THE CROSSLINKAGE THEORY OF AGING: CLINICAL IMPLICATIONS

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Before 1940, thousands of papers had been written about crosslinking as a means of stabilizing macromolecules for industrial purposes—for example, tanning hides to make them insoluble and resistant to microbial attack, and vulcanizing rubber to increase its stability. Controlled artificial crosslinking of gelatin made the photographic film possible. No one had yet connected this vast body of knowledge with the changes that occur with the passage of time in collagen, elastin, and other large, reactive, biological molecules.

In 1937, having studied under Nobel laureates Hans von Euler and A. I. Virtanen, the author began directing a group working on stabilizing industrial protein gels used in graphic arts. In 1942, he expressed the crosslinkage theory of aging as follows:

> The aging of living organisms I believe is due to the occasional formation, by tanning, of bridges between protein molecules, which cannot be broken by the cell enzymes. Such irreparable tanning may be caused by tanning agents foreign to the organism or formed by unusual biological side reactions, or it may be due to the formation of a tanning bridge in some particular position in the protein molecule. In either event, the result is that cumulative tanning of body proteins, which we know as old age.[1]

If we add the words "and nucleic acids" to "proteins," this concept still covers the basic tenets of the crosslinkage theory. Indeed, it applies to any large molecule that contains any sites reactive with any sites on any crosslinking agent to which it can be exposed under physiologic conditions. Most common of such reactive sites are amino-, hydroxyl-, and carboxyl-groups.

At the recent International Symposium, "Adducts to DNA: Their Significance to Aging, Carcinogenesis, and Radiation Biology," 23 of the 64 papers dealt directly with crosslinking, and the subject was included in at least 20 others.

The theory is that crosslinkage is responsible for many of the secondary and tertiary causes of aging. While many previous conceptions about aging have been discredited, the crosslinkage theory has withstood the test of time.

In his introductory paper to the session on relevance of DNA adducts to aging, R. G. Cutler[2] stressed the significance of crosslinkages as a primary cause of many aging processes including sclerosis, failure of the immune system, loss of elasticity, decline in the secretion of many hormones, and increase in the sensitivity to trauma. Thus, if crosslinkage can be prevented or countered, a great

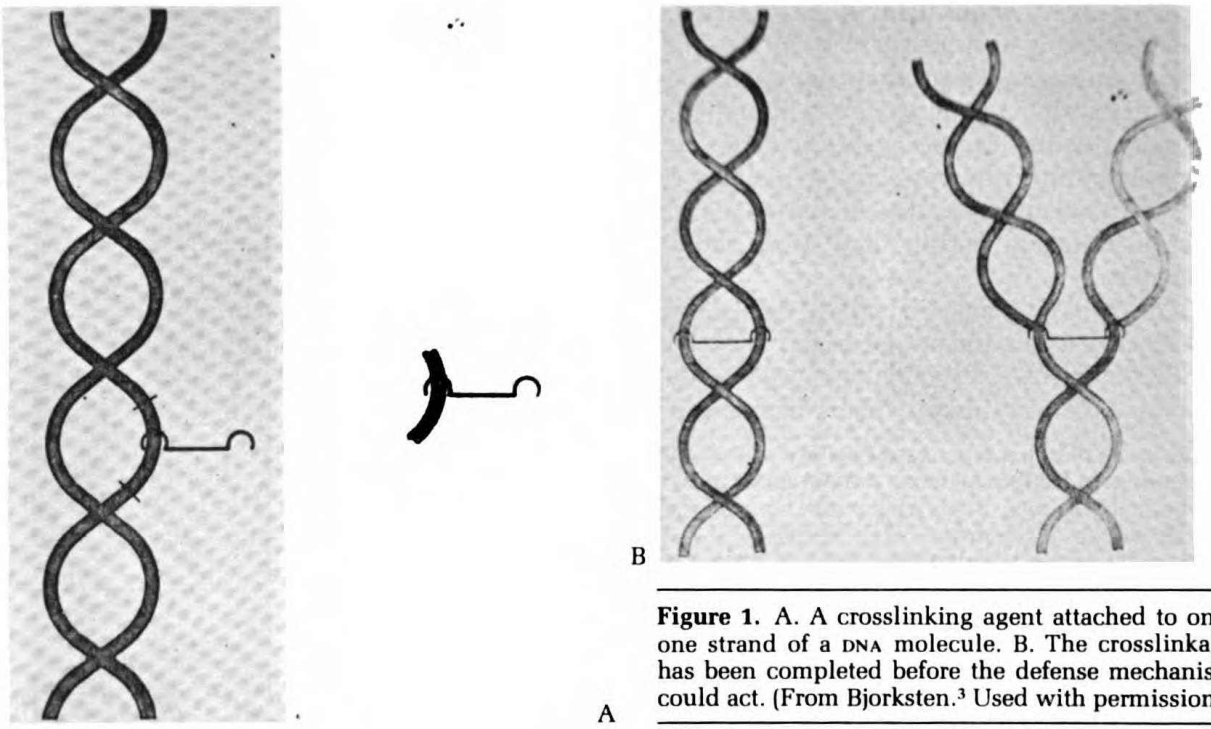

Figure 1. A. A crosslinking agent attached to only one strand of a DNA molecule. B. The crosslinkage has been completed before the defense mechanism could act. (From Bjorksten.[3] Used with permission.)

many other conditions will be beneficially affected, including the prognosis of any disease in the older age brackets.

CROSSLINKAGES

Crosslinkage reactions result in the union of at least two large molecules. A bridge or link between these is usually formed by a crosslinking agent: a small, motile molecule or free radical with a reactive hook or some other mechanism at both ends, capable of reacting with at least two large molecules. It is also possible for two large molecules to become crosslinked by the action of their own side chains or reactive groups present on one or both of them, or pathologically formed by ionizing radiation.

The double helix of the DNA molecule is easily recognized, as is the crosslinking agent with its two hooks. One of these attaches itself proximally to one strand only of the DNA helix. When the defense mechanisms of the body are unable to loosen the linkages (still only an incipient threat), they excise the linkage by cutting out the piece of the strand, thus removing the crosslinking agent as well. This is excreted (Figure 1A). The damaged strand is repaired, using the undamaged strand as a template.

This mechanism functions in a great many cases, but sometimes repair is too slow (Figure 1B). The crosslinking agent, with its proximal hook still attached to one DNA strand, connects with its distal hook to the corresponding site on the other, heretofore untouched DNA strand. This is fatal. If the corresponding sites on both strands are simultaneously cut out, no template remains for repair. On the other hand, if the defense mechanism simply leaves the crosslinkage in position, the strands cannot be parted at that point, as required in the next cell division. The resultant Y-shaped molecule is not viable. The cell involved will generally die, or in rare instances it might mutate.[3–7]

Thus it is readily understandable why crosslinking agents, all of which have two or more reactive sites, are much more destructive to the DNA molecule than are structural analogues with only one reactive site. Such structural analogues can attach themselves, but lack the second attachment point required for crosslinking.[3–7]

NONGENETIC MACROMOLECULES

In spite of the obvious importance and the proven sensitivity of DNA to crosslinkage, damage to the DNA molecule may not be the most

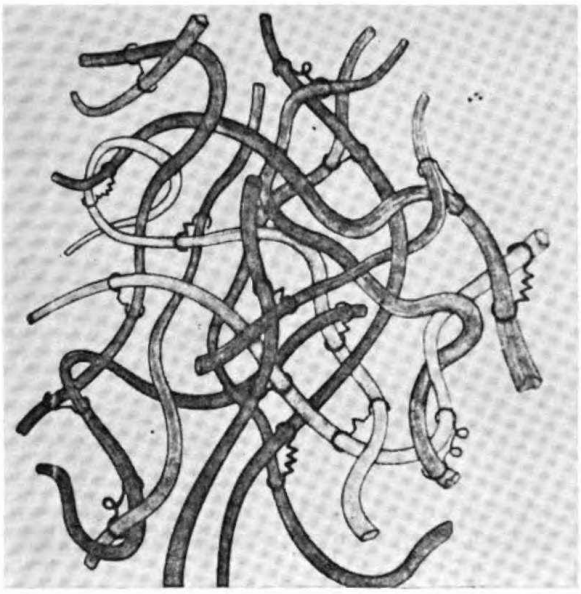

Figure 2. Random, accidental, mostly nonenzymatic crosslinkages accumulate over a lifetime to form dense aggregates of any available large molecules with any crosslinking agent. (From Bjorksten.[3] Used with permission.)

important factor in aging. In a lifetime, crosslinkages form between all types of large molecules, wherever one of the billions of crosslinking molecules in the organism accidentally locks onto a large molecule (Figure 2).

Although they are essentially similar, the genetic and the nongenetic molecules differ in sensitivity to crosslinkage. If a nongenetic aggregate is destroyed, the functional components can usually be replaced by normal anabolic reactions. The damage is primarily mechanical: crowding and interference with intracellular transport (Figure 2). A nongenetic molecule may suffer some crosslinkage without irretrievable damage, so long as access to repair (or scavenging) enzymes is not prevented. In such cases, these enzymes can break up the molecule into excretable fragments, which can be removed and replaced by the normal anabolic processes.

DNA molecules, however, are irreplaceable. Most higher organisms are diploid, containing one pair of DNA molecules in each chromosome. A lesion of one of the two DNA strands can be repaired, using the undamaged strand as template for exact replacement of building blocks. If the lesion extends to the corresponding site on the other strand, the damage is irreversible, and the molecule is destroyed.

Both the genetic and nongenetic crosslinkages are important. DNA is much more sensitive but is also far less abundant than the nongenetic proteins and enzymes.

Hart and Setlow[7] established a good correlation between the efficacy of the enzymatic excision-repair mechanism and the average life expectancies of man, elephant, cow, hamster, rat, mouse, and shrew. This correlation suggests that the speed with which the first step of DNA damage is repaired is crucial in determining life expectancy.

On the other hand, all these life expectancies reach a plateau at a certain level. In analogy with the DNA-repair situation shown in Figure 1, it seems possible that what is needed is an enzyme that can repair or remove the much more complex system shown in Figure 2.

When either of these crosslinking mechanisms has resulted in the spontaneous death of nondividing cells such as neurons, the damage is irreversible. The resultant disease knows no regression. This suggests that preventive therapy be initiated at the first indication of cerebral degenerative damage.

And although dividing cells such as connective tissue cells are readily replaced, their death may still lead to accumulation of foreign wastages, liposomes, or pigments.

SCLEROTIC CHANGES

The principal single cause of death—the gradual closure of the arteries—appears in many guises. Stroke, arteriosclerosis, circulatory disease, thrombosis, are all different facets of the same process.

An enormous amount of study has been devoted to the causes and therapy of circulatory disease. Duncan's basic work[8] and three of the more recent studies[9,10,11] establish quite clearly that some injury causing increased permeability of the intima is often an early occurrence. Some injury or deviation on the molecular scale obviously must precede or cause this increase in permeability. From the viewpoint of crosslinkage theory, the following appears to be the most plausible explanation:

The circulating blood plasma normally contains crosslinking agents, which over the years induce loss of elasticity, a primary effect of

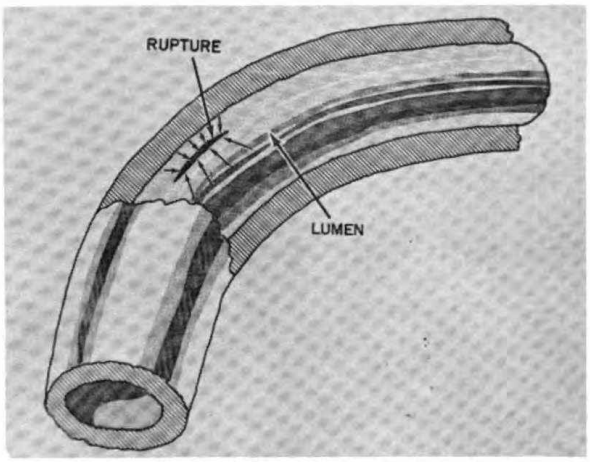

Figure 3. A rupture has formed in the intima; blood serum filters through and deposits particulate matter in the subintimal region.

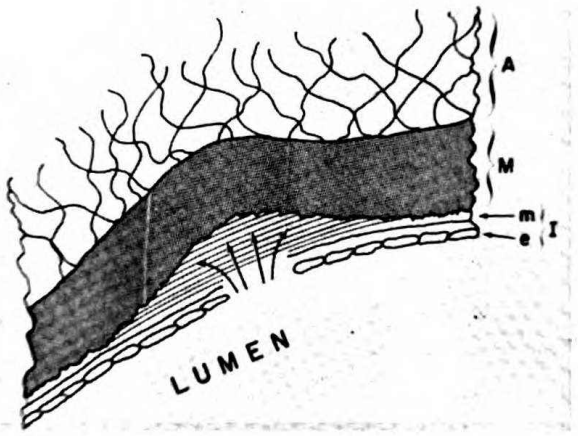

Figure 4. Diagram of the rupture in section. e =endothelium, m =membrane, I = intima, M = media, A = adventitia

crosslinking. This effect is most marked in the circulatory system, because this has the most direct surface contact with the bloodstream. The loss of elasticity, or embrittlement, which is inherent in multiple crosslinkage, is most strongly evident in the elastic tissues. It may also be important in the basement membrane, though studies of this are less complete.

In the early years of life the loss of elasticity is not so evident, but sooner or later, particularly in the tunica media, this loss will have progressed so far that the media yields less readily than before to the recurrent pressures of the pulse wave. The reduced yielding results in a larger force being required to effect the necessary displacement. Blood pressure rises, but even before this becomes evident, the intima is exposed to a hydrostatic stress at each heartbeat. This stress increases progressively as the media hardens. The intima is thus exposed every second to a hydrostatic squeeze between the hardening media and the noncompressible circulating blood.

When the crosslinkage has brought about a certain degree of hardening of the media and a reduced tolerance to pulsating deformation of the elements of the intima, the endothelium is damaged and becomes permeable, so that plasma flows through it, with plasma constituents diffusing into the subintimal region. This could occur by the formation of microfractures, degeneration of the endothelium, or failures of intercellular bonding in the endothelium. Be this as it may, it has been fully established that the damage that does occur leads to a greatly increased permeability of the endothelium. This damage occurs principally where the distension is maximal—in bends and branchings of the artery.

Blood corpuscles, chylomicrons, and any other particulate matter carried by the bloodstream are deposited in the subintimal region. Ingrowth of cells into the area of diffusion occurs. These cells take up plasma constituents, principally liquids, to form a cell resembling a phagocyte (a foam cell). Fibroblasts also grow into the region, and collagen is formed. Endothelial cells attempt to repair and recover the rupture. The degree of repair depends largely on the quantity and resorbability of the particulate lipid matter present. Figure 3 shows the initial lesion and infiltration, Figure 4 the next step.

Fatty degeneration can be expected to occur spontaneously, even without prior rupture, where crosslinkage has caused a substantial change toward lipophilicity in the surface orientation of susceptible protein (Figure 5). Thus, lipids can also be deposited in the media via diffusion from the vasa vasorum.

The accumulation and retention of lipids are further accentuated when, in the course of time, substantial parts of the macromolecules have become crosslinked, so that the more hydrophilic sides of the participating molecules are joined together. The lipophilic sides are then turned outward and exposed, attracting additional lipids.

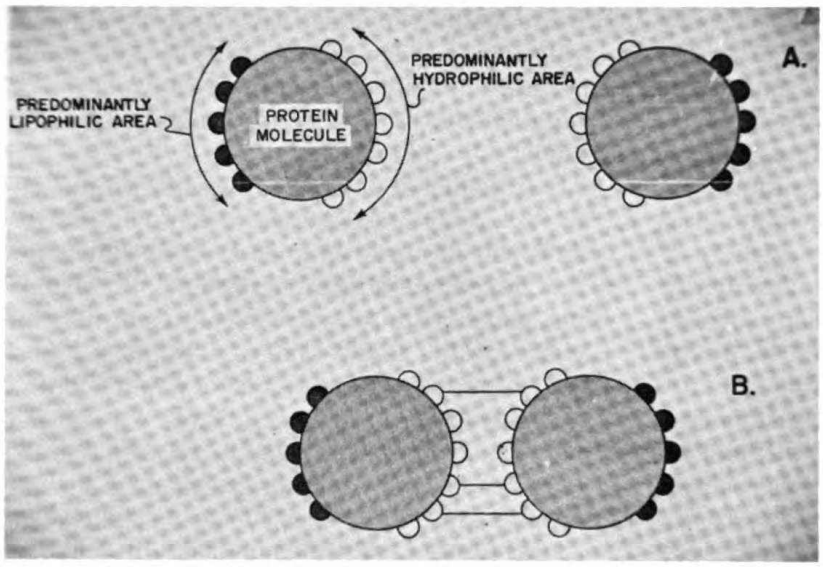

Figure 5. A. Two protein molecules having both lipophilic (dark) and hydrophilic (white) sites. B. The same molecules crosslinked preferentially at the hydrophilic sites, so as to turn the lipophilic sites outward, thus becoming lipophilic.

In diabetic metabolism, ketone bodies are formed, some of which are potent crosslinkers. This may explain the acceleration of sclerotic phenomena in uncontrolled diabetes. Milch et al.[12] have shown experimentally that conditions very similar to sclerotic and arthritic lesions can be induced by injections of crosslinking aldehydes.

CELLULAR EFFECTS

Visually observable precipitates may occur as crosslinking progresses with age. Some such precipitates are familiar to pathologists as lipofuscin, amyloid, vitamin E deficiency pigment, and the like. All contain a major proportion of matter not hydrolyzable under normal physiologic conditions. Their chemical composition varies within limits consistent with the view that they have been formed by random crosslinkage of whatever large molecules happened to become crosslinked by any one of numerous processes or reactants. The reaction time for this is more than 80 years—a lifetime.

Obviously, sufficiently large accretions of any physiologically inert material encroach on the space needed for life processes. Rudzinska's classical work with *Tokophrya*, a monocellular organism, shows the direct connection between intracellular precipitates and the lifespan of the organism—culture conditions that increase the precipitates also shorten the lifespan, and vice versa.

However, this is only the tip of the iceberg. For every aggregation that becomes visible, a far larger number of macromolecules have united to form a diffuse invisible cage or network within the cytoplasm (Figure 2). Techniques of visualization of DNA have shown its strands progressively snarled and netted with aging. Since the reactive groups are similar, and exposure to crosslinking agents is constant through life, similar aggregations are also formed by other, not yet visualizable large molecules including RNA, enzymes, and indeed all proteins. The presence of such reticular structures in a cell necessarily interferes with the mechanics of intracellular transport to a degree that results in progressive deterioration and finally becomes lethal.

Such progressively increasing interference with the normal transport within the cell might explain the observed deterioration of the immune system and the synapses with progressing age, the cybernetic effects stressed by Still, and the increasing spontaneous death rate of neurons and other nondividing cells.

PROPHYLAXIS AND THERAPY

The occurrence of unwanted random crosslinking cannot be prevented. The crosslinking agents normally present in the human body are far too numerous and reactive, and the reactions too diverse, to make practical any form of blockage and prevention of crosslinking reactions. However, some mitigation is possible.

Diet

All polyvalent metal ions are potential crosslinking agents. The human organism has developed defenses against adverse side effects from metal ions required in normal metabolism, particularly calcium, magnesium, iron, copper, zinc, and perhaps chromium. It is more defenseless against cadmium, which accumulates in soft tissues; aluminum, which increases with age in the brain, heart, and aorta; titanium, which also increases on aging; and possibly some additional, less common metals. The study of trace metals has concentrated on requirements, and on beneficial effects. However, equally important long-range negative effects have been neglected.

The presence of many polyvalent metal ions can be detrimental. Tyler,[13] for example, observed that mammalian sperm cells have up to 300% increased span of motility in water in which polyvalent metals have been selectively removed by treatment with ethylene diamine tetra-acetic acid (EDTA) or any other tolerated chelating agent. In any event, it seems wise to avoid unnecessary exposure to aluminum ion from the readily soluble aluminum compounds in antacids, coagulants, and baking powders.

Infusion of chelating agents has been approved as a therapeutic procedure for removal of polyvalent metals in the case of exposure to toxic quantities of lead or bone-seeking radioisotopes. It seems plausible to use them to remove some age-accumulated crosslinkages of a metallic nature, but this has not yet been sufficiently studied for any recommendations.

Oxidation of unsaturated fats is another controllable source of crosslinking agents. Numerous aldehydes, many ketones, and other reactive compounds such as peroxides and epoxides are thus formed. Many of these are known potent crosslinking agents.

These can be minimized by the use of supplementary antioxidants, of which vitamin E and selenium are the most thoroughly known, or by controlling the intake of unsaturated lipids. Since saturated fats are contraindicated in circulatory disease, alternatives would be to cut out virtually all fat, except a moderate amount of linoleic acid and lecithin, and to use carbohydrates as the principal energy source in the diet, or to use unsaturated fats with concurrent administration of vitamin E and lecithin in adequate dosage.[14] Selenium appears to be about ten times more potent than vitamin E on a weight basis but becomes toxic if given in excess. Vitamin E is far better tolerated.

A key question in the clinical or prophylactic use of antioxidants is how much antioxidant can be used without slowing down the normal oxidation reactions to the point of making metabolism sluggish? It appears that for vitamin E the safe-yet-effective range is generally somewhere between 100 and 500 IU (mg). Hillman reports that a healthy 41-year-old physician ingested 296,000 IU vitamin E in the form of alpha-tocopherol in 93 days as follows: 2000 IU/day for 37 days; then 4000 IU/day for 55 days, and 2000 IU additionally in one day. His serum level of plasma tocopherol rose to 2.2 ± 0.85 mg/100 ml, which was twice the control level. There was no change in excretion of creatinine or of ketosteroids, exercise tolerance, electrocardiogram or ballistocardiogram, serum cholesterol, liver function, or blood coagulation. A significant transient creatinuria was observed at the end of 93 days. This was interpreted as an early manifestation of an adverse metabolic effect, and the test was discontinued at this point. While large doses have thus been tolerated, minor disturbances such as aggravation of acne have been reported in isolated instances of sensitivity from as little as 600 IU daily.

Anoxia

The formation of crosslinking agents by random nonenzymatic spontaneous oxidation of unsaturated fats can be reduced by the employment of antioxidants, but the supply of oxygen must not be curtailed. Lack of oxygen may be due to insufficiency of air, low oxygen content, oxygen blockage by excessive dietary lipids, carbon monoxide (tobacco smoke), or deficiencies in the circulatory system. Any anoxia interferes with the final oxidation of ingested substances to innocuous water and carbon dioxide, so as to cause the accumulation of intermediate reaction products in the oxidation chain.

Many of these are potent crosslinking agents (aldehydes and free radicals). For example, it is not generally realized that formaldehyde, a well-known poison and an extremely potent crosslinking agent, is normally found in the

human body in at least eight fully established metabolic reactions.[3] With normal oxygen supply, formaldehyde is immediately removed by oxidation. The pathways for this must have evolved with life itself. Certainly no species could have survived unless its toxic metabolic intermediates were promptly removed. If oxygen is insufficient, it will react in any of a number of abnormal manners, many of which lead to crosslinkage of vital molecules. In *vitro*, formaldehyde will instantly destroy DNA, RNA, and almost any enzyme. The same holds true of uncontrolled formaldehyde molecules in *vivo*. This also applies to many other known metabolites. Some are slow-acting, yet over a lifespan they must be contended with. Though detoxification by oxygen is well known, its extent in controlling normal metabolites is not generally appreciated. The applications have been widely recognized empirically: exercise to develop collateral circulation, judicious use of vasodilatants, and good ventilation.

Dietary Excesses

Functionally analogous with anoxia is the effect of dietary excess. If the amount of any nutrient ingested at any time exceeds the quantity which can be immediately processed at any step of metabolism, this intermediate accumulates at the point of the bottleneck reaction in the metabolic pathway in question. Such an accumulation at any location in the organism cannot be absolutely controlled. More or less of the accumulated intermediate diffuses to the blood, where it has a systemic effect, or to other sites where it should not be, and where it may cause undesirable secondary reactions. Crosslinkages are among the potentially most damaging of such reactions. Crosslinking caused by metabolic intermediates could well be a principal reason why being overweight affects longevity so unfavorably. It is more judicious to take nutrition in many smaller meals than in a few large ones. This should help avoid overloading any metabolic step at any time and should reduce the amount of crosslinking agents formed.

Hydration

From a physio-mechanical standpoint alone, the probability of crosslinkage is high when the macromolecules are close to each other and when little extraneous material is around them to obstruct contacts. Therefore, it is not surprising that, other things being equal, the greater the dilution of a solution, or the higher the state of swelling or hydration of a colloid, the less probability of crosslinkages forming. This is easily demonstrated *in vitro*. Humans are largely composed of colloidal water gels. Therefore, this means of delaying crosslinkages might well deserve further exploration from the standpoint of aging prophylaxis. Several observations, each in itself unconvincing, will, in the aggregate at least, indicate that work along this line might prove fruitful:

1. Of the isolated communities in which an unusually high percentage of the population reaches lifespans in three figures, most are located in mountainous regions. It has been argued that this is so because of the effect of physical exercise in climbing, or because of isolation from contamination or the stresses of industrialized societies. This may all be true, but one factor these communities also seem to have in common is that the water they drink goes through a natural distillation process. It reaches these consumers at high altitudes with a minimum of dissolution or contamination.

2. Tyler showed[13] that the removal of polyvalent ions from tap water results in a manifold increase in the survival time of sperm cells suspended in this water. The removal was effected by treatment with nontoxic amounts of EDTA and with other chelating agents.

3. It is well known to everyone who has worked with pharmaceutical suspension products, or who has tried to use shaving cream in brackish water, that it is much easier to make stable suspensions in distilled water than in tap water. Loss of the ability to hold bound water is typical of aging tissue, and is readily duplicated *in vitro* by the addition of crosslinking agents to a gel.

On the other hand, it has been found that the incidence of coronary heart disease is lower where water is hard. This hardness results largely from high calcium content. This finding cannot be considered relevant to aging until a double-blind test compares the use of typical hard water with the use of distilled water while the subjects are receiving an adequate calcium supply from other sources. For all we know, the hard water might appear superior

because it offsets a lack of optimal calcium in the nutrition. Until this is resolved, the water question is ambiguous. In the meanwhile, the author uses in his household distilled water to which 10 ppm of magnesium and the same amount of zinc have been added, both in the form of soluble compatible salts.

CAN CROSSLINKAGE BE REVERSED?

Direct dissolution of crosslinkages as they form would be an attractive therapeutic approach. However, the crosslinkages are too many and too varied to make such an attack practical.

The next choice would be to excise the crosslinkages by enzymatically cutting out the intact crosslinkages, together with the pieces of the large molecules they bind together. Where crosslinking is far advanced, this may entail complete destruction and dissolution of the crosslinked inert aggregates that are accumulated over a lifetime and are resistant to all enzymes available to the organism—a formidable problem.

However, the use of extraneous enzymes has been considered.[15,16] Such enzymes might accomplish the destruction of aggregates by attacking the very backbones of the large molecules involved, particularly acting on the carbon-to-carbon or carbon-to-nitrogen bonds. The aggregates would thus be reduced to excretable fragments. Enzymes capable of accomplishing this must be available to some soil bacteria, for otherwise the earth would be covered with heavy sedimentary layers of crosslinked macromolecules. The success of such therapy is contingent on the patient's still having a functional anabolic metabolism, which would be capable of rebuilding the normal constituents after the enzymes have cleared the space.

What is needed is an enzyme of low specificity and very high capacity to reach and penetrate the tightly crosslinked aggregates formed and packed during a lifetime. Such enzymes are not available in the human organism beyond the very limited capacities of lysozymes and some collagenases active in parturition. However, microbes that produce enzymes having the desired lytic effects have been found.[15] The toxicity of at least one of these appears to be very low, although massive doses have caused hemorrhages in mice, evidently by lysis of capillary walls.

One of these bacterial enzymes, from a mutant strain of *Bacillus cereus*, was purified to a high degree and is now being produced commercially (Microprotease MPB). *In vitro* it dissolves the major portion of insoluble matter isolated from human brains, as well as hyalin deposits in human kidney autopsy material. The enzyme has a low specificity—it dissolves other tissue indiscriminately. This is not objectionable for our purpose, however, because the dosage can be modulated so that normal anabolic processes can keep pace in restoring the normal constituents replacing the crosslinked abnormalities as they are being dissolved. The crosslinked aggregates have been accumulated very slowly over a lifetime and thus will not be replaced nearly as rapidly as the normal tissue being dissolved concurrently.

SUMMARY

We have at our disposal means for direct attack on the aggregates of crosslinked, replaceable cell constituents. These include enzymes, RNA, all normal proteins, and the pervasive network structure shown in Figure 2.

They do not include the genetic matter, which is unique in each cell and thus irreplaceable. In this instance, the possibility of repair is strictly limited in time. As illustrated by Figure 1, the crosslinking agent initially attaches itself to only one strand of the DNA. At this point it can be excised without harm, for the other strand is still available to serve as template and blueprint for the repair. It is when the second strand is reached by the oscillating crosslinking molecule fixed to the first strand that the damage becomes irreversible and dooms the cell. Increasing the quantity or the efficiency of available relevant enzymes might thus have a beneficial effect. No experimental work has as yet been done to explore this possibility.

Observations on the physiologic activities of one potentially gerolytic enzyme are getting underway. It would be surprising indeed if the first relevant enzyme to become generally available should prove to meet all possible needs. Therefore, it is important to follow through now on the development of the other

similar but not identical enzymes that have been found in various spore-forming organisms; they would possibly provide answers to questions certain to arise as more information is gathered with the enzyme now available. The common denominator of these enzymes is an ability to overcome or circumvent steric hindrances of their access to the large crosslinked aggregates. The very density of crosslinkages can constitute such a hindrance. The ability to overcome it appears to be a function of the size and form of the enzyme molecule. Possibly all spore-forming organisms need enzymes of this type to dissolve the extremely resistant spore shells. So far, at least some of these enzymes have been found in all spore-formers studied.

Therapy would probably be directed first to the most accessible targets: endothelia directly exposed to circulating fluids, and the kidneys. However, much broader application does not seem excluded, since the literature reports some instances where orally administered enzymes (the protease bromelain and certain amylases) have reached tissues in an active state, penetrating all membranes. The lower the molecular weight of the enzyme, the higher is the probability that such a transit might be achieved. The form of the enzyme molecule also affects its ability to penetrate.

The only way of determining the molecular weight of an enzyme beyond all question is to define its exact chemical composition. This has not been done yet for the enzymes just mentioned. However, what matters is their ability to penetrate, and this can be observed under varying conditions and biological influences.

The place of the crosslinkage theory in cancer therapy is complex and not yet ripe for discussion. However, it might be appropriate to point out that cancer incidence increases logarithmically with age, and that most of the known carcinogens either are crosslinking agents or have metabolites that are.

While the life expectancy of women at age 60 has increased in the last decade, the life expectancy of white North American men at age 60 has remained virtually constant and is now only about two years higher than it was 187 years ago, when statistics first became available (Figure 6). The person who is saved from a coronary and evades cancer will still be so

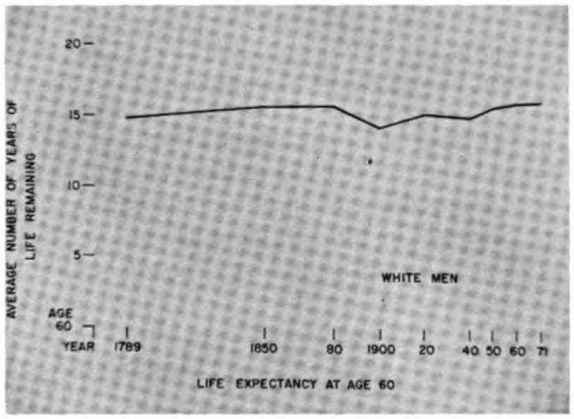

Figure 6. The life expectancy at age 60 for 1789–1971. (From data of Historical Statistics of the Metropolitan Life Insurance Company and from U.S. Vital Statistics.)

weakened at the age of 105 that even a slight stress is lethal.

A new approach is needed to overcome decisively the obstacles that have kept the life expectancy of white males at 60 nearly unchanged. The active consideration of the clinical corollaries of the crosslinkage theory might well prove useful guides for this approach.

Much present medical research is directed to the demonstrated correlation of cholesterol and triglycerides with atherosclerosis. The crosslinkage theory suggests that the effects of cholesterol and triglycerides are secondary, and that they consist of interferences with the processes of (a) healing of primary lesions of the intima, caused by loss of elasticity due to crosslinking; and (b) direct adsorption of lipids to macromolecules oriented by crosslinkages mainly to expose their more lipophilic sides, as shown in Figure 5. Retention of the elasticity and hydrophilic character of exposed tissues by prevention or reversion of crosslinkages would therefore strike hard at a primary cause of some major degenerative diseases. ▲

Acknowledgment

Because of the limitation of space allotted to references, it has been impossible to acknowledge all of the important contributions to this field made by numerous scholars and practicing physicians. References 5, 6, 14, and 15 have been cited for their more extensive bibliographies, in addition to other relevance.

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