done without an increase in size or aggregation of droplets; but boiling for sterilization was found to be not necessary. Tests with broth and blood agar plate cultures for bacteria and Littman plate cultures for molds were found negative. Apparently, the halogen acts as a sterilizing agent.

Summary. 1. A colloid sol of hexabromostearic acid was used successfully for hepatosplenography in the rat. This sol could be given intravenously or by repeated intragastric injections. Dibromindigo appeared in the urine after intragastric injection. 2. Half the minimum intravenous dose of several halogenated fat emulsions for hepatosplenography was effective when reinforced with injections by the intralymphatic and intragastric routes, but the total dose was greater than the minimum intravenous dose alone. 3. The emulsions were broken by boiling but this method of sterilization was not necessary since it was found that the halogens acted as sterilizing agents.

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Combination of Hydrochloric Acid and Sodium Hydroxide with Human Aortic Albuminoid.* (19583)

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Recent studies have revealed that the albuminoid (collagen and elastin) fraction of the human aorta undergoes significant quantitative aging and pathological changes. Though the percentage of elastin in this albuminoid fraction increases significantly only in markedly arteriosclerotic aortae(1), notable increases in the calcium content of aortic elastin have been demonstrated with progressive age(2). Likewise, important quantitative aging changes in the amino acid composition of elastin have been shown(3). It seemed of value, therefore, to investigate the acidic and basic properties of the human aortic albuminoid as part of an overall attempt to more fully evaluate the underlying pathological changes in this tissue.

Materials and methods. The albuminoids were prepared by previously described methods(4) with the exception that sodium chloride and dibasic sodium phosphate soakings were substituted for the calcium hydroxide treatments. The refrigerated tissues, subsequent to acetone and alcohol extractions, were soaked in 3 changes of 10% sodium chloride for a minimum of 24 hours during each extraction period. They were then treated with M/15 dibasic sodium phosphate for 2 soaking periods of at least 24 hours in the refrigerator. After five 24-hour washings in distilled water, the samples were dehydrated with several changes of absolute alcohol, dried in an oven at approximately 70°C, and desiccated for at least 24 hours. The 29 albuminoid specimens were each subdivided into 2 approximately equal samples ranging from 0.4 g to 2.6 g in weight. One aliquot of each tissue was treated with 30 ml of .10 N hydrochloric acid in saturated sodium chloride (25°C) per g of albuminoid, while the other fraction was soaked in 30 ml per g of tissue of .11 N sodium hydroxide in saturated sodium chloride (25°C) according to the method of Beek(5). After standing for 24 hours with occasional shaking,

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TABLE I. Combination of Hydrochloric Acid and Sodium Hydroxide with the Human Aortic Albuminoid.

Age group, yr	No. of samples	Mean mEq HCl combined/g albuminoid	Stand. dev.	t	P	Mean mEq NaOH combined/g albuminoid	Stand. dev.	t	P
0-39	7	.77	.10			.76	.05		
40-59	5	.95	.31	1.4	.18	.81	.02	1.8	.09
60-74	8	1.3	.48	3	.01	.79	.03	1.2	.24
75-90	9	1.7	.40	6.1	<.01	.79	.02	1.7	.11
Condition*									
+	4	.77				.78			
2+	4	.77				.75			
3+	5	1.1				.81			
4+	9	1.3				.79			
5+	7	1.8				.79			

* The aortae were divided on the basis of gross observations into five groups: Normal (+), few atheromatous plaques (2+), moderate atheromatous plaques (3+), atheromatous plaques and few calcified plaques (4+), and many plaques, atheromatous and calcified (5+).

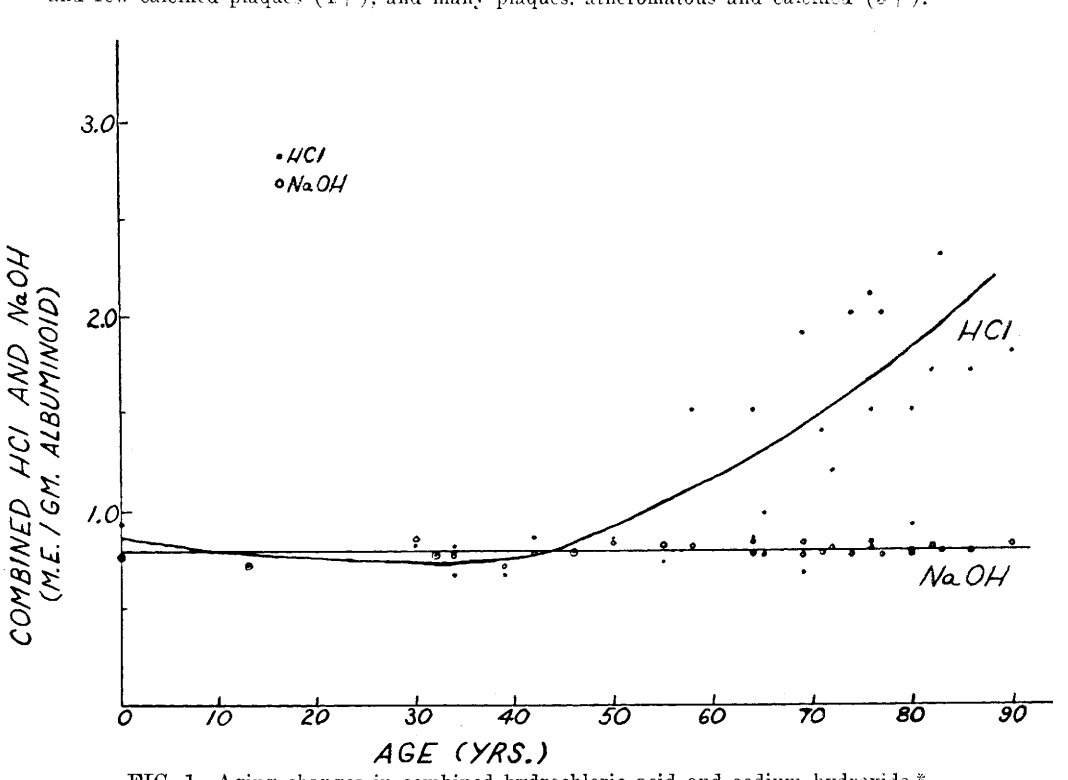

FIG. 1. Aging changes in combined hydrochloric acid and sodium hydroxide.*

a 5 ml aliquot of each supernatant solution was titrated against .10 N hydrochloric acid or .11 N sodium hydroxide, using methyl red as the indicator. All samples were treated simultaneously at a temperature of 26 ± 2°C. The results were expressed in terms of the number of milliequivalents of acid or base combined by one g of albuminoid.

Results. Aging changes. All samples combined with both sodium hydroxide and hydrochloric acid. The quantity of combined sodium hydroxide per unit weight of tissue remained constant in all age groups (Table I, Fig. 1). However, an approximately 2-fold increase in the quantity of combined hydro-

* Plotted by visual approximation.

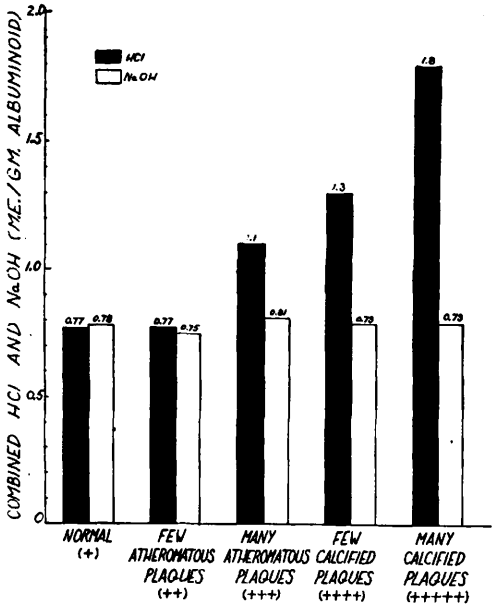

FIG. 2. Pathological changes and combined hydrochloric acid and sodium hydroxide.

chloric acid was observed to take place progressively between the ages of 40 and 90 years (Table I, Fig. 1). The statistical analyses (Table I) indicate that the increase in combined hydrochloric acid in the 60- to 90-year age groups is statistically significant, when compared with normal values. There are, however, no statistically significant age changes in the quantity of combined sodium hydroxide.

Pathological changes. The samples have been arbitrarily classified into 5 groups on the basis of gross pathological findings (Table I, Fig. 2). The amount of combined sodium hydroxide remained relatively constant in all groups. Markedly sclerotic aortae combined with larger amounts of acid than did normal or mildly atheromatous samples, while moderately sclerotic tissues yielded intermediate values. The chi square test showed that the differences in the number of milliequivalents of combined hydrochloric acid per gram of tissue for the various pathological groups cannot be accounted for by chance alone ($P = <.01$).

Discussion. If it is assumed that the calcium present in these tissues is in the form of tricalcium phosphate(3), this salt would certainly be an important factor in determining the above results. In addition, the presence of other minerals as well as the acid and base combining power of the protein itself must be taken into consideration. It is also difficult to determine whether the increased mineral deposition in the aorta with aging and pathological changes is due to changes in the albuminoid proteins themselves, or whether the protein changes are secondary to the increased mineral deposition.

Summary. 1. Human aortic albuminoid fractions combine with equal amounts of sodium hydroxide despite differences in age or pathological condition. 2. Arteriosclerotic and aged aortic albuminoids combine with larger quantities of hydrochloric acid than do relatively normal or younger samples.

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