I might also add that the observation of partial inactivation, as shown by Braams, has also been obtained with enzymes in solution.

3. RADIATION EFFECTS *in vivo*

Langendorff, Melching and Streffer[g]) deal in two papers with the disturbance of protein and amino acid metabolism in the irradiated animal (mice) or in organs taken from irradiated animals. The X ray doses applied were 690 r = LD 83/30 (the dose after which 83% of the animals die within 30 days). The course of taurine excretion in the urine is plotted against time up to 14 days after irradiation in Fig. 2. Especially pronounced is an increased excretion during the first 3 days with further irregular increases between 4 to 14 days.

The initial increase in taurine excretion is attributed to radiation induced activation of proteolytic enzymes and can largely be prevented by administration of histamine or by splenectomy prior to irradiation while the later irregularities are to a certain extent abolished by the administration of serotonin or of pyridoxal-5-phosphate together with 5-adenosine monophosphate. Clearly the applied procedures are involved. Theories on their action mechanism are proposed and discussed.

A second paper by the same authors[h]) deals with decarboxylation of amino acids in the spleen of irradiated mice immediately and up to 7 days after irradiation. An effect on decarboxylases was predicted on the basis of the involvement of pyridoxal-5-phosphate, a co-

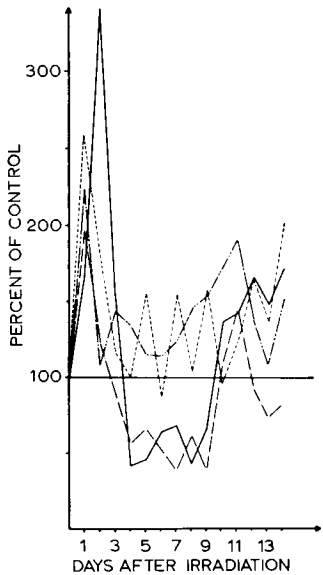

Fig. 2. The effect of hydroxytryptamine, histamine and of pyridoxal-5-phosphate together with AMP on the excretion of taurine in mice subsequent to total-body irradiation (690 r).
———— after irradiation
– – – – – after treatment with 5-hydroxytryptamine
— — — — after treatment with histamine
–··–··–··– after treatment with pyridoxal-5-phosphate plus 5′ AMP.
(Langendorff, Melching and Streffer.)

factor in decarboxylation, which had been observed in earlier experiments. Eleven different amino acids were tested. Their content in spleen tissue is generally diminished immediately after irradiation but rises after 3 hr and remains on the whole at a higher level than before irradiation except for glycine, alanine, glutamic and aspartic acid. Furthermore, the content of sulphydryl and disulphide groups was found to be diminished and the cystathionine synthesizing system, which requires pyridoxal-5-phosphate, is stated to be partially inhibited.

The paper by Lebez, Furlan, Antonijevic and Kopiter[1]) deals with the disturbance of protein metabolism, which results in an increased excretion of nitrogenous compounds in leucocytes or in isolated perfused spleen, liver and intestine of various animals (frog, rat, guinea pig, rabbit and ox). This is believed to be due to an increase of proteinase activity, which is accompanied by an increase in proteolytic activity in autolysing organs. The changes occur after comparatively low doses applied both *in vivo* and *in vitro* (100 to 900 r) and are considered to be a general irradiation phenomenon whereby the balance between synthesis and degradation of proteins is disturbed, leading to autolysis *in vivo*.

Antonijevic and Lebez[b]) deal with the dose and time dependence of nitrogen excretion in perfused frog's liver following irradiation. Here an increase in cathepsin activity, not apparent immediately after irradiation, develops gradually with time of perfusion. It is furthermore suggested that on whole-body irradiation cathepsin release occurs in the spleen and does so much earlier than in the liver, and that protein breakdown in the liver is the sequence to the transport of spleen metabolites to the liver by the blood stream. Some data are summarized in Table 1.

TABLE 1

Catheptic activity in different organs 1 hr after X irradiation. (Antonijevic and Lebez.)

Tissue	Dose (r)	% Increase of SA	
		AU/mg N	AU/Kg wt
Leucocytes[a]	100	33	16[b]
Spleen[c]	500	198	247
Liver[c]	900	10	12

SA = specific activity
AU = Anson unit
a = whole-body irradiation
b = Anson units/cell
c = organ is irradiated after extirpation.

Radiation response of auxin formation by animal tissues was studied by Gordon and Buess[e]). The plant enzyme which converts tryptophane to auxin (indolacetic acid, IAA) is almost unique in that it is readily depressed within minutes after irradiation of plant tissues with the comparatively low dose of several r only. An analogous enzyme exists in animal material, rich sources being kidney, liver, adrenals and testis of some animals. The radiosensitivity, however, differs strikingly from the plant enzyme, in that on whole-body irradiation much larger doses, of the order of Kr, are required to depress enzyme activity, while smaller doses, 50 to 100 r, cause a 5-fold rise in enzyme activity in the liver of mice. This radiation-induced rise in enzyme titre, however, is almost certainly a hormone-mediated stress syndrome. The possible involvement of the tryptophanepyrrolase system is discussed and rejected in view of known

comparative radiosensitivities which also form the basis for considerations of the biochemical pathways between tryptophane and auxin in the plant and in animal organs.

The release of RNAase bound in the nucleus of lymphoma cells following X irradiation was studied by Hamilton, Furlan and Alexander[1]), who examined the distribution of RNAase between nucleus and cytoplasm in mouse lymphoma cells. After X irradiation both *in vivo* and *in vitro*, the RNAase content is expressed as a multiple or fraction of the DNA content of either nucleus or cytoplasm. The normal distribution shows about 72 to 82% of the total RNAase content to be contained in the cytoplasmic fraction, the nucleus containing between 18 and 28%. The main result of irradiation consists in a slight rearrangement through loss or diffusion of RNAase from the nucleus through an apparently damaged nuclear membrane as can be seen in Table 2. This loss becomes apparent only

TABLE 2

Changes in distribution of RNAase between nucleus and cytoplasm of lymphoma cells following upon X irradiation. (Hamilton, Furlan and Alexander).

X irradiation (rad)	incubation (min)	% RNAase/mg DNA in	
		nucleus	cytoplasm
0	0	24–26	74–76
2000 *in vivo*	120 *in vivo*	16	84
0	60 *in vitro*	18	82
2000 *in vitro*	60 *in vitro*	10.4	89.6

with time, that is, if cells are incubated for 1 hr at 37° after *in vitro* irradiation or if they are left in the animal body for up to 2½ hr after *in vivo* irradiation. These prodecures led to a rise of RNAase by 10% in the cytoplasmic fraction with a corresponding drop between 30 and 39% in the nucleus. The total content of RNAase in irradiated cells, however, was found to be unaltered. Similarly, there is no significant change in total RNA in lymphoma cells after X irradia-

tion up to 2 Kr, although some relative changes between chromosomal and soluble RNA fractions are claimed. The authors couple these results with references to unpublished data concerning increased activity of ATPase, RNAase and DNAase in spleen and thymus in support of a theory concerning primary biochemical lesions, to which Bacq and Alexander have given the name "enzyme release theory".

I should like here to add a brief comment on the question of radiation-induced activation of enzymes which has been mentioned in some of the reviewed papers. It should not be thought that radiation can improve on nature. Enzyme activation has one definite meaning for the enzymologist, namely the initiating or raising of the turnover number by the addition of a necessary physiological factor, such as magnesium, iron, flavin, or a coenzyme and so on. Irradiation does not alter the chemical constitution or configuration of an enzyme in such a way as to increase its catalytic function, that is, to make it a better enzyme. All so-called activations are, therefore, at least to my mind, produced either by suppression of regulatory inhibitors, as in the induction of phage or of permease or of autolysis, or alternatively by diffusion phenomena which facilitate access of substrate to enzyme or *vice versa* through damaged membranes. Abscopal effects such as a possible enzyme synthesis under hormonal stimulus are, of course, not covered by this reservation.

Schwarz, Dreisbach, Barrionuevo, Kleschick and Kostyk[m]) directed their research towards another facet of the problem, namely the post-irradiation changes of mitochondrial lipids. Total lipids and total lipid phosphorus were found to have increased by 30% in rat liver mitochondria 24 hr after total-body irradiation with 1 Kr. This increase was mainly accounted for by a four-fold increase of one component of the nitrogen-free phosphatide fraction, i.e., the phosphatidyl glycerol. Other phosphatides have also been examined and found to be either unchanged or very slightly decreased. Continuation of this work, especially in view of the assumed participation of mitochondrial lipids in electron transfer, offers a promising avenue to radiobiological investigation.

The paper by Pauly[k]) forms a transition to the papers dealing with the irradiation of isolated enzymes. He records the effect of X rays on the synthesis of RNA and total protein, on the formation of an

inducible enzyme (lysin decarboxylase), and on colony formation in *B. cadaveris* after both aerobic and anaerobic irradiation. The doses given varied between 5 and 100 Kr. All parameters examined were exponentially inhibited with increasing dose, with the single exception of the synthesis of total protein after an aerobic irradiation, the dose-effect curve of which showed a shoulder in the initial part of the curve. The inhibitions of inducible enzyme protein and of RNA synthesis were identical and the oxygen enhancement ratio

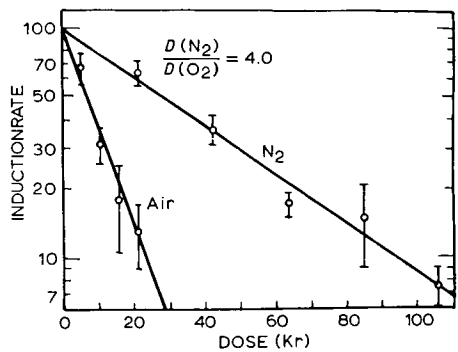

Fig. 3. Inhibition of enzyme induction (lysine decarboxylase) in *B. cadaveris* as function of the irradiation dose, applied under aerobic and anaerobic conditions. (Pauly.)

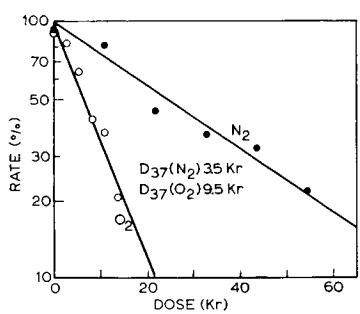

Fig. 4. Inhibition of RNA-synthesis in *B. cadaveris* as function of the irradiation dose applied under aerobic and anaerobic conditions. (Pauly.)

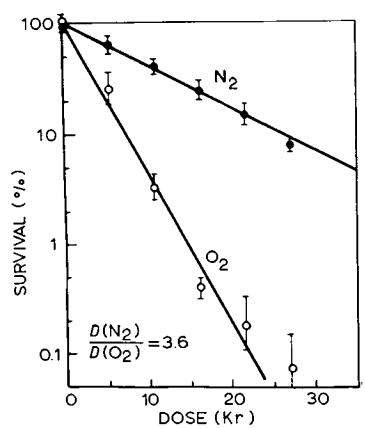

Fig. 5. Survival of *B. cadaveris* as function of the irradiation dose, under aerobic and anaerobic conditions. (Pauly.)

in all four systems examined was almost identical, namely 3.7 to 4.0. These results are illustrated in Figs. 3, 4 and 5. The clearly and convincingly presented data allow the justifiable conclusion that blocking of RNA synthesis, which, in turn, affects formation of the shortlived messenger RNA, is the cause of diminished protein and enzyme synthesis in irradiated bacteria, leading finally to arrest of bacterial multiplication.

4. ISOLATED ENZYME SYSTEMS

We come now to the experiments dealing with enzymes in solution, which throw a great deal of light on some important questions, such as: What is meant by inactivation, what has been changed and in which way? Is it the rate of substrate utilization? Is it the Michaelis constant and, if so, for what? For substrates, for some co-factors, for a terminal acceptor, as oxygen? And lastly is it an all-or-none effect?

Dose[d]) examined the effect of X and UV irradiation on lactic dehydrogenase in solution (1 to 2%). While no demonstrable changes in the amino acid composition of proteins are stated to occur at doses of up to 700 Krad he found that the electro-chemical properties of the enzyme were changed, probably by alterations of the secondary and tertiary protein structure. In fact, an electrophoretically distinct new form of enzyme protein was formed, which yet retains the properties of the original unirradiated enzyme. A large number of substances were tried as potential X ray protectors, amongst them cysteine, cysteamine and reduced glutathione, but only D-lactate offered pronounced protection.

Adelstein[a]) offers observations on the properties of X irradiated glutamic dehydrogenase and describes changes in the kinetics and physical properties. The main results consist in an increase of the Michaelis constant for substrates but not for coenzymes, in which the maximum velocity alone is affected. In addition, X irradiation of the enzyme destroys the capacity to enhance the fluorescence of reduced coenzyme. The decrease of fluorescence is directly proportional to the reduction in maximum velocity (Table 3). Experimental analysis to account for reduction of the enzymic activity has shown that the association constant of the site for reduced coenzyme binding is not affected. Coenzyme binding sites are lost, i.e., their number decreases and this is the proximate cause for the decreased

TABLE 3

Effect of X irradiation on the capacity of glutamic acid dehydrogenase to enhance the fluorescence of reduced DPNH. (Adelstein.)

Dose (rad)	per cent fluorescence	per cent max. velocity
0	100	100
40000	68	73
90000	27	25

capacity of irradiated enzyme to enhance DPNH fluorescence. The number of titratable sulphydryl groups furthermore decreased exponentially with dose in the same way as the enzymic activity.

Inactivation of enzymes by irradiation may justifiably be related to a change in the turnover number of substrate molecules or in the total number of catalytically active enzyme molecules. To distinguish between these two possibilities Mounter[1]) determined the esterase activity of α-chymotrypsin, using different substrates, namely the ethyl esters of acetyl tyrosine, acetyl tryptophane and acetylphenyl alanine, as well as the activity of acetylcholinesterase acting on 3 different substrates, acetyl- and acetyl β-methyl choline and isoamyl acetate. The results revealed considerable post-irradiation differ-

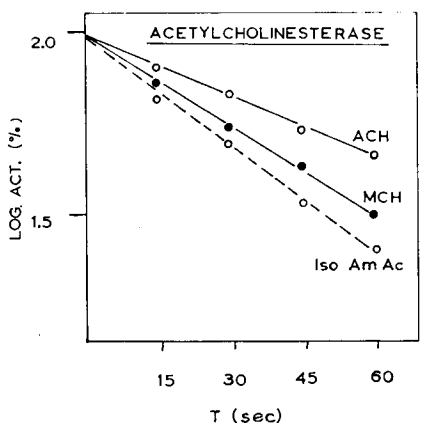

Fig. 6. Inactivation of acetylcholine-esterase, acting on different substrates, as function of X irradiation. (Mounter.)
ACH : acetylcholine
MCH: methylcholine
IsoAmAc: isoamylacetate.

ences in the relative rates of hydrolysis of different substrates which can be interpreted as signifying changes in specificity as shown in Fig. 6. They have, furthermore, revealed changes in the Michaelis constant for some substrates but not for others. Thus, alterations

in enzymes can occur which involve only partial loss of function and may to a certain extent depend on the assay conditions. Mounter's evidence supports the conclusion that irradiation causes a partial denaturation, resulting from the creation of disorder in the macromolecular protein structure and that this does not appear to be of the all-or-none type.

Finally Sanner and Pihl[1]) have put and answered the question "why is the dose-inactivation curve of enzymes exponential, if it is, and why is it not, if it isn't?" Their work emerged from the experimental observation of an exceptionally high yield of X ray inactivation of papain and of non-exponential dose-inactivation curves for this and other SH-enzymes. As a rule, the rate of inactivation of enzymes in dilute aqueous solution decreases with increasing radiation dose. This is due to the fact that inactivated enzyme molecules compete with active ones for the agents formed from water. However, a strictly exponential dose-inactivation can only be obtained if active and inactive molecules destroy the radiolysis products of water at the same rate. Experiments with a number of SH-enzymes, glyceraldehyde-3-phosphate dehydrogenase, alcohol dehydrogenase, and papain, suggested that in fact the active enzyme molecules react more readily with the radiolysis products of water than do inactivated ones.

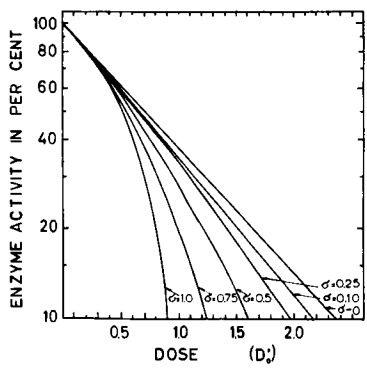

Fig. 7. Theoretical dose-inactivation curves calculated for different ionic yields (σ). (Sanner and Pihl.)

This led to a detailed theoretical reappraisal of the kinetics of enzyme inactivation which, in contradistinction to other theories in the field of radiobiology, was not built on *a priori* assumptions (Figs. 7, 8).

Since the ionic yield decreases during irradiation due to the fact

that inactivated enzyme molecules compete for radicals, the *initial* ionic yield is clearly the parameter of interest. From this consideration two terms have been derived: "true ionic yield" (σ), which is the initial ionic yield that would be observed in the absence of any interfering substances, and another term (Dp), which is the dose

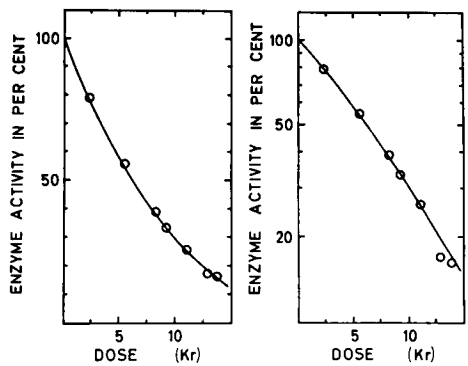

Fig. 8. X ray inactivation of papain. The experimental values are indicated by circles, the theoretical curves by the fully drawn lines. (Sanner and Pihl.)

required to inactivate the enzyme completely if the rate of inactivation were to remain constant and equal to the initial one. The "true ionic yield" is a property of the enzyme.

Lastly the conditions were analysed in which the presence of inactive enzyme molecules in preparations prior to irradiation will or will not affect the yield dependent on *initial* yield and enzyme concentration.

We have been presented with an "embarras de richesses" of experimental data, each of them trying to probe deeper towards the elusive target of primary biochemical lesion, into the critical neighbourhood of which we may soon enter.

DISCUSSION

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Tolbert[n]) thought the rapporteur's point that the doses used to irradiate dry systems could not be correlated with effects on living sys-

tems was debatable. We do not know whether radiation effects on living systems, including nucleus, mitochondria and cell walls, are more akin to effects in dilute aqueous solutions or in solids. However, we do know that these structures have an ordered and crystalline-like structure and for these reasons Tolbert approached the problem by irradiating isolated biochemical entities. He had found that approximately 5 to 10 eV are required to break chemical bonds in the dry state and this causes the disappearance of an amino acid or an amino acid residue from a peptide or some other structure of this type. Comparable amounts of energy produce the same effect in aqueous solutions. Thus the very large doses which are used by the chemist interested in solid state radiation effects do not represent unusual radiation stability, but rather the fact that the radiation energy is absorbed by very small amounts of material, and therefore doses in the Megarad range must be used.

Schwarz[m]) pointed out that, in his studies, phosphatylcholine, phosphatylserine and sphingomyelins contribute to the general increase of phospholipids in irradiated rat liver mitochondria. However phosphorylglycerol makes a substantially greater contribution to this increase, and increases by a factor of 4. This is counterbalanced by a decrease in phosphatyl ethanolamine. Characteristically these components contain fatty acids, 90% of which are unsaturated. (Linoleic acid constitutes about 70%.) This means that these substances must be isolated under nitrogen to prevent oxidation. Schwarz has isolated some of the combined nitrogen free fraction from solubilized mitochondria. These contain some crude enzyme systems like succinic dehydrogenase and it is possible that phosphatydyl glycerol is related to the electron transfer particles. This substance is unlikely to be a degradation product of other phospholipids because of some preliminary experiments performed with ^{32}P.

In addition to the remarks made by the rapporteur Melching[g,h]) pointed out that the increase in the taurine excretion in mice immediately after whole body irradiation until after the third day was due to inhibition of pyridoxal-5-phosphate and perhaps also of the coenzymes of decarboxylases. At the same time there are a certain number of changes in the spleen itself. The influence of substances which are known to protect the irradiated whole animal differ from case to case, whereas taurine excretion which results from proteolysis may also result from splenectomy or administration of histamine.

Adelstein[a]) did not wish to debate the rapporteur's statement that we cannot improve on nature, but did think that X rays may activate enzymes directly. Such a process may be brought about by an increase in substrate affinity and in this respect Okada[1]) has obtained some interesting and relevant results. With regard to the contribution of Sanner, Adelstein found that the inactivation of reduced DPN by radiation is neither linear nor exponential with dose. Since it is converted almost entirely to the oxidized form the explicit mathematical formulation of its inactivation can be derived. Thus the ionic yields can be calculated, not from an initial extrapolation value, but from a precise value which comes out to be about 18% of the initial concentration.

Gordon[e]) had no desire to enter into a discussion with Adelstein on whether enzymes can be activated by irradiation or not. As the rapporteur had mentioned, an increase in enzyme titre can be caused by several processes. There may be an increase in the concentration of the apo-enzyme, or the prosthetic group, or both, or an increase in the availability of co-factors. There may also be a decrease in the concentration of an inhibitor. Again, activation may result from a change in substrate-product equilibria. From the standpoint of the organism, the important question is whether the activation derives from the enzyme as a target or from an event elsewhere, the activation then being indirectly or humorally controlled. In regard to the discussion of his own work on the "activation" of the tryptophan-auxin enzyme of rodent liver, Gordon wished to make some comments. The inference that the post-irradiation increase in titre was an indirect manifestation is based on three observations. (i) The titre is increased by sham irradiation or injection of saline solution; (ii) the titre is enhanced by intraperitoneal injections of substance and (iii) the titre is high in foetal liver, drops precipitously at birth and rises slowly with age to foetal levels, a pattern suggestive of a maternal uterine stimulus and subsequent adaptation. Recent experiments show that the radiation-induced increase in titre in liver does not occur in the adrenalectomised animal. It therefore appears that the activity of the enzyme in liver is under humoral regulation and partly, at least, under control of the adrenals.

When the discussion was extended to the audience, Hutchinson (*Yale Univ., New Haven, Conn., USA*) asked Sanner[1]) whether he

calculated his results using a model involving two radicals of widely different reactivities or a single radical model. In solution it is reasonable to assume that there are both highly reactive radicals, such as OH; and radicals of very low activity which will react almost entirely with —SH groups. It is very difficult to fit a two radical model to experimental data satisfactorily because many different constants will fit the results. Sanner replied that the calculations were based on an average radical as used previously by Hutchinson himself and by Okada. When enzyme inactivation is almost exclusively due to efficient destruction of certain groups on the surface of the protein, the radicals responsible must react fairly specifically with the active groups. In papain, alcohol dehydrogenase and coenzyme A, to which the theoretical equations were applied, the SH groups are removed with rather high yields. Since the enzyme inactivation is due almost exclusively to the destruction of SH groups, it may be due predominantly to the action of $HO\cdot_2$ radicals which may react with a certain degree of specificity. On the other hand, the reaction between the OH· radicals and the proteins may be less specific and less important. In the above experiments catalase was present to prevent the action of hydrogen peroxide on the enzymes. In commenting on the remarks of Adelstein, Sanner stated that when the equations were applied to the experimental data, the calculation of the initial yield of inactivation was based on the whole dose-inactivation curve and not only on its initial slope.

Mouton (*Biol. Inst., Paris, France*) agreed with the conclusions reached by Antonijevic *et al.*[b]) that radiation-induced autolysis *in vivo* is very similar to the normal post-mortem autolysis and may be the essential feature of the radiation syndrome. In work on aseptic muscle which he had discussed at Moscow in 1960 he concluded that post-mortem autolysis is triggered off by anaerobic glycogenolysis when the blood circulation ceases. A subsequent drop in *p*H from 6.3 to 5.7 ensues. Therefore, it could be postulated that *in vivo* anaerobic glycogenolysis with a subsequent drop in *p*H takes place locally as a result of radiation injury. This can be observed and even controlled. Bate-Smith *et al.*[2]) found that the biochemical changes which occur on autolysis indicate a possible localized drop in *p*H in the tissues. In Mouton's view the use of microbeams of local high energy radiation along with appropriate histochemical tests, such as used by Zeman *et al.*[3]), would show whether the results ob-

tained by the Yugoslav workers are due to a mechanism of post-mortem autolysis. He would call this the "local *p*H drop theory" of radiation damage and pointed out that the *p*H drop results from the induction of anaerobic glycogenolysis and entails not only the freeing of endocellular proteases, but also disturbs the delicate balance of membranes, nuclei and cellular lysosomes.

Furlan[f]) replied that he had no intention of comparing the mechanism of liberation of cathepsin with what happens in irradiated and autolysing organs. However, there were two indications from their experiments which ruled out the possibility that a shift in *p*H to lower values is responsible for the release of intracellular proteinases in irradiated organs. In the first place the optimal conditions for extraction of the enzyme *in vitro* involved homogenization of the tissues followed by an adjustment of the *p*H to 3.5 and incubation at 37° for 24 hr. Secondly, the frog livers and guinea pig spleen were perfused with a fluid buffered at *p*H 7.3 and it was difficult to visualise a drop in *p*H under these conditions.

Van Bekkum (*Radiobiol. Inst., Rijswijk, The Netherlands*) asked Schwarz for further information on the nature of the effects in irradiated liver mitochondria. Were the changes in composition due to direct damage of the liver cells or to secondary effects on the liver caused by whole body irradiation? Schwarz said his data would not answer this question at the present time but the study would be expanded to include experiments on solubilized mitochondria and these might provide the relevant information.

Tabachnik (*Albert Einstein Med. Centre, Philadelphia, Penn., USA*) had two points for Hamilton. The first was that it must be difficult to obtain pure nuclear fractions from homogenates of lymphoma cells which probably consist mainly of nucleus with very little cytoplasm. Secondly, if the enzyme being studied is an alkaline RNAase had the author tested for an RNAase inhibitor by adding p-hydroxymercuribenzoate or another SH binding compound to the assay mixture? Hamilton agreed that there is only a small amount of cytoplasm in the lymphoma cell. The nuclei were prepared by first homogenizing the cells in the ionic medium described by Creasey and Stocken[4]) and then separating the nuclear fraction by centrifugation at 1000 × g for 7 min. The remaining cytoplasmic material was removed by suspending the preparation in 0.25 M sucrose, followed by layering of the nuclei in 0.34 M sucrose. On the second

point raised by Tabachnik, the cytoplasm does contain an RNAase inhibitor which is destroyed by the addition of 3×10^{-4} M p-chloromercuribenzoate. This leaves an RNAase with a single peak at *p*H 6.8 to 7.2. However no inhibitor was present in the nuclei of the five day old tumour.

Philpot (*MRC, Harwell, Berks., Great Britain*) pointed out that layering of the nuclei as a pure preparation can only be achieved after successful removal of the cytoplasm. Was a Waring blendor used in Hamilton's experiments, and if so, was it known if damage to the nuclear membranes had been prevented?

Hamilton said that the nuclei were prepared by homogenizing cells in the Dounce hand homogenizer in which the cytoplasm can be removed without damaging the nuclei. At each stage the preparation was examined under the light microscope and finally electron micrographs were made of the nuclei.

Okada (*Div. Exptl. Radiol., Univ. Rochester, NY, USA*) commented on Hamilton's conclusion that the changes in RNAase in spleen and thymus are due to an abscopal effect. He did not think it was justifiable to compare changes in enzyme activity in spleen and thymus, which consist of populations of mixed cells, with a homogeneous population of lymphoma cells. Hamilton agreed with these observations.

Okada also wished to know what explanation could be put forward to explain the decreased level of nuclear RNAase and the increase of low molecular weight RNA in the nucleus. Does it mean that the nuclear RNAase exists in an inactive form and is released as an active form after irradiation to act upon the nuclear RNA? In reply, Hamilton cited some of his experiments in which loss of RNAase from the nuclei after irradiation is accompanied by a transfer of RNA associated with the chromatin fraction to a fraction which can be extracted with neutral buffer. He added that he is currently investigating this degradation by studying the specificity of the nuclear RNAase. If this enzyme has similar properties to liver alkaline RNAase, it might be expected that the RNA which can be extracted will be rich in purine bases.

Romani (*Dept. Pomol., Univ. Calif., USA*) contributed some observations on his experiments on irradiated fruit tissues which can be treated with doses of 200000 rad. As these are relatively quiescent tissues, it is hoped that the initial cytoplasmic effects can be

picked out. He had found that a loss of particulate nitrogen occurred in irradiated cherries, which is in accord with the observations made by Antonijevic[b]). This loss is not apparent immediately after irradiation, but is maximal 24 hr later and is slowly reversed after this period. Measurements were also made on transaminase activity because these enzymes are involved in protein-carbohydrate exchange reactions. In keeping with Hamilton's results, no changes in the distribution of transaminases among the particulate and soluble fractions could be observed which were attributable to radiation effects. A general mild inhibition of transaminase activity was noted which agrees with the findings of Langendorff *et al.*[g,h]). Romani also observed that, when aldolase and alcohol dehydrogenase are irradiated, inactivation of the SH groups and the activity of the enzymes is not necessarily simultaneous. In the light of this was Adelstein able to differentiate between active and inactive SH groups, and secondly had he used an amperometric method to measure SH groups?

In reply, Adelstein stated that his titrations were done primarily at acid *p*H in the presence of 4M urea using p-hydroxymercuribenzoate. Repetition of the measurements using the Benisch amperometric silver titration method gave essentially the same results. Adelstein added that he could not say whether or not these SH groups are active. However there is a correlation between the binding of reduced DPN and the number of SH groups which have been oxidized or, at least, are not titratable.

Manoylov (*Acad. of Sci., Leningrad, USSR*) observed that irradiation of haem-containing enzymes results in a splitting of the porphyrin ring from the protein with accompanying loss of iron from the haem. The change is such that recombination of the haem and the protein is not possible.

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Biochemical and Physiological Effects

OBSERVATIONS ON MAMMALS

O. A. TROWELL

Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berkshire, Great Britain

a) G. DAVID, D. TANKA and B. FIAM,
Effect of whole-body X irradiation on liver.
National Institute of Rheumatism and Hydrology, Budapest, Hungary

b) K. HEUSS,
The age-dependency of the gastro-intestinal syndrome in irradiated white mice.
Max Planck Institut für Biophysik, Frankfurt am Main, Germany

c) J. R. MAISIN and J. DULCINO,
Absorption in the small intestine of X irradiated mice.
Radiobiology Department, Centre d'Etude de l'Energie Nucléaire, Mol, Belgium

d) B. M. MUNDAY and W. F. R. POVER,
Sodium loss and the gastro-intestinal syndrome.
Department of Medical Biochemistry and Pharmacology, Medical School, Birmingham 15, Great Britain

e) J. W. OSBORNE, K. N. PRASAD and G. R. ZIMMERMAN,
Induction of an intestinal neoplasm by X irradiation of the exteriorized intestine of the rat.
Departments of Radiation Research and Pathology, University of Iowa, Iowa City, USA

f) P. SCHAUER, S. SEBEK and S. JURECIC,
The changes in secretion activity of stomach and excretion of nitrogen compounds from stomach and ileum after whole-body irradiation of rats.
Nuclear Institute "Jozef Stefan", Ljubljana, Yugoslavia

g) M. F. SULLIVAN,
Radiation-diarrhoea modified by diverting the bile flow from the gastro-intestinal tract.
Medical Research Council, Radiobiological Research Unit, Harwell, Berkshire, Great Britain

h) M. F. SULLIVAN, S. MARKS and R. C. THOMPSON,
Delayed effects in rats of chronic yttrium-90 ingestion.
Biology Laboratory, Hanford Laboratories, General Electric Company, Richland, Washington, USA

i) D. TANKA, B. FIAM and G. DAVID,
Histochemical investigations of rat liver after whole-body irradiation.
National Institute of Rheumatism and Hydrology, Budapest, Hungary

1. INTRODUCTION

The papers in this session are concerned with the effects of radiation on the gastrointestinal tract or on the liver, and I shall consider them under the following sub-headings: the acute gastrointestinal syndrome: chronic effects on the intestine: and the liver.

2. THE GASTROINTESTINAL SYNDROME

Rats or mice exposed to whole-body irradiation in the range of dose which kills 50 per cent in 30 days (LD 50/30) die of bone marrow failure, but after higher doses (1000 r upwards) they die earlier, usually in 3 to 5 days, as a result of intestinal damage. The clinical picture associated with intestinal damage is known as the gastrointestinal syndrome. The first five papers to be considered are concerned in one way or another with this syndrome.

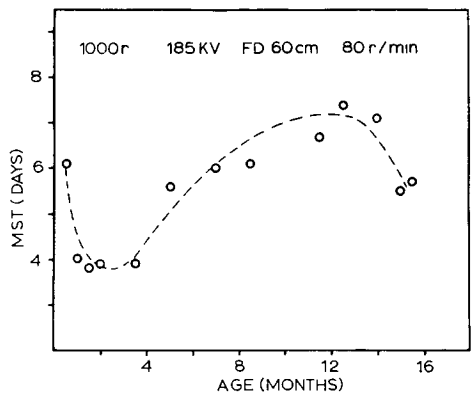

Fig. 1. The effect of age on the mean survival time (MST) of mice after 1000 r of whole-body X irradiation. (Heuss.)

Heuss[b]) exposed mice of varying age to a standard dose of 1000 r of X rays and measured the mean survival time. The survival time varied with age as shown in Fig. 1. If survival time is taken as a measure of radiosensitivity, then the mice were most sensitive at age 1 to 4 months and least sensitive at 12 months. In general trend this result is the same as had been found by Crosfill, Lindop and Rotblat[1]) using smaller doses, in the LD 50/30 range. The point of interest is that Heuss's animals died from the gastrointestinal syndrome whereas Crosfill's *et al.* died from bone marrow failure. This

suggests that the age-dependence of radiosensitivity in mice is some general property of the animal and not confined to one particular organ or mode of death.

In the gastrointestinal syndrome the presenting symptom is acute diarrhoea, and the underlying pathology is an almost complete loss of the intestinal epithelium. But the precise cause of death is not known. Several possibilities have been suggested, such as dehydration, electrolyte loss, bacteraemia, and release of proteolytic enzymes, and doubtless more than one may be implicated. Some of the possible factors have now been investigated in more detail. Munday and Pover[d]) investigated the loss of sodium from the gastrointestinal tract of irradiated rats and concluded that this was not a major cause of death. They gave intravenous ^{22}Na and found that the rate of loss of ^{22}Na from the intestine was not increased until just before death. They also found that intraperitoneal NaCl therapy prolonged survival by only about one day.

Maisin and Dulcino[c]) measured the intestinal absorption of fat in irradiated mice. This was done by feeding isotopically labelled palmitic acid or glyceryl tripalmitate and determining the amounts present in the wall and in the contents of the gastrointestinal tract of animals sacrificed at time intervals after feeding. Three days after a supralethal dose of radiation (1500 r) fat absorption was found to be subnormal, but the authors attributed this to gastric retention rather than intestinal failure. After smaller doses (600, 800 r) there was no change in fat absorption at any time and it was concluded that malabsorption of fat plays no part in the gastrointestinal syndrome.

As already mentioned, the chief and almost the only symptom in these animals is diarrhoea. Now Sullivan[g]) has found, very surprisingly, that the diarrhoea can be prevented simply by diverting the normal bile flow from the intestine. These experiments were done on rats given 1000 r of X rays to the abdomen; the bile was diverted by cannulating the bile duct and draining the bile outside the body. This is a most interesting discovery for it shows in the first place that the diarrhoea is not solely due to the effect of radiation on the intestine; some factor in normal bile is required for its manifestation. To discover what this factor was, further experiments were done in which, after the bile duct cannulation, various solutions were introduced into the intestine via a duodenal cannula. If the drained bile

was reintroduced, diarrhoea resulted, as would be expected. If an equal volume of isotonic saline was introduced no diarrhoea occurred. But if a solution of bile salts in isotonic saline was introduced through the duodenal cannula, diarrhoea resulted. These experiments show that the diarrhoea is due to the action of normal bile salts on the irradiated intestinal epithelium. Sullivan noticed that in the animals with diarrhoea the faecal matter consisted chiefly of mucus, and it seemed that the primary effect of biliary drainage was to prevent this discharge of intestinal mucus. He therefore postulated that bile salts act on the irradiated intestinal epithelium so as to produce a great secretion of mucin. Histological examination showed that this is in fact what happens. Fig. 2 is a histological section of normal intestine stained for mucin (black) and it shows the normal mucin-containing goblet cells in the epithelium. Fig. 3 is from an irradiated animal with diarrhoea and shows that the mucin has nearly all disappeared. Fig. 4 is from an irradiated animal with biliary drainage; it shows most of the mucin retained, and this presumably is why the animal had no diarrhoea. Apart from the radiation interest this work may have brought to light a hitherto unknown function of the bile salts. It looks as if the bile salts may be a normal physiological stimulus to the discharge of mucin from the goblet cells and that after irradiation either the goblet cells are more sensitive or else the concentration of bile salts in the intestine is higher than usual owing to some failure of reabsorption. If some substance which has the property of absorbing bile salts could be fed by mouth this might be a valuable therapeutic measure in treatment of the gastrointestinal syndrome and Sullivan is investigating this possibility.

Turning now to the stomach, Schauer, Sebek and Jurecic[f]) have found some very early changes in the gastric secretion of rats after exposure to 700 r of whole-body X irradiation. The rats had gastric fistulae and the volume and composition of the gastric juice were measured both basally and after ethanol stimulation. The secretion of pepsin (both concentration and total output) was greatly decreased one hour after irradiation (Fig. 5). The secretion then increased well above normal, reaching a peak at 24 hours, and thereafter it declined and tended to be subnormal for several days. The increased secretion at 24 hours was ascribed to discharge of the stored pepsinogen and the subnormal secretion afterwards to depression

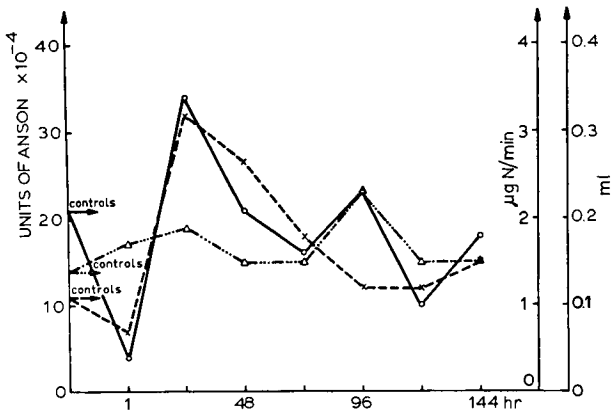

Fig. 5. Volume of gastric juice and pepsin activity (Anson units) in rats at daily intervals after 700 r whole-body X irradiation. (Schauer, Sebek and Jurecic.)
O—O activity of pepsin.
×—× nitrogen compounds in secretion liquid.
△--△ volume of gastric juice.

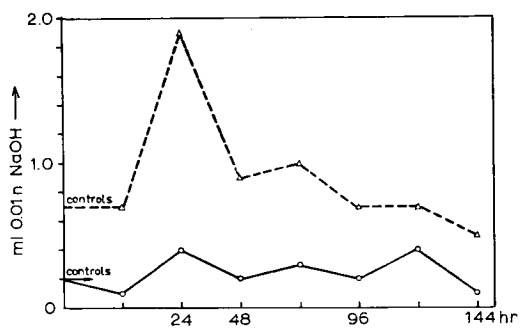

Fig. 6. Hydrochloric acid in gastric juice at daily intervals after 700 r whole-body X irradiation (Schauer, Sebek and Jurecic).
O—O free hydrochloric acid
△--△ total acid.

of pepsin synthesis. The secretion of HCl (Fig. 6) followed a similar sort of pattern. These changes of course play no part in death from the gastrointestinal syndrome. The interest is that they are early functional changes produced by a quite moderate dose of radiation.

3. CHRONIC EFFECTS OF LOCAL IRRADIATION ON THE INTESTINE

The two points of interest in this field of study are the remarkable

recuperative powers of the intestine after high doses of radiation, and the induction of tumours.

Sullivan, Marks and Thompson[h]) fed yttrium-90 to rats for 60 days and then followed the animals until death. ^{90}Y is virtually non-absorbed, it is a pure β-emitter, and its effect is therefore more or less limited to irradiating the walls of the alimentary canal as it passes through. As shown in Table 1 two different dose levels were used.

TABLE 1

Effect of intestinal β radiation from ^{90}Y in rats. (Sullivan, Marks and Thompson.)

^{90}Y	Accumulated β ray dose to large intestine	Survival time (days)	Weight loss	Tumours			
				Intestinal	Oral	Uterine	Other
0	0	626		0	0	0	3
90 μC/day	12000	434	no	1	7	0	2
410 μC/day	55000	350	yes	0	12	9	2

The lower dose reduced survival time and produced some tumours but there was no loss of weight and there were no other symptoms. The higher dose was more effective in life-shortening and tumour production and it also caused some loss of weight, but even in these animals it was found that the histological damage to the intestine had been completely repaired by the time of death. The fact that no residual histological damage could be detected is rather remarkable in view of the total accumulated dose of 55000 rad. This was the calculated dose to the large intestine; it was estimated that the dose to the small intestine would be about one quarter of this figure. The tumours induced were mainly in the mouth (squamous cell carcinoma) and the uterus (adenocarcinoma); only one intestinal tumour was found. In these small animals the uterine epithelium is not very far distant from the intestinal lumen; it is within range of the β rays from intestinal yttrium and the uterine tumours were explained in this way.

Carcinomata of the small intestine are very rare in normal rats and most workers, including those just cited, have failed to induce any significant number by radiation. Osborne, Prasad and Zimmer-

man[e]), however, have apparently been able to produce a large number of such tumours by giving higher doses of radiation. They X irradiated the whole exteriorized small intestine of rats and found that the threshold dose for tumour production was 1400 r, and with this dose about 50 per cent of the survivors developed an intestinal carcinoma which eventually killed the animal by intestinal obstruction. The histological appearance of these tumours is shown in Figs. 7 and 8. It is important to be sure that these really were malignant tumours and not just some non-malignant hyperplasia of epithelium trapped in the regenerating and fibrotic mucosa. The evidence on this point is fairly convincing, but not altogether conclusive. By clamping the mesenteric blood vessels during the period of irradiation, so that the gut was anoxic, it was possible to give even higher doses (1800 and 2000 r) without killing the animal, and after this procedure 60 to 70 per cent of the survivors developed intestinal tumours. Irradiation of a 7.5 cm length of intestine with 3000 r, or a 2.5 cm length with 5000 r produced what appeared to the naked eye to be tumours, and these killed the animals by intestinal obstruction. But histologically these "tumours" proved to be only an overgrowth of fibrous scar tissue.

4. HISTOCHEMICAL CHANGES IN THE LIVER

The liver has usually been regarded as a very radioresistant organ because morphological changes can be produced only by doses of 10000 r or more. But there is accumulating evidence of enzymatic and other biochemical disturbances after much more moderate doses and it is becoming clear that the liver is a good deal more sensitive than was formerly supposed.

David, Tanka and Fiam[a]) and Tanka, Fiam and David[i]) have found certain changes in the livers of rats two days after exposure to 800 r of X rays, which was about the LD 50/30 dose. The first change concerns the liver glycogen. After irradiation there was no change in the amount of glycogen which could be chemically extracted, nor was there any change in the amount demonstrated histochemically (by periodic acid Schiff staining) after ethanol fixation; but the amount demonstrable after formol fixation was considerably reduced. The interpretation was that radiation had partially depolymerized the glycogen, making it more water-soluble and therefore less well preserved by formol fixation. This was confirmed

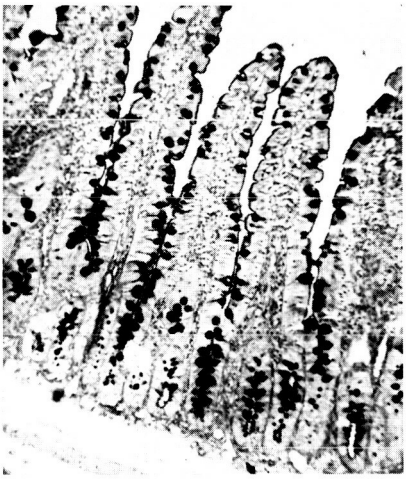

Fig. 2. Normal rat small intestine, stained for mucin. (Sullivan.)

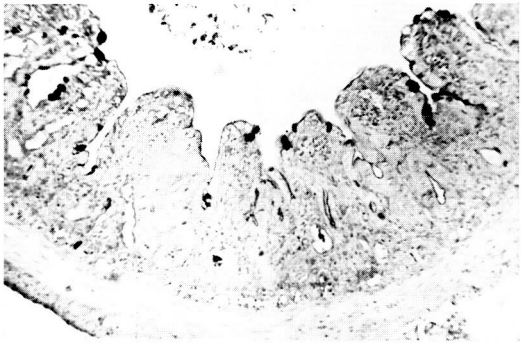

Fig. 3. Small intestine 4 days after 1000 r abdominal X irradiation, stained for mucin. (Sullivan.)

Fig. 4. Small intestine of bile-drained rat 4 days after 1000 r abdominal X irradiation, stained for for mucin. (Sullivan.)

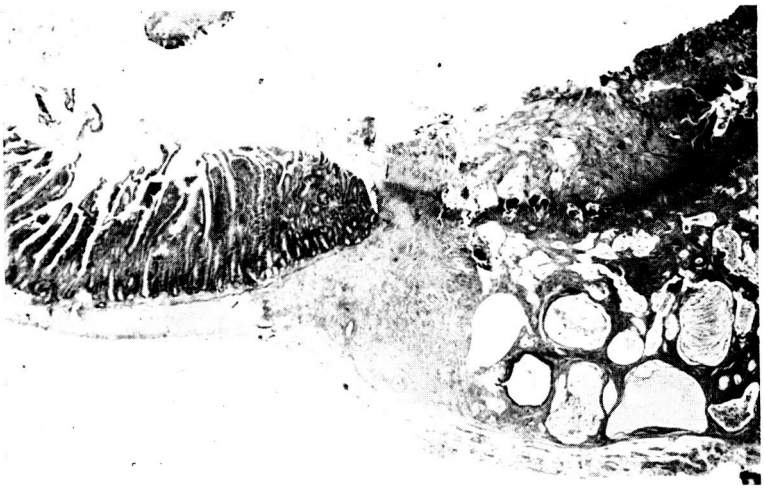

Fig. 7. Adenocarcinoma of the small intestine in a rat following irradiation of the exteriorized intestine with 1400 r of X rays, × 15. (Osborne, Prasad and Zimmerman.)

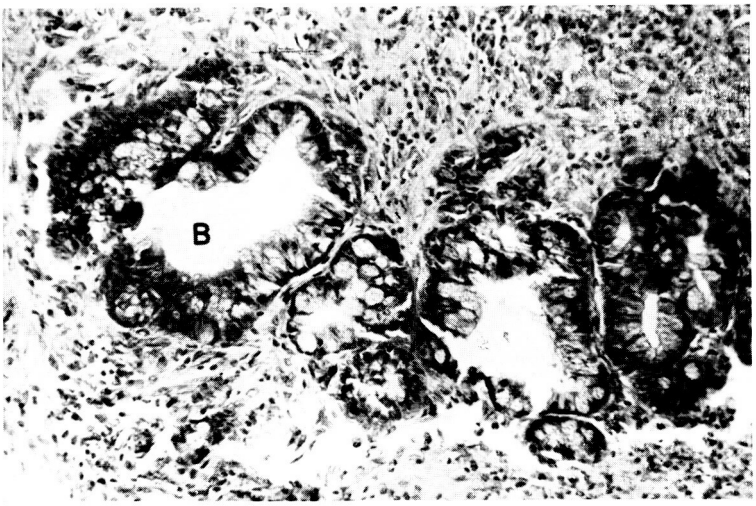

Fig. 8. A similar tumour to Fig. 7, × 170, to show malignant epithelium. (Osborne, Prasad and Zimmerman.)

by ultracentrifuge and viscometric studies on the extracted glycogen, which showed that the glycogen from the irradiated animals was less polymerized than that from the controls.

Next they found changes in the amounts, or at any rate in the activities, of certain enzymes in the liver. No quantitative study was made; the changes demonstrated were in the intensity of the specific histochemical staining reactions for the enzymes in question, using the usual azo-dye coupling methods. In the liver parenchymal cells, acid phosphatase, non-specific esterase and succinic dehydrogenase activities were all considerably reduced, while in the Kupfer cells and mast cells alkaline phosphatase was increased. These changes were well-marked 2 days after irradiation and complete recovery took place within 10 days.

Finally they report that the concentration of free tyrosine in the blood serum rose from the normal level of 10 μg/ml to about 20 μg/ml during the first 2 days and remained at this level for at least 10 days. From their experience with human patients suffering from various liver diseases, these authors contend that an elevated serum tyrosine level is a valid index of liver dysfunction, but the point remains to be proved.

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DISCUSSION

D. R. LUCAS

Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berkshire, Great Britain

Heuss[b]) thought that, though the similar age-dependency of lethality in the LD 50 range and survival-time at higher dose levels was intriguing considering the different lethal processes involved, the great difference in age-dependency between the mouse and the rat was much more exciting and urgently required substantiation. Observations on other species would also be of great interest, especially if they could be related to physiological differences, e.g. at the hormonal level, between the species.

In connection with his work, he wished to remind the audience

that Smith and Alderman (see report by Fowler, page 319) had reported that induction of the gastrointestinal syndrome in mice was also age-dependent.

Munday[d]) did not think that the total quantity of sodium lost was a major factor in gastrointestinal death. She and Pover had found that rats on a sodium-free diet could withstand a total loss of sodium through biliary secretion in excess of 8 milli-equivalents per kilogram of body weight. However, if loss of sodium and water occurred rapidly, shock was likely to ensue. She could confirm Sullivan's[g]) finding that diarrhoea did not occur if bile was prevented from reaching the intestine.

In reply to a question from the chair, Sullivan said that rats did appear to survive better at higher dose-levels when bile was diverted from the intestine, but he had not attempted to measure survival accurately because of technical difficulties.

Dykes (*Dept. of Exp. Path., University of Birmingham, Birmingham 15, Gr. Britain*) wondered whether Sullivan's findings were explained by an increase in the quantity of bile salts excreted in the stools or by a change in the sensitivity of the mucin-secreting cells to the salts. Sullivan replied that preliminary data obtained with ^{14}C-cholic acid suggested that absorption of this acid might be decreased by irradiation. On the other hand, if bile was introduced lower down the intestine, diarrhoea was induced in the irradiated animal, but not in the normal. This suggested that the sensitivity of the intestinal epithelium to the action of bile was increased by irradiation.

Vatistas (*Med. Res. Council, Hammersmith Hosp., London W12, Gr. Britain*) asked if Sullivan could exclude the possibility that changes in the nature and distribution of the microbial flora of the gut affected its content of bile. Furthermore, since the amount and composition of bile secreted might have been altered as a result of irradiating the liver, he wondered if any experiments had been carried out with the liver shielded.

In reply to the first part of the question, Sullivan stated that sterilizing the gut with antibiotics did not prevent the diarrhoea, but changes in the distribution of the intestinal flora might nevertheless be important. Thus, desoxycholic, which was not normally formed above the large intestine, was much more effective than cholic acid in inducing diarrhoea.

As to the second question, in all of his experiments four-fifths

of the liver was shielded. Moreover, Jackson and Entenman[1]) had shown that irradiation of the liver did not affect the volume or electrolyte composition of bile.

Vogel (*Nat. Lab., Argonne, Ill., USA*) said that the intestinal syndrome following X irradiation which had been described closely resembled that seen in rats and mice exposed to fast neutrons (200 to 350 rad). The early death (4 to 10 days after irradiation) of such animals could be prevented by daily injections of streptomycin. Furthermore, Wilson and Piacksek[2]) had shown that intestinal deaths at 3 to 4 days did not occur in "germ-free" mice after X irradiation (ca. 1000 rad). He thought both these approaches might help the present investigators.

With regard to tumours of hollow viscera, Unnewehr (*Max Planck Institut für Biophysik, Frankfurt am Main, Germany*) warned that cysts and abscesses could easily be mistaken for tumours unless very careful histological studies were made. In some recent work, he had found that some 8% of the "tumours" could be accounted for in this way.

Osborne[e]) replied that he fully agreed and thought the rapporteur had put his present position very clearly. They had tried to discover how epithelium became embedded in connective tissue. They considered that malignancy was indicated by piling up of the epithelial cells, increase in their nuclear-cytoplasmic ratio, loss of cell polarity, increased basophilic staining and the presence of mitotic figures.

While he did not wish to appear reluctant to defend his present position, he was quite prepared to admit that he might eventually have to accept that the epithelial inclusions were not carcinomatous. However, he felt that he must tentatively consider as carcinomatous those cases in which there were metastases in the mesentery, spleen and diaphragm and those in which the whole ileum and jejunum had received 2000 r or more. The incidence of tumours in the latter animals was over 50%.

Cole (*Naval Rad. Defense Lab., San Francisco, Cal., USA*) said that he and Nowell had published observations on mice[3]). They had found a relatively large number of true mucus-secreting adenocarcinomata of the intestine after a whole-body dose of 1100 r of X rays or 400 rad of fast neutrons, i.e. doses of the same order as those used by Osborne.

In reply to a question from Mendelsohn (*Dept. of Radiology, Hospital of the Univ. of Pennsylvania, Philadelphia 4, Penn., USA*) Tanka[i]) said that they had irradiated the whole body. The histochemical changes observed in the liver might therefore be either a direct or an abscopal effect of irradiation.

Replying to Kuzin (*Academy of Sciences, Moscow, USSR*), Tanka said that they had not established whether the changes in enzyme activity in the liver could be demonstrated as soon as one or two hours after irradiation. Their observations had been made 1, 2, 3, 7 and 10 days after irradiation.

In reply to Loutit (*MRC, Radiobiological Research Unit, Harwell, Berks., Gr. Britain*), Thompson[h]) said that the causes of early death, in the absence of tumours, in animals given the lower dose of ^{90}Y had not been established.

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INGESTED ISOTOPES

JANET VAUGHAN

Medical Research Council, Bone-Seeking Isotopes Research Unit, Churchill Hospital, Oxford, Great Britain

a) B. O. Biskis and Miriam P. Finkel,
Histopathologic changes in growing and mature dog bone after single or multiple injections of ^{90}Sr.
Argonne National Laboratory, Argonne, Ill., USA

b) W. R. Christensen and C. C. Smith,
Skeletal changes induced in dogs by radium, plutonium, mesothorium and radiothorium.
Department of Radiology, University of Utah College of Medicine, Salt Lake City 12, Utah, USA

c) S. H. Cohn, S. W. Lippincott, E. A. Gusmano and J. S. Robertson,
Short term kinetics of ^{47}Ca and ^{85}Sr in man.
Medical Research Centre, Brookhaven National Laboratory, Upton, NY, USA

d) T. F. Dougherty,
Comparison of carcinogenesis by single injections of five radionuclides.
Department of Anatomy, University of Utah College of Medicine, Salt Lake City 12, Utah, USA

e) J. I. Fabrikant and C. L. D. Smith,
Radiological changes in experimental animals following the administration of bone-seeking radionuclides.
Institute of Cancer Research, Royal Cancer Hospital, London, Great Britain

f) A. J. Finkel and C. E. Miller,
Patterns of radium retention in mice as related to man.
Argonne National Laboratory. Argonne, Illinois, USA

g) R. J. Hasterlik, A. J. Finkel and C. E. Miller,
The late effects of radium deposition in humans.
Argonne National Laboratory, Argonne, Illinois, USA and *Argonne Cancer Research Hospital, University of Chicago, Illinois, USA*

h) Norma Jacobs and F. W. Spiers,
The size-distribution of marrow spaces in trabecular bone: Its implications in problems of bone dosimetry.
Department of Medical Physics, The University of Leeds, Great Britain

i) W. S. S. Jee and Betsy J. Stover,
The long term effects of ^{239}Pu in adult beagles.
Division of Radiobiology, Department of Anatomy, University of Utah College of Medicine, Salt Lake City 12, Utah, USA

j) J. F. Loutit,
Movement of strontium in the human body.
Medical Research Council Radiobiological Research Unit, Harwell, Great Britain

k) T. NEUMARK,
Submicroscopical changes after local X ray treatment in the epiphyseal cartilage of young rats.
National Institute of Rheumatism and Medical Hydrology, Laboratory of Histopathology, Budapest, Hungary

l) A. NILSSON,
Long term effects of ^{90}Sr in bone tissue with special regard to carcinogenesis.
Research Institute of National Defence, Sundbyberg, Sweden

m) V. A. POLYAKOV, M. N. PAVLOVA and V. P. TORBENKO,
Biological effect of ionizing radiation on bony tissue.
The Central Institute of Traumatology and Orthopaedics under the USSR Ministry of Public Health, USSR

n) E. P. RADFORD, VILMA R. HUNT and D. SHERRY,
Analysis of teeth and bones for alpha-emitting elements.
Department of Physiology, Harvard School of Public Health, Boston, Massachusetts, USA

o) MARCIA W. ROSENTHAL, JOAN F. FRIED, J. SCHUBERT and A. LINDENBAUM,
Reduction of tumour incidence and tissue radioelement retention following DTPA therapy in plutonium-injected mice.
Argonne National Laboratory, Argonne, Illinois, USA

p) P. RUBIN, G. CASARETT and L. YOUNG,
Cysteamine protection against radiation injury of growing bone and cartilage.
Division of Radiation Therapy, Strong Memorial Hospital, Rochester, NY, USA

q) BETSY J. STOVER and W. S. S. JEE,
Some effects of long-term alpha-irradiation on the composition and structure of bone.
Division of Radiology, Department of Anatomy, University of Utah College of Medicine, Salt Lake City 12, Utah, USA

r) L. M. VAN PUTTEN,
Treatment of radiostrontium intoxication in mice.
Radiobiological Institute TNO, Lange Kleiweg 151, Rijswijk (ZH), The Netherlands

s) HELEN QUINCY WOODARD,
The effects of X rays on the composition of mouse bone.
Sloan-Kettering Institute, New York, USA

1. INTRODUCTION

The papers to be reported can be divided into two groups.

(1) Those that are concerned with the kinetics of calcium, strontium and radium metabolism.

(2) Those that are concerned with the observed effects of different isotopes on bone.

2. THE BEHAVIOUR OF CALCIUM, STRONTIUM AND RADIUM IN THE BODY

The alkaline earths are found in the same sites in mineralized tissue,

i.e. wherever the tissues are calcified. It is usually assumed that they are all associated with the apatite crystal and that their binding in bone might be described as at least secondary to that of calcium. Though their site of deposition is the same it is probable that the tissues do not handle the alkaline earths alike. There is evidence that calcium is more readily absorbed from the gut than strontium and that more strontium than calcium is excreted in the urine, probably due to differences in tubular reabsorption. Further, there is increasing evidence that discrimination may also occur in the exchange between blood and bone and bone and blood[1,2]). Quite apart from the general physiological interest of this problem of the movement of calcium, strontium and radium in the skeleton it is important from the point of view of evaluating the effect of ^{90}Sr from fall-out ingestion. Three papers presented in this session are concerned with this problem. Of equal importance, of course, is the metabolic pattern of the transuranic elements but here our ignorance is almost complete.

Cohn, Lippincott, Gusmano and Robertson[c]) have studied the behaviour of ^{47}Ca and ^{85}Sr over a 30 day period in six ambulatory patients with different clinical conditions (myeloma and osteoarthritis) reflecting various degrees of skeletal involvement. Measurements of whole-body retention were made with a whole-body gamma spectrometer. Simultaneous measurements of the tracers in the excreta and in samples of plasma give data necessary for the

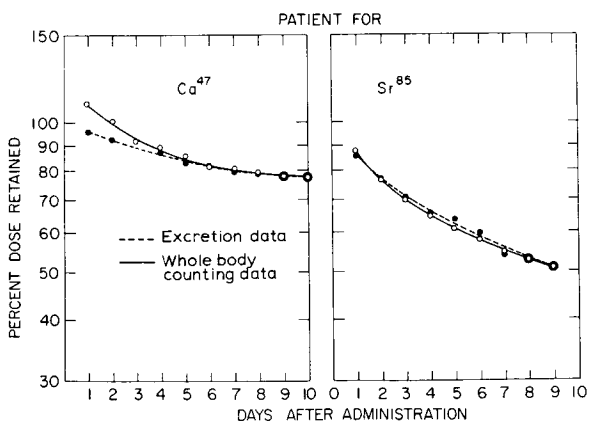

Fig. 1. Whole-body retention of ^{85}Sr and ^{47}Ca determined by excretion analysis and by whole-body counting. (Cohn, Lippincott, Gusmano and Robertson.)

determination of the skeletal accretion rate and exchange capacity using a four compartment model of the kinetic metabolic system, developed on an analogue computer, making the following assumptions, (i) that the $^{47}Ca/Ca$ ratio of the exchangeable bone fraction and of the plasma come rapidly into equilibrium, (ii) that the ^{47}Ca and the stable calcium are deposited in slowly exchanging bone in the same proportions that they simultaneously occur in plasma. These assumptions are, of course, open to question. The results of their calculations suggest to the authors that although strontium retention drops more rapidly than calcium during the first ten days, (Fig. 1) reflecting the preferential renal excretion of strontium, both elements are shown to be accreted into bone with the same time constant. The authors conclude from this that the initial exchange process and surface binding by the bone is similar for the two elements. However, after 10 days different rate constants are necessary

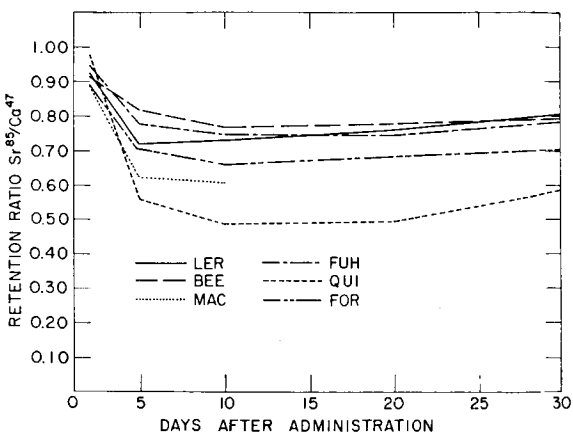

Fig. 2. Retention ratio of $^{85}Sr/^{47}Ca$ determined by whole-body counting. (Cohn, Lippincott, Gusmano, and Robertson.)

to express the observed retention values (Fig. 2). These rate constants for loss of activity may reflect the different modes of binding of the two elements in the crystal lattice. Strontium, they consider, is released more slowly from bone than calcium at a time interval greater than ten days after administration. Identical values for calcium and strontium tracers over long periods of time should not

therefore be postulated on the metabolic similarities observed in the short period after administration.

Loutit[1]) and his colleagues have also recently studied the relative metabolism of calcium and strontium following both the injection of ^{85}Sr into normal men and also the feeding of grain and milk into which ^{85}Sr has been incorporated[3-5]). The results of these studies together with the detailed figures for $^{90}Sr/Sr$ in the bones of the population in different age groups undertaken for the Medical Research Council monitoring programmes are discussed by Loutit for the present conference. Loutit emphasises that knowledge of the rate of turnover of bone salt and replacement of pre-existing strontium by stable strontium (and ^{90}Sr) from the diet is essential if the fall-out risk is to be evaluated. He goes on to show that turnover of bone mineral may be ten times or more greater than are replacement values. In balance experiments in normal subjects he found a mean retention of 6 per cent of ingested ^{85}Sr in the fourth week of a diet labelled with ^{85}Sr. At this period, though the level of plasma ^{85}Sr was constant there was still disequilibrium between various compartments — the specific activity of diet was far higher than that of plasma. He concludes, therefore, firstly that plasma strontium must be supplied from at least two sources, the diet and resorbing unlabelled bone: secondly that whereas more than half the absorbed dietary strontium is excreted daily, a much smaller fraction of "osteogenous" strontium is excreted since the specific activity of urine was found to be much higher than that of plasma.* From an analysis of data on $^{90}Sr/Sr$ in human bone over several years the figures for replacement appear somewhat lower than in the one month experimental period. He concludes therefore that strontium built into bone (as distinct from that on bone surfaces) is more tenaciously held and recycled than strontium entering the plasma from diet. The annual rate of replacement in the adult is thus only a few per cent per year when calculated from long term ingestion of ^{90}Sr, somewhat more according to short term experiments involving ingestion of ^{85}Sr and much less than the total turnover of bone mineral.

Both these papers then, though dealing with rather different as-

*) A correction of this finding has since been published. The specific activity of strontium in urine compared with plasma is much closer to unity. (T. E. F. Carr, G. E. Harrison, J. F. Loutit, and A. Sutton, B. M. J. ii (1962) 855).

pects of metabolism emphasize that the short term picture of strontium turnover may differ from the long term.

Our knowledge of the metabolism of radium in man has come from studies of either dial painters who ingested radium over fairly long periods as a result of licking their brushes or from patients who received injections of radium over short periods. Data on late excretion patterns and total retention only have been available, and though analysis of such data made by the Argonne and Boston groups has greatly aided our understanding of the late effects of radium poisoning, data covering the early period are urgently needed. Finkel and Miller[f]) have set out to correlate the retention observed using single intravenous injections of radium in mice with that observed on continuous feeding experiments. They have shown that the long term retention after finite periods of injection of radium (and by analogy that of strontium) can be regarded as essentially equivalent to multiple daily injections. For this purpose the retention after a single intravenous injection can be regarded as a power-function and integration of this function over a stated period of time can be used to predict the retention after continuous feeding.

The integrated curves after cessation of radium injection indicate that the radium content of the body falls much more slowly when a long time is required for the accumulation of the burden than when a comparable burden is acquired in a few days (Fig. 3), a

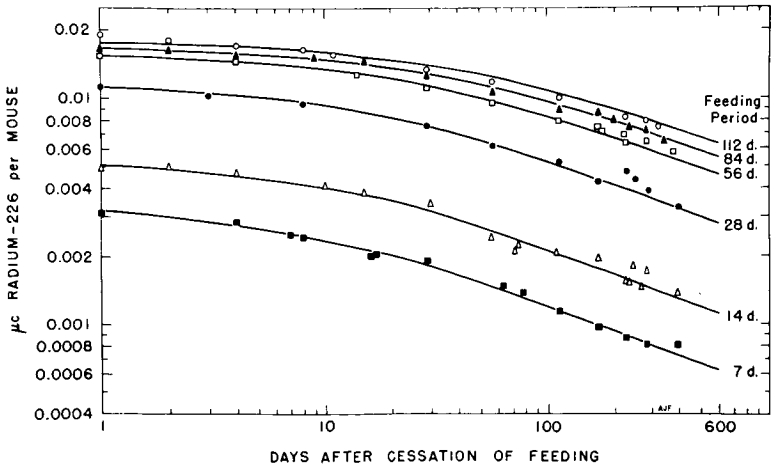

Fig. 3. Radium content of mice after continuous ingestion. (Finkel and Miller.)

fact that agrees to some extent with Loutit's observations of strontium turnover.

In connection with human radium metabolism the work of Radford, Hunt and Sherry[n]) may be mentioned. They have analysed human teeth and bone samples from different geological areas and consider that individual variations are not related to the soil but are probably dependent on food intake.

3. THE OBSERVED EFFECTS OF BONE-SEEKING ISOTOPES ON THE SKELETON

Under this heading come a large group of papers giving factual information about the effects of different isotopes upon the skeleton, particularly of mice and dogs. These effects differ in detail depending upon the character of the radiation, the site of deposition of the isotope, the age of the animal, the level of the administered dose and its method of administration whether single, divided or continuous. It is of course also important to have a measure of the dose-rate and accumulated dose at the point of damage. As a background to this section the summary of the present experience in the Utah experiment[6]) when five radionuclides were administered to beagle dogs, may be taken from the paper of Dougherty[d]). Retention of the injected radionuclide and its daughters was measured directly by whole-body counting and analysis of tissue samples at sacrifice,

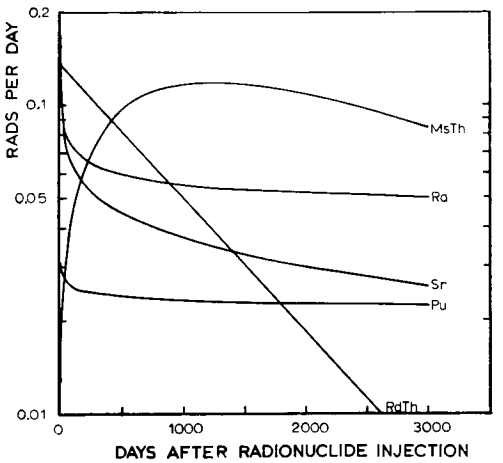

Fig. 4. Instantaneous mean dose-rates to the skeleton of 1-level beagle dogs. (Dougherty.)

and measured as the difference between injected activity and excretion. Knowing the disintegration energy, the instantaneous dose-rate (at t days after injection) was calculated taking the skeleton as 1/10 of the injection weight, the mean dose-rate only being given. The results for one injection level for the isotopes used are shown in Fig. 4. Initially the dose-rate from radiothorium is highest but after a few years it becomes the lowest due to its 1.9 year half life. In the mesothorium animals the dose-rate increases rapidly during the first few years due to the build up of its alpha emitting daughters and then decreases. By integrating the instantaneous dose-rate equations the cumulative dose has been computed as at the time of death. This value divided by time of death gives the average dose-rate to the skeleton throughout the entire exposure time.

Essentially all the tumours that developed can be attributed to radiation. Different radionuclides proved, as might be expected, both from previous work and on theoretical grounds, unequal in their effectiveness. In Table 1 are shown preliminary values for effectiveness using radium as a base line. A common time for cancer formation from the radionuclides considered was fixed and their dose-rates compared. Plutonium and radiothorium are far the most effective. Dougherty adds by way of caution that these relative effectiveness values may change at lower dose levels at which cancers have not yet appeared. In general mesothorium and radiothorium produce osteosarcomas at an earlier time than any other isotope. Plutonium, on the other hand, has been a more effective tumour producer over a wider range of dose level. ^{90}Sr has only produced tumours at the highest level so far.

TABLE 1

Effectiveness relative to radium in producing osteosarcomas

Nuclide compared	Days to death	Nuclide rad/day	Radium rad/day	Eff. rel. to radium
^{239}Pu	1500	0.7	3.7	5
^{228}Ra (MeTh)	1140	3.7	10.0	3
^{228}Th (RdTh)	1140	0.7	10.0	14
^{90}Sr	1140	8.5	10.0	1

These preliminary values will be revised as dosimetry is refined.
Relative effectiveness is expected to vary with time.

The probable explanation of the great toxicity of plutonium and radiothorium compared for instance with radium, also an alpha emitter, is the fact that the two former isotopes are concentrated on certain bone surfaces close to the sensitive osteogenic connective tissue while radium is distributed throughout mineralized bone, in hot spots it is true, but often away from the sensitive osteogenic tissue[7]). Support is given to this view by the careful study by Jee and Stover[1]) of the long term effects of ^{239}Pu in adult beagles of the Utah colony. They find that 95 per cent of the tumours arise from spongy bone (Fig. 5). The authors make some attempt to calculate

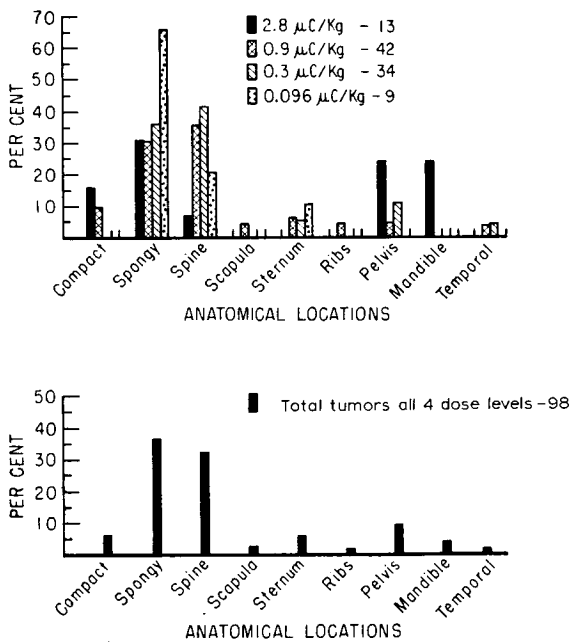

Fig. 5. Anatomical distribution of osteogenic sarcomas with various injection dose levels of ^{239}Pu. (Jee and Stover.)

the radiation dose received as an average skeletal dose in macroscopic regions of bone based on terminal values of ^{239}Pu per g wet bone. The dose to the shaft of the humerus on this basis in much less than that to the spongiosa. In one dog it was 2.2 Krad in the shaft compared with a maximum of 15.6 K rad in the spongiosa (Table 2). In view of this uneven distribution of dose it is not surprising

TABLE 2

Variation in average skeletal dose[a] in selected bone samples.

Dog number	M8P5	F9P5	F7P5	F5P5
Irradiation period (days)	1192	1145	1491	2059
Humerus	7.8	9.6	9.6	14.6
Proximal epiphysis	12.1	17.1	13.8	18.9
Proximal epiphyseal line	15.6	20.6	19.1	24.8
Proximal diaphysis	2.7	3.9	4.0	6.9
Mid diaphysis	2.2	2.6	3.3	4.6
Lumbar vertebra (3rd)	9.4	11.7	12.9	18.1
Body and transverse process	11.2	14.8	15.1	21.1
Skeleton[b]	4.8	4.8	6.6	8.7

[a]) Dose unit in Krad.

[b]) Calculated using fractional skeletal retention $= 0.72t^{-0.040}$ (ref.[6])).

that tumours in the shaft are restricted to the higher dose levels.

An elegant study of the size distribution of marrow spaces in trabecular bone and the implications for bone dosimetry by Jacobs and Spiers[h]) should in future facilitate precise dosage measurements in spongy bone. Their calculations of dose are for X rays but it should be possible to use their measurements of marrow spaces to calculate doses from alpha and beta particles knowing the isotope distribution and trabecular size. The sort of information obtained

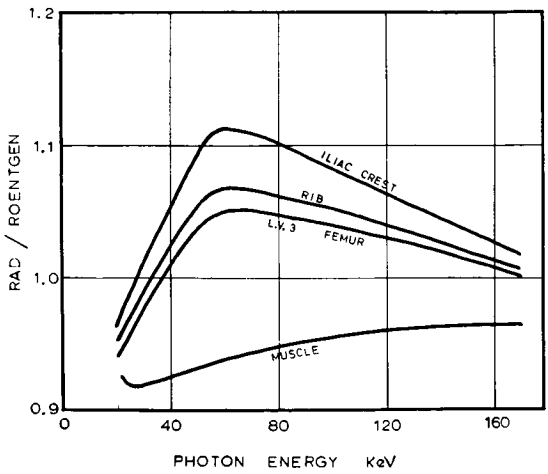

Fig. 6. Variation of mean rad/r factors with photon energy for iliac crest, rib, femur and lumbar vertebra. (Jacobs and Spiers.)

from their data is shown in Fig. 6 where the mean dose to trabecular marrow in bones of a 33 year old man are shown by averaging over the appropriate size distribution of the marrow spaces. The mean rad/r factors so obtained are shown together with the factors for soft tissue. The results for the femur do not differ significantly from those for the vertebrae.

The radiological changes in the 8 years of the Utah experiment have been closely followed by Christensen and Smith[b]). The changes seen are similar to those described in human cases of radium poisoning (Table 3). The earliest and most severe changes have occur-

TABLE 3

Radiographic lesions seen in beagle dogs receiving ^{226}Ra, ^{239}Pu, MsTh and RdTh

1. Structural changes in the mandible
2. Destructive changes in the teeth
3. Pathological fractures – with and without healing
4. Distortion of cortex of long bones
5. Disturbance of metaphyseal trabeculation
6. Osteolytic rarefaction
7. Rib end demarcation
8. Aseptic necrosis
9. Tumor formation

red in the highest dose level animals, though there is variation within a group. The first abnormality tends to occur in the mandible. Radiothorium and mesothorium appear to produce pathological changes more rapidly than plutonium and radium which, if the dose-rate curve shown by Dougherty is remembered, is not surprising but there is no distinctive pattern of radiation damage peculiar to any radionuclide. The relative rate of fracture injury is high and recovery poor with mesothorium. Pathological fractures and tumours may occur in osseous structures where gross radiological appearance is normal.

A similar serial study has been made on rat bones in animals receiving ^{239}Pu, ^{241}Am and ^{32}P by Fabrikant and Smith[e]). No attempt is made to estimate radiation dose received by the skeleton or even body burden at death so it is difficult to compare the results in the two animal species. Fabrikant *et al.* claim to be able to distinguish between the lesions due to ^{32}P and those due to the alpha emitters. Generalized osteoporosis and sclerosis they consider is

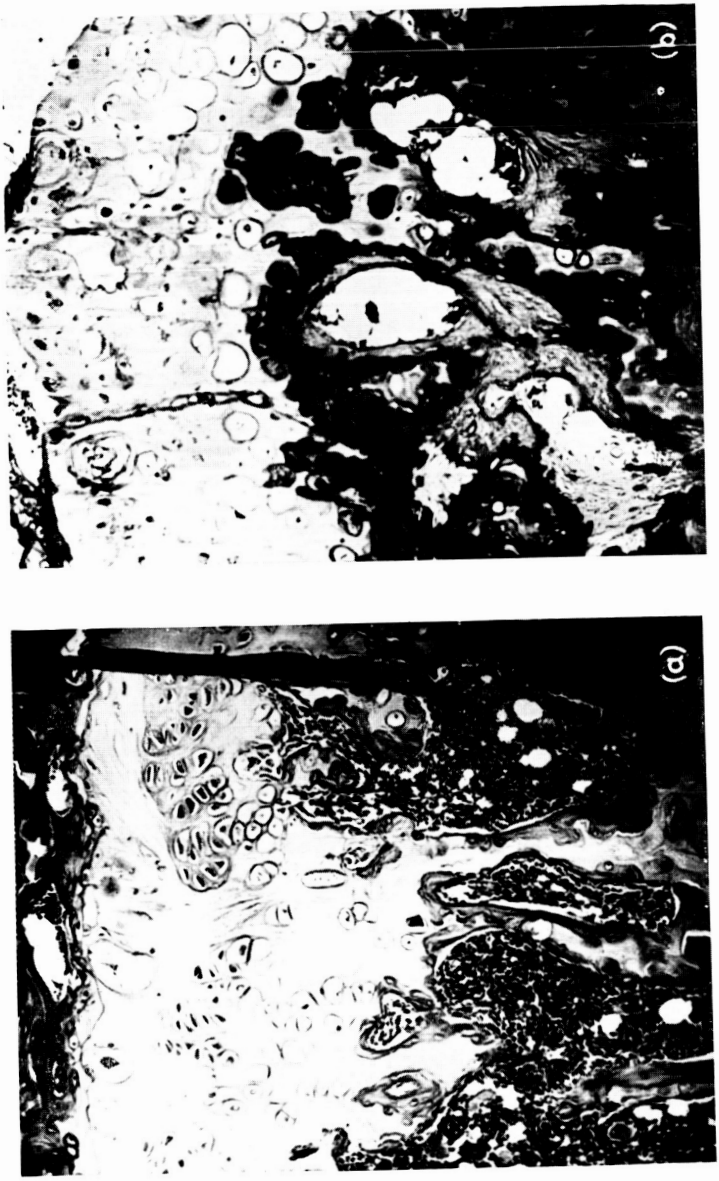

Fig. 9. Protective effect of cysteamine (150 mg/Kg) administered immediately before 2400 r (220 KV unit) on epiphyseal cartilage plate of rat bone (a) Cysteamine + 2400 r (b) 2400 r only. (Rubin, Casarett and Young.)

only produced by alpha radiation and not by ^{32}P though sclerotic bands occur following the latter. Alpha radiation tends to produce sclerosing tumours while beta radiation gives rise to osteolytic lesions almost entirely.

The gross radiological changes seen in these experimental animals with relatively high body burdens are in contrast to those found by Hasterlik, Finkel and Miller[g]) in a radiological study of 236 persons largely employed in the watch dial industry before 1925. Minimal radiographic lesions were seen with some degree of confidence when the radium level exceeded 0.01 μC. The frequency and severity of lesions progressed in the range of 0.1 to 1.0 μC and moderate, advanced and even malignant lesions were then observed. Above 1.0 μC advanced lesions were seen in the majority of patients (28/40) and malignant lesions in 14 out of the 40 (Table 4). Of course, as already mentioned in discussing Finkel and Miller's experimental work on rats fed or injected with ^{226}Ra, these terminal burdens may be related to very different initial burdens and therefore different accumulated and maximum dose-rates.

There is already in the literature a considerable body of work describing the histological picture that results from the ingestion of dif-

TABLE 4

Long term effects of radium deposition in man
(Correlation of clinical and radiographic findings with current body burdens of ^{226}Ra)

Body content (μC)	Number radio-graphed	Radiographic changes in skeleton attributable to radium deposition					
		None	Mini-mal	Mild	Moder-ate	Ad-vanced	(Malign-ant)
0.001	17	14	3	0	0	0	
0.001–0.01	28	28	0	0	0	0	
0.01–0.1	90	80	8	1	1	0	
0.1–1.0	61	25	13	9	9*	5***	(3)
1.0	40	1	2	5	4	28**	(14)
Total	236	148	26	15	14	33	(17)

* MsTh present in two cases.
** Based on film taken elsewhere in two cases.
*** Severe tooth changes only in one case.

ferent bone-seeking isotopes. An attempt is made here to pick out the observations that give us new knowledge in this field rather than to attempt to summarize the detailed studies that have been presented and which have indeed already been published in some cases. The difference in the histological picture in growing and mature dog bone after single or multiple injections of ^{90}Sr described by Biskis and Finkel[a]) are probably dependent on variations in radiation dose received due to variation in the biological activity of the tissues. There were no instances where tumours originate directly from heavily damaged bone but in many instances they arise from fibrous tissue replacing the resorbed damaged bone. On the other hand Jee and Stover[l]) emphasise that on reviewing their material in dogs given plutonium they wish to retract their previous statement that bone tumours arise from areas of dense peritrabecular fibrosis.

In a detailed study of 530 mice following ^{90}Sr injection Nilsson[l]) comments, as others have done, on the fact that the majority of tumours arise on the endosteal surface of the bone and are often multiple. He obtained no evidence that the osteosarcomas were, even indirectly, virus-induced. Metastases to soft tissues were rare from the original tumours but after tumours were transplanted metastases were common from osteosarcoma, but not from fibrosarcoma. The metastases readily took up ^{90}Sr injected immediately before death.

The histological damage to bone reported following all forms of radiation is associated with changes in mineral content, water content and enzyme content. Some of these changes have already been demonstrated in the case of rabbits given ^{90}Sr[8,9]) and in papers presented for this session they are recorded in the case of dogs after plutonium injection by Stover and Jee[q]), and by Woodard[s]), following X rays in mice. Woodard concludes that the catabolic processes are injured more severely by radiation than the anabolic processes but that effects can be modified by the thyroid hormone. Polyakov, Pavlova and Torbenko[m]) make the interesting observation that the size of the apatite crystal is affected and that the activity of amylase is also affected as well as alkaline phosphatase. Aldolase and phosphorylase show no change. Neumark[k]) reports decrease of the birefringence of collagen fibres and increase of metachromasia in the epiphyseal cartilage of young rats 24 hours after a single exposure to 2000 r from a 150 KV source.

Finally have we any hopeful information about the possible remo-

val of bone-seeking isotopes? Outstanding here is the use of DTPA (diethylenetriamine-pentacetic acid) in plutonium poisoning. Rosenthal, Fried, Schubert and Lindenbaum[o]) present us with an encouraging report of experimental work that shows that DTPA therapy, even if delayed until the radioelement has been deposited, significantly increases life expectancy and reduces the incidence of malignant bone tumours, while, as might be expected, the beneficial effect increases with the amount of plutonium removed (Figs. 7 and 8).

In the case of ^{90}Sr van Putten[r]) describes experiments on mice put on a low phosphorus diet 48 hours after injection. The survival time was increased and the tumour frequency reduced. Though the results are theoretically interesting the author himself doubts how far they are of practical application. In the case of local X irradiation, Rubin, Casarett and Young[p]) have evidence that in young rats if cysteamine is injected prior to irradiation, a significant degree of protection, approximately 50% is afforded (Fig. 9). This may well be of clinical importance.

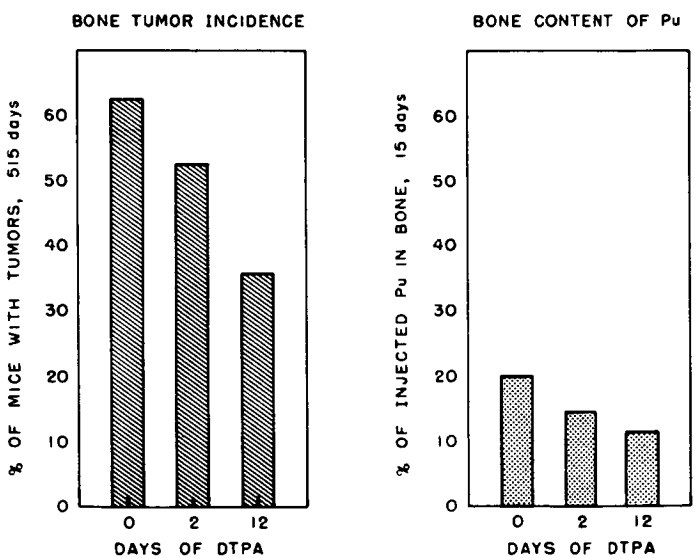

Fig. 7. Effect of DTPA therapy on bone tumour incidence and skeletal content of plutonium. Tumour incidence data are based on mice dying from 250 to 515 days after plutonium. This includes 99 in the control group, 38 and 75 in the groups treated 2 and 12 days respectively. (Rosenthal, Fried, Lindenbaum and Schubert.)

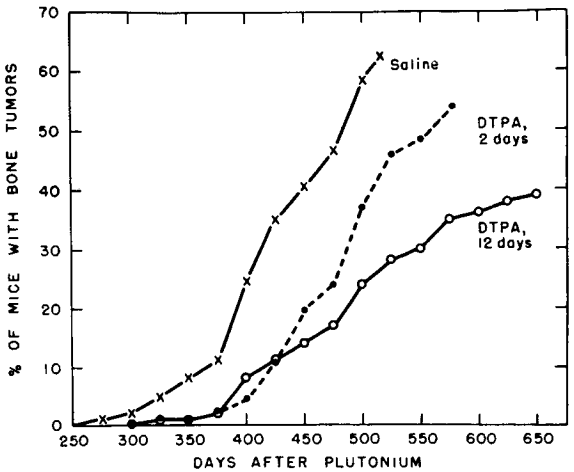

Fig. 8. Effect of DTPA on cumulative bone turnover incidence in plutonium-injected mice. (Rosenthal, Fried, Lindenbaum and Schubert.)

4. CONCLUSION

In summary it may be said that we now have much information about tumour production in animals from different bone-seeking isotopes. What we need to know is the relation of measured radiation dose to damage. Perhaps even more important is to recognize our ignorance of the physiological factors that control calcification and the regulation of calcium in the body, let alone factors controlling deposition of strontium, radium and the transuranic elements. We still lack elementary knowledge of bone physiology which we need to understand certain aspects of radiobiology as far as it affects the skeleton.

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DISCUSSION

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Nilsson[1]) commenced by presenting some additional material showing that at an early stage an osteosarcoma bud can be transplanted. One such transplant from the diaphysis of the femur to the neck of another CBA mouse had grown in five months to the size of a hazel nut, and was accompanied by numerous metastases to soft tissue and one to a cervical vertebra. He had not made any calculation of the radiation dose delivered in his experiments. Having used only male mice he had seen no evidence of a sex difference. Langendorff (*Radiol. Inst. Univ., Freiburg, Germany*) had found a greater uptake of strontium in castrated males than in castrated female mice; but Arghittu (*CAMAN, S. Piero a Grado, Pisa, Italy*) had never found any appreciable sex difference in strontium uptake and distribution in the numerous rats he had examined, and Mays (*Univ. Utah, Salt Lake City, USA*) had found no difference in retention between 30 male and 30 female dogs. Langendorff, in his own ^{90}Sr experiments, had found lung tumours in about 1% of cases, but they had not yet been examined histologically. Unnewehr (*Max Planck Inst. Biophysik, Frankfurt am Main, Germany*), on the other hand, found that bone tumours quite frequently produced metastases to the lungs, and that these metastases showed bony structures. Nilsson had never observed primary ^{90}Sr-induced tumours in the lung, but 8 to 9 months after injection of ^{90}Sr there were osteogenic osteosarcomas in lungs, liver, adrenals and other organs. Fibroblastic osteosarcomas, on the other hand, had never given metastases. This difference may be due to the more rapid growth of the fibroblastic tumour, which, in 3 to 4 weeks reaches the size attained by the osteogenic tumour in as many months. Metastases to skeleton were never seen without many metastases to soft tissue.

Neumark[k]) emphasised that in his experiments the polarizing microscope revealed changes very soon after a single large dose of X rays. These were changes in the fine structure of the long polymers in the epiphyseal cartilage of rats.

In reply to Stover[i,q]), Loutit[j]) reported that the ratio of specific activity of strontium in urine to that in plasma ranged from 1.7 to 4. * This range covered several modes of administration: a single injection or intravenous injection, an intravenous drip, or daily ingestions for a month. Cohn[c]) suggested that these observations must mean that strontium was presented to the kidney in two forms, differing in chemical composition or mode of binding. Loutit agreed that this was indeed his own view. It was possible to derive renal clearances for the two forms, and that for the dietary strontium was greater than that for the low specific activity material of, presumably, skeletal origin; he had no more direct evidence, however, of physicochemical difference between the two types, and their existence was as yet hypothetical.

Woodard[s]) recalled that in her work external irradiation had influenced both the anabolic and catabolic processes in bone, and she had since been able to manipulate conditions so that either an increase of bone density, no change, or a decrease would result, depending on the balance of these factors. She therefore suggested that where changes of density were produced by ingested radioactive materials the local isotope burden would determine whether the injury was manifested by an increase or a decrease of density. Hasterlik[g]) pointed out, however, that the lesions which appear radiographically as sclerotic lesions in the radium-bearing human are in fact calcium precipitates in bone which, from the lack of osteocytes in the lacunae among other evidence, can be seen to be dead. The situation described by Woodard in X irradiated mice could not apply in his α irradiated human bone. He also observed that in human patients bearing radium, where the effect is mainly due to α particles, the tumours seen are almost exclusively osteolytic. This is in contrast to the situation reported by Fabrikant[e]) in the mouse.

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*) A correction of this finding has since been published. The specific activity of strontium in urine compared with plasma is much closer to unity. (T. E. F. Carr, G. E. Harrison, J. F. Loutit, and A. Sutton, B. M. J. ii, (1962) 855.)

Lloyd (*Churchill Hospital, Oxford, Great Britain*) enquired whether Cohn had experimental evidence to support his assumption that the specific activity of ^{47}Ca in the exchangeable fraction of bone comes rapidly into equilibrium with that in plasma. In quantitative autoradiographic studies on rabbit bones, compared with specific activity measurements on plasma, she had seen evidence of a rapid uptake of ^{45}Ca, but none of the corresponding release that would be required to establish equilibrium. Cohn replied that this was only an assumption, but that, when applied to the four-compartment model he had used, it led to reasonable results.

Commenting on the existence of two phases in the loss of strontium from bone, one with a half period of days the other with a half period of months, Müller (*Strahlenbiol. Inst. Univ., Munich, Germany*) reported that in subjects saturated with stable strontium he had observed only the slow phase. He presumed that strontium was fixed in two different forms. Loutit replied that saturation with strontium had not changed the urinary excretion pattern from that observed with tracer quantities. However, Müller's observations were made directly on bone and not on urine. Mays asked for information on whether alkaline earth atoms other than calcium were situated within the hydroxyapatite crystals or only on the surface. This would have profound influence on the feasibility of removing isotopes fixed in the skeleton. He also warned against the trap of supposing that specific biological processes are associated with each of the rate constants of a retention equation that could be resolved into a series of exponentials. A two term equation had just been mentioned, but to fit the results on the Utah beagles after 6 years no less than five exponentials were required and he had no doubt that ultimately many more would be necessary. The equation represents the data but not the biological processes.

Replying to Sundaram (*Atomic Energy Establishment, Bombay, India*), Rosenthal[o]) stated that DTPA (diethylenetriamine pentacetic acid) is about as effective in removing plutionum even when given 40 days after administration as it is after 3 days. However, she had not studied the effect on tumour incidence when DTPA was given later than 3 days after administration of plutonium.

Dunjik (*Inst. Cancer, Louvain, Belgium*) enquired whether in Rubin's[p]) work there had been complications due to the toxicity of cysteamine, and whether there was not also risk of protecting the

tumour as well as other tissues. Casarett[p]) replying for Rubin, stated that the dose of cysteamine was only 150 mg per Kg body weight, and was below the toxic level in rats. While some tumours might be protected by cysteamine, there is a high concentration of cysteamine in cartilage and it is thought that the protection given might be useful in radiotheraphy.

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