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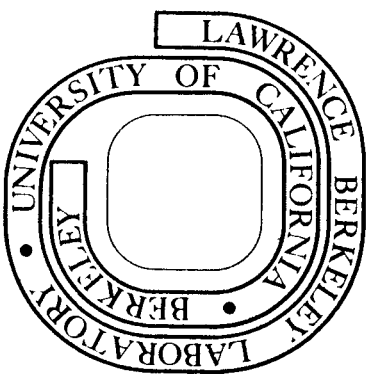

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DO FREE RADICALS SPEED AND ANTIOXIDANTS SLOW AGING AND CANCER?*

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At a recent conference on the chemistry of aging, the opening session devoted to defining the subject produced the following definition: "The beginning of aging is due to the accumulation of biological effects and events, the sum of which causes the function of a given organ system, either in whole or in part, to pass the point of optimum potential function in a given environment." Having satisfied ourselves with such a comprehensive definition, we were somewhat chagrined when someone erased the word aging and replaced it with disease.

We can paraphrase this type of definition of aging or disease as follows: In a given environment, cells suffer a certain amount of damage; if damage accumulates faster than cell division, biosynthesis, or repair can correct it, the damage will be cumulative. So we ask, "Where and how does the damage occur, and how can we protect against it in a given environment?"

A number of hypotheses have been advanced to account for cell aging. Many of these hypotheses of aging (free radical, mutation, error, crosslinking, and immunological) can be correlated under the more general umbrella of a genetic alteration theory. Within this theoretical framework, free radical damage plays a central role by causing molecular damage to informational macromolecules, which in turn amplify and spread the damage (Fig. 1). Damage to the biosynthetic and repair functions could result from free radicals originating from sources such as radiation, environmental pollutants, metabolically-generated radicals, or from genetic defects in peroxide-scavenging enzymes or from depletion of radical-scavenging molecules of the hydrophobic or aqueous phases of the cell (for review, cf. 1).

The World Health Organization has estimated that 75% of the incidence of human cancer is caused by environmental factors, and Boyland has suggested

that 90% of human cancer may result from endogenous and environmental chemical carcinogens (2). Free radicals are produced universally by many environmental agents and are implicated in experimental carcinogenesis, so it is essential to learn how free-radical species interact with tissue components to cause degeneration of the cells and, consequently, the organism. Here we shall review some evidence of damage caused by such free radicals and consider ways to eventually protect against that damage.

SOURCES OF FREE RADICALS

Certain environmental agents are themselves free radicals or serve *in vivo* to generate low, but continual, levels of radicals. The physical relationship between tissues and the agents that generate free radicals determines which cells will be affected. For example, wrinkles and change of skin texture, features that we commonly use as an index of chronological age, occur primarily on surface parts of the body that are exposed to visible and ultraviolet light.

Free radicals and peroxides are also generated internally. The stomach and small intestine are most likely to be exposed to lipid hydroperoxides originating from the oxidative rancidity of foods. The lungs, blood and heart are exposed to high concentrations of oxygen, the atmospheric pollutants nitrogen dioxide and ozone, all substances that generate free radicals *in vivo*. It can be demonstrated that air (which contains 20% oxygen) is somewhat toxic to cultured human fibroblast cells. If these cells are grown in an atmosphere similar to air but containing only 10% oxygen their lifespans are ordinarily 20-30% longer than cells grown in air (3).

Ionizing radiation produces many of its biological effects through the induced dissociation of water and the formation of H_2O_2:

$$H_2O \rightarrow H\cdot + HO\cdot$$

$$2\ HO\cdot \rightarrow H_2O_2$$

The hydroxyl radical (HO·) implicated here is also a product of lipid oxidation and is one of the most reactive free radicals to which man is exposed. When O_2 is reduced to water by the respiratory chain, the biological oxidation involves catalysts of hydrogen and electron transfer (flavins, quinones, non-heme iron-sulfur, and cytochromes) that exist as free radicals themselves during electron transport.

Free radical damage to membrane phospholipid results from several processes including abstraction of hydrogen from structural molecules by electron transport catalysts or production of superoxide ions (O_2^-) during biological oxidations. Such damage may be propagated by chain reaction in the lateral plane of a membrane (Fig. 2); e.g. where hydrogen abstraction leads to a free radical on a fatty acid of a membrane phospholipid, it may in turn form a peroxy compound by O_2 addition. These radical species are highly reactive and may damage adjacent proteins and lipids. One of the major decomposition products of the polyunsaturated fatty acids is a bifunctional aldehyde, malondialdehyde, which crosslinks and polymerizes lipids and proteins.

The cell has several endogenous mechanisms for protection against oxidative or free radical damage: free radicals are scavenged by antioxidants in both the lipid and aqueous phases of the cell by vitamins E and C, respectively. Peroxide byproducts of oxidative reactions are decomposed by catalases and peroxidases. Superoxide radicals are quenched by superoxide dismutase. When these protective mechanisms are lacking, major damage can result, as when severe or moderate deficiency of the enzyme glutathione peroxidase results in failure to decompose the peroxides that accumulate during metabolism. In this inherited disorder, red blood cells hemolyze and anemia results.

As this peroxidase requires selenium for normal activity, a deficiency in selenium can also interfere with this enzyme's activity.

Tocopherol prevents deleterious chain reactions and lipid decomposition by partitioning into the membrane lipid phase and donating electrons to reduce the lipid radicals. In stopping this radical propagation, vitamin E itself becomes a free radical but is a more stable radical because unpaired electrons are delocalized in the chromanol ring.

FREE RADICALS AND AGING

A number of changes associated with aging have been correlated with the free radical hypothesis. These include accumulative oxidative alterations in the long-lived molecules of collagen, elastin, and chromosomal material; breakdown of mucopolysaccharides through oxidative degradation; changes in membrane characteristics due to lipid peroxidation; accumulation of metabolically inert material such as ceroid and age pigment through oxidative polymerization reactions; and arteriolocapillary fibrosis. Although considerable evidence substantiates these changes, it is not certain whether they are of primary or secondary importance to organismic aging, or whether involvement of free radicals at the molecular level can be evaluated quantatively.

Crosslinking. New interest has been focused upon determining the mechanisms of crosslinking between DNA strands and other important aldehyde molecules caused by agents other than radiation (cf. Fig. 1). Crosslinking of macromolecules is thought to be important in mammalian aging because it is likely to cause macromolecular immobilization and loss of function. Evidence now exists that correlates age with increased binding of chromosomal proteins to each other and to DNA, and DNA-protein crosslinkage may partly account for the reported decreases in the percentage of DNA transcribed

with increasing age in mice and rats (4). DNA damage caused by crosslinking agents is sometimes irreparable because it can involve corresponding sites on both strands of the helix so that after excision, no template remains for DNA replication. Of the mechanisms that are able to induce crosslinking, lipid peroxidation by itself could cause many of the changes observed with age in the body proteins and nucleic acids (5).

The copper cation is a potent catalyst of lipid peroxidation. Total serum copper concentrations increase as a function of human age. Persons with atherosclerosis and arteriolocapillary fibrosis or with a history of myocardial infarction often have a significantly higher serum copper level. It is interesting that vitamin E has been reported to be useful to treat intermittent lameness by improving the microcirculation. Other antioxidants have been shown to prevent experimentally-induced atherosclerosis.

Lipopigments. Age pigments (lipofuscin and ceroid) have been identified in many animal cells. They often increase in quantity linearly with age, and may occupy as much as 50% of the cytoplasmic volume of some postmitotic cells (6). In human cardiac tissue, they accumulate at the rate of 0.6 to 1.0% of the total myocardial volume per decade.

The related pigments, lipofuscin and ceroid, have been implicated in many studies of cell senescence, and originate from subcellular organelles that are undergoing peroxidation reactions (7). Ceroid can accumulate in visible amounts within days to months, but lipofuscin formation generally occurs over months or years. Their composition, excitation and fluorescence emission spectra are similar. However, lipofuscin is richer in neutral lipid polymers, has a high concentration of zinc, and is resistant to chelating

agents or cation-exchange resins; ceroid contains a higher concentration of acidic lipid polymers, and can be dissolved by metal chelation.

It is difficult to imagine an efficiently functioning cell in which lipopigment occupies much of its cytoplasmic volume. Lipofuscin accumulation could impede the diffusion and transport of essential metabolites and macromolecules, and the resulting condition is reminiscent of glycogen storage diseases in which a genetic defect of a hydrolytic enzyme causes accumulation of intracellular glycogen particles, which bring about cell death and disease in infants. However, such a direct contributing role for lipofuscin has yet to be established.

Lipopigment accumulation occurs not only in aging cells but also in several conditions that involve peroxidative damage, such as Wilson's disease, thalassemia major, hemochromatosis, hypoxia, and vitamin E deficiency.

Vitamin E deficiency. Vitamin E deficiency has been related to aging in primates because such animals have accumulations of age pigment in long-lived postmitotic cells, a shortened lifespan, and increased susceptibility to disease. To what extent these changes are due to the loss of the antioxidative action of vitamin E is unknown.

In vitamin E deficiency, membranes become more labile because their polyunsaturated fatty acid content is decreased due to selective peroxidative destruction of polyunsaturated fatty acids, particularly arachidonic acid. Vitamin E deficiency is further exaggerated when the diet is high in linoleic acid or other polyunsaturated fatty acids that are suseptible to lipid peroxidative reactions, including lipopigment.

Thus, a hemolytic anemia characteristic of vitamin E deficiency in premature infants is effectively corrected by administering vitamin E and by lowering

dietary levels of linoleic and arachidonic acids. Circumstantial evidence, while not definitive, has shown that the lipopigment accumulating in the brain cells of chicks on a vitamin E-deficient diet contributes to the degeneration of cells (8).

At the cellular level, increased numbers of peroxisomes are observed in the liver of animals deficient in vitamin E. As this increase would improve H_2O_2 decomposition, it may be compensatory for the loss of vitamin E's antioxidative action. The membrane structure of mitochondria changes and after 150 days of vitamin E deprivation the average mitochondrial volume has increased 60 to 80%.

FREE RADICALS AND CANCER

Many chemical carcinogens and/or their intermediates may either be free radicals themselves or else may be activated by free radicals. Harmful environmental agents such as fumes, gas, and smoke often can be detected by their electron spin resonance (ESR) signals. Polycyclic hydrocarbons have a strong tendency to draw electrons from alkali metals to form relatively stable free radicals. Numerous studies have shed light on how carcinogenic transformation takes place: certain polycyclic hydrocarbons, which are initially active, become reactive carcinogens during oxidation by the liver microsomal electron transport system. Such carcinogens have been detected in tissue homogenates by ESR. For example, benz[α]pyrene is metabolized by a free radical mechanism to 6-OH-benzpyrene, with concomitant formation of H_2O_2, which reacts covalently with the nucleic acids of <u>in vitro</u> cell-free systems and induces strand breakage (9). This carcinogen causes morphological transformation of cultured Syrian hamster cells and rat fibroblasts. Interestingly,

transformation efficiency has a positive correlation with increasing age of the cell culture (10).

Carcinogens can also behave as photosensitizers when applied to animal skin by giving rise to reactive intermediates similar to those produced internally in liver from mixed-function oxidases acting on polycyclic hydrocarbons. The resulting, highly reactive intermediates can rapidly react covalently with DNA, and so can be potent frameshift mutagens (11).

Differences in ESR spectra in tumor and normal tissues. The experiments cited above show that free radical reactions often occur between carcinogenic chemicals and tissues that are becoming malignant. Thus, it has been suggested that ESR spectroscopy may become clinically useful for early detection of tumors. Rats were administered the carcinogens p-dimethylaminoazobenzene, thioacetamide and 2-acetylaminofluorene, and transitory signals at $g = 2.035 \pm 0.002$ were detected before recognizable tumors developed. In other experiments triplet signals were found in some tumors at $g = 2.07$ and at $g = 2.003$, but not in corresponding normal tissues. However the demonstration that a new, relatively stable paramagnetic complex, absent from normal tissues, is present and involved in the metabolism of tumor cells has not been unambiguously established. So far, ESR signals are not diagnostic of tumors.

ANTIOXIDANTS AND AGING

Studies that are directed toward extending the lifespan of animals are often carried out on model systems of lower animals or cultured cells where stochastic events are reduced, and death is more likely to result from cell senescence. Although the causes of aging in short-lived animals may differ from those in humans and other mammals, the fundamental mechanisms

of cellular aging are probably similar. For example, lipopigment accumulation, probably the most conspicuous age-associated subcellular change, has been found in cells ranging from primitive coelenterates and mollusks to humans and other mammals.

If free radical injury constitutes a major environmental cause of aging, then increased dietary concentrations of suitable forms of antioxidants and free radical scavengers should afford some protection against this kind of damage and could contribute to an organism's ability to realize its maximum lifespan. Several studies have shown that dietary supplementation with vitamin E or other antioxidants can, indeed, significantly increase lifespan and retard aging changes.

Cultured cells as model systems. The number of diploid mammalian and human cell replications is finite in vitro. Cultured human lung diploid cells (WI-38) have been documented by Hayflick (12) to have a lifespan of 52 ± 10 population doublings. The addition of hydrocortisone to the medium extends WI-38 cell lifespan by up to 40%. The number of population doublings has also been reported to increase when cells are cultured at reduced O_2 tension (10% O_2), when the culture medium is supplemented with vitamin E, and if a supplement of albumin (20 mg/ml) is added above the amount usually present in tissue culture medium. Although these last two observations are difficult to reproduce, they indicate that the mitotic potential of diploid fibroblasts in culture may not be rigidly limited and can be altered by dietary manipulation and environmental factors.

Experiments in which the effects of environmental stress and the protection afforded by antioxidants are examined are more easily reproducible.

Human diploid cells (WI-38) maintained confluently in a low serum medium accumulate considerable fluorescent material by seven weeks and look like old cells. However, the presence of 100 μg/ml vitamin E inhibits formation of malondialdehyde in cell lipids and prevents these changes. Addition of vitamin E to the culture medium also enhances the ability of WI-38 cells to survive stress induced by exposure to relatively high concentrations of oxygen (13).

The sensitivity of WI-38 cells to visible light may be induced by riboflavin and can be partially prevented by supplementing cells with 100 μg/ml vitamin E during growth preceding exposure to visible light. Our laboratory has found that liver mitochondria are implicated in flavin-photosensitized reactions; when rats were fed a vitamin E-deficient diet, the damage caused by flavin-linked enzymes was accelerated (14). α-Tocopherol also protects red blood cells against oxygen and light-induced deformation and abnormal budding by prohibiting the formation of cholesterol hydroperoxide which labilizes the membrane.

Animal studies. The free-living nematode Caenorhabditis briggsae, an organism composed mainly of postmitotic cells, is used as a model for aging studies. During its lifespan, pigment granules with acid phosphatase activity gradually accumulate, particularly in the intestinal epithelium. These granules eventually fill most of the cytoplasmic volume causing breakdown of the entire tissue. Near the end of the roundworm's life cycle, extensive damage to muscle cells and nerve complexes impair its motility. Addition of dl-α-tocopherol quinone to the medium prolongs its lifespan by 11 days over the 35 ± 2 days typical of control populations; the appearance of lipofuscin has been delayed, and the amount of detectable pigment reduced (15).

Life extension under the same static culture conditions has been confirmed in another species of nematode, Turbatrix aceti. In studies correlating enzyme changes with age, the specific activities of DNA polymerase and aldolase decreased, while there was a sharp increase in elongation factor 1 and RNA polymerase at 5 and 15 days, respectively, before the activities ultimately declined; inert enzyme molecules gradually accumulated as antigenically crossreacting material. Although such age-related evidence has been noted, but the causal relevance to aging is still in dispute. Inclusion of vitamin E in the medium prolonged the lifespan of the Turbatrix by 17% and extended the times before the specific activities of RNA and DNA polymerase reached their maximum (16).

When high concentrations of vitamin E were administered to mice by Tappel et al. (17) in conjunction with other antioxidants (selenium, butylated hydroxytoluene, methionine, and ascorbic acid), the age pigment accumulation decreased in heart and testes. This antioxidant mixture was used to obtain maximum protection against free radical damage because in certain tissues where exposure to oxidants is high, vitamin E alone cannot completely inhibit peroxidation. Of the other antioxidants, butylated hydroxytoluene was chosen for its chain-breaking synergism with vitamin E; ascorbate and methionine for scavenging free radicals in the aqueous phase of the cell; and selenium, a co-factor for glutathione peroxidase, for stimulating maximum activity of that enzyme system. The reduction of lipid peroxides to nontoxic hydroxy fatty acids by selenium-glutathione peroxidase spares the vitamin E and prevents decomposition of peroxides into free radicals capable of reinitiating peroxidation. Although the progressive accumulation of fluorescent lipopigment

could be slowed by increasing dietary antioxidants, there was no significant increase in lifespan (17).

It is possible that protection provided by antioxygenic nutrients and antioxidants could have been offset by nutritional imbalances from improper levels of methionine, vitamin E and selenium; or by difficulties in detoxifying the high doses of the synthetic antioxidant butylated hydroxytoluene; and/or by the increased susceptibility of old control and treated animals to infectious diseases.

AKR mice fed antioxidants other than vitamin E experienced a prolonged half-survival time, but not a prolonged lifespan: one percent w cysteine-HCl extended the time by 14.5%, 1% w hydroxylamine-HCl by 8.3%, and 2% w hydroxylamine-HCl by 17.0%. In LAF mice, the half-survival time increased 45% with 0.5% butylated hydroxytoluene and 29% with 2-mercaptoethylamine-HCl. Antioxidants seemed to increase the half survival time of mice only when the control lifespan values were suboptimal. This has been interpreted to indicate that anitoxidants retard aging indirectly by inhibiting some harmful environmental or nutritional factors such as the oxidation of essential dietary nutrients.

Two other antioxidants, nordihydroguaiaretic acid and ethoxyquin have also been reported to increase the half-survival time of mice. Antioxidant prolongevity may be related to the increased ability of these compounds to induce activity of the hepatic microsomal enzymes that are responsible for detoxifying nutrients. The activity and inducibility of the drug-metabolizing enzymes normally present decrease markedly with age. In mice fed nordihydroguaiaretic acid and ethoxyquin, the drug-metabolizing enzymes remained at about double the control level. N-propyl gallate, an antioxidant that does not extend the lifespan, did not induce these hepatic enzyme changes (18).

ANTIOXIDANTS AND CANCER

Populations in geographical areas naturally rich in selenium have a significantly lower death rate from cancer (19). Attempts to slow tumor growth in experimental animals by including vitamin E, selenium, or other antioxidants in the diet have produced several interesting developments, e.g. chemical transformation of hamster cells can be inhibited by antioxidants (20). The formation of cholesterol-α-oxide, a carcinogenic photoproduct of ultraviolet light is prevented in irradiated skin of mice fed a diet including a mixture of antioxidants. This type of finding may provide a basis for developing preventive measures against skin cancer induced by ultraviolet light. Of two experimental groups of rats that received 7,12-dimethyl-benz[α]-anthracene, those that received 20 mg vitamin E in addition had 40% incidence of tumors compared with a 73.6% incidence in animals receiving only 5 mg. Mice were similarly protected by vitamin E and selenium following topical application of this carcinogen. Dietary vitamin E also decreased the number of malignant growths resulting from feeding with the carcinogens 3-methyl-4'-dimethylaminobenzene and methylcholanthrene.

Other dietary antioxidants have shown some protection against cancer: inclusion of hydroxylamine-HCl markedly decreased the incidence of spontaneous tumors and gave a 20% extension of survival time to mice inoculated with Ehrlich ascites tumor. Butylated hydroxyanisole and ethoxyquin were effective in inhibiting carcinogenic effects of diethylnitrosamine and 4-nitroquinoline-N-oxide in mouse lung and benz[α]pyrene on the forestomach of the mouse.

Some possible modes of protection. The molecular mechanisms of antioxidant protection against cancer are not well understood. Antioxidants

may prevent various carcinogens from being activated to the more carcinogenic epoxides. Antioxidant agents would be expected to inhibit peroxidation reactions, which adversely affect DNA molecules, including the covalent reaction of carcinogens with DNA and destruction of pyrimidine moieties. It has been reported that cells cultured with DMBA had 63.2% more chromosome breakage than those cultures that contain both DMBA and vitamin E in the incubation medium (21).

Tumor levels of endogenous protectors. Tissues contain endogenous molecules that normally afford protection against free radicals. The amounts of these substances have been studied in tumor tissue, which generally possesses high antioxidant activity and low peroxidant activity. It has been shown experimentally that tumors generally have decreased catalase activity. Perhaps this reduction in catalase activity results from depressed synthesis; rats with ascites hepatoma cells incorporated less ^{14}C-leucine into liver catalase both *in vivo* and *in vitro* (22).

In persons with chronic leukemia and carcinoma, glutathione peroxidase is significantly less than normal (23). Correlation of low peroxidase levels with high putrescine levels in leukemia, Morris hepatoma, and Ehrlich ascites tumors has led to the interpretation that putrescine binding and inhibition of tissue peroxidase may be an important step in the transformation process. Decreased peroxidase would result in elevated tissue peroxides and these in turn would lead to increased numbers of free radicals that would ultimately damage nucleic acids, enzymes, and other cellular constituents and result in carcinogensis and/or mutagenesis (24).

CONCLUDING REMARKS

From an analysis of the current evidence we infer that:

Optimization of the cellular environment should delay some of the degenerative changes that accompany the aging process and may result in a modest extension of lifespan by enabling an organism to approach its full genetic potential. Further research is needed to identify the potential toxic effects of pollutants and other ambient factors in our environment such as oxygen and light, the components of our diet that contain damaging free radical substances, and the location and extent of organismic or cellular damage that is generated in a particular environment.

Results of experiments to clarify antioxidant protection against cancer and aging are highly promising and the subject warrants further investigation. Such studies could provide information on the combination of antioxidants that will afford maximum protection in a particular environment. Antioxidants eventually may be considered as essential dietary ingredients, much like vitamins.

REFERENCES

1. Walton, J., Packer, L., in "Vitamin E", L. Machlin, Ed., Marcell Dekker & Co., New York, 1977.
2. Boyland, E., Prog. Expt. Tumor Res. 11, 222, 1969).
3. Packer, L., Feuhr, unpublished results.
4. Cutler, R.G., Expt. Gerontol., in press.
5. Bjorksten, J., J. Am. Geriat. Soc., 16, 408, 1968.
6. Miquel, J., Tappel, A.L, Dillard, C.J., Herman, M.M., Bensch, K.G., J. Gerontol., 29, 622, 1974.
7. Chio, K.S., Reiss, U., Fletcher, B., Tappel, A.L., Science, 166, 1535, 1969.
8. Yu, W.A., Yu, M.C., Young, P.A., Exp. Mol. Pathol., 21, 789, 1974.
9. Ts'o, P.O.P., Caspary, W.J., Cohen, B.I., Leavitt, J.C., Lesko, S.A., Lorentzen, R.J., Schechtman, L.M., in "Chemical Carcinogenesis" Part A, P.O.P. Ts'o and J.A. DiPaolo, Eds., p. 113, Marcel Dekker, New York, 1974.
10. Lasne, C., Gentil, A., Chouroulinkov, I., Nature, 247, 490, 1974.
11. Ames, B.N., Durston, W.E., Yamasaki, E., Lee, F.D., Proc. Nat. Acad. Sci USA, 70, 2281, 1973.
12. Hayflick, L., Exp Cell Res. 37, 614, 1965.
13. Packer, L., "Protein and Other Adducts to DNA: Their Significance to Aging, Carcinogenesis, and Radiation Biology", Int. Symp. Proc., Williamsburg, VA, p. 519, 1975.
14. Pereira, O.M., Smith, J.R., Packer, L., Photochem. and Photobiol. 23, 237-242, 1976.
15. Epstein, J., Gershon, D., Mech. Age. Dev., 1, 257, 1972.

16. Bolla, R., Brot, N., Arch. Biochem. Biophys., 169, 227, 1975.

17. Tappel, A., Fletcher, B, Deamer D.W., J. Gerontol. 28, 415, 1973.

18. Walker, R., Rahim, A., Parke, D.V., Proc. Roy. Soc. Med., 66, 780, 1973.

19. Shamberger, R.J., Willis, C.E., Crit. Rev. Clin. Chem., 2, 211, 1971.

20. Piekarski, L., Konkiewicz, M., Neoplasma (Bratisl.) 22, 251, 1975.

21. Shamberger, R.J., Baughman, F.F., Kalchert, S.L., Willis, C.E., Hoffman, G.C., Proc. Nat. Acad. USA, 70, 1461 (1973).

22. Kashiwagi, K., Tobe, T., Higashi, T., Warabioka, K., Gann, 63, 57, 1972.

23. Hopkins, J., Tudhope, G.R., Br. J. Haematology, 25, 563, 1973.

24. Armstrong, D., Dimmitt, S., VanWormer, D., in "Protein and Other Adducts to DNA: Their Significance to Aging, Carcinogensis and Radiation Biology", Abst. p. 80, Int. Symp., Williamsburg, VA, May, 1975.

25. Smith, K.C., in "Cellular Aspects of Ageing: Concepts and Mechanisms", R.G. Cutler, Ed., S. Karger, Basel, 1976.

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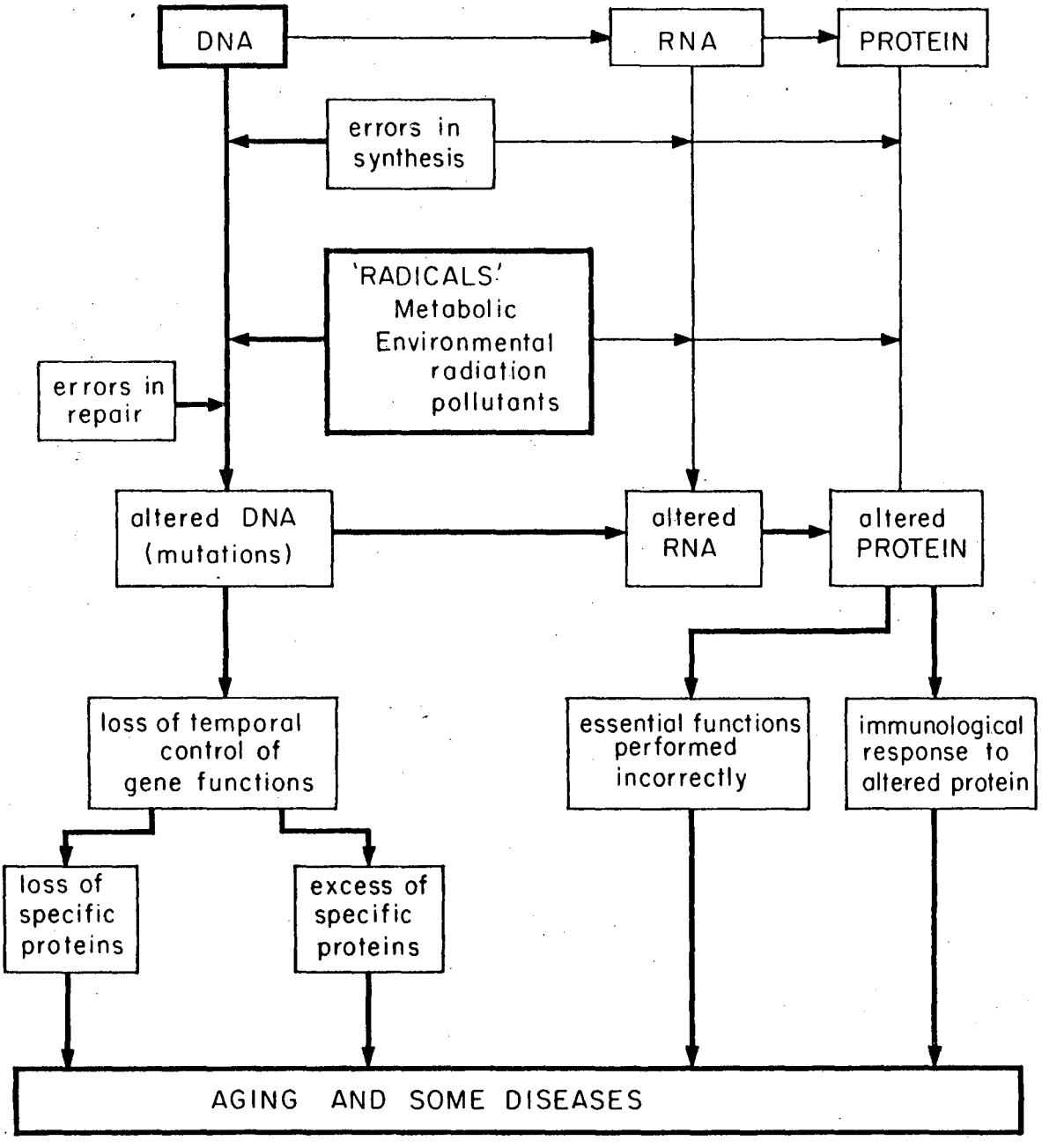

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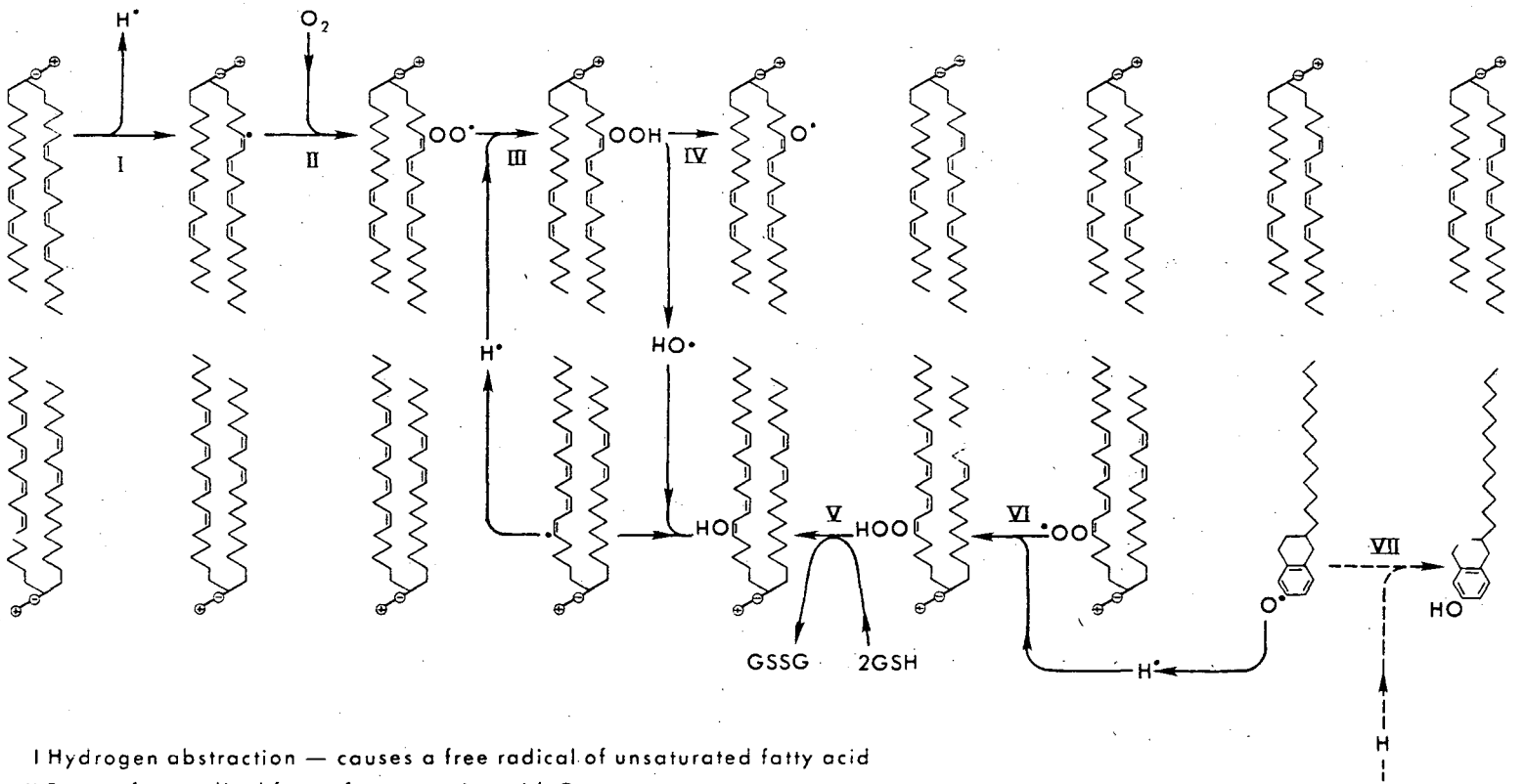

I Hydrogen abstraction — causes a free radical of unsaturated fatty acid

II Peroxy free radical forms from reaction with O_2

III Hydroperoxide forms by reduction of peroxy radical

IV Oxyradical arising by homolytic scission

V Hydroxy compound arising from action of glutathione peroxidase

VI Quenching of peroxy radical by dl-α-tocopherol

VII dl-α-tocopherol will decompose — possible reduction of radical by electron transport

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