Age Changes in Swelling Properties of Human Myocardium.* (24589)

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In aging humans there is a progressive decrease in cardiac output, and cardiac failure is precipitated with increasing ease. These and other age-related changes are usually considered a result of arteriosclerotic heart disease, although there is often a poor correlation between degree of coronary arteriosclerosis and cardiac function(*2*). This lack of correlation has led us to the hypothesis that there might be some underlying age-related change in cardiac muscle, probably at the molecular level. Studies on osmotic swelling properties of human collagen have shown that collagen passes through several stages of increasing rigidity with advancing age, and evidence was presented suggesting such changes are due to thermal denaturation at body temperature(3, 4). Since the same factors influencing collagen might be expected to act on fibrous proteins of muscle, a study of relationship of age to swelling properties of human myocardium was undertaken.

Methods. One hundred and forty-eight hearts were obtained from autopsies performed the day of death. Only samples of left ventricle showing no macroscopic lesions were used. Epicardium and endocardium were dissected away and the remaining myocardium was stored at −60°C until used. Control experiments showed that myocardium frozen 1 to 7 days did not differ in swelling ability from that of fresh unfrozen tissue. Each sample of myocardium was cut into three 25-40 mg fragments, washed briefly in 0.9% NaCl, blotted firmly dry and weighed. The fragments were then placed in 10 ml of distilled water adjusted to required pH levels by addition of NaOH or HCl where they were maintained at room temperature for varying time. After removal, the fragments were again blotted firmly dry and weighed. Percentage change in weight during incubation was then calculated, and used as a measure of swelling. pH values below 10 were determined with glass electrode pH meter, above 10 by titration with standard acid. Because changes in swelling might be due to a restricting influence of increased connective tissue, collagen content of hearts was determined by the method of Neuman and Logan(5). Hydroxyproline determined by this method was multiplied by 7.35 to obtain amount of collagen. As changes in water content would also influence swelling ability, dry weight of myocardium was determined after drying to constant weight at 110°C.

Results. Fig. 1 shows amount of swelling over wide pH range of samples from young and old heart, incubated in the various solutions for 3 hours. Values between pH 3 and 10 are not included because these pH's were very unstable during incubation of heart fragments and swelling was negligible in this range. There are small swelling peaks at pH 0.9 and 2.4, and maximal swelling at pH 11.2. A significant age difference is apparent at pH 11.2 where the young specimen shows more swelling than the old. This pH was used for subsequent studies. At pH 11.2 maximal swelling occurred on 3 hours incubation and this time was used for the following determinations. Swelling at pH 11.2 was completely reversed when fragments were returned to neutral salt solution. Triplicate determinations were usually within ± 3% of the mean value.

Swelling of heart muscle was determined for the entire age range at pH 11.2. Results are shown in Fig. 2. There is a sharp decrease in swelling between birth and approximately 8 years of age. Extent of swelling apparently remains at constant level between 8 and approximately 35 years of age, after which there is a decrease in swelling ability to a level which appears to remain constant until old age. Beyond the fourth decade the points show considerable scatter. The dashed line of Fig. 2 represents a subjective evaluation of changes which appear to take place as a function of age.

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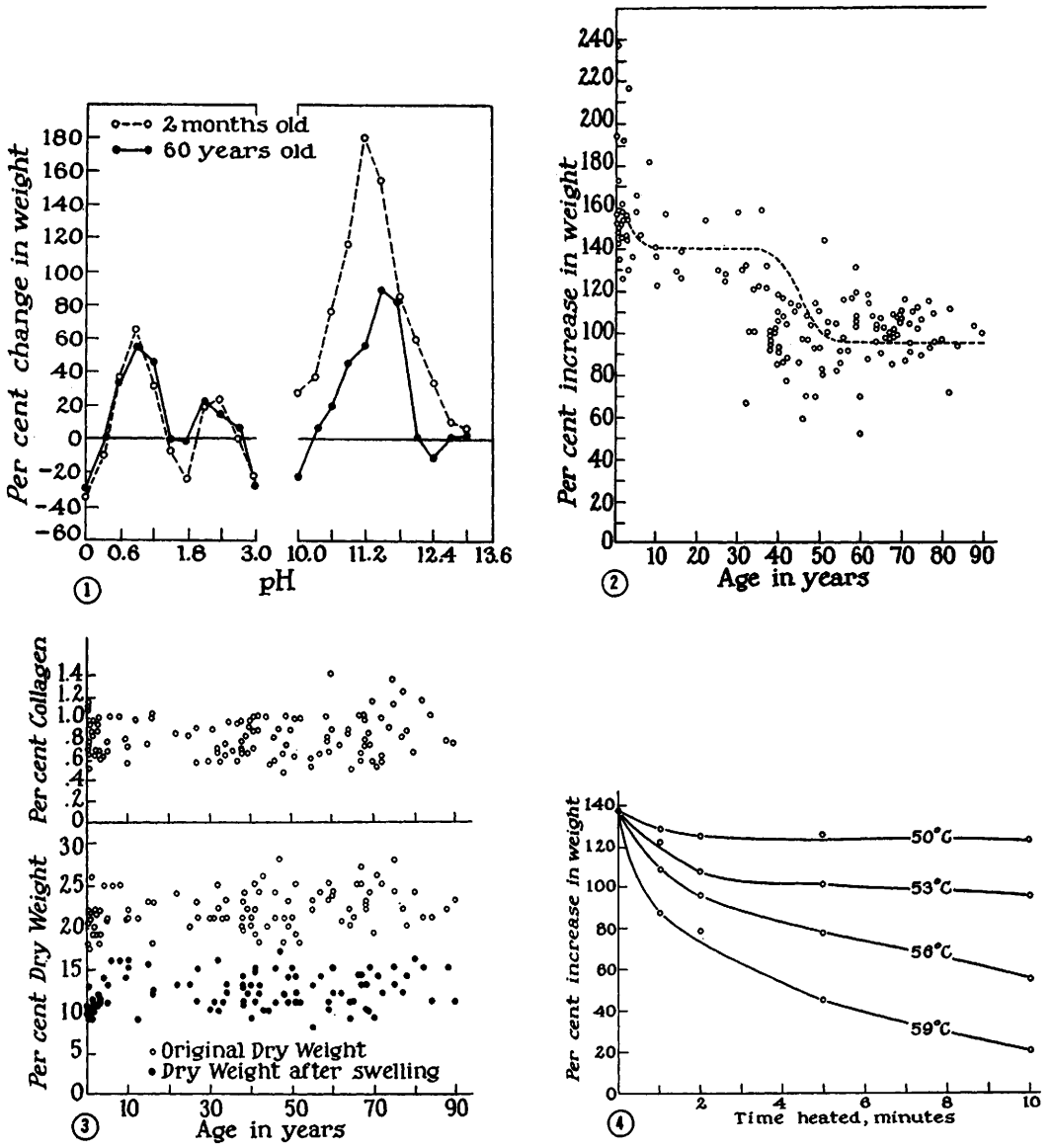

FIG. 1. Swelling of heart fragments from old and young individuals on exposure to acid and alkaline solutions for 3 hr.

FIG. 2. Swelling of myocardium from individuals of various ages at pH 11.2.

FIG. 3. Collagen content and dry wt of myocardium as a function of age; expressed as % of original wet wt.

FIG. 4. Effect of heating myocardium on subsequent ability to swell in a pH 11.2 solution at room temperature.

Data in Fig. 3 indicate that collagen content and percentage solids do not vary with age. Percentage dry weight, based on original weight, was also determined on fragments which had swollen at pH 11.2. This was done because of the possibility that old myorcardium swelled less than young because some substance responsible for swelling was more soluble in old muscle and was extracted by the alkaline solution. These determinations are included in Fig. 3 and show that about 45% of the solids are extracted during the swelling process. The amount does not vary with age.

To find if loss in swelling with age could be

caused by thermal denaturation, the effect of heat treatment on swelling was investigated. Heart fragments were incubated at elevated temperatures in mammalian Ringer's solution for various lengths of time. They were then cooled to room temperature and swelling ability at pH 11.2 was determined. Results of such an experiment with heart fragments from a 15-year-old individual are shown in Fig. 4. Heat has a marked effect in decreasing swelling ability. Control experiments showed that heat treatment did not result in extraction of solids beyond the amount extracted during swelling, and had no effect on amount of solids extracted during swelling. From data of Fig. 4 and similar data for other hearts, Arrhenius plots were constructed. These yielded activation energies of 40, 47 and 47 kcal/mol for myocardium specimens from individuals aged 15, 27 and 30 years respectively.

Discussion. Swelling properties of myocardium have apparently not been described previously. The swelling maximum at pH 2.4 is probably due to osmotic swelling of collagen in the heart, as previous studies have shown that human collagen swells maximally at this pH(3). Swelling at pH 0.9 and 11.2, in showing a decrease below pH 0.9 and above pH 11.2, is characteristic of Donnan equilibrium type of protein swelling described by Procter and Wilson(7). Collagen does not swell maximally at these pH levels and the small amount of collagen in the heart is not enough to cause swelling noted at pH 11.2. Thus, swelling at pH 0.9 and 11.2 probably results from formation of ionized myocardial protein salts.

Decrease in ability to swell at pH 11.2 in early years of life may represent part of a developmental process by which fibrous proteins become oriented and strengthened. The decrease beginning in the fourth decade, however, probably represents a degenerative process starting after maturity is attained. Loss of swelling with age implies development of some factor which increases rigidity of the protein structure. Since collagen content does not change with age, a finding in accord with the study of Oken and Boucek(6), it is possible that the change is in myocardial protein *per se*. As swelling is determined by 55% of solids which are not extracted in strongly alkaline solution, insoluble fibrous myocardial proteins may be the molecules which undergo change with age. Loss of swelling ability could be due to either a change in the type of proteins synthesized or a change in structure of relatively stable proteins. While there is no evidence that a different type of protein is or is not synthesized, there is some reason to believe that there are relatively stable molecules in the heart which might undergo changes with advancing age. Friedberg, Tarver and Greenberg, using labeled methionine, found turnover rate of myocardial proteins very low(1). Since their values applied to pooled proteins, some heart proteins must have extremely low turnover rates. Such molecules might be susceptible to the influence of various chemical and physical factors acting over many years.

A result of such influence might be an unfolding of protein molecules with formation of new cross links. Since heat treatment causes a decrease in swelling ability similar to that in aging, possibly the change in aging is due to thermal denaturation at body temperature. Using energies of activation experimentally determined at elevated temperatures and kinetics of a first order reaction, it was calculated that the loss in swelling between 35 and 55 years of age should take place in several hours at 37°C. Thus, the issue is not if body heat could cause the change, but rather what prevents the change from occurring at a more rapid rate.

A relationship between loss of swelling of myocardial proteins and decreased ability of heart to contract efficiently can be hypothesized on grounds that swelling ability is a measure of protein elasticity(7).

Summary. To detect changes with age in structure of myocardial proteins, swelling properties of human myocardium in solutions of HCl and NaOH were investigated. Fragments of myocardium were incubated in solutions of various pH values and increases in weight were used as measures of swelling ability. Maximal swelling occurred at pH 0.9, 2.4 and 11.2. Greatest swelling, probably of the Donnan equilibrium type, occurred at pH

11.2 and this value was used for subsequent studies. Swelling decreases rapidly in the first years of life, then appears to remain constant until 35 years of age after which it decreases, to apparently remain constant at a low level. Collagen content, dry weight and amount of solids extracted during swelling do not vary with age. Heating myocardium causes a decrease in swelling ability. The possible role of thermal denaturation in myocardial aging and physiological implications of swelling changes are discussed.

1. Friedberg, F., Tarver, H., Greenberg, D. M., *J. Biol. Chem.*, 1948, v173, 355.
2. Heller, B. I., *Med. Clin. N. Am.*, 1956, v40, 185.
3. Kohn, R. R., Rollerson, E., *J. Geront.*, 1958, v13, 241.
4. ———, *ibid.*, 1959, v14, 11.
5. Neuman, R. E., Logan, M. A., *J. Biol. Chem.*, 1950, v184, 299.
6. Oken, D. E., Boucek, R. J., *Circ. Res.*, 1957, v5, 357.
7. Procter, H. R., Wilson, J. A., *J. Chem. Soc.*, 1916, v109, 307.

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Red Cell Life Span in Newborn at Sea Level and High Altitudes. (24590)

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At sea level there is a marked decrease of erythrocytes in circulating blood of the newborn a few days after birth. The same process, to a lesser degree, has been described by Loret de Mola(1) in a study of newborns at high altitudes. While the decrease is chiefly due to depression in erythropoietic activity of bone marrow(2-5), a greater rate of cell destruction has been considered a contributing factor. Increased concentration of indirect bilirubin, especially during first few days after birth, has been interpreted by some investigators to favor the latter hypothesis. However, most authors believe that hyperbilirubinemia of early life has a hepatic origin. Hollingsworth(6) studied the life span of red cells in newborns at sea level, using radioactive chromium for "tagging" the blood taken at moment of birth, and before its injection into healthy adults. These studies demonstrated that red cells of newborns have a shorter life span than those of adult subjects. In Hollingsworth's opinion(6), this characteristic has no relation to quantity of fetal hemoglobin, degree of macrocytosis, or concentration of hemoglobin at birth; he suggested that the change from oxygen deficiency to normal supply might be a contributing factor. We, therefore, investigated newborns at high altitudes, where the hypoxic condition of intra-uterine life is not replaced, at birth, by normal oxygen pressure.

Methods. Two groups of 5 newborns were studied, one at Oroya, 3,730 meters (12,240 feet) and the other at sea level. About 15 cc of blood were obtained from umbilical cord at moment of birth, and placed in sterile flasks with a small quantity of heparin solution. After incubation at room temperature, with approximately 100 μc of Cr-51 in the form of Na^2Cr-5104, and washing twice with normal saline, the blood was injected into healthy adults, living at sea level. Fifteen minutes after transfusion, and then once a week thereafter, 4 cc of blood were obtained from recipients and degree of radioactivity measured in scintillation counter (well type). Radioactivity was expressed in percentage, considering the first sample as 100% (15 minutes after injection). All samples from each case were read simultaneously to avoid correction for physical decay of isotope.

Results. The results obtained, expressed in survival curves of the injected "tagged" red cells, are presented in Fig. 1 and 2, for newborns at sea level and at high altitudes, respectively. For comparative purposes we have also represented, in both diagrams, survival curves obtained in life span of red cells in 7 healthy adults at sea level, using auto-