Early Changes of Experimentally Induced Cerebral Aneurysms in Rats

Light-Microscopic Study

FUMITADA HAZAMA, MD, HIDEO KATAOKA, EIJI YAMADA, MD, KALULA KAYEMBE, MD, NOBUO HASHIMOTO, MD, MASAYUKI KOJIMA, MD, and CHIEGON KIM, MD

From the Department of Pathology, Shiga University of Medical Science, Otsu, Japan, and the Department of Neurosurgery, Kyoto University Medical School, Kyoto, Japan

The changes of the anterior cerebral artery/olfactory artery junction, one of the favorite sites of aneurysm formation, in rats treated with unilateral ligation of the common carotid artery and renal hypertension were investigated by light microscopy. The initial changes of aneurysm occurred not at the apex itself, but on the distal side of the major branch adjacent to the apex, at the intimal pad and the neighboring distal portion. Here the internal elastic lamina showed various degenerative changes and disappearance. The neighboring distal portion adjacent to the intimal pad showed a shallow depression associated with a thinning of the media due to a decrease of medial smooth muscle cells in number even in some control animals. Such degenerative changes of the internal elastic lamina and medial smooth muscle cells caused by hemodynamic stress due to branching structure, including intimal pads, augmented by the experimental treatment, are supposed to be the basis for aneurysm formation. (Am J Pathol 1986, 124:399–404)

CONCERNING the developmental mechanisms of cerebral aneurysms, particularly of saccular aneurysms, various hypotheses have been proposed, such as the medial defect theory,[1] the elastic lamellar theory,[2] degenerative theory,[3,4] congenital theories,[5,6] and others (see a review by Sekhar et al[7]).

We could experimentally induce cerebral aneurysms in rats by ligating the unilateral common carotid artery, making the animals hypertensive, and feeding them β-aminopropionitrile, one of the lathyrogens.[8] By this induction of cerebral aneurysms and analysis of the experimental conditions, it was proved that hemodynamic stress, hypertension, and a metabolic disorder of the connective tissue were of primary importance as etiologic factors in the development of cerebral aneurysms.[9–11]

This experimental model provides us now with an appropriate tool for the pathogenetic study of cerebral aneurysms. In order to obtain information about early changes of aneurysms for the better understanding of developmental mechanisms of aneurysms, we studied by light microscopy the changes of a branching site of the circle of Willis, the anterior cerebral artery/olfactory artery (ACA/OA) junction, which is one of the favorite sites of aneurysm development, in rats treated with unilateral ligation of the common carotid artery and mild renal hypertension. However, β-aminopropionitrile was not fed, because the lathyrogen itself has an injurious effect on the arterial walls, and we wanted to simplify the experimental conditions.

Materials and Methods

Twenty-two male rats of the Sprague–Dawley strain ranging in age from 25 to 30 weeks were used. In each animal, ligation of the left common carotid artery and the posterior branch of both renal arteries were performed under pentobarbital anesthesia. Ten age-

Supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (61570168).

Accepted for publication April 23, 1986.

Address reprint requests to Dr. Fumitada Hazama, Department of Pathology, Shiga University of Medical Science, Seta, Otsu 520-21, Japan.

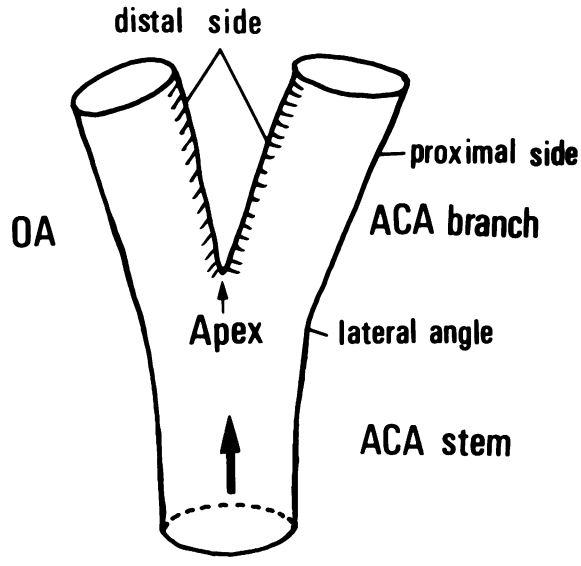

Figure 1—Schema of the anterior cerebral artery/olfactory artery junction. *Arrow* shows the direction of blood flow.

matched, untreated rats were used as control animals. The operated-on and untreated animals were given ordinary drinking water.

Three months later, the animals were perfused through the left ventricle with saline at 37 C, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The circle of Willis was carefully removed from the brain and immersed in the same fixative at 4 C for 12 hours. The ACA/OA junction was cut out from the circles. The specimens were then washed, postfixed in 1% osmium tetroxide in phosphate buffer (pH 7.4), dehydrated in graded ethanol, and embedded in Epon.

Semithin sections containing ACA, stem and branch, and OA were cut at 1 μ thickness and stained with 1% toluidine blue.

Blood pressure was measured by the tail-cuff autopickup plethysmographic method before sacrifice.

The nomenclature of the parts of the ACA/OA junction is shown in Figure 1.

Results

Control Animals

The blood pressure of the control rats was 96 ± 10 mmHg.

In the control animals, the ACA branch was larger than the OA in diameter (Figure 2A). The wall of the apex consisted of normal arterial components, that is, endothelial cells, internal elastic lamina, smaller-sized medial smooth muscles cells, and thin adventitial fibrous connective tissue (Figure 2B). Apical medial defects could not be found.

On the distal side of the ACA branch adjacent to the apex, a nodular intimal protrusion, an intimal pad, always existed, although there were variations in size (Figure 2A). At the lateral angle of the ACA, a similar intimal thickening was observed. When the section was cut longitudinally very near the face or dorsum, the intimal pads of the apex and lateral angle were seen continuously forming a bridgelike structure. The apical in-

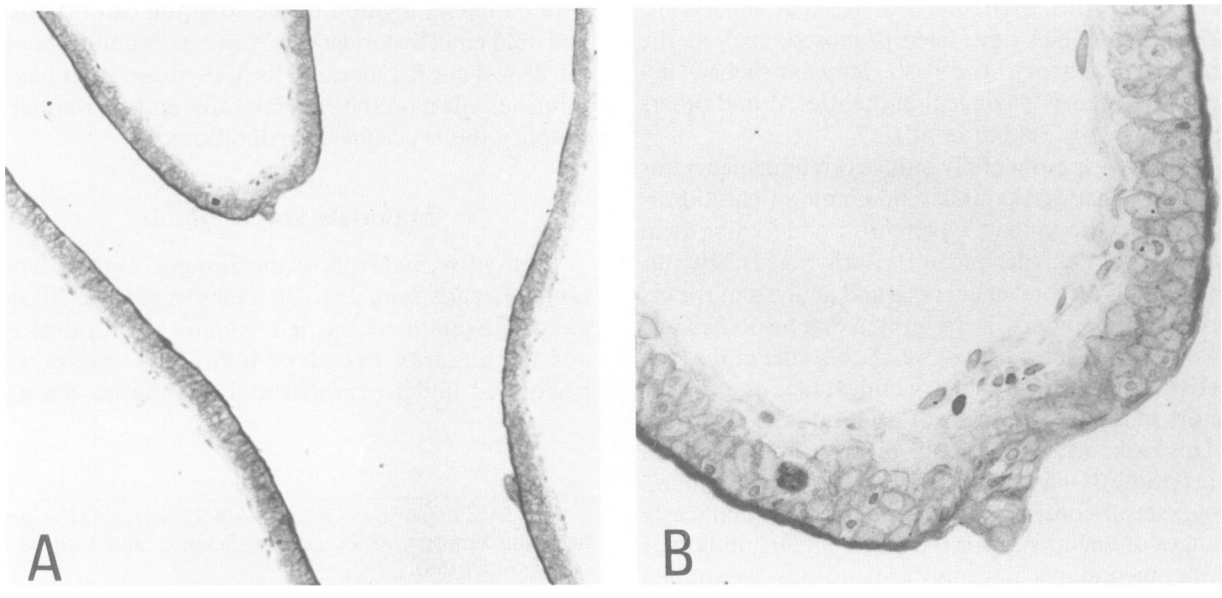

Figure 2—ACA/OA junction of a control rat. **A**—The ACA branch (*right*) is larger than the OA (*left*). Note apical and lateral intimal pads. (Toluidine blue, ×40) **B**—High-power view of the apical area, showing the intimal pad, juxta-apical depression, and no medial defect at the apex. The internal elastic lamina is thinned, faintly stained and partially fragmented under the intimal pad and at the juxta-apical depression. (Toluidine blue, ×200)

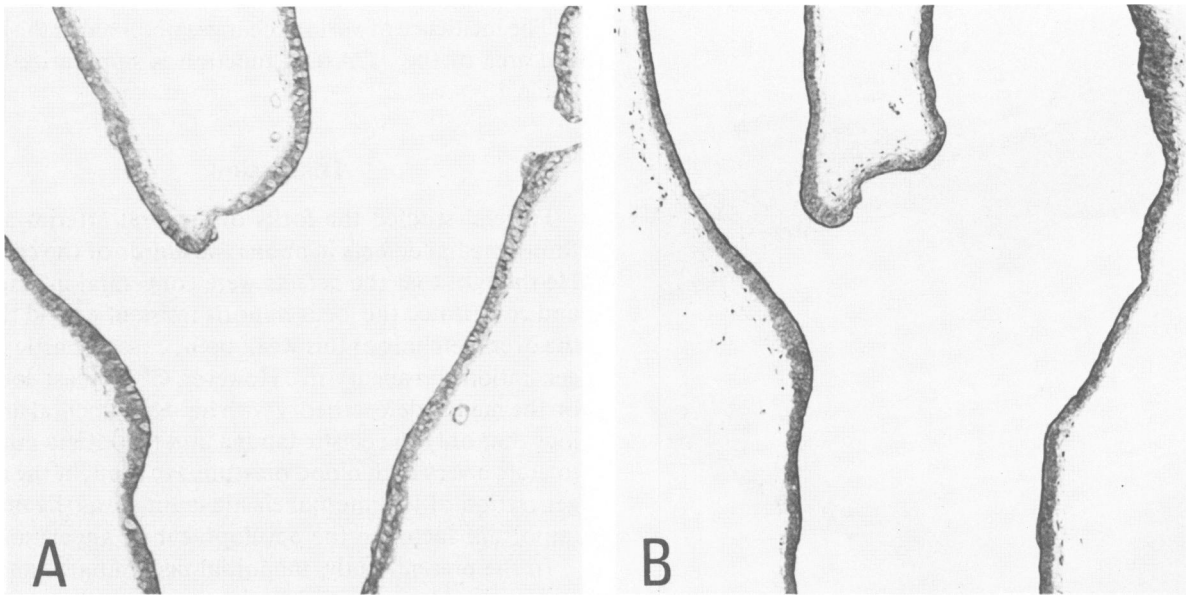

Figure 3—Early changes of cerebral aneurysms at the fork of the ACA/OA junction. (Toludine blue, ×40) **A**—The adjacent part distal to the intimal pad is thinned and dilated. **B**—The dilatation is more widened and involves the intimal pad.

timal pad consisted of spindle-shaped cells similar to medial smooth cells and fibrous connective tissue stroma. The cellular components were smaller than the ordinary medial smooth muscles and arranged longitudinally along the axis of blood flow, whereas the medial smooth muscle cells were oriented circularly. Beneath the intimal pad, the internal elastic lamina was thinned and faintly stained or had completely disappeared (Figure 2B). Sometimes the lamina was duplicated, and fine elastic fibers were observed among intimal cells.

Distal to the intimal pad, a small shallow depression of the luminal surface (juxta-apical depression) was occasionally observed on the distal side of the ACA branch (Figure 2B). In this area, medial cells were in some cases decreased in number. Here the elastic lamina was also thinned, faint in staining, and occasionally fragmented or had disappeared.

Experimental Animals

The blood pressure of the experimental animals was 172 ± 17 mmHg.

The most prominent feature of the ACA/OA junction in the experimental animals was thinning of the media with or without dilatation of the wall on the distal side of the ACA branch adjacent to the apex (Figure 3A and B).

Under a dissecting microscope, 8 of 22 cases showed a definite dilatation of the arterial wall at the ACA/OA junction. However, no well-developed aneurysms could be found. Microscopically, 4 of the remaining 14 cases also showed dilatation, and another 4 cases showed thinning of the media with a minute dilatation. Such thinning of the media and/or dilatation was located at the distal part adjacent to the apical intimal pad (Figure 4A and B), that is, corresponding in location to the juxta-apical depression observed in the control animals. In the advanced dilatation cases, however, the intimal pads were also involved in the outward bulging (Figure 4C). And in some mild cases, a small depression was also observed between the apex and intimal pad. In the remaining 4 cases, only a small depression was observed, like that seen in the control animals.

Both in the parts of the intimal pad and the thinned or dilated wall adjacent to the pad, the internal elastic lamina always showed similar changes. In the milder cases, the lamina was thinned, faintly stained, and sometimes discontinuous (Figure 4A). It had partially or completely disappeared in the more severe cases (Figure 4B and C).

Endothelial cells on the apex and intimal pads were taller than those in other parts, while on the depressions they were very flat (Figure 4B).

In the thinned or dilated parts of the wall, the media consisted of a decreased number of medial smooth muscle cells, which were in general elongated along the axis of the blood flow and sometimes vacuolated without any visible nuclei and fibrous connective tissue (Figure 4A). In the more severe cases, the thinned dilated arterial walls seemed to be composed only of fibrous connective tissue (Figure 4B and C). In such cases, the intimal pads were also involved in the outward bulging of the wall and seen as an island of partial intimal thickening (Figures 3B and 4C).

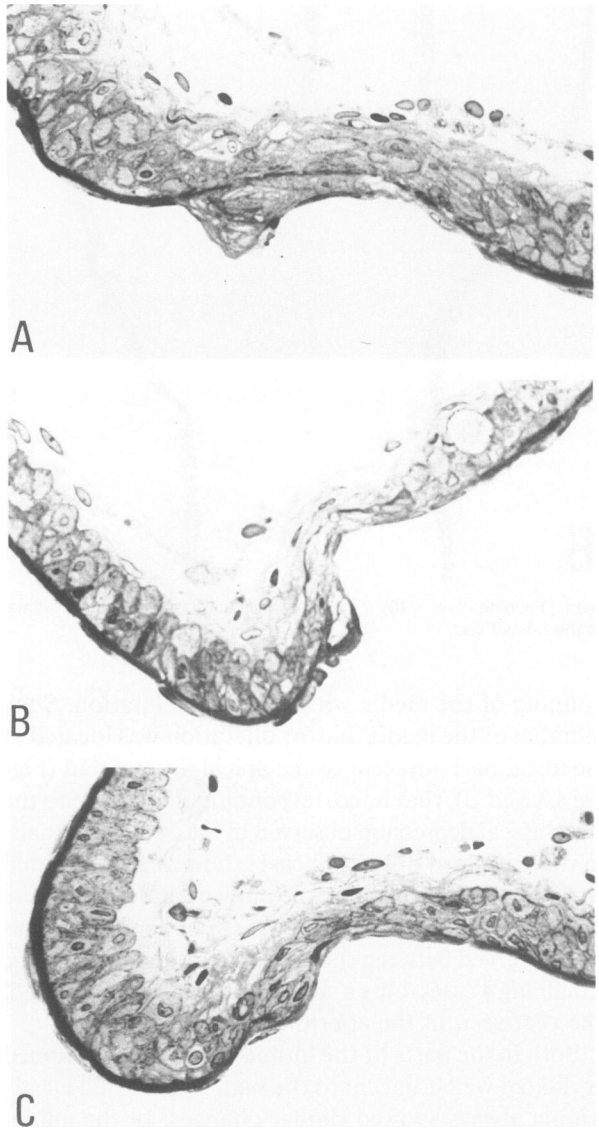

Figure 4—High-power views of the apical portion of the ACA/OA junction in the operated-on animals. (Toluidine blue, ×200) **A**—Note the depression of the distal part to the intimal pad. Under this and the intimal pad, the medial smooth muscle cells are degenerated and decreased in number, whereas the internal elastic lamina is still preserved but with mild degeneration. **B**—Note prominent thinning and dilatation at the juxta-apical depression. The internal elastic lamina is thinned and has partly disappeared. Note also degeneration of the medial cells distal to the dilatation. **C**—The apical pad is involved in the outward bulging. The internal elastic lamina has completely disappeared.

The adventitia of the dilated parts showed an increase of fibroblasts in number, compared with the remaining parts.

In the media on the distal side of the ACA branch distal to the thinning or dilatation, medial smooth muscle cells varied in size and were arranged in a disorderly fashion. Many enlarged smooth muscle cells with clear, faintly stained cytoplasms and with larger or no nuclei were seen intermingled with normal medial cells (Figure 4B and C).

The incidence of various changes observed in the apical area of the ACA/OA junction is summarized in Table 1.

Discussion

Forbus[1] studied the forks of cerebral arteries and found medial defects in about two-thirds of the cases. He thought that the defects were congenital in origin and constituted the locus minoris resistentiae and that the overstretching at this weak point caused elastic degeneration and aneurysms. However, Glynn[2] cast doubt on the medial defect theory with his experimental findings that only the elastic lamina and the intima could tolerate a very high blood pressure. He thought the degeneration of the internal elastic lamina was the most important factor in the development of aneurysms.

In the present study, the initial beginning of aneurysms proved to occur not at the apex itself, but on the distal side of the major branch at the intimal pad and the neighboring distal portion located adjacent to the apex. This fact, as well as the absence of a medial defect at the apex, disputes the medial defect theory[1] for the development of aneurysm.

The initial changes began with the degenerative changes of the internal elastic lamina at the intimal pad and the neighboring area distal to the pad. The internal elastic lamina showed thinning, fragmentation, duplication, and disappearance. The neighboring area distal to the apex often showed a shallow depression associated with a thinning of the media even in the control animals (juxta-apical depression). Such changes were augumented by the experimental treatment and developed with advancing age. Outward bulging or dilatation in the experimental animals developed at this juxta-apical depression (Figure 5). The changes in the elas-

Table 1—Incidence of Various Changes in the Apical Area of the ACA/OA Junction

	Control series* (n = 20)	Experimental series† (n = 22)
Intimal pad	17 (85.0%)	19 (86.4%)
Thinning and partial disappearance of elastic lamina	7 (35.0%)	13 (59.1%)
Disappearance of elastic lamina	1 (5.0%)	7 (31.8%)
Thinning of media	7 (35.0%)	15 (68.2%)
Fibrous displacement of media	0 (0.0%)	5 (22.7%)
Juxta-apical depression	8 (40.0%)	4 (18.2%)
Mild dilatation localized to juxta-apical depression	2 (10.0%)	10 (45.5%)
Marked dilatation with bulging of intimal pad	0 (0.0%)	6 (27.3%)

* Materials were taken from the both sides
† Materials were taken from the side not operated upon.

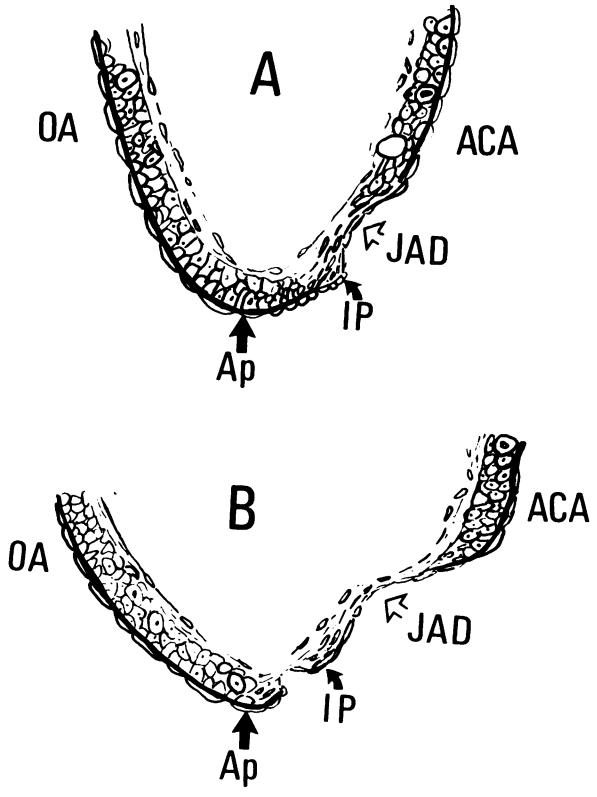

Figure 5—Schema showing the development of aneurysmal changes at the ACA/OA junction. **A**—A cerebral aneurysm in the very early phase. **B**—An advanced aneurysm in which the intimal pad is also involved in the outward bulging.

tic lamina seem to be the basis of aneurysm formation of the arterial wall, as others have already stressed.[2,4,25] However, degeneration and disappearance of medial smooth muscle cells in the juxta-apical depression may also play some role in the development of aneurysms. Carmichael[12] concluded that defects of both the medial and the internal elastic lamina must be present for aneurysm formation.

The part of the intimal pad was resistant to the blood pressure in the initial stage even though it showed elastic changes, but later it was involved in the aneurysm, remaining as a small protrusion of the intima near the orifice. Carmichael[12] has already expressed the opinion that the wall does not yield if the outer coat is supplemented by one substantial inner layer, whether composed of muscle, elastic tissue, or fibrotic intima.

In the advanced cases in which the intimal pad was involved in the aneurysmal change, the proximal part of the intimal pad, that is, between the apex and the intimal pad, also showed an outward bulging. Because this dilated portion showed various degrees of medial changes, from thinning to being completely defective, and was in direct contact with the apex, aneurysms looked as if they originated at the apex itself.

These early aneurysmal changes in our experiment closely resemble those in human cases. Stehbens[3] has reported that areas of thinning, one of the preaneurysmal changes, occur at the apex or on the adjacent part of the distal surface of the daughter branch and can coexist with the medial defect of Forbus. Though Stehbens' thinning areas have been reported by others[13,14] as large medial defects and are regarded as of congenital origin, our findings strongly support Stehbens' opinion that the area of thinning, or the large medial defect, at the apex and on the distal side of the branch adjacent to the apex is *acquired* and should be distinguished from medial defects.

Because the initial dilatation of the arterial wall always developed in the area distal to the intimal pad, the intimal pads are suspected of being the structure that causes hemodynamic strain in this portion. Although it has been said that the intimal pad plays a possible role in the pathogenesis of cerebral aneurysms,[15,16] the exact hemodynamic changes at this portion are not yet well known.

How do changes of the internal elastic lamina occur, which are the most basic changes in the development of aneurysms? By scanning electron microscopy, the endothelial cells on the intimal pad as well as on the juxta-apical depression showed very severe changes.[16] In the present study, medial smooth muscle cells in this portion also showed regressive changes. These findings indicate that this portion must be exposed to severe hemodynamic stress.

In our previous studies on hypertensive animals, we found an activitation of lysosomal enzymes[17-21] and proteases, including elastase, in the arterial walls.[22] We also demonstrated immunohistochemically the presence of elastase in the endothelial cells and subendothelial space in the hypertensive aorta, whereas in the control animals the enzyme was not found.[23] A decreased content of elastin has also been reported in the arterial walls in hypertensive animals.[24] From such findings, we concluded that the activated hydrolytic enzymes play some important role in the further regressive changes of arterial walls in hypertension. In the case of aneurysms, we may suppose that a similar mechanism is in operation. Cajander and Hassler[25] studied by electron microscopy the elastic component in the mouths of aneurysms and found extracellular lysosomal-like granules in close connection with the disintegrated elastic lamellas. They hypothesized that discharged leukocyte granules containing elastase help to destroy the elastic lamellas. In aortic aneurysms, activation of elastase has been demonstrated.[26] However, decreased synthesis of elastin due to degeneration of smooth muscle cells as well as endothelial cells cannot be ruled out.

Degenerative changes of endothelial cells and the medial smooth muscle cells were not restricted to the juxta-apical depression, but were also observed in the

arterial wall distal to the depression. Aneurysmal changes seemed to be extending to the distal parts.

We are now examining which kind of hemodynamic stress attacks the initial sites of aneurysm and which kinds of metabolic changes take place there.

In conclusion, we may hypothesize from the above-mentioned findings that hemodynamic stress due to the branching structure, including the intimal pad, augmented by hypertension and the ligation of the common carotid artery, causes degenerative changes of the elastic lamina and the medial smooth muscle cells on the distal side of the major branch adjacent to the apex, and that such degenerative changes are the basis for the formation of aneurysms.

References

1. Forbus WD: On the origin of miliary aneurysms of the superficial cerebral arteries. Bull Johns Hopkins Hosp 1930, 47:239–284
2. Glynn LE: Medial defects in the circle of Willis and their relation to aneurysm formation. J Pathol Bacteriol 1940, 51:213–222
3. Stehbens WE: Histopathology of cerebral aneurysms. Arch Neurol 1963, 8:272–285
4. Stehbens WE: Intracranial arterial aneurysms, In: Stehbens WE. Pathology of the Cerebral Blood Vessels. Edited by WE Stehbens. St. Louis, C.V. Mosby, 1972, pp 351–470
5. Bremer JL: Congenital aneurysms of the cerebral arteries. Arch Pathol 1943, 35:819–831
6. Agnoli AL: Vascular anomalies and subarachnoid haemorrhage associated with persisting embryonic vessels. Acta Neuropathol (Wien) 1982, 60:183–199
7. Sekhar LN, Heros RC: Origin, growth and rupture of saccular aneurysms: A review. Neurosurgery 1981, 8: 248–260
8. Hashimoto N, Handa H, Hazama F: Experimentally induced cerebral aneurysms in rats. Surg Neurol 1978, 10: 3–8
9. Hashimoto N, Handa H, Nagata I, Hazama F: Saccular cerebral aneurysms in rats. Am J Pathol 1983, 110:397–399
10. Hashimoto N, Handa H, Nagata I, Hazama F: Animal model of cerebral aneurysms: Pathology and pathogenesis of induced cerebral aneurysms in rats. Neurol Res 1984, 6:33–40
11. Handa H, Hashimoto N, Nagata I, Hazama F: Saccular cerebral aneurysms in rats: A newly developed animal model of the disease. Stroke 1983, 4:857–866
12. Carmichael R: Gross defects in the muscular and elastic coats of the larger cerebral arteries. J Pathol Bacteriol 1945, 57:345–351
13. Carmichael R: The pathogenesis of non-inflammatory cerebral aneurysms. J Pathol Bacteriol 1950, 62:1–19
14. Yamamoto Y, Yoshida Y: Kumomakuka shukketsuno byouri, tokuni berry doumyakuryuuno naritachito haretsuno kijonitsuite (Pathology and subarachnoid hemorrhage, with special reference to the morphogenesis of berry aneurysms of cerebral arteries and mechanisms of their rupture). J Jpn Coll Angiol 1977, 17:183–195
15. Hassler O: Morphological studies on the large cerebral arteries with reference to the aetiology of subarachnoid haemorrhage. Acta Psychiatr Neurol Scand Supp 1961, 154, 36(Suppl 154):1–145
16. Kojima M, Handa H, Hashimoto N, Choegon K, Hazama F: Early changes of experimentally induced cerebral aneurysms in rats: Scanning electron microscopic study. Stroke (In press)
17. Yamada E, Hazama F, Amano S, Hanakita J: Acid phosphatase activity of the brain in SHR. The first report of enzyme histochemical studies of spontaneously hypertensive rats. Jpn Circ J 1979, 43:285–292
18. Yamada E, Hazama F, Amano S, Hanakita J: Cytochemical investigation on acid phosphatase activity in cerebral arteries in spontaneously hypertensive rats. Jpn Circ J 1980, 44:467–475
19. Yamada E, Hazama F, Amano S, Sasahara M, Kataoka H, Kayembe K: Semiquantitative histochemical investigation of lysosomal enzyme activities in the aortic endothelial cells of spontaneously hypertensive rats. Atherosclerosis 1983, 47:19–26
20. Watanabe M, Yamada E, Hazama F, Nomura T: Acid phosphatase activity in the aorta of spontaneously hypertensive rats and the effect of various antihypertensive drugs. Atherosclerosis 1981, 40:167–174
21. Sasahara M, Hazama F, Yamada E, Kataoka H, Amano S, Kayembe K: Semiquantitative histochemical investigation of lysosomal enzymes in the aortic endothelial cells of rats with renal hypertension. Virchows Arch [Cell Pathol] 1983, 44:267–274
22. Yamada E, Hazama F, Kataoka H, Amano S, Sasahara M, Kayembe K, Katayama K: Elastase-like enzyme in the aorta of spontaneously hypertensive rats. Virchows Arch [Cell Pathol] 1983, 44:241–245
23. Yamada E, Hazama F, Amano S, Sasahara M, Kataoka H: Elastase, collagenase and catepsin D activities in the aortas of spontaneously and renal hypertensive rats. Exp Mol Pathol 1986, 44:147–156
24. Ito H, Yamamoto K, Okamoto K: The characteristics of aortic elastin in SHRSP. Jpn Heart J 1979, 20:240–242
25. Cajander S, Hassler O: Enzymatic destruction of the elastic lamella at the mouth of cerebral berry aneurysm? An ultrastructural study with special regard to the elastic tissue. Acta Neurol Scand 1976, 53:171–181
26. Basttil RW, Rinderbriecht H, Flesher A, Carmack C: Elastase activity: The role of elastase in aortic aneurysm formation. J Surg Res 1982, 2:214–217

Acknowledgments

Thanks are due to Mr. P. Schneider, Shiga University of Medical Science, for assisting in preparing the manuscript.