ACTIONS OF RADIATIONS
ON
LIVING CELLS

BY

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PREFACE

My intention in writing this book has been to give an account of certain of the simplest and most fundamental actions of X-rays and other ionizing radiations on living cells. I have not attempted to survey the whole field of the biological effects of radiations. Instead, I have thought that a useful purpose would be served by giving a rather detailed discussion of the mechanism of those actions of radiation which are sufficiently well understood for such a treatment to be profitable at the present time.

After introductory chapters describing the relevant physical properties and chemical effects of ionizing radiations, the bulk of the book is occupied by the discussion of the effects of radiations on viruses, and on the genes and chromosomes of higher cells. In the concluding chapter the killing of cells by radiation is discussed in so far as it can be understood in the light of the preceding chapters.

One of the difficulties in writing the book has been to decide what background to assume the reader to possess. In an endeavour to make the book to some extent understandable by all classes of reader, I have written the physical chapter in an elementary fashion, and where it seemed practicable, have prefaced my accounts of the various biological actions of radiation by a brief description of the properties and method of handling of the organisms concerned. While in general these introductory sections are brief, I have thought it advisable to provide a rather detailed introduction to the chapter on the genetical effects of radiation. The rather technical vocabulary of the geneticist is not well understood by non-specialists, and in consequence many workers studying the biological effects of radiation do not adequately appreciate the very considerable contributions which have been made to this subject by geneticists.

The study of the action of radiations on viruses, genes, and chromosomes has reached a stage where the experiments are mainly quantitative, and the interpretations therefore necessarily to some extent involve elementary mathematics. When developing such mathematical interpretations I have kept the algebraic detail in the background as far as possible, and have provided graphs and tables which enable the experimentalist to

interpret his results along the lines suggested without any technical mathematical ability being required.

I have been at pains in the physical chapter to provide an adequate amount of numerical data concerning the amount and spatial distribution of ionization in tissue exposed to the various radiations, such numerical data being constantly required in quantitative interpretations of the various biological actions of radiation. The tables in Chapter I and the Appendix have been specially computed for this book. While the physical principles involved in the dissipation of energy by ionizing radiations in their passage through matter are understood, numerical data of the sort tabulated are not so accurately known as one would wish. The tables are likely, therefore, eventually to need revision in the light of more exact information. It is hoped that in the meantime they will be found of value by workers in this field of research.

I should not have been in a position to write this book had I not, during the past ten years, been enjoying the collaboration of a number of friends and colleagues in studies of various biological effects of radiation. I cannot adequately express my indebtedness to these collaborators, namely Dr D.G. Catcheside, the late Dr R.B. Haines, Dr M.H. Salaman, Dr K.M. Smith, and my colleagues at the Strangeways Laboratory. I am especially indebted to Dr L.H. Gray and Dr F.G. Spear, in frequent discussions with whom, my ideas on the subject have taken shape.

For providing me with material for the plates, or for permitting the reproduction of published photographs, I am indebted to my collaborators already mentioned, and to Dr J.G. Carlson, Prof. P.I. Dee, Dr I. Lasnitzki, Dr R. Markham, Dr A. Marshak, Dr C.F. Robinow, Dr J.E. Smadel, Prof. C.T.R. Wilson, the British Institute of Radiology, the Radiological Society of North America, the Rockefeller Institute for Medical Research, and the Royal Society. Plate II E is a photograph taken at the National Physical Laboratory, and is reproduced by courtesy of the Director, Crown copyright reserved. I should like to thank Mrs D.E. Lea and Mr V.C. Norfield for preparing the figures and the plates respectively. Finally, I acknowledge gratefully the support of the British Empire Cancer Campaign and the Prophit Trust.

D. E. L.

Strangeways Laboratory
Cambridge, July 1944

CONTENTS

ILLUSTRATIONS

Chapter I

PHYSICAL PROPERTIES AND DOSIMETRY OF DIFFERENT RADIATIONS

Ionization, excitation, and 'point-heat'

The radiations with which we are concerned are the α-, β- and γ-radiations of radioactive substances, X-rays, protons and neutrons. These may be grouped together as ionizing radiations. Occasionally we deal with ultra-violet light which is a non-ionizing radiation. Since the term ionization will be used very frequently, we begin by explaining its meaning. An atom consists of a positively charged nucleus and a surrounding constellation of negative electrons, the whole being electrically neutral. The principal means of energy dissipation by an ionizing radiation in its passage through matter is the ejection of electrons from atoms through which it passes. An atom so *ionized* is left positively charged, and is referred to as an *ion*. It is possible that some actions of radiation of biological significance are due to this separation of electrical charge, but in most cases it is more plausible to attribute it to chemical change resulting from the ionization. For, *when an atom is ionized the molecule of which it is a part almost certainly undergoes chemical change*. Knowing that the chemical bonds which hold a molecule together are constituted by electrons shared between the two atoms joined by the bond, it is to be expected that the removal of such a bonding electron from a molecule will lead to its dissociation or other chemical change. The removal of electrons other than bonding electrons may also be expected to lead to chemical change, since the energy involved in ionization, 10 electron-volts[1] or upwards depending on the atom ionized and the level in it from which the electron is ejected, exceeds the energy required to remove an atom from the molecule.

A second method by which radiations dissipate energy in tissue is by *excitation*. This means the raising of an electron in an atom

1 The electron-volt (eV.) $= 1{\cdot}602 \times 10^{-12}$ erg, is a unit of energy of suitable magnitude for dealing with energy changes in single atoms or molecules. 1 eMV. $= 10^6$ eV.; 1 ekV. $= 10^3$ eV. 1 eV. per molecule $= 23{\cdot}05$ kilogram-calories per gram-molecule. Thus the statement that the energy of dissociation of the C—H bond is 94 kilocalories per gram-molecule means that the energy required to dissociate a single bond is about 4 eV.

or molecule to a state of higher energy, and is a less drastic process than the complete ejection of an electron. Ultra-violet light, as well as ionizing radiations, is capable of causing excitation. In simple reactions (e.g. inorganic gas reactions) ultra-violet-induced excitations are not much less effective than X-ray-induced ionizations in causing chemical change. There is some evidence, however, that in the decomposition of large organic molecules, an excitation is a good deal less efficient than an ionization.[1]

In biological actions of radiation in which quantitative data are available both for ionizing radiations and for ultra-violet light, particularly the inactivation of viruses and the killing of bacteria, an excitation by ultra-violet light is much less effective than an ionization. Thus it appears probable that when we are dealing with an ionizing radiation, excitation may usually be neglected as a cause of biological effect by comparison with ionization.

The electron which is ejected from an atom in the process of ionization eventually becomes attached to another atom and makes it a negative ion. As far as the physical measurement of ionization is concerned, the positive and negative ions are equally significant, and one usually speaks of the production of an ion-pair. But since the energy involved in the attachment of an electron to an atom to form a negative ion is usually even less than the energy of excitation, it is probably safe to neglect negative-ion formation as a factor of biological importance. Thus when we speak of an ionization we refer to the production of a positive ion by the ejection of an electron. This ejected electron may have sufficient energy to ionize on its own account before it is brought to thermal energy and finally attached, and this secondary ionization is important and will be discussed later. But again, it is the positive ions and not the negative ions which are of biological importance.

Practically all the energy dissipated by radiation in tissue ultimately becomes degraded to heat energy. Thus a dose of 10^5 roentgens is sufficient to raise the temperature about 0·25° C. This small temperature rise for a large dose of radiation means that temperature change is quite inadequate to explain the biological effects of ionizing radiations in the way in which tempera-

1 Cp. Jordan, P. (1938*a*).

ture rise accounts for most of the biological effects of, for example, short-wave wireless waves. On the other hand, the highly localized nature of the energy dissipation means that the energy which eventually causes a rise of temperature of 0·25° C. in the tissue as a whole is initially confined to a small proportion of the atoms, and might be considered to produce a large rise in temperature of these atoms. This is the basis of the 'point-heat' theory.[1] The concept of an ionization as a hot spot is less satisfactory than the concept of an atom ionized leading to chemical change in its molecule. However, in the course of the degradation of ionization and excitation energy to thermal energy, the possibility must be borne in mind of molecules near to a high concentration of ionization suffering chemical change even although not themselves ionized.[2]

Ultra-violet light

In quantitative experiments it is necessary to use monochromatic ultra-violet light, since the biological effectiveness per unit of energy varies very much with wave-length and in particular is very low above 3000 A. The physical measurement will usually give the energy incident upon the irradiated preparation in ergs per square centimetre. If we are irradiating small objects such as bacteria or viruses, and can obtain a suspension in a non-absorbing medium free from absorbing impurities, a satisfactory procedure is to use a stirred suspension sufficiently deep or sufficiently concentrated to absorb completely the incident ultra-violet light. The total incident energy may then be divided either by the total number of suspended organisms or by the total volume of protoplasm in suspension to obtain the absorbed energy either in ergs per organism or in ergs per cubic micron.

In other cases we may arrange to use a thickness of irradiated material sufficiently small to absorb only a small proportion of the incident radiation. If we know the absorption coefficient of the irradiated material, or better, the absorption coefficient of that part of it absorption in which we believe to account for the biological effect, we may calculate the energy absorption in this part in ergs per cubic centimetre. If I is the incident intensity in ergs/cm.^{2}, ρ the density of the absorbing substance in g./cm.^{3}, and μ the absorption coefficient in cm.$^{-1}$ defined by the relation

1 Dessauer, F. (1923). 2 Jordan, P. (1938*c*).

$\mu = \frac{1}{x} \log_e \frac{I_0}{I} = \frac{2{\cdot}3}{x} \log_{10} \frac{I_0}{I}$, where I is the intensity transmitted by a layer x cm. thick, I_0 being the initial intensity, then μI is the energy absorption in ergs/cm.^{3} and $(\mu/\rho)\, I$ the energy absorption in ergs/g. If (as happens, for example, in the irradiation of *Drosophila* sperm in the male) it is not possible to avoid excessive absorption, either by too great thickness of the irradiated material or of intervening tissue, then quantitative experiments are not possible unless measurement can be made of the intensity of radiation reaching the specimen under the conditions of the experiment.

To give some idea of the magnitude of the absorption coefficients, and the variation between different materials, we list in Table 1 the absorption coefficients[1] of a number of substances for 2536A., this wave-length being chosen since it is in the biologically most effective region, and also is readily obtainable nearly monochromatic. In the references cited the absorption coefficients for other wave-lengths may be found.

Ultra-violet light for biological experiments is usually obtained from a mercury lamp. If the efficiency of different wave-lengths is being compared, then a quartz mercury arc in conjunction with a large-aperture quartz monochromator is the usual equipment.[2] An intensity of about $2{\cdot}5 \times 10^4$ ergs/cm.^{2}/sec. is possible with a lamp dissipating 1 kW. If a monochromator is not available, or if one is not interested in the relative efficiency of different wave-lengths, the best type of lamp to use is the low-pressure discharge lamp[3] containing neon and emitting 85–95 %

1 If the absorption of a substance has been measured in solution, then the result will often be expressed as the extinction coefficient α in the formula $\log_{10}(I_0/I) = \alpha c x$, c being the concentration of solute in mg./cm.^{3} Or it may be expressed as the molar extinction coefficient ϵ in the formula $\log_{10}(I_0/I) = \epsilon c x$, where c is now the concentration in gram-molecules per litre of a substance of molecular weight M. In order to reduce the absorption coefficients to a common basis suitable for calculating energy absorption from knowledge of the ultra-violet intensity, all have been reduced to μ or μ/ρ, the absorption coefficient of the pure solute, by assuming that the absorption coefficient of a solution is proportional to the concentration of the absorbing solute. Thus $\mu/\rho = 2300\alpha = 2300\epsilon/M$.

2 Cp. for example, Gates, F.L. (1929*a*); Benford, F. (1936); Uber, F.M. & Jacobsohn, S. (1938); Uber, F.M. (1940); Cannon, C.V. & Rice, O.K. (1942).

3 Melville, H.W. (1936); Steacie, E.W.R. & Phillips, N.W.F. (1938); Heidt, L.J. (1939); Peel, G.N. (1939).

of its radiant energy in the line 2536A. The monochromatism may be further improved, if desired, by the use of gaseous filters of chlorine and bromine,[1] or liquid filters,[2] or by using the radiation from the lamp to excite resonance radiation.[3] A convenient lamp[4] takes the form of a 30 cm. length of quartz tubing about 8 mm. diameter wound in a close spiral of about 2·5 cm. diameter.

TABLE 1. Absorption coefficients for wave-length 2536A.

Material	μ cm.$^{-1}$	μ/ρ g.$^{-1}$ cm.^{2}	ϵ	% transmission	Reference
Plant virus protein	—	10,000	—	—	1
Tyrosine	—	3,800	300	—	2
Tryptophan	—	36,000	3,200	—	2
Bacterial protoplasm	3600	—	—	—	3
Bacterial nucleoprotein	—	32,000	—	—	4
Bacterial ribose nucleic acid	—	87,000	—	—	4
Trypsin	—	1,900	30,000	—	5
Ribonuclease	—	900	6,000	—	6
Maize-pollen contents	1900	—	—	—	7
Maize-pollen wall	—	—	—	30	7
Vitelline membrane of hen's egg	—	—	—	8	8
Abdominal wall of *Drosophila*	—	—	—	67	9

1 Bawden, F.C. & Pirie, N.W. (1938).
2 Holiday, E.P. (1936).
3 Gates, F.L. (1930).
4 Lavin, G.I., Thompson, R.H.S. & Dubos, R.J. (1938).
5 Uber, F.M. & McLaren, A.D. (1941).
6 Uber, F.M. & Ells, V.R. (1941).
7 Uber, F.M. (1939).
8 Uber, F.M., Hayashi, T. & Ells, V.R. (1941).
9 Durand, E., Hollaender, A. & Houlahan, M.B. (1941).

At 10 W. this gives an intensity of about 10^4 ergs/cm.^{2}/sec. at 10 cm. distance, or about 5×10^5 ergs/cm.^{2}/sec. on a specimen inside the spiral.

It is important to understand the difference in spatial distribution between the energy dissipation by ultra-violet light and by an ionizing radiation such as X-rays. With ultra-violet light, the absorption coefficient depends on the molecular structure, and is, for example, different for nucleic acid and for protein. The dose in ergs/cm.^{3} absorbed energy may be very different, for

1 Peskoff, N. (1919); Oldenburg, O. (1924); Heidt, L.J. (1939); Svedberg, T. & Pedersen, K.O. (1940); Mitchell, J.S. (1942).

2 A convenient filter is a quartz cell 1 cm. thick containing an aqueous solution of nickel sulphate (20%) and cobalt sulphate (6%). Houston, R.A. (1911); Bäckström, H.L.J. (1940); Bowen, E.J. (1942); Lavin, G.I. (1943).

3 Thomas, L.B. (1941).

4 Procurable from the Thermal Syndicate Ltd., London.

example, in different parts of an irradiated chromosome depending on the degree to which they are loaded with nucleic acid, and at different stages of the division cycle. No such differences exist with X-rays, apart from an increased energy absorption in and near to bone or other components containing atoms of elevated atomic number, since the absorption of X-rays by atoms is not affected by their chemical combinations. A further difference is that with ultra-violet light the excited atoms are distributed spatially at random in a homogeneous tissue irradiated by a uniform intensity, with no tendency for excited atoms to occur in groups, produced simultaneously, or concentrated in a linear path. This is because each excited atom is produced by the complete absorption of a single quantum of ultra-violet light and the quanta are emitted independently. With ionizing radiations the ionizations are localized along the paths of ionizing particles, and thus a number of ionizations may be concentrated in a *cluster* or a *column* of ionization.

The energy of a single quantum of ultra-violet light is connected with the wave-length (λ) in Angstroms by the relation: Energy in electron volts $= 12{,}400/\lambda$. Thus the wave-length 2536A. has quantum energy 4·89 eV.

X-rays

X-radiation like ultra-violet light is an electromagnetic radiation emitted in quanta, but the difference in wave-length (0·05–10A. for X-rays against 2000–3000A. for ultra-violet light) results in there being little similarity in practice. The absorption coefficient of X-rays depends not on the chemical combination of the absorbing atoms but only on their atomic number. On account of the greater penetrating power of X-rays it is not usually convenient to measure the total energy *incident* on a surface, but to measure the energy *absorbed* in a given volume. In practice the energies involved are too small for a thermal method of measuring energies to be used, and use is made of the fact that when the absorption takes place in air, the latter becomes conducting and the saturation current through a given volume of air is a measure of the rate of energy absorption in that volume of air. The *roentgen*—defined as 'the quantity of X- or γ-radiation such that the associated corpuscular emission per 0·001293 g. of air produces, in air, ions carrying 1 electrostatic unit of quantity of

electricity of either sign'—is the unit of dose employed.[1] It corresponds to the liberation of $2{\cdot}082 \times 10^9$ ion-pairs per cm.^{3} of air at 0° and 760 mm. pressure, involving an energy dissipation of 0·1083 erg/cm.^{3} of air (taking 32·5 eV. per ion-pair as the mean energy dissipation in air). Since the roentgen is already a unit of energy absorbed, there is no question of multiplying dose in roentgens by absorption coefficient. What is loosely spoken of as 'intensity' in roentgens per minute is strictly dose-rate, being a rate of energy absorption and not a rate of energy incidence.

For the convenience of the physical measurement the roentgen is defined in terms of energy absorption per unit volume of air. In the interpretation of experiments one is interested in the energy absorption per unit volume of tissue, which for the same incident intensity of radiation will be about 1000 times greater on account of the greater density of tissue. The actual factor varies with different wave-lengths, since the ratio of the absorption coefficients of tissue and air varies somewhat with wave-length. It also depends on the elementary analysis of the tissue though not on the chemical nature of the compounds in which the elements are combined. The way in which one calculates the amount of energy absorbed per gram of tissue per roentgen of radiation is explained in the Appendix, and in Table 2 the results of a calculation of this sort are given for water, for dry virus protein, and for an undried soft tissue. The percentage composition by weight assumed for the virus protein is H 7, C 49, N 16, O 25, P 1, S 0·5 and ash 1·5, the ash being treated as having an average atomic number of 16 for the purpose of the calculation. The wet tissue is taken as having a percentage composition by weight H 10, C 12, N 4, O 73, Na 0·1, Mg 0·04, P 0·2, S 0·2, Cl 0·1, K 0·35 and Ca 0·01. With most experimental materials it will be found satisfactory to use the figures given in Table 2, either for water or for virus protein or for wet tissue, according to which approximates best to the composition of the tissue actually being irradiated. Thus when irradiating a dried virus preparation the figures for virus protein will be used. When irradiating micro-organisms in aqueous suspension the figures for water are appropriate, while the figures for wet tissue may be used when one is dealing with undried tissue in bulk.

[1] 0·001293 g. of air occupies 1 cm.^{3} at 0° and 760 mm. pressure.