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68

Inflammatory Reaction

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Continuation of Ergebnisse der Pathologie

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Ultrastructure of Acute Inflammation

GÉRARD T. SIMON

I. Introduction

The gross morphological aspects of acute inflammation were known by the ancient world (*Majno,* 1975). One had, however, to wait for investigations of *Julius Cohnheim* (1867, 1873, 1889) and *Elie Metschnikoff* (1893, 1907) to get some insight, at the microscopic level, into the histologic changes observed during the first phase of inflammation.

This chapter deals only with the ultrastructural modifications observed during acute inflammation. Since the term ultrastructure is sometimes misinterpreted, a short de-

finition might be useful. Ultrastructural studies are concerned with the internal structure and its modifications of, in this particular case, biologic materials and use many types of analysis at levels of resolution beyond the resolving power of the light microscope.

Florey (*Marchesi* and *Florey,* 1961) initiated the ultrastructural investigation of inflammation. Even at this early time, *Florey* was very conscious of the limitations of this mode of investigation and insisted that this technique "does not explain how and why the sequence of events that follow injury take place" (*Florey,* 1970). Only correlation of the biochemical and morphological aspects of inflammation will enable us to understand the basic mechanisms involved. It is, however, not the purpose of this chapter to make such a correlation, for other chapters in this volume deal with the relationship between the biochemical and morphological aspects of inflammation.

In this chapter, it is our intention to discuss a) the changes observed during increased vascular permeability, b) the migration of circulating blood cells, particularly during the passage of polymorphonuclear neutrophils (PMN) and monocytes through the vessel walls, with subsequent enhancement of the phagocytic properties of PMN, macrophages, and platelets.

II. Increased Vascular Permeability

Although historically it is the migration of polymorphonuclear neutrophils which has been shown at the light as well as the electron microscopic level to be the first major feature of acute inflammation (*Metschnikoff,* 1893, 1907; *Marchesi* and *Florey,* 1961), this extravasation is preceeded by several modifications of the vascular wall which result in an increased permeability for fluids and large molecules in the capillaries and postcapillary venules (PCVs) (*Rous* et al., 1930; *Smith* and *Rous,* 1931; *Smith* and *Dick,* 1932; *Burke* and *Miles,* 1958; *Majno* et al., 1961, 1967; *Wells* and *Miles,* 1963; *Movat* and *Fernando,* 1963; *Majno,* 1964, 1965; *Cotran* and *Majno,* 1964b; *Cotran,* 1967a, b; *Cotran* et al., 1967; *Tasaki,* 1968). The term "increased permeability" refers to two different phenomena which should be strictly separated. This difference has been well established in the capillary loops of the renal glomeruli by nephrologists. They refer to 1) increased filtration and 2) increased permeability (*Hamburger* et al., 1972). Increased filtration enhances the passage of molecules which normally traverse the capillary barrier. In tissues, this enhanced filtration of fluids and small molecules is partly responsible for the initial oedema observed in inflamed territories. In contrast, increased permeability allows the passage through the wall of the vessels of particles which under normal conditions are not able to traverse the vascular wall (*Pappenheimer* et al., 1951; *Pappenheimer,* 1953; *Wallenius,* 1954). There is evidence that these two phenomena do not necessarily follow in sequence, and that even leucocyte migration can occur without increased permeability (*Hurley,* 1963, 1964). In order to visualize the modifications in structure related to these two types of vascular abnormality, a short analysis of the investigative methods will be given.

1. Tracers to Investigate Increased Filtration and Increased Vascular Permeability

In order to see at the ultrastructural level the increased passage of fluids and molecules through the vascular wall, tracers have to be used. For obvious reasons these substances have to be electron dense. Three different types of tracers can be used: (a) tracers that are electron dense by themselves, (b) tracers that can be coupled with an electron dense particle before injection, and (c) tracers that can be revealed by coupling with an electron dense substance in fixed or processed material. This necessity to use electron dense components considerably reduces the choice of tracers. Most substances which are electron dense are of large diameter, as for example, colloidal carbon, mercuric sulfide, or ferritin. Smaller tracers like horseradish peroxidase, heme peptide, or dextran have to be rendered electron dense by coupling them with appropriate heavy metals during the processing of the biologic material. This last method is inevitably associated with the possibility of a nonspecific deposition of the heavy metal. The coupling, prior to use, of electron dense components to small tracers increases their diameter, limiting their suitability to the study of the passage of molecules of low molecular weight.

a) Increased Filtration

According to *Pappenheimer* et al. (1951) and *Pappenheimer* (1953), molecules with a diameter of less than 30 Å pass freely through the vessel walls. This has been largely confirmed by recent studies, mostly done on normal vessels. Tracers of very small diameters have been introduced: horseradish peroxidase (50–60 Å, *Graham* and *Karnovsky,* 1966a, b; *Karnovsky,* 1967), cytochrome c (30 Å, *Karnovsky* and *Rice,* 1969), heme peptide (microperoxidase 20 Å, *Feder,* 1970, 1971), and Dextran 20 (40 Å, *Simionescu* and *Palade,* 1971). It is obvious that only small tracers of this size can be used to assess increased passage of the fluids and small molecules that normally traverse the vascular wall. The introduction of these tracers of small diameter is relatively recent, and to the best of our knowledge, no quantitative studies of their passage during acute inflammation have been done. Therefore, our understanding of the part played by increased filtration is very limited.

b) Increased Permeability

A great number of studies have been done on the increased permeability of the vascular wall which develops in acute inflammation, and most authors refer to it as "vascular leakage" (*Majno* et al., 1961, 1967; *Cotran* and *Majno,* 1964b; *Cotran,* 1967b).

The tracers used have a diameter greater than 30 Å, colloidal carbon (200–300 Å, *Majno* et al., 1961, 1967; *Majno,* 1964; *Cotran* and *Majno,* 1964b; *Cotran,* 1965, 1967b; *Ham* and *Hurley,* 1965, 1968; *Cotran* et al., 1967; *Walters* et al., 1968; *Hurley* and *Edwards,* 1969), horseradish peroxidase (50–60 Å, *Graham* and *Karnovsky,* 1966a; *Karnovsky,* 1967), colloidal gold (40–150 Å, *Palade,* 1960), mercuric sulfide (70–120 Å, *Majno* et al., 1961; *Majno,* 1964), or ferritin (100–120 Å, *Bruns* and *Palade,* 1968). Massive passage of these molecules indicates that the vascular wall has changed its pro-

perties to allow the extravasation of these molecules, which would not traverse the vascular wall under normal conditions (Fig. 1).

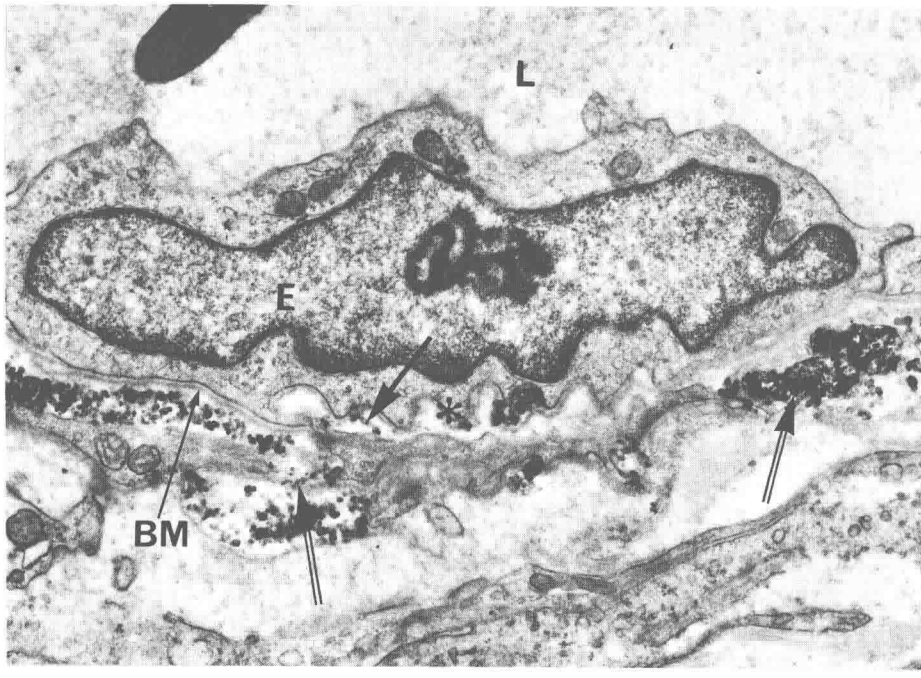

Fig. 1. After intravenous injection of colloidal carbon the particles are found between the endothelial cell (*E*) and basement membrane (*arrow*) or extravasated (*double arrows*). Note that when particles pass the basement membrane the latter appears to be fluffy and irregular (*). *L* = lumen, *BM* = basement membrane. × 7500, (reduced to 87%)

One of the concerns in using substances of this kind is that the tracer itself may increase the permeability of the vessel wall. In other words, the passage of this type of tracer does not always reflect the leakage induced by an inflammatory reaction, but may reflect injury caused by the tracers. For example, the injection of large quantities of colloidal carbon induces modifications in the lymph nodes which are similar to those observed in acute inflammation. Pletelets aggregate, fibrin filaments are observed, the carbon leaks massively through the wall of the PCV (Figs. 2 and 3) and other capillaries, and more monocytic cells migrate through the walls of these vessels (*Nopajaroonsri* et al., 1974).

Fig. 2. Massive administration of colloidal carbon induces an acute inflammatory reaction. In the lumen (*L*) of this vessel, the particles are admixed with fibrin. A monocyte (Mo_1) has already phagocytosed carbon and is in the process of migration through the wall. Another monocyte containing carbon (Mo_2) has already passed the vascular barrier. Note that free carbon has already been extravasated (*arrow*). × 3000, (reduced to 87%)

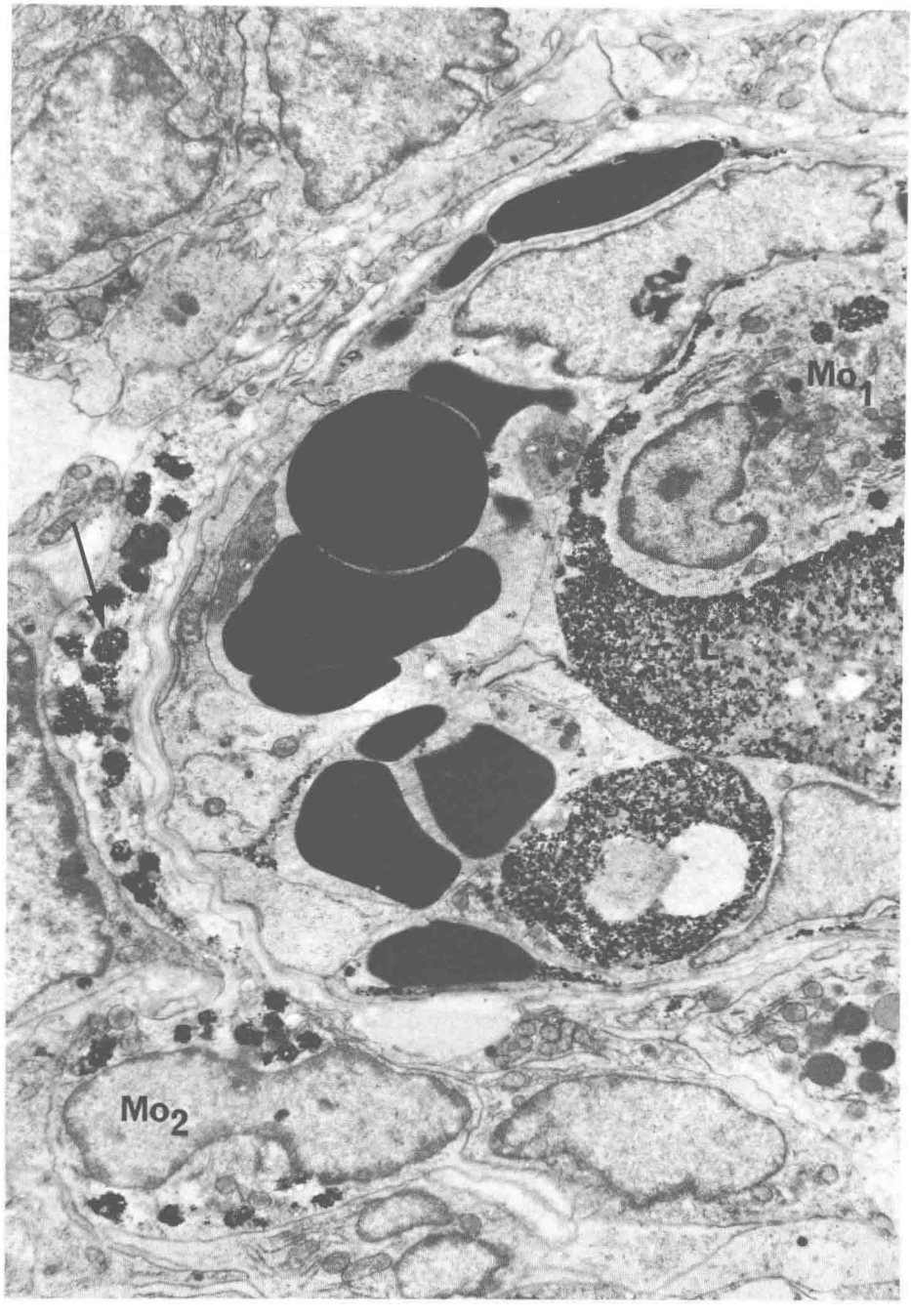

Fig. 2. Legend see page 4

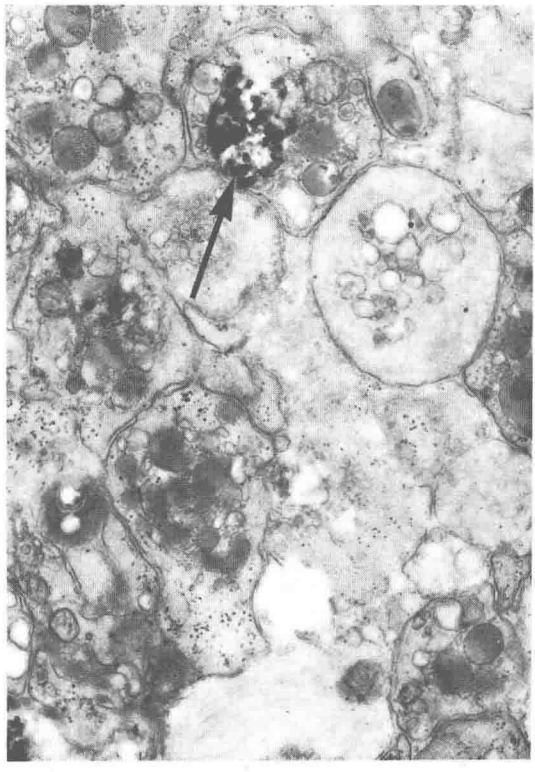

Fig. 3. Administration of a large dose of carbon involving an acute inflammatory reaction induces platelet aggregation. These platelets show phagocytic properties (*arrow*). X 28,700

Also, different tracers react differently. Colloidal carbon and peroxidase behave as amorphous particulate matter and their pathway proves to be essentially intercellular (*Graham* and *Karnovsky,* 1966b; *Majno* et al., 1967). Colloidal gold and ferritin, in contrast, are seen in large quantities in pinocytic vesicles of the endothelial cells. Controversy exists as to whether the transendothelial passage or the intercellular route represents the major pathway (*Palade,* 1960; *Bruns* and *Palade,* 1968).

The concentration of the tracer plays an important role in the interpretation of the results. For example, 10 mg colloidal carbon/100 g body weight injected intravenously in rabbits or rats will not be engulfed by the pinocytic vesicles of endothelial cells (*Burke* and *Simon,* 1970). However, at a higher dosage or if the concentration of the tracer in a particular vessel becomes high, the endothelial cells start to phagocytose the tracer (*Nopajaroonsri* and *Simon,* 1971; *Luk* and *Simon,* 1974) (Fig. 4).

Mercuric sulfide was used in early investigations (*Majno* and *Palade,* 1961; *Majno* et al., 1961; *Majno,* 1964), but has been almost completely abandoned because of its toxicity, raising doubt as to the relevance of the observations made.

It is doubtful whether ferritin can be used as an effective tracer to show leakage in the case of acute inflammation. In their paper on the permeability of the glomerular capillary walls, *Farquhar* and *Palade* (1961) showed clearly that ferritin molecules deposit randomly along the plasma membrane of the endothelial cells. This observation is confirmed in the study of muscle capillaries by *Bruns* and *Palade* (1968). Ferritin is phagocytosed where it attaches to the plasma membrane. In contrast, under the same conditions colloidal carbon is not engulfed by the endothelial cells.

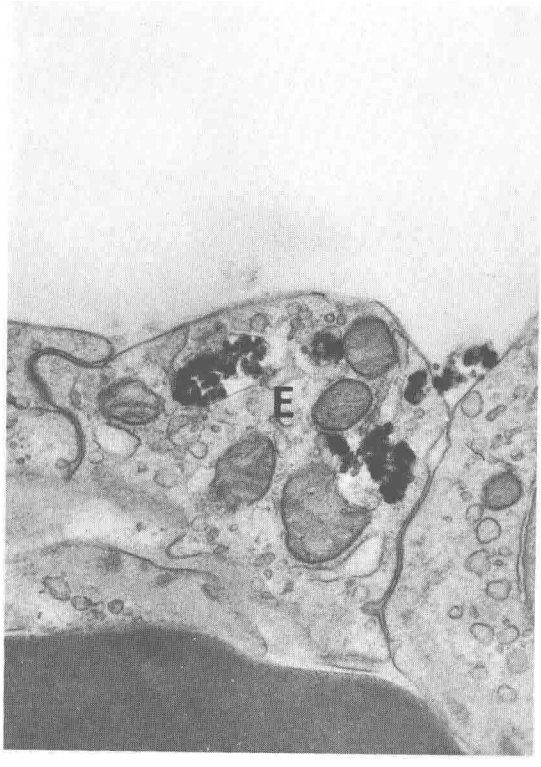

Fig. 4. An endothelial cell (*E*) engulfs carbon particles only in case of administration of large doses or when the particulate matter concentrates in a given vessel segment. X 26,800

2. Location

It is to the merit of *Majno* and *Palade* (1961) and *Majno* et al. (1961) that they first showed that the leakage in acute inflammation occurs mainly in the PCVs. As stated above, the demonstration of this leakage was made with rather large particles. It is still unclear if increased filtration of small molecules occurs concomitantly at the capillaries level. The fundamental observation of *Majno* and *Palade* (1961) concerning the site of leakage led to the belief that the PCV is the unique site of increased permeability and migration of PMNs and monocytes. Many authors have forgotten or overlooked that *Majno* et al. (1961) stated clearly that the leakage occurred mainly and not exclusively in the PCVs (Fig. 5). Further studies (*Majno* and *Palade,* 1961; *Cotran* and *Majno,* 1964a, b; *Majno,* 1964, 1965; *Cotran,* 1965, 1967b; *Majno* et al., 1967; *Majno* and *Leventhal,* 1967; *Cotran* et al., 1967; *Hurley* et al., 1967; *Ham* and *Hurley,* 1968) have shown that if the inflammation is severe, the leakage extends to capillary segments, most probably those adjacent to the PCVs (Fig. 1).

During the acute phase of inflammation, ultrastructural examination demonstrates that the vascular leakage occurs in vessels having all the characteristics of PCVs, namely, tall endothelial cells with prominent organelles and numerous pinocytic vesicles. Between these cells junctional complexes are rarely observed. This type of vessel has numerous pericytes (Fig. 6). In normal conditions, vessels with these characteristics

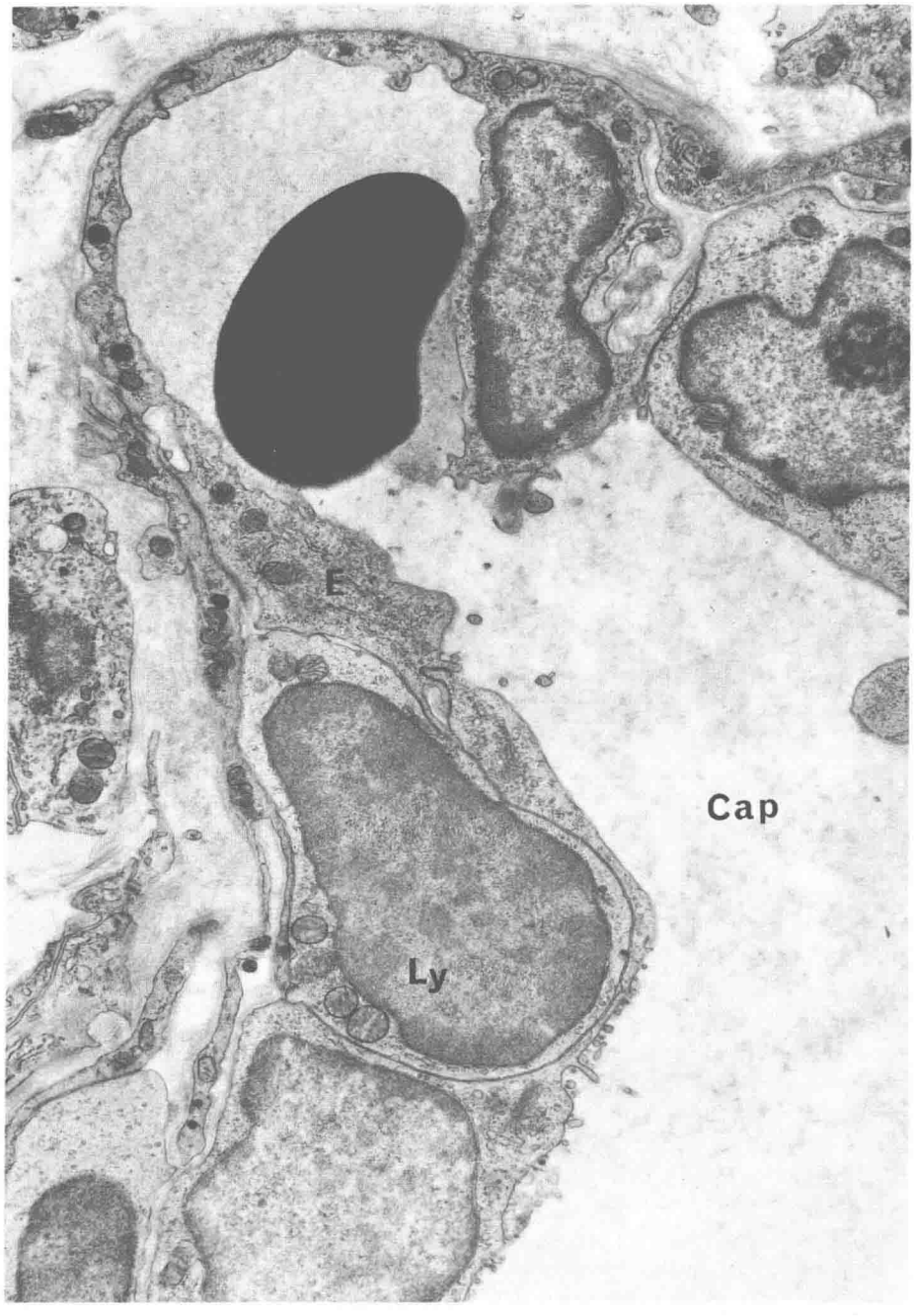

Fig. 5. Legend see page 9

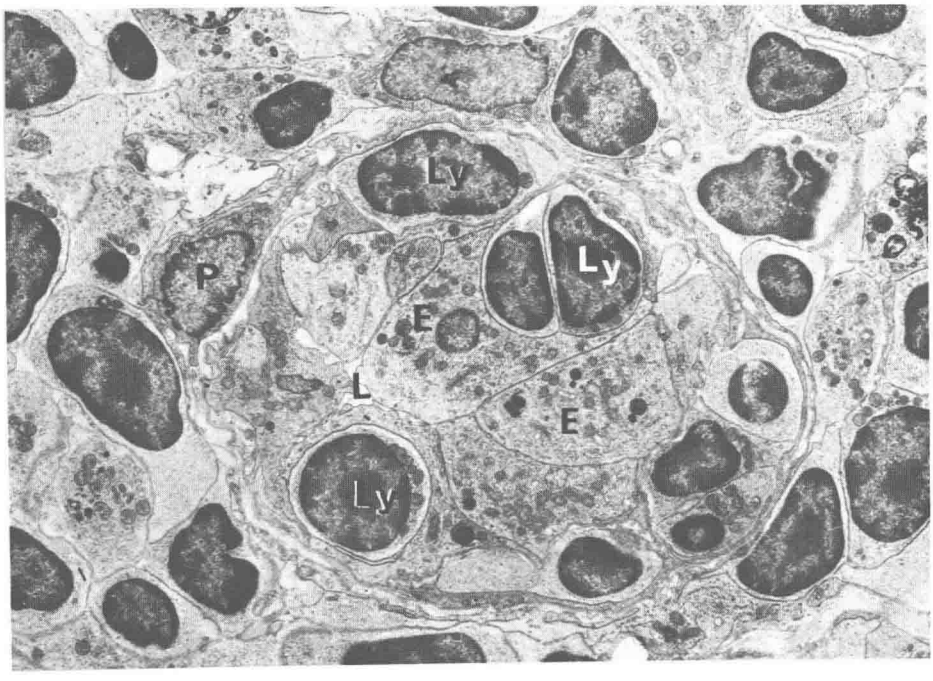

Fig. 6. A postcapillary venule (PCV) in a normal lymph node. The endothelial cells (*E*) are tall and their cytoplasm exhibits prominent organelles. In this PCV, lymphocytes (*Ly*) are crossing the wall. Note that the lumen (*L*) is reduced. *P* = pericyte. X 4000, (reduced to 87%)

are sometimes difficult to find, leading to the suggestion that the endothelial cells of the PCV react very rapidly to the inflammation by increasing the volume of their cytoplasm and the number of their organelles. It is therefore plausible that the endothelial cells of the capillary segment adjacent to the PCV could undergo similar modifications, thus giving this vascular segment all the appearances of a PCV (*Nopajaroonsri* et al., 1974).

In lymph nodes where the PCVs are very prominent and subject to a constant leakage corresponding to a kind of "a physiologic chronic stage of inflammation," experimentally induced acute inflammation will extend the leakage to capillary segments exhibiting low endothelial cells and few pericytes (*Nopajaroonsri* et al., 1974) (Fig. 7).

Although there is no doubt that the increased vascular permeability occurs mainly at the level of the PCVs, it should not be forgotten that according to the severity and type of injury, segments of the capillary network might also be involved.

Fig. 5. This capillary (*Cap*) in a lymph node shows the passage of a lymphocyte (*Ly*) through its wall. This vessel has a low endothelium (*E*) and does not correspond to the classical definition of a postcapillary venule. After injection of tracer the latter also passes the wall of such capillaries. X 12,300, (reduced to 87%)

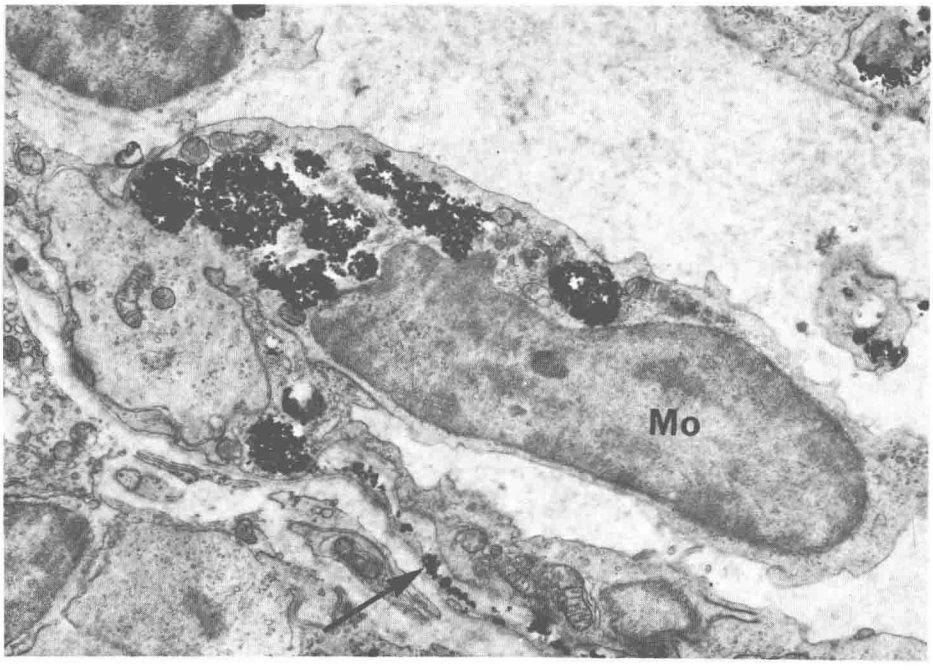

Fig. 7. This micrograph is another example to show that capillaries with low endothelial cells are sometimes the site of leakage during an acute inflammatory reaction. The monocyte (*Mo*) is in the process of passing the wall of this capillary. Note that free particulate matter has already been extravasated (*arrow*). × 10,400, (reduced to 87%)

3. Pathway of Tracers

As already mentioned, the behavior of tracers varies according to their nature and concentration. It is for this reason that some authors (*Palade,* 1960; *Farquhar* and *Palade,* 1961; *Bruns* and *Palade,* 1968) emphasize a transendothelial pathway and others an intercellular pathway (*Majno* and *Palade,* 1961; *Hurley,* 1963; *Movat* and *Fernando,* 1963; *Cotran,* 1967b; *Nopajaroonsri* et al., 1974).

a) Transcellular Passage

Before the observations of *Majno* and *Palade* (1961), it was thought that the increased permeability occurred mainly at the capillary level. The presence of junctional complexes between the endothelial cells (*Majno,* 1965; *Simon,* 1965) led to the belief that large molecules were transported through the endothelium by pinocytic vacuoles which have a diameter of 600–700 Å (*Palade,* 1961).

However, it has always been difficult to distinguish between segments of smooth endoplasmic reticulum, transport vesicles, "micro" phagosomes, and cytopemptic vesicles (*Palade,* 1953), and the idea that the passage of fluids and low molecular