Development of Intracranial Aneurysms as Revealed by Electron Microscopy

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There are many theories concerning the etiology of intracranial aneurysms. As to arterial aneurysms, much attention has been paid to a defective media in the wall of a vessel and their possible congenital or postnatal origin. Most of the defects of the media seem to be congenital, but some may have developed subsequently. The replacement of the defect by collagen does not seem to be a sufficient reason for the development of an arterial aneurysm, since numerous medial defects have been observed with no formation of aneurysm. Factors favoring fragmentation and disappearance of the elastic lamellae seem to be of great importance in the development of sacular aneurysms, but the changes caused by sclerosis also may play a primary part in the development of fusiform aneurysms, especially in older persons.

With reference to true arteriovenous aneurysms, much work has been done to clarify their pathogenesis. With the exception of the carotid-sinus cavernous aneurysms, most of the arteriovenous aneurysms are congenital in origin. The group has to be distinguished from other vascular malformations, such as angioma capillare et venosum calcificans (Sturge-Weber disease), telangiectases, cavernous angiomas (without brain parenchyma between the vessels), pure venous racemose angiomas, and pure arterial racemose angiomas. The existence of the last ones has been doubted. This opinion is contrary to reports by Hyland and Douglas, and by Asenjo and Uberall, who had observed such angiomas. The true arteriovenous aneurysm is characterized by the presence of both one or several feeding arteries and one or several draining veins, between which a direct connection exists either by means of one large vascular branch, several branches or a tangle of tortuous dilated veins, which take the place of the capillary bed. It is not always easy to demonstrate microscopically the connection between the arteries and the veins. Bergstrand et al. emphasized that the vessels in the angionoma may be so malformed that a pathological examination cannot determine finally whether they are arteries or veins, and that it may be difficult to find a histological difference between aneurysma arteriovenosum and angionia venosum. The difference may be purely physiological, since it is a question of whether there are large shunts, i.e., the vessels drain arterial blood, or if they drain venous blood which has passed through a normal capillary network.

Olivecrona and Ladenheim assumed that an arteriovenous aneurysm arises as a result of incomplete development at the fetal stage at which the differentiation of the primordial system into arteries, veins and capillaries occurs and richly anastomosing plexuses are formed (Streeter’s Stadium III). Zülch emphasized that it is Streeter’s Stadium V in which arteriovenous aneurysms originate.

Padget, in studying vascular development in man, found that crossing vessels may be separated only by a double layer of endothelial cells and suggested that a fistula is formed at the crossing point, this being the origin of a racemose angionia.

Hamby has described a typical arteriovenous aneurysm located in the right parieto-occipital lobe. Four different vascular types were found. Vessels of the first type were twisted spirally and located next to the plexus, and ran parallel to the axis of the aneurysm. Another type, empty of blood and
with a straighter course, but with a few sharp coils, was found in the same layer. There also was a blood-filled strongly convoluted type. The fourth type was more elongated. The diameter of the vessels varied from 0.15 to 0.7 mm. All four types of vessels provided drainage to the superficial cerebral or the deep cerebral venous system, through large, thin-walled vessels with a diameter from 1 to 5 mm. Traumatic hemorrhages were seen in the center of the dissection surrounding the vessels of the third type.

Microscopically the arteriovenous aneurysm appears as a confused mass of vessels and varies somewhat in type depending on whether the section has been made in the neighborhood of the feeding artery, more centrally in the specimens or near the draining veins. Sorgo found three types of vessels, i.e., thick vessels with endothelium and hyalin, nuclei-filled connective-tissue layers without elastin; vessels with intima, media and adventitia and a split-up elastica separating the endothelium from the muscular layer; and lacunar blood-filled vessels with thin endothelial walls. Pluvinage emphasized that the walls with a layer of media sometimes may be hypertrophic and sometimes very thin in the same vessel.

In the evaluation of the different factors in the development of intracranial arterial and arteriovenous aneurysms the numerous reports on the occurrence of these two types of aneurysm in one and the same patient are of great interest. Microscopically the arteriovenous aneurysm appears as a confused mass of vessels and varies somewhat in type depending on whether the section has been made in the neighborhood of the feeding artery, more centrally in the specimens or near the draining veins. Sorgo found three types of vessels, i.e., thick vessels with endothelium and hyalin, nuclei-filled connective-tissue layers without elastin; vessels with intima, media and adventitia and a split-up elastica separating the endothelium from the muscular layer; and lacunar blood-filled vessels with thin endothelial walls. Pluvinage emphasized that the walls with a layer of media sometimes may be hypertrophic and sometimes very thin in the same vessel.

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Material

The whole material studied consisted of 15 patients, of whom 7 had arterial and 3 arteriovenous aneurysms and 5 yielded tissue of origin. Two of the patients with arteriovenous aneurysm also had arterial aneurysms. The cases of arterial aneurysms were as follows:

Two patients, aged 36 and 52 years, the former with a bean-sized and the latter with a cherry-sized aneurysm of the anterior communicating artery; 1 patient 37 years old with two pea-sized aneurysms, one situated on the anterior communicating artery and the other on the left middle cerebral artery; 2 patients 45 and 60 years of age with a bean-sized aneurysm of the right middle cerebral artery; 1 patient aged 49 years with a bean-sized aneurysm of the internal carotid artery; and 1 patient aged 31 years with a cherry-sized aneurysm of the right superior cerebellar artery.

In the group of arteriovenous aneurysms a girl aged 10 had an egg-sized aneurysm in the frontal lobe which was supplied mainly by branches from the right pericallosal artery. One 44-year-old patient had a large arteriovenous aneurysm in the occipital lobe, supplied mainly by the right middle cerebral artery and the right posterior cerebral artery. A bean-sized arterial vascular aneurysm on the right middle cerebral artery was observed in the angiogram. A patient aged 45 had an egg-sized arteriovenous aneurysm of the frontotemporal region, supplied mainly by the right middle cerebral artery. The patient also had a vascular arterial aneurysm on both of the middle cerebral arteries.

There had been bleedings from the aneurysm in all cases. No extensive hypertension was observed. Because of the subarachnoid hemorrhage the patients with an arterial aneurysm were in a poor condition already on admission and 2 were semicomatose when operated on with a fatal result. All the arteriovenous aneurysms were removed completely and the arterial aneurysms in the same patients were also repaired with full recovery of the patients.

Methods

Specimens of the arterial aneurysms for light and electron microscopic studies were taken 4 to 10 hours post-mortem. The control specimens were taken from a site symmetrical to the site of the aneurysm.

Specimens of the arteriovenous aneurysms for light and electron microscopy were taken during the operation. The control material in this group consisted of 5 other patients who had undergone craniotomy, resection of some normal brain tissue having to be carried out incidental to different neurosurgical procedures.

Different parts of the aneurysms were fixed in osmic acid, stained with phosphotungstic acid and embedded in methacrylate and araldite. Some specimens were treated by adding uranyl nitrate in the final change of methacrylate. Ultra-thin sections were cut on a Porter-Blum microtome.
and examined with a Siemens Elmiskop I and a Philips 100 B electron microscope. The material was also analyzed with a light microscope using the following stains: Haematoxylin eosin, Delafield's haematoxylin solution, Weigert's haematoxylin, van Gieson's acid fuchsin, Sudan III dissolved in 70 per cent. alcohol, Lillie's alcolochrome connective-tissue stain, Weigert's resorcin fuchsin and neutral red in Hart's modification, and v. Kossa's calcium stain.

**Findings**

In light microscopy the control material showed typical walls of vessels with well preserved elastica interna and media. The endothelium seemed partly collapsed, but nothing remarkable could be observed, with the exception of some proliferation and fatty infiltration at the sites of atheromatous plaques in control vessels of 2 of the older patients with arterial aneurysms. In these cases some hyalinization of the connective tissue and infiltration of the media by macrophages also were observed. Regular layers of smooth-muscle cells surrounded by collagenous bundles and sparsely occurring elastic fibers generally were observed in the media. Some medial defects were organized by connective tissue. Nothing especially pathological was observed in the adventitia, which generally is developed weakly in the basal arteries. The elastica interna was homogenous and had a looping course. The Eversen fenestrations of elastica, i.e., rests of nuclei, were regular and somewhat larger in the smaller vessels which is typical of normal walls of vessels. In the smaller vessels of normal cerebral tissue nothing pathological was observed.

The walls of arterial aneurysms showed the following changes. Defects of the medial layers and splitting of the elastic lamina were frequent. In some cases the elastica was hypertrophied and split up. The elastica was lacking near the sites of the ruptures. The collagen seemed somewhat paler in the walls of the aneurysms than in the walls of the control vessels. The greatest differences consisted of the ones caused by atherosclerosis. These were found in the older patients aged 50 to 60 years, who exhibited similar changes, though of lesser degree, in the control vessels. Deposition of fat was most frequent in the fundus of the sac. Thickening of the intima and hyalinization of connective tissue were observed. Invasion of macrophages and branches of connective tissue into the media also were seen.

Typical changes were observed in the walls of the arteriovenous aneurysms. The arteries and veins in these malformations also showed sclerotic changes and hyalinized hypertrophic parts. Some of the vessels had a defective elastica. Extravasation of blood with fibroblastic proliferation and fibrosis were found. Macrophages occurred to some extent. The arteries as well as the veins were enlarged. Some lacunar vessels seemed to be built up of only a thin connective-tissue layer and could not be identified either as arteries or as veins. In 2 cases there also was rich proliferation of the perivascular capillaries.

In the electron microscopy the control material, i.e., normal vessels taken at autopsy from 7 patients with arterial aneurysm and vessels taken at operation from 5 patients yielding normal brain tissue showed no important differences. Some patients in the former group showed a slight tendency to atherosclerotic changes in the form of vacuolation of endothelial cells and infiltration of the media and adventitia by macrophages. In the autopsy specimens embedded in methacrylate there were post-mortem changes, but the autopsy specimens embedded in araldite seemed as well preserved as the material taken at operation. Typical normal walls of vessels were observed (Fig. 1).

Electron microscopic examination of the walls of the arterial aneurysms revealed several structures that were in accordance with the findings in the light microscopic examination. In the hypertrophic, well preserved endothelium and in the cytoplasm of other cells in the walls of arterial aneurysms, there frequently were vacuoles filled with lipid (Fig. 2) which were similar to those seen by some other workers and by the present author in the walls of cerebral vessels in cockerels with cholesterol-induced atherosclerosis. Walker and Allège have observed lipid-poor atheromatous intimal plaques in the walls of aneurysms by light microscopy.
In this study the lipid vacuoles were very rare in the control material.

The elastic lamina generally was split up and had lost its normal fibrillar structure. At the sites of the rupture of the aneurysmal sac the elastica was totally lacking. In the wall of some aneurysms the elastica was thickened and its fine structure differed from the normal elastica. The elastica was partly granulated and very electron-dense in relation to what generally may be observed in normal elastica (Fig. 3). At the sites where the Eversen fenestrations were enlarged, the granulated electron-dense elastica covered it in part. This unusual elastica occurred especially in the neighborhood of the ruptures. It consisted of a mass of irregular particles which were electron-dense at their margins and varied from 500 Å to 0.5 μ in diameter (Fig. 4).

In the enlarged Eversen fenestrations and in the perilaminar space, i.e., outside the elastica interna, there were numerous freely moving erythrocytes. Some of the wide fenestrations contained small well developed capillaries, which were *vasa vasorum* of the aneurysmal wall (Fig. 5). The capillaries occurred in the ground substance, which contained only a few bunches of collagenous fibrils. The capillaries had a normal basement membrane and a distinct endothelium. Generally they were small.

The layers of the media frequently were invaded by thick connective-tissue processes, which separated the rather stretched, elongated and irregularly arranged smooth-muscle cells from each other (Fig. 6). The ground substance in the media and adventitia showed single macrophages and groups of lipid vacuoles. Generally the fibrils of collagen seemed to be more evenly distributed in the ground substance of the aneurysmal wall than in the wall of the control vessels. Especially great interest has been paid to the inner-banding structure of the collagenous and reticular fibrils. Two kinds of periodicity were observed. The most frequent was the normal, i.e., 640 Å, but intermingled with this could be seen somewhat smaller fibrils which had a periodicity of about 800 Å (Figs. 7, 8 and 9). They were found in bunches of normal fibrils of collagen. Changes of the collagen have been observed by the present author also in vascular beds of brain tumors.15–17

The vascular walls of the arteriovenous aneurysms exhibited changes that were very similar to those in the arterial aneurysms. Generally the endothelium was not greatly hypertrophied. In all kinds of vessels vacuoles of lipid, as well as hook-like endothelial processes extending into the lumen, were present (Fig. 10). The subendothelial layer was partly hyalinized in arteries and veins and contained small bands of the above mentioned granular elastic matter. Outside this layer were several layers of granular elastic tissue separated by ground substance and interrupted by thin layers of smooth-muscle cells with lipid inclusions. In arteries the elastica interna was split up and the Eversen fenestrations were here and there very large and contained erythrocytes. Erythrocytes also were found in the space between the elastica interna and the media (Fig. 11), as was the case in the walls of arterial aneurysms. There also were erythrocytes in the perivascular region as observed in the light microscopic studies.

The muscular layer of the media showed distended smooth-muscle cells. Some lipid inclusions of muscle cells were observed. At sites with hypertrophic media the layer of muscle was overgrown with hyalinized connective tissue. In all kinds of vessels in the arteriovenous aneurysms the occurrence of collagenous and reticular fibrils in the ground substance was about the same as in the walls of arterial aneurysms. The periodicity seemed normal.

**Discussion**

Electron microscopy revealed several structures that were in accordance with the findings in the light microscopy, but could not be observed by the latter method.

The atherosclerotic changes, especially in the group of arterial aneurysms, could be observed by both methods. In the older patients atherosclerosis was present also in the
walls of the control vessels, although to a lesser degree. It seems probable that the atherosclerotic changes are a secondary process to the aneurysmal formation, the developing aneurysm being a *locus minoris resistentiae* for infiltration of macrophages, lipids, etc. Atherosclerotic changes also were seen in the vascular walls of arteriovenous aneurysms, but to a lesser degree. It is not a question of senescence, because, as observed by among others, Katz and Stamler, a tendency to atherosclerosis may occur also in young persons. Here it is a question of a purely local process. Both types of aneurysms contained enlarged Eversen fenestrations and a type of elastica differing from the normal. This was an insufficient and possibly immature elastica which was granular, electron-dense, and not so strong as the normal elastica. The weaker elastica may give way to blood pressure and begin to bulge and enlarge. Intermingled in the normal collagen in the aneurysmal wall there were fibrils of collagen, which may have been a type of
Fig. 7. Fibrils of normal collagen in wall of arterial aneurysm. Methacrylate, ×66,500.
Fig. 8. Distended "precollagen" in wall of arterial aneurysm. Methacrylate, ×80,000.
Fig. 9. Fibrils of collagen with normal periodicity in wall of a control artery. Methacrylate, ×66,500.
Fig. 10. Wall of a vessel in an arteriovenous aneurysm. l = lumen, va = vacuole, li = lipid, ep = endothelial process, el = elastica, col = collagen, smc = smooth-muscle cell. Epoxy, ×5,000.
Fig. 11. Wall of a vessel in an arteriovenous aneurysm. e = erythrocytes, ps = perilaminar space, ef = Eversen fenestration. Epoxy, ×5,000.
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distended precollagen. Distended precollagen was observed in the aneurysmal wall of a patient whose two angiograms, taken in 1954 and 1961, showed striking growth of the aneurysmal sac.

In keeping with the fetal origin of aneurysms, their different layers seem to preserve some fetal features during their postnatal development. The weak substance of the elastica in the arteriovenous aneurysms is a reminiscence of the Streeter Stadium in which the differentiation of vessels begins, while in the arterial aneurysms this substance is present at vascular points where there are remains of embryonic blood vessels. The fact that some elementary material in the walls of the vessels conserves certain properties during postnatal development hinders the body from organizing and eliminating some feeble points at an early stage of development. In this way we may understand the finding of a cherry-sized, thick-walled aneurysm bulging like a tumor from a small thin-walled vessel in the circle of Willis. On the other hand, organizing processes were found which have not been observed in the light microscopy. In the large Eversen fenestrations of the elastica there were several small capillaries embedded in a loose ground substance with sparsely occurring fibrils of collagen. In other, very large fenestrations and in the wide space between the elastica and the media were free-lying erythrocytes in both types of aneurysms. It may be supposed that splitting starting from the enlarged Eversen fenestrations is the beginning of the rupture, while the loose ground substance with capillaries is a sign of repair processes. The defects of the media generally seemed not to be complete, since often a thin, greatly distended layer of elongated smooth-muscle cells was present. Also the adventitia generally was thin in the aneurysmal wall. In some thick-walled aneurysms the thickness of the media was a result of broad connective-tissue processes invading this layer, and was not the result of an increase in the number of layers of smooth-muscle cells.

The features of the described type of aneurysm support the opinion that the malformation leading to an arterial or arteriovenous aneurysm is congenital in origin. However, the typical bulging and growth of the walls at the site of the malformation may be a postnatal process. At the sites with very thin layers of media, organizing processes develop which involve also the Eversen fenestrations. An immature elastic lamina is distended easily by the blood pressure. The enlarged fenestrations of the elastic lamina permit the penetration of erythrocytes into the perimembranous space between the elastica and the thin media. The pathological circulatory conditions give rise to changes in the different layers similar to those in atherosclerosis. The wall becomes weaker and more distended, in spite of simultaneous processes of repair. The rupture of the aneurysm follows the invasion of the various layers of the aneurysmal wall by macrophages and lipids. The rebleeding of an aneurysm seems to be the result of this process. The findings also are in accordance with the fact that in states of increased metabolism of lipids the occurrence of subarachnoid haemorrhage in patients with intracranial aneurysms seems to be more frequent than in states of normal metabolism of lipids.

The above findings and suggestions apply to the present small series, which contained some special cases. For example, 2 patients had both arterial and arteriovenous aneurysms. Many similar features were found in the two types of aneurysm in this series, but it is possible that other mechanisms are responsible for the development of other aneurysms, for example fusiform aneurysms in older patients.

Thus, partial ligation of the fundus or of an additional bulge in an aneurysmal sac, made necessary by anatomical difficulties, seems to have its value in preventing not only the pressure of blood, but also the deposition of macrophages and destructive lipids, which is most active in these weak regions of the sac. The antirupture effect of the ligation of the carotid artery is ascribable not only to the lower blood pressure obtained, but also to a lesser tendency of the circulating blood
to deposit destructive lipids in the walls of an aneurysm.

Summary

The walls of the arterial and arteriovenous aneurysms were studied by light and electron microscopy. The material consisted of 15 patients, 7 of whom had arterial and 3 arteriovenous aneurysms, while 5 patients served as controls. The specimens were fixed, embedded and sectioned in the routine manner for biological tissue. Examination was carried out with a Siemens Elmiskop I and a Philips 100 B electron microscope.

In electron microscopy the walls of cerebral aneurysms showed most of the structures, and in greater detail than may be found in light microscope examination. Vacuoles containing lipids were seen in the hypertrophied endothelium and in the cytoplasm of cells in the walls. The vacuoles were very rare in the control material. The structures may be regarded as a secondary result of the lesion. The splitting of an immature elastic lamina was very striking in the walls of the arterial and arteriovenous aneurysms. The Eversen fenestrations were very wide and occasionally contained small capillaries, which were eaca vasorum of the aneurysm wall, probably as a process of repair. Erythrocytes were abundant in the wide fenestrations and in the perilaminar space. The typical arrangement of collagenous and reticular fibrils into bundles was lacking in the walls of aneurysms, where the fibrils were distributed more evenly in the ground substance. The smooth-muscle cells in the thin media often were hyalinized. In the walls of arterial aneurysms a type of elongated precollagen was found intermingled with normal collagen. The possible significance of the present findings in the developmental process of aneurysms is discussed.

References

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