FOCAL INTIMAL PROLIFERATION IN THE CEREBRAL ARTERIES

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In investigating the pathogenesis of atherosclerosis, it is important to start with the earliest possible stage of the lesion, which as yet has not been carefully defined. Areas of intimal proliferation have been described in the aorta [1,2] and about the orifices of the large distributing arteries [3–5] in early life, but the cerebral arteries have not previously been examined in detail from this point of view. This intimal proliferation may be related to atherosclerosis, as Wilens [1] suggested. In the present work, serial sections of the cerebral arteries were made, rather than random sections as used by other workers. By this means, a more accurate localization of the thickenings in relation to the forks and a more thorough histologic study were made possible.

MATERIAL AND METHODS

The material consisted of 93 cerebral arterial forks from 22 human subjects, ranging in age from a fetus of 28 weeks to an infant of 8 months. All but 4 forks were bifurcations of the internal carotid and middle cerebral arteries. The tissues were fixed in 10 per cent formalin and embedded in paraffin. Serial sections were cut at 7 μ, in such a plane as to obtain Y- or T-shaped sections. From 5 to 9 consecutive sections were mounted on each slide. Toluidine blue, Mallory's phosphotungstic acid-hematoxylin (PTAH) or the McManus periodic acid-Schiff (PAS) technique were used to stain 2 nonconsecutive slides from near the center of the fork. All other slides were stained with Verhoeff's elastic tissue stain and counterstained with eosin.

The terminology used to define anatomic sites about the forks is the same as that used in a preceding paper.[6] The apex of the fork (Text-fig. 1) is the point at which the axial column of blood impinges on the vessel wall at the bifurcation. The apical angle is the angle between the two branches, and the lateral angles are those between the parent stem and each of the daughter branches. The face is the area on the uppermost part of the stem near the fork and between the entrances to the branches. The back (or dorsum) is the corresponding area on the under surface.

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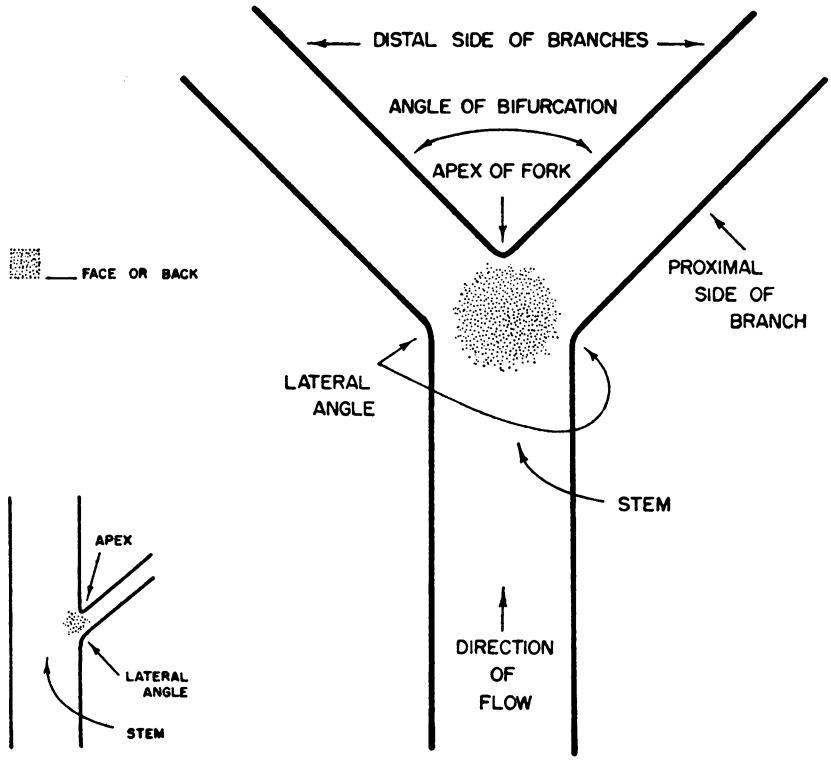
RESULTS

For the most part, the intima of the cerebral arteries of all sizes consisted of an endothelial layer and an internal elastic lamina, with no intermediate layer. The internal elastic lamina, when appropriately stained, appeared in transverse section as a darkly staining, frequently



TEXT-FIGURE 1. Diagram illustrating the terminology used for anatomic sites about the forks of vessels. (Reproduced from Stehbens [6] with permission of the publisher.)

wavy layer with sharply defined edges and of relatively uniform thickness. In tangential section it was a fenestrated membrane (Fig. 1). The fenestrae were mostly 2 to 3 μ in size, but varied from about 1 to 8 μ and were round or oval. They were fairly evenly distributed, though it was not uncommon for two to be very close together. The membrane, apart from the fenestrae, was fairly homogeneous. Variations from this structure occurred only in relation to the intimal thickenings or in those sites at which the thickenings were commonly found. Such changes took place in a regular sequence, both in location and in time, beginning in fetal life, even as early as 28 weeks.

Sequence of Development of Intimal Pads

Intimal thickenings were found only in relation to the sites of branching of the arterial tree. The intimal proliferation did not occur diffusely about each fork but occurred as separate pads which later coalesced. They were named apical, facial, dorsal, and lateral pads (Figs. 2 and 3) according to their position. The facial and dorsal pads were the earliest to be seen and the lateral pads next, while the apex was the last site to be affected. The sequence of the development and localization of these intimal pads was found to be constant. Lateral angle pads were never seen in the absence of facial and dorsal pads. It was only when facial, dorsal and lateral pads had enlarged and begun to coalesce (Fig. 2) that changes were found at the apex. Eventually, all these pads became continuous with one another. Without exception, the changes in all forks conformed to this pattern. Lateral pads occurred immediately beyond the site where the lateral wall began to curve into the proximal side of the daughter branch (Fig. 3). The extent of the alterations increased with increasing age of the subject and increasing size of the vessel. When there was considerable difference in the size of the two branches, there was only one lateral angle pad, as in Figure 3, on the side of the trunk from which the smaller branch took origin. Whatever the angle at which branches left the main stem, the position of the pads was the same, but the lateral pads were not always equal in size and extent on the two sides. At the bifurcation of the internal carotid artery, the lateral angle pad on the side of the anterior cerebral artery and elastic changes at the apex were sometimes found to develop earlier than the lateral angle pad on the side of the middle cerebral artery. There was also intimal proliferation at the orifices of smaller branches, but this was always less extensive.

Early Elastic Tissue Changes

Elastic tissue alterations were detected in the region of the face, dorsum, lateral angles and apex before the appearance of intimal proliferation. All intimal pads were associated with such changes, which were usually most prominent at the thickest portion or center of the pad but occasionally were seen beyond its edges. Sometimes the pad seemed to be slightly more extensive than the visible elastic tissue changes.

The changes in the elastic lamina consisted of loss in the depth and uniformity of staining which was associated with the lace-like and fibrillary appearances seen in Figure 4. In cross section, the lamina was often relatively straight and thin, or at times granular, segmented, or fuzzy (Fig. 5). Beading or a tendency to segmentation of the elastic lamina was also seen about the fork with or without some intimal thickening. The lamina, in places, failed to stain at all (Fig. 6).

Facial, Dorsal, and Lateral Pads

The earliest intimal pads found consisted primarily of smooth muscle fibers and some very fine elastic fibrils. In general, the smooth muscle fibers were less dense than in the media and were longitudinally arranged. In sections stained by PTAH, there appeared to be more intercellular matrix than in the media (Fig. 7), but with Verhoeff's stain (Figs. 8 and 9) the intercellular matrix appeared to consist of numerous elastic fibrils. The wavy elastic fibrils ran longitudinally in the pads and were probably thin laminas. These early pads were not areas of endothelial proliferation, for the endothelial cells were quite distinct from the underlying plain muscle fibers. Staining with toluidine blue and PAS revealed no metachromatic ground substance.

Larger and older pads had similar features. They were occasionally more than 200 μ in length, and often thicker than the media (Fig. 7). The underlying elastic lamina showed the changes (Fig. 4) described above. In addition, a new elastic lamina formed immediately beneath the surface (Fig. 10). This newly formed elastic lamina was usually most noticeable at the edge of the pad (Fig. 10), but it did often extend over the surface. The new lamina was thin, palely staining and very fibrillary (Fig. 11), though in older pads it was sometimes denser (Fig. 12). The lamina thus formed fused to the original lamina at the edges of the pad, and this gave the appearance of splitting of the original lamina. It seemed, however, that the duplication was caused not by splitting but by the formation of new elastic tissue beneath the endothelium. In Figure 12 the new lamina appears beaded in places, and near the arrow it is pale and lacy. Newly formed elastic laminas always showed these changes to some extent. In still more advanced plaques, musculo-elastic proliferation had proceeded on top of the second lamina (Fig. 13). The pad then appeared to consist of two strata, separated by a layer of elastic tissue. Other small laminas or fibrils in these pads were pale or indistinct.

These advanced pads with prominent accessory elastic laminas were found only in the forks of older subjects in which pads occurred at all areas about the fork.

Apical Pads

Apical pads occasionally projected well into the lumen, but usually were considerably thinner than those encountered elsewhere. The internal elastic lamina showed the same sort of alterations observed in other situations (Figs. 14 to 16). Apical pads also consisted mainly of muscle and elastic tissue. New elastic tissue tended to form beneath the endothelium, giving the elastic lamina a frayed or split appearance (Figs. 14

and 16). The pads sometimes appeared to be a part of the media, but elastic tissue stains revealed remnants of the original elastic lamina, distinguishing intima from media (Fig. 16). The outstanding feature of the apical pads was the general tendency for little intimal proliferation to take place, even in the presence of a fragmented or deficient elastic lamina. The apical pads were usually continuous with the facial and dorsal pads, and therefore might be merely the extension of the neighboring intimal thickenings.

Frequency and Severity of the Intimal Changes with Age

Grading the severity of the intimal lesions was technically difficult because the pads were cut in different planes of section, and the vessels varied considerably in size and angle of bifurcation. However, some indication of the frequency and severity of the alterations may be gained by dividing the specimens roughly into 3 groups. The first group consisted of 9 forks from 2 fetuses of 28 weeks' gestation. Changes in the elastic lamina with or without intimal proliferation were found in the facial and dorsal areas of 5 of these forks. The second group, consisting of 46 forks from fetuses of 36 weeks to infants of one month, revealed facial and dorsal pads in all cases. Some forks had lateral pads, but the apical regions were usually unaffected. A few of the pads contained two prominent elastic laminas. The remaining 38 forks from infants of 6 weeks to 8 months formed the third group. In these there were facial and dorsal pads in all cases and usually lateral and apical pads. These pads were generally larger and thicker than those of the other groups, and many of them contained 2 or 3 prominent elastic laminas.

Thus, the most marked alterations were observed in those instances in which there were pads at all sites about the fork; the least marked changes were observed in those in which there were only facial and dorsal pads.

Discussion

The nature of the intimal proliferation in the arteries of infants is unknown. There has been much speculation, and in general it has been assumed to be a normal structural component of an artery. Some authors,[1,7] however, have considered it to be the early stage or an integral part of atherosclerosis. The nature of the elastic tissue changes which preceded and were associated with intimal thickening is debatable, but they appeared to be steps leading to the eventual disappearance of the elastic lamina. Hass [8] said that the early stage of atrophy of elastica was characterized by the partial or complete loss of specific staining qualities, and the elastic tissue changes described here are probably degenerative.

In a recent study of intimal proliferation in the aorta,[2] atrophy of muscle and elastica was noted both in the intima and in the adjacent media.

If the elastic tissue alterations are degenerative, as it would appear, it follows that even at this early age there is some damaging factor at play. The intimal proliferation of the pads may be compensatory in nature because of the loss in tensile strength consequent upon the changes in the elastica. The pads continue to be associated with elastic tissue changes, and the new laminas also show similar features, suggesting that the hypothetical damaging factor persists. Recent evidence[9] suggests that turbulence may occur at the bifurcations and branchings of the cerebral arteries, and it may therefore be significant that the lateral and apical pads occur at stagnation points where the eddy currents are likely to occur. The facial and dorsal pads are not so easily related to flow patterns.

Summary

Intimal pads around bifurcations of the cerebral arteries of fetuses and infants were preceded by and associated with prominent elastic tissue changes, which might well be degenerative in nature. The pads appeared in a regular sequence. Facial and dorsal pads preceded the lateral angle pads. These had extended and had begun to coalesce before the apex was affected.

The pads consisted of musculo-elastic tissue. The apical pads were usually thinner than those at other sites, despite prominent changes in the elastic lamina. Successive laminas of elastica were formed, giving the intimal pads a stratified appearance.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. A tangential section of the internal elastic lamina. Note the fenestrae and the homogeneous appearance of the elastic tissue. Black dots are due to formalin pigment. Verhoeff's stain. × 500.

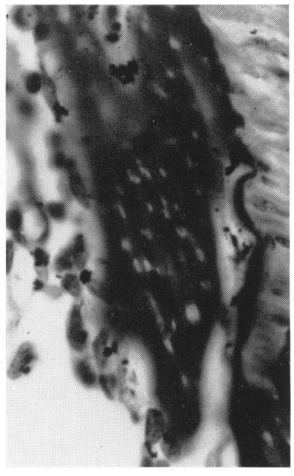
FIG. 2. An internal carotid artery, showing a facial pad (center) with prominent elastic tissue changes (center). Lateral pads are seen at L; that on the left is continuous with the facial pad. Verhoeff's stain. × 20.

FIG. 3. A lateral angle pad at branching of a middle cerebral artery in an infant. Note pallor of the elastic lamina beneath the pad. There is a defect of the media but no pad at the apex. Verhoeff's stain. × 35.

FIG. 4. An internal elastic lamina, pale and lacy, in tangential section. Endothelial cells and formalin pigment are manifest to the left. Compare with Figure 2. Verhoeff's stain. × 500.

FIG. 5. Slight intimal thickening associated with fragmentation of the elastic lamina at an apex. Verhoeff's stain. × 300.

FIG. 6. Slight intimal thickening but deficient elastic lamina at an apex. Verhoeff's stain. × 300.



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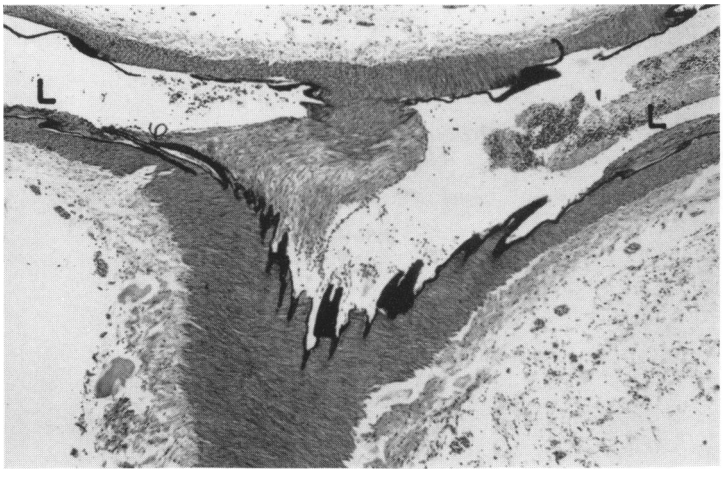

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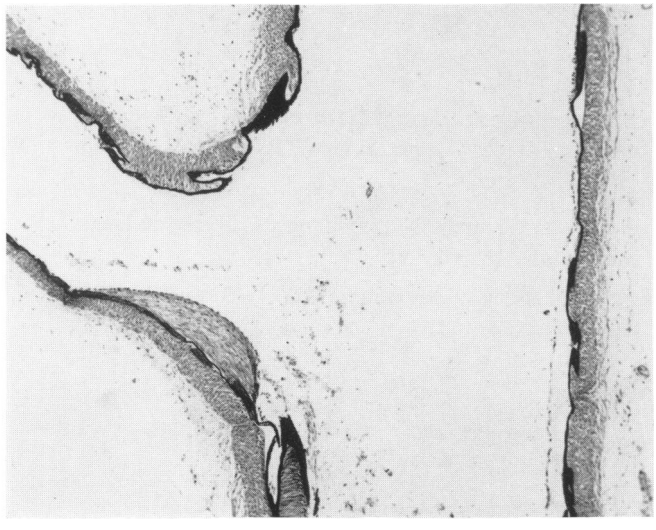

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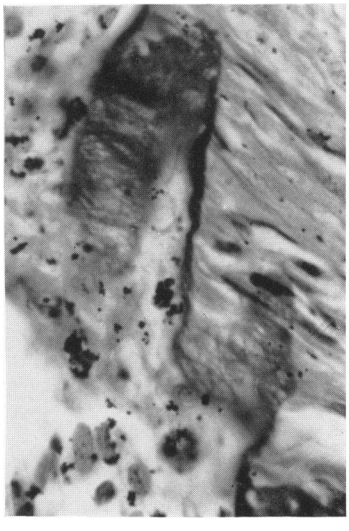

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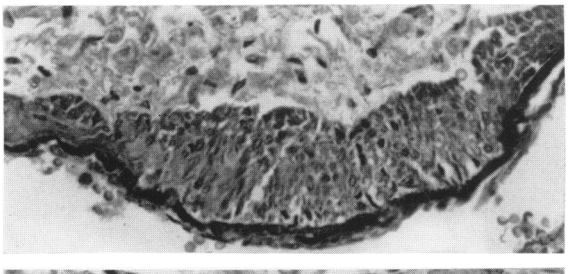

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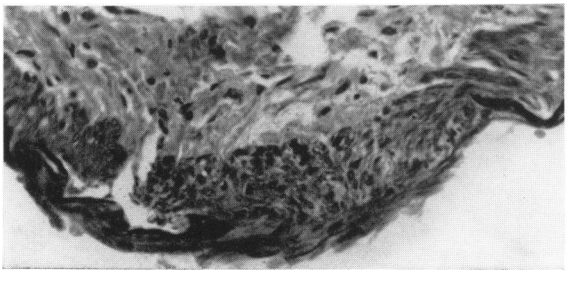

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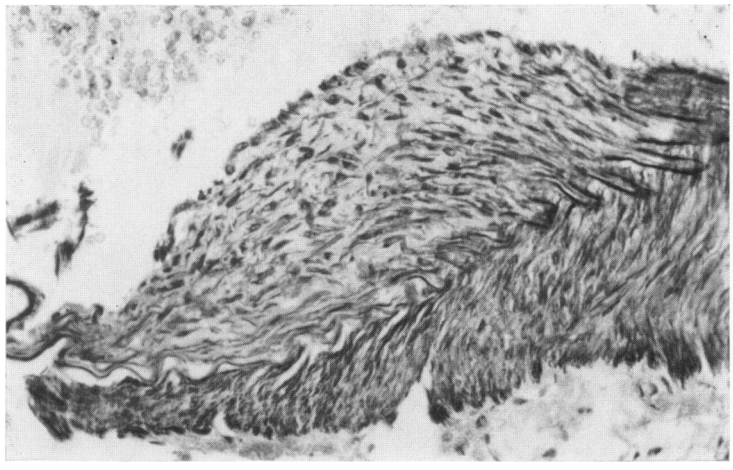
FIG. 7. A lateral angle pad containing muscle fibers more loosely arranged than in the media. Mallory's phosphotungstic acid-hematoxylin (PTAH) stain. × 300.

FIG. 8. A pad containing numerous elastic fibers. Verhoeff's stain. × 300.

FIG. 9. A larger pad than that shown in Figure 8. Numerous elastic fibers run longitudinally in the pad. Verhoeff's stain. × 300.

FIG. 10. A large pad with a palely staining elastic lamina. Newly formed laminas are seen at the edges of the pad (see arrows). Verhoeff's stain. × 75.



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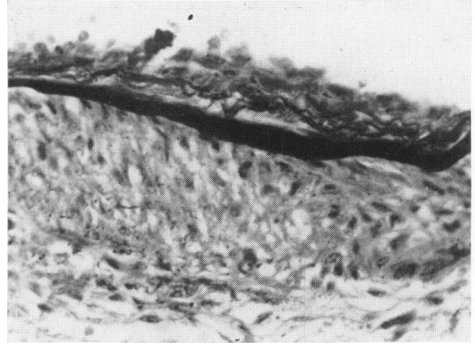

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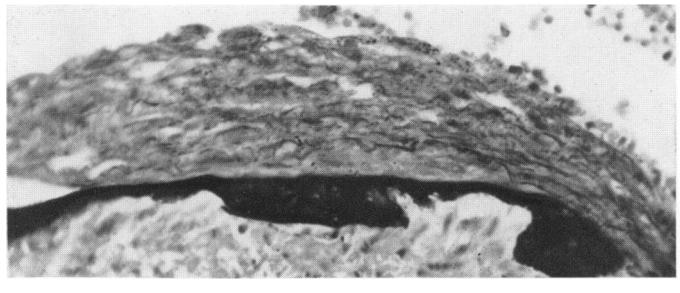

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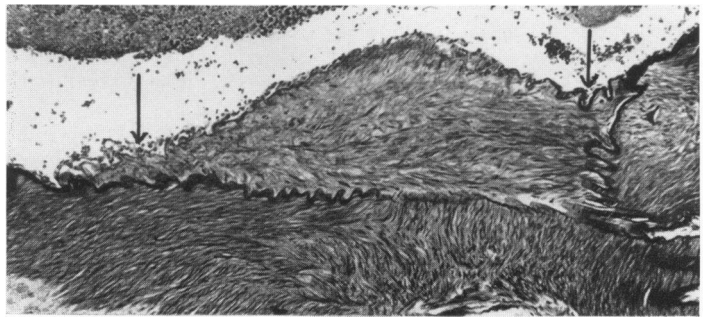

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FIG. 11. A pale, lacy elastic lamina beneath the endothelium at the edge of a pad. The original elastic lamina is palely stained when seen in tangential section. Formalin pigment appears on the surface of the pad. Verhoeff's stain. × 300.

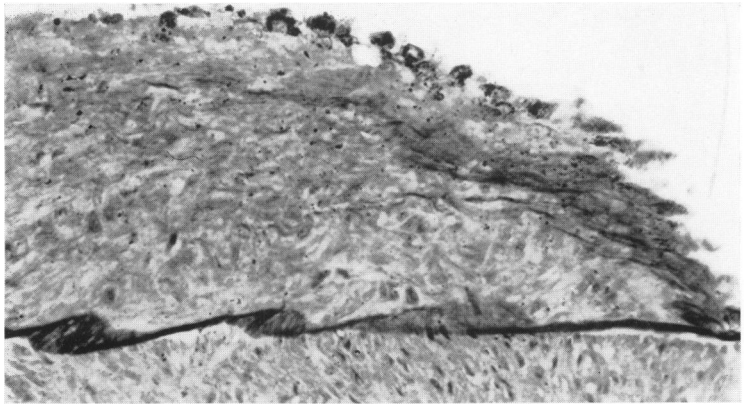
FIG. 12. Two thick elastic laminas in a facial pad, 6-months-old infant. The subendothelial lamina is beaded, though near the arrow it is palely stained and lacy. The original (deeper) lamina is also degenerated. Verhoeff's stain. × 150.

FIG. 13. The original elastic lamina is palely stained. A newly formed lamina in the middle of the pad is also pale and tends to divide the pad into two strata. Verhoeff's stain. × 75.

FIG. 14. Apparent splitting of the internal elastic lamina in an apical pad. Note the thinness of the original elastic lamina and subendothelial lamina at edges of the pad. Verhoeff's stain. × 300.

FIG. 15. An apical pad with gross degeneration of the internal elastic lamina, remnants of which separate the media from the intima. Verhoeff's stain. × 300.

FIG. 16. An apical pad with two elastic laminas. Note the gap in the deeper lamina and the irregularity of the superficial one. Verhoeff's stain. × 300.



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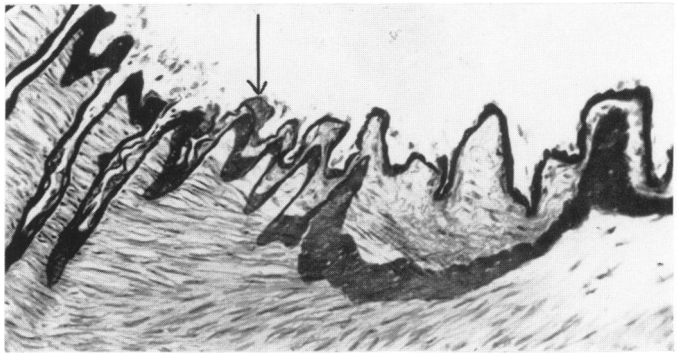

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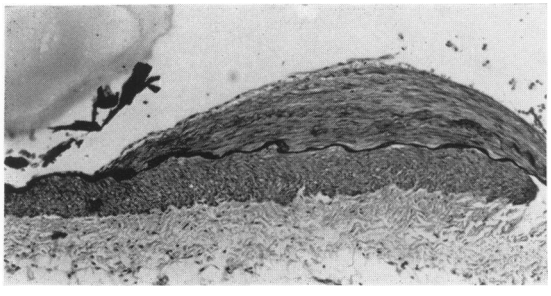

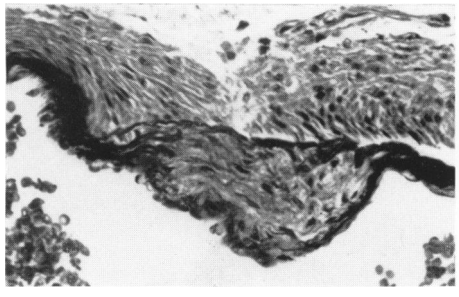

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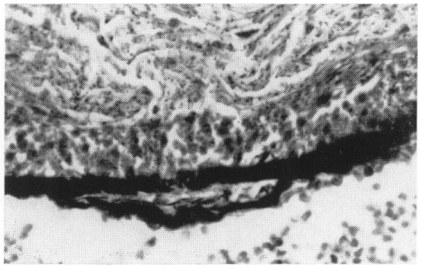

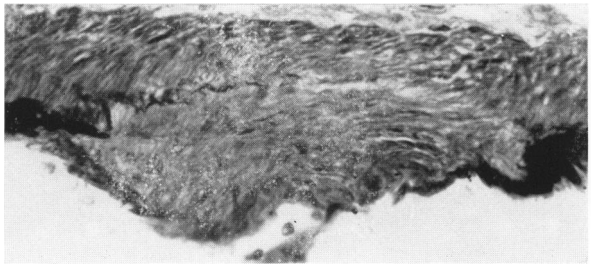

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