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Aortic Albuminoid Titration and Swelling Curves from Persons Differing in Age and Degree of Atherosclerosis. (20309)

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The present investigation is concerned with one phase of a study on the chemical changes associated with atherosclerosis and increasing age of the human aorta. Titration and swelling curves of the human aortic albuminoid (collagen, reticulin, and elastin) have been studied with reference to possible changes in protein constitution associated with atherosclerotic processes. Previously, the existence, in the human aortic albuminoid, of a carbohydrate fraction not quantitatively altered by pathological or aging changes has been demonstrated(1). However, albuminoids from aged and atherosclerotic aortae have been shown to combine with larger quantities of hydrochloric acid than do relatively normal or younger samples, although combination with sodium hydroxide remained constant in all cases(2). The mean percentage of elastin in this aortic albuminoid showed no significant change with age or atheromatous condition. However, a statistically significant increase in albuminoid elastin was apparent in samples showing visible calcification(3).

Materials and methods. The albuminoid samples were prepared from 23 human aortae by methods previously described(2). Following desiccation, each sample was ground in a Wiley mill (40 mesh) and replaced in the desiccator for a minimum of 24 hours. The volume swelling and titration curves were determined by the method of Highberger(4), which was slightly modified according to the special needs of the present problem. Eleven 100 mg samples from each aorta were weighed out on an analytical balance and placed in an equal number of graduated 15 ml centrifuge tubes. Ten ml of the appropriate standard solution was added to each tube and the contents shaken. Five samples were incubated in hydrochloric acid standards (0.0005N-0.05N), 5 in sodium hydroxide standards (0.00055N-0.055N), and one in distilled water. All samples were stoppered and incubated for 48 hours at room temperature. Following the incubation period, the tubes were centrifuged for 10 minutes at about 400 g. The volume of solid was estimated imme-

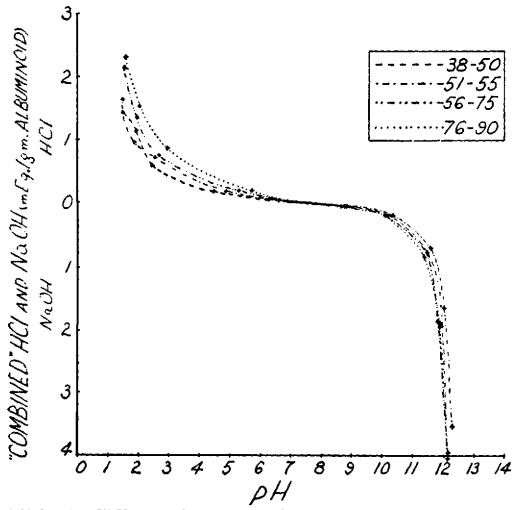

FIG. 1. Effect of age on titration curve of human aortic albuminoid (the 4 age groups contained, in chronological order, 4, 6, 6, and 6 samples).

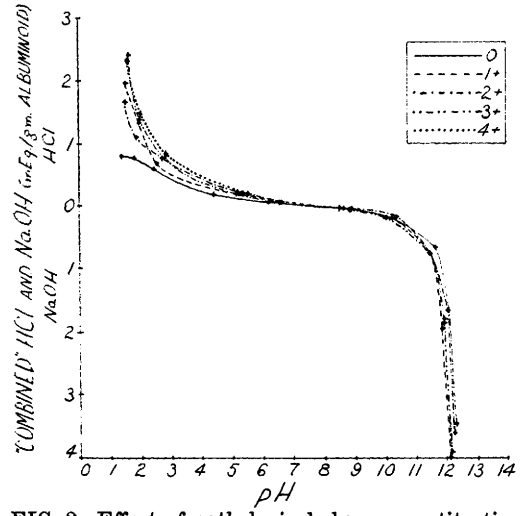

FIG. 2. Effect of pathological changes on titration curve of human aortic albuminoid. On the basis of gross observations, 0 = normal, 1+ = few atheromatous plaques, 2+ = moderate atheromatous plaques, 3+ = atheromatous plaques and few calcified plaques, and 4+ = many plaques, atheromatous and calcified. The groups contain 3, 6, 3, 6, and 5 samples, respectively.

diately following centrifugation. The pH of each supernatant solution was then determined with a Beckman pH Meter using the glass electrode. The alkaline pH readings were corrected for sodium ion errors and the number of milliequivalents of acid and base "combined" by each aliquot calculated from the above data.

Results. The data obtained from the aortae were arbitrarily subdivided into 4 age groups (Fig. 1, 3) and again into 5 groups graded according to the extent of atherosclerotic change (Fig. 2, 4). The latter were defined on the basis of gross observations. The average results within each age and pathological group were calculated and titration

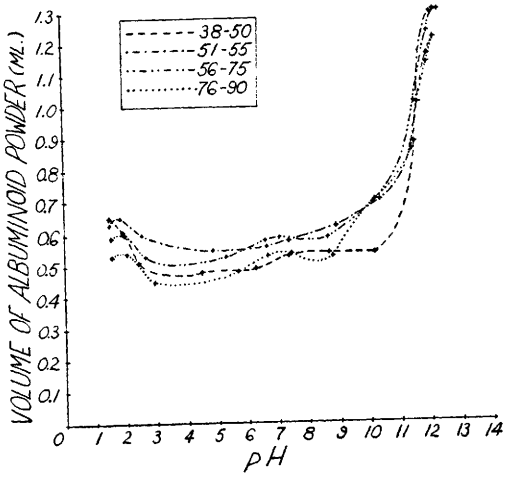

FIG. 3. Effect of age on swelling curve of human aortic albuminoid (the 4 age groups contained, in chronological order, 4, 6, 6, and 6 samples).

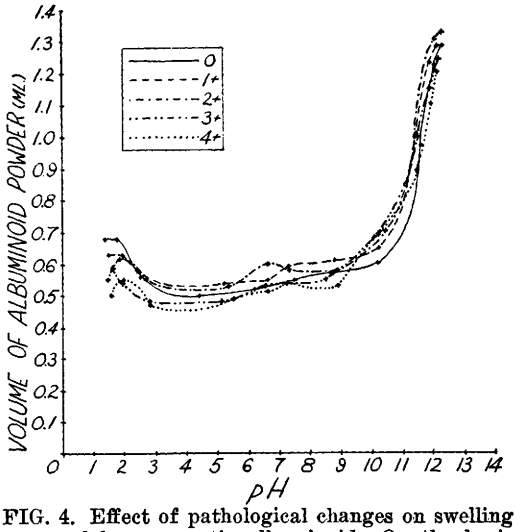

FIG. 4. Effect of pathological changes on swelling curve of human aortic albuminoid. On the basis of gross observations, 0 = normal, 1+ = few atheromatous plaques, 2+ = moderate atheromatous plaques, 3+ = atheromatous plaques and few calcified plaques, and 4+ = many plaques, atheromatous and calcified. The groups contain 3, 6, 3, 6, and 5 samples, respectively.

TABLE I. Swelling and Titration Properties of the Albuminoid of 23 Human Aortae.

Incubating solution	Mean vol ± stand. dev.	Mean pH ± stand. dev.	Mean combined HCl or NaOH (mEq/g) ± stand. dev.
N			
.05 HCl	.59 ± .09	1.54 ± .09	1.97 ± .70
.025 "	.59 ± .14	1.94 ± .17	1.26 ± .40
.01 "	.52 ± .11	2.68 ± .25	.75 ± .09
.002 "	.51 ± .05	5.19 ± .71	.20 ± .00
.0005 "	.54 ± .06	6.57 ± .21	.05 ± .00
Distilled H_2O	.56 ± .09	7.31 ± .52	—
.00055 NaOH	.57 ± .06	8.75 ± .67	.05 ± .00
.0022 "	.67 ± .13	10.21 ± .67	.20 ± .02
.011 "	.97 ± .19	11.50 ± .64	.76 ± .29
.0275 "	1.19 ± .18	11.95 ± .67	1.81 ± .27
.055 "	1.26 ± .09	12.19 ± .67	3.85 ± .47

and swelling curves plotted using these mean values (Fig. 1-4). The combined data were also computed (Table I). The titration curve (Fig. 1, 2) for the human aortic albuminoid was characterized by minimal acid and base combining power in the approximate pH range of 3-11. Below, and, especially, above this range, relatively large quantities of acid and base were combined. No marked effects of aging or pathologic change were observed, except for the apparent trend towards increased combination with hydrochloric acid with increasing age and pathology, consistent with previous findings(2).

The albuminoid swelling properties with respect to age (Fig. 3) and pathologic change (Fig. 4), were typified by pronounced increases in volume in the higher pH range (10-12). Again, no major changes resulting from increasing age or increasing pathologic change could be inferred from either curve.

Discussion. The problem of hydrolysis presents a complicating factor in attempting to interpret the observed data. It has been shown in similar experiments that the amount of protein nitrogen dissolving from steerhide collagen is greatest in the more basic solutions(4). This peak value, however, represents only 6.4% hydrolysis when calculated as an equivalent weight of collagen.

It is of interest to compare the present results with those obtained from steerhide collagen(4). The characteristic broadening of the isoelectric point into an isoelectric range observed in fibrous proteins can be shown in both cases. The results differ mainly in that steerhide collagen combines with more acid and less base at the extreme pH ranges than does the human aortic albuminoid. Likewise, steerhide collagen swells maximally at about pH 2.5, while aortic albuminoid reaches its swelling peak at approximately pH 12. In both cases, however, the similarity between titration and swelling curves of the same albuminoid sample is evident.

Though all the curves plotted are not everywhere identical in respect to aging and pathological changes, no consistent differences comparable to the marked changes observable in this tissue upon microscopic or visual examination can be found. The possible exception to this statement concerns those changes in acid-combining properties previously noted (2), which may be due to differences in ash content of the albuminoids. Though the conditions of the two sets of experiments were somewhat different, it seems of value to compare the results of the present investigation with those concerning the combination of hydrochloric acid and sodium hydroxide with the aortic albuminoid(2). The maximum amount of acid combined in the latter data was about 2.3 milliequivalents per g of albuminoid. The present work is essentially in agreement with that figure (1.97 mEq/g). On the other hand, 3.85 milliequivalents of base were bound maximally by the albuminoid in the present series, while the earlier data show a peak combination with only slightly above 0.8 milliequivalent per g of albuminoid.

This discrepancy is probably associated with the greater incubation time used in the recent experiments. Thus, a substantially larger amount of hydrolysis of the proteins incubated in sodium hydroxide may have taken place with prolonged standing.

Summary. 1. Titration and swelling curves of the human aortic albuminoid were determined and analyzed with respect to possible effects of increasing age and atherosclerosis. Except for previously determined differences in acid-binding, no marked changes were observed. 2. Titration curves were characterized by extension of the isoelectric point into an isoelectric range between approximately pH 5 and 10. Maximal swelling was observed at approximately pH 12.

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Cholesterol and Cholate Concentration of Perfusate and Bile of Isolated Liver Preparation.* (20310)

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A perfusion apparatus has been described by Brauer, Pessatti, and Pizzolato(1) in which the isolated rat liver can be maintained in a viable state for many hours, and its functional capacity demonstrated by the continuous formation of bile. The present report describes the cholesterol and cholate content of the perfusate, and of the bile, in 12 perfusion experiments on the isolated rat liver.

Methods. Young, male, non-fasted, Long-Evans rats in a weight range of 220-350 g were utilized. Ether inhalation anesthesia was employed in all instances (in some cases, intraperitoneal nembutal (12 mg) was given initially). After opening the abdomen, 1.0 mg of heparin was injected into the inferior vena cava. The bile duct, portal vein, and thoracic vena cava were cannulated in turn with polyethylene catheters. The liver was excised and placed in the perfusion apparatus, with perfusate entering the portal vein cather under a constant, non-pulsatile pressure of 21.5 cm. Clean, but not sterile, surgical technic was employed. The operative time during which circulation through the liver was interrupted, *i.e.*, from ligation of the portal vein until placement of the excised liver in the perfusion apparatus, was under 10 minutes in all cases, and averaged 5 minutes. The perfusate consisted of one part of heparinized whole rat blood diluted with 3 parts of salt solution, prepared according to Brauer(1). Dextrose sufficient to achieve a concentration of 80 mg % was added to the complete perfusate. In all but the first 4 experiments, an additional 4.0 cc of 10 "essential" amino acids was added, as prepared by White(2). In the last 4 experiments 4.0 cc of 9 of the B vitamin group, and 4.0 cc containing carotene, Vit. A, ascorbic acid, glutathione, and cysteine were added, also as prepared by White(2). An infusion containing the same salt and albumin† concentrations as the perfusate with the addi-

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