Electron Microscopic Study of Lymphatic Capillaries in the Removal of Connective Tissue Fluids and Particulate Substances*

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The removal of connective tissue fluids by the lymphatic capillaries is of fundamental importance in the normal maintenance of fluid balance for body tissues. In addition to restoring extravascular proteins back to the blood stream, the lymphatic capillaries are important in the response of the organism to infection and in the spread of disease to various parts of the body. The early studies of *Hudack* and *McMaster* (16) and *Drinker* and *Field* (10) clearly demonstrated that an intradermal injection of vital dyes or colloidal opaque particles readily passed from connective tissue spaces into lymphatic capillaries. More recent studies (6, 19, 20) indicate that fluids and particulate substances gain entrance into lumina of lymphatics via one of two pathways, i. e. (1) across the intercellular cleft of patent junction and (2) across the lymphatic endothelium within pinocytotic vesicles.

In order to gain additional information on the mechanisms involved in this transfer of fluids and particulate substances into lymphatics, the present study was designed to investigate this problem by studying the fate of particulate substances after their injection into the connective tissue. Since intradermally injected colloidal particles are not engulfed by endothelia of blood capillaries, but are rapidly ingested by those of the lymphatics, the latter are identified by the presence of opaque particles within the endothelium as well as their lumina. This communication is concerned with the fine structure of lymphatic capillaries and the transport of various colloidal particles across the lymphatic endothelium of dermal tissue.

Materials and Methods

Colloidal particles ranging in size of 80A to 0.088 μ (i. e. colloidal ferritin 80–100A; colloidal thorium 100–250A; colloidal carbon 200–350A and latex spheres 0.088 μ) are injected intradermally into the skin of *guinea pig ears and samples taken at* various time intervals (a few seconds up to 6 months). Tissues were fixed and processed as previously described by *Leak* and *Burke* (21, 23).

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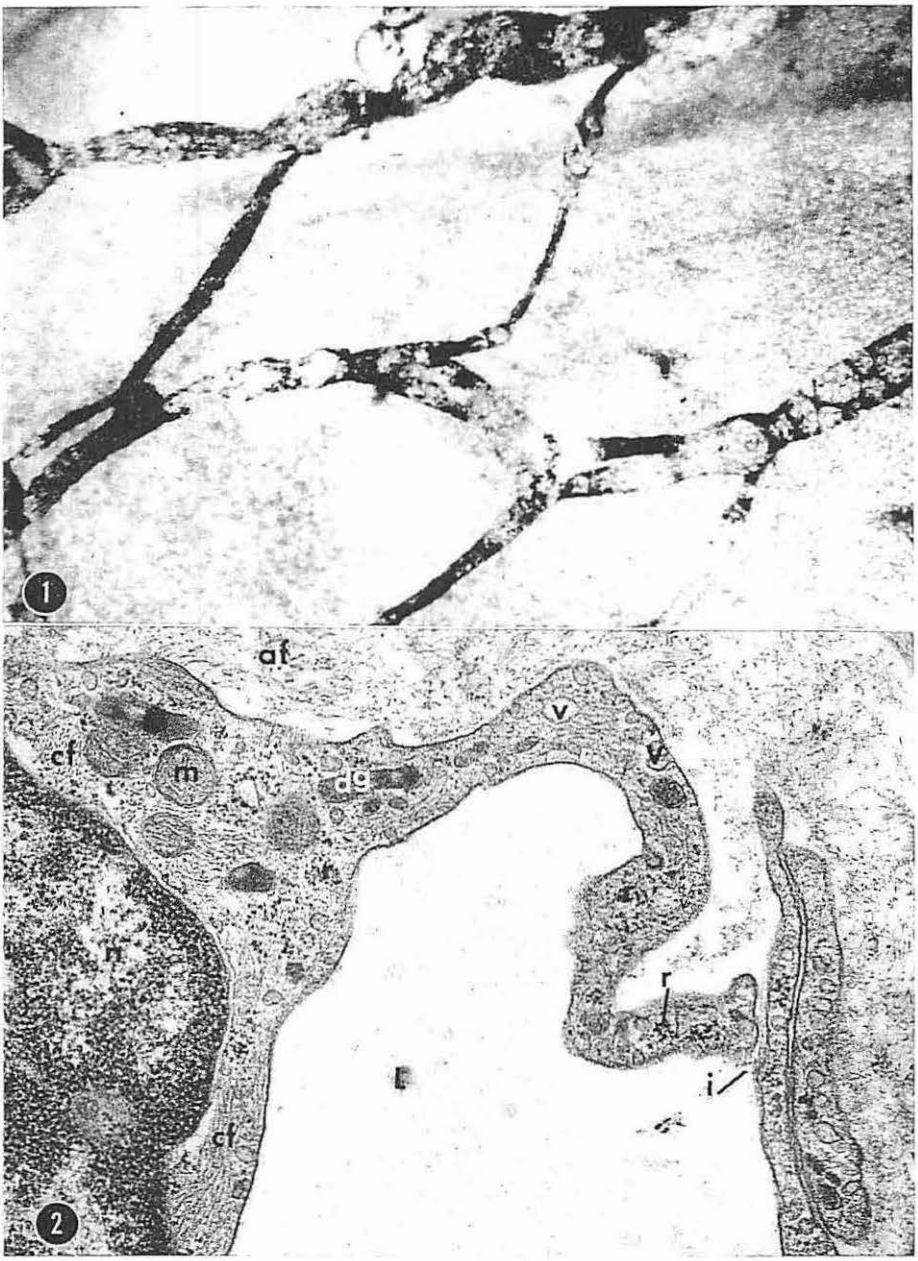


General Abbreviations Used in Figures

af. Lymphatic Anchoring Filaments
c. Injected carbon particles
cf. Cytoplasmic filaments
CT. Connective tissue area
ci. Coated invagination
cv. Coated vesicles
db. Electron dense material within vesicle
j. Cell junction
L. Lymphatic lumen
m. Mitochondria
n. Nucleus
r. Ribosomes
v. Pinocytotic vesicles

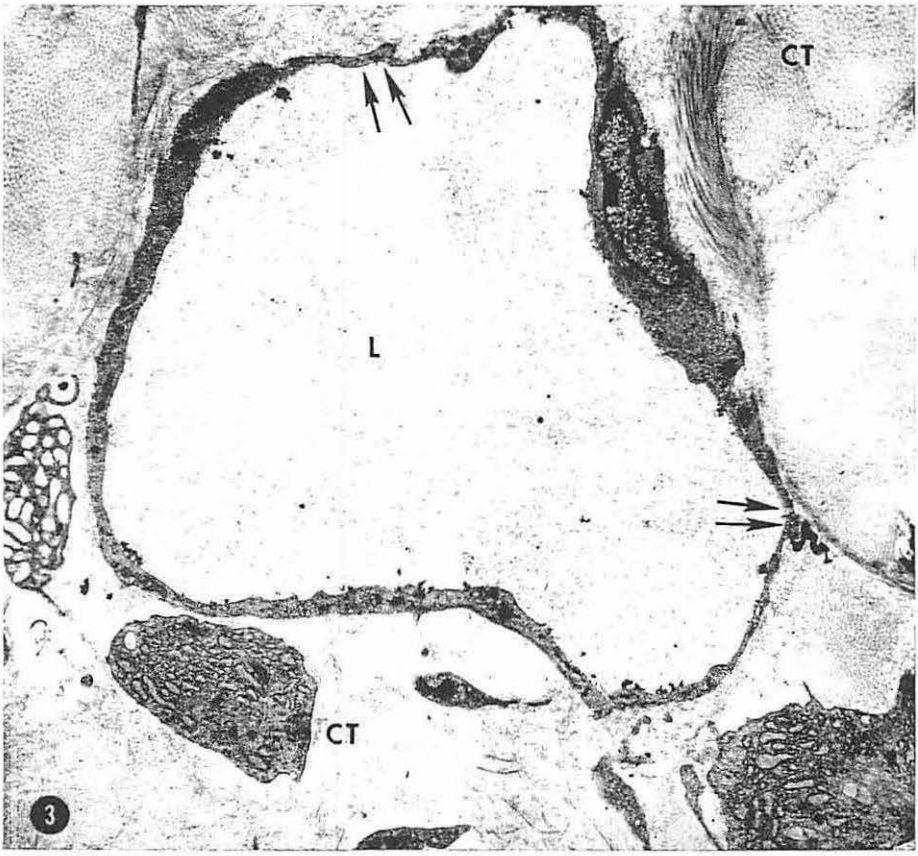


Fig. 3 Cross-section of lymphatic capillary illustrating the relation of vessel to adjoining tissue space (CT). Note cytoplasmic attenuation (double arrows) beyond the perinuclear (n) region, x 8,000.

Fig. 1 A whole mount preparation of lymphatic capillaries which demonstrates anastomoses of capillaries to form a fine plexus. Specimen injected with colloidal carbon prior to fixation and subsequent clearing, x 95.

Fig. 2 A cell junction (j) is demonstrated in this micrograph. The widths of the intercellular cleft measure 250 to 800 A along its length. Lymphatic anchoring filaments (af) occur along abluminal surface. Mitochondria (m), free ribosomes (r), vesicles (v) and dense granules (dg) are present in the cytoplasm. Cytoplasmic filaments (cf) are arranged in bundles and a portion of nucleus (n) is also shown. The lumen (L) is as marked, x 33,000.

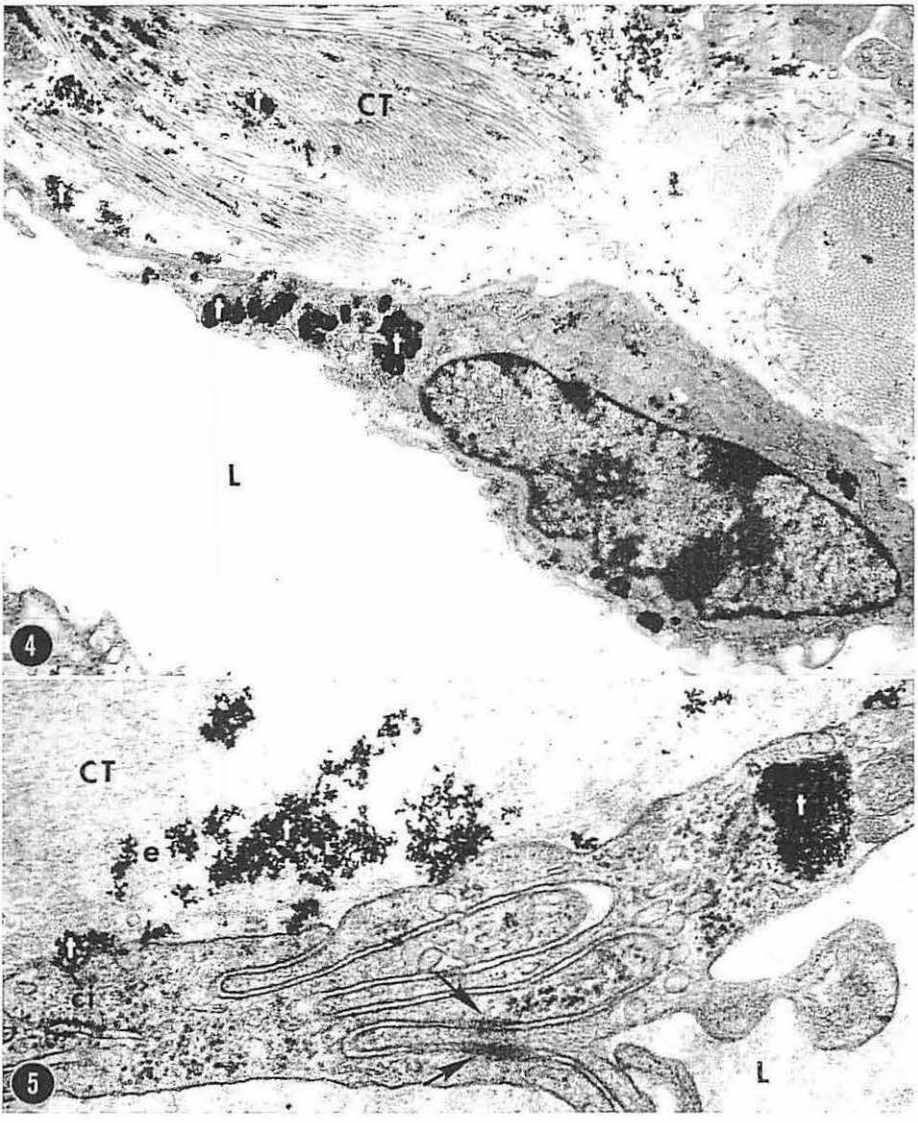


Fig. 4 Thorium (t) occurs in the connective tissue area (t) and in the lymphatic endothelium within vesicles, x 5,000.

Fig. 5 In this electron micrograph the terminal cell processes interdigitate. Points of close apposition are indicated by electron dense plaques (arrows). The marker particle (thorium – t) is present in the tissue area (CT) in coated invagination (ci) and in a vesicle within the central cytoplasm, x 50,500.

Results and Discussion

General Morphology of Dermal Lymphatic Capillaries

In our earlier studies of lymphatic capillaries, several fine structural features were described which may serve as criteria for differentiating them from the blood capillaries. The lymphatics can be distinguished from the latter by their 1) wider lumen, 2) a discontinuous basal lamina, 3) open intercellular junction and 4) the presence of numerous fine filaments at the abluminal surface which serve to anchor the lymphatic wall to the adjoining connective tissue. These delicate vessels ramify to form a rich plexus throughout the superficial layer of the dermis which extends to the peripheral margin of the ear (Fig. 1). In the deeper region of the dermis they merge, forming a coarser plexus that is continuous with the larger collecting vessels and lymph trunks.

The lymphatics are very irregular in outline and the endothelial wall varies from 0.1 μ to several micra in thickness (Fig. 2). The endothelial cells possess the usual complement of organelles, i. e. mitochondria, free ribosomes, a paucity of endoplasmic reticulum, and Golgi Complex with associated vesicles. In addition there are numerous cytoplasmic filaments, microtubules and coated vesicles throughout both the perinuclear cytoplasm and the attenuated cytoplasmic rims beyond the juxtanuclear region (Figs. 2 and 3).

The intercellular junctions of lymphatics vary in organization and structure, the endothelial cells overlap, or interdigitate at their margins, and are often held in close apposition without being imbricated to form junctions that are not very tightly apposed (Fig. 2). Although patent junctions occur with some regularity there are numerous areas along the intercellular clefts in which a close approximation of adjacent plasma membranes is observed. These regions of specialization occur at various points of the intercellular clefts and represent *macula adhaerens* (desmosomes). They may number up to several in regions where terminal cell margins imbricate (Fig. 5).

Uptake of Colloidal Particles by Lymphatic Capillaries

Immediately following a local injection of the above tracer particles lymphatics in the surrounding vicinity and those deriving from this region become filled with the colloidal suspensions. All marker particles investigated gain entrance into the lymphatics via one of two morphological pathways:

1. through intercellular cleft of patent junction, and
2. across the endothelium within vesicles.

Intracellular Passage of Colloidal Particles

Smooth surface vesicles: The transfer of intradermally injected particles across the lymphatic endothelium began within a few seconds after the injections. These particles appear within invaginations along the endothelial surface in addition to vesicles in the subjacent cytoplasm (Figs. 4–7). The latter are smooth surfaced and measure about 500A in diameter. Occasionally several of these vesicles appear to be in the process of fusing, thus pooling their content of marker particles, giving rise to larger vesicles which may reach measurements of up to 1 μ in diameter. Investigations of several workers have demonstrated the passage of electron opaque markers within

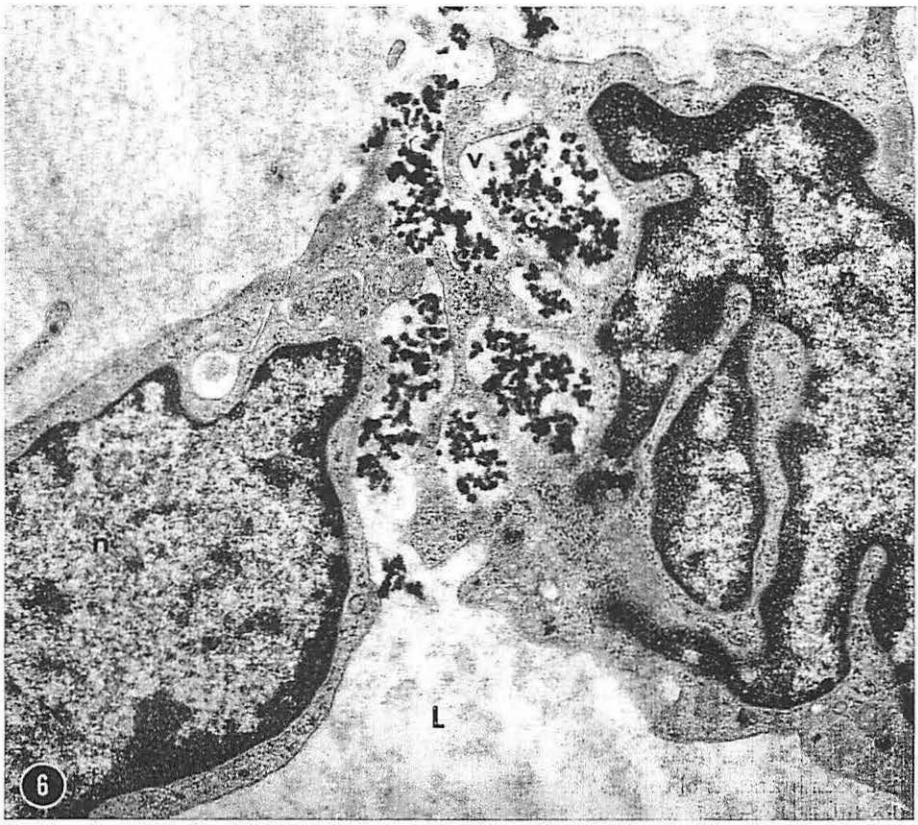


Fig. 6 The intercellular cleft is filled with colloidal carbon (c) which also occurs in vesicles (v). Parts of two nuclei (n) are also shown, x 20,500.

pinocytotic vesicles of a variety of cell types. These vesicles form by an invagination of the plasma membrane which contains the engulfed marker particles. They pinch off and form larger vesicles before traversing the cell and emptying their content on the opposite side (12, 13, 15, 17). Subsequent investigators have demonstrated similar results in the lymphatic endothelium (5, 6, 20, 21, 22).

In the long term injected specimens, it is common to find very large vesicles that are filled with the marker particles (Fig. 10), many of which measure several micra in diameter. A striking feature of the lymphatic endothelial cells in specimens that are injected for long periods of time is the occurrence of dense bodies and whorled membranous structures (Figs. 11 and 12). The whorled membranous structures are similar to myelin figures (29). These structures have been associated with both degenerative and regenerative changes brought about by chemical and mechanical treatments (2, 20, 27). Both the whorled membranous structures and dense homogenous bodies are topographically in close association with the ingested particles, and depict images that are similar to autophagic vacuoles or cytolysosomes which are involved in the intra-

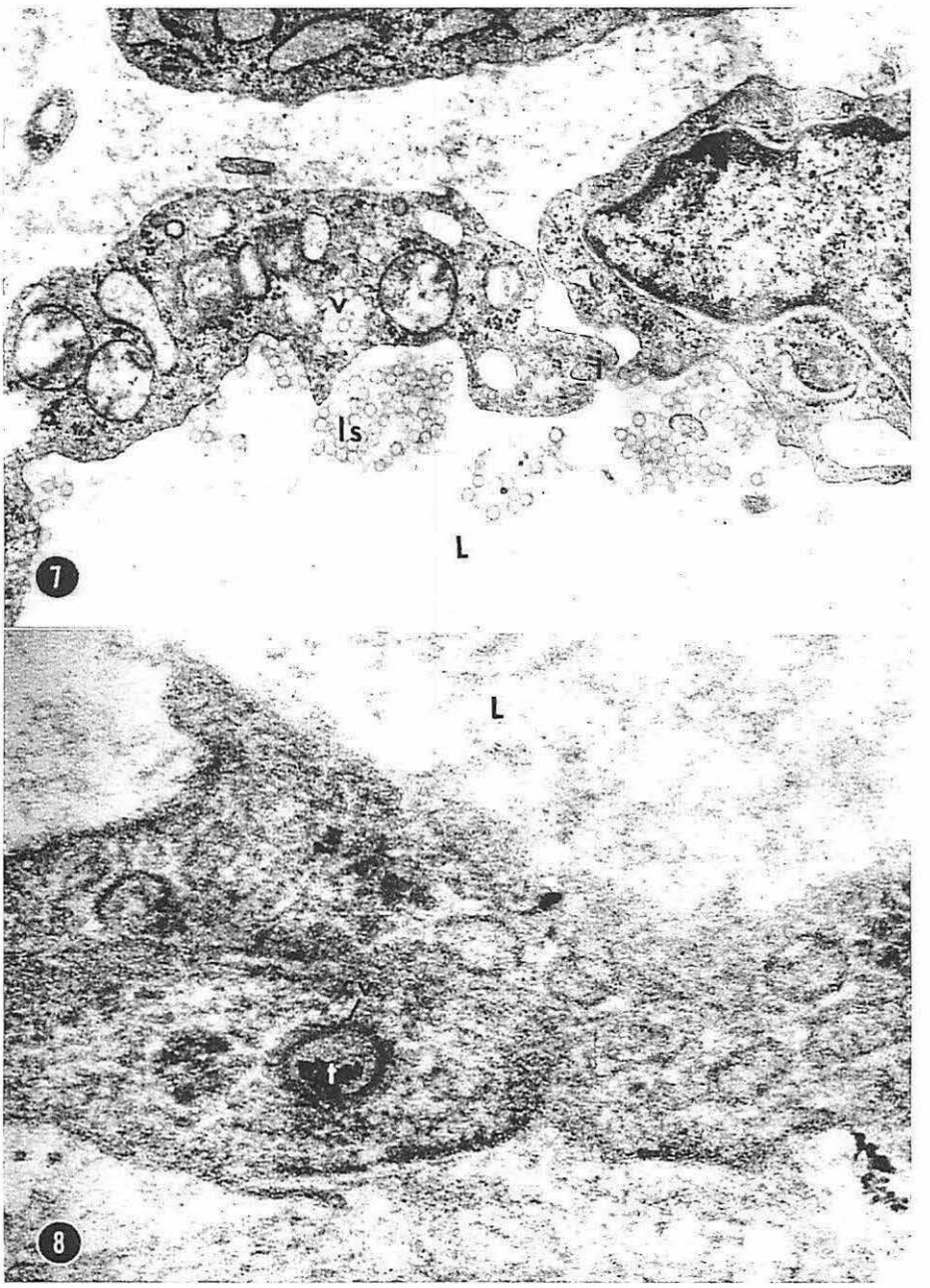


Fig. 7 Latex spheres (ls) are present in lymphatic lumen (L) and vesicles (v) within the endothelium. Intercellular junction (j) is as indicated, x 25,000.

Fig. 8 This micrograph demonstrates the occurrence of marker particle (Thorium – t) within a coated vesicle (cv), x 155,000.

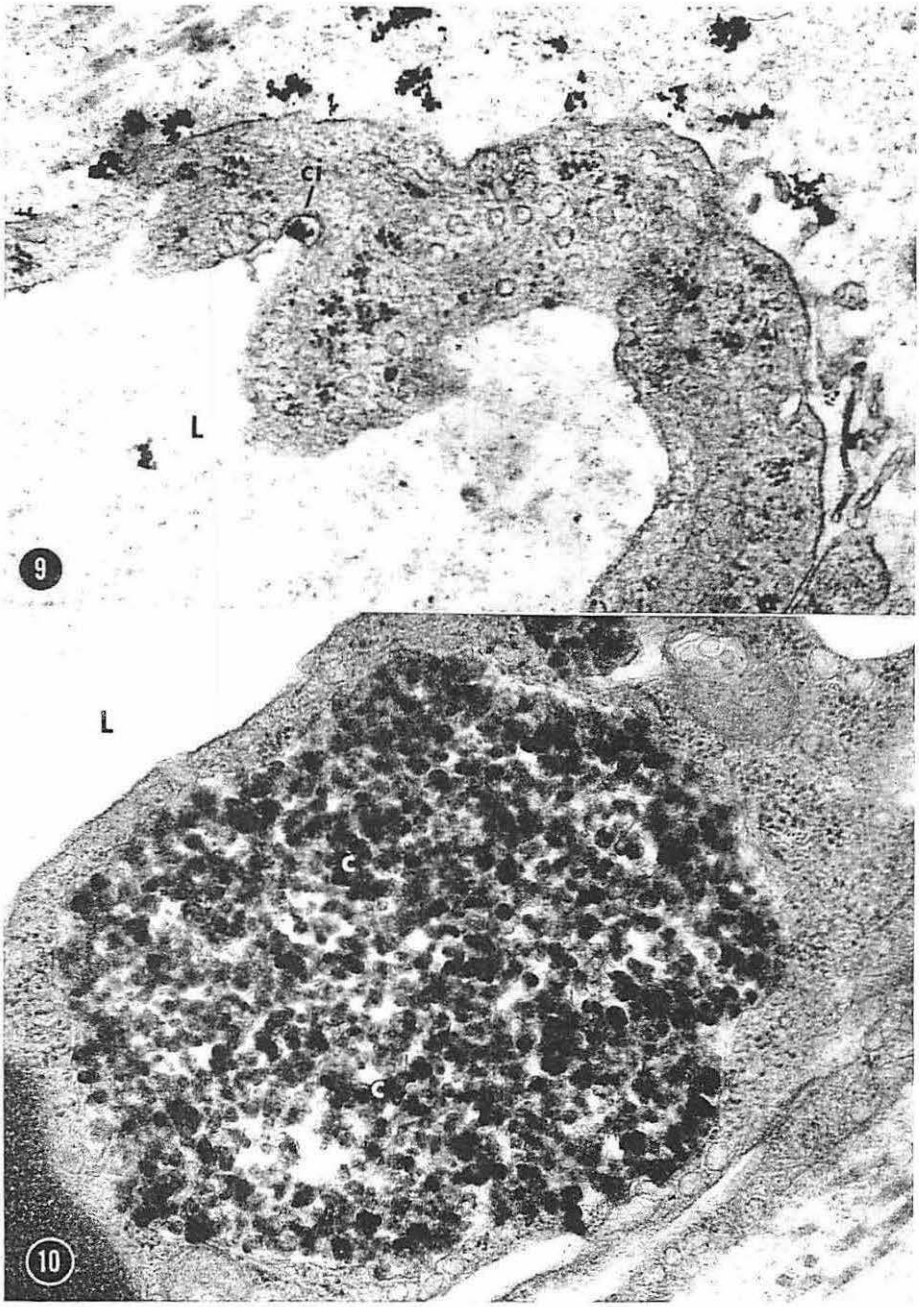


Fig. 9 The coated invagination (ci) occurs on the luminal surface in this micrograph, x 46,000.

Fig. 10 In long term injected specimens, the marker particle (carbon – c) accumulates in large vesicles as demonstrated in this micrograph, x 43,000.

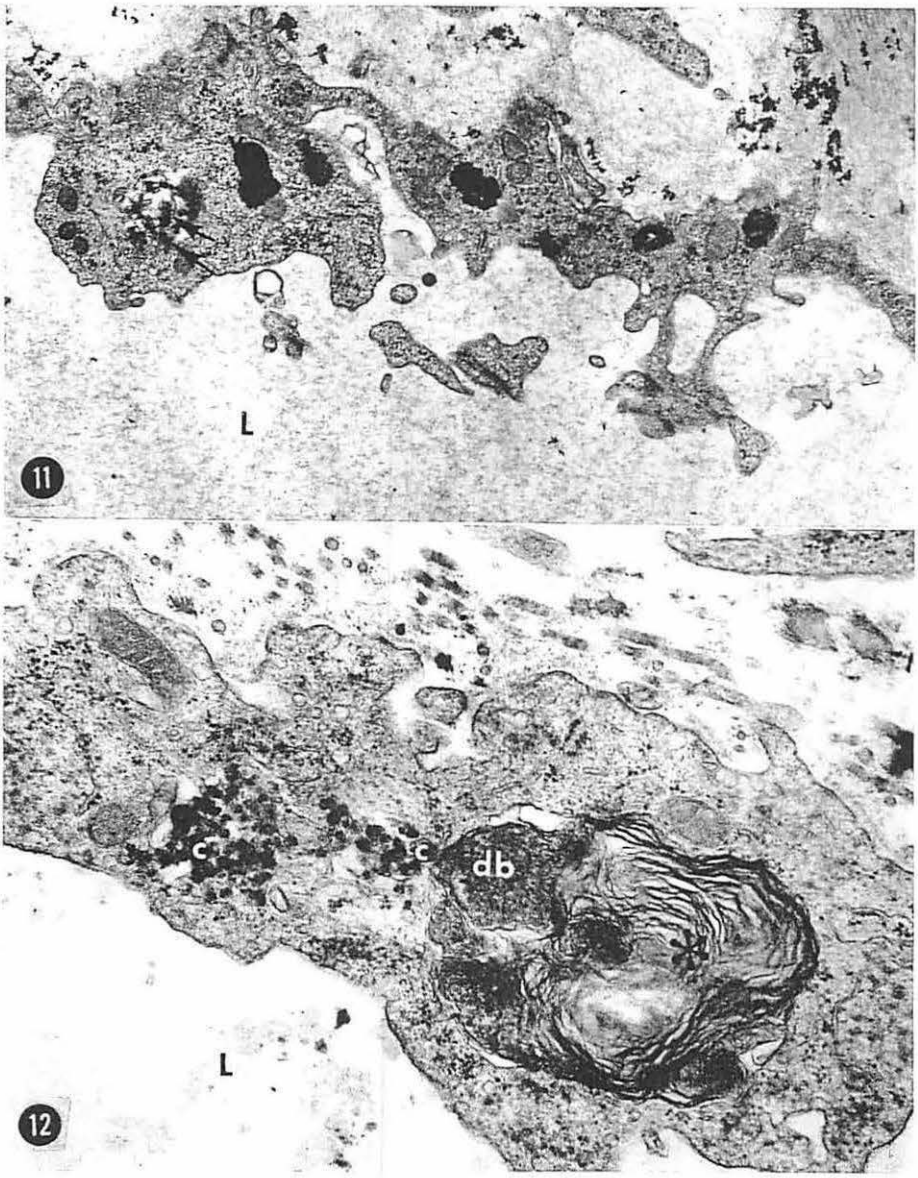


Fig. 11 Colloidal thorium also accumulates within large vesicle (v) after long periods of intradermal injections. In addition, dense material and membranes are also observed (arrows), x 12,000.

Fig. 12 Dense bodies (db) and a whorled membraneous structure (*) are in close association with the ingested carbon (c) particles (4 months after ingestion), x 35,500.

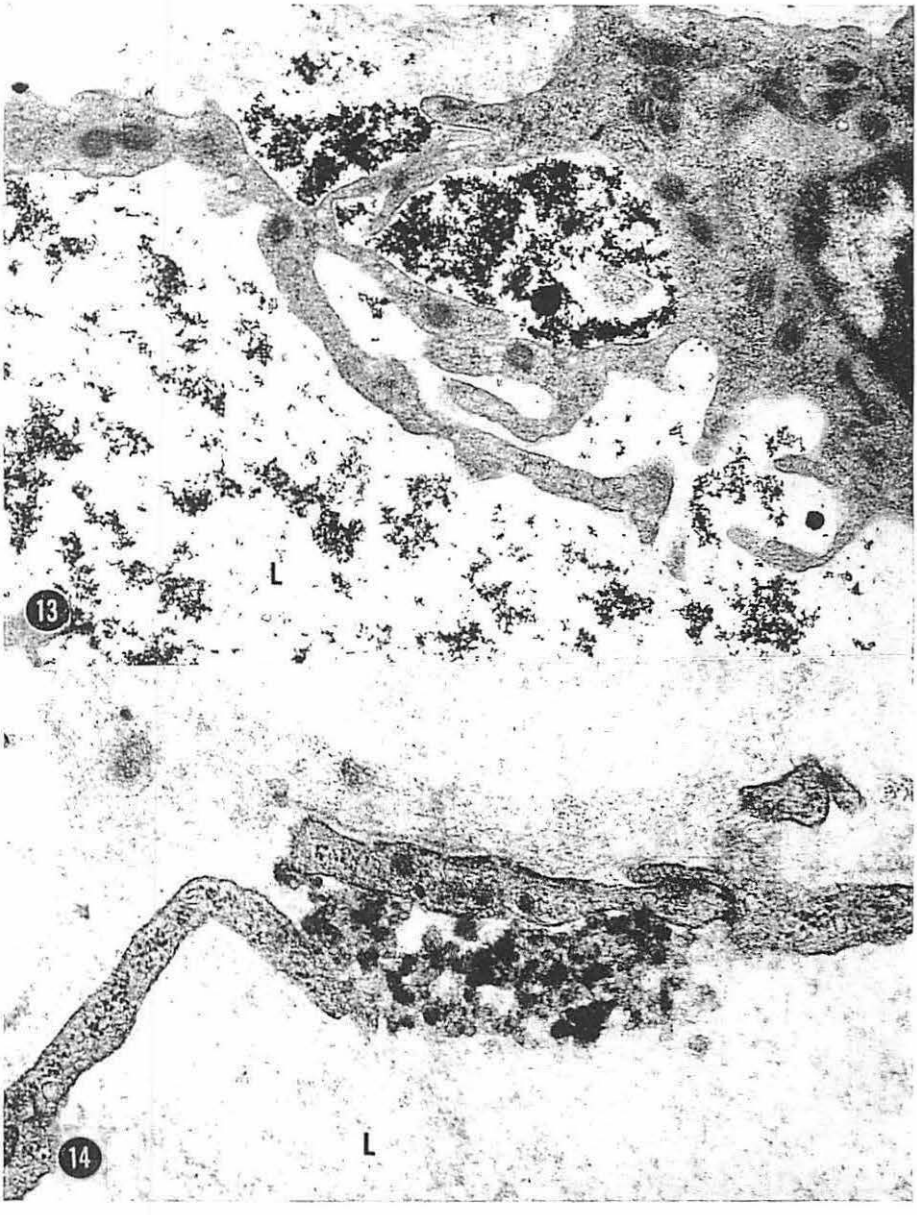
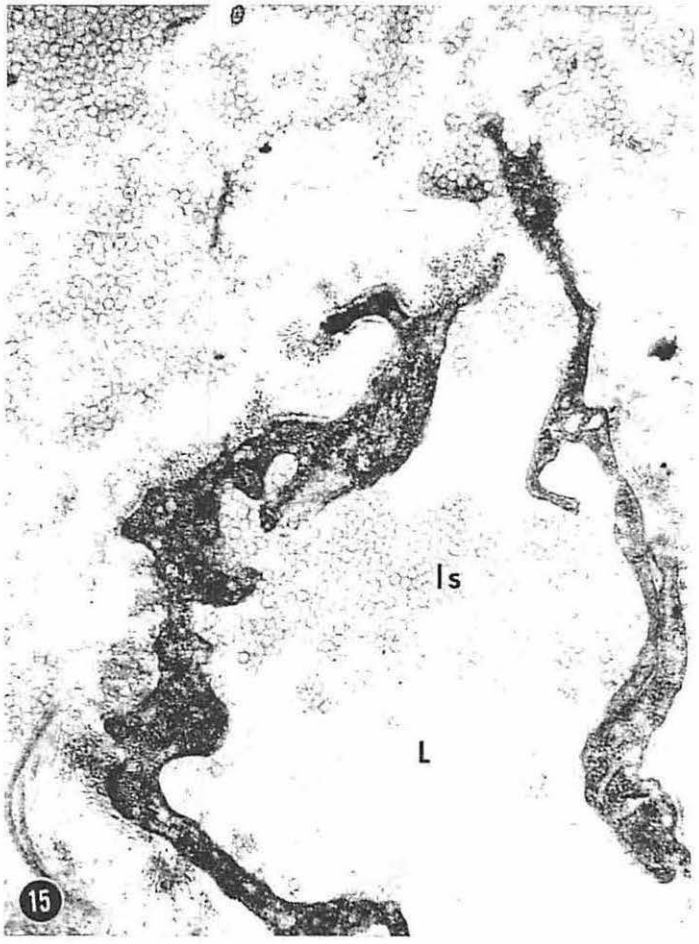


Fig. 13–15 Demonstrate the passage of the marker particles across the intercellular clefts of partially open and fully patent junctions. Fig. 13 ferritin, x 19,000. Fig. 14 carbon x 35,000. Fig. 15 Latex spheres x 27,500.



cellular digestion of foreign in addition to endogenous substances (24). Preliminary studies on the localization of acid phosphatase demonstrate that the reaction product occurs over many of these dense regions within the large vacuoles. Although much of the injected marker substance is transported across the lymphatic endothelium via the above mentioned vesicles, the present observations indicate that these endothelial cells are also capable of retaining large amounts of injected material for long periods of time.

Coated vesicles: The injected particles are also observed within coated pits and vesicles. These vesicles contain a spike-like material which covers their cytoplasmic surface (Figs. 5, 8, 9). The coated vesicles observed here are very similar to the spiked or bristle-coated vesicles observed in a variety of cells and tissue types (25). The studies of a number of investigators provide both experimental and circumstantial evidence which indicate that the bristle-coated vesicle is associated with selective uptake of protein from the extracellular environment (1, 14, 25, 28). Recent studies of lym-

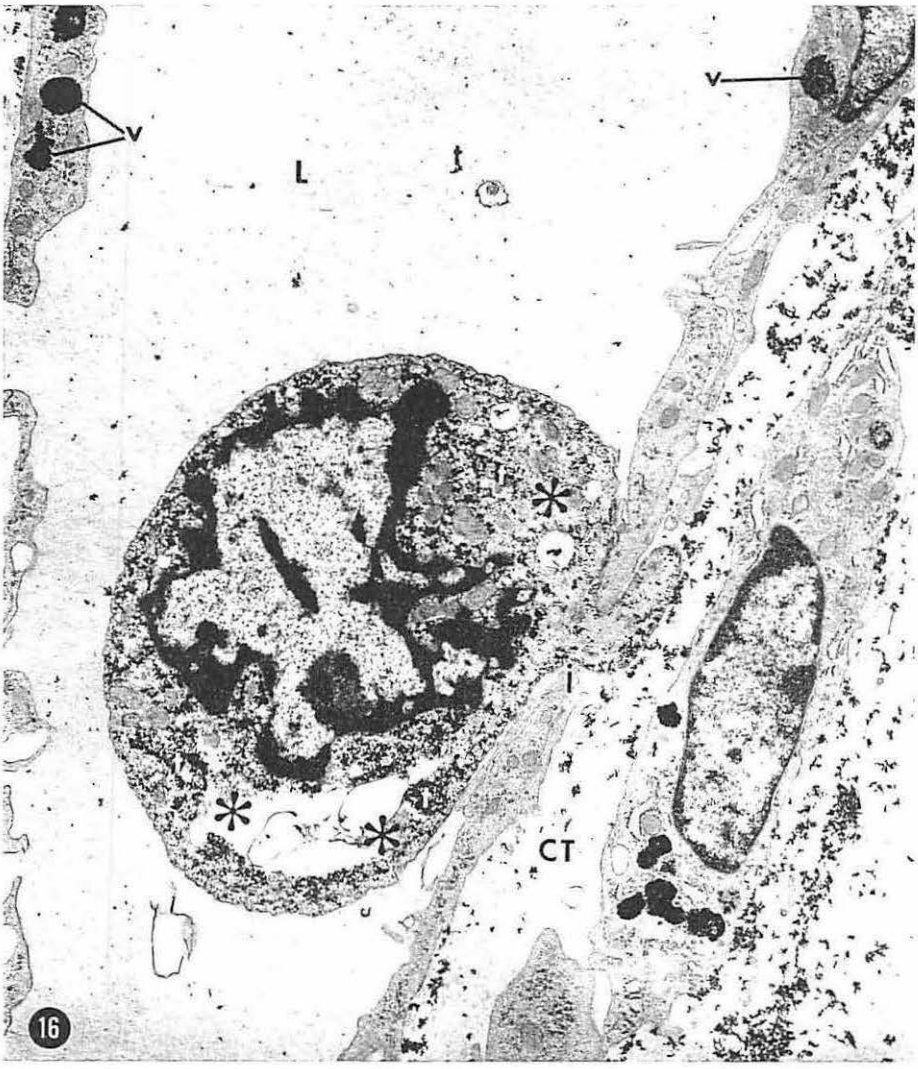


Fig. 16 Occasionally monocytes and leucocytes are observed in the process of traversing the lymphatic endothelium via intercellular clefts of patent junctions (j). Note the occurrence of the marker (thorium – t) within the cytoplasm of this damaged cell (*) lumen of vessel (L) in addition to the connective tissue area (CT) and vesicles (v) of the lymphatic endothelium, x 11,000.

phatics in amphibian larvae have demonstrated the uptake of colloidal carbon by coated vesicles (19). The uptake of carbon by amphibian lymphatics does not seem to be fortuitous, as a large number of the coated vesicles contained carbon. If these vesicles are specific for certain proteins (11, 26) it is possible that this marker particle is coated by a protein within the connective tissue prior to its engulfment by the coated vesicles, which would account for this uptake.

Since the lymphatics also serve the special function of absorbing proteins (7, 8, 9), the coated vesicles in the lymphatic endothelium, may also represent a transport mechanism for the transfer of extracellular protein across the endothelial cells of the lymphatic capillary.

Both carbon and latex spheres were enclosed within membrane-limited inclusion in all specimens observed. However in some specimens injected with ferritin and thorium, the marker particles were occasionally scattered randomly and free within the cytoplasmic ground substance. Since this condition is rarely observed it might be argued that these free particles represent fixation artifacts. Similar observations have been reported by other investigators for other cell types (3, 4). Recent studies of *Tormey* (30) suggest that OsO_4 fixed plasma membranes still retain some selectivity and filters out particles above a certain diameter (i. e. above 200A).

Intercellular Passage of Colloidal Particles

The absence of tight junctions *(zonula occludens)* and also desmosomes *(macula adhaerens)* from many areas of closely apposed membranes would also facilitate their separation, thus causing patent junctions along the wall of the lymphatic vessel. In these situations, the intercellular clefts are continuous with the adjoining connective tissue, thereby providing uninterrupted channels between lymphatic lumen and the adjoining tissue space.

Within the first few minutes after intradermal injection of the above colloidal marker particles (i. e. ferritin, thorium, carbon and latex spheres), a large number of these particles occur within the intercellular clefts of patent junctions (Figs. 14, 15), as well as junctions that are partially open as shown in figures 6 and 13. Occasionally cells are seen entering via patent junctions some of which appear damaged (Fig. 15). The occurrence of the injected particles within the intercellular clefts of these open junctions clearly demonstrates a direct entry of particulate material into the lymphatic lumen. The observed intercellular passage of large amounts of substances is also in keeping with the rapid removal of extravascular proteins and fluids from the connective tissue area (31).

Summary

The present observations on the lymphatic capillaries in both superficial and deep areas of guinea pig dermal tissue provide morphologic and experimental information which demonstrate the passage of fluids and various particulate substances, from the connective tissue areas into lymphatic lumina. This transfer occurs via two morphologically definable pathways: i. e. (1) Intercellular clefts of patent junctions, and (2) across the lymphatic endothelium within membrane bounded vesicles. In addition to the removal of fluids and particulate substances, the occurrence of cytolysosomes within lymphatic endothelium suggest that these cells may also be involved in the intracellular digestion of foreign materials that are removed from the adjoining tissues.

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Lymphatic Transport of Bacteria in Surgical Infection*

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The lymphatics have often been considered the final common pathway to the systemic circulation for tissue fluids and protein. Little significance has been placed on the system as the common pathway for delivery of bacteria to the systemic circulation and the reticuloendothelial system from an infection site or even from the normally contaminated organ manipulated at the time of a surgical procedure. In-

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