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Studies on the Healing of Arterial Lesions in Experimental Hypertension

I. An Electron Microscopy Study on the Healing Process of Intimal Fibrinoid Degeneration in Hypertensive Rats

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Summary. An electron microscopic study was carried out on the healing process of arterial lesions in hypertensive rats; these lesions resemble the plasmatic arterionecrosis seen in human hypertensive intracerebral hemorrhage. Fibrinoid substance deposited in the arterial intima of hypertensive rats disappeared with or without leaving cellulofibrous intimal tissue after continuous administration of antihypertensive drugs. Fibroblast-like smooth muscle cells, endothelial cells, and blood-derived mononuclear cells took part in the healing process of the intimal fibrinoid degeneration; the fibrinoid substance was phagocytosed by fibroblast-like smooth muscle cells and blood mononuclear cells or dissolved by fibroblast-like smooth muscle cells. Hyaline droplets, thought to represent phagocytized fibrinoid substance, were seen in the intimal smooth muscle cells. The cells also participated in the formation of ground substance and collagenous and elastic fibers. These intimal smooth muscle cells are considered to be partly derived from endothelial cells, because they were first found immediately beneath the endothelium during the healing process, because endothelial cells themselves showed myofilaments and fusiform dense bodies, because intimal cells were not found in the normal rat mesenteric arteries, and because degenerative changes were severe in the medial smooth muscle cells.

Fibrinoid degeneration induced in the arterial intima of hypertensive animals was healed with or without cellulofibrous intimal thickening left behind by removal of the cause of hypertension (Sekiguchi, 1964; Allison, 1967), or by continuous administration of antihypertensive drugs (Kojimahara, 1967). When, however, antihypertensive drugs were given discontinuously, blood pressure was fluctuated, producing marked dysoria (Schürmann and MacMahon, 1933) in the arterial intima, and exacerbating the arterial lesions (Kojimahara, 1967).

In the healing process, in which fibrinoid substance deposited in the arterial intima was absorbed, intimal cells were light-microscopically found to play a principal role. The fibrinoid substance around the intimal cells was markedly reduced in stainability with phosphotungustic acid hematoxylin (PTAH), Mallory's collagen stain, and hematoxylin and eosin stain, presenting a picture like that of the lacunar resorption of bone by osteoclasts; and the formation of hyaline droplets was often observed in the cytoplasm of the intimal cells (Kojimahara, 1967).

In the present study, electron microscopical investigation was performed on the healing process of the experimental hypertensive arterial lesions which resemble the "plasmatic arterionecrosis" (Ooneda, 1970), responsible for the human hypertensive intracerebral hemorrhage, especially on the absorption process of intimal fibrinoid substance after the continuous administration of antihypertensive drugs.

Material and Methods

Male Wistar rats, weighing 60–80 g, with the renal arteries bilaterally constricted with silver clamps were used. They were fed on ordinary diet (Oriental Kobo Ind. Co.) and given tap water *ad libitum*. After confirming the formation of nodular lesions in the mesenteric arteries at 2–8 weeks after the arterial constriction, the animals were given antihypertensive drugs—Apresoline (CIBA) 8 mg and Serpasil (CIBA) 0.08 mg per day—which were dissolved in drinking water and taken freely every day. At 3, 7, 10, 14, 20, 28 and 52 days of the administration, the animals were infused with 1 % osmium tetroxide (Caulfield, 1957) through the left ventricles or aortae, and the nodular lesions of the mesenteric arteries were removed to be fixed in the above mentioned fixative for 2 hours. After dehydration with ethanol, these specimens were embedded in Epon 812. Ultrathin sections were prepared with a Porter-Blum's ultramicrotome, and stained with uranyl acetate and Millonig's lead. The sections were observed under JEM-5G, JEM-7 and JEM-7A electron microscopes. Hypertensive rats, subjected to electron microscopy numbered 30.

Results

The early changes in the intima of the mesenteric arteries of hypertensive rats were swelling of endothelial cells with increased cell organelles and widening of the subendothelial space containing fine granular substance (plasma proteins) and fine fibrillar substance (basement membrane-like substance). They were followed by deposition of dense and compact fibrinoid substance, which sometimes showed periodic cross striations, in the subendothelial space.

1. Healing of the Early Arterial Lesions in Hypertension

In the subendothelial space were observed irregular pale areas, which indicated absorption of the fine granular substance (plasma proteins) deposited in the

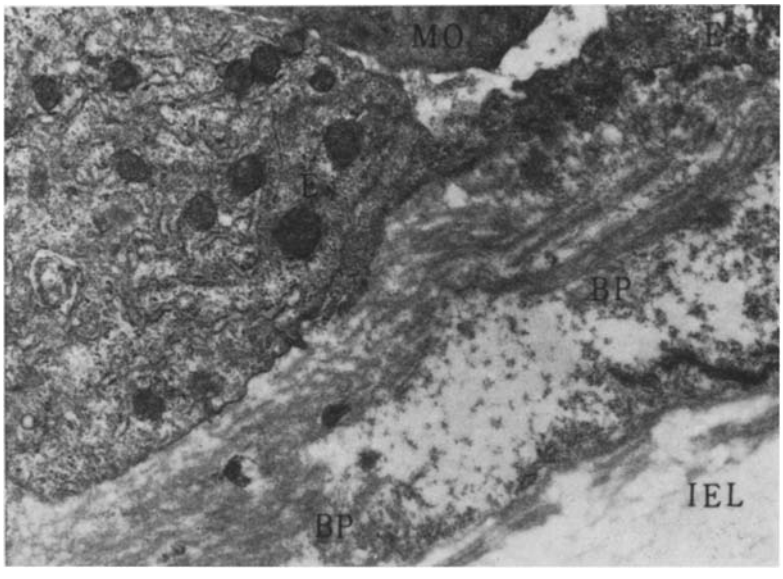


Fig. 1. Mesenteric artery of a hypertensive rat (7 days continuous administration from 4 weeks after the constriction). Absorption of blood plasma proteins (*BP*) deposited in the subendothelial space is seen. *E* endothelial cell; *MO* blood mononuclear cell; *IEL* internal elastic lamina. × 11900

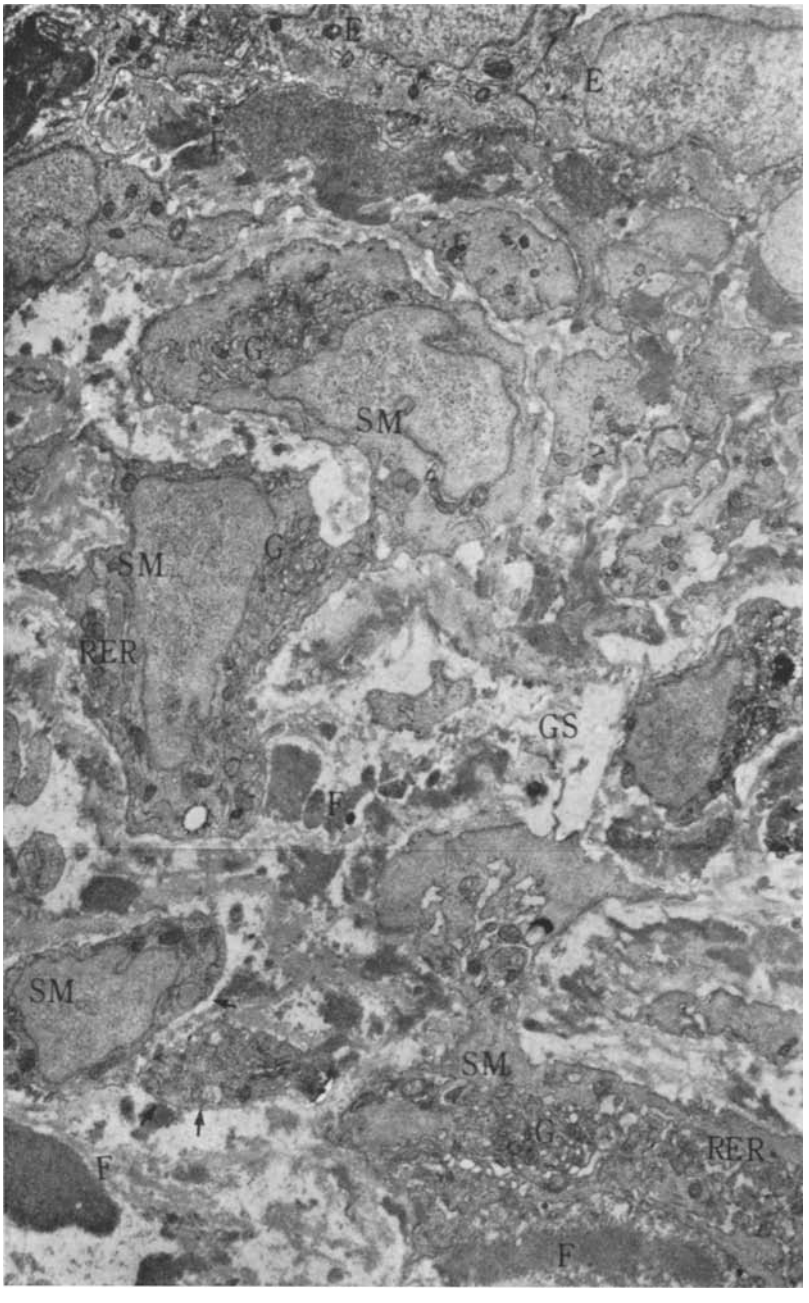


Fig. 2. Mesenteric artery of a hypertensive rat (10 days continuous administration from 8 weeks after the constriction). Intimal cells invading fibrinoid substance are fibroblast-like smooth muscle cells (*SM*). Their Golgi apparatus (*G*) are well developed. Single membrane-bounded absorption droplets are noticed in the cytoplasm (arrow). Fibrinoid substance (*F*) around the cell is dissolved, showing a picture like that of the lacunar resorption of bone. Myofilaments are seen in the endothelial cells (*E*). *RER* rough-surfaced endoplasmic reticulum; *GS* ground substance. × 5300

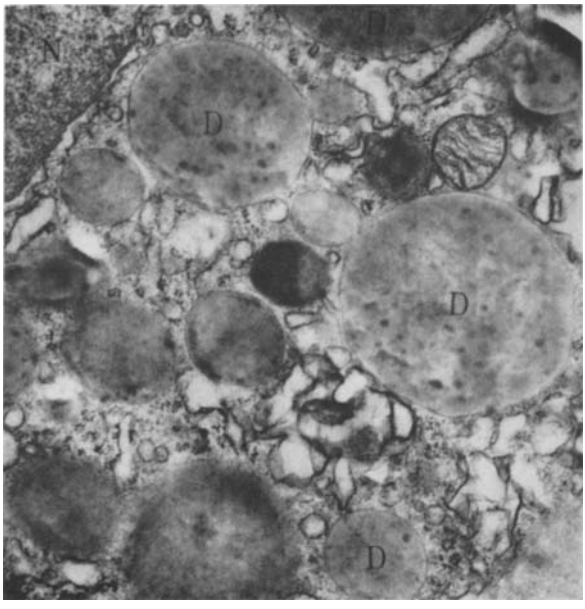


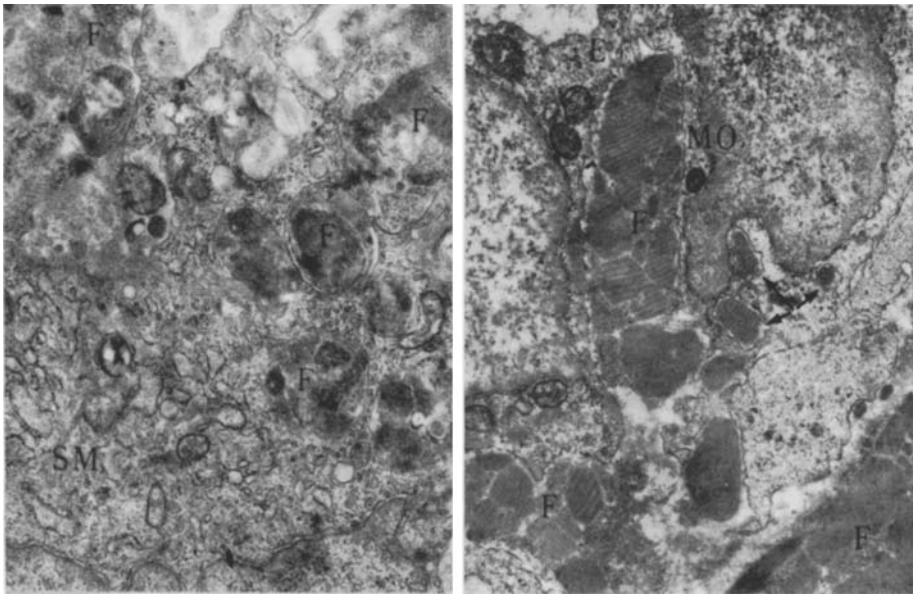
Fig. 3. Mesenteric artery of a hypertensive rat (20 days continuous administration from 7 weeks after the constriction). Hyaline droplets (*D*) are seen in an intimal fibroblast-like smooth muscle cell. They are single membrane-bounded spherical bodies. *N* nucleus. × 23200

space (Fig. 1). At this period, no fragmentation was seen in the internal elastic lamina, nor was observed any myofilaments in the cytoplasm of the endothelial cells.

2. Dissolution and Absorption of Intimal Fibrinoid Substance

In the segments without any fragmentation of the internal elastic lamina, many of intimal cells were first found immediately beneath endothelial cells, and they were resembling endothelial cells and fibroblast-like cells. In the basal cytoplasm of the endothelial cells, irregular myofilamentous structures were sporadically present.

Most of the intimal cells which invaded the intimal fibrinoid substance were fibroblast-like smooth muscle cells (modified smooth muscle cells) with dilated rough-surfaced endoplasmic reticulum, abundant free ribosomes and polysomes, and ectoplasm containing myofilaments. They were characterized by enlargement of the Golgi apparatus, and the Golgi vacuoles and vesicles contained substance which was relatively high in electron density. Fibrinoid substance around these cells was dissolved, and presented an appearance like that of the lacunar resorption of bone by osteoclasts (Fig. 2). Newly formed vacuoles of varied density ("absorption droplets") were noticed and the droplets were sometimes observed as hyaline droplets in the intimal cells invading fibrinoid substance (Figs. 2 and 3). And there was produced ground substance. Moreover phagocytosis of fibrinoid substance by the smooth muscle cells and blood mononuclear cells was observed



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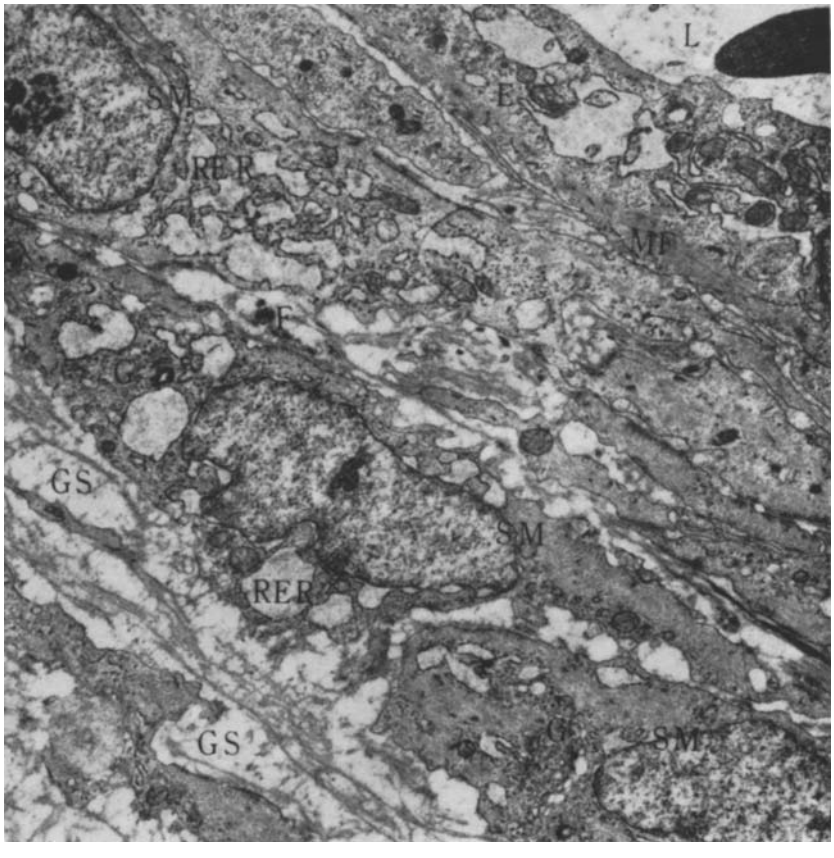

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Fig. 4 and 5

(Fig. 4). The ingested substance in these cells often showed periodicity of about 200 Å (Fig. 4). Some of the cells contained residual bodies of the substance.

When the fibroblast-like smooth muscle cells, which were increased in the intima, were actively participating in the dissolution and absorption of fibrinoid substance and in formation of intercellular ground substance and fibers, endothelial cells of the segments showed an active fibroblast-like appearance with dilated rough-surfaced endoplasmic reticulum and well developed Golgi apparatus. Furthermore the endothelial cells contained myofilaments of 50–100 Å in diameter and even bundles of myofilaments (fusiform dense bodies) mainly in the basal portion of the cytoplasm. Namely the cells showed the appearance of the so-called myoendothelial cells with the character of the fibroblast-like smooth muscle cells (Figs. 2, 5 and 7).

Elastic fibers were formed outside the basement membrane of the fibroblast-like smooth muscle cells or a little away from it; they showed aggregates of microfibrils, about 100 Å in diameter, or lucid central parts surrounded by microfibrils; and there were also collagen fibers in the ground substance (Fig. 6).

On the other hand, in the segments which showed fragmentation of the internal elastic lamina by blood plasma infiltration, many intimal cells were seen just beneath the endothelial cells and near the fragmented areas.

3. Formation of Cellulofibrous Intimal Thickening

When intimal fibrinoid substance had completely been absorbed, the intima was occupied by cellulofibrous tissue. The intimal cells of the tissue were all typical smooth muscle cells with organelles concentrated arround the nuclei, and were surrounded by abundant ground substance, elastic and collagen fibers (Fig. 8). Endothelial cells were flattened and did not contain any myofilaments.

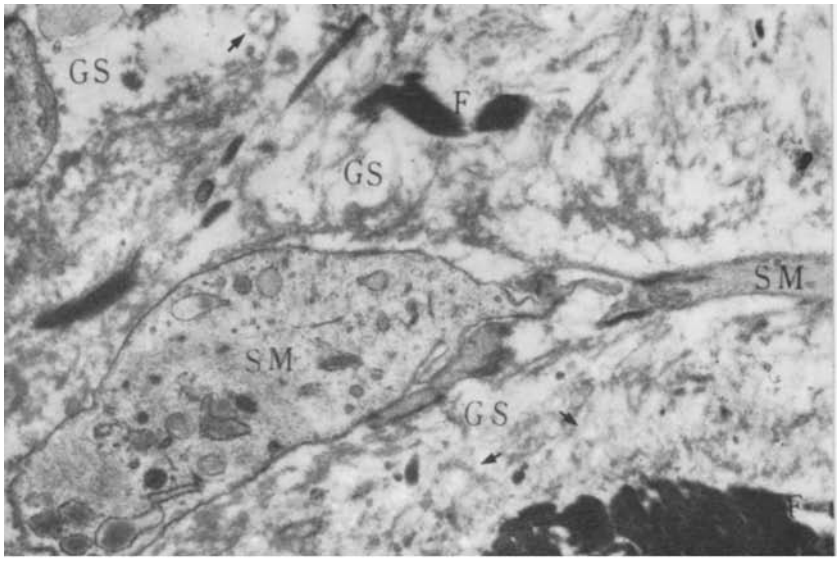
Discussion

When colloidal ^{198}Au was intravenously given to hypertensive rats, it permeated the arterial walls in a large amount; but when the blood pressure of the rats was lowered by continuous administration of antihypertensive drugs, the permeation was markedly decreased towards the normal level (Kojimahara, 1967). When insudation of blood plasma proteins into the arterial walls was decreased or stopped by the administration, intimal cells appeared.

The majority of cells which penetrated the intimal fibrinoid substance to perform actively the dissolution and absorption of the substance were fibroblast-like

Fig. 4. Mesenteric artery of a hypertensive rat (the experimental procedure is the same as Fig. 2). Fibrinoid substance (*F*) is phagocytized by an intimal smooth muscle cell (*SM*, left, × 11800) and by a blood mononuclear cell (*MO*, right). A stripe pattern is seen in the phagocytized fibrinoid substance (arrow). *E* endothelial cell. × 9300

Fig. 5. Mesenteric artery of a hypertensive rat (the experimental procedure is the same as Fig. 2). Increased intimal fibroblast-like smooth muscle cells (*SM*) with well developed rough-surfaced endoplasmic reticulum (*RER*) are seen. Between them ground substance (*GS*), microfibrils and remnants of fibrinoid substance (*F*) are noticed. Endothelial cells (*E*) is so-called myoendothelial cell showing myofilaments (*MF*) and fusiform dense bodies. *G* Golgi apparatus; *L* lumen. × 5700



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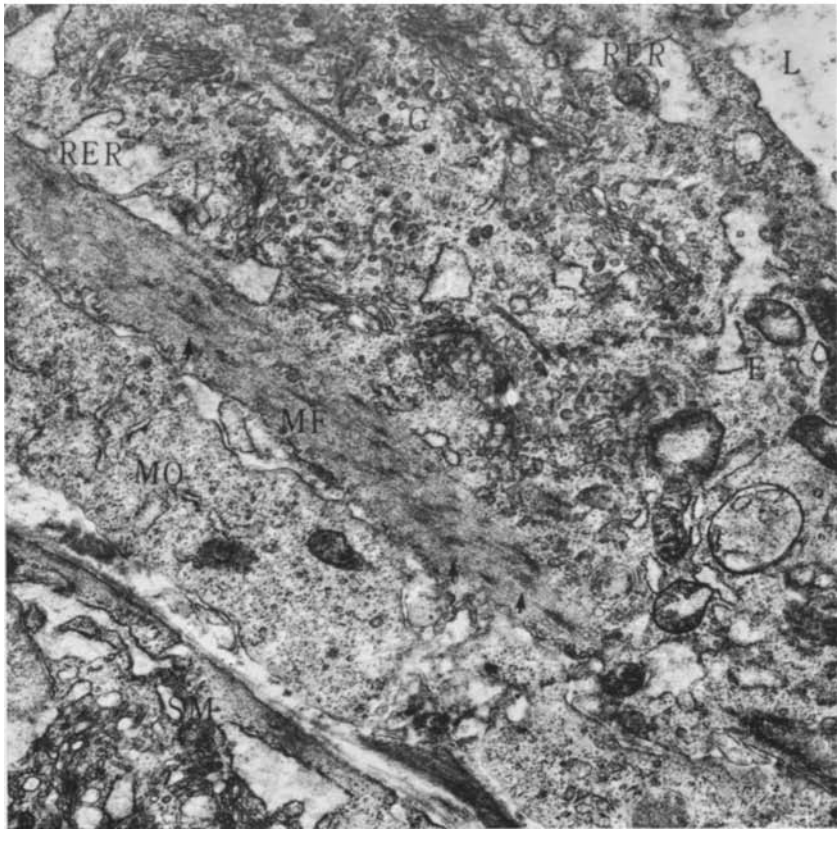

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Fig. 6 and 7

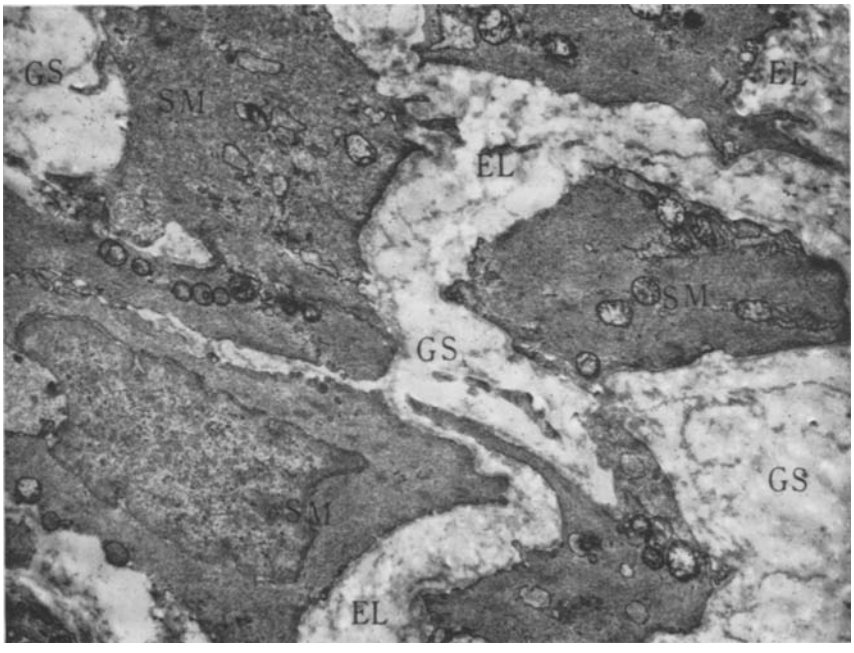

Fig. 8. Mesenteric artery of a hypertensive rat (52 days continuous administration from 7 weeks after the constriction). Intimal typical smooth muscle cells (*SM*) are surrounded by abundant ground substance (*GS*) and elastic fibers (*EL*). × 8400

smooth muscle cells. The rough-surfaced endoplasmic reticulum of the cells were dilated to contain floccular substance, and the vacuoles and vesicles of the hypertrophied Golgi apparatus contained relatively electron dense material. From these facts it is assumed that the fibroblast-like smooth muscle cells may synthesize and secrete some proteolytic enzyme, which will dissolve the surrounding fibrinoid substance, producing a picture resembling the lacunar resorption of bone. Furthermore, "absorption droplets" bounded by a single-layered membrane were newly formed in the intimal cells, invading fibrinoid substance and the droplets were sometimes observed as hyaline droplets, and they were assumed to be derived from lysosomes. This is nearly the same finding as observed in the tubular epithelial cells of the kidney (Miller and Palade, 1964). Therefore constituents of the

Fig. 6. Mesenteric artery of a hypertensive rat (the experimental procedure is the same as Fig. 3). Ground substance (*GS*), microfibrils, collagen fibers, and immature elastic fibers (arrow) are seen around the intimal fibroblast-like smooth muscle cells (*SM*). *F* fibrinoid substance. × 11000

Fig. 7. Mesenteric artery of a hypertensive rat (the experimental procedure is the same as Fig. 2). An endothelial cell (*E*) is a "myoendothelial cell" containing myofilaments (*MF*) and fusiform dense bodies (arrow) in the basal portion of the cytoplasm. *G* Golgi apparatus; *RER* rough-surfaced endoplasmic reticulum; *MO* blood mononuclear cell; *SM* intimal smooth muscle cell; *L* lumen. × 23500

fibrinoid substance, extracellularly dissolved, are considered to be absorbed by the intimal smooth muscle cells. Also ingestion of the intimal fibrinoid substance by blood mononuclear cells and the intimal smooth muscle cells was observed, and the substance thus engulfed by the cells often showed periodicity of about 200 Å (Ooneda *et al.*, 1965). Moreover, its residual bodies were noticed in the cells.

Fibrinoid substance, which is dissolved extracellularly may not only be absorbed by intimal cells but also be carried away into the adventitia by the flow of the tissue fluid. In the present study, however, there was no finding which positively indicated the flowing toward the arterial lumen of the subendothelial deposits (Olsen, 1969), although the dissolution and absorption of the deposits by endothelial cells were observed.

Nearly at the same time with the appearance of the intimal cells, changes were noticed in the endothelial cells of the segments. Thus in the same segment in which the intimal modified smooth muscle cells were found actively performing the dissolution of fibrinoid substance and formation of ground substance and fibers, the endothelial cells were shown to turn into cells resembling the fibroblasts of the active phase. Later they further came to show structural characteristics of smooth muscle cells, that is, the myofilaments (about 50–100 Å thick) and bundles of myofilaments (fusiform dense bodies) running parallel to the long axis of the cell chiefly in the basal portion of the cytoplasm. Namely, they became myoendothelial cells (Figs. 2, 5 and 7). The presence of filamentous structures in endothelial cells was reported by Hibbs *et al.* (1958), Hama (1961), Hackensellner and David (1961), Ooyama (1962), Piezzi *et al.* (1969) and Giacomelli *et al.* (1970). In the present experiment, these filamentous structures in the endothelial cells became indistinct when the formation of intimal tissue consisting of typical smooth muscle cells was completed. It is therefore considered that endothelial cells would produce myofilaments in the cytoplasm when, like intimal smooth muscle cells, they are in hyperfunctional state, synthesizing and releasing various substances. In other words, both endothelial cells and modified smooth muscle cells in the intima are assumed to have similar structure and function. It may be said that the cells can be nothing but intimal smooth muscle cells when the myoendothelial cells have fallen into the subendothelial space as the result of regenerative or reactive proliferation, while they can be regarded as fibroblast-like intimal cells when they have come to the same space in the preceding stage.

In general, medial smooth muscle cells showed marked regressive changes in the presence of fibrinoid degeneration in the intima even when the internal elastic lamina was intact. We can not, however, absolutely deny the possibility of medial smooth muscle cells migrating into the subendothelial space, since they are sometimes found active (Weiß, 1968).

As for the derivation of the smooth muscle cells proliferated in the intima, there are different views (Altschul, 1954; Parker, 1960; Haust *et al.*, 1960; Buck, 1961; Ooyama, 1962; Seifert, 1963; Spiro *et al.*, 1965; Still, 1966; Sawatari, 1966; Jørgensen *et al.*, 1967; Suzuki, 1967; Ooneda, 1968; Jurukova and Knieriem, 1970; De Faria, 1970). The present study indicated that some of the intimal cells would have been derived from endothelial cells, because 1) the intimal cells were first found immediately beneath the endothelium in the healing process, 2) the endothelial cells contained myofilaments and fusiform dense bodies, 3) the intimal cells

were not found in the normal rat mesenteric arteries, and 4) regressive changes were severe in the medial smooth muscle cells.

Many studies have been published concerning the formation of collagenous and elastic fibers (Karrer, 1960; Ooyama, 1962; Greenlee *et al.*, 1966; Takagi and Kawase, 1967; Kádár *et al.*, 1969). The present experiment revealed that the modified smooth muscle cells of the intima produced ground substance, in which were formed young and immature elastic fibers and collagen fibers, and that by and by ground substance, elastic fibers and collagen fibers were abundantly formed around typical smooth muscle cells in the intima.

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