Spontaneous Aortic Lesions in Rabbits
II. Relationship to Experimental Atherosclerosis

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ABSTRACT
Lesions in the rabbit aorta that occur spontaneously and contain no lipid consist of intimal mesenchymal proliferation and of medial sclerosis. Although the relationship of diet-induced atheroma to the intimal mesenchymal alterations is still not clear, our experiments have established that (a) the morphologic characteristics of diet-induced atheroma and of the spontaneous medial lesions are dissimilar at all stages of their development, and (b) the presence of spontaneous medial lesions clearly influences the development of atheroma. This influence is related to (a) an increased tendency for atheroma formation at the raised proximal and lateral borders of nodular medial lesions projecting from the luminal surface, (b) the absence of atheroma formation at sites of medial calcification, and (c) a propensity for lipid deposition in areas of acid mucopolysaccharide accumulation within spontaneous medial lesions.

ADDITIONAL KEY WORDS lipid deposition medial calcification
medial mucopolysaccharide diet-induced atheroma

Spontaneously occurring lesions of both the intima and media of the rabbit aorta have been described recently in detail. Since the rabbit is highly susceptible to dietary induced atherosclerosis, this animal has been used extensively as an experimental model to study various aspects of atherogenesis. The interpretation of morphologic data derived from such experiments depends, of course, upon a recognition of the nature and types of spontaneously occurring alterations. The present study was undertaken for the purpose of defining whatever relationships might exist between spontaneous aortic lesions and those induced by dietary means.

Methods
Forty-five New Zealand White (NZW) and 28 Dutch rabbits approximately 4 to 6 months old, were fed 2% cholesterol in addition to their regular diet* for 2 to 16 weeks. Animals of each breed were autopsied at 2-week intervals during this period. Six NZW rabbits were autopsied 8 weeks after the cessation of the cholesterol feeding. Complete autopsies were performed on all animals. The aorta was dissected free, washed in saline and opened longitudinally anteriorly. The entire intimal surface was examined under the dissecting microscope. All aortas were stained in oil red O and the intimal surface was re-examined under a dissecting microscope. The ascending aorta and arch were always sectioned at approximately 5-mm intervals and adjacent areas were prepared for paraffin and frozen sections. The descending and abdominal aorta were sectioned at approximately 1-cm intervals and prepared similarly. Frozen sections were stained with oil red O for lipid. Paraffin sections were stained with hematoxylin and eosin, Gomori-trichrome (for connective tissue), Verhoeff (for elastic tissue), PAS and alcian blue (for mucopolysaccharides) and von Kossa (for calcium).

Results
GROSS APPEARANCE
Focal yellow plaques, which stained red with oil red O, were present on the aortic


Circulation Research, Vol. XIX. July 1966
luminal surface in all of the rabbits after 4 weeks of cholesterol feeding. These were most prominent at the orifices of the branches arising from the arch and from the descending thoracic aorta. Cholesterol feeding for 8 to 16 weeks resulted in confluence of the focal plaques in the entire length of the aorta. At 16 weeks some, and at 24 weeks many, of the confluent plaques were grey rather than yellow and stained pink rather than bright red with oil red O.

In addition to the atheromatous plaques, spontaneous nodular type A lesions of the media were apparent in 32% of the NZW and 14% of the Dutch rabbits after two weeks and four weeks of cholesterol feeding. After eight weeks as the atheromatous plaques became larger and confluent it became increasingly difficult to define spontaneous lesions by gross examination. The distribution and gross characteristics of the spontaneous lesions were similar to those described previously. The spontaneous lesions were most prominent in the ascending thoracic aorta, a site where lipid plaques became prominent and confluent only after two or more months of cholesterol feeding. No spontaneous type B lesions of the media were encountered in any of the rabbits.

**MICROSCOPIC APPEARANCE**

Lipid deposition in the aorta occurred (a) in the form of atheroma and (b) within certain areas of the spontaneous medial lesions.

(a) Typical atheromatous plaques characterized by intimal accumulation of lipid-filled foam cells and lipid droplet accumulation in the subjacent media were present after two weeks, and well developed after four weeks of cholesterol feeding. No clear cut predilection toward atheroma formation was found at sites of spontaneous mesenchymal intimal thickening. At the end of the two-week cholesterol feeding interval, lipid accumulation was seen occasionally at sites of intimal mesenchymal thickening, but many of the early lipid deposits occurred in areas where there was no apparent underlying intimal alteration.

After 8 weeks of cholesterol feeding, the plaques showed evidence of connective tissue proliferation. At 16 weeks connective tissue was prominent both at the base of the plaques and beneath the endothelium. Two months after the cessation of cholesterol feeding the plaques contained, in addition to lipid, prominent connective tissue and some smooth muscle cells at the base and in the subendothelial region. A granular calcium deposition was seen occasionally at the base of the plaques. Both early predominantly lipid and older mixed lipid-fibrous plaques involved the media, but only minimally. Elastic tissue stains demonstrated, in every section examined, that the plaques remained superficial to and clearly and sharply demarcated from the media. Medial involvement, at most, consisted of extension of the plaque to the level of the third or fourth elastic lamina. The elastic laminae in these areas became separated and fragmented, but never calcified.

(b) Spontaneous type A medial lesions showed varying degrees of calcification, mucopolysaccharide accumulation, and cellular reaction. Early atheroma formation was seen frequently at the raised proximal and lateral margins of the spontaneous plaques (fig. 1). In addition to intimal foam cell accumulation, there was a prominent deposition of lipid as early as two weeks after the initiation of cholesterol feeding, within the medial lesions in areas of mesenchymal cell proliferation and acid mucopolysaccharide (MPS) accumulation (figs. 2, 4, 5). Atheromatous plaques were present at the base of the plaques, and some smooth muscle cells were present in the subendothelial region. A granular calcium deposition was seen occasionally at the base of the plaques. Both early predominantly lipid and older mixed lipid-fibrous plaques involved the media, but only minimally. Elastic tissue stains demonstrated, in every section examined, that the plaques remained superficial to and clearly and sharply demarcated from the media. Medial involvement, at most, consisted of extension of the plaque to the level of the third or fourth elastic lamina. The elastic laminae in these areas became separated and fragmented, but never calcified.

**FIGURE 1**

Aorta after cholesterol feeding, two weeks. A fatty plaque has developed at the raised margin of the spontaneous nodule. Oil red O, hematoxylin.

**FIGURE 2**

Cholesterol feeding, two weeks. There is lipid deposition in a noncalcified, acid mucopolysaccharide (MPS) containing portion of a spontaneous nodule in the aorta (see fig. 5). Oil red O, hematoxylin.

**FIGURE 3**

Cholesterol feeding, eight weeks. A calcified portion (dark blue staining) of a spontaneous aortic lesion still shows an inhibitory effect toward atheroma formation. Oil red O, hematoxylin.

*Circulation Research, Vol. XIX, July 1966*
FIGURE 4
Cholesterol feeding, sixteen weeks. Confluence of adjacent aortic atheroma has completely covered the spontaneous lesion, including the calcified portions. There is lipid deposition in the media within a non-calcified area of the spontaneous lesion. Oil red O, hematoxylin.

FIGURE 5
Spontaneous medial "type A" lesions in aorta. Acid mucopolysaccharide (MPS) stained with alcian blue is present between elastic lamellae. Areas of acid MPS accumulation in the spontaneous lesions appear to favor lipid deposition (see fig. 2).
plaques were never seen overlying areas of medial calcification, nor was there lipid deposition within these (figs. 1 and 2). After four and eight weeks of cholesterol feeding, areas of medial calcification still showed slight to no overlying atheromatous plaques (fig. 3). After twelve weeks, as the atheroma became larger and confluent, underlying medial lesions gradually began to be completely covered (fig. 4). In these, and in rabbits examined two months after the cessation of cholesterol feeding, combinations of intimal lipid plaques and areas of medial sclerosis were still distinguished easily from each other. The pure lipid and lipid-fibrous plaques remained superficial to the media and well demarcated from the medial lesions (figs. 6 to 9).

Discussion

Spontaneously occurring, non-lipid-containing alterations in the rabbit aorta include intimal mesenchymal proliferation, and two types of medial sclerosis. The present experiment was done for the purpose of evaluating the relationship of dietary-induced atheroma to these spontaneous changes. Of the medial sclerotic lesions, only type A was encountered. Type B lesions which involve the outer one-half of the media are apparently encountered only rarely in rabbits as young as the ones used in the present experiment.
SPONTANEOUS AORTIC LESIONS: RELATION TO ATHEROSCLEROSIS

No qualitative differences were found in either the NZW or Dutch rabbits.

Our experiments did not provide clear-cut evidence either for or against a predilection of atheroma formation at sites of pre-existing intimal mesenchymal thickening. The earliest dietary induced lesions seen after two weeks of cholesterol feeding consisted of lipid droplets in endothelial cells and in subjacent smooth muscle cells. The lipid-containing smooth muscle cells were situated usually in the media, but occasionally between the endothelium and innermost elastic lamina. There was obvious fine lipid droplet deposition in endothelial cells which rested directly on the inner elastic lamina. In all sites where even a small atheromatous plaque had developed, due to subendothelial accumulation of lipid-filled cells, it was no longer possible to evaluate whether or not these cells represented pre-existing intimal mesenchymal cells or an infiltrate. The only valid conclusion which we can make from our data at the present time is that lipid deposition in endothelium of the aorta can occur in areas where there is no apparent intimal mesenchymal thickening.

The existence of spontaneous medial lesions in the rabbit aorta has been known for many years and both Clarkson and Duff have emphasized that when these are present in rabbits subjected to cholesterol feeding, they can still be recognized as distinctly different from the lesions resulting from the feeding. Our findings agree completely with this. At no stage in the evolution of the atheroma from fatty plaque to fatty-fibrocalcific nodule, does its morphologic appearance ever resemble the spontaneous medial sclerotic lesion. Perhaps the most obvious differential features are (a) the superficial nature of the atheroma, which even when fibrosed never involves the media deeper than to the depth of the third or fourth elastic lamina, and (b) elastic lamina calcification, a prominent feature of the spontaneous lesion, is never seen in the atheroma. There was a conspicuous lack of any prominent involvement of the media by the cholesterol induced atheroma, either early or eight weeks after the cessation of cholesterol feeding. Although lipid deposition between elastic lamina in the upper media was a frequent finding, areas of medial alterations described by others were present only where an atheroma overlay a distinct spontaneous lesion.

While the two types of aortic lesions, dietary induced atheroma and spontaneous medial sclerosis, are morphologically distinct and most certainly unrelated pathogenically, the existence of the latter clearly influences the development of the former. Atheromata frequently develop at the raised borders of nodular medial lesions projecting from the luminal surface. The lipid accumulation is most prominent at the proximal and lateral margins of the nodular excrescences and this localization is consistent with present concepts of the role of hemodynamic factors in atheroma formation. In addition to these

FIGURE 9
Eight weeks cholesterol feeding. An aortic atheroma superimposed on a spontaneous medial lesion is distinct and separate from the media, although some lipid deposition in the media may occur in the spontaneous medial lesion (figs. 2 and 4).
sites, lipid deposition occurs early and prominently in areas of acid MPS accumulation or "lakes" and mesenchymal cell proliferation within nodular medial lesions. The relationship of acid MPS and lipid deposition has been and continues to be controversial. Abnormal intimal and medial acid MPS accumulation as a consequence of cholesterol feeding and as an event preceding lipid deposition, as well as an affinity of lipid for such MPS deposits, has been proposed as a mechanism of atheroma formation. On the other hand, Straus and Roberts in a recent review conclude that changes in acid MPS are incidental, secondary, and not causal to lipid deposition. In our experimental rabbits, lipid deposition at sites of MPS accumulation was seen only within spontaneous medial lesions. We did not observe any alteration in the ground substance preceding lipid deposition at other sites in the aorta. On the basis of these observations, we might suggest that proponents of the MPS concept of atherogenesis may possibly have misinterpreted the deposition of lipids in spontaneous medial lesions for the sequential induction of MPS accumulation followed by lipid deposition.

The increased tendency for lipid deposition at sites in the aorta where structural alterations have been produced traumatically is well known. In the present experiment, lipid deposition within and atheroma formation on the surface of spontaneous non-calcified plaques were prominent as early as two weeks after cholesterol feeding. It is apparent, therefore, that the increased tendency for lipid deposition at such sites is a process that depends upon alterations of the ground substance and connective tissue, rather than upon the etiologic factors which produced such alterations.

Areas of calcification within nodular medial lesions showed no lipid deposition at all. Lipid accumulation was not seen within the endothelium, intima or media in these areas even after prolonged cholesterol feeding. Calcified areas were gradually covered by atheroma but this occurred most probably as a result of confluence of adjacent plaques. The absence of lipid deposition in areas of medial calcification in rabbit aortas has been described by Hass et al. These workers produced, by means of hypervitaminosis D, calcific-degenerative medial lesions which are morphologically indistinguishable from the spontaneous ones. Of especial interest is the observation of Hass that areas of "active mesenchymal reaction" (which very probably contained increased acid MPS) were conducive to lipid deposition. We have studied the arterial lesions in the hypervitaminosis D-hypercholesterolemic dog and have been impressed by the similarity of at least some of the medial calcific and mesenchymal reactions to those which occur spontaneously in the rabbit. In the dog also, areas of medial calcification show little to no tendency for lipid deposition.

The findings presented and discussed in the present paper emphasize that investigators using the rabbit as an experimental model must be aware of the existence of spontaneously occurring aortic lesions and their relationship to dietary induced atheroma. Some of the data that have accumulated, and continue to accumulate in the literature, regarding the evolution and fate of the atheromatous plaque in the rabbit are, unfortunately, not based on such an awareness. Re-evaluations are needed especially with respect to (a) the experimental procedures designed to produce in the rabbit aorta, by so-called "chemical means," alterations resembling human atherosclerosis and (b) data pertaining to the nature of aortic medial involvement in the development and regression of the atheromatous plaque.

References

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