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Experimental Diffuse Intimal Thickening of the Femoral Arteries in the Rabbit

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Summary. The temporary placing of a polyethylene cuff around the superficial femoral artery leads to a diffuse intimal thickening without thrombosis. The evolution of the phenomenon during 16 months has been studied by light and electron microscopy. The intimal thickening that developed in the first 2 months, consisted mainly of modified smooth muscle cells, with few leukocytes and some cells of the endothelial type adhering to the endothelium. In the intercellular space, there is a significant production of elastic and collagen material.

The internal elastic lamina is interrupted over part of the vessel circumference for varying distances. The proliferation and dedifferentiation of the medial smooth muscle cells is seen until the 4th month. The adventitial reaction, characterized by fibroblast multiplication and the appearence of numerous histiocytes, disappears at about the 2nd month.

Over time, the heterogeneous cellular population is no longer identified and the intimal thickening is composed, after the 6th month, of well differentiated smooth muscle cells. After the 9th month, the elastic structures have a tendency to become rarefied, while the fibrous tissue increases at the expense of the cellular components. Signs of damage are noted within the intimal and medial smooth muscle cells. These long-standing modifications are related to ageing.

The origin of neointimal cells is discussed and possible participation of endothelial cells is suggested.

Key words: Endarteritis – Femoral artery – Smooth muscle – Endothelium – Elastogenesis – Elastolysis – Ageing

Intimal thickening of the arterial wall is of widespread occurence but unclear significance, in both man and animals. Bouissou et al. [5] studied its natural appearance and we have studied this phenomenon with regard to obstructive arteriosclerosis of the lower limbs and its relationship to ageing [45, 46].

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Twenty years ago, many authors attempted to reproduce the intimal thickening experimentally hoping to understand its histogenesis and to identify its possible links with early lesions of atherosclerosis. Many protocols were proposed using haemodynamic [38], mechanical [22, 28, 29, 34, 36, 43, 61], chemical [31, 37], thermal [7, 22, 59], hypoxic [6, 7, 15, 17, 22, 27, 58, 60], or immunological [18, 19, 20, 21, 40] approaches. Intimal proliferations induced by these methods are almost exclusively composed of smooth muscle cells and abundant fibrillar components. They express parietal remodelling accompanied by important histoenzymatic modifications [1, 47]. Unfortunately, they tend to regress and do not allow comparison with spontaneous lesions in man and animal.

A few years ago [52, 53], we demonstrated that the temporary placing of a polyethylene cuff around the superficial femoral artery of the rabbit at the level of the Scarpa triangle, leads to a diffuse intimal thickening without thrombosis. The advantage of this approach over others is that the lesions regress more slowly and are closer to what we see in human pathology during ageing.

The present work aims at adding some new elements to help us understand the phenomenon and its long term evolution.

Materials and Methods

I. Material and Operative Protocol

The present study included 39 male rabbits "Fauve de Bourgogne" 2.5 to 3 months old and weighing an average of 2,500 g at the begining of the experiment; the animals were fed by a standard food type U.A.R. and divided into two groups after an acclimatization period of 15 days.

a) Group 1. Twenty-seven rabbits were treated by the placing of a polyethylene cuff 10 mm long, on the right superficial femoral artery, according to the technique described by one of us with Rosnowski [53]. The cuff is systematically removed after one week (at the 7th day). Postoperative complications forced us to replace 5 animals.

b) Groups 2. Twelve untreated control rabbits were put in the same conditions.

II. Samples and Preparations of Tissues

A. Samples

One animal of Group 1 was sacrificed each week during the first month and then one every month for 8 months; 2 animals were sacrificed monthly between the ninth and the sixteenth months.

The first sample is therefore taken before removal of the cuff. Subsequently, guiding-marks on the adventitia were made with India ink at the edges of the cuff at the time of its removal; the left artery was systematically removed at the same level, and served as a control.

In Group 2, 4 rabbits were sacrificed on the 15th day of the experiment and the remaining 8, at intervals of 2 rabbits every 3 months. The two femorals were sampled at the same level as for the animals of Group 1.

B. Fixation

To assure the best conditions, fixation was always done in vivo, by perfusion, from the left ventricle, at the physiological pressure, of the anesthetized animal (injection in the ear marginal vein, of urethane 2 ml/kg from a 25% solution and nembutal 0.2 ml/kg from a 5% solution). The fixative solution was 2 % glutaraldehyde in 0.1 M cacodylate buffer pH 7. 2. The arterial fragments were

immediately resected. The treated segment of the right femoral was divided in 3 parts: the central part for electron microscopy, and distal parts for light microscopy. All the control segments were divided into 2 parts for electron and light microscopy.

For ultrastructural study, the fragments were cut into rings of about 1 mm long, returned to the fixative at +4° C for 1 h, washed for 18 h in 0.1 M cacodylate buffer at +4° C, and then postfixed at the same temperature in 2% osmium tetroxide.

For light microscopy, the fragments were counter-fixed in 10% formol – calcium.

C. Embedding and Staining

a) Electron Microscopy. The fixed fragments were dehydrated in ascending concentrations of alcohol followed by propylene oxide and embedded in Epon 812. Thick sections (1 μ) on Reichert Microtome OMU 2, are stained with toluidine blue or methylene blue and appropriate areas were selected for ultrathin sectioning (about 50 nm). After double staining with uranyl acetate and lead citrate (according to Reynolds), the sections are examined in a Siemens IA electron microscope using an accelerating voltage of 80 KV.

b) Light Microscopy. After embedding in paraffin, 5 μ sections were stained as follows: Masson trichrome; Silver impregnation of reticular fibers; Orcein; Periodic acid shiff (P.A.S.); 0.1% Alcian blue in acetic acid (pH 2.7) before and after digestion by testicular hyaluronidase.

Results

I. Macroscopic Observations

After perfusion, the previously cuffed femoral segment had a constantly higher diameter than the control femoral (1.5 to 1.8 mm). This may seem paradoxical. However, it should be noted that the diameter of the cuff (2.5 mm) had always been greater than that of the femoral artery (about 1 mm) in order to avoid mechanical stenosis and thrombosis.

II. Remarks on Normal Arterial Structure

The study of the contra-lateral and ageing control groups, yields the following results, which have been incompletely described in the literature [5, 7, 31, 52]: As of the 8th month, there is a constant appearance of a subendothelial space. Generally narrow, it contains a few myocytes perpendicularly oriented to the endothelium which have cytoplasmic extensions going into the widened fenestrations of the I.E.L. (Fig. 1). A few collagen fibers and microfibrils with two different diameters (3–4 nm and 11 nm) sometimes surrounding small aggregates of amorphous substance, appear between these cells.

More deeply, in the intima – media junction zone, the intercellular space includes, varying rounded formations: myelin figures, vesicles with a fibrillar content appearing like myofibrils and granules with more or less distinct boundaries at this time.

After the 15th month, a subendothelial space appears together with an architectural disorganisation of the internal third of the media, where the cells have variable orientations and are separated by an intercellular space rich in collagen fibers.

Thus, it is necessary to take into account modifications due to physiological ageing, in the interpretation of the experimental lesions.

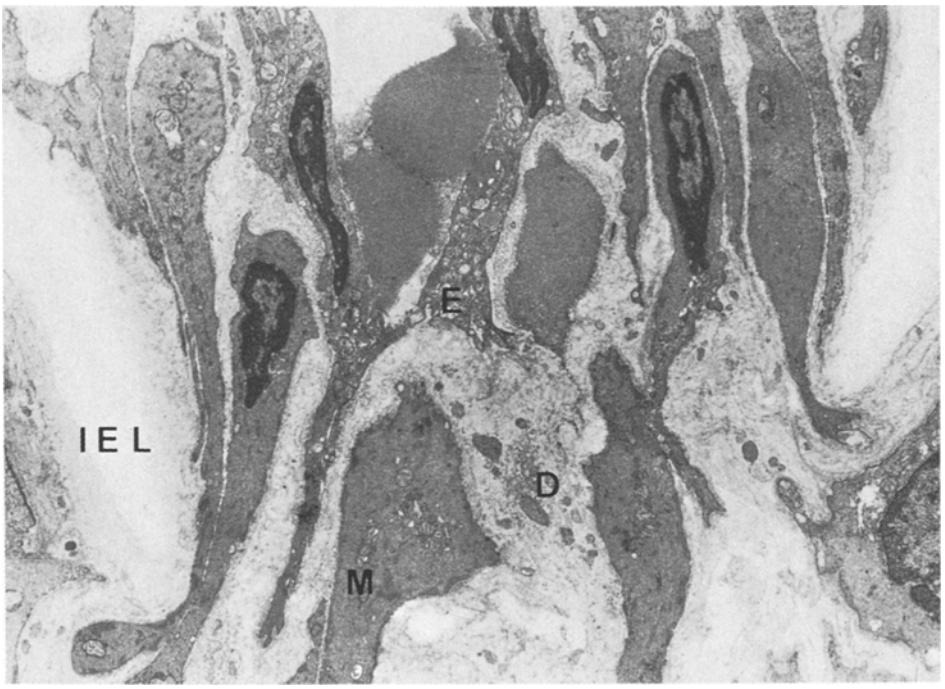


Fig. 1. Femoral artery of a 9 month old control rabbit. Myocytes (*M*) are seen in the widened fenestrations of the internal elastic lamina(*I E L*) – Endothelium (*E*) – Cellular debris (*D*). ×6,850

III. Short and Medium Term Evolution of the Lesions
(1st week to 8th month)

Our data confirm the observations of Roland and Rosnowski [52]. However, our study provides complementary data and more details.

This phase is characterized by the development and the relative stabilization of lesions. At 7 days (Fig. 2), the subendothelial space is populated with 4 to 8 layers of cells, of which the most discontinuous internal layer is formed of elements parallel to the endothelium and joined to it by an apparatus identical to the interendothelial junctions. Their electron density is similar to that of endothelial cells; their cytoplasm is less rich in organelles, but contains Weibel and Palade bodies (Fig. 3).

At a deeper level of this subendothelial space, the cellular composition is polymorphous at this stage. Ultrastructural study revealed two principal cellular types: (1) A few typical myocytes mainly oriented longitudinally. (2) Fusiform, rounded or stellate cells, with a radial or longitudinal orientation and an indented nucleus, sometimes very dense; they are smaller than the myocytes and their cytoplasm, bristling with multiple processes, contains numerous organelles (Fig. 3). These cells lying between myocytes and fibroblasts (myofibroblasts) have a morphology similar to modified myocytes which appeared at the same time in the internal third of the media (Fig. 4). Sometimes they show mitotic figures. Occasionally cells with the characteristics of monocytes are seen (Fig. 5).

The intercellular space includes granulofibrillar material constituting a varia-

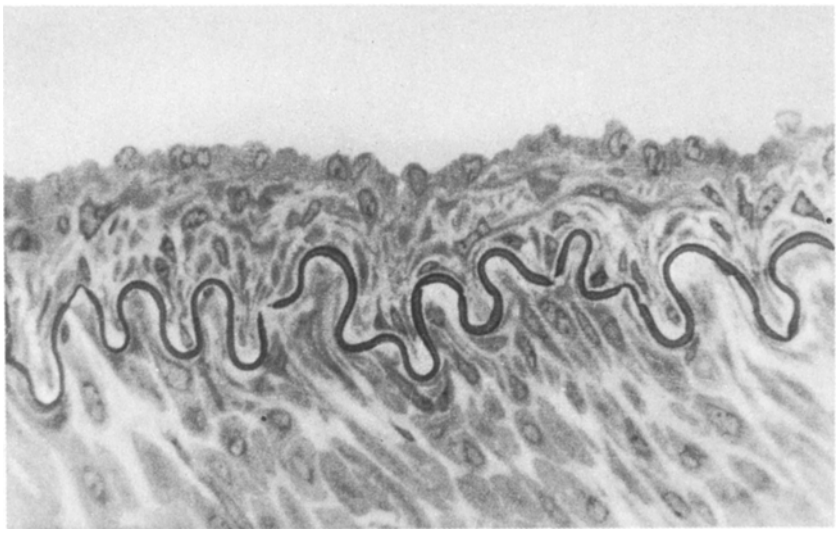


Fig. 2. Intimal thickening 7 days after placing the cuff, showing an abundant interstitial substance. In the inner media, the cells are disoriented. Toluidine blue ×650

bly loose irregular network. At high magnification, we can identify microfibrils with a diameter going from 3–4 to 11 nm (Fig. 6). Collagen fibers are sparse. Cellular debris of variable types, e.g., granular, vesicular or lamellar (myelin bodies) is scattered in this zone.

The evolution of endarterial plaque will be further characterized by the differentiation of the cellular population towards the myocytic type, and by the modifications of intercellular components.

Intimal thickening attains its maximum at the third month (10 to 15 layers); the ratio intima-media can reach 1/1 and in some places, even 2/1 (Fig. 7). Modified myocytes are few and the subendothelial cellular layer described previously is no longer seen. In some cells, we see areas of cytoplasmic lightening corresponding to myofilament rarefaction, or to their complete disappearance (Fig. 8).

In the intercellular substance reticulin fibers, fibrils and amorphous elastin appeared (Fig. 9). The smallest elastin units are seen in the neighbourhood of the endothelial basal membrane or in contact with glycocylyx sheathing myocytes and myofibroblasts. These elastic aggregates merge to form imperfect lamella on the inside and on the outskirts of which numerous microfibrils persist. This elastic neogenesis prevails over the superficial layers, especially beneath the endothelium, where a continuous lamella can appear as early as the 4th month, rather than from the 8th month as we had observed initially (Figs. 10, 11, and 12). Conversely, collagen fibers became more numerous in the deep intima in the proximity of the I.E.L. (Fig. 13).

Finally, the I.E.L. shows interruptions which might attain 7/8 of the circumference in some sections. The media which had had a slight hyperplasia at first (19 ± 3 cell's layers), probably due to the activation of its internal layers (Fig. 4), decreased to 7–8 layers. Modified myocytes are still present and some

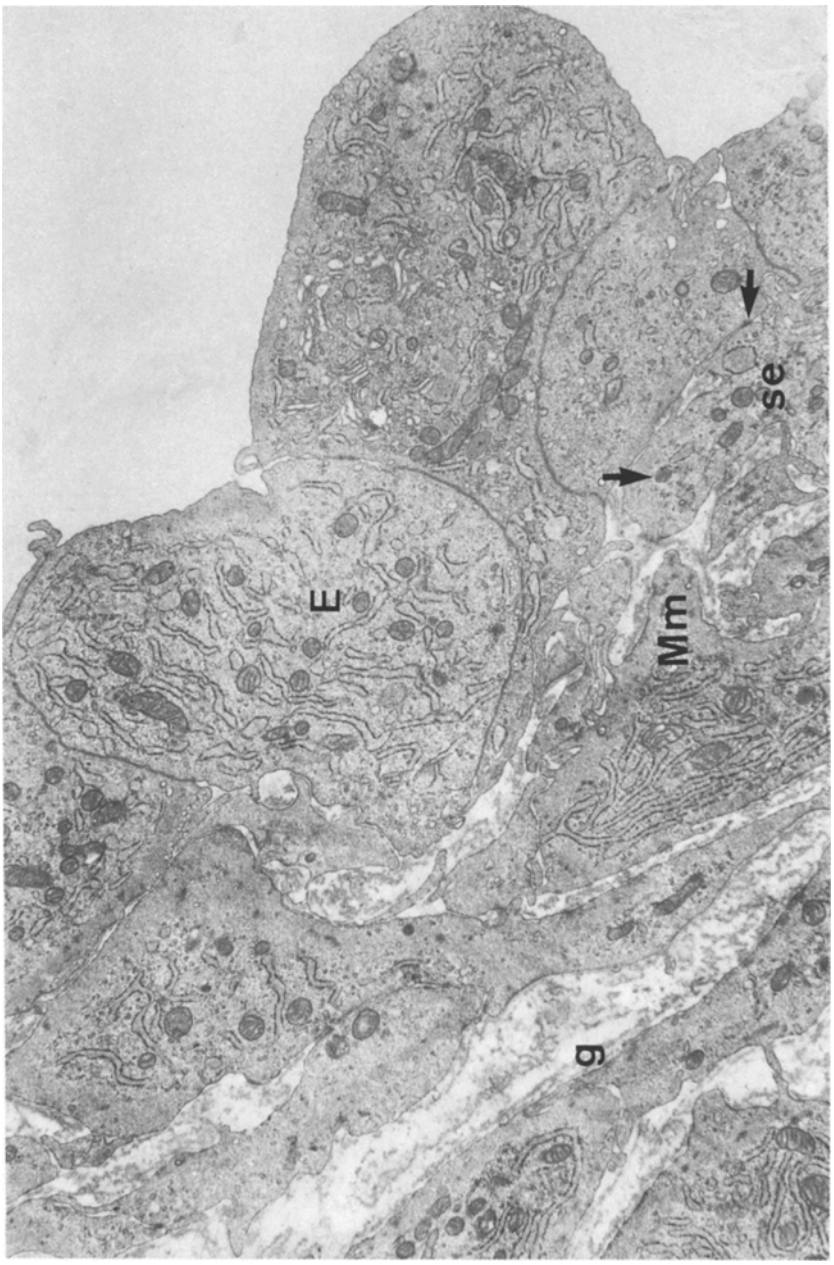


Fig. 3. Femoral artery 7 days after placing the cuff. The endothelium (*E*) shows numerous organelles. The subendothelial cells (*se*) are joined to the overlying endothelium by typical endothelial junctions (*horizontal arrow*) and, although they are less rich in organelles, contain Weibel and Palade Bodies (*vertical arrow*). Modified myocytes (*Mm*) have a discontinuous glycocalyx (*g*). ×11,500

of them are situated in the fenestrations of the I.E.L. From the 6th to the 8th month, the endarterial plaque regresses partially and includes no more than 2 to 8 celllular strata. We can still see signs of endothelial activity in some cells, along with occasional monocytes or undifferentiated cells beneath the endothelium.

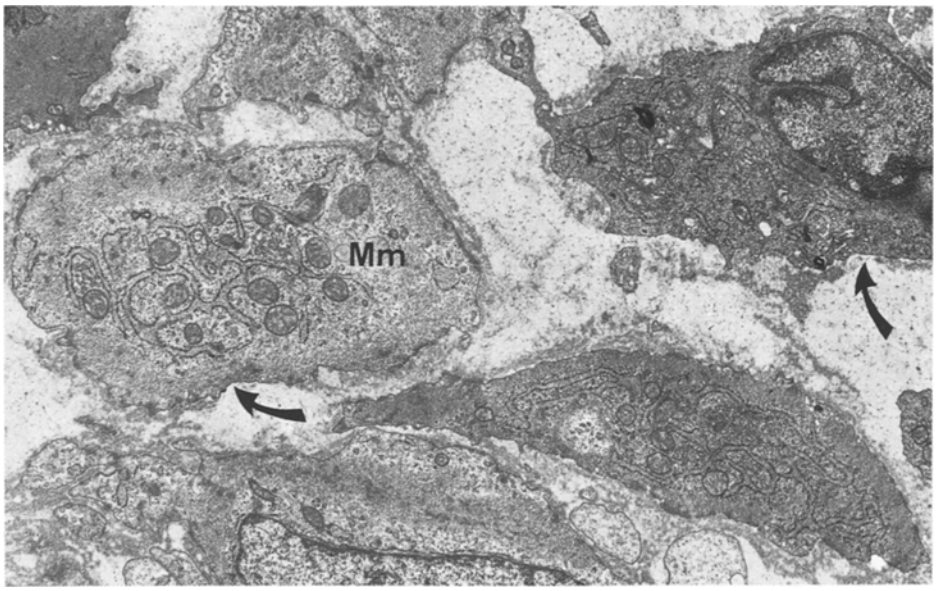


Fig. 4. Internal media of an artery, 7 days after placing the cuff. The myocytes are dedifferentiated (*Mm*), showing numerous organelles and a partially deficient glycocalyx (*arrows*). × 10,500

So, in the short and medium term, the evolution of the experimental intimal thickening shows undeniable resemblance to what we observe during ageing. But the lesions appear from the 7th day and, at 3 months, acquire an intensity which is never realised in physiological ageing. What is interesting is the discontinuity between intimal and internal medial lesions on the one hand and the adventitial reaction on the other hand. The latter is characterized by fibroblast multiplication and the appearance of numerous histiocytes. Vasa vasorum are sometimes dilated into wide lacunae crammed with blood cells and lined by pericytes in mitosis. Collagen fibers are numerous. The adventitial reaction persists for several weeks, decreases afterward and then disappears at about the 2nd month. During this period, cells of the external two-thirds of the media keep their circular orientation, their size and their well differentiated myocytic structure.

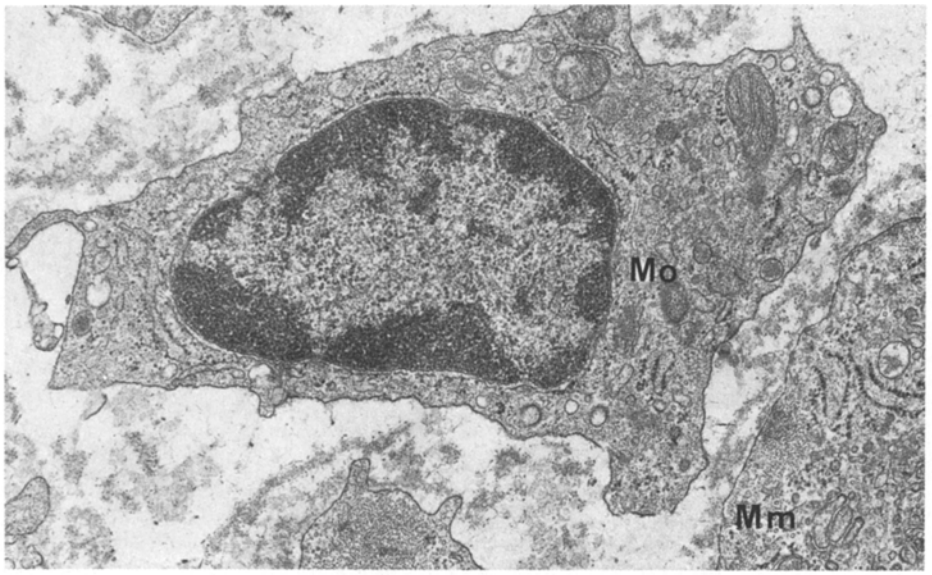
The intimal thickening process shows notable individual variations in different sections as well as differences within the same animal. There may also be circular and longitudinal variations within the treated arterial segment:

The plaque is sometimes discontinuous or of unequal thickness with healthy regions subsisting along the cuffed zone.

Elastogenesis is variable depending on the level observed and, in a single section, shows variable development depending on the arterial circumference; the same is true for the intensity of fibrosis.

The I.E.L. shows more or less extensive and scattered interruptions without obvious systematization.

These findings complete our previous observations and show that, for valid



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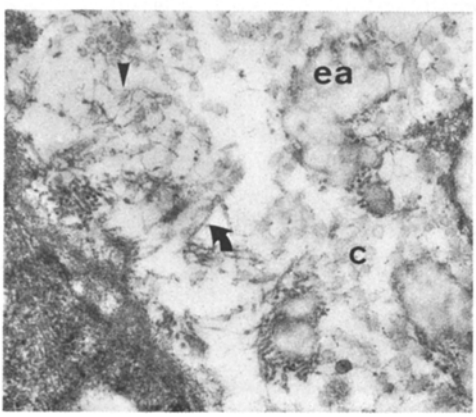

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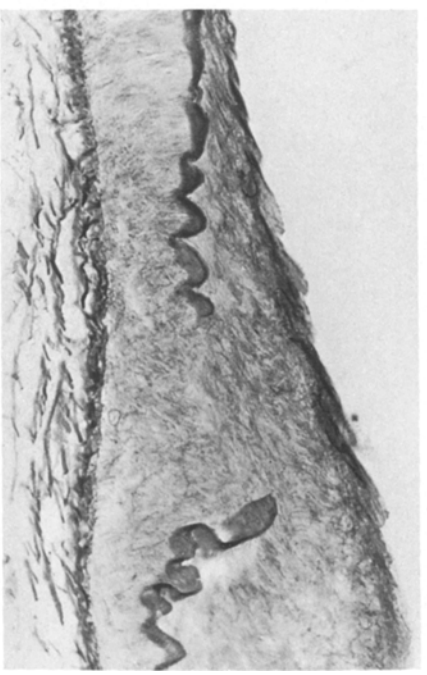

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Fig. 5. 7 Days after placing the cuff, the intimal thickening includes some cells with the characteristics of monocytes (*Mo*). Modified myocytes (*Mm*). × 26,250

Fig. 6. Microfibrils with a diameter of 3–4 nm (*arrow head*) or 11 nm (*arrow*) in an intimal thickening of an artery, 5 months after placing the cuff. Collagen fibers (*c*). Elastic aggregates (*ea*). × 33,600

Fig. 7. After 3 months, the femoral artery shows an increased intimal thickening at the expense of the media. The IEL is disrupted. Orceine. × 250

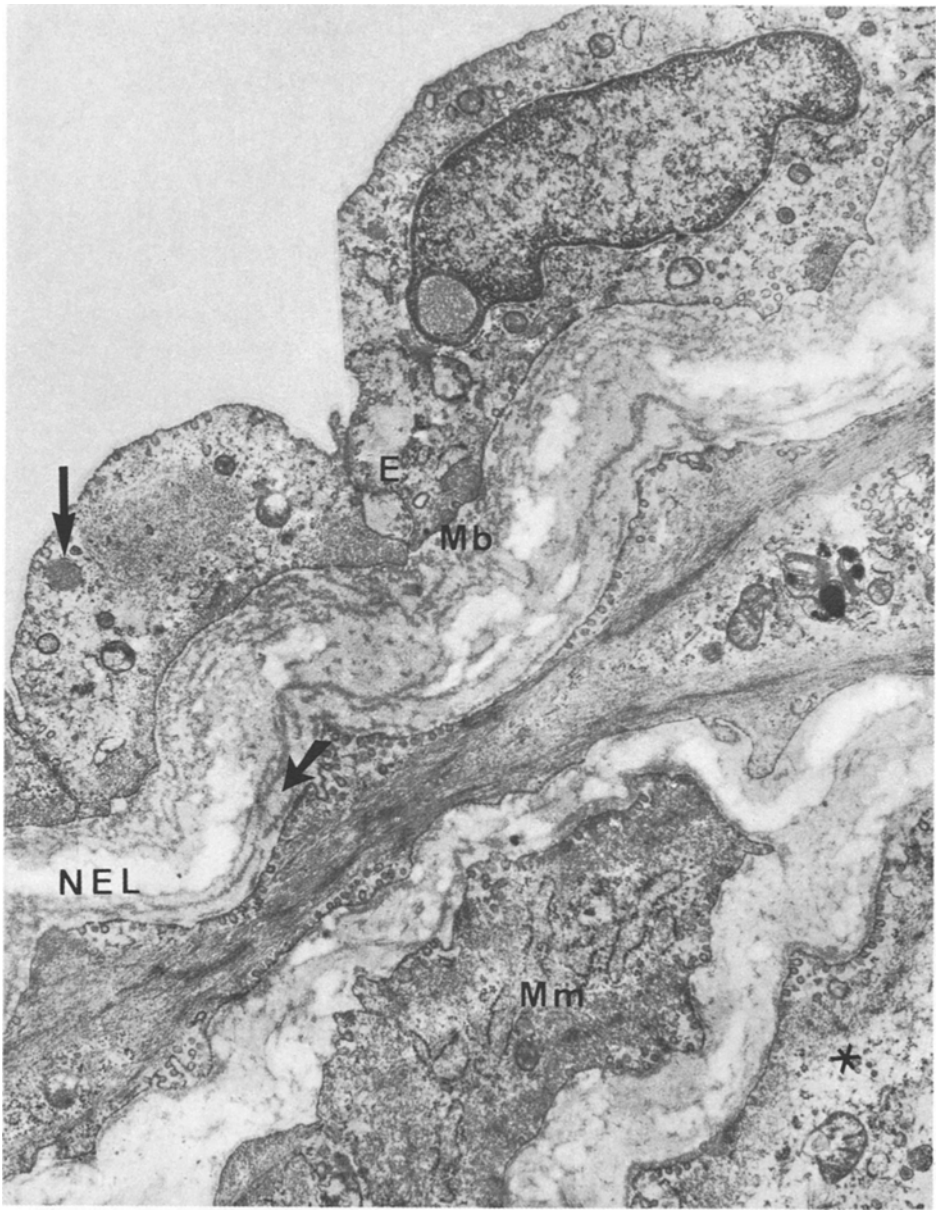
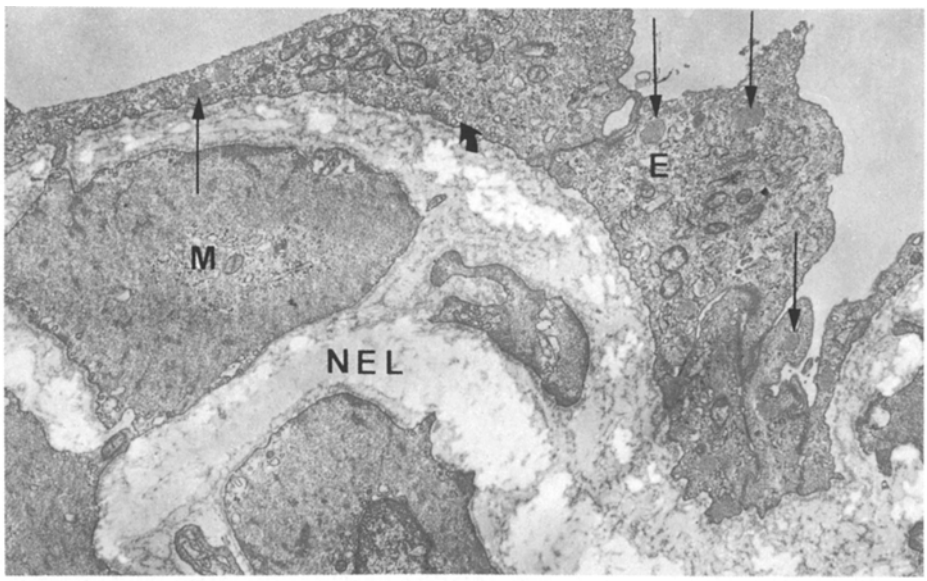


Fig. 8. Three months after placing the cuff, beneath the endothelium (*E*), the intimal thickening includes modified myocytes (*Mm*) showing rarefaction of myofilaments (*) and a glycocalyx which is sometimes split (*arrow*). The endothelial basal membrane (*Mb*) is fragmented and continuous with the underlying microfibrils. New elastic lamelia (*NEL*). Weibel and Palade bodies (*vertical arrow*). × 16,600



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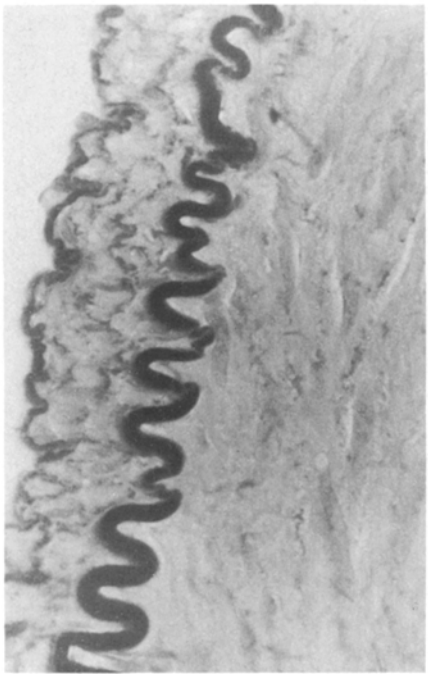

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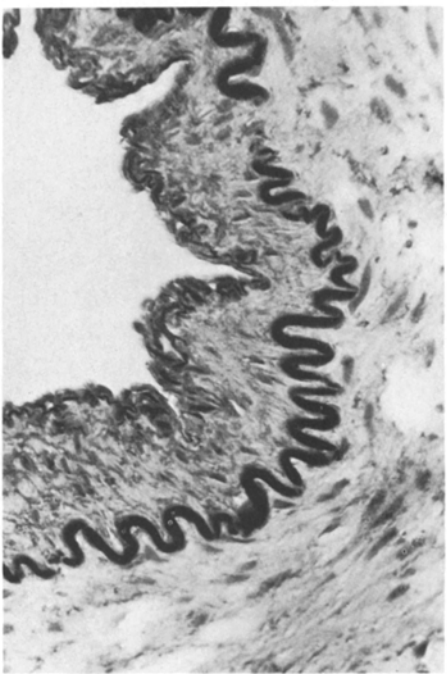

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Fig. 9. Two months after placing the cuff, the elastic aggregates merge to form the still discontinuous lamellae (*NEL*). Endothelium (*E*) shows numerous vesicles of pinocytosis (*thick arrow*) and Weibel and Palade bodies (*vertical arrows*). Myocyte (*M*). ×16,800

Fig. 10. After 4 months: subendothelial newly formed elastic lamella is thinner than the IEL. Orceine ×1,000

Fig. 11. Five months after placing the cuff, elastic neogenesis is predominant in the superficial layers. The IEL shows interruptions. Orceine ×900

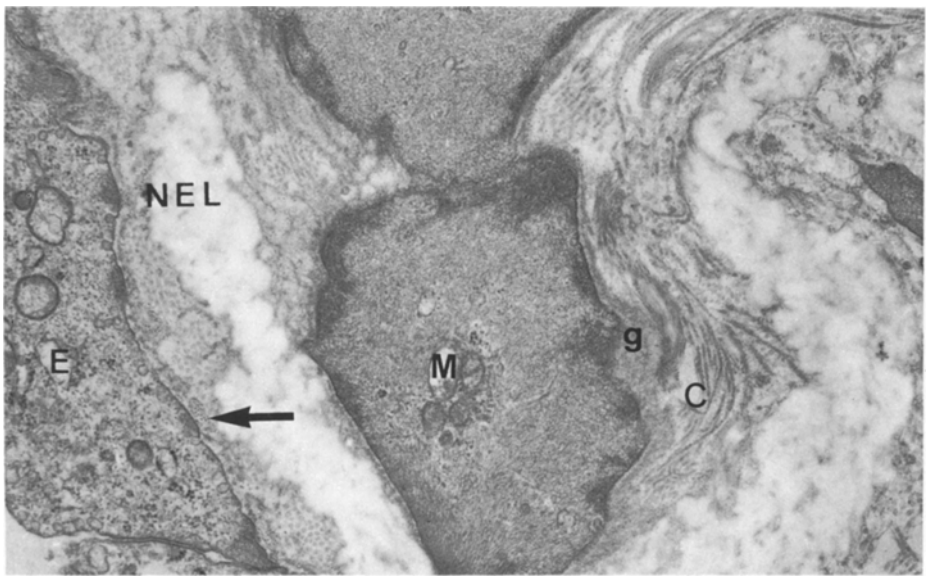

Fig. 12. Intimal thickening of an artery, 4 months after placing the cuff. The continuous new elastic lamellae (*NEL*) contain and are lined by numerous microfibrils. Endothelium (*E*). Microfibrils with a diameter of 11 nm (*arrow*). Collagen fibers (*C*). Myocytes (*M*). Glycocalyx (*g*). ×25,000

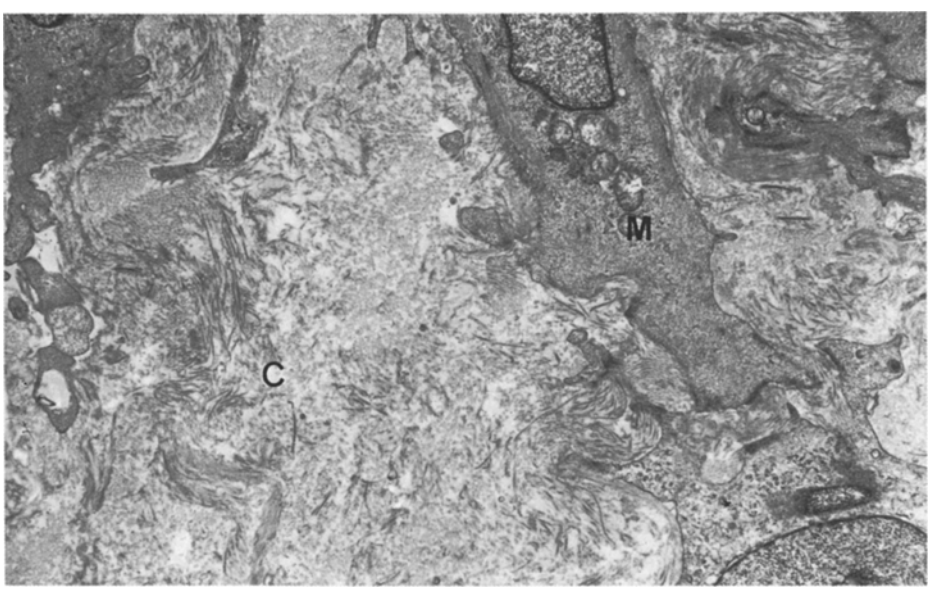

Fig. 13. Four months after placing the cuff: Deep in the thickened intima, the collagen fibers (*C*) are very numerous. Myocytes (*M*). ×12,500

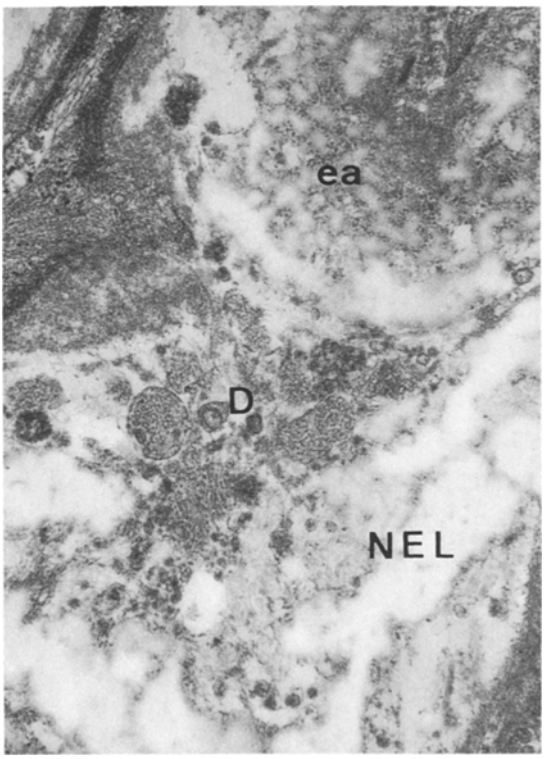

Fig. 14. Intercellular space in the intima of an artery 16 months after placing the cuff. Cellular debris (*D*) of variable size and structure. Elastic aggregates (*ea*). New elastic lamella (*NEL*). ×23,000

and reliable comparative studies, it is necessary to work on data collected from many sections of the same arterial segment.

IV. Long Term Evolution (9th to 16th month)

a) Light Microscopic Study. Over time, the diffuse intimal thickening still remains unequally circumferential (1 to 10 cell layers). The cells which populate the thickening maintain variable size and do not show a fixed orientation with respect to the arterial lumen. The organization of the newly formed elastic elements does not become clearly defined. After about 12 months, we note an increase of fibrous tissue at the expense of the cellular component.

The condition of the media is variable. Its structure is always heterogeneous with myocytes of different orientation, form and density. Enlarged and alcianophilic intercellular spaces include, in addition to elastic fibers, abundant reticulin and collagen fibers.

b) Ultrastructural Study. Systematic ultrastructural study of all samples removed corroborates previous data.

Beneath an endothelium, no longer showing signs of metabolic hyperactivity, the plaque is populated by well differentiated smooth muscle cells after the

9th month. Some of them exhibited signs of damage showing rarefaction or localised disappearance of myofilaments. In the widened intercellular spaces, the collagen fibers are increased, while the elastic structures have a tendency to become rare. The elastolysis is distinct in the I.E.L., but it is much more difficult to see on newly formed elastic structures. Comparative analysis of successive samples suggests that elastogenesis and elastolysis phenomenon are associated until the last stages of evolution. A quantitative study of elastic structures will doubtless allow us to clarify this point. At the level of the I.E.L., the enlarged interruptions are characterized by a fragmentation of the lamina in the shape of aggregates which are mixed with cellular debris and membranous formations (Fig. 14).

In one case, images of myocytes going into the fenestrations of the I.E.L. were still identified at the 14th month.

In the media the ground substance is occupied by many fibers; some of them have collagen periodicity. The others which are contiguous to muscle cells do not have a defined periodicity. They appear to be more delicate and in close relation to elastic material. The enrichment with collagen fibers increases at the 8th month, and occurs along with a considerable diffusion of intercellular debris.

Discussion

The placing of a polyethylene cuff around the rabbit's superficial femoral artery causes a persistent metabolic activation of the wall, with a permanent intimal thickening of variable size.

The unequal induction of plaque in the same animal is explicable by structural differences observed in the I.E.L.. Indeed, when studying the structure of the I.E.L. on the scanning electron microscope, Kerenyi et al. [33] note a non-uniform distribution of fenestrations which have an effect on parietal nutrition and, probably, condition the intensity of transparietal cellular exchanges.

The process starts by endothelial hyperactivation associated with remodeling of internal media and important modifications of the subendothelial space. We have previously evoked this particular topography of parietal reaction. It seems largely related to the operative protocol, which deprives the external two thirds of the media of its nutrition by the vasa vasorum. In order to compensate for this, there is an exacerbation of trans-parietal transfer mechanisms from the blood.

I. Endothelium Modifications

Endothelial reaction is early and transitory, similar to observations in atherosclerosis induced in the rabbit [6, 49] or in the hypertension induced in the rat [23]. Our observations are similar to those of Hatt [23] for whom the great development of organelles is related to an active endothelial hyperplasia. Although we could not observe mitosis, the presence beneath the endothelium of a cellular layer similar to endothelial cells but less rich in organelles, suggests that we are seeing young post-mitotic endothelial cells. Such mitotic activity

is admitted by Altshul [2], Hoff and Gottlob [28], Webster et al. [59], and is in agreement with work concerning the capacity of endothelial regeneration [24, 60].

Our histoenzymatic studies during the experimental endarteritis [1, 47] support the point of view that there is a very clear enzymatic activation since the endothelium and the underlying cells are rich in acid phosphatase, phosphorylase and aerobic oxydoreductases. This suggested a functional relationship with endothelial cells and differences with elements deeply situated in the intimal thickening. As in rapid endothelial regeneration following vascular trauma, this hyperplastic reaction is transitory. At 2 months, we can only see one layer of endothelial cells, in which the organelles remain abundant until the 4th month.

Activation of endothelial cells poses the problem of the part played by them in the population of endarterial plaque and in the elaboration of intercellular substances.

II. Origin of Neointimal Cells

The most widely agreed source is the media. Medial cells from its internal third show early enzymatic activation similar to that of most of the intimal plaque cells [1, 47]. This coincides with a marked development in the intracytoplasmic organelles, along with a partial regression of myofilaments. The presence of such modified smooth muscle cells entering gaps in the I.E.L., suggests that they migrate through this membrane [6, 7, 15, 28, 49]. Autoradiographic studies, using tritiated thymidine [22, 59], show maximal incorporation into the internal media preceding that of the intima. However, these intimal cells continue to divide and mitotic figures are noted, which are more frequent after colchicine blockade [50]. According to other authors, the origin of the cells of the thickening is endothelial [25] or intimal from subendothelial elements [30, 38, 56, 57], or both endothelial and medial [20, 22, 51], or both medial and leukocytic [13], or of triple origin Hatt et al. [23]. We should be able to exclude the hypothesis of intimal development from preexistant elements in the young rabbit, because the subendothelial space contained no cells. The presence of monocytes is reported by various authors following different injuries [6, 15, 23, 28, 29, 51, 59], or as spontaneous lesion [48]. Webster et al. [59], using labeled molecules, demonstrated an increase of endothelial permeability after electrocoagulation or suture placement. Following O'Neal et al. [44], Glagov and Ts'ao [15], we must consider the possibility of a myocytic transformation of the blood cells. In our observations, the presence of leukocytes is not constant and transitional forms are missing. The velocity of blood flow in the arterial lumen probably limits the importance of the phenomenon, and assigns it a feature that is accessory, although real.

The participation of endothelial cells should be considered more seriously. Hatt [23] and Hassler [22] noticed slight participation of endothelial cells in the formation of intimal smooth muscle cells. In our cases, we could not show transitional forms between these cells. However, the observations discussed

above along with the histoenzymatic data suggest that at the beginning of the process there is incorporation within the plaque of sub-intimal cells of endothelial origin.

Therefore, we can state that in the initial stages endothelial cells and, perhaps, blood cells, contribute to the building up of the thickening. But it seems that modified myocytes, coming from the inner media play a major role.

III. The Development of Intercellular Substances

The development of intercellular substances occurs very early in the endarterial plaque (1st week) and shows histochemical and histoenzymatic modifications, suggesting an active fibrillogenesis [1, 47]. The first visible fibrils seem to be related to the development of elastic structures. Our observations concur with those in the literature. There are two types of microfibrils: the most delicate have a diameter of 3 to 5 nm [31, 39, 41], while the largest are going from 7–10 nm [16, 31, 41] to 12 [39] and even 14 nm [26].

The nature and signification of these microfibrils are still under discussion. Haust, Low and Montaldo [26, 39, 41] consider them to be the structural common denominator of elastic and collagen tissue. For Jellinek [31], the largest are of elastic nature and the finest might be associated with elastic fibers. In our experimental findings fibrils appear grouped around small amorphous areas, with which they form "units" in close connection with glycocalyx, surrounding modified myocytes. The role of these myocytes in elastin development is demonstrated by autoradiographic studies [9, 12, 36, 54, 55].

However, it seems that the endothelium also participates in the elaboration of elastic material [8, 11]. In the experimental endarterial plaque, endothelium is very active until the 4th month. Vesicles of micropinocytosis abound on its basal face and the basal membrane is in close relation with the underlying microfibrillar structures. In any case, the first neoformed lamella prevail in this zone.

In most experimental intimal proliferation the synthesis of collagen fibers is attributed to dedifferentiated myocytes [6, 31, 49].

There is a real radial gradient in the distribution of elastic and collagen elements, also noted by Poole et al [50]. The density of the former decreases in the deep intima, whereas the opposite happens for the latter. This distribution recalls that in ageing [4, 42] where the concentration of collagen increases in the intimal tissue, going from the endothelium to the I.E.L.

IV. Long Term Evolution of Endarterial Plaque

It is difficult to assess the role of ageing in the late evolution of lesions. The placing of a polyethylene cuff surely simulate the physiological process of ageing, which occurred more quickly and with greater intensity. The histogenetic mechanisms set in action in both cases are comparable. The accentuated and extensive signs of damage of smooth muscle cells have been described following various experimental processes [15, 23, 35, 43, 51, 56]. If in the first stages the rarefaction

of myofilaments is due to fibroblastic regression [23], their subsequent persistance on typical myocytes would, more likely, be related to the ultimate vulnerability of the cells of intimal thickening. Indeed, the histochemical behaviour of these cells shows failing biogenesis and metabolic instability [18].

In the 9th experimental month, the elastic degradation increases and the newly formed lamella are subjected to major fragmentation, which is also seen in atherosclerosis and hypertension [3]. In addition, the zones reshaped by fibrosis, especially deep in the thickening, enclose few cells such as the intima of arterial allografts [51]. Along with elastolysis, we still see elastic units beneath the endothelium and in contact with modified myocytes. This indicates a continuous synthesis of elastin. From the biochemical point of view these morphologic data suggest a decreasing of desmosines and highly reticulated elastin. The continuous synthesis of elastin is explained by the relative stability of elastin which is weakly reticulated and associated with glycoproteins, compared to highly reticulated elastin [42]. These variations in elastin during ageing are in contrast to the variations which happen during ontogenesis [10].

The newly formed elastin never attains the structural perfection of physiological elastin. This suggest a limit in the potential of arterial construction. The development of functional elastic lamella is limited to ontogenic phase [5, 52].

This long term evolution of endarteritic process explains the persistance of remodeling phenomena in the arterial wall. This is confirmed by the passage of myocytes through the gaps of I.E.L. at the last stages.

Conclusion

The diffuse intimal thickening obtained by the transitory placing of a polyethylene cuff on the superficial femoral artery of the rabbit is part of the general framework of the arterial response to various assaults. The development of the endarterial plaque sets in the activation of mechanisms which are exacerbated by the new conditions. They start by an activation of the endothelium and the internal zone of the media, with colonization of the subendothelial space by modified myocytes, largely coming from the media, through the gaps of the I.E.L.. It is probable that there is also participation of the endothelium at least at the beginning. In the intercellular space, the modalities of elastogenesis recall the development of elastic fibers during ontogenesis. But this elastogeneis never leads to the formation of highly individualized lamella. Moreover, probably due to the effect of ageing, there is elastolysis along with an accentuation of fibrosis and sitgmata of cellular damage. As in human lesions, there is an adaptability of the wall to new biological conditions.

The experimental model does not reveal any parietal lipid infiltration of the atheromatous type. Measurement of cholesterol and triglyceride, made in another set of experiments, does not show characteristic modifications (work in progress). The induced alterations can be regarded as a model of purely proliferative alteration comparable with spontaneous arteriosclerosis.

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