AGING: A THEORY BASED ON FREE RADICAL AND RADIATION CHEMISTRY

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The phenomenon of growth, decline and death—aging—has been the source of considerable speculation (1, 8, 10). This cycle seems to be a more or less direct function of the metabolic rate and this in turn depends on the species (animal or plant) on which are superimposed the factors of heredity and the effects of the stresses and strains of life—which alter the metabolic activity.

The universality of this phenomenon suggests that the reactions which cause it are basically the same in all living things. Viewing this process in the light of present day free radical and radiation chemistry and of radiobiology, it seems possible that one factor in aging may be related to deleterious side attacks of free radicals (which are normally produced in the course of cellular metabolism) on cell constituents.*

Irradiation of living things induces mutation, cancer, and aging (9). Inasmuch as these also arise spontaneously in nature, it is natural to inquire if the processes might not be similar. It is believed that one mechanism of irradiation effect is through liberation of OH and HO_2 radicals (12). There is evidence, although indirect, that these two highly active free radicals are produced normally in living systems. In the first place, free radicals are present in living cells; this was recently demonstrated in vivo by a paramagnetic resonance absorption method (3). Further, it was shown that the concentration of free radicals increased with increasing metabolic activity in conformity with the postulates set forth some years ago that free radicals were involved in biologic oxidation-reduction reactions (11, 13). Are some of these free radicals OH and/or HO_2, or radicals of a similar high order of reactivity, and where might they arise in the cell?

The most likely source of OH and HO_2 radicals, at least in the animal cell, would be the interaction of the respiratory enzymes involved in the direct utilization of molecular oxygen, particularly those containing iron, and by the action of catalase on hydrogen peroxide. This follows from the fact that it has been known for many years that iron salts catalyze the air oxidation of organic compounds (5, 6, 14, 15); OH radicals are believed to be involved in these reactions (13). Iron salts also catalyze the decomposition of hydrogen peroxide to water and oxygen—a reaction that involves OH and HO_2 radicals (16). Further, recent studies in this laboratory on the inactivation of rat liver catalase suggest that the OH radical is involved. The catalase activity of the homogenates both in the presence and absence of hydrogen donors such as sodium bisulfite, sodium hypophosphite, pyrogallol, and mercaptans remains relatively constant under an atmosphere of nitrogen. However, in the presence of air, catalase activity rapidly decreases and the rate of decrease is accelerated in the presence of the hydrogen donors. In addition, methanol, ethanol, and sodium formate (compounds (2) which are oxidized by hydrogen peroxide in the presence of catalase) stabilize the enzyme in the presence of air. A free radical mechanism involving the OH radical has been implicated in the analogous degradation of hemoglobin and myoglobin (7).

Thus, although the evidence is indirect, there are good reasons for assuming that the changes produced by irradiation and those which arise spontaneously in the living cell have a common source—the OH and HO_2 radicals. These arise on the one hand through the dissociation of water and on the other largely by the interaction of the oxidative enzymes with oxygen and hydrogen peroxide. (It is not unlikely that other metal-containing enzymes, such as vitamin B_{12}, which contains cobalt, also contribute.)

The manner in which a highly reactive radical such as OH would exert its effect on a cell is obscure. However, it would be expected to react for the most part near the area where it

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was produced and to react with the more easily oxidized substances such as $DPNH_2$ or the reduced form of the flavoproteins. They would also be expected to react to a certain extent with other cellular constituents including the nucleoproteins and nucleic acids. The organic radicals formed in this manner (by removal of a hydrogen atom) could then undergo further reactions, e.g., addition of oxygen leading to the formation of peroxides and other oxygenated compounds, degradation into smaller units, dimerization, etc., such as has been observed in simpler free radical and polymer systems (4). In this manner the functional efficiency and reproductive ability of the cell could eventually be impaired. In addition, since genes would be expected to be attacked occasionally it would be anticipated that mutations and cancer would result every now and then.

In a multicellular organism such as man the effects of cells on each other are superimposed on the above. Some cells are more important than others in maintaining life. For example, as degenerative changes occur in the cells of the circulatory system, the flow of oxygen and metabolites to other cells is interferred with, thus leading to further degenerative changes in them.

In addition to the reactions within the cells themselves, one would expect that there would be also a slow oxidation of the connective tissue by molecular oxygen catalyzed by metals such as iron, cobalt, and manganese.

This theory is suggestive of chemical means of prolonging effective life. For example, maintenance of an increased cellular concentration of an easily reduced compound such as cysteine, which affords some radiation protection, would be expected to slow down the aging process and thereby put off the appearance of the diseases associated with it. As a side effect radiation resistance would be enhanced. Further studies of the effect of hydroxy and other radicals, in the presence and absence of oxygen and easily oxidized substances, on cellular constituents such as DNA and RNA may be quite productive. Some of the implications of this theory both as it pertains to aging and to cancer are now under study. Groups of mice of the AKR and C3H strains, which spontaneously develop lymphatic leukemia and mammary cancer, respectively, are daily being given various radiation protection compounds in their diet. These animals will be followed to determine if the average age at which they develop leukemia or cancer is greater than in the controls. Mice in each group are also being sacrificed at periodic intervals for histologic study.

Consideration of the biochemistry of cancer cells and of the systematic effects in cancer from the standpoint of the theory presented in this paper led to the conclusion that hydrogen donors, such as cysteine for example, might be of benefit in the fields of cancer chemotherapy and nutrition. Preliminary work with ascites tumor and LCS cancer in mice supports this conclusion.

SUMMARY

Aging and the degenerative diseases associated with it are attributed basically to the deleterious side attacks of free radicals on cell constituents and on the connective tissues. The free radicals probably arise largely through reactions involving molecular oxygen catalyzed in the cell by the oxidative enzymes and in the connective tissues by traces of metals such as iron, cobalt, and manganese.

REFERENCES

1. Brody, S.: *Bioenergetics and Growth.* Reinhold Publishing Corp., New York, 1945.
2. Chance, B.: The Iron-Containing Enzymes. The Enzyme-Substrate Compounds and Mechanism of Action of the Hydroperoxidases, Chapter 56 C. In *The Enzymes*, edited by Sumner, J. B. and Myrbäck, K., Vol. 2, Part 1. Academic Press, Inc., New York, 1951.
3. Commoner, B., Townsend, J., and Pake, G. E.: Free Radicals in Biological Materials. *Nature, 174:* 689-691, 1954.
4. D'Alelio, G. F.: *Fundamental Principles of Polymerization.* John Wiley and Sons, Inc., New York, 1952.
5. Fenton, H. J. H.: Oxidation of Tartaric Acid in Presence of Iron. *J. Chem. Soc. 65:* 899-910, 1894.
6. Fenton, H. J. H., and Jackson, H.: The Oxidation of Polyhydric Alcohols in Presence of Iron. *J. Chem. Soc., 75:* 1-11, 1899.
7. George, P.: The Specific Reactions of Iron in Some Hemoproteins. In *Advances in Catalysis*, edited by Frankenburg, W. G., Komarewsky, V. I., and Rideal, E. K., Vol. 4, 367-428. Academic Press, Inc., New York, 1952.
8. Heilbrunn, Z. V.: *Outline of General Physiology,* 2nd Edition. W. B. Saunders, Philadelphia, 1943.
9. Hempelmann, L. H., and Hoffman, J. G.: Practical Aspects of Radiation Injury. *Ann. Rev. Nuclear Sci., 3:* 369-389, 1953.
10. Lansing, A. I., editor: *Cowdry's Problems of Ageing,* 3rd Edition, Williams and Wilkins Co., Baltimore, 1952.

11. Michealis, L.: Theory of Oxidation-Reduction, Chapter 44 in *The Enzymes*, edited by Sumner, J. B. and Myrbäck, K., Vol. 2, Part 1. Academic Press, Inc., New York, 1951.

12. Stein, G., and Weiss, J.: Chemical Effects of Ionizing Radiations. *Nature, 161:* 650, 1948.

13. Waters, W. A.: *The Chemistry of Free Radicals.* Oxford University Press, London, 1946.

14. Wieland, H., and Frage, K.: Über den Mechanismus der Oxidationsvorgänge. XX. Bernsteinsäure-Dehydrase. *Ann., 477:* 1-32, 1930.

15. Wieland, H., and Franke, K.: Über den Mechanismus der Oxydationsvorgänge. XII. Die Aktivierung des Hydroperoxyds durch Eisen. *Ann., 457:* 1-70, 1927.

16. Uri, N.: Inorganic Free Radicals in Solution, *Chem. Rev., 40:* 375-454, 1952.