Department of Forensic Medicine, University Medical School, Budapest
(Director: Prof. S. Ökrös)

THE ULTRASTRUCTURE OF MYOCARDIAL CAPILLARIES ON THE BASIS OF ELECTRONMICROSCOPIC HISTOLOGICAL STUDIES*

L. Takácsy

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In recent years there have been numerous reports on the electronmicroscopic histology of capillaries. For example, Ekholm [3] has described the capillaries of the thyroid, Groodt, Lagasse, Lebruyns [4] those of the ovaries, and Morato, Fereira [8] those of various viscera.

The capillaries of the mammalian and human heart have been discussed in detail by Kisch [6], who in agreement with Moore, Ruska [9], Edwards [2] emphasizes that although research has answered many problems, there still remain numerous unsolved questions as regards the ultrastructure of heart capillaries.

Their data in the literature have induced us to study the electronmicroscopic histology of the capillaries of the heart.

Methods

Mature albino rats specimens were immediately excised from their heart and were killed by decapitation. The specimens were fixed in 1 per cent, buffered, pH 7.2, isotonic osmium tetroxide solution, embedded in butyl-methyl-methacrylate, cut by means of a glass knife in a Jung ultramicrotome, and the pictures were photographed on Agfa plates in an electromagnetic electronmicroscope of the 1957 WF (Werk für Fernmeldwesen) type.

Results

Several capillaries were detected in the muscle layer under the external membrane of the left ventricle of the rat's heart.

In the electronmicroscopic picture the capillaries appeared as round, or more or less elongated loop-like structures (Figs. 1, 2, 3, 4).

The shape of the capillaries is known to depend on many factors, first of all upon the following ones.

a) Depending on their blood content, the capillaries appear as round, oval or elongated loop-like structures. When empty, detailed analysis is

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required to show that the many continuous membranes are actually parts of a collapsed capillary. This view is shared also by KISCH [6]. To identify the structures, the various layers must be followed accurately with a careful scrutiny of the cytoplasm.

b) The shape of the capillaries depends also on the angle of section. The nearer it is to the transversal, the more round or oval the capillary will appear. If the cut is transversal and the capillary was filled with usually an endothelial cell bulging into the lumen (Figs. 5, 6). Sometimes a perithelial cell cap may also occur on the capillary wall (Fig. 8).

The space relation of capillaries and muscle fibres had been a controversial issue in histology. Before the advent of electronmicroscopy numerous authors thought that capillaries were penetrating into the muscle fibres, in between the myofibrils. Electronmicroscopy has finally decided the question. In agreement with other authors [6, 9], I, too, found clear-cut evidence that the capillary never passes through the sarcolemma of the muscle fibre, nor does it penetrate in between the myofibrils; there is invariably some clearly outlined sarcolemma between the capillary and the muscle fibre (Figs. 1, 2, 3, 4, 5, 8).

Endothelial cells constitute the most important component of the capillaries. It is known that the capillary wall is formed by closely packed, spirally distributed [1, 7] endothelial cells and by the perithelial cells around them, together with their cytoplasm. The finer structure of the capillary wall is composed of the nucleus and cytoplasm of the endothelial cell, and the various structures in the cytoplasm.

The nucleus of the epithelial cell is big, bulges into the capillary lumen and contains osmophile granula. At the border of the nucleus the double contours of the nuclear membrane composed of parallel layers is clearly visible. According to HAM [5] the distance between the two layers is 200 Å and the membrane as a whole is 400 Å thick. Irregularly distributed pores are visible in the nuclear membrane (Fig. 6).

Around the nuclear membrane the cytoplasm is basket- or cistern-wise widened [6], with usually many plasmosomes in the basket (Figs. 6, 7). This is so much the rule that such a widening of the cytoplasm will tell the expert (even though no nucleus may be visible) that the cut has been made near the nucleus. According to KISCH [6] the endothelial cell has a high metabolism; this is made most probable by the size of the nucleus, its marked osmophile granulation and the numerous plasmosomes around it.

The cytoplasm of the endothelial cell is usually delineated by a straight, sharp, double-contoured external membrane (Figs. 5, 6, 7, 8). According to data in the literature [9, 5, 6] the thickness of this cell membrane varies from 70 to 300 Å. It is very thin in the lungs, thicker in the heart and still thicker in the kidney. The changes in thickness from organ to organ have been ascribed

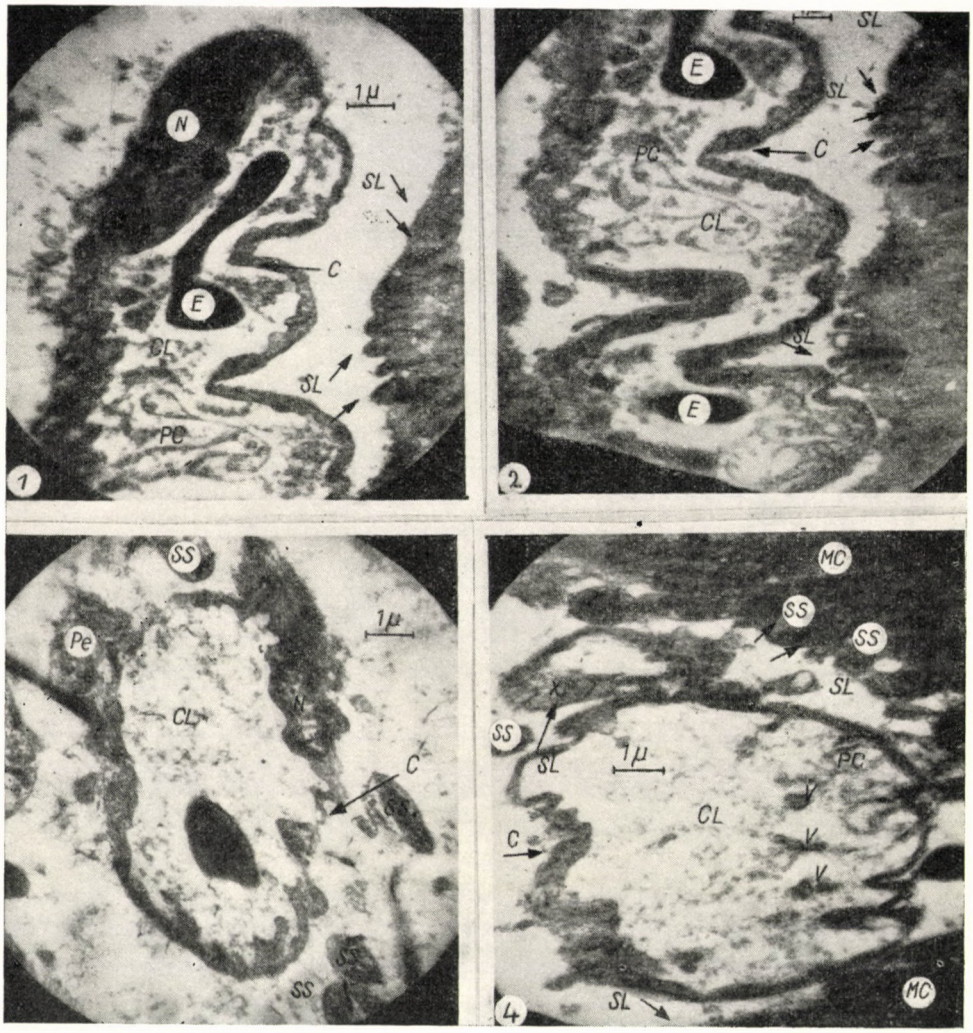

Fig. 1. Typical oval capillary (C). The nucleus (N) of an endothelial cell and in the capillary lumen (CL), part of an erythrocyte (E) and plasma coagulates (PC) are visible. Right to the capillary the marked sarcolemma of the muscle fibre (SL) is seen in its entire length. Magnification ×6000

Fig. 2. Another part of the capillary (C) (shown in Fig. 1). In the lumen (CL) two parts of erythrocytes (E), one above and one below, surrounded by many plasma coagula (PC) are visible. Below on the right, the marked sarcolemma is visible next to the capillary (SL). Magnification ×6000

Fig. 3. Capillary with a roundish lumen (C). In the lumen part of an erythrocyte (E), the nucleus of an endothelial cell (N) and a percyte (Pe). In the vicinity of the capillary many sarcosomes (SS) can be seen. Magnification ×6000

Fig. 4. Round capillary (C). In the lumen (CL) many blood plasma coagulates (PC) and cross-sections of villi (V). The structure marked X may be considered to be another part of the contorted capillary. SL: sarcolemma, MC: myocardial tissue, SS: sarcosomes. Magnification ×6000

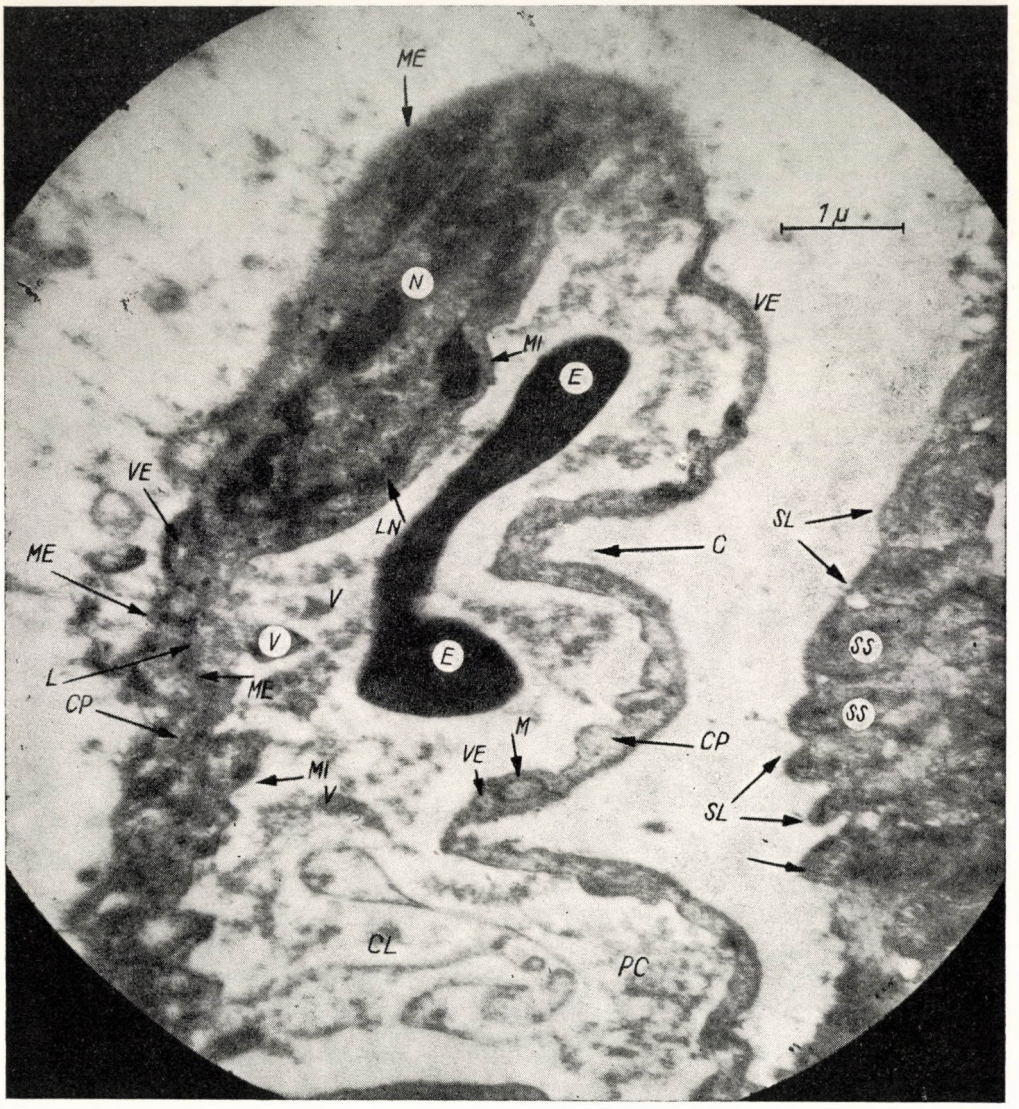

Fig. 5. Part of a capillary (C) of elongated shape. The ultrastructure of the nucleus (N) of the endothelial cell, of the erythrocyte (E) and of the cytoplasm (CP), mitochondria (M) and vesicles (VE) are visible, but a greater magnification would be needed to visualize finer details. In the cytoplasm (CP) on the left several marked lamellae (L); these are similar to the membrana interna (MI) and externa (ME), and apparently constitute parts of the cytpolasm of an endothelial cell adhering to the capillary wall. Magnification ×20 000

to differences in active physiological capillary function. For example, the comparatively great thickness of the membrane in the kidney would indicate that in this organ the membranes are suitable not only for carrying out physicochemical functions, but together with the cytoplasm they would also perform

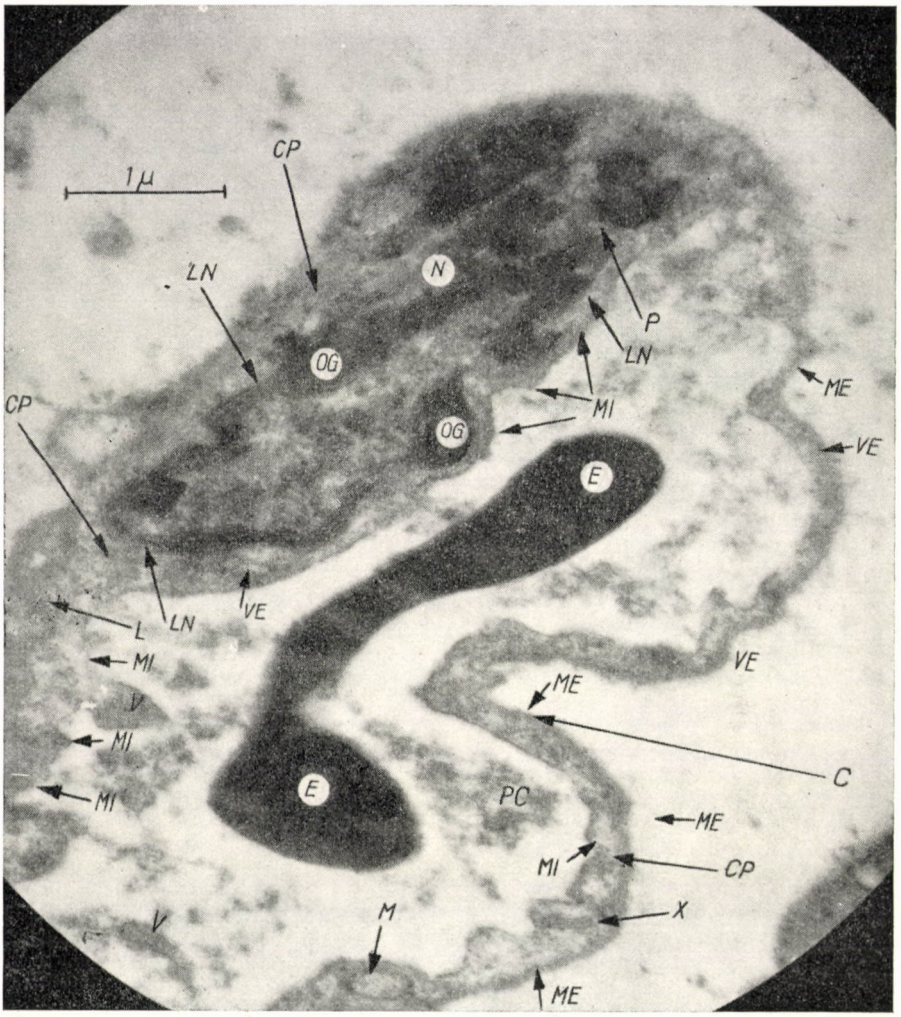

Fig. 6. Same as Fig. 5, at a magnification of 25 000. The nucleus of the endothelial cell (N), the nuclear membrane (LN), the pore (P) in it (indistinct), and the marked osmophile granulation are better visualized. C: capillary. E: erythrocyte taking the shape of the capillary, showing characteristic structure and containing neither nucleus nor plasmosome. PC: blood plasma coagulates. V: villi. CP: cytoplasm. ME: membrana externa. MI: membrana interna. M: mitochondria. VE: vesicles.

active selection and transportation. This applies in some measure to the capillaries of the heart muscle, too.

The cytoplasm is separated from the lumen by a notched internal membrane, usually 100 to 150 Å thick. The indented, wavy internal membrane is clearly visible in Figs. 5, 6, 7 and 8. In agreement with other authors [6, 9] I, too, have found the villi-like processes of the internal membrane (Figs

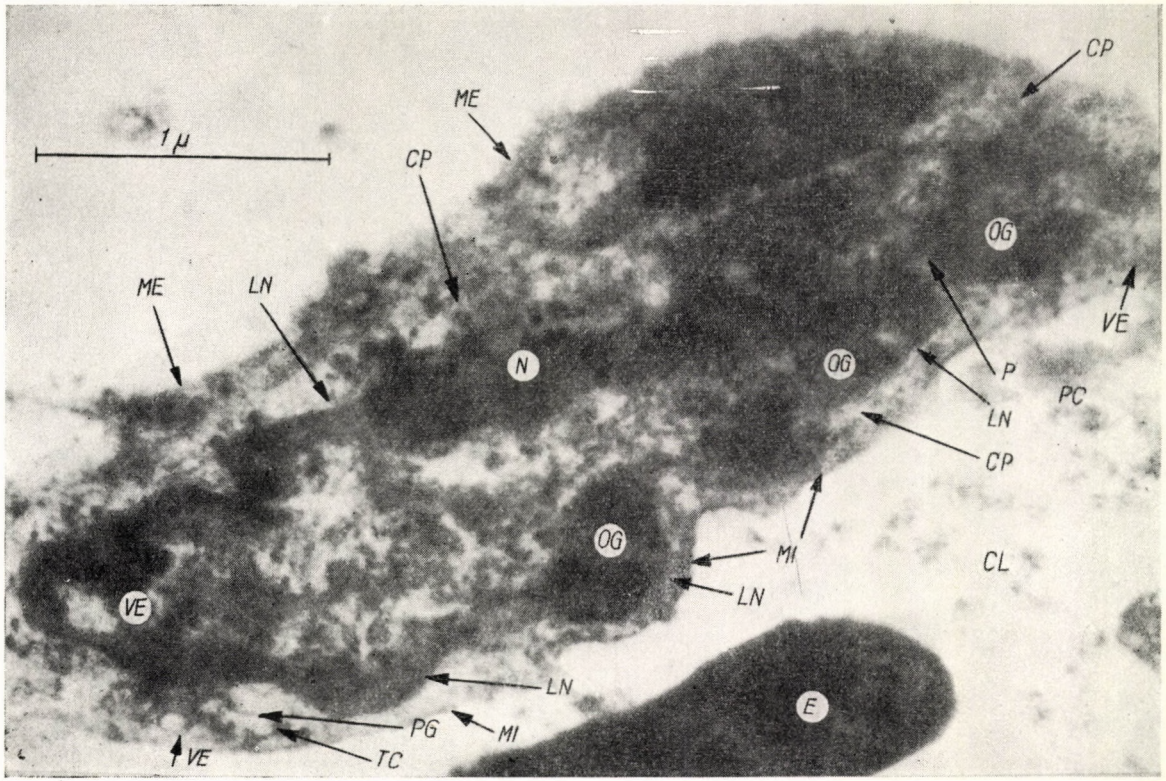

Fig. 7. Nucleus of an endothelial cell (N). OG: osmophilic granulation. LN: nuclear membrane. P: pore. MI: membrana interna. ME: membrana externa. CP: cytoplasm. VE: vesicles. TC: cross sections of tubules. PG: Palade granulation not characteristic in the cytoplasmic basket (CP) under the nucleus, on the left and next to the membrana interna. CL: capillary lumen. E: part of an erythrocyte, surrounded by blood plasma coagulates (PC). Magnification ×50 000

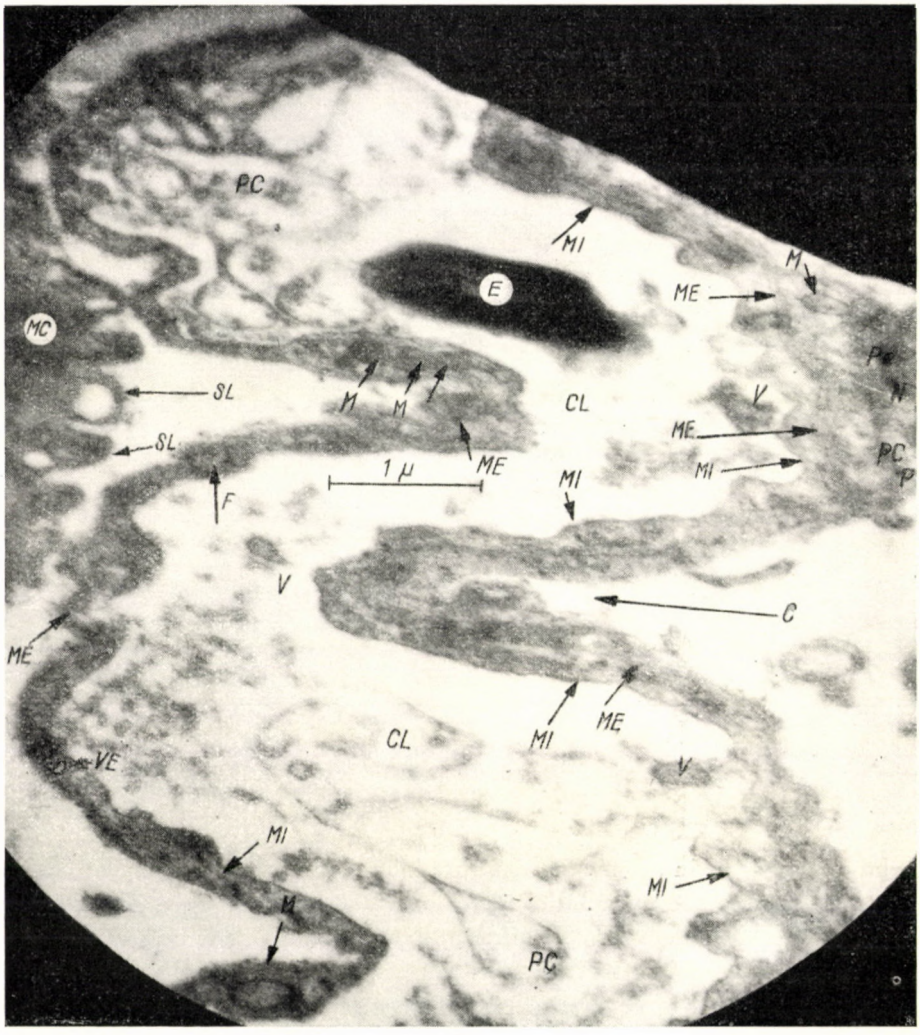

Fig. 8. Cytoplasm (CP). ME: membrana externa. Pe: pericyte, with a mitochondrium (M) in its cytoplasm and near it the vague shape of a nucleus (N)

The cytoplasm of the pericyte (PCP) ensheathes the endothelial cell. In the right wall of the capillary (C) the components of the cytoplasm separated sharply by double lamellae but closely packed can be followed. In the cytoplasma mitochondrium (M), cross sections of several cavities and vesicles (VE), while in the lumen part of an erythrocyte (E) and several blood plasma coagulates (PC) are visible. F marks the site of a mural window. Sections of villi in the capillary lumen (CL). MI: membrana interna. V: villi. SL: sarcolemma. MC: myocardium. Magnification ×25 000

4, 6, 8). KISCH [6] and KROGH [7] have suggested the attractive hypothesis that the wavy course of the internal membrane would serve to increase the size of the inner surface and that the processes protruding into the blood stream

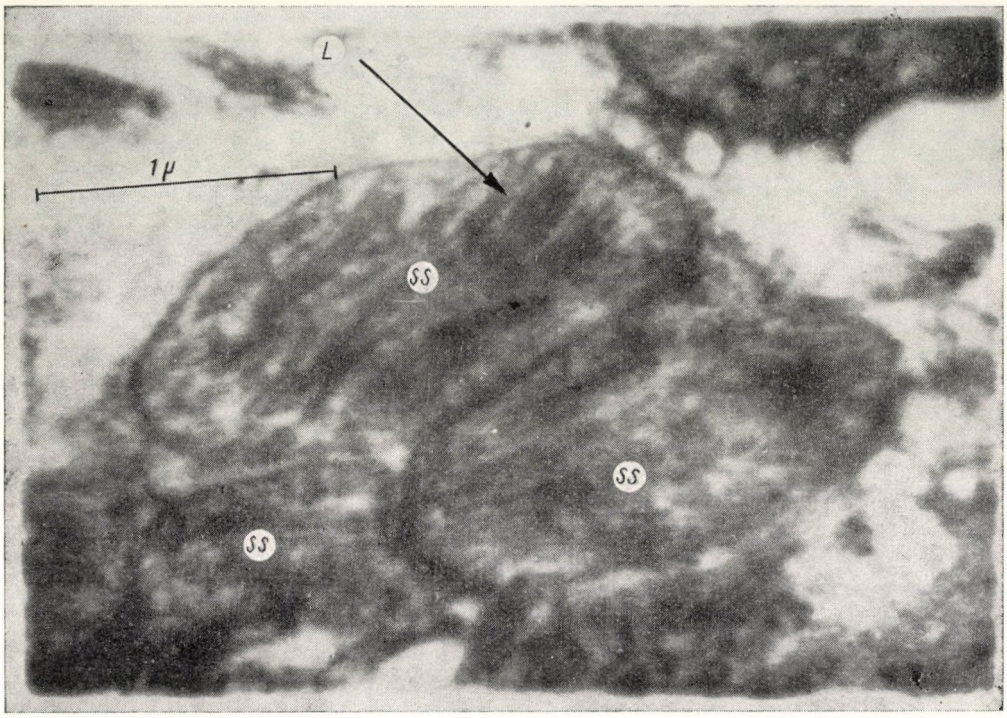

Fig. 9. Sarcosome (SS) with parallel or slightly concentrical osmophile lamellae of characteristic shape and distribution. Magnification ×50 000

may have some role in the mechanism of blood clotting slowing down the rate of blood flow near the vascular wall.

The cytoplasm between the inner and outer layers may be 3 or 4 microns to 300 or 400 Å wide. Many structures can be found in this considerable mass, especially perinuclearly or in the pouch near the nucleus, but also in other areas. Numerous mitochondria, vesicles of various sizes, tubular cavities may be seen (Figs. 5, 6, 7, 8). KISCH [6] considers them to be endoplasmic reticulum, signs of active cytoplasmic function. MOORE, RUSKA [9], PALADE [10] suggest that these vesicular structures of various sizes, but especially the transversal sections of the tubular cavities with Palade granulation on their outer surface are the histological signs of active cell function. They think that these small vesicles would be formed first on the cytoplasmic membranes, then, penetrating deeper and deeper they would merge to form bigger and bigger vesicles and would transfer their contents to the perinuclear cistern or to the nucleus. Accordingly, the tubular structures would serve transportation. Sections of such cavities are clearly visible in the cistern around the nucleus in Fig. 7; some of them, near the outer margin, show Palade granulation, not

very distinctly unfortunately. The oval structure marked by X in Fig. 6 is thought to be a compressed cross section of such a cavity, a structure I have never seen demonstrated.

The lamellae in the cytoplasm should also be dealt with. According to KISCH [6], they are cytoplasmic membranes pressed against each other due to the spiral distribution of endothelial cells. If we see many lamellae side by side, the presence of a pericyte should always be suspected. This is the case in Fig. 8, in which the several parallel lamellae are components of the capillary wall and the pericyte on it. Similar lamellae are visible in Fig. 5, in the cytoplasm under the nucleus. These may be lamellae of the cytoplasm of an adjacent endothelial cell, or, which is less probable, part of a pericyte. Such a side-by-side occurrence of the lamellae representing the cell borders has been mentioned also by MOORE and RUSKA [9]. A transverse, or zigzag development of lamellae is visible in the cytoplasm, in the area of the so-called mural spaces or windows. Between the cell membranes lying close to one another and through these spaces the vascular lumen communicates freely with the pericapillary space. Such a window can be seen in Fig. 8. In agreement with KISCH [6] we think that these spaces serve as gateways, ducts for the cells and fluids passing in and out of the lumen. Thus, in the case of diapedesis the erythrocytes and leucocytes would leave the capillary not through the capillary wall, but by way of these ducts. Such ducts have been described also by MOORE and RUSKA [9].

In proportion to their blood content, the capillary lumens contain corpuscular blood elements (Figs. 5, 6, 7), coagulated blood plasma (Figs. 5, 6, 7, 8), as well as parts of the inner membrane villi (Fig. 8). The cells in the lumen (Fig. 8) are definitely erythrocytes; they are typical in appearance, and possess neither a nucleus nor plasmosomes. The circumstance that the erythrocytes assume the shape of the capillary suggests that the inner membrane of the capillary may closely embrace them. — There is, however, a wide interspace between the erythrocyte and the capillary wall in the pictures. This might be an artefact resulting from shrinkage, inevitable with fixation. The close connexion between the erythrocyte membrane and the capillary wall may play an important role ir giving off oxygen.

Finally, Fig. 9 shows sarcosomes alongside the capillary, within the distinctly visible sarcolemma. They possess long vertical rows of oval, parallel, and at sites slightly concentric, osmophile lamellae. According to the extensive studies of KISCH [6], sarcosomes occur in great numbers, in long rows in the heart muscle fibres, in the interspace between sarcolemma and myofibrils. They contain metabolistic enzymes important in heart function. Their presence in great numbers may be explained by the constant, extreme physiological activity of the heart; there are less of them in the skeletal muscles. Changes in the number of lamellae and in the intensity of granulation would signify different phases of enzyme function.

Discussion

I have undertaken to describe the ultrastructure of mammalian capillaries in order to compare normal and pathological conditions in subsequent studies.

Many capillaries are found in the muscle layer of the left ventricle, under the pericardium.

By the method described electronmicroscopic pictures of adequate thinness and giving satisfactory contrast have been obtained, in which the fine ultrastructure of heart capillaries could be studied and illustrated, in accordance with data in the literature.

The evidence obtained supports the opinion of numerous authors [6, 9, 3, 10] in that the capillaries of the heart cannot be considered to be a system of semipermeable membranes depending on physico-chemical effects, but we should think of the capillary wall and its structures as constituting a living cytoplasmic system of complex structure and of mostly unknown function, the actual condition of which has always a decisive influence upon the function and metabolism of the capillaries and through them on the organism as a whole. This view is borne out by the electronmicroscopic findings in pathological conditions of the capillaries.

Summary

The electronmicroscopic ultrastructure of the capillaries of the normal mammalian (rat) heart has been studied.

Many capillaries were found to be present in the subpericardial muscle layer of the left ventricle.

Depending on their blood content and the plane of section, the capillaries are round or oval in shape. Capillaries were never found to penetrate through the sarcolemma of the muscle fibre and never extended in between myofibrils.

The most important buildstones of the capillaries are the peculiar macronuclear endothelial cells showing strong osmophile granulation and possessing a characteristic cell membrane with a double contour. Many vesicles, sections of cavities are visible in their cytoplasm. On the outer surface of the cytoplasm there is the double line of the external membrane and at the surface facing the lumen there is the notched internal membrane. The finer ultrastructure of the capillary is composed of the cytoplasm, with the endoplasmic reticulum in it, as well as the inner and outer membranes, with the windows and mural interspaces.

Adjacent to the capillaries and inside the sarcolemma many sarcosomes can be found in the heart muscle.

Like those of other organs, the capillaries of the heart are a living cytoplasmic system of complex structure, the actual condition of which has an active effect on the metabolism of the capillary and the organism as a whole. This influence is not merely one of physico-chemical nature.

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УЛЬТРАСТРУКТУРА КАПИЛЛЯРОВ СЕРДЕЧНОЙ МЫШЦЫ НА ОСНОВЕ ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОГО ГИСТОЛОГИЧЕСКОГО ИССЛЕДОВАНИЯ

Л. ТАКАЧИ

Настоящая статья посвящена электронномикроскопическому исследованию ультраструктуры капилляров сердечной мышцы здоровых млекопитающих (крыс).

В мышечном слое под наружной оболочкой левого желудочка можно обнаружить много капилляров.

Форма капилляров оказалась в зависимости от их наполнения кровью или же от плоскости среза круглой, или более или менее овальной. Капилляры никогда не проникали через сарколемму мышечного волокна и не распространялись среди миофибрилл.

Самые важные основные элементы капилляров представляют своеобразные крупноядерные эндотелиальные клетки, обладающие, показывающей сильную осмофильную грануляцию, ядерной оболочкой с характерным двойным очертанием, и в цитоплазме которых видно много пузырьков, пересеченных полостей. На наружной поверхности цитоплазмы наблюдается наружная оболочка с двойной линией, и на поверхности в сторону просвета волнистая внутренная оболочко. Цитоплазма, находящаяся в ней эндоплазматическая сетчатка, далее внутренняя и наружная оболочка плазмы вместе с окнами и щелями стенки образуют тонкую ультраструктуру капилляров.

Наряду с капиллярами в сердечной мышце наблюдается внутри сарколеммы много саркосом.

Капилляры сердца, подобно капиллярам прочих органов, представляют из себя живую протоплазматическую систему сложной структуры, состояние которой всегда активно проявляется в обмене веществ капилляров и всего организма даже сверх физико-химических действий.

ÜBER DIE ELEKTRONENMIKROSKOPISCHE ULTRASTRUKTUR DER MYOKARDKAPILLAREN

L. TAKÁCSY

Die Ultrastruktur der Myokardkapillaren gesunder Ratten wurde elektronenmikroskopisch untersucht.

In der Muskelschicht unter der Außenhülle der linken Herzkammer befinden sich zahlreiche Kapillaren. Ihre Form ist in Abhängigkeit von dem Blutgehalt, bzw. der Schnittfläche rund, oder mehr oder weniger elliptisch. Sie durchdringen nie das Sarkolemma und gelangen nicht zwischen die Myofibrillen.

Die wichtigsten Grundelemente der Kapillaren bilden bizarre, großkernige Endothelzellen, die eine starke osmiophile Granulation zeigende charakteristische doppelkonturierte

Kernmembran aufweisen, und in deren Zytoplasma zahlreiche Bläschen, Höhlendurchschnitte zu sehen sind. Auf der äußeren Oberfläche des Zytoplasmas kann die verdoppelte Membrana externa und gegen den Lumen die wellige Membrana interna beobachtet werden. Das Zytoplasma, das endoplasmatische Retikulum, ferner die innere und äußere Plasmamembrane bilden zusammen mit den Fenstern und Wandspalten die feineren Details der Ultrastruktur der Kapillaren.

Neben den Kapillaren befinden sich innerhalb des Sarkolemmas zahlreiche Sarkosome im Herzmuskel.

Die Myokardkapillaren stellen — ähnlich den Kapillaren der anderen Organe — ein kompliziertes lebendes Protoplasmasystem dar, desen jeweiliger Zustand im Stoffwechsel der Kapilaren und des ge-amten Organismus auch über physisch-chemische Wirkungen hinaus aktiv in Erscheinung tritt.

Dr. László Takácsy, Budapest, IX. Üllői út 93. Hungary.