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Age-Induced Changes of Cerebral Arteries in Rats

An Electron Microscope Study

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Summary. Age-related changes of the anterior cerebral arteries of Wistar rats were studied by electron microscope. Medial smooth muscle cells (SMC) in the young rats often made membranous contact. Myo-endothelial junction was also observed. In the 4-week-old arteries, development of cerebral arteries was almost complete. In the 3-month-old arteries, necrotic changes of the SMC appeared in the media. In aging rats, medial SMC necrosis was characteristically observed in the outer media. The outer surface of the SMC showed irregular shape and necrotic substance derived from the SMC was increased with advancing age. Increase of collagen fibers was seen only in the outer media and the adventitia.

Age-related changes in the arterial walls of experimental animals have chiefly been observed electron microscopically in the aorta (Karrer, 1960, 1961; Paule, 1963; Stein *et al.*, 1969; Cliff, 1970; Gerrity and Cliff, 1972). But little is known regarding the age changes of cerebral arteries in rats.

It is the purpose of this paper to report electron microscopic findings of anterior cerebral arteries in young and aged rats.

Material and Methods

A total of 50 Wistar rats ranging in age from newborn to 20 months were used. They were fed on ordinary diet (Oriental Kobo Ind. Co.). The animals were killed at adequate times and the stem of the anterior cerebral arteries were observed. At sacrifice, blood pressure of the rats was measured, under ether anesthesia, by direct manometric method in the abdominal aorta and the animals were perfused with 5% buffered (pH 7.4) glutaraldehyde through the left ventricles. The brains were removed quickly and immersed in the same fixative. The stem of the anterior cerebral arteries with optic chiasm were cut away. Then they were postfixed with 1% osmium tetroxide buffered with 0.2 M cacodylate (pH 7.4) at 4° C 2 hours. After dehydration with graded ethanol, all the tissues were embedded in Epon 812. All the sections were examined with JEM-7 type and JEM-7A type electron microscopes.

Results

Blood pressure was 100–130 mm Hg in the rats ranging in age from 4 weeks to 20 months.

In the newborn to 10-day-old arteries, endothelial cells were arranged in a single layer and contained relatively numerous cell organelles. Cytoplasmic filaments, each having a diameter of about 50–100 Å, mainly at the basal surface were observed (Fig. 1). The cell processes sometimes extended through narrow fenestrations in the internal elastic lamina (IEL) and made contact with the

medial smooth muscle cells (SMC) (Fig. 1b). The media consisted of 1–3 layers of SMC. They were embryonic and resembled endothelial cells. The SMC contained a few myofilaments with scattered fusiform densities. There was immature IEL between the endothelial cells and the SMC. Intercellular spaces were narrow and the medial SMC made often membranous contacts (Fig. 1a). Mitosis was observed in the SMC.

In the 4-week-old arteries, endothelial cells were sometimes overlapping. Cytoplasmic filaments were not clearly visible. Under the endothelial cells, mostly close contact with them, there was a mature IEL (Fig. 2). The media consisted mainly of 5 layers of SMC and development of the cerebral arteries were almost completed (Fig. 2). The intercellular spaces with few elastic and collagen fibers were nearly uniform in width. There was a basement membrane (BM) on the external side of the SMC (Fig. 2). The media did not contain any nerve fibers, nor was seen the external elastic lamina between the media and the adventitia. There were small number of fibroblasts, microfibrils, collagen fibers and unmyelinated nerve fibers in the adventitia.

From about 3-month-old arteries on, focal cytoplasmic necrosis (coagulation or/and liquefaction necrosis) came to be appeared in a small number of the SMC in the outer media. But most of the SMC had a similar appearance and outer surface of the cells showed smooth. Cellular debris, which is described below, was present around the cells.

In the 10 to 20-month-old arteries, changes of the SMC were conspicuous in the outer media. Most of the necrotic parts in the SMC were limited by a single membrane (Fig. 3). Outer surface of the SMC was markedly irregular and often showed bizarre form. Around the SMC, which were reduced in size, there were abnormal substances such as dense granules, vesicles and dense homogeneous masses (Fig. 4). Accumulation of thick or fibrillary BM-like substance was also observed (Figs. 5 and 6). Granulo-vesicular profiles were always intermingled in this substance (Figs. 5 and 6). These substances seen around the cells increased with advancing age. Necrosis, however, involving the whole cytoplasm of the SMC was rarely found. Edematous cytoplasmic alteration with decreased myofilaments was also seen in the SMC. There were not any medial cells showed structural characteristics of fibroblast-like SMC (modified SMC). The nucleoplasm of the nuclei of the SMC generally tended to become lucent with age and space was often observed around the nucleus. Increase of collagen fibers was only seen in the outer media and the adventitia. Neither any marked changes in the endothelial cells nor subendothelial spaces were noticed in the stem of the cerebral arteries. Medial damage was also observed in the branching of the cerebral arteries in aging rats. Their subendothelial spaces were slightly distended with BM-like substance and granulo-vesicular profiles.

Fig. 1. a (rat at 3 days of age): Intercellular space is narrow and medial SMC (*SM*) show membranous contacts (※). Internal elastic lamina (*IEL*) is immature. ×16500. b (rat at 9 days of age): Endothelial cells (*E*) make contact with underlying SMC (*SM*) through fenestrations of the IEL. ×20000

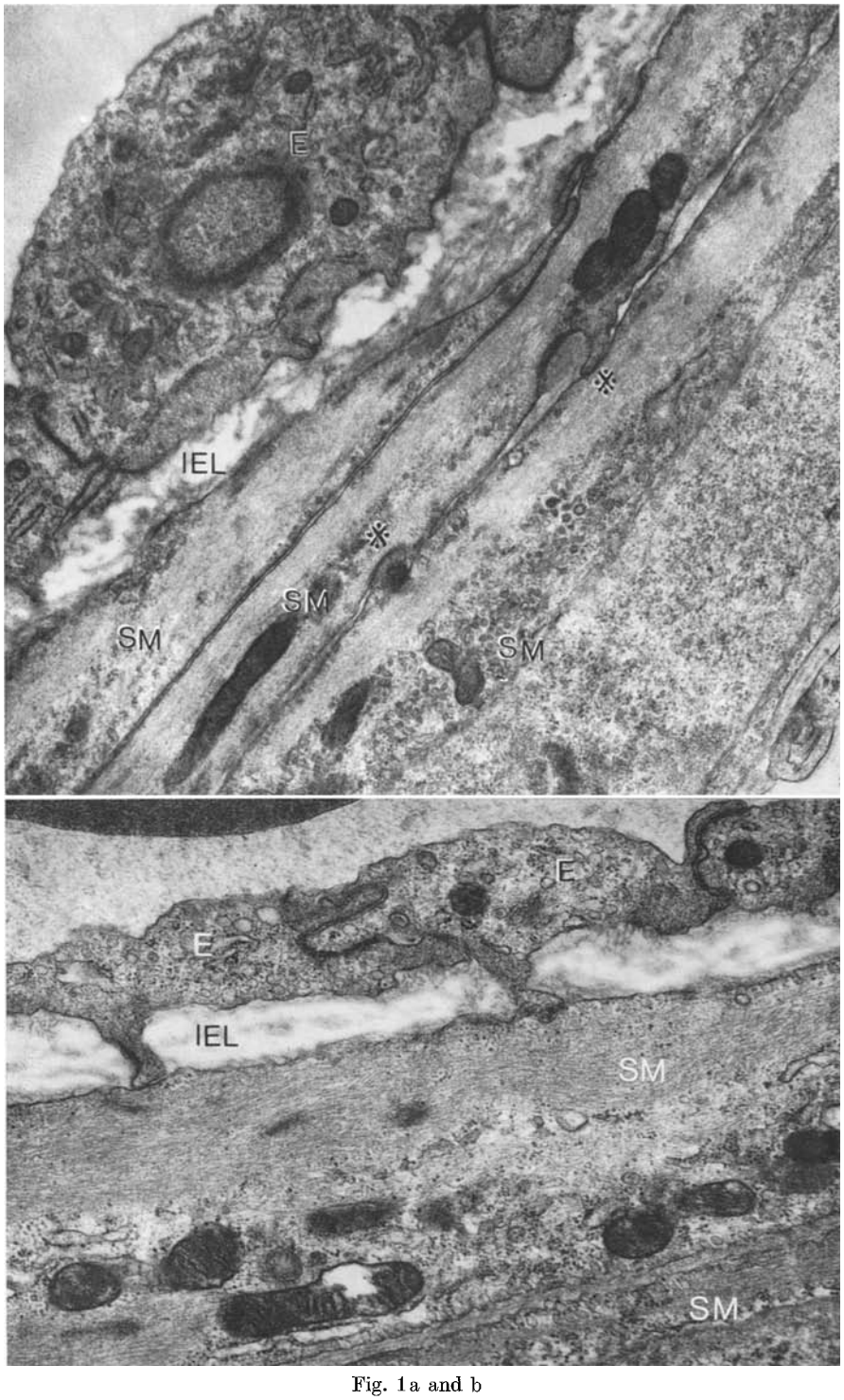


Fig. 1a and b

Intimal cells were observed only at branching points (intimal pads) in young and aging rats.

Discussion

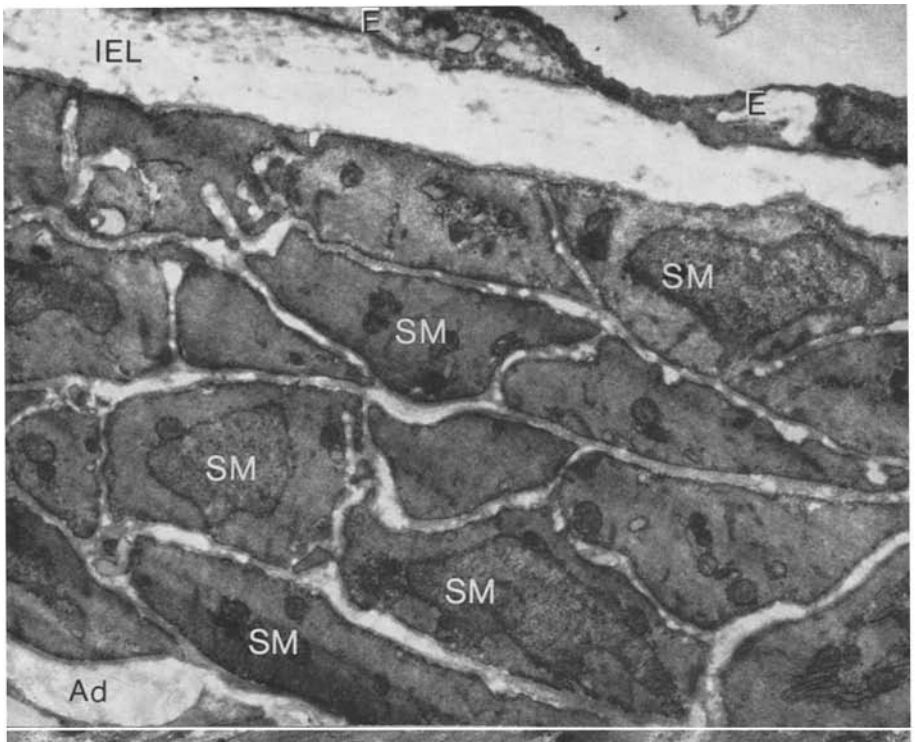
Medial SMC in the young rats made often membranous contact (Fig. 1a) and endothelial cells made also contact with medial SMC (myo-endothelial junction) (Fig. 1b). A system of myo-endothelial junctions was observed in the dog heart (Moore and Ruska, 1957) and in terminal arterioles and the pericapillary sphincters (Rhodin, 1962, 1967). In the 4-week-old arteries, there were typical SMC in the media having nearly uniform intercellular spaces, where elastic and collagen fibers were contained (Fig. 2).

In aging rats, medial necrosis was characteristically observed in both stem and branches of the anterior cerebral arteries. The lesions were, in general, localized in the outer media. The abnormal substance increased in the extracellular spaces with age were necrotic one derived from the medial SMC. In the substance were found the following ultrastructural forms: 1) Membrane bounded bodies with pleomorphic profiles. They included vesicles, granules and vacuoles with varying density (200–1500 Å) (Figs. 4 and 5). These bodies were, in principle, limited by a single membrane. Sometimes multimembrane bounded bodies and myelin figures were noticed. These profiles were seen also in the necrotic cytoplasm of the SMC (Fig. 3). It was light microscopically (Altmann, 1955) and electron microscopically (Hruban *et al.*, 1963; Ericsson *et al.*, 1965; Takebayashi, 1970) observed that focal cytoplasmic necrosis occurred in the cells was generally segregated sooner or later by membrane to be disposed within it; 2) Relatively homogeneous masses with high or moderate electron density. They were fragments of necrotic cytoplasm (Fig. 4); and 3) BM-like substance. The substance was either thick or fibrillary one. The substance frequently showed a lamellar structure (Fig. 6). It was suggested that the substance might be SMC-derived necrotic residue, which was gradually deposited as the result of repetitive segregation of the cell surface of the SMC.

Cellular debris observed in our materials was also seen in tunica media of aging rat aorta (Cliff, 1970) and of arterioles in experimental hypertension (Takebayashi, 1970). Original BM in the atrophic SMC was often seen (Takebayashi, 1970) (Figs. 4 and 6). There was no cellular reaction to above-mentioned necrotic substance (Cliff, 1970). Medial calcification of the cerebral arteries (Wilens and Sproul, 1938) was not seen in our materials.

Fig. 2. (Rat at 4 weeks of age). The internal elastic lamina (*IEL*) is in close contact with the endothelial cells (*E*). There are SMC (*SM*) in the media having nearly uniform intercellular space. Basement membrane and elastic fibers are seen around the SMC. *Ad* adventitia. ×7200

Fig. 3. Focal cytoplasmic necrosis of a SMC (*SM*) (rat at 18 months of age). There are membrane bounded bodies such as vesicles, granules and vacuoles in the necrotic cytoplasm. Relatively homogeneous masses and myelin figures are also observed in the cytoplasm. Around the SMC, basement membrane-like substance intermingled with small granulovesicular profiles is noticed. Newly formed membrane (↑) is seen between the cytoplasm and the necrotic part of a SMC. *Ad* adventitia with collagen fibers and fibroblasts. ×8800



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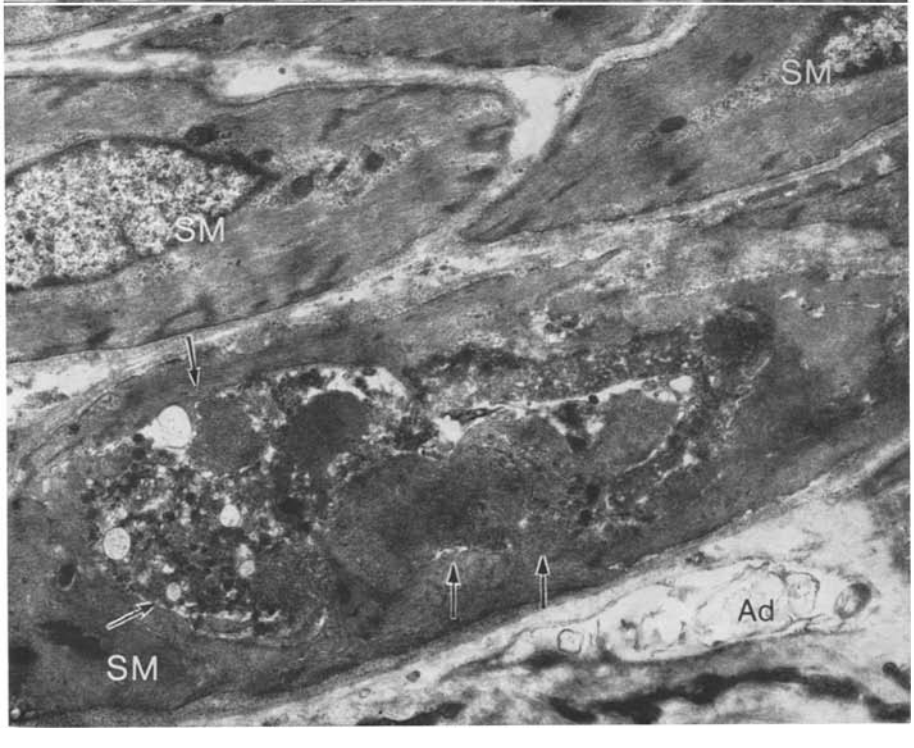

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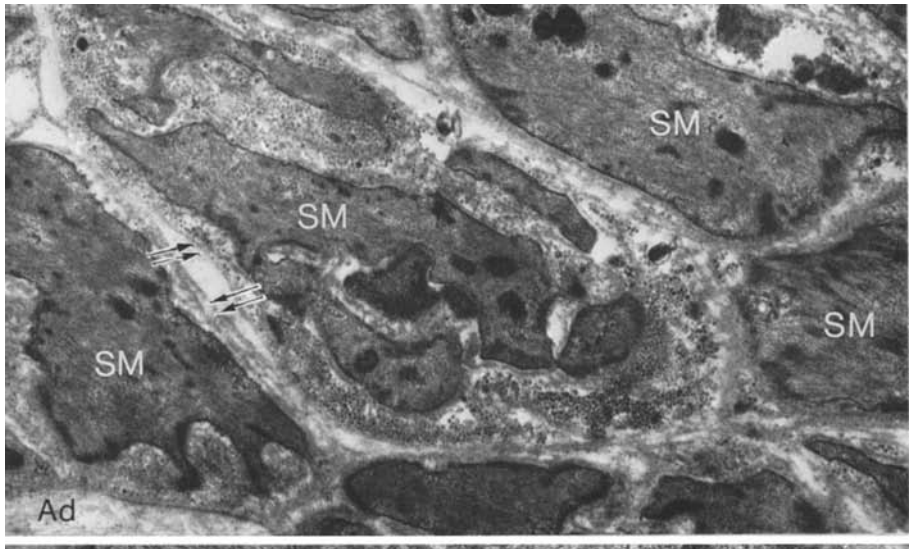
Figs. 2 and 3



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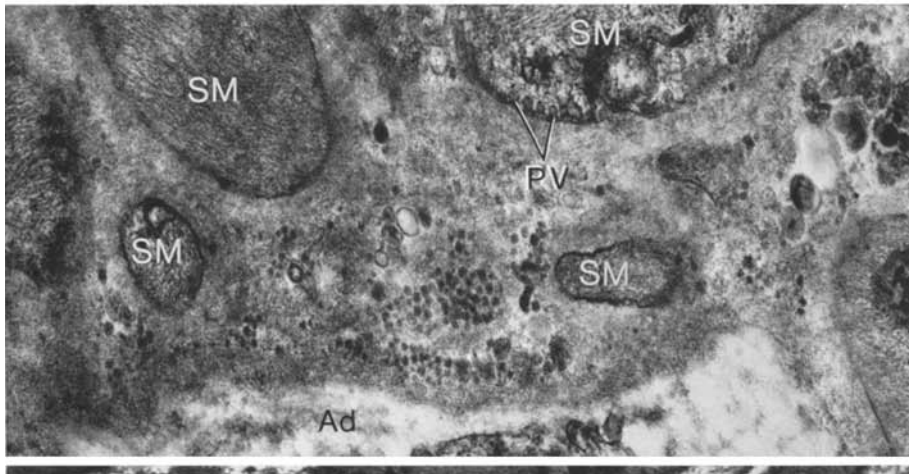

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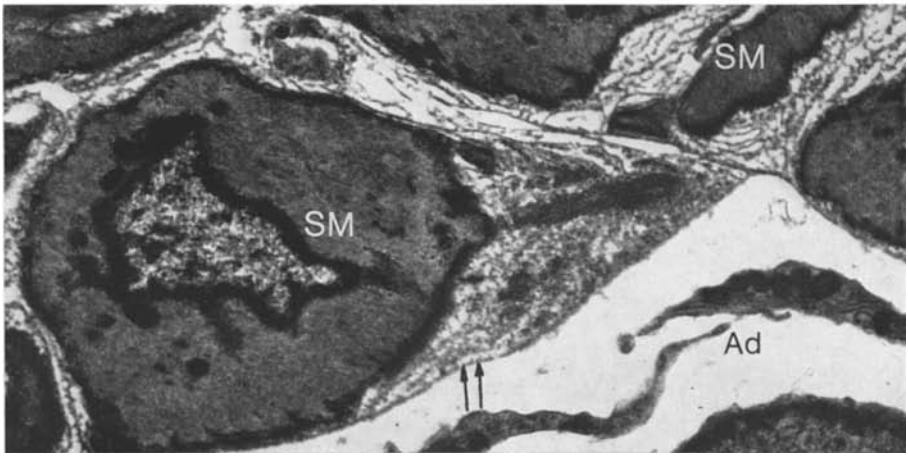

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Figs. 4—6

Gerritiy and Cliff (1972) reported diffuse intimal thickening with granulo-fibrillar material and cellular component in aging rat aorta. In our materials, the stem of cerebral arteries showed only SMC-damage in outer media without any intimal alteration. Subendothelial spaces of the branches, however, were slightly distended with BM-like substance and granulo-vesicular profiles. Intimal cells were observed only at branching points (intimal pads). The cellular component of the pads was SMC (authors' observation). Majority of the collagen fibers which increased in aging rats in the outer media would be derived from the adventitia (Aikawa and Koletsky, 1970).

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Fig. 4. Cellular debris is seen as granules in varying density around the SMC (*SM*). Basement membrane-like substance and dense masses are noticed around the cells. Original basement membrane (↑↑) is seen on the external side of cell debris (rat at 18 months of age). *Ad* adventitia. ×9600

Fig. 5. Thick basement membrane-like substance deposited in the extracellular space. Cluster of pinocytotic vesicle-like profile is intermingled with the substance (rat at 16 months of age). *PV* pinocytotic vesicles in a SMC (*SM*); *Ad* adventitia. ×27000

Fig. 6. Cytoplasmic necrotic lesion in a SMC (*SM*) is replaced by basement membrane-like fibrillary substance which shows lamellar fashion. Cluster of dense granules is noticed in it (rat at 20 months of age). ↑↑ Original basement membrane of a SMC; *Ad* adventitia. ×10300

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