Spontaneous Aortic Lesions in Rabbits
I. Morphologic Characteristics

By Eric A. Schenk, M.D., Elizabeth Gaman, and A. S. Feigenbaum, Ph.D.

ABSTRACT
A number of morphologically distinct, spontaneously occurring lesions are present in the rabbit aorta. Intimal atheromata occur in newborn rabbits, decrease in incidence as the rabbit becomes weaned, and are a rarity in the adult. Intimal mesenchymal thickening is first seen in weanling rabbits and is almost universally present in the adult. Medial sclerotic changes involving exclusively the inner one-half of the media and termed "type A" lesions are present in weanling and adult, but not in newborn rabbits.

Type A lesions have a variable gross appearance, but a fairly uniform microscopic appearance, which shows principally fragmentation and calcification of elastic lamina and accumulation of acid mucopolysaccharides.

"Type B" lesions, medial sclerosis involving exclusively the outer half of the media, usually occur throughout the entire aorta, and only in adult rabbits.

ADDITIONAL KEY WORDS
lipid deposition aortic intimal lesions spontaneous regression of atherosclerosis aortic calcification intimal smooth muscle proliferation aortic mucopolysaccharide deposition newborn and adult rabbits

Various types of spontaneously occurring lesions in rabbit aortas have been described during the past 80 years. However, there still exists lack of knowledge as to their etiology, and perhaps more important, confusion as to their morphologic characteristics. Since the rabbit has been and continues to be widely used in experimental vascular research, an appreciation of these lesions is mandatory for a meaningful interpretation of experimentally produced alterations. During the past six months alone a number of articles have appeared in the literature dealing with aortic lesions in rabbits produced by various experimental techniques, all of which illustrate typical spontaneous lesions. The term "spontaneous" is used by us to refer to those lesions that occur in the aortas of healthy rabbits that have not received any special experimental diets, drugs or undue stresses.

The purpose of the present study was to define the morphologic characteristics of these spontaneous lesions in two breeds of rabbits at various ages.

Methods
Forty-one New Zealand White (NZW) and 25 Dutch, 1- and 3-day-old newborn, 21 NZW and 16 Dutch 3-week-old, and 21 NZW and 27 Dutch 5-week-old weanlings, and 210 NZW and 44 Dutch adult rabbits older than six months, were used. Approximately one-half of the adult NZW aortas were obtained from the Pel-Freez Corporation.* All of the newborn, weanling, and adult Dutch, and the remainder of the adult NZW aortas came from rabbits housed in our animal facility in Rochester and obtained from commercial breeders. All received rabbit pellets† and water ad libitum. None was used for any other experimental purposes. Complete autopsies were performed on all of these animals. The entire

*Pel-Freez, Rogers, Arkansas.
aorta was dissected free, washed in saline and opened longitudinally anteriorly. The entire intimal surface was examined under a dissecting microscope. All aortas were stained in oil red O and the intimal surface was re-examined under a dissecting microscope. All lesions or abnormal appearing areas were prepared for both frozen and paraffin sections. 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), and PAS-alcian blue (for mucopolysaccharides), von Kossa (for calcium), Gomori methenamine silver (for fungi), Lisa (for bacteria), and PTAH (for myofibrils). All aortas, whether or not they showed gross lesions, were sectioned circumferentially at approximately 5 mm intervals, and adjacent areas prepared for frozen and paraffin sections as described above. A minimum of 20 sections from each aorta was examined microscopically. Six each of the newborn and 5-week weanling NZW and 18 of the adult NZW aortas were examined by means of Hautchen preparations, 7 and silver nitrate and phosphotungstic acid-hematoxylin (PTAH) were used, in addition to the above described stains.

Results

NEWBORN RABBITS

Twenty-two per cent of the NZW and 16% of the Dutch showed scattered foci of intimal lipid accumulation in both the thoracic and abdominal aorta. These were not apparent under the dissecting microscope even after the entire aorta was stained with oil red O. Microscopically there was a slightly elevated plaque-like structure composed of intimal lipid-containing foam cells, and less prominent, fine lipid droplet accumulation in subjacent smooth muscle cells (fig. 1). Occasionally, lipid droplets could be identified within endothelial cells. These early lipid accumulations were seen in one-day-old rabbits, all of which had begun to nurse as evidenced by the presence of curdled milk in the stomach.

No medial lesions were found in any of the newborn rabbits.

WEANLING RABBITS

Only 10% of the NZW and 6% of the Dutch 3-week-old and 4% of the NZW and 3% of the Dutch 5-week-old rabbits had fatty intimal plaques. Areas of intimal thickening due to accumulation of smooth muscle cells and undifferentiated mesenchymal cells between the endothelium and the internal elastic lamina were found in 24% of the NZW and 25% of the Dutch 3-week-old and in 74% of the NZW and 72% of the Dutch 5-week-old weanlings. These areas contained no stainable lipid, and could be identified only on microscopic section. The smooth muscle cells were identified on the basis of PTAH staining myofilaments and typical elongated blunted nuclei. Cells with similar nuclei but no myofilaments were arbitrarily considered to be undifferentiated mesenchymal cells (fig. 2).

Five per cent of the 3-week-old NZW and 6% of the 3-week-old Dutch showed grossly apparent nodular elevations on the luminal surface. These were found exclusively in the ascending thoracic aorta and in the area of the arch of the aorta, and were oil red O negative. On microscopic section they appeared as focal accumulations of predominantly acid mucopolysaccharide between elastic lamellae of the inner one-third to one-half of the media (figs. 4 and 5). There was no subintimal proliferation or foam cell accumulation and no stainable lipid was present. At five weeks, 13% of the NZW and 8% of the Dutch showed similar but more severe lesions in the media of the ascending aorta and arch. At this time, elastic lamina fragmentation was prominent and there were interlamellar lakes of acid mucopolysaccharide (MPS). Focal calcification of elastic tissue was now apparent. Stainable lipid was again absent from these lesions. There was no apparent relationship to the less frequently found intimal lipid plaques, nor was there any transitional state between these two noted.

ADULT RABBITS

Mesenchymal intimal thickening due principally to smooth muscle cell accumulation between the endothelium and inner elastic lamina was present in 84% of the NZW and 81% of the Dutch. Häutchen preparations illustrated the circumferential orientation of these smooth muscle cells at right angles to the endothelial cells (fig. 3). The smooth muscle cells were embedded in a loose network of elastic fibers which differed in ap-
pearance from the more dense inner elastic lamina.

Typical intimal lipid plaques were found in only one adult NZW. There were no findings at autopsy to explain why this lone animal had fatty plaques.

Nodular medial sclerotic lesions were found in 40% of the NZW and 18% of the Dutch rabbits. On the basis of their gross appearance, six types of lesions have been identified (fig. 13). Frequently, two or three different types were found in a single aorta. Type 1 is a longitudinally striated lesion with the striations limited to a discrete area or in the form of long longitudinal furrows. Type 2 is a raised, hard, flat plaque. Type 3 is a raised, flat plaque with a variably sized central depression which gives an umbilicated appearance. Type 4 consists of numerous adjacent, small, round, hard nodules. Type 5 is a composite of the first three types; namely, raised, flat and umbilicated plaques admixed with longitudinal striations or furrows. Type 6 lesions consist of deep, circumferential, hard furrows associated with an extensively brittle aortic wall, having an "egg shell" consistency. Type 1 to 5 lesions were confined principally to the ascending aorta, arch and upper descending aorta. The type 6 lesion invariably involved the entire length of the aorta.

Microscopically, type 1 to 5 lesions all showed minor variations of a similar basic morphologic process. To simplify classification and evaluation of their relative incidence, the type 1 to 5 lesions have been designated as types A1, A2, A3, A4 and A5. These lesions were always confined to the inner one-half to one-third of the media (fig. 6). There was always MPS accumulation, either between elastic lamellae or in the form of "lakes" where elastic lamellae had been disrupted. Elastic lamina calcification was frequently but not invariably present. In the umbilicated lesions, the central depressed area consisted of compressed, usually calcified, elastic lamellae with little or no remaining inter-lamellar tissue. An inflammatory reaction to these changes was quite variable in extent and location.

When present, this reaction consisted of an accumulation of histiocytes, plasma cells, lymphocytes, and occasionally giant cells. Lipid was not present in any of the lesions. Special stains for bacteria and fungi showed no organisms (figs. 7 to 11).

The type 6 lesion microscopically was seen to involve principally the outer one-half of the media, the inner one-half being intact and unremarkable. These lesions have been designated as type B. There was massive calcification of the media (fig. 12). An inflammatory reaction was only rarely present. Thirty per cent of these lesions had focal nodular areas of cartilaginous metaplasia. In four rabbits with this lesion there were associated type 1 to 5 lesions in the inner one-half of the media.
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Circulation Research, Vol. XIX, July 1966
Discussion

Our findings demonstrate a number of morphologically distinct and probably pathogenetically unrelated lesions in normal rabbit aortas. Their incidence is listed in Table 1. Intimal lesions in the newborn and weanling rabbits consist of small typical atheroma. Spontaneously occurring atheroma were found with decreasing frequency in weanlings and were a rarity in adults. In the weanlings and adults, intimal alterations consisting of smooth muscle and elastic tissue proliferation were seen. Since the incidence of the intimal lipid lesions decreases as that of the mesenchymal proliferation increases, it is tempting to speculate that the mesenchymal reaction evolves from and is the residuum of the fatty plaque. Thus far, however, we have not been able to identify transition forms between these two lesions. The much higher incidence of the adult mesenchymal intimal changes, as compared with the incidence of atheroma in newborns, also argues against any such relationship. It is of course possible that our method of analysis of the aortas missed atheromatous lesions in the newborn animals and

FIGURE 13
Gross appearance of spontaneous aortic medial lesions (see text for description).

FIGURE 7
An inflammatory cell infiltrate is sometimes present. Hematoxylin and eosin.

FIGURE 8
Von Kossa stain shows calcification of elastic lamina.

FIGURE 9
Alcian blue stain shows acid mucopolysaccharide accumulation between the elastic fibers in a non-calcified area.

FIGURE 10
Verhoeff stain shows the marked disruption and fragmentation of elastic laminae.

FIGURE 11
Oil red O stain shows the characteristic and invariable absence of lipid from these lesions.

FIGURE 12
Adult rabbit. Type B medial lesion. Extensive calcification of aorta is present in the outer one-half of the media.
TABLE 1

Types and Incidence of Spontaneous Aortic Lesions in Rabbits

<table>
<thead>
<tr>
<th>Age of rabbits and lesions</th>
<th>New Zealand White</th>
<th>Dutch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn (1 to 3 days old)</td>
<td>(41)*</td>
<td>(25)</td>
</tr>
<tr>
<td>Intimal: atheromatous plaques</td>
<td>22%</td>
<td>16%</td>
</tr>
<tr>
<td>Medial</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Weanling</td>
<td>(21)</td>
<td>(16)</td>
</tr>
<tr>
<td>Intimal: atheromatous plaques</td>
<td>10%</td>
<td>6%</td>
</tr>
<tr>
<td>mesenchymal thickening</td>
<td>24%</td>
<td>25%</td>
</tr>
<tr>
<td>Medial: nodular</td>
<td>5%</td>
<td>6%</td>
</tr>
<tr>
<td>5 weeks old</td>
<td>(47)</td>
<td>(27)</td>
</tr>
<tr>
<td>Intimal: atheromatous plaques</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>mesenchymal thickening</td>
<td>74%</td>
<td>72%</td>
</tr>
<tr>
<td>Medial: nodular sclerotic</td>
<td>13%</td>
<td>8%</td>
</tr>
<tr>
<td>(type A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>(210)</td>
<td>(44)</td>
</tr>
<tr>
<td>Intimal: atheromatous plaques</td>
<td>0.5%</td>
<td>0%</td>
</tr>
<tr>
<td>mesenchymal thickening</td>
<td>84%</td>
<td>81%</td>
</tr>
<tr>
<td>Medial: nodular sclerotic</td>
<td>40%</td>
<td>18%</td>
</tr>
<tr>
<td>types A 1-5</td>
<td>37%</td>
<td>16%</td>
</tr>
<tr>
<td>type B</td>
<td>3%</td>
<td>2%</td>
</tr>
</tbody>
</table>

*Figures in parentheses indicate the number of rabbit aortas examined.

weanlings, and that serial sectioning of the entire aorta would show a higher incidence of these small and focal lesions. Solowjew⁸ found lipid in the intima in 6 of 10 35- to 48-day-old suckling rabbits. Bragdon⁹ reported lipid in the intima of 10 of 12 suckling rabbits. This was absent in fetuses and in rabbits 12 hours old which had not yet begun to suckle. The degree of lipid deposition correlated well with an increased plasma lipid concentration found in suckling rabbits. Both Solowjew⁸ and Bragdon⁹ found that the lipid deposited during the suckling period tends to disappear in the weeks following weaning. Neither of these authors mentioned residual intimal alterations following regression of the lipid deposits in weaned rabbits. An examination of the ultrastructure of the aorta of normal adult rabbits by Bierring and Kobayasi¹⁰ elegantly demonstrates a subendothelial layer of "appreciable width" and consisting of branching elastic fibers, sparse collagen fibers and spindle shaped cells resembling smooth muscle cells, embedded in a homogenous ground substance. These workers suggest that "degenerative transformation" of the inner tunica media associated with aging is the origin of this alteration. Our data do not clarify the problem as to whether these changes are lesions (i.e., pathologic) or an expression of normal growth and aging.

Spontaneous medial lesions have not been found in the newborn, but are apparent in 3-week-old, and well developed in 5-week-old weanlings. A clear progression from early acid MPS accumulation and elastic lamina separation and fragmentation to MPS lake formation, elastic tissue calcification and fibrosis, is obvious as the rabbit ages. The gross appearance of these lesions is highly variable but five more or less distinct types are recognizable. Microscopically, this differentiation of gross appearance is seen to depend more on the degree of calcification and inflammatory cell reaction than on different pathologic processes. The exclusive localization in the inner one-half of the media predominantly in the ascending aorta and arch, prominent MPS deposition, occurrence in weanling rabbits, and high incidence in adult rabbits, serve to distinguish this type of medial lesion which we have termed type A. A second type of medial sclerosis (type B) found much less frequently and only in adult rabbits, involves ex-
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Inclusively the outer one-half of usually the entire aorta. Cartilaginous metaplasia, which is rarely present in the type A lesion, is common in the type B one. Mucopolysaccharide accumulation has not been a feature of the type B lesion, although, even with the relatively large number of rabbit aortas we have thus far examined, we have seen only fully developed and calcified lesions of this type.

Spontaneously occurring medial lesions in rabbits have been described and summarized on a number of occasions since Israel's11 original description. The pathogenesis of these lesions is as yet unknown. A recent histochemical analysis by Kobernick et al.19 has failed to shed light on this problem.

The spontaneous occurrence of medial sclerotic lesions is not unique to the rabbit aorta. Similar changes, especially those of the type A lesion, have recently been described in the horse,20 sheep,21 pigeon,22 cat,23 cow,24 rat,25 swine,26 and various other animals.27-28 Medial sclerotic lesions in breeder rats25 bear a remarkable morphologic resemblance and similarity in distribution to those seen in the rabbit. It is difficult to see what common pathogenetic mechanism may exist in the weanling rabbit, female breeder rat, and the variety of species of other animals in which these types of lesions have been found. The major purpose of the present communication, however, is neither to review nor to speculate on pathogenesis, but rather to re-emphasize the occurrence and morphology of the types of lesions which have been presented and discussed. The importance of an awareness of these factors is self evident. In the span of six months, descriptions and illustrations of typical spontaneous medial lesions have been presented as evidence for the effects on aortic structure of glucosamine,1 lead,2 vasodilation,3 fibrinolysis inhibitors,4 copper deficiency,5 and phosphate, caseinate, or powdered milk.6 The list of previous publications implicating various chemicals, especially epinephrine, in the production of aortic medial sclerosis in rabbits is a lengthy one and will not be summarized here. It is obvious that future experiments of this nature will have to demonstrate either significant variations in the morphology of the lesions from those which occur spontaneously or a statistical evaluation of a change in incidence between experimental and control groups. Since the incidence of the spontaneous lesions appears to vary considerably among different breeds and perhaps according to geographic location (to be published) a careful selection and detailed documentation of the breeds and source of the rabbits used will be mandatory.

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ERIC A. SCHENK, ELIZABETH GAMAN and A. S. FEIGENBAUM

Circ Res. 1966;19:80-88
doi: 10.1161/01.RES.19.1.80

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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