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Ultrastructural Characteristics of Peripheral Arteriovenous and Venous Angiodysplasias

K. A. Pavlov*, I. A. Chekmaryova*, A. I. Shchyogolev*,, and O. D. Mishnyov*,****

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Comparative histological and electron microscopic study of arteriovenous and venous angiodysplasias revealed specific features of their structure, presumably reflecting differences in their morphogenesis. Specific ultrastructural characteristics of angiodysplasias are modified shape of endotheliocytes, impaired structure of the basal membrane, and reduced count of pericytes.

Key Words: *angiodysplasias; pericytes; ultrastructure; endotheliocytes*

Angiodysplasias or vascular malformations (VM) form a rare group of diseases resultant from disorders of vessel formation during the embryogenesis. The incidence of these diseases in the population varies from 0.3 to 0.5% [6]. The pathogenesis and development of angiodysplasias remain not quite clear and attract much attention [1]. Study of the ultrastructure of VM will presumably help to solve many problems, *e.g.* detect the details of individual components of the pathogenesis and disease course and evaluate the efficiency of therapeutic interventions [2]. Unfortunately, studies of this problem are scanty, and only few studies were devoted to ultrastructural changes in peripheral angiodysplasias.

We compared the histology and ultrastructure of arteriovenous and venous angiodysplasias of peripheral location.

MATERIALS AND METHODS

The operation material from 7 patients (4 women and 3 men) aged 17-57 years with peripheral VM, treated at A. V. Vishnevskii Institute of Surgery in 2006-2008, is analyzed. Venous malformations were diagnosed in 4 cases (3 women and 1 man), arteriovenous malformations in 3 cases (1 woman and 2 men).

Tissue fragments were fixed in 10% neutral formalin. Histological studies were carried out on paraffin sections (5 μ) stained with hematoxylin and eosin. Specimens for electron microscopy were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, dehydrated in ascending alcohols, and embedded in epon and araldite mixture. Ultrathin sections were examined under a Philips CM-10 electron microscope.

RESULTS

Histological studies of operation material (specimens of arteriovenous angiodysplasias) showed chaotic accumulation of arterial and venous capillaries (5-15 μ in diameter). The intimal elastic membranes of these vessels were characterized by uneven distribution of elastic fibers with sites of fiber destruction. There were subintimal foci of hypertrophic smooth-muscle cells and focal sclerosis in the venous walls. All formations were sur-

*Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery; **Department of Pathological Anatomy, Therapeutic Faculty, Russian State Medical University, Moscow, Russia. ***Address for correspondence:*** pavlovka@ixv.comcor.ru. K. A. Pavlov

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rounded by a zone of fibrous connective tissue (Fig. 1, *a*).

Microscopic studies of venous angiodysplasias revealed fine-walled venous caverns of different size and shape. The walls of these caverns were of different thickness: the number of myocytes was reduced in thin sites and increased in thick ones (Fig. 1, *b*). Areas of compact fibrous connective tissue and foci of hemorrhages of different size were seen around these formations.

Electron microscopy of peripheral venous malformations showed that medium-sized and small veins and caverns were lined with round functionally active endotheliocytes. These cells had nuclei of irregular shape and numerous processes of different length (from medium to long) (Fig. 2, *a*). Large caverns were lined mainly with flat endotheliocytes of low functional activity, with just few short processes and oval nuclei. Signs of functional activity (numerous vacuoles in the cytoplasm, cytoplasmic membrane outgrowth, finely dispersed chromatin, numerous ribosomes) were more often observed in endotheliocytes lining small vessels, whose walls consisted of just one layer of endothelial cells and solitary pericytes (Fig. 2, *b*). The basal membrane in large caverns had gaps and local thickenings.

Tight junctions between endotheliocytes were solitary, their number being somewhat higher in large caverns. In one case we detected large caverns with impaired endothelial lining and erythrocyte extravasation (Fig. 2, *c*). The walls of these caverns consisted of mature collagen fibers with fibroblasts between them. Solitary pericytes were detected in the vascular wall in all preparations. The vascular wall was thick in large caverns mainly because of mature collagen fibers and fibroblasts, which were more often oval or spindle-shaped and rarely irregularly shaped (Fig. 2, *d*). Some fibroblasts was functionally active.

Electron microscopy of the peripheral arteriovenous angiodysplasias showed that small arteries and veins were lined with functionally active endothelial cells with numerous medium-sized and long processes (Fig. 3, *a*). The nuclei of these endotheliocytes were oval, with irregular nuclear membranes. Large arteries and veins were lined with mainly flat endothelial cells with low functional activity, few short processes, and oval nuclei. The walls of small vessels consisted of just one layer of endotheliocytes with signs of high functional activity and solitary pericytes. The number of tight junctions between endotheliocytes was minimum in vessels of different diameter (Fig. 3, *b*). The vascular wall was significantly thickened in large veins and moderately thickened in medium-sized arteries and veins mainly at the expense of mature collagen fibers and oval and spindle-shaped fibroblasts. The basal membrane of large arterial walls seemed to have gaps or looked multilamellar with thick sites (Fig. 3, *c*). The number of pericytes was reduced (Fig. 3, *d*). Foci with fiber destruction in collagen bundles were seen in the walls of large arteries. Some fibroblasts were functionally active.

Studies of the effects of various factors on angiogenesis showed that any disorders in this process led to the development of vascular malformations, including contacts between endotheliocytes [8]. Cell-cell contacts regulate endothelial adhesion and processes of intracellular transport of substances. The following types of contacts between endotheliocytes were distinguished: intermediate, gap junctions, and tight junctions. Reduced number of tight junctions can attest to failure of the blood-brain barrier associated with CNS VM and liability of angiodysplasia relapse [7]. Reduction in the num-

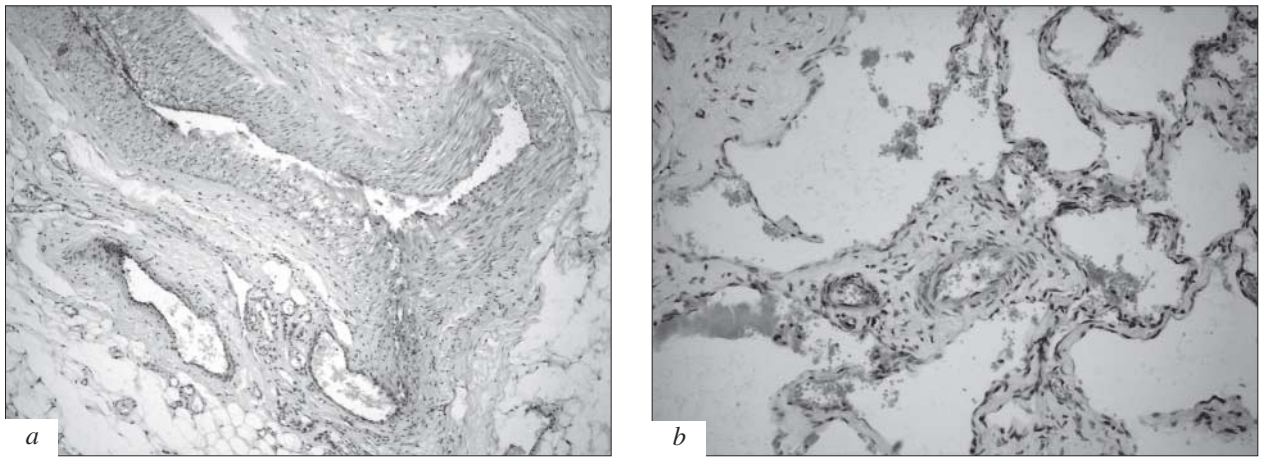

Fig. 1. Histological characteristics of arteriovenous (*a*) and venous (*b*) malformations. Hematoxylin and eosin staining, ×100 (*a*), ×200 (*b*).

ber of close contacts between endotheliocytes in angiodysplasias is usually paralleled by an increase in the number of gap junctions. Gap junctions between endotheliocytes in dysplastic vessels in these cases are much wider than in normal cerebral vessels [11]. Our analysis of arteriovenous and venous angiodysplasias revealed few tight junctions between endotheliocytes. The number of tight junc-

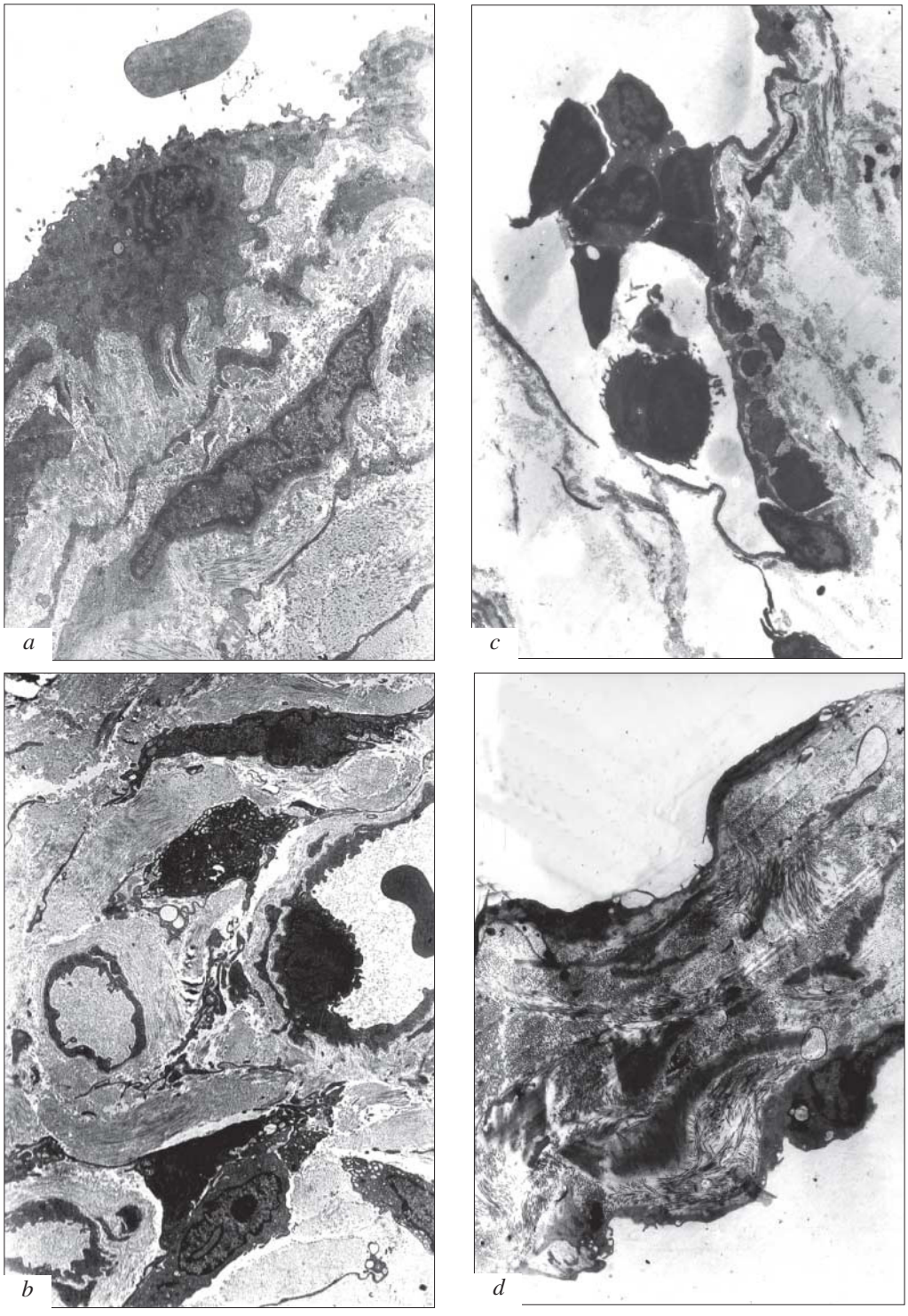

Fig. 2. Ultrastructural characteristics of venous malformations. *a*) round endotheliocyte with numerous processes; part of processes are directed towards the basal membrane, ×6500; *b*) round functionally active endotheliocyte lining the wall of a small vessel, ×3500; *c*) fragment of a large cavern wall with impaired endothelial lining and erythrocyte extravasation, ×4200; *d*) fragment of thick vascular wall of a large cavern with numerous mature collagen fibers and fibroblasts, ×4000.

tions in arteriovenous angiodysplasias was somewhat lower, which presumably explains more active growth and greater liability of these formations to relapsing.

One more manifestation of disorders in the vasculogenesis and angiogenesis processes of different nature is imbalance of the so-called accessory components of the vascular wall, seen from a lesser count or complete absence of pericytes in its structure [5]. Shortage of these cells, whose function is to stabilize the structure of the vascular wall and, presumably, phagocytosis, is essential for the pathogenesis of some angiodysplasias [9]. No pericytes were detected in the walls of the cavernous

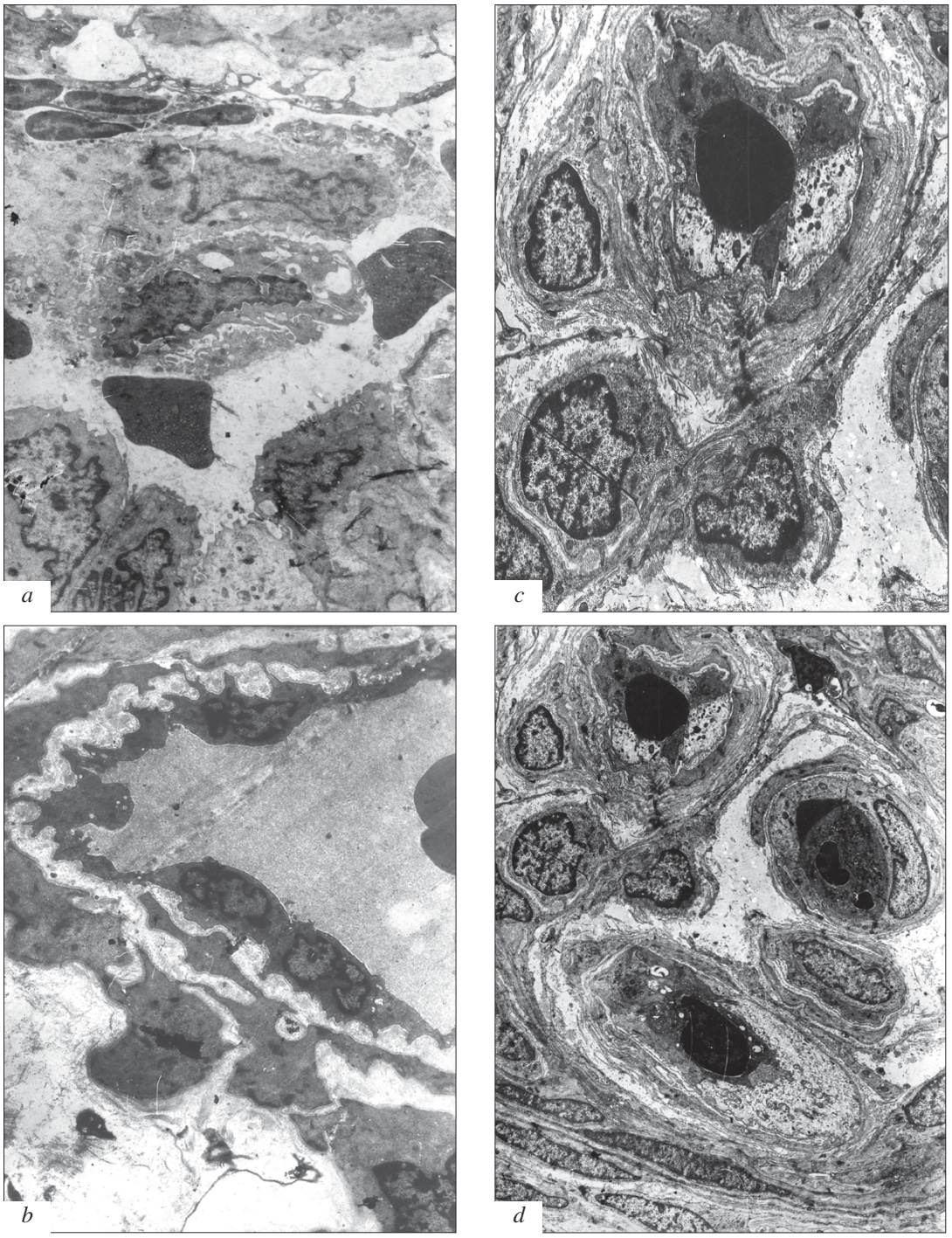

Fig. 3. Ultrastructural characteristics of arteriovenous malformations. *a*) round functionally active endotheliocytes, lining small arteries and veins, ×7000; *b*) solitary close contacts between endotheliocytes, ×6000; *c*) thickened and multilamellar basal membrane, ×3500; *d*) solitary pericytes in vascular walls of small arteries and veins, ×3500.

malformations in CNS [4]. We detected solitary pericytes in the vascular walls of arteriovenous and venous angiodysplasias; these cells were completely absent in some vessels of arteriovenous malformations.

A characteristic ultrastructural sign of the cerebral VM is impaired structure of the basal membrane of the vascular wall [4,12], which is multilamellar, of uneven thickness, with deposition of hemosiderin granules. Partial or complete absence of the basal membrane in the vascular walls of the cerebral arteriovenous and cavernous VM has been described [10,11]. Our studies of the peripheral angiodysplasias also showed gaps and locally thickened sites of the basal membrane in large caverns and arteries and virtually complete absence of the basal membrane in some venous malformation caverns. The basal membranes of small and medium-sized capillaries looked normal.

According to a previous study [3], cultured endotheliocytes, isolated from cerebral cavernous angiodysplasias, formed three populations: cells of common shape and size, large spindle cells, and large round cells. Solitary large round endotheliocytes were detected by electron microscopy of cavernous malformations of the brain [12].

We found that small and medium-sized veins and caverns in venous angiodysplasias and small and medium-sized veins and arteries in arteriovenous VM were lined by round and irregularly-shaped endotheliocytes with numerous cytoplasmic processes of medium and great lengths. The majority of endotheliocytes lining small vessels and some cells lining medium-sized vessels were functionally active. The nuclei of these endotheliocytes were oval, with irregularly shaped nuclear membrane. Endothelial cells lining the caverns, large veins and arteries had typical (flat) shape with few short cytoplasmic processes, and were predominantly functionally inert.

On the whole, the walls of medium-sized and large vessels in arteriovenous and venous angiodysplasias consisted of mature collagen fibers with fibroblasts between them. The walls were considerably thicker in large caverns, mainly at the expense of mature collagen fibers and fibroblasts, which were more often oval and spindle-shaped and rarely irregularly-shaped.

Hence, the detected ultrastructural features of the peripheral angiodysplasias reflect the differences in their morphogenesis and can be used for the differential diagnosis of VM and hemangiomas.

REFERENCES

1. V. N. Dan and S. V. Sapelkin, *Angiodysplasias (Congenital Vascular Malformations)* [in Russian], Moscow (2008).
2. O. D. Mishnyov, K. A. Pavlov, E. A. Dubova, and A. I. Shchyogolev, *Angiodysplasias (Vascular Malformations)* [in Russian], Moscow (2008).
3. N. I. Baev and I. A. Awad, *Stroke*, **29**, No. 11, 2426-2434 (1998).
4. R. E. Clatterbuck, C. G. Eberhart, B. J. Crain, and D. Rigamonti, *J. Neurol. Neurosurg. Psychiatry*, **71**, No. 2, 188-192 (2001).
5. J. Folkman and P. A. D'Amore, *Cell*, **87**, No. 7, 1153-1155 (1996).
6. M. C. Garzon, T. J. Huang, O. Enjolras, and I. J. Frieden, *J. Am. Acad. Dermatol.*, **56**, No. 3, 353-370 (2007).
7. A. Peters, S. L. Palay, and H. D. Webster, *The Fine Structure of the Nervous System: Neurons and Their Supporting Cells*, New York (1991), pp. 344-355.
8. C. Suri, P. F. Jones, S. Patan, *et al.*, *Cell*, **87**, No. 7, 1171-1180 (1996).
9. J. C. Tille and M. S. Pepper, *Arterioscler. Thromb. Vasc. Biol.*, **24**, No. 9, 1578-1590 (2004).
10. J. Tu, M. A. Stoodley, M. K. Morgan, and K. P. Storer, *J. Neurosurg.*, **103**, No. 5, 903-909 (2005).
11. J. Tu, M. A. Stoodley, M. K. Morgan, and K. P. Storer, *Neurosurgery*, **58**, No. 5, 961-970 (2006).
12. J. H. Wong, I. A. Awad, and J. H. Kim, *Ibid.*, **46**, No. 6, 1454-1459 (2000).