Blood Capillaries of the Heart and Other Organs

By GEORGE E. PALADE, M.D.

The article is a review of work recently carried out on blood capillaries by the author in collaboration with Drs. M. G. Farquhar, G. Majno, and S. L. Wissig.

It reviews the morphology of these vessels at the electron-microscope level and confirms the existence of at least 3 distinct types of blood capillaries in small laboratory mammals. It shows that the capillary wall consists of 3 concentric layers (endothelium, basement membrane, and adventitia), and indicates that the basement membrane forms a continuous layer in all capillaries so far studied.

Experiments in which colloidal gold particles were used as a tracer have shown that, in capillaries with a continuous endothelium (muscle capillaries), the particles are transported across it by "pinocytic" vesicles. At the end of this step they must still transverse the basement membrane.

Experiments on glomerular capillaries, which typically have a discontinuous endothelium, were carried out on normal and nephrotic rats using ferritin as a tracer. By its accumulation on the luminal side of the basement membrane, the ferritin has identified this layer as the main filtration barrier.

A similar function of the basement membrane was demonstrated in muscle venules and venous capillaries by experiments in which the endothelium was rendered discontinuous by local treatment with histamine and serotonin.

THERE ARE, I believe, a good number of reasons for a renewed interest in problems of capillary permeability. To begin with, it is clear that we are dealing with a basic process in the physiology of metazoa. In these complex organisms, the life of the multitude of cells in the intimacy of tissue depends, in ultimate analysis, on the ample and continuous exchanges that take place across the wall of capillary vessels between the blood plasma on one side, and the interstitial fluid on the other. To continue, many pathologic conditions can be traced back to circulatory disturbances in general, and to variations in capillary permeability in particular, an outstanding example being the inflammatory process. And to finish, the mechanisms involved in the exchange of large quantities of water and solutes across the capillary wall are still poorly understood.

Studies on the structural aspects of the problem, carried out over many years by light microscopy, have revealed only a few general morphologic features that facilitate the exchanges: one could list under this heading the small diameter of the vessels, their organization into a tight meshwork that ensures a high surface-to-volume ratio for the circulating blood, and finally the extreme tenuity of the capillary wall. A description of these features, together with an attempt to quantitate them in terms of capillary volume and capillary surface per unit volume in various tissues, figured prominently—for instance—in August Krogh’s book "The Anatomy and Physiology of Capillaries," which summarized what was known in 1928 about the morphology and physiology of blood capillaries.1 In this book, which had considerable influence on the subsequent development of the field, Krogh assumed that the capillary wall consisted only of a layer of endothelial cells* but, aside from stressing their extreme thinness, did not further inquire into structural devices directly

*The existence of a second layer, called basement membrane ("Grundhütcchen"), was described, however, by many histologists. See, for instance, Benninghoff.2
involved in capillary permeability. With this in mind, it would be fair to say that we inherited from our light-microscope predecessors a good knowledge of the general layout of the capillary vessels, but practically no knowledge regarding their fine structure and especially the structural details involved in capillary permeability.

By comparison, the physiologic aspects of the problem have been more thoroughly investigated and seem to be better understood. Ever since Starling’s 1896 hypothesis, it has been assumed that the force that drives the fluid out of or into the capillary vessels is the difference between the hydrostatic and osmotic pressures of the blood plasma.† More recently, however, it has been realized that exchanges that operate on this basis are rather modest (a few per cent of the total) and that the mechanism in question may be more important for maintaining the blood volume than for carrying through adequate exchanges between the blood and the tissues. In this respect it is generally agreed at present that diffusion plays the major role, but difficulties are encountered when physiologic results are interpreted in structural terms. Pappenheimer and his collaborators,‡ for instance, conclude that the permeability characteristics of the capillary wall could be explained by assuming that the wall is a rigid partition provided with patent permanent pores of ~ 60 Å effective diameter; whereas Chinard and his colleagues§ believe that the wall behaves like a laminar gel permeated by a continuous aqueous phase—fibrils and interfibrillar spaces in the gel being of molecular dimensions.

It should be pointed out that in all these structural extrapolations it is assumed that the capillary wall consists of a single cellular layer, the endothelium. In Pappenheimer’s formulation, the postulated pores occupy a small part of the wall surface (~ 0.1 per cent) and are presumably located along the cell junctions, cutting—so to say—through the intercellular cement, a hypothetical substance that fills all the intercellular spaces of the endothelium. The hypothesis according to which the exchanges between blood and tissues are carried through these cement-filled spaces was originally advanced by Chambers and Zweifach. Before ending this short review of the physiologic aspects of the problem, I should add that the capillary wall is more permeable to lipid than to water-soluble substances. For this reason Pappenheimer and Renkin and Pappenheimer have actually postulated a double pathway across the wall: via the pores for water and solutes, and via the cells proper for lipid soluble substances.

The Fine Structure of Blood Capillaries

This was the general state of our knowledge before the electron microscope was used to investigate the structural aspects of the problem. The reinvestigation has already provided a large body of information and has established firmly at least two points.† ‡ §

First: The capillary wall is a multilayered structure. In addition to a cellular endothelial layer, which could be described as an internal tunic, it comprises an acellular layer—the basement membrane (middle tunic)—and an outer, discontinuous stratum of cells and fibers that constitute an adventitial tunic.

Second: Although similar in their general construction, capillary vessels differ constantly and characteristically in their structural details from tissue to tissue, or, rather, from groups of tissues to groups of tissues. The differences affect primarily the cellular layers, the endothelial and the adventitial, which could be discontinuous or even absent, whereas the basement membrane generally persists as an uninterrupted layer. This continuity of the middle tunic, or basement membrane, emerges then as a common structural feature for practically all types of capillary vessels so far examined.*

† Actually the algebraic sum of the following terms: hydrostatic pressure of the blood plasma, osmotic pressure of same, hydrostatic pressure of interstitial fluid ("tissue pressure"), and osmotic pressure of same.

*See, however, Bennett et al. and Wood on the problem of hepatic blood sinuses.
With this preparation, we can start reviewing the electron microscopic evidence. Figure 2 illustrates the type of capillary encountered in a skeletal muscle and in other tissues of the soma but also present in certain viscera, e.g., the myocardium and the smooth muscle of the digestive and reproductive tract. It is characterized by a continuous endothelium, 0.5 to 0.2 μ thick, and a continuous basement membrane. The endothelial cells are extremely flat but otherwise similar in organization to other animal cells. They possess a nucleus, a centrosphere region with 2 centrioles and a few small piles of smooth-surfaced cisternae.

*The morphology of blood capillaries was studied in a number of small mammals (rats, hamsters, guinea pigs, rabbits) by electron microscopy, using tissue specimens fixed in OsO₄ (buffered at pH 7.4 to 7.6) and embedded in methacrylate.

The experimental work was carried out exclusively on rats, using the same preparative procedures for electron microscopy.

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**Explanation of Plates**

All figures represent electron micrographs of rat blood capillaries. The corresponding tissues were fixed in osmium tetroxide and embedded in methacrylate. The sections were stained with lead hydroxide and "sandwiched" with carbon or formvar films before examination. General Abbreviations: BM, basement membrane; EN, endothelium; EP, epithelium; L, capillary lumen.

**FIGURE 1**

Wall of blood capillary in a skeletal muscle (rat). The lumen, which contains precipitated plasma proteins, is marked L and the pericapillary space PS. The capillary wall consists of an inner or endothelial tunic (EN), a middle tunic or basement membrane (BM), and an adventitial tunic represented here by a few collagen fibrils seen in longitudinal (c₁) or transverse (c₂) section. Small vesicles in the cytoplasm of the endothelial cell appear aligned behind the luminal and tissue fronts of the cell. Some of them (v₁) are open to the cell surface and could be described as invaginations of the cell membrane; others (v₂) are closed and appear located deeper in the cytoplasm. In this case, the number of vesicles is greater on the tissue than on the luminal front of the endothelium. × 73,000.

**FIGURES 1 and 2**

General view of a transversely sectioned blood capillary in a skeletal muscle (rat). The lumen is marked L, and the pericapillary spaces PS. Parts of 3 endothelial cells, EN₁, EN₂, EN₃, form the inner tunic at this level; their junctions appear at j₁, j₂, and j₃. The nucleus of one of the endothelial cells can be seen at n.

The middle tunic or basement membrane is marked BM. It is relatively well outlined toward the endothelium, from which it is separated by a shallow subendothelial space (ss), and frays into distinct fibrils (tf) toward the pericapillary spaces.

The adventitial tunic is represented by part of a pericyte (P) characteristically enwrapped between 2 leaflets of the basement membrane. × 35,000.

* Circulation, Volume XXIV, August 1961
Figures 1 and 2 (See legends on opposite page)
tissue front of the cell (fig. 3), and a few simple computations indicate that the vesicles represent a considerable amount of membranous material: about } 2 \mu^2 \text{ of membrane behind each } \mu^2 \text{ of cell front, and account for a sizeable part of the total volume of the cell: } \sim 1/3.

The spectrum of appearances encountered could be explained by assuming that the vesicles are formed by invaginations of the cell membrane that are pinched off and become closed elements in the cytoplasm, carrying an imprisoned droplet of extracellular fluid. A reverse process would evidently produce the same series of appearances, but this time the sequence would start with a fluid-filled vesicle in the cytoplasm, which moves to the surface, where its membrane coalesces with the cell membrane, and where its content is discharged in the extracellular medium by the orifice created at the site of the coalescence. If the 2 processes are combined, the vesicles could transport fluid from one front of the cell to the other in small, more or less equal portions or quanta. The diameter of such a pocket is } \sim 650 \text{ to } 750 \text{ Å and its volume } \sim 100,000 \mu^3 \text{ to } \sim 180,000 \mu^3.

This hypothesis has been tested experimentally but, before presenting the appertaining results, I should like to close the morphologic inquiry by reviewing information obtained on the cell junctions and on the basement membrane in this type of capillary. At the level of the cell junctions (fig. 4), there is a narrow intercellular space } \sim 100 \text{ Å that separates 2 symmetric densifications of the apposed cell membranes. The latter are sometimes backed by a condensation of the subjacent cytoplasmic matrix. What seems to be important is the fact that the narrow intercellular gap is occupied by a material of moderate density frequently condensed into a denser intermediary layer or lamina. In other words, the narrow intercellular gap is not an unobstructed passage from the lumen to the pericapillary spaces. The material in the gap does not represent, however, the cement substance postulated by light-microscope studies. That cement was supposedly characterized by its ability to reduce silver ions to metal and become impregnated by it. In electron microscopy the silver deposits appear spread over a broad band centered on the junction, but preferentially concentrated within the zones of densification of the adjacent cytoplasm.\textsuperscript{13} The presence or absence of pores in the intercellular spaces will be discussed later.

The second layer, the middle tunic, of the capillary wall is represented by the basement membrane, which appears as a continuous

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**FIGURE 3**

Grazing section of a blood capillary of the myocardium (rat) showing the large number and irregular distribution of vesicles \(v_1, v_2\) on the tissue front of the endothelium. The "stoma" of some of these vesicles, i.e., their opening to the cell surface, shows clearly as a light, circular area \(v_2\).

The basement membrane appears as a densely matted felt of fine fibrils (f), with sparse, slightly thicker fibrils in its peripheral layers (tf). A few collagen fibrils of the adventitia are visible at ef. Adjacent muscle fibers are marked mf. \(\times 55,000\).

**FIGURE 4, a and b**

Epithelial cell junctions in blood capillaries of skeletal muscle (rat). The thickening of the opposed cell membranes is visible in Fig. 4b in between the short arrows. The companion densification of the subjacent cytoplasm shows more clearly in Fig. 4a at d. The intercellular space (is) is occupied by a material of higher density than that filling the pericapillary spaces.

Note in both cases the oblique or sinusoidal course of the junctions, the pseudopodia (ps) that flank them on the luminal front, and the fact that the basement membrane passes without interruption or infolding over the junction (long arrows). The fibrillar texture of the basement membrane can be distinguished at f. Collagen fibrils are marked ef. \(\times 71,000\).
Figures 3 and 4 (See legends on opposite page)
coat of moderately dense material 200 to 500 Å in thickness. The limits of the coat are rather sharp toward the endothelium, from which it is separated by a narrow subendothelial space, and more poorly outlined toward the pericapillary spaces. In this direction the basement membrane comes in contact with various adventitial elements—collagen and elastic fibrils, and cells of varied type, pericytes, macrophages, fibroblasts, and others.

In view of the tenuity and moderate density of this basement membrane, one may wonder whether we are dealing here with an independent structural element of the wall or merely with a condensation of the ground substance of the connective tissue, which could disperse easily once the endothelial substrate is removed. The answer is found in damaged specimens which show extensive retraction of the endothelium: although left behind, the basement membrane subsists as a distinct layer; apparently it is cohesive enough to resist the various mechanical and chemical insults involved in our preparation procedures.

In specimens fixed by osmium tetroxide, the basement membrane appears as an amorphous layer of more or less homogenous density. In preparations stained by heavy metals, however, fine fibrillar elements of higher density can be demonstrated therein (fig. 5). In fact, 2 types of fibrillar elements can be recognized: one finer and tightly meshed in the inner parts of the layer; another coarser, less abundant, and less intertwined in the outer parts of the structure. Grazing sections through the capillary wall are particularly favorable for demonstrating these fibrillar components, both of which appear to be distinct from mature collagen fibrils: they are thinner and do not show the characteristic periodic pattern of the latter. The coarser of the fibrils found in the basement membranes look morphologically similar to a special type of fibril usually encountered around elastic fibers. Needless to say, the chemical nature of all these fibrillar elements is unknown.

On the strength of this evidence we can conclude that the basement membrane is a felt of fine fibrils and that the meshes of the felt seem to be filled by another material, a matrix, which still appears amorphous at the present level of resolution.

So this is the type of construction encountered in most capillaries: between the blood plasma and the interstitial fluid in interspersed a succession of barriers consisting of a continuous endothelium, a continuous basement membrane, and an adventitia that, in this case, is discontinuous enough to be negligible as a true barrier.

**Tracer Experiments on Blood Capillaries in Striated Muscles**

What is the functional role of these layers in blood-tissue exchanges? What structures participate and what mechanisms are involved in the transport of various substances across the wall?

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**Figure 5**

*Section through a bent blood capillary of the myocardium (rat). The lumen, cut in 2 places (L₁, L₂), is marked by colloidal gold particles. The endothelial cytoplasm contains small vesicles (v) and RNP particles (r). In the middle of the field, a grazing cut through the basement membrane exhibits its poorly resolved, felt-like texture (f). × 82,000.*

**Figure 6**

*Blood capillary of the myocardium (rat) 10 minutes after an intravenous injection of colloidal gold (90 mg. in 1.5 ml.). The lumen (L) is marked by gold particles (g₁), which are also present in the endothelium—within small vesicles (g₂, g₃), in the basement membrane (g₄), and in the pericapillary spaces (g₅). Note the sharp decrease in particle concentration from the lumen to the endothelium, and the fact that within the latter the tracer is restricted to vesicles and does not have access to the cytoplasmic matrix. At the level of this section, the endothelium is covered by the long process of a pericyte (P). 73,000.*
Figures 5 and 6 *(See legends on opposite page)*

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To find an answer to such questions we injected into the general circulation a tracer small enough to give meaningful information and dense enough to be seen individually in the electron microscope. Ferritin molecules $\sim 100$ Å in diameter\textsuperscript{14} and micelles of colloidal gold ranging from 30 to 250 Å\textsuperscript{15} proved to be useful for this type of work. At intervals ranging from 2 to 60 minutes after the injection of the tracer into the general circulation, specimens for electron microscopy were collected from the heart or tongue to take advantage of the high concentration of capillary vessels in the muscle of these organs. Colloidal gold particles were found in large number in the lumen and in considerably smaller numbers in the endothelium, basement membrane, and pericapillary spaces (fig. 6). In the endothelium they were as a rule restricted to the vesicles described in the vicinity of the cell membrane, the only other structure in which they were encountered being the so-called multivesicular bodies. Only occasionally were particles found in the cytoplasmic matrix (fig. 7). Since a relatively large number of micelles were detected in the pericapillary spaces, it can be concluded that they had been ferried across the endothelium by vesicles. In these experiments only an occasional, or no accumulation of particles was found against the basement membrane; apparently it allowed practically all micelles that crossed the endothelium to pass. After longer time intervals, one hour for instance, the situation was comparable, except that most of the particles in the pericapillary spaces were found ingested by macrophages. It should be stressed that no tracer was found at any time in the intercellular spaces of the endothelium. The results obtained with ferritin molecules were less clear cut.\textsuperscript{14} Frequently the tracer was found restricted to vesicles within the endothelium, but sometimes ferritin molecules occurred freely dispersed in the cytoplasmic matrix with no indication of how they reached this location.

On the strength of the results obtained with colloidal gold, it can be concluded that the vesicles of the endothelium do function in transendothelial transport. It is clear that they ferry the marker across the endothelium and it is highly probable that, together with

**Figure 7**

Blood capillary of the myocardium (rat) 10 minutes after an intravenous injection of colloidal gold (90 mg. in 1.5 ml.). The irregular lumen is marked by circulating particles (g1). The endothelium contains a number of similar particles enclosed singly (g2, g3) or severally (g4) into vesicles. One tracer particle (g5) appears against a slightly dense patch which could correspond to the top of a sectioned vesicle; another particle (g6) is definitely free in the cytoplasmic matrix. A few tracer particles (g7) have reached the pericapillary spaces.

Note that many other vesicles (arrows) contain small particles that seem to be attached to the inner surface of their membrane. Their identification as gold colloidal micelles is uncertain because their density is low and their form irregular. $\times 77,000$.

**Figure 8**

Small blood vessel (venule) in the cremaster of a rat, 2.5 minutes after a local subcutaneous injection of histamine. The tracer, colloidal mercuric sulfide, was previously injected in the general circulation.

The lumen is occupied by 3 erythrocytes (RB1, 2, 3). The endothelium (EN1, EN2) is discontinuous; through the gap marked by arrows, tracer particles (t1), chylomicrons (ch) and a red blood cell (RB4) have penetrated and dissected the wall up to the basement membrane (BM) which, in this case, appears particularly thin and poorly outlined. Note that most of the particles are retained by the basement membrane, and that the intramural deposit is highly concentrated, presumably as a result of water and solute escape through the basement membrane. Note also that, of the few particles which have reached the pericapillary spaces (t2), some have already been incorporated (t3) by a phagocytic element (PC). $\times 46,000$. 

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Figures 7 and 8 (See legends on opposite page)
the marker, they transport water and solutes, since there is room enough for a few hundred thousand molecules of corresponding size, in addition to the tracer particle, in each vesicle of 650 Å diameter. If this modality of transport seems to be well established by our experiments, it should be pointed out that its relative importance in the overall exchanges between blood and tissue fluids remains unknown: transport by vesicles may account for all, or only for part, of these exchanges and only future quantitative work will tell us whether we are dealing here with the main mechanism of transport or with an accessory one of limited importance.

In any case it should be realized that transport across the endothelium represents only one step in the entire operation. There is a second barrier to be crossed, the basement membrane, and if in the experiments so far reported it did not markedly affect the passage of the tracer, this does not mean that it will behave in the same way under different conditions or in respect to other tracers. In fact there is good ground to assume that the basement membrane should play an important role in such exchanges, the principal reasons being the following: it is difficult to ascribe specificity to a transport in quanta and, moreover, there are types of capillaries in which the endothelium is discontinuous or fenestrated and in which the blood plasma gains direct access to the basement membrane, which appears to be the only continuous barrier in the wall of the vessels.

Tracer Experiments on Renal Glomerular Capillaries

Capillaries with a fenestrated endothelium are encountered in many viscera,\textsuperscript{8, 16} and the fenestration of their endothelium becomes particularly extensive in the glomerular capillaries of the kidney. In addition to an extensively fenestrated endothelium, these capillaries are characterized by the existence of a third cellular layer—made up of the pseudopodia of the visceral epithelium. Their basement membrane is also thicker and apparently more substantial than in other capillaries, visceral or somatic. Glomerular capillaries represent a favorable object of study because in their case we are dealing with one-way transport only—i.e., from the lumina to the capsular space—and because the nature of the capsular fluid is relatively well known. It has been collected by direct micropuncture in a number of species and its analysis has shown that it is a protein-free, or almost free, filtrate of the plasma.\textsuperscript{17}

In the experiments on glomerular capillaries, carried out in collaboration with Drs. M. Farquhar and S. Wissig, the tracers used were again ferritin and colloidal gold.\textsuperscript{18-20} After short time intervals (3 to 15 minutes), the ferritin was found in high concentrations in the lumen. From the lumen it appeared to gain free access, through the fenestrae of the endothelium, to the basement membrane. Within the latter the tracer was found in noticeably lower concentrations and more or less evenly distributed in surface and in

Figure 9

Renal glomerular capillary of a nephrotic rat one hour after the intravenous injection of 50 mg. ferritin.

The basement membrane (BM) crosses obliquely the field separating the epithelium (EP) at left from the endothelium (EN) at right. The lumen is hardly visible at the extreme right. Large deposits of ferritin infiltrate the spongy areas (sa) and the luminal layers of the basement membrane. Such deposits identify the basement membrane as the main filtration barrier. Fewer particles penetrate the peripheral layers of the filter and reach the epithelium where they can be seen in invaginations of the cell membrane (t1), in closed vesicles and small vacuoles within the cytoplasm (t2), and in dense bodies or absorption droplets (t3).

At this relatively late time point, the endothelium contains membrane-bound vacuoles (pb) filled with packed ferritin—an indication of the phagocytic activity by which the filtration deposits are removed. × 73,000.
Figure 9 (See legend on opposite page)
depth. Few ferritin molecules reached the foot processes of the epithelial layer, and those that did appeared to be caught either in small invaginations of the cell membrane or in small vesicles within the cytoplasm. After longer time intervals (30 to 60 minutes), 2 new noteworthy features emerged: first, there was a gradual increase in the concentration of the marker in the luminal strata of the basement membrane and finally extensive piling up of ferritin molecules against its luminal surface; second, the number of ferritin molecules captured by the epithelium increased and, in addition to those located in membrane invaginations and small vesicles in the foot processes, the tracer appeared in the cell body proper in large vacuoles and in dense bodies.

To confirm these findings, we also administered the tracer to rats rendered nephrotic by treatment with the aminonucleoside of puromycin.21 In such cases, the permeability of the glomerular capillaries is increased and substantial amounts of blood proteins are lost during glomerular filtration. In nephrotic animals, the piling of ferritin against the basement membrane was about as striking as in normals after long time intervals (fig. 9), but the amount of ferritin in the basement membrane and in the epithelium was considerably greater (fig. 10*). The epithelium showed its typical response to the nephrotic condition: the extensive disappearance of the foot processes. In these altered epithelial cells, as in normal ones, the marker was restricted to membrane-limited spaces, i.e., vesicles, vacuoles, and dense bodies. In nephrotic animals there was a definite reduction in the extent of the fenestration of the endothelium.

From these experiments, it can be safely concluded that in this type of capillary the main filtration barrier is the basement membrane. The tracer is retained by it as by a filter. It is clear, however, that the filter is imperfect. Even under normal conditions, it leaks a detectable amount of the tracer, which is recovered, at least in part, from the filtrate by the epithelium that seems to function as a monitor of the filter proper. As expected, operations connected with this recovery are considerably enhanced when the filter becomes more leaky in nephrotic animals. Turning now to the filter proper, it should be pointed out that the ferritin molecules that escape through it were found distributed at random within the membrane. There was no preferred relationship to the slits of the epithelium, for instance. Moreover, and probably more important, no pores were seen in the basement membrane. The tracer molecules were found embedded in its substance without channels ahead or trails behind. Pores of simple geometry allowing the passage of a ferritin molecule would have a diameter in excess of 100 Å and should be visible. Since they are not, we are led to conclude that they are either extremely tortuous and consequently difficult to see in sections of the thickness used (~ 500

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**Figure 10**

Part of a renal glomerulus in a nephrotic rat, 2 hours after the intravenous injection of 50 mg. ferritin.

Most of the field is occupied by part of an epithelial cell (EP), which contains the marker (ferritin molecules) in small vesicles (t₁), small vacuoles (t₂), large vacuoles with a light content (t₃), structures of intermediary appearance (t₄), and—finally—typical dense bodies (t₅, t₆, t₇). It is assumed that all these forms represent progressive stages in the segregation and concentration of the marker and other materials incorporated by the cell from the glomerular filtrate.

The basement membrane (BM) of the capillary, covered by this epithelial cell, appears in the upper left corner infiltrated by numerous ferritin molecules.

The lumen is not visible in this field, but some urinary spaces can be seen (US). X 73,000. (From Farquhar and Palade: J. Biophys. & Biochem. Cytol. 7:297, 1960.)

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Figure 10 (See legend on opposite page)
to 800 Å, or that permanent pores do not exist. One has the impression that the marker moves through a yielding gel, creating a channel as it moves. As far as the activities of the other layers are concerned, they appear to be ancillary, if we restrict our interest to the filtration process proper and disregard morphogenetic processes for the moment. The endothelium seems to function as a valve that regulates the amount of plasma which gains direct access to the filter, whereas the epithelium behaves like a monitor which partly compensates for the imperfections of the filter. Analogies with the endothelium and the adventitial cells (primarily macrophages) of muscle capillaries are quite evident.

Effects of Histamine and Serotonin upon the Structure of Small Blood Vessels

In the light of these experiments on glomular capillaries, should we conclude that the basement membrane is also the main filter in muscle capillaries, i.e., in capillaries with a continuous endothelium, and that in the latter the vesicles represent only a more refined "valve" than the fenestrae of the glomular capillaries?

The last experiments to be reported suggest that such a conclusion is justified, at least in part. In collaboration with Dr. Guido Majno, and Miss Gutta Schoeff, from the Department of Pathology at Harvard, we tried to find out what changes are introduced in the structure of blood capillaries by local histamine or serotonin treatment which is known to increase the permeability of small vessels markedly. The experimental device was simple and relied primarily on the discovery of a favorable specimen: the cremaster of the rat, a thin layer of striated muscle—2 muscle fibers thick—located under the skin of the scrotum. The marker, this time a coarser colloidal particle of mercuric sulfide (200 to 500 Å in diameter), was introduced into the general circulation; and histamine or serotonin, in doses of 50 µg. and 5 µg. respectively, was injected locally between the skin and the cremaster. The latter was excised and fixed at various time points thereafter either in toto, for light microscopy, or in small blocks, for electron microscopy. The effect of the 2 amines becomes visible under the dissecting microscope in approximately 3 minutes, and spectacular in 10 to 15 minutes. It consists in a spotty blackening of the vessels that otherwise retain their usual appearance at low magnifications. The electron microscope provides a satisfactory explanation for the blackening. The "lesion" produced by the amines is a local discontinuity in the endothelium, apparently caused by the pulling apart of 2 endothelial cells over longer or shorter distances. A gap is thus produced through which the plasma, loaded with the marker, gains direct access to the basement membrane. The latter apparently lets the water and many of the solutes pass, but as a filter retains the marker. In time, relatively large deposits of HgS particles accumulate within the wall of the vessel and start to dissect its layers. I should point out that the amines affect preferentially the small venules, as clearly indicated by the examination under the light microscope of cremasters mounted in toto, but the lesions extend on the vascular tree toward the capillaries, at least toward their venous ends. In view of the preferential localization of the lesions, in itself a very intriguing finding, it is not surprising that the morphology of the affected vessels is sometimes extremely complex. Successive and unequal deposits are found in the thickness of the wall cleaving its various layers, which in the case of a venule can be more numerous than in the case of a capillary. In addition to the marker, many other circulating particles are retained by the filter. Frequently chylomicrons accumulate within the wall, together with deposits of proteins, some of which polymerize into tactoids with the periodicity of fibrin. Finally cellular elements, i.e., platelets, erythrocytes, and leukocytes, find their way into these dissecting aneurysms of the wall of the vessels. What is remarkable is the fact that, with all its tenuity and poor definition, the basement membrane of these vessels is capable of retaining the large deposits formed by the residues of filtration. Relatively few particles reach the pericapillary space and those that

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do are rapidly picked up by phagocytic elements located along the vessels (fig. 8). The effect of histamine on the structure of blood capillaries has been surveyed by Alksne, who arrived at the conclusion that the amine increased the pinocytotic activity of the endothelium in addition to causing the formation of channels across the endothelial cells. The differences in results and interpretation between his and our experiments are due to differences in specimens and time points examined. In his case the specimen (skin) was less favorable and the timing inadequate. The results of the histamine and serotonin experiments indicate clearly that the basement membrane of the venules and capillaries of the cremaster behaves, when denuded, like that of glomerular capillaries: it proves its ability to function as a filter by accumulating a conspicuous filtration residue. Yet it is too early to conclude that the endothelium does not screen what it transports at all, for so far only a very small number of various kinds of particles have been tested.

General Comments

Admittedly much remains to be done before the relation between the structure and the function of various capillary vessels is clearly understood, but the findings so far recorded already suggest distinct roles for the successive layers of the capillary wall and point to the basement membrane as a functionally important component. This layer appears to be the best candidate for the role of selective filter. It remains to be seen, however, whether its selectivity can be explained by simple devices, such as pores of fixed geometry, or by more complex properties. In this respect the higher permeability of all capillaries to lipid-soluble substances (when molecules of similar diameter are considered) should not be forgotten. With this in mind, it is to be regretted that we know so little about the chemistry of the filter. Whatever we know is derived from histochemical tests which indicate that the basement membrane consists of a mucopolysaccharide, probably conjugated or associated with proteins. More knowledge will undoubtedly be helpful. I hope that the work presented may act as a stimulus in this direction.

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