Morphological classifications of vertebrate blood capillaries

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Bennett, H. Stanley, John H. Luft and James C. Hampton. Morphological classifications of vertebrate blood capillaries. Am. J. Physiol. 195(2): 381-390. 1959.—Morphological features of blood capillaries from various vertebrate forms and organs are presented. Simple classifications are proposed, based on presence or absence of continuous basement membrane, on the nature of the endothelial cell and on the presence or absence of a complete investment of pericapillary cells. It is suggested that these varying capillary structural features may be relevant to problems relating to exchange of materials between blood plasma and parenchymal cells. Simple three-digit notation systems are presented for characterizing and designating the type of any capillary with respect to the classifications submitted.

The mechanisms whereby materials exchange across capillary walls have long engaged the attention of physiologists, who have recognized the importance of the structure of the capillary wall as related to function. For the past few years, the thinking of physiologists in this field has been dominated by the important work of Pappenheimer and his co-workers (1-4). Featured in these studies have been attempts to deduce structural characteristics of capillaries from measurements of rates of passage of various materials across the capillary barrier.

Meanwhile, as a result of entirely different approaches involving electron microscopy, a great deal of new information relating to the structure of capillaries has been accumulated. For the most part the physiological implications of these new morphological findings remain to be assessed. But it is already evident that the structure of blood capillaries is quite complex and varied. It is desirable to attempt a correlation of these structural features with the diverse functional characteristics recognized by physiologists who have worked on this problem (1, 4).

This paper is submitted with the full realization that the vertebrate body is as yet incompletely explored with respect to capillary structure, and that further work may necessitate modification or extension of the concepts here advanced.

Materials and Methods

The electron micrographs presented as data were prepared by methods of fixation, dehydration, embedding, sectioning and microscopy which have become routine in many cytological laboratories. The basic procedures have been reviewed by Porter and his co-workers (5, 6). The fixing fluids and methods of tissue processing have not been the same for all the micrographs presented. In some cases the 'standard' Palade (7) veronal buffered 1% osmic acid was used. The variant figuring in this study is the veronal-sucrose osmic mixture of Caulfield (8). The fixative used is indicated in the legend of each micrograph.

The section thicknesses have not been measured but are presumed to lie in the range of 300-600 Å.

The electron micrographs were taken on RCA EMU-2 or Siemens Elmiskop I electron microscopes fitted with compensated objective pole pieces and objective apertures. Efforts were made continually to achieve high resolution, but the success of these efforts varied from time to time, so that the resolutions actually attained are not uniform. In the best of the pictures the resolution appears to be better than 30 Å, and hence would be adequate for visualization of channels 60 Å in diameter as postulated by Pappenheimer, Renkin and Borrero (1).

Definitions of Capillary and of Endothelium

In this paper we apply the word, 'capillary' to any small blood vessel, including sinusoids, that serves for the major part of the exchange of materials and sub-
stances between parenchymal cells and blood plasma. When using the word 'endothelium,' we refer to the lining of any blood or lymphatic channel. A cell lining such a channel is spoken of as an endothelial cell, whether it has conspicuous phagocytic properties or not.

**Basement Membrane of Capillaries**

Figures 1 and 5, which are electron micrographs of blood capillaries from electric organs of electric fish, show that there are two structural components interposed between the blood plasma and the intercellular space. One of these components is the endothelial cell (G). The other is an extracellular connective tissue structure corresponding to the basement membrane of capillaries. This basement membrane (B) has long been recognized as an external investment surrounding the endothelium of capillaries. In the past many have thought of this basement membrane as a rather loose meshwork of fibers with large apertures between them. Hence, one might deem such a network to be unimportant from the point of view of capillary permeability. But in these capillaries one can see that, at the resolution presented, the basement membrane appears to be nearly homogeneous and to surround the capillaries completely. Holes in a meshwork are not apparent.

Since the resolution in these micrographs is acceptably good, one should not overlook the fact that the close structure of this basement membrane may have an appreciable effect on the functional characteristics of these capillaries. This is also evident in the case of the mammalian glomerular capillary (4, 9-11). In addition to capillaries displaying a continuous component of basement membrane, as shown in figures 1 and 5, there are ones which lack such a continuous investment (fig. 2). Such capillaries have been described in mammalian liver and spleen, where they are often called sinusoids or sinuses (12-14). In these capillaries most of the outer endothelial cell surface is bare of any resolved structural investment, and seems to present directly to the fluid surrounding the capillary. Thus over most of the capillary wall substances exchanging between pericapillary fluid and intracapillary blood plasma can accomplish a passage without encountering any discernible basement membrane.

One can find apposed to the outer surface of endothelial cells of spleen or liver here and there occasional strands of connective tissue material which histologists have long recognized in the form of a loose plexus of reticular or 'argyrophil' connective tissue fibers. These can be seen in electron micrographs (12) as isolated strands of extracellular material perhaps .25-5 μ in diameter. These resemble basement membrane material in texture, being almost structureless at levels of resolution usually achieved, yet showing unclear evidence of a very fine fibrillar consistency. But the spaces between these isolated strands are so vast that these structures need not be considered in relation to the permeability characteristics of these capillaries, though they may contribute to mechanical strength.

**Classification of Capillaries on Basis of Basement Membrane**

The considerations in the preceding section have led to the conclusion that there are capillaries with a continuous investment of basement membrane and, in addition, other capillaries without such a continuous investment, but fitted with a very loose open meshwork of connective tissue strands. This permits a very simple classification of capillaries, which can be presented as follows:

**Capillary type A.** Capillaries with a complete continuous investment of basement membrane.

**Capillary type B.** Capillaries without a complete continuous investment of basement membrane.

Capillaries with basement membranes (type A) have been described in skeletal muscle (15), in heart muscle (16, 17), in the nervous system (18, 19), in glomerular and tubular region of mammalian kidney (9-11, 20-22), in avian kidneys (93), in the parathyroid (24, 25), in the hypophysis (26), in the adrenal (27), in the lung (28-30).

In this paper capillaries from the electric organ (figs. 1 and 5) and from the lamina propria of mammalian intestine (figs. 3 and 4) are shown to possess basement membranes, and hence to belong to type A. In the intestine, as seen in figures 3 and 4, the basement membrane is very delicate, but can be seen within the pores perforating the endothelial cells and on the luminal surface of the endothelium, as well as on the outer surface.

Capillaries of type B have been clearly described so far only in liver and spleen of mammals (12-14). However, judging from the incomplete study of Pease (31), it is possible that the capillaries of bone marrow may also belong in this group.

As will be developed in subsequent passages of this paper, capillaries can also be classified on the basis of other characteristics.

**Structure of the Endothelium of Blood Capillaries**

Figure 2 is an electron micrograph of a cross section of a capillary or sinusoid in the liver of a rat. It is typical
FIG. 1. Capillary from electric organ of electric eel, Electrophorus electricus. This capillary is representative of type A-I-a. Capillaries of this type are also found in muscle and nervous tissue. Portions of four endothelial cells are seen, separated from each other by dense attachment belts (A) and areas of mutually contiguous but unattached endothelial cell membrane (U). Surrounding completely the endothelial cells are the irregular but almost structureless densities of basement membrane (B), which can be regarded as occupying part of the intercellular space (I). External to this basement membrane are seen the cytoplasmic projections of several cells (P). Cells belonging to this pericapillary cytoplasm are not clearly identified, and may be adjacent endothelial cells of the same capillary, or cells with some other function. Mitochondria (M) and a nucleus (N) can be recognized. Of special interest are numerous vesicles (V) in cytoplasm of the endothelial cells, and caveolae intracellularis, or caves or pockets (E) seen along inner and outer endothelial cell border. These were first recognized by Palade (15), who suggested that they might have a role in capillary function. Vesicles and caveolae are 200-600 Å in diameter. No images suggest that they are sections through continuous tunnels, but rather, they appear to represent numerous isolated spheroidal or ellipsoidal vesicles unconnected to each other by membrane-lined channels. Attachment belts (A) are dense areas of firm mechanical adhesion between cells. Thus no perforations of the capillary wall can be seen, either through or between cells. Palade's buffered osmic fixation. Biopsy without anesthesia. Mag. 21,000 X. Inset shows a portion of intercellular attachment at A. Plasma membranes of adjacent endothelial cells are seen, with an area of increased density at A'. Mag. 55,000 X.

of vertebrate liver capillaries. The borders of the endothelial cells (G) are clearly delineated. Between profiles of cell borders are large gaps (f) which permit blood plasma to have direct access to the surface of the liver parenchymal cells and the subendothelial space (of Disse) (S). These features are confirmatory of the initial findings of Fawcett (13) and of more elaborate analysis of Hampton (14). These capillaries are characterized by large intercellular gaps or fenestrations between processes of the endothelial cells. These gaps may be several thousand Angstroms across, and thus can exercise no selective filtering effect on blood plasma constituents smaller than platelets. Similar intercellular gaps have been described by Weiss (12) in splenic blood vessels. There are only occasional strands of basement membrane interposed between blood plasma and liver parenchymal cells. The capillary surfaces of the liver parenchymal cells are thus bathed directly with blood plasma and the endothelium can be regarded as being freely
permeable to cell plasma constituents, with passage occurring between the cells.

Now attention is invited to figures 3 and 4, which show a capillary from the lamina propria of the intestine of a mouse. The endothelium is seen to be perforated by numerous fenestrations or pores ($K$) 300–600 Å in diameter. In these capillaries the pores contain a delicate dense material representing the basement membrane ($B$), which, in this instance, also extends over the luminal surface of the endothelial cells. The pores penetrate completely through thin cytoplasmic sheets of endothelium and are in no sense intercellular. The intracellular fenestrations or pores are too large in themselves to exercise any selective filtration effect at the molecular level, though the finer structure of the basement membrane may well be significant in this respect.

Similar endothelial perforations have been described in the glomerular and tubular capillaries of the mammalian kidney (9–11, 20, 21). Capillaries with much less abundant perforations of comparable size have been described in parathyroid gland (25) and hypophysis (26). In these endocrine capillaries the fenestrations
FIG. 3. Portion of a capillary and pericapillary space in the lamina propria of a villus of intestine of a mouse. Capillary is of the type A-2-a. A thick portion of an endothelial cell is seen at C. In this region of cell one sees mitochondria (M) and vesicles (V). One can identify inner endothelial cell membrane (C) and outer endothelial cell membrane (D). In lower portion of capillary wall the endothelium is very much thinner and is fenestrated with numerous pores about 500 Å in diameter, separated by cytoplasmic densities measuring about 0.1-1 μ. Interfenestrual endothelial cytoplasmic structures are about 500 Å thick overall, and are surrounded completely by plasma membrane. A thin basement membrane (B) is discernible as a diffuse shadow adjoining outer endothelial cell membrane, and spanning and even filling the fenestrations (K) which perforate the endothelial cell. Portion of a pericapillary cell appears at (P). Dense black bodies in the pericapillary intercellular space (I) may represent fat which has been absorbed from the intestinal lumen. An erythrocyte is seen at R, permitting identification of this structure as a blood capillary. Fixed by intra-intestinal injection of Caulfield's buffered sucrose-osmic into an unanesthetized animal. Mag. 20,000 X.

FIG. 4. Enlarged view of lower portion of intestinal capillary shown in fig. 3. Basement membrane densities show more clearly here, and can even be identified over luminal or inner surface of endothelial cell. Mag. 46,000 X.

occupy only a small portion of the capillary surface area, so that one can suspect that the physiological functioning of these capillaries might be somewhat different from those of the mammalian kidney and intestine.

In micrographs of capillaries with endothelial fenestrations, one can identify places where one endothelial cell comes into contact with another. Near the intersection of these contact surfaces with the plasma surfaces of the cell one can find attachment belts or attachment surfaces, where the cell membrane of one cell appears to be firmly anchored to the apposed area of the cell membrane of its neighbor. Yamada (9) has figured these attachment areas, which appear to be identical in structure to the ones depicted in figure 14 and 14'. The attachment surfaces seem to surround each endothelial cell completely like a girdle or belt. Perforations or passages between the cells have not been recognized.

A third type of endothelial cell is represented in
In this type, at the resolutions achieved, the endothelium is continuous all around the capillary, without intercellular gaps or intracellular perforations tunneling through the cytoplasm. Where one endothelial cell makes contact with another, near the plasma surface one can find dense attachment belts (fig. 1A and A') similar to those described in capillaries with intracellular fenestrations. No continuous passages between plasma and pericapillary intercellular fluid can be recognized in these capillaries. It is evident that capillaries of the type shown in figure 1 are different in important respects from the fenestrated types shown in figures 2, 3 and 4. They are here spoken of as unfenestrated or unperforated capillaries, or as possessing a continuous investment of endothelium without intercellular or intracellular pores. By this it is meant that no pores perforating the endothelium from plasma to interstitial space have been recognized.
These endothelial cells do, however, show structural features which were not appreciated before such vessels were examined with the electron microscope. Very conspicuous in the cytoplasm are numerous small vesicles (V) and caveolae (E) about 200–600 Å in diameter (figs. 1 and 5). These were first reported by Palade (15) and have since been described further (16, 17, 19, 32). The vesicles are most concentrated along the inner and outer endothelial cell borders, where they often appear in section as if lined up in rows. Smaller numbers of vesicles can be encountered deeper in the cytoplasm of the endothelial cell (figs. 1 and 5). It is not our purpose here to discuss these structures in detail. Yet we wish to emphasize that the capillary endothelial vesicles do not appear to represent tunnels or pores extending through the endothelial cell, nor is there reason to believe they may represent, or may function in the manner postulated for, the hypothetical pores proposed by Pappen-
heimer, Renkin and Borrero (1). Nevertheless, Palade (16), Bennett (33, 34), Moore and Ruska (17) and others have suggested that these vesicles might have an important physiologic role in the functioning of the capillary, though the mechanism invoked is quite different from any discussed by Pappenheimer, Renkin and Borrero (1). The speculations relating to the possible physiological importance of these capillary vesicles are now under active experimental test in several laboratories, and nothing is to be gained by attempting to foreshadow here the outcome of this work.

Attention is drawn further to the portions in the micrographs which represent areas of contact between adjacent endothelial cells, each of which is united around its entire circumference to a neighboring cell by a belt comprised of a specialized attachment structure (fig. 1A and 4A). These structures appear to be barriers between capillary lumen and pericapillary space, and bonds between adjacent endothelial cells. They are to be regarded as corresponding in structure and in function to the 'terminal bars' of columnar epithelium (35), or the 'desmosomes' or 'nodes of Bizzozero' of the intercellular bridges of stratified squamous epithelium (36-39), or the interrelated discs of heart muscle (39, 40-42). They represent areas where the cell membrane is specialized, of greater density than elsewhere, and firmly attached to its opposite member. No gaps, defects, openings, pores or slits in these attachment belts have been discerned, although many hundreds have been examined. All components of the attachment belts are denser than blood plasma. Thus no evidence for channels containing plasma passing between endothelial cells has been observed, although Chambers and Zweifach (43) have suggested that preferential filtration passages might exist between endothelial cells.

Unfenestrated capillaries were first described by Palade (15). They have been reported in cardiac muscle (16, 17), in lung (28-30, 44) and in the nervous system (18, 19, 45). They are here demonstrated in mammalian skeletal muscle (fig. 6) and in electric organ (figs 1 and 5).

Classification of Capillaries on the Basis of Endothelial Cell Type

The considerations presented in this section permit us to formulate a second classification of capillaries based on the endothelial cell structure and relations. The classification is proposed as follows:

Capillary type 1. Capillaries without fenestrations or perforations (described in muscle, electric organ, central nervous system, lung, etc.).

Capillary type 2. Capillaries with intracellular fenestrations or perforations (described in mammalian kidney and intestinal villi and in some endocrine glands).

Capillary type 3. Capillaries with intercellular fenestrations or gaps (described in liver and spleen and perhaps in bone marrow).

Pericapillary Cellular Investments

In each capillary pictured in the figures accompanying this article, the parenchymal cells of the organs supplied fuse directly on the extracellular space through which the capillary tunnels. Variable percentages of capillary endothelial surface are likewise directly exposed to this extracellular space, the balance of the endothelial surface being shielded by the cytoplasm of pericapillary cells (P). The percentage thus shielded is relatively large in figures 1 and 5, small in figures 3 and 6, and indeterminate in figure 2. This designation may include several types of cells, such as overlapping endothelial cells, epithelioid fibroblasts, or 'pericytes' or 'Rouget cells,' if these latter cells really exist. In the tissues so far examined, these pericapillary cells obscure less than 50% of the endothelial surface, and there is little doubt that the sheath formed by these cells is incomplete. In such situations, we would infer that the pericapillary cells would have relatively little effect on the permeability characteristics of the capillary.

However, in the case of the glial cells of the central nervous system, a different situation may exist. Dempsey and Wislocki (18) and Luse (46) have reported that here processes of glial cells attach themselves closely to the outer surface of the capillary basement membrane. These glial cell processes were said to invest the capillary completely so that there is virtually no recognizable outer capillary surface free from this glial covering. According to the view of these authors, glial cells are interposed between capillary and neurone, and must mediate exchanges between them.

This glial cell investment has been deemed by van Breeeman and Clemente (45) and by Dempsey and Wislocki (18) to constitute the so-called 'blood-brain barrier,' for it is at the cell border of the pericapillary glial cell processes that particles containing silver or trypan blue are stopped after emerging from central nervous system capillaries.

More recently Maynard, Schultze and Pease (19) have reported their study of cerebral capillaries. Here they state that glial cells cover most—but not all—of the outer surface of the capillaries of the corpus callosum. About 15% of the outer capillary surface is judged by these authors to be bare of glial investment.

The technical aspects of these three studies are not entirely satisfying, and one is left in doubt as to the situation with respect to the completeness of the glial investment of capillaries in the central nervous system. It may be that there are regional variations in this respect, or that technical imperfections may have led one or another of the above authors to erroneous conclusions. Nevertheless, the reporting of capillaries with complete or incomplete pericapillary cellular investments interpolated between parenchymal and endothelial cells suggests a classification of capillaries on this basis.
CLASSIFICATION OF CAPILLARIES

Classification of Capillaries on Basis of Presence or Absence of Pericapillary Cellular Investment

Capillary type α. Capillaries without a complete pericapillary cellular investment interposed between parenchymal cell and capillary (as in liver, muscle, kidney, etc.).

Capillary type β. Capillaries with a complete pericapillary cellular investment interposed between capillary and parenchymal cell (as described in central nervous system by several authors). The residual uncertainty about the completeness of this pericapillary investment in the central nervous system makes it difficult to evaluate the usefulness of this classification at the present writing.

Proposed Notation for Designating Type of Any Capillary

Since it is desirable to correlate physiological variations in capillary function with variations in structure, it may prove to be convenient to have a simple method of designating the morphological type of a capillary involved in an experiment, just as the classification of nerve fibers into A, B, and C fibers has provided a useful basis for correlation of form and function in the field of neurophysiology. In order to lead into a proposed system of three-digit notation which may prove to be useful, let us set forth once more the three classifications of capillaries presented in earlier sections of this paper.

Capillary type A. Capillaries with a complete continuous basement membrane.

Capillary type B. Capillaries without a complete continuous basement membrane.

Capillary type 1. Capillaries without fenestrations or pores.

Capillary type 2. Capillaries with intracellular fenestrations or perforations.

Capillary type 3. Capillaries with intercellular fenestrations or perforations.

Capillary type 4. Capillaries without a complete pericapillary cellular investment interposed between parenchymal cells and capillary.

Capillary type 5. Capillaries with a complete pericapillary cellular investment interposed between parenchymal cells and capillary.

Any capillary can be characterized simply by noting the letter or number appropriate to it in each classification. Thus a liver capillary (fig. 2) lacks a continuous basement membrane (type B), has intercellular gaps (type 3), and lacks a pericapillary cellular investment interposed between capillary and parenchymal cell (type α). Such a capillary can be symbolized as to types by the designation B-3-α. By a similar argument, a muscle capillary (fig. 6) possesses a continuous basement membrane (type 4), has unperforated endothelial cells (type 1), and lacks a pericapillary investment (type α). It would be designated as A-1-α, whereas a cerebral capillary would be characterized by the notation A-1-β. A mammalian intestinal (figs. 3 and 4) or metanephric glomerular capillary (9) would be assigned the symbols A-2-α.

Discussion

It is appropriate here to comment on some possible implications of certain observations of Luft and Hechter (27). These authors described the endothelium of capillaries in the adrenal cortex of glands removed from cattle at a slaughterhouse and transported to a laboratory some distance away. Samples of such glands fixed one or two hours after death of the animal showed capillaries with fenestrated endothelial cells, probably best classified as type 2. If such a gland were perfused with warm oxygenated beef blood for an hour or so after reaching the laboratory, a change in the character of the endothelium resulted. Samples of glands fixed after such perfusion revealed unfenestrated capillaries of type 1 when examined with the electron microscope.

These observations suggest that capillary endothelial cells may be labile and may change structural characteristics under the influence of circumstances such as anoxia, or under the influence of various regulating or pharmacological mechanisms. One can conceive that these alterations might under some conditions be sufficient to change substantially the type and classification of the capillary. At present we do not know the extent of the structural permutations of which a capillary is capable. It is possible that some of the reports and descriptions cited in this paper may deal with capillaries whose structure has been influenced by anoxia or anesthetic agents or other factors, or that isolating and perfusing the hind leg of an animal may introduce significant structural changes in the capillaries. In preparing original material for this study, we have so far as possible avoided the use of anesthetics and anoxia. Figures 1, 3, 4 and 5 are from specimens fixed very freshly in unanesthetized animals. Capillaries shown in figures 2 and 6 are from anesthetized animals.

In this study particular attention focuses on the structural characteristics of capillaries of type A-1. These are found in muscle, skin, and connective tissue, and dominate the vascular bed of the legs of mammals, which served as experimental material in much of the work of Pappenheimer and his co-workers (1, 2, 4). These investigators considered that their measured rates of exchange of substance between blood plasma and extracellular space could be explained by postulating pores traversing the capillary wall. These pores were deemed to have an ‘effective radius’ of about 30-45 Å, with a population density of 1-2 X 10^9/cm^2 of capillary wall. The length of the pores was presumed to correspond to the thickness of the endothelial cell, which was taken to average 0.3 μ. Bennett (34) has calculated that pores of these dimensions, occupying the proportion of the capillary surface postulated above, should be present in reasonable frequency in electron micrographs of sectioned capillaries. Thus in a section 220 Å thick, of a
capillary 3 µ in diameter, two to four pores with such a population density should be present. For example, in figure 1 of this paper, assuming a section thickness of 500 Å, four to six pores would be expected according to the Pappenheimer calculations, if electric organ capillaries are to be regarded as similar in structure and function to those of the mammalian hind leg.

We deem it significant that we have not visualized such passages either through or between endothelial cells of capillaries of type A-I in muscle and connective tissue, although we have studied many hundreds of pictures of such capillaries from this laboratory and from others. For these reasons, we doubt the pore model of capillary structure and function proposed by Pappenheimer (2) as applied to capillaries in general, and especially to capillaries of the A-I group. We recognize the much larger holes in renal glomerular and intestinal endothelium as a separate category (A-2).

The variations in structure in capillaries of different organs are so fundamental that one must caution against using measurements made on one capillary bed to support a permeability hypothesis with respect to a capillary bed of a different type. Moreover, the variations and complexities of capillary structure, the presence of components such as the intraendothelial vesicles and caveolae, which were not known a few years ago, and the failure to find pores or perforations which could serve the functions postulated by Pappenheimer, lead us to the view that a reconsideration and reinvestigation of concepts of capillary physiology is in order.

REFERENCES


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