THE EFFECT OF HYPERCHOLESTEROLEMIA UPON INTIMAL REPAIR OF THE AORTA OF THE RABBIT FOLLOWING EXPERIMENTAL TRAUMA *

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Relatively little information is available concerning the onset and pattern of development of atherosclerosis. In a previous study from this laboratory,[1] it was pointed out that aortic intimal fibro-elastic plaques are a constant finding in all children over 2 weeks of age. Although lipid deposition was not a prominent feature of these early lesions, sudanophilic droplets were demonstrated both intracellularly and extracellularly within the deeper portions of these plaques. A morphologically identical lesion has been described by Moon and Rinehart[2] within the coronary arteries of young individuals who died suddenly without preceding debilitating illness. It seems reasonable to assume that these intimal alterations may be the earliest phase in the development of the adult atherosclerotic plaque. We have never observed changes within these intimal thickenings indicating that they are undergoing "spontaneous regression."

The cellular composition of these plaques in children suggested to us that they might represent reparative activity following injury to the intima. This concept was of additional interest since most authorities concede that there is an element of local injury or damage within the intima associated with lipid precipitation as an initial stage in the pathogenesis of the human plaque. Pertinent also to this hypothesis is the recent re-emphasis of the concept that atherosclerotic plaques may be mural thrombi which have undergone organization and degenerative phenomena.[3-7] Accordingly, Prior and Hutter[8] traumatized the aortic intima of rabbits to study vascular injury and repair, and to contrast the changes observed in these mural thrombi with the fibro-elastic plaques of infants. A lesion grossly resembling an early plaque in the human was noted 35 days after intimal injury in the experimental animal; and this lesion was histologically identical with the fibro-elastic thickenings of children. Although lipid material and macrophages were never a prominent feature of this experimentally produced lesion, calcification was seen as early at 49 days after injury

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although its location and appearance differed from that observed in the human atherosclerotic lesion.

The present investigation was an attempt to study the effect of hypercholesterolemia upon segments of the previously traumatized rabbit aorta. Although there are points of similarity between human and cholesterol arteriosclerosis, Duff and McMillan[9] have pointed out that great caution should be exercised in transposing interpretations derived from studies of experimental cholesterol atherosclerosis into terms supposedly applicable to the human disease. The present experiment was designed with the hope that the combination of intimal trauma and hypercholesterolemia would produce lesions similar to the human process and add some knowledge of the pathogenesis of the atherosclerotic plaque.

Methods

Twenty-seven New Zealand white rabbits, approximately evenly divided as to sex, were used in this study. Their age at the beginning of the experiment was 8 to 10 weeks and their average weight was 2,200 gm. They were housed separately in wire cages and fed Purina rabbit chow supplemented with 1 gm. of cholesterol per day. The cholesterol was administered as the purified substance dissolved in ether, mixed with the food pellets, and allowed to evaporate. Blood cholesterol values at the beginning of the study ranged between 20 and 110 mg. per 100 cc. The cholesterol supplement was instituted on the same day that intimal trauma was carried out.

The animals were anesthetized with pentothal and ether, and the peritoneal cavity was incised under sterile precautions. The abdominal viscera were retracted from a segment of lumbar aorta and the latter was freed from the adjacent connective tissue with a minimum of trauma. The aorta was then pierced at an oblique angle with a 24-gauge needle, the point of which was deliberately angulated to provide a barbed instrument. The aortic intimal surface was then traumatized by repeated vertical and horizontal motions. The needle was withdrawn and hemostasis affected by means of direct pressure over the puncture site. Before closing the peritoneal cavity the traumatized area was marked by silk sutures placed within the peri-aortic connective tissue.

The animals were sacrificed at intervals ranging from 2 days to 8½ months following intimal injury. As will be pointed out later, it was necessary to sacrifice the animals at approximately 1 week intervals during the first 3 months of the experiment. The entire aorta was removed and areas at a distance from the traumatized segment were considered to serve as controls for cholesterol effect only. Blood cholesterol determinations were obtained on all animals at the time of

sacrifice. These ranged between 600 and 800 mg. per 100 cc., levels of 600 mg. being present as early as 14 days after commencement of the cholesterol feeding.

Pathologic Findings

The technique did not permit control of the depth of the trauma, so that the tissue injury varied from an intimal defect only to foci in which nearly the entire thickness of the vessel wall had been traumatized. In general, we were particularly interested in those areas in which the trauma had been more superficial, i.e., had involved the intima and inner media only.

In gross appearance, the injured intimal surface 2 days following trauma presented a pitted area with raised, swollen margins. Microscopically, wide disruption of the intimal and medial coats was present. The medial cellular elements in the involved areas showed nuclear variation and shrinkage and there was evidence of edema and fibrin deposition. A moderate number of polymorphonuclear leukocytes were noted within the adventitia. Fibroblastic activity was prominent at the margins of the lacerated intima and also in the adventitial coat. No new capillaries could be identified although there was some attempted endothelial bridging of the denuded intima. Frequently the lacerated vessel lining protruded into the lumen as a polypoid mass (Fig. 1). At 7 days the core of these polypoid areas still consisted of fibrin and cellular débris, although the surface was now completely endothelized (Fig. 2). Where depressions had been present in the vessel lining, the gaps were replaced by active fibroblastic tissue growing perpendicular to the long axis of the vessel and covered by a new endothelial lining. Broad zones of hyaline material persisted within the damaged media and tended to be surrounded by proliferating fibroblasts. A few new capillaries extended from the vasa vasorum into the inner media.

Lipophages were first observed 14 days following the initial injury (Fig. 3). They were concentrated about the junction of the intact media and newly formed fibrous cap. This cap was very cellular and the fibroblasts were oriented in a plane parallel to the long axis of the vessel. Weigert-van Gieson's staining showed that the intercellular material was composed of large amounts of elastic tissue and scant collagenous fibers (Fig. 4). In areas where the trauma had involved the adventitia, calcification of the necrotic débris was noted. The vasa vasorum were prominent and new endothelium-lined channels could be traced lumenward from these vessels. While the source of the lipophages cannot be stated categorically, it is our impression that they were altered wandering cells which apparently entered through the vasa vasorum, since their migration from the vessel lumen through

the dense avascular fibro-elastic cap is extremely unlikely. It is impossible to deny, however, that some may arise *de novo* through transformation from fibroblasts.

The location of the lipophages in the traumatized segment at this stage is in marked contrast to their position in the earliest spontaneous (hypercholesterolemia) or non-traumatized lesions noted in these animals. The earliest microscopic spontaneous lesions were seen at 27 days and consisted of a tightly packed row of lipophages located between the internal elastic membrane and the endothelium (Fig. 5). We were impressed by the similarity between the experimentally produced lesion at 14 days and moderately advanced human lesions observed in coronary arteries and aorta. This similarity is readily apparent by referring to MacCallum's[10] Figure 185 which is an illustration of a human coronary artery encroached upon by a thick, atherosclerotic plaque. Common to both are raised hillocks composed of dense fibro-elastic tissue, some destruction of the musculo-elastic layer, and collections of large lipophages in the basilar portion of the plaque.

At 27 days after injury, when the first spontaneous lesions which have been referred to, were appearing, the traumatic lesions were showing some hyaline change within the fibrous tissue cap and occasional plaques disclosed beginning necrosis within the subendothelial zone. The spontaneous lesions showed fibrosis and some necrosis at 36 days.

Forty-six days after trauma the aorta showed grossly such a number of lesions of the spontaneous type that it was sometimes difficult to identify the traumatized segment. The spontaneous plaques were oval and elongated in the long axis of the aorta. They were definitely raised above the intimal surface, light yellow, and showed some tendency for fusion of adjacent plaques (Fig. 6). The traumatized areas presented as transversely oriented, plateau-shaped elevations which had a darker yellow color than the spontaneous type (Fig. 7). Small polypoid excrescences frequently could be identified along the margin of the transverse ridges. There were, however, striking similarities between the microscopic appearance of lesions of the spontaneous and of the traumatic type at 46 days. The spontaneous variety appeared as a conchoidal intimal thickening which was very rich in lipophages (Fig. 8). The latter were most concentrated along the internal elastic membrane although they were generally diffuse. A few foci of pyknosis and karyorrhexis were present, but this was not impressive. The Weigert-van Gieson stain showed the intercellular material to be devoid of elastic or collagenous elements. The internal elastic membrane was intact and no evidence of medial disease was

noted. The traumatically induced lesions were larger, flatter, and had a much thicker intima than the spontaneous variety (Fig. 9). The lipophages were fewer and were also concentrated in the basal portion of the plaque. Necrosis was prominent throughout the plaque and extended medialward so that no well defined internal elastic membrane could be identified.

This process continued until at 97 days after injury it was no longer possible to separate the advanced spontaneous lesions from those traumatically induced (Figs. 10 and 11). Both were composed chiefly of acellular necrotic débris in which some lipophages could still be identified. We were not impressed with either reparative connective tissue or significant dystropic calcification within the spontaneous or traumatic lesions over long periods, i.e., up to 8½ months after trauma. The animals which were maintained on oral cholesterol over this long period tended to show minimal calcification of the necrotic fatty material which was more noticeable in lesions of the traumatic type.

Discussion

The sequence of events following cholesterol feeding in the rabbit and resulting in the experimental atherosclerotic plaque has been studied carefully.[11,12] In young rabbits fed 1 gm. of cholesterol daily in Purina rabbit chow, the earliest lesions were foam cells and intercellular lipids located between the internal elastic membranes and the endothelium. These early lesions were arranged parallel with the vessel axis but increased in width until they extended completely around the vessel lumen. Between the foam-laden cells were fine strands of fibroglia, elastic fibrils, and conspicuous ground substance. The foam cells in the deep portion became necrotic and left the fatty material free in the finely granular débris. Fibroblasts proliferated extensively in the deeper layers of the intimal thickening and there was an increase in fibroglia and elastic fibers. The pool of free fatty substance became calcified and calcification might extend to involve the underlying media.

Duff[11] described focal necrosis of muscle fibers in the media before any intimal changes were observed. Medial damage of the more severe degrees always had associated a more or less marked change in the overlying intima. With progression of the medial lesions there occurred destruction of muscle and elastic tissue and an increasing infiltration of lipids. The internal elastic membrane eventually underwent complete disintegration and the inner layer of the media, packed with fat-laden foam cells, became almost indistinguishable from the intima.

While most of our observations have been concentrated upon rab-

bits fed cholesterol over a period of 3 months, fibroblastic activity seemed minimal in the spontaneous lesions throughout this period. There was, however, active fibroblastic proliferation in the traumatized segments reaching a maximum at 14 days. This lesion, which so closely resembled the human atherosclerotic plaque, showed prominent necrosis 27 days after injury. From this time on the traumatic lesion was characterized by progressively increasing necrosis, until 46 days after injury it was difficult to separate the traumatic from the spontaneous lesions on a histologic basis. Traumatic lesions alone, however, in animals maintained on a cholesterol-free diet, showed no inhibition of connective tissue repair and a dense fibro-elastic plaque was produced in 35 days after injury.[8] Degenerative changes, other than rare calcific deposition, were unusual in these intimal thickenings and lipid deposition was never a prominent feature, minute amounts being occasionally seen extra-cellularly.

A review of the mechanically induced vascular traumatic lesion has been presented in a previous publication.[8] Pertinent to the present investigation is a brief mention of the results of traumatic procedures carried out in the experimental animal maintained on a high cholesterol diet. The present study has confirmed the observation that traumatized areas accumulate lipid at a much faster rate than those not traumatized, the earliest spontaneous lesions being noted at 27 days while those of the traumatic variety were rich in foam cells 14 days after the injury.

Ssolowjew traumatized the rabbit aorta by adventitial cauterization[13] and also studied changes in the carotid artery which had been subjected to an exteriorization procedure.[14] It is of interest that in the former experiment abundant lipid deposition in the injured media was observed after 14 days, when cauterization was performed coincidentally with the commencement of cholesterol feeding. Extracellular and intracellular fat deposition was noted in the damaged media of the exteriorized vessel while the adjacent normal vessel was free of fat. Displacement of the carotid artery into a skin bridge produced transverse lacerations of the elastic lamellae. Where tears of the elastic tissue occurred, there was increased permeability to substances present in the blood (trypan blue). After some time, however, the local increased permeability was counteracted by reparative processes. The lipid substances circulating in the blood after induction of lipidemia invaded the arterial wall exactly like the stain and accumulated wherever its permeability had been increased (where the elastica was torn). During the later stages of the reparative process (after intimal thickening had developed), no further lipids were deposited in sites originally occupied by tears in the elastic lamellae.

Kelly *et al.*[15] studied the combination of induced intimal proliferation secondary to medial degeneration and localized lipid deposition from prolonged hypercholesterolemia in both old and young rabbits. The daily cholesterol intake in these animals was 0.06 gm. per lb. of body weight. After 22 weeks on the diet, localized lesions in the abdominal aorta and renal artery were produced by the freezing procedure of Taylor *et al.*[16] Animals in the hypercholesterolemic series were sacrificed at intervals of 2 to 52 weeks after freezing. The frozen areas underwent the same sequence of medial degeneration and fibroblastic intimal regeneration as described by Taylor in animals not fed cholesterol. The lesions were predisposed to lipid accumulation and with this there was interference with intimal regeneration. The localization of lipids was conspicuous in the degenerated media of the renal artery but not in the aorta. The importance of an intact internal elastic membrane in preventing lipid infiltration of the degenerated media was apparent.

Harrison's[17] experimental arterial disease produced by cholesterol and vitamin D is pertinent to this discussion. He carried out two experimental procedures: in the first, cholesterol sclerosis was induced in the rabbit aorta as a preliminary lesion and vitamin D sclerosis was later superimposed; in the second, vitamin D sclerosis constituted the preliminary lesion and cholesterol was then added. In both cases the second lesion was confined to the parts of the vessel left unaffected by the first lesion. Both cholesterol sclerosis and vitamin D sclerosis render the affected parts of the vessel wall relatively immobile and this immobility appears to make them less susceptible to the subsequent lesion. He concluded that the movements of the vessel determine the localization of both vitamin D and cholesterol lesions, and probably the human disease.

Summary

The reaction to a form of mechanically induced trauma to the intimal surface of the rabbit aorta in the presence of hypercholesterolemia has been described. The injured segment showed increased permeability to cholesterol and 14 days after trauma a plaque composed of lipid material and dense connective tissue was produced. This lesion was histologically identical with early human atherosclerotic plaques as seen in the coronary arteries and aorta. This experimentally produced lesion subsequently was characterized by necrosis and inhibition of connective tissue so that 46 days after injury the spontaneous and traumatically induced plaques were structurally similar. This inhibitory effect of cholesterol upon connective tissue repair may in part explain why the histologic counterpart of the advanced human atherosclerotic lesion cannot be duplicated experimentally by cho-

lesterol administration. A brief review of the results of other combinations of vascular trauma and hypercholesterolemia has been presented.

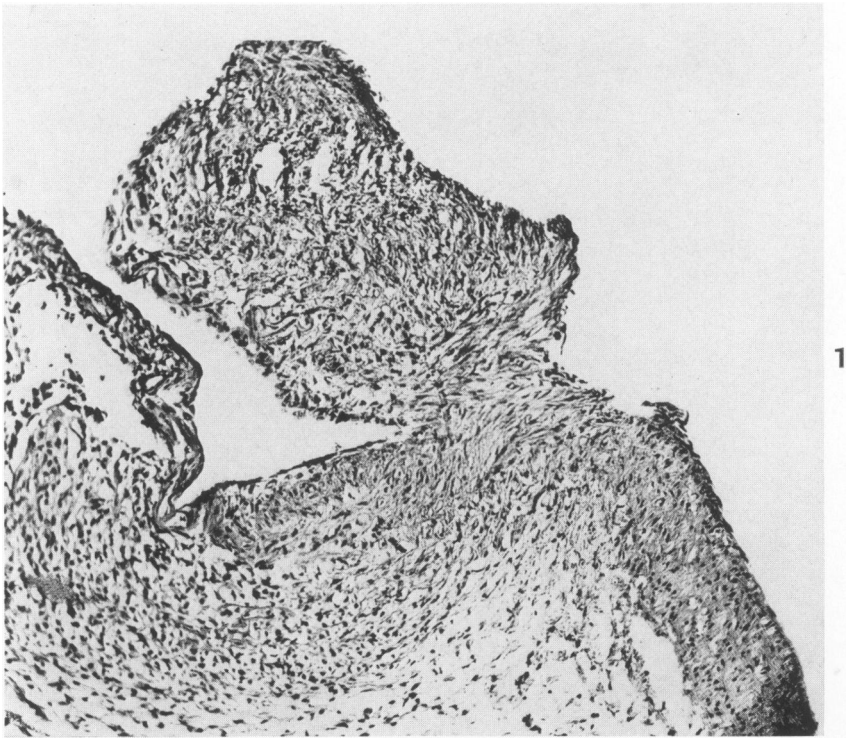
REFERENCES

1. Prior, J. T., and Jones, D. B. Structural alterations of the aortic intima in infancy and childhood. *Am. J. Path.*, 1952, **28**, 937–951.
2. Moon, H. D., and Rinehart, J. F. Histogenesis of coronary arteriosclerosis. *Circulation*, 1952, **6**, 481–488.
3. Duguid, J. B. Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. *J. Path. & Bact.*, 1946, **58**, 207–212.
4. Duguid, J. B. Thrombosis as a factor in the pathogenesis of aortic atherosclerosis. *J. Path. & Bact.*, 1948, **60**, 57–61.
5. Duguid, J. B. Pathogenesis of atherosclerosis. *Lancet*, 1949, **2**, 925–927.
6. Duguid, J. B. The arterial lining. *Lancet*, 1952, **2**, 207–208.
7. Crawford, T., and Levene, C. I. The incorporation of fibrin in the aortic intima. *J. Path. & Bact.*, 1952, **64**, 523–528.
8. Prior, J. T., and Hutter, R. V. P. Intimal repair of the aorta of the rabbit following experimental trauma. *Am. J. Path.*, 1955, **31**, 107–123.
9. Duff, G. L., and McMillan, G. C. Pathology of atherosclerosis. *Am. J. Med.*, 1951, **11**, 92–108.
10. MacCallum, W. G. A Text-book of Pathology. W. B. Saunders Co., Philadelphia, 1936, ed. 6, p. 327.
11. Duff, G. L. Experimental cholesterol arteriosclerosis and its relationship to human arteriosclerosis. *Arch. Path.*, 1935, **20**, 81–123.
12. Kuntz, A., and Sulkin, N. M. Lesions induced in rabbits by cholesterol feeding, with special reference to their origin. *Arch. Path.*, 1949, **47**, 248–260.
13. Ssolowjew, A. Experimentelle Untersuchungen über die Bedeutung von lokaler Schädigung für die Lipoidablagerung in die Arterienwand. *Ztschr. f. d. ges. exper. Med.*, 1929–30, **69**, 94–104.
14. Ssolowjew, A. Über experimentell hervorgerufene Elasticarisse der Arterien und deren Bedeutung für die Lipoidablagerung. *Virchows Arch. f. path. Anat.*, 1932, **283**, 213–230.
15. Kelly, F. B., Jr.; Taylor, C. B.; Haas, G. M., and Craig, J. M. Arteriosclerotic lesions in areas of vascular injury in hypercholesterolemic rabbits. *Circulation*, 1951, **4**, 463.
16. Taylor, C. B.; Baldwin, D., and Haas, G. M. Localized arteriosclerotic lesions induced in the aorta of the juvenile rabbit by freezing. *Arch. Path.*, 1950, **49**, 623–640.
17. Harrison, C. V. Experimental arterial disease produced by cholesterol and vitamin D. *J. Path. & Bact.*, 1933, **36**, 447–453.

LEGENDS FOR FIGURES

FIG. 1. Traumatized segment of a rabbit's aorta after 2 days. The core of the polypoid mass is composed of avulsed, degenerating media and intima. × 138.

FIG. 2. Traumatized segment at 7 days showing endothelization of the polypoid mass and fibroblastic replacement of a V-shaped defect above it. × 138.



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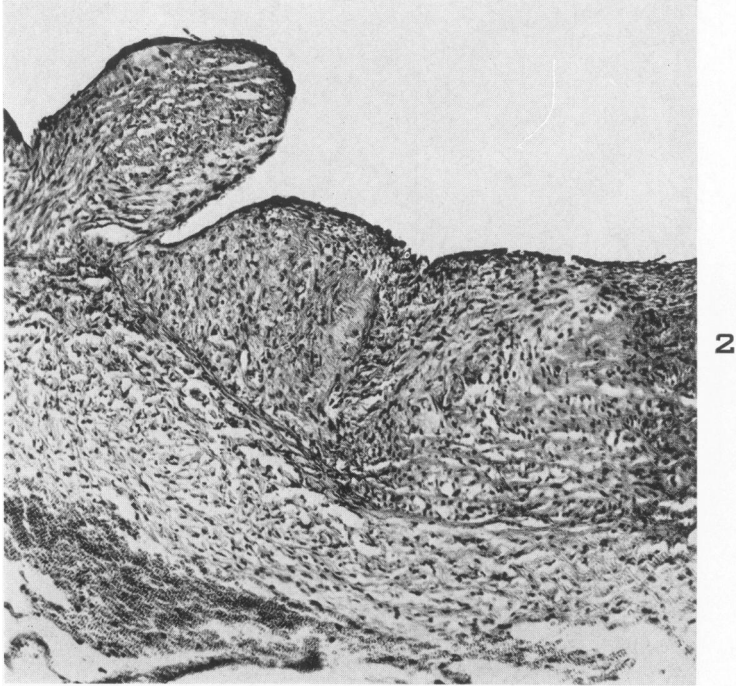

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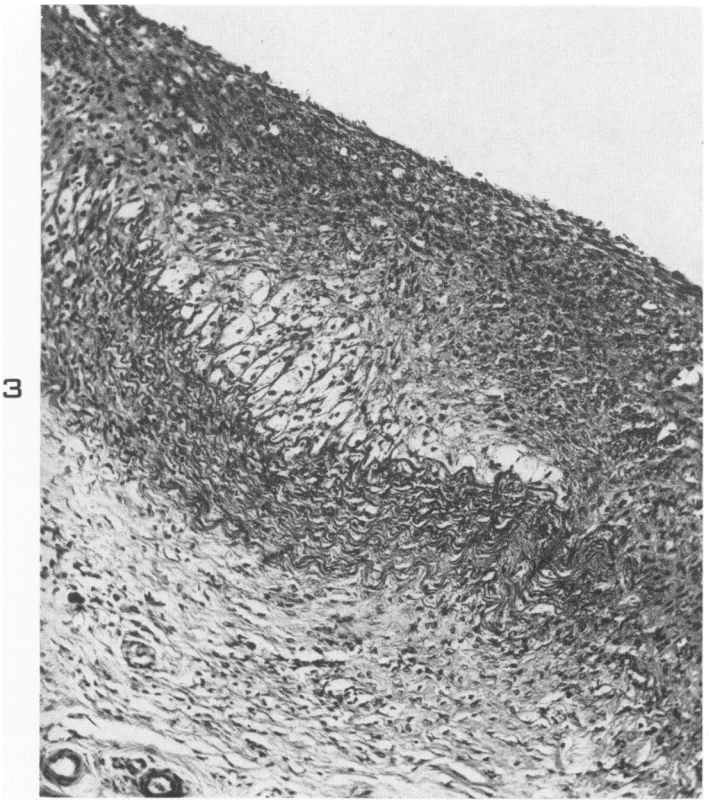
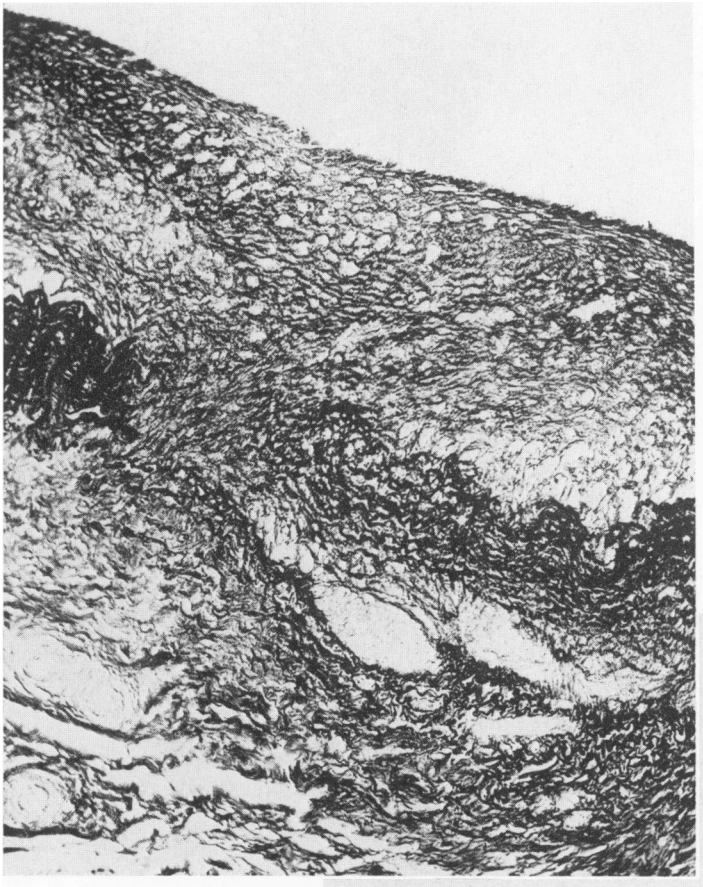


FIG. 3. Traumatized segment 14 days after injury. The collection of lipophages in the deeper tissue covered by a thick fibro-elastic cap presents a striking similarity to the early human plaque. × 138.

FIG. 4. Weigert-van Gieson's stain from the same block as the section shown in Figure 3. The intimal thickening is rich in elastic tissue, with scant collagen being present. × 138.

FIG. 5. Non-traumatized segment of rabbit aorta 27 days after institution of cholesterol feeding. × 227.



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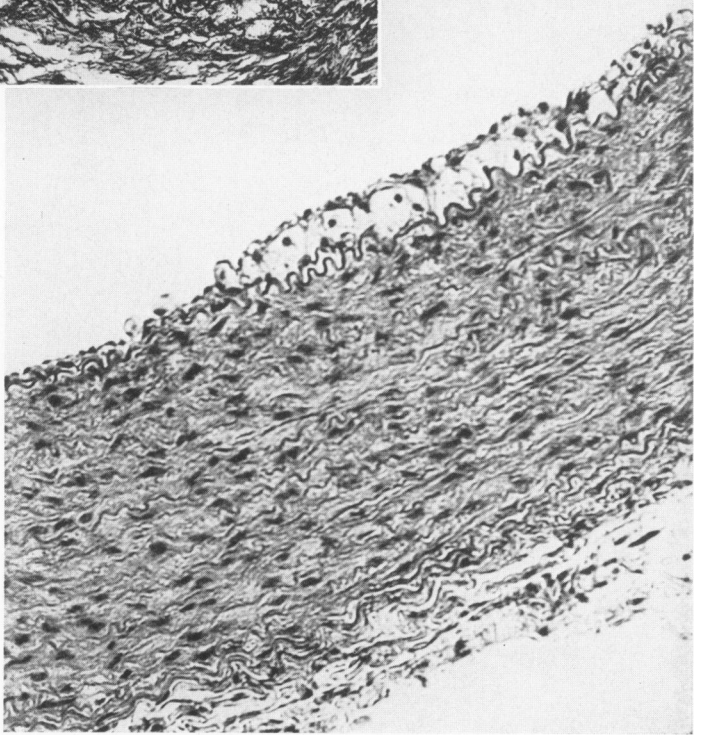

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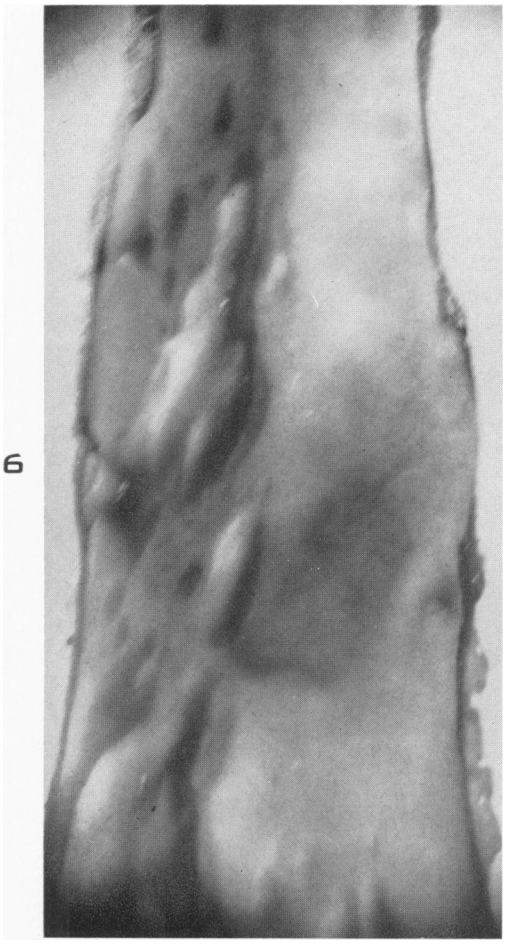

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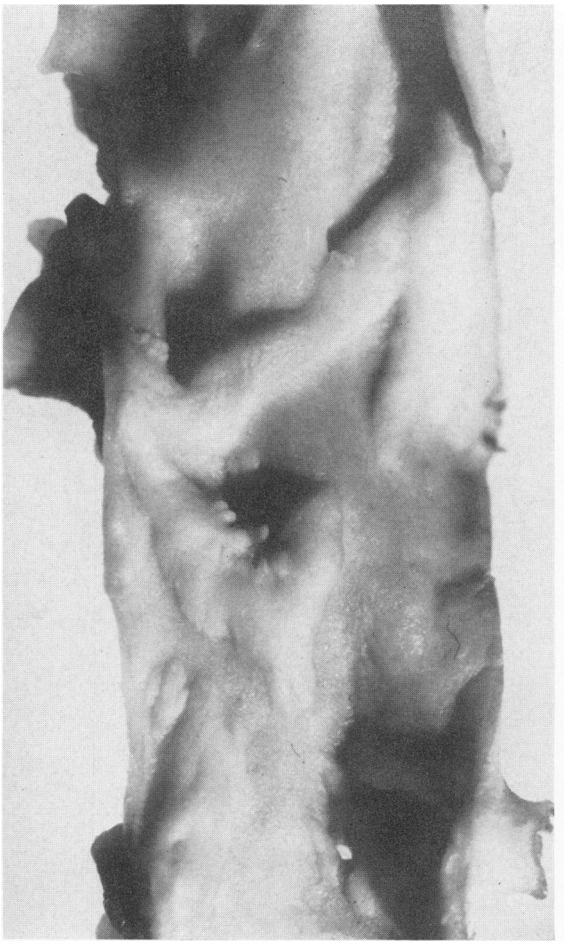

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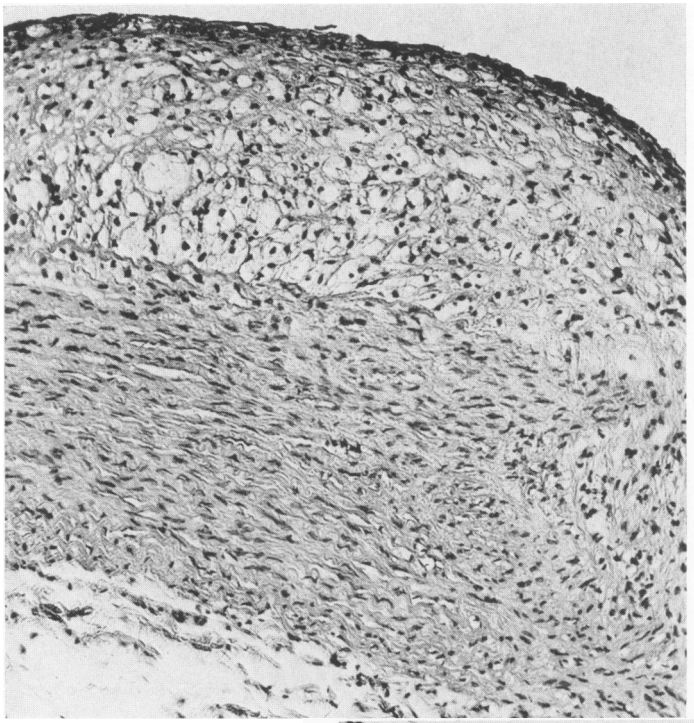
FIG. 6. Spontaneous lesions (non-traumatized) at 46 days. The oval-shaped plaques are elongated in the long axis of the aorta. × 3.5.

FIG. 7. Traumatized segment at 46 days. The transverse plateau-shaped elevations show small polypoid nodules along one margin. × 7.

FIG. 8. Spontaneous lesion at 46 days. The lesion is rich in lipophages and shows a moderate degenerative change. × 160.

FIG. 9. Traumatic lesion at 46 days showing extensive necrosis, some lipophages, and an ill defined internal elastic membrane. × 160.



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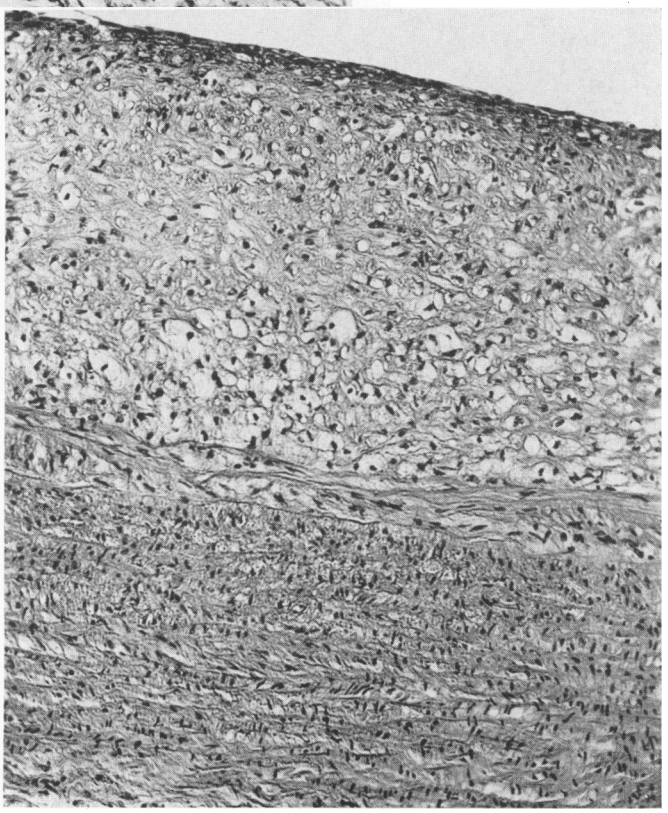

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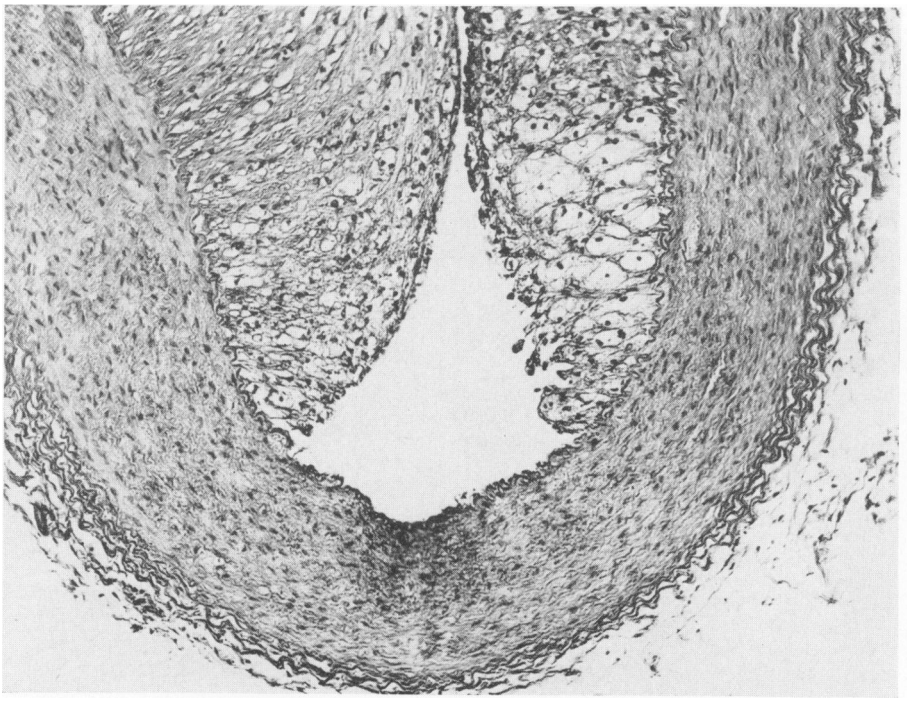
FIG. 10. Spontaneous lesions at 97 days. The younger lesion occupies the right half of the lumen. × 141.

FIG. 11. Traumatic lesion at 97 days composed of acellular necrotic débris and some lipophages. × 179.



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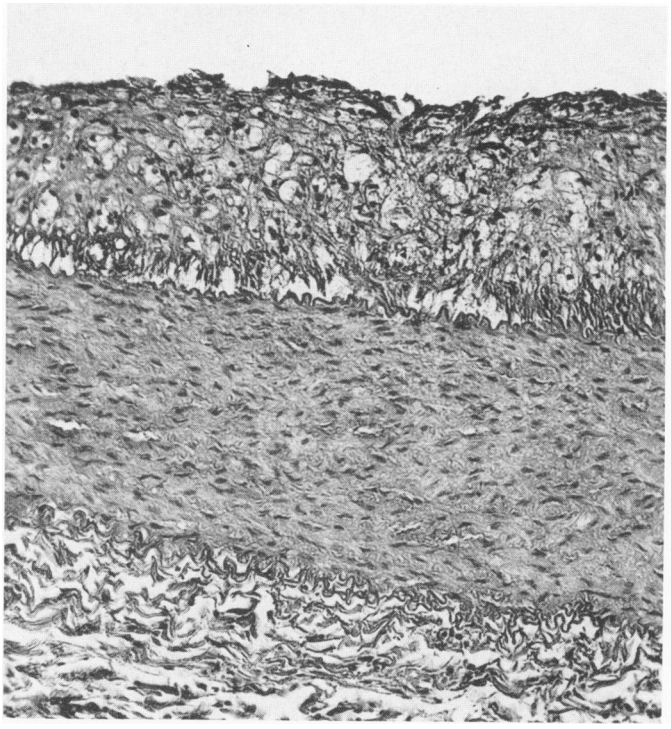

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