# STRUCTURAL MAKEUP OF CAPILLARY WALL*

*By* Benjamin W. Zweifach
*Department of Biology, New York University, New York, N. Y.*

Changes in the functional behavior of the smallest subdivisions of the vascular tree have been related, in the past, only in broad generalities to particular structural elements. This is unfortunate, since the basic function of the circulatory system, the establishment of tissue homeostasis, resides primarily in processes unique to the capillary bed proper. The entire series of structural elements encountered in exchanges between the blood and the tissue cells are collectively referred to as the *hematoparenchymal* barrier. This ubiquitous entity is concerned with a wide spectrum of physiological and pathological processes ranging, on the one hand, from its role as a semi-permeable membrane in the exchange of fluid and solutes between the blood and tissue compartments and, on the other, to its participation in local defense mechanisms, hemostasis, leukocytic invasion, *etc.* An adequate understanding of these fundamental processes will depend, in large part, upon our ability to relate them to particular constituents of the barrier. A singularly rewarding approach to the problem was found to be direct microscopic visualization of the smaller blood vessels in the living animal, using a combination of histochemical, micromanipulative, and biological indices to delineate the locus of the derangement involved.

There has been a tendency to ascribe the functional attributes of the blood-tissue interchange to properties of its most obvious constituent, the endothelial cell. More careful analysis indicates that the endothelial membrane *per se* represents only a skeletal framework onto which are superimposed, on either side, discrete structural entities equally important as determinants of the functional characteristics of this complex barrier.[1] Materials permeating from the bloodstream to the tissue cells encounter five separate structural components: (1) an adsorbed layer of protein lining the inner surface (presumably a plasma constituent and/or blood platelets enmeshed in the pores of the intercellular cement); (2) the endothelial membrane proper, whose surface represents a dual entity, a composite of living cells, and (3) the intercellular cement substance, a small fraction of the total surface; (4) a condensation of fine connective tissue fibrils enmeshed in a dense amorphous ground substance, referred to as the pericapillary sheath; and (5) a layer of connective tissue about 25 to 50 micra in depth intervening between the cell and the vessel proper.

The movement of materials across this barrier is governed primarily by physicochemical forces, with the net exchange across a unit area of capillary surface being determined by intrinsic factors, such as the nature of the pores in the separate structures, the relative thickness of each component, and the lipid solubility of the material involved. The actual surface across which the exchange occurs consists of an extensive cellular area with a small nonliving intercellular zone (less than 1 per cent). There is some disagreement

* This investigation was supported by a research grant from the Life Insurance Medical Research Fund, New York, N. Y.

whether the bulk of the exchange occurs across the entire surface area[2] or is restricted for most molecules to the more pervious intercellular cement.[3] The evidence is clear that the penetration of large molecular aggregates and formed elements probably occurs through the intercellular portion of the wall. In essence, the basic structure involved is a porous network of a complex cement substance, presumably a calcium proteinate. Superimposed on this network either by electrical, chemical, or surface tension forces is a large molecular component which plugs most of the large pores in the cement, considerably reducing the over-all perviousness of the structure. Whether the secondary layer represents a component of the blood-clotting system, as believed by different investigators,[4] or is a combination with blood platelet elements,[5] remains speculative. The increased capillary permeability that develops in conditions involving blood platelet depletion or a defect in the blood-clotting system[6] is suggestive of a direct relationship between these processes and the perviousness of the capillary wall.

The intercellular cement, a structure analogous to the extraneous coats observed in unicellular marine forms, is secreted continuously by the endothelial cell, a major contribution to the integrity of the vascular barrier. In experiments where the capillary was exposed to a weak solution of silver nitrate introduced with a micropipette, the cement material was precipitated, apparently as a silver proteinate. When done carefully, this staining can be achieved without interrupting the blood flow through the capillary vessel. The blackened cement is gradually washed away by the flow of the blood. Since this occurs without affecting the integrity of the vessel wall, it is reasonable to assume that the intercellular material is being replaced by the endothelial cells. In instances where the endothelial cell is damaged either chemically or by mechanical handling, the progressive washing away of the nitrate blackened cement is accompanied by a disruption of the wall and the outward penetration of red blood cells. Stasis then ensues rapidly.

The properties of the cement will vary with changes in electrolyte concentration. For example, the presence of calcium has been found to be important for the maintenance of the normal adhesiveness and tensile strength of this material. In perfusion experiments where the calcium content of the circulation is artificially reduced, the cement appears to go into solution and is washed away. In tissue-culture preparations, the growth of endothelium in the form of sheets or tubes is likewise dependent on the presence of adequate amounts of calcium.[7] For example, when the calcium content is reduced, endothelial sheets begin to fall apart, the cells round up and assume ameboid characteristics or lie rounded in the clot. With the reintroduction of calcium, these cells again flatten out and join with one another to form continuous sheets or tubes. Apparently, a small amount of calcium (about 10 per cent of normal) is sufficient to maintain cellular adhesiveness. In the calcium-deficient experiments, the endothelial cells could readily be detached from one another with microneedles. This is in contrast with experiments using normal concentrations of calcium, where it is almost impossible to separate two contiguous endothelial cells with microneedles without destroying the cells.

The substitution of magnesium or strontium is not adequate to replace calcium with regard to this function. In perfusion experiments, where the concentration of calcium was minimal or absent and the amount of potassium was increased, the intercellular cement of the blood capillaries underwent swelling and became unusually prominent. Under these conditions, the vessel was highly permeable to the extent that edema developed. Extravasation of blood cells was present but not excessive. The use of magnesium ions as a substitute for calcium results in the transformation of the cement into a jelly-like, clear, extracellular material on both the surface of the cell and along the intercellular margins. The presence of excess calcium in perfusion media results in a heavy precipitation along both the intercellular margins of the endothelial cells and the inner surface in contact with the perfusate. It is interesting to note that excessive amounts of calcium will result in the transformation of extraneous coats of various cells from gelatinous materials into a brittle, opaque substance which cannot be stretched by microneedles. Actually, in perfusion experiments with excess calcium (two times normal) the vessels are more easily ruptured by stretching with microneedles than in experiments with normally balanced perfusates.

The intercellular cement material is difficult to visualize under normal conditions. It can be stained, of course, with silver nitrate. In the living vessel, the cement can best be visualized by virtue of its adhesive properties through the intravenous administration of an inert particulate substance, such as carbon or graphite. Under normal conditions, the sticking of carbon is confined to the intercellular cement material (FIGURE 1). Reports indicate that it is possible to stain the intercellular cement with indigo tetrasulfonate methylene blue.[8] Whether this represents a staining of some blood protein which is adherent to the cement or the cement substance *per se* cannot be ascertained with this procedure.

The evidence with respect to the behavior of the cement substance to electrolytes and changes in pH would appear to indicate that the material is a

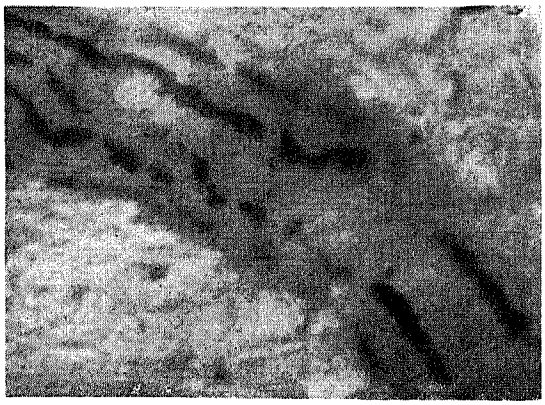

FIGURE 1. Blood capillary in living mesenteric preparation showing intercellular lines following intravenous injection of suspension of fine carbon particles. Carbon adheres only to cement between cells. ×450.

complex protein, possibly a calcium proteinate, differing in chemical and physical properties from that of the connective tissue ground substance.[3] The introduction of a proteolytic enzyme such as crystalline trypsin results in a breakdown of the intercellular cement substance. This is in contrast to the effects of a testicular extract with hyaluronidase activity, where an effect can be demonstrated only on the connective tissue ground substance and on the perivascular sheath.[9] The interendothelial cement appears intact, as evidenced by its reaction to particulate matter and its response to stretching with microneedles. Thus far, in all experiments where the permeability of the capillary as a whole has been increased so as to facilitate the permeation of blood proteins or blood cells, a concomitant change in the physical characteristics of the cement substance has been noted. In general, factors that tend to loosen the cement or to cause it to swell increased the perviousness of the vessel wall, whereas factors which tend to insure the stability of the cement reduce the tendency toward abnormal capillary permeability.

The endothelial cells proper obviously constitute an important structural element from a variety of considerations.[10] First, the tone or elasticity of the capillary wall is, in part, a consequence of the tone of the endothelial cell proper. The tone of nonmuscular cells varies in large part by changes in the water content. Under different conditions, the endothelial cell becomes swollen in appearance and loses its normally elastic properties. This deficiency can be demonstrated by micromanipulative means. When examined under high microscopic magnification, the endothelial cell can be seen to exhibit a constant ameboidlike activity. In conditions where the cell tone is deficient, this activity is lost.

A second phenomenon in which the endothelial cell participates is the continuous replacement of the intercellular cement, as noted previously. Third, the exchange of lipid-soluble materials would appear to be a function of the endothelial cell proper. It is obvious that factors that interfere with the functional state of the endothelium will influence this type of exchange. The requirement of the tissue cells for oxygen and the effective removal of $CO_2$ are probably referable to this particular phenomenon.

Previous studies have tended to emphasize the extreme thinness of the endothelial cell as an important factor in the exchange of materials between blood and tissues. The fact by itself cannot account for the type of permeability exhibited by the vessel wall. Histochemical studies have indicated that the endothelial cells have an unusual content of glycogen.[11] Recent histochemical investigations with tetrazolium salts have indicated that the endothelial cells are metabolically active (FIGURE 2), the relative order of their activity being comparable to that of vascular smooth muscle.[12] The enzymatic reduction of tetrazolium by endothelium apparently varies with the functional state of the vessel.

Endothelium therefore appears to represent an extremely labile tissue even on a metabolic level. The endothelial component of the blood-tissue barrier is affected by a wide variety of conditions, especially by changes in local tissue metabolism. Hypoxia *per se* does not appear to represent an important con-

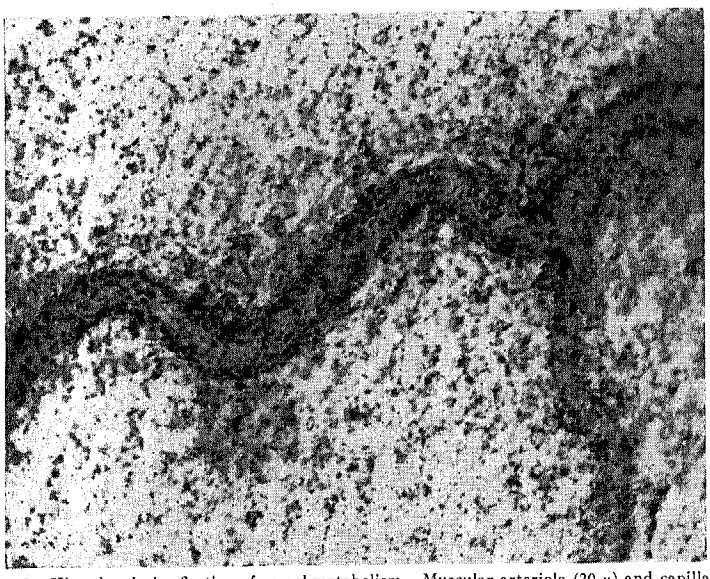

FIGURE 2. Histochemical reflection of vessel metabolism. Muscular arteriole (20 $\mu$) and capillary branch (right-hand side of photo) in mesentery of rat. Vessels colored by reduced neotetrazolium (NT) crystals in smooth muscle and endothelial elements. Living mesentery incubated in NT for three hours at 37.5° C., fixed in neutral formalin. ×100.

sideration, since the oxygen tension of perfusion mixtures must be reduced to almost zero before an effect on the integrity of the endothelium becomes apparent. Other factors, such as the tonicity of the extracellular fluids and pH would appear to be of direct importance. For example, the local introduction of hypertonic solutions (NaCl, glucose, sucrose, albumin) will result in a shrinkage of the endothelial elements and a consequent pulling apart of contiguous surfaces of endothelial cells. This result, of necessity, is accompanied by increased outward loss of fluid and even by extravasation of red cells.

An aspect of capillary structure which has been almost completely neglected is the presence on the inner surface of the endothelial wall of a thin layer closely adherent to the endothelium and the cement.[1] This structure is so thin as to be invisible through the microscope in all but exceptional circumstances. The lining material would appear to represent some constituent of the blood which is deposited onto these surfaces. The exact factor that is involved has not been identified. Because of the intimate relation of the inner-vessel surface to local thrombus formation, together with the adherence of blood platelets to the intercellular cement, the blood proteins concerned with the clotting mechanism have been suspected in this regard. Perfusion experiments, in which the blood colloids were replaced by various synthetic or naturally occurring colloidal agents, indicate that the permeability characteristics of the capillary wall can be altered in this manner. This change occurs independently of the precise osmotic properties of these colloidal agents. The substitution of different colloidal substances as the material plugging the interstices or pores of the capillary membrane will affect its permeability characteristics. Such a role has been

demonstrated not only for a native protein such as albumin, but for substances such as gelatin and gum acacia. In experiments where the capillary system was perfused with colloid-free mixtures, the naturally occurring lining of the vessel could be washed out and the progressive edema studied. The addition of different proteins or colloids to the medium could then be used to study the restoration of normal permeability characteristics. Crystalloidal solutions were completely ineffective.[13] Small amounts of colloid produced an effect on permeability disproportionate to their osmotic pressure values. The addition of blood platelets to the perfusion fluid likewise served to restore partially the permeability of the capillary wall.[14] The platelet factor has been suggested to operate by virtue of a true plugging of the capillary pores. The mechanism, however, is not a simple mechanical effect, since the platelet action is dependent on the presence of other serum proteins and, apparently, upon the physical characteristics of the vessel surface. Thus, this phenomenon could be regulated not so much by the total number of platelets, as by a precise physicochemical relationship between these elements and the vessel wall.

Other investigators have shown that it apparently is possible to displace the normally present lining by the administration of surface active materials.[15] Among the agents found to exert their effect by this particular mechanism are basic proteins, such as clupein. The displacement of the protein lining by these agents results in an altered permeability of the vessel wall. Experiments with heparin also point to an action on the lining proteins leading to an increased capillary permeability.

A prominent feature of the capillary wall is the presence of a sheath of delicate fibrils in the form of a membraneous structure adherent to the outer endothelial surface. This sheath varies in consistency and thickness in different regions as well as under various conditions. Being a condensation of the ground substance of the connective tissue proper, it presents a definite barrier to the passage of substances to and from the capillary lumen (FIGURE 3). It is interesting to note that leukocytes, upon passage through the capillary wall, at first lie in the space between the endothelium and the pericapillary sheath. Red blood cells usually enter the tissue spaces under conditions which weaken this supporting structure. Actually, the most common forms of increased capillary fragility are a consequence of a disturbance in the capillary sheath.

The perivascular sheath undergoes changes in its physicochemical characteristics, in addition to those in the tissue ground substance, and is profoundly altered by enzymes in extracts with hyaluronidase activity. In vitamin C deficiency states in the guinea pig, this structure is likewise found to be deficient. The intravenous injection of snake venoms results in numerous petechial hemorrhages, again believed to be due to alterations in the perivascular sheath.

The final structural component involved in blood-tissue exchange is the connective tissue proper. This structure presumably has no major influence on diffusion processes. The injection of diffusible dyes into the ground substance of the connective tissue results in a progressive, even diffusion similar to that observed when such dyes are introduced into a block of gelatin. Attention should be given to a number of features which could limit the free movement of

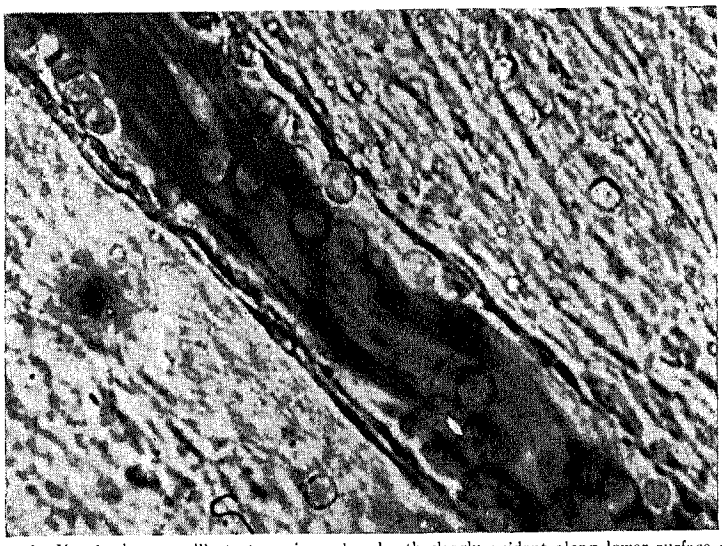

FIGURE 3. Venule shown to illustrate perivascular sheath clearly evident along lower surface of vessel. Photograph of untreated mesentery of rat. ×200.

water and dissolved substances in this medium. It is well known that in hypothyroidism, mucoproteins appear which have an avidity for water and result in a typical tissue edema. It is probable that the avidity of the various fibrous elements and the ground substance for cations may change under different conditions. This change, in turn, can conceivably affect the adsorption of substances such as the hyaluronate compounds to the connective tissue matrix and thereby alter the permeability characteristics of this barrier. Apparently, ascorbic acid deficiencies[16] and X radiation[17] will give rise to abnormalities in the ground substance that lead to increased capillary fragility and edema. The principal influence of a change in the connective tissue proper on capillary permeability would appear to be indirect, resulting from the subsequent disturbance in the metabolic activity of the parenchymal cells proper or of the capillary endothelium.

*References*

1. ZWEIFACH, B. W. 1954. Connective Tissues. 5th Conf. Josiah Macy, Jr. Found., New York, N. Y. : 38.
2. FLEXNER, L. B., D. B. COWIE & G. J. VOSBURGH. 1948. Cold Spring Harbor Symposia Quant. Biol. **13:** 88.
3. CHAMBERS, R. & B. W. ZWEIFACH. 1940. J. Cell. Comp. Physiol. **15:** 255.
4. TOCANTINS, L. M. 1947. Ann. Surg. **125:** 292.
5. ROSS, M. H., J. FURTH & R. R. BIGELOW. 1952. Blood. **7:** 417.
6. CRONKITE, E. P. & G. BRECHER. 1952. Blood Clotting. 5th Conf. Josiah Macy, Jr. Found., New York, N. Y. : 171.
7. CHAMBERS, R., G. CAMERON & C. G. GRAND. 1949. Acta Unio Intern. contra Cancrum. **6:** 696.
8. SAMUELS, P. B. & D. R. WEBSTER. 1952. Ann. Surg. **136:** 422.
9. ZWEIFACH, B. W. & R. CHAMBERS. 1950. Ann. N. Y. Acad. Sci. **52:** 1047.
10. CHAMBERS, R. & B. W. ZWEIFACH. 1947. Physiol. Revs. **27:** 436.
11. GOMORI, G. 1952. Microscopic Histochemistry. Univ. Chicago Press. Chicago, Ill.

12. FRIED, G. H. & B. W. ZWEIFACH. 1955. In press. Anat. Record. **121:** 97.
13. ZWEIFACH, B. W. 1940. Anat. Record. Suppl. **78:** 83.
14. DANIELLI, J. F. 1940. J. Physiol. **98:** 109.
15. DANIELLI, J. F. & A. STOCK. 1944. Biol. Revs. Cambridge Phil. Soc. **19:** 81.
16. LEE, R. E. & N. Z. LEE. 1947. Am. J. Physiol. **149:** 465.
17. EDGERLEY, R. H. 1953. A . J. Physiol. **174:** 341.

*Discussion of the Paper*

DOCTOR SZENT-GYÖRGYI: Have you ever tried to work to higher magnification, as with the electron microscope?

DOCTOR ZWEIFACH: We have not, but others have performed studies on muscle, especially since muscle tissue does lend itself to electron microscopy in terms of sectioning. Other tissues are more difficult to prepare, and one can identify the cement and the so-called bridges that hold the cells together. Other cellular details are not too illuminating with respect to the particular problem.

DOCTOR BRUNO KISCH: Working in recent years on electron microscopy, especially of the heart muscle, we could not avoid seeing the capillaries and their structure and, even though that is only a beginning of the investigation, we can say that the capillary itself has a wall that is very complicated in its structure. Sections through a capillary show one wall of the capillary, not the capillary itself. You see here an inner structure, a wall, and an outer structure enclosing a kind of material that, up to now, I have not been able to analyze clearly with the electron microscope, but it always shows in good pictures of the capillaries. You have here, then, the nuclei of the endothelium and the other nuclei and, outside of the wall, very definite sheaths can sometimes be seen running parallel with the capillary; so that a capillary wall is not simply a sheet of tissue, but a very complicated structure. A long time will be needed before we really know this structure and its changes in abnormal conditions of the capillaries. This is a big project that is beginning to show how the electron microscopic structure of the capillary wall changes under certain conditions, especially in vitamin C deficiency.