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Relationship between Aortic Histamine Formation and Aortic Albumin Permeability in Atherogenesis (40969)[1]

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Abstract. The relationship between aortic histamine-forming capacity (HFC) and albumin permeability was examined in intima–media preparations of ascending aorta and aortic arch segments of male, Dutch-belted rabbits maintained on Purina Rabbit Chow containing 0.5% cholesterol for 2- and 4-week periods; pair-fed controls were maintained on Purina Rabbit Chow. Aortas from rabbits maintained on the 2-week cholesterol regimen showed no evidence of atherosclerosis, while those from rabbits on the 4-week regimen had substantial subendothelial lipid, as determined by oil red O staining of fresh-frozen sections. Aortic albumin permeability, measured in terms of aortic content of bovine serum albumin conjugated to fluorescein isothiocyanate (FITCBSA) under steady-state conditions, was highest in the 2-week treatment group (43.8 ± 6.3 ng/mm^2, $P < 0.05$) than in either control segment (34.4 ± 1.86 ng/mm^2) or similar segments from the 4-week treatment group (31.4 ± 3.38 ng/mm^2). Similarly, the ascending aorta and aortic arch segments of this same 2-week treatment group had a significantly higher ($P < 0.05$) HFC (16.82 ± 0.8 dpm/mm^2) than corresponding control (15.13 ± 0.87) and 4-week (13.85 ± 0.99 dpm/mm^2) treatment groups. Linear regression analyses of the intraaortic FITCBSA content against the aortic HFC yielded a significant correlation coefficient ($r = 0.59$, $P < 0.05$) between these parameters in the 2-week treatment group only. These results indicate that the rate of aortic histamine synthesis is increased during early stages of the atherogenic process, and that a causal relationship may exist between the rate of this histamine synthesis and the increased aortic transmural albumin permeability which occurs under these preatherosclerotic conditions.

It is generally accepted that the initial event in atherogenesis is an increase in endothelial permeability to macromolecules, which subsequently evokes a variety of reactive processes within the arterial wall, and that endothelial injury occupies a key role in this process. In this respect, we have been examining relationships between aortic *de novo* histamine formation and a variety of atherogenic stresses, including hypertension (1, 2), elevated shear stresses (3, 4), experimental diabetes (5), and dietary-induced hypercholesteremia (6). A consistent finding has been that aortic histamine formation is significantly elevated in each of the above conditions, and that in hypercholesteremia the increase is transient and restricted to the endothelium (7, 8). Since inflammatory processes have been recognized as being associated with atherosclerosis for in excess of 100 years, and on the basis of our previous work, we have proposed that these initial permeability increases may be mediated, at least in part, by the increase in aortic histamine formation, especially that of the involved endothelial cells. Therefore, the present study has been designed to examine what relationship exists, if any, between aortic histamine formation and aortic permeability to albumin under normal, preatherosclerotic, and early atherosclerotic states.

Materials and methods. Seventy-two male Dutch-belted rabbits, 6–8 weeks old, were randomly divided into three treatment groups of 24 animals each; these received either a control diet of Purina Rabbit Chow or an experimental diet of Purina Rabbit Chow containing 0.5% (w/w) cholesterol for either a 2- or a 4-week period. Food and water were administered *ad libitum*, and feeding periods were arranged so that all animals completed their prescribed dietary regimen at 10–12 weeks of age. All animals were housed in individual stainless-steel

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cages under controlled environmental conditions.

At the end of the prescribed dietary period, each animal was injected via marginal ear vein with bovine serum albumin (BSA) conjugated to fluorescein isothiocyanate (FITC) at a dose of 100 mg/kg. This was done in order to subsequently quantify albumin uptake by the ascending aorta and aortic arch under steady-state conditions, as previously described (9). To insure that this steady-state condition was present (9, 10), six animals in each treatment group were killed by stunning at 1, 3, 6, and 12 hr after FITCBSA injection. The thoracic aorta was measured, and the entire length of the aorta (from aortic valve to diaphragm) was excised, stripped of the periadventitial sheath, opened longitudinally, and rinsed with phosphate-buffered saline (PBS, pH 7.4, 4°C) to remove adhering blood. The aorta was reextended to its *in vivo* length and traced for measurement of surface area. The ascending aorta and aortic arch was removed, a 1-mm sample of this region was excised for histological evaluations, and the remaining tissue was homogenized in 20 vol of PBS (pH 7.4, 4°C). The supernatant solution (10,000*g*, 4°C, 20 min) was used for determination of the histamine-forming capacity (HFC) and FITCBSA content, both of which were standardized on the basis of aortic segment surface area.

The HFC of the ascending aorta and aortic arch was determined using modifications of the method of Levine and Watts (11), as previously described (3, 7, 8). The incubation medium consisted of 1.85 ml enzyme supernatant solution, 0.05 ml 1 m*M* pyridoxal 5-phosphate, and 0.1 ml L-[^{14}C]-histidine (carboxyl-labeled, 1 μCi/ml). Radioactivity was counted via standard liquid scintillation procedures. Results were corrected for background, compared against blanks containing supernatant treated with trichloroacetic acid (TCA), and expressed in terms of HFCs standardized on the basis of surface area (dpm/mm^2).

The intraaortic FITCBSA content was measured by quantitative fluorometric procedures previously described (9). Briefly, this involves conjugation of FITC to BSA,

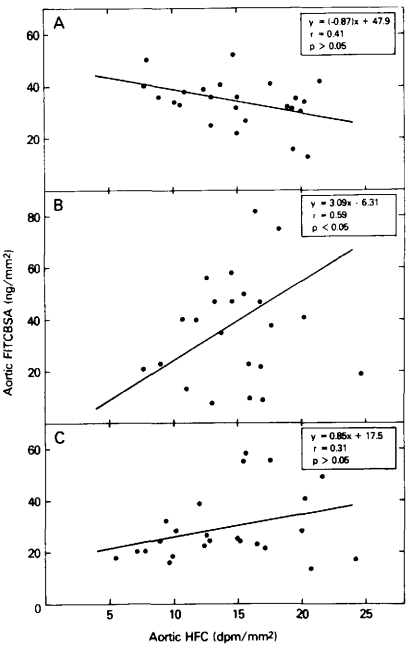

FIG. 1. Regression analysis of relationships between aortic histamine-forming capacity (HFC) and intraaortic content of bovine serum albumin conjugated to fluorescein isothiocyanate (FITCBSA) in ascending aorta and aortic arch segments. Control values are plotted in panel A; panels B and C represent similar plots from rabbits maintained on a diet containing 0.5% cholesterol for 2 and 4 weeks, respectively. Each point corresponds to one aortic segment from one animal.

purification by means of Sephadex G-25, verification of purity by measuring non-TCA-precipitable FITCBSA following TCA treatment of the FITCBSA solution, and measurement of fluorescence of aliquots of the 10,000*g* supernatant solution (at 490-nm excitation and 530-nm emission) in relation to that obtained from a standard curve. Results were corrected for dilution and likewise standardized on the basis of aortic surface area.

Food and water intake were monitored throughout the duration of each feeding period. During this time, weekly serum cholesterol determinations were made using procedures described by Levine and Zak (12), adapted for use with the Technicon autoanalyzer.

Results. There were no apparent differences in feeding behavior among animals maintained on the cholesterol diet with re-

spect to those on the control diet. Typical food consumption was approximately 200 g/day for animals in each group. All animals maintained on the cholesterol diet exhibited hypercholesteremia. Mean values (±SEM) were 56 ± 12 mg/dl (control), 639 ± 74 mg/dl (2 weeks of cholesterol feeding), and 917 ± 47 mg/dl (4 weeks of cholesterol feeding). With respect to histological evaluations, frozen cross sections from specimens obtained from each ascending aorta and aortic arch region of animals on the 2-week cholesterol regimen failed to exhibit any oil red O staining; similarly stained sections from aortas of animals on the 4-week cholesterol feeding period, however, contained subintimal oil red O. Similar observations and photomicrographs have been previously published (6).

There were no significant differences in the intraaortic FITCBSA content over the 1-, 3-, 6-, and 12-hr postinjection times between animals of a given treatment group, a finding consistent with previously reported results for both rats (9) and rabbits (10). Accordingly, all results were pooled, and mean data (±SEM) calculated from FITCBSA contents of all animals in each of the three treatment groups are given in Table I. Comparison between the treatment groups indicates that the intraaortic FITCBSA content of the ascending aorta and aortic arch of the 2-week treatment group was 27% higher than that of the control group and 40% higher than that of the same aortic region from the 4-week treatment group. The mean HFC data of these same aortic sections show that a similar change (but of lesser magnitude) occurred in *de novo* histamine synthesis. Specifically, the HFC of this region from the 2-week cholesterol group is significantly higher ($P < 0.05$) than that of the corresponding control segment. In the case of the 4-week treatment group, the mean HFC of this aortic region is significantly lower ($P < 0.05$) than corresponding control segments.

Regression analysis of individual intraaortic FITCBSA and HFC data in each of the three treatment groups indicates that a significant correlation coefficient ($P < 0.05$) exists between these parameters in the case of the 2-week cholesterol-fed group. In both other cases, i.e., control and following 4 weeks of cholesterol feeding, the correlation coefficients are not significant.

Discussion. Both Gaman and Feigenbaum (13) and Adams *et al.* (14) have shown that Dutch-belted rabbits are more lesion resistant than New Zealand white rabbits, although over time both rabbit breeds show essentially similar hypercholesteremic responses with cholesterol feeding. They have likewise shown that in these animals the ascending aorta and aortic arch is the first region to develop atherosclerotic lesions. Both of these findings, i.e., a preferential site for atherosclerosis development and relative lesion resistance, are necessary components of the experimental design of the present investigation, since the intent has been to examine both histamine formation and albumin permeability (as measured by analysis of the intraaortic albumin content under steady-state conditions) in both a preatherosclerotic state as well as in an early stage of atherosclerosis. The histological appear-

TABLE I. INTRAAORTIC CONTENT OF BOVINE SERUM ALBUMIN CONJUGATED TO FLUORESCEIN ISOTHIOCYANATE (FITCBSA) AND AORTIC HISTAMINE-FORMING CAPACITY (HFC) OF THE ASCENDING AORTA AND AORTIC ARCH OF RABBITS FED 0.5% CHOLESTEROL FOR 2 AND 4 WEEKS

Treatment group (duration)	Intraaortic FITCBSA[a] (ng/mm²)	HFC (dpm/mm²)
Control (24)	34.4 ± 1.86	15.13 ± 0.87
2 Weeks (24)	43.8 ± 6.25[b]	16.82 ± 0.80[b]
4 Weeks (24)	31.4 ± 3.38	13.85 ± 0.99[b]

[a] 100 mg/kg body wt, iv, 1–12 hr before sacrifice. All values are group means ±SEM. Numbers in parentheses indicate number of animals in a treatment group upon which these statistics have been calculated.

[b] Difference from control significant ($P < 0.05$, Student's t test).

ances of aortas in each treatment group indicate that both states have been achieved, since aortas from animals fed cholesterol for 2 weeks lacked visible lesions while those from similar animals on the 4-week dietary regimen showed histologically identifiable subintimal lipid deposition, a typical and widely used index of manifestations of early atherosclerosis.

Results of the present study indicate the permeability of the aortic arch region to albumin in rabbits maintained on 0.5% cholesterol for 2 weeks is 27% higher than control values and 40% higher than that of the 4-week treatment group. This is indicative of a transient increase in albumin permeability occurring early in the atherogenic process and is consistent with findings of Robertson and Khairallah (15), who have likewise shown that aortic permeability changes during atherogenesis are in many cases transient.

In experiments involving examination of endothelial and smooth muscle histamine synthesis under conditions identical to those of the present study, we (8) reported that endothelial HD activity increased approximately 40% with respect to both endothelial cells from control aortas and from aortas of rabbits maintained on a similar cholesterol diet for 4 weeks. The change in HFC over this 4-week period in the present study thus presumably reflects the increased HD activity of the endothelial component which is also transient; the lower magnitude of the increase in HFC seen in the present study reflects the influence of the smooth muscle component of the vessel wall in which no increase in HFC occurs (8).

That a relationship between aortic *de novo* histamine synthesis and aortic permeability is causally related is suggested both by corresponding significant increases in the aortic HFC and FITCBSA uptake and by regression data which indicate a significant and positive correlation between these parameters in the aortic arch region of the 2-week treatment group which were in a preatherosclerotic state. No significant relationships between the HFC and intraaortic FITCBSA content were apparent in either the control group or that group of animals maintained on cholesterol for the 4-week period. These findings are also consistent with the concept and further support for the premise that initial transient increases in aortic permeability occurring during atherogenesis are mediated, at least in part, by increased aortic *de novo* histamine synthesis mediated by increased activity of the aortic wall histidine decarboxylase system, and involve a process similar to the microcirculatory prolonged phase of inflammation (16).

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