REVIEWS OF MODERN PHYSICS VOLUME 31, NUMBER 2 APRIL, 1959

32

General Features of Radiobiological Actions*

RAYMOND E. ZIRKLE

Committee on Biophysics, The University of Chicago, Chicago 37, Illinois

THIS paper and the three that follow (Pollard, p. 273; Wood, p. 282; Tobias, p. 289) are concerned with radiation biology. The potential scope of this field—effects of all types of radiations on all types of biological systems—and its relation to other areas of radiation research are indicated in Fig. 1, where an energy spectrum of radiations is plotted as abscissae and various inanimate and biological systems are "plotted" as ordinates in ascending order of presumed complexity. At the level of macromolecules, radiation biology shades imperceptibly into radiation chemistry and physics, the basic sciences on which it draws for necessary facts and concepts. At the level of groups of multicellular organisms, it approaches "radiation sociology," which is needed to deal with the problems of group behavior in irradiated populations.

Radiations below about 1 ev produce biological effects, if any, through heating. These effects usually are regarded as cognate to, but not included in, radiation biology. The region from 1 to 6 ev, comprising the near infrared, the visible, and the familiar ultraviolet wavelengths, is readily available for investigations as is the region from about 1000 ev upward. These two accessible portions of the spectrum conveniently are termed low-energy and high-energy, respectively. The intervening "transition" region is absorbed in air and biological material so readily that it can be used only on very small objects *in vacuo*. It is of great theoretical interest, in view of the great differences in basic response of biological systems to the low- and high-energy spectra.

Figure 1 shows that radiation biology potentially ramifies throughout biology as a whole. So does biophysics. What is the relationship, if any, between the two? In my personal view, each contains some of the other. The biophysical content of radiation biology is probably about as great, both potentially and actually, as that of general biology.

It is clearly impracticable, in four short papers, to cover all of radiation biology. Accordingly, the coverage is narrowed as follows. First, attention is concentrated on the high-energy radiations, with occasional comparative references to the ultraviolet. Second, specific samples are selected from the spectrum of biological systems (Fig. 1); after these brief general remarks, Pollard describes experiments on certain macromolecules and viruses (p. 273), Wood speaks on certain cellular effects that have been investigated intensively (p. 282), and Tobias discusses selected studies on cell populations and multicellular organisms (p. 289).

By and large, the effects of high-energy radiations, as well as those of the ultraviolet, are injurious to all or part of the irradiated system. Nevertheless, considerable effort goes into the study of these effects, for various reasons. Some persons find a fascinating field of research in the general problem of how very small amounts of radiation energy produce such drastic effects. I believe that the real attraction here is the peculiar combination of various portions of physics and chemistry and of many aspects of biology that must be brought to bear on any serious investigation of radiobiological mechanism. This peculiar versatility demands so much of a human life span that a radiation biologist is necessarily a specialist, much as he may strive to not become too differentiated.

However, most of the interest in radiation effects stems from their applications. In many branches of basic biology, they have long been used as powerful research tools. For example, the development of genetics has been accelerated immensely by the use of radiations as mutagenic agents; partial body irradiation has found fruitful application in embryology; partial cell irradiation is used to get information about the properties and functions of various cell parts. As basic biological tools, the various radiations have two properties that, in many situations, are of critical advantage: they do not disrupt membranes and other structures grossly, and the dosage usually is reproducible.

Radiation biology also has some important practical applications. The oldest, and still one of the most important, is radiation therapy. Another is its use in dealing with the widespread and multifarious problem of radiation hazards that, within the last decade and a half, have increased so explosively that they even figure in national and international politics.

Regardless of one's motivation to study or use radiation effects, there is obvious need to know as much as possible about their mechanisms. There is nothing basically unique about current methods of investigating these mechanisms; they are essentially those of the physical sciences and of the analytical biological sciences, with some special variations that stem from the unique physical properties of the high-energy radiations.

Like any story, a radiation action has a beginning, an end, and a middle. The beginning is the act of irradiation, and the end is the effect observed; there is considerable information about these, and the prospects of

---

* The following data are used in this paper: The usual energy unit is the electron volt (ev); 1 ev$=1.6\times10^{-12}$ erg$=3.8\times10^{-20}$ cal. For any radiation, energy times wavelength is 1.235×10^{4} ev A. "Dose" is radiation energy transferred to unit mass of irradiated object; 1 rad$=100$ ergs/gram$=66$ ev/μ^3, assuming density of object to be 1.05; 1 roentgen (r) *usually* is equivalent to 0.93 rad.

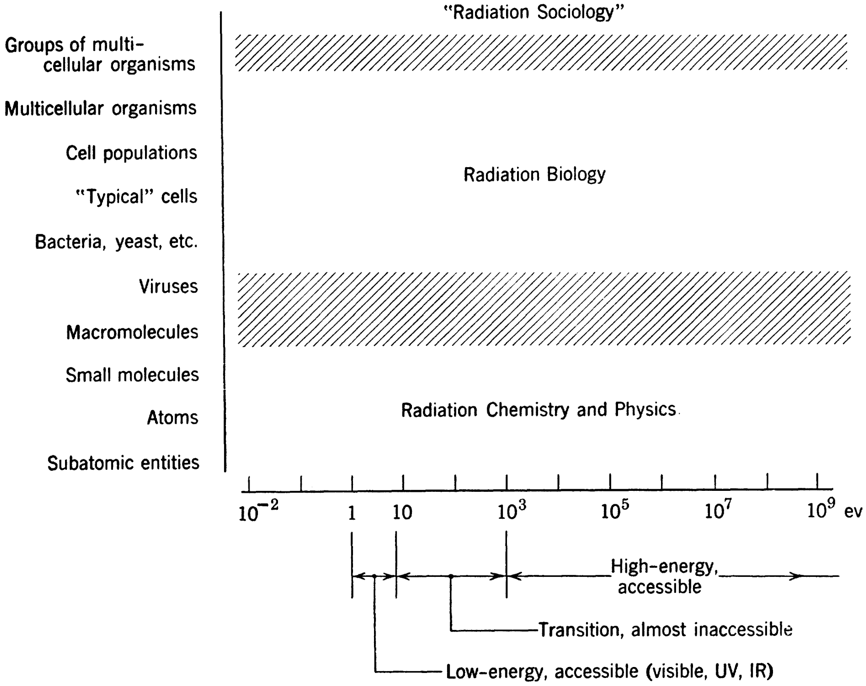

FIG. 1. Scope of radiation biology and its relations to other areas of radiation research. Abscissae, partial radiation energy spectrum in electron volts; ordinates, "spectrum" of inanimate and biological objects in order of presumed complexity.

getting more are good. The middle, frequently miscalled the "latent period," is essentially a domain of ignorance wherein most of the problems lie. The remainder of this article is devoted to an attempt to indicate some of the ways in which current research is directed to reduction of this ignorance.

Clearly, the end effect must be defined as accurately as possible. This implies information about the state of the investigated system *before* it is irradiated. The incompleteness of such information is demonstrated well by the need for a large conference such as the Study Program in Biophysical Science and by the remedial attempts so vividly described by earlier speakers. High-energy radiation in sufficient amount is capable of producing significant changes in practically any biological structure or function. In view of the great diversity and complexity of these structures and functions, it is not surprising that the list of known end effects is long. Some vigorously investigated examples are aberrations in chromosome structure, inhibition of cell division, inactivation of enzymes and other macromolecules, gene mutations, and induction of tumors in animals.

It is also essential to know as much as possible about the process of irradiation and the immediately consequent events. Here, one encounters facts and concepts that are not basic to other areas of biological research. The high-energy radiations differ in two important features from all other physical and chemical agents, including the low-energy radiations. First, a large fraction of the molecules that receive energy from the radiation are activated to very high states, even to ionization. As a result, in most biological material, radiation energy is transferred to the individual molecules of the many species usually present in a nonselective fashion that is chiefly dependent only on the molecular mass. Chromophores do not enter into consideration, as they do in work with low-energy radiations. This circumstance presents both advantages and disadvantages to the investigator.

Second, the energy transfers to individual molecules do not occur singly in space and time but in more or less linear groups. All of the high-energy radiations are either charged particles in motion (e.g., electrons or α-particles) or are agents (neutrons; x-rays and γ-rays) that set such particles in motion. These charged particles typically have kinetic energies of thousands or millions of electron volts, whereas the average energy that can be accepted by an individual molecule is some tens of electron volts. Thus, successive energy transfers occur along the path of the high-energy particle. Some of these transfers are capable merely of exciting the molecules to higher energy states. Others involve enough energy to produce ionization, e.g., to eject electrons, some of which have kinetic energies sufficient to eject electrons from other molecules (secondary ionization). The ions produced in gases are well demonstrated by means of the Wilson cloud chamber. The trail of ions produced by a single primary charged particle is termed an ionization track. Similar tracks are recorded on photographic plates (linear sequences of blackened grains) and in suitable liquids at low temperatures (trails of bubbles). This, and other physical evidence, makes it highly probable that the distribution of energy transfer in a biological object is essentially the same as that displayed in the cloud chamber, except that the dimensions should be reduced by a factor of about eight hundred because of the difference in density.

These two properties—the high average value of the individual energy transfers and the grouping of these transfers into tracks—make the high-energy radiations unique in their mechanisms of action, even though these mechanisms, in some cases, produce end effects that are superficially indistinguishable from those of other agents.

To students of mechanism, probably the most significant single feature of high-energy radiation actions, especially on cells and on even more-highly organized systems, is the small amount of energy required. Of the many end effects that are known, most are produced to a significant degree by 10 000 rad (or less). This dose equals about 0.02 cal/g of biological material.

For some actions, the dose-effect relations ("kinetics") are suggestive. When samples of certain small cells (bacteria, haploid yeast, etc.) are given graded doses, the resulting survival curves are exponential; for more-complicated cells and organisms, the curves are sigmoid. The exponential curves suggest that the action on each cell is owing to some single primary event (formation of an ion pair, passage of a single ionizing particle, etc.). Sigmoid curves are correspondingly ascribed to multi-event types of action. In some cases, as Wood points out, the curve is exponential or sigmoid, depending on known properties of the cells. This is useful in devising models for the action.

Macromolecules and viruses typically exhibit exponential curves, although their shapes may be modified by various factors. By making simple assumptions concerning the nature of the hypothetical single event, the size of the "target" relevant to the radiation action can be calculated[1,2]; Pollard describes this procedure in detail (p. 273).

In dilute inorganic aqueous solutions, the alteration of the solute (e.g., oxidation of ferrous ion) typically follows zero-order kinetics, which indicates that the energy is absorbed principally by the solvent and that chemical intermediates, formed from solvent molecules, react with the solute. If two or more solutes are present, they compete for the intermediates and thus "protect" each other. Actions involving intermediates are termed "indirect."[3] "Direct" actions are those in which the radiation energy must be transferred to the solute molecules themselves.[4]† Current radiation chemistry identifies some of the intermediates[4,5]; they are OH and H radicals, H_2O_2 molecule, and, in the presence of molecular oxygen, HO_2 radical.

All of these concepts, derived from work on simple solutions, ramify throughout radiation biology. Indirect action has been demonstrated in many radiation effects on macromolecules in solution.[3] If a preponderant concentration of protective substances is present, a residual effect is observed that is ascribed to direct action. Indirect action has been invoked in analysis of various cellular effects[6]; the concept has been extremely fruitful in suggesting experiments, although in no case has its correctness been established for systems as complex as cells.

Many physical, chemical, and biological factors are known to modify the amount of absorbed radiation energy necessary to produce a given degree of effect. A few that have been found to operate in a wide variety of systems are mentioned here.

One is the physical parameter called linear energy transfer (LET), which is the amount of energy transferred per unit length of track of an ionizing particle to the molecules it traverses. This parameter varies with the square of the charge on the particle and increases as the velocity decreases. With the various kinds of radiation currently available, one can obtain LET values ranging from 0.025 to 25 ev/A, corresponding roughly to average spacings between primary ionizations that range from 4000 down to 4 A. Thus, it is possible to give the same dose to a biological system in various ways: a few ionizing particles with high LET, many particles with low LET, and intermediate values. Results are detailed elsewhere.[7] Not only does LET significantly influence the dose-effect relations in practically all radiobiological actions that have been investigated thoroughly in this respect, but it also operates in "simple" chemical actions, such as the formation of H_2O_2 in pure water. Thus, LET not only gives some geometrical notions about mechanism, but also gives some encouragement to use radiation chemistry as a basis for interpretation of radiobiological actions.

Another factor quantitatively influencing a wide variety of actions is molecular oxygen.[8] In all but a few of these actions, radiosensitivity increases with concentration of O_2 until a plateau is reached, usually at a value two or three times that observed when the O_2 concentration is zero. The basis of the "oxygen effect" is still controversial (cf. Wood, p. 282), but I think it significant that, like LET, it is encountered in radiation effects on simple aqueous solutions. There is also an interrelationship between O_2 and LET: the greater the LET produced by the radiation, the less is the influence of O_2.

In the foregoing, I have tried to communicate a concept of the present scientific state of basic high-energy radiation biology. Many effects on many diverse biological systems are known and cataloged; however, there is encouraging evidence that the mechanisms leading to these diverse effects have some strong resemblances. No one mechanism has been elucidated yet. On the other hand, several have been investigated intensively by means of the general approaches indicated, and, in a few cases, observed facts have been used as bases for theories which have been successful; i.e., they

---

† If no solvent is present, the action must be direct. Target theory, in its strict sense, presupposes direct action.

have suggested experiments which in turn have yielded new, significant facts. Good examples of such investigations are given in the three papers which follow.

BIBLIOGRAPHY

[1] D. E. Lea, *Actions of Radiations on Living Cells* (Cambridge University Press, London, 1955), revised edition.

[2] E. C. Pollard, W. R. Guild, F. Hutchinson, and R. B. Setlow, Progr. in Biophys. and Biophys. Chem. **5**, 72 (1955).

[3] W. M. Dale in *Radiation Biology*, A. Hollaender, editor (McGraw-Hill Book Company, Inc., New York, 1954), Vol. I, p. 255.

[4] A. O. Allen, J. Phys. & Colloid Chem. **52**, 479 (1948).

[5] J. L. Magee, Ann. Rev. Nuclear Sci. **3**, 171 (1953).

[6] R. E. Zirkle and C. A. Tobias, Arch. Biochem. Biophys. **47**, 282 (1953).

[7] R. E. Zirkle in *Radiation Biology*, A. Hollaender, editor (McGraw-Hill Book Company, Inc., New York, 1954), Vol. I, p. 315.

[8] Z. M. Bacq and P. Alexander, *Fundamentals of Radiobiology* (Academic Press, Inc., New York, 1955).