THE DEVELOPMENT OF THE PSEUDOINTIMA LINING FABRIC GRAFTS OF THE AORTA

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IN a previous communication (Florey, Greer, Poole and Werthessen, 1961) it was shown that knitted " Dacron " grafts inserted into the lower abdominal aortae of East African baboons (*Papio doguera*) had become lined by a layer of tissue (the pseudointima) 10 weeks after operation. The innermost layer of the pseudointima consisted of endothelium which extended over the whole inner surface of the graft and was continuous with the endothelium lining the real aorta. The rest of the pseudointima consisted of cells and fibres: the predominant cell type was the smooth muscle cell and the fibrous components comprised collagen, elastic tissue and some unidentified fine fibres. As the healing process had reached such an advanced stage it was impossible to draw any conclusions about the sequence of events which had led up to this state of affairs.

The present communication reports the findings in baboons with similar grafts which were killed at intervals of from 1 week to 3 months after operation.

MATERIAL AND METHODS

All techniques are essentially as described in the previous paper (Florey *et al.*, 1961). Fourteen baboons had tubes of knitted " Dacron " 3 cm. long inserted in the lower abdominal aorta by S. J. G. and were killed between 7 and 22 days after operation. Four further baboons had been operated on similarly by J. K. and R. T. with the insertion of knitted " Dacron " tubes 10 cm. in length ; these 4 animals were killed 12 weeks after operation. The surgical operations were carried out, and the animals were killed, at Darajani. Certain microscopical preparations were made there by J. C. F. P. and further material was brought to Oxford for histological examination and for electron microscopy. The specimens for electron microscopy were treated with phosphotungstic acid and imbedded in Araldite at Darajani and transported as completed blocks. In the previous study material had been sent from San Antonio in Texas to Oxford in 70 per cent alcohol after fixation. The tissues examined in the present investigation were in general much better preserved and it seems clear that when material for electron microscopy has to be transported over considerable distances it is desirable, if possible, to arrange for the imbedding to be carried out at the place where the animals are killed, even when, as in the present case, the conditions under which imbedding has to be carried out are far from ideal.

RESULTS

The initial lining of the graft

In animals killed at about 1 week after operation the fabric tube was found to be lined by a thin layer, which in the electron microscope, was seen to consist of agglutinated platelets, leucocytes and some densely-staining fibres which are probably fibrin although it was not possible to demonstrate the characteristic

banding (Fig. 1 and 2). These appearances closely resemble those of the initial stages of thrombus formation (Bizzozero, 1882). Platelet agglutinates in thrombi have been examined in the electron microscope by Policard, Collet and Giltaire-Raylte (1955), Pease (1956), Levene and Levene (1957), Poole and French (1961) and French (1962) who illustrated appearances similar to those found in the present study.

In places non-granular bodies could be seen at the surface of platelet aggregations (Fig. 3). These non-granular bodies closely resemble those seen in other studies with the electron microscope on the appearance of platelet aggregations *in vitro* (Parmeggiani, 1961 ; Rodman, Mason, McDevitt and Brinkhous, 1962 ; French and Poole, 1962) and are possibly the same structures as can be seen around platelet clumps *in vitro* with the phase-contrast microscope (see Sharp, 1961). The present observations, by indicating that these non-granular bodies can occur *in vivo* add interest to the *in vitro* studies.

Formation of the endothelial lining

In 12 of the animals, the aorta was stained by silver nitrate and fixed by perfusion and the graft and adjacent parts of the aorta were slit open, pinned out flat and cleared so that the endothelial " cement-line " pattern could be examined *en face*. In no case was the endothelial lining complete. At each end of the graft, the endothelium lining the graft was continuous with the endothelium lining the real aorta and its appearance did not change at the suture line. The extent of that part of the endothelial lining which was continuous with the lining

EXPLANATION OF PLATES

FIG. 1.—Platelets (P), leucocytes (L) and fibrin (dark bands) lining the graft one week after operation as seen in section in the electron microscope. ×5500.

FIG. 2.—Platelets and fibrin from the same specimen as Fig. 1. ×24,000.

FIG. 3.—Platelets and non-granular bodies from the same specimen as Fig. 1. ×20,000.

FIG. 4.—Häutchen preparation of growing edge of endothelium. Silver nitrate and Weigert's iron haematoxylin. ×360.

FIG. 5 (*a*).—Mouth of a small vessel connected with the lumen of the graft. Two weeks after operation. Specimen stained by silver nitrate and cleared in clove oil. ×145.

FIG. 5 (*b*).—Drawing of the area shown in Fig. 5 (*a*).

FIG. 6 (*a*).—Montage of photomicrographs of an endothelial island. From the same specimen as Fig. 5. Numerous mouths (M) of small blood-vessels are seen. ×50.

FIG. 6. (*b*).—Drawing of the area shown in Fig. 6 (*a*).

FIG. 7.—Diagram showing area covered by endothelium 12 weeks after operation. Endothelium is represented white and the bare area black. The zigzag lines mark the ends of the graft. The specimen had been opened longitudinally and pinned out flat.

FIG. 8.—Electron micrograph of section through normal baboon aortic endothelium. J : cell junction ; arrow : fibrillar region ; S : stacks of vesicles to be compared with those in a cell in Fig. 11. ×15,600.

FIG. 9.—An area adjacent to and partly overlapping that shown in Fig. 8. CI : caveolae intracellulares. Other symbols as in Fig. 8. ×18,000.

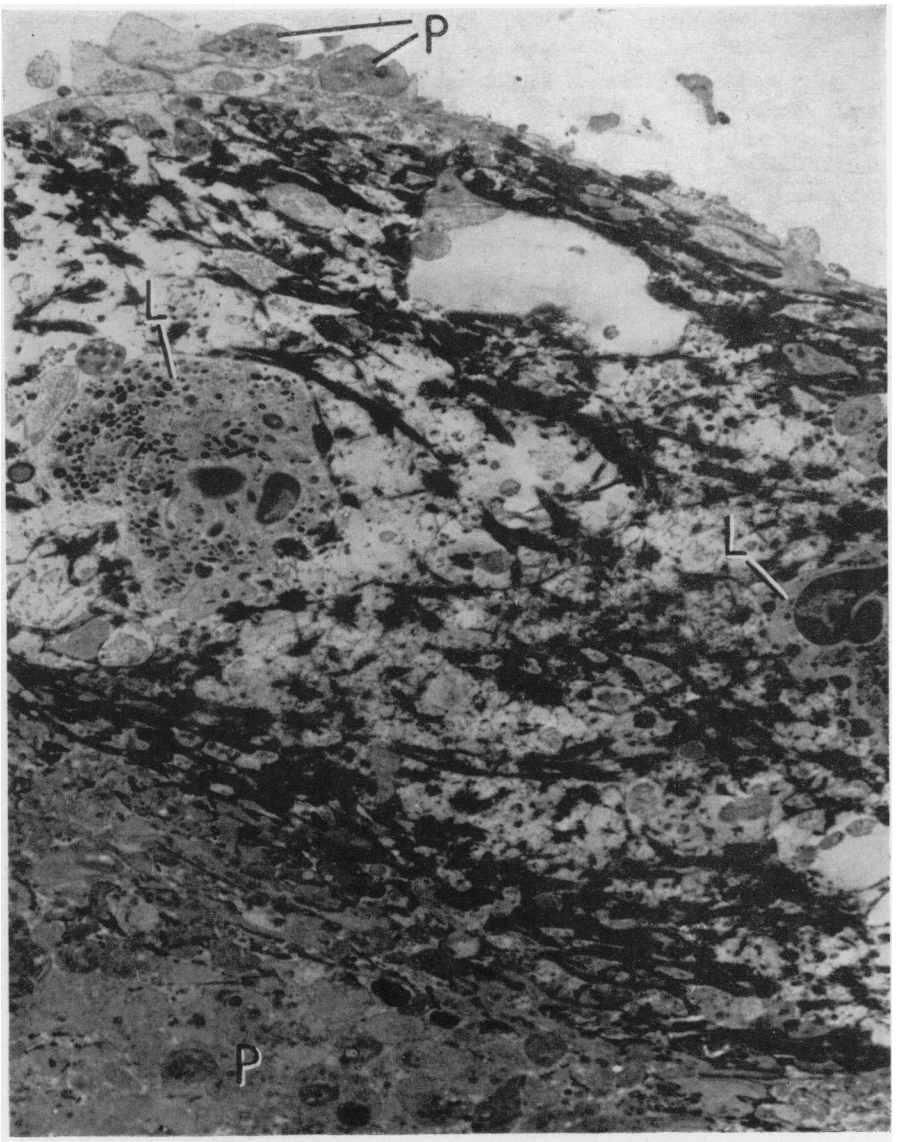
FIG. 10.—Endothelium lining the graft. ER : endoplasmic reticulum. Other symbols as in Fig. 8. ×17,500.

FIG. 11.—Part of an endothelial cell lining the graft (above) and parts of three fibroblasts deep to it. M : mitochondria ; ER : endoplasmic reticulum. Note stacks of vesicles (S) in endothelial cell and compare with those in Figs. 8 and 9. ×16,000.

FIG. 12.—The deep surface of an endothelial cell lining the graft to show vesicles (V) and caveolae intracellulares (CI). ×46,000.

FIG. 13.—Smooth muscle cells (SM) and fibrous elements in the pseudo-intima. COL : collagen fibres. ×14,000.

FIG. 14.—Fibres in the pseudointima. COL : collagen fibres ; EL : elastic tissue. ×47,000.



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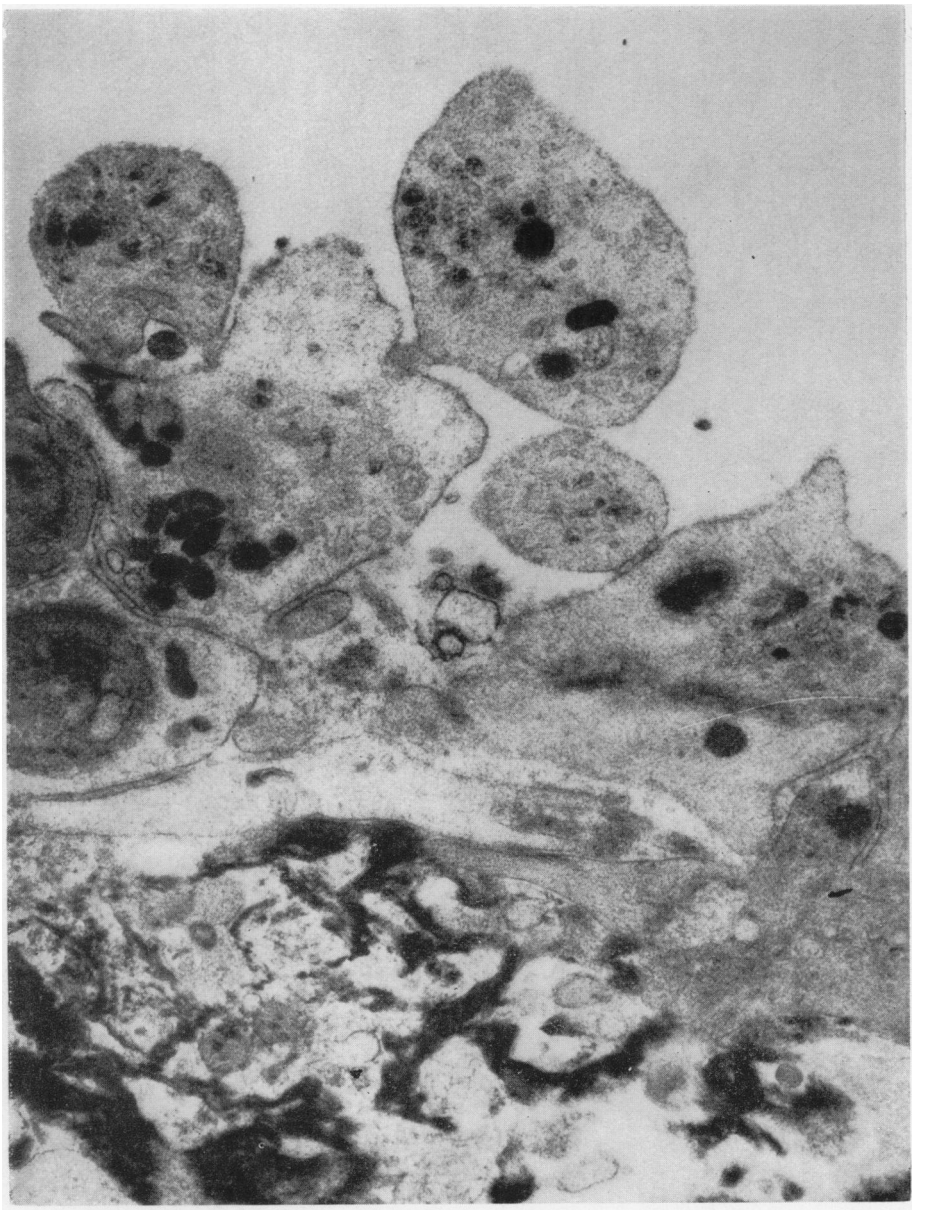

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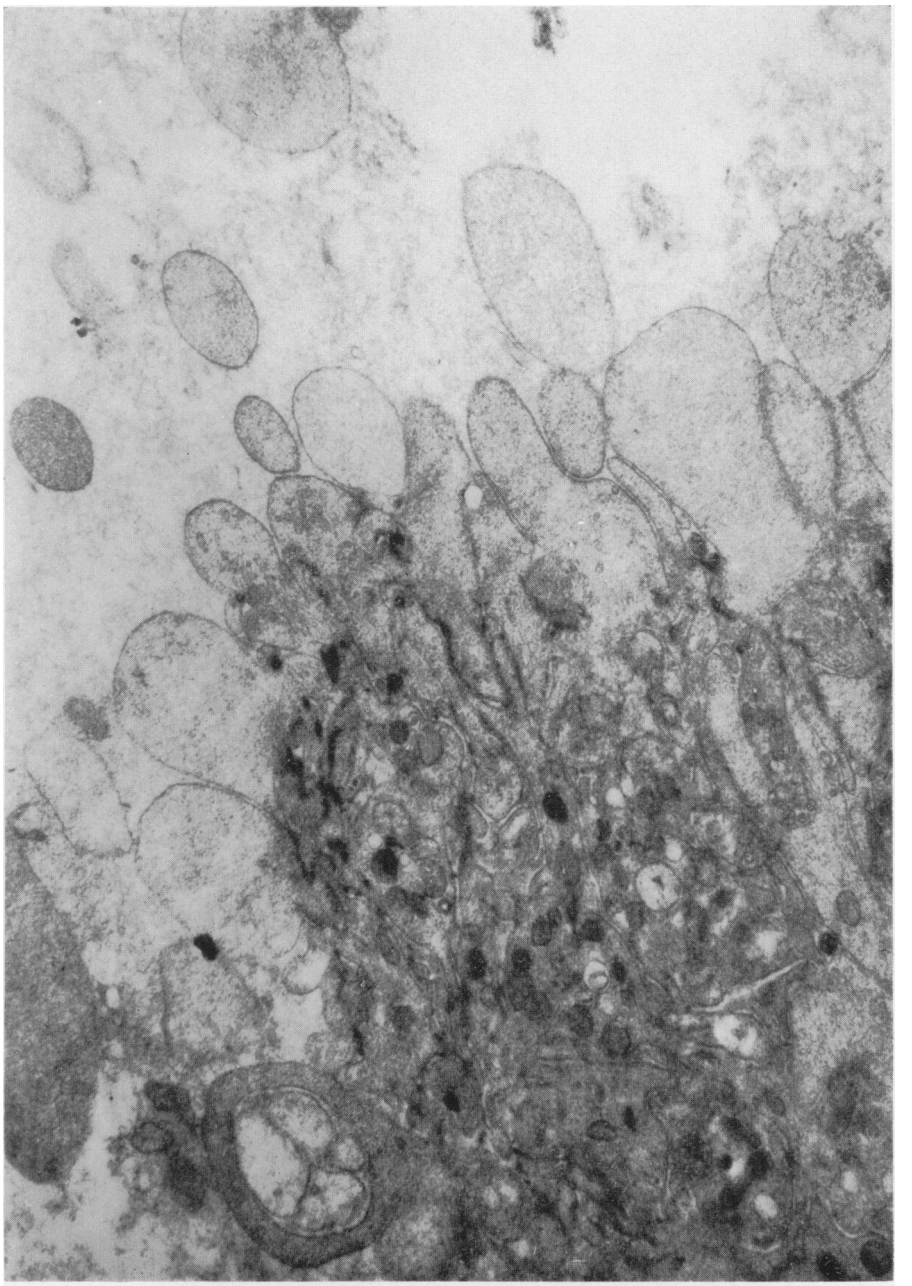

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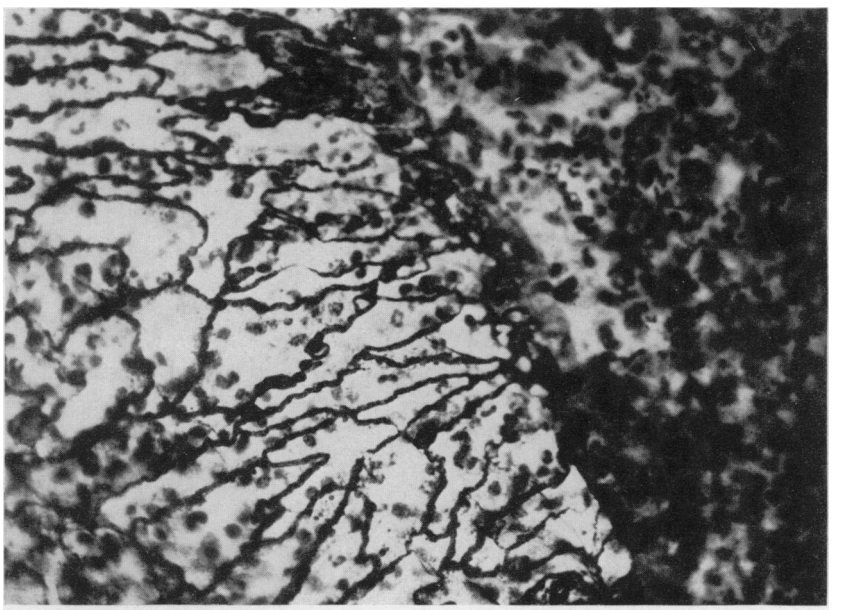

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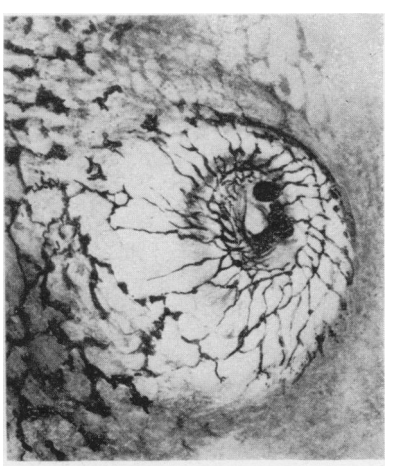

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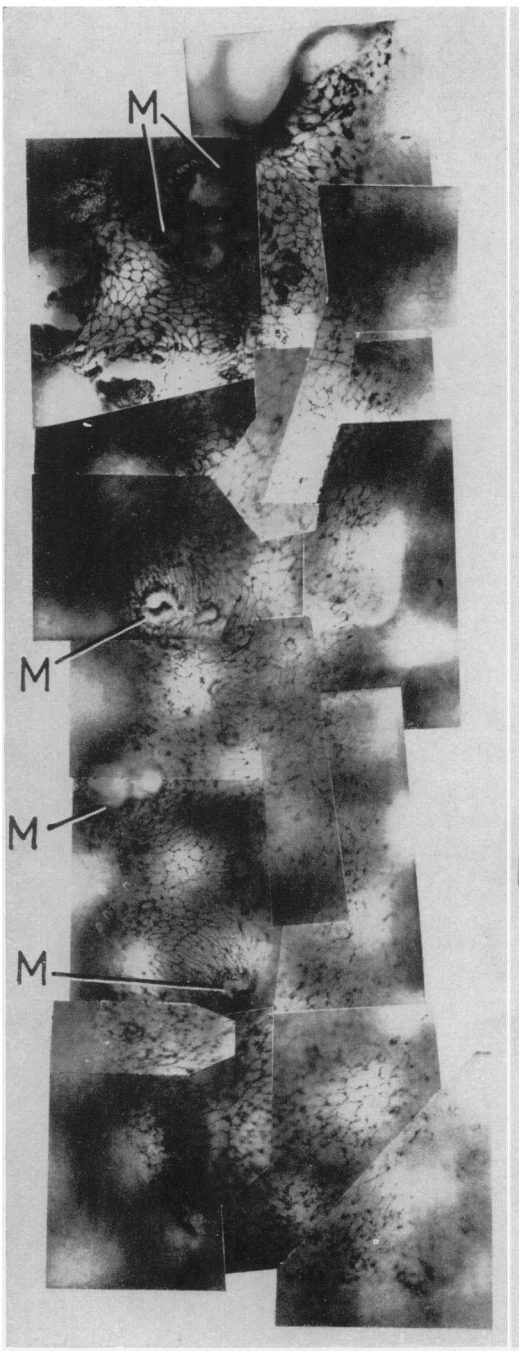

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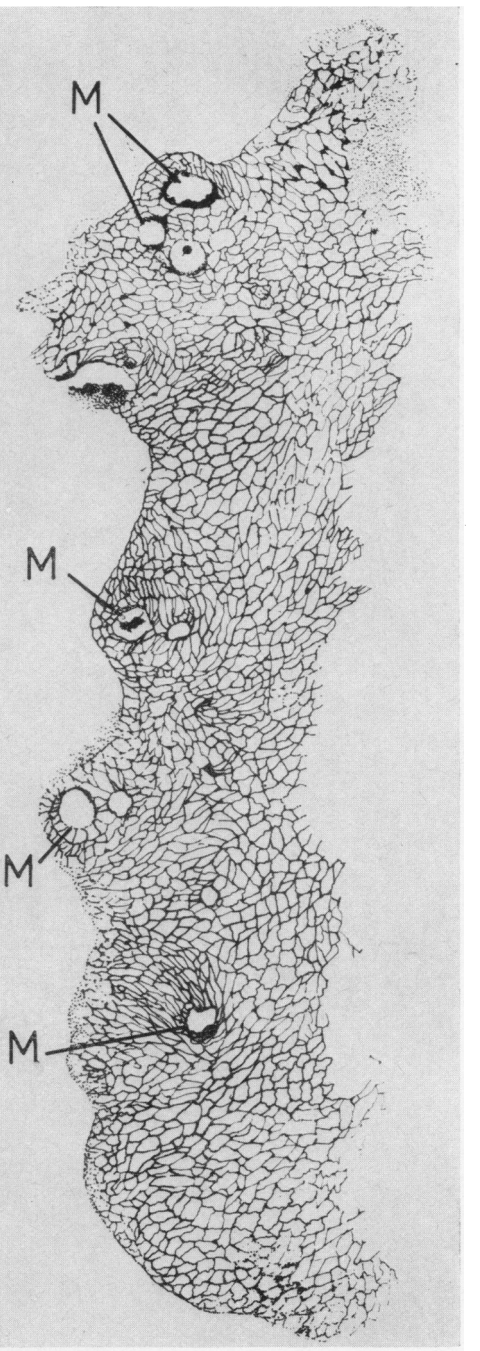

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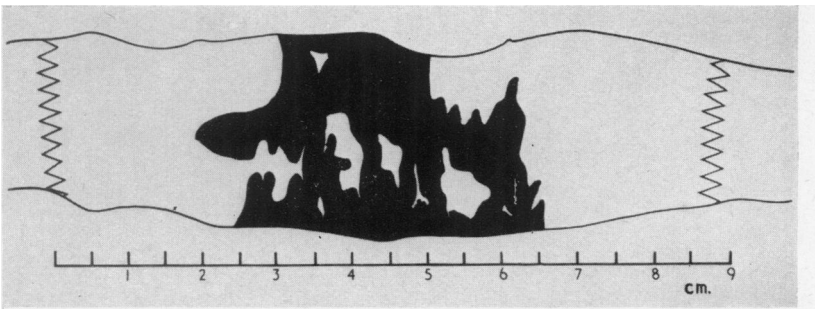

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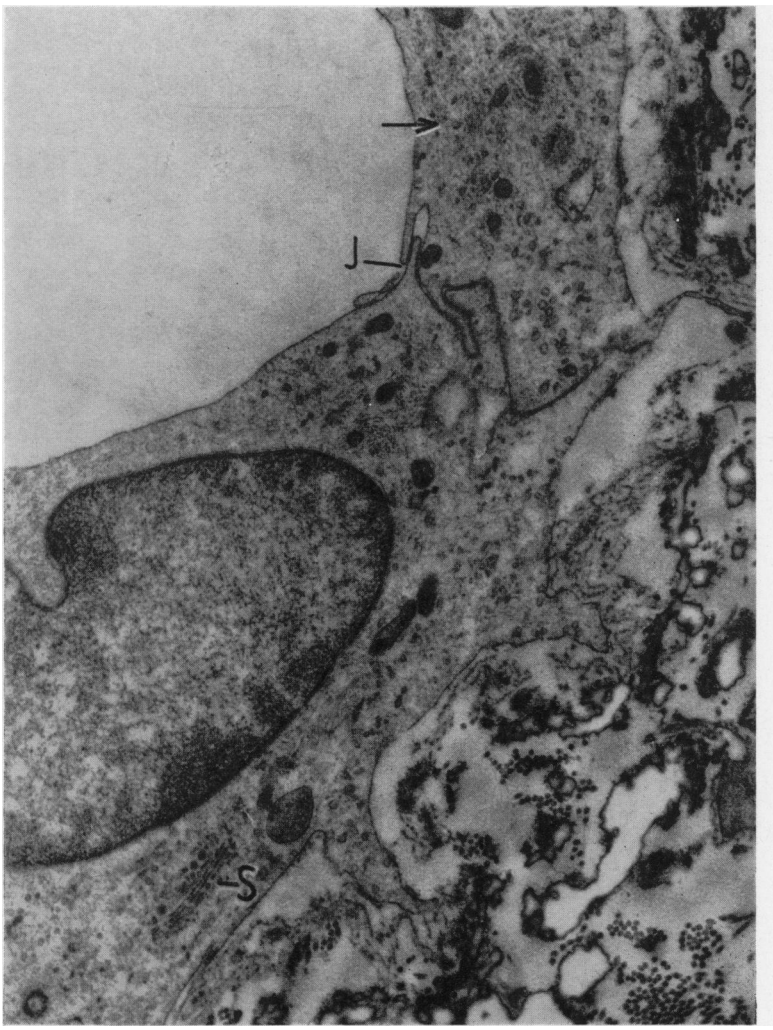

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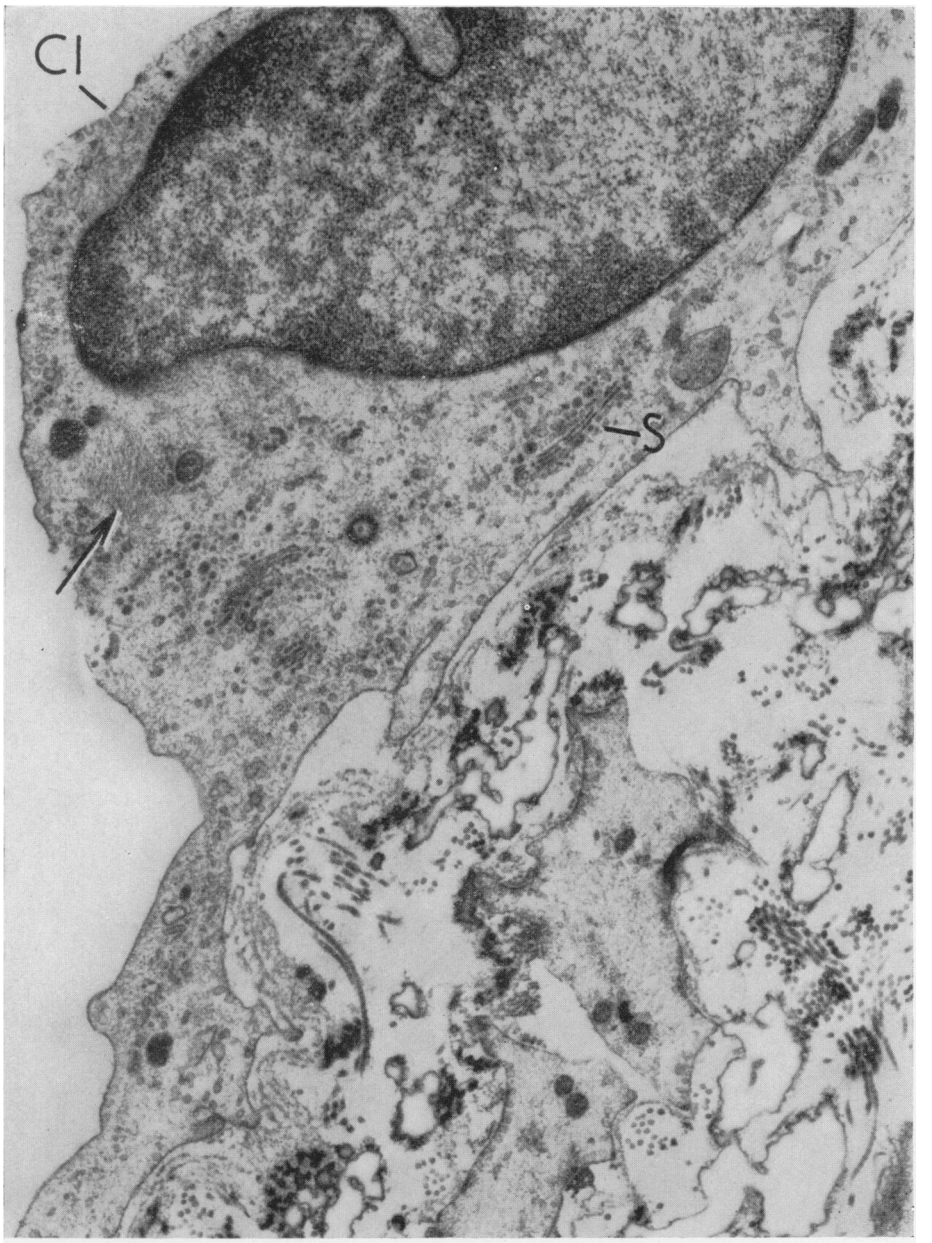

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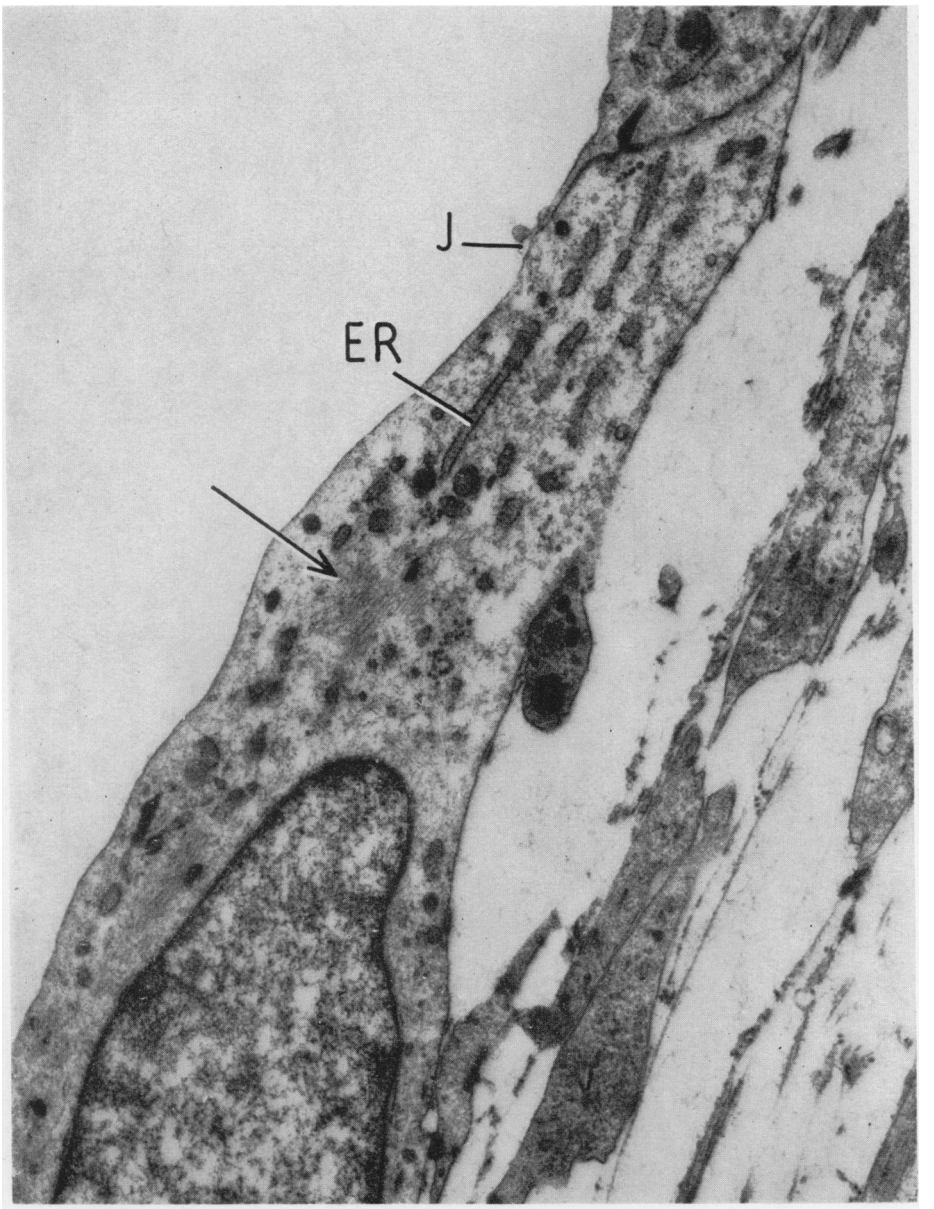

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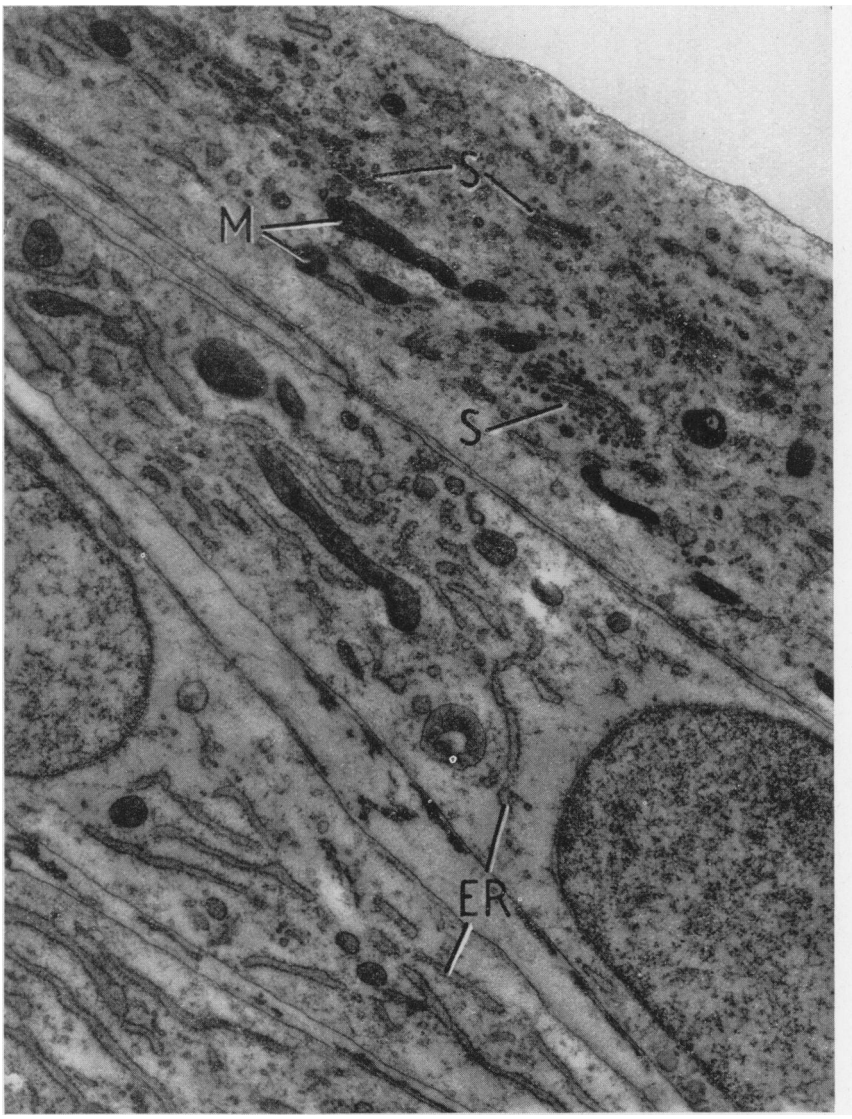

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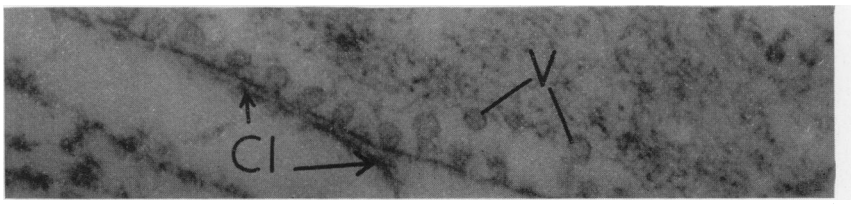

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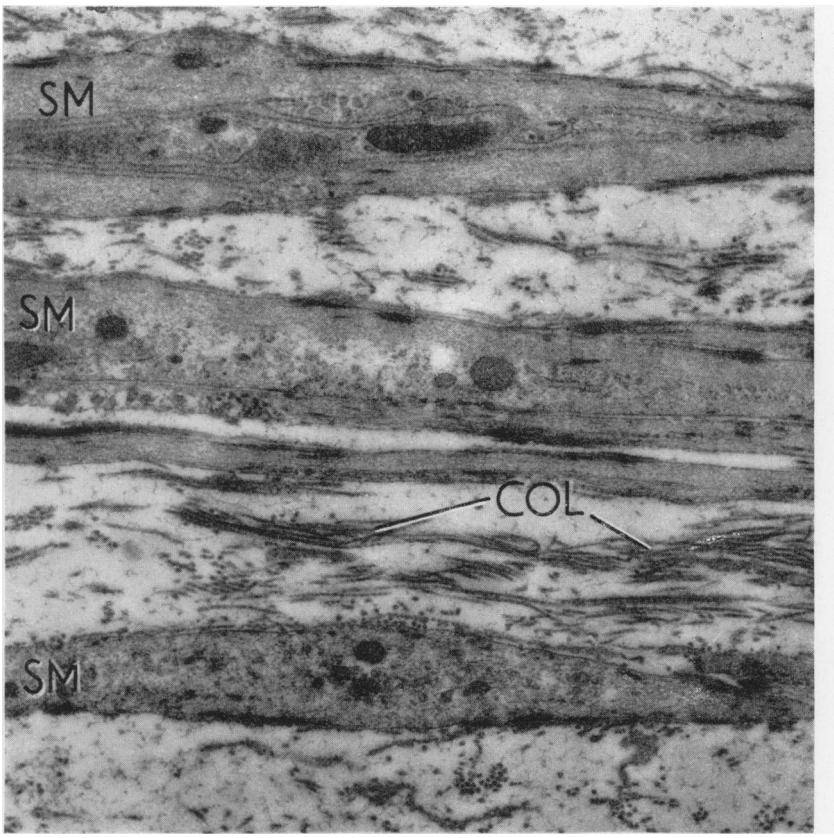

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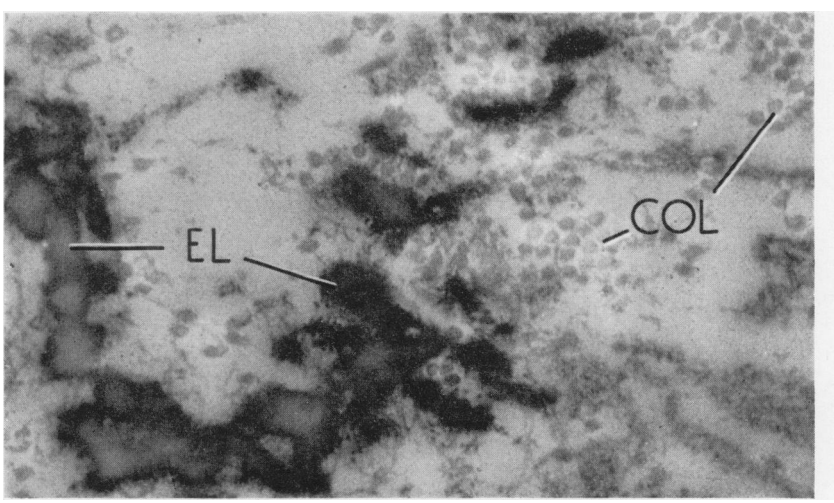

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of the aorta increased with increasing time since operation. The margins of the newly formed endothelium showed large, irregularly shaped cells (Fig. 4) reminiscent of those seen in healing rabbit aortic endothelium (Poole, Sanders and Florey, 1958).

The endothelial lining of the graft was not, however, formed only by extension of the lining of the real aorta at the ends of the graft, since, from about 2 weeks after operation onwards, there were detached islands of endothelium to be seen scattered irregularly over the inner surface of the graft, and these islands increased in size with the passage of time. Within the area of each island one or more mouths of vascular channels could always be seen. At an early stage (Fig. 5) the island consisted of no more than a rim of endothelium surrounding the mouth of the channel. A more developed island is shown in Fig. 6. While it is clear that growth of endothelium from the margins of the mouths of these branches makes an important contribution to the endothelial lining of the graft, it is not clear how the branches themselves develop. They often appear to pass through interstices in the fabric and are probably connected with vessels in the connective tissue surrounding the fabric tube though it cannot be shown that they certainly arise from this layer.

The distribution of endothelium in one of the specimens obtained 12 weeks after the operation is seen in Fig. 7. The configuration observed suggests that there has been growth of endothelium not only inwards from the ends but also from the extension of islands. A number of islands may have been incorporated in the continuous sheets of new endothelium adjacent to the suture lines.

The appearance of newly formed endothelium in the electron microscope

Figs. 8 and 9 show electron micrographs of normal baboon aortic endothelium. The appearances resemble those of aortic endothelium in other mammalian species. Figs. 10, 11 and 12 illustrate new endothelium lining the graft. Densely fibrillar regions similar to those indicated by arrows in Figs. 8, 9 and 10 have been described before by several workers (see Altschul, 1961, for references) in endothelial cells. Typical caveolae intracellulares were observed in the newly formed endothelium. They are illustrated at a high magnification in Fig. 12. Stacks of vesicles (S in Figs. 8, 9 and 11) were seen in normal endothelial cells and in the newly formed endothelium. No similar structures could be made out in any of the other cell types encountered in this study.

Other cellular and fibrous components of the pseudointima

Abundant collagen could be found in the pseudointima 3 weeks after operation. This was presumably formed from fibroblasts, and cells with the usual structural features of fibroblasts could be demonstrated in the same regions (Fig. 11). At this stage, neither elastic tissue nor smooth muscle cells could be found. Both were, however, present in graft tissue which had been developing for 12 weeks (Figs. 13 and 14).

Occasional polymorphonuclear leucocytes and macrophages were seen both 3 and 12 weeks after operation. Many cells and parts of cells were seen in the electron micrographs which could not be identified with certainty and it is impossible to say whether or not cells occur which belong to categories other than those enumerated above.

DISCUSSION

Development of the endothelial lining

When endothelium is removed mechanically from the rabbit's lower abdominal aorta (Poole, Sanders and Florey, 1958, 1959) a defect about 2 cm. long is relined by endothelium in about 12 months. The new endothelium is formed by mitosis from endothelial cells just behind the edge of the endothelium which remains, and growth gradually extends from each end of the bare area until the new lining is complete, after which no further endothelial growth takes place. Meijne (1959) and Mackenzie and Loewenthal (1960) made microscopical observations on surface preparations of the tissue lining fabric grafts and showed that a layer of cells resembling endothelium was formed by extension inwards from both ends of the graft. The work of Florey *et al.* (1961) confirmed the belief that the lining layer was true endothelium in so far as it can be identified by morphological studies. But whereas the healing of the lesion in the rabbit aorta was complete only after about 12 months the " Dacron " graft in the baboon was covered with endothelium in 10 weeks or less. The results of the present study make it probable that the main, and perhaps the only reason for the difference is that the endothelial lining of the graft develops not only from the adjacent endothelium of the aorta at the ends of the graft but also from islands of endothelium which appear at a number of different places over its inner surface. These islands grow from the mouths of small vascular channels in the developing pseudointima. How these small vessels are formed is not at present clear.

The possibility that there is a species difference between the rabbit and the baboon in respect of rate of growth of endothelium cannot be excluded. Nor can one exclude the possibility that endothelium grows more rapidly over the inner surface of a fabric graft than over the normal tunica media. The present observations are not sufficiently extensive to permit any precise estimate of the time taken for the endothelial lining to become complete. In the previous study, grafts which had been in place for 10 weeks were found to be completely lined whereas in the present study, some which had been present for 12 weeks were not. This may be due to the fact that the 12-week grafts examined were of greater length than the others but it may merely indicate that the time taken is variable. It is known that fabric grafts can take much longer than this to acquire an endothelial lining. Kinmonth (1960) described an experiment in which an " Orlon " graft in a dog's aorta was not completely lined by endothelium after 3 years. Possibly the fabric used makes a considerable difference to the result.

Various workers have suggested that in fabric grafts the endothelial lining is formed by fibroblasts (Griffith, Eade, Zech and Harkins, 1955 ; Sanger, Taylor, McCall, Duchesne and Lepage, 1956 ; Wesolowski and Sauvage, 1956 ; Petry and Heberer, 1957). The simplest hypothesis to account for the appearance of new endothelial cells in any situation is that they are formed from pre-existing endothelial cells by the ordinary processes of cell division. The experimental evidence now available is entirely consistent with the idea that this is what in fact happens in the grafts.

Origin of fibroblasts, smooth muscle cells, collagen and elastic tissue

The present observations have not added much to our previous knowledge of the origin and development of the other cellular and fibrous components of the

pseudointima. It has been possible to demonstrate cells which are probably fibroblasts. In the previous study on more mature pseudointima no fibroblasts could be identified with any confidence. Fibroblasts are usually supposed to originate from fibrocytes which are believed to be stimulated to divide by injury. The present observations are consistent with this general hypothesis but do not contribute any fresh support for it. Smooth muscle cells and elastic tissue appear at a later stage than do fibroblasts and collagen, but their origin remains obscure.

SUMMARY

Knitted polyethylene glycol terephthalate (" Dacron ") grafts were inserted into the lower abdominal aortae of baboons (*Papio doguera*). The animals were killed at intervals of from 1 to 12 weeks after operation. The fabric grafts were initially lined by a thin layer of platelets, leucocytes and fibrin. Later, endothelium developed, growing partly from the endothelium lining the real aorta at the ends of the graft and partly from the mouths of small vascular channels whose openings into the lumen of the graft are scattered over its inner surface. Islands of endothelium form by growth from the margins of the mouths of these small vessels. Fibroblasts, collagen, macrophages and polymorphs can be identified deep to the endothelium 3 weeks after operation. Other cell types may be present at this stage but none can be identified with any confidence. Smooth muscle cells and elastic tissue are present only at a later stage.

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