INTIMAL REPAIR OF THE AORTA OF THE RABBIT FOLLOWING EXPERIMENTAL TRAUMA *

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Despite the fact that arteriosclerosis is the leading cause of morbidity and mortality today, one cannot avoid the conclusion that our knowledge of its pathogenesis is shamefully deficient. As we have suggested previously,[1] this is in a large measure due to overemphasis of the rôle and mechanism of lipid deposition, directly attributable to the production of experimental "atherosclerosis" by the administration of cholesterol to a variety of animals. With the main interest focused upon the method of lipid transport through the vascular endothelium, it is understandable why the basic processes of injury and repair within the arterial subendothelial zone have been ignored.

The present investigation was stimulated by our observation of microscopic thrombi associated with intimal thickening in the aortas of 50 consecutive necropsies from the pediatric service.[1] Originally, these thrombi were an accidental microscopic finding and were noted primarily in those cases in which there had been a bacteremia (Fig. 1). Although Rokitansky[2] had originally pointed out that endothelial thrombi might be the basis of plaque formation in atherosclerosis, the idea was soon discarded and only recently has there been a reversion to this early concept. Duguid[3-6] and Crawford and Levene[7] have shown that mural thrombosis is extremely common in the aorta and that appearances identical with those seen in atherosclerosis may result from organization of such thrombi. In view of these observations it seemed pertinent to study the reaction to artificially-induced aortic intimal trauma in the experimental animal and to trace the sequence of repair eventuating in complete healing.

METHODS

Thirty New Zealand white rabbits, approximately evenly divided as to sex, were used in this study. Their average age at the beginning of the experiment was about 10 weeks and average weight was about 3500 gm. They were housed separately in wire cages and were fed Purina rabbit chow and water ad libitum.

* Presented at the Fifty-first Annual Meeting of the American Association of Pathologists and Bacteriologists, Philadelphia, April 8, 1954.
Received for publication, April 23, 1954.

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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 appearance. The intimal surface was then traumatized by repeated vertical and horizontal motions. The needle was withdrawn and hemostasis effected by means of direct pressure over the puncture site. Before closing the peritoneal cavity the area of trauma was marked by silk sutures in the periaortic connective tissue.

The animals were sacrificed at approximately weekly intervals ranging from 3 hours to 220 days following intimal injury. Within the experimental series, the entire aorta was removed and areas at a distance from the traumatized segment were considered normal and to serve as controls.

Pathologic Findings

The technique followed in this experiment did not permit control of the depth of the trauma, so that in any vessel the injured tissues might vary from the intimal coat only to the entire vessel thickness including the adventitia. In general, we were more interested in areas in which the trauma had been superficial, i.e., intimal only.

Normal rabbits of this age possess an unusually thin intimal coat, the endothelial lining appearing to lie directly upon a well developed internal elastic membrane. Isolated fibroblasts can, however, be recognized within the subendothelial zone. Animals sacrificed 3 days following intimal injury disclosed pitted lesions grossly and showed no evidence of intimal thickening. Microscopically, in those areas in which the trauma was relatively superficial, there was deposition of loosely arranged fibrin in which nuclear débris and rare mononuclear cells could be recognized (Fig. 2). The endothelial cells adjacent to the injured area were swollen and hyperchromatic and, where the injured segment was narrow, beginning endothelization was apparent. Below the fibrin layer fibroblasts were arranged in a parallel fashion, generally perpendicular to the endothelium. Polymorphonuclear leukocytes (amphophils) were abundant within this zone. The deeper, intact media showed edema and the adventitia invariably disclosed early fat necrosis, presumably the result of the pressure applied for hemostasis.

At 7 days the intimal surface was characterized by multiple, shallow, eroded areas giving the vessel a pebbly appearance. Microscopi-

cally, endothelial activity was much more prominent and it was not possible to distinguish these cells morphologically from the active fibroblasts growing toward the surface from the inner media (Figs. 3 and 4). Cells of both types were arranged in a disorderly fashion and were characterized by cytoplasmic swelling and nuclear hyperchromatism without evidence of mitosis. Beneath this zone in some areas the young fibroblasts had completely replaced the fibrin while in other areas the fibrin, although covered, had a peculiar pale pink "fibrinoid" appearance (Fig. 3). Calcification of these fibrinoid zones sometimes followed, being observed as early as 49 days in one case (Fig. 6). About the margins of the fibrinoid areas a mononuclear cellular reaction was noted frequently. Intramural hemorrhage was observed at this stage but new capillaries were not in evidence. In the adventitial foci of fat necrosis a giant cell reaction and large numbers of lymphocytes and plasma cells were present. Occasionally, the non-organized fibrin protruded into the vessel lumen as an endothelium-covered polypoid excrescence (Fig. 5). Although there was evidence of fibroblastic proliferation at the base of such nodules, calcification occasionally supervened before organization was completed (Fig. 7).

At 18 days following intimal trauma, the same pitted lesions as have been described were seen on the intimal surface, but beginning thickening of the adjacent margins was present. Microscopically, the lesions showed no significant changes from those described at 7 days. The fibroblastic reparative process was nearly completely avascular and no unusual changes within the vasa vasorum were apparent.

At 35 days following injury, the aortic intima showed raised, somewhat yellow foci above and below the lacerated segment. These superficially resembled the plaques of adult atherosclerosis. Microscopic examination of these thickenings revealed them to be conchoidal and composed of loose connective tissue (Fig. 8). The cells comprising the thickened focus were slender, spindle-shaped, and hyperchromatic, and showed moderate variation in polarity. The internal elastic membrane at the base of the thickening frequently showed fraying, fragmentation, and splitting. The portion of the intima which was contiguous with the internal elastic membrane disclosed considerable edema and nuclear débris. With Verhoeff's technique these intimal plaques were found to be composed predominantly of elastic tissue (Figs. 9 and 10), which appeared as delicate fibrils arranged parallel to the internal elastic membrane and which seemed to be most dense in the more superficial portion of the intimal thickening. Masson's trichrome stain revealed only minute amounts of collagenous material.

It is this stage of the reparative process which is morphologically inseparable from the fibro-elastic intimal thickenings described by Prior and Jones[1] and which occurred so consistently in children over the age of 2 weeks that we postulated that these might actually represent the earliest phase in the development of the atheromatous plaque in man. An impressive gross lesion on the intimal surface of the rabbit's aorta was not noted until 61 days following injury. At the site of trauma numerous transverse lacerations, each measuring 0.1 cm. long, were observed. The intimal surface about the margins of these lacerations presented a raised, slightly yellow, rolled appearance. The entire area was placed in a solution of 50 per cent alcohol to which several drops of Herxheimer's scarlet red had been added, but these yellow areas failed to stain positively for lipid material. The microscopic picture of these rolled, raised areas was similar to that of the thickenings noted at 35 days (Fig. 7), although the trauma had been deeper so that the internal elastic membrane was partially replaced by dense fibrous tissue. Wherever the medial elastic lamellae were injured they were replaced by non-specific dense scar tissue with a reaction of mononuclear type.

At longer intervals the gross appearance of the aortic intima was striking, the typical surface alterations being shown in Figure 11. Large plaque-like elevations were located about the margins of previously injured segments. Although these areas had a yellowish discoloration, they also failed to stain positively with the alcoholic Herxheimer's scarlet red solution. The plaques averaged about 0.1 cm. in diameter with the largest measuring 0.3 cm., and their elongation was invariably in the longitudinal plane. Microscopically, the thickenings were composed of mature connective tissue which was rich in elastic tissue. Such an area of thickening 148 days following injury is shown in Figure 12. The reduplication or splitting of the internal elastic membrane was striking and minute amounts of lipid material were visible as demonstrated by osmic acid preparations. The fat was sparse and located within the more superficial portion of the thickening. No foam cells were recognized and this fatty material appeared to be entirely extracellular. Of considerable interest is the fact that only the transverse lacerations remained as depressions and became associated with these fibro-elastic thickenings and that no residual scarring from the vertical lacerations could be identified. Although reasons for this are not clear, one is tempted to suggest that the transverse lacerations may have been associated with disturbances in the flow, such as eddy currents.

Discussion

The pathogenesis of atherosclerotic plaques is a neglected subject in the standard textbooks of pathology. The historical background of their development is a succession of theories ranging from a simple chronic inflammatory concept to one in which the physicochemical changes within the intimal extracellular colloids are paramount, and, finally, to the present concept centered upon aberrations of serum lipids and lipoproteins. It is of interest that Duguid[3] has pointed out that Rokitansky[2] first held that the atheroma was produced by the deposition of an endogenous product derived from the blood and, for the most part, from the fibrin of the blood. He noted the metamorphosis of the deposit into a pulpy mass consisting of crystals of cholesterin, fatty globules, and of molecules of albumin and calcareous salts. Virchow[8] later refuted this concept, holding that the connective tissue proliferation was initially subendothelial, and referred to the endothelium as if it were a fixed landmark by which the tissues of the vessel could be identified.

Duguid's views[3-6] are in essence a reversion to the teachings of Rokitansky[2] and appear to contrast sharply with the present day concept of the genesis of the plaque. Karsner,[9] for example, in discussing the pathogenesis of atherosclerosis, stated that the first lesion is damage in the lower intima with splitting of the elastic fibers, some destruction of fibrous connective tissue, and lipid deposition. In response to the injury, connective tissue is formed in excess to produce intimal plaques. This overgrowth of fibrous tissue is succeeded by hyalinization, and either before, or coincident with hyalinization, there may be mucoid degeneration of connective tissue. Duguid[3] pointed out that when a thrombus forms in an artery it adheres to the wall, the endothelium disappears, and there is an invasion of the mass by connective tissue. There then follows a progressive transformation of the outer layers of the thrombus into fibrous tissue, so that an advancing zone of fibrosis is formed which overruns and obliterates the original line of demarcation between thrombus and intima—an appearance similar to that seen in pleural or pericardial inflammation. Organization of the thrombus then follows, although the progress of this change depends to a great extent upon the composition of the mass. Pure fibrin, which is relatively firm, is readily organized, but collections of corpuscles, such as are present in red thrombi, undergo softening and fatty degeneration which interfere with organization. The corpuscles break down and globules of fat appear among them

and accumulate until they obscure the entire semi-fluid or paste-like mass. Such masses, having no firm structure on which granulation tissue can build, tend to persist as areas of fatty degeneration and it appears that this is one of the ways in which atheromatous plaques are formed. Fatty changes also occur in the fibrinous parts of the thrombus, but in these areas the thrombus usually is not associated with softening and therefore is not so permanent. With transformation of fibrin into fibrous tissue the fats are taken up by phagocytes which tend to occur in clusters around the zone of organization, and with canalization and further organization they appear to be embedded in a thickened intima. Duguid[6] contended that these observations can be confirmed by inducing experimental thrombosis in animals by such methods as pricking the central artery of the rabbit's ear or by passing silk threads a short distance along the lumina of the femoral or carotid arteries of the dog. He also called attention to Harrison's[10] study in which fibrin particles were injected into the veins of rabbits. The particles which lodged in the smaller pulmonary arteries were converted into fibrous thickenings indistinguishable morphologically from the vascular changes in human pulmonary hypertension. Finally, it would appear that the study of aortic fibrin deposits by Crawford and Levene[7] adds strong support to Duguid's hypothesis.

Our main interest has been with the non-lipid-induced variants of arteriosclerosis. Reviewing the literature, one finds a kaleidoscope of experimental methods of inducing vascular lesions, both similar and unlike those found in human blood vessels. Broadly, these may be classified under chemical methods (including macromolecular substances), and physical (including direct and indirect trauma). Anitschkow,[11] in his survey of the literature (1890–1924), cited many examples of mechanically induced arterial lesions. All of these procedures—ligating, pulling, pinching, wounding, cauterizing with galvanic current or silver nitrate—produced inflammatory responses which were not similar to human arteriosclerosis. These procedures did, however, result in increased permeability of the vascular intima to intravenous trypan blue dye, and subsequent intimal thickening. Ssolowjew[12] showed that the course of the arterial lesion depends upon the degree of injury to the elastic framework. With severe injury the adventitia initiates granulation tissue replacement of the structure. With the elastic framework intact (i.e., only the cellular parts destroyed) the regenerative proliferation begins in the adjacent intact media. Contributions by the intima and intra-luminal circulating cells are also said to have a rôle. Ssolowjew demonstrated that

isolated transverse lacerations of the elastic lamellae of the inner media, produced by fixation of the carotid artery into a bridge of skin, rendered the overlying intima more permeable to substances circulating in the blood (trypan blue and lipid substances in cholesterol hyperlipemia). The reparative processes consisted of subendothelial thickening by cells migrating from the intima. Altschul[13] spoke of migration of smooth muscle cells across the elastic lamina to the intima. He also referred to Krafka,[14] who contended that there is a migration of intimal cells to the media through herniations in the internal elastic lamellae, and that in the later stages of repair associated with new elastic and collagen fibers, lipids are no longer deposited at sites of intimal tearing.

Dill and associates[15,16] studied aortas of rabbits with constricting rings located both proximally, and proximally and distally, to the renal arteries. The added factor of pregnancy was present in some of these animals. They noted in the thoracic and abdominal aorta atheromatous plaques which were composed of amorphous intimal material which stained positively for fat, and fibrillation of the underlying elastic lamella. They concluded that the incidence of plaques was closely related to the level of systemic blood pressure and to the length of time such pressure acts. No lesions were seen below the level of the constricting band nor were any present in those animals with the band but without elevated blood pressure. Plaque formation was observed much more frequently in animals which were pregnant. They concluded that an elevation in aortic pressure transmitted through the depth of the wall would interfere with filling of the vasa vasorum and bring about an anemia of the subintimal layers—a hypothesis based upon the observations of Winternitz, Thomas, and LeCompte.[17] Mehrotra,[18] who recently investigated changes that occur in ligated arteries and veins, noted that double ligation of arteries results in obliteration of the lumen by intimal proliferation, with subsequent medial atrophy. Double ligation of veins also stimulates intimal proliferation; however, a new lumen with connection to other veins and the re-establishment of circulation always occurs. It was believed that the relative ease of venous repair contrasted with arterial repair could be explained by structural differences in the vessels, veins being composed chiefly of fibrous tissue while arteries are rich in the more highly differentiated muscle and elastic tissues.

Searing the adventitia of the ascending aorta of dogs produced changes in the outer and middle portions of media.[19] These consisted of necrosis, liquefaction, cyst formation, and ultimate replacement by

collagenous tissue. The rate of necrosis was determined by the extent of the collateral circulation. In one animal there was spontaneous rupture and subsequent dissection of an aneurysm. In an attempt to induce atheromas or dissecting aneurysms, blood was injected into the vessel walls of dogs with the result that after 3 months there was either complete healing or medial scarring without evidence of hemorrhage or dissection.[20] Closely allied to this discussion of injury and repair of arterial walls is the progression of events seen in arterial homografts. Swan, Robertson, and Johnson,[21] in a study of aortic transplants in dogs, observed that the adventitial and intimal tissues were totally replaced by the host. In grafts stored less than 40 days, up to 50 per cent of the muscle of the media survived, while none survived in grafts stored over 40 days although the elastic tissue survived. They also noted that after 5 months the intima showed spotty areas of calcification and some lipid deposition, presumably cholesterol.

Finally, a pertinent study is that of Sheehan[22] upon the effects of irradiation on small arteries (100 to 500 μ in diameter). He described foam cell plaques in the intima of irradiated human uterine, ovarian, and rectal arteries. The plaques were located between the endothelium and the internal elastic lamina and were composed of either foam cells alone or foam cells in combination with other cells, fluid, fibrin, and hyaline material. Pathologic changes in the media and adventitia were not constant. The plaques produced marked narrowing or occlusion of the vessel lumen, "thrombosis, fibroblastic proliferation of the intima or deposition of elastic tissue in the thickened intima seldom result." Nevertheless, Sheehan illustrated segments of a uterine artery with necrosis adjacent to the radionecrotic area at one end, a plaque at the other, and a thrombus in the intervening portion. He proposed the theory that radiation altered the permeability of the endothelium, facilitating migration of lymphocytes, monocytes, and erythrocytes into the intima. With red cell degeneration, liberation of their lipids and subsequent ingestion of lipids, foam cells are produced. No stains for fat were done, but since crystals, presumably cholesterol, accompanied the foam cells, it was inferred that the foam cells contained cholesterol and were really xanthoma cells.

Summary

The reaction to a form of mechanically induced trauma to the intimal surface of the rabbit aorta has been described. The injured segment heals chiefly by means of marked fibroblastic activity, resulting in the production of a localized fibro-elastic thickening within which,

in the later stages, minute amounts of lipid were demonstrated. Although the gross and microscopic appearances of these intimal "plaques" showed little similarity to human atherosclerotic plaques, the lesions were histologically inseparable from the fibro-elastic aortic thickenings observed in infants, which are considered by some to be precursors of the adult atheromatous lesion. A brief review of the results after other forms of mechanically induced vascular trauma has been presented.

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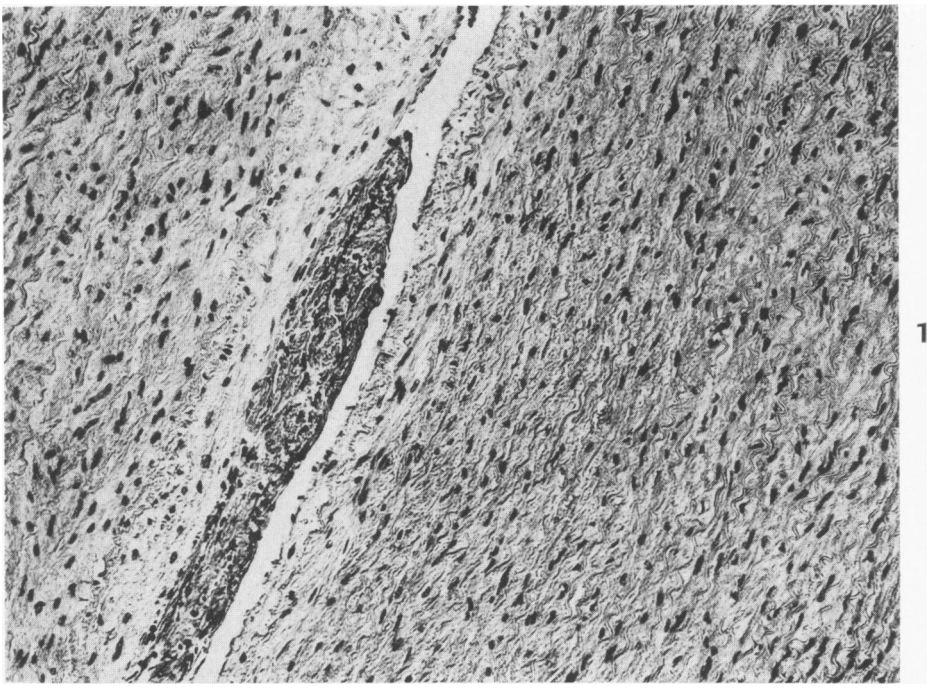
LEGENDS FOR FIGURES

FIG. 1. Aortic lumen of an 8-year-old child showing, on the right, intima of normal thickness, and, opposite this, a fibro-elastic plaque with superimposed thrombus. × 175.

FIG. 2. Traumatized segment of a rabbit's aorta after 3 days. A meshwork of fibrin and proliferating fibroblasts is growing toward the lumen. × 465.



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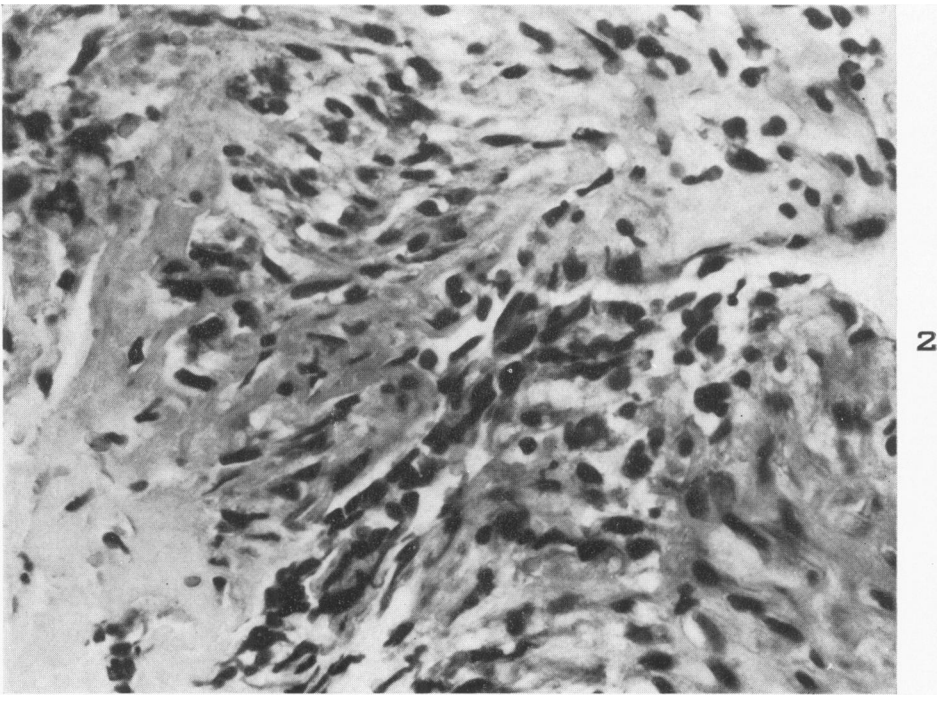

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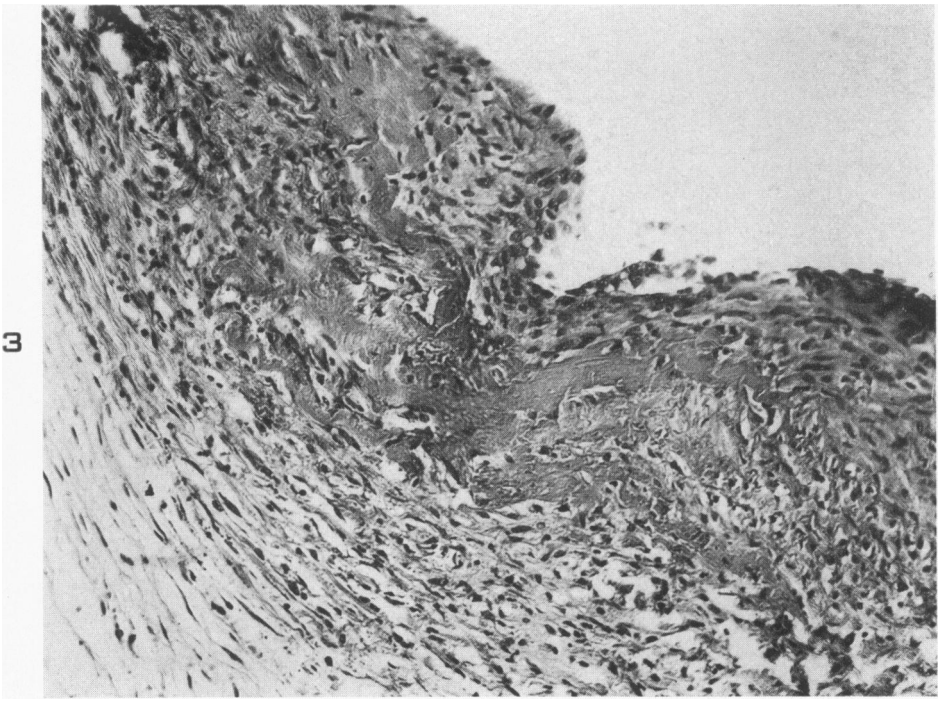
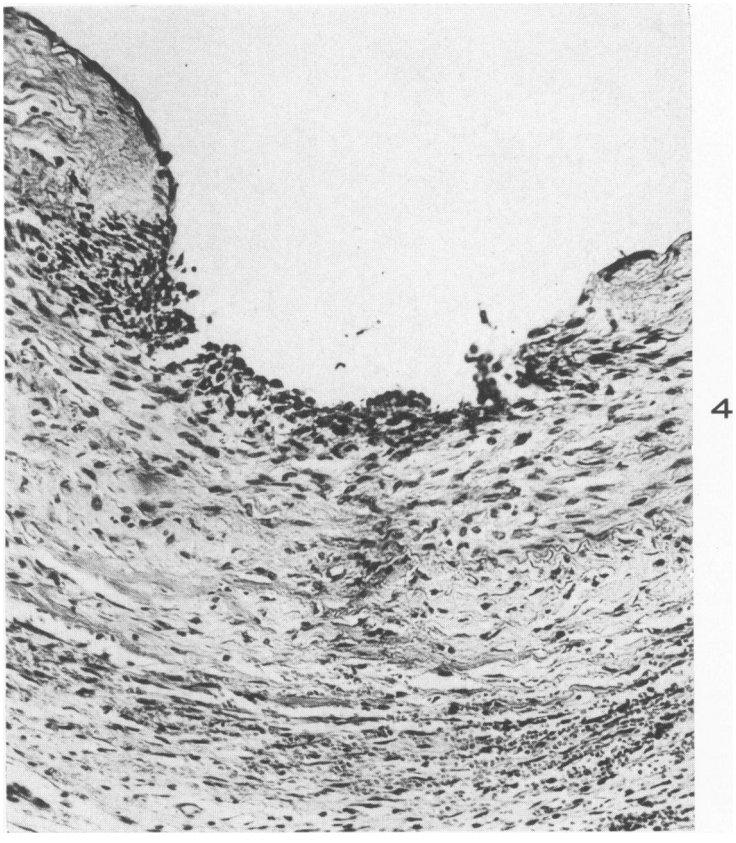


FIG. 3. Traumatized segment at 7 days, showing fibroblastic hyperplasia on either side of the central depression. Fibrinoid material is present within the media. × 160.

FIG. 4. Traumatized segment at 7 days, showing extensive fibroblastic and endothelial cell proliferation. × 160.

FIG. 5. Traumatized segment at 7 days, revealing the polypoid non-organized mass at one margin of the depressed area. × 160.



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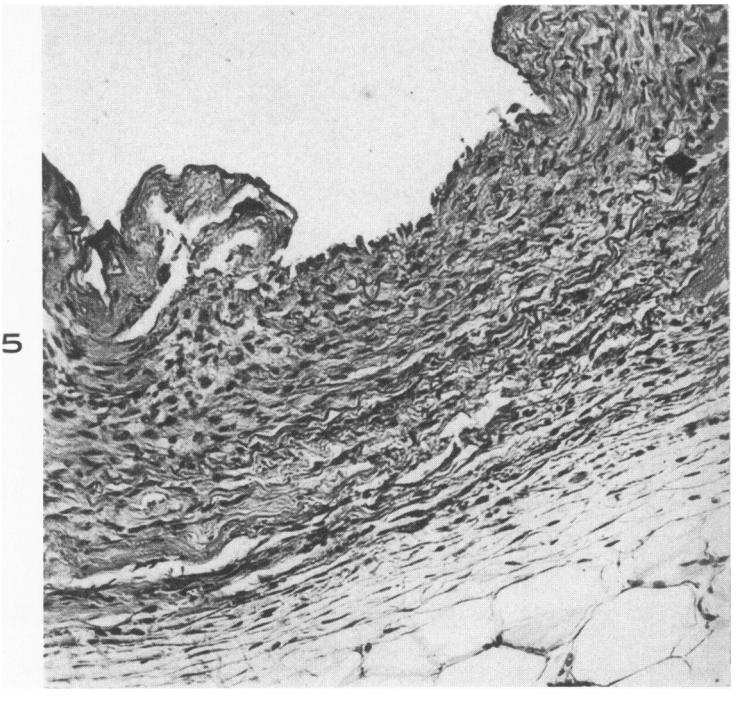

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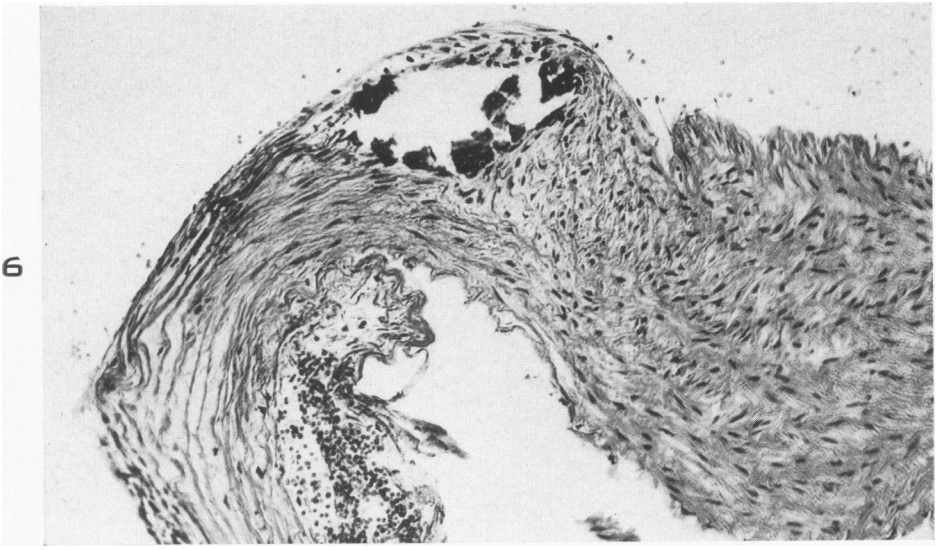


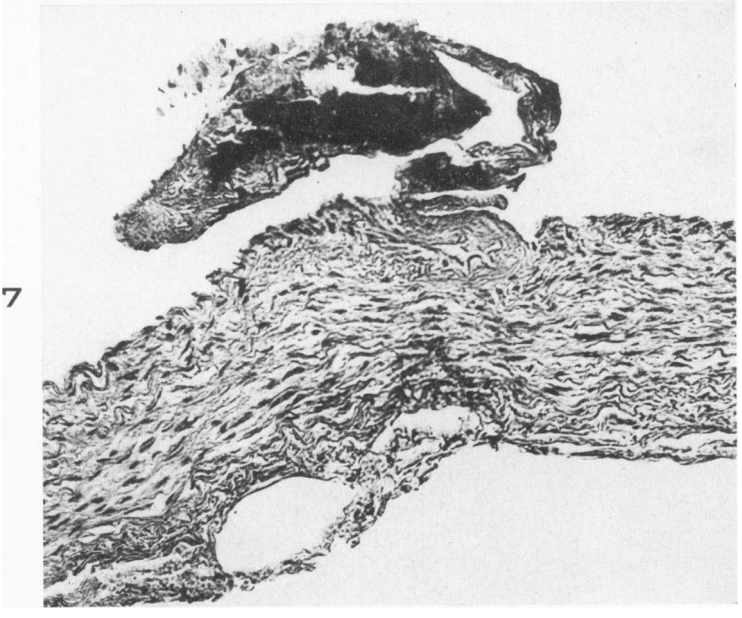
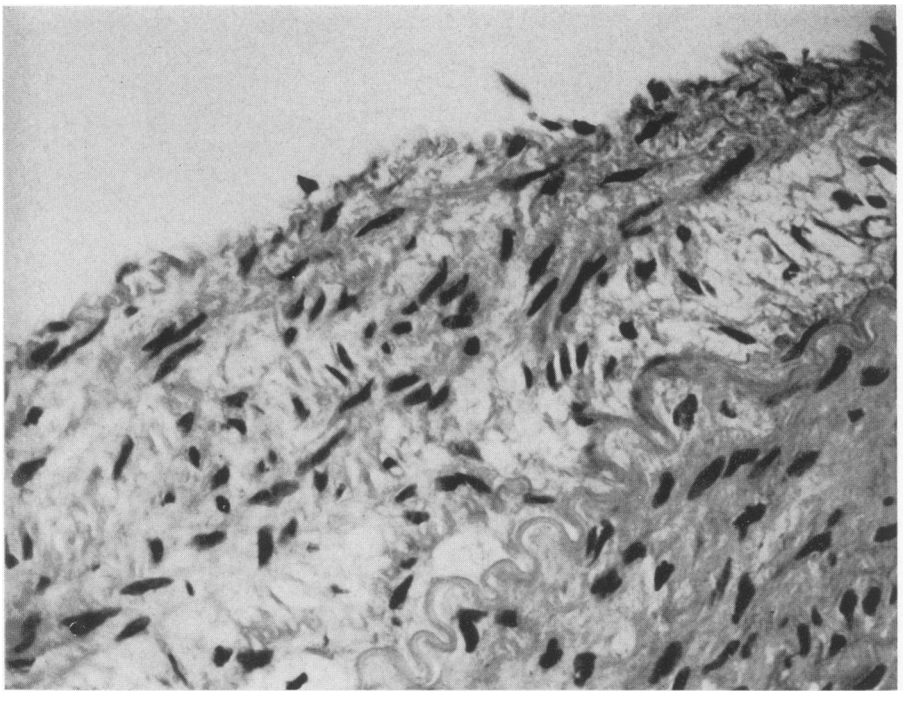


FIG. 6. Traumatized segment at 49 days, with calcification of the non-organized material. × 160.

FIG. 7. Calcification of a polypoid mass similar to that shown in Figure 6, at 111 days. × 160.

FIG. 8. Thickened fibrous intimal plaque 35 days after injury. The internal elastic membrane shows fraying toward the right. × 465.

FIG. 9. Elastic tissue stain of a plaque similar to that shown in Figure 8. × 165.



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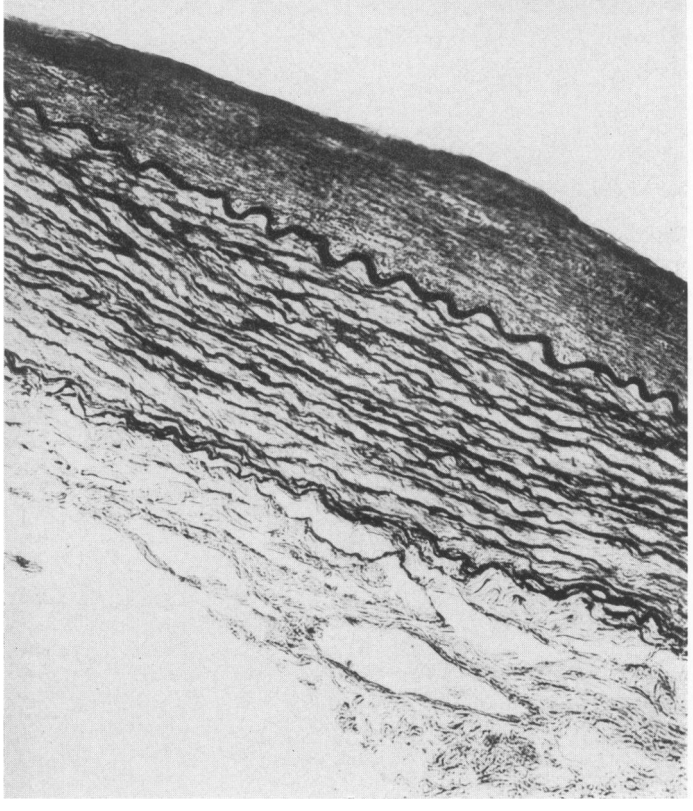

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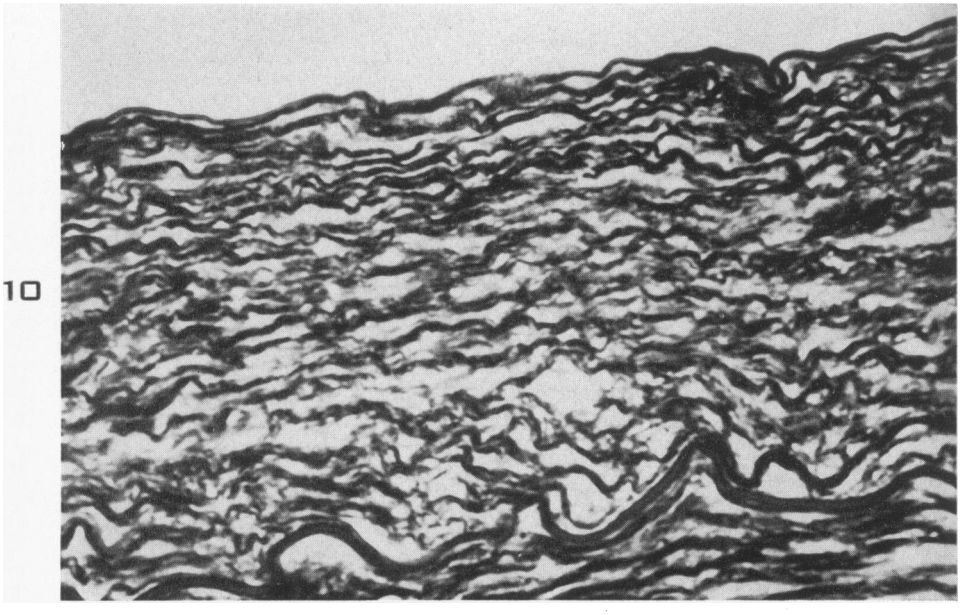

FIG. 10. High-power magnification of the plaque shown in Figure 9. × 600.

FIG. 11. Intimal surface of the aorta 163 days after injury, showing the intimal thickenings adjacent to the transverse fissures. × 5.

FIG. 12. Preparation stained with osmic acid to show the distribution of lipids in the subendothelial zone 148 days after injury.

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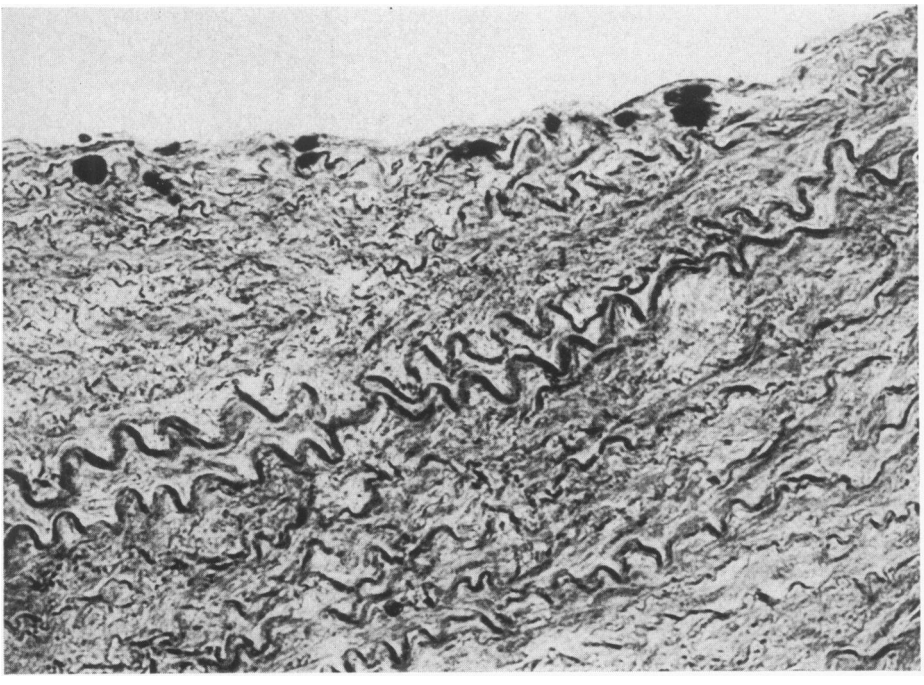

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