# SURVEY OF BIOLOGICAL PROGRESS

G. S. Jr. Avery

## VOLUME II

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GEORGE S. AVERY, JR.

*Editor-in-Chief*



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# PREFACE

It was originally our intention that the SURVEY OF BIOLOGICAL PROGRESS would appear each year. The numerous "annual review" volumes successfully follow such a pattern.

In corresponding with authors it soon became evident that such a publication schedule could not be the primary goal if they were to attempt synthesis and integration in the broad field of biology. To write for the student in another field is an exacting task; terminology, almost the mechanics of thought for the specialist, must be reduced to the minimum for the non-specialist or for the specialist in another of the many disciplines of science. Such writing is arduous and time consuming, and we have tried to free the authors from undue pressure in meeting a deadline. Hence Volume II appears about two and one-half years after Volume I.

The extent to which authors have been successful in writing for others than those in their own fields must be left to the judgment of the reader. It is their hope and ours that the objective has in fair measure been realized.

The original goal of the SURVEY remains unchanged. The long-term trend toward specialization continues, and in direct proportion the need becomes more acute for all of us to gain and retain an understanding of what occurs in fields marginal to and sometimes even unrelated to our own.

Ours is a serious attempt to help in the seemingly hopeless task of integrating the field of biology.

GEORGE S. AVERY, JR.
*For the Advisory and Editorial Boards*

*New York, New York*
*August 1952*

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# Effects of Radiations on Biological Systems

BY A. H. SPARROW AND B. A. RUBIN

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## I. Introduction

For the first twenty years of this century a great many types of biological effects, mainly qualitative, were seen to result from crudely measured doses of radiation. The asserted range of variations in magnitude of effect was the result of a lack of understanding of the characteristics of the radiations themselves, as well as the lack of satisfactory dosage measurements. Nevertheless, a great many interesting, useful, and provocative facts were discovered. One of the most important was the differential action of radiation on certain types of malignant cells, substantiated as early as 1904. There was also some insight into the mechanism by which the radiations produced their effects.

After 1920, radiobiology grew to a science in which physical dose and biological response could be more accurately measured. An international system of units for x-rays was agreed upon and dose measuring instruments became more reliable. A landmark was reached in 1937-38 with the 5th International Congress of Radiology and the publication of Duggar's two volume "Biological Effects of Radiation." A great deal of experience was available, and agreement on principles had been reached in certain major areas.

The quantum concept of biological effect, adopted from the developing quantum concepts in chemical kinetics, led to the search for an "event," to which could be ascribed the biological effect of radiation. Thus, the importance of "ionization" was emphasized, together with the characterization of "ionizing radiations."

The radiations were detected by the ions produced in measuring devices, and also were thought to be effective by virtue of their ionizations produced within the living systems through which they passed. The interesting kinetics of the effect of radiation on living systems were interpreted in terms of a "target theory," i.e., it was conceived that the actual occurrence of only a single "event" within some definite living volume, was required for a biological change (Giese, 1947; Lacassagne, 1950).

## II. Types of Radiation

Although many very early experiments in radiobiology were quite sophisticated, the recent interest in "Atomic Energy" established an important new point of departure. For the biologist, there was a new level of interest and support and also a great increase in the variety and availability of sources of radiation (Smyth, 1947).

The range of wavelengths of electromagnetic radiations now available for experimental radiobiology has been greatly expanded. Recent work has claimed biological importance not only for infrared rays, but also for the high frequency radio waves. The ultraviolet region has been intensively investigated, with the development of better (and cheaper) sources of both monochromatic and continuous spectra The measurement of these radiations (in ergs per gram or photon flux per centimeter squared) has greatly improved with the development of a better understanding of photocells, bolometers, thermopiles, etc. Both total energy and photon flux can be measured with readily available equipment. Monochromatic radiation can be obtained from special lamps (i.e., mercury) having strong characteristic emissions, or from the selection of particular wave lengths from continuous emitters, such as tungsten or hydrogen lamps. A high degree of spectral purity can be obtained by the use of prism and grating monochromaters. Efficient interference filters are available which transmit as much as 45% of incident radiation in a band whose width at half maximum intensity is less than 200 A. (Ellis and Wells, 1941; Loofbourow, 1948).

There is now available x-ray equipment of very high and stable output. Gas filled x-ray tubes, though they had the advantage of self-rectification. have been generally replaced by vacuum tubes, which afford better control, easier maintenance and longer life. The cooling of targets and the improvement of target materials permit longer continuous operation. The beryllium window Machlett tube shows the fine results of imaginative design: short target-to-window distance, effective cooling, and newer materials produce tremendous output at low cost (Sproull, 1946; Ham and Trout, 1950; Trout and Gager, 1949).

For higher voltages the "Sloan" equipment is readily available. By making use of an oscillator circuit, somewhat similar to a short-wave broadcasting set, the Sloan tube can deliver energies in the order of millions of volts. Still higher voltages are available from machines; betatrons, synchrotrons and Van de Graaff generators can deliver from 10 to 100 million volt x-rays (Trump and Van de Graaff, 1948). Fantastically high radiation doses can be supplied by some of the high energy machines. Doses of $10^8$ r per microsecond have been produced by "pulsed electronic discharges," notably the "capacitron," and as much as $10^7$ r per second at a fairly steady rate can be produced by cathode ray tubes (Brasch and Huber, 1947).

High energies and appropriate filters make it possible to obtain roughly monochromatic bands from x-ray tubes. The general rule in this technique is to use as a filter a substance whose atomic number is just under that of the target. For studies not requiring great intensity or a large field, monochromatization may be obtained by means of crystals or gratings. Present equipment is much more effective than formerly, because of the improved output of the tubes. Generally, however, studies of biological effect as a function of x-ray wavelength leave much to be desired. Good monochromatization can be obtained only by sacrificing intensity. Moreover, in their passage through biological material the radiations are degraded, so that quality varies with depth of penetration. Radioactive isotopes can sometimes provide monochromatic x-rays (gamma rays) of high purity, but there are important limitations to this type of source in terms of difficulty of handling, relatively few suitable emitters and, in many cases, short half-life (Uber, 1950).

Many new sources and types of charged particles are now available. Nuclear reactors create dozens of beta and gamma emitters of a wide range of energies and chemical characteristics. In some cases, nuclides can be selectively incorporated into specific biological sites where they may apply large doses of radiation to restricted areas. Similar effects may be produced with the betatron, an instrument whose high energy electron beam can be somewhat concentrated in small areas and at specified depths (Paul and Schubert, 1950; Trump, Van de Graaff and Cloud, 1940).

The variety of "heavy charged particles" has greatly increased since the discovery of alpha rays. Not only are protons and deuterons (as well as alphas) available from the many cyclotrons, Van de Graaffs, synchrotrons, etc., but much heavier particles are produced in the fission process. Such particles have great biological significance on account of their tremendous ionization densities.

The nuclear reactor, certain isotope combinations and some large machines provide beams of uncharged particles—neutrons of a wide range of energies. These have been shown to produce their effects in a manner somewhat different from that of charged particles. Fast neutrons physically can bump atoms out of their positions in molecules, while slower ones can cause them to be ejected by a recoil mechanism (Szilard-Chalmers process), (Conger and Giles, 1950; Curtis, 1951).

Finally, there has been a great increase in the understanding of the biological effect of cosmic radiation and natural radioactivity. That these can and do produce biological effect is illustrated by Haldane's (1948) recent calculations which would indicate that natural radiation constitutes an appreciable source of human mutation; and also in studies revealing the carcinogenic effects of these rays. Machines which will produce radiations of energies comparable to cosmic rays (and capable of producing mesons) will soon be available and may provide an exceedingly important tool for the study of radiation effect, related perhaps to natural evolutionary processes (Morris and Nickerson, 1948; Hess and Eugster, 1949).

## III. Radiation Dosagl Units

Since the energy absorption by a biological system in the usual radiation experiment is sufficient to raise its temperature only about .01°C., dosage measurements have had to be based upon other effects, usually ionization. In practice, the radiation dose "r" (roentgen) is determined from ionization produced in some measured gas volume; it is very difficult to measure ionization in a liquid or a solid. That this method is inadequate for energy measurement is widely recognized. The observed reading depends on the wall material of the meter and on the quality and uniformity of the radiation.

Radiation chemists prefer to use the "G" scale which is the number of changed molecules per 100 electron volts absorbed. This scale is superseding ionic yield (M/N) measurements—the number of reacted molecules per ion pair produced by the radiations. More recently radiation measurements have been made by means of graded chemical changes (Day and Stein, 1949), but these are only valid for a limited range of radiation qualities, since the efficiency (effect-energy absorbed) of a radiation is dependent not only upon the energy but upon its distribution within the absorbing material.

The measurement of neutron intensity is a problem of even greater complexity. Neutrons cause ionization only indirectly. The extension of the definition of the roentgen (as has been done for charged particles effects) is quite unsuitable because the energy conversion of neutrons varies very greatly with the absorbing material, even for light elements. Air is no longer even a remote approximation to tissue. Energy absorption in water has also been used, but with less reliability than for charged particle measurements. The unit employed in this country is called the "n", which is defined as a neutron flux that provides the same number of ionizations as a roentgen ($1.61 \times 10^{12}$) in a specific (Victoreen 100 r) air-filled ionization chamber. Its biological effectiveness is of the order of ten times as great as an r obtained from beta or gamma radiation. The actual amount of energy produced by one "n" in water is about 2-2.5 times that of the "r". The English system uses the $\nu$, equivalent to about .4-.5 n, to provide the same ionization dose *in water* as the r. When the energy lost in tissue is the same as that lost in air by one r, the dose is spoken of as one roentgen-equivalent-physical (r.e.p.). This unit is applied to charged and uncharged particle radiations as well as to x-rays and gamma-rays (Uber, 1950; Neary, 1946; Evans, 1947; Rainwater and Wu, 1947).

Another dosage problem is related to the use of radioactive isotopes as sources of radiation, where direct measurements are frequently impossible. Here the dose must be determined from a knowledge of the radioactive characteristics of the isotope, and from its concentration and distribution within the biological system. Upon these questions are superimposed the problems of decay, biological elimination, and redistribution. Formidable mathematical considerations have not completely resolved these difficulties, even when one neglects the uncertainties of isotope measurement and of geometrical distribution (Richards and Rubin, 1950).

## IV. Radiation Energy Transfer Mechanisms

Not only is there a vast range of possible responses of biological materials to radiations, but the radiations themselves differ both qualitatively and quantitatively. From the point of view of the biologist, it is of more than academic interest to understand the differences among the radiations, for these differences should be kept in mind during the design and interpretation of radiobiological experiments.

Considering the range of energies which occur within the category of electromagnetic radiation, a variety of energy transfer mechanisms can be expected. Radiations whose energies are below 1 electron volt (e.g., infrared and radiowave) rarely cause changes at a chemical level, because their energy is below that of most chemical (electron) energy states. And although many biological effects have been claimed for these radiations, it has been difficult to prove that these changes do not result from purely thermal responses.

By consulting Fig. 1, it may be observed that in the region of visible light, energies are reached which affect chemical structure. As the energy increases (into the ultraviolet) more changes become possible and the effects can become more drastic. It is important to keep in mind the factor of absorption since energy not absorbed obviously is ineffective. The strong dependence of absorption on wavelength may be circumvented at times by adding a photosensitizing substance to the absorbing medium. The existence of strong absorbing groups in a molecule makes it more sensitive, but the point of absorption is not necessarily the point of chemical change (Uber, 1950). The photochemical reaction which takes place may be regarded as occurring in three rather indistinct stages. The radiation is (1) absorbed to form an "excited" molecule, which then (2) either decomposes, or re-emits the energy by fluorescence, by collision or by chemical reaction. The products of this second step may be reactive atoms or radicals, which may (3) cause secondary reactions, e.g., molecular rearrangements, chain reactions, polymerization (or depolymerization), etc., (McLaren, 1949).

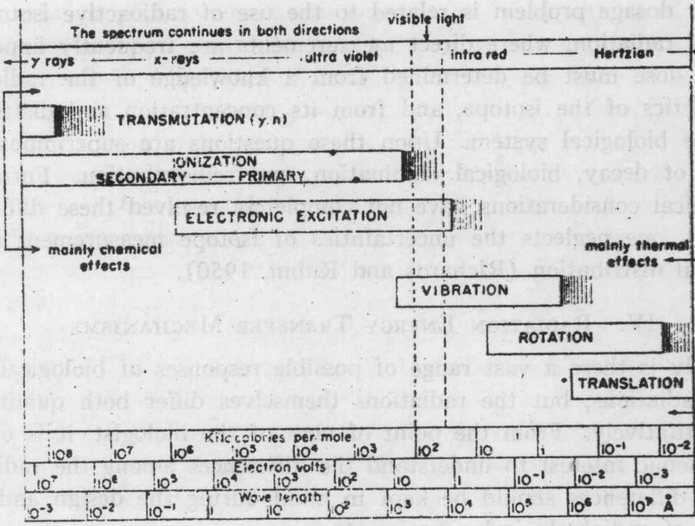


FIG. 1

The primary processes of the reaction of electromagnetic radiation with matter.

The energy imparted to a molecule by ultraviolet light is sometimes sufficient to ionize it by completely ejecting an orbital electron. The significance of the ionization process is best discussed below, but it should be noted here that although this is not the usual energy transfer mechanism, short wavelength ultraviolet can affect the ionization of some absorbing materials. The exclusion of ultraviolet from the family of ionizing radiation is quite arbitrary since electromagnetic radiations form a continuum with no natural divisions as to energy transfer or penetration properties (Fig. 1).

The energy of an x-ray (or gamma) quantum is ultimately distributed in a variety of ways. A fraction goes into "excitation," giving rise to processes that are essentially photochemical. That these form an important source of certain radiochemical events is illustrated by experiments in which ions can be removed as rapidly as formed, with but minor change in the reaction rates (Smith and Essex, 1938). Ionization, however, has generally been considered responsible for most of the radiobiological effect. The mechanisms by which x-rays and gamma-rays produce ionization are multiple and complex, and must be understood in evaluating their effects. As has been described above, even electromagnetic radiations of energies much below the x-ray level can produce ionization by imparting enough energy to a molecule to eject completely an orbital electron (the photoelectric effect). With higher energy x-rays this process also takes place, but actually accounts for a very small fraction of the total energy. Most of the ionizations result from secondary processes. In ejecting an electron from an atom or molecule, an x-ray quantum usually imparts so much energy to that particle that it goes on to produce a number of secondary ionizations. At relatively low energies (10 kev) almost all x-ray quanta are completely absorbed by molecules which then eject electrons with all the initial energy of the quantum minus the binding energy of the ejected electron.

As the energy level increases, another energy transfer process appears. The impinging x-ray quanta may now collide directly with orbital electrons to lose only a fraction of their energy (the Compton effect). Among x-rays of from 0.1 to 1.0 Mev, the mixture of photoelectric and Compton electrons is such that there is essentially no difference in average energy. Above 3 Mev, two new types of interaction become important—the absorption of the gamma ray by a nucleus with the emission of a neutron, and the reaction in the neighborhood of a nucleus in which the gamma energy is transformed into a "pair" (electron and positron). Although these latter processes have been largely ignored by biologists, they must be seriously considered when high energy sources of radiation become more prevalent.

The effect of x-rays or gamma-rays is due largely to energetic electrons that are produced in one of the ways described above. Many of the characteristics of these radiations can best be understood by the study of electrons in motion. The energy per unit path length given up by a charged particle in its passage through matter depends upon the speed of the particle and the square of its charge. As a charged particle passes close to an orbital electron of the absorbing material, some of the energy is transferred. If this energy is less than the ionization potential of that atom or molecule, the orbital electron is "excited" to a state of higher energy. When the energy is equal to or greater than that required for ionization, the electron is completely ejected from its atom or molecule, and may go on, if it has enough residual energy, to excite or ionize some other molecule (delta days). Because of their very small mass, beta particles (electrons) suffer a somewhat different fate from that of heavier particles. For a given energy, they travel at much greater velocity and have much longer paths than other charged particles. Alpha particles, protons and deuterons dissipate energy in *straight* lines of high ionization density, while beta particles scatter their energy more thinly over a tortuous path. The energy loss of x-rays is even less regular than that of beta particles; very little is produced along the actual path of the x-ray quantum, but rather in short paths at right angles to the photon path. The particulate radiations slow up as they give up energy, producing greater ionization densities. This characteristic pattern of energy loss is only slightly dependent upon the absorbing material. In x-rays, however, the process of absorption is a complex matter, increasing exponentially with the atomic number of the absorbing material (Crowther, 1949; Pollard and Davidson, 1951).

Neutrons are uncharged particles which cannot interact to release energy directly in any manner described above. In their passage through matter they give rise to secondary ionizing particles by means of a direct collision or by absorption into nuclei. In this respect, fast neutrons are somewhat analogous to high energy x-rays. While the energy of x-rays is converted by means of electrons, the energy of the fast neutrons is mainly lost through the agency of recoil hydrogen nuclei (protons). Slow neutrons are captured by nuclei of various absorbing materials which then release some gamma radiation.

When the source of radiation is within a biological system in the form of radioactive isotopes, the concentration of the radioactivity may produce extremely great effects not only by means of the selective concentration of the ionization, but also by a mechanism similar to that mentioned for neutrons. In the process of emitting an ionizing particle, the nucleus may change to another chemical species, or may have a great deal of recoil energy. In either (or both) cases, a direct chemical change results, the effect of which is added to that of the ionizations and excitations caused by the emitted particles (Libby, 1947; Rubin, 1948).

## V. The Distribution of Energy
## (The Importance of "Specific Ionization")

Radiations may be administered in a variety of ways. Using the same source, the dose may be given slowly or rapidly, continuously or intermittently. Using different sources (or a machine capable of producing radiations of various energies) one may vary the character of the radiation (different energies, charges, masses) or compare the effect of different radiations given simultaneously with that of each radiation type used alone. All of these procedures have been tried with many variations, on a wide range of test systems. The variety of results produced by such investigations is not necessarily contradictory. Theoretical explanations of such results have appeared which may reconcile some of the differences.

In the case of "ionizing radiation" the energy lost is distributed along the path of the charged particle in the various manners described above. The atoms or molecules which receive this energy have no way of distinguishing its origin. The variations in effect which are observed must then be attributable to the different spacings of the energy exchanges. As has been pointed out, several types of energy transfer are found, but this problem is usually visualized by considering only ionization, assuming that the other processes are proportionate in some simple linear fashion. The density of ionizations along a track is usually discussed in terms of "specific ionization" or "ion density," i.e., the number of ions per unit length of track.

Certain effects of radiation (killing of viruses, gene mutations) are thought to be caused by a single ionization while some other events (breaking whole chromosomes, killing metazoa) require much more energy—many ionizations per event. In the first group, the most effective radiation would be the one which spaces its energy losses most widely and randomly. In the second category, large groups of ions produced together would be more effective when the total energy loss is small. In this latter case, dose rate, and the continuous or discontinuous character of radiations can be important since healing will occur (Witte, 1950).

In some experiments, ionization density appears to be of no importance. Such results may be attributed to several causes. Many early workers did not measure dosage too well, and could not distinguish between the factors of quality and quantity. Other investigations were limited to the x-ray wavelengths from .07 to .4 A.—where there is little difference in specific ionization. A third group of experiments involving skin measurements on humans was frequently inadequate because of the uncertainty in the determination of the actual energy absorbed. There are cases in which there is really no sensitivity to ionization density—or the different densities used happened to have the same effectiveness. This last group is composed mostly of tests on rather complex metabolic processes, development of embryos, inhibition of respiration, hatching of eggs (Zirkle, 1943).

There are a number of clear cases of decreased effectiveness with increased specific ionization. These experiments are most striking when the test systems are chemicals in solution. In these cases where a single ionization suffices, a dense track can only waste its energy in a unit (molecule or virus) already changed.

Increase in effectiveness with increased specific ionization is usually seen in studies of higher forms—seed plants, mammals, and insects. Such a complex system might be expected to consist of many similar units (cells), many of which must be destroyed before a whole effect can be seen.

In systems where a very low number of ionizations is effective, higher ionization densities are less efficient. If some definite larger number is required, ionizations in excess of that number are wasted. Indeed, if an infinite range of ionization densities were available, every system would have a peak effectiveness at one ionization density. Two such cases among multihit effects are known—the inhibition of division in yeast and the inhibition of mitosis in the broad bean (Zirkle, 1943). Recently, extremely high ionization density has been achieved with fission fragments and the effectiveness in producing complex (multihit) effects still increases, failing to show a theoretically expected peak effect (Tobias *et al.*, 1948).

## VI. The Target Theory

The above effects have been considered from the point of view of what may be considered the classical "target theory." This theory focuses attention on the discontinuous nature of radiation adsorption and assumes that an effect is seen when a biological structure is directly ionized by a quantum "hit" of radiative energy. A multihit effect is one requiring some definite number of ionizations to occur in a sensitive volume, within some definite time interval. Within an irradiated system, each reactive unit has an equal and random chance of reacting, which in the case of "single-hit" systems provides the frequently observed monomolecular kinetics

$$S = N_0(1 - e^{-aD})$$

where $S$ is the number of transformed units, $N_0$ is the original number of reactive units, $D$ the radiation dose, and $a$ is a constant reaction probability.

If we turn back now to the effect of specific ionization, there are several experimental observations which are difficult to explain on the basis of this theory. First is the occasionally seen phenomenon of an infinite difference in the effectiveness of two radiations. There seem to be effects which occur with one type of radiation which do not occur at all (as far as can be measured) with another type. One case is the production of $H_2O_2$ in water free of $O_2$. Here dense ionizations from alpha rays produce a marked effect while x-rays produce none. Another case is that of abnormal development in Drosophila larvae induced by neutrons but not at all by x-rays (Zirkle, 1943). While in the second experiment it might be argued that an extremely large number of ions is required and the recovery may be rapid between x-ray ionization hits, no such case can be made out for the simple water system. There is also the case of an ionization density which is *less* effective than either a higher or lower density, seen in the production of abnormal embryos from Ascaris eggs. This can be explained by assuming the existence of two separate processes, one of which is responsive to the effect of single or low ionizations and one affected by high ionization. The third kind of experiment which provides a difficulty for the target theory is one in which the simultaneous administration of two kinds of radiation proves to be non-additive in effect (Zirkle, 1950). In some of these instances the absorption of the radiations may be different enough to cause the measured effect (usually death) by different mechanisms. On the whole there is a strong suggestion that a simple picture of the target theory is inadequate.