Aspects of Acute **INFLAMMATION**

SCOPE® Monograph on

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formerly of the Faculty,
University of Michigan, School of Medicine;
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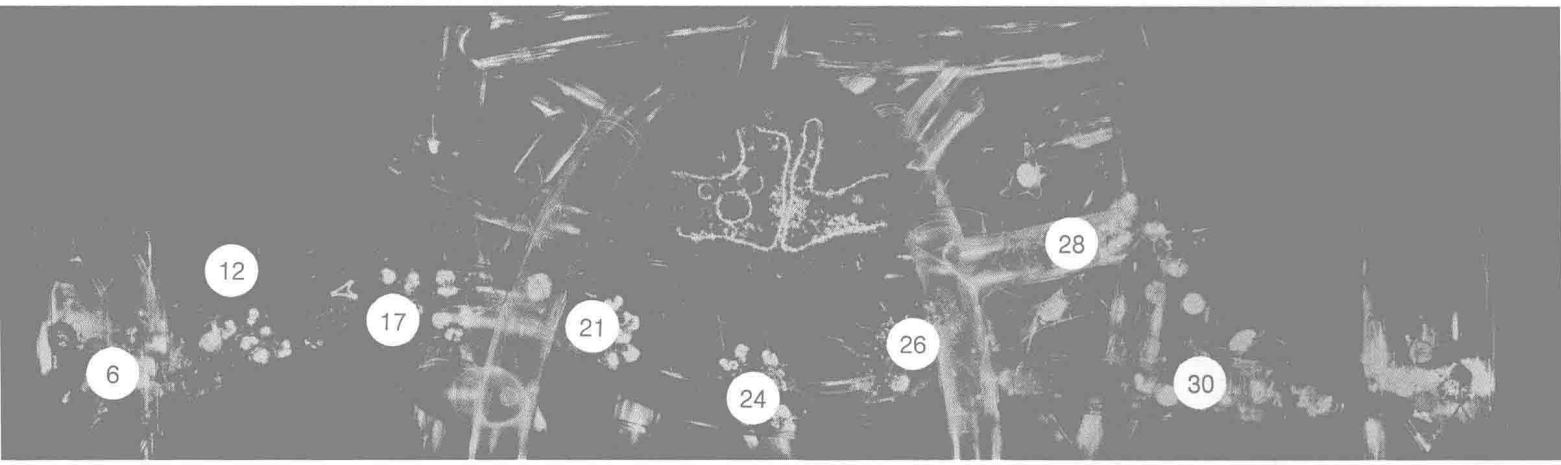

Table of contents

LIBRARY OF CONGRESS CARD NUMBER 78-112744

Published by The Upjohn Company, Kalamazoo, Michigan

Baird A. Thomas, *Editor*

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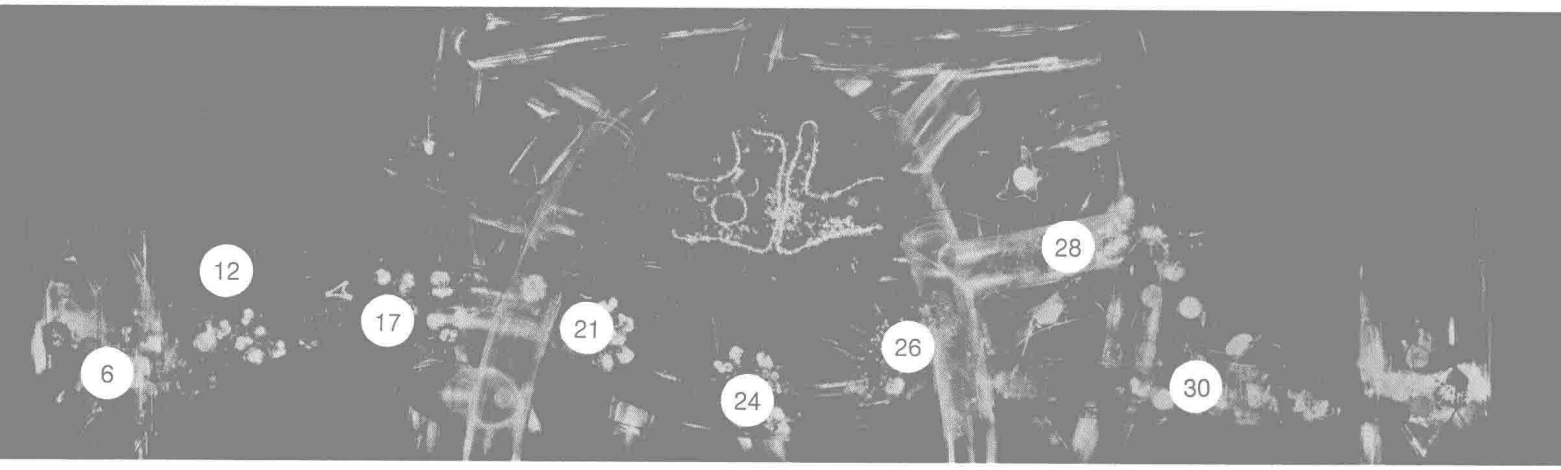

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Historical background

In the past The Upjohn Company has produced a number of exhibits with accompanying monographs featuring subjects of particular current interest in medical science. They have featured the discoveries made by the introduction of a revolutionary new research technique such as electron microscopy (The Cell), *or an important new concept such as the structure of the DNA (desoxyribonucleic acid) molecule* (Genes in Action). *The subject featured in this year's exhibit and monograph, however, relies on neither a new technique nor an epoch-making discovery to give it interest but depends on its importance both to clinical medicine and biology in general. The subject is the organism's reaction to injury in defense of its existence—inflammation.*

There have not been extraordinary, pivotal discoveries concerning inflammation in the past decade; only the conscientious application of every new device or method of approach to any aspect of the subject on which they might shed light. The exhibit and this booklet, therefore, celebrate no significant major scientific discovery, but hopefully will be a guide through a vast accumulation of discoveries which are more confusing than illuminating individually but of importance as an interrelated whole.

In any subject made up of so many small pieces, it is impossible to trace out the origin of each idea and give due credit for every contribution. Not only does the available space not allow it, but the reader would be led along so tortuous a path that he might well lose his way. Instead he will be guided along what currently seem the important paths.

Figure 1:
Cornelius Celsus,
(approximately 30 B.C.-38 A.D.),
though probably not a physician, was the first author to enunciate the four cardinal signs of inflammation.

The title of this essay implies that inflammation is primarily a defensive reaction which is important in restoring the organism to health following injury. As will be seen, it ordinarily is successful in eliminating or neutralizing the noxious agent and bringing about the repair of the damaged tissue. Thermodynamically, it is the means that the organism has for diverting a part of its energy and substance to repel a threat to its existence. It fails when more energy is required than can be supplied.

This concept of inflammation as a defense mechanism has not always been understood. The early physicians were familiar with the manifestations of inflammation. In fact, Celsus (1st Century A.D.) enumerated four of its cardinal signs: *tumor* (swelling), *rubor* (redness), *calor* (hotness) and *dolor* (pain), to which Galen (130-200 A.D.) added the fifth, *functio laesa* (loss of function). They and most of their successors, however, regarded it as simply a lesion, i.e., a manifestation of injury. It was perhaps John Hunter (1728-1793) who first recognized that inflammation was indeed a defense reaction. This idea was, of course, implied in the concept of laudable pus—a clinical indication that the body was winning the battle for recovery.

It was not until the development of histologic techniques and an understanding of normal microanatomy had been obtained that inflammation could be accurately described in cellular terms and rational theories developed as to its mechanism and purpose. This occurred largely during the last quarter of the 19th Century.

It is not possible here to delve deeply into the historical development of our present concepts of inflammation, but a little background will be helpful.

Rudolf Virchow (1821-1902) understood the histologic manifestations of inflammation and its recurrence with modifications in many pathologic situations. The theory he developed concerning its mechanism did not gain a wide acceptance. He believed that in some manner the body supplied the cells in the injured region with a superabundance of nutrition, and that in consequence they were increased in number and activity. This theory was too general, did not help in understanding many of the observable phenomena and lacked objective proof. It did, however, explain the local evolution of heat which subsequent theories have tended to neglect.

A considerable advance in understanding inflammation was made by S. Samuel and J. Cohnheim who focused attention on the small blood vessels. In fact Cohnheim believed that the entire process was explainable on the basis of the effect of injury on the vascular wall. It was his belief that some noxious material emanating from the site of injury weakens the vessel wall and causes it to leak both fluid and blood corpuscles. Subsequent work has indicated that this theory is certainly true so far as it goes, but there are also other aspects of inflammation which have to be considered, in particular the behavior of the white blood cells. Cohnheim like most of his predecessors regarded inflammation as a manifestation of injury and not an active defense reaction.

Another important phase to the investigation of inflammation was added by Elie Metchnikoff (1845-1916) before the close of the 19th Century. Metchnikoff was a zoologist and approached the subject from the standpoint of the evolution of the reaction to injury from its simplest manifestations in unicellular organisms to the complex process of inflammation in higher mammals. The protozoa, lacking for the most part tough or resistant cell walls to keep invaders out or organs usable for offense, must rely on their ability to destroy organisms by engulfing and digesting them and finally extruding the harmless debris. Since this is an active process, Metchnikoff emphasizes throughout his work on inflammation the idea of active defense against injury. As he progressively studies more and more complex organisms, he points out that with few exceptions each has cells which react actively to injury by attempting to

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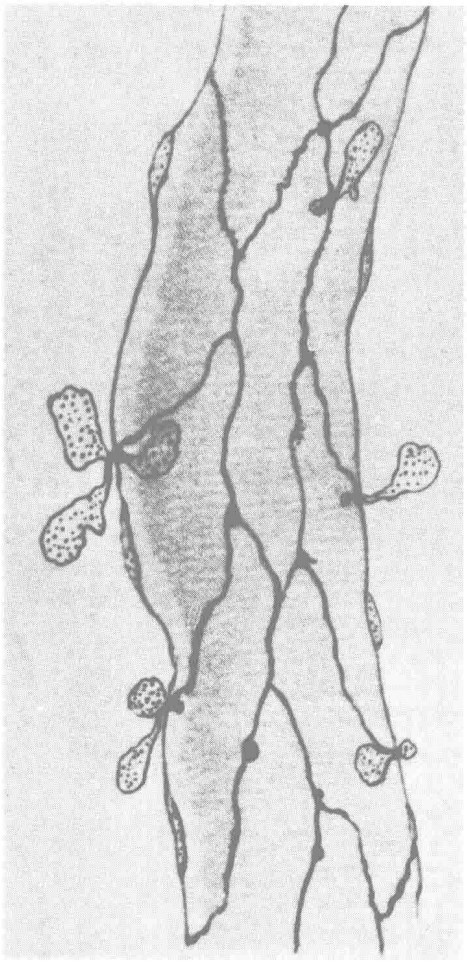

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Figure 2:
Rudolf Virchow, (1821-1902), was the great progenitor of cellular pathology, stressing the idea that changes in disease are due to fundamental alterations in the cell.

Figure 3:
Leucocytes emigrating from a small inflamed vessel in frog mesentery was observed by Julius Arnold in 1875 and reported in Virchow's Archiv für pathologische Anatomie.

engulf and digest or simply to wall off the offending organism or object. It is of interest that in those organisms in which in the course of their development there is a mesoderm, the defending cells are derived from this.

In primitive organisms which do not have blood vessels, the defending cells make their way to the site of injury by amoeboid motion, and in some instances primitive connective tissue cells in the injured area take up the defense. When describing the reaction of mammals to injury, he describes the inflammatory process much as we understand it today but strongly emphasizes the role of the polymorphonuclear and mononuclear phagocytes, which he called microphages and macrophages respectively. But once again, inflammation has proved to be more than just phagocytosis. Much as Metchnikoff advanced the subject, he did its advancement a disservice in overemphasizing phagocytosis to the point that other aspects of the subject were neglected. He did, however, clearly indicate that inflammation is an active defense mechanism which is usually beneficial.

In the early part of the 20th Century there was a renewed interest in the microcirculation in connection with inflammation. A. Krogh made extensive studies of small blood vessels. He was particularly interested in their function in the exchange of metabolites. He showed that the number of capillaries carrying blood at any one time was subject to sensitive adjustment. In addition, Sir Thomas Lewis, in his later years, turned his attention from the heart to the microcirculation, inflammation, and its chemical mediators.

In the passing generation the great exponent of the study of inflammation was Valy Menkin. It is to him that we largely owe the modern concept of the process as a whole. He brought together the vascular reactions emphasized by Cohnheim, the phagocytic activities discovered by Metchnikoff, and the importance of the biochemical mediators and immune phenomena that are the principal subjects of research today. It was particularly his work on the humoral substances that trigger and govern the process that has been valuable. While the biochemical mediators Menkin described and named are no longer adequate to explain the accumulated experimental data, they pointed the direction which modern research is following. For many years Menkin kept alive interest in inflammation when few other investigators were concerned with it.

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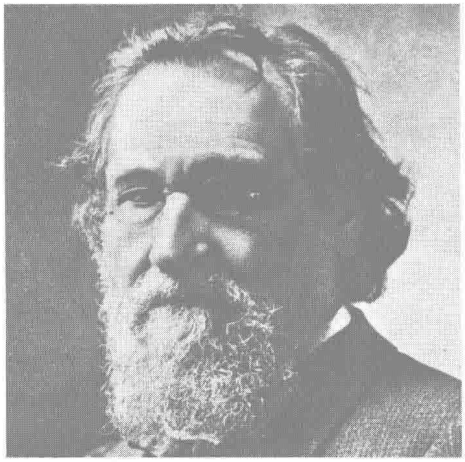

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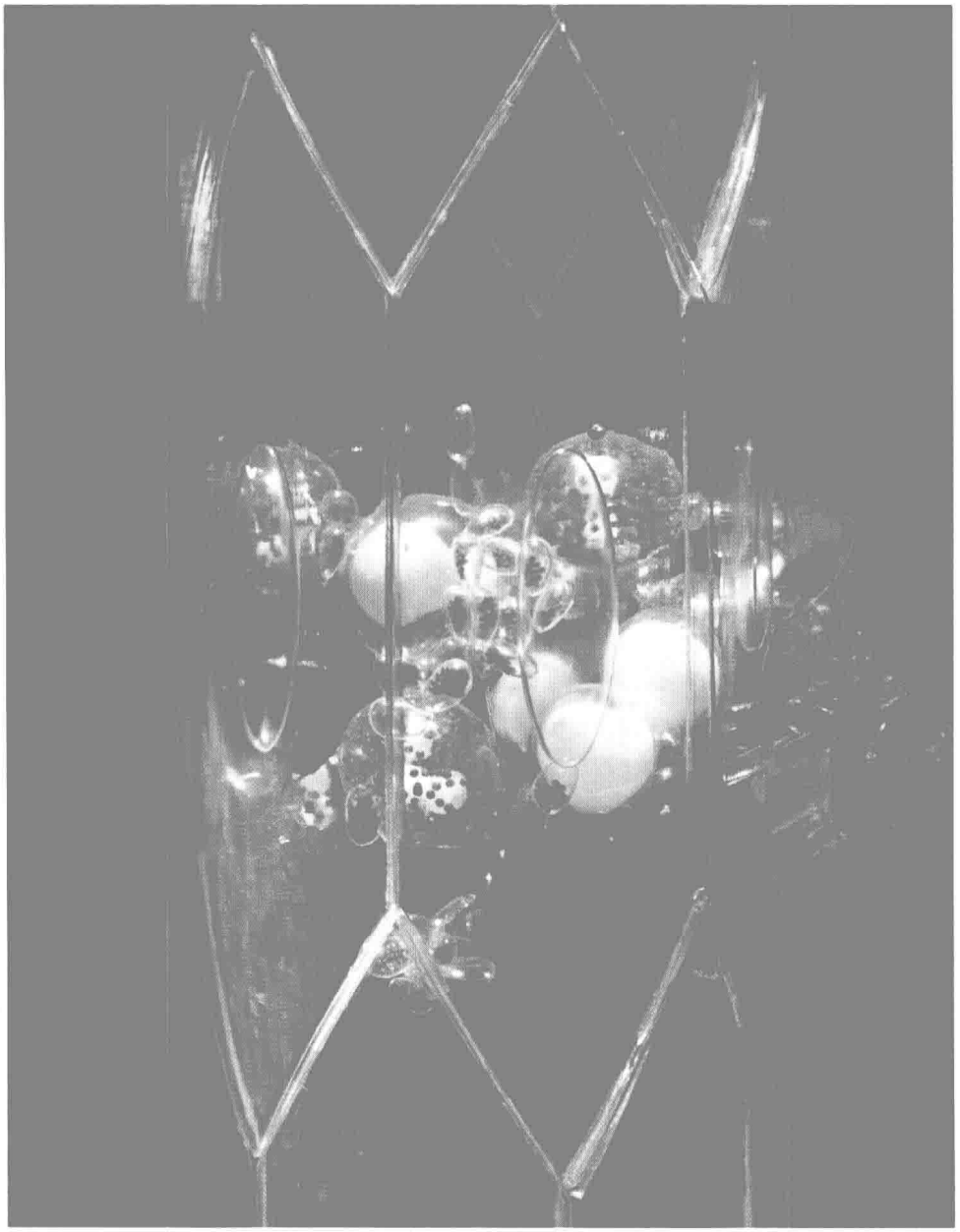
Figure 4:
Julius Cohnheim, (1839-1884), in his studies on the pathology of inflammation, pointed out the important part played by the small blood vessels. He emphasized that venules rather than capillaries merited most attention.

Figure 5:
Elie Metchnikoff, (1845-1916), demonstrated the nature of phagocytosis as a defense mechanism that engulfed and destroyed invading microorganisms.

Figure 6:
Valy Menkin, (1901-1960), carried on persistent research into the cellular nature of inflammation at a time when few others were interested. Although recent opinion considers his biochemical work unsatisfactory, he was the first to suggest polypeptides as mediators of inflammation.

Structure of the microcirculation

Figure 7:
The microcirculation appears to be the locus of greatest interest in acute inflammation. Shown here is a schematized venule in the normally healthy state, with its endothelial cells closely joined. In the lumen are lymphocytes (with large white nuclei), platelets (small ovoid shapes), erythrocytes (red), and neutrophils (with purple granules).



While it is no longer possible to believe as Cohnheim did that the entire process of inflammation is explainable on the basis of abnormalities of the small blood vessels, nevertheless, other than the initial injury itself, the earliest events in the inflammatory process involve the endothelium of the small venules. Consequently, an understanding of the anatomy of the microcirculation, i.e., the microvasculature, particularly the fine structure of the endothelial cells is essential.

The microcirculation *(Fig. 47, p. 44)*, for the purpose of this discussion, is that through arterioles, venules and their intercommunications, the arteriovenous communications and the true capillaries. Even before Henryk Hoyer (1872) first visualized them it was realized that there are fairly direct communications between arterioles and venules and that many of the true capillaries arise from these channels. B. W. Zweifach believed that since many of these intercommunicating vessels have occasional smooth muscle fibers in their walls, often near capillary junctions, it is probable that neurogenic adjustment of the flow of blood through the shunts helps govern the flow through the capillaries, because it is generally agreed that so far as adjustment of blood flow is concerned, capillaries respond passively to the pressure gradient between their two ends.

As long ago as 1896, E. H. Starling's observations led him to conclude that water and other substances left the circulation through the fine cracks between the endothelial cells of the capillaries. On the other hand, R. Heidenhain even earlier had suggested that the endothe-

lium might be actively secretory and not act as a passive porous membrane. As will be seen, these two opposing concepts in modern dress are still with us today.

In the 1950's, J. R. Pappenheimer and his associates made extensive studies of the diffusion of various substances from the circulation. They approached the subject from the physicochemical point of view with particular attention to the diffusion rates of molecules of different sizes. They employed both physiologic experiments and various types of models. It emerged that the rates of diffusion were roughly proportional to the molecular size. They concluded that their results were best explained by the assumption that a small percentage of the capillary surface consisted of cylindrical pores or narrow slits. If pores, their diameter was about 30 Angstroms, and if slits, their width was about 37 Angstroms. Pappenheimer's studies have stood the test of time; consequently, the studies of morphologists directed toward locating the sites of transport of material from the capillary lumen to the tissue spaces must take them into account. Later work by G. Grotte indicated that in addition to the

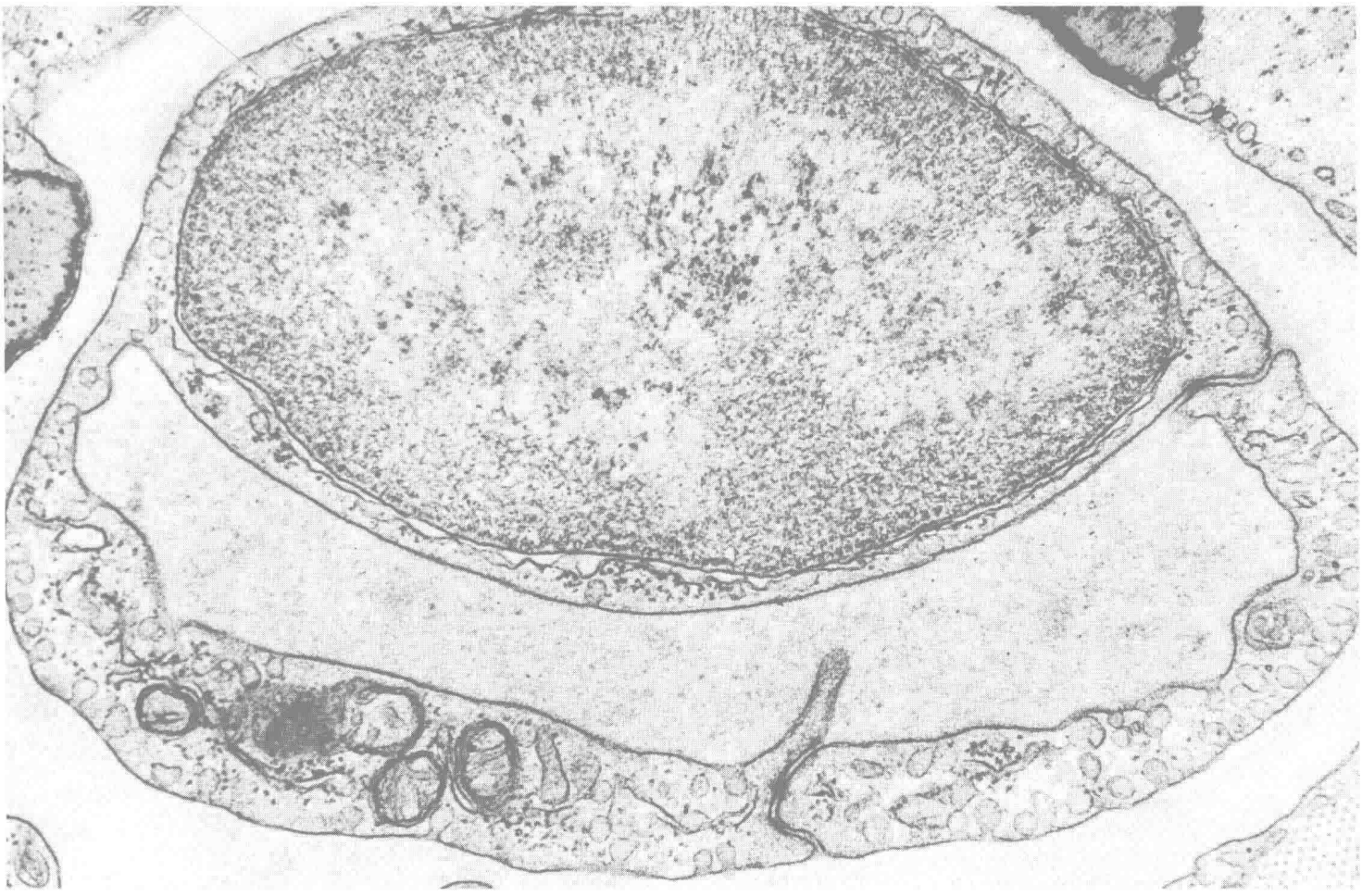


Figure 8:
The capillary in this electron micrograph displays two clearly demarcated endothelial cells and their junctions.
The nucleus of one is especially conspicuous.
An interesting feature, the function of which is not known, is the marginal fold—the long process visible at the junction at lower center.

pores postulated by Pappenheimer there were also sparsely distributed "leaks" (140 Angstroms) which passed much larger molecules.

In recent years the latest techniques of electron micrography have been utilized in depicting the fine structure of the endothelial cells and their junctions. Two other matters have also been considered by electron micrographers, the existence of an intercellular cement and an endocapillary layer both of which were hypothesized by R. Chambers and B. Zweifach from their extensive observations of the passage of blood through living capillaries.

Occasional cross sections of capillaries have been described in which there have been no junctions between cell processes. Usually, however, there are one or more junctions. In other words, the capillary is made up of a simple mosaic of flattened endothelium. Its diameter is small enough so that the cytoplasm of a single cell frequently extends completely around it. Scattered along this simple tube and closely applied to it are occasional cells, pericytes *(Fig. 9)*, whose function is still not clear, except perhaps in the retina of the eye where they are much more numerous and where they seem to have a supportive function for the endothelial cells.

Early electron micrographs of capillary endothelium indicated that these cells contain all the usual organelles found in tissue cells—mitochondria, endoplasmic reticulum, and Golgi complexes—and are bounded by the usual three-layered unit membrane. Nothing, however, corresponding to cylindrical pores was revealed, but it seemed possible that the cell junctions might be the hypothesized slits. But their width seemed in general too great for them to act like any kind of molecular sieve.

Close examination of the junctions has revealed some interesting features. Most conspicuous are the marginal folds which extend along the luminal aspect of the junctions, sometimes extending from one lip, sometimes from both *(Fig. 8)*. In cross section these, of course, appear like fingers. At high magnification the junctions are of considerable length, throughout most of which the components are a fair distance apart, but over one small segment they are closely approximated. *(Fig. 10)*. Since these tight junctions are visible in every section, it appears that there is a gasket extending completely along each of the junctions between cells. The character and consistency of these gaskets is obviously of great im-

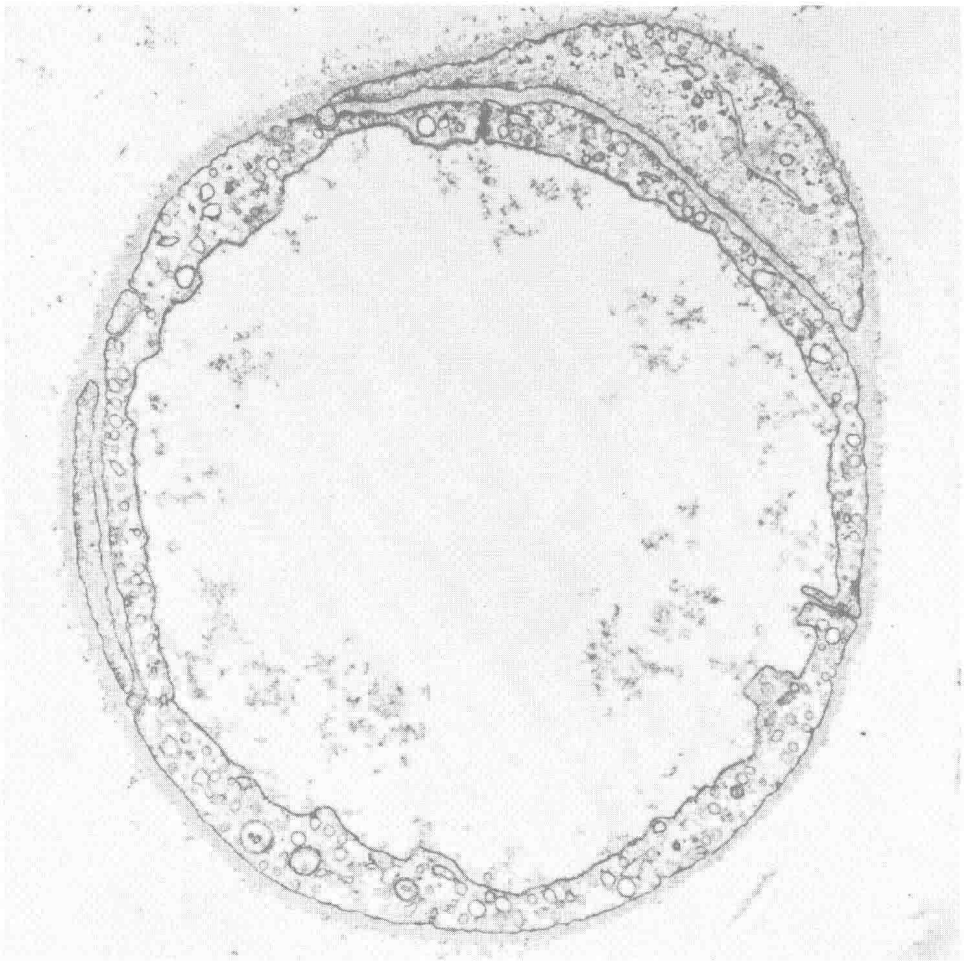


Figure 9:
A capillary, from mouse diaphragm, shows two endothelial cells partially enfolded by the processes of two pericytes The basement membrane is clearly exhibited, extending completely around the vessel and the pericytes.

portance in understanding the transport of molecules of various sizes from the capillary lumen to the tissue spaces. In sections where the three layers of the unit membrane of the endothelial cells can be distinguished, it appears that the outermost layers from each component of the junction are fused at these tight junctions.

Another interesting feature of electron micrographs of endothelial cells is the vesicles which are most conspicuous along their luminal and external margins. That most of these vesicles open onto the cell surface is indicated by the fact that electron-opaque material injected either into the vessels or the tissue spaces can be observed in the vesicles on either the inner or outer surfaces respectively *(Fig.13)*. Some vesicles appear not to be connected to either surface. These observations have led to the idea that if these vesicles travel from one cell surface to the other, they might pick up fluid from the blood and transport it through the cell.

In suitably prepared specimens electron micrographs reveal a somewhat felt-like finely fibrillar layer surrounding the external surface of the capillary. This is

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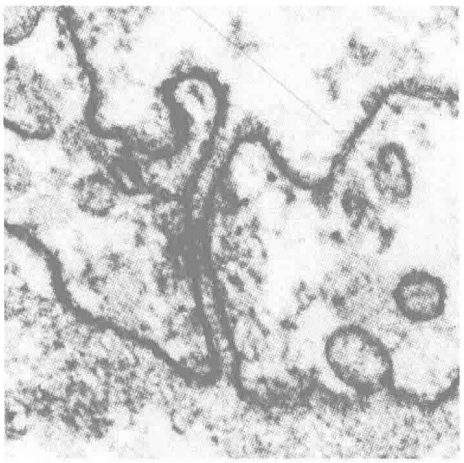

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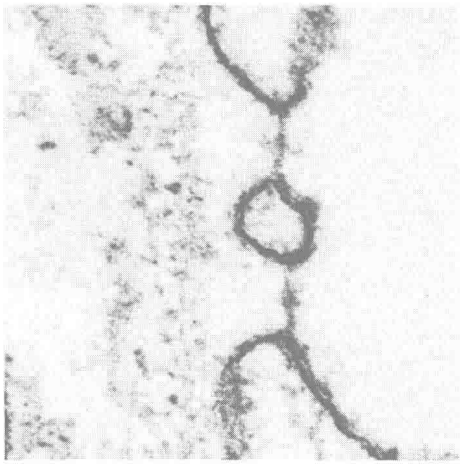

Figure 10:
A normal endothelial junction, as shown in this electron micrograph, varies from about 200 Angstroms wide to about 140 Angstroms in the region known as the "tight junction."

Figure 11:
This detail shows fenestrations in the endothelium bridged by diaphragms that appear to be continuous with the outermost layer of the endothelial cell's unit membrane.

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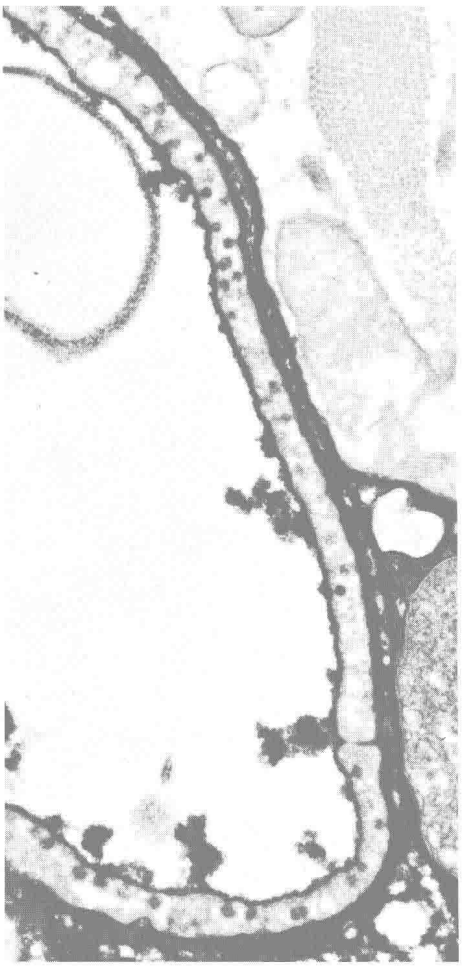

Figure 12:
A section of capillary from mouse diaphragm treated with ruthenium red, which stains mucopolysaccharides. The basement membrane and an apparent endocapillary layer are well labelled.

Figure 13:
Also labelled with ruthenium red are vesicles or apparent invaginations of the endothelium which are contiguous to the basal and luminal borders.

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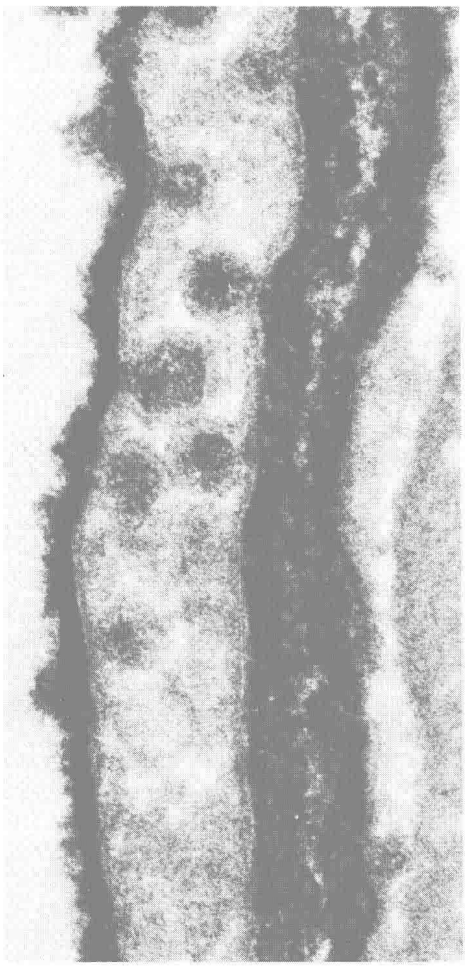

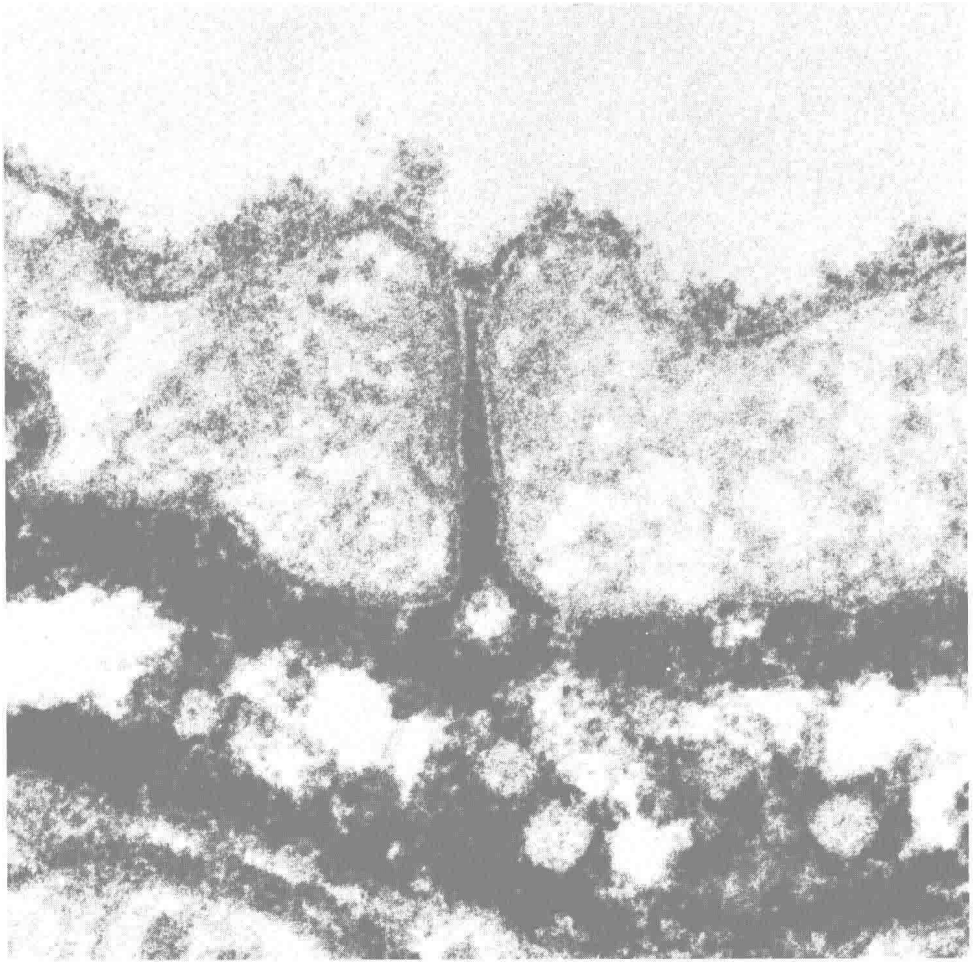

Figure 14:
The endothelial junction in this electron micrograph is filled, from the extravascular side, with material labelled by ruthenium red. It has penetrated through the "tight junction" and is diffusing along the luminal surface of the endothelium.

the basement membrane which consists largely of collagen fibers. It has been thought that this might act as a differential filter of material that had passed through the intercellular spaces. More recent studies make it seem unlikely that much filtering is done by the basement membrane except for large particles.

Electron microscopy is just as dependent upon proper methods of fixation and staining as light microscopy, and consequently, as these methods improved, more and more of the fine structure of the cells has been revealed. Furthermore, certain "staining" techniques which may not be suitable for revealing the general structure of cells may, however, be of great value in showing the presence of a particular material and indicating where in the cell it is located. An example of such a technique is the use of ruthenium red to demonstrate the presence of mucoproteins (mucopolysaccharides).

By the use of ruthenium red J. H. Luft has not only demonstrated the presence of mucopolysaccharides in the basement membrane of the endothelium in addition to the collagen fibers but the presence of a thinner layer of a similar material lining the luminal surface of these cells which probably represents the endocapillary layer described by Chambers and Zweifach *(Fig. 12)*. It further appears that similar material is present in the junctions between the cells and might constitute an intercellular cement.

Luft was also interested to see if the ruthenium red could penetrate the tight junction between the endothelial cells. In at least one preparation where the dye was permitted to diffuse from the tissue spaces toward the lumen of the vessels, it appeared to penetrate beyond the tight junction and stain a small amount of material on the luminal side of it *(Fig. 14)*. While, because of technical difficulties not yet circumvented, unequivocal proof that these junctions can be penetrated by ruthenium red is still lacking, it seemed likely to Luft that it is the mucoprotein material in these narrow isthmuses that acts as the differential filter that is responsible for the molecular sieving described by Pappenheimer. It is also of interest that estimates of the dimensions of these tight junctions correspond roughly to those of the pores or slits postulated by Pappenheimer.

A still clearer demonstration that these intercellular spaces can transmit molecules of considerable size has been furnished by M. J. Karnovsky. He used the enzyme horseradish peroxidase which has a molecular weight of about 40,000.

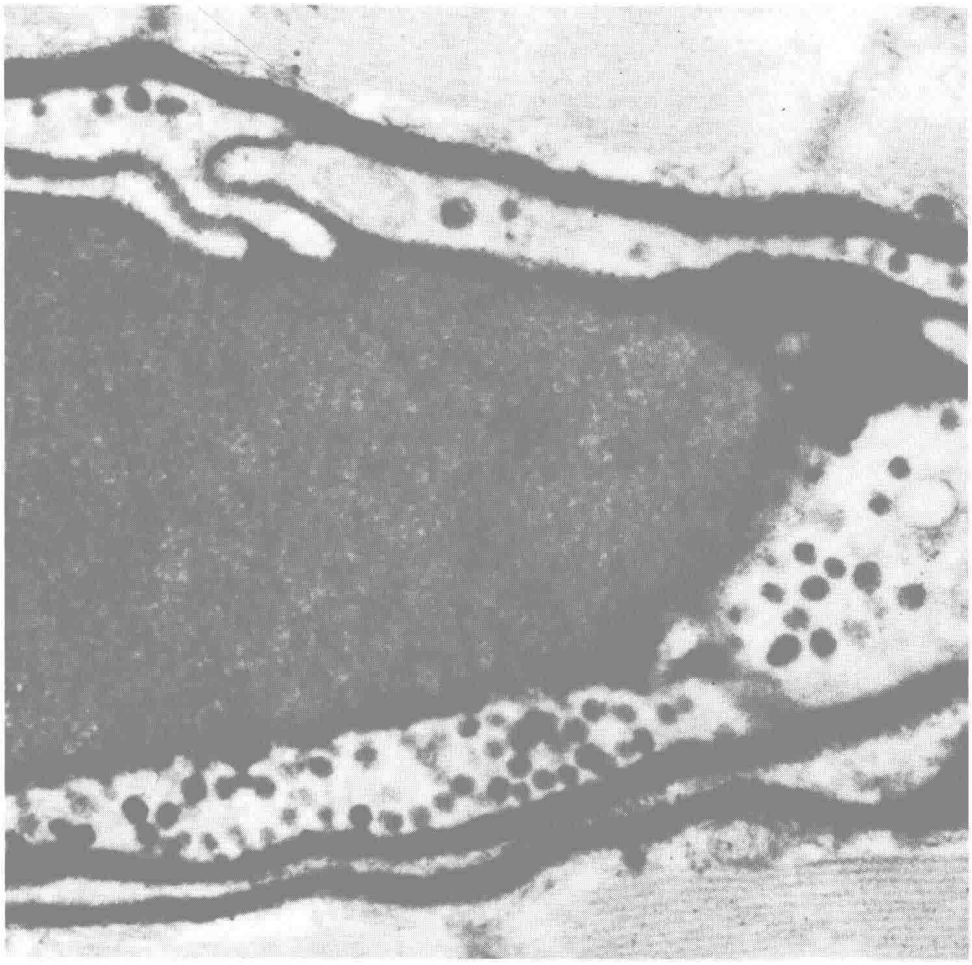

Figure 15:
Horseradish peroxidase, used in this electron micrograph, oxidizes a heavy benzidine compound and produces an electron dense substance (black). Here it has spread from the lumen, through the endothelial junctions, into the extravascular region. It has also penetrated into many vesicles, implying that they are contiguous with the endothelial surfaces.

The presence of the enzyme can be detected in the electron micrograph by its ability to oxidize a heavy benzidine compound. The experiments of this author clearly show that this enzyme when introduced into the circulation can gain access to the tissue spaces via the intercellular junctions *(Fig. 15)* since it can be visualized actually in them.

In Karnovsky's pictures there is also staining of the intracellular vesicles, those opening on the luminal surface, some with no apparent contact with either surface, and those opening on the outer surface. Consequently, some material might well have been conveyed across the endothelial cytoplasm by this means. The author, however, regarded this as a slower and less important route. That material can indeed be conveyed across the capillary wall by way of these vesicles has been shown by G. E. Palade using ferritin particles. In his opinion this is the major means of transport because he believes that the intercellular junctions are for all practical purposes impermeable under normal circumstances and that the vesicular route is entirely adequate.

Up to this point we have been considering as typical the capillaries of skeletal muscle and, of course, these make up a very large part of the total capillary bed. It should be noted, however, that in certain tissues there are capillaries whose endothelial junctions are very different from those just described, notably endocrine glands, kidney and intestinal mucosa where there may be very large gaps between endothelial cells. For example, Fig. 11 shows the gap between two endothelial cells of a capillary of mouse intestine. While the gap itself is very wide, it is bridged by a thin membrane which Luft has indicated is probably of the same composition as and continuous with the outermost layer of the unit membrane of the endothelial cell. It would appear to be this diaphanous diaphragm that performs the sieving function in these special situations.

In summary, it appears that the electron microscopists have shown two possible means for substances to leave the capillaries and gain access to the tissue spaces. At the moment the intercellular spaces seem the most probable site of the small pores capable of molecular sieving and the vesicular transport probably accounts for the passage of large particles—the leaks of Grotte. Another possibility for the leaks is a large opening that may occur at the junction of several endothelial cells.

Initial events: Increased permeability

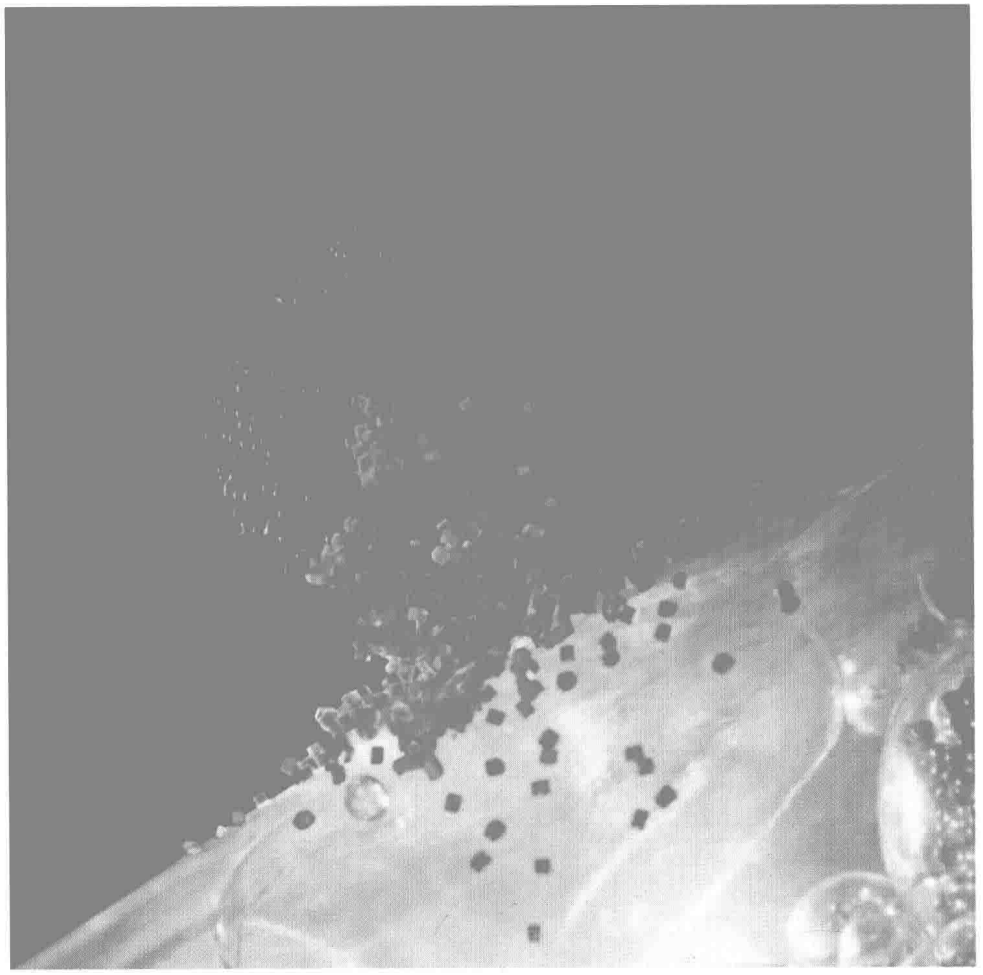

Figure 16:
The model of a mast cell, near a blood vessel, is shown discharging vasoactive substances (pink), in reaction to an injury. These substances, streaming toward the vessel wall, may be among the mediators of the inflammatory response.

Acute inflammation

There are clearly two phases to the acute inflammatory response, a brief early increase in vascular permeability of short duration and then, after a short interval, a much more prolonged second phase consisting of the following events:

1 Increased permeability;
2 Sticking of white blood cells to the vessel wall;
3 Diapedesis of white cells through the wall, sometimes accompanied by some red cells;
4 Accumulation of white cells in the injured area;
5 Phagocytosis of bacteria or other material by the white cells with the death of many of them;
6 Possible intensification of the reaction by materials released from the white cells;
7 Leakage of fibrinogen and platelets from the vessel;
8 Fibrin deposition in the area of injury;
9 Intravascular clotting with destruction of vessels;
10 Disposal by macrophages of most of the necrotic debris;
11 Migration of fibroblasts and formation of connective tissue;
12 Ingrowth of capillaries.

In time past the early phases of acute inflammation were attributed to neurogenic reactions influencing vascular flow in the affected region. In most recent work, these phenomena are almost completely neglected, although a recent paper by J. H. Brown and associates has indicated that the first phase of the acute inflammatory response does not occur in denerv-

ated tissue. The reason for this neglect of the role of nerve impulses in inflammation is that more or less typical inflammatory reactions can be produced in tissue devoid of nerves.

Whatever the role of the nervous system, it has been recognized for a long time that mediation of most of the phenomena of inflammation must be humoral arising from some substance released by injury of tissue cells. It also seems likely that once the response is set in motion by the initial injury, subsequent events are linked to each other. The search for these chemical mediators has, however, been one of the most frustrating in modern biological science. There have been no clearcut breakthroughs, and each experiment seems to raise more questions than it answers.

Histamine

For many years histamine has attracted the attention of investigators who have in many ways tried to connect it with the inflammatory process. While histamine unquestionably has the ability to increase the permeability of small blood vessels, its action is very evanescent and it does not bring about conspicuous leucocyte sticking to the vessel wall. Current work seems to confirm, however, that it is probably largely responsible for the initial, transient phase of vascular permeability. This concept largely depends on the fact that this phase can be inhibited by antihistamines. Histamine may be released from mast cells *(Figs. 16, 17).*

Vessels involved

While at one time it was thought that inflammation involved the capillaries, it is now clear that the process starts in the small venules and only later involves the true capillaries, spreading from the venous ends toward the arterioles. While this may be because of the delicacy and susceptibility of the endothelium of the venules, it also seems to have some relation to the venous nature of the blood itself, because if the flow of blood is reversed in the capillary by mechanically altering the pressure gradient, what was previously the arterial end of the vessel becomes the preferred site for inflammatory change (Zweifach).

This suggests that the fall in hydrostatic pressure from the arterial to the venous end of the small blood vessel renders the access of materials diffusing from the site of injury into the blood vessel easier at the low pressure end.

Although as has been indicated earlier there is considerable difference of opinion as to how fluid and solutes of various kinds leave the normal capillary, there is

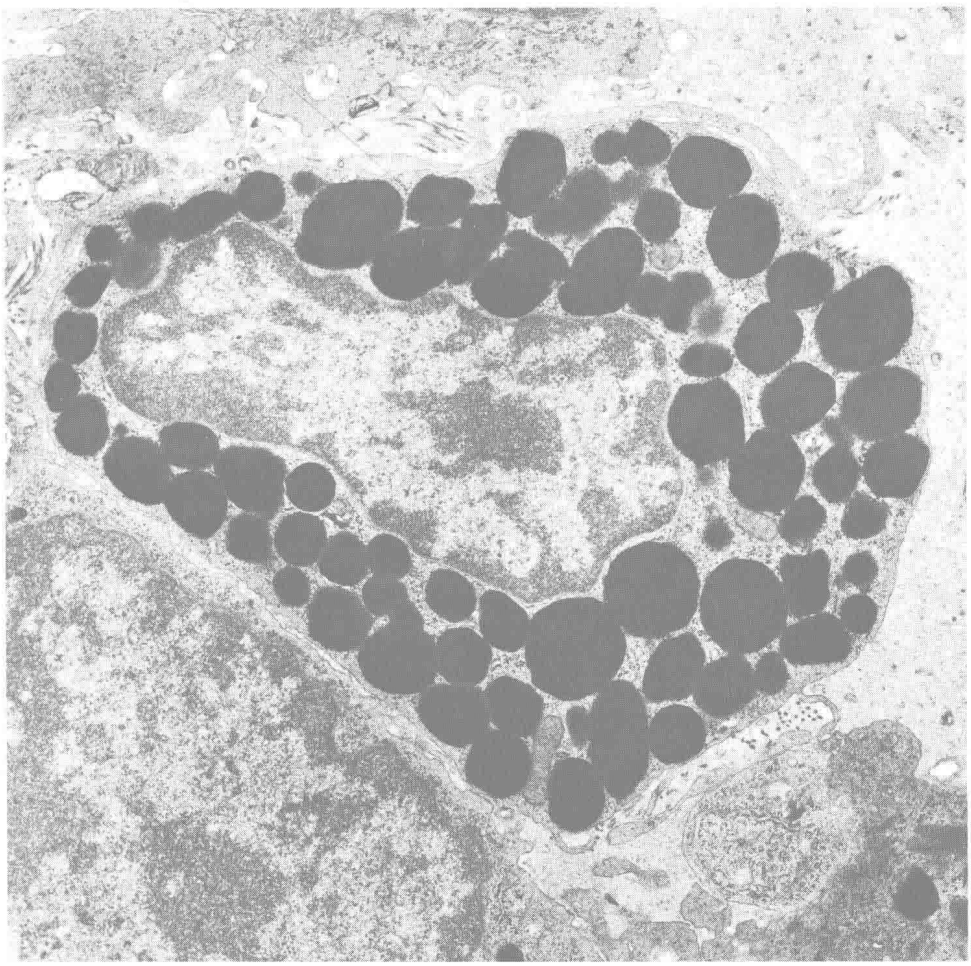

Figure 17:
An electron micrograph of a mast cell in rat intestine shows the nucleus surrounded by a cluster of conspicuous granules. Mast cells are rich in mucopolysaccharides, among which is heparin. Bioactive amines and many enzymes are also contained in mast cells and are probably released from their granules.

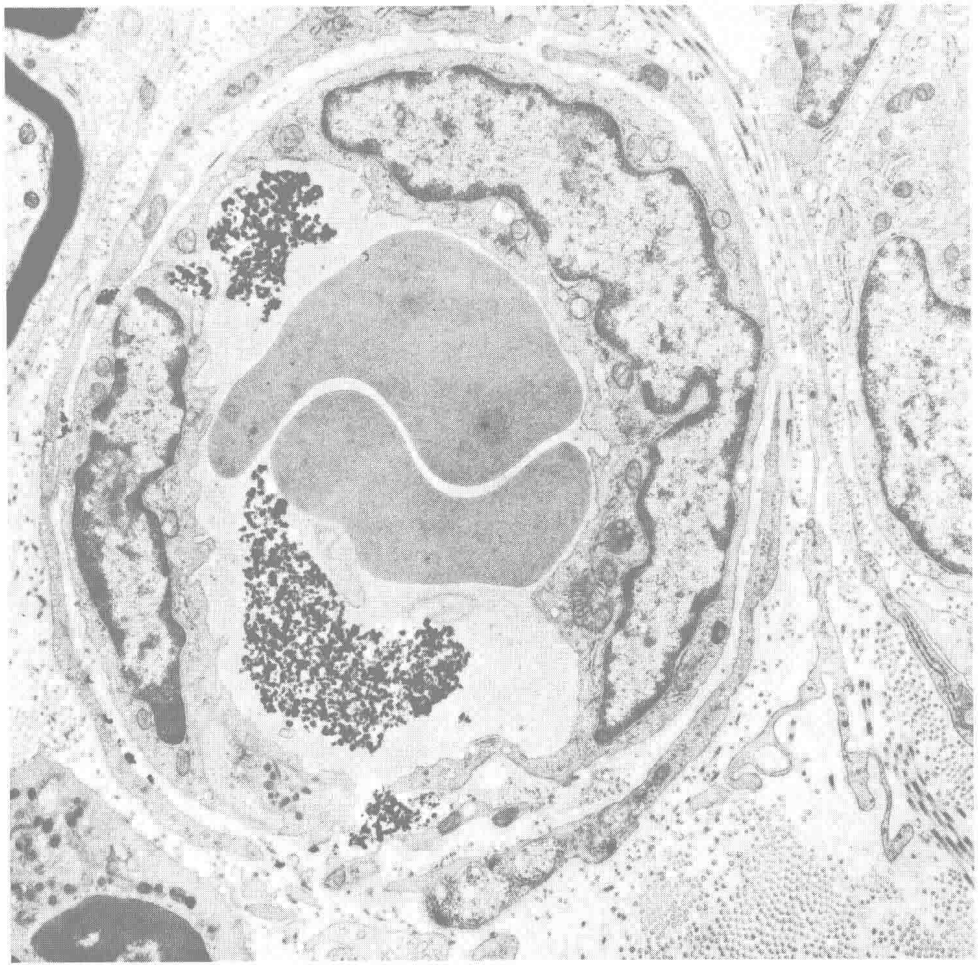

Figure 18:
Carbon particles, which have been injected into a capillary in an inflamed area of rat skin, may be seen in the lumen (in which are also two erythrocytes and two platelets).

no mystery as to how elements of the blood leave the permeable capillary in an inflamed area. The endothelial cells seem to round up, thereby pulling away from their attachments to each other and leaving sizable gaps through which both fluid and cells can escape *(Figs. 18-20).*

Kinins

In considering the mediators of the delayed increase in permeability in acute inflammation it is as well to state at the beginning that no thoroughly satisfactory candidate has yet been found.

Years ago, Valy Menkin felt that the substance which he isolated from inflammatory exudate and called leukotaxin was this mediator. Current investigators have found that this extract is not a pure substance as Menkin believed it to be, but a mixture of various materials, and that its activity is not due to the polypeptide which he believed to be the active substance but probably to other materials present in smaller amounts. Nevertheless, his work did call attention to the fact that the mediator was probably not a simple substance but a proteinaceous material of some molecular size and complexity. Current work seems to indicate that probably the mediators responsible for the increased permeability of blood vessels and for leucocyte sticking followed by emigration are separate and distinct substances and not a single substance as was at one time thought.

To date the most likely candidate for the mediator of the prolonged phase of the acute inflammatory process is a kinin. These substances are exceedingly potent inducers of increased permeability in venules and to a lesser extent in capillaries. While they are normally destroyed very rapidly in tissue by kininases, it is possible that they can be quite as rapidly released at the site of an acute inflammation. They are not nearly so potent at promoting the sticking of leucocytes and their subsequent migration through the vascular wall as they are at increasing permeability. It is now likely that these two manifestations of acute inflammation are mediated by different substances. Because there are no specific antagonists of the kinins, experiments similar to those done with antihistamines are not possible. There are, however, antagonists to the formation of kinins, and these substances are anti-inflammatory.

Kinins are polypeptides with powerful pharmacologic actions. They strongly affect the contraction of smooth muscle, produce hypotension, cause increased vascular permeability in small blood vessels, and induce severe pain when in-