Notions on Sensitivity of Cells to Radiation

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The observed sensitivity of cells to ionizing radiation is undoubtedly the product of several factors that are related to structure, metabolism, reproduction, and environment. In spite of a large body of experimental and speculative work, the nature of these factors and their interaction in causing the various types of response and the wide range of sensitivity observed in different kinds of cells are obscure. This is hardly surprising when it is realized that radiation may induce a large number of chemical and physical changes in both the nucleoplasm and the cytoplasm. Some of these changes may have little effect on any kind of cell under any conditions. Others may have a more or less drastic effect, depending on the type of cell and its environment.

In the hope of clarifying some of the significant factors contributing to the radiation sensitivity of cells, we have focused our attention on some of the most radiosensitive cell types (*1*). These cells are certain cells of the embryo, especially those that are undergoing differentiation (*2, 3*); differentiating oocytes (*4*); mature and immature lymphocytes (*5, 6*); certain tumor cells (*6, 7*); spermatogonia of mammals (*8*); subependymal cells (*9*); and various others, such as specific cells of the small intestine (*10*).

The response of these cells to radiation is characterized by the following: (i) the end-point is cell death; (ii) death occurs in the interphase or early prophase stage; (iii) death of a large fraction of the population is caused by extremely small radiation doses—10 to 100 roentgens; and (iv) the cells appear to be highly sensitive both *in vivo* and *in vitro,* and death occurs relatively quickly, largely eliminating environmental factors from consideration.

Consideration of the properties of these cells and correlations with recent findings in radiation chemistry and cellular physiology have led us to several notions related to the cause of the unusual sensitivity of these cells to radiation. Briefly stated, these are as follows. (i) During ordinary biooxidations, many loci in the cytoplasm of aerobic cells are constantly exposed to oxidizing radicals (probably in complex molecular form) identical to those produced in water by ionizing radiations. (ii) As might be expected, most cytoplasmic systems appear to function normally after moderate doses of radiation. (iii) Unlike the cytoplasm, the nucleoplasm is not exposed to the same degree to oxidizing radicals or their complexes. (iv) The nucleoplasm may lack adequate defenses against these foreign oxidizing agents, and sensitive systems within the nucleus may be considerably injured by radiation. (v) All the aforementioned extremely radiosensitive cells seem to be uniquely dependent on an active nucleus for maintenance and integrity, even during the nondividing phase. (vi) These considerations suggest that the high radiation sensitivity of these cells is the result of the action of strongly oxidizing, radiation-produced substances on the normally anaerobically metabolizing systems of nuclei in cells in which the nucleus plays an unusually active role in cellular metabolism. (vii) The death of these cells, then, is the result of a sequence of unidentified biochemical changes that occur within the cell and are initiated by damage to an as-yet-unknown system in the nucleus.

(Although in the cells under consideration this "metabolizing nucleus" sensitivity factor plays a predominant role in determining radiation sensitivity, in other cells it must play a more or less important role, depending on the importance of nuclear metabolism to the cell as a whole and on the importance of other factors (*11*). To take an extreme example, this factor can have no importance whatsoever in the nonnucleated erythrocyte.)

A search of the literature does not answer unambiguously the question of whether death of these cells results from injury to the cytoplasm or nucleoplasm. Numerous studies have been carried out on other cells (*12*), presumably with such objectives, but only one of these has given what can be considered a conclusive result. Whiting (*13*) irradiated female wasps with up to 50,000 roentgens, then mated them with unirradiated males. Normal androgenic development followed, presumably mediated by the unirradiated nucleus. No development of diploid individuals occurred following such a large radiation dose. On the basis of this work she concluded that cytoplasm may function normally after an irradiation dose many times greater than that lethal to the nucleus.

The same conclusion may be drawn from the *in vitro* radiation studies of Barron (*14*) and others (*15*) on isolated enzymes. These studies show that, although certain sulfhydryl-containing enzymes are inactivated by fairly low doses of radiation, this inactivation can be reversed by reducing agents such as glutathione, and the enzymes themselves can survive relatively high doses of radiation without irreversible damage. Cytoplasm and tissue fluids normally contain antioxidants and reducing agents of several types in more than adequate concentrations to accomplish this.

Why the cells under consideration differ in radiation sensitivity from other nucleated cells is suggested (i) by morphologic and metabolic comparisons, both of which indicate a considerable nuclear activity on the part of these cells and (ii) by theoretical reasons which lead us to propose that the active nucleus is the most radiovulnerable cellular system.

Morphologic and Metabolic Comparisons

Morphologically, these cells all have a large nuclear volume in comparison with their cytoplasmic volume (*3, 4, 16*). Another feature is the presence of relatively few cytoplasmic particles and, in particular, a marked paucity of mitochondria (*3, 4, 16*). A dramatic illustration of how such cellular organization can be correlated with radiation sensitivity is provided by the work of Tahmisian on grasshopper eggs (*4*). In this insect, the eggs differentiate as they pass down the oviduct, so that the cells closest to the external opening have many cytoplasmic particles, whereas cells higher up the duct have larger nuclei and fewer mitochondria. As little as 100 roentgens will cause the nondifferentiated eggs to disintegrate, whereas approximately 500 roentgens are required to destroy eggs that are about to be laid.

The extraordinary participation of the nuclei of these cells in synthesis and cell maintenance is borne out by their high glycolytic as compared with oxidative metabolism (*17*). This has been measured by Beck (*5*) for chronic lymphatic

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leukemic cells and normal leukocytes. The data for leukocytes indicate rather strongly that normal lymphocytes must also have a higher glycolytic rate. Mendel, Rudney, and Bowman (*18*) have reported high glycolytic rates for the small intestine. Gal *et al.* (*19*) offer indirect evidence that such a metabolic pattern obtains in many of the tissues of the embryo. They were able to correlate low rhodanese activity with a high rate of glycolysis and found that embryo tissues are extremely low in rhodanese. Potter and DuBois (*20*) have shown that tumors and embryonic tissues are exceptionally low in cytochrome *c* and cytochcrome oxidase. Hicks (*3*) has shown that the neuroblasts of the rat will tolerate more than 1 hour of total anoxia without breakdown (a finding that also indicates a well-developed glycolytic mechanism), whereas the mature neuron is destroyed by such exposure in only a few minutes.

Thus the available evidence indicates that the extremely radiosensitive cells carry out a relatively large portion of their metabolism via nuclear glycolytic pathways.

Theoretical Reasons

Theoretical reasons, suggesting a relative insensitivity of many cytoplasmic loci and sensitivity of major portions of the nucleus, are provided by current theories on metabolic processes and on the mechanisms by which radiation acts. Convincing evidence has been obtained that, in any oxygenated aqueous system, the primary oxidative irradiation products are H_2O_2 and the free radicals HO_2 and OH (*21*). These then react with any substances available by conventional oxidation reactions. In irradiated biological systems, these substances are formed, probably along with a smaller number of organic ions and free radicals. Current theories on oxidation-reduction reactions indicate that identical substances, probably as complexes, are produced in cytoplasm during reduction of oxygen to water by the cytochrome oxidase system in normal cell respiration (*22*). Presumably this reduction takes place in a series of 1-electron steps, giving in turn O_2^- (the anion of the ionized HO_2 free radical), H_2O_2, $OH + OH^-$, and finally $2OH^-$, or complexes thereof. Since these substances are normally present in respiratory systems, it is not surprising that the introduction of additional small amounts by radiation has little effect. Means for utilizing them in normal metabolic processes are available, and antioxidants such as catalase are present to destroy any that escape normal metabolic routes.

Circumstances in the nucleus are somewhat different. Radiation produces the same substances as it does in the cytoplasm, but in most of the nucleus they are comparatively foreign. In a recent review, Stern (*23*) has arrayed a large body of evidence supporting the view that systems of the nucleus exist in an environment in which aerobic oxidation reactions are essentially absent. He points out that, in the many tissues studied, cytochrome oxidase and flavoproteins are absent from the nuclei. Thus it is reasonably certain that the system responsible for the bulk of terminal oxidations is present mainly in the cytoplasm and that the oxygen, which is known to be present in the nucleus, takes little if any part in its metabolism.

This means that many systems of the nucleus, apparently relatively lacking in oxygen-activating enzymes, may be exposed to at most very low concentrations of oxidizing radicals during ordinary existence. Further, it suggests that there is something in the nucleus that is highly susceptible to strong oxidizing agents (*24*). It might be suspected that nuclei would be low in antioxidants on grounds that seem to have considerable precedence in biological systems—namely, that a defense does not exist where danger is minimal. Supporting this notion are the findings of several investigators that a major antioxidant, catalase, is present only in low concentrations in tumor and embryonic cells (large nuclei) as well as in lower organisms that lack cytochrome systems (*25*). In this connection, Barron's *in vitro* studies may have bearing on the radiation-induced reaction. In discussing the sensitivity of cytoplasm to radiation, it was pointed out that the sulfhydryl enzymes are inactivated *in vitro* but that they are readily reactivated by a number of substances such as the antioxidants found in the cytoplasm. In the nucleus, in the absence of appreciable amounts of antioxidants, such reactivation probably would not occur. Any sulfhydryl groups inactivated by the radiation might be permanently lost to the nucleus.

The extent to which such impairment from this or any other cause would be apparent in the whole cell during the interphase stage would depend on the relative importance of nuclear metabolism to the cell. Cells that are especially dependent on the nucleus for existence, as are the cells under consideration, would be expected to be unusually sensitive to radiation.

Conclusion

In presenting these notions, we are aware of the lack of sound experimental foundation in several instances. Data showing whether each cell listed conforms in all respects to the notions are not available. It is possible to interpret some of the available data as showing that cytoplasmic enzymes may be directly inhibited or destroyed by low doses of irradiation. It could then be argued that certain cytoplasmic enzyme systems present in these predominantly nuclear cells are (i) necessary to the continued functioning of the cell, (ii) vulnerable because of low concentration, and (iii) are thus the critical systems affected by radiation. However, such an interpretation does not seem to be as consistent with the totality of the available observations and with the basic theories as does the interpretation presented here.

Certain cells that seem to belong to the nuclear-dependent group do not show the expected sensitivity. For example, the cells of myeloid leukemia appear to be morphologically and metabolically similar to those in the group characterized by radiosensitivity, but they are apparently not killed in interphase by moderate doses of radiation, although mitosis is prevented (*26*). Assay of the distinguishing properties of these and other exceptional cells should be of first importance for developing a further understanding of cell sensitivity to radiations.

References and Notes

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Zeno Payne Metcalf, Distinguished Entomologist

Zeno Payne Metcalf was a tireless worker to the very last, for he was in his office working on the catalog of the Homoptera of the world until noon of the day he died. He devoted his whole professional life to North Carolina State College and brought international repute to that institution. He died at his home at Raleigh, N.C., 5 January 1956. Even though he had suffered poor health for many months, he died quite suddenly and unexpectedly while talking to his wife and daughter.

Dr. Metcalf was the author of nine books and an active member of 36 learned and professional societies. He was a key speaker at the International Congress of Zoology, which convened in Paris in July 1948, and at the International Congress of Entomologists, which met in Stockholm in August 1948. In addition, he was president of three major national scientific organizations, the Entomological Society of America, the Ecological Society of America, and the American Microscopical Society—a distinction that is accorded few scientists in the United States.

Dr. Metcalf also served on the editorial boards of four national professional journals and was the author of 96 professional publications. At the time of his death he was engaged in preparing a 42-volume catalog of the Homoptera of the world. Fifteen volumes had been or were in press at the time of his death, and several more volumes are almost ready to go to press. An attempt is being made to provide means of completing the entire set of 42 volumes. Dr. Metcalf has spent much of the past 40 years collecting notes for the series. In an effort to obtain material, he read and checked more than 20,000 books and papers dealing with insects and visited all the principal libraries in the United States and England. The order Homoptera comprises about 4000 described genera and 30,000 described species. The catalog now contains 512,000 references, probably the greatest catalog of any order of insects to be found anywhere in the world.

A native of Lakeville, Ohio, Dr. Metcalf was educated at Ohio State University, where he received his A.B. degree in 1908, and at Harvard University, where he earned his D.Sc. degree in 1924.

Prior to joining the North Carolina State College Faculty in 1912 he was an instructor in entomology at Michigan State College (1907–08) and was on the staff of the North Carolina State Department of Agriculture (1908–12). He joined the North Carolina State College faculty as entomologist with the experiment station and as professor of zoology and entomology. He was visiting professor in the summer session at Ohio State University in 1916 and 1918 and in the summer session at the University of Michigan in 1926. During the school year of 1935–36 he served as visiting professor of zoology at Duke University. Dr. Metcalf was head of the department of zoology and entomology, North Carolina State College, from 1912 to 1950. He was director of instruction in the School of Agriculture at North Carolina State College during the years 1923–44; director of graduate studies at the college, 1940–43, and associate dean of the Graduate School of the Consolidated University, 1943–50. He retired from administrative duties in 1950 and later devoted his full time to teaching, research, and writing.

He was active in both civic and professional affairs and was a former president of the North Carolina Academy of Science. He was also a fellow of the American Association for the Advancement of Science and the Entomological Society of America.

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Natural laws there probably are, rigid and unchanging ones at that. Understand them and they are beneficent; we can use them for our purposes and make them the slaves of our desires. Misunderstand them and they are monsters who may grind us to powder or crush us in the dust.—HENRY A. ROWLAND.