Lack of intimal hyperplasia response in an experimental model of non-endothelial vascular wall damage

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Summary. The endothelial and medial layers are generally presumed to play an important role in the appearance and development of intimal hyperplasia.

We have carried out a short-, media- and long-term study of the morphological changes taking place in the common iliac artery of rats after surgical removal of the adventitial layer. Our aim has been to assess the likely role played by this layer in the development of intimal hyperplasia.

Our results show recurrent periods of cellular desquamation and almost complete absence of hyperplastic response during the first two months. After three months three is a slow process of endothelialization which is completed by the 6th month and persists one year after adventitial resection.

Thus, adventitial resection seems to cause instability at the subendothelial bed level, not allowing the junction and embedding of endothelial cells nor the development of intimal hyperplasia.

This lack of hyperplasia might also result from the fact that the endothelial desquamation process does not involve cellular rupture, which would prevent mitogenic-factor release.

After morphological repair of the endothelium, a slow morphofunctional recovery of the artery takes place.

Key words: Endothelial cells, Neointima, Adventitia, Intimal hyperplasia

Introduction

The arterial wall is known to be particularly responsive to any stimuli that implicate alterations in its structure or function through repair mechanisms still not elucidated (Reidy, 1985).

The investigations performed on the etiopathogenesis of vascular repair have resulted in a great number of works with different experimental designs. Thus, arterial or venous vascular reconstruction models (autografts) (Larson et al., 1978; Henderson et al., 1986; Dilley et al., 1988; Kholer et al., 1990; Mii et al., 1990), or lesions produced through angioplasty with balloon catheters (Clowes et al., 1989; Fingerle et al., 1989; Weindinger et al., 1990) performed in animals, have had as a major aim the study of alterations and effects on the endothelial and media layers.

On the contrary, there are few references related to lesion models on the adventitial layer, which is considered by many to be the mechanical and nutritional support of the other components of the arterial wall (Gebrane et al., 1982; Chignier and Eloy, 1986).

In our experimental works on vascular wall repair (Buján et al., 1992), carried out on arterial autografts, we have emphasized the importance of the role played by this layer, perhaps greater than had previously been recognised.

Thus, the aim of this study has been to assess, by means of the morpho-cellular events taking place after its experimental removal, the role that the adventitial layer might play on the vascular wall repair process.

Materials and methods

Female Sprague-Dawley rats, with an average weight of 250 grs, were used for the experiment; 86 out of 110 animals were considered valid for the study.

Surgery was performed on animals previously anaesthetized with a mixture of ketamine hydrochloride (0.5 mg/100 g body weight) and atropine (0.05 mg/100 g body weight) administered into the peritoneum.

A midline laparatomy was performed to isolate the right common iliac artery. This was followed by resection, with a WILD M-650 operating microscope, of a 10 mm tubular adventitial segment. No microsurgical clamps were needed. The limits of adventitial resection were marked with two stitches in both its proximal and

distal ends. Neither sterile surgical technique nor antibiotic coverage were used.

Timing designs and sacrifice intervals after surgery were performed to cover short-, medium- and long-term studies. Each study group was made up of a minimum of 3 animals to a maximum of 5.

Sacrifice intervals were organized as follows: 10 minutes; 24; 48 hours; 5; 7; 9; 11; 14; 18; 21; 30; 50; 70; 90; 120; and 180 days. The study control group was formed by animals which were only submitted to a sham laparatomy with isolation of the vascular area chosen for each time interval.

Prior to the sacrifice, the animals were perfused with a glutaralformaldehyde solution, at 100 mm Hg of pressure.

The specimens, which always included the marking stitches, were then processed in accordance with the study to be performed on each sample.

The samples for optical microscopy were included in paraffin, obtaining transverse and longitudinal sections, 5 µm thick. They were stained with haematoxylin-eosin, VOF, orcein and MASSON trichrome.

The samples for ultrastructural study were fragmented into small sections, placed in 7.3 MILLONING buffer and postfixed in 2% osmium tetroxide. They were then dehydrated in a gradual series of acetones and embedded in Araldite for thin cuts. Afterwards, they were contrasted with lead citrate, and observed through a ZEISS 109 transmission electron microscope.

For scanning studies, after a short perfusion of the animals, the samples obtained were longitudinally opened under an operating microscope and placed in 3% glutaraldehyde. They were then laid in a pH 7.3 MILLONING buffer for an hour, dehydrated in a gradual

series of acetones, obtaining the critical point in an E-3000 Polaron with CO_2, metallized with gold-palladium and observed through a ZEISS 950 DSM scanning electron microscope.

Results

After resection of the adventitial tunica, an aneurysmatic dilatation of the vascular segment took place for two weeks, and then a neoadventitial layer was formed, allowing the recovery of the former calibre.

The first observations made ten minutes after adventitial resection did not show alterations of the luminal surface of the iliac artery. The endothelium formed a continuous and well-preserved layer. The medial layer did not have any signs of aggression; this was limited to the irregular border which could be seen in transverse sections through light microscopy (Fig. 1).

One hour after surgery the first morphological alterations appeared. The endothelial cells began detached, with adhesion of platelets on a single layer onto a subendothelial bed, in small areas irregularly distributed along the resected segment (Fig. 2).

Paradoxically, the increase in endothelial denudation areas which was observed after 24 hours was not accompanied by platelet deposits.

After 48 hours, the endothelial loss in some areas covered 40% of the arterial circumference. Endothelial cells detached from their substrate, presenting fusiform profiles and desquamating at the vascular lumen. At both ends of the adventitial removal site a discrete inflammatory infiltrate was observed (Fig. 3).

After 5 days, a neoadventitial layer, coming from healthy adjacent segments and formed by a fibrous-like scar tissue, invaded the operated arterial segment.
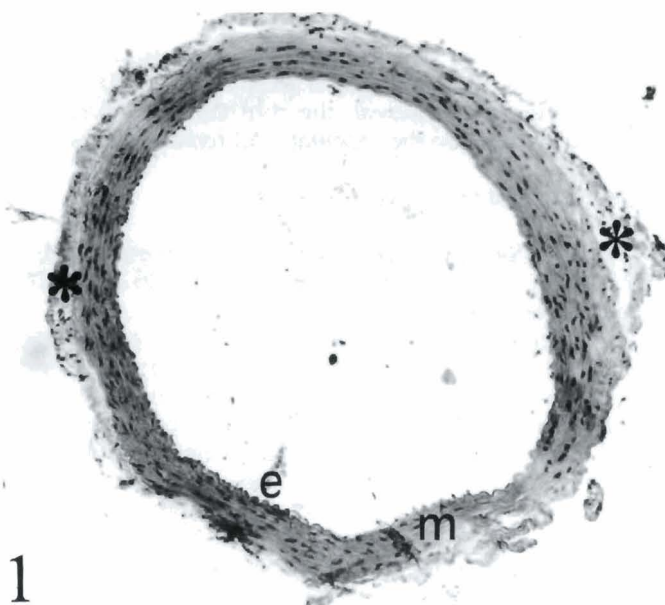

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Fig. 1. Transverse section of the iliac artery, ten minutes after adventitial resection. Endothelium (e); medial layer (m); adventitial residues (*). Haematoxylin-eosin. x 6.5
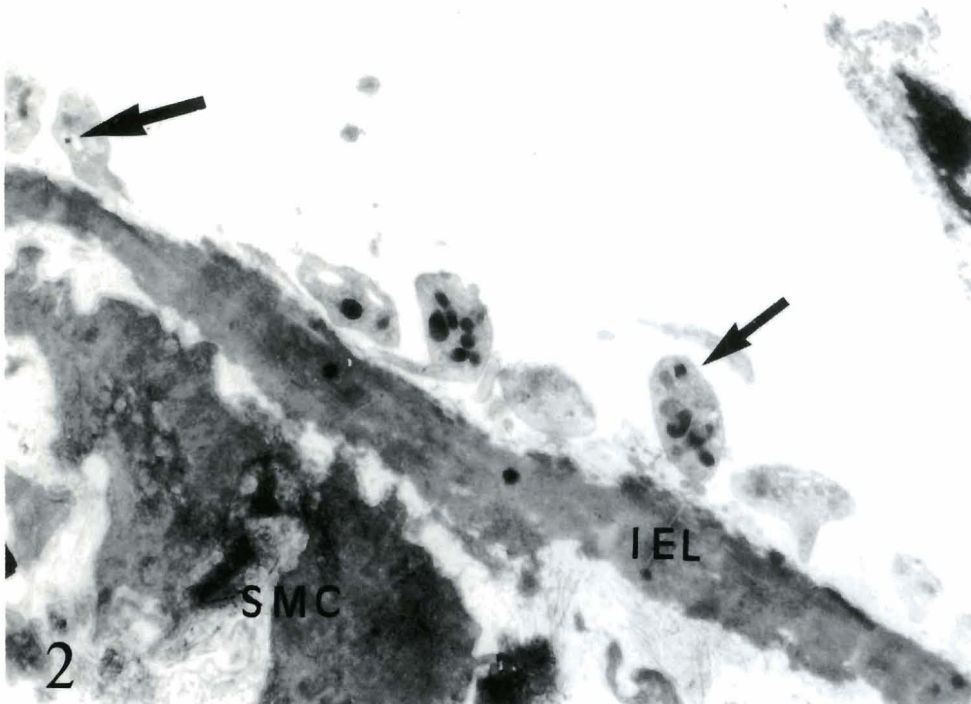
Fig. 2. Adhesion of platelets (arrows) on a single layer onto a subendothelial bed, one hour after adventitial resection. Internal elastic lamina (IEL); smooth muscle cells (SMC). X 2,500

Fig. 3. 48 hours after resection, transverse section showing denudation area of the luminal surface lumen (arrows) and some inflammatory infiltrates in the resected adventitia (*). Haematoxylin-eosin. x 6.5

Electron microscopy showed endothelial lesions in areas with irregular borders and with exposure of the subendothelial matrix. No platelet adhesion was seen, despite the extension of the lesions (Fig. 4).

At the seventh day we found a luminal surface covered by an even layer that showed practically no damage. This endothelialization was clearly visible in transverse sections by light microscopy (Fig. 5).

This endothelialization did not continue, and, after 9 days, a cellular desquamation phenomenon, which had increased by the eleventh day, could be noticed. Fourteen days after the experiment, one could observe

images of the endoluminal surface entirely similar to those found after 5 days of adventitial resection (Fig. 6a). This happened in spite of the other two layers of the

arterial wall showing normal (Fig. 6b) characteristics in the presence of a «neoadventitia» with many vasa-vasorum.
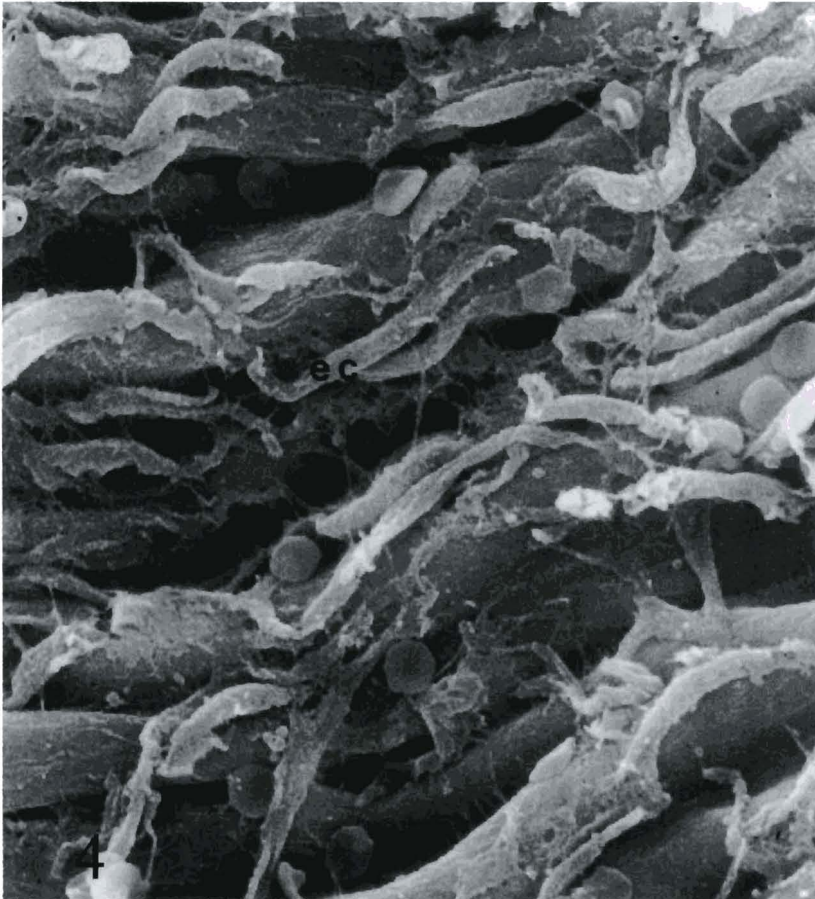

Fig. 4. The endothelial surface, with a mottled appearance, five days after adventitial resection. Endothelial cells (ec). x 1,000
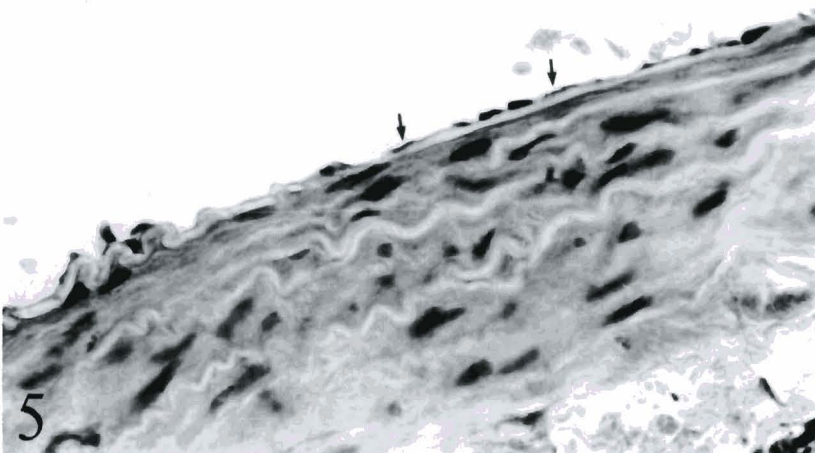

Fig. 5. Seven days after resection; transverse section by light microscopy, showing a luminal surface covered by an even endothelial layer (arrows). Haematoxylin-eosin. x 16

On the eighteen day a small myointimal hyperplasia, not surpassing more than a bicellular stratum over the elastic lamina, was observed (Fig. 7).

Desquamation, denudation and repair processes were predominant at the luminal surface level, during the 21-, 30- and 50-day study intervals. After three weeks, we observed the appearance of a fibrillar component at the subendothelial bed level, with a flocculous material deposit, as well as platelet deposits in different areas of a non-thrombogenic surface (Fig. 8a,b).

After a seventy-day period, desquamation was not important, although through scanning microscopy a bubbling aspect in the lining areas could be observed (Fig. 9a). This phenomenon could be corroborated by transmission electron microscopy, through which the cover cell was seen attached to the subendothelial site by cytoplasm bridges; this appeared among these prolongations, showing little density to electrons, and with a fibrillar component decrease (Fig. 9b).

Attachment of cells to the subendothelial bed slowly took place between 90 and 120 days. Cellular detachment decreased, thus leading to progressive covering of the sub-

Fig. 6. 14 days after adventitial resection: **a)** instability at the luminal surface. x 1,000. **b)** hyperplastic areas (*) with degranulated platelets (arrows) and macrophage (M) below the internal elastic lamina (IEL). x 2,500

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endothelial matrix. This was evident by 180 days, at which time stabilization of the repair process was achieved (Fig. 10a,b).

Discussion

In vascular wall repair processes, two outstanding facts are the endothelial response and the appearance of intimal hyperplasia.

In our experimental model, after surgical removal of the adventitia, some modifications of the endothelial monolayer have been observed. These changes started appearing 10 minutes after surgery, when endothelial cells began to show the first signs of cellular alteration. These cells presented a fusiform aspect and detached from the luminal surface, starting a desquamation process with exposure of the subendothelial bed. In the first moments there was platelet adhesion in monolayer on the denuded surface; this fact was not recurrent in later phases.

These observations differed from those by Chignier and Eloy (1986), who found massive denudation between 6 and 12 hours after surgery in a similar experimental model.

As of the fifth day, the adventitial repair process started with a large profusion of capillaries that could be seen macroscopically after 9 days, very much alike to what happens in other vascular reconstruction processes (Díaz-Flores and Domínguez, 1985; Clowes and Reidy, 1987). However, this process of adventitial repair was not followed by stabilization of the endothelial layer. From the fifth day to two months after adventitial resection, the artery luminal surface presented a similar aspect, with cellular desquamation, exposure of an athrombogenic subendothelial matrix of fibrillar characteristics and deposit of flocculous material.

These findings could be explained on the basis of the likely existence of recurrent episodes of desquamation and repair of the intima layer, after adventitial removal (Chignier and Eloy, 1986; Govyrin et al., 1988). Similar endothelial desquamation findings have been observed due to the action of endotoxins (Reidy and Schwart, 1983; Nayyar et al., 1989), although there is no exposure of subendothelial matrix in these cases.

In our view the desquamation phenomenon is produced by the lack of an adhesion substrate adequate to the lining cell; consequently the subendothelial matrix seems to play an important role. The repair process may be responsible for the alteration in the synthesis and disposition of some types of collagen (Houdijk et al., 1985; Farquharson and Robins, 1989).

The appearance of type IV collagen could thus justify the non-adhesion of neither lining cells nor platelets, since this fact was not observed despite the large extension of denuded areas. When platelet adhesion appeared sporadically from the 30th day onwards, this adherence was not directly produced on the matrix but on lining cells, and this fact may suggest that these could be endothelial cells with cellular dysfunction (Cross et al., 1988), or that these lining cells are not really endothelial cells (Form et al., 1986).

After 2 months, and when morphological formation and stabilization of the neoadventitia were noticeable, a recovery process of the luminal surface took place. Such a process seems to be very closely related to a change on the subendothelial matrix which allowed the junction and embedding of the cells.

This fact would be in accordance with Form and co-workers (1986), who emphasized the importance of the extracellular matrix during angiogenesis processes.

On the other hand, we must also think of the role that stress fibres might play in cellular adhesion, (White and
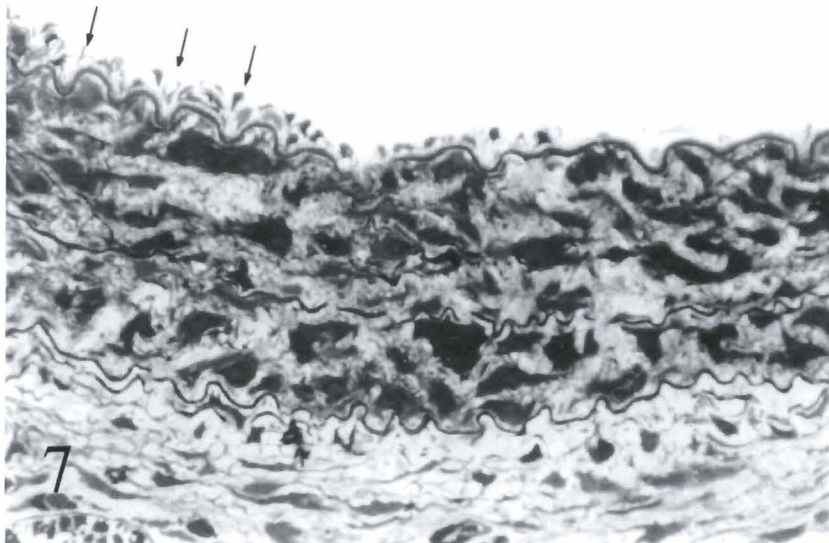

Fig. 7. 18 days after resection; transverse section of a semithin cut showing areas with some hyperplasia not surpassing more than a bicellular stratum (arrows). Toluidine blue. x 16
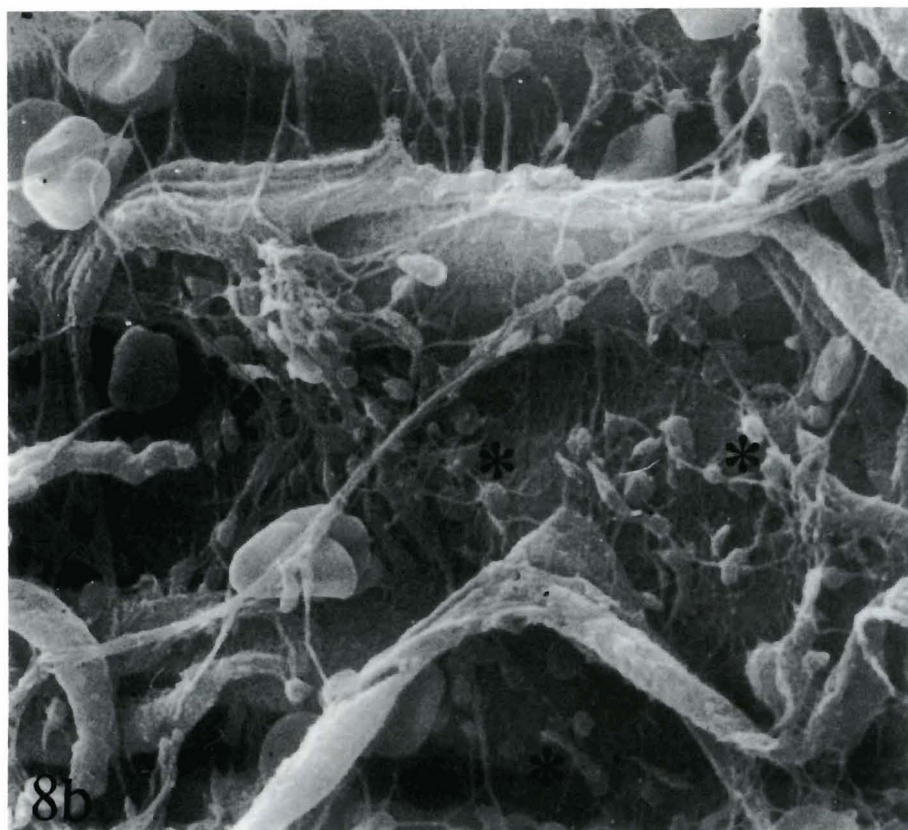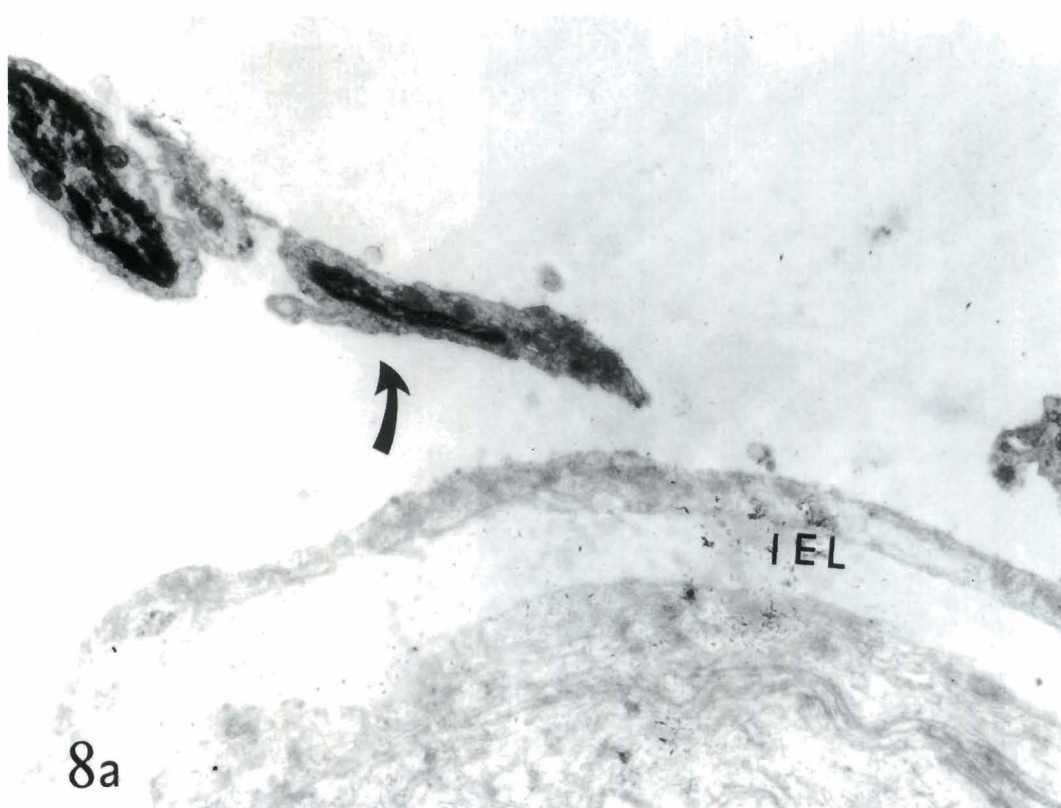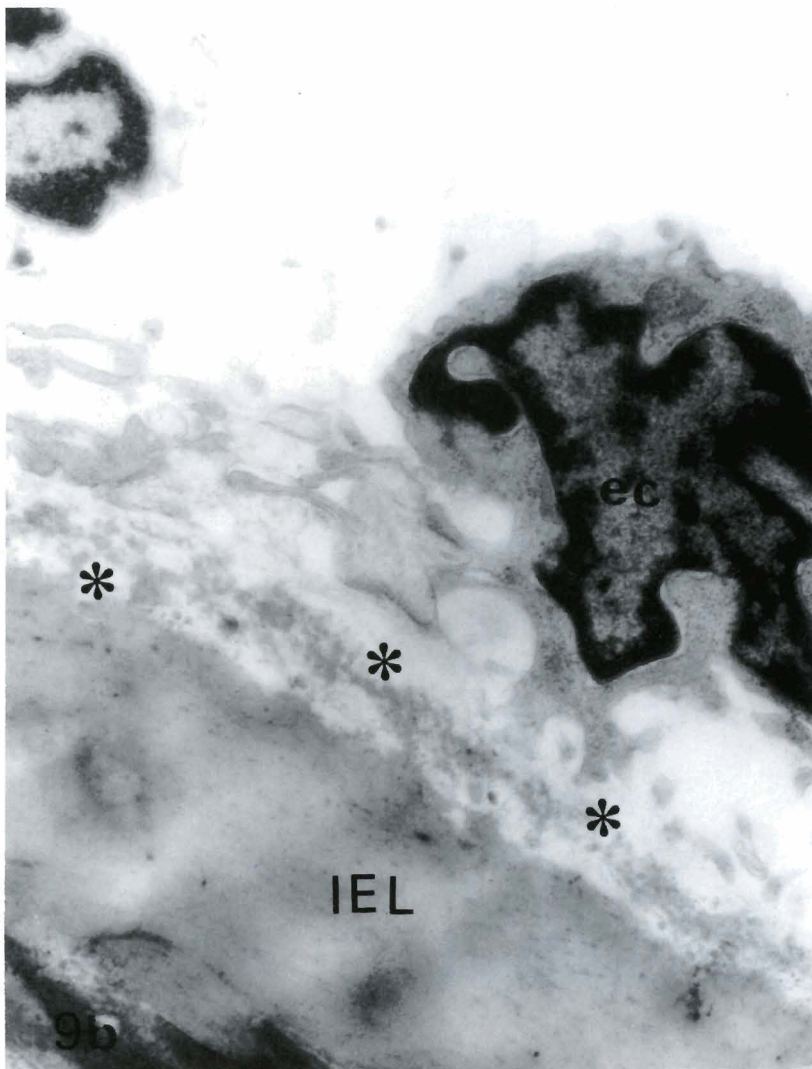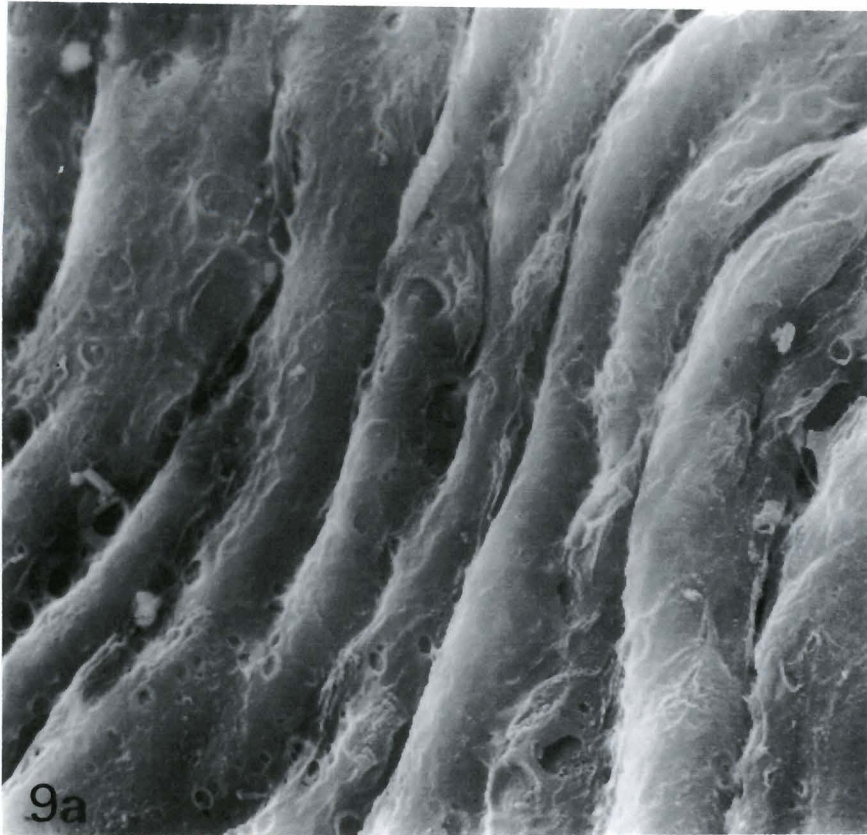
Lack of intimal hyperplasia response

Fig. 8. 21 to 50-day interval after resection:
a) cellular desquamation (arrows). Internal
elastic lamina (IEL). x 4,400. **b)** scanning of
the luminal surface with platelet deposits (*).
x 2,000

Fujiwara, 1986; White et al., 1988; Guyton et al., 1989). The stabilization process developed very slowly and would not be definitely stable till over six months after surgery. These observations differ from those stated by other authors (Chignier and Eloy, 1986; Govyrin et al., 1988), who find a stable surface a month after adventitial resection.

Another important aspect in our experimental model is the almost complete absence of intimal hyperplastic response, which was nevertheless found in the form of small patches between 7 and 18 days after surgery.

Other authors (Chignier and Eloy, 1986) found hyperplastic phenomena in a similar model, although they chose a vascular site different from ours and provided with collaterals. This fact had already been reported by Haudeschild and Schwartz (1979), who showed that the existence of collaterals would favour the appearance of hyperplasia.

Our results regarding the absence of hyperplastic response are in agreement with those achieved by Spaet et al. (1982) who, after causing selective lesions of the

Fig. 9. 70- to 90-day interval after resection: **a)** scanning of the luminal surface, showing a cellular lining. x 1,000. **b)** bubbling aspect of the endothelial cell (ec) over the subendothelial matrix (*) at 90 days. Internal elastic lamina (IEL). x 7,000
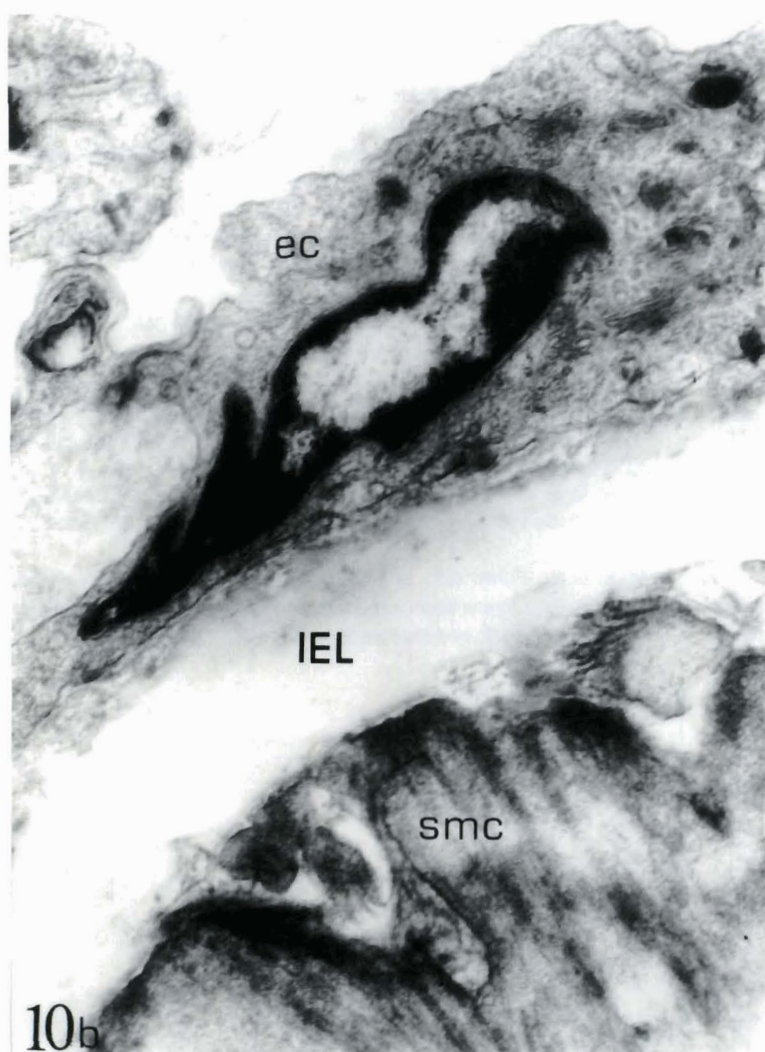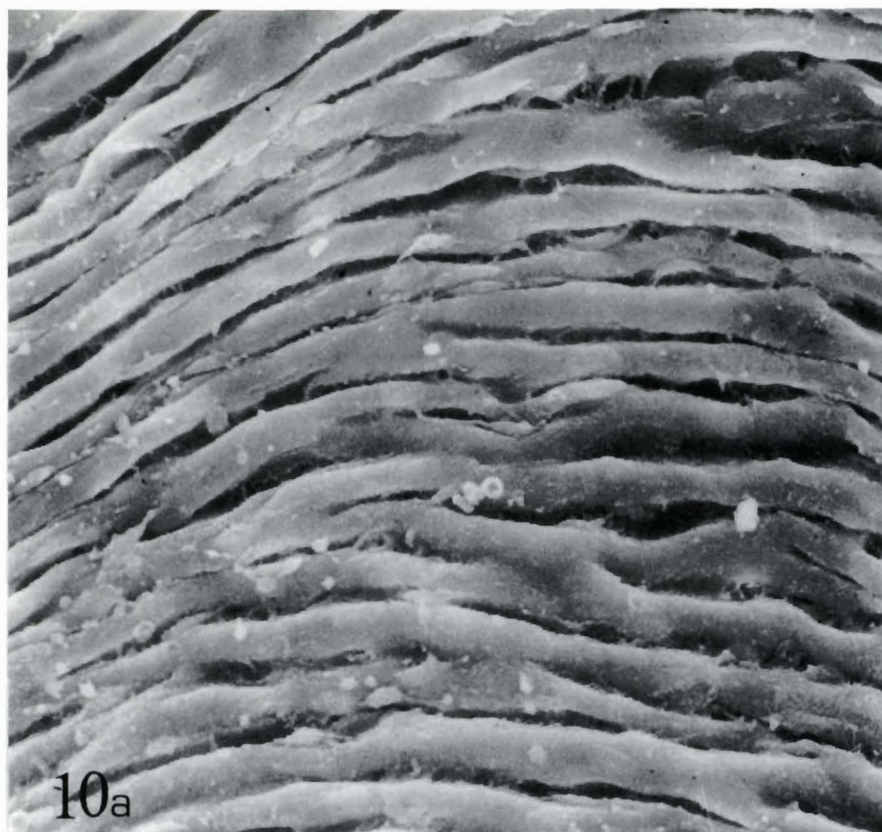
tunica media, did not obtain such a response. The hyperplastic phenomenon is neither obtained in other models with minimal endothelial lesions (Tada and Reidy, 1987). These authors justify the lack of hyperplasia by stating that the minimal endothelial lesion is quickly covered by endothelial cells that would inhibit smooth muscle cell migration to the subintimal site.

In our model, and despite endothelial instability during the first three months, there was a minimal hyperplastic response. Perhaps this was due to the fact that the endothelial desquamation process did not involve cellular rupture which would prevent mitogenic factor release. On the other hand, the medial layer had not been damaged, this being one of the basic facts to produce hyperplasia (Chervu and Moore, 1990).

No participation of blood cells in the evolution of intimal repair, was seen, and we only detected polymorphonuclear infiltrates at adventitial level after surgery. Therefore, one of the mechanisms involved in the genesis of hyperplasia, namely the mitogenic factors released by leucocytes, would not act in this aggression to the vascular wall. This could also explain the absence of a well-determined hyperplastic phenomenon as seen in other experimental models of vascular wall lesion.

Fig. 10. 120-180-day interval after adventitial resection. a) normal lining of luminal surface. x 500. b) electron microscopy of endothelial cell (ec) attachment at subendotelial matrix and internal elastic lamina (IEL). x 12,800

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