Histol Histopath (1992) 7: 189-197

Histology and Histopathology

Intimal thickening in arterial autografts. Role of the adventitial layer

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Summary. In the present work, the repairing response of the iliac arterial wall is studied after carrying out autografts in segments of these vessels.

The formation of the intimal hyperplasia, which occurred in all the cases, was followed at the biochemical level (tritium thymidine incorporation) and with light and electron microscopy.

The adventitial layer showed great activity during the repairing process. We believe that it plays an important role not only in neoadventitial formation, but also in myointimal raising.

Key words: Arterial injury, Endothelial cell, Adventitia, Autograft, Intimal hyperplasia

Introduction

The arterial wall, which is considered to have a low turn-over rate in normal conditions, is very susceptible to any aggression. Its repairing response is shown in many cases by the appearance of a new layer called myointimal, intimal thickening or intimal hyperplasia. This is of great importance because its appearance is the cause of complications and/or vascular failure.

Intimal hyperplasia has been the object of many studies, but there remain doubts in important aspects such as: a) factors initiating this response; b) the origin of this layer; and c) its evolution in time.

Regarding the first point, many factors seem to be involved in this process, principally the injury to the endothelium.

The role of the vascular endothelium has changed with time from being considered a simple barrier in the interphase blood-vascular wall, to being either a morphological or functional layer of great importance. For instance, as a semipermeable barrier which actively controls coagulation, cellular growth and vasoreactivity (Weidinger et al., 1990).

The endothelial denudation, which exposure of the thrombogenic subendothelial matrix, causes immediate platelet adhesion. For many authors (Block et al., 1980; Steele et al., 1985; Ross et al., 1986), the platelet factors start the proliferative response, while for others (Fingerle et al., 1989; Clowes et al., 1989) they act in a second phase after vessel de-endothelialization and distention.

There are other factors also involved in this process: blood and plasma factors (angiotensin II, thrombospandin, catecholamines), and those belonging to cell elements (monocytes, leukocytes, etc.) which, working directly or indirectly, stimulate the proliferative response of the arterial wall (Badimon et al., 1990; Singh et al., 1990; Jonasson et al., 1988).

From this point of view, numerous authors, such as Ross and Glomset (1973); Ross et al. (1974); Beneditt (1977); Clowes et al. (1983a); Clowes and Schwartz (1985) agree about the platelet role in cellular migration and intimal proliferation, the middle layer cells being responsible for the myo-intima formation.

However, other authors propose different origins for this layer. For example from mesenchymal cells (Feigl et al., 1985), from blood circulation (Buck, 1961), or from adventitial pericytes (Díaz-Flores and Domínguez, 1985; Díaz-Flores et al., 1990).

Possibly, the mitogenic stimulus reaches all the cells of the vascular wall, resulting in a multiple response to this stimulus (Fig. 1).

The third aspect or step is the evolution of the myo-intima. Firstly, it originates as a consequence of a hyperproliferative response, but, progressively, the number of cells that form this layer stabilizes. Then, the quantity of extracellular material secreted by the myo-intimal cells is responsible for the subsequent increase of thickness and causes, in many cases, the

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complete occlusion of the vessel (Clowes et al., 1983b). In other cases, the appearance of complications of a lipidic degenerative kind or calcification, as occurs in atherosclerosis, is responsible for this occlusion.

Finally, this layer may involute, and the artery acquires its original or primary conformation.

The aim of this work is to study the vascular wall in an experimental model of arterial autograft. Our results suggest, in addition to all the factors above indicated, that there is a specific response of the adventitial layer, which may model the size and characteristics of the myo-intimal layer.

Materials and methods

The animals used for this study were female Sprague-Dawley rats each weighing approximately 250 g. Ninety grafts were performed in order to have a minimum of 4-6 animals per study group. Anesthesia was induced with intraperitoneal ketamine hydrochloride (0.5 mg/100 g body weight) and atropine (0.5 mg/10 g body weight).

The surgical procedure was always performed in the same vascular area, the right common iliac artery, through a midline laparatomy. Using a Wild M-650 operating microscope, a 1.5 - 1.7 cm segment of the iliac artery was dissected free from its aortic origin; 5 mm of this segment were removed, after clamping and cutting both ends, and placed in a Ringer solution for 10 minutes. Thereafter, it was reimplanted with end-to-end anastomosis using a double microclamp and 10/0 monofilament interrupted microsutures.

The animals were sacrificed 1, 2, 5, 7, 9, 14, 21, 30, 50 and 90 days after surgery. At the time of sacrifice, the autograft was removed together with its undisturbed proximal and distal vascular segments. The cases with complications, early bleeding, thrombosis and aneurysm, less than 10%, were excluded.

Rats which had been subjected only to midline laparatomy formed the control group.

The samples for optical microscopy were fixed by immersion in a 10% formol solution and embedded in paraffin, transversal and longitudinal sections 5 micron thick being obtained. As staining hematoxylin-eosin, VOF, orcein and Masson trichrome were used. For the autoradiographic study, the animals were injected with 1mCu/g tritium thymidine (Nuclear Iberica - 5 Cu/mMol) directly into the inferior vena cava, an hour before sacrifice. The samples were embedded in paraffin, obtaining 5 micron thick, on which a Kodak AR 10 film was placed. They were then kept for 30 days in the dark, at 4° C. The slides were developed in Kodak D-19 developer and fixed. The sections were then stained with hematoxylin and evaluated at high power (X1000) under oil. All cell nuclei showing 5 or more overlying silver grains were considered labelled. The thymidine labelling index was given by the number of labelled nuclei out of a thousand.

The animals destined for electron microscopy were perfused at 100 mm Hg with a 3% glutaraldehyde

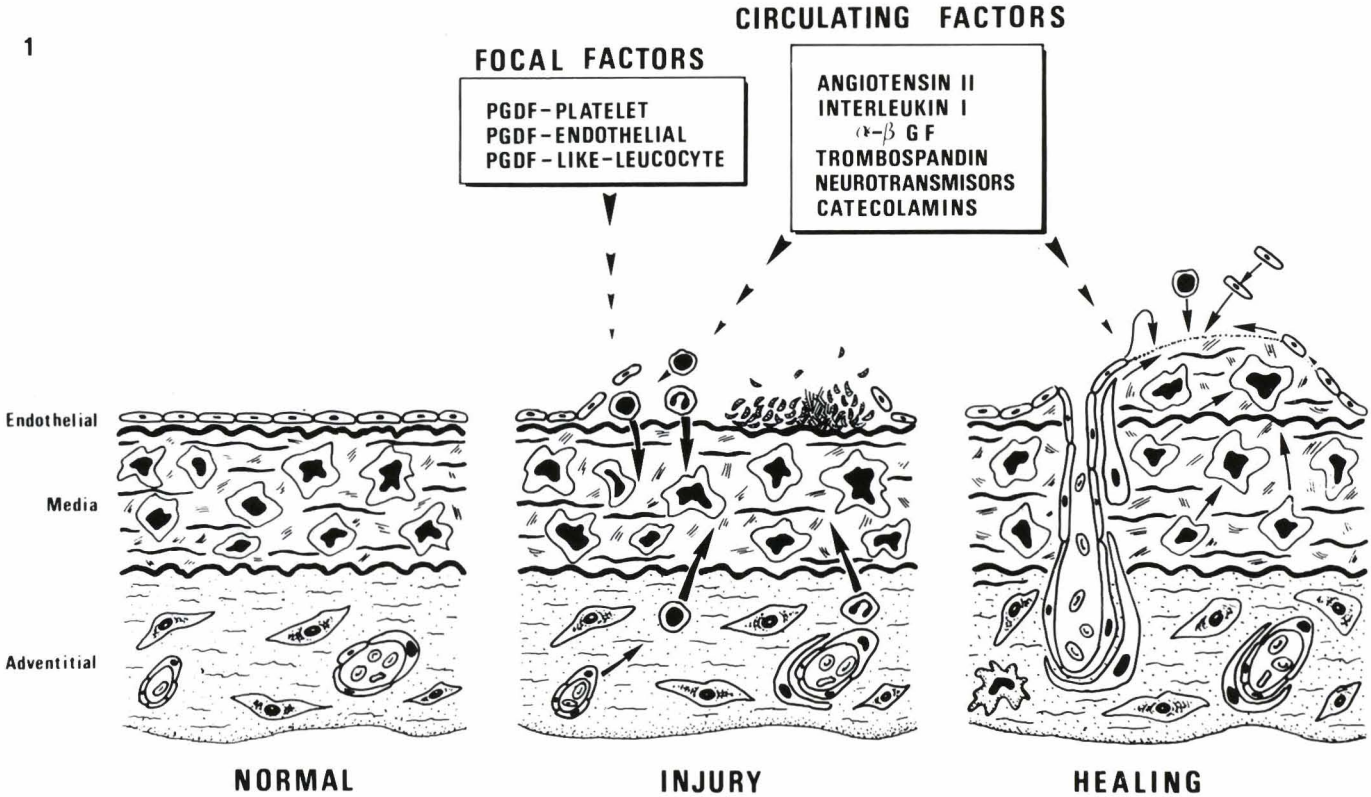


Fig. 1. Scheme of the repairing response process.

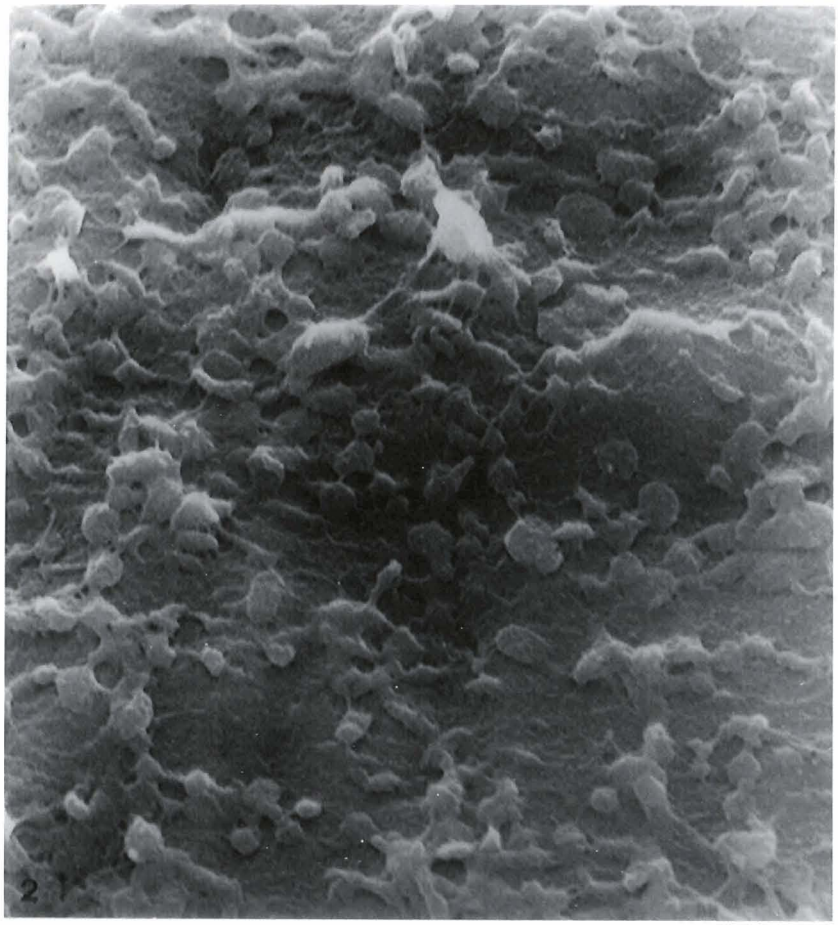


Fig. 2. 48 hours autograft. Platelet monolayer. × 2,000

solution. The samples destined for ultrastructural study were fragmented in small sections, placed in Milloning buffer (pH 7.3), postfixed in 2% osmium tetroxide, dehydrated in a gradual series of acetones and embedded in Araldite for semithin cuts. Afterwards, they were contrasted with lead citrate, and observed through a ZEISS 109 transmission electron microscope.

For the scanning studies, after a short perfusion of the animals, the samples obtained were opened longitudinally under the operating microscope and submerged in 3% glutaraldehyde, placed in Milloning buffer (pH 7.3) for an hour, dehydrated in a gradual series of acetones, obtaining critical point in an E-3000 Polaron with CO2, metallized with gold-palladium and observed through a Zeiss 950 DSM scanning electron microscope.

Because of the characteristics of our experiment, the study data were analyzed using the Gauss-Markoff and the Fisher and Yates p 1/2 Logit regression methods.

Results

During the first post-surgical stages (24 and 48 h.), the arterial luminal surface was covered by a fibrin network, with platelet aggregates and white cells adhering to the subendothelial spaces. The fibrillar network progressively disappeared. The affected arterial surface showed different aspects, with de-endothelialized areas, presence of cells which signs of degeneration, regions of endothelialization covered by a platelet monolayer (Fig. 2), and white blood cells. Infiltration of the wall by macrophages loaded with lipids, either in the subtintimal or the adventitial zones, was frequently observed. In this early stage, the thymidine incorporation showed low intensity in all its values.

On the fifth day after surgery the optical microscope showed de-endothelialization of the luminal surface, where white cells and little fibrin patches could be observed. A single layer of platelets was present in ultrastructural sections, and polymorphonuclear infiltrates could be seen in both the middle and neoadventitial layers, being more intense in the latter.

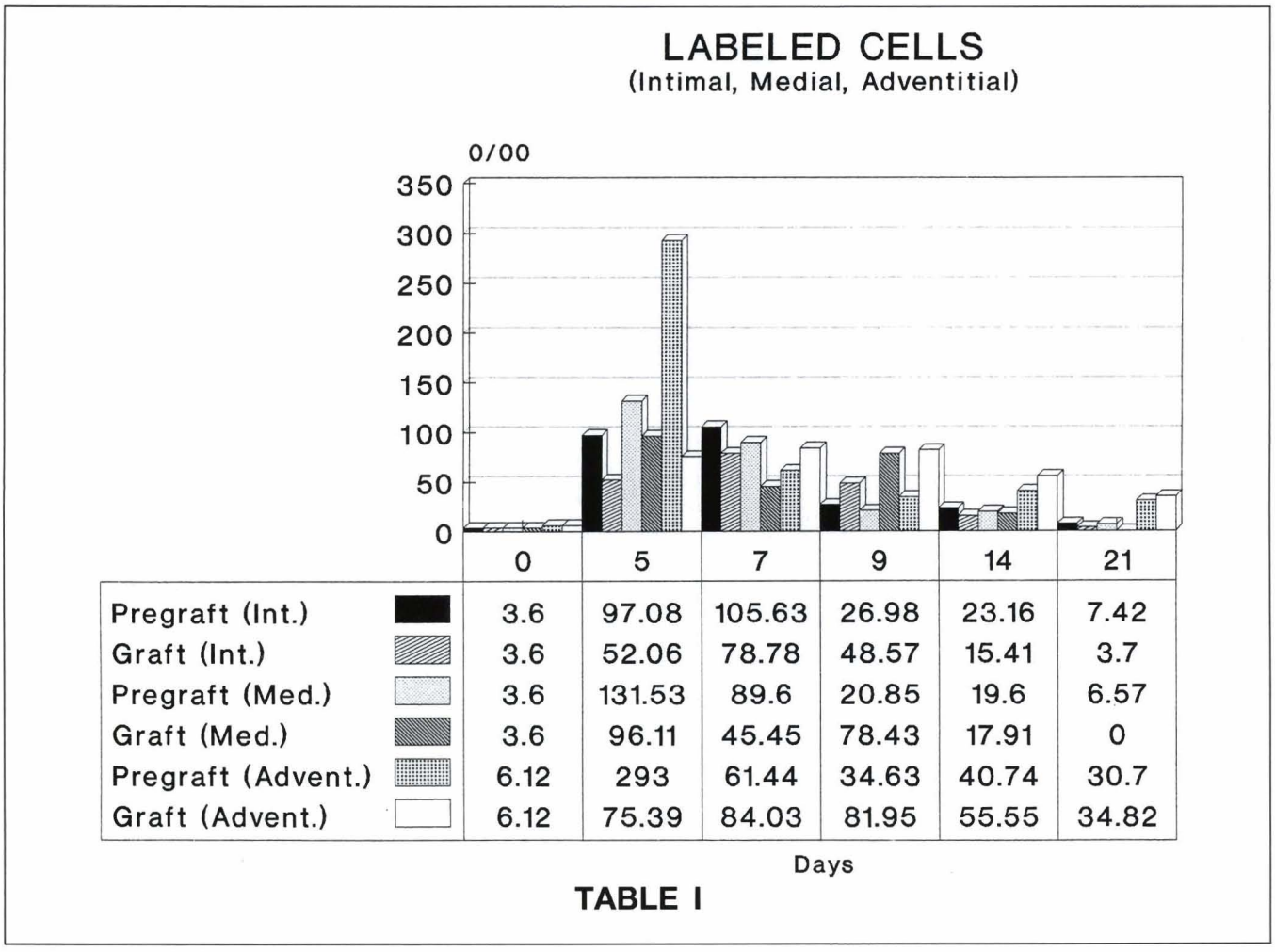
Incorporation of tritium thymidine was important, above all, in the non-injured areas close to the graft, with a high rate of labelling in the adventitial layer, where the values reached 293 per 1000 (Table 1) (Fig. 3).

Scanning electron microscopy (Fig. 4a) showed, in the areas next to the implanted graft, the non-affected endothelium (Fig. 4b). In the anastomotic areas, the endothelium rose in sheets due to the presence of white cell infiltrates and of platelet aggregates (Fig. 4c-d). In the suture areas (Fig. 4e), as in previous stages, an intense accumulation of white cells and platelets, appeared. Finally, in the central area of the autograft (Fig. 4f), de-endothelialization and some monocytes and macrophages were observed.

In the second week, the light microscope showed a polystratified myointimal layer (Fig. 5). The orcein staining revealed the elastic compound secreted by those cells without laminal organization. Myointimal ultrastructural features were arranged typically in those cells, with their greater axis parallel to the blood flow. The extracellular matrix was rich in filamentous and amorphous material.

Ultrastructurally, the middle layer showed signs of great activity with the presence of «contractile» myocites, in its most superficial areas, and «secretory» myocites, in its deepest areas. The latter were characterized by a great development of the endoplasmic reticulum and large deposits of amorphous material, probably a precursor to elastine.

Table 1. Tritiated thymidine incorporation in the arterial wall. The reimplanted segment (graf) and undisturbed neighbouring areas (pregraft).



The thymidine incorporation was inverted with respect to the previous stage, with the appearance of values that were higher in the area of autograft than in the non-injured adjacent areas (Table 1).

At 21 days, the myo-intimal layer reached its maximum thickness, 60 μm. in the grafted segment and 160 μm. in the anastomotic areas, which constituted the most relevant fact of this stage (Fig. 6). This intimal thickening was formed by a multicellular layer whose cells had muscular characteristics, with fibrillar cytoplasm and secretory deposits in some of them. The extracellular matrix showed a clear fibrillar component that stained well with orcein. The cells of this layer were initially perpendicular to the blood flow and, later, their main axis took on a parallel direction to the elastic sheets. In the transveral sections, it was possible to observe the presence of small vessels in the intima thickness (Fig. 7). The well-defined middle layer showed a stable composition, with a greater secretory activity of the myocites next to the myo-intima (Fig. 8).

The adventitial layer was characterized by a prominent neo-vascularization in all the affected arterial segments, with the formation of many vascular buds which made up a wide microcirculatory net. Also, numerous macrophages loaded with lipids were observed (Fig. 9). In this stage, the three arterial layers showed a high morphological activity that contrasted with the decrease of thymidine labelling.

By scanning electron microscopy, the luminal surface appears uniform, being completely covered by endothelial cells of apparently normal morphological characteristics.

Between 30 and 90 days, involution signs were observed, with the following findings: a) recuperation of turn-over basal levels of the arterial wall cells (Table I.); b) stabilization and organization of the myo-intima layers.

Discussion

The behaviour of the vascular territory studied has been satisfactory for our purposes. The iliac artery, with good calibre and without vasospasm, showed few

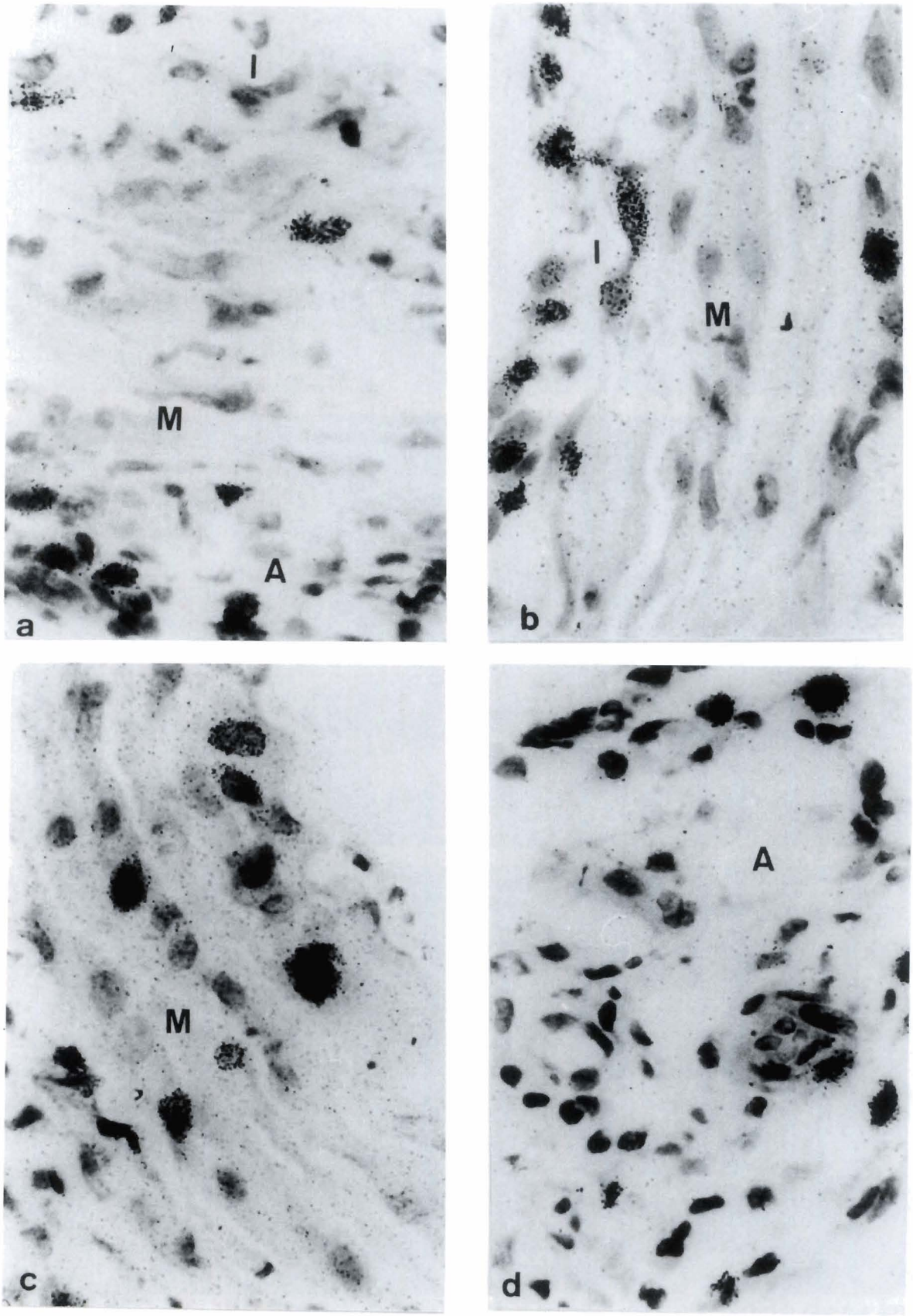


Fig. 3. Tritium thymidine autoradiographic images in the first week. (I) intimal layer. (M) medial layer. (A) adventitial layer. × 100. **a.** Labelled cells of adventitial layer. **b.** Intense label of the intimal layer. **c.** Labelled cell pointing out in the middle layer. **d.** Adventitial reaction with labelled cells.

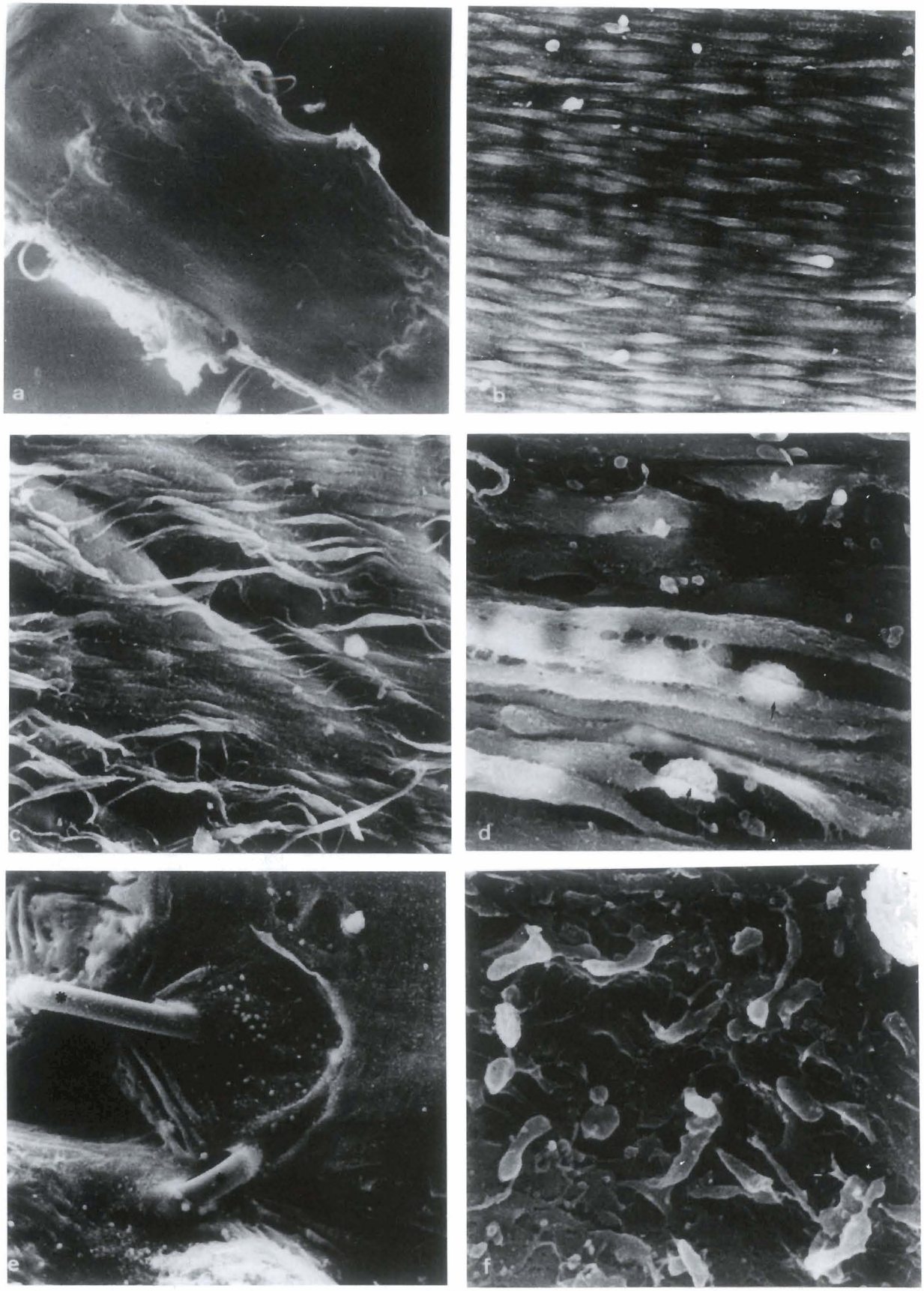
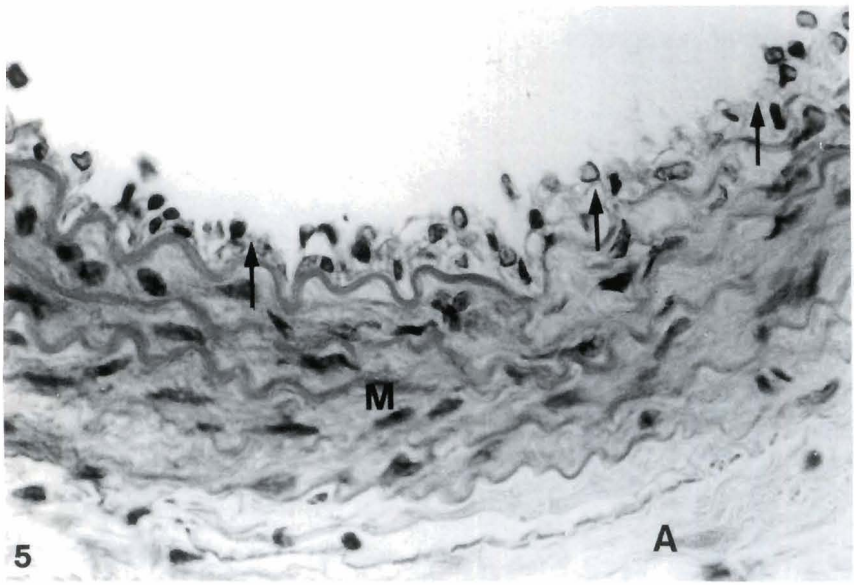
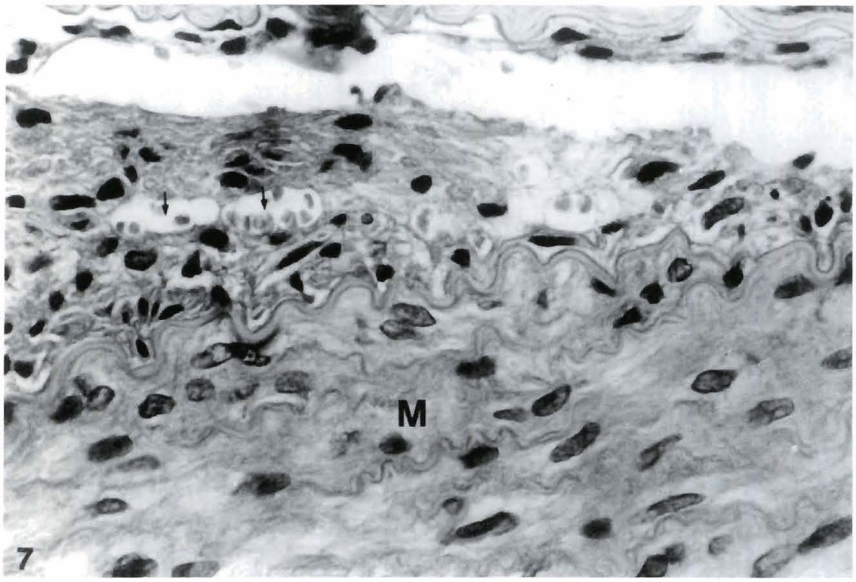


Fig. 4. 5 days micrograft. **a.** Scanning panorama. × 22. **b.** Non-injured endothelium. × 500. **c.** Endothelium rises in sheets. × 500. **d.** White cell (◗) infiltration. × 2,000. **e.** Suture stich (*). × 2,000. **f.** Autograft area. × 5,000





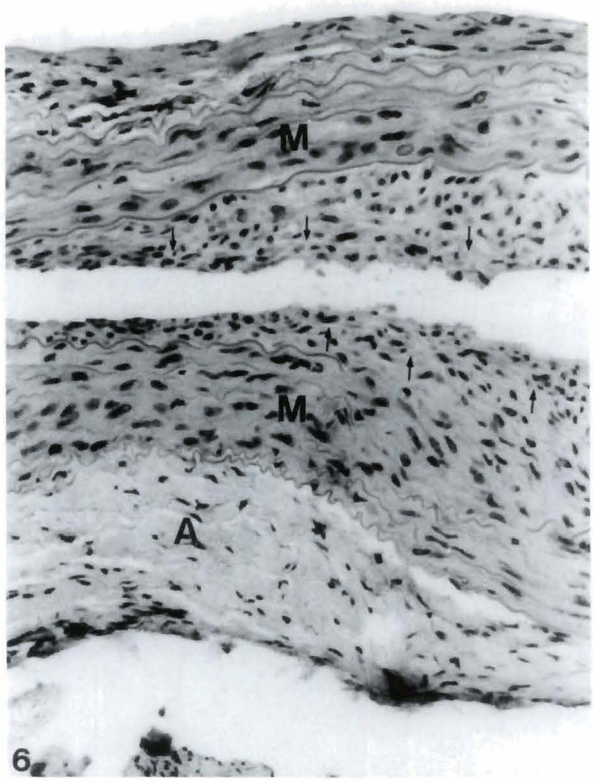


Fig. 5. 9 days microfaft (M) medial layer. (A) adventitial layer. Small patches of hyperplasia. (') × 40

Fig. 6. 21 days autograft. Hyperplasia. ('). (M) medial layer (A) adventitial layer. × 16

Fig. 7. Myointimal vessels (') are present in the third week. (M) medial layer. × 40

problems after surgery. The main complications, early bleeding, thrombosis and aneurysm, did not surpass 10%. The luminal arterial obstruction due to intima hyperplasia, with calcification and necrosis of the myointima, similar to that produced in atheroma patch (Baxter et al., 1972; Acland, 1980), was not observed.

During the first stages after the autograft, the luminal surface showed endothelial lesions, and fibrin and platelet deposits. It is known that the loss of endothelial integrity leads to platelet adhesion phenomenon (Badimon et al., 1990), partially responsible for the beginning of thrombogenic complication and hyperplastic response. Another possible initial mechanism of aggression could be discrete aneurysmatic distention of the injured segment and the initial manipulation (Clowes et al., 1989).

Our observations in the proximal and distal suture areas, where the suture thread and the middle layer are exposed to vascular lumen, agree with those of Isogai et al. (1988) in whose repair process four sequential steps were established.

The white cell adhesion and infiltration under the endothelium, especially monocytes and macrophages, although little reported in cases of autografts, were prominent in our observations.

The findings may indicate a similar process to those observed in the initial stages of the atherosclerotic lesions (Faggiotto et al., 1984; Davies, 1986; Camilleri et al., 1987; Stein and Stein, 1990).

In canine autograft models, Larson et al. (1978) reported changes in the lipid composition in the absence of visible deposits, which could be the base substratum for atherogenic behaviour. In our morphological observations, macrophages loaded with lipids were only found in the stages previous to the repair processes, which may indicate the absence of an atherogenic morphological substratum.

The study of the regeneration by means of tritium thymidine incoroporation, has shown a high capacity of response of the non-injured tissues of the proximal and distal arterial ends adjacent to the graft, where very significant values were observed on the fifth day. Later, intense thymidine incoroporation appeared in the grafter vascular segment. The latter finding agrees with other observations in either autologous (Abbott et al., 1974) or heterologous autografts (Timmermann and Thiede, 1985; Henderson et al., 1986; Galumbeck et al., 1987), as well as in artificial prostheses (Zacharias et al., 1987; Clowes and Reidy, 1987).

In our work, a great activity of the adventitial layer has been revealed, which seems to indicate that this

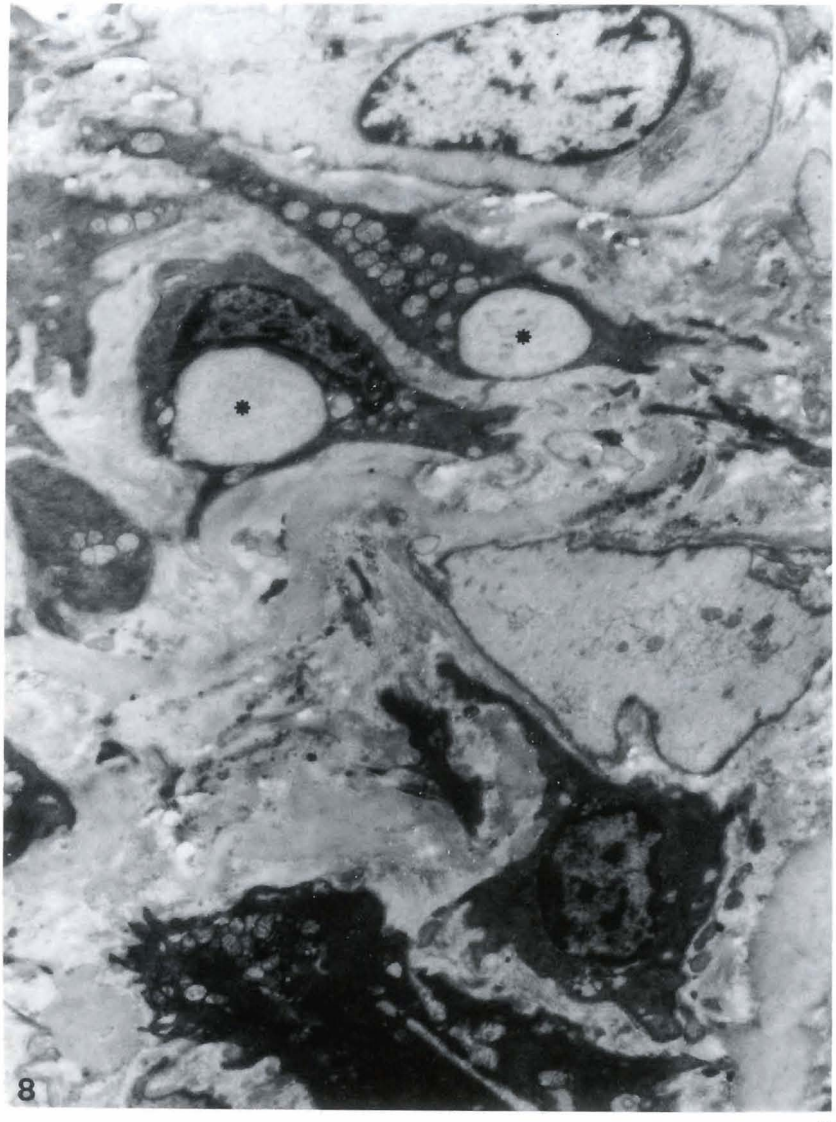


Fig. 8. 21 day autograft. Secretory myocites. (*). × 3,350

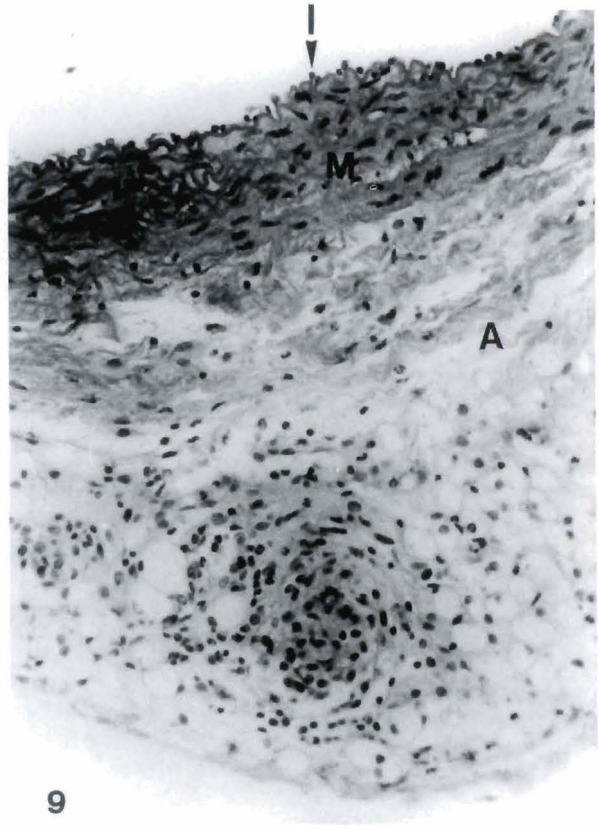


Fig. 9. Adventitial activity at 21 days.(I) intimal. (M) medial layer. (A) adventitial layer. × 16

layer may play role as a source of cells for the neo-adventitia and/or the myointima formations, agreeing with other morphological observations (Díaz-Flores et al., 1990). Indeed, in the myo-intima formation, other kinds of cells may also participate besides the smooth muscle cells. For example perycites of the adventitial microcirculation, which could emigrate and differentiate to contribute to the myointimal cells and even to the vessel reendothelialization (Díaz-Flores et al., 1990).

In our experimental model, in the histogenesis of the intimal thickening, several factors may be involved as has been previously indicated in other cases (Clowes and Clowes, 1980; Munro and Contran, 1988; Weidinger et al., 1990; Gómez Gerique, 1990). Indeed, intimal lesion, with de-endothelialization, platelet adhesion, which releases mitogenic factors, and white cell adhesion (Davies, 1986), with macrophage proliferation and infiltration are the most likely factors. The action of other factors like B fibrinogen, that would justify the observed macrophagic adhesion, and the releasing of diverse leukines by the cells of the vascular wall and the circulating cells, could also contribute to the middle vascular layer proliferation.

Acknowledgements. The work was supported by a grant from the FISS 091/0175 and Alcalá de Henares University No 89/B6-M40.

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Accepted October 4, 1991