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An Electron Microscopic Study of Plasmatic Arterionecrosis in the Human Cerebral Arteries*

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Summary. An electron microscopic study of the intracerebral arteries from 9 hypertensive cases was performed in order to elucidate the morphogenesis of the plasmatic arterionecrosis which was considered to be the direct cause of hypertensive intracerebral hemorrhage. In the preceding stage of the arterial lesions, marked necrosis of medial smooth muscle cells and increase of basement membrane-like substance in the intima and media were observed. The lumina of these arteries were slightly dilated. The dilatation and hemodynamic factors were supposed to cause endothelial injury resulting in blood plasma insudation into the intima through the opened spaces between endothelial cells. The insudated blood plasma dispersed and dissolved the basement membrane-like substance, collagen and elastic fibers in the arterial wall, leading to the development of the plasmatic arterionecrosis.

Key words: Cerebral hemorrhage — Cerebral artery disease — Plasmatic arterionecrosis — Electron microscopy.

It was reported that hypertensive intracerebral hemorrhage was caused by rupture of the plasmatic arterionecrosis in the intracerebral arteries and of intracerebral microaneurysms resulted from the arterionecrosis, and that medial smooth muscle cell necrosis and blood plasma insudation were important in the morphogenesis of the arterionecrosis (Ooneda *et al.*, 1970, 1973). There is no exact knowledge, however, how endothelial permeability increases and what kinds of factors take part in the histolysis of arterial wall in the development of the lesions. The authors have investigated the fine structure of the plasmatic arterionecrosis in the human cerebral arteries, and discussed its morphogenesis.

Material and Methods

Materials were obtained at autopsy within 3 hours after death from 9 hypertensive cases aged from 48 to 78 years—5 with intracerebral hemorrhage and 4 without it.

The two criteria that were used to determine whether the patient was hypertensive or not were the systolic blood pressure and heart weight. Hypertension was defined as a systolic blood pressure of 150 mm Hg or over. The second criterion was a ratio of the heart weight (g) to body height (cm) of 2 or more.

The cerebral arteries were fixed by perfusion of a buffered 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) containing a 0.2 M sucrose through the bilateral internal carotid and vertebral arteries of the brain removed at autopsy. Then 1% trypan blue in the same fixative was also perfused for staining of the intracerebral arteries. From the cerebral cortices and basal ganglia, small tissue blocks, 2 mm thick, were obtained, refixed with the above-

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mentioned fixative for 4–8 hours and then cleared with pharmacopeial glycerin for several days. Microaneurysms of the intracerebral arteries observed in the cleared slices were excised. After through washing with the phosphate buffer, they were postfixed in the phosphate buffered 1% osmium tetraoxide for 2 hours, dehydrated with graded ethanol, and embedded in Epon 812. Ultrathin sections were prepared and double-stained with uranyl acetate and lead solution, and observed under a JEM-7A type electron microscope. Moreover sections for light microscopy were cut from the Epon-embedded materials, and stained with 2% toluidine blue.

Results

1. Preceding Changes of Plasmatic Arterionecrosis

Light Microscopy. The lesions of this stage were characterized by the extreme loss of medial smooth muscle cells and hyaline and fibrous thickening and edematous swelling of the intima. The internal elastic laminae of these arteries were stretched and the lumina were slightly dilated.

Electron Microscopy. Neither endothelial desquamation nor the opening of intercellular junctions of endothelial cells was observed. And a single layer of endothelial cells was closely attached to the subendothelial tissue (Fig. 1). The intima failed to show any evident deposition of blood plasma constituents, but revealed that basement membrane-like substance was increased in a reticular or multilaminar arrangement. Between the substance were sporadically distributed cell debris and necrotic intimal cells, which were surrounded by the basement membrane-like substance and elongated (Fig. 1). The substance was continuous with the same substance in the media through the fenestrations or disruptions of the internal elastic lamina.

Intercellular spaces between medial smooth muscle cells were enlarged, where broad and laminar basement membrane-like substance was increased in a laminated or reticular fashion (Fig. 1). Many of the smooth muscle cells had undergone necrosis and their cytoplasm was turned into small masses or vesicles, which were scattered between the basement membrane-like substance (Fig. 1). Survived smooth muscle cells were elongated and poor in myofilaments. Large fat vacuoles bounded by limiting membrane were often seen in them. The basement membrane-like substance directly beneath the endothelium was lower in electron density than that surrounding medial smooth muscle cells (Fig. 1).

In the adventitia, fibroblasts were observed in which cytoplasmic organelles and nuclei were well preserved, and around which were formed relatively large amount of collagen fibers.

2. Plasmatic Arterionecrosis

Light Microscopy. In the affected artery swollen endothelial cells projected into the lumen. In the deep layer of the slightly thickened intima with fibrous tissue, collagen fibers were swollen and lowered in staining, and sometimes a small number of foam cells were noticed, whereas in the upper layer, being deeply stained with toluidine blue, collagen fibers were indistinct and the tissue was homogeneous (fibrinoid degeneration). The internal elastic lamina was stretched, fragmented and lost here and there. Medial smooth muscle cells disappeared, so that the intima came directly in contact with the adventitial connective tissue (Fig. 2).

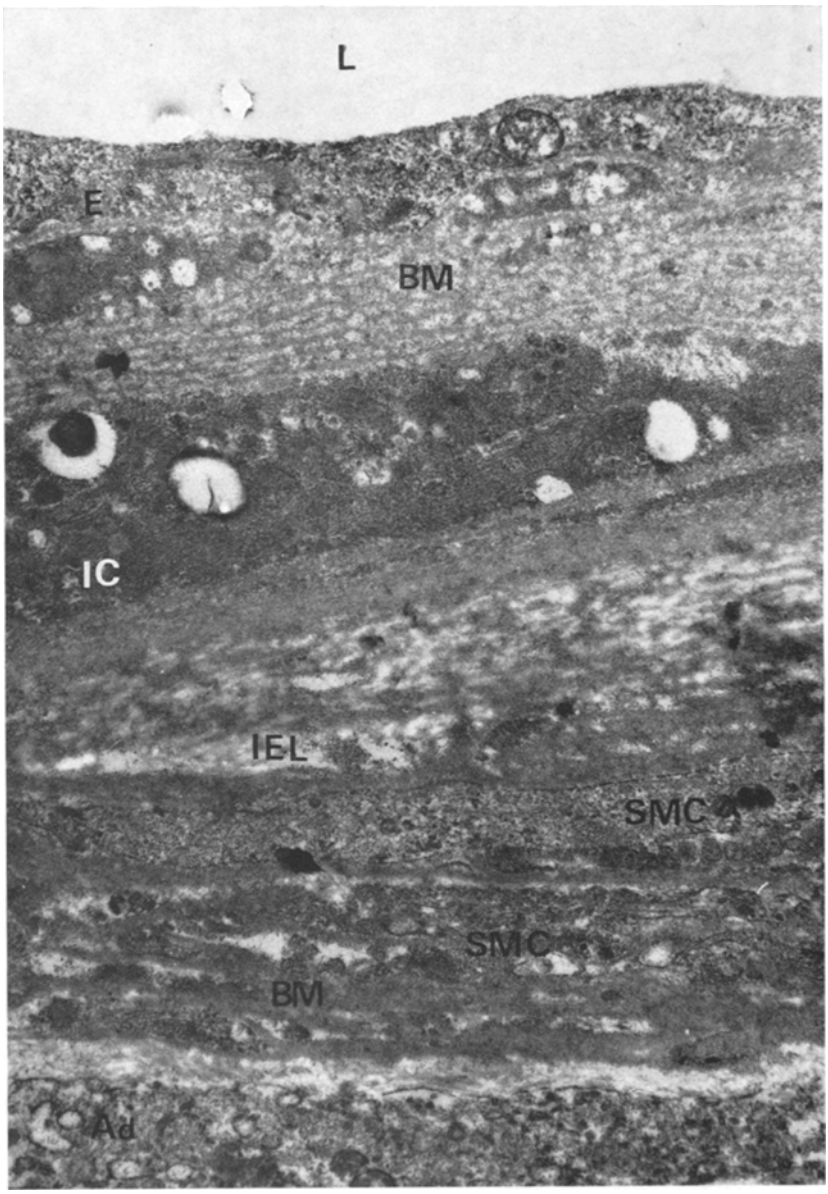

Fig. 1. Lesions preceding the plasmatic arterionecrosis. There are increased basement membrane-like substance (*BM*) and necrosed smooth muscle (*SMC*) and intimal cells (*IC*) in the intima and media. *Ad*, adventitia; *E*, endothelial cells; *IEL*, internal elastic lamina; *L*, arterial lumen. The medullary artery in the cerebral cortex from a hypertensive case, aged 57 years. ×18,200

Electron Microscopy. Endothelial cells of the affected segments were swollen with cytoplasm of low electron density, in which ribosomes and cell organelles were decreased. Filaments in the endothelial cells tended to be rather more numer-

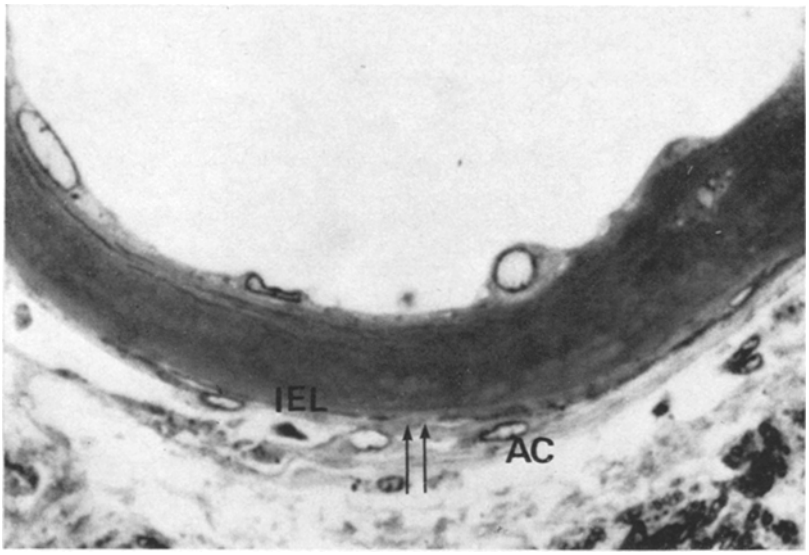

Fig. 2. Light microscopic picture of plasmatic arterionecrosis. Fibrously thickened intima is swollen by insudated blood plasma. Note the marked swelling of endothelial cells, disruption of the internal elastic lamina (*IEL*; arrows) and disappearance of medial smooth muscle cells. Adventitial cells (*AC*) are well preserved. A branch of the lateral striate arteries (140 μ in diameter) in the putamen from a hypertensive case, aged 56 years. Toluidine blue stain

ous in the basal portion of the cytoplasm, and were running mainly in parallel with the basal cell membrane (Fig. 3). Enlarged vacuoles (6,000–9,000 Å in size) in the endothelial cells contained homogeneous substance of medium electron density or hollow minute droplets (approximately 270 Å in diameter) of high electron density regularly arranged on several straight lines (assumedly lipoprotein; Fig. 3). Endothelial pinocytotic vesicles opening into the subendothelial space were filled with fibrillar or granular substance of the same electron density with that of subendothelial fibrin and blood plasma protein (Fig. 4). On the luminar surface of endothelial cells deposition of platelets and fibrin was not noticed. Some of endothelial cell junctions were opened to 3,800–4,700 Å spaces, and in the subendothelium, particularly beneath the opened junctions, fibrin, appearing as a rod-shaped mass of high electron-dense fibrillar substance, and blood plasma protein, appearing as flocculent and granular materials were deposited (Figs. 3 and 4). Subendothelial basement membrane-like substance was almost lost, but sometimes it remained there in several thin layers, and blood plasma protein was accumulated between them. Fibrillar, flocculent and granular substance resembling blood plasma protein (Fig. 3) tended to fused each other and formed an electron dense rod-shaped substance having regular cross-striations with a period of 138 to 270 Å (Fig. 4). This periodicity nearly agreed with that of fibrin.

In the deep intima, besides fibrin and blood plasma protein, lipoprotein-like hollow granular substance in various sizes (about 250–1,600 Å), cell debris, foam

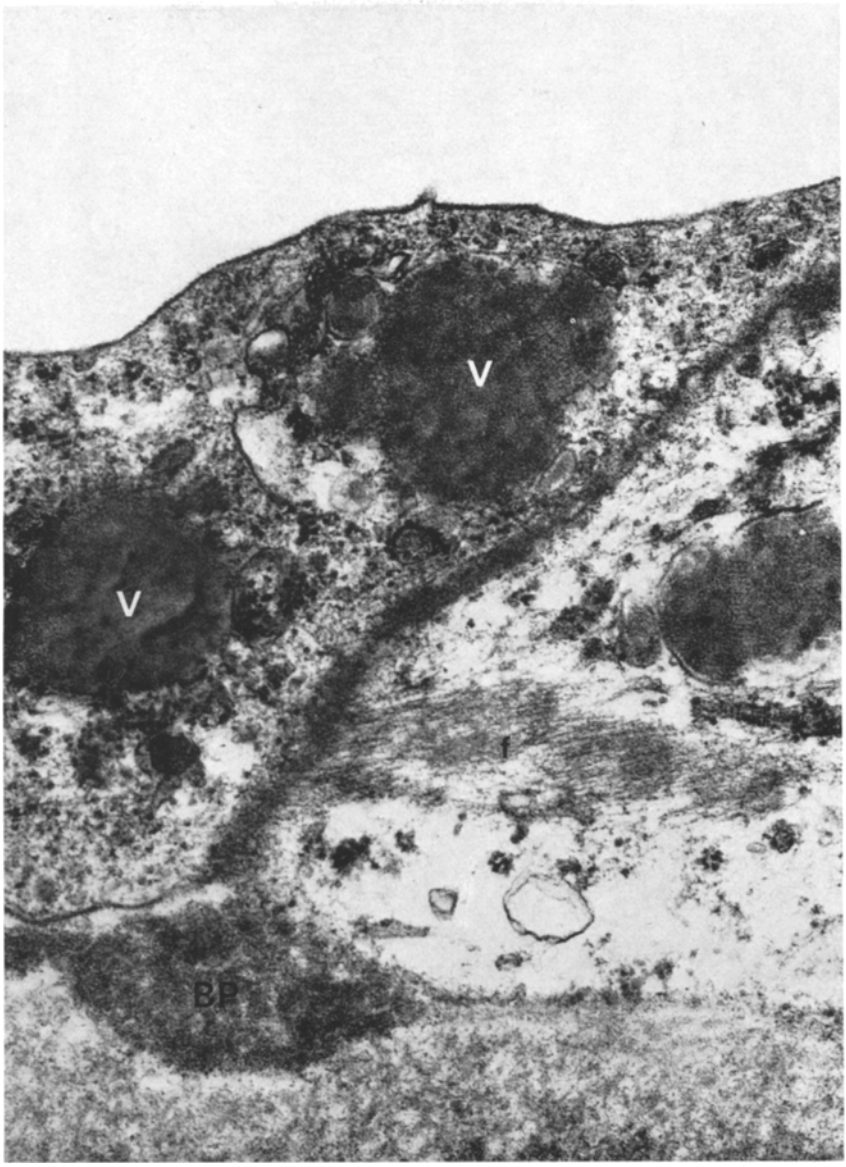

Fig. 3. Electron microscopic picture of the same artery in Fig. 2. Large vacuoles (*V*) in endothelial cells contain either electron dense homogeneous substance or fine hollow granules nearly even in size (270 Å in diameter). Electron dense blood plasma protein (*BP*) is accumulated just beneath the endothelial cell junction and numerous filaments (*f*) are seen in the endothelial cytoplasm. ×18,600

cells and fat droplets possively derived from necrotic foam cells were seen (Fig. 5). Electron microscopic observation of swollen collagen fibers in the deep intima disclosed that they were dispersed and fragmented by the deposition of a large

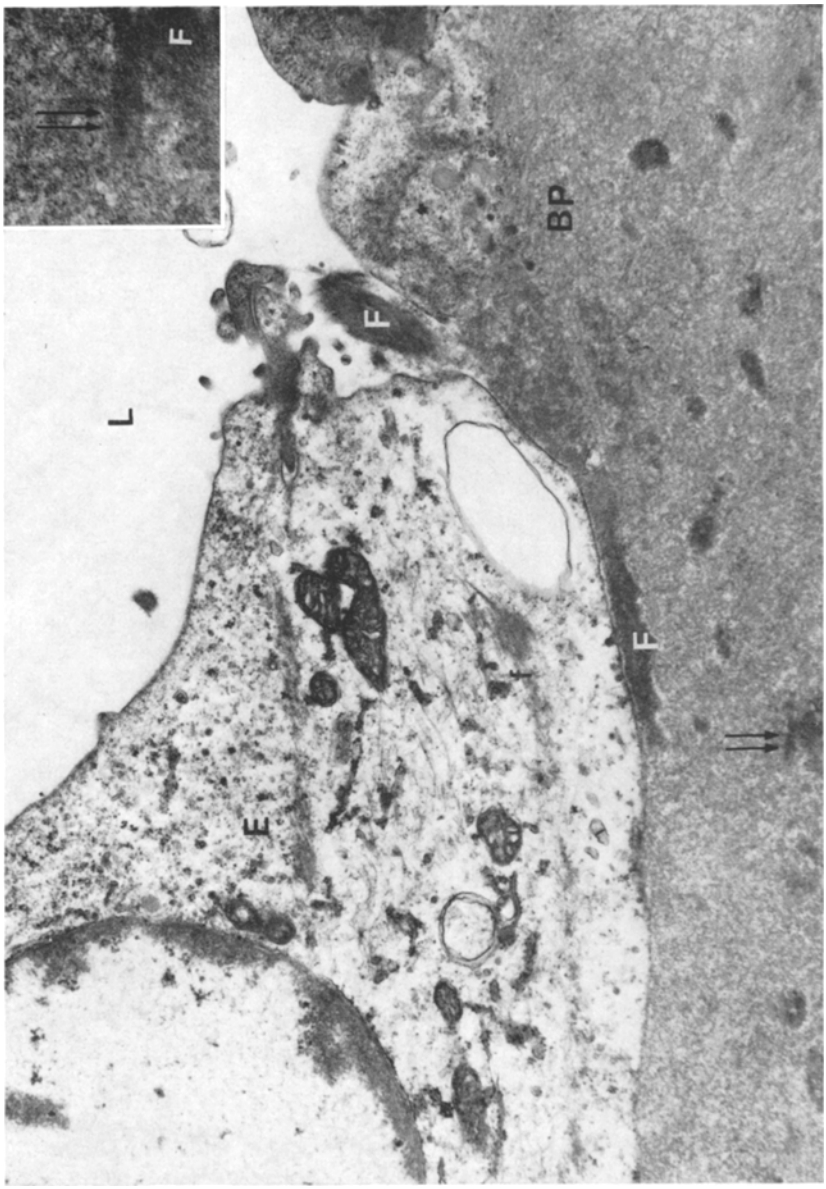

Fig. 4. Electron microscopic picture of the artery shown in Fig. 2. Endothelial cell junction is open (4,700 Å), allowing fibrin (*F*) and the other blood plasma protein (*BP*) to insudate into subendothelium. Endothelial cells (*E*) are swollen, and filaments (*f*) are seen in the translucent cytoplasm. *L*, arterial lumen. ×19,400. Inserted picture: Subendothelial fibrin (*F*) having regular cross-striations with a period of 138 Å (arrows). ×58,500

amount of blood plasma protein, lipoprotein-like granular substance and cell debris between them (Fig. 5).

The internal elastic lamina was stretched fragmented and lost allowing the accumulated substance in the intima to flow out into the media and adventitia (Fig. 5).

Beneath the sites of intimal deposition, the media was invariably damaged

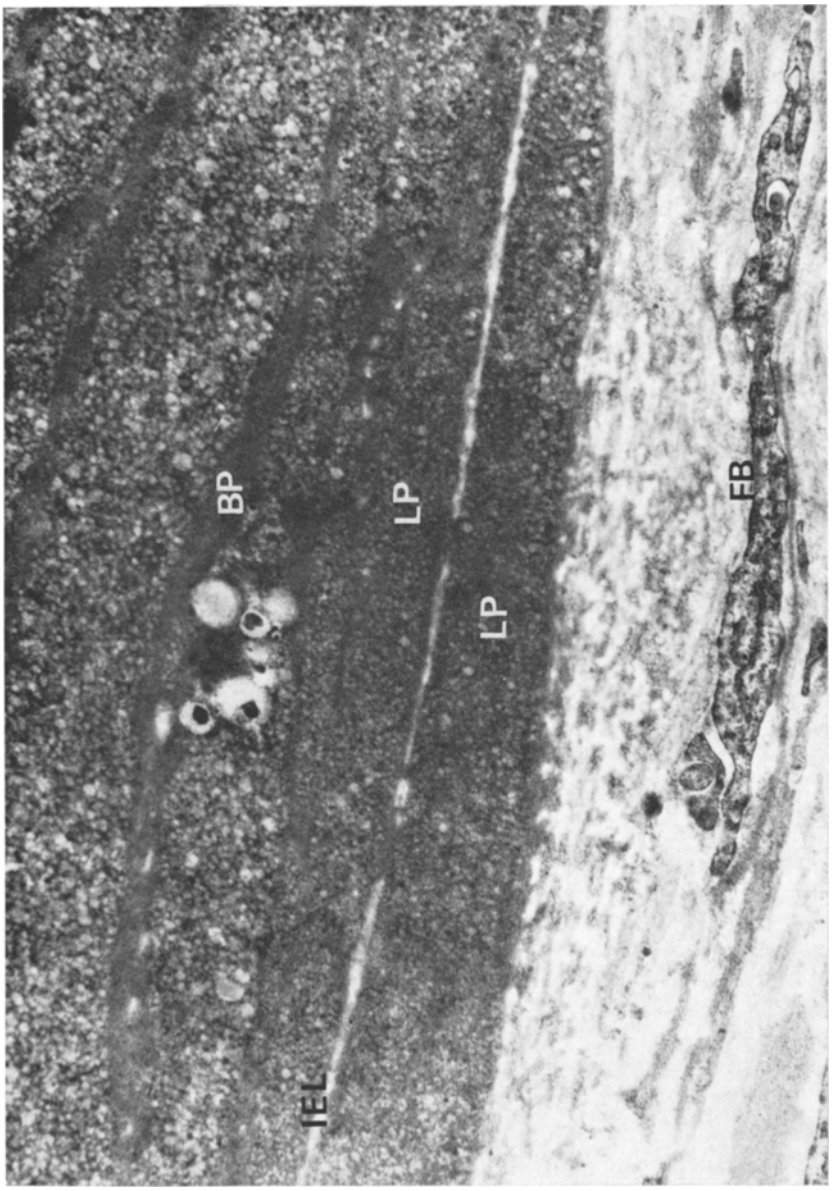

Fig. 5. Deep intima of the artery shown in Fig. 2. Accumulated fibrin, blood plasma protein (*BP*; frayed and fragmented collagen fibers due to blood plasma protein infiltration) and lipid (*LP*) in the intima are seen flowing into the media and adventitia through interrupted parts of the internal elastic lamina (*IEL*). Medial smooth muscle cells are lost. *FB*, fibroblast in the adventitia. ×21,700

severely, and in almost cases smooth muscle cells were entirely lost (Fig. 5). But occasionally necrotic smooth muscle cells and broad basement membrane-like substance were remaining.

In the adventitia were seen collagen fibers and fibroblasts containing well-developed rough-surfaced endoplasmic reticulum and mitochondria. These cells did not show any degeneration or necrosis (Fig. 5).

Discussion

1. Preceding Changes of Plasmatic Arterionecrosis

Electron microscopic observation of arterial wall which was assumed to be involved in the earliest changes of plasmatic arterionecrosis revealed that medial and intimal smooth muscle cells were markedly necrosed and lost, and reticular or lamellar basement membrane-like substance was increased (Fig. 1).

On the basis of the results of studies with human autopsy cases, the necrosis of medial smooth muscle cells was attributed to aging, hypertension and hemodynamic stresses (Ooneda *et al.*, 1973). Experimentally were assumed, as the cause of the smooth muscle cell necrosis, aging (Kojimahara *et al.*, 1973), hypertension (Jellinek *et al.*, 1966), angiospasm (Terry *et al.*, 1970), physiological saline extract of the renal cortex (Asscher and Anson, 1963), hypoxidosis (Garbarsch *et al.*, 1969; Terry *et al.*, 1970), and angiotensin (Wiener and Giacomelli, 1973). Observation of the arteries which showed blood plasma infiltration and fibrinoid deposition in the intima disclosed that medial smooth muscle cells were necrosed far more advanced, leading to their complete disappearance. Although it is impossible to exclude the possibility that the blood plasma infiltration may have enhanced the necrosis of medial smooth muscle cells, it appears more probable that the blood plasma insudation may have occurred in the arterial wall where medial smooth muscle cell necrosis was especially severe. In the morphogenesis of arterial fibrinoid degeneration in hypertensive rats, the necrosis of medial smooth muscle cells preceded the blood plasma insudation (Kérényi *et al.*, 1966; Suzuki *et al.*, 1972).

From the above described, it can be concluded that the earliest and most important change in the morphogenesis of plasmatic arterionecrosis is the necrosis of medial smooth muscle cells.

2. Increase of Endothelial Permeability

The plasmatic arterionecrosis of the human intracerebral arteries is characterized by insudation and deposition of blood plasma constituents and histolysis in the arterial wall. The insudation is considered to take place through opened endothelial cell junctions (Fig. 4). The lumina of the segments of arteries with marked necrosis of medial smooth muscle cells were found somewhat dilated. As the result the tension of the arterial wall will be increased according to the Laplace's law. Not only increased tension but also hemodynamic factors are supposed to cause endothelial injury, resulting in opening of endothelial cell junctions, eventually leading to increased permeability. Moreover endothelial cells of the human cerebral arteries contain numerous filaments in the cytoplasm. These filaments are believed to have the same antigenecity as the myosin of smooth muscle cells (Wakamatsu *et al.*, 1973). Accordingly it is also considered that endothelial cells are contractile and the space between endothelial cells will be open by their contraction.

Blood plasma insudation into the arterial wall of hypertensive rats is assumed to take place mainly through opened endothelial cell junctions and partly by vesicular transport (Suzuki *et al.*, 1971). Insudation of lipid in the human arterial wall may also take place by vesicular transport, since fine lipid droplets, assumedly

low density lipoprotein, were seen in large vacuoles in endothelial cells and deposited between collagen fibers in the deep intima of the present study.

3. Histolysis of Arterial Wall

In the arterial wall involved in marked plasma infiltration, the previously increased basement membrane-like substance in the intima almost disappeared. Collagen fibers in the deep intima with marked blood plasma infiltration were frayed, fragmented and dispersed, and a large amount of lipid, blood plasma protein and cell debris were deposited between them. And the internal elastic lamina was stretched, showing disruption and dissolution here and there.

Thus where the blood plasma infiltration was marked, dispersion, disruption and disappearance tended to occur not only in the basement membrane-like substance but also in collagen and elastic fibers. Pancreatic elastase in the circulating blood (Hall, 1966; Janoff and Scherer, 1968; Loeven, 1969), collagenase (Lazarus, 1968) and elastase (Janoff and Scherer, 1968), both derived from leucocyte granules in the intima, are considered as the causative factors for them. Though the intima and media with marked histolysis failed to show any cells with normal structure, the adventitia manifested an active protein synthesis by fibroblasts and well developed collagen fibers. It seems therefore necessary to consider that the disappearance of fiber-synthesizing cells, namely, smooth muscle cells in the intima and media may also be concerned with the disappearance of fibers in the affected arterial wall.

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