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The Biologic Clock: The Mitochondria?

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ABSTRACT: The author suggests that the maximal life span of a given mammalian species is largely an expression of genetic control over the rate of oxygen utilization. The latter determines the rate of accumulation of mitochondrial damage produced by free radical reactions, the rate increasing with the rate of oxygen consumption, which ultimately causes death.

The mean life span in the United States has remained virtually constant at around 70 years since the mid 1950's (1), so it is likely that the rate of increase in the future in the United States, as well as in other developed countries, will be low unless new ways are found for inhibiting the rate of biological degradation in man. The changes in an organism leading ultimately to death can be regarded as due to a basic aging process(es) on which disease states are superimposed and intertwined. From this point of view, disease serves merely to hasten the death made inevitable by the aging process(es). The age at death, then, of any given organism (ignoring accidental deaths not associated with aging) is determined by the combined deleterious effects of the aging process(es) and disease states; and the maximal life span of the species is the value observed when these effects are minimized.

The degradative changes associated with aging and disease processes may be due in part to free radical reactions (2, 3) — reactions that are ubiquitous in living systems (4). Thus decreasing the level of deleterious free radical reactions in an organism might be expected to result in a decreased rate of biological degradation with an accompanying increase in the years of useful, healthy life. This approach offers the prospect of an increase in the average life expectancy to beyond 85 years (the maximal mean life span to be expected (5) if overt diseases such as cancer and cardiovascular disorders were eliminated as causes of death) and a significant increase in the number of people who will live to well beyond 100 years.

Experiments based on this hypothesis — the free radical theory of aging — have been encouraging (6-13). For example, a number of inhibitors of free radical reactions have been

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found to increase the mean life span of mice when added to the daily diet; 2-mercaptoethylamine (2-MEA), butylated hydroxytoluene (BHT) and a quinoline derivative (Santoquin) were the most effective (10-11). Likewise, decreasing the amount and degree of unsaturation of the dietary fat of female C3H mice resulted in a decrease in the mortality rate (12).

However, although a number of attempts to decrease the level of more-or-less random free radical reactions in mice have resulted in significant increases in the average life span, there has been little, if any, increase in the maximal life span. Why?

Is the hypothesis that more-or-less random free radical reactions contribute to the degradation of biological systems wrong, and/or these reactions make only a relatively small contribution to degradation? Have the attempts thus far to decrease the rate of free radical-induced deleterious changes been ineffective in areas that determine maximal life span? That the latter is probably correct derives from a consideration of the relation between oxygen consumption and the life span; animal species with high basal metabolic rates (BMR's) have short life spans (14). This focuses attention on the mitochondria: here oxidative reactions involving O_2 proceed continuously at a more-or-less constant high rate as compared to elsewhere in the body; over 90 per cent of the oxygen reacting in mammalian tissues is reduced in the process of mitochondrial electron-transport while most of the remaining 10 per cent or less is involved in the microsomal oxidase system.

It is suggested that the maximal life span of a given mammalian species is largely an expression of genetic control over the rate of O_2 utilization which, in turn, determines the rate of accumulation of damage produced by free radical reactions in the mitochondria, the rate increasing with the rate of O_2 consumption. The damaging free radical reactions may arise by both enzymatic and nonenzymatic processes. Studies with many of the classical oxidative-phosphorylation uncoupling agents suggest the presence of free radicals in the respiratory chain during oxidative phosphorylation (15, 16); free radicals are also probably present during the utilization of O_2 in the respiratory chain in the absence of oxidative-phosphorylation. Such intermediates are present in the mechanisms proposed for two recent model oxidative-phosphorylation systems (17, 18). Conceivably, substrate molecules in the mitochondria could reduce respiratory-chain free radical intermediates giving rise to "free" free radicals which could then initiate more-or-less random damaging reactions. "Free" free radicals could also arise through reactions of O_2 with respiratory-chain free radical intermediates.

Free radicals "escaping" from the respiratory chain or formed otherwise in the mitochondria would be expected to produce deleterious effects mainly in the mitochondria because of their high chemical reactivity. Is this the cause of the increased fragility (19) of mitochondria with increasing age as well as the decrease in the number (20, 21) of mitochondria per cell? Are these effects mediated in part through alteration of mitochondrial DNA functions?

The apparent failure of the free radical reaction inhibitors studied thus far to increase the maximal life span of mice can now be tentatively ascribed to a combination of a high rate of "free" free radical initiation, short chain lengths of free radical reactions, and possibly a low concentration of added anti-oxidant in the mitochondria. If the chain lengths were long, then it would be expected that some of the anti-oxidants studied thus far would have increased the maximal life span irrespective of whether or not the chain initiation rates were high or low. If the chain lengths were short and chain initiation low, the rate of accumulation of chemical damage in the mitochondria might be too low to serve as a suitable clock from an evolutionary standpoint.

Thus the observed increases in the average life span of mice produced by adding anti-oxidants to the diet may be attributed to a significant decrease in the level of deleterious free radical reactions throughout the body, except in the mitochondria. The situation would appear to be somewhat analogous to that in radiation biology where it is relatively easy to protect against the acute effects of whole-body ionizing radiation with a number of inhibitors of free radical reactions, whereas little if any protection is observed against delayed effects [such as life-shortening (22)] which, in view of the foregoing, may be due to a failure to protect the mitochondria from radiation-induced free radical reactions.

As the rate of utilization of O_2 increases in

different mammalian species the necessary higher rate of O_2 transport is facilitated by an increasing ease of dissociation of O_2 from oxyhemoglobin (14) and an increased Bohr effect (14), i.e., more O_2 is disassociated from oxyhemoglobin by a given decrease in pH. The increased exposure of cellular and extracellular areas to O_2 resulting from higher O_2 transport rates should lead to an increase in the rate of oxidative change in these areas, "explaining", for example, the correlation between the age changes in long-lived molecules such as collagen (23), elastin (24, 25), and probably also in DNA, and the life span. Likewise, a correlation between tissue oxygen concentration and the overall level of O_2 consumption may largely account for the fact that the rates of development of the degenerative diseases are, roughly, inversely proportional to the life span. Free radical reactions have been implicated in atherosclerosis, hypertension, cancer and amyloidosis (1, 3, 10, 12, 13, 26).

This extension of the free radical theory of aging focuses attention on the mitochondria. It is hoped that it will lead to fruitful experiments directed toward increasing the healthy human life span.

REFERENCES

1. Harman, D.: Prolongation of life: role of free radical reactions in aging, J. Am. Geriatrics Soc. 17: 721-735, 1969.
2. Harman, D.: Aging: a theory based on free radical and radiation chemistry, J. Gerontol. 11: 298-300, 1956.
3. Harman, D.: Role of free radicals in mutation, cancer, aging and the maintenance of life, Radiation Res. 16: 753-763, 1962.
4. Isenberg, I: Free radicals in tissue, Physiol. Rev. 44: 487-517, 1964.
5. Woodhall, B., and Joblin, S.: Prospects for further increases in average longevity, Geriatrics 12: 586-591, 1957.
6. Harman, D.: Prolongation of the normal life span by radiation protection chemicals, J. Gerontol. 12: 257-263, 1957.
7. Harman, D.: The free radical theory of aging: the effect of age on serum mercaptan levels, J. Gerontol. 15: 38-40, 1960.
8. Harman, D.: Prolongation of the normal life span and inhibition of spontaneous cancer by antioxidants, J. Gerontol. 16: 247-254, 1961.
9. Harman, D.: The free radical theory of aging: effect of age on serum copper levels, J. Gerontol. 20: 151-153, 1965.
10. Harman, D.: Free radical theory of aging: effect of free radical reaction inhibitors on the mortality rate of male LAF_1 mice, J. Gerontol. 23: 476-482, 1968.
11. Harman, D.: Free radical theory of aging: effect of free radical inhibitors on the life span of male LAF_1 mice — second experiment, Gerontologist 8: III, 13, 1968.
12. Harman, D.: Free radical theory of aging: effect of the amount and degree of unsaturation of dietary fat on mortality rate, J. Gerontol. 26: 451-457, 1971.
13. Harman, D.: Atherogenesis in minipigs: effect of dietary fat unsaturation and of copper, *in* Atherosclerosis: Proceedings of the Second International Symposium, ed. by R. J. Jones. New York, Springer-Verlag, 1970, pp. 472-475.
14. Schmidt-Nielsen, K.: Energy metabolism, body size, and problems of scaling, Fed. Proc. 29: 1524-1532, 1970.
15. Szent-Gyorgyi, A.: Bioelectronics. New York, Academic Press, 1968, p. 15.
16. Pullman, B. (Ed.), Electronic Aspects of Biochemistry. New York, Academic Press, 1963.
17. Wang, J. H.: Oxidative and photosynthetic phosphorylation mechanisms, Science 167: 25-30, 1970.
18. Painter, A. A., and Hunter, F. E., Jr.: Phosphorylation coupled to oxidation of thiol groups (GSH) by cytochrome C with disulfide (GSSG) as an essential catalyst. IV. Stability of intermediates and possible mechanism of reaction, Biochem. Biophys. Res. Communs. 40: 387-395, 1970.
19. Weinback, E. C., and Garbus, J.: Oxidative phosphorylation in mitochondria from aged rats, J. Biol. Chem. 234: 412-417, 1959.
20. Tauchi, H., and Sato, T.: Age changes in size and number of mitochondria of human hepatic cells, J. Gerontol. 509-512, 1968.
21. Herbener, G. H., and Thu, T. M.: Quantitation of mitochondrial structure in heart and liver of very old mice. Gerontologist 10: II, 24, 1970.
22. Bacq, Z. M.: Chemical Protection against Ionizing Radiation. Springfield, Illinois, Charles C Thomas, Publisher, 1965, p. 144.
23. LaBella, F. S., and Paul, C.: Structure of collagen from human tendon as influenced by age and sex, J. Gerontol. 20: 54-59, 1963.
24. LaBella, F. S.; Vivian, S., and Thornhill, D. P.: Amino acid composition of human aortic elastin as influenced by age, J. Gerontol. 21: 550-555, 1966.
25. Sinex, F. M.: Chemical changes in irreplaceable macromolecules, *in* Biological Aspects of Aging, ed. by N. W. Shock. New York, Columbia Univ. Press, 1962, p. 307-312.
26. Harman, D.: Atherosclerosis: a hypothesis concerning the initiating steps in pathogenesis, J. Gerontol. 12: 199-207, 1957.