Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation

Edited by
R. J. C. HARRIS
Division of Experimental Biology and Virology
Imperial Cancer Research Fund, London

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PREFACE

In this volume are published the papers and the discussion of the third joint UNESCO and IAEA sponsored Symposia on topics of radiobiology related to fundamental cell biology.

The first of these symposia was held in Venice in June 1959, and the second in Moscow in October 1960. Both concentrated on the nature of the initial processes at the sub-cellular level which are responsible for initiating the chain of events that leads to cell damage. In this, the third of these symposia, it was decided to enter a less extensively studied area of radiobiology and one to which cell biology has not so far made a very large contribution.

This symposium deals with the mechanisms which are responsible for the late somatic effects of ionizing radiation; emphasis was placed on the discussion of the nature of early biological changes (i.e. occurring within days of irradiation) which are responsible for the eventual appearance of cancer, leukaemia and non-neoplastic late effects, particularly those which bring about the observed shortening of life-span. At the present time very little is known about the aetiology of these disturbances. Somatic mutations, activation of latent viruses and anatomical and morphological changes (e.g. in blood vessels) due to cell killing at the time of irradiation, as well as modification of the structure of extra cellular connective tissue components may all contribute. In radiation induced cancer, one of the many unresolved problems is whether the cell (or cells) which starts the tumour must actually be irradiated or whether radiation damage in the surrounding tissue can initiate malignancy.

A knowledge of the cellular basis of late effects of radiation is of more than academic interest since only by understanding mechanisms will it be possible to predict with confidence the magnitude of the hazards to human beings from low doses of radiation. Late somatic effects are, of course together with genetic changes, the principal hazards of the peaceful uses of atomic radiations. In deciding the level of exposure that is acceptable, considerations of the late somatic effects are predominant. It has frequently been pointed out that prediction of the risks from very small doses of radiation and, in particular, the problem of whether there is a threshold cannot be decided from animal experimentation even if attempted on a vast scale. Only an understanding of the basic mechanisms will make it possible to decide the shape of the dose response curves at low doses. These considerations seemed to justify devoting a whole symposium to this narrow and relatively unexplored field. We hoped to gather a group of some thirty scientists actively engaged in this field and I believe that with the exception

of workers in the U.S.S.R. who found themselves unable to participate, the group was fairly representative both on the basis of discipline and nationality.

The symposium was divided into six three-hour sessions each of which dealt with specific topics. The final and seventh session was devoted to general discussion and was led by Professor Z. M. Bacq. For this general discussion the technique was adopted of posing a number of controversial statements and asking only those to speak who disagreed with the formulation of this statement. This method of running the general discussion was first adopted by Professor Bacq in Moscow where it proved eminently successful. In each of the first six sessions there was only one formal presentation, which was in the form of an introduction to the subject, and which took not more than 30 minutes. The remaining time was devoted to free discussion. The participants were asked beforehand to give an indication of specific scientific communications which they wished to make in the various sessions, and the titles of these contributions were known to the Chairman of each session. These contributions usually occupied between five and ten minutes and consisted of presentation of new data directly relevant to the subject under discussion. The Chairman's task was to plan each session around the list of contributions. In this book the specific contributions have been written up in the form of papers and are not printed in the form in which they were given. The general discussion which surrounded these contributions was tape recorded and after suitable editing is reproduced here.

Although no formal committee was set up to help in the planning of this conference, my task of organizing it was greatly assisted by the advice of colleagues and friends, of whom I particularly wish to mention Dr. Vladimir Zeleny, Professor P. C. Koller and Professor L. F. Lamerton. As already stated, the organization of the individual sessions was left to the Chairmen, all of whom took great trouble and discharged their duties splendidly. The success of the meeting was in a large measure due to the efforts of the Chairmen. In the general organization, I received a great deal of help from the staff of the IAEA and I am particularly indebted to Dr. Zeleny, who was indefatigable in straightening out many difficulties. The IAEA also carried out the arduous task of transcribing the tape recording. It is a particular pleasure to thank the many members of the staff of the Chester Beatty Research Institute and, in particular, Mr. N. P. Hadow, Mr. K. Moreman, and Miss M. Samuel, all of whom put in a great deal of work in the general running and administration of the meeting. We must also acknowledge with gratitude the help of the National Science Foundation of the U.S.A. who generously made available travel funds to some of the U.S. participants.

December 1962 P. ALEXANDER

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LEUKAEMOGENESIS: QUANTITATIVE ASPECTS AND CO-FACTORS

R. H. MOLE

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INTRODUCTION

Leukaemogenesis has been singled out from carcinogenesis and given twice the time in the programme for the reasons, I suppose, that even small doses of radiation, as received in diagnostic radiology, appear to be leukaemogenic in man, that enough radiation-induced human leukaemia has occurred to suggest quantitiative dose-response relationships, and that the vast amount of experimental work which has been done might have been expected by now to have turned up something definite and important about mechanisms.

THE PHENOMENOLOGICAL DEFINITION OF LEUKAEMIA

Leukaemia, like many other indispensable terms in current use in medicine, and indeed in biology generally, is merely a descriptive word for a group of phenomena whose nature is not properly understood but which can readily be recognized by a trained observer. The gross symptoms of leukaemia may be highly variable and the underlying common feature is a progressive and ultimately lethal proliferation of cells which are morphologically not too unlike one or other (or more than one) of the different kinds of cells which make up the normal haematopoietic, lymphopoietic and reticular tissues of the body. Individual cases of leukaemia are usually named according to the predominant cell-type and it is a basic assumption that the particular cell (or cells) from which the leukaemia started is (or are) related to the predominant cell in the same way as stem-cells are related to their differentiated progeny, although in leukaemia, both generally and in specific cases, it is always uncertain just how far back in the cell lineage one has to go to find the cell (or cells) in which the leukaemia originated. The varieties of leukaemia are given specific names but, as in taxonomy in general, the boundaries between named species and even the grouping of particular sets of species into genera or families is often somewhat arbitrary.† If this is forgotten it becomes all too easy to talk

† The demonstration of hybridization between somatic cells in tissue culture (Sorieul and Ephrussi, 1961) may make it possible to apply to the problems of classification of leukaemia a similar criterion to that used in the classification of species, the possibility or otherwise of interbreeding.

about leukaemia as if it were one "thing" and thus to asume that there must necessarily be a common basic mechanism for every case of leukaemia. In this and many other ways the problems of leukaemia are just the same as the problems of cancer and it is generally, though not quite universally, accepted that leukaemia is in fact cancer of the haematopoietic tissues.

It is indeed possible to imagine that all leukaemias originate in totipotent cells which normally are capable of giving rise to fully differentiated daughter-cells as different as small lymphocytes, granulocytic leucocytes, erythrocytes and macrophages. The predominant cell in different cases of leukaemia would then depend on the particular route of biological development of the daughter-cells of the "leukaemic" stem-cell and how far along that route their mock-differentiation has proceeded. There might then be a single basis for all leukaemias, the particular morphological features of the predominant cells of a particular case being an "accidental" consequence of some factor determining cell differentiation. On the other hand, most workers nowadays would believe that there are several really different kinds of leukaemia. The epidemiological evidence certainly suggests that the causes of acute leukaemia, of chronic myeloid leukaemia and of chronic lymphatic leukaemia in man are distinct (Court Brown and Doll, 1959) and it is important to note that radiation has been shown to increase the first two kinds of leukaemia but not the third.

BIOLOGICAL CHARACTERS OF DEVELOPED LEUKAEMIA

Grafting leukaemic cells into genetically acceptable donors is followed by their multiplication. The cell in developed leukaemia has permanent and inheritable properties and it must be genetically different from normal cells.

Much more interesting is the progressive transformation of these properties as the leukaemic cells continue to multiply. This occurs not only on transplantation but also in the primary host of origin where leukaemic cells are not by any means always identical (cf. Hauschka, 1961). In this respect leukaemic cells are qualitatively different from normal cells for not only do biochemical and metabolic properties change but the chromosomal constitution can also change. There is a characteristic plasticity of chromosomal number and morphology which just does not, and indeed could not, occur in normal cells if ordinary ideas of genetic determination are true.

Individual cases of leukaemia often have a quality of uniqueness. The experimenter with inbred strains of mice can easily forget this but a competent clinical haematologist in a hospital with, say, a dozen cases of leukaemia can usually tell by looking at the cells in a blood smear from which case it came. No cytologist, however competent, can do this with a set of blood smears from normal people.

Thus the cells of developed leukaemia have a genetic character different from the normal and the unsolved question is how this difference is acquired. The cause of a genetic change is not necessarily primarily genetic.

It is as well to note that the evidence suggests that leukaemic cells of chronic leukaemias divide more slowly (not faster) than the corresponding normal cells, and that the accumulation of abnormal cells in these varieties of leukaemia is due to their abnormally long survival time. As is true of all forms of neoplasia, the body's control of the rate of cell division of leukaemic cells is an imperfect control. It is a false definition of cancer to say that it is uncontrolled growth.

LEUKAEMIA AS A RARE EVENT

Leukaemogenesis, like carcinogenesis, must be a rare event in cellular terms. If leukaemia originates in one cell—and, since a developed leukaemia can sometimes be transmitted by grafting a single cell, this hypothesis cannot be dismissed out of hand—then only one cell in the 1·5 kg of active bone-marrow in a human adult need be changed in order that myeloid leukaemia should develop. If all 10^{11} myeloid cells capable of proliferation are susceptible of leukaemia induction then the probability of action of an inducing agent causing a 100% incidence of leukaemia is 10^{-11} per cell (Brues, 1959). If only one in 10,000 of these cells is susceptible of a leukaemic change, the probability would be 10^{-7}: how far back one goes in a cell lineage before finding "susceptible" cells is quantitatively very important. If leukaemia is thought to originate not in a single cell but in a field of cells, the required probability for a whole field of 1 mm^3 would still be about 10^{-6}.

There is also an element of rareness about the kind of leukaemogenic (or carcinogenic) events which follow irradiation. Single doses of 5 to 50 r may produce gross degrees of physiological damage (Table I) but doses of several hundred to several thousand r or more are needed to produce large (~50%) incidences of leukaemia or cancer. Radiation must be much more efficient in killing cells or in interfering with their ability to multiply (their so-called reproductive integrity) than in causing malignant transformation of individual cells or of small foci of cells.

This observational fact should make one very cautious about applying to leukaemogenesis and carcinogenesis the results and ideas derived from the radiobiological experiments on cell populations. In cell-survival experiments information on the effect of dose-rate and LET and oxygen tension is derived from the whole population of cells which retain the ability to divide. On the other hand, if irradiation of cells can induce leukaemic (or cancerous) changes directly, what is relevant to leukaemogenesis (or carcinogenesis) is the information coming from the small fraction of the whole surviving population in

which these particular changes have occurred. Until there is some experimental means for distinguishing this fraction of cells separately, there will be no way of knowing if conclusions based on reproductive integrity also apply to the postulated malignant transformation.

TABLE I. *Some somatic effects in mammals of small doses of X- or γ-radiation* (from Mole, 1962)

Organ	Species	Age at exposure	Dose (r) approx.	Structure or function examined	Magnitude of effect
Ovary	Mouse	10 days	10	Primitive oocytes	50% Depletion
		7–14 days	85 (0·5 r/hr)	Fertility when adult	Reduced to ~10%
		Adult	50	Reproductive capacity	More than halved
Testis	Mouse	Adult	20	Spermatogonia (late A—early B)	50% Depletion
Thymus	Rat	Weanling	20	Cortical lymphocytes	50% Depletion
Bone marrow	Rat	Adult	40	Mature normoblasts	50% Depletion
		Adult	50	24-hr uptake of ^{59}Fe in blood	Halved
Eye	Mouse	Adult	15–30	Lens opacities	Detectable
Stomach	Rat	Adult	40	Emptying time	Doubled

The vast amount of information on chromosomal changes after irradiation is also of uncertain relevance. To be observed, cells have to be dead yet it is only viable cells which are relevant; if the carcinogenic change is a rare event the only relevant chromosomal change will be a rarely occurring one and again there is no way of knowing which it is.

HUMAN RADIATION-INDUCED LEUKAEMIA

No species has ever been, or ever can be, examined so intensively and in such large numbers as the human. Not only does this provide a sound foundation of fact but the human evidence also covers a range of effect which is inaccessible to experiment and therefore uniquely significant to any academic study of leukaemogenesis. Acute leukaemia and chronic myeloid (or granulocytic) leukaemias can be caused by radiation. There is no evidence that chronic lymphoid leukaemia and other kinds of haematosarcoma can be so caused but this could be merely because these conditions have a longer latent period.

The quantitative aspects of human leukaemogenesis by radiation have been thoroughly considered by many authorities (Brues, 1958; Court Brown, 1958; Cronkite *et al.*, 1960; Heyssel and Brill, 1961; Murray and Hempelmann, 1961), especially the distinction between the linear dependence of leukaemia incidence on dose (Lewis, 1957) and the curvilinear which relates incidence to