Intimal Reaction of the Rat's Aorta to Intraortic Thrombus Formation.* (27378)

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Following the production of a thrombus in the aorta of a normal rabbit, a plaque ensues (1) that eventually bears a striking resemblance to the pearly white but lipid-poor plaque frequently found in the diseased human aorta. Furthermore, if the rabbit bearing this thrombosclerotic process is made hypercholesteremic by oral administration of cholesterol, the plaque quickly becomes cholesterol and triglyceride laden(2,3), *i.e.*, a thromboatherosclerotic process which in turn markedly resembles the human arterial atherosclerotic plaque.

Earlier we observed(1) that insertion into the aorta of a rat of the same alloy spiral used in the rabbit studies would induce in this species, too, a thrombus which in turn elicited an aortic hyperplastic intimal response. However, it also was observed that the intensity and magnitude of this response at the end of 21 days was much less than that observed in the rabbit aorta in a comparable period.

Since it is just this intensity and magnitude of intimal response which governs the gravity of pathological consequences of plaque formation, it seemed important to compare the type of plaque occurring in the rat fed an excess of cholesterol and fat with that occurring in the rabbit similarly fed. The results will be described below.

Material and methods. A. *Production of thrombosis in rats.* Twenty male rats (Long-Evans strain) were anesthetized with ether, the abdominal aorta below the renal arteries visualized and a zinc chloride-treated magnesium alloy wire spiral (1.5 cm long, 0.6 mm in diameter) was inserted therein as previously described(1). After the operation, half of the rats were allowed to ingest a high fat-cholesterol diet slightly modified† from that described by Hartroft(4) and the remaining half were continued on Purina Lab Chow. Blood samples were obtained initially and then monthly for serum cholesterol analysis(5). All rats were killed 3 months after insertion of the spiral. The aorta was opened, and the plaque inspected. A section of each plaque was obtained for histological study, then the remainder of each plaque and a separate segment of the adjacent abdominal aorta were analyzed for total cholesterol content (5). Nine of each group of 10 rats survived the total period.

B. *Production of thrombosis in rabbits.* Ten young male rabbits were anesthetized with pentobarbital and a zinc chloride-treated magnesium alloy wire spiral was inserted into the abdominal aorta as previously described (1). Five of the rabbits were allowed to ingest Wayne rabbit chow diet plus added cholesterol (2%) and cottonseed oil (2%), and the remaining 5 only the chow diet for the experimental period of 3 months. The same blood and post mortem studies described above were performed upon these rabbits.

Results. The thrombotic process developing in the hypercholesteremic rat does not differ from that in the normocholesteremic rat but it does differ markedly from the thrombotic plaques forming in both the normo- and hypercholesteremic rabbit. At the end of the 3 month period, the processes in both types of rats on gross inspection appeared (Fig. 1) essentially similar in size, shape and failure to become adherent, except at one of their terminal ends, to the aortic wall. In the rat, therefore, cholesterol feeding failed to effect an increase in size of the mass as it usually does in the rabbit(2) (Table I). When the

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† Butter, 40%; casein, 20%, cholesterol, 5%; cellulose, 4%; salt mixture, 4%; vitamin mixture, 2%; choline chloride, 1%; thiouracil, 0.2%; cholic acid, 0.3%; sucrose sufficient to make 23.5%.

TABLE I. Cholesterol Content of Aortic Plaques in Rat and Rabbit.

No. of animals	Avg wt (g) Beginning	Avg wt (g) End	Avg serum cholesterol* (mg/100 ml)	Aorta cholesterol (g/100 g)	Aortic plaque Wt (dry) (g)	Aortic plaque Cholesterol (g/100 g)	Plaque/Aorta cholesterol ratio
A. Rats fed excess cholesterol and oil							
9	308	262	489 ± 22 † (368-595)‡	1.6 ± .15 (1.1-2.2)	.002 (.001-.002)	4.8 ± .3 (3.6-5.6)	3.0 ± .5 (2.1-5.1)
B. Rats fed stock diet							
9	323	432	52 ± 3.2 (42-62)	.5 ± .03 (.3-.6)	.002 (.001-.002)	2.3 ± .25 (1.2-3.4)	4.6 ± .8 (2.4-6.8)
C. Rabbits fed excess cholesterol and oil							
5	2820	3840	982 ±120.0 (673-1411)	5.9 ±2.2 (.8-14.0)	.020 ±.002 (.015-.025)	21.9 ±2.6 (13.7-31.6)	3.7 ±1.1 (2.3-6.2)
D. Rabbits fed stock diet							
5	2780	3460	54 ± 2.8 (48-55)	.5 ± .02 (.4-.6)	.010 ±.002 (.008-.012)	1.6 ± .07 (1.5-1.9)	3.2 ± .4 (2.9-3.6)

* Avg of initial and monthly serum cholesterol values.
† Stand. error of mean
‡ Range.

thrombotic lesions of both types of rats were compared grossly with that of the hypercholesteremic rabbit (Fig. 2), the dense, fibrous, plaque-like nature of the latter lesion contrasted strongly with the rat's thin-walled, flexible, soft, rather sac-like structure.

Upon microscopic examination also, a very marked difference was found between the lesions of the rat and rabbit. The arterial lesion in both normo- and hypercholesteremic rats was not a true plaque, but rather an agglomeration of residual, unabsorbed metallic salts skimpily covered and enclosed by a thin rim of hyperplastic intimal tissue (Fig. 3, 4). This failure of rat intimal tissue to proliferate and to remove the original metallic salts, contrasted strongly with the plaque with its heavy fibrous rim and abundant intimal hyperplasia invariably observed in both the normo- and hypercholesteremic rabbit (Fig. 5). However, the arterial lesion of the hypercholesteremic rat resembled that of the hypercholesteremic rabbit in one respect, namely, in the occurrence of slight to moderate Sudanophilic infiltration at the base of the lesion, usually at the point where intimal tissue arose from the artery wall to cover the total mass. This type of accumulation, however, never was found in the lesion of the normocholesteremic rat. The absence of Sudanophilia was the only histological difference noted between this latter lesion and that of the hypercholesteremic rat. No hemorrhages were observed in any of the rat thrombotic processes.

On chemical analysis, both the adjacent intact segment of aorta and the thrombotic process of the hypercholesteremic rat were found to contain significantly more cholesterol (Table I) than their counterparts in the control animals. But when the arterial lesions of the hypercholesteremic rats were compared with those of the hypercholesteremic rabbit, it was seen that a far greater accumulation of cholesterol had occurred in the lesions of the latter species (Table I). In part this could have been a consequence of the greater average serum cholesterol content of these rabbits during development of the plaque. The higher serum cholesterol also might explain the higher average cholesterol

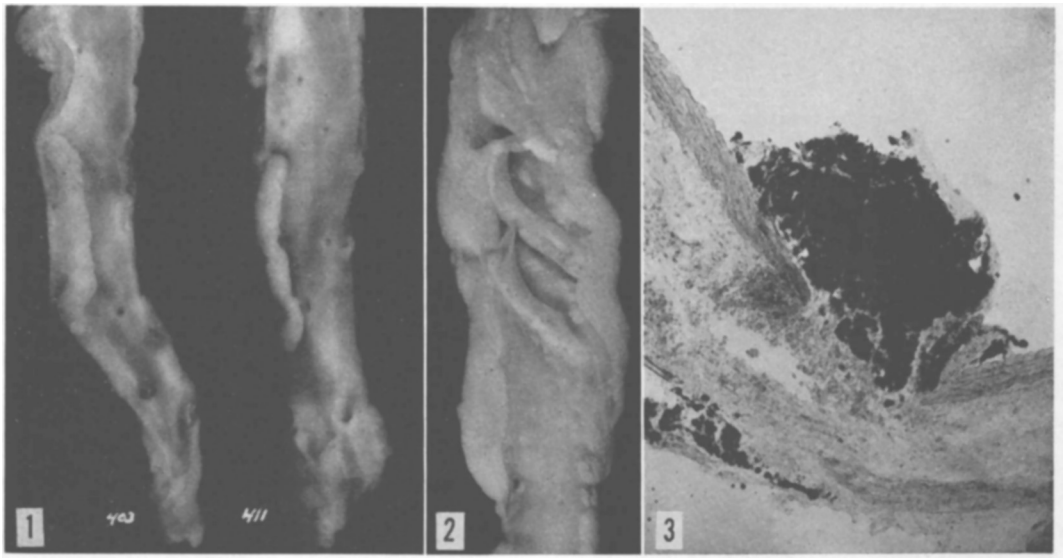

FIG. 1. Aortic thrombotic process in hypercholesteremic (#403) and normocholesteremic (#411) rat 3 mo after insertion of the spiral. Size, shape and general appearance of the 2 lesions are essentially the same. The sac-like structure attached at only one end to the aorta contrasts strongly with the dense, fibrous scar-like masses observed in the rabbit aorta (Fig. 2).

FIG. 2. Thromboatherosclerotic plaque in abdominal aorta of a rabbit fed excess cholesterol during the 3 mo following insertion of the spiral. The plaque in this animal was actually a spirally shaped, continuous process but was cut into 3 sections on incision of the aorta. Areas of spontaneous atherosclerosis between, above and below the plaque segments are discernible.

FIG. 3. Aortic thrombotic process in hypercholesteremic rat 3 mo after insertion of the spiral (Sudan IV Stain × 28). The section was obtained at point of attachment of the mass to the aorta. Note complete disruption of the media and internal elastic membrane immediately beneath the central mass. Many cells in this area exhibited Sudanophilia. The thin rim of intimal tissue encircling the mass has been partially fragmented on section. These cells failed to exhibit Sudanophilia. The relative failure of the aortic intima to respond to the original thrombus is shown by comparison with Fig. 5.

content of the adjacent aortic segment.

Discussion. Earlier(1,2,3) we presented evidence that an arterial thrombus induced in the rabbit quickly led to an intimal reaction which, in the presence of hypercholesteremia, became an atherosclerotic plaque. This latter lesion developed because the new intimal tissue growing in response to the presence of the thrombus possessed both new capillaries (derived from adventitia sources) that "leaked" large quantities of lipid and cholesterol, and cells that ingested and retained these escaping moities. Furthermore, it became clear that not only a thrombus but also any other incident or agent (probably including excess cholesterol itself) that elicited a new growth of intimal cells and capillaries could serve as an atherogenic agent, given also the appropriate quantity, and possibly quality, of cholesterol in the serum. In other words, given an excess of cholesterol, a leaking vessel and a very phagocytic cell, an atherosclerotic reaction ensues.

The present study of the rat and the reaction of its aorta to a spiral-induced thrombus tended to confirm this hypothesis, both in its positive and negative aspects. Thus, following induction of the intra-aortic thrombus in the cholesterol fed rat, an intimal reaction did occur and at the basal area of this new intimal tissue (as was similarly found in the rabbit aorta) excess triglyceride (and presumably cholesterol too) accumulated. Also, when the total plaque was analyzed it contained an excess of cholesterol but not as much as was found in the rabbit plaque. Unlike the rabbit aorta, the rat's aorta responded to the induced thrombus with the scantiest of intimal hyperplasia. Apparently just enough new tissue formed to encircle and isolate the total thrombus mass. So scant was this intimal reaction that the major part of the

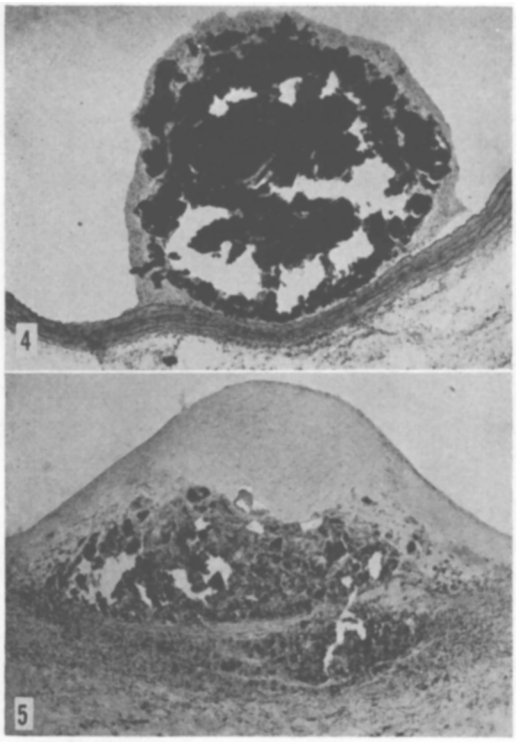

FIG. 4. Aortic thrombotic process in normocholesteremic rat 3 mo after insertion of the spiral (Sudan IV Stain × 24). The section was obtained near to but not precisely at point of attachment. As in Fig. 3, the central mass consists essentially of residual metallic salts, covered by a thin, lightly staining rim of new intimal tissue. The media and the internal elastic membrane subjacent to the process appear to be intact. No Sudanophilic staining was present.

FIG. 5. Typical thromboatherosclerotic plaque in abdominal aorta of the cholesterol fed rabbit 3 mo after insertion of the spiral (Sudan IV Stain × 24). In contrast to Fig. 3, only scattered fragments of metallic salts remain in the central mass, the latter area being chiefly composed of invading intimal cells that were impregnated with Sudanophilic material. Note very thick fibrous layer overlying the central area, the areas of necrosis in the central mass and complete disorganization and cellular infiltration of the media subjacent to plaque.

original residue of metallic salts remained unabsorbed. Accordingly, a true atherosclerotic plaque similar to that observed in the rabbit aorta or in the human artery did not occur, but rather the formation of a polypoid mass consisting of a sac-like structure of cholesterol-rich intimal cells whose peripheral extensions enveloped an unresolved mass of metallic salts and possibly other organic debris. In short, although hypercholesteremia was present, the growth response of intimal cell and capillary was so slight that little significant atherosclerosis ensued.

This seeming inertia of response on the part of the rat's aortic intima to the foreign body represented by our thrombus probably explains the failure of this same segment of the arterial system to respond in an atherogenic manner to the feeding of even the cholesterol-fat rich diet of Hartroft. For although this diet does lead to atherosis of the coronary vasculature as Hartroft(4) maintains, neither he nor we have observed that the same diet induces a similar change in the aorta.

Summary. Intraortic thrombi were induced in rats fed stock and high cholesterol fat diets. Similar thrombi were induced in rabbits fed similar diets for comparative purposes. The thrombi in rats fed either type of diet evoked an intimal cellular hyperplasia that led to encirclement of the thrombus. The new intimal tissue in the hypercholesteremic rat also exhibited an excess of lipid and cholesterol. However, the intensity of cellular response, of lipid and of cholesterol accumulation was much less in the hypercholesteremic rat's aorta than in that of the hypercholesteremic rabbit.

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