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Protective Mechanisms in Ionizing Radiation Injury

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KNOWLEDGE OF THE FACTORS governing radiosensitivity is a fundamental aspect of the actions of radiations on living systems. Considerable emphasis has been placed, therefore, upon methods of prevention and reversal of radiation effects. This sort of endeavor has become even more popular in recent years with the realization that many of the radiation-induced reactions are indirect, a consequence presumably of energy transformations in water.

Interest in the modification of radiosensitivity is by no means new, however. Apart from academic curiosity and the obvious ramifications to biology in general there is the practical matter of preventing radiation sickness, first described by Walsh (353) a few months after the discovery of x rays, and of protecting normal tissue while enhancing the sensitivity of diseased tissue during therapeutic irradiation. The former has been minimized somewhat by advances in exposure techniques and by greater attention to clinical detail. The latter remains as a limiting factor to radiation therapy. As early as 1909, Schwarz (293) reported the protective role of ischemia in cutaneous radiosensitivity, and many of the effects of temperature (183), hydration (266), oxygen, and related factors (78, 183, 194, 240) were described some years before the concept of indirect action by activated water was promulgated.

We are concerned here with the many protective agents and situations that have been reported for the high energy or ionizing radiations. Only those papers that have particular relevance to basic mechanisms in radiation biology and from which reasonable data and conclusions could be obtained have been included. No doubt, important papers have escaped the writer's attention for one reason or another. Before discussing the various modifying factors and their possible modes of action, it is appropriate to consider briefly the nature of radiation effects and the general problem of radiosensitivity. Some attention will also be given to the radiochemical events in water that are relevant to interpretation of the protective mechanisms. For a more detailed survey of the biological effects of ionizing radiations, the following publications may be consulted (40, 54, 82, 151, 214, 245, 248, 268, 313, 375).

REMARKS CONCERNING THE NATURE OF RADIOBIOLOGICAL EFFECTS

Whatever the ultimate mechanism may be, it is clear that the biological actions of the various ionizing radiations are related in some manner to direct local release of free energy. It has been inferred, largely from studies with gases since the high conductivity of water precludes conventional measurement, that the initial transfer of energy in tissue is accomplished by ionization and excitation of the constituent molecules, probably with a relative frequency of two to three excited molecules for every ion pair (117, 239). Radiation dose may thus be defined as energy absorbed in the form of ionization and excitation per unit of tissue or in absolute terms as ergs per gram. The energy transfer is chemically nonspecific and may be contrasted with the highly specific molecular excitation by ultraviolet radiation. Biological effective-

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ness of the different ionizing radiations has been shown to depend upon the rate of energy loss to the medium, a quantity that is determined primarily by the velocity of the ionizing particle and the magnitude of its effective charge, and that is roughly proportional to the linear density of ions along the particle track (150, 152, 340, 370, 373). A significant aspect of energy absorption is the infinitesimal total energy required to produce a change in the biological machinery; in contrast, effects due to elevated temperature require several thousand times as much absorbed energy. Even with lethal dosages, the molecules that are transformed by the randomly distributed primary effects of irradiation must represent an exceedingly small fraction of those present within a cell. It may be recalled further that water is by far the most abundant substance in biological material, and therefore that it must serve as the major repository of the initial energy transfer. How then is the physical act of irradiation amplified and translated into one or another of the many radiobiological effects that have been described?

Two main concepts of radiation action have been formulated. These are at best only generalizations but they have provided a useful and frequently fruitful working hypothesis. Direct action postulates that the critical energy transfer, i.e. ionization or excitation, occurs in an especially sensitive volume of a cell, e.g. a gene or an enzyme molecule. This idea in its classical sense is compatible with the single-hit type of effect that is characterized by its exponential relationship to radiation dosage, its independence of dosage rate and the inverse relationship between its efficiency and ion density (214). It is perhaps best expressed in the effects on dried biological materials. In the presence of water, however, it seems clear that biologically important ionizations and excitations may also occur at some distance from the critical molecules or sensitive regions of the cell. In this instance water is believed to be the most important site of energy transfer, the absorption of radiation by water molecules giving rise to chemically active entities, e.g. H, OH, HO_2, H_2O_2 (62, 359). These radicals are formed in high concentration along the tracks of individual ionizing particles. According to this concept of indirect action, localization of an effect within the biological system would depend upon the nature of the acceptors as well as upon the spatial distribution of the ionizations and excitations, the number of reactive substances formed, and the kinetics of their diffusion. The indirect type of action is dose rate dependent and, in general, its efficiency varies directly with ionization density.

Reactive intermediates other than those derived from water may also be of some consequence. Ammonia formed from irradiated proteins, amino acids, nucleic acids (88, 360) and H_2S derived from cysteine and glutathione (91) could have profound effects on cellular processes, as could histamine (102) and other toxic breakdown products (347). The local changes in pH that probably occur in the vicinity of an ionization track (62) may also play a role in the development of injury. On theoretical grounds, the probability of modifying the sequence of events would seem to be considerably greater on the basis of indirect than of direct action. While it appears that many of the biological effects of ionizing radiations are mediated by the decomposition products of water and possibly by other reactive intermediates, it should be remembered that direct effects of radiation on solutes also play a role, but one which at present is not as well defined. The relative importance of direct and indirect effects is often difficult to equate; it is clear however that direct action and indirect action are complementary.

Radiation responses in general conform to the familiar concepts of threshold, summation, intensity-duration, and adaptation (38, 107, 214, 248, 312). The interval

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between irradiation and its biological expression is variable; it may be a matter of minutes for suppression of cell division, hours for lymphopenia, days for neutropenia, weeks for anemia, or months or years for neoplasia. This latent period depends not only upon the character of the radiation and manner of exposure but in some instances may also be related to the life span, mitotic rate, and metabolic activity of the cells concerned with the effect. Little is known of the biophysical and biochemical changes that intervene between the primary ionizations and excitations and the recognizable end result. Effects on respiration (12, 26, 28, 77, 126, 298), enzymes (31, 32, 85, 119, 123, 332), nucleic acids (171, 172, 220, 221, 237, 309), redox potential (344), viscosity (270, 365), permeability (57, 339), and pH (140, 247) have been described. It is not known however whether such changes are the cause or the effect of cell injury and death. It is noteworthy that effects on growth occur at dosages considerably below those required to influence metabolism (44, 120, 147, 184, 247, 294).

In view of the ubiquitous nature of energy absorption, and hence of the wide variety of atomic and molecular changes that are possible within the framework of biological complexity, it is not surprising that the effects of irradiation are manifested in many ways and that many factors have the capacity for influencing radiation responses. This diversity of action increases the probability of nonspecificity, and thus no single response has been found to be unique for radiation injury, while a number of agents (nitrogen mustards, urethane, benzol and hydrogen peroxide) are capable of mimicking radiation effects (50). Since it is almost impossible for a radiomimetic agent to attain the uniform distribution of penetrating radiation, no single agent would be expected to duplicate all of the radiation-induced reactions. From the many factors that are known to influence radiation sensitivity, it is clear that the development of injury and of repair from such injury depends, at least in degree, upon a number of biological conditions.

Problem of Radiosensitivity

In general, radiosensitivity as discussed here will refer to the destruction or degeneration of cells as living entities or to the overall lethal effect on the whole organism. Damage of a more restricted nature, e.g. to genetic materials, and interference with more discrete processes, e.g. cell division, growth and differentiation, will, however, also be considered in specific instances. The great difference in sensitivity among biological objects is rather astonishing (248, 312). Tolerance to growth inhibition or lethal action differs by a factor of about 8000 (312). The dose range required to produce various biological responses extends moreover from about 10 to 10^6 roentgens. There are no satisfactory explanations for these differences. Factors that may be related to the radiosensitivity of the various cells, tissues and organisms include the structural and chemical components of protoplasm; physical conditions such as molecular orientation, viscosity, conductivity and temperature; the level of activity, for example the rate of metabolism and growth; and the stage in the life cycle as manifested by the phase of mitosis, the degree of differentiation and aging.

It is well known that the nucleus is a more sensitive indicator of damage than the cytoplasm. Perhaps not as obvious is the fact that nuclear damage depends in large measure upon direct irradiation of the nucleus. This is true for both ultraviolet and x-radiation (42, 167, 288, 348, 368). Cytoplasmic change is not necessarily less important, however. There is in fact evidence that nuclear pyknosis in amphibian eggs may be a result in part of toxic factors originating in irradiated cytoplasm (100). It has also been suggested that release of heparin-like substances from combination

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with proteins may be responsible for part of the antimitotic effect of irradiation (161, 162). There appears to be a physiological requirement for the production of visible nuclear damage, since irradiation of isolated nuclei results in negligible change in nuclear structure (100) and in the properties of nucleoproteins (349). The sensitivity of nuclei may be related to changes produced in nucleoprotein by radiation (159, 220, 221, 310, 311, 335, 361).

An indication of the importance of metabolism in its broadest sense may be the fact that the cell in mitosis is usually more susceptible to injury than the cell at rest. Even in mitosis there are differences; cells in prophase or metaphase are generally the most sensitive to radiation injury. The phase of peak sensitivity varies in the different biological materials (151, 309). It is noteworthy that degenerative changes are prone to occur at the time of cell division regardless of the period of irradiation, which is also strongly suggestive of a physiological influence on the development of injury (146, 206, 314, 334). The sensitivity of the cell during division may reside in some facet of its instability. At present, it is possible only to implicate some of the differences that exist between the interphase and mitotic cell in, for example, chromosomal mass and surface, viscosity, permeability, conductivity and energy requirements. Numerous investigations have been directed toward an elucidation of this fundamental problem. These studies have been reviewed recently by Sparrow (309, 310) and Gray (151).

The inherent difference in sensitivity of various cells and tissues attracted early attention, and in 1906 Bergonié and Tribondeau (36) formulated the principle that actively proliferating tissues are the most sensitive to radiation and that the radiosensitivity of a tissue varies inversely with the degree of differentiation. In accord with this concept, it is generally true that hematopoietic tissue, testes, gastrointestinal epithelium, skin, lens epithelium, embryonic germinal centers and plant meristematic tissues are the most readily damaged by ionizing radiations. It is interesting to note that the acute radiation syndrome may be causally related to cytological damage in areas of rapid cell turnover, e.g. the hematopoietic and intestinal tissues. There are, however, many departures from the simple condition relating radiosensitivity to growth and differentiation (39, 56, 168, 186, 241, 242, 246, 318, 343, 354). The reasons for this are largely a matter of conjecture, but it is clear that other factors must influence the responsiveness of cells during their life history.

There is no obvious relationship between susceptibility of different tissues and their basal oxygen consumption. Brain and kidney have higher rates of respiration than spleen; yet the former are relatively radioresistant while the latter is radiosensitive. Although it has been shown that polyploidy protects certain simple organisms against radiation damage (70, 210), there is little reason to believe that this is an important factor in radiosensitivity of the several animal tissues. Polyploid cells occur in the liver, but they are the exception rather than the rule and probably do not account for the apparent resistance of this organ to radiation.

Recovery phenomena have been invoked to explain differences that are frequently observed in the response to X rays of slowly and rapidly dividing cells (171). The argument is made that the slowly dividing cell has a greater chance to recover since the death of a cell frequently occurs at mitosis from changes induced sometime before (146, 206). This sort of dependence on cell division is not as characteristic with alpha and neutron irradiation or with high dosages of X-radiation (207, 314, 334). Certain types of recovery may actually be faster in cells that are presumed to have a more rapid turnover (186, 203). Temperature studies reveal that primary damage is usually not repaired in metabolically depressed tissues.

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Although the lethal dose of ionizing radiation varies among the different animal species (97) it is of interest that some physiological and histological changes, e.g. lymphopenia and lymphoid involution, are more nearly independent of species and reflect the amount of radiation instead of the lethal effect (39, 55, 93). Species sensitivity is not well correlated with body size or with metabolic rate although these factors may be important in individual animals. There is little difference, for example, in the basal heat production of the guinea pig and rat, although the LD_{50} of the former is lower by a factor of about two. Sensitivity may be related to differences in rates of recovery from radiation injury rather than to differences in the extent of the initial injury; the mean survival time after median lethal irradiation is greater for the more sensitive species (283). The lethal response may, of course, simply reflect the particular susceptibility of different species to the diverse mechanisms leading to morbidity, e.g. leucopenia, bacteremia, hemorrhage, impaired nutrition and shock.

The shapes and slopes of dose effect curves frequently yield useful information about mechanism (214, 376). It is unlikely that an exponential curve is due to biological variation; on the other hand, the significance of a sigmoidal curve is less obvious. Haploid yeast cells show an exponential dose-response curve, indicative of the inactivation of a single site, while polyploid cells present a sigmoidal curve, suggestive of multiple sensitive sites (210, 340). Curves relating dose to mortality in animals are of the sigmoid type and are quite steep, especially for mammals (43, 101). Variations in mortality of from 0 to nearly 100 per cent may occur in the LD_{50} range (71). The dose-dependence curve in this instance is almost certainly a complex function of a number of reactions.

Apart from these perhaps more subtle mechanisms, other factors are known to influence the radiation responses. Radiosensitivity of the embryo varies with its age (199, 321, 363, 364); the fetus is also more susceptible than either the young or adult animal. Malformations of the skeleton and destruction of the developing nervous system are prominent sequelae of irradiation during the latter two-thirds of gestation (175, 280). Adult bone and nervous tissue, on the other hand, are relatively radioresistant. The newborn mouse is less sensitive to lethal action than the puberal animal, perhaps because of protective influences associated with suckling (1). It may be stated that maximal susceptibility to radiation lymphoma in the mouse also occurs during puberty (196). There is little difference in the acute lethal response among adult animals (377). However, the amount of X-radiation required for minimal stunting following local exposure of the epiphyseal region of rats is linear with age at least up to 6 months (176). The role of body weight in the radiosensitivity of a given species is not well defined, although it appears that heavier animals tend to be less sensitive (104, 154, 269). It is not always clear, however, whether this is a reflection of weight or of age. At any rate neither age nor weight effects are attributable to a simple relationship between the rate of growth and sensitivity. Differences in absorption and distribution of the radiation, for example, because of the low effective atomic number of fat (315), could account conceivably for small differences in sensitivity due to body weight. Sex differences are fairly negligible; females may be slightly more resistant but this is not apparent in all species (1, 154, 155).

Intermediary Radiochemical Events

The reactions induced by ionizing agents in aqueous solutions have been subjected to rather intensive scrutiny during the past decade. Such endeavors have profoundly influenced the trend in radiobiological thought with the result that a large part of our attention has been given to the role of water in the development of radia-

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tion injury. Water, because of its great abundance in living tissues and its susceptibility to activation by ionizing radiation, constitutes a prime biological target. The significance of water resides largely in its role as an amplifier of the absorbed energy. This is not to say, however, that water may not contribute to radiation effects in other ways, as for example by influencing metabolism or structure. Since the reactions of irradiated water are responsible for many of the chemical effects of the high energy radiations and form the basis for many of the protection studies, it will be profitable at this time to summarize the events in water that appear to be relevant to radiobiology. The reader is referred to recent papers in this field (10, 62, 83, 151, 359, 374).

Irradiated water constitutes an oxidation-reduction system. As postulated by Weiss (358), ionizing particles are believed to form free hydrogen atoms and hydroxyl radicals along their tracks. According to Burton (62) free radicals may be produced in the ambient liquid even when ionization occurs directly in a solute molecule. The initial spatial distribution of the H and OH radicals appears to be a decisive factor in the chemical and biological effects of the various ionizing radiations and indeed probably represents the essential difference between radiochemical and photochemical decomposition of water. Although it is generally believed that ionization is the primary physical event in the aqueous phase (62, 214, 216) ionization is not always adequate to explain the observed transformations, and in such instances it is necessary to consider excitations and chain reactions. The dissociation of ionized water molecules (H_2O^+ and the H_2O^- formed by capture of the ionized electron) forms H and OH radicals with all types of ionizing particles. The ultimate effects vary, however, and depend mainly upon the nature of the incident radiation (essentially its rate of energy loss or ionization density) and the presence of dissolved oxygen and other solutes. These factors influence markedly the recombination or further reaction of the free radicals.

With high ion density radiation, e.g. alpha particles, each ionization track is believed to consist of a dense central core of H^+ and OH ($H_2O^+ \rightarrow H^+ + OH$) and an envelope of H and OH^- ($H_2O^- \rightarrow H + OH^-$) (48, 62, 92, 152, 214, 216). Interaction between OH radicals to form H_2O_2 is quite probable in view of their close proximity. Likewise, H atoms combine readily to form hydrogen gas. Oxygen is another byproduct, presumably from decomposition of H_2O_2. As the free radicals diffuse, the chances for collision between H and OH and H^+ and OH^- to reform H_2O increase and may be presumed ultimately to balance the frequency of combinations between like radicals. This picture may be contrasted with the situation following low ion density radiation, e.g. x- or gamma radiation, where the initial distribution of radicals along the ion track appears to be a more random event and there is a relatively small over-all change in pure water unless oxygen or anions of low electron affinity are present (4, 48, 62, 134). These differences are related to the velocity of the impinging particles; slow electron irradiation with tritium beta rays has been shown to simulate the effects observed with alpha particles (138).

Oxygen has at least a twofold influence on the radiochemical effects of fast particle or low ion density radiation. First, it converts free hydrogen atoms to the hydroperoxyl radical (HO_2), a powerful oxidizing substance which in turn may be degraded to H_2O_2. Second, by virtue of the reduction in hydrogen atom concentration, the presence of oxygen would be expected to increase the survival of the highly reactive free OH radicals, and therefore to facilitate their reaction with other substances that may be present. As a consequence, oxygen may be expected to increase, and oxygen acceptors to decrease, the radiosensitivity of biological systems sensitive to

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oxidation. This is indeed the case for many of the effects of x- and gamma radiation. If the oxidizing OH radicals derived from the action of either high or low ion density radiations on water are important radiotoxins, reducing agents in general might improve the situation with all of the ionizing radiations. Even here, however, some difference in protective efficiency may be anticipated because of the initial spatial distribution of the radicals.

It may be noted that H_2O_2 is an important product of the radiodecomposition of water. As demonstrated by Bonet-Maury and Frilley (47), H_2O_2 production is determined by the energy dissipated in water. The yield, commonly expressed as molecules transformed per ion pair, corresponds to 0.54 or, in other words, to approximately 50 μg. of H_2O_2 per cc. per million roentgens. The yield of hydrogen peroxide with x rays depends somewhat on dose rate (49). Peroxide formation may be greater with x rays than with alpha rays in oxygen-saturated water (48). It is greatly reduced, however, with the low ion density radiations in the absence of oxygen (4, 48, 134). With radiations of high ion density, e.g. alpha rays, the yield of H_2O_2 is essentially identical in the presence and absence of oxygen (46, 48, 61). This implies that with alpha rays the hydrogen atoms do not make an appreciable contribution to peroxide formation. Although H_2O_2 formation depends upon oxygen concentration with x ray dosages of the order of 200,000 r and above, the yield with lower dosages may be independent of oxygen tension (9, 151). This is perhaps understandable since there may still be a considerable excess of oxygen atoms relative to the hydrogen atoms produced by low dosages of x rays (92). This point merits careful attention since exceedingly small dosages of radiation are important biologically, and radiosensitivity has been shown to vary with oxygen tension.

From the work of Bonet-Maury and Lefort (48), it is apparent that changes in temperature compatible with most living systems do not affect the yield of H_2O_2 with either alpha or x-rays. Peroxide formation is decreased by about 50 per cent at $-4°$C. However, with alpha rays there is no difference between $-4°$C. and $-190°$C., whereas with x rays hydrogen peroxide formation decreases progressively and can no longer be detected at $-116°$C. The pH of the solution appears to exert an effect, the yield of H_2O_2 with x rays declining abruptly above pH 8 and rising sharply below pH 3 (49). The amount of H_2O_2 found in biological fluids may be considerably less than that formed in water because of the presence of catalase. This situation may, nevertheless, be compensated, since peroxide formation is increased by other biological components such as cysteine, ascorbic acid and aldehydes (225). It is obvious that in a nonhomogeneous system the spatial relationships of the various constituents would be of crucial importance.

It is not to be inferred from these considerations that H_2O_2 is necessarily the primary radiotoxin. On the contrary, as emphasized by Latarjet (208) and others, the formation of H_2O_2 may represent an important detoxifying mechanism in the reactions of irradiated water. No doubt, part of the emphasis on hydrogen peroxide is related to the convenience of its application to biological studies and to the existence of suitable direct methods for its detection. This is not the situation with the free hydroxyl and hydroperoxyl radicals. It is noteworthy that the action of H_2O_2 may be enhanced by addition of ferrous ions or by ultraviolet irradiation (74); these agents are believed to convert H_2O_2 to free OH radicals. The relative contributions of the decomposition products of water will be discussed in connection with the protective effects of anoxia and chemicals. Although the emphasis is more on oxidation as a mechanism of inducing radiation injury, it must be remembered that reduction reactions due to free hydrogen atoms may also play a role (123). It may be noted,

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moreover, that local changes in pH must also occur in the vicinity of an ion track; pH probably decreases in the track itself and increases in the immediately adjacent areas (62). Shifts in pH toward increasing acidity have been detected in irradiated water containing oxygen (134). Protein solutions irradiated with alpha rays also show an increased acidity, provided that the initial pH of the solution is above the isoelectric point (17). Changes in pH have not been seen in X-irradiated beef broth (174).

The activated water concept of radiation action has received ample confirmation in a variety of aqueous systems since the classic observations of Risse (277) and Fricke (128). An essential feature of these reactions is the independence of the ionic yield (molecules affected/ion pairs formed) from the concentration of solute over a wide range of concentrations. This has been termed the 'dilution effect' by Dale (85–87) who made extensive experiments on the effects of X and alpha rays on purified enzymes in aqueous solutions. In effect, this phenomenon substantiates the idea that solute molecules are transformed indirectly by energy that is transmitted as a result of the change in solvent molecules. The dilution effect is not observed at very low or high concentrations of solute. In the former instance, it is generally considered that the ratio of activated water molecules to solute molecules is much higher and hence favors competitive recombination of radicals rather than interaction with sparsely distributed solute. On the other hand, as solute concentration increases, or with increase in radiation dosage, there is a gradual transition from the activated water type of reaction to the direct hit type characteristic of nonaqueous systems. Under these circumstances, the ionic yield, in general, increases to some optimum value, since both types of action prevail. In contrast with the direct effect, the ionic yield for the indirect effect diminishes with increase of molecular weight (214, 215). The yield will also be influenced by the distribution of ions and free radicals. Although there are relatively few data relating to the efficiency of different radiations on solutions, alpha particles have been found to be less effective than X rays in initiating certain chemical reactions (89, 92, 152, 214). This follows from the relative probabilities of recombination of radicals and of their collisions with randomly distributed solute molecules. It is not to be inferred, however, that chemical changes induced in cells will necessarily manifest a similar dependence. In fact, this picture may be contrasted with the situation in living systems where the effectiveness varies directly with ionization density for a number of radiation-induced changes.

Of considerable theoretical significance is the 'protection effect' described by Dale (87). This refers to competition between various solutes, or between breakdown products of a single solute, for activated water molecules. The protective power of different solutes is proportional to their molecular weight. Specificity is indicated, however, in that protection has also been shown to depend upon specific atomic groupings, thiourea being one of the most effective agents for the protection of carboxypeptidase in aqueous solution (88, 90). Protection cannot always be attributed to simple competition for free radicals, since it has been observed that protective power may decline with increase in concentration of the protector. It is necessary, therefore, to postulate more involved energy-sharing mechanisms.

A word about the temporal aspects of this type of protection is appropriate. The main energy transfers, including the events in water (free radical formation, recombination or further reaction), occur undoubtedly within a small fraction of a second after passage of an ionizing particle. One would expect, therefore, that protective agents that depend on altering the mechanisms of energy dissipation must be brought into play at the time of irradiation in order to be effective. This is the usual

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situation. It is well to recall, however, that as an aqueous system becomes contaminated, the possibility of longer-lived toxic products and chain reactions becomes greater. The contaminants, on the other hand, may also exert a protective effect. Indeed, one part of a macromolecule may protect another as was effectively demonstrated by Dale (86) for the enzyme, *d*-amino acid oxidase. A further manifestation of the protection effect is the exponential curve obtained when the proportion of biologically active enzyme remaining after irradiation is plotted against radiation dose.

Many types of radiochemical reactions have been described: oxido-reduction of inorganic and organic molecules (15, 29, 123, 129, 130, 139, 359), deamination of amino acids (88, 360), denaturation of proteins (29), depolymerization of nucleoproteins (159, 220, 221, 311, 335, 361), and polymerization and aggregation of organic molecules (84, 267). Similar effects have been induced by chemically generated free radicals and radiomimetic agents such as the nitrogen mustards (3, 64, 74, 220, 279). Each of these phenomena could be relevant to radiobiological processes.

The ramifications and implications of the protective effect in biological systems and in molecules of biological importance will be reviewed in the following sections. It is at once obvious that there are a number of obstacles to the *in vivo* extrapolation of these *in vitro* effects of irradiated water. We should anticipate a marked protective action by the bulk of protoplasm; yet, in general, there is a relatively greater effectiveness of ionizing radiations on living systems. Weiss (361) has suggested that a small primary attack on an important macromolecule followed by hydrolysis may provide the amplification factor, while small differences in activation energies may account for its selective reactivity in the presence of protective substances. Although it may be inferred from the effects in simple solution that an enzyme present in minute amounts in a homogeneous cell system would be no more sensitive than the same enzyme irradiated in concentrated solution, the situation may be altered if the cell constitutes a nonhomogeneous system. Moreover, if enzymes or other critical entities form surface films, the chances for inactivation may be greatly enhanced. As shown by Mazia and Blumenthal (234, 235), x-irradiation with only 100 r of pepsin-albumen films spread on the surface of water leads to 50 per cent inactivation of the enzyme. A single radiation event may, therefore, affect a large number of molecules. Since the efficiency of enzyme inactivation varies with the surface pressure of the film, it is believed that inactivation may be related to the physical configuration of the enzyme molecules. Further work in this area should add greatly to our understanding of the biological actions of radiation.

Modifying Factors

A few general comments are in order before discussing the various modifying factors. Protection or potentiation of radiation action must be defined in terms of specific parameters. These include, in addition to the biological effect under consideration, the quantitative and temporal aspects of the radiation and of the modifying agent or situation. It is desirable that a given agent or situation be evaluated in terms of the dose-response curve for the effect in question. This is true for several reasons. In the first place, this is necessary in order to arrive at a reliable estimate of the efficiency of a protective or potentiating agent. Secondly, knowledge of the dose dependence affords a standard for comparison with other effects or with the same effect determined elsewhere. And finally, information regarding mechanism may be derived from comparison of the dose-response curves obtained under control and experimental conditions. As discussed by Zirkle (376), a change in slope probably

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reflects a change in the relative probabilities of competing reactions, while a change in shape may imply a change in the number of relevant reactions or events that are required for the particular biological effect.

The biological end point also deserves mention. Protection against acute lethal action, for example, does not necessarily imply protection against the more chronic effects insofar as different mechanisms may be responsible for their development. Likewise, protection against one or another of the acute sequelae does not signify protection against all of them. No doubt a number of pathways exist for the expression of injury in a heterogeneous and highly integrated biological unit. Uniformity of protection against a number of radiation effects suggests that the chain of events is being intersected at a common and presumably early stage in their development. Protection against a single event, on the other hand, may well imply an intervention farther downstream or a change in a specific biological target.

Water

It was recognized long ago that desiccation of biological systems favors radioresistance. Effects can be induced, however, even in dried materials such as bacteria (94). The dependence of sensitivity on water content has been demonstrated in spores (177), seeds (136, 266), seedlings (168, 362), tumor cells and isolated tissues (67, 115). About 50 per cent of barley seeds soaked in water for 23 hours show fragmentation and bridging of chromosomes after 10,000 r (136). With 15 per cent water content the frequency of chromosomal aberrations decreases to 28 per cent and with 10 per cent water content, to 13 per cent. Since it is apparently necessary to remove most of the water in order to induce these effects, it is obviously difficult to determine whether a similar dependence exists for the intact animal. Frogs that are kept in dry, individual containers for 3 days lose about 35 per cent of their water; yet they are no more resistant to x-irradiation (263). Mice deprived of drinking water for 24 to 40 hours tend to live somewhat longer, but the proportion surviving is not altered materially (125).

In the case of seeds, the water effect is apparently related mainly to processes associated with germination. Although, as noted above, a 5 per cent increase in water content doubles the number of chromosomal aberrations in barley seeds (136), other effects, e.g. mutations, growth inhibition, may not increase until germination is underway (168, 316). The onset of germination represents an abrupt transition in the life of the seed in terms of water content, respiration and cell division. However, there is no clear relationship between these factors and the rapidly increasing radiosensitivity of germinating wheat seedlings with age (168). Wheat seedlings appear to become slightly more resistant with onset of visible mitosis.

Since cells exposed to ionizing radiation may swell, especially after large dosages, and this can be a contributing factor in cell death (57, 114, 115), it may be argued that dehydration can decrease sensitivity by minimizing cell swelling. In support of this contention, it may be noted that the growth capacity of irradiated mouse sarcoma 180 is reduced by immersion in isotonic or hypotonic Locke-Ringer solutions and enhanced by similar treatment with hypertonic solutions (326). Injection of small quantities of distilled water into this tumor also enhances its regression following local x-irradiation *in vivo* (116). Equivalent water injection per se does not affect tumor growth. While local changes in osmotic pressure due to partitioning of ions and molecular fragments, the so-called fluid flow hypothesis advanced by Failla (114, 115), is an interesting notion, increase in cell size, especially after moderate irradiation, can also be attributed to absence of cell division while growth continues

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at a normal rate (51, 214). The possible importance of shifts in
cannot be dismissed, however. Nuclear volume may be increase
tion (159) and this may be due to an increase in water content

One is tempted to inquire whether these effects of water are rela-
ner to the activated water concept of radiation action. A decrease in radio-
with relative dehydration could be explained by assuming a transition from mainly
indirect to direct action when water content is low enough. To this may be added
the probable increase in concentration of naturally occurring protective substances.
While there is rather convincing evidence, admittedly indirect, in support of *in vivo*
effects of activated water, it is not possible from available data to appraise the relationship between water content and radiosensitivity in these terms. To do so, one must contend not only with the technical problem of removing sufficient water from the cell without inducing injury or a contributing physiological change, e.g. in respiration or growth, but also with the uncertainty regarding the spatial distribution and state of water within the cell. Differences in water content of various subcellular elements may be relatively more important than the over-all content. Likewise, a water effect may depend more on solvent water and perhaps on water of hydration than on nonsolvent or bound water.

It is appropriate to say a word about dilution effects in simple biological systems. Direct and indirect actions and dilution and protection effects are well demonstrated with preparations of viruses and bacterial toxins (105, 132, 133, 217, 230). The relative importance of direct and indirect actions depends on the ratio of water content to solid content in the suspension. It may be remarked in this connection that there are distinct differences in the properties of bacteriophage inactivated by the two types of effect (355, 356). Sensitivity of the virus suspended in water is in general greater than that of the dry virus preparation. This is so because in addition to direct ionization of the virus particle, there is also the possibility of inactivation by reactive intermediates formed in irradiated water. Lea (215) has shown, however, that an ionization in water is about one-thousandth as effective as an ionization in the tobacco mosaic virus. It is necessary, therefore, to achieve a water-solid ratio greater than 1000 in order to decrease the radiation dose required to inactivate a given percentage of viruses by a factor of two (215). In keeping with this it will be recalled that, in general, the ionic yield for the indirect effect is lower than for the direct effect in the case of very large molecules. The prime factor in determining whether the action of radiation on the virus is direct or indirect appears to be the total protein content of the suspension and not the virus concentration (215). Apropos of the distinction between direct and indirect effects, it should be pointed out that part of a so-called direct effect could result conceivably from absorption of radiation in the water of hydration or in the water envelope surrounding a biological particle. Moreover, an ionization in the particle itself may be effective because of free radicals formed in the ambient water layer (62). Water intimately associated even with a so-called dry substance may, therefore, be of some consequence. It has been shown, however, that X rays are unable to depolymerize DNA in the dry state as well as in ethylene glycol or in a frozen aqueous solution (220).

Dilution effects have also been demonstrated for *Arbacia* sperm (111) and yeast (299). The X-ray-induced reduction in fertilizing capacity of sperm and in anaerobic CO_2 production by yeast is diminished when the concentration of the suspensions is increased. The protective effect of concentration in these cases is not entirely comparable with that observed with enzymes and viruses. One is concerned here with two aqueous environments, namely the extra- and intracellular fluids, and the contribu-

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tion of each to indirect action must be assessed. The effect of rather close packing of cells may reflect the decreased surface available for attack by toxic products produced in the extracellular phase. As shown by Evans *et al.* (111), cleavage delay of *Arbacia* ova successfully fertilized by irradiated sperm does not depend upon a medium effect. Likewise, there is only a weak effect of the irradiated medium (phosphate buffer) on the formation of visible colonies by yeast cells (299). It is not to be inferred from this that these effects are necessarily a result of direct ionization or excitation of biological targets other than water, since ample water is present within the cells. It is noteworthy that closely packed thymocytes are about twice as resistant to the lethal action of x rays as cells suspended in buffered plasma (251). In this instance, the protective effect is attributed to the hypoxia resulting from cell packing. Whether this effect of hypoxia is related to the events presumed to occur in cellular water upon irradiation is another matter.

Oxygen

The relationship between oxygen and radiosensitivity has been the subject of considerable investigation and much speculation. In essence, the oxygen effect refers to the diminution of many of the changes induced in chemical and biological systems by x and gamma rays when irradiation takes place under conditions of relative oxygen deficiency. This obviously basic phenomenon was recorded as early as 1921 by Holthusen (183), although it may be recalled that a presumably related protective effect, that of ischemia, was described by Schwarz in 1909 (293). Several papers bearing on the oxygen effect appeared during the next two decades, notably those by Mottram (240–244) and Crabtree and Cramer (78). Emphasis in the early investigations was placed mainly on the physiological aspects of oxygen deficiency as it related to radiosensitivity. The more or less latent interest in the contribution of oxygen to radiation processes has since been profoundly influenced by our understanding, incomplete as it may be, of the events that transpire in irradiated water. The protective role of hypoxia has been established for a variety of biological materials; a corollary to the diversity of objects so protected is the variety of radiation effects that are subject to the oxygen influence. It must be emphasized that all radiation reactions are not affected by oxygen, a fact that is not inconsistent with the concepts of direct and indirect actions discussed in the preceding pages.

Oxygen deprivation during exposure to x or gamma rays has been shown to decrease the following radiation effects: oxidation of quinhydrone (224), auxin (301), glutathione (34), and other substances in aqueous solution; deamination (289) and delayed depolymerization of solutions of thymus nucleic acid (63); inactivation of bacteriophage suspended in buffer solution (11); cleavage delay in *Ascaris* eggs (183); mitotic inhibition in grasshopper neuroblasts (135) and in *Vicia faba* meristems (271, 272); chromosomal aberrations in barley seeds (160), *Tradescantia* microspores (142, 144, 145), and *Vicia faba* root tips (271, 272, 336); sex-linked lethal mutations in *Drosophila* (25) and biochemical mutations in *E. coli* (14); growth reduction in germinating seedlings (160, 168) and *Vicia faba* roots (271, 272, 336); developmental abnormalities in mouse embryos (281); and lethality of bacteria (178), yeast (16), tumor cells (78, 157), mice and rats (98, 205). The degree of protection is rather similar for these different effects. In more specific terms, the radiation dose required to induce a comparable degree of change in the absence or near absence of oxygen is generally increased by a factor of 2 to 3, at least in those instances where sufficient data are available to allow such comparison. It may be remarked that an increase in ambient oxygen tension above the level in air has a relatively small effect

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in most systems. We will return to this point later, as well as to the biological implications of the oxygen effect.

Oxygen is not always a determinant of radiation sensitivity. The transformations in carboxypeptidase (92) and in ribonuclease (182) are not influenced by removal of oxygen from the solution. An oxygen effect is not apparent in the case of bacteriophage suspended in a protein rich broth (174). In this instance, the radiation effect is attributed to direct ionization of the phage particles since it is assumed that sufficient protein is present to neutralize the radicals that are formed. The selective potassium accumulation by X-irradiated erythrocytes suspended in plasma is likewise unaffected by oxygen tension (297), and radiation induction of dominant lethals in mice also appears to be independent of oxygen (282). The reason for this is less obvious unless direct ionization or interaction with OH radicals is the primary mechanism for these changes. Specificity of the oxygen effect is clearly demonstrated by the experiments of Anderson (14), who has found that oxygen exerts a negligible effect on X-ray induction of back mutations in a streptomycin-dependent strain of *E. coli* and a striking effect on the back mutation rate of a purine-dependent strain. Yet there is a similar decrease in sensitivity of both strains to the lethal effect of X rays in the absence of oxygen. The dose reduction by anoxia is identical for the lethal and genetic effects in the purineless mutant. Quite apart from the demonstration of negative oxygen effects, it may be noted that a decrease in oxygen tension during X-irradiation potentiates the radiation-induced aggregation of hemocyanin molecules *in vitro* (267). This situation may perhaps be explained by assuming that hemocyanin aggregation is a result of some reduction process, although other explanations are possible. The destructive effect of X rays on catalase in dilute aqueous solution is also facilitated in the absence of oxygen (123).

Mode of Action. When we turn to the mechanism of the oxygen effect, we are immediately faced with the proposition of determining whether the mechanism is essentially radiochemical or biological in nature. It is clear that oxygen may modify radiation injury by influencing the pathways of energy transfer in water, and hence the production of active radicals and peroxides. This is almost certainly the case for the oxygen effect on solutes in simple solution and on other systems in which the external aqeuous environment contributes a substantial part of the radiation effect. In systems of increasing complexity, albeit still a single cell, the possibilities of interpretation multiply in proportion to the uncertainties regarding the morphological and physiological relationships of the cellular constituents and the biochemical processes induced by the radiation. It is at this point that one must inquire about a more specific biological effect of oxygen, perhaps on the physico-chemical relationships of the various biological targets in the cell, or on some aspect of metabolism, cell division, or recovery.

We may recall the observation by Loeb in 1910 (223) that suppression of cellular oxidation in *Arbacia* eggs prevents the toxic action of a number of agents. It is well known that diminished oxygen tension shifts metabolic pathways, leads to accumulation of acid metabolites with corresponding changes in tissue pH, and stabilizes the organic sulfur system in favor of high sulfhydryl concentration. Cell division also depends on the energy supply and therefore on the availability of oxygen (173). Respiratory peaks have been described during the mitotic cycle and are related presumably to synthetic activity. It is necessary, however, to distinguish the qualitative from the purely quantitative changes in the biochemical machinery; as will be discussed in the section on temperature effects, moderate differences in metabolic rate during irradiation do not appear to influence radiosensitivity. In view of the

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marked effect of the ionizing radiations on mitotic processes and of the somewhat obscure relationship between sensitivity and cell division, it is well to keep these possibilities in mind, if not as alternatives, at least as complements to the presently more attractive radiochemical hypothesis.

Effects on Solutions and Suspensions. That oxygen endows x-irradiated water with the properties of an active oxidation system is firmly established. The redox potential of an irradiated aqueous solution of quinhydrone moves progressively in the direction of oxidation in the presence of oxygen; in its absence, oxidation does not take place (224). Inactivation of auxin solutions (301) and oxidation of glutathione (34) are dependent upon oxygen. With 10,000 r x-radiation there is 26 per cent oxidation in an aerated dilute solution of glutathione and about 9 per cent oxidation in an oxygen-free solution, a reduction of 67 per cent. Addition of catalase to an aerated solution of glutathione reduces the oxidation by about 25 per cent. From this it has been concluded by Barron and Flood (34) that hydrogen peroxide contributes 25 per cent to the oxidation of glutathione, the hydroperoxyl radical about 42 per cent, and the free hydroxyl radical 33 per cent.

Comparable experiments have not been reported for sulfhydryl enzymes. It may be remarked, however, that catalase has been shown by Barron and Dickman (32) to reduce by approximately 50 per cent the inhibitory action of alpha radiation on phosphoglyceraldehyde dehydrogenase in dilute aqueous solution. This suggests a more formidable effect of hydrogen peroxide with alpha rays than with x rays as described for glutathione (34). Yet an even greater degree of protection by catalase has been observed when phospholgyceraldehyde dehydrogenase is exposed to beta rays, which in terms of ionization density are more nearly comparable to hard x rays (32). The apparent inconsistency may possibly be attributed to the very low dose rates employed in their experiments with both alpha and beta radiation. It may be noted that catalase decreases the beta-ray oxidation of BAL by only 17 per cent, which is similar to the situation with x rays (34). In this instance, however, the total dose was delivered in 2½ hours in contrast with the 1-day exposure with alpha rays and the 4-day exposure with beta rays for the sulfhydryl enzyme. It remains to be determined, therefore, whether the contribution of hydrogen peroxide to thiol oxidation differs with radiation quality. The ionic yields reported for phosphoglyceraldehyde dehydrogenase (32) should also be re-evaluated in view of the wide differences in the rates of irradiation. Although the production of hydrogen peroxide increases somewhat over a narrow range of dose rates (49), the contribution of a long-lived reactant, such as hydrogen peroxide, must be relatively greater as the time required to deliver a given dose is increased. On the other hand, the action of a short-lived radical, e.g. OH, should be relatively less dependent upon dose rate. These considerations emphasize the importance of dose rate, which in a real sense complements ionization density, in the interpretation of indirect actions.

Relevant to the contribution made by various oxidants in irradiated water, Collinson *et al.* (74) have shown that ribonuclease is inactivated primarily by OH radicals. This enzyme is not protected by catalase nor inactivated by hydrogen peroxide. The absence of an oxygen effect has also been established (182). Hydroxyl radicals produced during the reduction of ferric ions by ultraviolet radiation, the decomposition of hydrogen peroxide by ultraviolet radiation, or the reaction of ferrous ions with hydrogen peroxide, inactivate ribonuclease. The degree of inactivation varies exponentially with the number of radicals formed. There is reason to believe that other nonsulfhydryl enzymes in aqueous solution, e.g. carboxypeptidase, are inactivated mainly by free OH radicals formed upon irradiation (92). This is

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consistent with the low ionic yield obtained for carboxypeptidase irradiated with alpha particles as against x rays. The rate of recombination of OH radicals should be greater with alpha rays than with the more randomly distributed low ion density radiations. Oxidation of reduced cytochrome *c* by x rays is also unaltered by the presence of catalase or the absence of oxygen and may be presumed to be a consequence of interaction with OH radicals (30).

The immediate fall in viscosity of x-irradiated thymonucleic acid solutions is independent of oxygen concentration, but may be attributed, nevertheless, to an indirect mechanism (63, 151, 335). On the other hand, the delayed depolymerization occurring over a period of several hours after x-irradiation with 7,000 r is influenced by oxygen, and it is perhaps significant that the protection is of the same order of magnitude as described for the oxygen effect in living systems (63). The agent responsible for the delayed effect may be the HO_2 radical, since sufficient hydrogen peroxide cannot be detected in the solution. Moreover, hydrogen peroxide in concentrations that exceed those postulated for x-irradiated water do not affect the viscosity of thymus nucleic acid solutions (118, 335).

These results stand in sharp contrast with the radiation after-effect on bacteriophage suspended in phosphate buffer. Alper (11) has observed that the greater part of the delayed inactivation of the dysentery phage, S13, is the result of a change occurring during x-irradiation, which makes it more susceptible to the action of formed or added hydrogen peroxide. The enhanced sensitivity of irradiated phage to peroxide and the immediate inactivation of some of the phage particles are due presumably to OH radicals. These effects are independent of oxygen. Oxygen increases the overall inactivation, however, because it favors the formation of hydrogen peroxide. The possibility of a change in threshold for hydrogen peroxide effects as a consequence of irradiation adds a further complication, in addition to that of cell barriers, to comparison of the actions of radiation and of hydrogen peroxide. This may well account for the negative peroxide effect described for thymonucleic acid (335) and for the variable contribution of the irradiated medium and of added hydrogen peroxide observed in other situations (33, 106, 118, 251, 352, 367). As far as nongenetic effects in paramecia are concerned, it appears that directly irradiated animals are not influenced to a greater degree than would be expected from the irradiated medium alone (200). Nongenetic death and division delay in paramecia that are x-irradiated in a dilute medium can be accounted for to a large extent by hydrogen peroxide or organic peroxides. Genetic effects are apparently independent of the medium. The production under certain conditions of relatively stable reactants in irradiated media and the occurrence of delayed effects again emphasize the importance of reaction time and hence of attention to the circumstances of irradiation.

Effects on Cells. When we turn to the oxygen effect in cells and tissues, a second aqueous environment enters the picture and we must contend with both intra- and extracellular water as well as with the physiological state of affairs. It is significant that the resistance of bacteria, *Tradescantia* microspores, and broad bean roots to x rays is the same whether anaerobiosis is induced under vacuum or by nitrogen, helium, argon, hydrogen or carbon dioxide (142, 144, 145, 178, 271, 272). Neither the type of gas nor its pressure has a demonstrable effect. Moreover, the removal or addition of oxygen immediately after x-irradiation under either aerobic or anaerobic conditions is without influence. The important consideration is clearly the availability of oxygen during irradiation.

The quantitative aspects of the oxygen dependence for x rays have been studied by varying the oxygen concentration in the aqueous medium of *Vicia faba* root tips

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and of bacteria or in the gas phase surrounding *Tradescantia* inflorescences and *Drosophila*. A rather similar dependence on oxygen concentration has been found in the different organisms; the radiation effects increase linearly up to about 10 to 15 per cent oxygen in the gas phase or 5 to 10 mg. of oxygen per liter in the ambient fluid (58, 59, 142, 178, 271, 272). As pointed out by Read (272) and by Hollaender and his associates (178), oxygen concentration within certain limits acts as a multiplying factor on the radiation dose. Read (273) has also made the interesting observation that the biological additivity of two briefly spaced x-ray doses given with different oxygen concentrations is determined solely by the oxygen levels during each exposure. Extreme manipulation of this sort is obviously impossible in adult mammals. Definite protection against radiation lethality is seen in rats and mice breathing, respectively, 5 and 7 per cent oxygen during x-irradiation (98); sensitivity is unaffected, however, in animals breathing 10 or 100 per cent oxygen (98, 252).

In general, protection is not enhanced at levels below 1 per cent oxygen. This may be due to the fact that at these low levels oxygen is already depleted in critical loci within the cell. An explanation is less obvious, however, in the case of bacterial suspensions where much of the oxygen effect may be presumed to reside in the medium. It is well to recall the uncertainty concerning the dependence of hydrogen peroxide formation on oxygen tension with low dosages of x rays (151). In the case of water, as long as some oxygen atoms are present there may be an excess relative to the hydrogen atoms produced by such low dosages. A similar situation may not exist, however, in a dynamic system in which oxygen is in constant demand, and the observed dependence of radiosensitivity on oxygen concentration is not unreasonable.

In general the oxygen effect plateaus at levels approaching the physiological or normal ambient oxygen tension (142, 178, 271, 272). A notable exception is the marked potentiation of chromosome-breaking efficiency when *Tradescantia* pollen grains are exposed to x rays in pure oxygen (75). But, as shown by Conger and Fairchild (75), oxygen per se has considerable effectiveness in producing chromosomal aberrations. This raises the question of a possible sensitization even by physiological oxygen concentrations to certain forms of radiation injury. That such a mechanism may take place has been suggested by King and his collaborators (202). They have observed that exposure of *Tradescantia* microspores to carbon monoxide during x-irradiation increases the frequency of chromosome aberrations by as much as 70 per cent. Carbon monoxide alone is ineffectual as far as chromosome changes are concerned. Since the increase in aberration frequency depends on the time of exposure to carbon monoxide prior to irradiation, with a maximum effect reached after 1 hour of pretreatment, one may inquire to what extent carbon monoxide influences cell division under these conditions. Carbon monoxide potentiation is reversed by oxygen in high concentration; oxygen per se under positive pressure results in a small increase in the frequency of x-ray-induced chromosome aberrations. These findings have been interpreted by postulating the inhibition of cytochrome oxidase by carbon monoxide, which in turn facilitates the action of flavoproteins as terminal oxidases. This action is believed to produce excessive amounts of hydrogen peroxide that sensitize the chromosomes to irradiation. Accordingly, oxygen antagonizes sensitization by carbon monoxide by reversing the inhibition of cytochrome oxidase. It is suggested further that the oxygen effect may also be mediated by way of the flavoproteins, low oxygen tensions decreasing and high oxygen tensions increasing their activity as terminal oxidases with a corresponding decrease or increase in production of hydrogen peroxide. Whatever the ultimate mechanism may be, this significant contribution focuses attention on the biochemical processes of the cell, which have

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been somewhat neglected by the emphasis on oxygen as an amplifier of activated water reactions.

Dependence on Radiation Quality. Perhaps the most impressive evidence in support of the *in vivo* action of activated water is derived from experiments by Thoday and Read (336, 337). These investigators have confirmed and extended the earlier observations of Mottram (241), in which oxygen deprivation was shown to decrease the growth reduction of *Vicia faba* roots following gamma irradiation. Exposure of bean root meristems to x rays under anoxic conditions decreases mitotic inhibition, the frequency of chromosome aberrations, and the magnitude of growth reduction (271, 272, 336). It has been observed, on the other hand, that oxygen lack does not afford significant protection against the effects of alpha irradiation (337). These findings parallel the radiochemical reactions involving oxygen in aqueous solution, since oxygen would be expected to exert a minor influence on peroxide formation by alpha rays. It is assumed that the primary action of irradiation with regard to water takes place in the roots themselves, rather than in the tap water in which they are irradiated.

While these results are suggestive of an important role of peroxides in radiation action, the effect of the presence of oxygen in the case of x or gamma rays would be also to increase the life span and possibly also the production of free OH radicals. Although, as we have seen, oxygen has a negligible influence on the sensitivity of substances believed to be inactivated by OH radicals in simple solution, the situation may be quite different in the aqueous milieu of the cell where the survival and reactivity of such radicals are doubtless subject to many influences including local oxygen concentrations. With alpha rays, OH radicals are presumed to be formed close together and their chances for reaction with an essential molecule in the immediate vicinity are correspondingly greater. The absence of an oxygen effect with alpha radiation is consistent with such a scheme. At any rate, it is clear that valuable information may be gained in protection studies by taking advantage of differences in the spatial distribution of the various ionizing radiations. Such data are urgently needed.

Giles and Beatty (142) have, in fact, attempted to evaluate the role of OH radicals on the production of chromosomal aberrations in *Tradescantia* inflorescences exposed to x rays. Little difference is seen in interchange frequencies when inflorescences are exposed in an atmosphere of hydrogen at normal pressure or at 3 atmospheres above normal. One might anticipate that the back reaction of hydrogen atoms and hydroxyl radicals to form water would be enhanced at high pressures of hydrogen (4). The lack of an effect of hydrogen pressure may indicate, as suggested by Giles and Beatty (142), that even the residual aberrations in the absence of oxygen are due to direct ionization of chromosomes rather than to OH radicals. This conclusion obviously rests on the assumption that reactions in the virtually anoxic cell and in oxygen-free water are identical.

Biological Factors in the Oxygen Effect. From the biological point of view, it is well to ponder the greater dependence of low doses of x rays than of alpha rays on mitotic conditions (206, 314, 334) and also the greater restitution of chromosome breaks with the high ion density radiation (151). If the oxygen effect is related to mitotic phenomena or to restitution of broken chromosomes, such differences could complicate the interpretation of the relative effectivesness of oxygen for the different radiation qualities. However, there is no obvious correlation between the change in x-ray sensitivity of broad bean roots induced by anaerobiosis and the proportion of cells in divison at the time of irradiation (242). Moreover, the time lag for altering

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the sensitivity of the roots after changing the oxygen concentration of the water is not greater than 1 minute (271, 272). Giles *et al.* (141, 143, 145) have concluded that restitution of broken chromosomes is not an important factor in the oxygen effect on *Tradescantia*. In this preparation, rejoining of breaks does not take place for several minutes and the only critical parameter is oxygen tension during irradiation. The oxygen effect on *Tradescantia* chromosomes appears also to be independent of the intensity of x-irradiation, which further supports the view that restitution of breaks is not an important factor (276). A similar conclusion may be drawn from consideration of the oxygen effect on sex-linked lethals in *Drosophila* (178).

Interpretation becomes more involved, however, when we consider the influence of temperature on the oxygen effect. The yield of dominant lethal mutations in *Drosophila* (25) and of chromosome aberrations in *Tradescantia* (143) is increased when x-ray exposure is performed at low temperatures in the presence of oxygen. The increased yield may be attributed to the greater solubility of oxygen and to its diminished utilization at low temperatures. In the absence of oxygen, however, the effect is reversed; more aberrations are noted in *Tradescantia* chromosomes irradiated at high than at low temperatures. The reversal has been taken to suggest that the oxygen effect is probably not related to recombination of the split chromosomes. By way of contrast, it has been found recently that the percentage of autosomal recessive lethal mutations induced in *Drosophila* by ultraviolet radiation is actually greater when the exposures are made in the absence than in the presence of oxygen (236). Moreover, this difference is especially marked when the *Drosophila* are incubated at elevated temperature after irradiation. The picture with *Tradescantia* may also be contrasted with the temperature dependence noted for thymic cells suspended in plasma. Anoxia protects such cells against killing by x rays, but protection does not occur if the cell suspension is chilled while oxygen is being removed (37). Even when the anoxic suspension is prepared at room temperature, chilling for a brief period immediately after irradiation partially reverses the oxygen effect. Similar conditions of temperature in the presence of oxygen do not affect the lethal response of thymocytes to x rays. Other data also emphasize the difficulty of interpreting the oxygen effect solely in terms of immediate oxidative reactions in irradiated water. It has been observed, for example, that anoxic conditions after exposure to x rays exert almost as great a protective effect on the hematopoietic cells of tadpoles as similar treatment before and during the exposure (7). Similar protective effects have been noted in the case of poisoning with colchicine. It has been concluded that the protective influence of anoxia against both colchicine and x rays is in this instance related to changes in cell division.

It is appropriate to take cognizance of another form of oxygen sensitization to x-ray damage. Microorganisms capable of living either aerobically or anaerobically are more resistant in anaerobiosis. This phenomenon has been investigated with *E. coli* by Hollaender and his group (178, 181), who find that bacteria grown anaerobically but x-irradiated in the presence of oxygen are about one and one half times as resistant as their aerobic counterparts. On the other hand, bacteria grown and irradiated anaerobically are about 10 times as resistant as those grown and irradiated with oxygen available. It is noteworthy that the oxygen tension of the buffer solution in which the bacteria are irradiated determines the slope of the survival curve, while its shape is influenced by the environmental condition in which the organisms are grown (178, 181). The former suggests a change in the relative probabilities of competing reactions such as might be anticipated from the effect of oxygen on the com-

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binations of free radicals. The change in order of the survival curve undoubtedly reflects the altered cell physiology.

Influence of Blood Supply. Finally, we may include in our consideration of the oxygen effect the influence of blood supply on the radiosensitivity of the various animal tissues. Sensitivity of embryos increases as their blood supply is established (312). It is also well known that radiation injury to specific sites, e.g. skin, lymph node, ovary and tumor, parallels the blood flow during exposure (66, 109, 121, 194, 240, 243, 244, 293). Thus, damage to a limb is greatly diminished when the limb circulation is blocked, and tumor sensitivity varies with its vascularity. Since a factor common to all of these examples is a reduced oxygen tension, it is believed that hypoxia accounts for the modification of sensitivity. While this seems reasonable from the preponderant evidence in support of anaerobiosis cited previously, it has not been proved.

Epinephrine and Pitressin administered prior to x-irradiation have been shown to protect against local effects as well as against lethality (149, 265, 293). The influence of epinephrine and of Pitressin may be presumed to be a consequence of the decreased availability of oxygen, perhaps mainly because of changes in the blood supply. Effects of some other pharmacological agents may be mentioned at this time. The resistance of mice and rats to lethal doses of x rays is enhanced when *p*-aminopropiophenone is administered before irradiation, a possible explanation being the hypoxia resulting from methemoglobin formation (320). Maximum methemoglobinemia occurs, however, about 30 minutes after injection of this chemical, while optimum protection is obtained when the injection is made just prior to irradiation. Sodium nitrite, which produces a rather similar methemoglobinemia, gives equivocal protection (72, 170, 320). It has been suggested that a positive action of sodium nitrite when observed may be related to the accelerated decomposition of hydrogen peroxide (72). However, the importance of hydrogen peroxide in mammalian radiation toxicity remains to be demonstrated.

Several points of general interest may be noted in concluding the discussion of oxygen effects. We recall particularly the similarity in the degree of protection by anoxia against the production of chromosome aberrations, the inhibition of cell division and growth, and the death of the organism. Although this by itself does not establish causality, the coincidence is striking. It can be inferred from the uniformity of the protection that the oxygen effect takes place at a very early stage in the chain of radiation events, presumably on a common mechanism. These studies reveal further that genetic effects may result from indirect actions, a conclusion that is consistent with the demonstration of chemical mutagens and in particular with the mutagenic action of the inorganic and organic peroxides. Whether point mutations originate as a consequence of the indirect effects of irradiation is questionable. Oxygen dependence and independence with regard to radiation-induced mutations in the various organisms and in mutants of a single organism indicate that different mechanisms lead to the genetic effects of irradiation.

While it is almost axiomatic to search for an all-embracing mechanism, and, for the most part, the oxygen effect can be interpreted in terms of the diminished yield of oxidants in irradiated water, it is abundantly clear that this is by no means a certainty for all of the examples detailed in the preceding paragraphs. It is well, therefore, to continue to look for other effects of oxygen in regard to radiation sensitivity and at the same time to take advantage of the unique dependence of radio-

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chemical and biological effects on the rate of energy loss of the different ionizing radiations.

Chemical Protection

Numerous examples of chemical protection have appeared in the recent literature. The findings support the premise that many of the biological effects of ionizing radiations are indirect, a consequence mainly of the transfer of energy from water molecules to essential biological components. Interest in this section will be confined to substances that are believed to act at the level of the primary injury. Needless to say, the complexities of interpretation encountered in the oxygen effect, broadly defined as radiochemical versus biological, are even more in evidence in the case of protection by various chemicals. It is possible, nevertheless, to arrive at a reasonable approximation of the protective mechanism in some instances.

The simplest case is one in which two solutes compete for the active radicals formed in irradiated water. The protection of solutes, including enzymes and viruses, by bulk protein has already been considered. Although the protective power for equimolecular concentrations of different solutes is roughly proportional to their molecular weight, specificity also plays an important role in the protection. Thiourea is a very effective agent for the protection of carboxypeptidase in aqueous solution, while the closely related urea has little protective activity (87, 88, 90). In fact, sulfur compounds in general, i.e. colloidal sulfur and thiosulphate, have considerable effectiveness in protecting this enzyme. Another example of specificity in the protection of carboxypeptidase is the finding that the protective power of oxalate is increased by a factor of 200 when it is converted to formate (87, 88). Simple competition for free radicals is not always adequate to account for these effects, since the protective efficiency of thiourea, formate and glucose declines as their concentration increases (88, 90). More involved energy sharing mechanisms have been postulated to explain this phenomenon, e.g. chain reactions between free radicals and protector molecules, or temporary activation of protector molecules by the free radicals with subsequent transfer of the energy to the protected molecules.

Sulfhydryl and Related Substances. Considerable interest has been manifested in sulfhydryl substances as protectors against radiation injury. This is attributed in part to the demonstration by Barron *et al.* (31) that sulfhydryl enzymes are quite sensitive to oxidation when x-irradiated in dilute aqueous solution and that it is possible to protect and to reactivate such substances *in vitro* by addition of glutathione. On the other hand, emphasis has also been placed on the simple thiols as potential indicators of, and buffers for, the oxidants formed in irradiated water without regard to the question of whether the —SH group represents the sensitive site from the standpoint of radiation toxicity. We shall return to these considerations later. It is well to remember that sulfhydryl addition is similar to oxygen removal in the production of a number of biological effects.

Turning first to solutes in solution, glutathione added prior to x-irradiation reduces the inactivation of phosphoglyceraldehyde dehydrogenase (31), a sulfhydryl enzyme, and of ribonuclease (182), a nonsulfhydryl enzyme. Protection of the —SH enzyme by glutathione can be interpreted to mean that the oxidants formed in water are shared by the —SH groups of each substance. Yet, addition of glutathione immediately after irradiation with 200 r is nearly as effective as its addition prior to the exposure, even though the amount employed is only one-ninth of that added before irradiation (31). Reversal by glutathione indicates that enzyme inactivation is

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partly due to oxidation of sulfhydryl groups to disulfides. The efficiency of reactivation suggests that the protective effect of glutathione added before irradiation can also be attributed to a similar recovery process as the exposure continues rather than to competition for free radicals. The degree of reactivation diminishes with increase in radiation dosage; it has been inferred from this that nonspecific protein denaturation becomes progressively more important (31). With 500 r, the activity of phosphoglyceraldehyde dehydrogenase is 94 per cent inhibited, and there is only 10 per cent reactivation by glutathione. It is not known whether glutathione pretreatment will protect —SH enzymes against x-ray dosages of this magnitude, which, incidentally, are in the biologically effective range. Reactivation by glutathione is relatively slight in the case of alpha rays and is apparently independent of the radiation dose; reactivation has not been seen after beta radiation (31, 32). These differences may possibly be attributed to the great variations in the dose rates employed rather than to fundamental differences in action of the three radiations.

Glutathione has been shown to protect the nonsulfhydryl enzyme, ribonuclease, which requires about 13,000 r for 50 per cent inactivation in dilute solution (182). Protection in this case is most likely a result of the competitive sharing of free OH radicals. X-ray inactivation is not influenced by the absence of oxygen or the presence of catalase and can be induced by chemically generated OH radicals but not by hydrogen peroxide. This enzyme is not reactivated by glutathione and is protected only if —SH groups are present during exposure to x rays.

In contrast to these protective effects, cysteine and reduced glutathione have been shown to enhance the destructive effect of x rays on catalase in dilute aqueous solution from which oxygen has been removed (123). Catalase is protected, however, by cystine and oxidized glutathione. It is assumed that catalase is sensitive to reduction and that free hydrogen atoms are responsible for this action. In support of this, it may be noted that iodide ions, which are believed to decrease the amount of free hydrogen, also protect catalase against inactivation by x-radiation.

Cysteine added to thymus nucleoprotein solutions just before or after 50,000 r x-irradiation affords almost complete protection against the immediate decrease in viscosity (118). Of interest is the large increase in viscosity observed 3 to 4 hours after the addition of either cysteine or glutathione to nonirradiated alkaline solutions of nucleoprotein. Thiocyanate, cyanide and ascorbic acid also lead to some increase in viscosity of nonirradiated nucleoprotein, while BAL, thiourea, thiouracil, uracil, urea and cystine are ineffectual. It is significant that cysteine added prior to x-irradiation gives approximately the same viscosity increase at 3 hours as that observed in the nonirradiated cysteine nucleoprotein solution (118). On the other hand, the viscosity increase is greatly diminished when cysteine is added immediately after irradiation. These studies, which are based upon a single experiment, require amplification since they introduce the possibility of multiple actions by cysteine in regard to radiation sensitivity, namely as a buffer for radiotoxins and as a modifier of molecular orientation and structure. We may recall particularly the profound influence of molecular orientation on the radiosensitivity of pepsin (234, 235).

It is worthwhile to contrast the effects of cysteine with those of thiourea and anoxia. The immediate changes in x-irradiated nucleoproteins are independent of the presence of oxygen, but are prevented by addition of protein and are presumed to result mainly from indirect action. The delayed effects require oxygen during irradiation and may be attributed to either HO_2 or H_2O_2. Interpretation of the cysteine effect on irradiated nucleoprotein solutions is complicated by the dual nature of its

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action as noted above. Among other things, it is important to determine whether viscosity will be increased when cysteine is added subsequent to irradiation under anaerobic conditions. The immediate radiation effects on nucleoproteins *in vitro* are prevented by thiourea (220) as well as by cysteine, but the former does not affect the viscosity of nonirradiated nucleoprotein. Thiourea does not modify the initial changes in nucleoproteins *in vivo* (221); it does protect, however, against the more delayed radiation effects. These considerations illustrate the difficulties inherent in extrapolation from the simple to the more complex systems.

It is well known that the inactivation of viruses, cells and tissues depends on the nature of the medium in which they are exposed. Inactivation is less in a nutrient broth than in water or in a solution of inorganic salts. A number of organic substances—cysteine, cystine, glutathione, thioglycollic acid, tryptophan, glucose, ascorbic acid, alanine, gelatin and egg albumin—have been shown to protect bacteriophage suspended in aqueous solution (105, 133, 209, 230). The residual inactivation in the presence of broth or extraneous matter is generally believed to result from a direct action of radiation on the virus particles (230). It is noteworthy, therefore, that cysteine and BAL have been shown in preliminary studies to reduce the rate of x-ray inactivation of phage T2H suspended in nutrient broth or in a gelatin solution (96). It is not known whether the protective action in the presence of broth indicates a direct effect of cysteine or BAL on the virus particles or an interaction of these substances with toxic agents produced in the water of hydration. This finding is especially interesting in view of the effect of cysteine on the viscosity of nucleoproteins described previously. Ethanol and glycerol may also protect the virus in the presence of broth. Ethanol in very low concentrations has been shown to protect dilute solutions of trypsin against inactivation by x rays; the shape of the inactivation curve is altered (231), possibly indicative of a change induced in the trypsin molecule by ethanol.

Effects on cells. Cysteine, BAL (2,3-dimercaptopropanol), mercaptosuccinic acid and mercaptopyruvic acid have been shown by Burnett *et al.* (60) to increase the resistance of *E. coli* B/r suspended in phosphate buffer to the lethal effects of x-radiation. Protection of *Propionibacterium pentosaceum* by cysteine and to a lesser extent by thioglycollic acid and thiourea has also been reported (124); these results are not as detailed nor as impressive as the findings with *E. coli.* The sulfhydryl compounds must be present during irradiation in order to protect the bacterial suspensions; they apparently do not affect the growth rate of nonirradiated bacteria in the concentrations used. A word about the criterion is appropriate. Lethality is determined by transferring the x-irradiated suspensions after suitable dilution with nutrient broth to agar plates and counting the average number of colonies developing after 24 hours incubation at 37°C. (60). This procedure does not allow a distinction between immediate killing, delayed killing and delayed cell division. The dosages generally employed are in excess of 15,000 r or in other words are above the LD_{99} for *E. coli* as determined in this manner.

As noted previously for the oxygen effect, protection by sulfhydryl compounds is characterized by a change in the slope of the survival curves. The dose of x rays required to produce equivalent lethality in the presence of BAL is increased by a factor of approximately 4. Protection is greater with equimolar concentrations of BAL, a dithiol, than with the monothiols (60). Although propanol is also protective, much higher concentrations are required, and it is unlikely that the greater efficiency of BAL is in any way related to the propanol portion of the molecule. Protection of *E. coli* has been shown to vary directly with the concentration of —SH

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compounds in the aqueous medium up to an optimum of about 0.02 M. Cysteine and BAL are additive in their action at concentrations below 0.01 M (180).

It can be assumed that part of the effect on bacterial cells originates in the phosphate buffer medium. If this is true, protection by the sulfhydryl substances may also reside to a degree in the medium. The —SH compounds might then protect against the medium effect in two ways: by direct competition with bacterial cells for free radicals (OH and HO_2) and hydrogen peroxide that are formed in the medium; and secondly, by competition with free hydrogen atoms for the oxygen present in solution. That the first mechanism is operative, although not to the exclusion of the second possibility, is perhaps indicated by the claim that —SH compounds give some protection in oxygen-free suspensions (178), and that the dose reduction with BAL is slightly greater than with oxygen removal by nitrogenation (59, 60). Yet a similar situation exists with sodium hydrosulfite, which is believed to act exclusively by removal of dissolved oxygen (59). BAL and sodium hydrosulfite are also reported to give some additional protection when they are combined (180). The reasons for this are not obvious.

Hollaender (177, 181) has stated that a heavily irradiated medium is not toxic to *E. coli* added subsequently. Moreover, added hydrogen peroxide is not toxic to the bacteria unless the concentration is considerably in excess of that formed by reasonable amounts of radiation. The hydroperoxyl radical (HO_2) may thus be implicated as the major radiotoxin for lethal effects in bacterial suspensions. We recall, however, that the influence of an irradiated medium or added hydrogen peroxide may be conditioned by a change in the responding system in consequence of its irradiation (11). While the presumption is that oxidants are also formed within bacterial cells, their contribution is difficult to evaluate, since indirect effects from the medium undoubtedly play a substantial role in a system of this sort. It seems important to determine whether sulfhydryl compounds added to a medium containing an excess of protein will protect bacteria. If this is the case, the above arguments with regard to oxygen removal and competition for oxidants by sulfhydryl substances can be applied equally well to an intracellular site of action. In this instance, however, one must contend also with the possibility that part, if not all, of the protection may have a biological rather than a radiochemical basis.

There are, in fact, indications that these substances may protect in other ways or at least that the protective phenomena cannot be interpreted completely in terms of immediate oxidative reactions. Patt *et al.* (251) have observed that rabbit thymic cells suspended in a medium consisting of equal parts of homologous plasma and phosphate buffer are protected against x-radiation by cysteine. Thymic cells maintained in this manner almost never divide; surviving cells can be estimated by the method of unstained cell counts using eosin (291, 324). As with bacteria, sensitivity of thymic cells is decreased by a factor of about 2 when cysteine is added to the suspension. Protection depends upon cysteine concentration and time of administration but, significantly, is not a simple function of the sulfhydryl level during exposure (251). Cysteine addition 15 to 30 minutes before irradiation is optimum. Moreover, unlike the situation with bacteria and animals, there is a definite protective effect of cysteine added immediately after irradiation. This can be accounted for only to a slight extent by the persistence of toxic substances in the medium. It may perhaps be attributed to the reversal of a chain reaction or to the reconstitution of an injured site. A postirradiation effect of cysteine has also been described for onion epidermis cells (35).

The plasma medium apparently makes a negligible contribution to the toxic

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effect of x rays on thymocytes. The resistance of packed thymic cells and the failure of cysteine to protect them may be attributed to their hypoxic state. Cells equilibrated with oxygen before packing by centrifugation appear to be as sensitive as cells in suspension and are readily protected by cysteine (251). This may be interpreted as evidence that cysteine acts at the level of the cell, although whether on the cell surface or intracellularly cannot yet be stated. A cellular site of action is also implicated from studies with tumor fragments (158). In contrast to the report that —SH compounds give some protection to bacteria in oxygen-free phosphate buffer (178), it may be noted that removal of oxygen from the thymic cell suspension is as effective as cysteine but that the two are not additive (251). These results are consistent with the observations on packed thymic cells and suggest perhaps that cysteine action may be related to diminished availability of intracellular oxygen. If this is true, suboptimal deprivation of oxygen and suboptimal concentrations of cysteine may prove to be additive in their protection.

Other complications enter the picture, however, and it appears that oxygen deprivation alone is not the sole decisive event in the protection of thymic cells by cysteine. It has been observed that protection is dependent upon temperature during the first 30 minutes or so after irradiation (250). A brief period of chilling at 2°C. immediately after x-ray exposure completely reverses the protection. Temperature dependence of cysteine action before irradiation has also been shown (250, 292). Brief periods of chilling either immediately before or after irradiation do not affect thymocyte sensitivity. These findings may be contrasted with the report that sulfhydryl compounds protect bacteria equally at 2° and at 37°C. (317). We recall also that *Tradescantia* microspores are more resistant in the absence of oxygen at low than at high temperatures (143), but that thymic cells are not protected when chilled during the period of oxygen removal (37). There is no satisfactory interpfetation of these differences.

Effects on animals. Considerable information is available concerning sulfhydryl protection against radiation injury in mammals. Cysteine has been shown to protect mice and rats against acute lethality, i.e. deaths occurring within 30 days after irradiation (259, 264, 303); glutathione protection has been observed in mice, rats and dogs (68, 69, 259), and BAL protection in rats (302). These substances must be given before irradiation to be effective (256, 259). The optimum time of cysteine administration is immediately before exposure but injection as long as 1 hour before irradiation is still protective. The report (227) that several injections of cysteine and ascorbic acid during the first two postexposure hours improves the survival of rabbits has not been verified in either rabbits or mice (253). There is no satisfactory explanation for the apparent discrepancy in the time course of the protection in certain plant and animal cells as described previously and in the whole animal. The kinetics of the reactions with cysteine and the time constants for development of irreversible injury may differ in the isolated cells and in the intact animal.

It is significant that pretreatment with cystine or methionine does not alter radiation toxicity in animals (259). Although the amount of cystine that can be given in solution is limited by its relative insolubility, comparable dosages of cysteine afford definite protection (256, 303). It may be noted also that all sulfhydryl-containing substances and reducing agents do not modify sensitivity. In contrast to the results with bacteria described previously, neither mercaptosuccinate, mercaptopyruvate, nor hydrosulfite are effective protectors to irradiated mice (254). Ascorbic acid (259) and borotetrahydride (252) are also ineffectual. These differences may be a consequence of the temporal and spatial distribution and biological life of the various substances. Hydrosulfite and borotetrahydride, for example, are oxidized almost

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instantaneously upon intravenous injection. On the other hand, protection has been seen with thiourea (222, 238) and dithiophosphonate (238); the degree of protection by these substances is rather small, however.

Cysteinamine (mercaptoethylamine produced by decarboxylation of cysteine) is highly effective in protecting mice, and, in fact, has been shown by Bacq and Herve (23) to have greater effectiveness than cysteine in comparable dosage. It is somewhat more toxic, however, and as a consequence a greater radiation dose reduction may be achieved with cysteine (252). In view of the negative action of cystine, it is interesting to note that cystinamine also enhances the survival of mice when it is administered before irradiation (23). This can be attributed to the rapid formation of histamine after cystinamine injection. Large doses of histamine and of other amines have also been shown to protect mice (22, 23), possibly because of the decreased tissue oxygen tension resulting from vasodilatation and hypotension. Cysteinamine, on the other hand, does not liberate histamine. A more fundamental action of this compound is indicated also by the fact that it protects pea roots against the growth reduction induced by x rays; this is not the case with cystinamine (23).

In addition to lethality, a number of more specific radiation sequelae are diminished by prior injection of cysteine. These include lymphopenia, granulocytopenia, and anemia in mice and rats (256, 258, 278), splenic atrophy in mice (256), epilation in guinea pigs (124), greying of hair in mice (204), and lenticular opacities in rabbits (350, 351). Cysteine has also been shown to protect implants of the Walker 256 mammary carcinoma in rats against growth reduction following total-body x-irradiation (322). It would appear, therefore, that cysteine may raise the threshold for x-ray effects generally with the probable exception of those attributable to direct ionization or excitation. The action of cysteine and related compounds against repeated low dose irradiation has not been evaluated; protection against the more chronic sequelae of irradiation is also largely undetermined.

It may be remarked that cysteinamine is reported by Herve (169) to ameliorate clinical radiation sickness, which brings to mind an earlier observation (300) that several injections of glutathione can relieve the discomfort sometimes seen after therapeutic irradiation. Evaluation of the many prophylactic and therapeutic agents that have been recommended for the clinical syndrome, which represents only a part of the total radiation picture, does not fall within the scope of this review. We may inquire, however, about the advisability of employing agents that manifest a more or less universal action against radiation effects to prevent clinical radiation sickness. Unless selective protection can be accomplished, the purpose of the therapy may be defeated in that the area to be treated may also show an increased radioresistance.

The degree of protection by cystine or glutathione is dependent upon the dose that is injected (79, 256, 303). The gradual decrease in effectiveness with increase in the time between administration and irradiation is doubtless related to the rapid clearance and metabolism of these sulfhydryl compounds. There is a rather uniform distribution of cysteine throughout the body during the first few minutes after its injection intravenously (260). With time after injection, both cysteine and glutathione tend to concentrate in the kidneys, liver, spleen and intestine (81, 260). There is no obvious relationship between the difference in concentration in the various tissues and the time course of the protection (260); it will be recalled that injection immediately before irradiation is optimum. It is of interest that cysteine protection against acute lethality is more efficient in abdomen-shielded than in abdomen-irradiated rats (329); the reason for this is not apparent.

It has been suggested that sulfhydryl substances, specifically glutathione, may

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protect animals by promoting regenerative mechanisms rather than by preventing initial cellular destruction (79, 80). Data upon which this supposition is based do not, however, constitute proof of the failure to prevent injury. Histological changes, in general, reflect the amount of radiation and not the lethal or morbid effect on the animal (39, 93). Furthermore, the threshold point of maximal or near maximal change for a number of radiation effects is below that required for acute lethality. It is also generally recognized that quantitation of hematological and organ weight changes is exceedingly difficult in the narrow lethal dose range. By way of illustration, the difference in splenic and thymic involution between 600 and 800 r is only about 5 per cent (65). These considerations can explain the failure to observe a protective effect of glutathione, which in the concentration used results in a dose reduction of about 25 per cent, on the early changes in organ weights, blood and tissues (79, 80).

As contrasted with the above view, there is a body of evidence, which has already been alluded to, indicating that sulfhydryl compounds can diminish cellular destruction incident to x-ray exposure. It has been observed, moreover, that cysteine affords a fairly uniform protection over a wide range of radiation doses against lethality, splenic atrophy, leucopenia, lymphopenia and granulocytopenia (256). Uniformity of the protection by cysteine against a number of radiation sequelae in the intact animal points to a true dose reduction in the sense that it alters a common pathway. This suggests that the decisive action occurs at an early stage in the chain of events but does not necessarily imply protection against all radiation changes insofar as different primary mechanisms may be involved in their development.

The cysteine effect is manifest by a change in the slope of the radiation dose-response curve for all of the effects that have been studied, or, in other words, the dose reduction consists of the deletion of a proportional amount of the radiation effect (256). The magnitude of the change depends upon the cysteine dose; sensitivity is decreased by a factor of about 2 with the maximally tolerated cysteine dose. Similar proportionalities have been seen with bacteria (60) and thymic cells (251). Patt *et al.* (256) have observed that the biological additivity of two briefly spaced x-ray doses in mice is a direct function of the cysteine dose preceding each exposure. A similar additivity has been observed for the oxygen effect on broad bean roots (273).

In general, the observations on animals are consistent with the assumption that sulfhydryl substances serve as absorbing material for the oxidants presumably formed in intracellular water. Competition for the decomposition products of water may be indirect in that cysteine may act by diminishing the availability of oxygen and hence the yield of certain of the oxidants, e.g. HO_2. Protection by cysteine is enhanced significantly when mice are allowed to breathe 10 per cent oxygen during irradiation or are injected with dinitrophenol prior to the exposure (252). Neither 10 per cent oxygen nor dinitrophenol alone is protective; however, when animals receive both treatments radiation lethality is diminished. If cysteine protection is indeed related to decreased availability of oxygen, we should perhaps anticipate differences in its effectiveness against radiations differing in ionization density. There are no available data concerning this point.

With regard to a more specific effect of the sulfhydryl substances, e.g. in terms of protection or reactivation of sulfhydryl enzymes, there is no direct evidence, and little reason to assume, that the —SH group represents the sensitive spot from the standpoint of radiotoxicity. While sulfhydryl enzymes may be inhibited by ionizing

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radiations under certain conditions, evidence for their selective inhibition *in vivo* is equivocal (28, 95, 99, 218, 308). As to reactivation of these enzymes, it is well to point out that —SH enzymes can be reactivated *in vitro* only after low dosages of x rays (31). Enzyme inactivation after dosages comparable with those required for lethality in animals has been attributed to protein denaturation (31); there is, however, no evidence for the latter with dosages of this magnitude, e.g. 500 r.

It is of interest that x-irradiation has no immediate effect on sulfhydryl levels in a number of tissues (122, 127, 257, 260, 295, 332). This is consistent with theoretical considerations; the available sulfhydryl in tissue exceeds by several orders of magnitude the amount that could be oxidized directly by lethal dosages of irradiation even in the absence of the naturally occurring protective substances. More delayed changes in sulfhydryl concentration have been observed in irradiated tissues, but these appear to be a consequence rather than a cause of the injury (295, 332, 366). Similar effects have been reported for a variety of conditions and are apparently nonspecific (296). Although some of the cellular effects of sulfhydryl reagents, e.g. heavy metals, and of x rays are similar (29), sulfhydryl poisons in general do not reproduce the radiation syndrome. Radiation toxicity is not affected when *p*-chloromercuribenzoate, a sulfhydryl complexing agent, is administered to mice either before or after whole-body x-irradiation (255). Postirradiation cysteine can reverse the effect of a toxic dose of mercuribenzoate given prior to exposure, but radiation toxicity is unaltered under these conditions. In evaluating these results, it must be remembered that a sulfhydryl reagent almost certainly does not attain the uniform distribution of penetrating radiation.

The toxicity resulting from nitrogen mustards (52, 357) or alloxan (212, 213) in rats and rabbits is also prevented by the simultaneous administration of cysteine. As with x rays, treatment after injection of these toxic agents is generally ineffectual; a slight protective effect does occur when cysteine is injected within 1 minute after alloxan injection. The protective effect against nitrogen mustard poisoning is reported to be specific for cysteine (52), whereas glutathione, thioglycollic acid and BAL are also protective against alloxan (212, 213). Alloxan toxicity may perhaps be attributed to the selective inactivation of —SH groups in the islet cells of the pancreas (212, 213). Although nitrogen mustards may form alkylated compounds with thiols, a variety of atomic groupings react with the nitrogen mustards, and interpretation of the protective effect of cysteine is not readily apparent (52).

In concluding, it should be emphasized that as in the case of the oxygen effect we cannot dismiss readily the idea that several modes of action may prevail in the protection of the various systems by sulfhydryl substances. The possibility remains that these substances may protect by altering the nature of the biological targets or the biochemical pathways as well as by modifying the reactions of activated water. Information relating to the relative effectiveness of these protective substances for the different qualities of radiation should aid materially in the elucidation of their mechanisms of action.

Metabolites. A number of metabolites are reported to afford protection against x-ray effects. It is generally believed that such substances are effective because they serve as oxygen acceptors. The inactivation by x rays of strychnine in dilute aqueous solution is diminished by glucose as well as by other oxygen acceptors including sodium nitrite, ferrous sulfate and stannous chloride (208). Solutes that do not accept oxygen, e.g. sucrose, sodium chloride, sodium nitrate, ferric sulfate and stannic chloride, are not protective. It is of interest that massive amounts of glucose given prior to x-irradiation diminish cutaneous effects in rabbits (19) and mortality in rats

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(226). Conversely, pretreatment with insulin augments cutaneous radiosensitivity in the rabbit, presumably because of hypoglycemia (346). Some degree of protection has been achieved in mice by the injection of large amounts of ethanol (249) and of glycols (284). These protective effects may also be related to relative oxygen deficiency.

The lethal and mutagenic effects of both ultraviolet and x-radiations are reduced when pyruvate is added to bacteria suspended in broth (338). Protection ensues only when pyruvate is present during irradiation. Pyruvate also protects bacterial cells from the lethal action of hydrogen peroxide, presumably because of the rapid reaction between these two substances. The absence of an after-effect of pyruvate has been interpreted as further evidence that hydrogen peroxide is not the mutagenic agent in this biological system. Protection of *E. coli* B/r against the lethal effects of x rays has also been achieved by the addition of either formate, succinate, pyruvate, serine or ethanol to the bacterial suspension prior to irradiation (317). Unlike the previously cited experiments where the presumption is that preliminary incubation of bacterial cells with pyruvate is not necessary for protection, in the latter experiments, the effect has been shown to depend upon a period of incubation. Definite protection occurs only when the metabolites are incubated (37°C.) with the bacterial cells for 30 minutes; incubation at 2°C. results in a negligible effect (317). A notable exception is the finding that large quantities of ethanol protect *E. coli* without preliminary incubation. The actions of cysteine, BAL and hydrosulfite on bacterial suspensions are also temperature independent.

These findings suggest some metabolic intervention in the protection by formate, succinate, serine, ethanol in small quantities, and perhaps pyruvate. It may be noted that β-alanine, which is only slightly utilized as an oxidative substrate by *E. coli*, is not effective, whereas α-alanine, which is oxidized reportedly at a rapid rate, affords good protection (317). The effectiveness of some of these metabolites is greatly diminished in the presence of either cyanide or iodoacetate (317). Neither cyanide nor iodoacetate alone exerts an appreciable influence on the radiosensitivity of *E. coli*. The generalization has been made that loss of protection is related to respiratory inhibition and, therefore, that oxygen removal from the bacterial cells or their immediate environment is the primary mode of action by these metabolites (317). Inspection of the data, however, reveals inconsistencies in the computed percentage loss of protection by cyanide and iodoacetate and in the relationship of respiratory inhibition to loss of protection. One may inquire about the probability of achieving an effective diminution of cellular oxygen as a consequence of metabolism in a system of this sort, since the oxygen gradient between the small bacterial cells and the suspending medium is probably relatively slight. Protection by pyruvate has been shown to occur in a bacterial suspension that is aerated continually during the period of irradiation (338).

Latarjet (208) has expressed the view that some of the oxygen acceptors, e.g. glucose, may protect not by removal of oxygen but rather because they serve as hydrogen donors. The hydrogen is presumed to combine with certain free radicals, e.g. HO_2, to form hydrogen peroxide, which is considered to be a subsidiary detoxification product. It is interesting to note that cysteine, ascorbic acid and aldehydes are also reported to increase the yield of hydrogen peroxide in water exposed to x rays (225). The idea that an increase in hydrogen peroxide may signify a decrease in the yield of a biological reaction stands in sharp contrast to the importance that has been attached to this substance as a primary radiotoxin.

Enzyme Inhibitors. Cyanide exerts some protective action against x rays in the

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mouse (20, 21, 23, 24). Survival is enhanced significantly only when it is injected prior to exposure. Cyanide does not appear to be as effective as the sulfhydryl-containing substances in terms of radiation dose-reduction. In contrast to the protective action in mice, a cyanide effect has not been observed in rats (98) and frogs (262). Bacq *et al.* (20, 23, 24) have suggested that cyanide may protect the mouse by inhibiting the formation of peroxides by X rays and perhaps by forming a loose bond with sulfhydryl groups to prevent their oxidation. Nitrides have also been shown to afford a small degree of protection to mice (21); the heavy metals, fluorides, fluoroacetate, malonate and mercuribenzoate are ineffectual, however (20, 254, 255). Simultaneous administration of cyanide and cysteine to rats does not modify the protective action of the latter (302). The possibility that the cyanide effect in the mouse may be a consequence of anoxia cannot be dismissed readily; species specificity to cyanide may be related to its actions on pulmonary and tissue respiration which work in opposite directions.

Cyanide has, in fact, been shown to potentiate radiation effects in *Vicia faba* roots (241) and in tumor cells (78). Iodoacetate and sodium fluoride, which like cyanide and oxygen deficiency inhibit aerobic glycolysis, do not affect the sensitivity of tumor cells (78). Potentiation of tissue sensitivity by previous treatment with cyanide may be due to an increase in cellular oxygen tension as a consequence of inhibition of the cytochrome system or to inactivation of catalase. The former seems more likely. Hall (157) has observed that cyanide does not alter the radiosensitivity of tumor fragments in the absence of oxygen and, moreover, that the cyanide effect is related directly to depression of oxidative metabolism in tumor slices. In further support of this, the sensitivity of tumor fragments in air is an inverse function of their size, the implication being that the more centrally located cells are relatively anoxic and that the anoxic area varies directly with the size of the tumor fragments (157). We may recall that cyanide does not influence appreciably the sensitivity of bacterial suspensions. In this instance it may be assumed that there is a very small oxygen gradient from the medium to the individual cells.

PH Effects. In contrast to the large body of information on the effects of oxygen and related chemicals, there are few data concerning the influence of PH on tissue radiosensitivity. The frequency of chromosome aberrations in plant cells is reduced when dilute solutions of ammonium hydroxide are applied prior to X-ray exposure (233). This may perhaps be related to the decreased yield of hydrogen peroxide at alkaline PH (49). Germinating fern spores, *Drosophila* eggs and *Paramecia* have been exposed to X rays in the presence of different concentrations of carbon dioxide and ammonia (369, 371, 372). Radiosensitivity increases to a maximum and then declines as acidity is increased by carbon dioxide. With increase in PH, sensitivity first declines to a minimum value and then increases. Zirkle (369, 371, 372) has compared this behavior with the effect of irradiation on proteins where maximal flocculation is observed at a PH near the isoelectric point. He considers the effects of PH to be consistent with the belief that total sensitivity is due to the added effects of several reactions having maximum yields at different hydrogen ion concentrations. Further experimentation is needed in this rather neglected area. In addition to PH effects on radiochemical reactions (29, 49, 91), changes in hydrogen ion concentration per se may contribute to radiation injury.

Temperature and Related Phenomena

The influence of temperature on radiosensitivity has received considerable attention. Temperature studies are especially prominent in the early radiobiological

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literature. Many of the reports are conflicting and virtually every conceivable effect has been described. From a physical point of view, there is no reason to believe that the primary energy transfer resulting in ionization is dependent on temperature. For example, the splitting of hemocyanin molecules by irradiation is temperature-independent (328). Reactions of injury and of recovery must follow rapidly, however, and these may appear in some degree even before irradiation is terminated. It is perhaps not surprising that many types of temperature responses have been described, since the time course and temperature coefficients of the secondary reactions must vary for different effects and different biological systems. Although it is not possible to review here the voluminous literature on this subject, it is appropriate to attempt some interpretation of the temperature effects in the light of our present understanding of the mechanisms of radiation action.

For reasons that are already apparent, it is necessary to distinguish the events occurring during irradiation from those taking place after the exposure. Sensitivity of *Ascaris* eggs (183) and *Drosophila* eggs (246) and of infant mice and rats (108, 110, 165, 205) is increased by elevation of temperature during irradiation. Yet temperature is without influence on *E. coli* (214), eggs of *Nereis*, frog, and hen (13, 100, 274), wheat seedlings (168), thymic cells (290), tadpoles (5, 6) and frogs (262). Moreover, a cold environment is reported to increase sensitivity of broad bean roots (241) and of tumor tissue (78) to the growth-retarding effects of irradiation and also to increase the number of dominant lethal mutations in *Drosophila* (25).

Certain temperature responses during irradiation, e.g. on *Ascaris* and *Drosophila* eggs, may be attributed to concomitant changes in cell division (183, 246). Other effects appear to be a consequence mainly of changes in blood flow or in oxygen tension. For example, the beneficial effects of chilling the skin (66, 108, 110) can be ascribed to changes in the vascular bed. The early observation (240) in which cooling increased the sensitivity of rat tail epithelium is consistent with this explanation, since irradiation took place during the hyperemic reaction to cooling. Decreased lethality of the chilled newborn mammal (165, 205) may, likewise, be due to lowered oxygen tension resulting from the relatively greater depression of breathing than of tissue respiration in the cold. A definite temperature effect has not been observed in infant animals breathing low oxygen mixtures (164). In contrast, the increase in dominant lethal mutations in *Drosophila* (25) and the enhanced sensitivity of plant and tumor tissue (78, 241) when irradiation is performed in the cold can be related to an increase in cellular oxygen tension. The *in vitro* sensitivity of tumor tissue is not affected by low temperatures in the absence of oxygen (157).

Other effects of temperature during X-ray exposure have been attributed to an influence on recovery processes. Fewer chromosome aberrations appear in *Tradescantia* pollen irradiated at normal or above normal temperatures than at temperatures around 0°C. (112, 286, 287). It is believed that there is little influence of moderate temperature change on the production of chromosome breaks, but that reconstitution of the broken ends is temperature dependent. We may recall that there is essentially no difference in the yield of hydrogen peroxide with X-rays between 0° and 20°C. (48). On the other hand, hydrogen peroxide production declines abruptly at temperatures below 0°C. and can no longer be detected at about −120°C. It is of interest that the number of chromosome breaks induced by X rays in *Tradescantia* pollen is diminished below 0°C. and then declines gradually as temperature is decreased further (113). Chromosome aberrations are still observed, however, at −192°C. Whether the relationship between the yield of hydrogen peroxide and the

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production of breaks in this instance is real or fortuitous requires amplification. The magnitude of the residual aberrations induced by x rays in *Tradescantia* at −192°C. or in the absence of oxygen is rather similar (113, 142, 143).

The inactivation efficiency of dried bacteriophage exposed to x rays in vacuo is decreased slightly when temperature falls from 37° to −196°C. (18). Physical contraction of the virus particles is apparently not sufficient to account for this effect. The small decrease in the inactivation rate with temperatures below 37°C. is of the order that would be anticipated from an interaction with highly energetic free radicals. Some water is doubtless present even in a dried preparation of this sort. A residual inactivation, calculated by extrapolating the results to −273°C., may be considered as a measure of the direct inactivation of the particles. Whether such extrapolation reveals the true picture at the absolute zero is another matter.

Although radiosensitivity may be influenced by extreme temperature changes, perhaps because of altered formation and diffusion of free radicals or of some alteration in configuration of the various biological targets, changes of temperature compatible with most living systems do not modify directly the immediate radiation reactions, at least within the limits of experimental error. Temperature independence over this range is consistent with the concept of indirect as well as of direct action, since the free radicals must have zero or nearly zero energies of activation.

The events subsequent to energy transfer, on the other hand, appear to be rate-sensitive. Since recovery may also be influenced, the ultimate outcome is not always changed (6, 76, 262). Such considerations are not confined to radiation injury. Temperature depression after irradiation has been shown to decrease the rate of development of injury in a variety of cells and tissues (70, 100, 299, 321, 327, 331) in amphibians (5, 6, 262) and in hibernating mammals (304). A rather specific effect of temperature on recovery has been observed in bacteria (179). Postirradiation incubation for 24 hours at certain reduced temperatures increases survival; the optimum reduced temperature varies for different strains of bacteria. This type of recovery is reported to occur only in the presence of certain media.

Other findings agree with the concepts discussed in connection with the effects of temperature. An increased metabolic activity after exposure to ionizing radiation induced by thyroid extract or dinitrophenol (41, 307), a cold environment in non-acclimatized mammals (165, 306), or exhaustive exercise (301) enhances lethality. Thiouracil and thyroidectomy are without appreciable influence on adult mammals (41, 156, 165, 307). Desiccated thyroid fed to mice both before and after x-irradiation leads to the same potentiation of lethality as treatment begun after exposure (307). Pretreatment alone fails to modify lethality and, moreover, does not alter the mitotic changes induced in irradiated epidermis (305).

The results with anesthetics and depressants are somewhat conflicting. Animals irradiated while anesthetized with Nembutal may (232) or may not (165, 249, 252) show a decrease in radiosensitivity. It is not known whether protection, when observed, can be attributed to depression of the respiratory center and consequent anoxia. A similar situation may apply also to the protective action of large doses of morphine in mice (195). The protective effect of alcohol in mice noted earlier is apparently unrelated to its anesthetic action (249). Urethane anesthesia has been found to increase lethality in x-irradiated rats (166) but not in mice (249). While urethane is generally considered to be a radiomimetic agent, the reason for this specificity in its action is not apparent.

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Miscellaneous Physiological States

A number of observations suggest that injury and recovery are, to some extent, dependent upon an interaction between irradiated and nonirradiated areas. The many effects produced at sites distant from an irradiated area are obvious departures from a purely local action. As might be anticipated some physiological interplay is evident between irradiated and adjacent nonirradiated areas and, in fact, the latter may play an important role in recovery processes. The severity of cutaneous erythema, for example, is related to the size of the irradiated field and two fields a distance apart show less injury than areas that are closer together (190, 191, 193). A protective effect of nonirradiated tissue is also apparent in corneal epithelium when areas of different size are exposed to X rays (325). When tumors are irradiated *in vivo* and then transplanted into nonirradiated animals, the percentage of successful transplants varies inversely with the time the tumors remain in the original host after exposure (326). Experiences with irradiation of tumors through various sized grids indicate that fractionation of the dose in space as well as in time influences tumor destruction (192). Nutritional studies are also revealing; a low protein diet enhances the initial inhibition of tumor growth by X rays, while a high protein diet facilitates reconstitution of the irradiated region (103). Development of ovarian tumors in mice requires irradiation of both ovaries (219) and induction of lymphoma in the same species also requires irradiation of a large part of the susceptible tissue (197).

Injury to specific regions, e.g. a lymph node or a tumor, is generally more severe after a total-body exposure than after local irradiation. This may be attributed to the liberation of nonspecific toxic materials from irradiated tissue and/or to a sparing action of nonirradiated tissue. While some evidence for circulating factors has been obtained, their significance is not fully appreciated. It is significant, however, that parabiosis, cross-circulation, and early blood transfusion have been shown to diminish radiation toxicity (27, 53, 285, 330, 345); early *in vivo* dialysis appears to be ineffectual at least in the dog (211). Potentiation of local radiation effects by total-body exposure may be contrasted with the protection afforded against the latter by shielding small volumes of tissue. Shielding of the spleen, liver, head, extremities, or other relatively small areas will decrease mortality from an otherwise complete irradiation (2, 8, 137, 185, 187). Recovery of hematopoietic tissue and of the antibody response is also more rapid after subtotal irradiation (45, 185, 188, 275). It may be noted that a radioisotope with osseous distribution and one with reticuloendothelial distribution are synergistic with respect to lethality (131).

The role of the shielded spleen has been investigated most intensively, especially by Jacobson and his collaborators (185). These studies have been conducted chiefly with mice, for it is in this species that the most striking effects of spleen shielding have been observed, possibly because of the spleen's primitive role with regard to hematopoiesis. Spleen shielding offers relatively little protection to the rat and rabbit against radiation lethality, although qualitatively similar protective effects are observed with regard to the blood-forming tissues (185). Likewise, spleen homogenates and transplants are highly effective in the mouse when given during the first hours after exposure (73, 185, 189); whereas homogenates exert only a slight influence in the rat (73).

Even in the mouse there are revealing age and strain differences. Spleen shielding is relatively ineffective in the puberal mouse (185), a fact that cannot be attributed simply to the increase in radiosensitivity during this stage in development.

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Transplantation of spleens from mice of the same age level may or may not reduce the mortality of irradiated adult mice (185, 319). The effectiveness of spleen shielding also depends upon the animal strain; there is a relatively small effect in adult C-57 black mice as compared with adult strain A mice (198). Strain specificity is also apparent when splenic transplants are made in x-irradiated mice. It has been reported that adult spleens are effective only when the donor belongs to the same inbred strain as the recipient (319). Specificity is lost, however, when the donor mice are less than 10 to 15 days old. This suggests that the age effect noted above may be a consequence of some intrinsic difference in the spleens themselves; in view of the conflicting evidence (185), this point must be regarded as unsettled.

It has been shown recently that intravenous or intraperitoneal injection of homologous bone marrow immediately after x-irradiation improves survival in the mouse and guinea pig and, in fact, that heterologous marrow also has considerable effectiveness (228, 229). Heterologous spleen transplants are also reported to facilitate recovery of hematopoietic tissues (185), but a protective influence has not been seen against lethality (319). Positive effects with heterologous tissues do not necessarily implicate a humoral factor, since it is known that normal tissue can survive and proliferate in an x-irradiated heterologous host (341). Moreover, strain and species differences are also in evidence here; homologous transplants of bone marrow do not influence recovery appreciably in certain mouse strains (319), dogs (275) and rats (333).

It has been suggested that shielded tissues and transplants elaborate recovery principles, and that such activity may occur *in situ* or after preliminary colonization of other areas by living cells (185). The postulation of a humoral recovery mechanism is challenged, however, by species and strain differences already alluded to and by the conflicting evidence with regard to the efficacy of heterologous transplants. Moreover, cell-free spleen extracts have shown no activity under the conditions in which they have been used (185). It is also of interest that marrow suspended in distilled water is not effective in contrast to the effectiveness of an isotonic saline suspension (323). Under the circumstances, colonization of injured sites by non-irradiated cells, or a more transient functional existence of these cells either *in situ*, in the blood, or in the reticulo-endothelial system must also be regarded as possible mechanisms.

It is worthy of emphasis that the modifying factors discussed in this section are apparently concerned with events responsible for injury to specific physiological systems or to recovery from such injury. The protection afforded to animals by pretreatment with large doses of estrogens (261, 342), foreign protein (148, 163) and terramycin (153) are other examples of rather specific procedures. Such protective devices may be contrasted with other agents or situations, e.g. oxygen and certain chemicals, which exert a more universal and presumably primary influence against the biological actions of ionizing radiations.

General Implications of Protective Phenomena

Interpretation of the various protective phenomena is necessarily incomplete, when we reflect that there is no direct evidence concerning the early physical and chemical events in irradiated tissue. It is possible, however, to make some generalizations about the mechanisms of radiation effects on the basis of present understanding of the modifying factors. The more important implications may be restated in concluding.

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It is recognized that ionizing radiations may act in several ways depending upon the biological object, its environment, and the conditions of irradiation. Much attention has been focused on reactions in irradiated water, and the tenuous extrapolation from water to protoplasm has been fruitful. The existence of free radical mechanisms *in vivo* as well as *in vitro* is suggested by the rather parallel influence of oxygen and of protective substances on the behavior of simple aqueous systems and of living systems to x rays. This is not to say that all of the protective effects are necessarily related to modification of the pathways of energy dissipation in water nor that all biological systems are influenced in the same manner. There is, in fact, evidence that cannot be interpreted solely in terms of immediate oxidative reactions by activated water, and it is necessary to think of other actions of the modifying agents. We recall that radiosensitivity may be affected profoundly by molecular configuration and distribution and by the quality of the metabolic pathways. The ultimate expression of injury and of recovery is also related to the physiological state of affairs. The rapidity with which biological processes take place after the primary injury is inflicted may be of decisive importance in certain instances.

It is worthwhile also to reiterate a few of the basic problems and questions that remain unanswered or deserve attention. Foremost among these is the determination of the relative effectiveness of the various protective factors for the different qualities of radiation. The complementary parameter of dose rate should be included in such comparisons. Although many of the protective factors probably act intracellularly, the site of action is not easily resolved. Differentiation in cell suspensions would perhaps be facilitated if sufficient protein were present to neutralize indirect effects from the medium. This type of medium has the further advantage of being more nearly comparable to extracellular fluid *in vivo*. The effects of enzyme inhibitors, chemical analogues and temperature can provide further information along these lines. The desirability of evaluating the more impressive protective agents in terms of the totality of radiation effects in a given organism rather than of a single radiation effect is also clear. Further understanding in these areas should contribute materially to the elucidation of the various protective mechanisms.

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REFERENCES

1. Abrams, H. L. *Proc. Soc. Exper. Biol. & Med.* 76: 729–732, 1951.
2. Abrams, H. L. and H. S. Kaplan. *Stanford M. Bull.* 9: 165–166, 1951.
3. Alexander, P. and M. Fox. *Nature, London* 169: 572–574, 1952.
4. Allen, A. O. *J. Phys. Colloid Chem.* 52: 479–490, 1948.
5. Allen, B. M., O. A. Schjeide and L. B. Hochwald. *Proc. Soc. Exper. Biol. & Med.* 73: 60–62, 1950.
6. Allen, B. M., O. A. Schjeide and L. B. Hochwald. *J. Cell. & Comp. Physiol.* 38: 69–82, 1951.
7. Allen, B. M., O. A. Schjeide and R. Piccirillo. *Univ. California at Los Angeles Report No. 149*, 1951.[1]
8. Allen, J. G. *Argonne National Laboratory Report No. 4625*, 60, 1951.[1]
9. Allsopp, C. B. *Brit. J. Radiol.* suppl. 1: 43–45, 1947.
10. Allsopp, C. B. *Brit. J. Radiol.* 21: 72–74, 1948.
11. Alper, T. *Nature, London* 169: 964–965, 1952.
12. Altman, K. I., J. Richmond and K. Saloman. *Univ. Rochester Report No. 169*, 1951.[1]
13. Ancel, P. and P. Vintemberger. *Compt. rend. Soc. de biol.* 97: 796–799, 1927.

[1] Information concerning the availability of this document may be obtained by addressing inquiries to the Office of Technical Service, Department of Commerce, Washington 25, D. C.

0018380

14. ANDERSON, E. H. *Proc. Nat. Acad. Sc.* 37: 340–349, 1951.
15. ANDERSON, R. S. AND B. HARRISON. *J. Gen. Physiol.* 27: 69–75, 1943.
16. ANDERSON, R. S. AND H. TURKOWITZ. *Am. J. Roentgenol.* 46: 537–541, 1941.
17. ARNOW, L. E. *J. Biol. Chem.* 110: 43–59, 1944.
18. BACHOFER, C., C. EHRET, S. H. MAYER AND E. L. POWERS. Unpublished data.
19. BACLESSE, F. AND J. LOISELEUR. *Compt. rend. Soc. de biol.* 141: 743–745, 1947.
20. BACQ, Z. M. *Experientia* 7: 11–19, 1951.
21. BACQ, Z. M. AND A. HERVE. *Brit. J. Radiol.* 24: 617–621, 1951.
22. BACQ, Z. M. AND A. HERVE. *Nature, London* 168: 1126, 1951.
23. BACQ, Z. M. AND A. HERVE. *Bull. Acad. roy. de méd. de Belgique* 17: 13–58, 1952.
24. BACQ, Z. M., A. HERVE, J. LECOMTE AND P. FISCHER. *Science* 111: 356–357, 1950.
25. BAKER, W. K. AND E. SGOURAKIS. *Proc. Nat. Acad. Sc.* 36: 176–184, 1950.
26. BANCROFT, G. AND V. E. KINSEY. *Biochem. J.* 31: 974–979, 1937.
27. BARNES, W. A. AND O. B. FURTH. *Am. J. Roentgenol.* 49: 662–681, 1943.
28. BARRON, E. S. G. *Atomic Energy Commission Document, No. 2316*, 1946.[1]
29. BARRON, E. S. G. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 216–240.
30. BARRON, E. S. G. AND V. BONZELL. *Argonne Nat. Lab. Report No. 4467*, 1950.[1]
31. BARRON, E. S. G., S. DICKMAN, J. A. MUNTZ AND T. P. SINGER. *J. Gen. Physiol.* 32: 537–552, 1949.
32. BARRON, E. S. G. AND S. DICKMAN. *J. Gen. Physiol.* 32: 595–605, 1949.
33. BARRON, E. S. G., V. FLOOD AND B. GASVODA. *Biol. Bull.* 97: 51–55, 1949.
34. BARRON, E. S. G. AND V. FLOOD. *J. Gen. Physiol.* 33: 229–241, 1950.
35. BELLOCK, S. AND A. T. KREBS. *Army Medical Research Laboratory Report No. 6-64-12-06-(43)*, 1951.
36. BERGONIÉ, J. AND L. TRIBONDEAU. *Compt. rend. Acad. Sc.* 143: 983–985, 1906.
37. BLACKFORD, M. E. AND H. M. PATT. *Argonne Nat. Lab. Report No. 4840*, 1952.[1]
38. BLOOM, M. A. *Radiology* 55: 104–115, 1950.
39. BLOOM, W. *Radiology* 49: 344–348, 1947.
40. BLOOM, W. (editor). *Histopathology of Irradiation from External and Internal Sources*. New York: McGraw-Hill, 1948.
41. BLOUNT, H. C., JR. AND W. W. SMITH. *Science* 109: 83–84, 1949.
42. BLUM, H. F., J. C. ROBINSON AND G. M. LOOS. *J. Gen. Physiol.* 35: 323–342, 1951.
43. BOCHE, R. D. AND F. W. BISHOP. *Manhattan District Declassified Document No. 250*, 1946.[1]
44. BOELL, E. J., M. RAY AND J. H. BODINE. *Radiology* 29: 533–540, 1937.
45. BOFFIL, J. AND O. MILETZKY. *Ann. méd.* 47: 220–244, 1946.
46. BONET-MAURY, P. *Sixth Internat. Cong. Radiol. Abst.*, 1950, p. 42.
47. BONET-MAURY, P. AND M. FRILLEY. *Compt. rend. Acad. Sc.* 218: 400, 1944.
48. BONET-MAURY, P. AND M. LEFORT. *Nature, London* 162: 381–382, 1948.
49. BONET-MAURY, P. AND M. LEFORT. *J. Chem. Physiol.* 47: 179–183, 1950 or *Nature, London* 166: 981–982, 1950.
50. BOYLAND, E. *Ann. Rev. Biochem.* 18: 217–242, 1949.
51. BRACE, K. C. *Proc. Soc. Exper. Biol. & Med.* 74: 751–755, 1950.
52. BRANDT, E. L. AND A. C. GRIFFIN. *Cancer* 4: 1030–1035, 1951.
53. BRECHER, G. AND E. P. CRONKITE. *Proc. Soc. Exper. Biol. & Med.* 77: 292–294, 1951.
54. *British Medical Bulletin. (Radiobiology: Experimental and Applied)*. 4, 1946, 80 pp.
55. BRUES, A. M. AND L. RIETZ. *Argonne Nat. Lab. Report No. 4227*, 183–187, 1948.[1]
56. BRUES, A. M. AND L. RIETZ. *Ann. New York Acad. Sc.* 51: 1497–1507, 1951.
57. BUCHSBAUM, R. AND R. E. ZIRKLE. *Proc. Soc. Exper. Biol. & Med.* 72: 27–29, 1949.
58. BURNETT, W. T., JR., cited by A. Hollaender and G. E. Stapleton. *Physiol. Rev.* 33: 77, 1953.
59. BURNETT, W. T., JR., M. L. MORSE, A. W. BURKE, JR. AND A. HOLLAENDER. *J. Bact.* 63: 591–595, 1952.
60. BURNETT, W. T., JR., G. E. STAPLETON, M. L. MORSE AND A. HOLLAENDER. *Proc. Soc. Exper. Biol. & Med.* 77: 636–638, 1951.
61. BURTON, M. *Sixth Internat. Cong. Radiol. Abst.* 1950, p. 42.
62. BURTON, M. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, p. 117–138.
63. BUTLER, J. A. V. AND B. E. CONWAY. *J. Chem. Soc.* p. 3418, 1950.
64. BUTLER, J. A. V., L. A. GILBERT AND K. A. SMITH. *Nature, London* 165: 714–716, 1950.
65. CARTER, R. E., P. S. HARRIS AND J. T. BRENNAN. *Los Alamos Nat. Lab. Report No. 1075*, 1950.[1]

0018381

66. CARTY, J. R. *Radiology* 15: 353–359, 1930.
67. CHAMBERS, R. J. *J. Applied Physiol.* 12: 336–337, 1941.
68. CHAPMAN, W. H. AND E. P. CRONKITE, *Proc. Soc. Exper. Biol. & Med.* 75: 318–322, 1950.
69. CHAPMAN, W. H., C. R. SIPE, D. C. ELTZHOLTZ, E. P. CRONKITE AND F. W. CHAMBERS, JR. *Radiology* 55: 865–873, 1950.
70. CLARK, A. M. AND E. M. KELLY. *Cancer Research* 10: 348–352, 1950.
71. CLARK, W. G. AND R. P. UNCAPHER. *Proc. Soc. Exper. Biol. & Med.* 71: 214–216, 1949.
72. COLE, L. J., V. P. BOND AND M. C. FISHLER. *Science* 115: 644–646, 1952.
73. COLE, L. J., M. C. FISHLER, M. E. ELLIS AND V. P. BOND. *Proc. Soc. Exper. Biol. & Med.* 80: 112–117, 1952; *Naval Radiological Defense Lab. Document 370*, 1952.
74. COLLINSON, E., F. S. DAINTON AND B. HOLMES. *Nature, London* 165: 267–269, 1950.
75. CONGER, A. D. AND L. M. FAIRCHILD. *Genetics* 36: 547–548, 1951.
76. COOK, E. V. *Radiology* 32: 289–293, 1939.
77. CRABTREE, H. G. *Biochem. J.* 29: 2334–2343, 1935.
78. CRABTREE, H. G. AND W. CRAMER. *Proc. Roy. Soc., London, s. B.* 113: 238–250, 1933.
79. CRONKITE, E. P., G. BRECHER AND W. H. CHAPMAN. *Mil. Surgeon* 109: 294–307, 1951.
80. CRONKITE, E. P., G. BRECHER AND W. H. CHAPMAN. *Proc. Soc. Exper. Biol. & Med.* 76: 396–398, 1951.
81. CRONKITE, E. P., W. H. CHAPMAN AND G. BRECHER. *Proc. Soc. Exper. Biol. & Med.* 76: 456–459, 1951.
82. CURTIS, H. J. *Advances in Biological and Medical Physics*, edited by J. H. Lawrence and J. G. Hamilton. New York: Academic Press, Inc., 1951, vol. 2, pp. 1–51.
83. DAINTON, F. S. *Ann. Rep. Chem. Soc.* 45: 5–33, 1948.
84. DAINTON, F. S. *J. Phys. Colloid Chem.* 52: 490–517, 1948.
85. DALE, W. M. *Biochem. J.* 34: 1367–1373, 1940.
86. DALE, W. M. *Biochem. J.* 36: 80–85, 1942.
87. DALE, W. M. *Brit. J. Radiol.* suppl. 1: 46–50, 1947.
88. DALE, W. M. *J. Cell. & Comp. Physiol.* 39: 39–55, 1952.
89. DALE, W. M. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 177–188.
90. DALE, W. M., J. V. DAVIES AND J. W. MEREDITH. *Brit. J. Cancer* 3: 31–41, 1949.
91. DALE, W. M. AND J. V. DAVIES. *Biochem. J.* 48: 129–132, 1951.
92. DALE, W. M., L. H. GRAY AND W. J. MEREDITH. *Phil. Tr. Roy. Soc. London A* 242: 33–62, 1949.
93. DE BRUYN, P. P. H. *Anat. Rec.* 101: 373–405, 1948.
94. DEVI, P., G. PONTECORVO AND C. HIGGENBOTTOM. *Nature, London* 160: 503–504, 1947.
95. DINNING, J. S., I. MESCHAN, C. K. KEITH AND P. L. DAY. *Proc. Soc. Exper. Biol. & Med.* 74: 776–777, 1950.
96. DOERMANN, A. H. *Oak Ridge Nat. Lab. Report No. 1026*, p. 20, 1951.[1]
97. DOWDY, A. H. *NEPA-1019-IER-17*, 1949.[1]
98. DOWDY, A. H., L. R. BENNETT AND S. M. CHASTAIN. *Radiology* 55: 879–885, 1950.
99. DUBOIS, K. P., K. W. COCHRAN AND J. DOULL. *Proc. Soc. Exper. Biol. & Med.* 76: 422–427, 1951.
100. DURYEE, W. R. *J. Nat. Cancer Inst.* 10: 735–795, 1949.
101. ELLINGER, F. *Radiology* 44: 125–142, 1945.
102. ELLINGER, F. *Schweiz. med. Wchnschr.* 3: 61–65, 1951.
103. ELSON, L. A. AND L. F. LAMERTON. *Brit. J. Cancer* 3: 414–426, 1949.
104. ELY, J. O. AND M. H. ROSS. *Neutron Effects on Animals*. Baltimore: Williams & Wilkins, 1947, pp. 142–151.
105. EPHRATI, E. *Biochem. J.* 42: 383–389, 1948.
106. EVANS, T. C. *Biol. Bull.* 92: 99–109, 1947.
107. EVANS, T. C. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 393–413.
108. EVANS, T. C., J. P. GOODRICH AND J. C. SLAUGHTER. *Proc. Soc. Exper. Biol. & Med.* 47: 434–437, 1941.
109. EVANS, T. C., J. P. GOODRICH AND J. C. SLAUGHTER. *Radiology* 38: 201–206, 1942.
110. EVANS, T. C., W. A. ROBBIE, J. P. GOODRICH AND J. C. SLAUGHTER. *Proc. Soc. Exper. Biol. & Med.* 46: 662–664, 1941.
111. EVANS, T. C., J. C. SLAUGHTER, E. P. LITTLE AND G. FAILLA. *Radiology* 39: 663–680, 1942.

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112. FABERGE, A. C. *J. Genetics* 39: 229–248, 1940.
113. FABERGE, A. C. *Genetics* 35: 104, 1950.
114. FAILLA, G. *Some Fundamental Aspects of the Cancer Problem*, edited by H. B. Ward. New York: Science Pr., 1937, pp. 202–214.
115. FAILLA, G. *Am. J. Roentgenol.* 44: 649–664, 1940.
116. FAILLA, G. AND K. SUGIURA. *Science* 89: 438–439, 1939.
117. FANO, U. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 13–24.
118. FEINSTEIN, R. N. *Proc. Soc. Exper. Biol. & Med.* 76: 646–649, 1951.
119. FEINSTEIN, R. N., C. L. BUTLER AND D. D. HENDLEY. *Science* 111: 149–150, 1950.
120. FENN, W. O. AND W. B. LATCHFORD. *Am. J. Physiol.* 99: 454–462, 1931–32.
121. FERROUX, R., J. JOLLY AND A. LACASSAGNE. *Compt. rend. Soc. de biol.* 95: 646–649, 1926.
122. FISCHER, P., L. DE LANDTSHEER AND J. LECOMTE. *Bull. Soc. chim. biol.* 32: 1009–1011, 1950.
123. FORSSBERG, A. *Nature, London* 159: 308–309, 1947.
124. FORSSBERG, A. *Acta radiol.* 33: 296–304, 1950.
125. FRANCE, O. *Metallurgical Project Report CH-3889*, 1946.[1]
126. FRANKENTHAL, L. AND A. BACK. *Biochem. J.* 38: 351–354, 1944.
127. FREDERIC, J. *Brit. J. Radiol.* 25: 43–44, 1952.
128. FRICKE, H. *Cold Spring Harbor Symp., Quant. Biol.* 2: 241–248, 1934.
129. FRICKE, H. AND E. J. HART. *J. Chem. Physiol.* 3: 60–61, 1935.
130. FRICKE, H. AND B. W. PETERSEN. *Am. J. Roentgenol.* 17: 611–620, 1927.
131. FRIEDELL, H. L. AND J. H. CHRISTIE. *Proc. Soc. Exper. Biol. & Med.* 76: 207–210, 1951.
132. FRIEDEWALD, W. F. AND R. S. ANDERSON. *Proc. Soc. Exper. Biol. & Med.* 45: 713–715, 1940.
133. FRIEDEWALD, W. F. AND R. S. ANDERSON. *J. Exper. Med.* 74: 463–487, 1941.
134. FRILLEY, M. *Brit. J. Radiol.* suppl. 1: 50–55, 1947.
135. GAULDEN, M. E. AND M. NIX. *Genetics* 35: 665–666, 1950.
136. GELIN, O. E. *Hereditas* 27: 209–219, 1941.
137. GERSHON-COHEN, J., M. B. HERMEL AND J. Q. GRIFFITH, JR. *Science* 114: 157–158, 1951.
138. GHORMLEY, J. A. AND A. O. ALLEN. *Oak Ridge Nat. Lab. Report No. 128*, 1948.[1]
139. GIERLOCH, Z. S. AND A. T. KREBS. *Am. J. Roentgenol.* 62: 559–563, 1949.
140. GIESE, A. C. *Physiol. Rev.* 30: 431–458, 1950.
141. GILES, N. H. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 267–284.
142. GILES, N. H. AND A. V. BEATTY. *Science* 112: 643–645, 1950.
143. GILES, N. H., A. V. BEATTY AND H. P. RILEY. *Genetics* 36: 552–553, 1951.
144. GILES, N. H. AND H. P. RILEY. *Proc. Nat. Acad. Sc.* 35: 640–646, 1949.
145. GILES, N. H. AND H. P. RILEY. *Proc. Nat. Acad. Sc.* 36: 337–344, 1950.
146. GLÜCKSMANN. A. AND F. G. SPEAR. *Brit. J. Radiol.* 12: 486–498, 1939.
147. GOLDFEDER, A. AND J. L. FERSHING. *Radiology* 31: 81–88, 1938.
148. GRAHAM, J. B., R. M. GRAHAM AND A. J. GRAFFEE. *Endocrinology* 46: 434–440, 1950.
149. GRAY, J. L., E. J. MOULDEN, J. T. TEW AND H. JENSEN. *Proc. Soc. Exper. Biol. & Med.* 79: 384–387, 1952.
150. GRAY, L. H. *Brit. M. Bull.* 4: 11–18, 1946.
151. GRAY, L. H. *Progress in Biophysics and Biophysical Chemistry*, edited by J. A. V. Butler and J. T. Randall. New York: Academic Press, Inc., 1951, vol. 2, pp. 240–305.
152. GRAY, L. H. *J. Cell. & Comp. Physiol.* 39: 57–73, 1952.
153. GUSTAFSON, G. E. AND S. KOLETSKY. *Proc. Soc. Exper. Biol. & Med.* 78: 489–490, 1951.
154. HAGEN, C. W., JR. AND G. SACHER. *Manhattan District Declassified Document No. 1252*, 1946.[1]
155. HAGEN, C. W., JR. AND E. L. SIMMONS. *Manhattan District Declassified Document No. 1210*, 1947.[1]
156. HALEY, T. J., S. MANN AND A. H. DOWDY. *Science* 112: 333–334, 1950.
157. HALL, B. V. *Federation Proc.* 11: 63, 1952.
158. HALL, B. V. *Cancer Research.* In press.
159. HARRINGTON, N. J. AND R. W. KOSA. *Biol. Bull.* 101: 138–150, 1951.
160. HAYDEN, B. AND L. SMITH. *Genetics* 34: 26–43, 1949.
161. HEILBRUNN, L. V. *Proc. Soc. Exper. Biol. & Med.* 70: 179–182, 1949.
162. HEILBRUNN, L. V., W. L. WILSON AND D. HARDING. *J. Nat. Cancer Inst.* 11: 1287–1298, 1951.
163. HEKTOEN, L. *J. Infect. Dis.* 22: 28–33, 1918.
164. HEMPELMANN, L. H. *Personal communication*, 1952.

0018383

165. HEMPELMANN, L. H., T. T. TRUJILLO AND N. P. KNOWLTON, JR. *Nucl. Sc. Abstr.* 3: 46, 1949.
166. HENRY, J. A. *Nature, London* 163: 134–135, 1949.
167. HENSHAW, P. S. *Am. J. Cancer* 33: 258–264, 1938.
168. HENSHAW, P. S. AND D. S. FRANCIS. *J. Cell. & Comp. Physiol.* 7: 173–195, 1935.
169. HERVE, A. *Rev. méd. Liège* 7: 276–279, 1952.
170. HERVE, A., Z. M. BACQ AND H. BETZ. *Sixth Internat. Cong. Radiol. Abst.*, 1950, p. 169.
171. HEVESY, G. *Revs. Modern Physics* 17: 102–111, 1945.
172. HEVESY, G. *Nature, London* 163: 869–870, 1949.
173. HEVESY, G. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 189–215.
174. HEWITT, H. B. AND J. READ. *Brit. J. Radiol.* 23: 416–423, 1950.
175. HICKS, S. P. *Proc. Soc. Exper. Biol. & Med.* 75: 485–489, 1950.
176. HINKEL, C. L. *Am. J. Roentgenol.* 47: 439–457, 1942.
177. HOLLAENDER, A. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 285–295.
178. HOLLAENDER, A., W. K. BAKER AND E. H. ANDERSON. *Cold Spring Harbor Symp., Quant. Biol.* 16: 315–326, 1951.
179. HOLLAENDER, A. AND G. E. STAPLETON. *Physiol. Rev.* 33: 77, 1953.
180. HOLLAENDER, A., G. E. STAPLETON AND W. T. BURNETT, JR. *Isotopes in Biochemistry*, edited by J. N. Davidson. London: A Ciba Foundation Conference, Churchill, 1951, pp. 96–113.
181. HOLLAENDER, A., G. E. STAPLETON AND F. L. MARTIN. *Nature, London* 167: 103–104, 1951.
182. HOLMES, B. *Nature, London* 165: 266–267, 1950.
183. HOLTHUSEN, H. *Arch. f. d. ges. Physiol.* 187: 51, 1921.
184. HUBERT, R. *Arch. f. d. ges. Physiol.* 223: 333–350, 1929.
185. JACOBSON, L. O. *Cancer Research* 12: 315–325, 1952.
186. JACOBSON, L. O., E. K. MARKS, E. O. GASTON, E. L. SIMMONS AND M. H. BLOCK. *Science* 107: 248–250, 1948.
187. JACOBSON, L. O., E. K. MARKS, M. J. ROBSON, E. O. GASTON AND R. E. ZIRKLE. *J. Lab. & Clin. Med.* 34: 1538–1543, 1949.
188. JACOBSON, L. O., E. L. SIMMONS, W. F. BETHARD, E. K. MARKS AND M. J. ROBSON. *Proc. Soc. Exper. Biol. & Med.* 73: 455–459, 1950.
189. JACOBSON, L. O., E. L. SIMMONS, E. K. MARKS, E. O. GASTON, M. J. ROBSON AND J. H. ELDREDGE. *J. Lab. & Clin. Med.* 37: 683–697, 1951.
190. JOLLES, B. *Brit. J. Radiol.* 14: 110–112, 1941.
191. JOLLES, B. *Brit. J. Radiol.* 23: 18–24, 1950.
192. JOLLES, B. AND P. C. KOLLER. *Brit. J. Cancer* 4: 77–89, 1950.
193. JOLLES, B. AND R. G. MITCHELL. *Brit. J. Radiol.* 20: 405–409, 1947.
194. JOLLY, J. *Compt. rend. Soc. de biol.* 91: 351–354, 1924.
195. KAHN, J. B., JR. *Proc. Soc. Exper. Biol. & Med.* 78: 486–489, 1951.
196. KAPLAN, H. S. *J. Nat. Cancer Inst.* 9: 55–56, 1948.
197. KAPLAN, H. S. *Cancer Research* 11: 261–262, 1951.
198. KAPLAN, H. S. AND J. PAULL. *Proc. Soc. Exper. Biol. & Med.* 79: 670–672, 1952.
199. KARNOFSKY, D. A., P. A. PATTERSON AND L. P. RIDGWAY. *Am. J. Roentgenol.* 64: 280–288, 1950.
200. KIMBALL, R. F. AND N. GAITHER. *Proc. Soc. Exper. Biol. & Med.* 80: 525–529, 1952.
201. KIMELDORF, D. J., D. C. JONES AND M. C. FISHLER. *Science* 112: 175–176, 1950.
202. KING, E. D., H. A. SCHNEIDERMAN AND K. SAX. *Proc. Nat. Acad. Sc.* 38: 34–43, 1952.
203. KNOWLTON, N. P., JR. AND L. H. HEMPELMANN. *J. Cell. & Comp. Physiol.* 33: 73–91, 1949.
204. KULWIN, M. H. *Personal communication*, 1952.
205. LACASSAGNE, A. *Compt. rend. Acad. sc.* 215: 231–232, 1942.
206. LASNITZKI, I. *Brit. J. Radiol.* 16: 61–67, 1943.
207. LASNITZKI, I. *Brit. J. Radiol.* 16: 137–141, 1943.
208. LATARJET, R. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 241–258.
209. LATARJET, R. AND E. EPHRATI. *Compt. rend. Soc. de biol.* 142: 497–499, 1948.
210. LATARJET, R. AND B. EPHRUSSI. *Compt. rend. Acad. sc.* 229: 306–308, 1949.
211. LAVIK, P. S., J. R. LEONARDS, G. W. BUCKALOO, C. HEISLER AND H. L. FRIEDELL. *Western Reserve Univ. Report NYO-1637*, 1952.[1]
212. LAZAROW, A. *Proc. Soc. Exper. Biol. & Med.* 61: 441–447, 1946.

0018384

213. LAZAROW, A. *Proc. Soc. Exper. Biol. & Med.* 66: 4–7, 1947.
214. LEA, D. E. *Actions of Radiations on Living Cells.* New York: Macmillan, 1947.
215. LEA, D. E. *Brit. J. Radiol.* suppl. 1. 35–40, 1947.
216. LEA, D. E. *Brit. J. Radiol.* suppl. 1: 59–63, 1947.
217. LEA, D. E., K. M. SMITH, B. HOMES AND R. MARKHAM. *Parasitology* 36: 110–118, 1944.
218. LEMAY, M. *Proc. Soc. Exper. Biol. & Med.* 77: 337–339, 1951.
219. LICK, L., A. KIRSCHBAUM AND H. MIXER. *Cancer Research* 9: 532–536, 1949.
220. LIMPEROS, G. AND W. A. MOSHER. *Am. J. Roentgenol.* 63: 681–690, 1950.
221. LIMPEROS, G. AND W. A. MOSHER. *Am. J. Roentgenol.* 63: 691–700, 1950.
222. LIMPEROS, G. AND W. A. MOSHER. *Science* 112: 86–87, 1950.
223. LOEB, J. *Science* 32: 411–412, 1910.
224. LOISELEUR, J. AND R. LATARJET. *Compt. rend Soc. de biol.* 135: 1530–1532, 1941.
225. LOISELEUR, J. AND R. LATARJET. *Bull. Soc. chim. biol.* 24: 172–181, 1942.
226. LOISELEUR, J. AND G. VELLEY. *Compt. rend. Acad. Sc.* 231: 182–184, 1950.
227. LOISELEUR, J. AND G. VELLEY. *Compt. rend. Acad. sc.* 231: 529–531, 1950.
228. LORENZ, E., C. C. CONGDON AND D. UPHOFF. *Radiology* 58: 863–877, 1952.
229. LORENZ, E., D. UPHOFF, T. R. REID AND E. SHELTON. *J. Nat. Cancer Inst.* 12: 197–201, 1951.
230. LURIA, S. E. AND F. M. EXNER. *Proc. Nat. Acad. Sc.* 27: 370–375, 1941.
231. MCDONALD, M. R. cited in *Cold Spring Harbor Symp., Quant. Biol.* 16: 315–326, 1951.
232. MACK, H. P. AND F. H. J. FIGGE. *Science* 115: 547–548, 1952.
233. MARSHAK, A. *Proc. Soc. Exper. Biol. & Med.* 38: 705–713, 1938.
234. MAZIA, D. AND G. BLUMENTHAL. *Proc. Nat. Acad. Sc.* 34: 328–336, 1948.
235. MAZIA, D. AND G. BLUMENTHAL. *J. Cell. & Comp. Physiol.* 35: 171–186, 1950.
236. MEYER, H. V. AND H. J. MULLER. *Records Genetic Soc.* 21: 48, 1952.
237. MITCHELL, J. S. *Brit. J. Exper. Path.* 23: 285–295, 1942.
238. MOLE, R. H., J. ST. L. PHILPOT AND G. R. V. HODGES. *Nature, London* 166: 515, 1950.
239. MORRISON, P. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 1–12.
240. MOTTRAM, J. C. *Brit. J. Radiol.* 29: 174–180, 1924.
241. MOTTRAM, J. C. *Brit. J. Radiol.* 8: 32–39, 1935.
242. MOTTRAM, J. C. *Brit. J. Radiol.* 8: 643–651, 1935.
243. MOTTRAM, J. C. *Brit. J. Radiol.* 9: 606–614, 1936.
244. MOTTRAM, J. C. AND A. EIDINOW. *Brit. J. Surg.* 19: 481–487, 1932.
245. NICKSON, J. J. (editor). *Symposium on Radiobiology.* New York: Wiley, 1952.
246. PACKARD, C. J. *Cancer Research* 14: 359–369, 1930.
247. PACKARD, C. *Science of Radiology*, edited by Otto Glasser. Springfield, Illinois: Thomas, 1933, pp. 319–331.
248. PATERSON, E. *Treatment of Malignant Disease by Radium and X-ray.* R. Paterson. Baltimore: Williams & Wilkins, 1949, pp. 550–600.
249. PATERSON, E. AND J. J. MATTHEWS. *Nature, London* 168: 1126–1127, 1951.
250. PATT, H. M. AND M. E. BLACKFORD. *Argonne Nat. Lab. Report No. 4713:* 48–52, 1951.[1]
251. PATT, H. M., M. E. BLACKFORD AND R. L. STRAUBE. *Proc. Soc. Exper. Biol. & Med.* 80: 92–97, 1952.
252. PATT, H. M. AND S. H. MAYER. To be published.
253. PATT, H. M., S. H. MAYER AND D. E. SMITH. *Argonne Nat. Lab. Report No. 4625:* 51, 1951.[1]
254. PATT, H. M., S. H. MAYER AND D. E. SMITH. *Argonne Nat. Lab. Report. No. 4625:* 50, 1951.[1]
255. PATT, H. M., S. H. MAYER AND D. E. SMITH. *Federation Proc.* 11: 118, 1952.
256. PATT, H. M., S. H. MAYER, R. L. STRAUBE AND E. M. JACKSON. *J. Cell. & Comp. Physiol.* In press.
257. PATT, H. M., D. E. SMITH, M. E. BLACKFORD, AND R. L. STRAUBE. *Argonne Nat. Lab. Report No. 4401:* 75, 1950.
258. PATT, H. M., D. E. SMITH AND E. JACKSON. *Blood* 5: 758–763, 1950.
259. PATT, H. M., D. E. SMITH, E. B. TYREE AND R. L. STRAUBE. *Proc. Soc. Exper. Biol. & Med.* 73: 18–21, 1950.
260. PATT, H. M., R. L. STRAUBE, M. E. BLACKFORD AND D. E. SMITH. *Am. J. Physiol.* 163: 740, 1950. Also unpublished data.
261. PATT, H. M., R. L. STRAUBE, E. B. TYREE, M. N. SWIFT AND D. E. SMITH. *Am. J. Physiol.* 159: 269–280, 1949.
262. PATT, H. M. AND M. N. SWIFT. *Am. J. Physiol.* 155: 388–393, 1948.

0018385

263. PATT, H. M. AND E. B. TYREE. Unpublished data. 31
264. PATT, H. M., E. B. TYREE, R. L. STRAUBE AND D. E. SMITH. *Science* 110: 213–214, 1949. 31
265. PENDERGRASS, E. P. Unpublished observations. 32
266. PETRY, E. *Biochem. Ztschr.* 128: 326–353, 1922. 32
267. PICKELS, E. G. AND R. S. ANDERSON. *J. Gen. Physiol.* 30: 83–99, 1946. 32
268. Plutonium Project, The. *Radiology* 49: 269–365, 1947.
269. QUASTLER, H. *Am. J. Roentgenol.* 54: 457–461, 1945. 3
270. RAGAN, C., C. P. DONLAN, J. A. COSS, JR. AND A. F. GRUBIN. *Proc. Soc. Exper. Biol. & Med.* 66: 170–172, 1947. 3
271. READ, J. *Brit. J. Radiol.* 25: 89–99, 1952. 3
272. READ, J. *Brit J. Radiol.* 25: 154–160, 1952. 3
273. READ, J. *Brit. J. Radiol.* 25: 336–338, 1952. 3
274. REDFIELD, A. C., E. M. BRIGHT AND J. WERTHEIMER. *Am. J. Physiol.* 68: 368–378, 1924. 3
275. REKERS, P. E., M. P. COULTER AND S. L. WARREN. *Arch. Surg.* 60: 635–667, 1950. 3
276. RILEY, H. P., N. H. GILES AND A. V. BEATTY. Unpublished data. 3
277. RISSE, O. *Ergebn. d. Physiol.* 30: 243–293, 1930. 3
278. ROSENTHAL, R. L., L. GOLDSCHMIDT AND B. I. PICKERING. *Am. J. Physiol.* 166: 15–19, 1951. 3
279. ROSS, C. J. *Nature, London* 165: 808–809, 1950. 3
280. RUSSELL, L. B. *J. Exper. Zool.* 114: 545–601, 1950. 3
281. RUSSELL, L. B., W. L. RUSSELL AND M. H. MAJOR. *Anat. Rec.* 111: 455, 1951
282. RUSSELL, W. L., J. C. KILE, JR. AND L. B. RUSSELL. *Genetics* 36: 574, 1951.
283. SACHER, G. Unpublished observations, 1951.
284. SALERNO, P. R., P. A. MATTIS AND H. L. FRIEDELL. *Federation Proc.* 11 387–388, 1952.
285. SALISBURY, P. F., P. E. REKERS, J. H. MILLER AND N. F. MARTI. *Science* 113: 6–7, 1951.
286. SAX, K. *Genetics* 32: 75–78, 1947.
287. SAX, K. AND E. V. ENZMANN. *Proc. Nat. Acad. Sc.* 25: 397–405, 1939.
288. SCHLIEP, W. *Arch. Zellforsch.* 17: 289–367, 1923.
289. SCHOLES, G. AND J. WEISS. *Nature, London* 166: 640–642, 1950.
290. SCHREK, R. *J. Cel & Comp Physiol.* 28: 277–304, 1946.
291. SCHREK, R. *Radiology* 46: 395–410, 1946.
292. SCHREK, R. Personal communication, 1952
293. SCHWARZ, G. *München med. Wchnschr.* 56: 1217–1218, 1909.
294. SCOTT, C. M. *Medical Research Council* (British) Special Report Series No. 223, 1937.
295. SHACTER, B. *Cancer Research* 2: 277–278, 1951.
296. SHACTER, B., H. SUPPLEE AND C. ENTENMAN. *Naval Radiological Defense Lab. Document, AD-333(B)*, 1951.[1]
297. SHEPPARD, C. W. AND M. STEWART. *Federation Proc.* 10: 125, 1951.
298. SHERMAN, F. G. AND H. B. CHASE. *J. Cell. & Comp. Physiol.* 33: 17–26, 1949.
299. SHERMAN, F. G. AND H. B. CHASE. *J. Cell. & Comp. Physiol.* 34: 207–219, 1949.
300. SHIRAI, M. *Nagoya Igakkai Zasshi* 54: 183, 1941.
301. SKOOG, F. *J. Cell. & Comp. Physiol.* 7: 227–270, 1935.
302. SMITH, D. E., H. M. PATT AND E. B. TYREE. *Argonne Nat. Lab. Report No. 4401*: 72, 1950.[1]
303. SMITH, D. E., H. M. PATT, E. B. TYREE AND R. L. STRAUBE. *Proc. Soc. Exper. Biol. & Med.* 73: 198–200, 1950.
304. SMITH, F. AND M. M. GRENAN. *Science* 113: 686–688, 1951.
305. SMITH, W. W. *J. Cell. & Comp. Physiol.* 38: 41–49, 1951.
306. SMITH, W. W., B. J. HIGHMAN, J. R. MITCHELL, AND H. C. BLOUNT, JR. *Proc. Soc. Exper. Biol. & Med.* 71: 498–501, 1949.
307. SMITH, W. W. AND F. SMITH. *Am. J. Physiol.* 165: 639–650, 1951.
308. SONNENBLICK, B. P. *Biol. Bull.* 101: 230, 1951.
309. SPARROW, A. H. *Ann. New York Acad. Sc.* 51: 1508–1540, 1951.
310. SPARROW, A. H., M. J. MOSES, AND R. STEELE. *Brit. J. Radiol.* 25: 182–189, 1952.
311. SPARROW, A. H. AND M. ROSENFELD. *Science* 104: 245–246, 1946.
312. SPEAR, F. G. *Brit. M. Bull.* 4: 2–10, 1946.
313. SPEAR, F. G., Editor. *Brit. J. Radiol.* Suppl. 1, 1947.
314. SPEAR, F. G. AND K. TANSLEY. *Brit. J. Radiol.* 17: 374–379, 1944.
315. SPIERS, F. W. *Brit. J. Radiol.* 19: 52–63, 1946.
316. STADLER, L. J. *J. Hered.* 21: 3, 1930.
317. STAPLETON, G. E., D. BILLEN AND A. HOLLAENDER. *J. Bact.* 63: 805–811, 1952.

0018386

318. STEWART, F. W. *Arch. Surg.* 27: 979–1064, 1933.
319. STORER, J. B. *Atomic Energy Declassified Document, AECU-2095*, 1952.[1]
320. STORER, J. B. AND J. M. COON. *Proc. Soc. Exper. Biol. & Med.* 74: 202–204, 1950.
321. STRANGEWAYS, T. S. P. AND H. B. FELL. *Proc. Roy. Soc., London, s. B* 102: 9–29, 1927.
322. STRAUBE, R. L., H. M. PATT, D. E. SMITH AND E. B. TYREE. *Cancer Research* 10: 243–244, 1950.
323. STRAUBE, R. L., H. M. PATT, E. JACKSON AND D. E. SMITH. *Am. J. Physiol.* 171: 770, 1952.
324. STRAUBE, R. L., G. SVIHLA, M. E. BLACKFORD AND H. M. PATT. *Am. J. Physiol.* 167: 829, 1951.
325. STRELIN, G. S. *Nucl. Sc. Abstr.* 4: 953, 1950.
326. SUGUIRA, K. *Radiology* 29: 352–361, 1937.
327. SUGUIRA, K. *Radiology* 37: 85–93, 1941.
328. SVEDBERG, T. AND S. BROHULT. *Nature, London* 143: 938–939, 1939.
329. SWIFT, M. N., S. T. TAKETA, AND V. P. BOND. *Federation Proc.* 11: 158, 1952.
330. SWISHER, S. N. AND F. W. FURTH. *Proc. Soc. Exper. Biol. & Med.* 78: 226–229, 1951.
331. TAHMISIAN, T. N. *J. Exper. Zool.* 112: 449–464, 1949.
332. TAHMISIAN, T. N. AND D. ADAMSON. *J. Exper. Zool.* 115: 379–397, 1950.
333. TALBOT, J. M. AND E. A. PINSON. *Mil. Surgeon* 108: 412–417, 1951.
334. TANSLEY, K., L. H. GRAY AND F. G. SPEAR. *Brit. J. Radiol.* 21: 567–570, 1948.
335. TAYLOR, B., J. P. GREENSTEIN AND A. HOLLAENDER. *Arch. Biochem.* 16: 19–31, 1948.
336. THODAY, J. M. AND J. READ. *Nature, London* 160: 608, 1947.
337. THODAY, J. M. AND J. READ. *Nature, London* 163: 133–134, 1949.
338. THOMPSON, T. L., R. B. MEFFERD, JR. AND O. WYSS. *J. Bact.* 62: 39–44, 1951.
339. TING, T. P. AND R. E. ZIRKLE. *J. Cell. & Comp. Physiol.* 16: 189–195, 1940.
340. TOBIAS, C. A. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 357–392.
341. TOOLAN, H. W. *Proc. Soc. Exper. Biol. & Med.* 78: 540–543, 1951.
342. TREADWELL, A. DE G., W. U. GARDNER, AND J. H. LAWRENCE. *Endocrinology* 32: 161–164, 1943.
343. TULLIS, J. L. *Arch. Path.* 48: 171–177, 1949.
344. UCHIMURA, T. *J. Biochem.* 25: 207–217, 1937.
345. VAN DYKE, D. C. AND R. L. HUFF. *Proc. Soc. Exper. Biol. & Med.* 72: 266–269, 1949.
346. VELLEY, G. AND J. LOISELEUR. *Compt. rend. Acad. Sc.* 230: 2132–2133, 1950.
347. VENTERS, K. D. AND E. E. PAINTER. *Federation Proc.* 9: 129, 1950.
348. VINTEMBERGER, P. *Compt. rend. Soc. de biol.* 99: 1968–1971, 1928.
349. VON EULER, H. AND L. HAHN. *Acta. radiol.* 27: 269–280, 1946.
350. VON SALLMANN, L. *Tr. Am. Ophth. Soc.* 49: 391–417, 1952.
351. VON SALLMANN, L., Z. DISCHE, G. EHRLICH AND C. M. MUNOZ. *Am. J. Ophth.* 34: 95–113, 1951.
352. WAGNER, R. P., E. H. HADDOX, R. FUERST AND W. S. STONE. *Genetics* 35: 237–248, 1950.
353. WALSH, D. *Brit. M. J.* 2: 272–273, 1897.
354. WARREN, S. *Arch. Path.* 43: 443–450, 1942.
355. WATSON, J. D. *J. Bact.* 60: 697–718, 1950.
356. WATSON, J. D. *J. Bact.* 63: 473–485, 1952.
357. WEISBERGER, A. S., R. W. HEINLE AND J. H. CHRISTIE. *Western Reserve Univ. Report, NYO-1611*, 1950.[1]
358. WEISS, J. *Nature, London* 153: 748–750, 1944.
359. WEISS, J. *Brit. J. Radiol.* suppl. 1: 56–59, 1947.
360. WEISS, J. *Sixth Internat. Cong. Radiol. Abst.* p. 165, 1950.
361. WEISS, J. *Nature, London* 169: 460–461, 1952.
362. WERTZ, E. *Strahlentherapie, Sonderbände* 67: 700–711, 1940.
363. WILSON, C. W. *Acta. radiol.* 16: 719–734, 1935.
364. WILSON, J. G. AND J. W. KARR. *Anat. Rec.* 106: 259–260, 1950.
365. WILSON, W. L. *Protoplasma* 39: 305–316, 1950.
366. WOODWARD, G. E. *Biochem. J.* 29: 2405–2412, 1935.
367. WYSS, O., J. B. CLARK, F. HAAS AND W. S. STONE. *J. Bact.* 56: 51–57, 1948.
368. ZIRKLE, R. E. *J. Cell. & Comp. Physiol.* 2: 251–274, 1932.
369. ZIRKLE, R. E. *Am. J. Roentgenol.* 35: 230–237, 1936.
370. ZIRKLE, R. E. *J. Cell. & Comp. Physiol.* 16: 221–235, 1940.

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371. ZIRKLE, R. E. *J. Cell. & Comp. Physiol.* 16: 301–311, 1940.
372. ZIRKLE, R. E. *J. Cell. & Comp. Physiol.* 17: 65–70, 1941.
373. ZIRKLE, R. E. Metallurgical Project Report, CH-946, 1943.[1] *Radiation Biology*, edited by A. Hollaender. New York: McGraw-Hill, vol. 1. In press.
374. ZIRKLE, R. E. *Radiology* 52: 846–855, 1949.
375. ZIRKLE, R. E. (editor). *Effects of External Beta Radiation.* New York: McGraw-Hill, 1951.
376. ZIRKLE, R. E. *Symposium on Radiobiology*, edited by J. J. Nickson. New York: Wiley, 1952, pp. 333–356.
377. ZIRKLE, R. E., E. ANDERSON, E. F. RILEY AND H. J. CURTIS. *Manhattan District Declassified Document No. 418*, 1946.[1]

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