THE PATHOGENESIS OF CHOLESTEROL ARTERIOSCLEROSIS IN THE RABBIT

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(PLATES CIV-CVIII)

IT has been shown by one of us (Duguid, 1946 ; 1948) that in man lesions indistinguishable from atherosclerosis may result from the organisation of mural thrombi. When fibrin is deposited on the internal surface of an artery it becomes covered with endothelium and thus incorporated in the vessel wall, so that when it is subsequently organised, it forms a fibrous thickening of the intima. In the course of this process it may also undergo fatty change, and thus the characteristic picture of atherosclerosis is produced. On enquiring into the incidence of mural thrombi we have been impressed by the frequency with which minute deposits of fibrin are to be found in association with the familiar superficial fatty streaking of the aorta in quite young individuals, and it was suggested (Duguid, 1948) that these fatty streaks might constitute the primary lesions on which fibrin deposits were formed.

In considering the possible ways in which this idea might be put to the test it seemed to us that the nearest experimental counterpart to human fatty streaking was cholesterol sclerosis in the rabbit, and accordingly we decided to review this condition in order to see if fibrin deposits occurred in relation to it also. Our results in this respect were negative but they were none the less interesting in that they provided another, and to us quite unexpected, example of the way in which surface deposits come to be incorporated in the walls of arteries, the deposits in this instance being cholesterol-bearing cells.

Experiments

Twenty young rabbits, weighing about 2 kg. each, were given 1·5 g. of crystalline cholesterol daily and killed after periods ranging from 5 to 45 weeks. All of them developed vascular lesions of varying severity depending more or less on the length of time they were under treatment, but none of them showed mural deposits of fibrin. We do not propose to describe the lesions in detail, since they correspond with what has already been reported by many other observers, but we wish to draw attention to certain features which we think throw fresh light on the genesis of cholesterol deposits in arteries.

Histology

Rabbits killed in the second and third months of the treatment showed only slight lesions, chiefly in the aorta and pulmonary arteries, some of them consisting of nothing more than minute clusters of cholesterol-bearing foam cells lying on the internal surface of the vessels (fig. 1). The identification of these cells, which has long been a matter of controversy, was the first problem to which we directed our attention, and we were fortunate in obtaining some evidence from certain sections of the pulmonary arteries. In some of the rabbits with relatively advanced lesions the pulmonary arteries and in one case the aorta were found to be filled with blood *post mortem*, and we were able to cut frozen sections of these with their contents *in situ* (figs. 2-5). In them we found that the foam cells were not only deposited in the vessel walls, but were also scattered amongst the blood in the lumen (figs. 2 and 3), from which it was obvious that they were circulating in considerable quantities in the blood. In many areas the walls were thickly covered with foam cells and a notable feature of these formations was the variability of the endothelial lining. In parts this was missing, so that quite large masses of foam cells were exposed to the blood stream. This appearance at first sight suggested erosion of the endothelium over the cholesterol deposits with discharge of their contents into the blood, but further examination convinced us that this was not the case, for in other parts, where the endothelium was intact, occasional foam cells could be seen adhering to its inner surface (figs. 4 and 5). Such a picture, it seemed to us, could be explained only on the assumption that the cells in question were being deposited on the surface of the vessel from the blood stream (see also fig. 8). Accordingly we concluded that the picture shown in fig. 1 represented a deposit of this kind, and was in fact the initial stage in the development of the cholesterol lesion. This appearance was a comparatively rare finding, for in the great majority of lesions the foam cells were covered with endothelium, as is commonly described (figs. 6-10). Sometimes it was a rather delicate and incomplete covering, as in figs. 6-8, but usually it was better formed, the cells being thicker and more numerous than in the normal vessel (fig. 9), whilst occasionally there was in addition a well-defined sub-endothelial layer of connective tissue (fig. 10).

In studying these appearances we were reminded of the process occurring in human arteries, where mural thrombi, on being covered with endothelium, become part of the vessel wall, and we were convinced that the same natural process was in operation here. Cholesterol-bearing cells circulating in the blood were settling out and adhering to the vessel walls and, by a readjustment of the endothelial lining, were being incorporated in the walls. All our subsequent findings seemed to fit in with this idea.

In some of the prolonged experiments, lesions of extreme severity

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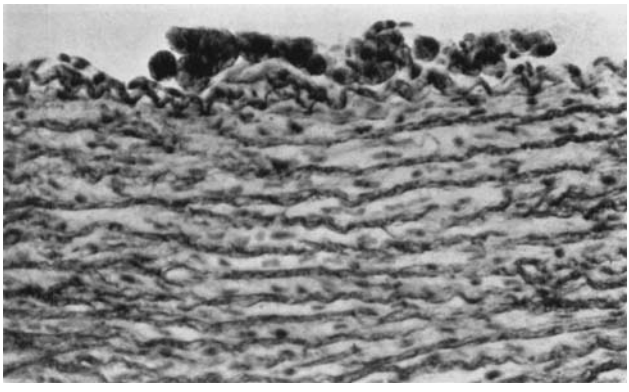

FIG. 1.—Frozen section. Rabbit N. 3, 6 weeks. Aorta showing clusters of foam cells lying on the intimal surface. Hæmalum and Sudan IV. ×230.

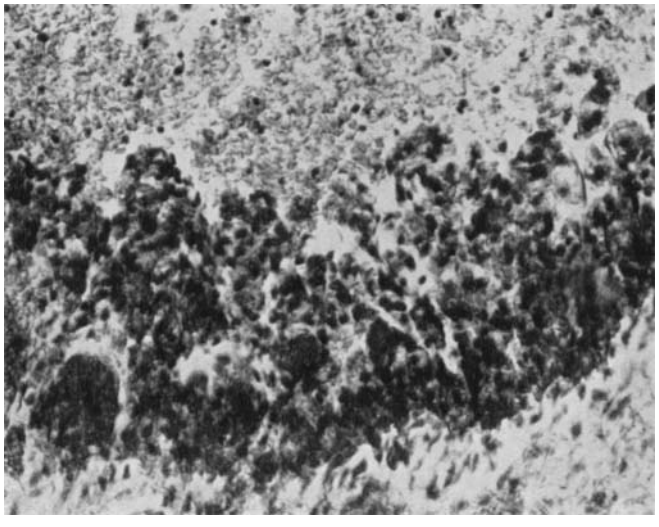

FIG. 2.—Frozen section. Rabbit N. 1, 7 weeks. Pulmonary artery showing intimal surface with a thick layer of foam cells lying in contact and partly intermingled with the blood in the lumen (above). Scattered foam cells are also seen in the blood (see fig. 3). A few endothelial cells can be made out on the surface towards the left. Hæmalum and Sudan IV. ×230.

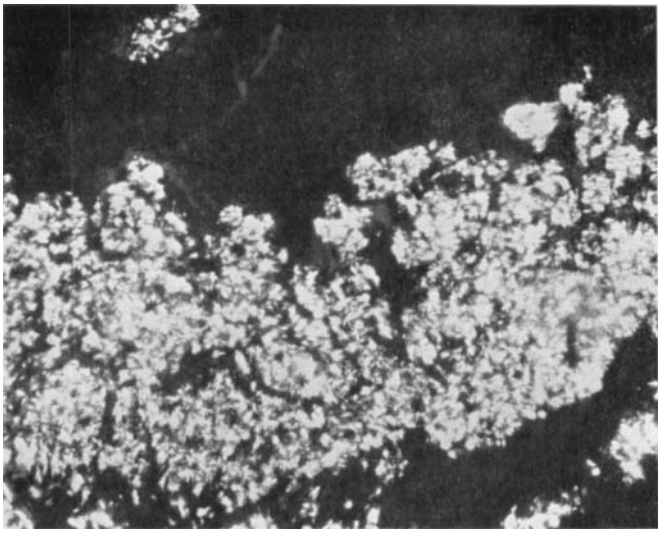

FIG. 3.—As fig. 2, viewed with polarised light.

were found, with great thickenings of the arteries, especially the aorta, where the intima was sometimes increased to several times the thickness of the media (fig. 11). These thickenings were of course extremely variable, but it was noticeable that, in the superficial zones, layers of foam cells similar to those already described were nearly always in evidence, whereas in the deeper zones, towards the media, there was often a state of extreme disorganisation, the foam cells having disintegrated, leaving free cholesterol mixed with fatty debris in the form of a more or less amorphous ground substance. These appearances seemed to indicate that the formative part of the process was on the surface, whilst in the deeper layers the lesions were of longer standing and had undergone degeneration.

Another significant feature of the superficial zones was the tendency for the foam cells to be distributed in layers (figs. 12 and 13). This was not always very distinct, since post-mortem contraction of the vessels tended to throw the intimal surface into folds which obscured the formation, but careful examination usually showed a fairly distinct stratification, the layers of foam cells being separated from one another, sometimes by layers of fibrous tissue and sometimes by sheets of flattened cells which bore a striking resemblance to endothelium (fig. 12). In some instances these sheets were seen to be continuous with the surface endothelium, and there could be little doubt that they were in fact endothelium which had originally been on the surface but had later become buried under further deposits of foam cells. Even where thick bands of connective tissue occurred, it was notable how closely the connective-tissue cells resembled those of the endothelium, so that it was difficult to avoid the conclusion current among the early observers (Baumgarten, 1902-04) that endothelium may play a part in connective tissue formation.

The above is a very brief account of some of the salient features of the experimental lesions. Many interesting variations have been passed over without mention, but none of them seemed to conflict with the idea we have put forward, namely that the cholesterol lesion represents a process of recurring deposits of foam cells on the surface of the vessel. Each deposit becomes covered with endothelium and the endothelium in its turn covered by further deposits, while, as the intima thickens, the older deposits, deeply buried under the surface, tend to degenerate and form atheromatous foci.

Discussion

The production of arterial lesions in animals by cholesterol is not likely to throw much light on human atherosclerosis until we can decide exactly how the experimental lesions are produced, and there is as yet no agreement on this point. Some observers, including Duff (1936), Hueper (1944) and Altschul (1950) believe that the foam cells are derived from the intima, and represent endothelial or

connective tissue cells which have taken up cholesterol from the body fluids or tissues. A different view has been put forward by Leary, and upheld by him (1949) through years of opposition. Leary holds that the foam cells originate in the liver and are transported to the arteries by the blood. He suggests that they adhere to the vessels and creep through the endothelium so as to invade the intima, and our interpretation coincides with this in all but the final point. To us it seems that the foam cells play no active part in penetrating the vessel walls but become included in them by a simple process of readjustment of the endothelium. Thus the cholesterol lesions fall into line with our findings in human atherosclerosis, and illustrate once again the principle that solid matter deposited in the vessels becomes incorporated in their walls.

Our failure to find mural thrombi in relation to these lesions is not without significance, since it adds some negative information to the pathology of thrombosis. It has been assumed that the integrity of the endothelium is a factor in the prevention of intravascular thrombosis, but we have frequently found the endothelium to be missing without any sign of thrombosis, and we must conclude that the vessel lining is of less importance in this respect than we have been led to suppose.

Summary

Experimental cholesterol lesions in rabbits' arteries are formed by cholesterol-bearing cells which circulate in the blood and are deposited on the intimal surface of the arteries. These deposits become covered with endothelium and in this way are incorporated in the intima. In prolonged experiments, successive deposits are heaped up on top of one another and produce great thickenings, whilst the older deposits, as they become deeply buried, tend to degenerate and form atheromatous foci.

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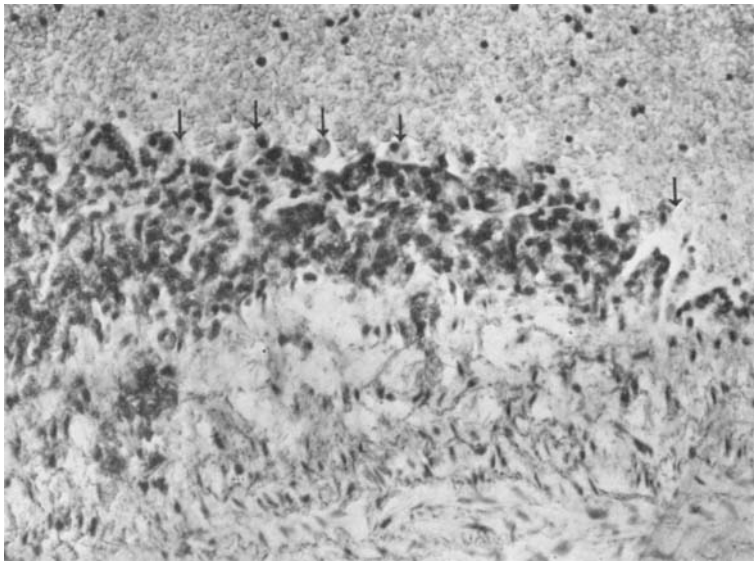

FIG. 4.—Frozen section. Rabbit N. 1, 7 weeks. Aorta with blood in the lumen (above). Here the endothelium, though not well shown, is intact, but several isolated foam cells are seen lying on its surface as indicated. Hæmalum and Sudan IV. ×230.

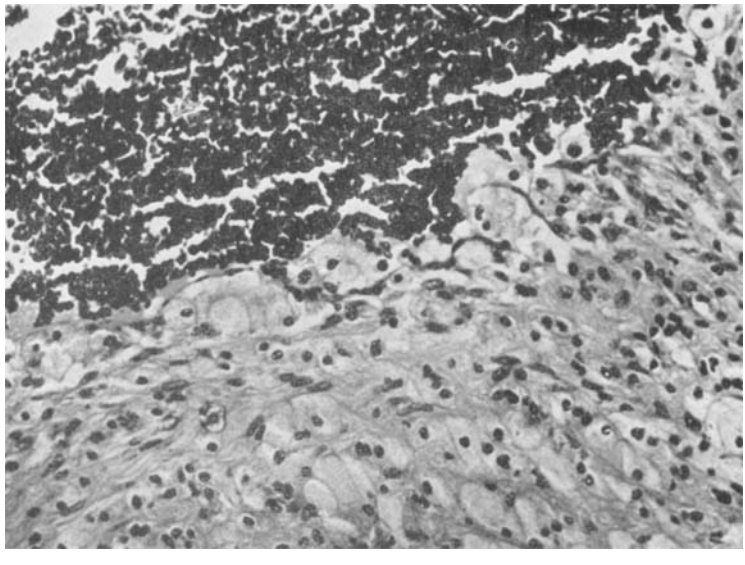

FIG. 5.—Paraffin section. Rabbit N. 19, 5 weeks. Here the endothelium can be traced out over most of the section but in several places foam cells are seen lying singly or in clusters on its intimal surface. Near upper left-hand corner a large foam cell is lying free in the blood. Hæmalum and eosin. ×230.

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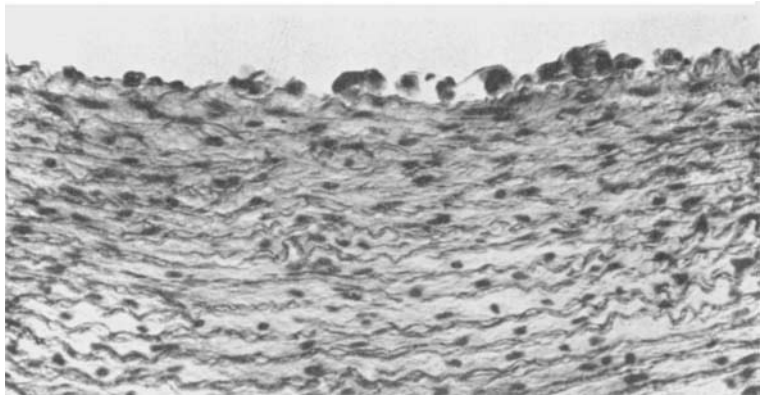

FIG. 6.—Frozen section. Rabbit N. 3, 6 weeks. Aorta showing foam cells incompletely covered by endothelium. Hæmalum and Sudan IV. ×230.

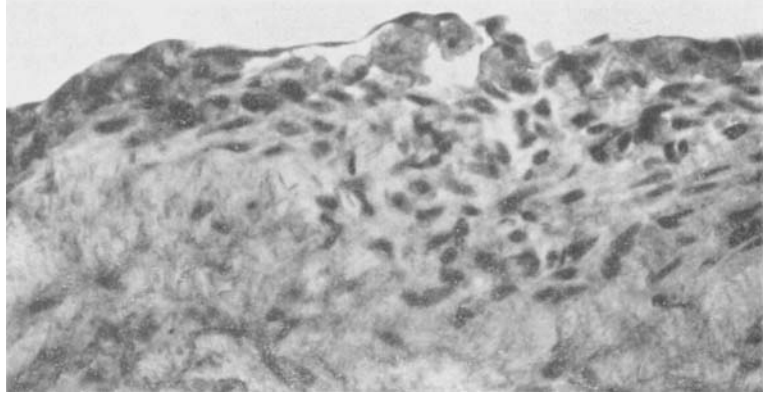

FIG. 7.—Frozen section. Rabbit N. 1, 7 weeks. Aorta showing foam cells incompletely covered by endothelium. Hæmalum and Sudan IV. ×350.

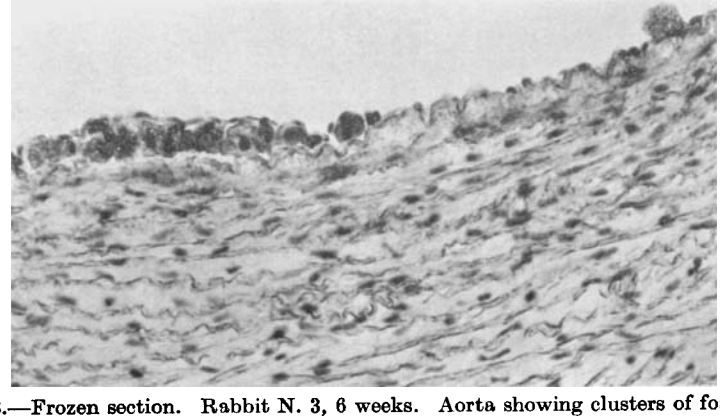

FIG. 8.—Frozen section. Rabbit N. 3, 6 weeks. Aorta showing clusters of foam cells on left covered by endothelium and, towards the right, a solitary foam cell adhering to the surface. Hæmalum and Sudan IV. ×230.

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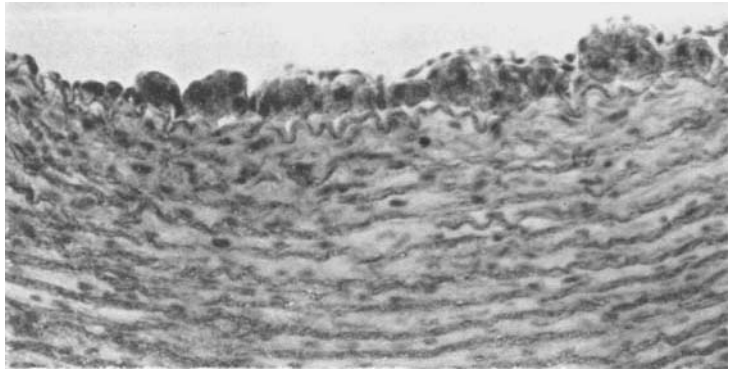

FIG. 9.—Frozen section. Rabbit N. 3, 6 weeks. Aorta showing a cluster of foam cells on the intimal surface covered by a more pronounced layer of endothelium. Hæmalum and Sudan IV. ×230.

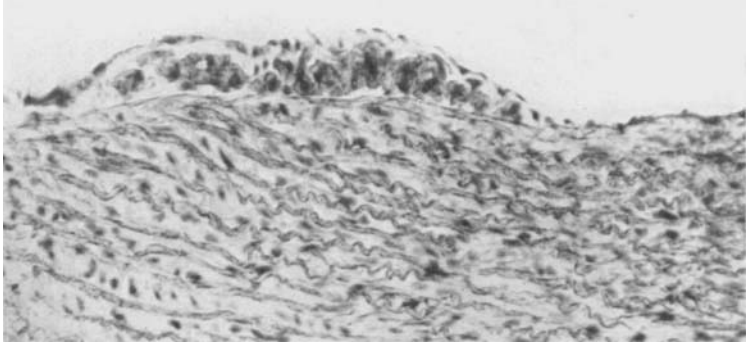

FIG. 10.—Frozen section. Rabbit N. 3, 6 weeks. Aorta showing a cluster of foam cells covered by endothelium and a well-defined subendothelial layer of connective tissue. Hæmalum and Sudan IV. ×230.

FIG. 11.—Frozen section. Rabbit N. 14, 45 weeks. Aorta showing great intimal thickening. The dense black surface layer represents foam cells. Beneath it the tissue is degenerate and consists of layers of fatty debris alternating with layers of connective tissue. The dark area near the centre is a focus of calcification. The vessel in this case was enormously dilated, but the media (below), though stretched, was still intact. Hæmalum and Sudan IV. ×40.

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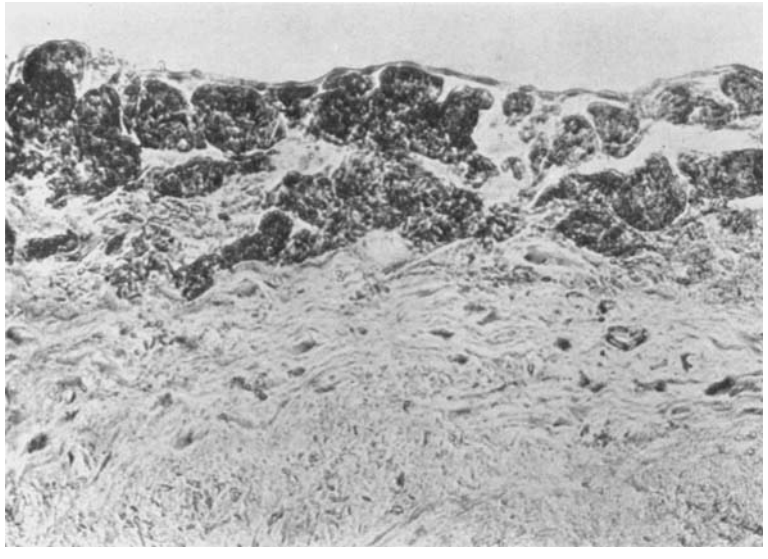

FIG. 12.—Frozen section. Rabbit N. 14, 45 weeks. From the same animal as fig. 11 but another part of the aorta, showing stratification of the foam cells in the surface zone of the intima. In the subjacent layer free cholesterol crystals and other debris are mixed with the bands of fibrous tissue. Hæmalum and Sudan IV. × 230.

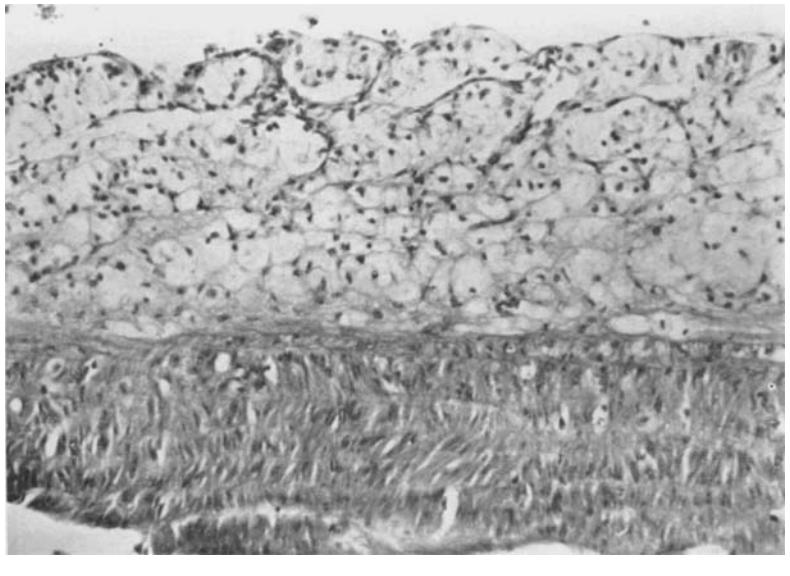

FIG. 13.—Paraffin section. Rabbit N. 8, 8 weeks. Pulmonary artery showing layers of foam cells with sheets of spindle cells between them. At one point at least continuity can be traced between the surface endothelium and the sheets of spindle cells. In the deeper layers adjacent to the media there is commencing disintegration of foam cells. Hæmalum and eosin. × 180.