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Electron Microscopic Study on the Medial Defect at the Apex of Human Cerebral Arterial Bifurcations

Keiji Suzuki, Sadao Hori, and Genju Ooneda

Second Department of Pathology, School of Medicine, Gunma University, Maebashi, Showamachi 3-39-22, Japan

Summary. It is a well known fact that the berry aneurysms, which are the direct cause of subarachnoid hemorrhage, develop at the apices of bifurcations of larger cerebral arteries. In order to elucidate the morphogenesis of the aneurysms in these sites, electron microscopic observation was made on one of their predilective regions, that is, the bifurcation of the first temporal branches from the middle cerebral arteries. Human autopsy cases from newborn and elderly patients were studied.

The apices of the cerebral arterial bifurcations exhibited a medial defect immediately after birth, where collagen fibrils, ground substance and increased basement membrane-like substance were observed, suggesting that the defect might have resulted from necrosis of medial muscle cells. The tunica media adjacent to the defect became tapered into a wedge-like shape with the thin and towards the defect and the medial muscle cells were decreased in number. In young cases, medial muscle cells near the defect displayed focal cytoplasmic necrosis, and granulovesicular cell debris and lamellar increase of basement membrane-like substance were seen around the muscle cells with irregular profiles. With aging the defect gradually enlarged and the adjacent part of the media composed of lamellarly or reticularly increased basement membrane-like substance and granulovesicular cell debris, without muscle cells. In the media away from this area muscle cells were irregular and surrounded by granulovesicular cell debris, similarly increased basement membrane-like substance, and increased collagen fibrils, with enlarged intercellular spaces.

The internal elastic lamina at the apices of bifurcations showed fragmentation and lumpy degradation both of which increased with age.

Necrosis of medial muscle cells and subsequent enlargement of the medial defect together with degenerative changes in the internal elastic lamina, which are age-induced and presumed to be due to haemodynamic factors, are all considered to be important in the formation of berry aneurysms.

Key words: Berry aneurysm – Medial defect – Medial muscle cell necrosis – Degeneration of internal elastic lamina – Cerebral arterial bifurcation.

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Introduction

It is well-known that berry aneurysms, the cause of subarachnoid bleeding, are formed at the apices of bifurcations of the larger cerebral arteries. Forbus (1930) considered that the medial defect and degeneration of the internal elastic lamina at the apex were important in pathogenesis but Glynn (1940) and Stehbens (1959, 1972) did not regard the medial defect as a necessary factor for the formation of the aneurysms, because they are uncommon despite the high incidence of medial defects in the bifurcations of the cerebral arteries. These authors placed more emphasis on changes in the internal elastic lamina. The change in the internal elastic lamina, however, is said to coincide with the area of the medial defect (Yamamoto and Yoshida, 1977), and an outward bulging is more readily produced with a large medial defect (Carmichael, 1945). Consequently the medial defect is thought to be the most important single pathogenic factor for the cerebral aneurysms. In view of this difference of opinion, electron microscopic observations were made on one of the sites of predilection for berry aneurysm, i.e. the apex of bifurcation of the first temporal branches from the middle cerebral arteries.

Materials and Methods

From 19 normotensive autopsy cases including the old, aged 75, as well as newborns (Table 1), brains were removed at 1–5 h after death, and the middle cerebral arteries were perfused with 2.5% glutaraldehyde (phosphate buffer, pH 7.4) for 5–10 min. The bifurcations of the first temporal branches from the middle cerebral arteries were dissected out, and fixed with 2.5% glutaraldehyde for 4 h. After washing with phosphate buffer (pH 7.4), the specimens were postfixed in 1% osmic acid for 2 h. After dehydration, they were embedded in Epon 812, and their ultrathin sections were doubly stained with uranyl acetate and lead hydroxide for electron microscopy.

In this studies, human autopsy cases examined within 5 h after death, because the cytoplasm or cell organelles of endothelial and medial cells were well preserved. Cases examined over 5 h after death showed vacuolar and liquefaction changes, a process known as autolysis.

Results

The medial defect in the bifurcations of the larger cerebral arteries is said to be found in embryonal stages (Yamamoto and Yoshida, 1977), and was seen here at the apices of the bifurcations immediately after birth.

Table 1

Number	Age	Sex	Hypertension	Number	Age	Sex	Hypertension
1	5 h	male	–	11	52 years	female	–
2	3 days	male	–	12	52 years	male	–
3	5 months	male	–	13	57 years	male	–
4	3 years	male	–	14	58 years	male	–
5	6 years	male	–	15	60 years	male	–
6	17 years	male	–	16	63 years	male	–
7	34 years	female	–	17	65 years	male	–
8	36 years	male	–	18	74 years	male	–
9	44 years	male	–	19	75 years	male	–
10	46 years	male	–				

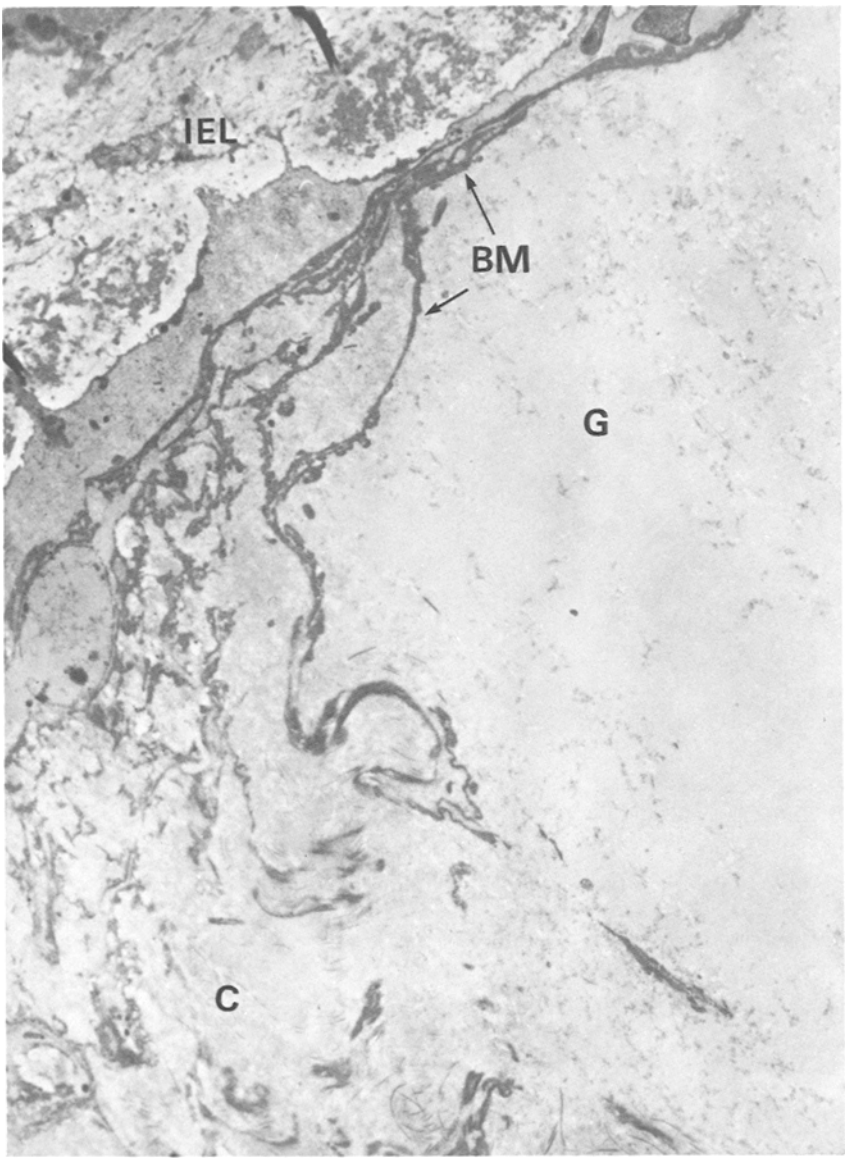


Fig. 1. Apex of cerebral arterial bifurcation from a male infant, aged 5. Medial muscle cells are absent beneath the internal elastic lamina (*IEL*), while basement membrane-like substance (*BM*), ground substance (*G*) and collagen fibrils (*C*) are seen. ×4,700

In newborns and infants up to 6 years of age, lumpy elastin, basement membrane-like substance and a small number of intimal muscle cells were observed subendothelially at the apices of the bifurcations. The internal elastic lamina was not homogeneous but mottled in appearance, and frequently showed fragmentation (Fig. 1). No muscle cells were noticed in the medial defect immediately beneath the internal elastic lamina, and there was basement membrane-like substance, collagen fibrils and medium electron-dense ground substance

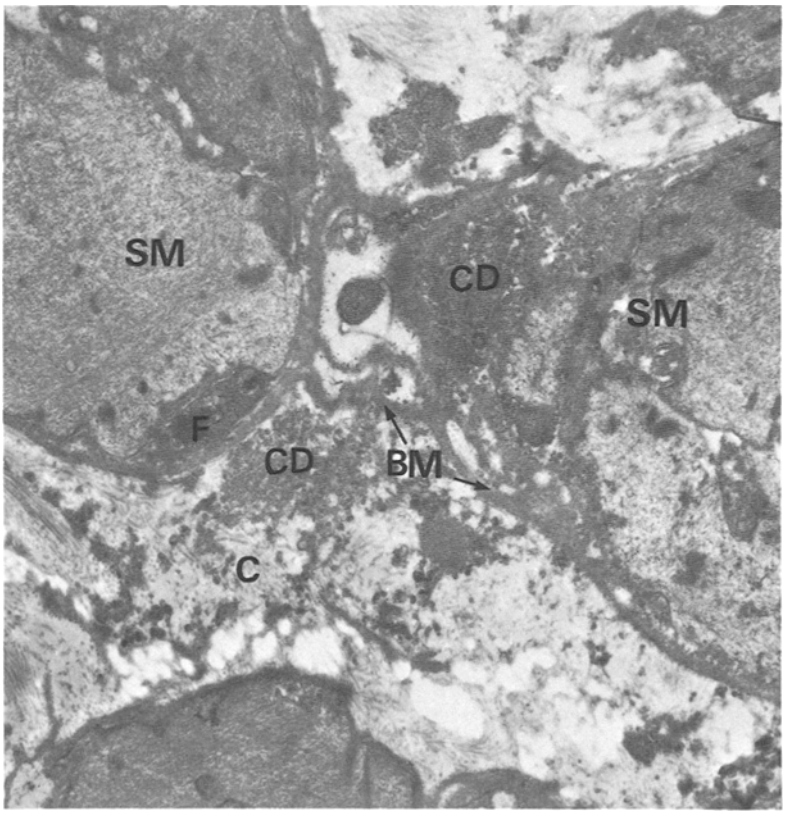


Fig. 2. Apex of cerebral arterial bifurcation from a male infant, aged 3. The media in close vicinity to the defect shows focal cytoplasmic necrosis (*F*) of muscle cells (*SM*), granulovesicular cell debris (*CD*) and an increase of lamellar basement membrane-like substance (*BM*) surrounding the muscle cells. Not only cell debris (*CD*), but also collagen fibrils (*C*) are observed between muscle cells. ×15,400

(Fig. 1). Granulovesicular cell debris was infrequently observed there. The basement membrane-like substance in the medial defect was continuous with the basement membrane which encircled muscle cells close to the defect. The findings suggested that the membrane might have remained after the necrosis and disappearance of muscle cells. In the media away from the defect, many muscle cells were closely packed, covered with a thick basement membrane, and separated by ground substance and collagen fibrils. These cells were decreased in number and the media attenuated as it approached the apex, presenting a wedge-shape toward it. Focal cytoplasmic necrosis of muscle cells was seen near the medial defect, and around these cells granulovesicular cell debris and lamellary increased basement membrane-like substance were noticed (Fig. 2).

In one case, aged 17, there was a marked increase in collagen fibrils in the medial defect, and a small amount of basement membrane-like substance and granular cell debris were seen immediately beneath the internal elastic lamina. Muscle cells adjacent to the defect showed a wedge-shaped decrease

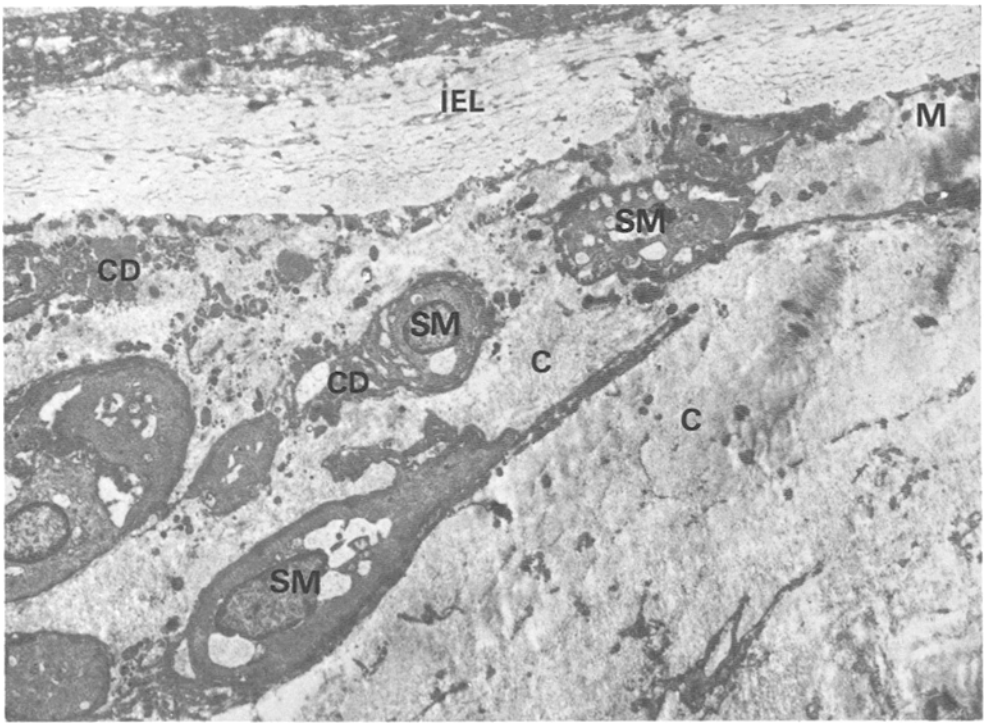


Fig. 3. Apex of cerebral arterial bifurcation from a young male, aged 17. The surface of the internal elastic lamina (*IEL*) is smooth on its medial side and irregular on the intimal side. Medial smooth muscle cells (*SM*) are decreased in number in the vicinity of the medial defect (*M*) on the right. Intercellular space between smooth muscle cells is enlarged, where collagen fibrils (*C*) and cell debris (*CD*) are increased. × 3,700

in number toward the defect, around which they were irregular in shape, and basement membrane-like substance was increased around them. The intercellular space was widened where granular cell debris and collagen fibrils were markedly increased (Fig. 3). The internal elastic lamina was not homogeneous, and showed a tendency to fragmentation and degradation into masses.

In adults and aged cases (34–75 years of age), slight intimal thickening was noted in the apices of bifurcations, where the cytoplasm of endothelial cells was generally electron dense and frequently had many filaments. In the slightly thickened intima there were muscle cells, basement membrane-like substance, electron dense cell debris, collagen fibrils and lumpy elastin. Around the intimal muscle cells, lamellar or homogeneous basement membranes were increased.

The internal elastic lamina at the apex presented fragmentation and degradation. Its surface was smooth on the medial side but showed a marked trend of degradation of the intimal side, presenting a lumpy appearance. In severely injured cases degradation into masses was observed on the medial and intimal

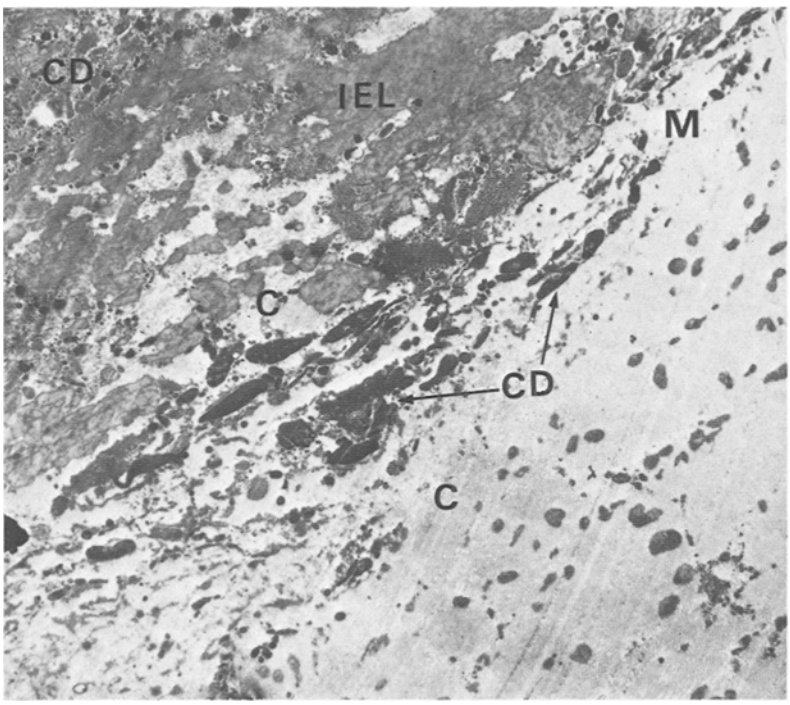


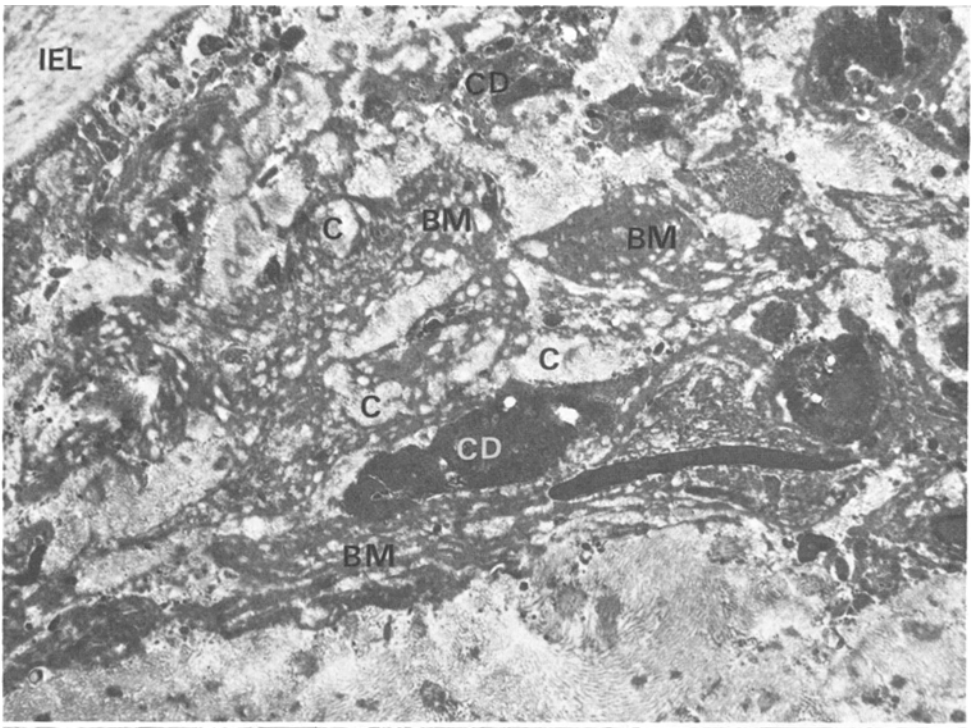
Fig. 4. Apex of cerebral arterial bifurcation from a male, aged 75. Cell debris (*CD*) and collagen fibrils (*C*) in the tapering media close to the defect (*M*). Internal elastic lamina (*IEL*) undergo degradation into masses, between which collagen fibrils (*C*) and cell debris (*CD*) are seen. × 5,800

sides of the internal elastic lamina. Between elastin that displayed degradation cell debris and collagen fibrils were noted (Fig. 4).

Collagen fibrils were numerous in the medial defect, where electron dense cell debris was found infrequently. Medial muscle cells showed an attenuation in a wedge-shape toward the defect, and in the tip of the wedge-shaped media muscle cells with normal appearance almost disappeared, leaving only a small number of atrophic or necrotic cells (Fig. 5). Around the cell debris basement membrane-like substance was increased lamellarly or reticularly and collagen fibrils were found. Medial cells near the defect exhibited focal cytoplasmic necrosis and irregular profiles, around which basement membrane-like substance was increased lamellarly or reticularly (Fig. 6). In the muscle cells enclosed

Fig. 5. Apex of cerebral arterial bifurcation from an adult male, aged 46. In the media close to the defect, basement membrane-like substance (*BM*) was increased in a lamellar or reticular fashion intermingled with cell debris (*CD*) just beneath the internal elastic lamina (*IEL*). Where cell debris is absent, empty basket-like figures are present. Collagen fibrils (*C*) are noted in and around the increased basement membrane-like substance (*BM*). × 5,500

Fig. 6. Apex of cerebral arterial bifurcation from an adult male, aged 46. Tunica media near the defect. Muscle cells (*SM*) are irregular in shape. Increased basement membrane-like substances (*BM*), either lamellarly or reticularly, intermingled with cell debris (*CD*) and collagen fibrils (*C*). × 5,200



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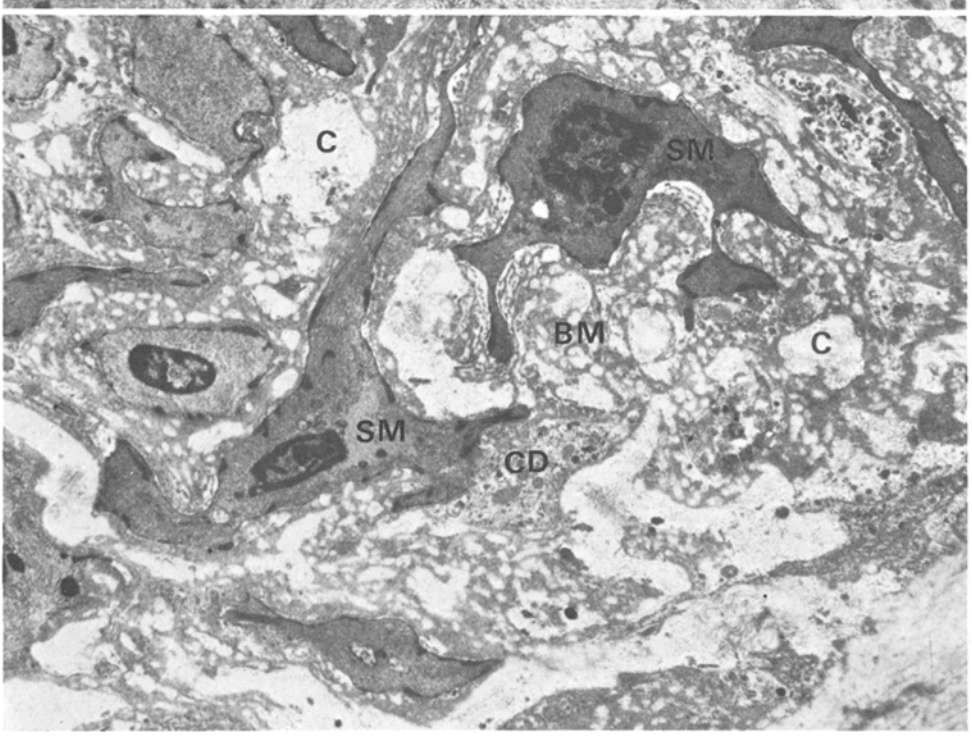

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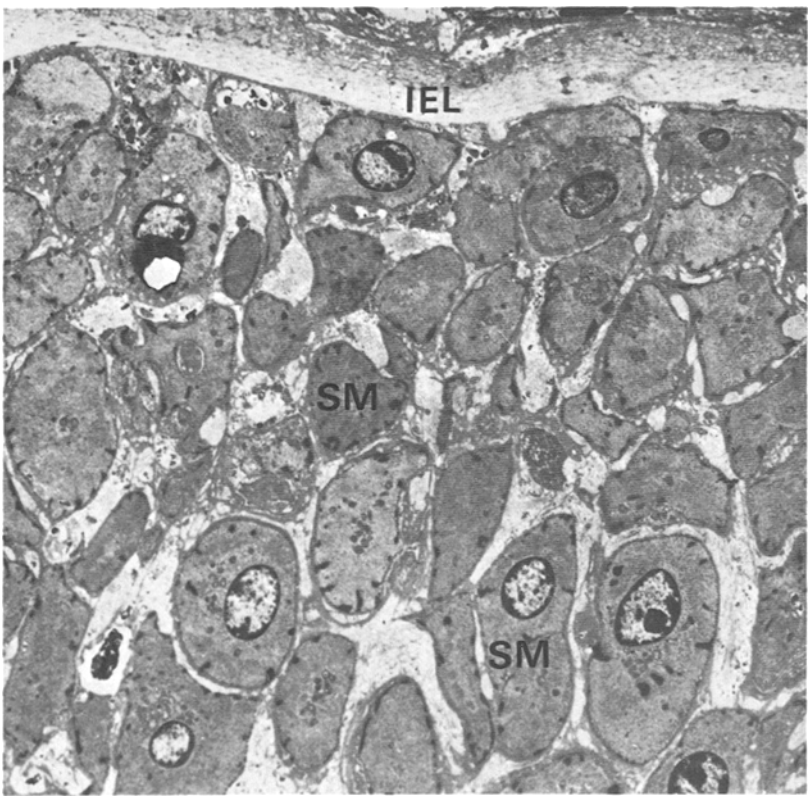

Fig. 7. Tunica media away from the apex of the cerebral arterial bifurcation, from an adult male, aged 46. Medial muscle cells (*SM*) are closely packed and regular in shape having relatively smooth profiles. Around them there is little debris or basement membrane-like substance. Internal elastic laminae (*IEL*) are well preserved. ×3,800

by the increased substance focal cytoplasmic coagulation necrosis was seen, and large and small dense granular or vesicular cell debris derived from the necrotic cell bodies were observed (Fig. 6). This debris was also seen between the increased basement membrane-like substance and between collagen fibrils around muscle cells. In the media away from the defect, muscle cells were compactly arranged and surrounded by slightly increased amounts of basement membrane. Irregularly-shaped muscle cells and cell debris were few (Fig. 7). In these areas the internal elastic lamina was smooth both on the medial and intimal sides (Fig. 7).

Discussion

Various opinions exist as to whether the medial defect at the apex of cerebral arterial bifurcations is congenital or acquired. Smith and Windsor (1961), who examined 509 bifurcations of cerebral arteries from infants, discovered the medial

defect in 31.1% of them and considered the defect to be congenital. Stehbens (1959, 1972), however, regarded it as acquired, since its incidence increased with age. According to Yoshida (1969) medial defects at the apex were noted at 6 gestational months, though incomplete, became well-developed at 10 gestational months and in newborns, and thereafter enlarged with age. Electron microscopically we have observed that within the medial defect in infant cases, basement membrane-like substance, collagen fibrils and ground substance are found immediately beneath the internal elastic lamina, but there are no muscle cells (Fig. 1). Around muscle cells in the vicinity of the defect, cell debris and basement membrane-like substance was increased (Fig. 2). Accordingly the medial defect at the apex is considered to have been formed a posteriori and enlarged as the result of necrosis of muscle cells in early life.

The width of the medial defect gradually increases with age (Yoshida, 1969; Yamamoto and Yoshida, 1977). Electron microscopically, the media in the apex tapered toward the defect in a wedge-like shape, the terminal part of which contained basement membrane-like substance (increased in a lamellar or reticular fashion), cell debris and small muscle cell bodies (Figs. 3 and 5). In the media near the defect, there were irregular-shaped muscle cells resulting from focal cytoplasmic necrosis (Takebayashi, 1970), cell debris, and increased basement membrane-like substance (Figs. 5 and 6). The formation of the cell debris has been observed in the arterial media of hypertensive rats (Esterly and Glagov, 1963; Kojimahara and Ooneda, 1970; Suzuki and Ooneda, 1972), in the media of old rat cerebral arteries (Kojimahara et al., 1973) and in the intima and media of human cerebral arteries (Stehbens, 1975; Shinkai et al., 1976). This cell debris is considered to be produced from focal or massive necrosis of muscle cells and to be characteristic of muscle cell damage. The lamellar or reticular formation of basement membrane-like substance around intimal or medial muscle cells was noted in the arterial media of hypertensive rats (Spiro et al., 1965; Suzuki and Ooneda, 1972), in the cerebral arteries of old rats (Kojimahara et al., 1973), and in human cerebral arteries (Lang and Kidd, 1965; Stehbens, 1975a, b; Shinkai et al., 1976). These findings were attributed by Stehbens (1975a) to decrease in cohesion between muscle cells and their basement membranes, and considered by Vracko and Benditt (1970) to have resulted from damage to muscle cells, because cell debris was found between basement membranes. Suzuki and Ooneda (1972) thought that the increase in basement membrane-like substance was the consequence of repeated damage to muscle cells and repeated formation of basement membrane by these damaged cells. An increase in basement membrane-like substance observed in the present study (Figs. 1, 2, 5 and 6) is considered to be attributable to repeated focal cytoplasmic necrosis of muscle cells and repeated regeneration of the substance.

Mural damage at the apex was thought by Stehbens (1959, 1975b) to have resulted from haemodynamic stress, and the medial defect was reported to be more frequent in an obtuse than in an acute angle bifurcation (Stehbens, 1972). Yamamoto and Yoshida (1977) stated that the medial defect was apt to be formed at the side of a larger branch of a bifurcation. The apex of the bifurcation is the site which is directly struck by blood flow, and this

haemodynamic stress, which is enhanced by hypertension, is considered likely to damage the muscle cells of this site. Yamamoto and Yoshida (1977) stressed that hypertension plays an important role in the genesis of berry aneurysms.

Necrosis of cells at the apex of bifurcations is accompanied by increase of collagen fibers in the media (Figs. 3, 4, 5 and 6). Carmichael (1945) observed concomitant occurrence of medial fibrosis and muscle cell degeneration. Suzuki et al. (1975) noted ^{3}H-proline uptake by medial muscle cells of the mesenteric arteries of hypertensive rats which indicates that muscle cells damaged by hypertension still retain the activity to produce connective tissue fibers. Cell debris and collagen fibrils were observed between basement membrane-like substance increased around the medial muscle cells at the apex (Figs. 5 and 6). This seems to have resulted from repeated focal cytoplasmic necrosis of muscle cells and formation of basement membrane and collagen fibrils. It is considered that collagen fibrils in the defect may have been produced by the remaining damaged muscle cells.

The internal elastic lamina at the apex which did not undergo change in the fetal stage shows duplication and fragmentation in newborns, which increased with age (Yamamoto and Yoshida, 1977). Electron microscopy in infants revealed that the internal elastic lamina at the apex was mottled in appearance and showed large fenestrae and occasional fragmentation (Fig. 1). In adults fragmentation was frequent, and degradation into masses was noted (Fig. 4). Such degenerative changes in the lamina was observed by Forbus (1930), and was considered to be an important causal factor for the formation of berry aneurysms. In the degeneration haemodynamic factors (Forbus, 1930; Stehbens, 1972) and the absence of maintenance by muscle cells were thought to be responsible. The lamina was well found to be well preserved in arterial segments where medial muscle cells were not deficient (Fig. 7).

Enlargement of medial defects resulting from smooth muscle cell necrosis and age-induced degeneration of the internal elastic lamina are considered to be important pathogenic factors in berry aneurysms. Both changes occure concomitantly with age.

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References

Carmichael, R.: Gross defect in the muscular and elastic coat of the larger cerebral arteries. J. Path. Bact. **57**, 345–351 (1945)

Esterly, J.A., Glagov, S.: Altered permeability of the renal artery of the hypertensive rats. An electron microscopic study. Am. J. Pathol. **43**, 619–638 (1963)

Forbus, W.D.: On the origin of miliary aneurysms of the superficial cerebral arteries. Bull. Johns Hopkins Hosp. **47**, 239–284 (1930)

Glynn, L.E.: Medial defects in the circle of Willis and their relation to aneurysm formation. J. Path. Bact. **51**, 213–222 (1940)

Kojimahara, M., Ooneda, G.: Electron microscopic study on the middle cerebral artery lesions in hypertensive rats. Acta Pathol. Jap. **20**, 399–408 (1970)

Kojimahara, M., Sekiya, K., Ooneda, G.: Age-induced changes of cerebral arteries in rats. An electron microscope study. Virchows Arch. Abt. A Path. Anat. **361**, 11–18 (1973)

Lang, E.R., Kidd, M.: Electron microscopy of human cerebral aneurysms. J. Neurosurg. **22**, 554–562 (1965)

Shinkai, H., Yoshida, Y., Ooneda, G.: An electron microscopic study of plasmatic arterionecrosis in the human cerebral arteries. Virchows Arch. A Path. Anat. and Histol. **369**, 181–190 (1976)

Smith, D.E., Windsor, R.B.: Embryologic and pathogenic aspects of the development of cerebral saccular aneurysms. In: Pathogenesis and treatment of cerebrovascular disease, W.S. Fields (ed.), pp. 367–386. Chicago, Illinois: C.C. Thomas Publisher 1961

Spiro, D., Lattes, R.G., Wiener, J.: The cellular pathology of experimental hypertension: 1. Hyperplastic arteriosclerosis. Am. J. Pathol. **47**, 19–49 (1965)

Stehbens, W.E.: Medial defects of the cerebral arteries of man. J. Path. Bact. **78**, 179–185 (1959)

Stehbens, W.E.: Intracranial arterial aneurysms. In: Pathology of the cerebral blood vessels, W. Stehbens (ed.), pp. 351–470. Saint Louis: C.V. Mosby Co. 1972

Stehbens, W.E.: Cerebral atherosclerosis. Intimal proliferation and atherosclerosis in the cerebral arteries. Arch. Pathol. **99**, 582–591 (1975a)

Stehbens, W.E.: Ultrastructure of aneurysms. Arch. Neurol. **32**, 798–807 (1975b)

Suzuki, K., Ooneda, G.: Cerebral arterial lesions in experimental hypertensive rats: Electron microscopic study of middle cerebral arteries. Exp. Mol. Pathol. **16**, 341–352 (1972)

Suzuki, K., Hori, W., Ooneda, G.: Derivation of intimal cells appearing in spontaneous healing process of hypertensive arterial lesions. Exp. Mol. Pathol. **23**, 402–416 (1975)

Takebayashi, S.: Ultrastructural studies on arteriolar lesions in experimental hypertension. J. Electron Microsc. **19**, 17–31 (1970)

Yamamoto, Y., Yoshida, Y.: Pathology of subarachnoid hemorrhage, with special reference to the morphogenesis of berry aneurysms of cerebral arteries and mechanism of their rupture. J. Jap. Coll. Angiol. **17**, 183–195 (1977) (in Japanese)

Yoshida, Y.: Pathology of cerebral vascular diseases. J. Jap. Coll. Angiol. **9**, 115–118 (1969) (in Japanese)

Vracko, R., Benditt, E.P.: Capillary basal lamina thickening. Its relationship to endothelial cell death and replacement. J. Cell Biol. **47**, 281–285 (1970)

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