Inflammation, Immunity and Hypersensitivity

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Preface

Some three or four years ago I was asked to coedit a large treatise on general pathology, with emphasis on new developments, particularly ultrastructural changes. Although the complete text as originally planned did not materialize, the authors contributing to the portion of the book for which I was responsible, dealing with inflammation and immunity, encouraged me to complete that section as a separate monograph. Thus INFLAMMATION, IMMUNITY AND HYPERSENSITIVITY came into being.

This monograph covers the acute inflammatory process and most of what is known as "immunopathology." The book, we trust, will be of particular interest to experimental pathologists, immunologists, allergists, and graduate students. While it is not intended as a text for medical students, they may find it useful as a source of reference.

The contents are organized into five parts. The first one, dealing with the inflammatory reaction in general, encompasses the entire field of acute inflammation. Emphasis is focused on the changes which occur at the blood-tissue barrier and on the chemico-pharmacological mediators of the vascular phenomena of inflammation. The second part deals with cellular immunology and includes development of the antibody-forming apparatus and antibody formation, delayed hypersensitivity, transplantation immunity, and autoimmunity. Immediate hypersensitivity, covered in the third part, consists of chapters dealing with anaphylaxis, the Arthus phenomenon, and serum sickness. Although alluded to repeatedly in every chapter, the complement system is reviewed briefly in the fourth part. Two chapters comprise the fifth part. The first, dealing with the local Shwartzman reaction, represents a special form of acute inflammatory process. Its relation to immunity and hypersensitivity, although repeatedly proposed, has not been established. The last chapter is entitled "The Reaction of the Blood to Injury." I was taught many years ago that *"Blut ist ein ganz besonderer Saft"* ("blood is a very special juice," Mephistopheles, in Goethe's *Faust*). However, it was not until much later that, through my association with Dr. Fraser Mustard, I learned of the very special role that blood plays in the inflammatory process.

The writing of this monograph would not have been possible had it not been for the encouragement and work of the contributors. With some, Drs. Irvin Broder, Douglas McGregor, Fraser Mustard, Norton Taichman, and Keiji Udaka, I have been associated for some time. The others I have known for many years. Dr. Robert McCluskey was particularly helpful in reorganizing portions of the original text into this monograph. I am grateful to all for invaluable help.

TORONTO

Henry Z. Movat

Contents

Chapter 1

The Acute Inflammatory Reaction

Henry Z. Movat

Supported by the Medical Research Council of Canada, the Ontario Heart Foundation, the Canadian Arthritis and Rheumatism Society, and the J. P. Bickell Foundation.

"Inflammation" is the reaction of living tissue to injury which comprises the series of changes of the terminal vascular bed, of the blood, and of the connective tissue which tends to eliminate the injurious agent and to repair the damaged tissue.

A SHORT HISTORY OF INFLAMMATION

Inflammation was known as *phlogosis* to the Greeks and as *inflammatio* to the Romans. The four cardinal signs and symptoms of inflammation—*rubor, calor, tumor,* and *dolor* (redness, heat, swelling, and pain)—are said to date back to Celsus (c. A.D. 178).* To these Galen (c. A.D. 130–200) is believed to have added *functio laesa* (loss of function). It was not until the nineteenth century that the precise mechanism of signs and symptoms was elucidated. According to Marchand (1924) the ancient and medieval concept of inflammation was that it represented an accumulation of heat originating in the heart, followed by an influx of blood, mucus, and bile.

This *humoral theory* of inflammation was gradually replaced in the eighteenth century by the *vascular theory* (Marchand, 1924; Ehrich, 1956). It was John Hunter (1728–1793) who showed that the rubor of inflammation is due to increased blood flow through dilated vessels. He was the first to recognize that inflammation is associated with exudation of plasma and that suppuration is due to extravasation of small globules. In the early part of the nineteenth century the vascular changes were attributed to neurogenic factors (see Ehrich, 1956).

Following the discovery of the cell by Schleiden (1838) and Schwann (1839), the present concept of inflammation gradually evolved. Virchow (1821–1905) made the observation that inflammation is preceded by cell and tissue injury and that the vascular changes occur secondary to such cell injury (Virchow, 1858). However, it was Virchow's pupil, Julius Cohnheim (1839–1884) (Fig. 1-1), who described in great detail the vascular changes in inflammation, including the emigration of leukocytes (Cohnheim, 1867, 1873, 1889), although the latter had been observed before by Dutrochet (1824), Addison (1843), and Waller (1846). Using the frog's tongue and mesentery and the rabbit's ear, Cohnheim described the circulatory changes which take place in the terminal vascular bed, i.e.,

* For a criticism of Celsus, see G. Majno. Mechanisms of Abnormal Vascular Permeability in Acute Inflammation. In *Injury, Inflammation and Immunity,*" p. 58 (L. Thomas, J. W. Uhr, and L. Grant, eds.). Williams & Wilkins, Baltimore, 1964.

FIGURE 1-1. Julius Cohnheim (1839–1884).

hyperemia, followed by slowing of the blood and, with more severe injury, complete standstill. Cohnheim's in vivo observations included also the margination and sticking of leukocytes and their migration through the vascular wall. He also described circular zones showing varying intensities of circulatory changes around a central point of injury.

While Cohnheim stressed the local circulatory disturbances in inflammation, Metchnikoff (1845–1916) (Fig. 1-2) emphasized phagocytosis and elimination of the inflammatory agent by phagocytic cells (Metchnikoff, 1893, 1906). In a study of the comparative pathology of inflammation he showed that lowest animals, e.g., sponges, reacted to injury only with phagocytosis by mesenchymal cells. A vascular reaction with emigration of leukocytes was observed in animals with a vascular system consisting of arteries, veins, and capillaries. He pointed out the relatively late phylogenetic participation of the blood and the vascular system in the inflammatory response. Metchnikoff was criticized, particularly by German pathologists of the late nineteenth and early twentieth centuries (see Ehrich, 1956). However, although in higher animals phagocytosis alone, without a vascular reaction, is not looked upon as an inflammatory reaction, removal of the inflammatory agent by inflammatory cells is one of the principal functions of inflammation. This, and the demonstration that the complex inflammatory response of man and higher animals developed gradually from the primitive digestive ability of the mesenchyme, are the contribution of Metchnikoff (who was a zoologist) to medical science.

Parallel to the work of Metchnikoff and Cohnheim, a new science, that of immunology, was developing. Beginning with the observations of Jenner (1749–1823) on vaccination, and reaching a peak toward the end of the nineteenth and the beginning of the twentieth centuries with Pasteur (1822–1895), von Behring (1854–1917), Ehrich (1854–1915), and Landsteiner (1868–1943), this new branch of medicine and biology was also concerned with the body's defenses. Immunologists accumulated evidence that the body fluids and in particular the blood serum (serology) had a protective effect against invasive microorganisms which was equal to, or even greater than, that of cells.

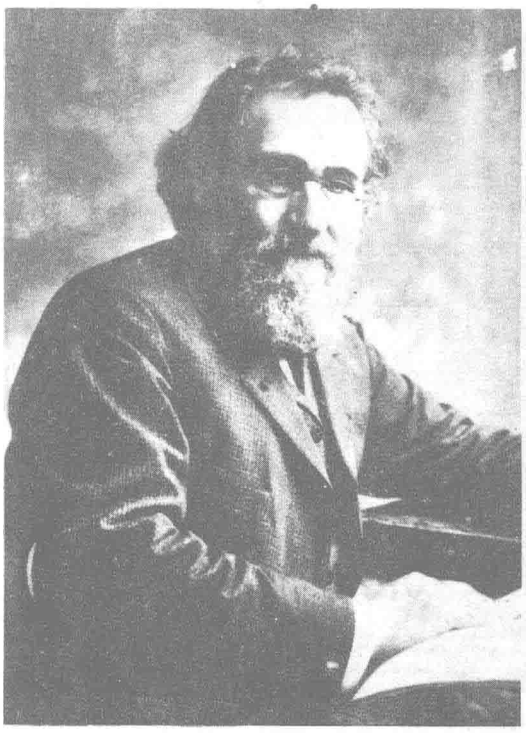

FIGURE 1-2. Eli Metchnikoff (1845–1916). (Courtesy Dr. James Hirsch and the Rockefeller University.)

The various views on inflammation have gradually been synthesized into a general doctrine. Thus in 1923, when Rössle (1923) reviewed the problem, he concluded that in inflammation in higher animals the vascular system facilitates the rapid accumulation of large quantities of phagocytes and antibodies. The principal aim of the inflammatory reaction, Rössle concluded, is parenteral digestion. Rössle also coined the concept of "allergic inflammation," as the inflammatory reaction which follows interaction between a foreign protein or antigen

FIGURE 1-3. Lord Florey (1898–1968).

and antibody in an animal which had previously been in contact with that particular antigen. This reaction, according to Rössle, varies only quantitatively but not qualitatively from ordinary or "normergic" inflammation. Since it is more intense than normergic, he referred to it as "allergic-hyperergic." Rössle (1944) also introduced the concept of "interstitial serous inflammation."

The most outstanding among those who investigated the chemistry of the inflammatory reaction are Schade, Lewis, and Menkin. Schade (1923, 1935) studied the physical chemistry of inflammation and stressed the initial local alteration of cells and tissues (see also Virchow, 1858), particularly the increase in hydrogen ion concentration and osmotic pressure. Lewis (1927) presented evidence that the vascular phenomena of the inflammatory reaction are initiated by histamine or other similar "H-substances," and Menkin (1940, 1956) isolated thermolabile and thermostable substances from inflammatory exudates which caused increase in vascular permeability and emigration of leukocytes. These studies initiated an era in which the mediators of inflammation still are actively being pursued.

In recent years extensive studies have been carried out on the ultrastructure of the vascular alterations during exudation and leukocyte emigration. These investigations were initiated by Florey (Fig. 1-3) (Marchesi and Florey, 1960; Florey and Grant, 1961) and Majno (Majno and Palade, 1961). "At present," said Lord Florey (1962) "the electron microscope with its great magnification and resolving power is being used to extend earlier observations, while at the same time much interest is being taken in the chemical and physical mechanisms involved in the many changes that occur. [However,] we are still far from being able to give a complete picture of what happens in the tissues or to explain how and why the sequence of events that follows injury takes place."

THE TERMINAL VASCULAR BED (MICROCIRCULATION)

We owe the most thorough study of the terminal vascular bed, or microcirculation, to Zweifach (1961) and Illig (1961). Most investigations were done on the mesentery, omentum, and mesoappendix by direct observation. The terminal vascular bed consists of the vessels located between a terminal arteriole and a venule and comprises a central or thoroughfare channel and a network of true capillaries, in addition to the terminal arteriole and venule (Fig. 1-4) (Zweifach, 1961).

ANATOMY

Arteries and veins are paired in close association with one another, but this rela-

tion is lost when arterioles are given off. Small arterial vessels, particularly *arterioles*, have been given various definitions, according to the method used for their study (see Movat and Fernando, 1963a). Based on in vivo observation, Zweifach (1961) described arterioles as 30 to 40 μ in diameter, coiled, and giving off numerous side branches along their course. He also stated that the major physical resistance to blood flow is probably encountered in these vessels.

The arterioles subdivide several times into narrower structures—the *terminal arterioles* (Chambers and Zweifach, 1944). These measure about 20 to 30 μ in the partially constricted state. The terminal arteriole is lined by endothelium resting on basement membrane and has one layer of circularly arranged smooth-muscle cells. Like larger arterial vessels, it exhibits periodic dilations (about one-fourth of its diameter) which are more regular than those of the metarteriole described below. Zweifach (1961) has suggested that "terminal arteriole" be used to designate vessels up to the point where they begin to distribute smaller side branches, although the "arterial vessel" follows a variable course beyond this point and its end point is difficult to establish. Terminal arterioles have often been referred to as "precapillary arterioles."

Although the organization of the terminal vascular bed in other parts of the body often varies from that in the mesentery (Illig, 1961), it is here that most in vivo observations were made. One of its main characteristics is the presence of *central, thoroughfare,* or *preferential channels* (Chambers and Zweifach, 1944). Such a channel has a proximal portion, known as *metarteriole*, and a distal segment or *a-v capillary*. The metarteriole is 5 to 7 μ in diameter when contracted and about 12 to 20 μ when dilated. Its lining endothelium rests on basement membrane which is surrounded discontinuously by smooth-muscle cells. Unlike the terminal arteriole, its vasomotion (described below) is readily abolished by irritation of the tissues. The segment immediately distal to the typical metarteriole is said to have a few "atypical muscle cells" (Chambers and Zweifach, 1944). The most distal part of the thoroughfare channel or a-v capillary is free of smooth-muscle cells and is noncontractile.

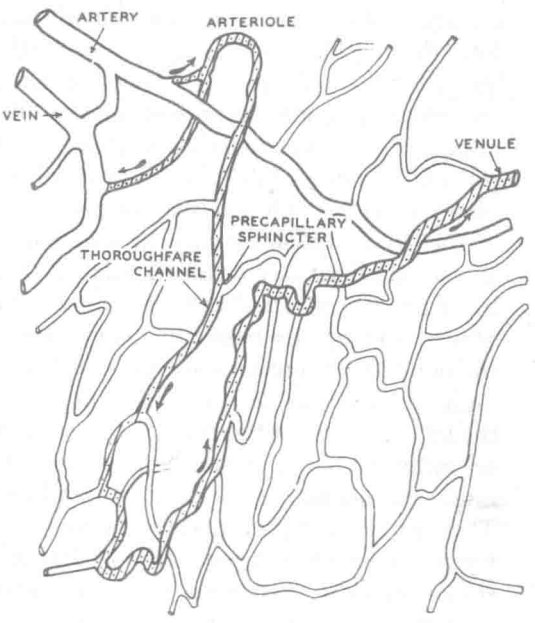

FIGURE 1-4. The microcirculation. (From B. W. Zweifach. *Functional Behavior of the Microcirculation*, 1961. Courtesy of Charles C Thomas, Publisher, Springfield, Ill.)

According to Zweifach (1961), the need to differentiate between terminal arterioles and metarterioles has more than academic significance, since the relative importance of humoral and neurogenic agents in the regulation of blood flow, reactivity thresholds, and the influence of local tissue factors differ in these two segments of the terminal vascular bed.

The capillaries are connected with the metarterioles or terminal arterioles through *precapillary sphincters* or *precapillaries* (15 to 20 μ in diameter) which branch off from the metarterioles or terminal arterioles at an abrupt angle. This junctional segment is surrounded by several smooth-muscle cells which coil several times around it. The precapillary phincters display vasomotor

changes which interrupt the blood flow into the capillaries independently of the metarterioles and terminal arterioles. Even the slightest contraction can narrow the orifice of the precapillaries sufficiently to prevent entrance of cellular elements of the blood, allowing only plasma to enter.

The *true capillaries* vary in size. Often a true capillary has a lumen smaller than the diameter of a red cell (Fernando and Movat, 1964b). Its basement membrane is surrounded discontinuously by cells varyingly referred to as pericytes, periendothelial cells, adventitial cells, clasmatocytes, or undifferentiated mesenchymal cells. The most commonly used term is pericytes. They probaby represent incompletely differentiated smooth-muscle cells. As already stated, there is a variation in the organization of the terminal vascular bed from tissue to tissue, and this is most pronounced in the capillary patterns, e.g., in mesentery, skeletal muscle, skin. For a detailed description including arteriovenous shunts, the reader is referred to the monographs of Zweifach (1961) and Illig (1961).

A *venule*, often referred to as a collecting or postcapillary venule, measures 30 to 40 μ in diameter and is nonmuscular, but as it leaves the capillary bed, a venule acquires a smooth-muscle coat and elastic elements in its wall. In the mesentery, thoroughfare channels become confluent with the distal ends of capillaries, giving rise to venules. In other regions, e.g., the skin or the hamster cheek pouch, arterial vessels, after passing through most of the capillary bed, lose their anatomical distinction and empty, via several capillary offshoots, into venules.

FUNCTION

Regulation of the peripheral blood flow is the primary function of the microcirculation, the blood being distributed in accord with the metabolic needs of the tissues and the effective removal of cellular by-products. This type of homeostasis is accomplished by intrinsic vasomotor adaptations, by pressure-flow and pressure-diameter relations within the terminal vascular bed, and by selectively changing the number of "active" capillaries (Zweifach, 1961).

Under normal conditions the thoroughfare channels are always open. However, there is a variation in the amount of flow through the channels, a phase of relative constriction alternating with one of dilation. This is referred to as "vasomotion." While the recurrent vasomotion of the metarterioles is the factor which regulates the flow through the thoroughfare channels, the alternate dilation and constriction of the precapillary sphincters control the intermittent flow through the true capillaries.

When the tissue supplied by a terminal vascular bed is in a state of relative rest, the blood flows, except for a sporadic flow through the capillaries, only through the thoroughfare channels. Thus resting tissue is relatively ischemic. Upon irritation, e.g., mechanical stimulation, the vasomotion ceases and the metarterioles and precapillary sphincters dilate. This is followed by flooding of the capillaries with blood—hyperemia. "The metarterioles and precapillaries form an integral part of the capillary bed proper and their reactive muscular elements are so disposed as to be fully exposed to chemical changes in the environment" (Chambers and Zweifach, 1944).

With respect to vascular reactivity, Zweifach (1961) states:

> Any attempt to reconstitute our knowledge concerning vascular reactivity into a working schema, must place primary emphasis on the interaction between humoral factors of local origin and neurohumoral regulatory influences. The only neurohumoral agents which have been unequivocally identified are norepinephrine and acetylcholine, and it has been presumed that the modulating effect of local chemicals is achieved by altering the response of the smooth muscle cells to these substances. Local shifts in the redox potential seem to be an important environmental factor influencing

the effectiveness of the catechol amines, either through an anti-oxidative mechanism or an effect on the enzymic handling of the substances. Local release of polypeptides and amines of the 5-hydroxytryptamine type would appear to be the means by which reactivity thresholds of the separate components of the microcirculation may be modified. To date, it has not been possible to determine the precise local antagonist of cholinergic stimuli.

ULTRASTRUCTURE OF THE VASCULAR WALL: THE BLOOD-TISSUE BARRIER

The ultrastructure of the vascular wall varies somewhat along the terminal vascular bed. However, it appears sufficient to discuss the vascular wall as a whole and merely point out the differences. Three types of capillaries can be recognized at the fine structural level: continuous, discontinuous (sinusoids), and fenestrated. These will be referred to only briefly in connection with the endothelium. Most of the description to follow deals with vessels which have a continuous endothelium, as encountered in the connective tissue of structures that have been used to study inflammation experimentally, e.g., mesentery, skin, hamster cheek pouch. For a detailed discussion of the ultrastructure of capillaries and the vascular wall in general, the reader is referred to the review of Majno (1965).

ENDOTHELIUM

The Endothelial Cell (Fig. 1-5)

In capillaries endothelial cells are 0.1 to 0.3 μ in thickness and bulge up to 3 to 4 μ at the site of the nucleus. The endothelial lining of the remainder of the terminal vascular bed is slightly thicker. The nuclei are oval or elongated, depending on how much the vessels are distended, and often have indentations of varying size. The cytoplasm contains the usual organelles: mitochondria, rough-surfaced endoplasmic reticulum, ribosomes, centrioles, a small Golgi region, smooth-surfaced vesicles, multivesicular bodies, and a system of small vesicles (Palade, 1953, 1961). Lysosome-like structures are encountered particularly in endothelial cells of venules (Movat and Fernando, 1964a). The hyaloplasm contains filaments (50 to 100 Å in thickness), often in bundles (Fernando and Movat, 1964a).

The system of small vesicles, generally referred to as pinocytic vesicles (Fig. 1-5), occupies about one-third of the cell volume of the capillaries of rat myocardium (Palade, 1961). The vesicles are uniform and measure 600 to 700 Å in diameter. Those in the depth of the cell lie free, whereas those facing the lumen or the adventitial aspect of the endothelial cell are continuous with the cell membrane. The pinocytic vesicles have small stomata (220 to 250 Å) which point toward the lumen on the luminal, and toward the basement membrane on the adventitial, side of the endothelial cell (Fernando and Movat, 1964a). Palade (1953) had suggested that the pinocytic vesicles, which form as an invagination of the endothelial cell membrane, pinch off and lie free in the cytoplasm, traverse the cell, make contact with the cell membrane of the opposite side, and empty their contents, which they picked up on the luminal side. It was suggested that fluid could be carried across the cell in this manner (see under "Pathways Across the Blood-Tissue Barrier").

The number of endothelial cells which line the various segments of the terminal vascular bed varies. In small capillaries a single cell may constitute the entire lining, at least in one plane of sectioning. In small postcapillary venules the lining tube con-

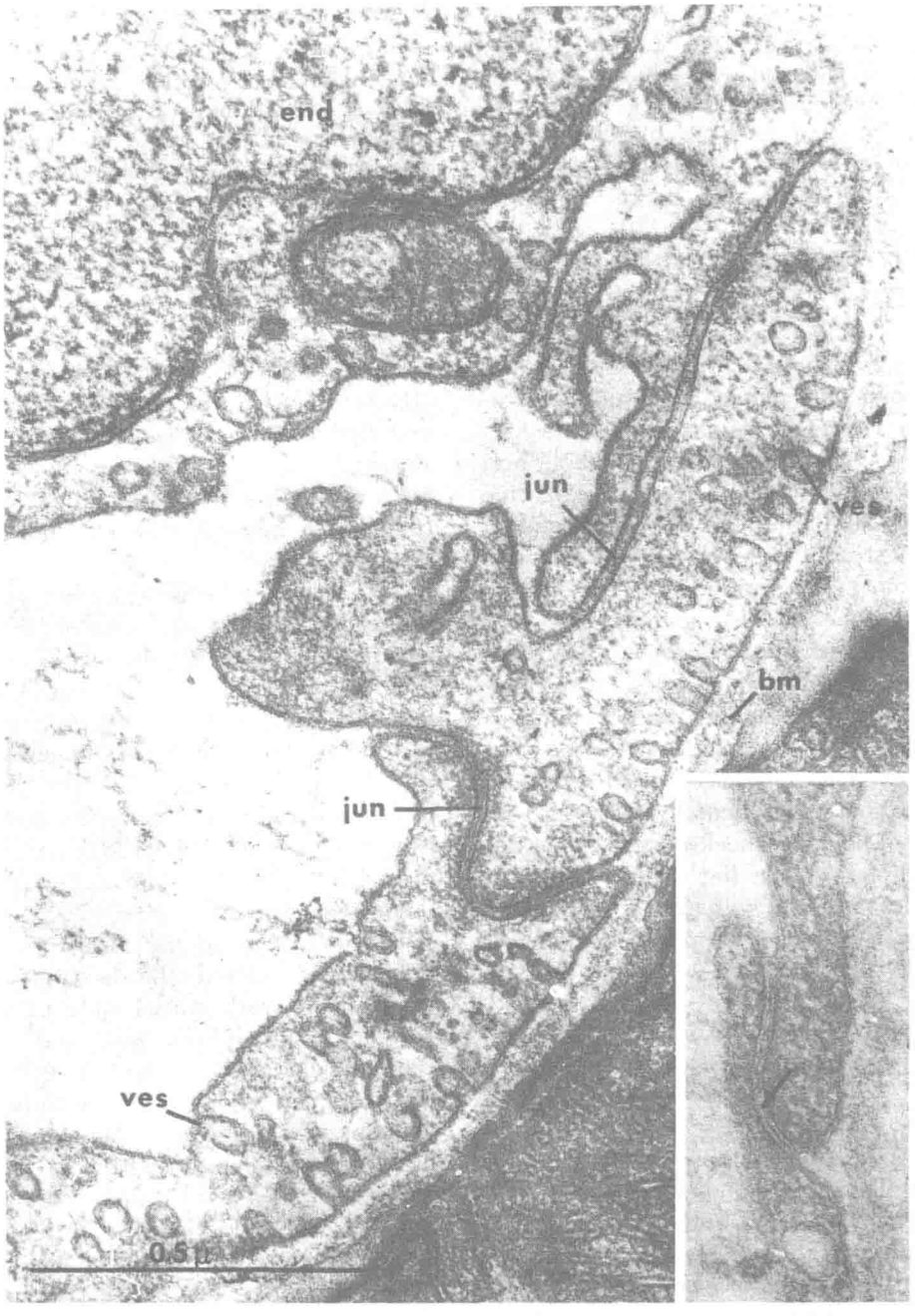

FIGURE 1-5. Portion of a capillary of the rat. Note intercellular junctions (*jun*), and pinocytic vesicles (*ves*). Some of the latter open to the lumen, others toward the basement membrane (*bm*). Inset shows a tight junction with apparent obliteration of the intercellular space. *end* = endothelium. (From J. Wolff. *Z. Zellforsch.* 73, 143, 1966; labels added.)

sists of three to five cells in a cross section. Five to nine endothelial cells can be counted in a terminal arteriole and three to five cells in a thoroughfare channel.

Intercellular Junctions

With the aid of certain techniques, particularly impregnation with silver nitrate,

the outlines of endothelial cells can be demonstrated (Florey, Poole, and Meek, 1959). When viewed *en face*, these *Häutchen*-preparations show a line of reduced silver (about 1 μ in thickness), seemingly separating endothelial cells at sites of intercellular "cement" (*Kittsubstanz*, Arnold, 1876). For some time it was believed that this cement substance played an important role in transcapillary exchange and increase in vascular permeability (Chambers and Zweifach, 1947). Ultrastructural studies (Palade, 1953; Karrer, 1956; Moore and Ruska, 1957; Bargmann, 1958; Bennett, Luft, and Hampton, 1959; Fawcett, 1959; Palade, 1961) could not demonstrate an intercellular cement between adjacent endothelial cells. The silver lines thought to represent cement are due to precipitation of silver in the endothelial cytoplasm close to the junctions (Buck, 1958; Florey, Poole, and Meek, 1959). Recent studies of Luft (1966) indicated that an intercellular cement might be present between adjacent endothelial cells. The material cannot be visualized in ordinary sections, but stains with ruthenium red and is continuous with similar material in the lumen and in perivascular connective tissue (Fig. 1-6).

With the aid of the electron microscope a space of 100 to 150 Å can be seen to be present between adjacent endothelial cells. At certain points along the intercellular junction the cell membrane and adjacent cytoplasm appear darker. The dark material was believed not to bridge the intercellular space and was first interpreted as a zone of reinforcement of the junction and referred to as "attachment belt" (Bennett, Luft, and Hampton, 1959), to indicate that it surrounded the entire cell. Subsequently, Robertson (1958) (having demonstrated that each cell membrane was composed of two osmiophilic layers separated by an osmiophobic space) showed that partial fusion of the outer osmiophilic layers occurs between many cells. Because of this fusion one can no longer demonstrate four osmiophilic and two osmiophobic layers, i.e., six layers, and therefore Karrer (1960) introduced the term "quintuple-layered junction." The structure known as attachment belt was subsequently described by Muir and Peters (1962) as a quintuple-layered "tight junction" toward the luminal end of the interendothelial junction. Farquhar and Palade (1963) examined the intercellular junctions of several epithelia and those of endothelium.

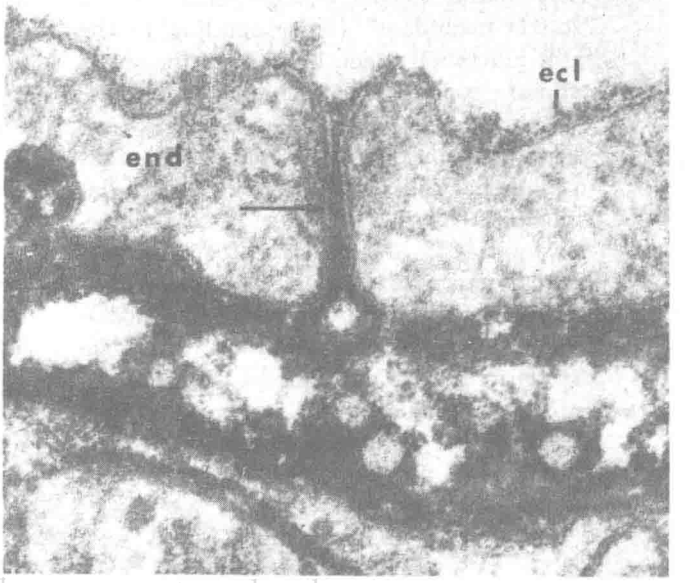

FIGURE 1-6. Capillary of mouse diaphragm. Black-staining (ruthenium red) material is seen on the surface (*ecl* = endocapillary layer) and in the tissue space below the endothelium (*end*), continuous with similar material in the junction (*arrow*). (From J. H. Luft. *Fed. Proc. 25*, 1773, 1966; labels added.)