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FOREWORD

EMERITUS PROFESSOR S. H. ROBERTS, C.M.G.

Opening remarks to the Conference, August 15, 1960

IT is a particular honour to be invited to open this important conference, and I feel this, more especially, since I am perhaps the only one present whose scientific knowledge of your particular field of investigation is largely derived from the rather terrifying popular accounts of the effects of ^{90}Sr, and other numbered symbols. Although, more happily, as one concerned with the administration of scientific departments, I am constantly made aware of the rapid advance of studies in your subject and in the related physical fields, which has inherent, in its fruition, untold benefits to mankind.

It is also a pleasure for me to be able to subscribe, personally, to the welcome that has been expressed to you, on behalf of the University, by the Dean of the Faculty of Medicine, Sir Edward Ford. We are happy that our University has been selected as the venue for your conference, and I can assure you that our welcome is especially warm and sincere. I know, of course, that the First Australasian Conference on Radiobiology was convened by Dr. J. H. Martin of the Cancer Institute in Melbourne five years ago. The Second Conference was held at the University of Melbourne in 1958.

It is especially interesting to me, as a historian, to regard the tremendous changes which have occurred since the discovery of radioactivity by Bequerel in 1896, and of x-rays by Roentgen in 1895. An extraordinary thing, looking back over the perpetuity of time, is what an amazingly short time ago that was. The study of the effect of ionizing radiations on the tissues of the body, commencing at the time of those epoch-making discoveries, progressed steadily for almost fifty years, and I venture to think as a comparative historian that this is one of the greatest developments of modern time. Then, with the coming of the nuclear age, radiobiology received such an impetus, and achieved such sudden importance, that to most people it now appears as an entirely new subject. Its future progress is intertwined with one of the great changes in human history.

It is a happy portent that, amid the world-wide apprehension and fear of the destructive use of atomic energy, there shines the enormous potential of the beneficial uses of ionizing radiation for the welfare of man.

As tokens of the inestimable value of such uses, we see about us its increasing utilization in industry, medicine, agriculture, veterinary science and research.

The papers to be presented to this Conference indicate the importance of radiobiology in this new world. It must, of necessity, continue to be the basis of medical radiology. It is concerned with the pressing need for assessment of the hazards of nuclear and other radiations—the great public health problem of today. It is an important adjunct to all the basic sciences. But

it is platitudinous, in such a gathering, for me to continue with the lengthy list of the important associations of your own subject. I shall merely add that, as the task of this conference is to stimulate further research in radiobiology in this country, it is of very special consequence to us all.

I am especially pleased on behalf of the University and the Chancellor to congratulate the workers in this new, and important, and indeed, very exciting field who are gathered here today.

I want to thank the distinguished scientific visitors, particularly from overseas, whose presence graces the University on this occasion and to wish you all well in your most important deliberations.

It is proper for me to add that, on account of the tremendous implications of the studies you pursue, and of their immeasurable importance to the future welfare and safety, and development of the peoples of all races, there can be, at this time and in this country, no more important gathering of scientific workers.

It is an honour, and a great pleasure, to declare this Third Australasian Conference on Radiobiology open.

ACKNOWLEDGEMENTS

A LARGE part was played by the Post Graduate Medical Foundation within the University of Sydney in meeting the expenses of the Conference. The Post Graduate Committee in Medicine made available its facilities and valuable secretarial help. Other bodies supporting the meeting were the New South Wales State Cancer Council, the Science and Industry Endowment Fund of the Commonwealth Scientific and Industrial Research Organization, the Anti-Cancer Campaign Committee within the University of Adelaide, the Anti-Cancer Council of Victoria, the Australian Atomic Energy Commission and numerous firms as well as many private individuals.

CONTENTS

		Page
	Foreword	v
	Acknowledgements	xi
1.	The Nature of Radiation Damage at the Subcellular Level. PETER ALEXANDER	1
2.	Effects of Gamma Radiation on Ribonuclease. SIDNEY RUBENFELD and EVELYN SLOBODIAN	17
3.	Chromosome Damage Produced by Radiations. JOHN READ ...	27
	Discussion. Chairman: Dr. G. M. Watson	37
4.	Cytogenic Analysis of Cell Populations in Radiation Chimaeras. C. E. FORD	39
5.	Haemopoietic Homografts Retransplanted Through Radiation Chimaeras. C. E. FORD, P. L. T. ILBERY and S. M. WINN ...	46
6.	Experiments in Skin Homograft Survival. MAXWELL KENT ...	54
7.	Preservation of Viable Bone Marrow by Freezing. PHAN THE TRAN and M. A. BENDER	59
8.	Effect of Radiation and Tissue Transfer on the Immune Response. P. C. KOLLER, S. M. A. DOAK and A. J. S. DAVIES	65
	Discussion. Chairman: Dr. S. Fazekas de St. Groth	84
9.	Components of Radiation Carcinogenesis. JACOB FURTH and KENJIRO YOKORO...	86
10.	Application of the Two-Stage Mechanism of Carcinogenesis to Leukaemogenesis with Low Doses of X-Rays as Initiating Factor. I. BERENBLUM and N. TRAININ	98
11.	Radiation in the Aetiology of Sporadic Human Leukaemia. F. W. GUNZ	102
12.	Radiation-Induced Aging in Mice. HOWARD J. CURTIS ...	114
	Discussion. Chairman: Emeritus Professor H. K. Ward ...	123

13. Mechanisms of Radiation Protection by Cysteamine. PETER ALEXANDER 129

14. Comparative Evaluation of Radioprotective Efficacy of Cysteamine against 500 r Whole Body Irradiation with Different Biological Reference Systems. P. DE, R. CHATTERJEE, A. BOSE and S. BOSE 138

15. Radioprotective Effect of Chlorpromazine *in Vivo* in Relation to Tissue Anoxia. DANA JAMIESON and H. A. S. VAN DEN BRENK 142

Discussion. Chairman: Professor J. L. Still 158

16. Electron Microscopic Studies of a Transferable, Radiation-Induced Mouse Leukaemia. H. HOFFMAN and N. DARVENIZA 161

17. Results of ^{90}Sr Determinations on Samples From Lucas Heights and Richmond, N.S.W. 1959–60. L. E. SMYTHE and P. S. DAVIS 170

18. Estimation of ^{90}Sr by an Ion-Exchange Method. P. S. DAVIS ... 184

Discussion. Chairman: Professor J. L. Still 191

19. Delayed Effects of Radiation on Seeds. HOWARD J. CURTIS ... 193

Discussion. Chairman: Professor Th. Dobzhansky 203

20. Radiation-Induced Quantitative Variation in Subterranean Clover. R. D. BROCK and B. D. H. LATTER 205

21. Modification of Genetic Response to X-Irradiation in *Drosophila*. A. M. CLARK 216

Discussion. Chairman: Professor Th. Dobzhansky 229

22. Influence of Ultra-Violet Irradiation on General Transduction in *Pseudomonas aeruginosa*. B. W. HOLLOWAY and MARILYN MONK 231

23. Some Effects of X-Irradiation on the Endogenous and Auxin-Induced Growth of Etiolated Pea Stem Tissue. JOHN W. KING and ARTHUR W. GALSTON 238

Discussion. Chairman: Professor Th. Dobzhansky 243

24. The Use of Ionizing Radiation in the Study of Immunity to Parasitic Nematodes. W. MULLIGAN and D. F. STEWART ... 245

25. Results of Assessment of Irradiation Response in the Treatment of Carcinoma of the Uterine Cervix by Evaluation of Serial Biopsies. K. A. MCGARRITY and J. M. GARVAN 250

26. Experiments in Photosensitivity. V. J. McGovern 270

Discussion. Chairmen: Dr. H. J. Ham and Professor B. J. F. Ralph 276

27. Comparison of the Biological Effects of X-Rays and Radiomimetic Chemical Agents. P. C. Koller 281

28. Mechanisms of the Cytotoxic Action of the Radiomimetic Agents. Peter Alexander 287

29. A Comparison of the Effects of Radiomimetic Drugs and Radiation on Haemopoiesis in the Rat. D. A. G. Galton ... 298

Discussion: Chairman: Professor R. H. Thorp 303

Index 309

1

THE NATURE OF RADIATION DAMAGE AT THE SUBCELLULAR LEVEL

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INTRODUCTION

IN this paper I propose to discuss the problems of the initial lesions, that is those radiochemical reactions that initiate the complex process which eventually leads to cell injury and death. The general pattern of injury development is quite well understood and the stages are listed below:

(*i*) As the radiation passes through the cell it deposits energy, the amount left behind being given by the dose in rads.

(*ii*) Part of this energy is used up in chemically altering some of the cell constituents.

(*iii*) Most of these chemical reactions are trivial and do not harm the cell, but some of them, the *initial* (*chemical*) *lesions*, occur at a vital point and act as a focus for the development of damage by subsequent cell processes.

(*iv*) After a certain period of active metabolism, biochemical lesions can be observed.

(*v*) These biochemical lesions lead to anatomical lesions (*i.e.* biological end-effects).

We have very little definite information about these very early chemical events. The U.N. Committee on radiation hazards concluded in its report (1958): 'The nature of the initial step of radiation damage remains to be determined.'

The early biological lesions to cells can conveniently be classified into: (*a*) physiological effects, (*b*) cell lysis or interphase death, and (*c*) delayed death often requiring mitosis before being manifested.

PHYSIOLOGICAL EFFECTS

These are only now being studied since it has become clear that there are numerous disturbances in mammalian radiopathology which occur almost immediately after irradiation (*i.e.* minutes to hours) and which must be attributed to an interference with the physiological function of nerve and muscle cells. Particularly striking demonstrations of such immediate effects have been provided by Brinkman and Lamberts (1960) and Hug (1960) with snails and earth-worms. All these physiological changes are characterized by extremely rapid repair and they are therefore very dose-rate dependent and may have to be studied while the irradiation is going on. At the cellular level

instantaneous changes in the transport of ions have been encountered following irradiation (*cf.* review by Bacq and Alexander, 1961) and it seems likely that reactions of cell membranes are involved. These physiological effects may follow directly on the 'chemical lesion' without there being an intervening biochemical and subcellular lesion, an aspect to which I return at the end of the paper.

INTERPHASE DEATH

Cell lysis is one of the first forms of radiation damage to be discovered. The susceptibility of cells to death during interphase without going into division

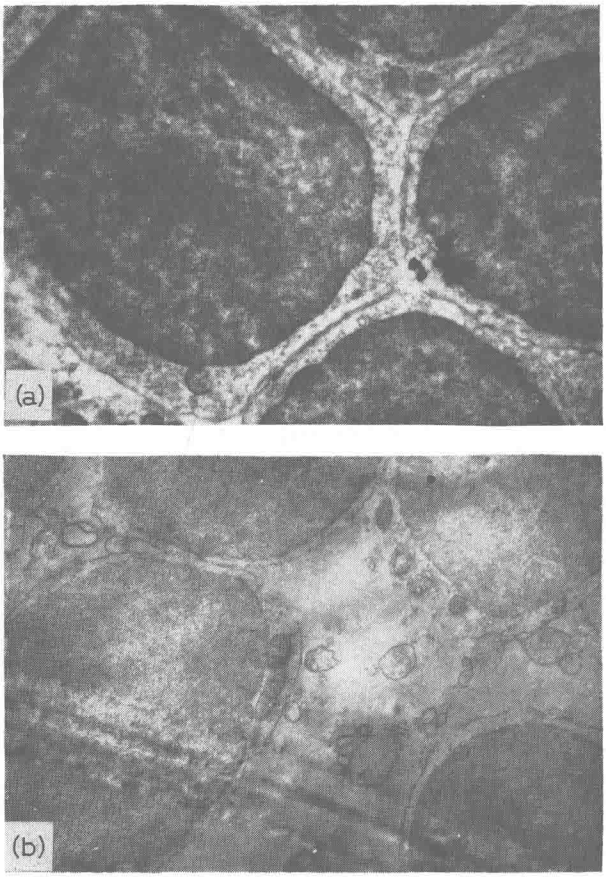

Figure 1. Electron photomicrograph of rat thymocytes

(*a*) Unirradiated; (no pycnosis can be seen in histological preparation)
(*b*) From an animal 4 hours after the thymus was irradiated with 1,000 rads of x-rays (pycnosis evident on histological examination in nearly every cell)

(Kindly provided by E. M. C. Birbeck)

or attempting division varies greatly. The best known example of very sensitive cells are lymphocytes, but they are not unique; oocytes and late spermatagonia also 'fade away' after small doses of the order of 100 rads. For most other mammalian cells thousands of rads are necessary to attain interphase death and if these cells can be induced to divide they will be killed by a process of mitotic death at lower doses. Interphase death is probably not important in the radiation damage of rapidly proliferating tissue. Some workers, for example Read (1958) maintain that interphase death plays no part in radiotherapy, but I do not share this view since the rate of cell division in many cancers is very low yet the destruction of the tumour mass is not delayed.

The mechanism of interphase death is still unknown and has received surprisingly little study. Duryee's beautiful work (1949) with amphibian eggs has focused attention on the role of the cytoplasm while Trowell (1953) in his detailed investigations of lymphocytes has used pycnosis of the nucleus as the criterion for injury. The occurrence of an altered appearance of the nucleus following staining and fixing does not, however, imply that the lesion is in the nucleus. It is quite possible that the typical pycnotic changes such as the clumping of nucleoprotein are due to a difference in the response of the cell to the fixative (and/or stain). For example, the clumping may be due to the interaction of the heavy metal in the fixative with DNA and the effect of irradiation may be to allow the metal to gain access. Support for this view comes from an electron microscope examination (see *Figure 1*) in which no difference could be seen between sections of the thymus of a rat before irradiation and 4 hours after 1,000 rads when histologically 90 per cent of the cells showed marked pycnosis. Biochemical investigations (Alexander and Scaife, 1961) also failed to reveal any difference between the DNA from normal thymuses and from those that had received 1,000 rads 4 hours earlier and which already showed marked pycnosis. While we would not have expected to observe, by the methods used, the amount of damage in the DNA that results from the initial radiochemical effect we would have expected to detect the amount of damage needed to alter the staining characteristic of the nucleoprotein. It would appear that the pycnotic appearance of the nucleus is not due to damage of the DNA occurring by some biochemical processes after irradiation, but due to another factor; that factor may be altered permeability.

MITOTIC DEATH

Frequently cells, following irradiation, will divide once or several times (*i.e.* form microcolonies) before they die out in the sense that they cease to proliferate further. *Figure 2* illustrates this well-known effect for leukaemia cells growing in suspension as independent cells in a culture medium in which they divide once every 12 to 15 hours (Alexander and Mikulski, unpublished). After 18 hours little difference can be seen between the irradiated and unirradiated cultures; in both, the cell number is more than doubled during this period. Only after 18 hours do the 90 per cent of the irradiated cells stop dividing. There are some 10 per cent uninjured cells that are growing up normally and which are responsible for the increase in cell numbers seen after 3 days. Of the 90 per cent of cells that are killed

about a quarter form giants which eventually break up (see *Figure 3*). The majority of the damaged cells however just 'fade away'.

The cytogeneticist's explanation of mitotic death is that the radiation has produced chromosome abnormalities which result in loss of genetic material which the daughter cells cannot survive. According to this view the delay between irradiation and cell death is due to the need for chromosome damage to express itself by mitosis. There can be little doubt that this is *one* cause of mitotic death, but there may well be others that do not involve a genetic mechanism. Both Oakberg and Minno (1960) and Bender (1960) have found that radiosensitivity and the incidence of chromosome abnormalities do not go hand in hand. The possibility must be envisaged that the time

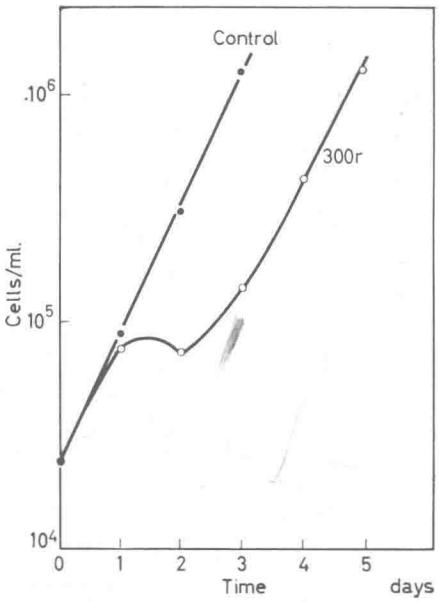

Figure 2. Effect of 300 rads of x-rays on growth of mouse leukaemia cells in tissue culture

interval between irradiation and cell death is needed for the metabolic development of the injury as is the case for all radiation effects. The fact that in rapidly dividing cells mitoses occur during this essential time interval need not imply that mitosis itself is a necessary step for the process of mitotic death; the mitosis may be coincidental. Evidence for this view is derived from experiments in which cells are prevented from division after irradiation by a treatment that does not suppress metabolism. If leukaemia cells are kept after irradiation at 22 °C for 18 hours and then returned to their normal temperature of 37 °C, cell death takes place without intervening mitosis (Alexander and Mikulski, unpublished). At the lower temperatures the cells still metabolize although no division occurs (95 per cent of the unirradiated cells survive 18 hours at 22 °C). The recent literature (see Bacq and Alexander, 1961) is in agreement that the biochemical lesion for mitotic death is not the inhibition of DNA synthesis and interference with one of the unknown processes that controls mitosis, has to be postulated.

The mechanism leading to chromosome abnormalities ('breaks') is equally obscure. The concept that the ionizing particle severs the chromosome thread on passing through it finds no support from *in vitro* studies of DNA (Alexander, Lett, Moroson and Stacey, 1960) nor does it explain the biological data (Revell, 1959). A time interval is always necessary between irradiation and the appearance of the chromosome 'break'. To see a break the cell has to be studied in metaphase or anaphase, yet the irradiation has to be carried out hours earlier while the cell is in the resting stage or in early

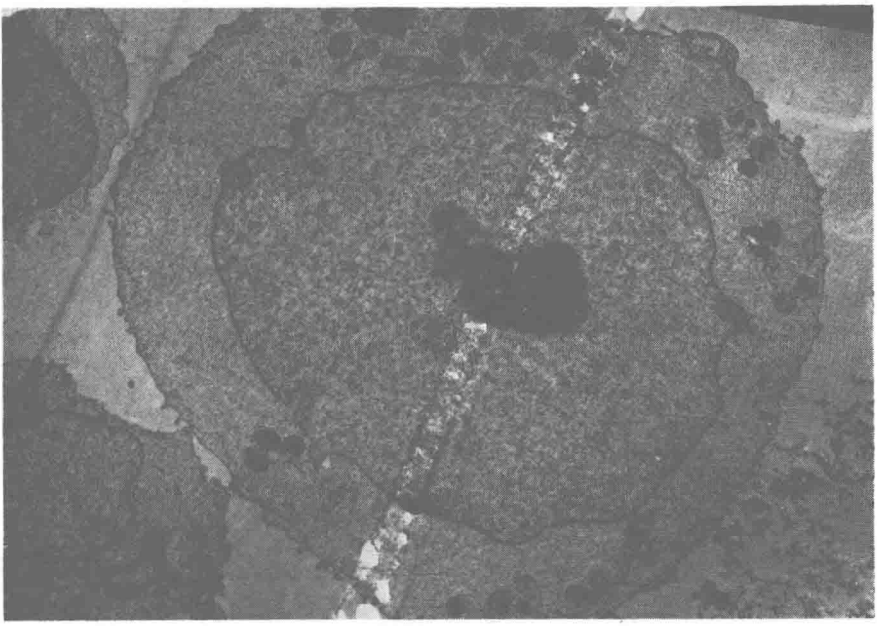

Figure 3. Electron photomicrograph of mouse leukaemia cell grown in tissue culture 42 hours after 300 rads of x-rays. About 10 per cent of the cells turn into giants and an example of these is seen in the centre. The majority of the cells only double in volume (*i.e.* diameter increases by less than 30 per cent) and two of these are seen in the bottom left.

(Kindly provided by E. M. C. Birbeck)

prophase. The explanation that the interphase chromosomes are more slender structures, that can be severed more easily by an ionizing particle than the visible chromosomes of mitosis, is invalid since cells irradiated during mitosis show chromosome 'breaks' at a high frequency in the next mitosis (Sparrow, 1951). Chromosome 'breakage' must therefore be considered as a typical biological end-effect caused by the metabolic development of an, as yet, unknown primary chemical lesion.

VARIATIONS IN RADIOSENSITIVITY AND INTRACELLULAR PROTECTION

The radiosensitivity of different mammalian cells, normal and malignant, is very similar so long as they are proliferating in tissue culture, their LD_{63} being between 100 and 300 rads. However, other cells vary very greatly in their radiation response and the LD_{63} of vegetative bacteria (assessed by

inability to form colonies) ranges from 1,900 rads to 30,000 rads. The radioresistance of some spores is still higher while amoebae and certain infusoria require hundreds of thousands of rads to be killed. The cause for the variations in radiosensitivity could be sought either at the level of the initial chemical lesions or in the processes of injury development by metabolism, but this latter factor will not be discussed here.

During his extensive study of the radiation response of plants, Sparrow (private communication) has found that radiosensitivity is inversely related to the size of the cell nucleus in diploid strains. In a rather limited series (Alexander and Dean, unpublished) the radiation resistance of bacteria has been found to be inversely proportional to their DNA content. Whether any significance can be attached to these relationships remains to be seen. One interpretation would be that the cell is killed when a given number of DNA molecules have been damaged. The more DNA there is per cell the smaller will be the dose needed to produce the given number of lesions (*i.e.* the number of changes within a certain cell substance by a given dose expressed in rads is to a first approximation proportional to the amount of this substance that is present in the cell). This explanation could, however, be at best only part of the truth since the DNA content of *Escherichia coli* and its radiation resistant mutant B/r is the same (Harold and Ziporin, 1958).

Another aspect of the problem is the presence of intracellular protective agents. Radiochemical experiments (see p. 129 of this volume) have shown that added substances and, in particular, sulphydryl compounds reduce the effect of radiation on proteins and nucleic acids. The magnitude of the primary lesion could depend on the extent of this protection and the concentration and localization within the cell of substances that protect may in part determine the relative radiosensitivity of the cell. We are testing this hypothesis by comparing the dose needed to damage the same 'marker' molecule within different cells. In these experiments large doses have to be used since a radiochemical reaction unmagnified by metabolism is being studied. Initial results (Alexander and Dean, unpublished) indicate that the dose of radiation needed to reduce the molecular weight of the DNA in the cell to one-half does not vary greatly between thymocytes, leukaemia cells, the bacteria, *Pseudomonas* (with a LD_{63} of 1,900 rads) and a micrococcus (with a LD_{63} of 30,000 rads). Yet the dose needed to inactivate the enzymes of the Krebs cycle is some four times greater in the micrococcus than in *Pseudomonas fluorescens*.

If there is chemical protection by intracellular substances the possibility of sensitization of cells to radiation becomes a distinct possibility. Treatment of cells with substances that combine with —SH groups would be expected to reduce protection by intracellular sulphydryl compounds. Boccacci and Quintiliani (1960) have found that intestinal damage is enhanced if mice are treated before irradiation with iodoacetate, a classical sulphydryl-reacting substance.

Leukaemia cells in tissue culture exposed to iodoacetate at a concentration of 10^{-5}M for 30 minutes prior to irradiation are somewhat more sensitive and 300 rads produce an effect for which 375 rads would otherwise be necessary (Alexander and Mikulski, unpublished). To make this experiment easily interpretable the treatment with iodoacetate alone must not interfere with the