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Pathways to the Decisive Extension of the Human Specific Lifespan*

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ABSTRACT: Three approaches to reversal or removal of gerogenic aggregations of macromolecules have shown promise. Of these the *enzyme approach* is the most gentle, and can be made specific. Aside from this, the lower the molecular weight of an enzyme, the better chance it will have to be immunologically tolerated as well as replicated synthetically in whole or in part. The *chelating approach* provides a powerful means for removing a single class of unwanted, random crosslinkages, i.e., those due to extraneous polyvalent metals such as lead, cadmium and aluminum. The *free hydroxyl radical approach* is the most penetrant and most versatile means for removing otherwise insoluble aggregates, but its very lack of specificity will demand great foresight in control and use. Together, these three methods, when properly applied, might bring some principal objectives of gerontology within closer range.

The following steps are required to effect a breakthrough for extending the specific maximum lifespan attainable by humans, with concurrent improvement in health and vitality:

1) To define the objective.
2) To define the principal adverse mechanism.
3) On the basis of 1) and 2), to define the targets.
4) To find ways to attack these targets effectively.
5) To demonstrate results.

After 36 years of work it is beginning to appear that steps 1-4 are now behind us and step 5 well under way, held up only by lack of funding.

1. *Definition of objective*

At present the life expectance of a man at age 60 is less than 2 years more than it was 188 years ago, in 1789. A positive change is overdue, and would appear to be a worthy prime objective. The number of years remaining to the average white man at age 60 (1) is represented in Figure 1.

2. *Principal adverse mechanism*

The principal adverse mechanism is crosslinkage, because no other mechanism can cause as much damage with as small an input as a random, unwanted crosslinkage at the wrong time and place. The components and conditions for this process are unavoidably present in all living organisms (2-13).

3. *The targets*

Specific targets for correction are represented in Figure 2, A and B, showing crosslinking of two strands of DNA and various crosslinkages in the course of a lifetime.

4. *Ways to attack the targets*

These are:

A. To develop a lytic enzyme of small molecular weight which can reach the desired sites: 1) to overcome the steric hindrances which block the access of larger enzymes, so that enzymatic removal of gerogenic crosslinked aggregates becomes possible (4, 14); and 2) to shorten the time necessary for excision repair of DNA (10, 15). The effects of such an enzyme that dissolves the "insoluble" are shown in Figure 3.

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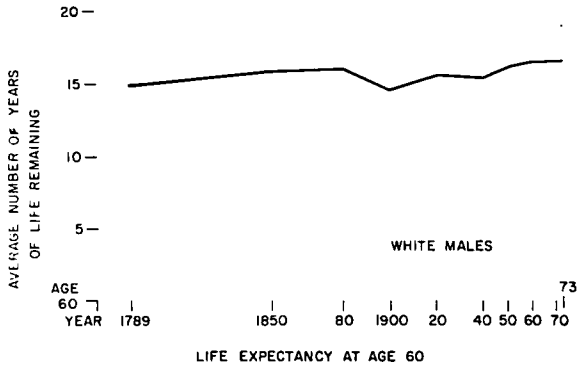

Fig. 1. Number of years remaining to the average white man at age 60. (Prepared from data obtained from the Metropolitan Life Insurance Company and the U.S. Bureau of Vital Statistics.)

B. To select and test chelation agents and techniques for removing metal-based crosslinkages (17). Although limited to metal-containing bonds, this may be important, particularly in countering senile mental disintegration (18, 19).

C. To use free hydroxyl radicals (OH) for depolymerizing the gerogenic aggregates (Fig. 2A) and possibly effect gradual renewals in DNA molecules before the crosslinking agent (Fig. 2B) has connected to the second strand.

REDUCTIVE-OXIDATIVE DEPOLYMERIZATION

Pathways A and B (enzymes and chelation) have been covered in detail in previous publications. Here, we shall discuss chiefly reductive-oxidative depolymerization, which apparently has not before been stressed in this context.

Like many others interested in aging, we felt the need for a quick assay method to determine in a few hours whether or not any given treatment was effective in eliminating the gerogenic insoluble materials. For this purpose we prepared assay animals, for example, as follows:

A pregnant rat received 40 millicuries of tritiated acetate at the time of giving birth; the offspring thus received tritium from their mother, and no more of it during their lifetime. Their tritium was initially excreted very fast, but finally excretion leveled out so that after about two years very little was excreted, while the residual radioactivity remained firmly locked into immobile compounds (20, 21). Anything that would remobilize these compounds would immediately be apparent from a sudden peak in the radioactivity of the urine of the animals. This litter served its purpose, but the mother, which had the highest tritium content of them all, died after 23 months. We isolated from her liver an insoluble fraction by repeated extractions with water (acidic and alkaline), acetone, methanol-chloroform, repeated digestion with a huge excess of

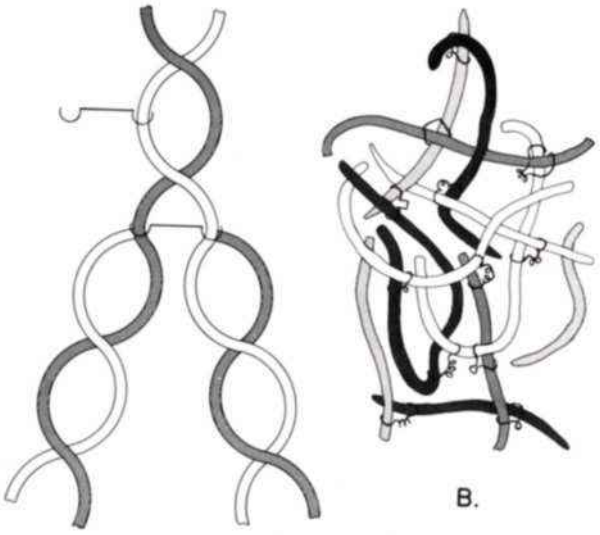

Fig. 2. **A.** Two strands of DNA have become cross-linked at the corresponding sites. This cannot be repaired, because the same site is involved in both strands. The cell will die or mutate in the next mitosis (10, 11).

B. In the course of a lifetime, numerous large molecules will randomly become tied up by crosslinkages so tightly that repair enzymes are excluded. They will form nets or cages which impede transport within the cell and reduce the space available for normal functions (10, 11).

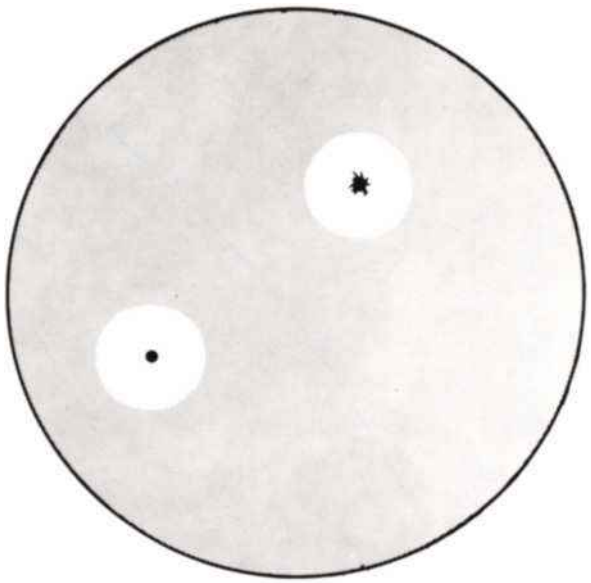

Fig. 3. Petri dish containing transparent agar gel in which is suspended an aggregate of Figure 2B material, isolated from old human brains. Two bacterial colonies are shown, from which enzyme is diffusing. This enzyme has dissolved the "insoluble" in a halo-like zone surrounding the colonies (10, 16).

"Pronase" (a proteolytic enzyme-aggregate), plus a final solvent; and then exposed the aggregate to *free hydroxyl radicals* from oxidizing ferrous salt, which was being continually reduced back by .01 mol. ascorbic acid and so used over and over. This method of depolymerization by oxidative-reductive processes was first indicated by Skanse and Sundblad for the specific application to hyaluronic acid (22); it was explored in great detail and applied to carbohydrates broadly by Pigman and co-workers (23-28). It was *re*discovered by Orr in the context of depolymerizing the enzyme catalase (29), and was found by Robinson, Richheimer, Westall et al (30-32) to be active on proteins, particularly transferrin, an encephalitogenic protein, and a pentapeptide. Richheimer and Robinson (32) showed that this involved direct breakage of peptide linkages.

The study of this reaction had been carried out with a minimum of cross-references between the groups. The similarity of findings, nonetheless, indicated the identity of the reaction. It appeared that this distinctly nonspecific reaction should have a chance to release the gerogenic frozen metabolic pool (17), so we tried it on the radiotagged insolubles from the described 40-millicurie mother rat, with the result shown in Figure 4.

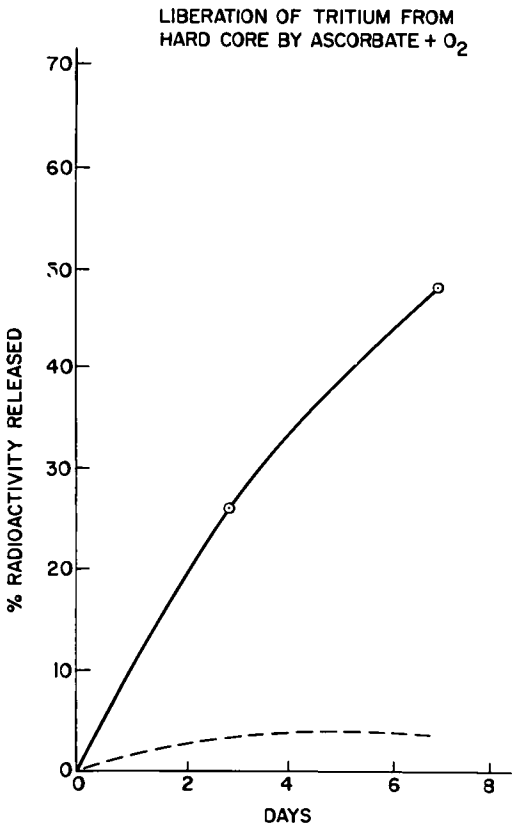

Fig. 4. Liberation of tritium from hard core by ascorbate + O_2. (Experiment on radio-tagged mother rat.)

This figure shows that a rapid liberation of the radioactivity, which had defied other means for release, immediately took place.

When it comes to penetration of dense gerogenic aggregates, we could hardly wish for a smaller, more mobile and penetrant agent than the free hydroxyl radical, which is readily generated and used under physiologic conditions, by a ferrous salt and any enediol containing the sequence (33, 34):

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   |
 C.OH
   ||
 C.OH
   |
 C=O
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What it lacks in specificity it makes up in mobility.

Each of the three outlined pathways has its limitations and advantages. Together they represent an arsenal which, properly used, should enable us to extend decisively the specific lifespan of rats and, upon completion of the requisite testing, perhaps also of man.

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