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Predisposing Effect of Spontaneous Mesenchymal Intimal Thickenings of Rabbit Aorta to Early Lipid Deposition

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Summary. Ten rabbits received by gastric tube 0.3 to 0.8 g pure cholesterol every second day for 4 to 8 weeks. Ten animals were used as control. Total serum cholesterol was determined weekly in experimental and control groups. The mean value of the total serum cholesterol in the experimental group was 482 ± 79.6 mg/100 ml, about 2.5 times more than the cholesterol value of the control group (206 ± 51.0 mg/100 ml).

Early and selective lipid deposition was present in spontaneous mesenchymal thickenings of all aortas of the animals receiving cholesterol. The deposition failed to be observed in the aortic intima without that thickening. The intimal thickening is a change of the arterial wall that predisposes to lipid deposition.

This observation supports the view that the proliferative changes seen in human atherosclerosis precede the lipid deposition and are a predisposing factor to this deposition.

Spontaneous mesenchymal thickening in the intima of rabbit aortas is frequent (Schenk, Gaman, and Feigenbaum, 1966, I). A similar thickening is seen in the human aorta and coronary arteries (de Faria, 1965, 1968) preceding the lipid accumulation. In rabbit aorta, however, the latter is rare spontaneously (Schenk, Gaman, and Feigenbaum, 1966, I). In dietary atherosclerosis Schenk and collaborators (Schenk, Gaman, and Feigenbaum, 1966, II) observed occasional lipid deposition at sites of intimal mesenchymal thickening. The purpose of this report is to demonstrate the role played by the spontaneous intimal thickenings on lipid deposition in aorta of rabbit submitted to a mild hypercholesterolemia of short duration. So far as we know such an experiment has not been made.

Material and Methods

Twenty rabbits were used, 10 experimental and 10 control. Most animals were mixed of Chinchilla and New Zealand White, and few New Zealand White, 4–10 months of age, ranging in weight between 2.4 and 4.02 kg (Table 1 and 2). All animals were fed a standard diet of commercial rabbit pellets, to which green vegetables were added. Ten experimental animals received by gastric tube about 0.300 to 0.800 g pure cholesterol (cholesterin, Merck) each two days, during 4 to 8 weeks. The cholesterol quantity given weekly was controlled by the cholesterol value in serum.

Blood was obtained from fasting animals by puncture of ear veins to determine the level of serum cholesterol. The cholesterol was determined by the method of Ferro and Ham (1960). All of the analyses were performed in duplicate.

One to three days after interrupting the cholesterol feeding the test and control animals were killed by a blow on the neck followed by cutting of the neck vessels. Autopsies were performed on all of the animals. In each case, the aorta was opened ventrally along its entire

Table 1. *Total cholesterol in serum of control rabbits*

Age (initial) months	Animal no.	Weight (initial) kg	Sex	Total serum cholesterol mg/100 ml[a]								
				initial	1st week	sec. week	3rd week	4th week	5th week	6th week	7th week	8th week
5	9	3.15	♀	175	207	210	195	195	158			
5	10	3.88	♀	230	222	172	179	164	113			
4	13	2.80	♂	157	222	149	154	135	92			
7	20	4.02	♀	236	163	155	118	116	144	129	238	155
7	27	3.85	♀	277	233	235	136	129	110	129	304	230
7	31	3.68	♂	255	200	89	111	77	62	77	238	160
10	303	3.25	♂	250		327	382	184				
9	486	3.05	♂	200	200	272	286	192	240			
9	494	2.95	♂	266	281	263	278	200				
10	601	3.75	♂	250	281	281	278	232	288			

[a] Mean value and standard deviation 206 ± 51 (mean calculated from the mean of each animal).

Table 2. *Total cholesterol in serum of rabbits fed cholesterol*

Age (initial) months	Animal no.	Weight (initial) kg	Sex	Duration of cholesterol feeding days	Total amount of cholesterol fed (g)	Total serum cholesterol mg/100 ml[a]								
						Precholesterol feeding	1st week	sec. week	3rd week	4th week	5th week	6th week	7th week	8th week
±8	1	2.40	♂	26	12.3	116	293	331	450	317	—	—	—	—
5½	1B	2.90	♂	40	12.85	157	458	428	365	557	317	—	—	—
5½	2B	2.85	♂	40	12.80	169	365	353	413	742	703	—	—	—
5½	7	2.84	♀	39	7.50	290	859	470	358	764	361	—	—	—
7	14	3.80	♂	58	10.86	211	382	433	281	320	234	348	628	520
7	17	3.48	♂	48	8.36	218	459	668	533	322	689	658	—	—
7	18	3.65	♂	58	10.11	192	262	439	518	290	400	509	628	570
7	21	3.80	♀	60	9.61	211	394	668	511	361	482	503	666	500
7	26	3.35	♀	58	11.11	191	284	305	398	335	351	490	876	740
8	47	2.50	♂	41	9.25	275	512	709	582	720	552	507	—	—

[a] Mean value and standard deviation: before cholesterol feeding 203 ± 51.8; after cholesterol feeding 482 ± 79.6 (mean calculated from the mean of each animal).

length, removed and examined before and after staining with sudan IV (Hollman *et al.*, 1958). At the intimal areas stained positively or dubiously with sudan IV, one transverse specimen of the aorta was taken for frozen sections and then paraffin sections; in addition frozen and paraffin sections from transverse specimens were taken from ascending aorta, arch, descending thoracic aorta (2–3 pieces) and abdominal aorta (2 pieces) normal in appearance and in areas with spontaneous medial sclerosis. The remaining parts of both latter segments were rolled and prepared for paraffin sections. The fixative was 20 per cent neutral formalin with 0.7 per cent sodium chloride (Birge and Tibbits, 1961). Sections cut from frozen tissue were stained with sudan IV propylene glycol (Woolfrey and Pearson, 1962). Paraffin sections were stained with hematoxylin and eosin, Weigert for elastic tissue and van Gieson, toluidine blue (0.05 per cent buffered solution at pH 4.1, using tertiary butanol as dehydrating reagent) and Mallory's phosphotungstic acid hematoxylin.

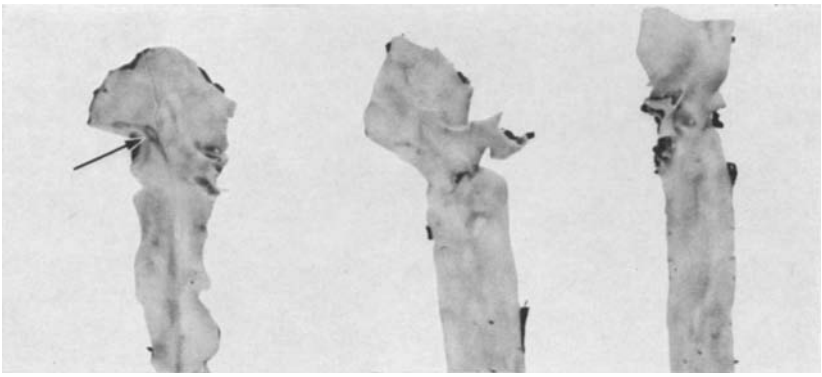

Fig. 1. At left, aorta from rabbit no. 1B fed cholesterol, showing a lipid streak (arrow). The aortas, at right (control no. 13) and in the middle, do not present intimal lipid spot. Gross staining for fat (6)

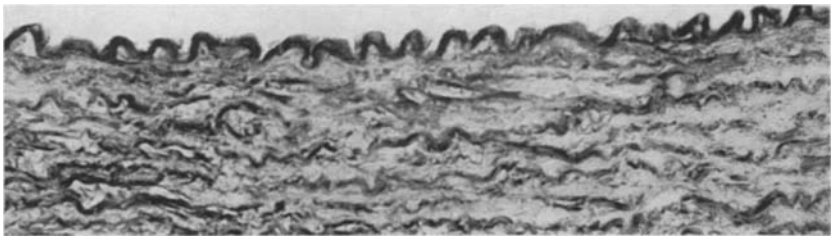

Fig. 2. Aorta from control rabbit no. 486. At the top, aortic intima without thickening. Weigert's elastic tissue and van Gieson's stains (nuclei not stained). ×400

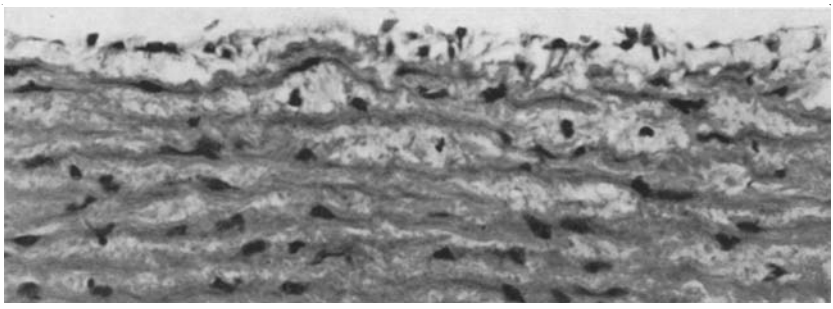

Fig. 3. Aorta from control rabbit no. 27. At the top, notice the light edematous appearance of the thickened intima. Hematoxylin and eosin stain. ×400

Results

A. Control rabbits. Grossly the aortic intima did not present any area stained with sudan IV, except for one rabbit (no. 31) showing two inconspicous areas, less than 0,1 cm in diameter, localized at the aortic arch. Microscopically, except for aorta from one rabbit (no. 31) no lipidic substance was present in the aortic intima and media of frozen sections stained with sudan IV. In rabbit no. 31 the lipid deposition in the intimal thickening was only extracellular.

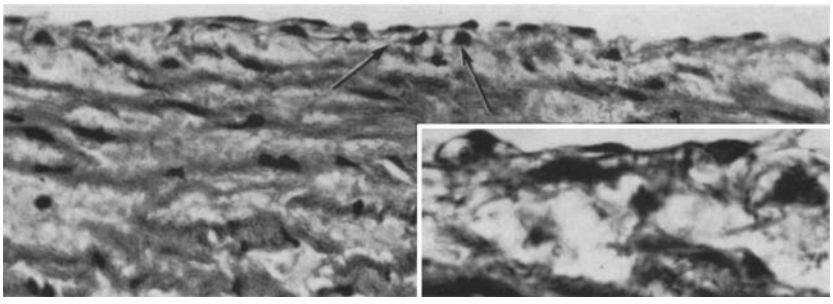

Fig. 4. Aorta from rabbit no. 26 fed cholesterol. At the top, notice the light thickened intima showing subendothelial cells with cytoplasmic vacuoles (arrows). Intimal thickening to be compared with that of Fig. 3 from control rabbit. Hematoxylin and eosin stain. ×400. Inset shows detail of the endothelial (at left) and subendothelial vacuolated cells. ×1000

Fig. 5. Frozen section from the same specimen of Fig. 4 stained with sudan IV, showing lipid-filled cells (arrows) in the thickened intima. Sudan IV-propylene glycol. ×231

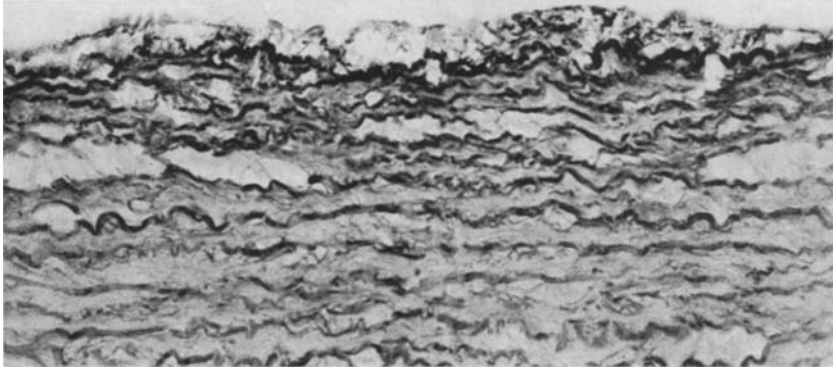

Fig. 6. Control rabbit no. 27. The same aorta as shown in Fig. 3 in immediate section. At the top, notice the thickened edematous intima. Weigert's elastic tissue and van Gieson's stains. ×400

In paraffin sections the aortic intima was thin with thickened areas, changing in width and appearance, being 6–64 μ thick, light and loose (edematous aspect) or dark and dense (hematoxylin and eosin stain; Figs. 2 and 3). Generally the thickenings were slight, long, so-called diffuse, and rarely cushion-like. They

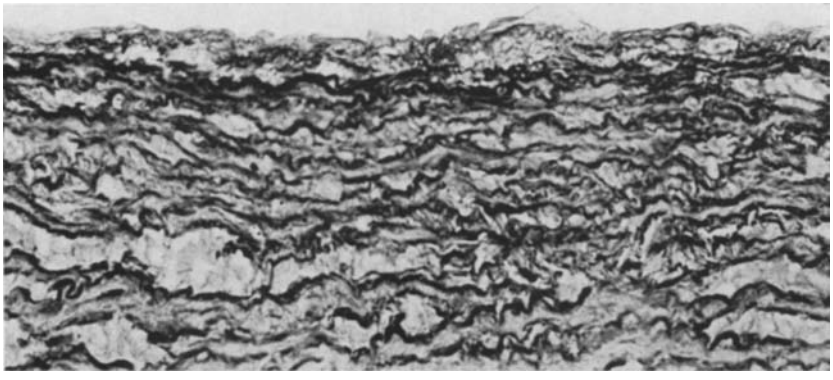

Fig. 7. Rabbit no. 26 fed cholesterol. The same aorta as shown in Figure 4 and 5 in immediate section. At the top, intimal thickening with lipid deposition in frozen section prepared from the same specimen, to be compared with the thickening of Fig. 6 (control rabbit). Weigerts' elastic tissue and van Gieson's stains. ×400

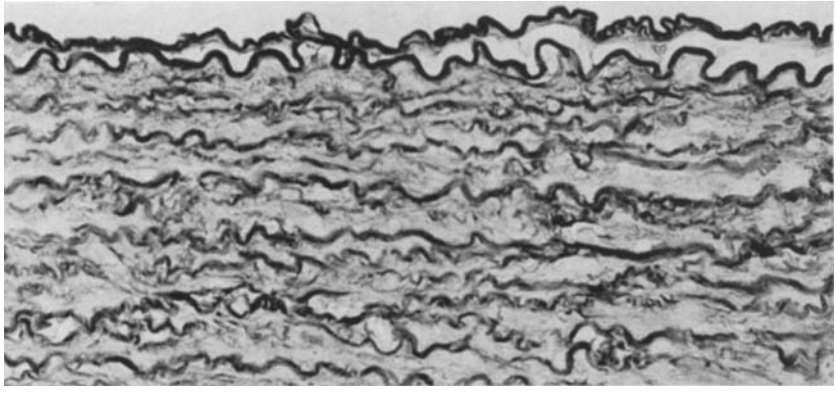

Fig. 8. Aorta from control rabbit no. 31. Notice the intimal thickening with duplication of the internal elastic membrane (in this area lipid deposition was absent). Weigert's elastic tissue and van Gieson's stains. ×400

consisted of few cells, elastic and collagenous fibers and scarce metachromatic substance. The cellular elements were undifferentiated fibroblast-like mesenchymal cells and smooth muscle cells (de Faria, 1970). The latter failed to be observed in the slight thickenings and formed one to four rows of cells in the more severe thickenings; generally the muscle cells were arranged longitudinally.

The elastic fibers showed a varied thickness, being very thick, moderately thick or very thin (Figs. 6, 8 and 10). The thick fibers were single, appearing as a duplication of the internal elastic membrane, or various (up to four) and parallel to each other. The thin fibers were arranged loosely in the light thickenings and densely in the dark ones. One row of muscle cells was separated from the neighbouring one by a thick elastic fiber, which looked-like an elastic lamella of the media (media-like structure). The collagenous fibers were thin and not numerous.

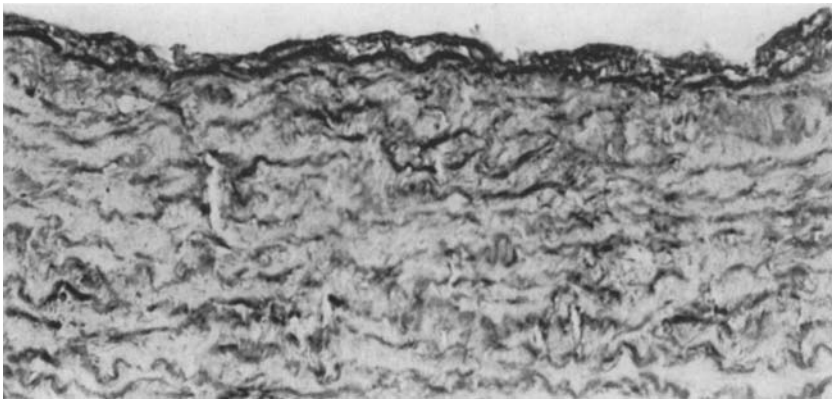

Fig. 9. Rabbit no. 1 fed cholesterol. At the top, notice the intimal thickening with lipid deposition in frozen section prepared from the same specimen, to be compared with the thickening of Fig. 8 (control rabbit). Weigert's elastic tissue and van Gieson's stains. ×400

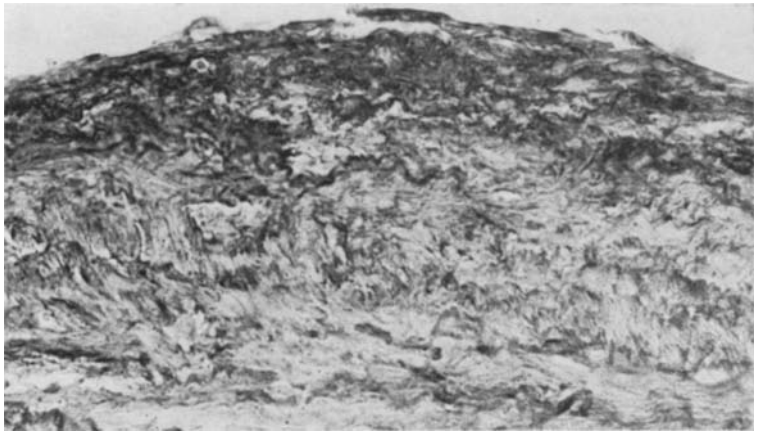

Fig. 10. Aorta from control rabbit no. 486. At the top, notice the intimal thickening stained darkly, consisting mainly of very thin, densely packed, elastic fibers. Weigert's elastic tissue and van Gieson's stains. ×400

B. Test rabbits. Grossly the aortic intima of all animals but two (no. 1 and 14) presented rare areas stained with sudan IV (Fig. 1). These areas were small, conspicuous in half of the cases and localized mainly in the intima of the aortic arch (inferior convex surface), then in decreasing order of frequency in the ascending aorta, descending thoracic and abdominal aorta. The latter was involved in only one case (no. 17) with more frequent lipid deposition. Inconspicuous lipid spots were seen in 4 animals involving the ostium of innominate, left common carotid, left subclavian and celiac arteries. The fat stained areas were rounded, about 0.1 cm in diameter, or elongated, streaky, 0,5–2.0 cm in length.

Microscopically, frozen sections stained with sudan IV confirmed the lipid nature of the intimal areas seen grossly (Figs. 5 and 12). Also the aortas of 2 animals without gross fat spots (no. 1 and 14) revealed such deposition on micro-

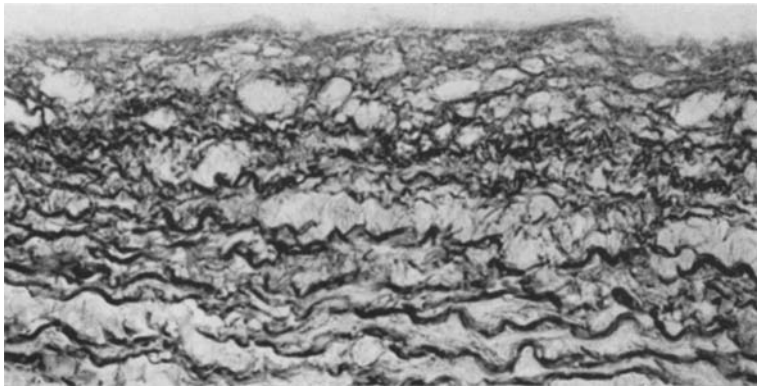

Fig. 11. Aorta from rabbit no. 17 fed cholesterol. At the top, notice the intimal thickening consisting mainly of very thin elastic fibers with many rounded vacuoles, which were sudan IV positive in frozen section prepared from the same specimen. (Compare with Fig. 10). Weigert's elastic tissue and van Gieson's stain. ×400

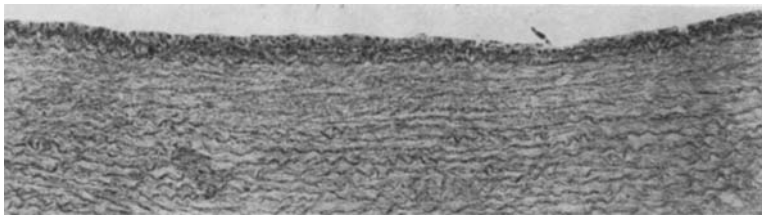

Fig. 12. Aorta of rabbit no. 17 fed cholesterol. At the top, notice the intimal thickening stained darkly, due to lipid deposition. Sudan IV—propylene glycol stain. ×92

scopical examination. Generally, however, the intimal areas unstained grossly for fat did not show lipid deposition on the microscopical examination. The lipid substance was free in the intersticial spaces and within endothelial and subendothelial cells. This substance was more rare in the subjacent media. In 4 aortas (no. 1B, 14, 18 and 47) with few tiny spontaneous sclerotic changes in the media, the lipidic substance was not seen in the adjacent thickened intima.

In paraffin sections the intimal thickenings were similar in width and composition to those of control rabbits (Figs. 4, 7, 9 and 11). There was no disorganization or modification of the intimal structure. The elastic fibers did not present degenerative changes and the collagenous fibers were not more numerous. The thickenings, however, presented conspicuous, enlarged, endothelial cells and were more cellular due to macrophages beneath the endothelial layer. Endothelial and subendothelial cells (macrophages) presented cytoplasmic vacuoles, changing in number and size, being small and numerous (foam cells) or large and single (Fig. 4). The vacuoles were due to lipid deposition, as seen in frozen sections of the same piece stained for fat (Fig. 5).

The intima adjacent to spontaneous sclerotic changes in the media presented, as well as in controls, a thickening essentially similar in width and composition to that not related to medial changes.

Discussion

The spontaneous mesenchymal intimal thickenings present a clear cut predilection toward lipid deposition. Such deposition was not observed in the intimal areas without this thickening. Further research is needed to elucidate why the intimal thickening adjacent to sclerotic calcified medial changes does not predispose to lipid accumulation, as reported by Gore and Goodman (1967). There is, however, a predisposition of the intima at the margins of the medial lesions (Schenk, Gaman, and Feigenbaum, 1966, II).

Grossly the early lipid spots presented a distribution like that of late more severe dietary atherosclerosis (Duff, 1935), being involved mainly the aortic arch and less the abdominal aorta.

The intimal thickenings seen in the test rabbits presented the same histological components and structure as the thickenings observed in control animals, except for more macrophages with lipid droplets. (The figures are to be compared). Therefore, the view that the proliferative changes in the intima followed the lipid deposition is not supported by the histological findings.

Our results aid to understand atherosclerosis in man and support the view that in human atherogenesis the proliferative changes in the intima precede the lipid deposition (de Faria, 1965, 1968) and are a predisposing factor to the latter. It is possible that studying the precocious lipid deposition in the intimal thickenings we can determine the unknown critical plasma cholesterol levels (Ho and Taylor, 1968), which initiate the cholesterol accumulation in rabbit aorta.

The spontaneous thickenings in the aortic intima of rabbits are similar to those seen in the human aorta and called diffuse thickenings (de Faria, 1965). Possibly the thickenings in rabbits are related to changes in the tunica media, as in man (Doerr, 1963; de Faria, 1965).

The total serum cholesterol values in this series of control rabbits (206 ± 51 mg/100 ml) were higher than commonly seen in reports (Cox *et al.*, 1961). This may be due to the method used for the cholesterol determination, that gives values 12 to 20% higher than other methods (Ferro and Ham, 1960). Our values approximate to those of Giordano, Spraragen and Hamel (1970) (183 ± 25 mg/100 ml) in one group of animals.

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