Some Elementary Physical and Chemical Processes in Radiobiology[1]

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Some significant facts of radiobiology may be summarized in the following simplified terms. Irradiation of biological material under suitable circumstances causes mutations. These mutations are changes detectable in terms of the tests we apply. For lower organisms the test most applied is lethality or inability of the entity to procreate. A negative test means that at least one function of the entity responsible for reaction to the test applied is adversely affected. In turn, adverse response reflects damage in the particular part or parts of the total chemical structure of the biological entity responsible for the effect observed. A mutation observable by a particular test signifies damage affecting a particular prosthetic group. Unless the damage affects that group, the test indicates no damage to the entity. The latter conclusion is, of course, incorrect. A negative test means only no perceptible damage detectable by the test applied.

The effect of the radiation is not necessarily directly on the biological entity adversely affected thereby. On the macroscopic scale the radiation may produce persistent products poisonous to the organism. On a microscopic scale the radiation may produce transient phenomena or chemical entities in the ambient fluid which are potentially highly damaging to the biological entity.

In this paper we confine ourselves to elucidation of the simpler phenomena of radiobiology in terms of elementary physical and chemical processes. Consequently, our considerations are applicable only to very simple biological entities or particles, e.g., minimum fragments of virus responsive to test, bacteriophage, etc. Furthermore, we limit ourselves to effects labelled "microscopic" in the preceding paragraph, where the radiation acts either directly in the biological particle or, indirectly, chemically or physically in an interval of time not much greater than a microsecond. We are not concerned, for example, with radiation-induced changes in the nutritional environment of a biological particle.

Even on such a rudimentary scale of radiobiology, however, there are apparently secondary effects. If oxygen is removed from a biological fluid, the material becomes less sensitive to x or gamma radiation; the mean lethal dose may be many times increased. A similar effect is obtained by incorporation in the fluid of certain reagents such as cysteine, certain sugars, and hydrogen cyanide. (11). The existence

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of such "protective agents" and the mechanism of their protection is a matter of primary concern.

Mathematical analysis of the effects of irradiation on a biological suspension indicates that the individual effect of the irradiation particle is principally via ionization rather than excitation, that a single hit within a sensitive volume or "target" is in many cases sufficient to produce a detectable (e.g., lethal) effect, that the target is in general smaller than the biological entity (as measured in electron microscopic examination of the dry entity), that this negative deviation of target size increases with increased size of entity, but that nevertheless low-velocity bombarding particles give a larger computed target than fast particles. It is the purpose of this paper to show that our present knowledge of elementary processes of radiation physics and radiation chemistry is consistent with these facts of radiobiology and that parallel to the purely mathematical model of the "target theory" a consistent chemical model can be constructed.

Physical Effects

The elementary physical effects of radiation are summarized in Table I. The major effect of x or gamma radiation is produced by the fast electrons ejected. A single 1 Mev electron ejected in a Compton recoil may be responsible for more than 30,000 subsequent ionization and 30,000 excitation processes. Thus, the primary effect of the radiation is chemically insignificant. A similar statement applies to the primary effect of the neutrons. Most of the resultant chemical effects in water, for example, are explicable in terms of action by the proton expelled from a parent molecule by the neutron.

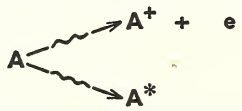
TABLE I. Physical Effects of Radiation

Radiation	Process	Primary Effect	Distance between Primary Effects
X or gamma	Principally Compton effect	Electron expulsion	Only 1 effect per photon
Neutrons	Nuclear collision	Atom expulsion plus ionization plus excitation	Dependent on nuclear cross-section for neutron of velocity v
Charged particles	Interaction with electronic atmosphere	Ionization and excitation	For slow particles: $\backsim$ 5-10 molecules. For fast particles: $\backsim$ 100-500 molecules

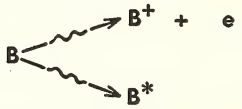
A charged particle interacts with the electron cloud around an atom or molecule and produces quantized displacement corresponding to some state of excitation or ionization. The degree of interaction depends on the time available for the process and the distance of closest approach. The greater the time available, the greater the distance at which perceptible interaction occurs. For slow particles of high energy (such as protons, deuterons, or alphas) the cross-section for interaction is much greater than for fast particles of equal energy (i.e., electrons). Consequently, the distance between successive ionizations (or excitations) may be about 5-10 molecules for the former and about 100-500 molecules for the latter. In the radiation chemistry of water these primary physical effects are reflected in essential differences in chemical effects.

Many physical effects are secondary but, nevertheless, more important contributors to the total effect than the primary process. Thus, electrons ejected in photon absorption or by Compton recoil are responsible for most of the effect of x or gamma rays. Electrons (delta rays) ejected in charged particle interaction with atoms or molecules are directly responsible for a major part of heavy particle effects. Protons ejected by neutron bombardment in water are responsible either directly or indirectly (i.e., via energetic electrons) for most of the effects observed.

Two other important classes of phenomena must be mentioned in the physical category. They are ionization transfer and excitation transfer. We write schematically as possible alternative first steps[2]



where either positive ions or excited molecules are produced. Consider a mixture containing also the species B. Then, also



If the ionization potential of A is greater than the ionization potential of B (i.e., $I_A > I_B$), then the reaction

$$A^+ + B \rightarrow A + B^+ + \text{energy}$$

occurs with high probability particularly in condensed systems. On the other hand, the possibility of energy transfer such as

$$B^* + A \rightarrow A^* + B$$

or the reverse is determined by considerations which may be more restrictive.

Chemical Effects

We are concerned principally with effects of significance in biological systems. Consequently, we shall treat successively water, organic substances and aquo-organic systems.

Water.—The radiation chemistry of water has been summarized principally by Allen and his co-workers (1, 2). We follow the ideas employed by them. The principal reactions are

$$H_2O \rightsquigarrow H_2O^+ + e \qquad 1.$$
$$H_2O^+ + aq \rightarrow H_3O^+ + OH \qquad 2.$$
$$H_2O + e \rightarrow H + OH^- \qquad 3.$$
$$OH + OH \rightarrow H_2O_2 \qquad 4.$$
$$H + H \rightarrow H_2 \qquad 5.$$
$$OH + H \rightarrow H_2O \qquad 6.$$

The excitation processes in water are not important in biological systems. As we have seen, the distance between successive ionizations in water is small for slow-moving particles, large for fast-moving particles. In consequence, under deuteron irradiation for example, free OH radicals are formed relatively close together and reaction 4 is probable. On the other hand, under electron or x-irradiation production of H_2O_2, or of oxygen formed by its decomposition, is perceptible only with great difficulty.

Free OH radicals disappear not only by formation of hydrogen peroxide and in the back reaction 6 but also by a chain process involving the products of reactions 4 and 5.

$$OH + H_2 \rightarrow H_2O + H \qquad 7.$$
$$H + H_2O_2 \rightarrow H_2O + OH \qquad 8.$$

Any process which interrupts the repeated sequence 7, 8 promotes the yield of hydrogen peroxide. Thus, although hydrogen peroxide cannot be formed in conveniently measurable concentration in radiolysis of pure water by electrons or x rays, introduction of a small amount of oxygen promotes such production because of the reaction

$$H + O_2 \rightarrow HO_2 \qquad 9.$$

This reaction is not only a first stage on the path to formation of H_2O_2 (e.g., by H atom capture) but also interrupts chains such as 7, 8 responsible for back reactions in which H_2O_2, H and OH revert to water without appearance of detectable products.

For our present purpose our interest in reaction 9 is that thereby a very active entity, free H, is removed from solution and replaced by a less active (reducing or oxidizing) entity HO_2. Simultaneously, reactions available for disappearance of the active entity OH are more restricted and that active oxidizing entity consequently persists longer.

Long persistence of a radical such as HO_2, or OH in its presence, means that it has a greater diffusion range. We shall see that in radiobiology many of the effects produced by radiation occur via free radicals. Thus, any process such as reaction 9, which increases diffusion range of radicals produced in the elementary chemical processes, effectively increases the size of the sensitive volume or "target" involving the biological entity. The H_2O_2 formed in reaction 4 is itself more persistent than the parent OH radicals and may consequently be responsible for greater target size in heavy-particle irradiation.

Organic substances.—Let us consider first the reaction

$$A \rightarrow A^* \qquad 1.$$

The molecule A^* may subsequently decompose but the locus of decomposition, as shown by photochemical studies, e.g. of aldehydes and ketones, is not necessarily the locus of primary excitation (12). It is not necessarily true that reaction 1 lead to a decomposition

$$A^* \rightarrow R + X \qquad 2.$$

In particular, where product radicals are large, a Franck-Rabinowitch cage effect (7) may reduce yield and even prevent it in condensed systems. Furthermore, there is evidence (such as in the alkyl benzenes) that excitation in a labile group may be transferred into a resistant portion of the molecule and dissipated without chemical effect (5, 8, 13).

An excited molecule is produced not only directly, as in reaction 1, but indirectly, as in the sequence,

$$A \rightsquigarrow A^+ + e \qquad 3.$$

$$A^+ + e \rightarrow A^{*\prime} \qquad 4.$$

Although the states of excitation of A^* and $A^{*\prime}$ differ, cage effects and protection effects, by resistant groups to which energy may be transferred, can still occur.

Where we deal with a mixture of organic substances we must not neglect the possibility of ionization transfer. Thus, the reaction

$$A^+ + B \rightarrow A + B^+ + \text{energy} \qquad 5.$$

already mentioned can transfer ionization from a labile to a non-labile part of a biological entity or vice versa. In other words, the site of the chemical effect need not be the site of the absorption act. Conversely, the existence of an inert group may serve to protect a labile group at a distance. Evidence for such phenomena has been adduced for interaction between groups in alkyl benzenes (8, 13).

Thus, we find that in organic compounds and mixtures of organic compounds such as may exist in biological entities, energy absorbed at a particular locus may be effective at a more remote point or may be made ineffective by transfer to, and dissipation in, a less reactive group (e.g., a benzene ring). It follows, since a good portion of a complex mixture may be relatively inert to high-energy radiation that the sensitive volume may be much less than the total volume. Furthermore, if the reaction involves a rupture process 2, perceptible product formation may occur only when cage effects are unimportant; i.e., near the surface of the aggregate involved. We must also take into consideration the fact that although a chemical change may occur a prosethetic group may not be involved. From these elementary considerations, it follows not only that the radiation-sensitive volume (measured by a particular test) of a molecular aggregate may be less than the true volume but that the ratio of radiation-sensitive volume to true volume may decrease with size of aggregate. The latter effect will in turn be exaggerated in any case where there is a natural tendency for the relative amount of inert material to increase with size of the aggregate.

Aquo-organic systems.—The ionization potential of liquid water is most probably <7.4 ev (4) while organic compounds have somewhat higher ionization potentials in aqueous dispersion. It has been shown (3) that under such conditions, if we designate the organic material by A, an important sequence of reactions involving primary ionization of A in aqueous suspension is

$$A \rightsquigarrow A^+ + e \qquad 1.$$

$$A^+ + H_2O\ aq \rightarrow A + H_3O^+ + OH \qquad 2.$$

Thus, the result of a primary ionization in A may be the formation in immedately adjacent water of an H_3O^+ ion and the active hydroxyl radical. Furthermore, after thermalization of the electron, the neutralization process

$$A^+ + e \rightarrow A^* \qquad 3.$$

competes with the process (3, 10)

$$H_2O + e \rightarrow H + OH^- \qquad 4.$$

which also occurs immediately adjacent to the organic aggregate. Thus, even when the primary radiation process occurs in an organic aggregate, the chemical process may involve interaction of the aggregate with one of the highly active entities H, OH, H_3O^+, or OH^- characteristic of the radiation chemistry of water.

Radiobiology

We have seen from the properties of water and aqueous systems that radiation absorbed in a region sufficiently close to a biological entity may cause damaging effect because of interaction with that entity of free hydrogen atoms or hydroxyl radicals resultant in an early stage of the radiolysis of water. Since heavy particles produce high local concentrations of hydroxyl radicals in water (with resultant increased probability of hydrogen peroxide production) the diffusion range of the active entities may be somewhat greater than in electron, x or gamma irradiation of water. Thus, the sensitive volume or target size is somewhat greater for heavy-particle bombardment.

When oxygen is present in water, it traps atomic hydrogen by the reaction

$$H + O_2 \rightarrow HO_2$$

The hydroperoxyl radical thus produced is less active, and therefore, more persistent, than H or HO_2 and consequently has a much greater diffusion range. Thus, oxygen tends to increase the sensitive volume (in the neighborhood of a biological entity) or the target size and the lethality of the radiation. However, this effect is significant only in light-particle irradiation, not in heavy-particle phenomena. The explanation is given by Dainton (6) and by Magee (9) who concluded that in order for a solute to be effective in reactions with intermediates produced in the ionization column (of high-energy radiation) it must

be present in concentration of the same order as that attained by the intermediates in the column. Such a concentration of oxygen is easily achieved in fast-particle irradiation, where radical products are about 100-500 molecules apart. It is practically unattainable when the radicals are produced only 5-10 molecules apart.

The method of testing the effect of radiation on biological material ensures that a significant fraction of the effects may be undetected. Only those effects in a prosthetic group, responsive to the method of testing, are found. Furthermore, not all parts of the entity are equally sensitive to radiation, some parts may be inert, and cage effects may prevent observable chemical change in other parts. The total effect is to make the mathematically calculated target size considerably less than that of the entity. This situation is true in spite of the fact that the sensitive volume includes a portion of the aqueous medium adjacent to the entity. Thus, the calculated target is not to be identified with the biological entity and, in spite of the fact that the sensitive volume includes water, is smaller than the entity.

Summary

Simple observations of radiobiology, particularly relating to target theory, the effect of oxygen, and inhibition of oxygen effects, are explicable in terms of elementary physical and chemical processes of radiation chemistry. The target is not identified with the biological entity. The latter and the ambient fluid are included in the target but the biological entity is not uniformly sensitive. The sensitive volume of fluid is determined by the range of effective radicals produced therein. The free radicals H and OH are extremely active but, consequently, have a small diffusion range. Presence of oxygen causes entrapment of free H to give the less active, and therefore longer range, free radical HO_2. Oxygen increases target size for, and therefore lethality of, x or gamma radiation but no oxygen effect is possible with heavy-particle bombardment within the ordinary range of oxygen pressures. Lethality of oxygen-containing solutions may be decreased by introduction of protective agents, oxidizable by HO_2 but not by oxygen.

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