

# STUDIES ON THE PERMEABILITY OF LYMPHATIC CAPILLARIES

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## ABSTRACT

The passageway for interstitial fluids and large molecules across the connective tissue lymph interface has been investigated in dermal lymphatic capillaries in the ears of guinea pigs. Numerous endothelial cells overlap extensively at their margins and lack adhesion devices at many points. The observations suggest that these sites are free to move as a result of slight pressure changes. Immediately following interstitial injections of tracer particles (ferritin, thorium, carbon, and latex spheres), many of the overlapped endothelial cells are separated and thus passageways are provided between the interstitium and lymphatic lumen. Tracer particles also occur in plasmalemmal invaginations along both connective tissue and luminal fronts. All of the tracer particles accumulate within large autophagic-like vacuoles. Very few particles of ferritin are observed in the endothelium after 24 hr; however, the vesicles containing the nonprotein tracer particles (carbon, thorium, and latex) increase in size and content and remain within the lymphatic endothelial cells up to 6 months. The role of vesicles in the transport of large molecules and particles is discussed in relation to the accretion of tracer particles within large vesicles and autophagic-like vacuoles in the endothelial cytoplasm.

Much interest has been concentrated on the problem of how substances pass from the luminal side of small blood vessels to the abluminal surface, (Palade, 1960; Palade and Bruns, 1964), with subsequent distribution into the connective tissue space. Although the removal of these interstitial substances by lymphatic capillaries is of vital importance in the normal maintenance of body fluids, this problem has not excited as much interest as the one concerned with substances passing across the endothelium of small blood vessels.

Earlier studies on the delicate lymphatics demonstrated that vital dyes, as well as colloidal carbon, readily passed from the connective tissue into the lymphatic capillaries (Hudack and McMaster, 1932). Numerous physiological studies have since demonstrated the varied permeability

pattern of the lymphatics to proteins and chylomicrons (Drinker et al., 1940; Grotte et al., 1951; Wasserman and Mayerson, 1954; Hall et al., 1967), and cells, i.e. lymphocytes, are regular constituents of the lymph that is collected from normal animals (Courtice and Steinbeck, 1950, 1951; Korner et al., 1954; Morris, 1956; Wasserman et al., 1955). It is clear that physiological studies on the lymphatics have advanced at a far greater pace than studies on their precise distribution and fine structure.

In our previous studies of these vessels (Leak and Burke, 1965) colloidal carbon was used as a marker particle to label lymphatics in order to identify them with some degree of certainty at both the light and ultrastructural levels. Despite a number of ultrastructural observations on delicate lymphat-

TABLE I  
*Time Interval of Sacrifice after Injection*

No. animals per sample time	Marker particles	Time interval of sacrifice after injection				
		<i>min</i>	<i>min</i>	<i>hr</i>	<i>hr</i>	<i>hr</i>
3	ferritin	5	30	2	8	24
4	thorium dioxide	5	30	2	8	24
3	carbon	5	30	2	8	24
2	latex spheres	5	30	2	8	24
2	controls (saline injection)	5	30	2	8	24

ics (Fraley and Weiss, 1961; Casley-Smith and Florey, 1961), a consistent interpretation of their structure and of permeability has not yet evolved.

The purpose of this report is to describe the results obtained from experiments with interstitially injected tracer substances which facilitate the localization of pathways through which connective tissue fluids and large molecules pass across the lymphatic capillary wall.

#### MATERIALS AND METHODS

##### *General*

In studies designed to delineate more precisely the pathways by which connective tissue fluids and large particulate components enter the lymphatic capillary lumen, suspensions of vital dye (viz., trypan blue) and colloidal particles have been utilized to investigate the uptake of connective tissue fluids by lymphatic capillaries. Likewise, these tracers have been useful in determining the organization and distribution of lymphatics within the superficial and deep regions of the dermis at the light microscope level. The electron-opaque particles in the suspensions used in these studies covered a wide range of sizes (approximately 80–1000 Å in diameter). The particles were injected interstitially and their progress across the lymphatic capillary wall was observed in the living animal and subsequently in thin sections obtained from specimens that were processed for electron microscope studies.

The tissues studied included ears of guinea pigs, rats, and mice which contain a rich plexus of lymphatic capillaries that drain connective tissue fluids from both the superficial and deep areas of the dermis. The observations presented in this report are based primarily upon examinations of lymphatics in the ears of guinea pigs. Before the injection of electron-opaque tracer substances, the ears were depilated with Surgex (Crookes-Barnes Labs, Inc., Wayne, N. Y.). The interstitial injections were carried out with either quartz micropipettes mounted on a

modified Chambers micromanipulator or with a 27 or 30 gauge needle mounted on a 0.5 ml syringe. The animals were anesthetized with ether or pentobarbital, after which samples were taken at various time intervals as indicated in Table I.

##### *Methods*

**EXPERIMENTS WITH VITAL DYES:** Ears of young adult guinea pigs were perfused with solutions of trypan blue in physiological saline, by microinjection techniques, through portions of lymphatic capillaries in the outer margins and tips of the ears. A direct observation of the substances injected was followed with the aid of a custom-made Leitz stereomicroscope assembly. The rapid engulftment of dye by the lymphatics and the egress of fluids from the injection site were recorded by time-lapse cinéphotography with a Bolex H 16 M camera.

**EXPERIMENTS WITH ELECTRON-OPAQUE MARKER PARTICLES:** Colloidal carbon, thorium, and ferritin possess the ability to scatter electrons and can be easily identified throughout the various tissue components when viewed in the electron microscope. Advantage is taken of this inherent property to follow the passage of these marker substances across the lymphatic capillary endothelium at the ultrastructural level.

**FERRITIN:** Cadmium-free ferritin was obtained from Nutritional Biochemical Corporation. Interstitial injections (0.05 ml) were made in ears of anesthetized animals, and samples were obtained as indicated in Table I.

**COLLOIDAL THORIUM:** A 25% solution of stabilized colloidal thorium dioxide (Thorotrast) was obtained from Fellows-Testagar Div., Fellows Mfr. Co., Inc., Detroit, Mich.

**COLLOIDAL CARBON:** A shellac-free suspension of carbon (Biological ink type 11/143/2) was obtained from John Herschel and Company, Inc. Before injection, carbon suspensions were diluted with 10 parts of saline and filtered with Whatman No. 1 filter paper.

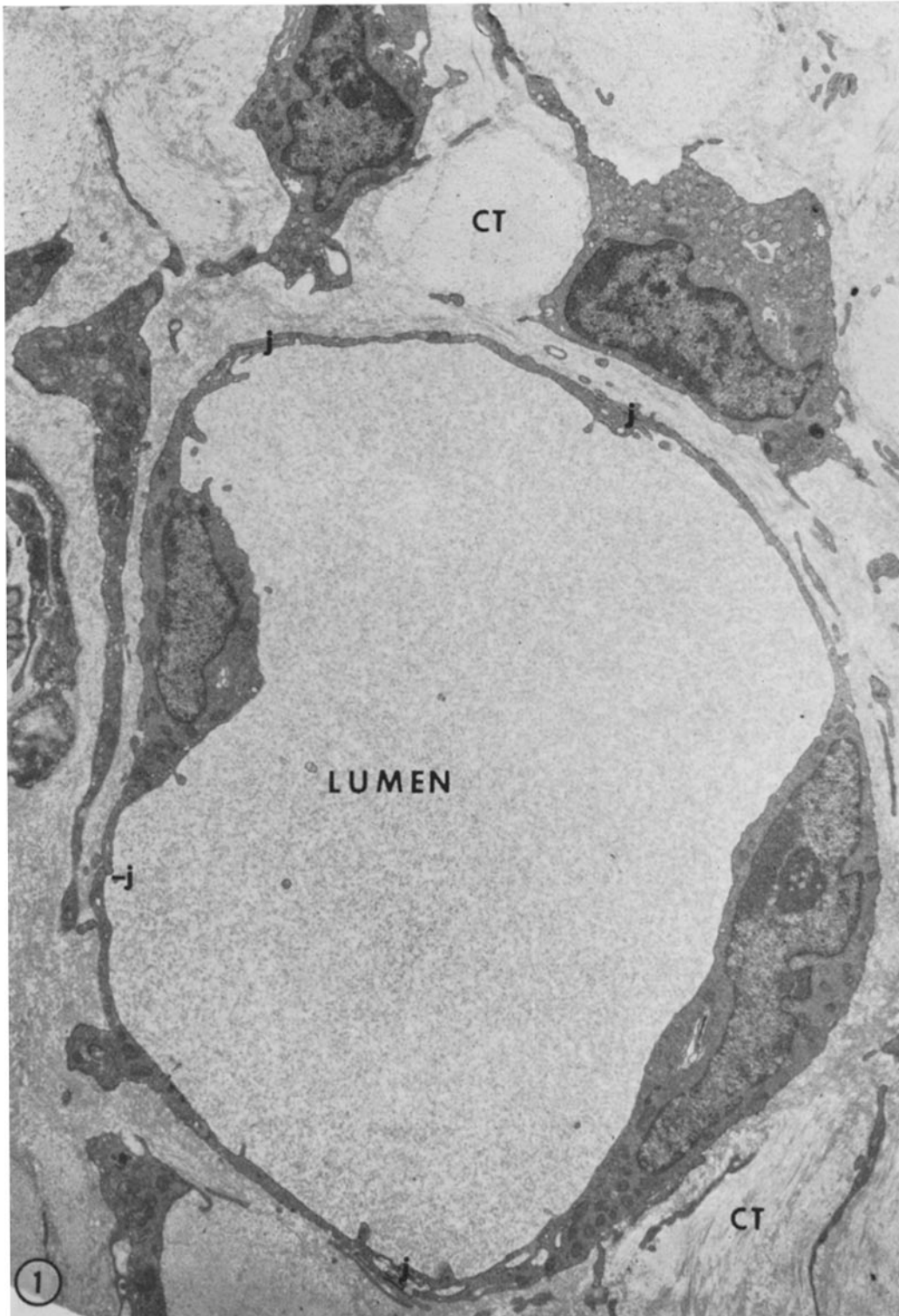
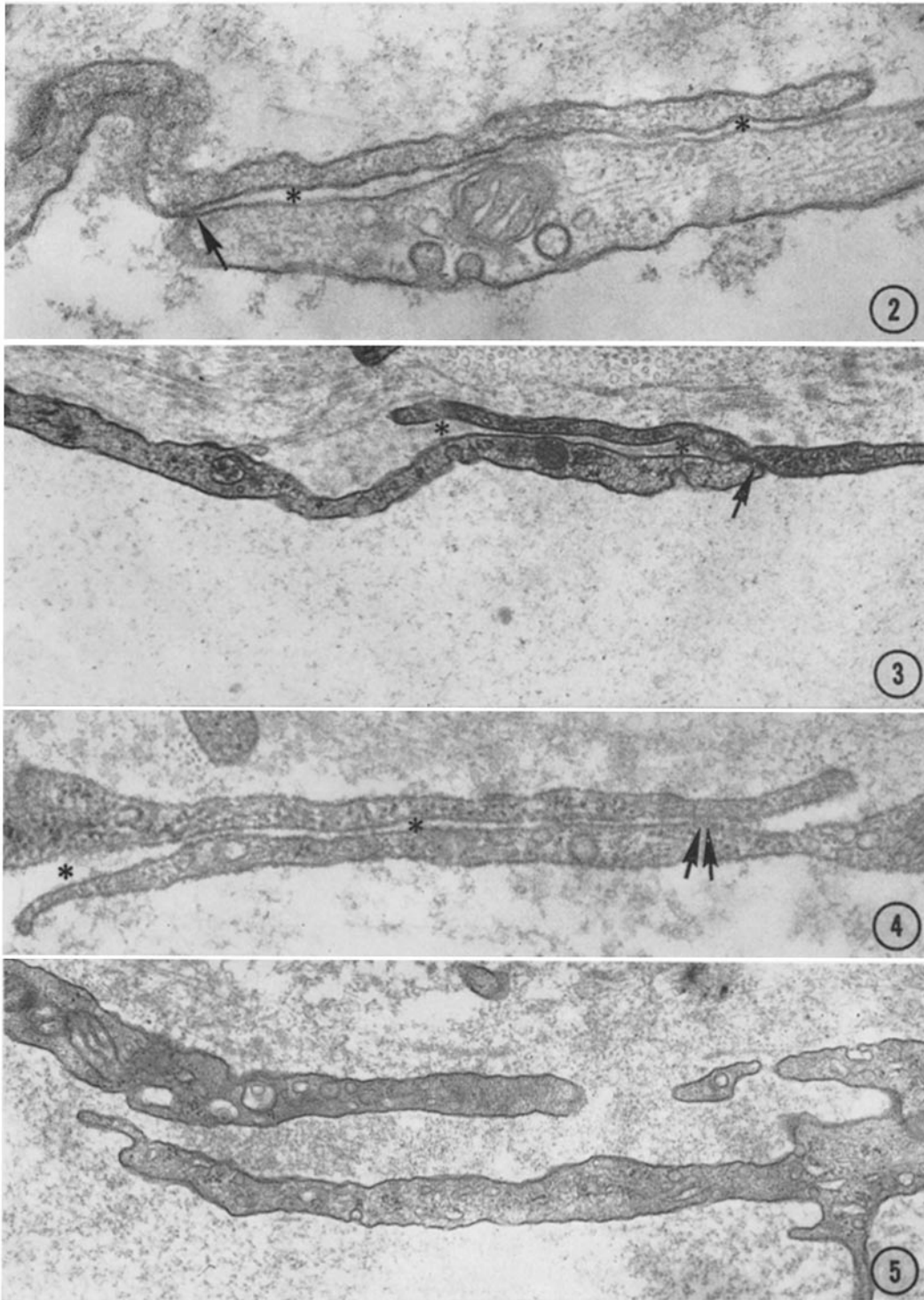


FIGURE 1 Cross-section of a lymphatic capillary from the ear of a guinea pig. The irregular outline of the capillary is demonstrated, in addition to attenuations of endothelium beyond the perinuclear region. A flocculent material of moderate electron opacity occupies its lumen while a close association of the abluminal surface of the endothelium is maintained with the adjoining connective tissue elements (*CT*). Intracellular junctions are as indicated (*j*).  $\times 7000$ .



FIGURES 2-5 These micrographs illustrate the overlap of endothelial cells. In Figs. 2 and 3 there is at least one point of close apposition between apposing membranes (arrows) while there is variability in the width of intercellular clefts along its length (\*). In Fig. 4 the intercellular cleft is also variable but narrow near the abluminal surface (double arrows). In Fig. 5 a patent junction is illustrated. Lumina are at bottom of figures. Fig. 2,  $\times 89,500$ ; Fig. 3,  $\times 21,000$ ; Fig. 4,  $\times 39,000$ ; Fig. 5,  $\times 28,000$ .

**LATEX SPHERES:** Polystyrene latex suspensions were generously supplied by the Dow Chemical Company (Midland, Mich.). The measurement given for the average particle size was  $0.088 \mu$  with a standard deviation of  $0.0080 \mu$ . Latex spheres do not scatter electrons as effectively as the above tracers (i.e. ferritin, thorium, and carbon) and are thus visualized in the electron microscope as translucent spheres.

**LANTHANUM HYDROXIDE:** A suspension of lanthanum was also used as a tracer. Blocks of tissue from ears of guinea pigs with and without injected colloidal tracer particles were fixed in formaldehyde-glutaraldehyde (Karnovsky, 1965), washed in buffer, and postfixed in osmium tetroxide collidine containing a suspension of lanthanum according to the recommendations of Revel and Karnovsky (1967). This procedure proved to be unsatisfactory for dermal tissue. However, satisfactory results were obtained by adding a 6% solution of dimethyl sulfoxide<sup>1</sup> (DMSO) to the formaldehyde-glutaraldehyde solution as well as the osmium solution. Some tissues were fixed in 2% glutaraldehyde containing alcian blue in 0.1 M cacodylate buffer, and then postfixed in osmium tetroxide-collidine with lanthanum according to Behnke (1968) and Shea and Karnovsky (1969). Other tissues were fixed in 2% glutaraldehyde with 0.1% ruthenium red in 0.1 M cacodylate buffer and postfixed in osmium tetroxide with 1% lanthanum.

**CONTROLS:** In order to determine if artifacts were produced by tracer particles, animals were injected with 0.05 ml saline without tracer particles and the tissue was fixed and processed for ultrastruc-

<sup>1</sup> Ethel Lipner, personal communication.

tural studies. Noninjected animals were also examined.

**FIXATION OF TISSUE:** At the appropriate time interval after the injection of marker substances (Table I), the tissue was cut into small blocks in 3% glutaraldehyde with 2% acrolein buffered to pH 7.4 with phosphate buffer (Leak and Burke, 1966). After 30 min the partially fixed blocks were cut into small strips of about 1 mm thickness, and fixation was continued for 2–12 hr. The tissue was then rinsed in phosphate buffer, and postfixed in 1% osmium tetroxide buffered with 0.1 M sodium phosphate (pH 7.2) for 1–2 hr at 4°C. Some tissues were fixed in 2.5% glutaraldehyde without the acrolein while others were fixed in the glutaraldehyde-formaldehyde fixative of Karnovsky (1965) or in buffered formalin (Pease, 1962) and then postfixed in osmium tetroxide. Some tissue was stained en bloc, before dehydration, with 0.5% uranyl acetate in Michaelis buffer (pH 5) in order to enhance contrast in membranes (Farquhar and Palade, 1965). Dehydration was accomplished in a graded series of ethanol followed by propylene oxide and Epon embedding (Luft, 1961). Sections were cut on a Porter-Blum microtome with a du Pont diamond knife and stained with uranyl acetate and lead citrate (Venable, and Coggeshall, 1965). Observations were made with a Philips EM 300 electron microscope.

## OBSERVATIONS

### General

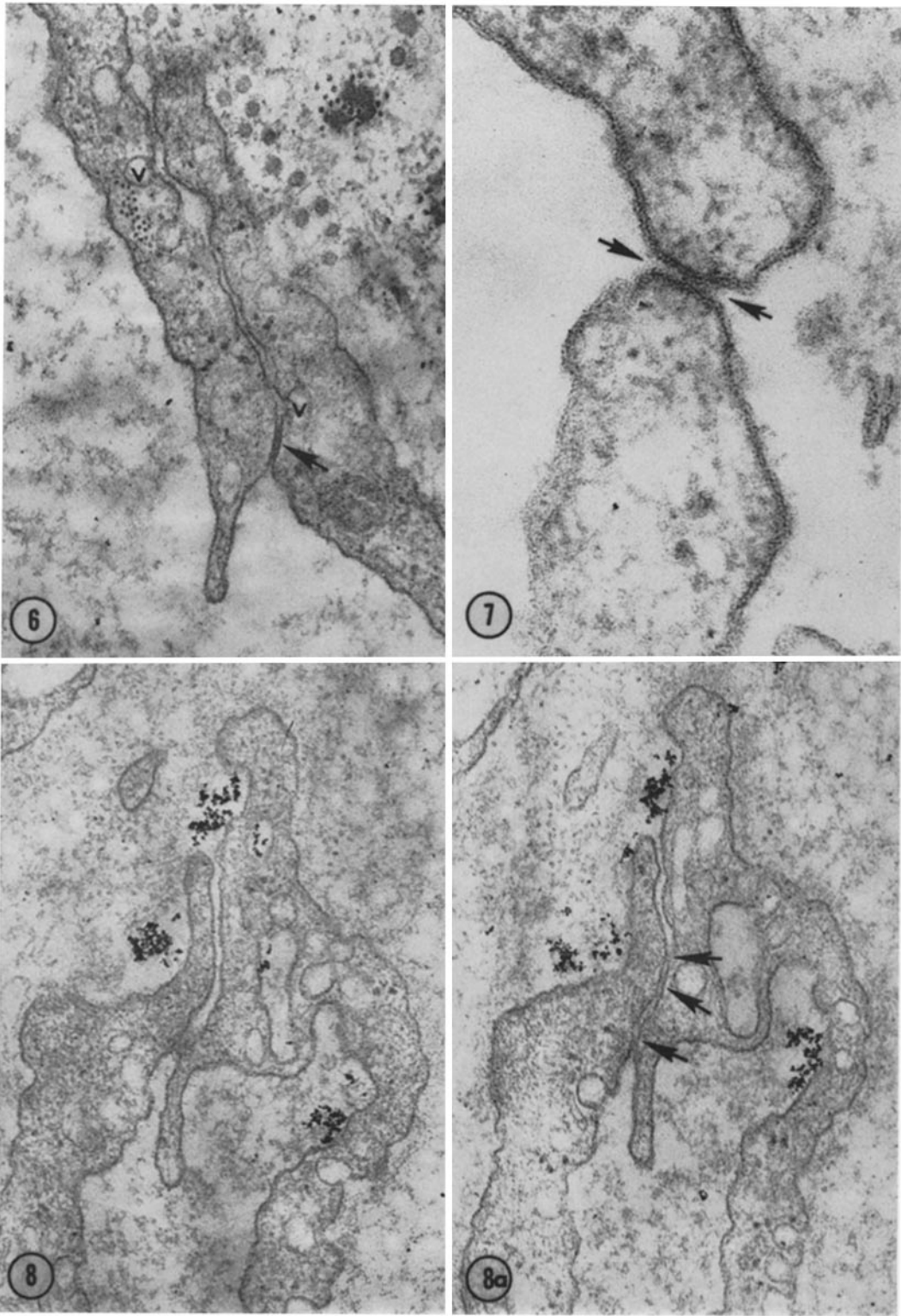
No major differences were noted in the over-all pattern of the lymphatic capillaries in the ears of the various animals studied. The descriptions that

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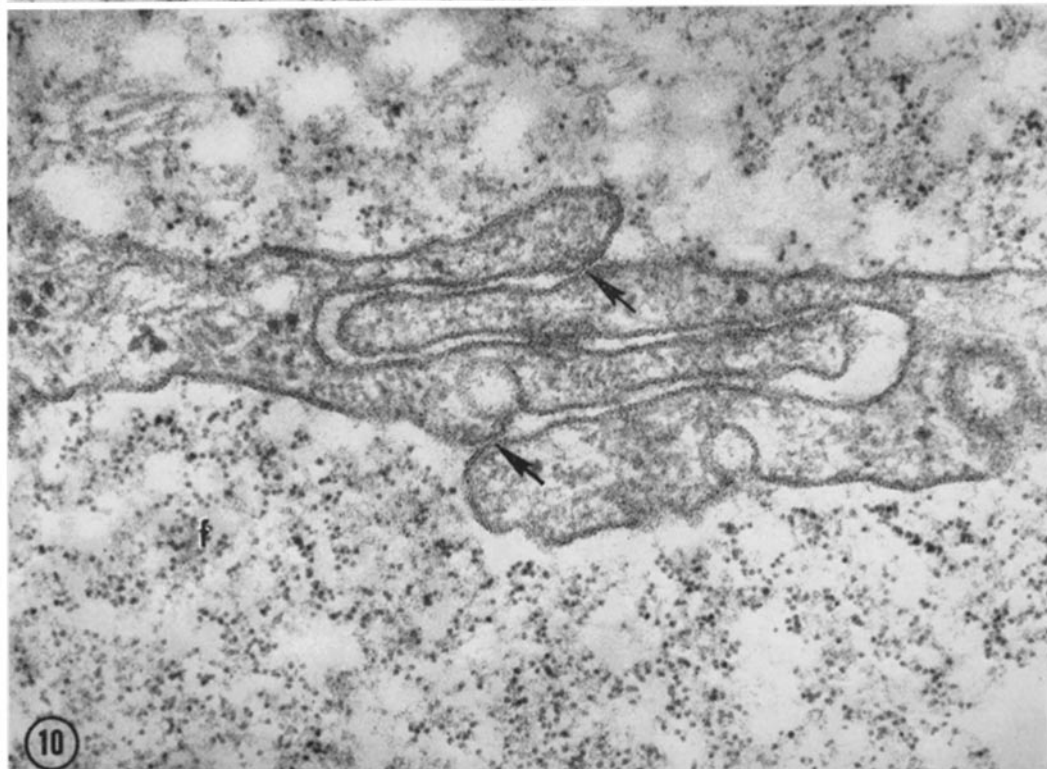
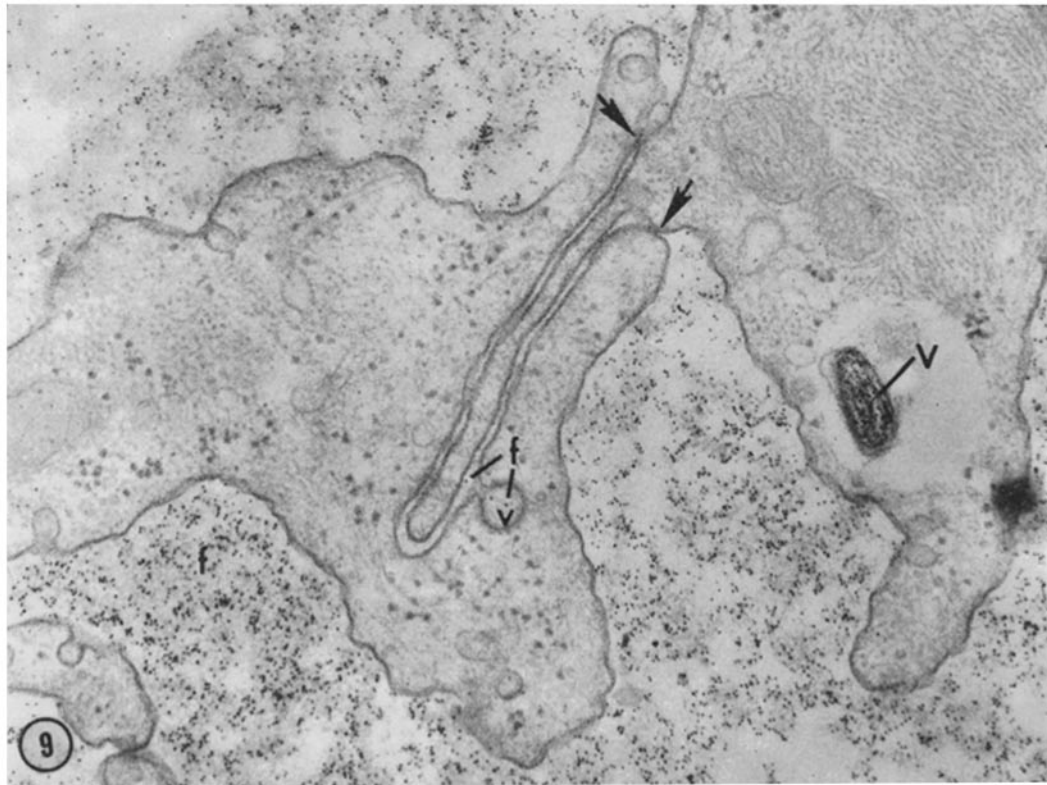
**FIGURE 6** Although the cleft in this junction is quite variable, its width near the lumen of the capillary is relatively constant for a short distance. This short segment contains a dense line (arrow) resembling a membranous component which is believed to be different from that which is formed by a fusion of the two outer leaflets of adjacent cell membranes, especially since this magnification is not sufficient to reveal the three components of the unit membrane. Plasmalemmal invaginations (*v*) also occur in the region of overlap between cells. Lumen is to left.  $\times 62,600$ .

**FIGURE 7** The intercellular junction depicted in this micrograph illustrates an abutment of two apposing endothelial cells. The intercellular cleft contains a small area of moderate electron opacity (arrows) which occupies an area approximately  $680 \text{ \AA}$  in length. The width of the intercellular cleft at the closest point measures approximately  $40 \text{ \AA}$ . Lumen is to left.  $\times 168,600$ .

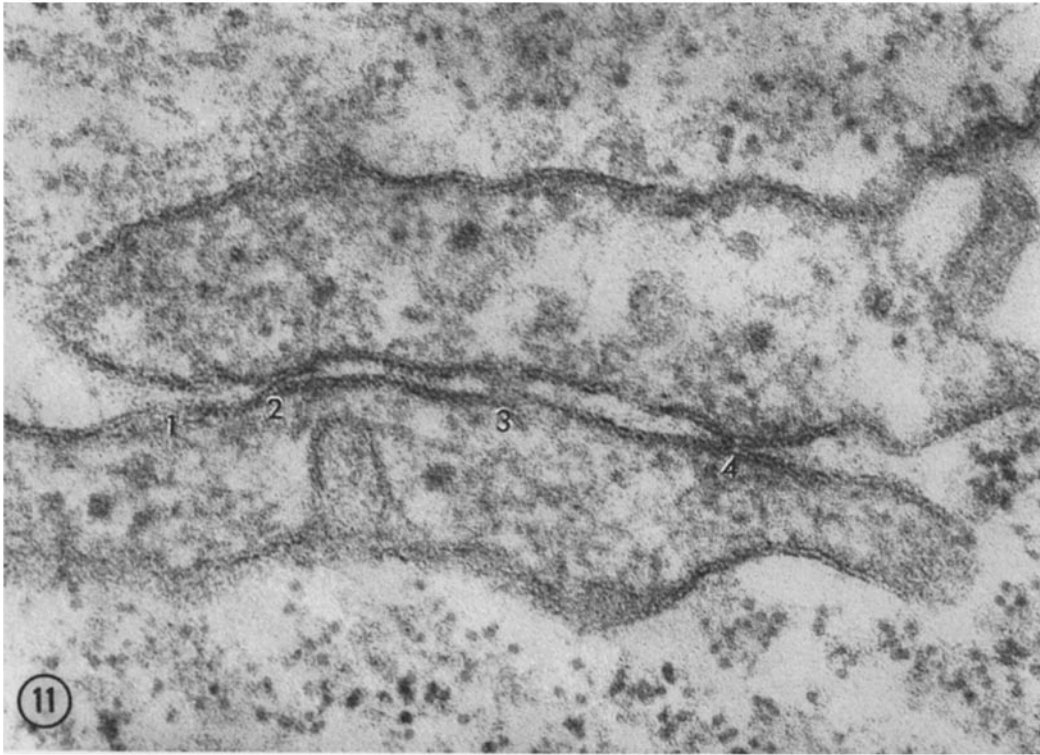
**FIGURES 8 and 8 a** These two electron micrographs depict semi-serial sections of an intercellular junction. In Fig. 8 the closest point between apposing endothelial cells occurs near the lumen, while the intercellular cleft is approximately  $150 \text{ \AA}$  wide throughout its length. Fig. 8 a demonstrates a section through the same intercellular junction at another level. Note the irregularity in the width of the intercellular cleft as indicated at the arrows. Specimen injected with colloidal thorium 3 hr before fixation. Lumina are at bottom of figures.  $\times 62,700$ .







FIGURES 9 and 10 Overlapping and interdigitations of terminal margins of adjacent endothelial cells are demonstrated in these electron micrographs. In Fig. 9, ferritin particles (*f*) occur in the intercellular cleft as well as in a plasmalemmal invagination that is partially open to the intercellular cleft (*v*), and in a large vesicle containing membranes (*V*). Note the various points of close apposition between adjacent endothelial cells (arrows) near the luminal front as well as the connective tissue front in both Figs. 9 and 10. Specimen injected with colloidal ferritin 5 min before fixation. Lumina are at bottom of figures. Fig. 9,  $\times 70,500$ ; Fig. 10,  $\times 100,000$ .



FIGURES 11 and 11 a The semi-serial sections depicted in these electron micrographs demonstrate areas of close apposition between endothelial cells (areas 1, 2, 3, and 4 in Figs. 11 and 11 a). Lumina are at bottom of figures. Figs. 11 and 11 a,  $\times 172,000$ . Inset in 11 a,  $\times 500,000$ .



follow will consider mainly the observations made on the ears of guinea pigs. Details of the fine structure of dermal lymphatic capillaries have been given elsewhere (Leak, 1970; Leak and Burke, 1966, 1968). It is not necessary to discuss the basal lamina here, especially since it is irregular or absent from the dermal lymphatic capillary (Leak and Burke, 1966, 1968) and, therefore, cannot serve as a primary filtration barrier to large molecules as suggested for transendothelial exchanges in blood capillaries (Farquhar et al., 1961).

**THE ULTRASTRUCTURE OF ENDOTHELIAL CELL JUNCTIONS IN LYMPHATIC CAPILLARIES OF NONINJECTED SPECIMENS:** Frequently, margins of apposing endothelial cells overlap for several microns. The width of intercellular clefts formed by such an extensive overlap of apposing endothelial cells is often extremely variable. Occasionally, points of close approximation (30–40 Å) are observed, but in many cases widths of up to 0.5  $\mu$  may extend the total length of the intercellular cleft to form patent junctions (open junctions) as demonstrated in Figs. 1–5.

In some of the junctions formed by the extensive overlapping of endothelial cells, there may be one point and, occasionally, several points along the length of the intercellular cleft where plasma membranes are closely approximated. Observation of these areas at higher magnifications reveals that the apposing membranes are held together by a macula adherens (Figs. 2–4, 6, 8). Occasionally, areas of close apposition were observed where points of the intercellular cleft are indeed obliterated by the fusion of external leaflets, as illustrated in Figs. 11 and 15 *a*. In Fig. 11, the clarity of membranes is enhanced by en bloc staining with uranyl acetate (Farquhar and Palade, 1965).

Some intercellular junctions are formed by a

simple abutment of apposing endothelial cells (Fig. 7). Others interdigitate to form complex imbrications of the endothelial cell margins as illustrated in Figs. 9 and 10. Focal surface specializations are also observed along the junctions, which presumably permit apposing cells to maintain cell-to-cell contact. These specialized sites do not form continuous belts, as sections taken at another level of the same junction fail to show them (cf. Figs. 8, 8 *a*, 11, 11 *a*, 15).

#### *Experiments with Electron-Opaque Tracer Substances*

**GENERAL OBSERVATIONS:** In the living animal, within seconds after a local injection of suspensions of carbon, thorium, and ferritin, lymphatics in the vicinity of the injection site and those deriving from it are clearly delineated by their content of the colloidal particles. In ciné-photographic studies of interstitial injections, lymphatics are clearly outlined (cf. Figs. 16 *a*, 16 *b*) and the tracers can be seen to advance very rapidly from the much smaller lymphatic capillaries toward the larger collecting lymphatic vessels located near the base of the ear. The filling of both superficial and deeper lymphatic capillaries after interstitial injections and the egress of the marker substances from the injection site take place with surprising rapidity. Subsequent examination of the intradermally injected specimens with the electron microscope shows marker particles within the interstitial areas, within the cytoplasmic vesicles of the lymphatic capillary endothelium, within the intercellular clefts (patent junctions), and within the lumina of the lymphatic capillaries (Figs. 9, 10, and 17).

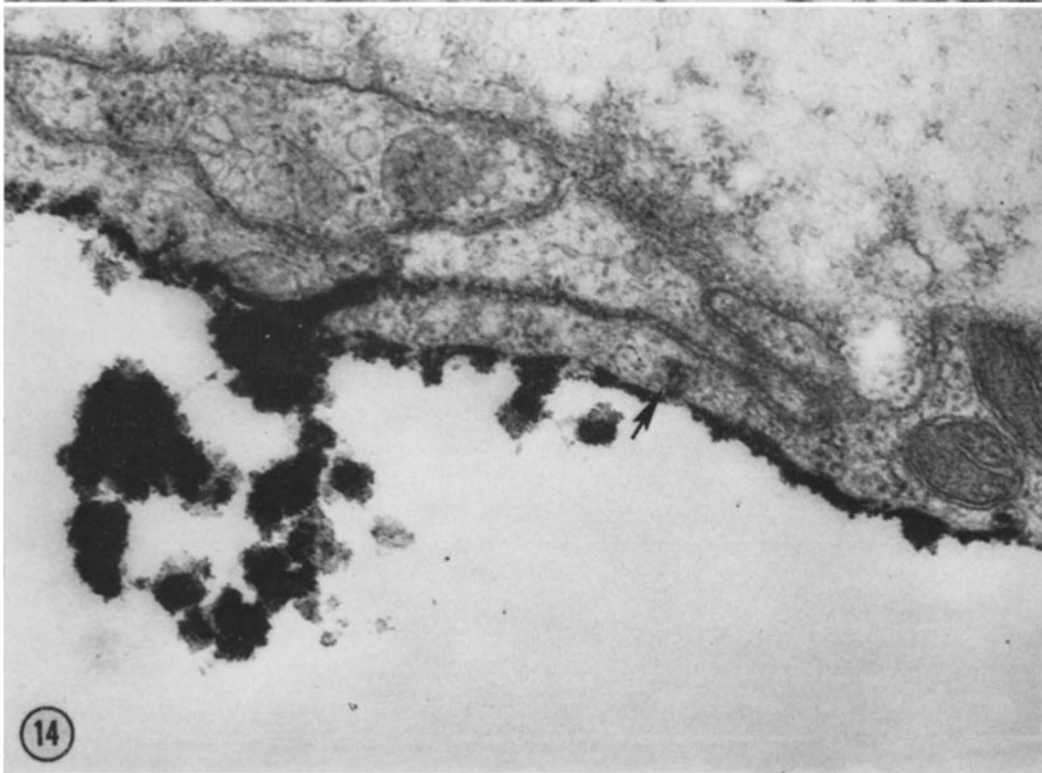
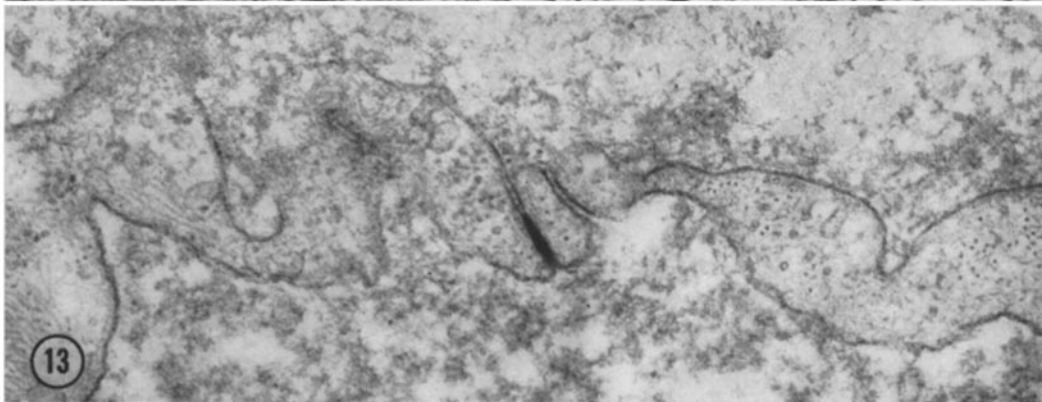
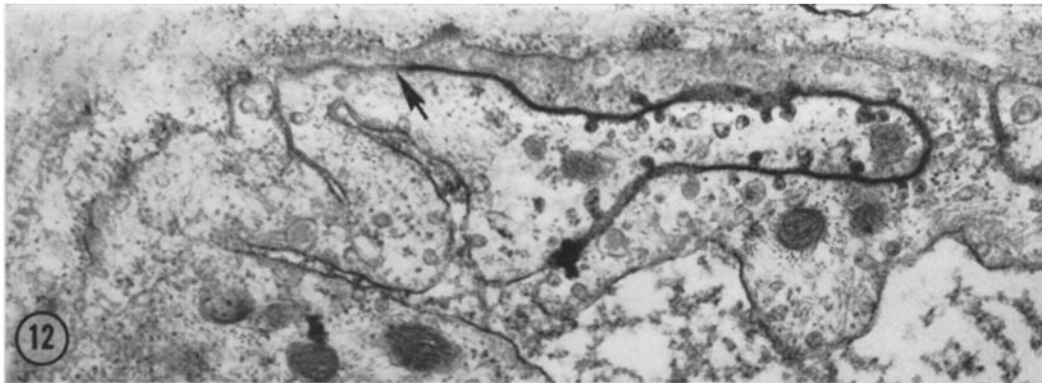
**LANTHANUM STUDIES:** Electron microscope

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**FIGURE 12** Intercellular junctions in specimen processed with lanthanum hydroxide. Lanthanum is located along most of the length of the intercellular cleft, including the plasmalemmal invaginations that are exposed to the intercellular cleft. Note that lanthanum is restricted from the area near the connective tissue front where there seems to be an area of close apposition (arrow).  $\times 28,000$ .

**FIGURE 13** Micrograph showing lanthanum trapped in the intercellular cleft, while in an area close by there is a distance of approximately 200 Å between the endothelial cells which is free of lanthanum.  $\times 48,600$ .

**FIGURE 14** Lanthanum is found along the luminal surface of the endothelial cells, in areas of the intercellular cleft, as well as in plasmalemmal invaginations (arrow). Tissue processed with ruthenium red plus lanthanum hydroxide.  $\times 56,700$ .



observations of thin sections from tissues processed with lanthanum revealed that lanthanum readily traversed the intercellular junctions of endothelial cells of lymphatic capillaries.

In intercellular clefts that are formed by closely apposed endothelial cells, the passage was unrestrained and tracer substance could be followed through most of its length (Figs. 12 and 13). However, in some areas the presence of surface specializations on the apposing endothelial membranes prevented the passage (Fig. 15 *a*). High magnifications of these sites indicate that they represent tight or occluding junctions, i.e., a fusion of the external leaflets of apposing membranes (Fig. 15 *c*).

In tissues processed with ruthenium red plus lanthanum, the tracer substance was frequently found in plasmalemmal invaginations along the intercellular clefts and occasionally the luminal surface of the endothelium (Fig. 14). A similar reaction product was also observed along the luminal surface when lanthanum was used in combination with Alcian blue.

**INTERCELLULAR PASSAGE OF ELECTRON-OPAQUE MARKER PARTICLES:** Examination of specimens fixed within minutes after injection of the above tracer particles reveals the presence of these substances within the intercellular clefts that occur between surface specializations which serve to maintain cell-to-cell adhesion (Figs. 17, 20, 23). On the other hand, in cell junctions devoid of adhesion devices, i.e. maculae occludentes and maculae adherentes, the marker particles were often seen to form a continuous path through the entire length of the intercellular cleft (Figs. 19, 22, 24, 25, 27).

From about 30 min onward after intradermal injections, fewer particles were observed within the intercellular cleft. The size of the injected particles had no apparent effect on their ability to penetrate the intracellular cleft. The occasional presence of connective tissue cells within intercellular junctions that are apparently in the process of traversing the

intercellular cleft points to the ability of lymphatics to accommodate very large particles.

In specimens examined at 8 hr or longer after the injection of tracer material, relatively little marker substance remained in the lymphatic lumen since it evidently had been drained off to the much larger collecting vessels for filtration by the regional lymph nodes or movement toward lymphatic ducts for delivery to the general circulation.

**INTRACELLULAR PASSAGE:** In addition to being present within the cleft between endothelial cells, the marker particles were also observed in vesicles which were located along both the luminal and connective tissue fronts (Figs. 8, 18, and 20).

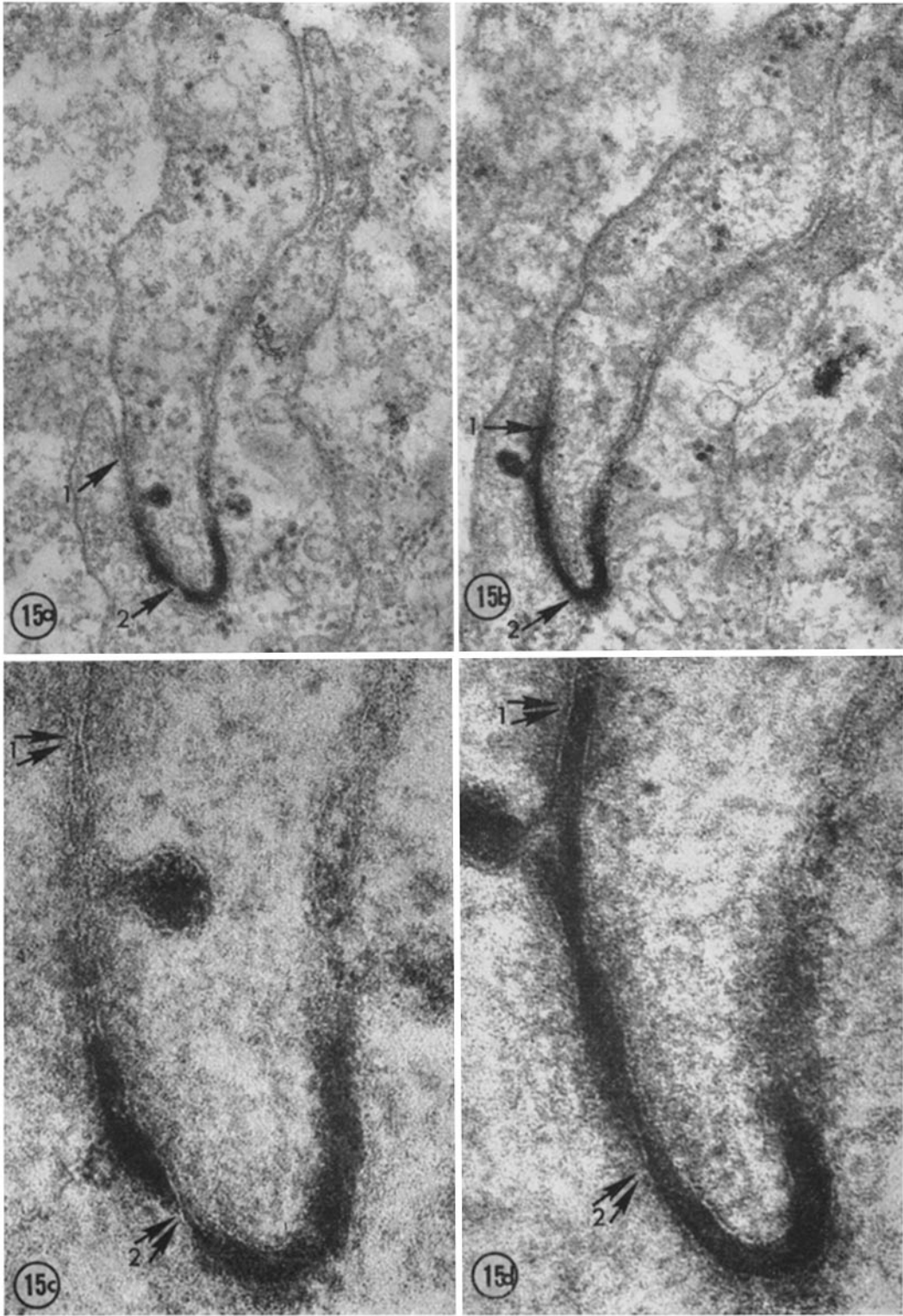
In specimens injected 5 min–8 hr before fixation, many vesicles, both large and small, contained tracer particles in varying concentrations (Figs. 8, 9, 17, 18, 20, and 21). By 24 hr, very little ferritin was observed in cytoplasmic vesicles; however, a significant increase in the number and size of dense bodies and vacuoles with tracer particles from specimens injected with thorium, carbon, and latex was observed (Figs. 26–29). Beyond 24 hr, thorium, carbon, and latex were found extensively concentrated in autophagic-like vacuoles, many of which contained membranous structures and dense bodies that are suggestive of intracellular digestion as demonstrated for other cell types (de Duve, 1963).

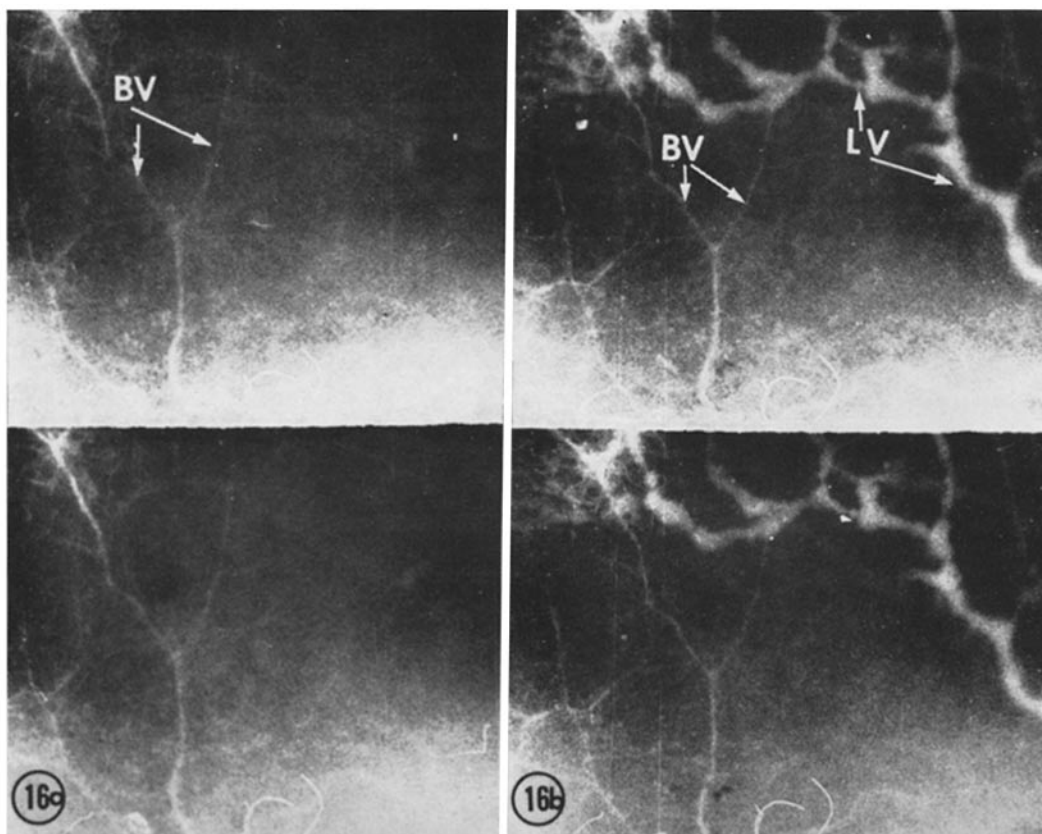
## DISCUSSION

The primary direction of transport in the blood capillary is from the lumen toward the connective tissue front (Palade, 1953, 1960; Karnovsky, 1967), while in the lymphatic capillary the direction is just the opposite (i.e. for the most part, fluids and particulate components pass from the interstitial area of the connective tissue into the lumina of the lymphatic capillaries), as depicted in the diagram in Fig. 30. The observations presented here provide morphological data in support of the many physiological studies which suggest the lymphatics as the primary system that serves to clear the interstitium

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**FIGURES 15 *a*–15 *d*** Another example of lanthanum found in the intercellular cleft and plasmalemmal invaginations. Figs. 15 *a* and 15 *b* represent semi-serial sections. Note that very little lanthanum occurs within the intercellular cleft at the arrows for points 1 and 2 which indicate an area of close apposition. See Fig. 15 *c* which is an enlargement of the same area (double arrows). In Fig. 15 *b*, the site of close apposition observed in Fig. 15 *a* is not seen in this adjacent section of the same junction (1). Similarly, an area of close apposition at point 2 in Fig. 15 *b* is also missing in the adjacent section in Fig. 15 *d*. Figs. 15 *a* and 15 *b*,  $\times 58,800$ ; Figs. 15 *c* and 15 *d*,  $\times 109,500$ .





FIGURES 16 *a* and 16 *b* Cinéphotomicrographs of blood (BV) and lymphatic (LV) vessels. Fig. 16 *a* illustrates an area before labeling of lymphatics. Fig. 16 *b* shows the same area as Fig. 16 *a* after intradermal injection of a mixture of trypan blue and colloidal carbon.  $\times 70$ .

of excess fluids and proteins, as well as other substances which might accumulate within the interstitium and offset the balance of forces upon which homeostasis depends (Mayerson, 1962, 1963).

The observations in the present study demonstrate that the intercellular cleft between adjacent endothelial cells provides the major route for the passage of fluids as well as large particulate substances. In addition, injected tracer particles are

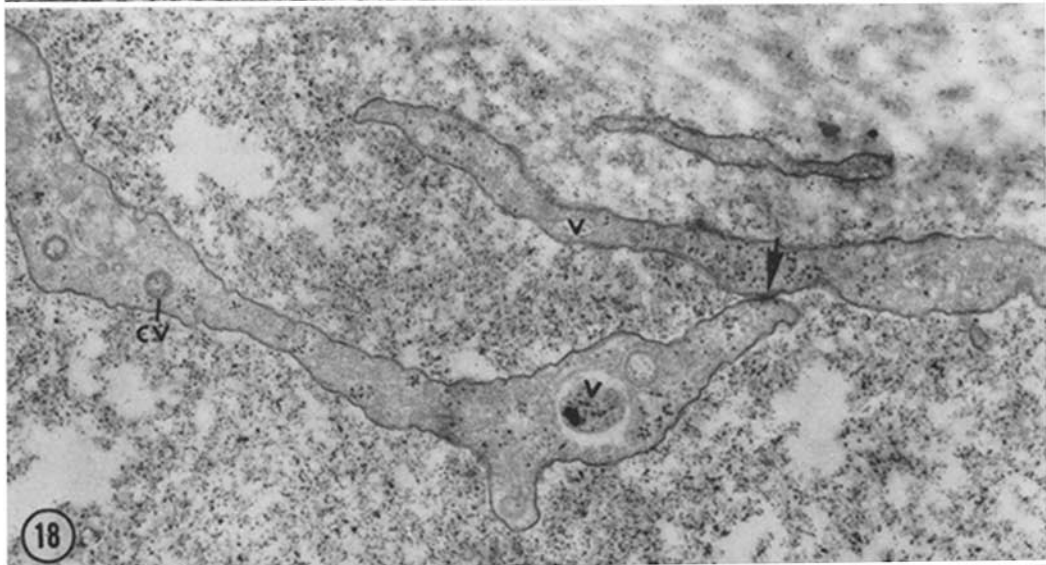
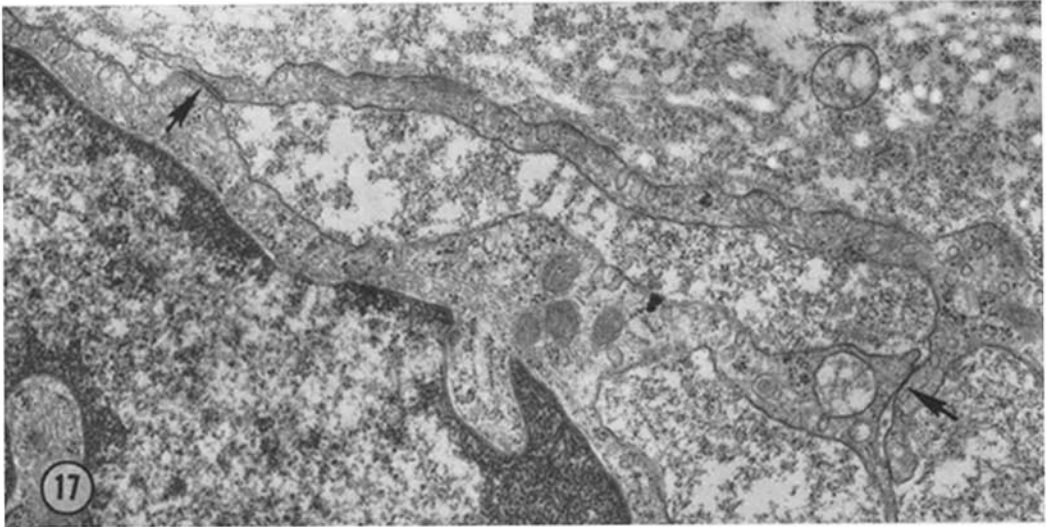
removed from the connective tissue front within plasmalemmal vesicles.

#### *The Intercellular Cleft as a Major Passageway*

Specimens observed within the first 5 min after interstitial injection of marker substances revealed a large number of patent junctions which con-

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FIGURES 17-19 Micrographs showing the various configurations of the intercellular junctions in specimens injected with ferritin 5 min before fixation. In Fig. 17 there are two areas along the intercellular cleft where apposing cells are held in close apposition (arrows) forming a macula adherens. In Fig. 18, a close apposition between apposing cells is maintained at one point (arrow). Note the presence of tracer particles in vesicles of the endothelial cells, both coated (*cv*) and smooth surfaced (*v*). In Fig. 19, apposing endothelial cells are still overlapped for quite a distance. However, the intercellular cleft is extremely wide, forming an uninterrupted passageway from the connective tissue into the lymphatic lumen (patent junction). Fig. 17,  $\times 22,600$ ; Fig. 18,  $\times 26,200$ ; Fig. 19,  $\times 27,000$ .





tained the tracer, while the samples that were observed at a much later time period showed fewer particles. The rapid egress of the injected substances from the lymphatic capillaries toward the larger collecting vessels clears the interstitium of excess fluids in a relatively short time and would account for the difference. It is evident that the rapid removal of connective tissue fluids by the lymphatic capillary is not entirely a passive filtration process but also involves the participation of specific morphological structures located at the connective tissue-lymph interface, i.e. intercellular clefts of patent junctions (Leak and Burke, 1968; Leak, 1970), as well as other forces such as muscular and cardiac activities (French et al., 1960; Beck, 1924).

It is recognized that even the injection of minute quantities of fluids into the connective tissue increases the interstitial fluid pressure within the immediate vicinity of the injection site. However, with improved microinjection techniques, the topographical relationship between the lymphatic endothelium and adjoining connective tissue of specimens injected with such techniques is not different from that of noninjected specimens (Leak, 1970). Therefore, the observation of extremely overlapped adjacent endothelial cells in both the injected and noninjected specimens reveals many sites that are free to move as a result of slight pressure changes between the interstitial space and lymphatic lumen. Thus, a separation of endothelial cells at these sites provides passageways (i.e., open channels) through which fluids and particles may pass into the lymphatic lumen without having to encounter a structural barrier (see diagram, Fig. 30).

Although the distance between endothelial cells in many instances may be quite wide, there are specialized regions which occur at regular intervals along the intercellular clefts. These regions would presumably permit cell-to-cell contact by focal

areas where the membranes are more closely approximated, i.e. intercellular clefts can be as narrow as 40 A and in some cases they are obliterated by the fusion of the external leaflets of plasma membranes. Similar structures have recently been reported in the endothelium of blood capillaries in heart muscle (Karnovsky, 1967; Bruns and Palade, 1968).

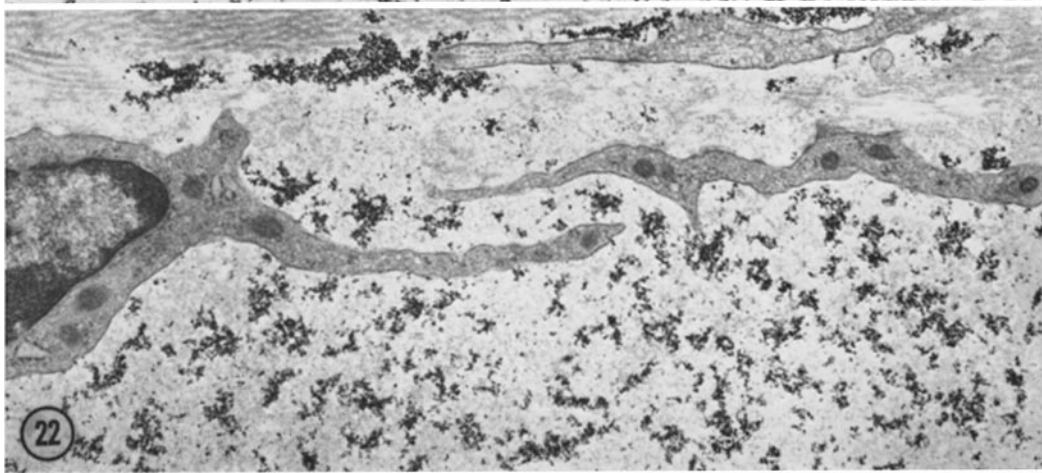
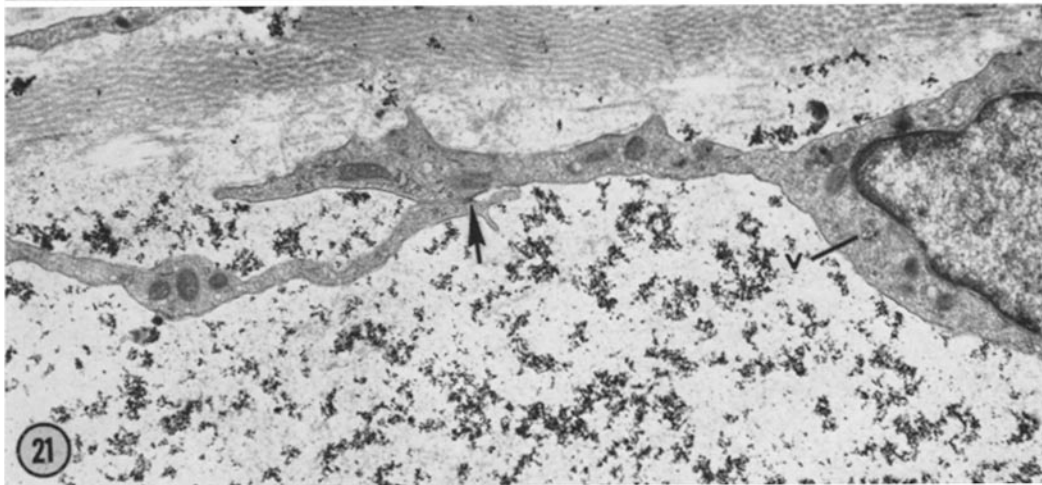
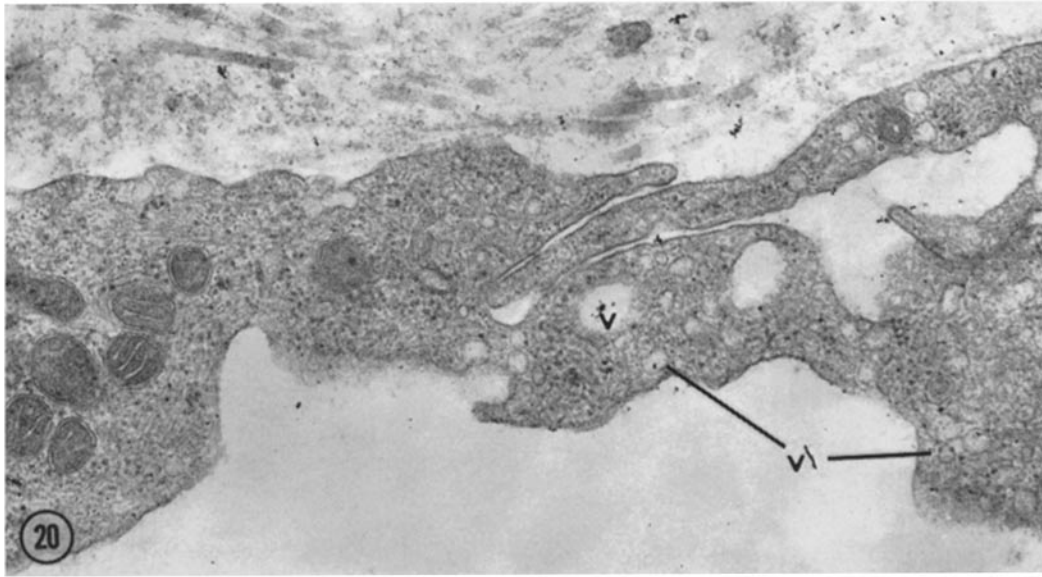
#### *The Role of Vesicles in the Removal of Large Molecules and Tracer Particles*

Although vesicles with varying concentrations of the injected tracers are observed at what appears to be the deeper levels of the endothelium, several studies with the tracer lanthanum demonstrate that many of the vesicles that appear to be centrally located in the endothelial cytoplasm are, in essence, still open to either the connective tissue or the luminal front of the endothelium. Similar results have been obtained for blood capillaries (Karnovsky, 1967) as well as other cell types (Revel and Karnovsky, 1967; Shea and Karnovsky, 1969). Therefore, once tracer has entered the lymphatic lumen (which is instantaneous as determined by our cinéphotographic studies), the vesicles along the luminal front that contain tracer particles may, in fact, represent plasmalemmal invaginations of the luminal endothelial surface and not necessarily vesicles that were shuttled from the connective tissue front.

The accretion of ferritin in large vesicles after short time periods (18–24 hr) and the accumulation of nonproteinaceous injected colloidal particles (i.e. carbon, thorium, latex) in very large autophagic-like vacuoles after extensive time periods, i.e. up to 6 months (Leak, 1970), negate the suggestion that all colloidal particles that are taken up by endothelial cells are directly transported from the connective tissue front to the lymphatic lumen within pinocytotic vesicles. Instead,

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FIGURES 20–22 Micrographs showing the appearance of intercellular clefts 5 min after the injection of colloidal thorium. In Fig. 20, apposing cells interdigitate to form an intercellular junction. Thorium is found in the connective tissue in the intercellular cleft as well as in vesicles (*v*) of various sizes. In Fig. 21, adjacent endothelial cells maintain close contact at the region indicated by the single arrow. Thorium occurs in the connective tissue and the lumen. Thorium is also observed in a vesicle (*v*) located in the perinuclear region. In Fig. 22, an uninterrupted passageway containing colloidal thorium extends from the connective tissue into the lymphatic lumen. Lumina are at bottom of figures. Fig. 20,  $\times 36,800$ ; Fig. 21,  $\times 13,600$ ; Fig. 22,  $\times 28,800$ .



the plasmalemmal vesicles containing tracer particles tend to move toward the central cytoplasm where they merge with large autophagic-like vacuoles, presumably to be digested by the lysosomal enzymes (de Duve, 1963). This presumably holds for the ferritin molecule (Granick, 1946; Farrant, 1954; Richter, 1958; Florey, 1967; Bruns and Palade, 1968), in which case the cell has no difficulty in the digestion. This would account for the rapid elimination of ferritin in comparison to the other tracer particles used in the present study (i.e. carbon, thorium, and latex) which are non-proteinaceous and therefore are not easily digested by lysosomal enzymes for reutilization and/or discharge from the cell.

In studying the passage of particles and fluids across the lymphatic endothelium, Casley-Smith (1964) suggested that substances passed (*a*) across the lymphatic endothelium within vesicles of various sizes (i.e., small vesicles about 50 m $\mu$  and larger vesicles about 500 m $\mu$ ), (*b*) directly across the endothelial cell as free particles within the cytoplasm, and (*c*) via open intercellular junctions. In contrast, our observations did not reveal the marker substances free in the cytoplasm in tissues doubly fixed with aldehydes and osmium tetroxide (Sabatini et al., 1963).

The studies of Tormey (1965) suggested that plasma membranes fixed in osmium tetroxide alone still retained some selectivity and filtered out particles above a certain diameter, i.e. around 150–200 A. It is of interest that the free particles within the cytoplasm observed by Casley-Smith (1964) were found in specimens that were fixed in

the osmium tetroxide alone after injections of colloidal ferritin and thorium. These tracers fall within the size range indicated by Tormey (1965). The observations of Tormey (1965), of Bruns and Palade (1968) and of our studies suggest that the free particles within the cytoplasm represent fixation artifacts and are not indicative of a condition that would occur *in vivo*.

### Conclusion

The present study confirms the early observations that the major passageway for the removal of connective tissue fluids and large molecules by the lymphatic capillary is the intercellular cleft. Although plasmalemmal vesicles occur at both connective tissue and luminal fronts, the present observations suggest that the movement of ingested particles is toward the central cytoplasmic matrix where engulfed particles are deposited in autophagic-like vacuoles for digestion and possible distribution within or from the lymphatic endothelium.

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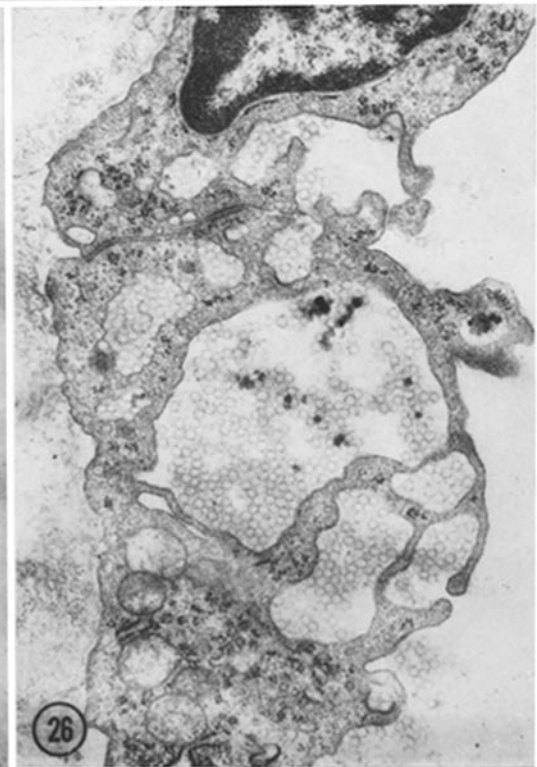
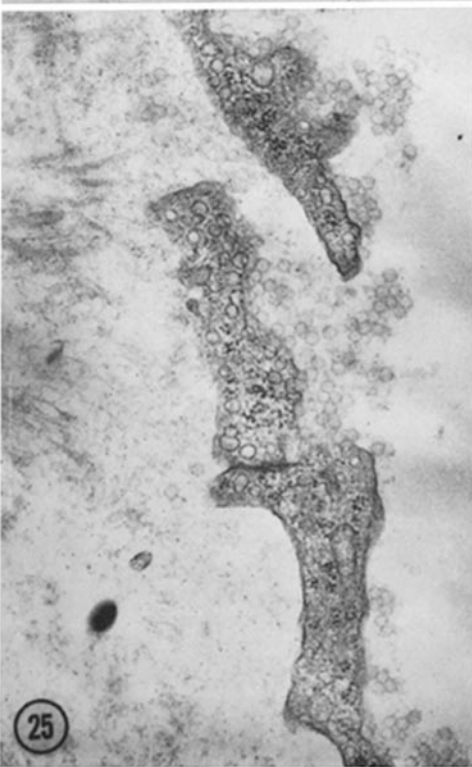
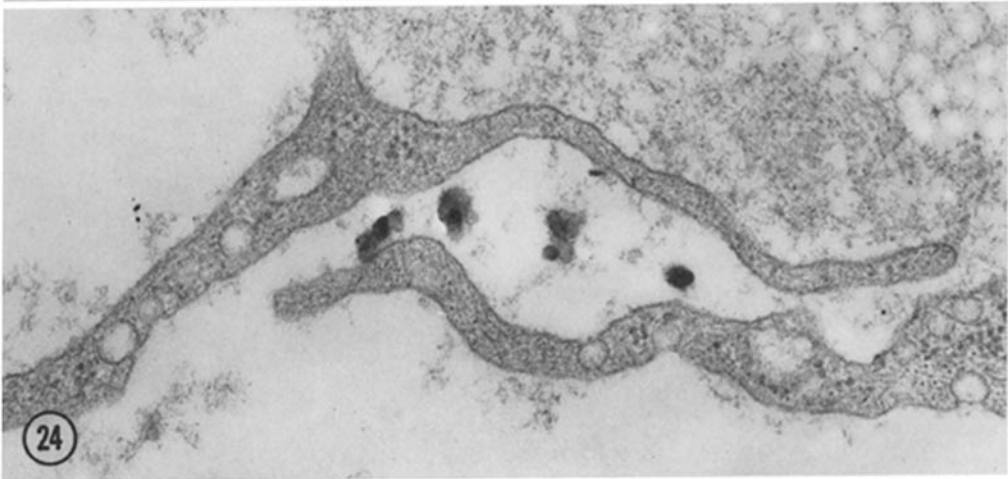
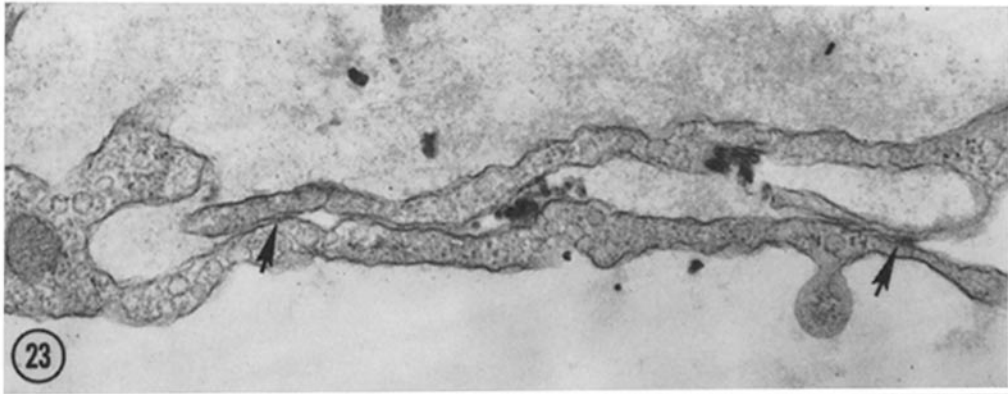
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**FIGURES 23 and 24** These electron micrographs illustrate the passage of colloidal carbon via the intercellular cleft. In Fig. 23, several areas of close apposition are found along the intercellular cleft (arrows), while in Fig. 24 the intercellular cleft forms an uninterrupted passageway. Specimens were injected with colloidal carbon 5 min before fixation. Lumina are at bottom of figures. Fig. 23,  $\times 28,800$ ; Fig. 24,  $\times 55,000$ .

**FIGURE 25** Another example of the passage of large particles via the intercellular cleft. Latex spheres fill a continuous passageway from the connective tissue into the lymphatic lumen. Specimens were injected with latex particles 30 min before being fixed. Lumen is to right of figure.  $\times 24,000$ .

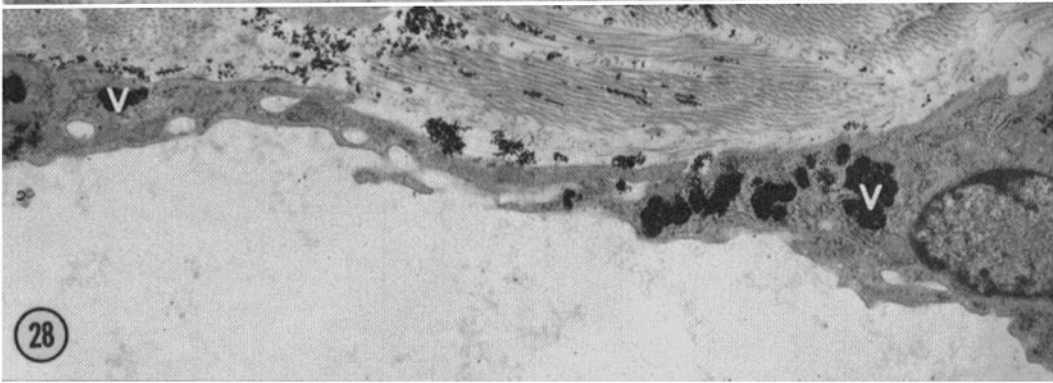
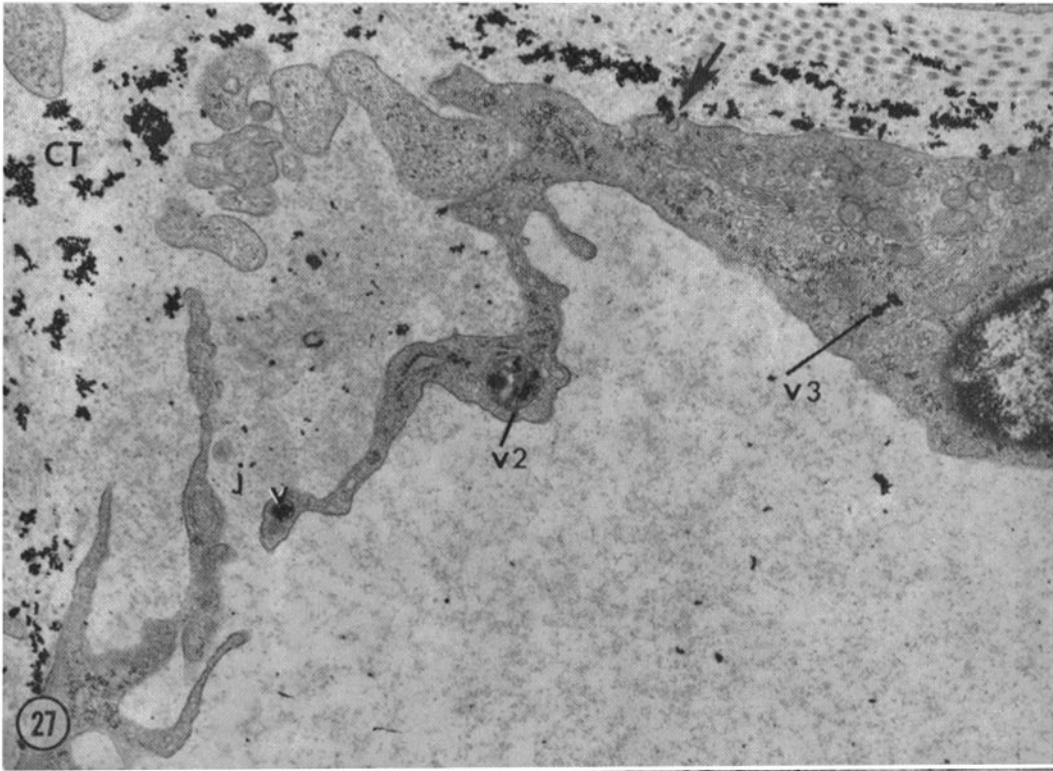
**FIGURE 26** This micrograph demonstrates the accumulation of latex spheres and carbon in large vesicles of the endothelial cell. The specimen was injected 24 hr before fixation. Lumen is to right of figure.  $\times 18,000$ .



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FIGURE 27 This micrograph demonstrates the appearance of colloidal thorium in the connective tissue (*CT*), in the intercellular cleft of a patent junction (*j*), associated with a plasmalemmal invagination (arrow), and in vesicles (*v*), two of which are similar in structure to autophagic vacuoles (*v2*, *v3*). The tissue was injected with colloidal thorium 18 hr before fixation. Lumen is at bottom of figure.  $\times 17,200$ .

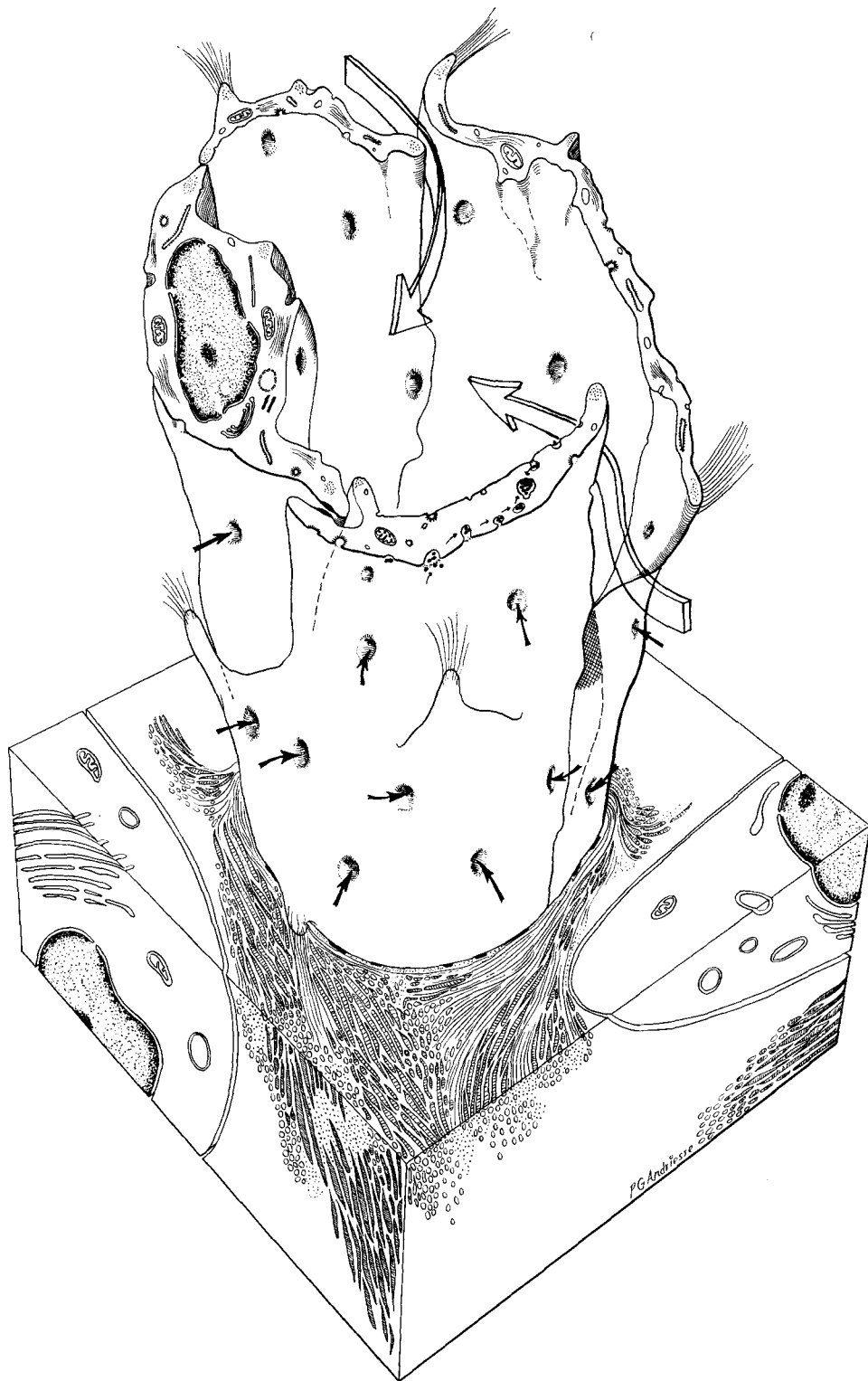
FIGURES 28 and 29 The accumulation of tracer particles in large vesicles (*V*) within the lymphatic endothelium is demonstrated in these electron micrographs. Lumina are at bottom of figures. In Fig. 28, the specimen was injected with thorium 18 hr before fixation.  $\times 5300$ . In Fig. 29, carbon was injected 24 hr before fixation.  $\times 6200$ .





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FIGURE 30 A three-dimensional diagram of a short segment of a lymphatic capillary that was reconstructed from collated electron micrographs. The major passageway for the transport of fluids and particulate substances from the interstitium into the lymphatic lumen is the intercellular cleft (long white arrows). The uptake of particulate components from the connective tissue front is indicated by the sequence of vesicles containing the particles. It is suggested that the movement of particles proceeds from the connective tissue front of the endothelium via vesicles (small arrows). These vesicles seem to merge or they fuse with autophagic vacuoles where intercellular digestion occurs for proteinaceous molecules for subsequent utilization or discharge. Whether all vesicles containing large molecules and tracer components take this route is not known at present.



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