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# Studies on the Healing of Arterial Lesions in Experimental Hypertension

## II. An Electron Microscopy Study on the Origin of Intimal Smooth Muscle Cells in the Intima with Marked Dysoria

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*Summary.* Rats made hypertensive by bilaterally constricting their renal arteries were intermittently given antihypertensive drugs to cause their blood pressure to fluctuate; the intima of their mesenteric arteries was then investigated electron microscopically. The intimal fibrinoid degeneration showed a much weaker tendency to heal than that of the previously reported rats treated continuously with antihypertensive drugs (Kojimahara *et al.*, 1971), and the arteries showed marked dysoria associated with endothelial injury and thrombus formation. Endothelial cells that had migrated from the endothelium into the subendothelial space became intimal cell, which after proliferation by mitosis, formed myofilaments in their cytoplasm, turning into fibroblast-like smooth muscle cells (modified smooth muscle cells) in the intima. Thus some of the smooth muscle cells that proliferated in the intima and took part in the organization of the intimal fibrinoid substance were considered to be derived from endothelial cells.

When blood pressure in hypertensive rats was lowered by the continuous administration of antihypertensive drugs, fibrinoid substance, deposited in the intima was absorbed by intimal fibroblast-like smooth muscle cells and replaced by cellulofibrous tissue as previously reported (Kojimahara *et al.*, 1971). When, however, the blood pressure was fluctuated by the discontinuous administration of the drugs, the intimal fibrinoid degeneration showed less tendency of healing, and there were bleeding and ulceration in the intima, and marked thrombus formation, that is to say, the findings of severe dysoria (Schürmann and MacMahon, 1933; Kojimahara, 1967).

The object of this paper is to describe electron microscopic findings in the intima with marked dysoria and to discuss the derivation of intimal smooth muscle cells, seen in such intima.

### Material and Methods

The methods were the same as described in the previous report (Kojimahara *et al.*, 1971) except that the antihypertensive drugs were given every other week in the present study. After macroscopically confirming the formation of nodular lesions in the mesenteric arteries of hypertensive rats at 2–8 weeks after the constriction of the bilateral renal arteries, the drugs were given for the 1st and 3rd weeks, and tap water without the drugs was given for the 2nd and 4th weeks. The animals were killed at 4 weeks after the start of the drug administration. Hypertensive rats, subjected to electron microscopy numbered 15.

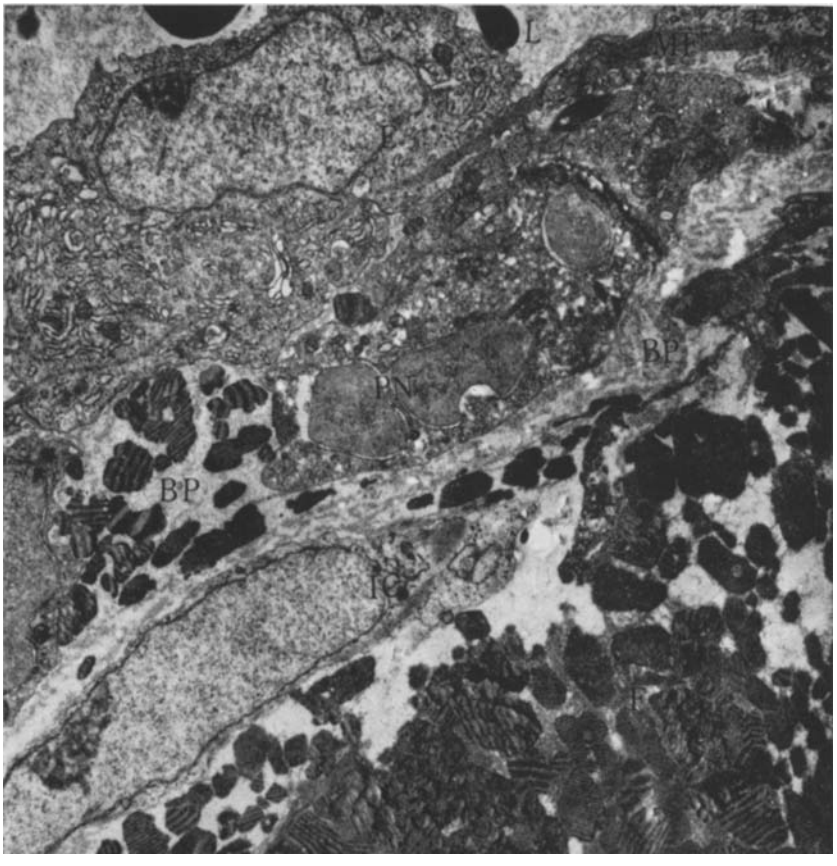


Fig. 1. Mesenteric artery of a hypertensive rat (4 weeks discontinuous administration from 7 weeks after the constriction). In the subendothelial space, are seen fine granular blood plasma proteins (*BP*) and fibrinoid substance (*F*) with various stripe patterns. Myofilamentous structure (*MF*) is noticed near the junction of endothelial cells (*E*). *PN* polymorphonuclear leucocyte; *IC* intimal cells; *L* lumen. ×5600

## Results

Healing tendencies of the intimal fibrinoid degeneration of the animals in the present study were very much weaker than in rats which were continuously given antihypertensive drugs in the previous study (Kojimahara *et al.*, 1971). There were thus many fresh hypertensive arterial lesions (Fig. 1), and endothelial injuries were prominent in the segments in which the lesions were found or their organization started. These were demonstrated by such pictures of exacerbation—widening of interspaces between endothelial cells, endothelial cell necrosis, intimal hemorrhage and intimal ulceration. The internal elastic lamina was fragmented and dissolved as the result of blood plasma infiltration.

There was marked thrombus formation. Intimal cells, which were proliferated in the inner layer of the intima with widened spaces between endothelial cells, were fibroblast-like cells or fibroblast-like smooth muscle cells (Fig. 2).

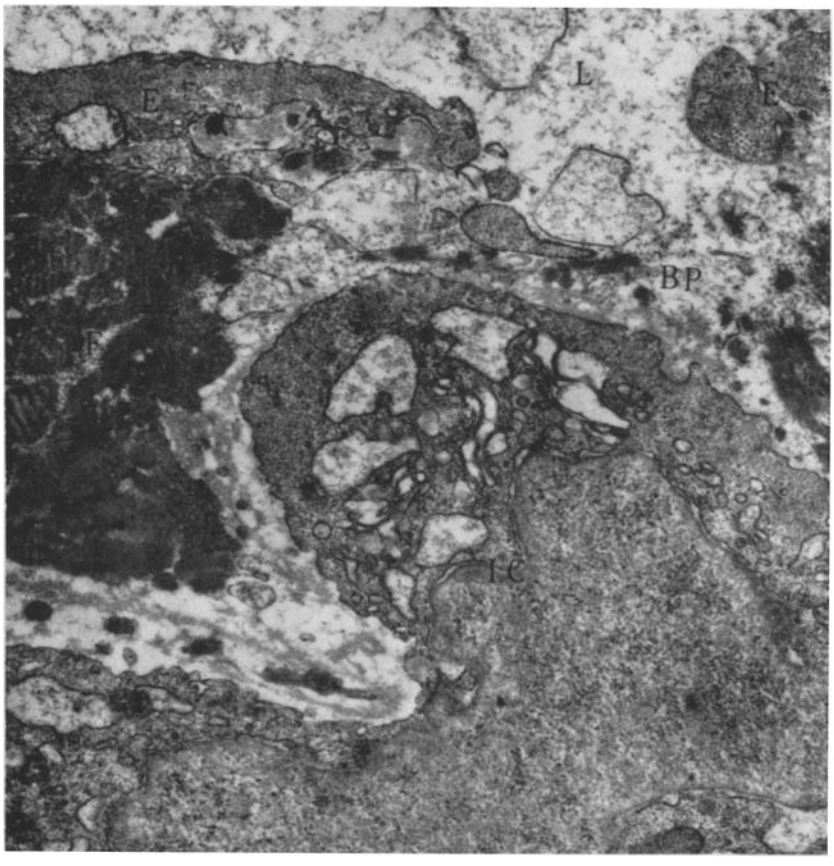


Fig. 2. Mesenteric artery of a hypertensive rat (4 weeks discontinuous administration from 7 weeks after the constriction). Interspace between endothelial cells (*E*) was widened, and an intimal cell (*IC*) and fibrinoid substance (*F*) are found facing the lumen. *BP* blood plasma proteins; *L* lumen. × 9300

In the intima where ulceration or extensive disappearance of endothelial cells were seen, the endothelial cells, sporadically found, were necrotic ones with dark granular cytoplasm, very active ones with fibroblast-like appearance, and myo-endothelial cell-like ones with myofilamentous structures in their cytoplasm.

In the endothelial cells, which covered fibrin and granular substances, deposited on the denuded intima, the Golgi apparatus were well developed, and the basal parts of the cytoplasm were characterized by the presence of myofilamentous structures (Fig. 3).

Two neighbouring endothelial cells were drawn together so that an intervening cell between them was driven out to fall into the subendothelial space (Fig. 4). Another swollen endothelial cells were covering these cells (Fig. 4). The endothelial cells which were driven out into the subendothelial space showed a fibroblast-like appearance, and intercellular junctions were observed between the cells

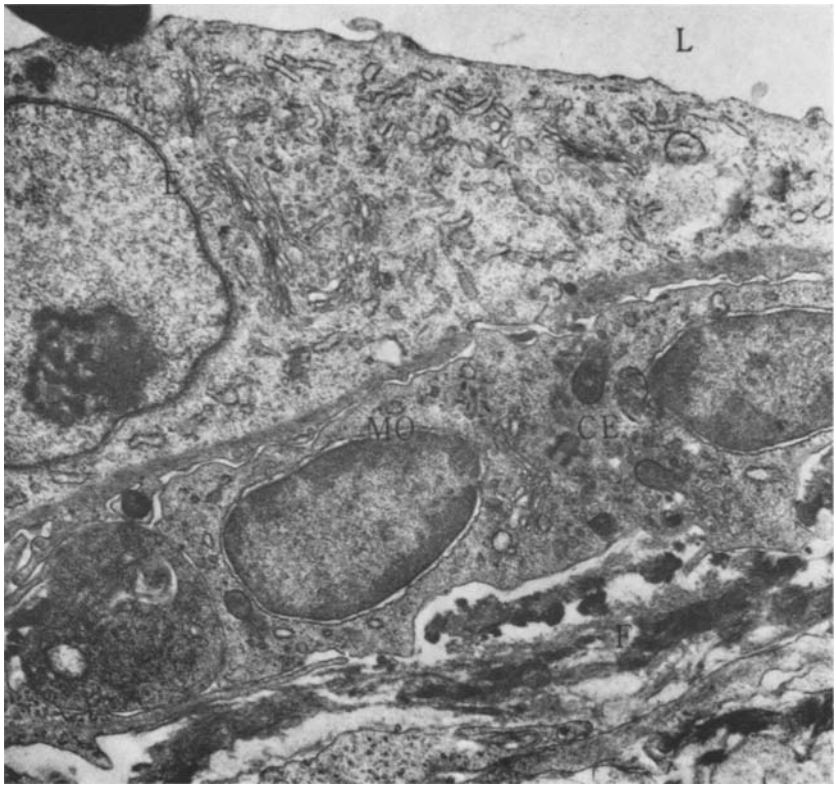


Fig. 3. Mesenteric artery of a hypertensive rat (4 weeks discontinuous administration from 7 weeks after the constriction). Myofilamentous structure is noticed in the basal cytoplasm of an endothelial cell (*E*). *MO* blood mononuclear cell; *CE* centriole; *F* fibrinoid substance; *L* lumen. × 8200

and lining endothelial cells (Fig. 4). When isolated from blood stream, the driven out cells were freed from the intercellular junction, and became intimal cells. Mitotic figures were observed in these endothelial cell-derived intimal cells. In the intimal cells which were proliferated in this way, the myofilamentous structures came to be seen more conspicuously, and by and by differentiated into fibroblast-like smooth muscle cells provided with abundant organelles and myofilaments in their cytoplasm (Fig. 5).

The penetration of neutrophils and circulating mononuclear cells into the sub-endothelial space was more marked than in rats which were continuously given antihypertensive drugs.

There were segments in which the intima showed cellulofibrous thickening, which consisted mainly of smooth muscle cells, without fibrinoid substance. It was assumed that there must have been fibrinoid degeneration preceding the cellulo-fibrous intimal thickening, because the internal elastic lamina was fragmented or disappeared in the segments.

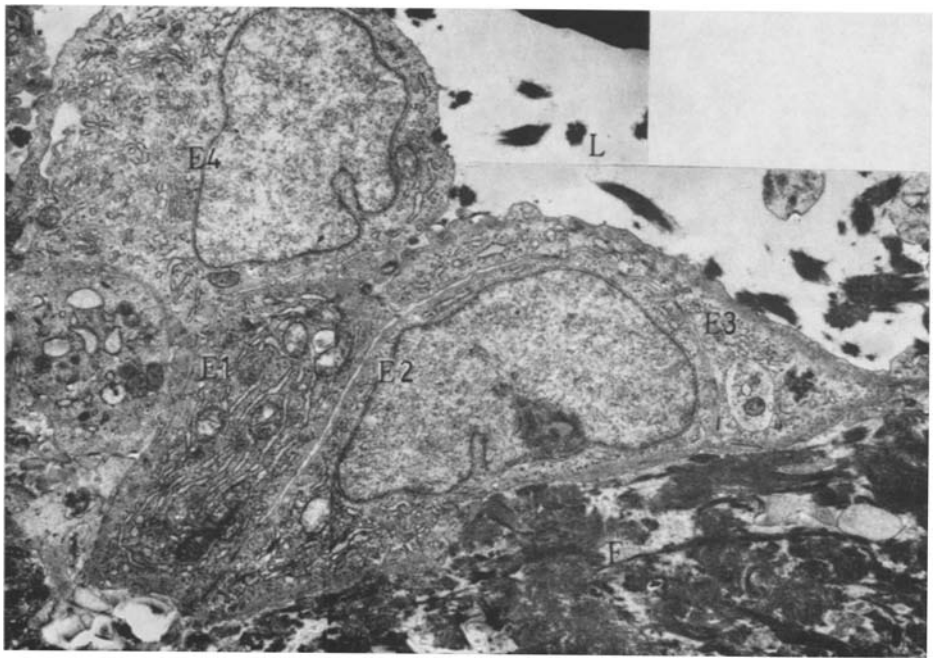


Fig. 4. Mesenteric artery of a hypertensive rat (4 weeks discontinuous administration from 7 weeks after the constriction). Regenerative proliferation of endothelial cells (*E*). Two endothelial cells (*E1*and *E3*) are considered to have driven out an *E2* endothelial cell into the subendothelial space. Junctions are seen between *E1* and *E2* and between *E2* and *E3*. Another endothelial cell (*E4*) is covering these cells. All are deficient of the features of blood cells. *E3* shows an autophagic vacuole. *F* fibrinoid substance; *L* lumen. ×7000

## Discussion

The fibrinoid substance which was observed in the intima of hypertensive rats was composed of blood plasma proteins especially fibrinogen insudated into the intima owing to the increased permeability; fibrin which is produced by polymerization of the fibrinogen and which had periodic cross striation of about 200 Å was the main constituent. The substance showed irregular stripe patterns (Ooyama, 1962; Ooneda *et al.*, 1965; Hüttner *et al.*, 1968). In the present experiment, however, in which blood pressure was fluctuated, fibrinoid degeneration was produced not only through insudation of blood plasma proteins but also likely through incorporation by endothelial cells of fibrinoid thrombus (Duguid, 1959) formed in the part of severe dysoria.

Endothelial cells which underwent severe degeneration owing to fluctuation of blood pressure, were eventually lost. But those spared from degeneration or receiving only minimal injury were regeneratively proliferated later. In the present experiment, an endothelial cell was found driven out by the approximation of the two neighbouring cells around it into the subendothelial space where it was converted into an intimal cell (Fig. 4).

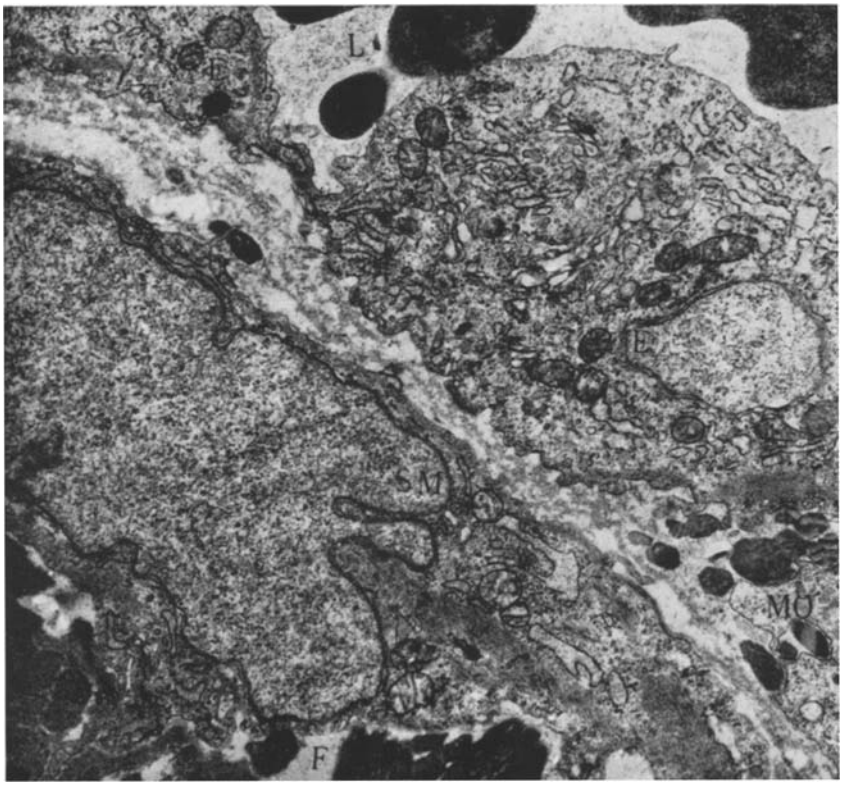


Fig. 5. Mesenteric artery of a hypertensive rat (4 weeks discontinuous administration from 7 weeks after the constriction). Fibroblast-like smooth muscle cell (*SM*) and blood mononuclear cell with phagocytosed fibrinoid substance (*MO*) are seen beneath the endothelial cells (*E*). In the endothelial cells, the Golgi apparatus is hypertrophied, and myofilamentous structures are seen in the basal cytoplasm. *F* fibrinoid substance; *L* lumen. × 6900

The regenerative proliferation of injured endothelial cells is said to be performed through amitosis (Kunz and Sulkin, 1949), or through mitosis (Pool *et al.*, 1958; Suzuki *et al.*, 1970) or through either of these (Sinapius, 1952, 1966). In the present experiment, mitosis was observed in those derived from endothelial cells which had fallen into the subendothelial space.

Endothelial cells, when separated from the endothelial lining and isolated from blood stream, are said to become fibroblasts (Silberberg, 1930; Altschul, 1954; Ooyama, 1962). Though intimal cells just beneath the endothelial cells were fibroblast-like cells in the present as well as in the previous study (Kojimahara *et al.*, 1971), most of the cells showed myofilamentous structures in the cytoplasm. When these cells began to participate in the absorption of intimal fibrinoid substance, they turned into fibroblast-like smooth muscle cells (modified smooth muscle cells) provided with abundant cell organelles, especially rough-surfaced endoplasmic reticulum and with myofilaments in their ectoplasm. It was thus confirmed that the modified smooth muscle cells, which played an active role in the

healing of the intimal lesions of the arteries in experimental hypertension, were partly derived from endothelial cells (Altschul, 1954; Ooyama, 1962; Ooneda, 1968). The following can be mentioned as the reasons of this confirmation; 1) In the healing process of the intimal fibrinoid degeneration, as previously reported (Kojimahara *et al.*, 1971), intimal cells were first observed just beneath the endothelial cells; 2) endothelial cells, like intimal cells, came to have myofilaments in their cytoplasm and thus turned into myoendothelial cells resembling the intimal modified smooth muscle cells; 3) as revealed in the present study, endothelial cells fell into the subendothelial space to turn into intimal cells, which, through mitotic proliferation, became the modified smooth muscle cells; and further 4) the intimal cells were absent in the mesenteric arteries of normal control rats.

As for the cause of the dissolution of the internal elastic lamina, one of the characteristics of the arterial lesions of hypertensive rats, blood plasma infiltration is considered most important. Owing to this infiltration the lamina became serrated and fragmented, and then it was dissolved. It was assumed that in this case the action of elastase, derived from the pancreas and granules of neutrophils (Janoff and Scherer, 1968; Loeven, 1969), might be involved.

There is a view that blood cells would become intimal smooth muscle cells (Still, 1966; Jørgensen *et al.*, 1967). We can not absolutely deny a possibility that the endothelial cell-derived cells, which were found in the present experiment to fall into the lumen, may be intermingled among circulating blood cells.

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