ULTRASTRUCTURE OF THE VEINS IN *Rana temporaria*

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It has been shown by histochemical methods that the frog's veins vary widely in the extent of their supply with adrenergic fibers [1]. Besides such richly innervated veins as the abdominal vein and posterior vena cava, there are others which have no adrenergic innervation. Comparison of the ultrastructures of the innervated and noninnervated veins in order to discover any differences between them was the object of the investigation described below. No data could be found in the literature on electron-microscopic investigation of the veins in amphibians.

EXPERIMENTAL METHOD

Autumn and winter specimens of *Rana temporaria* were used. Segments of the veins were fixed with 3.5% glutaraldehyde solution in phosphate buffer (0.1 M, pH 7.4). A 1% solution of OsO_4 was used for postfixation. Ultrathin sections were stained on the grids by Reynold's method [6]. The sections were studied in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

The ultrastructure of the frog's abdominal, pulmonary, and femoral veins was studied.

The intima of all the veins studied consisted of endothelial cells and an inner elastic membrane (Fig. 1a). The endothelial cells in different veins differed in shape and size: In the abdominal vein they were cubical in shape and 0.3-2.2 μ in diameter, in the pulmonary vein they were circular and 0.6 μ in diameter; in the femoral vein they were flat and 0.1-0.7 μ in diameter. The nuclei occupied a central position in the endotheliocytes or were shifted into the apical part of the cells. The cytoplasmic membrane was periodically invaginated into the cytoplasm, to form micropinocytotic vesicles 50-100 nm in diameter. The endotheliocytes of all the veins were characterized by the presence of a large number of intracellular vesicles, the dimensions of which corresponded to those of the pinocytotic vesicles. High micropinocytotic activity of the endothelium is considered to indicate the mobility of its cell surface [3], whereas the presence of numerous intracellular vesicles is evidence of intensified transport of materials into the cell [7]. Channels and cisterns of the smooth and rough endoplasmic reticulum run between the vesicles, where ribosomes, most frequently grouped closely together, also are to be found. There are few mitochondria, round or oval in shape depending on the plane of section, and the cristae in them are arranged parallel or at a small angle to one another. Numerous cytoplasmic granules surrounded by elementary membranes, 50-200 nm in diameter, can be observed. The contents of the granules are distinguished by their high electron-optical density. The cytoplasm of all endothelial cells contains filaments. Sometimes the endotheliocytes form junctions of desmosome type, but more frequently they are joined together by gap junctions. The width of the gap between the cytoplasmic membranes of adjacent cells does not exceed 18 nm. From the basal part of the cells many thin cytoplasmic processes are given off, in contact with one another and with processes of neighboring cells. Often the processes perforate the internal elastic membrane and reach the bodies and processes of myocytes to form intermediate contacts with them. In the femoral vein sometimes the cytoplasmic membranes come close together to form five-layered nexus structures (Fig. 1b).

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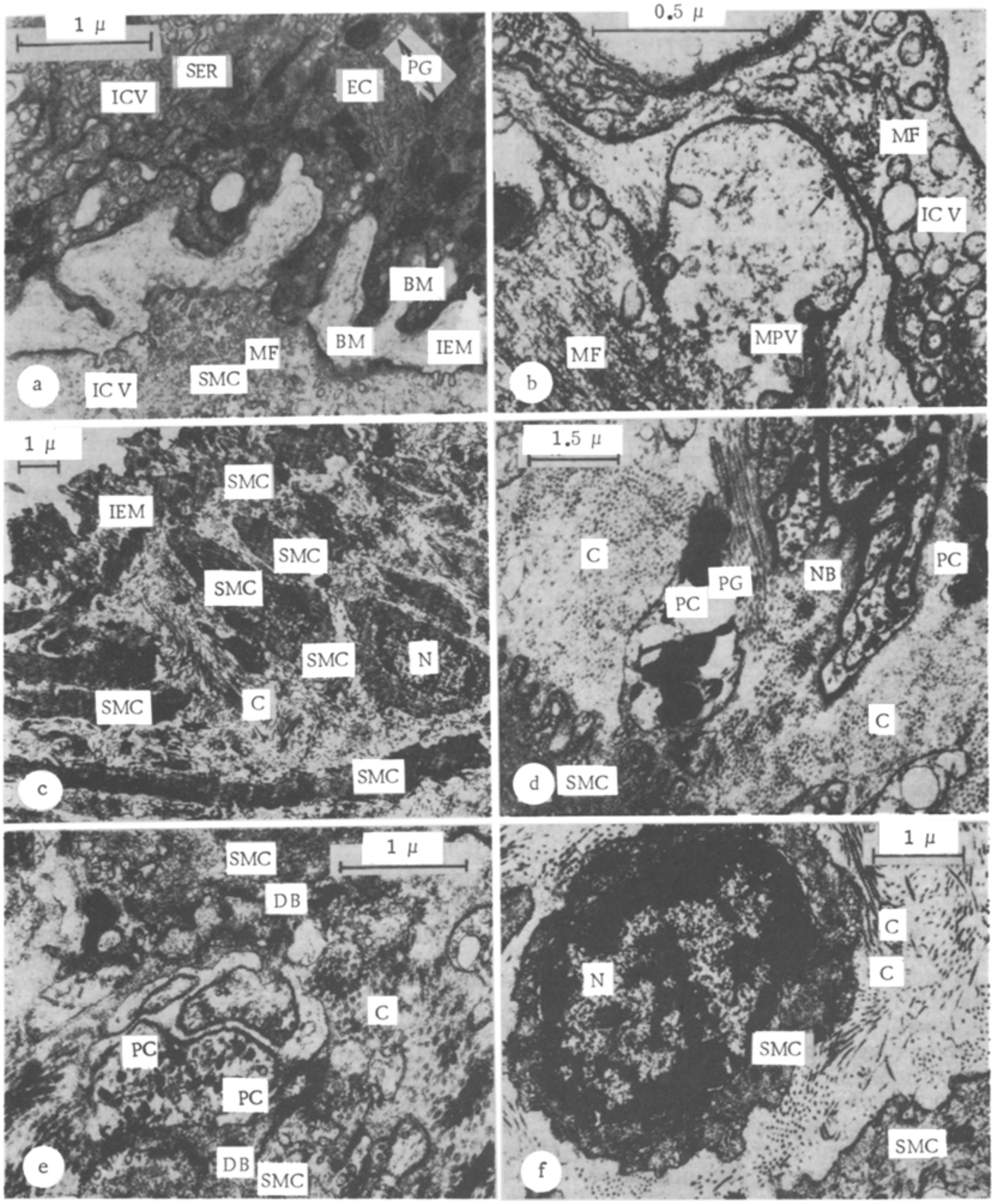

Fig. 1. Ultrastructure of frog's veins: a) intima of pulmonary vein (22,500 ×), b) myoepithelial junction in intima of femoral vein (60,000 ×), c) area of wall of abdominal vein (5250 ×), d) adventitia of abdominal vein (15,000 ×), e) neuromuscular junction in abdominal vein (22,500 ×), f) giant myocyte in adventitia of pulmonary vein (15,000 ×), BM) basement membrane, IEM) inner elastic membrane, ICV) intracellular vesicles, SMC) smooth-muscle cell, SER) smooth endoplasmic reticulum, GV) granular vesicles, C) collagen, MPV) micropinocytotic vesicles, MF) myofilaments, MCF) microfilaments, M) mitochondria, NB) nerve bundle, PSMC) process of smooth-muscle cell, DB) dense bodies, PG) pigment granules, PC) pigment cell, EC) endothelial cell, N) nucleus. [Omission as in Russian original — Consultants Bureau.]

The inner elastic membrane, which follows the endothelium, consists of thin filaments running both from the basement membranes of the myocytes and from the endothelium, and of collagen fibers and a few elastic plates. The thickness of the inner elastic membrane differs in different parts of the cross section of the vessel and varies in the abdominal vein from 70 to 500 nm, in the pulmonary vein from 200 to 500 nm, and in the femoral vein from 600 to 3000 nm.

Media. In all veins the myocytes are always mononuclear and surrounded by a basement membrane; their nuclei occupy a central position. The cytoplasm of the smooth-muscle cells can be divided into two parts: central, where most of the organelles are located in the perinuclear zone, and peripheral, filled with contractile structures. Groups of mitochondria, round, oval, elongated, or coiled in shape and 100-200 nm in diameter, are concentrated in the perinuclear zone. The mitochondrial cristae are separated by spaces filled with material of varied electron-optical density, with osmiophilic granules embedded in it. In the peripheral part of the cell there are few mitochondria and sometimes giant, curiously-shaped mitochondria may be seen. The mitochondrial membranes are closely linked with channels of the smooth endoplasmic reticulum, and the latter is linked with membranes of the intracellular vesicles and micropinocytotic vesicles. In the perinuclear zone channels and cisterns of the smooth and rough endoplasmic reticulum pursue their course, and sacs and vesicles of the lamellar complex and free and grouped ribosomes are found. The peripheral part of the cytoplasm is filled with contractile filaments. By the fixation method used, mainly thin (actin) filaments are revealed, with only a few myosin filaments, possibly because of the difficulty of their detection [4]. Dense bodies, located mainly along the plasmalemma, and measuring 25-50 nm are closely connected with the myofilaments. The myolemma, because of invagination into the cytoplasm, forms numerous micropinocytotic vesicles or primary vesicles, from which secondary vesicles, connected to the primary by thin bridges, radiate into the depth of the cytoplasm. Invaginations of the myolemma lead to the formation of numerous thin, short cytoplasmic processes. Processes of one smooth-muscle cell form junctions with processes or invaginations of the myolemma of another cell. The length of the junctions is 1-10 nm and the mean width of the gap between the membranes is 50 nm. No muscle junctions of nexus type could be observed. Whereas no distinguishing features of any particular vein could be found in the ultrastructure of the smooth-muscle cells of all the veins studied, the mutual arrangement of the myocytes differed in the different veins. For instance, the muscular coat of the abdominal and pulmonary veins consists of three layers of smooth-muscle cells whose orientation relative to the long axis of the vessel differs: The first (inner) layer consists of obliquely arranged myocytes, whereas the second and third layers consist of cells oriented to it at a large angle (Fig. 1c). Only two layers of obliquely oriented smooth-muscle cells are present in the femoral vein. The myocytes of the tunica media of the veins are separated from one another by large spaces filled with extracellular material, consisting of intricately interwoven collagen fibers and a few elastic fibers.

Adventitia. The muscular coat gradually gives way to the adventitial coat, consisting of bundles of circular and longitudinal collagen fibers, thin elastic fibers, pigment cells, fibroblasts, occasional chromaffin and mast cells, lemmocytes, and single smooth-muscle cells scattered throughout the adventitia (Fig. 1d). In the pulmonary vein, unlike the abdominal and femoral veins, pigment cells are found also in the muscular coat among the myocytes. A special feature of the adventitia of the pulmonary vein is that it contains giant myocytes, arranged parallel to the long axis of the vessel; their diameter is 3 times greater than that of the myocytes of the media (Fig. 1f). Of the three veins studied, unmyelinated nerve bundles and single axons close to the outer layer of smooth-muscle cells were found only in the abdominal vein. In the pulmonary vein large nerve bundles ran a considerable distance from the muscle cells. In the femoral vein, no nerve fibers could be seen. These observations confirm the results of histochemical investigations [1]. Just as in mammals, the axoplasm of the nerve fibers contains neurofilaments, a few microtubules, mitochondria, and glycogen granules. Material with high electron-optical density occupies a mainly central position in granular vesicles 30-100 nm in diameter; the diameter of the agranular vesicles is 40-90 nm. Sometimes varicose expansions were observed in the femoral vein between the second and third layers of smooth-muscle cells. The minimal distance between the outer membranes of such a varicose expansion and the myocyte was 50 nm (Fig. 1e). These tight neuromuscular junctions were not a frequent phenomenon in the frog vein, although they are characteristic of certain veins in mammals [2]. Adrenergic nerves in the abdominal vein also run to pigment cells of the adventitia. As a rule these are small bundles consisting of four or five axons, accompanied by a lemmocyte; the distance between the outer cytoplasmic membranes of the pigment cell and the axon cross section does not exceed 50 nm.

Analysis of the data thus showed that all the veins studied have a common structural plan and the same ultrastructure of the tissue components of the vessel wall. Veins in frogs have a simpler histological structure than those in mammals. For instance, in the intima there is no subendothelial layer of longitudinal smooth-muscle cells, although in the adventitia of the pulmonary vein single giant myocytes appear.

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