STUDIES ON INFLAMMATION

I. The Effect of Histamine and Serotonin on Vascular Permeability: An Electron Microscopic Study

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ABSTRACT

The mechanism, whereby histamine and serotonin increase the permeability of blood vessels, was studied in the rat by means of the electron microscope. The drugs were injected subcutaneously into the scrotum, whence they diffused into the underlying (striated) cremaster muscle. An intravenous injection of colloidal HgS was also given, in order to facilitate the identification of leaks by means of visible tracer particles. After intervals varying from 1 minute to 57 days the animals were killed; the cremaster was fixed, embedded in methacrylate, and examined with the electron microscope.

One to 12 minutes after the injection, the blood vessels of the smallest caliber (3 to 5 micra as measured on electron micrographs) appeared intact. Numerous endothelial openings were present in blood vessels with a diameter of 7 to 8 micra or more. These gaps were 0.1 to 0.8 micra in width; portions of intercellular junctions were often present in one or both of the margins. The underlying basement membrane was morphologically intact. An accumulation of tracer particles and chylomicra against the basement membrane indicated that the latter behaved as a filter, allowing fluid to escape but retaining and concentrating suspended particulate matter of the size used.

Uptake of tracer particles by endothelial vesicles was minimal. Phagocytosis by endothelial cells became more prominent at 3 hours, but as a secondary occurrence; the pericytes were actively phagocytic at all stages. At the 3-hour stage no leaks were found.

The changes induced by histamine and serotonin were indistinguishable, except that the latter was more potent on a mole-to-mole basis. In control animals only small accumulations of tracer particles were found in the wall of a number of blood vessels.

With regard to the pathogenesis of the endothelial leaks, the electron microscopic findings suggested that the endothelial cells become partially disconnected along the intercellular junctions. Supporting evidence was provided at the level of the light microscope, by demonstrating—in the same preparation—the leaks with appropriate tracer particles[1], and the intercellular junctions by the silver nitrate method. The lipid nature of the chylomicron deposits observed in electron micrographs was also confirmed at the level of the light microscope, using cremasters fixed in formalin and stained *in toto* with sudan red.

Almost a century has elapsed since Cohnheim's "New Studies on Inflammation" (1): the monograph in which Virchow's former assistant announced, for the first time, that acute inflammation must involve an increased permeability of the blood vessels. This concept was slow in gaining acceptance (2) perhaps because it did not fit

[1] See accompanying paper.

with Virchow's own concept of inflammation (3, 4). It was only after the discovery of histamine and of its effects on vascular permeability (5, 6) that Cohnheim's theory was revived and generally accepted. At this time, largely through the work of Rich (7) and Lewis (8), a more precise concept of acute inflammation began to take root: injured tissues liberate histamine or similar substances, which act upon the surrounding vessels and cause them to become abnormally permeable.

In recent years, many other endogenous substances have been identified which share with histamine its leak-promoting effect, and which may also be demonstrated, or presumed to appear, in injured tissues (see 9, 10). Hence, if we attempt to summarize our progress since Cohnheim's original statement, we find that it consists mainly of having identified a large number of chemical mediators which may be responsible for the abnormal exudation of fluid.

On the other hand, very little has been learned about the mechanism of action of all these permeability - increasing factors. Two important questions, for instance, are still unanswered. In the first place, the exact portion of the vascular tree that is "made more permeable" has never been directly identified, though it is generally thought to be the capillary; this problem will be dealt with in the next paper of this series. Furthermore, the cellular (endothelial) changes whereby the vascular permeability is increased are not clearly understood. Several mechanisms could be conceived: an acceleration of the normal transport or filtration processes; the development of leaks within endothelial cells (11); or also a reversible dissociation of the endothelial cells along their margins, as suggested by a number of early workers (12–15). The problem seemed to be particularly suited for an electron-microscopic approach.

In the present study we will demonstrate—by means of the electron microscope—the effects of histamine and serotonin, applied topically, on the endothelium of blood vessels in a striated muscle. In order to facilitate the tracing of the path followed by the fluid in leaking across the endothelial wall, some of the experimental animals were given an intravenous injection of colloidal particles dense enough to be visualized by electron microscopy (colloidal mercuric sulfide).

MATERIAL AND METHODS

The animals were young male rats of the Sprague-Dawley (Holtzman) strain, weighing 200 to 350 gm, and fed *ad libitum*. All our observations were made on the cremaster muscle, a laminar expansion of the internal oblique of the abdominal wall (16). In the rat, as in other laboratory rodents, each cremaster forms a pouch containing one testis. Though partly joined by loose connective tissue, the two cremasters are anatomically separate. They are loosely adherent to the skin of the scrotum on the ventral and lateral sides, and to the perineal region on the dorsal aspect; the visceral surface is lined by an extremely thin serosa, continuous with the peritoneum. When isolated and gently stretched the cremaster becomes a membrane of very uniform thickness (about 250 micra) and 2 to 3 cm in diameter.

A number of reasons induced us to make use of this particular tissue. In the first place, the injurious agents to be tested can be injected into the subcutaneous tissue of the scrotum, whence they will diffuse into the underlying muscle; thus the muscle itself is not submitted to direct mechanical trauma as a result of the injection. Also, because of its thinness, the cremaster lends itself very well to rapid fixation *in situ* for electron microscopic study. Lastly, the entire muscle can be dissected out very easily, stretched, fixed and cleared for study by transillumination with the light microscope. In this state, the preparation is uniquely suited for topographic studies of the vascular tree, as will be explained in further detail in the following paper. Other laminar muscles, thoracic and abdominal, were also examined but none proved to be as convenient as the cremaster.

Under ether anesthesia the rats were given an intravenous injection of colloidal HgS, and immediately thereafter a single subcutaneous injection of histamine or serotonin in the scrotum. The suspension of colloidal HgS was prepared from a dry powder obtained from Hille & Co., Chicago, Illinois. This dry material (batch No C11/1411A) contained about 25 per cent of black HgS and 0.2 per cent cresol, the remainder being accounted for by a suspending agent. The powder was dissolved in 0.85 per cent NaCl, to give a 3 per cent concentration of HgS[2]; this suspension was injected into the saphenous vein in the amount of 0.5 cc per 100 gm of body weight. There

[2] The particles in this suspension varied in diameter between 70 and 350 A; about 50 per cent were in the 70 to 160 A range (see Fig. 17, insert). These figures refer to the electron-opaque granules visible on electron micrographs; *in vivo*, however, the effective size of these bodies may be increased by a layer of absorbed material of low contrast, not directly visible on electron micrographs.

was an occasional indication of transient lung edema; otherwise no ill-effects were observed.

The histamine and serotonin solutions were injected subcutaneously on the ventral aspect of the scrotum using a gauge 30 needle. In our first experiments we used a tuberculin syringe and injected the solutions in the constant volume of 0.02 cc; with this dosage the lesion in the cremaster was sometimes very faint and difficult to localize, particularly in the early stages. In later experiments we used a Hamilton microsyringe made to deliver 0.05 cc with an accuracy of ±1 per cent; with this dosage the lesions were more easily identified. *Histamine* was prepared as a 1:1000 solution of the diphosphate (Abbott Laboratories, North Chicago, and Eli Lilly & Co., Indianapolis) in 0.85 per cent NaCl. *Serotonin* (creatinine sulfate, Nutritional Biochemicals Corp., Cleveland) was dissolved in saline immediately prior to use at the concentration of 1:10,000. In delivering the subcutaneous injections great care was taken not to pinch or otherwise traumatize the skin of the scrotum.

At the time of sacrifice, with the rat under ether anesthesia, the spermatic cords were clamped with hemostats, a fold of scrotal skin was cut off, and the exposed cremaster quickly flooded with chilled fixative: 2 per cent OsO_4 in veronal buffer at pH 7.3 to 7.4 (17), with 0.4 M sucrose (18). One cc of the fixative was also injected into the spermatic cords cavity. The fixed portion of the cremaster was then excised and stretched with pins on a plate of dental wax, under several drops of fixative. After 3 or 4 minutes the superficial fascia (if still present) was gently removed and the tissue was cut into squares about 1 mm in diameter. These were further fixed for 2 to 4 hours at 3°C, transferred directly to 70 per cent ethanol, dehydrated in graded alcohols at room temperature, and embedded in a mixture of 80 per cent butyl and 20 per cent methyl methacrylates. The last change of absolute alcohol, and the methacrylate, contained uranyl nitrate in the amount of 75 mg per 100 cc (19). Sections were cut on a Porter-Blum microtome equipped with a glass knife, and mounted on Effa (200 mesh) grids coated with a collodion film stabilized with a very thin layer of carbon. After staining with lead hydroxide (20) the sections were covered with a membrane prepared from 0.1 per cent solution of parlodion in amyl acetate (21) and examined in an RCA type EMU-2 electron microscope at instrumental magnifications of 1,400 and 15,000.

RESULTS

A total of 25 rats, which had received an intravenous injection of colloidal HgS, and a local injection of histamine or serotonin, were sacrificed after the following intervals: 1, 1½, 2½, 3, 4, 5, 12 minutes, 3 and 3½ hours, and 57 days. Controls (22 rats) were of four kinds: (*a*) animals which received an intravenous injection of colloidal HgS, and a local injection of 0.85 per cent NaCl; (*b*) animals which received only a local injection of histamine or serotonin, and were killed after 4 minutes; (*c*) animals which received only the intravenous injection of colloidal HgS, and were killed at intervals of 6, 12, and 30 minutes; (*d*) normal animals which had not received any local or intravenous injection.

GROSS OBSERVATIONS

Two or 3 minutes after the local injections of histamine or serotonin a slight local edema became apparent; at 12 minutes the muscle was also found thickened. When colloidal HgS had been administered in conjunction with the drug, the injection site appeared as a faintly gray patch about 10 mm in diameter, somewhat larger on the underside of the skin than on the cremaster. Within this patch, from the 4-minute stage onwards, it was often possible to distinguish very fine branching patterns of vessels which had been blackened by the HgS; under the dissecting lens these vessels appeared to be venules. Blackened capillaries could not be seen. In the animals killed after 57 days there was no grossly distinguishable trace of the injection site. Low-power studies with the light microscope will be reported in the following paper.

Comparison of Histamine- and Serotonin-Induced Lesions

If the diameter of the gray patches produced in the skin is taken as a basis for comparison, serotonin may be said to be roughly 100 times more active than histamine, on a mole-to-mole basis, in that it produced a patch of a given diameter with $\frac{1}{100}$ the molar dose required for histamine (calculated as base; see also (22)). This was, in fact, the only difference found in our study between the effects of the two drugs; the results were identical at the electron microscopic level, hence they will be described together.

ELECTRON MICROSCOPIC OBSERVATIONS

1. *Normal Blood Vessels*

The morphologic features of the small blood vessels in normal, control animals were essentially

the same as in previous accounts (23–27). The endothelial cells of the small blood vessels appeared to have the same characteristics, independent of the type of vessel which they lined. It is possible, of course, that an accurate comparison on the basis of quantitative criteria may bring out some differences between the endothelial cells in different types of vessels; however, this was not suggested by simple examination of the electron micrographs. Intracellular structures include vesicles 500 to 700 A in diameter (23), scanty elements of the endoplasmic reticulum of the rough surfaced type, a few mitochondria, streams of very fine filaments (23), pairs of centrioles (Fig. 2), and a Golgi complex. Where two endothelial cells come in contact, the juxtaposed cellular membranes give rise to the well known structure appearing in cross-section as a set of two parallel lines, about 90 A apart, and winding a more or less sinuous course (25) (Fig. 1); at

Key to Abbreviations on Figures

B, basement membrane
Bs, septum arising from the basement membrane
E, endothelial cell
G, gap in the endothelium
H, histiocyte
J, intercellular junction
L, lumen
M, fiber of striated muscle
N, nucleus
P, platelet
Pe, pericyte
R, red blood cell
X, extraneous material in the vascular wall
ch, chylomicron, or cluster of chylomicra
m, mitochondrion
pr, endothelial process
ve, vesicle

All the figures except Figs. 21 to 25 represent electron micrographs of rat cremaster muscle, fixed in osmium tetroxide and embedded in methacrylate with added uranyl nitrate (19). The sections were stained for 2½ to 4 minutes with lead hydroxide (20) and subsequently 'sandwiched' (21).
Scale for all electron micrographs: 1 micron.

FIGURE 1

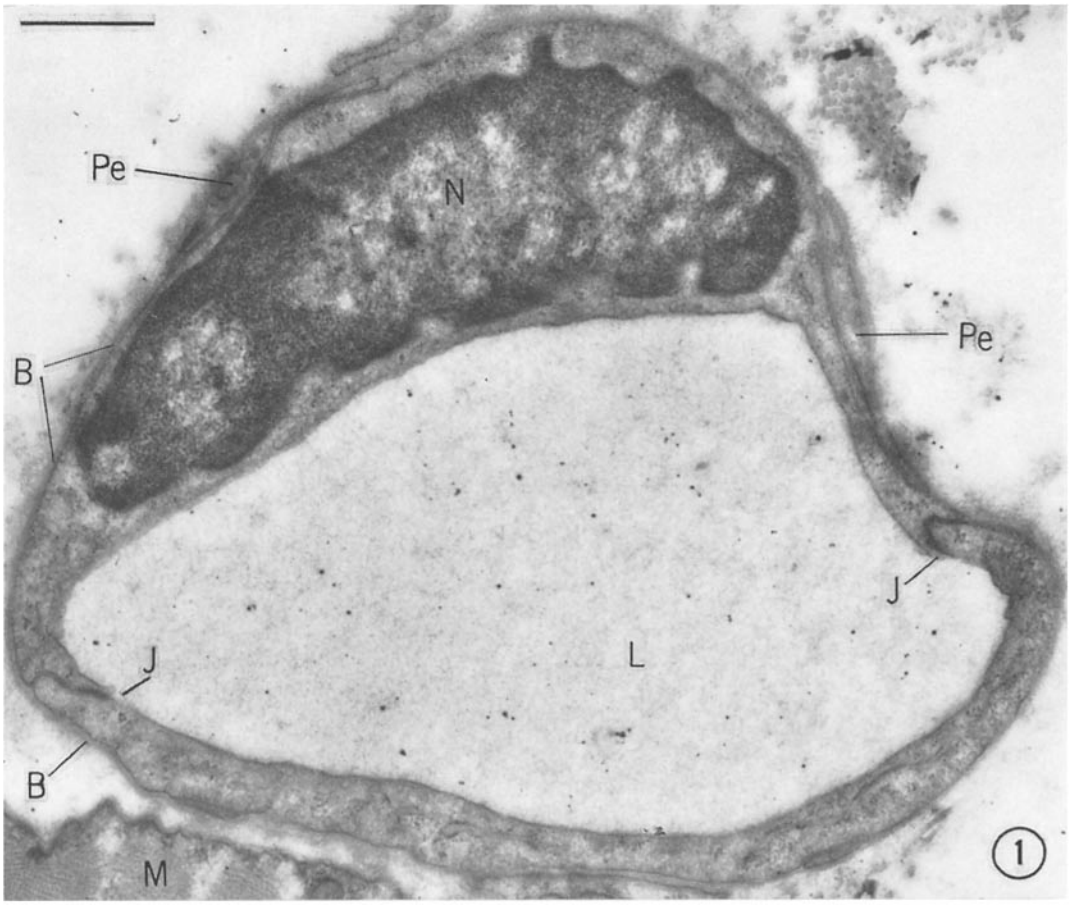
A capillary, 4 minutes after the local injection of serotonin and intravenous injection of colloidal HgS. There is no evidence of injury. Particles of HgS are visible in the lumen; none are present in the vascular wall.

This illustrates an important point; namely, that the finest blood vessels as a rule did not develop leaks under the effect of histamine or serotonin. The wall of these "non-leaking capillaries" consists of only one cell-layer, except for the thin expansion of pericytes (*Pe*) contained within the basement membrane (*B*). The lumen, in this case, is encircled by only two endothelial cells, as shown by the two intercellular junctions (*J-J*). Magnification, 18,000.

FIGURE 2

Wall of a larger vessel, 1 minute after a local injection of histamine and an i.v. injection of HgS. Example of the most common image observed in the leaking vessels: tracer particles forming an intramural deposit (*X*) not connected with the lumen. The particles have presumably penetrated through an endothelial gap missed by the section. Amorphous material is also present. Though extremely thin, the basement membrane appears to be very effective in retaining the tracer particles (see, *e.g.*, at *B*).

Note that the wall of this leaking vessel is thicker and more complex than that of the non-leaking capillary shown in Fig. 1; this is a larger vessel, presumably a venule (51). *Arrows:* a pair of centrioles. Magnification, 17,000.


Pe
N
Pe
B
J
L
J
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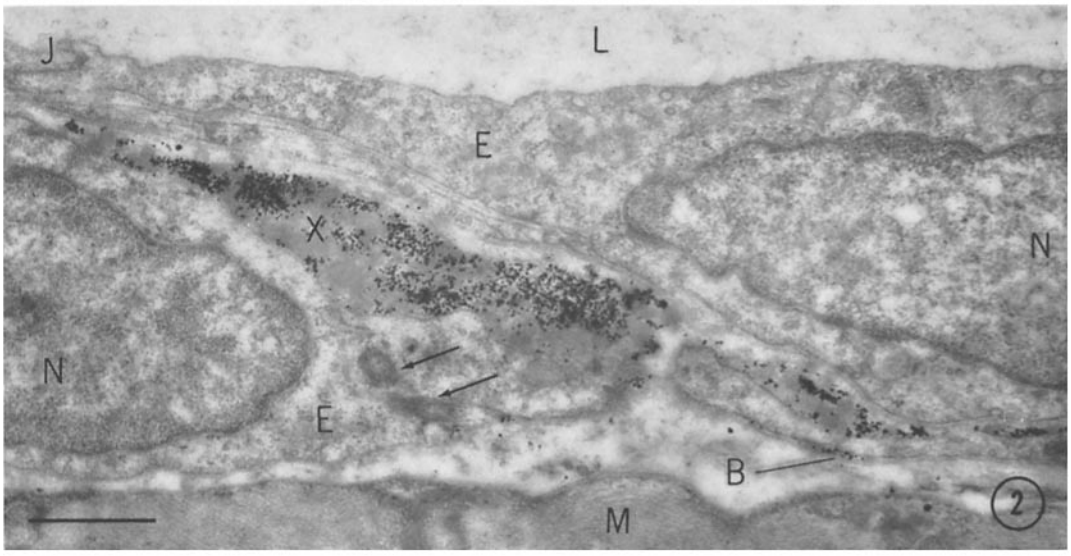
1


J
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2

irregular intervals the two lines appear denser or reinforced over a stretch of 1000 A or more, constituting a "desmosome" or "adhesion plate" (26) (Fig. 4). It is sometimes possible to see a few short bristles projecting from either side of the adhesion plate into the cytoplasm of the cells. The capillaries, and the vessels of similar structure but larger diameter, show a definite basement membrane, which appears in cross-section as a rather fuzzy band 400 to 600 A in thickness, sometimes faintly fibrillar after staining with lead. It can often be seen to split in order to envelop the body of a pericyte (Fig. 5). Sometimes a pericyte sends into the vascular wall a prolongation which penetrates *through* the basement membrane and comes in direct contact with the outer surface of an endothelial cell. A detailed description of muscle capillaries in the rat can be found elsewhere (28). Structures which could be definitely classified as lymphatics (29) were not encountered.

2. *Effect of Histamine and Serotonin*

a) *Changes Observed between 1 and 12 Minutes*

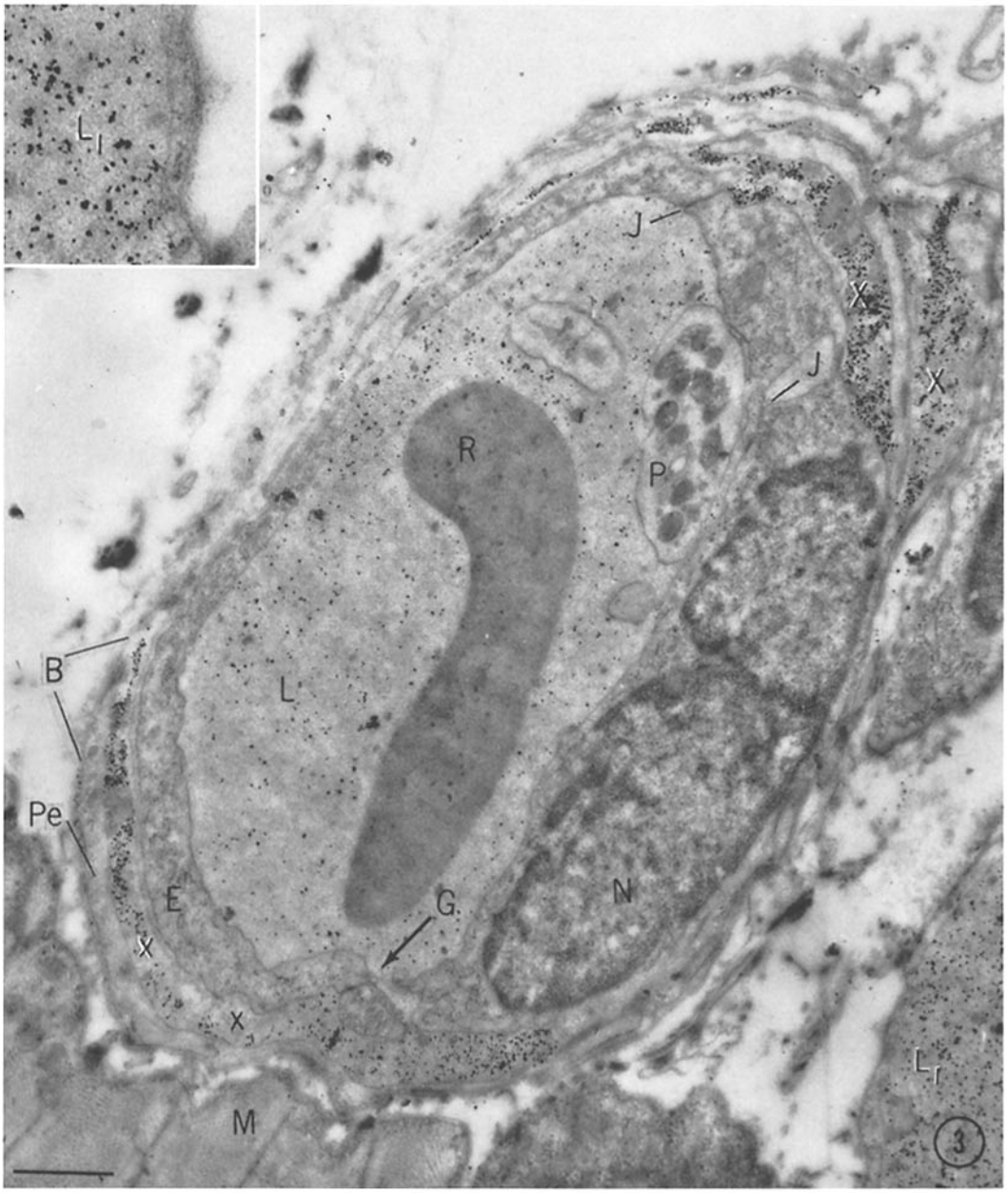
There is no substantial difference between the preparations examined within this time lapse, hence they will be described as a group. An obvious finding from scanning the sections at relatively low magnifications (1400 to 2500) is that the vessels of the smallest caliber (and hence identifiable as true capillaries) appear normal; the lumen contains blood cells as well as plasma, with particles of HgS in suspension (Fig. 1). Many other vessels of a somewhat larger caliber (10 to 12 μ) are completely filled with red blood cells, so tightly packed that their outlines are scarcely recognizable: this is the condition known as *stasis* (30) and at low powers it represents the most striking difference from the controls. Another important difference is that most of the congested vessels contain deposits of HgS within their wall; the particles are often tightly packed, to form a layer that separates the endothelium from a pericyte, or cleaves the space between endothelium and basement membrane (Figs. 2 to 4). Many particles are present in the circulating plasma, whereas very few are found within the endothelial cells. This general picture clearly suggests that plasma must be leaking out, in discrete areas, through gaps in the endothelium; and that the basement membrane, acting as a filter, retains the particles while it allows the fluid to seep through. In fact, by examining more closely the endothelial lining, it is possible to identify a certain number of true leaks through which the lumen communicates directly with the intercellular spaces of the vascular wall (Figs. 3 to 10).

Anatomy of the endothelial leaks. In typical instances

FIGURE 3

General view of a leaking vessel, 3 minutes after the local injection of serotonin and the i.v. injection of colloidal HgS. A gap (*G*) is visible in the endothelium; through this gap, plasma has escaped into the vascular wall, and is now dissecting a space (*x-x*) between the endothelium (*E*) and a pericyte (*Pe*). As the intramural layer of plasma reaches the basement membrane (*B*), the particles of HgS become more concentrated, suggesting that the basement membrane acts as a filter, retaining particulate matter while allowing fluid to escape. At the opposite pole of the vessel, a platelet (*P*) adheres to the surface of the endothelium at the site of a junction between two cells. Within the wall, this junction widens to form a clear space, suggesting that the two cells may be in the process of separating. However, the endothelial gap which has led to the formation of the deposit *X-X* is not present in the section. In the lumen of this vessel (*L*) the concentration of protein HgS, and probably also of chylomicra is greater than normal, indicating that there has been loss of fluid (stasis; compare with the contents of the vessel shown in Fig. 1). Magnification, 14,000.

Lower right corner: a smaller vessel (L_1) (true capillary; caliber 4 to 5 micra) which shows considerable plasma concentration. The tracer particles, however, are retained, indicating that this capillary—as is usually the case for all blood vessels of this caliber—has not developed endothelial leaks. The *inset* shows, at a higher magnification, the simple structure of this non-leaking vessel (a single cell layer) as compared with the more complex organization of the larger, leaking vessel shown in the center. The latter vessel is presumably a venule (51). Inset, magnification, 32,000.


L
J
X
J
X
R
P
B
L
Pe
E
G
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x
x
M
L
3

these appear as open gaps in the endothelial lining, 0.1 to 0.8 micra in width. When the endothelium is not distended and flattened by packed red blood cells, the details of the lesion can be studied more easily. The lips of the opening are outlined by a cellular membrane. Plasma and particles of HgS can be seen inside the opening and thence penetrating into the vascular wall (Figs. 4, 5). In some instances a few irregular, membrane-limited projections arise from the endothelial cytoplasm on both sides of the gap; some are filamentous, others rounded or wart-like. When these projections are cut in cross-section, they appear as bodies lying free within the gap or at its margins; it is possible that some of these may represent filamentous pseudopodia bridging the opening (Figs. 6 to 8). Another common feature in one or both of the lips is the presence of an intercellular junction in the immediate proximity of the gap (Figs. 4, 5, 9, 11, 14, 15).

Most leaks are found in vessels of 10 to 20 micra in diameter. None were observed in the smallest vessels (3 to 5 micra) and none in vessels clearly identifiable as arterioles. The frequency of the leaks is difficult to quantitate; when a vessel is labeled as "leaky" by the presence of HgS within its wall, an endothelial gap is not necessarily found in the same section. On the other hand, the cross-section of a single vessel may show two or three leaks. A fair estimate might be one leak for every three to four cross-sections of leaking vessels.

Abnormal elements in the vascular wall. With wide open gaps in the endothelial lining, it is not surprising that the walls of the vessels should contain a variety of elements not normally present. These include (apart from the HgS) red blood cells, leukocytes, platelets, chylomicra, fibrin, and amorphous material, presumably plasma protein.

The deposits of mercuric sulfide generally appear, in cross-section, as narrow bands of particles running inside the vascular wall, and encircling part or all of the lumen. When the related leak is present on the same cross-section, it is obvious that the material tends to spread circumferentially from the point of entrance (Figs. 3, 4, 6 to 9); the dissection occurs between the endothelial cell on the luminal side, and the basement membrane (Fig. 6) or another cell (Figs. 5, 9) on the outside. Distal to the leak, the deposit terminates rather abruptly against the basement membrane (Fig. 16). At 12 minutes, however, when the deposits are more extensive, the basement membrane is often lifted away over several micra of the circumference by large masses of particles; there results a bulging pocket which recalls the image of an aneurysm (Fig. 17).

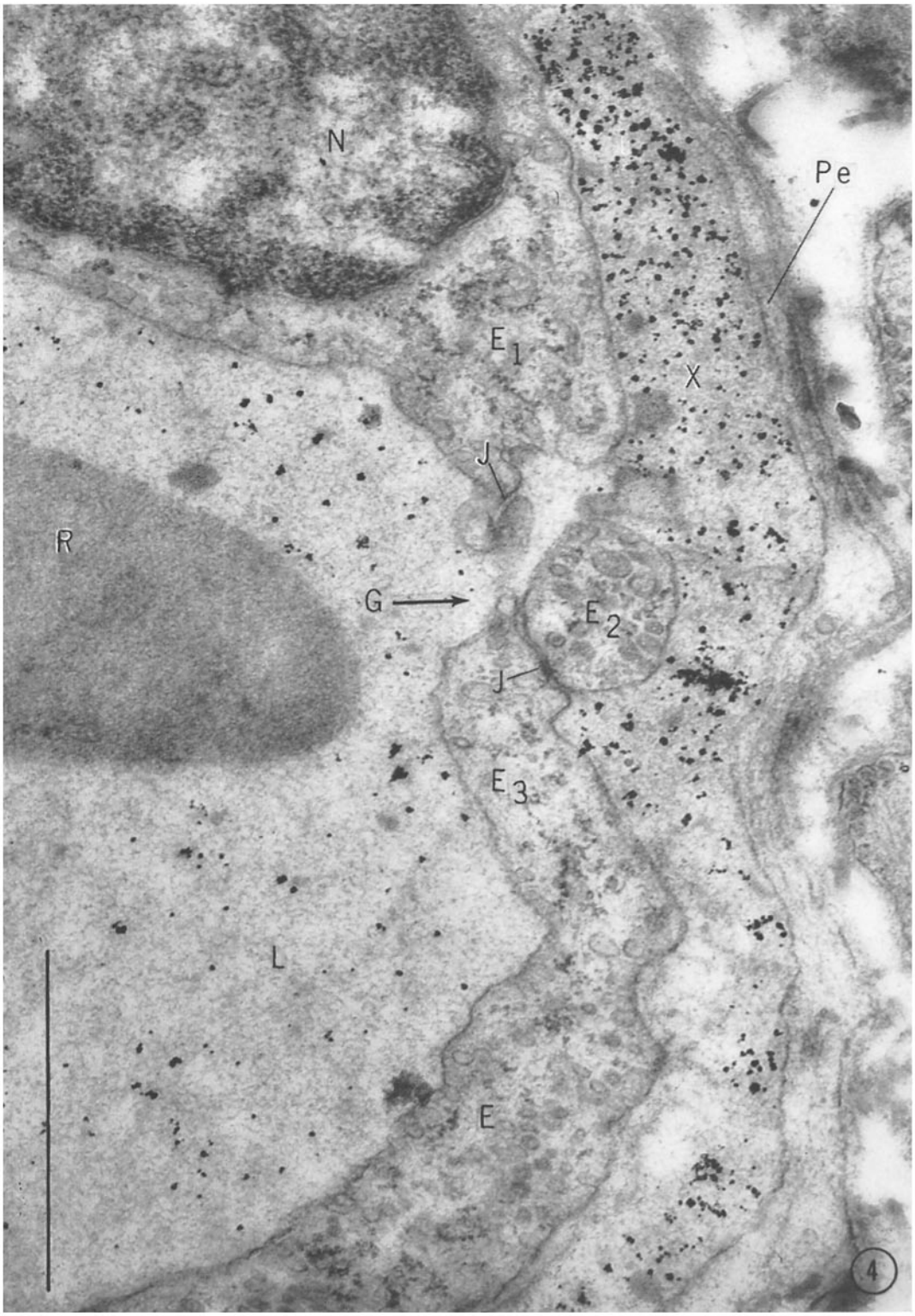
If different leaks are compared, it is obvious that the concentration of particles therein varies considerably. In leaks which have presumably just opened (especially between 1 and 4 minutes) the sheet of material dissecting the vascular wall appears essentially as plasma, somewhat enriched in tracer particles (Figs. 4, 6); the amorphous material in the background (plasma protein) also tends to show an increased density by comparison with that contained in the vascular lumen (Figs. 4, 6). In other leaks, presumably of longer

FIGURE 4

Detail of the preceding figure (serotonin, 3 minutes). G = gap in the endothelium, with segments of intercellular junctions (J) on either side. At first sight the image suggests that the endothelial cells E_1 and E_3 have torn apart in such a manner that a sizable fragment of E_1 has remained attached to E_3 (the fragment would be E_2). However, there is no evidence that the cytoplasm has been torn, and there are other more plausible interpretations.

E_1 and E_3 could be parts of the same cell, and E_2 a finger-like extension of the neighboring cell, protruding into the side of E_1–E_3. A third possibility is that this area represents the junction of several endothelial cells, and what appear to be fragments (*e.g.* E_2) are in reality parts of different cells.

The layer of plasma (X) which has escaped between the endothelium and the pericyte (Pe) has undergone concentration (compare with the contents of the vascular lumen (L), as described for Fig. 3). Magnification, 53,000.


N
Pe
E1
X
J
R
G
E2
J
E3
L
E
4

standing, the particles are packed almost solid, though pockets of other materials may appear among them, as described below.

Chylomicra[3] were present in almost every leak. They appeared as rounded bodies 800 to 1500 A in diameter, with a pale center and an indistinct outline of greater density; they tended to "burn off" rather rapidly in the electron beam, and appeared much more distinct after we adopted the sandwich technique (21). In recent leaks the chylomicra were often single (Fig. 5); later they tended to aggregate, and formed large clusters in which each element still maintained its identity (Figs. 15, 16). The final identification of these deposits as actually representing fatty material was accomplished by means of lipid-soluble stains applied to whole mounts of cremaster, fixed in formalin (see below).

At the 12-minute stage it was fairly common to observe filaments of fibrin, recognizable by the characteristic transverse striation with a period of 220 A (Fig. 17). The filaments were usually lying among masses of mercuric sulfide, and occasionally in proximity to a disintegrating platelet. Scattered among the filaments of fibrin were small masses or pools of granular material (Fig. 17); whether these represented, in part at least, fibrin at an earlier stage of polymerization could not be determined. No fibrin filaments were seen outside the basement membrane, in the connective tissue spaces.

Platelets were found frequently and at all levels: sometimes bridging a gap, sometimes plugging it (Figs, 12 to 14) or lying deeper inside the vascular wall. Images of platelet lysis were not uncommon. Fig. 12, for instance, shows a platelet lodged in a gap; towards the lumen the platelet is still intact, whereas at the opposite pole its membrane seems to have disappeared. We sometimes observed rounded or oval granules, 0.25 to 0.4 micra in diameter, trapped within the vascular wall; they resembled the larger type of granules contained in the platelets, and are probably to be interpreted in this fashion.

Portions of red blood cells trapped in the wall were a very frequent finding, leukocytes were seen only occasionally. Sometimes it was possible to see an erythrocyte projecting into a gap, as though flowing through it and into the wall of the vessel (Fig. 11). The images of diapedesis of red and white blood cells, observed by us, were very similar to those published by Marchesi and Florey (31).

Phagocytic activity of the endothelium. The presence of tracer particles in pinocytotic vesicles was uncommon; it was not observed in the smallest vessels (capillaries), but only in such vessels as also showed evidence of leakage. The first scattered instances were observed at the 2½-minute stage, but the phenomenon was definitely more pronounced at the 3-hour stage (see below).

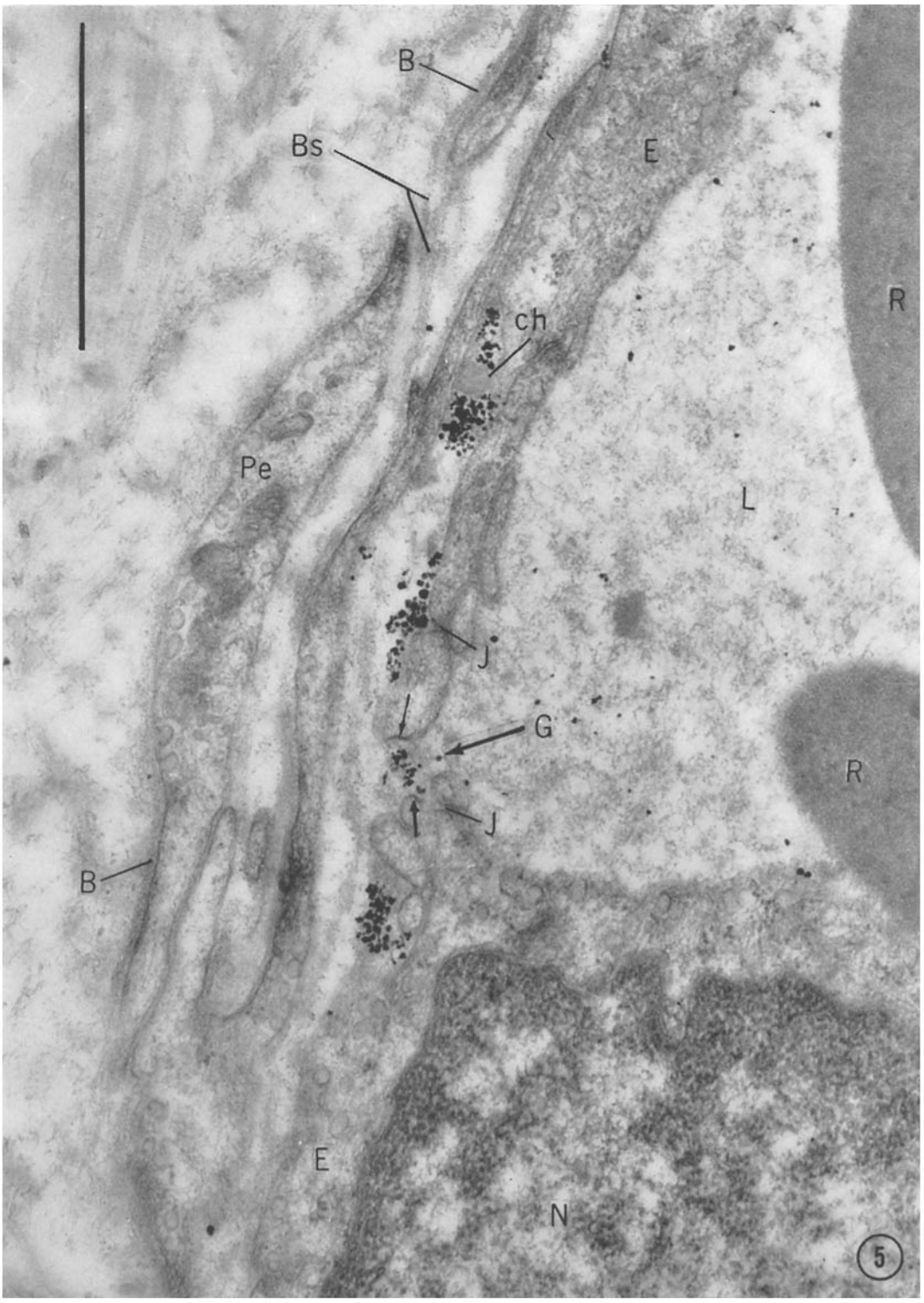
Behavior of the basement membrane. The key role of the basement membrane in the morphogenesis of the lesions has been referred to in earlier paragraphs. It should be further noticed that the basement membrane never showed changes that could be considered primary. Even when stretched to perhaps twice its surface in the "aneurysmal" pockets, it retained its capacity to withhold the tracer particles (Figs. 16, 17). As for the septa which the perivascular basement membrane sends between the cells of the vascular wall, their behavior also indicates a passive role: as plasma and other materials penetrate into the vascular wall and dissect its layers, the cleavage plane

[3] The term "chylomicra" is used, throughout this paper, in a broad sense, *i.e.*, without implying that these bodies necessarily represent recently absorbed lipid.

FIGURE 5

Part of a leaking vessel, 4 minutes after the local injection of histamine and i.v. injection of HgS. Example of an endothelial gap at an early stage. Tracer particles are present between the margins of the gap (*G*), while other particles have penetrated between the layers of the wall, together with a small body which probably represents a chylomicron (*ch*). The small arrows point to dense segments of the cellular membrane, which may represent the two halves of an adhesion plate now cleaved apart to form the gap.

Note that the structure of this leaking vessel is more complex than that of typical capillaries as exemplified in Fig. 1; there are two to three layers of cells, with a basement membrane (*B*) from which arise septa (*Bs*) which penetrate between the layers. Magnification, 50,000.


B
Bs
E
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ch
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L
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5

usually passes along one face of the septum; in other words, the septum is not destroyed, but rather becomes detached along one of its surfaces. In rare instances a septum which originally separated two cells is dissected off on both surfaces, and remains as a tenuous membrane separating two pockets of tracer particles (Fig. 16).

Perivascular phagocytes and other cell types. The tracer particles which escaped through the vascular basement membrane were rapidly engulfed by the perivascular phagocytes and sequestered in vacuoles (Figs. 13, 15). Mast cells and fibroblasts were fairly numerous; both cell types appeared normal. The striated muscle fibers were also normal in structure.

b) *Stage of 3 to 3½ hours (Fig. 19)*

No open leaks were found at this stage, and correspondingly the deposits of tracer particles in the vascular wall appeared very "dry," or tightly packed. Some of the deposits were still extracellular, but now the cytoplasm of both endothelial cells and pericytes contained clusters of particles sequestered into rounded bodies 0.1 to 1 micron in diameter, limited by a membrane, and often containing small masses of amorphous material together with the HgS. At this stage it was also relatively frequent to observe endothelial vesicles containing single tracer particles, particularly where the vascular wall contained heavy intra- and extracellular deposits of HgS.

The fragments of red blood cells entrapped in the vascular walls were apparently unchanged, judging from their structure and density. The same can be said of other erythrocytes lying free in the extravascular spaces, and of the fragments contained in perivascular phagocytes. Platelets or fragments of platelets could not be identified at this stage, in the wall of the injured vessels.

c) *Stage of 57 days*

Scattered histiocytes, mostly perivascular, contained large amounts of HgS. The vessels appeared normal; it was still possible, however, to find an occasional vessel with large clusters of tracer particles inside an endothelial cell.

3. *Controls*

In the animals which received a local injection of histamine or serotonin, but no intravenous injection of tracer particles, typical endothelial leaks developed. Some were plugged by platelets (Fig. 12) and large amounts of chylomicra had accumulated inside the vascular wall. When the colloidal HgS was administered alone (in the absence of any local injection) leaks were not found, but intramural accumulations of HgS were observed in a number of vessels (Fig. 20). These deposits were small, and had to be searched for carefully; they did not compare, in extent, with the intramural accumulations of treated animals. In the rats which received NaCl locally and HgS intravenously, the findings were similar to those of the preceding group, but occasional leaks were found which were indistinguishable from those of the treated animals.

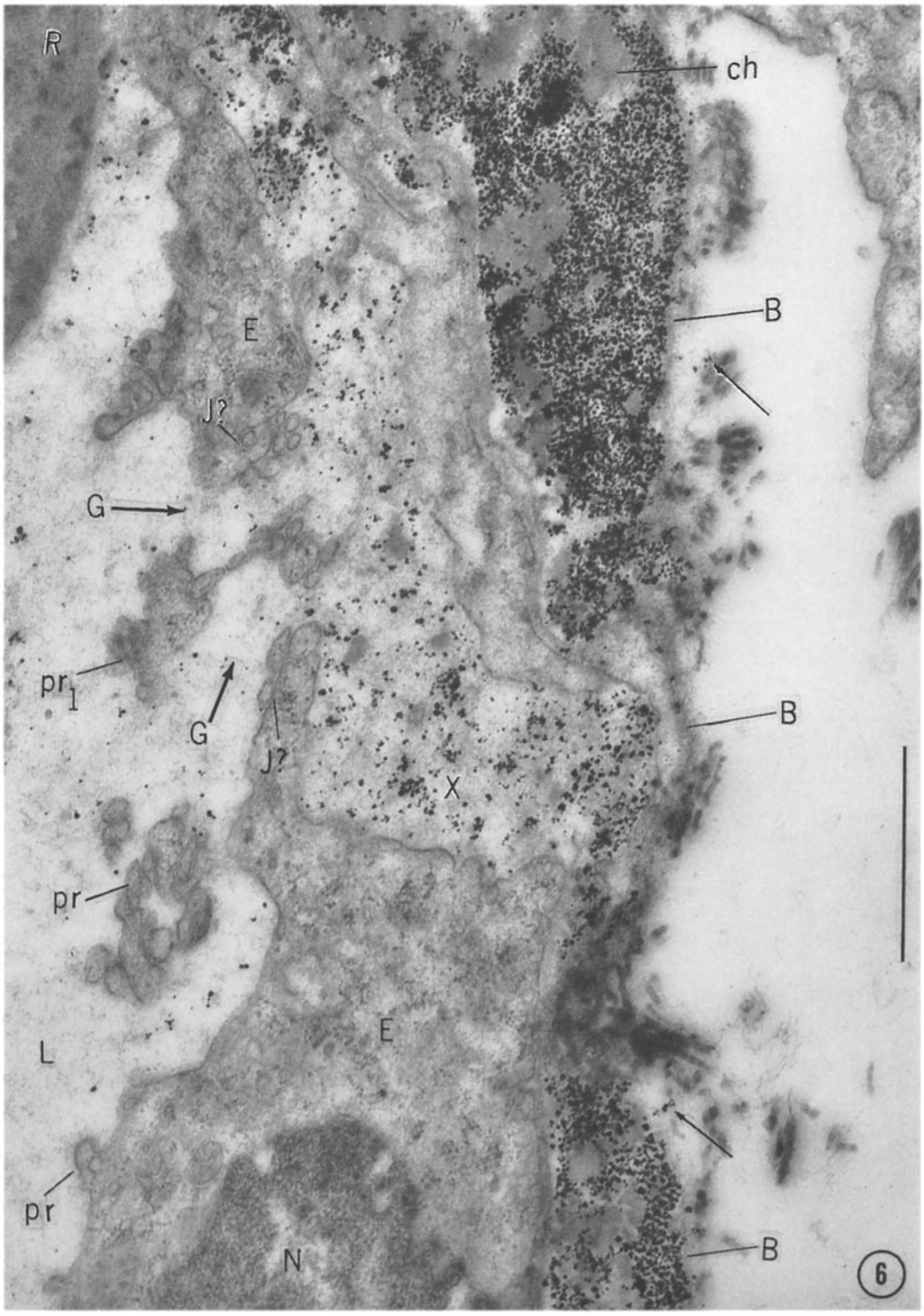
STUDIES WITH THE LIGHT MICROSCOPE

Combined Demonstration of the Vascular Leaks and of the Intercellular Junctions

For obtaining additional evidence that the leaks represent a separation of contiguous endothelial cells, we resorted to a topographic preparation at the level of the light microscope. It is well known that the intercellular lines in vascular endothelium can be demonstrated with the use of silver nitrate

FIGURE 6

Wall of a leaking vessel (outer diameter 10 to 11 μ) 3 minutes after the local injection of serotonin and i.v. injection of HgS. There is a broad gap in the endothelium (*G-G*) containing a fragment of cytoplasm (pr_1) which has no visible connections with the margins. This is probably not a free-floating structure, but either part of a cytoplasmic bridge still connecting the cells *E-E* across the gap, or part of another cell (*cf.* Figs. 7, 8). In the immediate neighborhood of the gap, the endothelial cells give rise to irregular processes (*pr*); this is not a constant occurrence. Note the condensation of the plasma (*X*) inside the wall of the vessel, as described for Figs. 3 and 4. The basement membrane (*B*) has been raised by masses of HgS and chylomicra; very few particles, however, have reached the extravascular space (arrows). Magnification, 33,000.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(see 32); it is also possible to visualize the leaking vessels, with the light microscope, by injecting intravenously a particle of better optical properties than HgS (*e.g.*, carbon black). We therefore superimposed the two procedures, as follows.[4] A rat received an injection of histamine in the scrotum, as usual, and an intravenous injection of carbon black (a suspension especially prepared by Guenther-Wagner Pelikan-Werke, Hannover, Germany, for use in experimental animals). An hour was allowed for the carbon circulating in the blood stream to be cleared by the reticulo-endothelial system; then the animal was killed, its vascular system was rapidly perfused with warm 10.2 per cent sucrose solution, and thereafter with 0.5 per cent silver nitrate. The cremaster was excised, fixed in 10 per cent formalin, exposed to light before and during fixation, cleared in glycerin, and examined *in toto* (by transillumination) at enlargements of 300 to 450. The "silver lines" appeared in brown, whereas the carbon deposits were distinctly black. The result was clear cut: wherever the intramural deposits of carbon could be observed at an early stage of development (allowing a clear resolution of their topographic relations) they were situated along a silver line (Figs. 21–24). In vessels in which the carbon deposit was very extensive, the pattern of silver lines was obscured (Fig. 21).

Demonstration of Fat in the Walls of the Vessels

In order to confirm the fatty nature of the amorphous masses identified as chylomicron deposits by electron microscopy, whole cremasters (from normal and histamine-injected animals) were fixed in formalin, stained *in toto* with sudan red, cleared in glycerin, and examined by transillumination. Control vessels remained unstained. At the site of the histamine injection many small vessels took up a diffuse red stain, and their walls also contained numerous red droplets about 1 micron in diameter. Further details will be given in a later publication.

[4] For these preparations we are indebted to Miss Gutta I. Schoefl.

DISCUSSION

The essence of our findings may be stated as follows. Within 1 minute after the local injection of histamine or serotonin, gaps appear in the endothelial lining of certain blood vessels. Tracer particles become concentrated in these gaps and accumulate against the basement membrane, while very few are seen outside the vessel. These images suggest that the endothelial gaps are true leaks through which plasma gains access to the basement membrane, which behaves like a filter and retains the tracer particles.

A. PATHOGENESIS OF THE VASCULAR LEAKS

A gap in the endothelium could develop, conceivably, in two ways: either by the separation of contiguous cells, or as a fenestration piercing the cytoplasm of a single endothelial cell. The mechanism is best studied in specimens taken at very early stages (1 to 3 minutes after the application of the drug). In some instances it is clear that the cells are separating along the intercellular

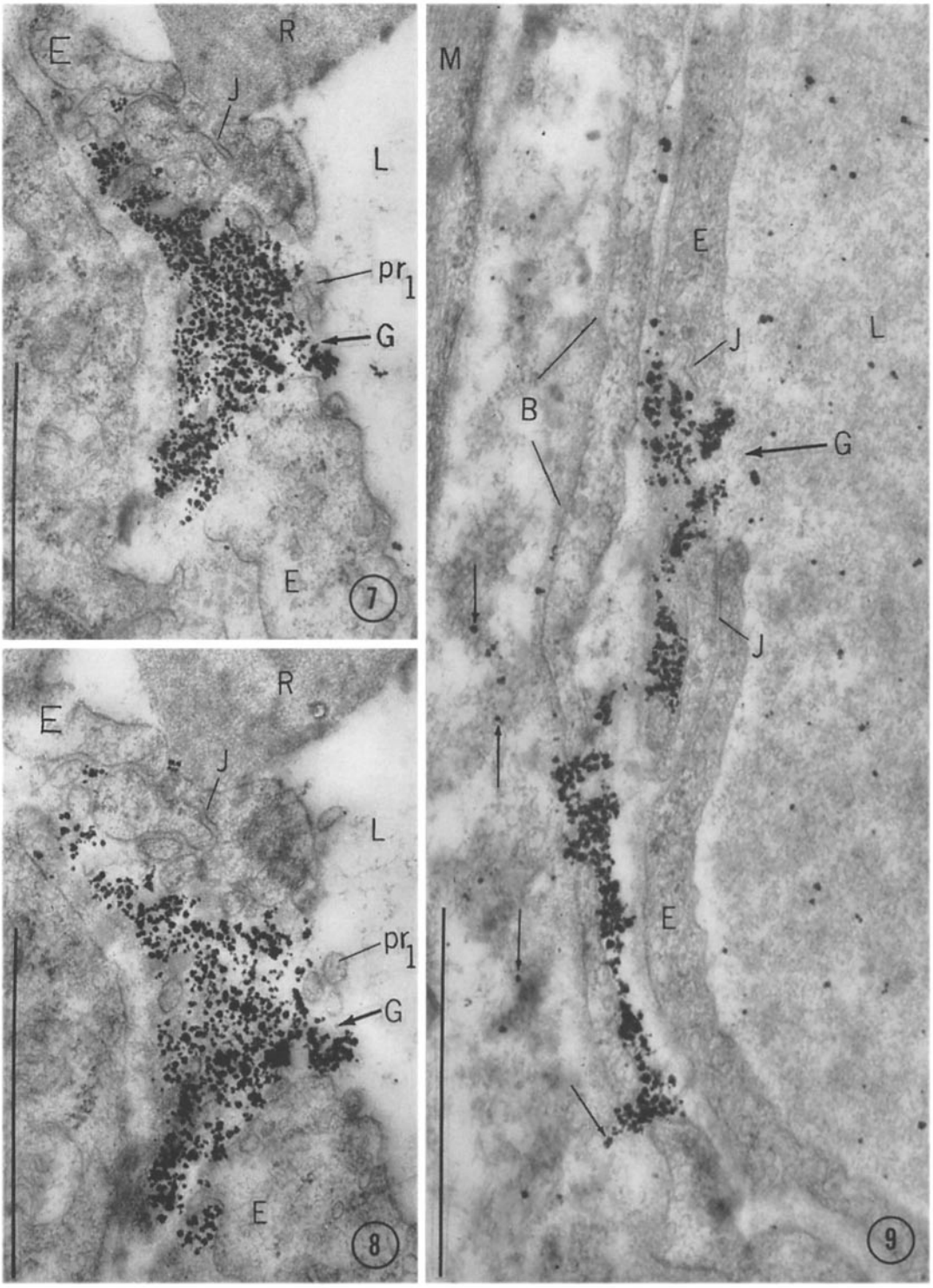
FIGURES 7 AND 8

Serial sections of a leak (*G*) in the wall of a vessel, 3 minutes after the local injection of serotonin and the i.v. injection of HgS. The three rounded bodies which appear to lie free between the margins of the gap (pr_1) may be either cross-sections of cytoplasmic bridges connecting the cells *E-E* or part of another cell (*cf.* Fig. 6). Magnifications, 41,000 and 53,000.

FIGURE 9

Wall of a venule, 4 minutes after the local injection of histamine and the i.v. injection of HgS. Endothelial gap (*G*) with intercellular junctions (*J*) in both margins. A few tracer particles have escaped into the extravascular spaces (arrows).

The wall of this leaking vessel consists, as usual, of two cell layers. Magnification, 56,000.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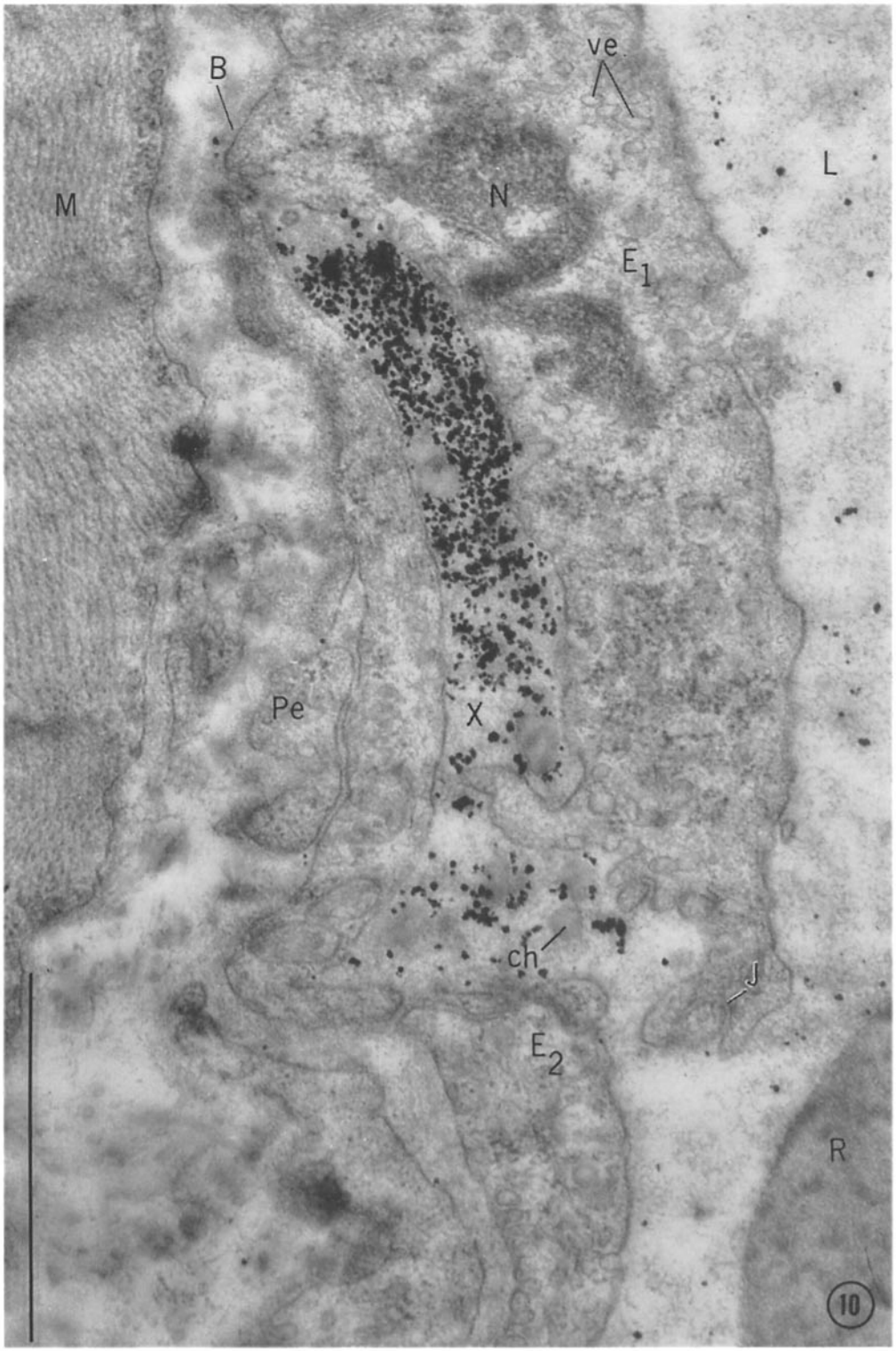
junction, as exemplified in Fig. 10. Here an endothelial cell is lifted off from the underlying cell, and the intervening fissure is becoming the site of a leak, as demonstrated by the local concentration of tracer particles. In many other instances the mechanism of formation of the leak is not as clear, because there is no overlap of cells to suggest a detachment; the appearance is that of a short channel, punched perpendicularly through the endothelium. In most of these cases, however, there is evidence of an intercellular junction close to one or both of the lips (Figs. 4, 5, 7 to 9, 11, 14, 15). This image—an intercellular junction very close to a gap—could arise as follows: either the cells have separated principally along the intercellular junction, but in such a manner that small parts of cytoplasm of one cell tore off and remained attached to the other cell; or, more likely, the suggestion of a tear is a false appearance brought about by the plane of section. If the endothelial lining of a venule is studied with the light microscope in preparations obtained with the silver nitrate method as described above, it is obvious that adjoining cells meet along a wavy or jagged line (Figs. 22, 24). Now, if a small gap developed at one discrete point along this line (as demonstrated in Figs. 21 to 24), a microtome section falling along the general direction of the line —perpendicular to the endothelial surface— would show an endothelial layer, with one or more intercellular junctions in close proximity to a gap. This is the case in Figs. 4 and 5. A third possibility is that the opening, in some cases, occurs at the site of junction of three or more cells. This would allow one to explain the fragments of cells attached to the margins of the gap (Figs. 4, 5, 9 to 11) as parts of a third endothelial cell interdigitating with two others. The hypothesis that openings may develop where three cells unite (44) is supported by our preparations in which the intercellular junctions and the leaks are demonstrated simultaneously. Fig. 24 shows at least four leaks occurring in this fashion. The isolated fragments of cytoplasm which are occasionally found in relation to a gap, as if suspended between the lips (Figs. 6 to 8), probably represent the cross-sections of fine cytoplasmic bridges crossing the gap.

Our views on the pathogenesis of the endothelial leaks are in contrast with those of Alksne (11) who studied the effect of histamine on the dermal vessels of the mouse. Alksne described openings in the endothelium very similar to those observed by us, but interpreted them as "channels perforating endothelial cells." Particles of HgS, injected intravenously, were observed in "folds, vesicles, clefts and caveolae"; the author concludes that histamine "accelerates the normal transport process" by stimulating the "membrane activity" of the endothelial cells: a view inspired by the membrane flow hypothesis of Bennett (33).

In support of his view, Alksne provides the following evidence. (*a*) In a capillary wall, the intercellular junction may appear normal, whereas tracer particles may be present beneath the endothelium, having "already crossed the endothelial cell." This argument is not conclusive, for the particles could have reached the outer surface of the endothelium through an opening not shown in the section, without necessarily crossing the endothelial cell. (*b*) "No cell membrane thickenings or signs of intercellular contacts or attachment belts were associated with the membrane infoldings and perforating channels." This statement could only bear the weight of negative evidence; furthermore, in the single published illustration of a "channel," segments of intercellular junctions may well be present in both the lips (ref. 11, Fig. 19). (*c*) The cytoplasm of endothelial cells affected by histamine contained "more and larger" vesicles than it did in normal capillaries. The illustrative material

FIGURE 10

Wall of a leaking vessel (outer diameter 11 micra) 3 minutes after the local injection of serotonin and the i.v. injection of HgS. The edge of an endothelial cell (E_1) has become detached from the neighboring (E_2) as well as from the underlying cell. The fissure thus created has become the site of a leak, as indicated by the accumulation (X) of tracer particles and chylomicra (*ch*). Note how the HgS particles become progressively more concentrated in the neighborhood of the basement membrane (B), suggesting that the latter behaves as a filter. The endothelial vesicles (*ve*) do not contain tracer particles. Magnification, 55,000.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refers to animals killed 20 minutes after the application of histamine (in other cases the stage is not indicated). At these relatively late stages secondary changes may occur in the endothelium, as shown by the mitochondrial swelling of our Fig. 18. We have not seen evidence of such "vesiculation" in our material; our only finding which could possibly be interpreted as evidence of membrane stimulation was the formation of pseudopodia close to the leaks, but this too was not constant, and probably represented a secondary occurrence. (*d*) Red blood cells of histamine-treated animals were said to contain membrane-lined vesicles which were absent in controls. We have observed a number of such vesicles in normal as well as in treated animals; they appeared to be remnants of cytoplasmic structures, with no relationship to the experimental procedure.

Thus it appears that Alksne's interpretation, and his concept of endothelial perforation, are not clearly supported by the evidence. Endothelial openings of a different origin were described by Marchesi and Florey, in their electron microscopic study of diapedesis in the rat mesentery (31). These authors came to the conclusion that the emigrating cells pass through openings which may appear either between endothelial cells, or actually through them. However, it is interesting to note that in the figures which should exemplify the case of an opening *through* the cell, an intercellular junction is apparent in the immediate neighborhood of this opening.

The electron microscope, therefore, provides strong evidence to suggest that the endothelial openings induced by histamine and serotonin occur primarily along the intercellular junctions; and if this indication is combined with the findings at the level of the light microscope, the concept of intercellular leaks becomes difficult to deny, at least as the major modality in the formation of the endothelial openings. We cannot exclude, of course, minor disruptions at the margins of the cells, as a secondary occurrence. A further contribution of the light microscopic preparations, in which both the leaks and cellular junctions are demonstrated, is to show that the points of convergence of three cells are particularly vulnerable; and to confirm that the leaks develop as discrete defects, not as a generalized loosening of the endothelial lining.

In reaching this conclusion we find ourselves in line with a number of very old observations, made soon after the discovery of the silver nitrate method (12–15). Arnold especially upheld for many years the theory that damaged vessels develop intercellular "stomata" (14). He found that particles of red mercuric sulfide (cinnabar), injected intravenously, accumulate along the intercellular junctions (13). One of Arnold's original drawings is reproduced in our Fig. 25; it is obviously reminiscent of our own preparation, obtained in similar fashion (Fig. 24). More recently Chambers and Zweifach (34), Samuels and Webster (35), Gottlob and Zinner (36), Robertson, Moore, and Mersereau (37) and others have emphasized the fact that the earliest signs of endothelial damage—consequent to injuries of various kinds—occur at the level of the intercellular junction.

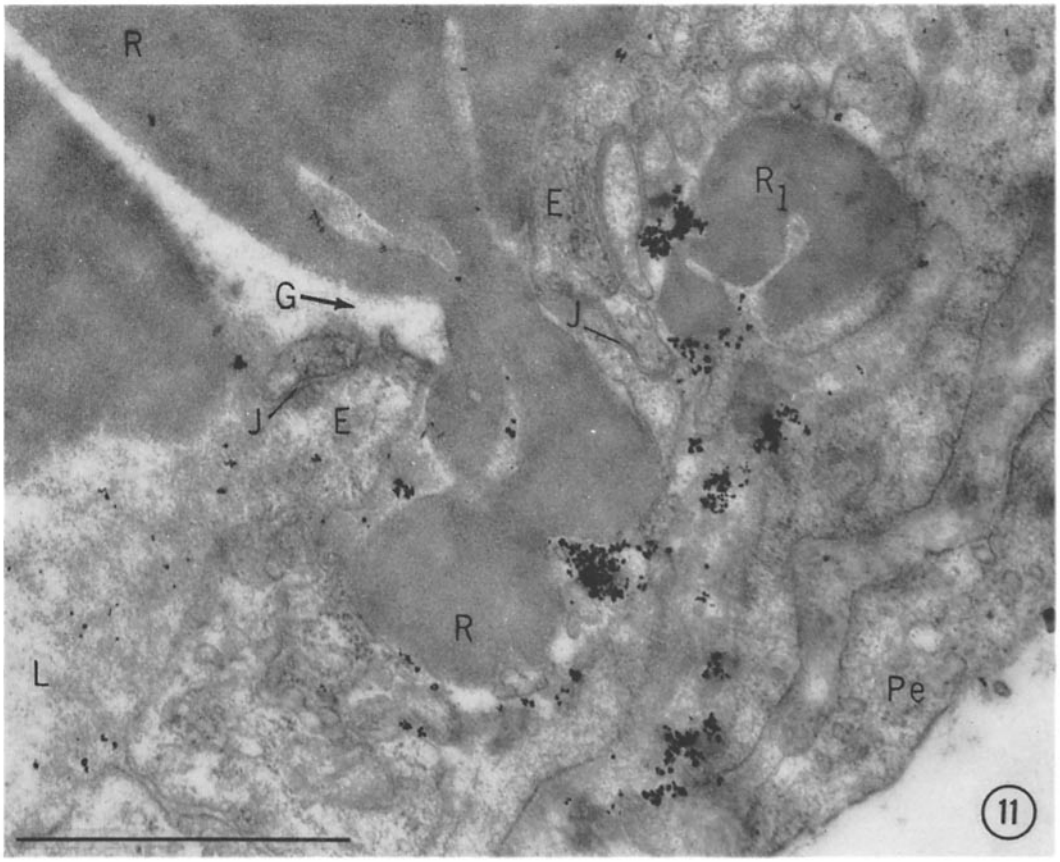
While this point seems reasonably well established, several related problems remain unsolved. We do not know, for instance, why the drugs should affect the endothelium of certain vessels, and not of others; and why the endothelial cells should separate only at certain points, rather than diffusely. We are equally ignorant as

FIGURE 11

Wall of a leaking vessel, 4 minutes after the local injection of serotonin and i.v. injection of HgS. A portion of a red blood cell (*R-R*) is engaged in an endothelial gap (*G*). There is an intercellular junction (*J*) on either side of the gap. R_1 = part of the same or of another red blood cell, lodged inside the wall of the vessel which consists of two to three layers of cells. Magnification, 44,000.

FIGURE 12

Wall of a leaking vessel, 4 minutes after the local injection of histamine. No tracer particles were administered. A platelet (*P*) is shown in the act of plugging an endothelial gap (*G*); the membrane is well defined in the portion of the platelet which is protruding into the lumen of the vessel, but in the intramural part the membrane is no longer visible, suggesting that the platelet may be disintegrating. Magnification, 42,000.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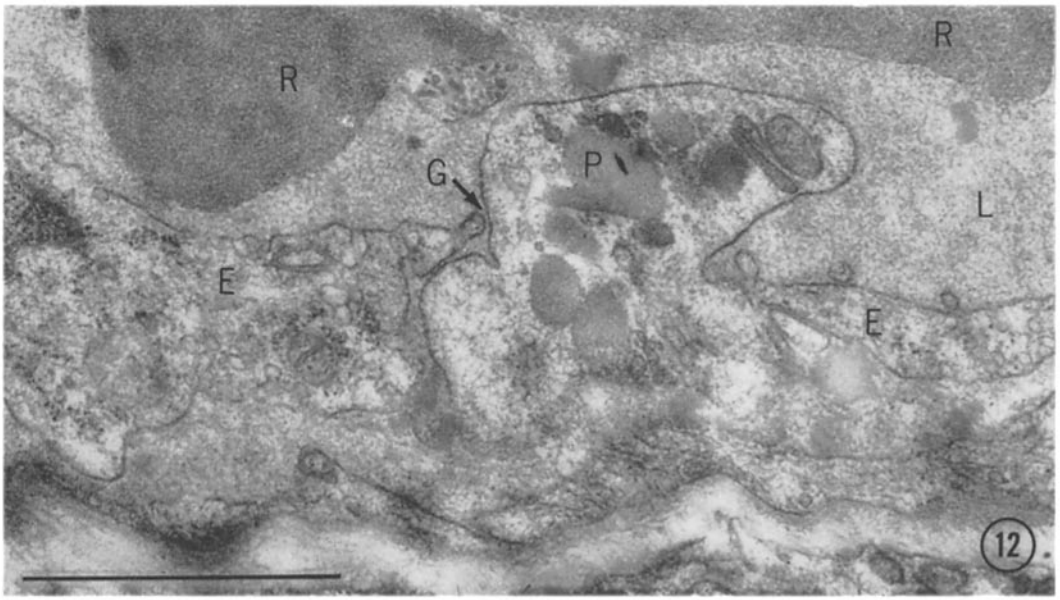
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to the mechanism whereby histamine and serotonin cause the cells to separate. The possibility of cellular contraction comes to mind (10): both substances—like many others which increase vascular permeability—are also notable for their capacity to induce contraction of smooth muscle (9, 10); other cell types can also be made to shrink (38, 39). Our evidence, however, rather points to a primary loosening of the intercellular junction, in discrete areas. Since there is little electron microscopic evidence for an intercellular cement in the traditional sense (34), it may be that the drugs act directly on the cellular membrane, and possibly on particular areas of this membrane (40, 41).

B. THE FILTERING EFFECT OF THE BASEMENT MEMBRANE

Where endothelial gaps leave the basement membrane uncovered, it is obvious that the latter behaves as a filter. Though some of the HgS particles may find their way into the extracellular spaces (Fig. 9) the great majority are retained. A similar phenomenon is observed (under normal conditions) in the glomerular capillaries of the kidney, where the basement membrane is able to retain molecules of ferritin (42) and particles of colloidal gold (43).

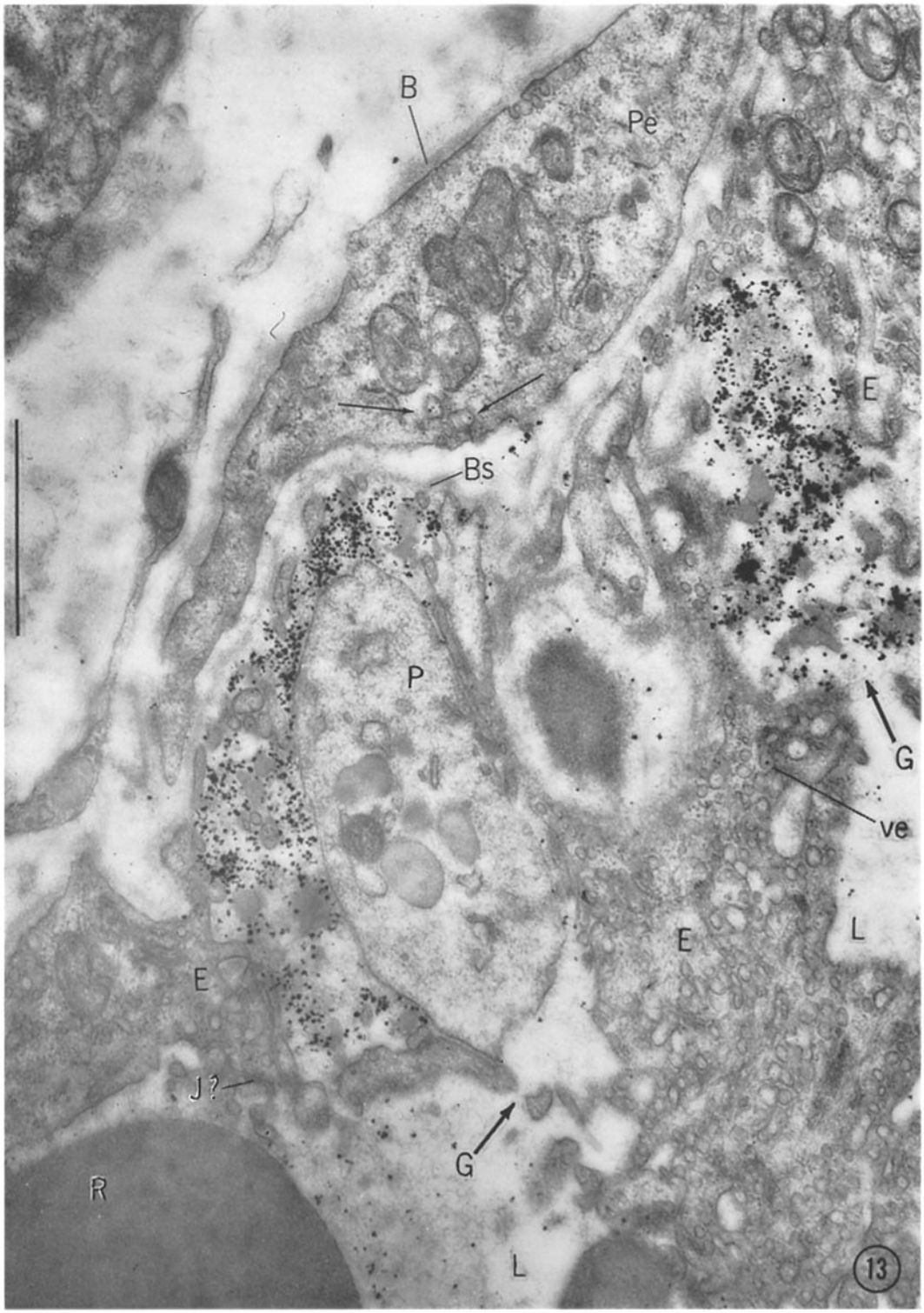
It is remarkable that very fine particulate material, such as colloidal HgS, is retained, whereas erythrocytes manage to reach the extracellular spaces in considerable numbers. It is conceivable that larger, pliable bodies, such as red blood cells, might be more easily pushed through by the intravascular pressure. Krogh (44) mentions that red blood cells may pass through an ordinary filter capable of retaining particles of much smaller size. It must further be assumed that as soon as the erythrocyte has punched a hole through the basement membrane, this hole promptly closes up again, for there is little leakage of particles into the extracellular spaces. This closure would presuppose a considerable degree of elasticity by the basement membrane; and for this there is indeed clear evidence. In many leaking vessels the basement membrane stretches to form deep pockets, (*e.g.* Fig. 17); and furthermore, capillaries are said to be extremely resistant and elastic by micromanipulation, a property which Nagel attributed principally to the basement membrane (45).

The density of the plasma contained in recent leaks is constantly greater than in the lumen of the corresponding vessel (Figs. 4, 6), suggesting that water has been able to filter off at a faster rate than the proteins it contains. The average diameter of the blood proteins is close to 40 A, and if the basement membrane is capable of retaining almost all the particles of HgS down to a diameter of about 70 A, it is clear that the filtration of protein molecules should encounter some difficulty. The retention of plasma protein probably accounts for the intramural pools of amorphous material in which filaments of fibrin polymerize (Fig. 17). Platelets are often caught in the act of penetrating into a leak, and occasionally of disintegrating inside the vascular wall (Fig. 12). This could presumably trigger the clotting mechanism.

The retention and accumulation of chylomicra (see footnote[2]) was one of the most striking features of the leaking vessels (Figs. 5, 10, 14, 15). A peculiar characteristic of the chylomicra is that they tend to form clusters, inside the vascular wall, rather than to mix uniformly with the particles of HgS (Fig. 16). It can only be inferred that the surface properties of the chylomicra cause them to adhere to each other, but not to the particles of HgS. As far as we know, it has never been

FIGURE 13

Wall of a leaking vessel, 2½ minutes after the local injection of histamine and i.v. injection of HgS. The grazing section of an endothelial cell, which appears as if it were protruding into the lumen (lower right), indicates that the vessel has been cut obliquely. Two endothelial gaps are shown (*G*, *G*); tracer particles, chylomicra, and a platelet (*P*) have penetrated into the wall of the vessel. The particles appear to be retained (left center) at the level of a faint gray line which represents an intramural expansion or septum of the basement membrane (*Bs*). There are no tracer particles in the endothelial vesicles, with one exception (*ve*); a few have been taken up by a pericyte (arrows). Magnification, 33,000.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noticed by histologists that histamine (or acute inflammation) induces fat to appear in vascular walls. One reason for this oversight may be that the amount of fat involved is relatively small, and while obvious on whole mounts of tissue, it is quite elusive on stained sections of frozen tissue.

We have little information regarding the later fate of the materials accumulated inside the vascular wall. It is clear, however, that the tracer particles eventually find their way out of the vessel and into the surrounding histiocytes. It is possible that the pericytes may play a role in this emigration, since they are sometimes seen to straddle the basement membrane (see Results).

C. PHAGOCYTOSIS BY THE ENDOTHELIUM

The uptake of tracer particles by the endothelial cells is very slow. A few vesicles containing particles can be found, particularly in the neighborhood of the leaks, but there is no evidence to support the notion that histamine stimulates either phagocytosis by the endothelium (46–48) or the "normal transport process" (11). Phagocytosis by the endothelial cells becomes somewhat more prominent in later stages, *e.g.* after 3 hours, when the abnormal materials spilled into the vascular wall have become concentrated in rounded bodies reminiscent of the "phagosomes" described by Straus (49).

The pericytes appear to be definitely more phagocytic than the endothelial cells, at least under the conditions of our experiments. In the neighborhood of a leak, for instance, it is common to observe that particles of HgS are present in the pericytes and not in the endothelial cells (Fig. 13).

D. RELATION OF ABNORMAL FINDINGS TO THE CALIBER OF THE VESSELS

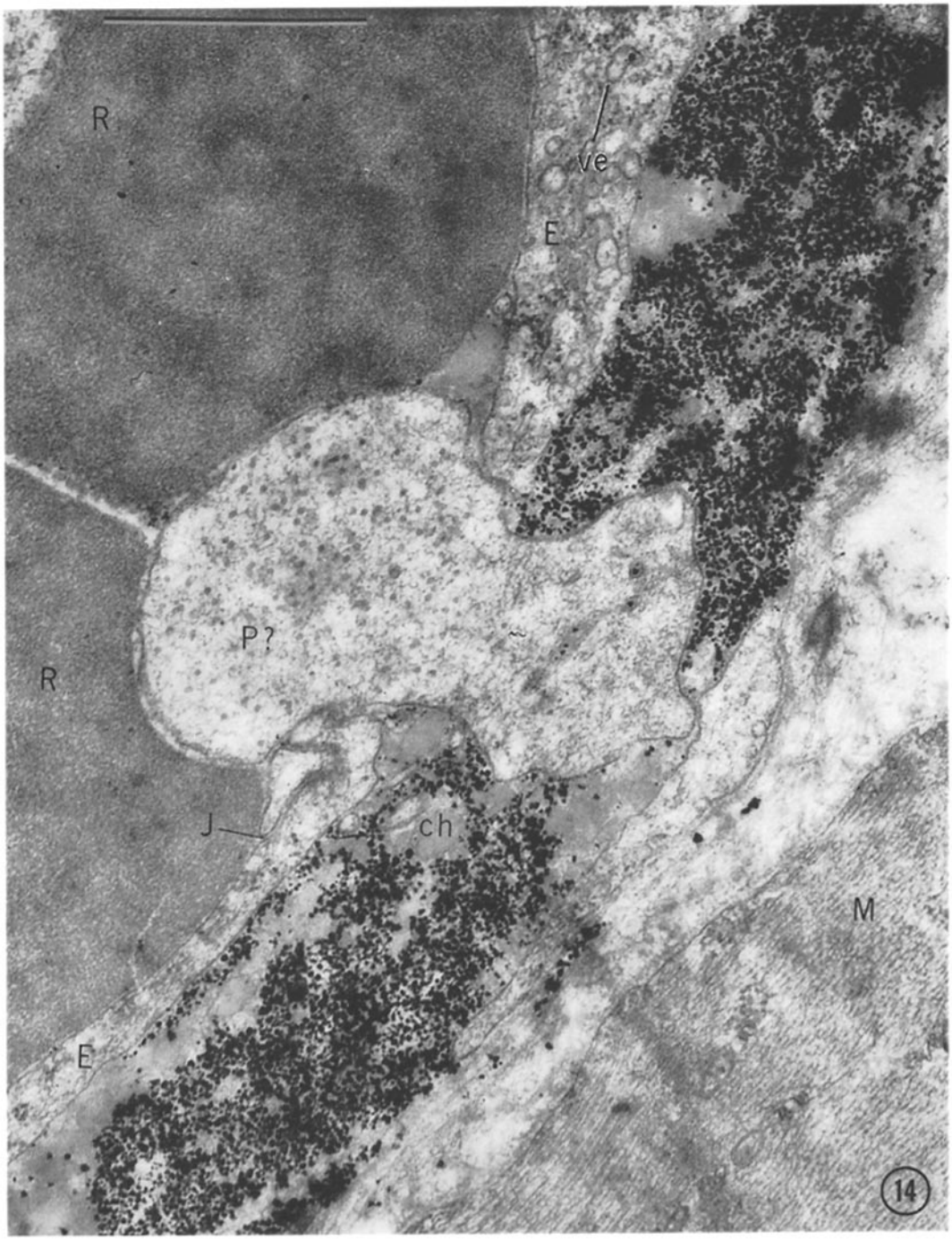
It is striking to observe, in the tissue sections, that the smallest vessels are as a rule devoid of leaks. These vessels, measuring 3 to 5 micra in diameter[5] and undoubtedly representing true capillaries, showed only one occasional abnormality: that is, a concentration of the luminal contents. Of this there was threefold evidence: a greater density of tracer particles, of chylomicra, and of the granular background representing plasma proteins (Fig. 3, insert). The latter finding is probably the morphologic equivalent of the increase in plasma protein concentration which is characteristic of stasis, as described by physiologists (50). It was observed more often in larger, leaking vessels (Fig. 16).

The leaks usually occurred in vessels with a diameter of 7 to 10 micra or more. The difference in caliber between leaking and non-leaking vessels,

[5] The figures mentioned in this paragraph are outer diameters (the basement membrane being taken as a reference point) of vessels in sections of osmium-fixed, methacrylate-embedded tissue. As such they do not directly apply to the situation *in vivo*. Our figures are smaller than those usually quoted in the literature; the discrepancy is due in part to the shrinkage caused by embedding, which is known to amount to approximately 10 to 15 per cent (55), and possibly to contraction of the vessel at the time of fixation. It should be realized, however, that there is no systematic study of capillary diameter by light microscopy; the data available are scarce, and indicate wide scatter (this point will be treated more extensively in the accompanying paper). At present we have the impression that the diameter of these vessels has been overestimated in the past.

FIGURE 14

Wall of a leaking vessel, 12 minutes after the local injection of serotonin and i.v. injection of HgS. In the center, a portion of a cell (possibly a platelet) engaged inside an endothelial gap, in the immediate neighborhood of an intercellular junction (*J*). The thickness of the vascular wall is increased severalfold by a large accumulation of material, consisting mainly of tracer particles, but also of an amorphous material very smooth in texture, with occasional faint outlines of rounded bodies (chylomicra) (*ch*). In the endothelium, one vesicle contains a tracer particle (*ve*). In the cytoplasm of the cell engaged in the gap, there are two or three vesicles with particles of HgS; discrete images of phagocytosis such as these were not exceptional in platelets. Magnification, 39,000.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expressed in micra, appears slight, but in a given section there are many more vessels of the smallest caliber; hence it may well happen that none of the vessels, in a single section, shows a leak. This observation is rather intriguing, because the vessels affected by histamine and serotonin are generally believed to be capillaries. From our sections it would appear that the damage is localized in vessels of a somewhat larger caliber and more complex wall (see, *e.g.*, Figs. 2, 3, 5). The relative position of the leaking and non-leaking vessels in the vascular tree is of course extremely difficult to establish in tissue sections; the problem will be dealt with in the subsequent paper (51) in which it will be shown that the leaking vessels are on the venous side of the vascular tree.

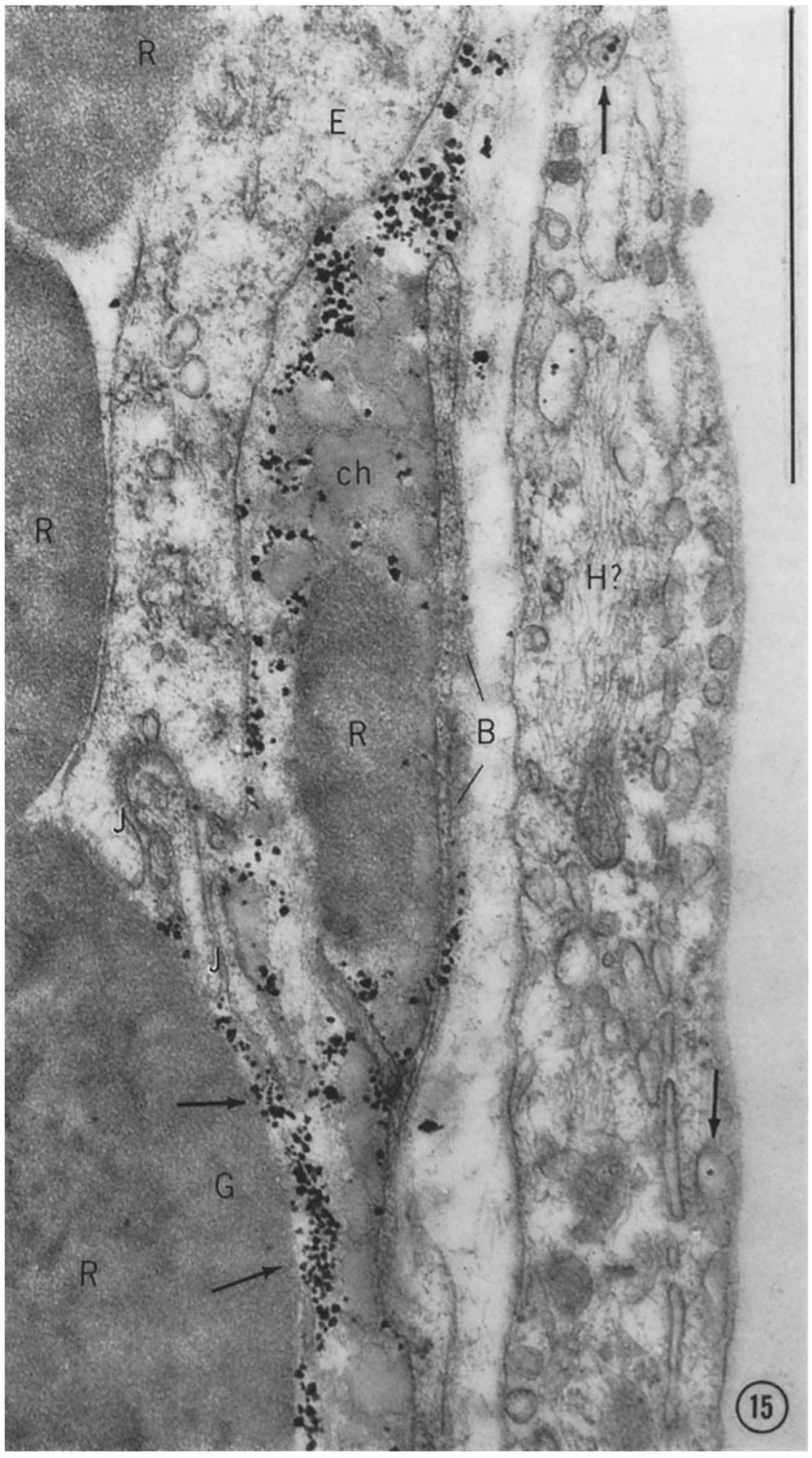
It should be mentioned here that there was evidence of leaking also in the untreated controls, again in vessels larger than the finest capillaries. We are referring here to animals which received an intravenous dose of HgS, but no local injection of histamine or serotonin. The intramural accumulations consisted of few tracer particles; chylomicra were absent (Fig. 20). Similar observations were made by Alksne (11). How the particles reached the intramural space was not established; it could have been by pinocytosis, through occasional small leaks, or by a combination of these mechanisms. We cannot rule out the possibility that the very injection of tracer particles (inclusive of a large amount of protective colloid) may have liberated some endogenous histamine, a property common to many macromolecules (52). On the other hand, it is interesting that Grotte (53), through studies with dextrans of various molecular weight, postulated the existence of a system of relatively large leaks existing under normal conditions.

E. CONCLUDING REMARKS

In order to explain the increased vascular permeability induced by histamine, and by drugs of similar properties, a number of mechanisms have been suggested. According to one traditional view, these substances have the property of increasing the permeability *of the cells*; this mechanism seems *a priori* rather unlikely, for if the endothelial cells were to become suddenly more permeable, to the point of being literally washed through by plasma, their inner environment would be destroyed and they would almost certainly not survive (34). A simple acceleration of any normal pinocytotic transport mechanism could not, of course, suffice to bring about a large and sudden spurt of exudate; and we have already mentioned that the concept of cellular perforation, in the sense proposed by Alksne (11), has little to support it. If our conclusions are valid, the mechanism which operates to increase the vascular permeability—the partial dissociation of the endothelial sheet—is possibly the simplest, and also recalls other situations in which a sheet of cells is required to become more permeable. In referring to Arnold's concept of intercellular stomata (14), a concept which is essentially the same as ours, Metchnikoff states (54): " . . . These pores between endothelial cells, which open to allow the passage of the corpuscles and fluid of the blood, and which close after the passage thereof, could be more properly compared to the pores of the ectoderm of the sponges, which open and close for the passage of the corpuscles suspended in the surrounding water."

FIGURE 15

Wall of a leaking vessel, 2½ minutes after the local injection of histamine and i.v. injection of HgS. In the lumen, packed red blood cells, indicative of stasis. Between the smaller arrows, a gap (*G*) in the endothelial lining in the immediate neighborhood of an intercellular junction (*J*). Various materials have accumulated inside the vascular wall: particles of HgS, a portion of a red blood cell (*R*), and chylomicra forming a cluster (*ch*) in which the outlines of the individual droplets are still recognizable. The basement membrane (*B*) has retained most of the tracer particles, but some have escaped; a few have been phagocytized by a perivascular cell, possibly a histiocyte (*H*), and can be seen inside vesicles (arrows). None of the endothelial vesicles contain particles. Magnification, 66,000.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The data presented in this and in the following paper have already been published in abstract form (*Fed. Proc.*, 1961, **20,** 119). Fig. 15 was reproduced in *Circulation*, 1961, **24,** 368.

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FIGURE 16

Wall of a leaking vessel, 12 minutes after the local injection of serotonin and i.v. injection of HgS. In the lumen there is clear-cut evidence of plasma condensation: increased density of plasma protein, of tracer particles, and of chylomicra, some of which have aggregated to form a cluster (ch_1). In *G*, an endothelial gap; just beyond the entrance of this gap, a cluster of chylomicra about 1 micron in width (ch_2). (Clusters of this size were easily identified as lipid with the light microscope, using formalin-fixed material stained with sudan red). The vascular wall consists (top right) of two layers of cells; as these were cleaved apart a septum of basement membrane (Bs) was lifted away from the surrounding cells and now appears as an isolated structure. Note the efficiency of the basement membrane (*B*) in retaining the particles of HgS (top). A few particles have been phagocytized by a pericyte (Pe); none are contained in endothelial vesicles. Magnification, 28,000.

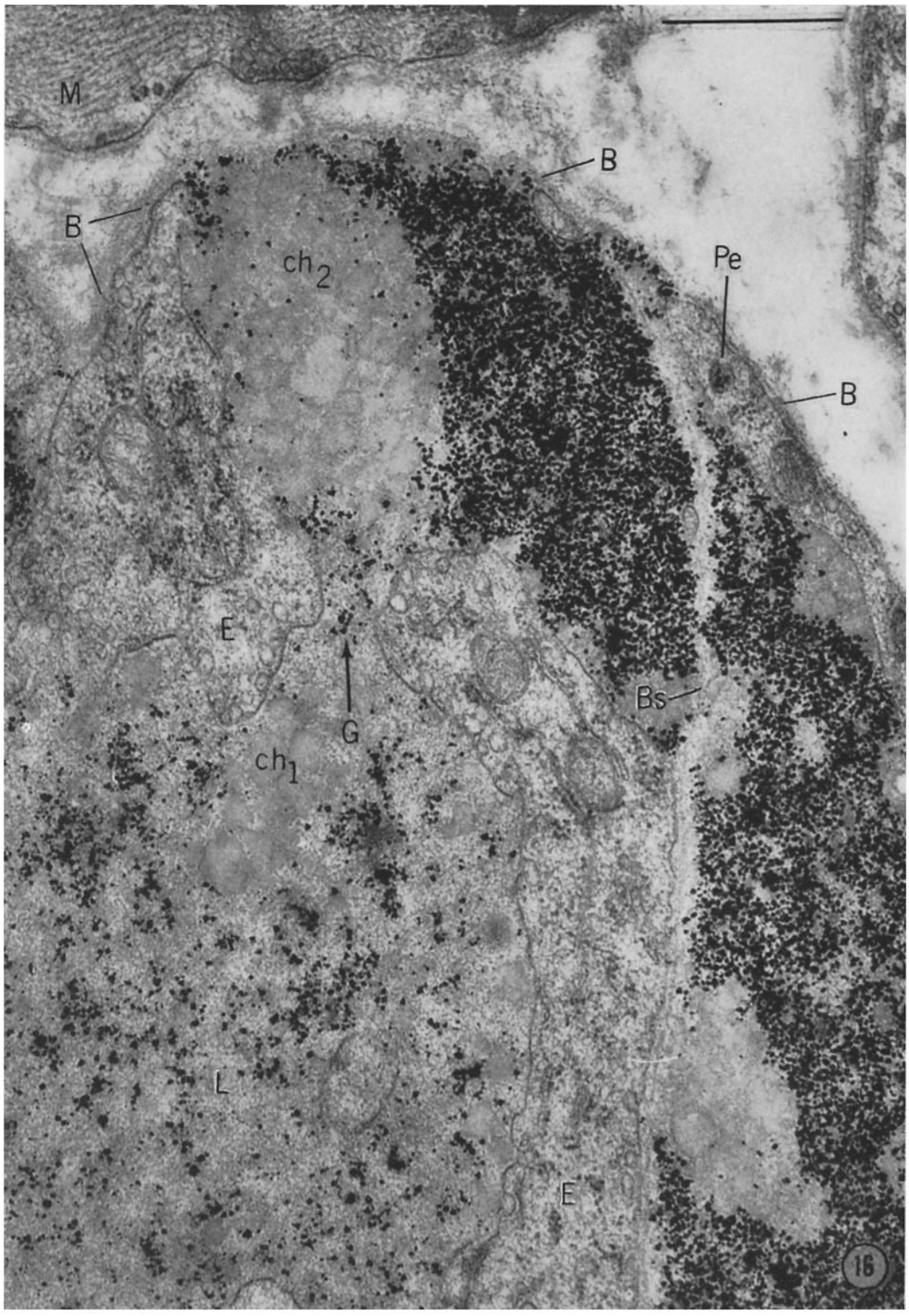
M
B
B
B
Pe
B
ch2
E
G
Bs
ch1
L
E
16

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FIGURE 17

Wall of a leaking vessel, 12 minutes after the local injection of histamine and i.v. injection of HgS. The wall contains extracellular particles of HgS at different levels, but the endothelial gap through which the particles reached this location is not visible. The basement membrane (*B*) has been dissected away from the underlying cell by a deposit of extraneous material which appears to be almost 3 micra in thickness; the resulting image recalls a dissecting aneurysm. Within the mass of HgS particles, several filaments exhibit the typical 220 A periodicity of fibrin. In the endothelium, a few particles are contained within membranous structures (arrows). Magnification, 26,000.

Insert: an enlargement of the longest fibrin filament. By comparison with the 220 A period it is possible to estimate the size-range of the HgS particles (70 to 350 A). Magnification, 64,000.


B
R
E
N
R
B
17

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FIGURE 18

Wall of a leaking vessel, 12 minutes after the local injection of serotonin and i.v. injection of HgS. The wall consists of at least two layers of cells, and these layers are cleaved apart by a deposit of HgS and other materials (*X-X*). *J* = intercellular junction, containing a few particles of HgS (arrows); the main leak, however, is not apparent at this level. No particles are present in the endothelial vesicles. Note the swelling of mitochondria (*m*) and vesicles; this is not a constant occurrence. The sarcosomes in the surrounding muscle fibers were normal in appearance. Magnification, 32,000.

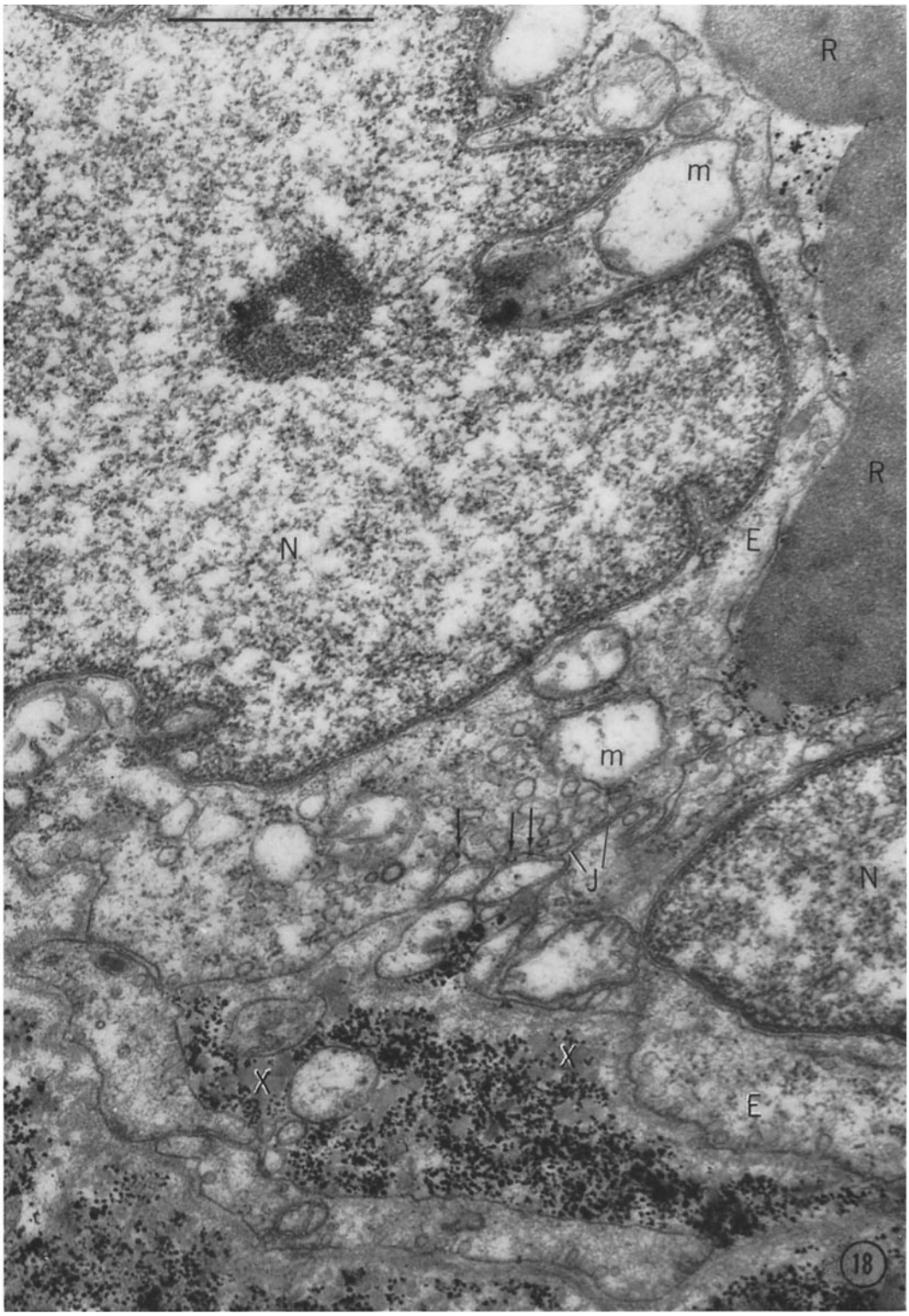
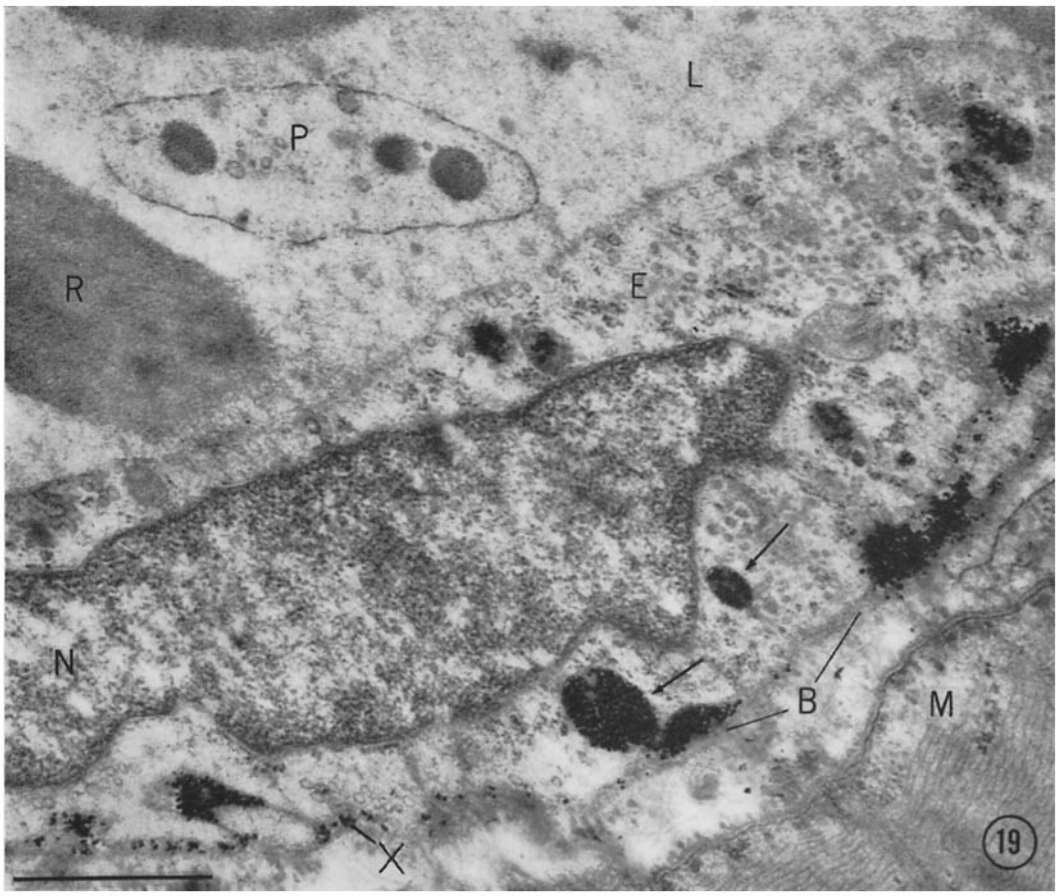

R
m
R
N
E
m
J
N
X
X
E
18

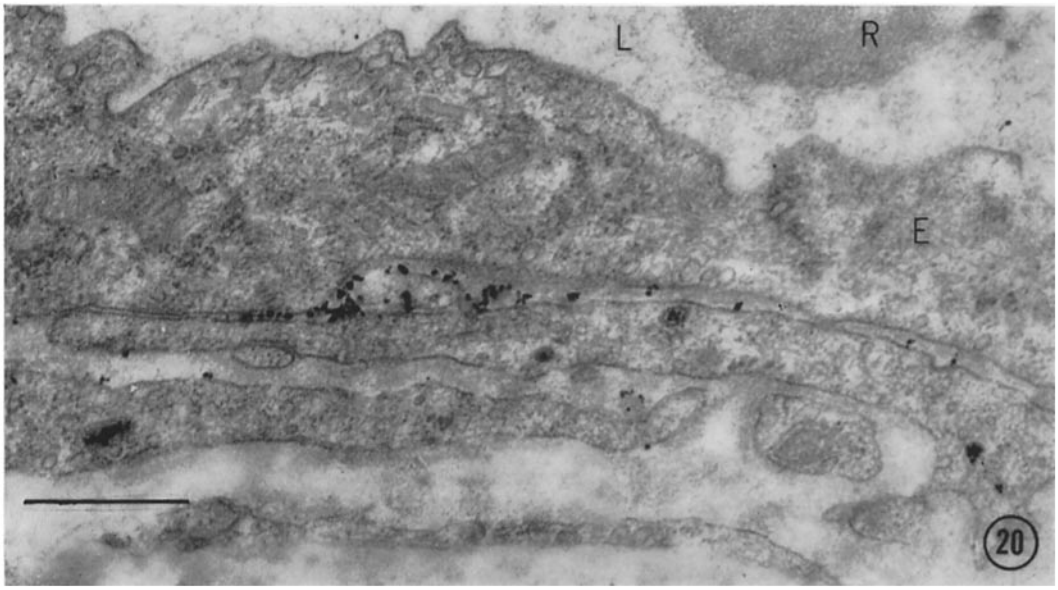
FIGURE 19

Wall of a vessel 3 hours after the local injection of histamine and i.v. injection of HgS. No open leaks were observed in this or any other vessel at this stage, but from the amount of HgS particles present in the wall, it can be safely assumed that this vessel contained—soon after the application of histamine—endothelial leaks. Some of the particles are collected in rounded bodies (arrows) limited by a membrane. Other particles are extracellular (*X*), mostly aligned against the basement membrane (*B*). Magnification, 27,000.

FIGURE 20

Control: vessel of a normal animal, which was given an i.v. injection of HgS 30 minutes previously. There was no local injection. The wall of this presumably normal but leaking vessel consists of two to three layers, indicating that it is larger than a true capillary; possibly a venule. Small but significant deposits of tracer particles are present between the cellular layers of the wall; a few are present inside vesicles. The mode of entrance of these particles (by phagocytosis, or through intercellular leaks similar, on a smaller scale, to those induced by histamine and serotonin) could not be established. Magnification, 34,000.


L
P
R
E
N
B
M
X
19


L
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E
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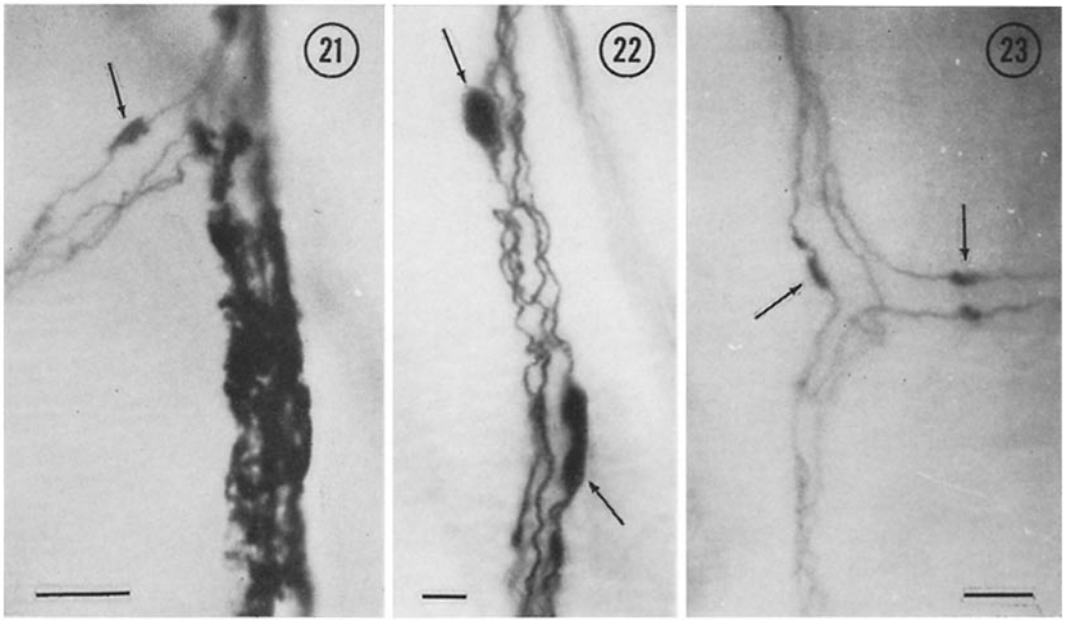
FIGURES 21 TO 24

Microphotographs of rat cremaster muscle: *combined demonstration of the endothelial leaks (with a carbon suspension, in vivo) and of the intercellular junctions (post mortem, with silver nitrate).* The animals received an intravenous injection of a carbon suspension, and a local injection of histamine over the cremaster; an hour later they were killed. By this time the leaks induced by histamine in the blood vessels had become "labeled" in black (51), the particles of carbon being small enough to penetrate into the leaks, but large enough to be retained by the basement membrane. Thereafter, the animal was killed, the blood vessels were perfused with a silver nitrate solution (see Methods), the cremaster was excised, exposed to light, fixed, cleared in glycerin, and examined *in toto* by transparence at enlargements of 300 to 450. In Figs. 21 to 24 the intercellular junctions appear as fine wavy lines, the carbon deposits as heavy black masses. Where a single discrete leak can be identified (arrows) it falls on a "silver line," indicating that the endothelial gaps occur along the intercellular junctions, as had been suggested by the electron micrographs. In Fig. 24, the arrows point to accumulations of carbon at the junction of three cells. *Scale: 10 micra.*

FIGURE 25

Reproduction of a drawing from one of Arnold's papers on diapedesis (1875) (13), illustrating an experimental observation almost identical to that of our Fig. 24 above. Small vein from the mesentery of a frog; the animal was given a suspension of cinnabar intravenously, then the blood vessels were perfused with a solution of silver nitrate. The granules of cinnabar are shown to coincide with the silver lines. The author concludes: ". . . There is an alteration in the state of aggregation of the cement substance, which becomes looser . . ." (13).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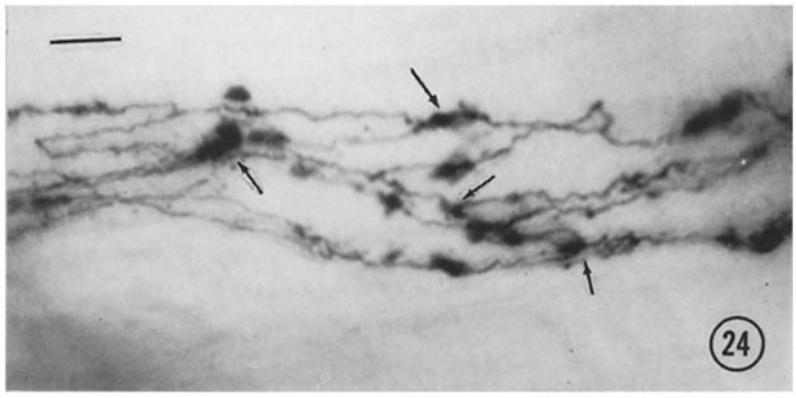
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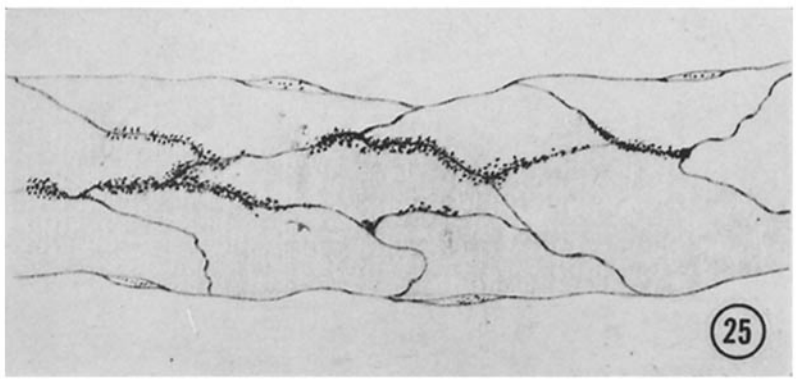
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