FUNDAMENTALS OF AGING: A COMPARISON OF THE MORTALITY CURVE FOR HUMANS WITH A VISCOSITY CURVE OF GELATIN DURING THE CROSS-LINKING REACTION

JOHAN BJORKSTEN, PH.D. AND FRED ANDREWS, M.S.

Bjorksten Research Foundation, Madison, Wisconsin and Houston, Texas

The mortality curve is an expression of the rate of aging, for it may be assumed that the rate of exposure to infections, accidents and other traumata is in the same order of magnitude in the various periods of life, and does not increase logarithmically or even linearly with age. Thus the rate of traumata being roughly constant, the mortality curve is an expression of the decreasing ability of the aging organism to overcome all kinds of stress. This decreasing ability to overcome stress is the principal expression of aging and is the basis for the shape of the mortality curve.

In view of the mounting evidence in favor of the theory that biologic aging is largely caused by the immobilization of active large molecules, it appeared of interest to compare the shape of a time/viscosity curve of protein immobilization by cross-linkage *in vitro* with the shape of the mortality curve for humans.

MATERIALS AND METHODS

A 25-Gm. aliquot of Graylake gelatin having an isoelectric point of 8.0 and a Bloom value of 250 was dissolved in 250 ml. of distilled water and the pH was adjusted to 3.3 with sulfuric acid. The viscosity of the gelatin solution (at 45°C.) was measured with a Brookfield viscometer and found to be 65 centipoises, before addition of the cross-linking agent. Then 7.5 Gm. of a commercial basic chrome sulfate (Tanolin W2XD)[1] was added to the composition after the gelatin was placed in a delta blade Brabender Plastograph.[2] The mixing speed of the instrument was 50 rpm, the reaction temperature was 45°C., and the lever ratio was 1:1. The revolving rate of the chart was 1 cm. per minute.

RESULTS

A typical Brabender viscosity tracing obtained under the conditions described is shown in Figure 1, and a plot of the data redrawn to linear coordinates is shown in Figure 3. If aging and the concomitant decreased resistance are indeed results of immobilization of proteins, it would be expected that a curve expressing a simple chemical reaction leading to such immobilization would have the same shape as the mortality curve. That this can be the case is shown by Figures 2 and 3.

DISCUSSION

The theory that aging is due to immobilization of large active intracellular molecules (previously expressed by one of the present authors (2, 3)) has not

[1] Tanolin W2XD was obtained from Diamond Alkali Company, Kearny, N. J.

[2] The Brabender Plastograph is manufactured by the C. W. Brabender Instruments, Inc. of South Hackensack, N. J. It subjects the test material to the kneading action of two revolving blades, and records the power required, which increases with viscosity.

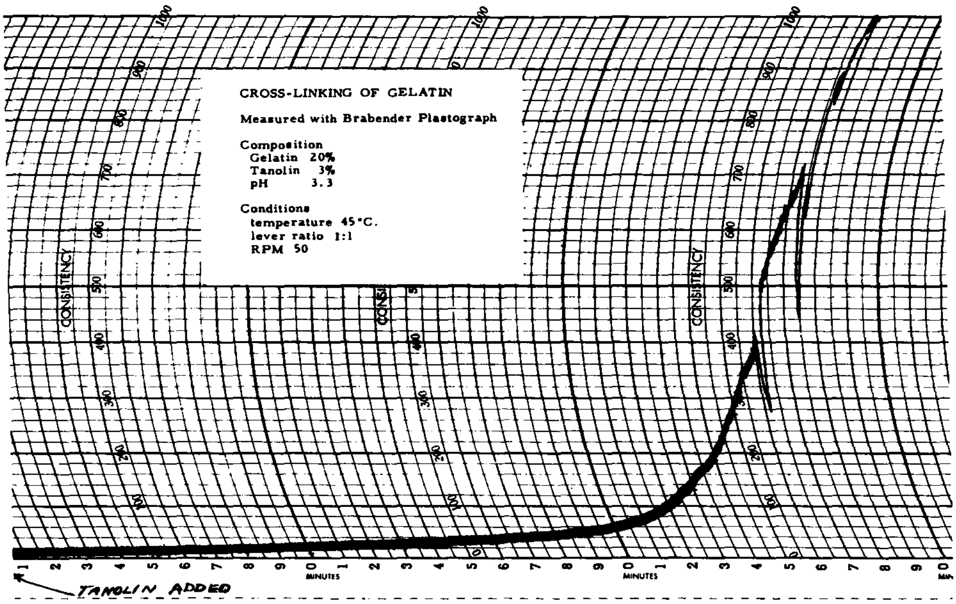

FIG. 1. Brabender Plastogram of the gelatin-Tanolin W2XD reaction

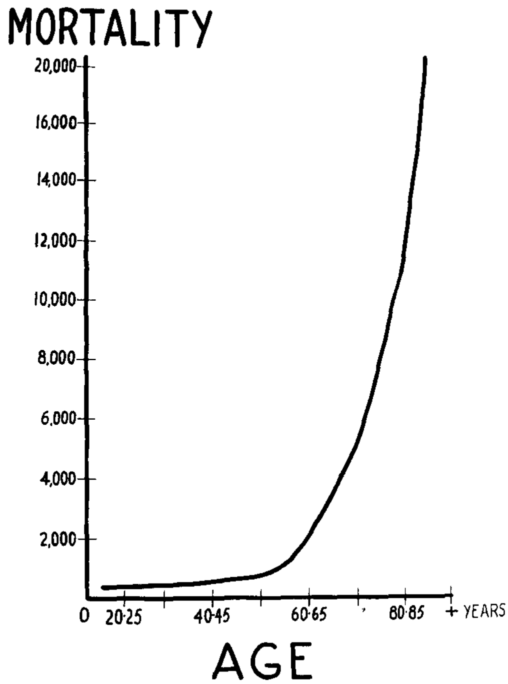

FIG. 2. Mortality rate plotted against time (age) for white American males (1)

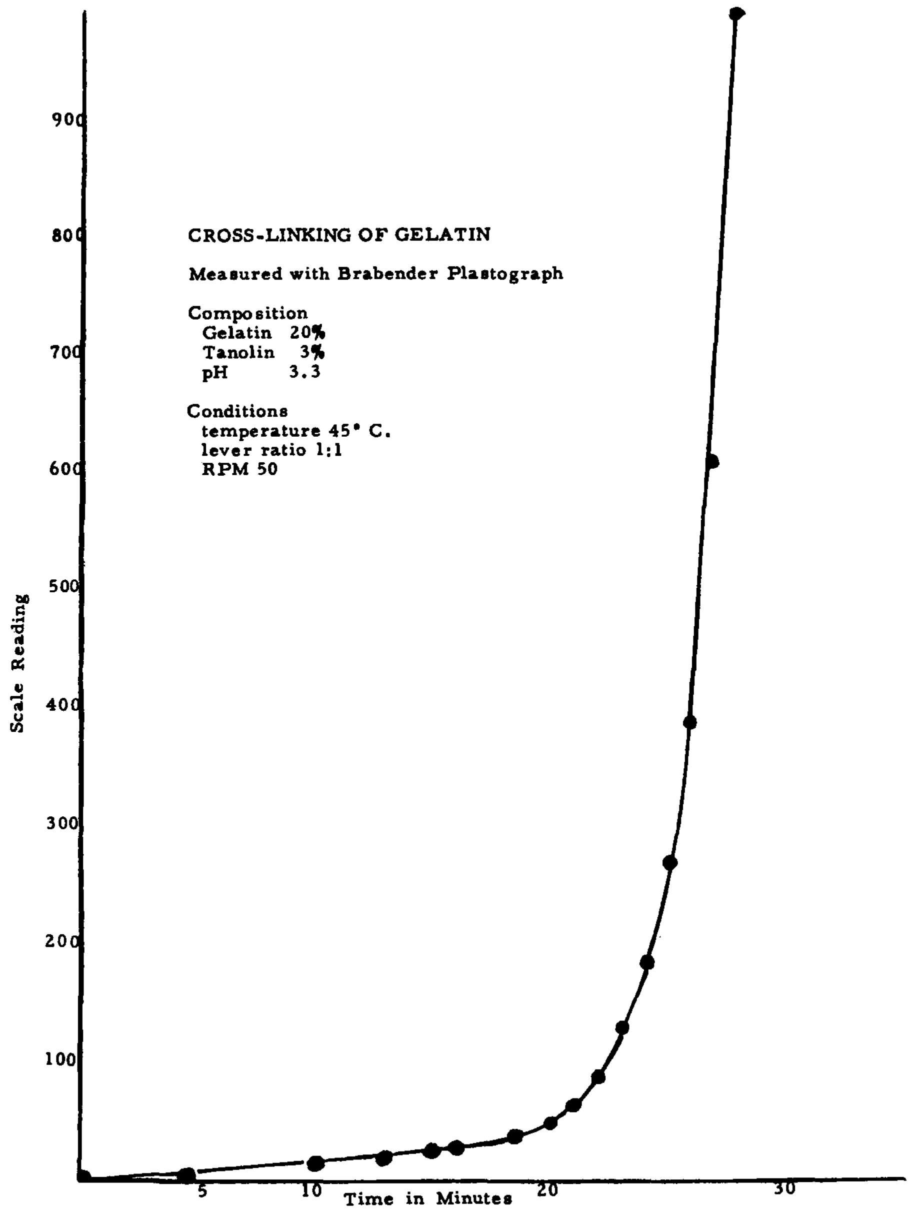

FIG. 3. The rate of progressive immobilization of gelatin under the influence of a cross-linking agent, as recorded on the Brabender Plastograph—transcribed from Figure 1 to straight-line coordinates.

been disputed. Various possibilities have been presented as to the way in which this immobilization could take place. Cross-linkage appears the most likely major mechanism, however, because through cross-linkage a very small amount of reactant can have a very large effect on the large molecules, and it is as uni-

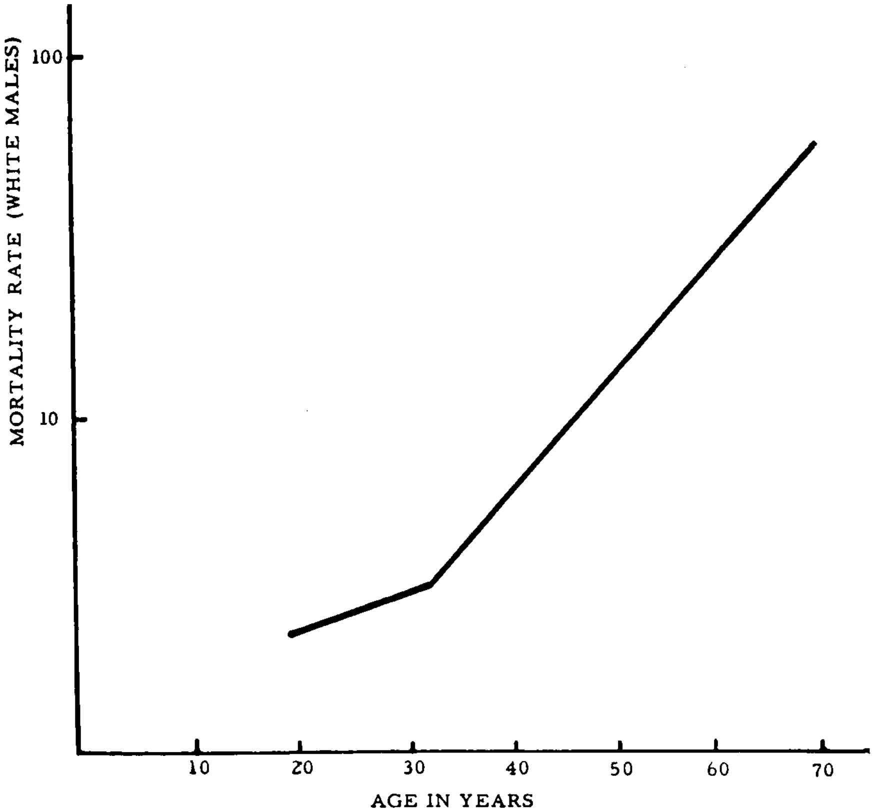

FIG. 4. Mortality rate plotted against time (age) for white American males, using semi-logarithmic coordinates.

versal as aging itself (4, 5). Cross-linkages can immobilize proteins in a way which renders them irreversibly inert toward body enzymes (6). Since cross-linking agents have been shown to be normally present in the organism (5), these highly reactive compounds are bound to interact with the body proteins. The recent work of Alexander and Stacey (7) has shown that cross-linkages of deoxyribonucleic acid may similarly occur.

The finding of Bjorksten and Gottlieb (6) that enzymatic digestion of a protein (gelatin) is blocked or severely distorted by cross-linkage, has been recently confirmed by Kohn and Rollerson (8) using collagen cross-linked with formaldehyde. These authors state: "The data suggest that the decrease (in digestibility of collagen) noted with advancing age may be due to a chemical reaction between collagen and some metabolite functioning as a tanning agent."

Radiation effects have been proposed or implied by Harman (9, 10) and by Szilard (11). Although radiation will break off free radicals which can cause cross-linkages, this can hardly be a major mechanism in aging, because even ten times the amount of radiation a person receives from cosmic sources during a lifetime is insufficient to cause the amount of immobilization of proteins observed in old animals (12).

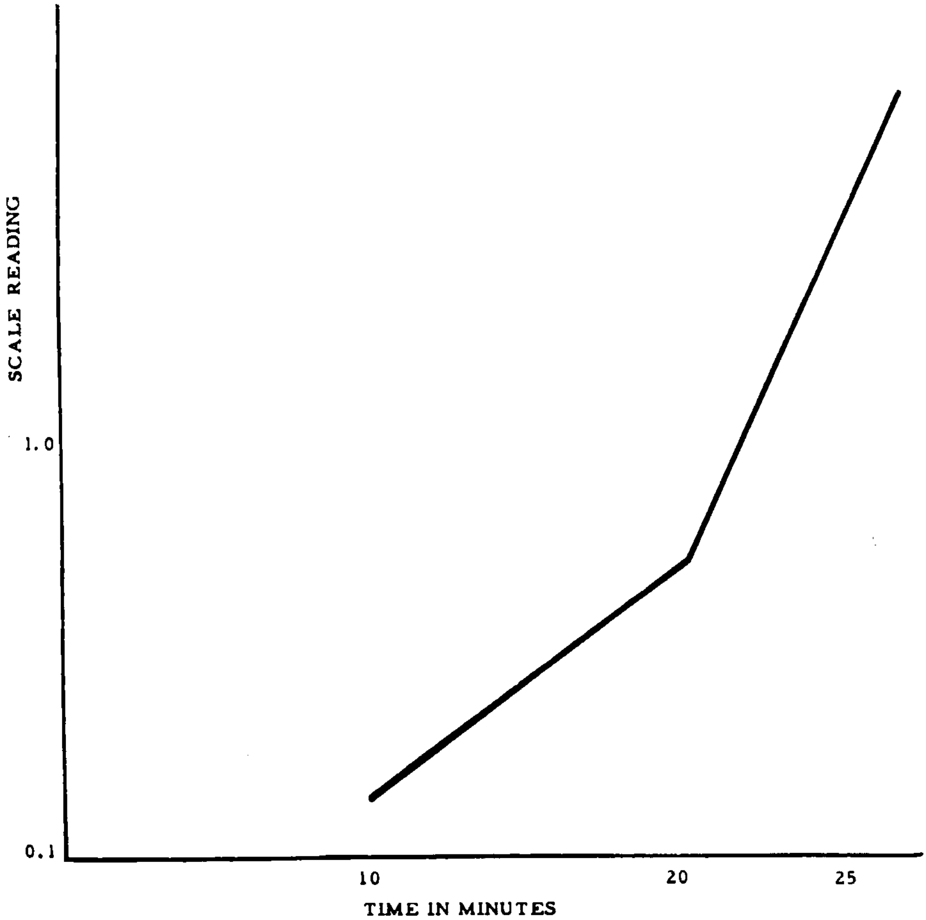

FIG. 5. The rate of progressive immobilization of gelatin under the influence of a cross-linking agent (from Figures 1 and 3) plotted on semi-logarithmic graph paper.

Thermal denaturation has been proposed as a form of immobilization of protein (13, 14) but this is also unlikely to be a major mechanism because denatured proteins are usually easily attacked by body enzymes and thus returned to the metabolic cycle.

It is not our wish to attempt any analysis or conclusion on the basis of the present limited data, but it is interesting that curves (Figs. 2 and 3) plotted on a logarithmic scale show similar breaks in the slope. This is apparent from Figures 4 and 5. The latter parts of these curves proceed in accordance with a steeper exponential slope. The reason in Figure 5 probably is, and in Figure 4 perhaps is, that the initial cross-linking fixes the protein chains in spatial proximity to each other, thereby increasing the probability of additional cross-linking and consequent final immobilization.

SUMMARY

The data of the present study demonstrate that a similarity exists between the curve expressing changes in mortality with time in humans and the curve expressing congelation of protein being cross-linked under the specified conditions. Plotted on semi-log paper, both curves show similar breaks and rate changes. This adds further support to the theory that immobilization of large molecules is a controlling factor of the aging mechanism.

Acknowledgment

The authors express thanks to Abbott Laboratories and the Markus and Bertha Coler Foundation Inc. which have contributed to the financing of this study.

REFERENCES

1. U. S. Bureau of Vital Statistics, Washington, D. C., 1951.
2. BJORKSTEN, J., AND CHAMPION, W. J.: Mechanical influences on tanning, *J. Am. Chem. Soc.* **64:** 868–869 (Apr.) 1942.
3. BJORKSTEN, J.: Chemistry of duplication, *Chemical Industries* **49:** 2, 1942.
4. BJORKSTEN, J.: Cross-linkages in protein chemistry, *in* Advances in Protein Chemistry, ed. by Anson, Edsall and Bailey. New York, Academic Press Inc., 1951, vol. 6, pp. 343–381.
5. BJORKSTEN, J.: A common molecular basis for the aging syndrome, *J. Am. Geriatrics Soc.* **6:** 740–748 (Oct.) 1958.
6. BJORKSTEN, J., AND GOTTLIEB, H.: Protein structure and aging—cross-linking in gelatin. I. Formaldehyde-induced. Air Force Office of Scientific Research, Technical Report No. OSR-TN-54-304, (Nov.) 1954, p. 68.
7. ALEXANDER, P., AND STACEY, K. A.: Cross-linking of deoxyribonucleic acid in sperm heads by ionizing radiations, *Nature* **184:** 958–960 (Sept.) 1959.
8. KOHN, R. R., AND ROLLERSON, E.: Aging of human collagen in relation to susceptibility to the action of collagenase, *J. Gerontol.* **15:** 10–14 (Jan.) 1960.
9. HARMAN, D.: Aging: a theory based on free radical and radiation chemistry, *J. Gerontol.* **11:** 298–300 (July) 1956.
10. HARMAN, D.: Prolongation of the normal life span by radiation protection chemicals, *J. Gerontol.* **12:** 257–263 (July) 1957.
11. SZILARD, L.: On the nature of the aging process, *Proc. Nat. Acad. Sc.* **45:** 30–46 (Jan.) 1959.
12. BJORKSTEN, J.; ANDREWS, F.; BAILEY, J., AND TRENK, B.: Fundamentals of aging: immobilization of proteins in whole-body irradiated white rats, *J. Am. Geriatrics Soc.* **8:** 37–47 (Jan.) 1960.
13. SINEX, F. M.: American Chemical Society meeting, Boston, Mass., 1959.
14. SINEX, F. M.: Aging and the lability of irreplaceable molecules, *J. Gerontol.* **12:** 190–198 (Apr.) 1957.