ELECTRON-MICROSCOPIC INVESTIGATION OF PERMEABILITY OF LYMPHATIC WALLS BY MEANS OF A NEW MARKER

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The ultrastructure and permeability of the wall of small lymphatics from the upper third of the rabbit leg were investigated 10 min after injection of 5-10 ml of the substance FeLEK (particles size 50-250 Å), used for the first time as marker, into the plantar pad of the animal's hind limb. It was found that the wall of the small lymphatics consists of endothelium, a discontinuous basement layer, and infrequent smooth-muscle cells. High-contrast particles were found in small and large vesicles of the cytoplasm of the endothelial cells and in the intercellular junctions of the endothelial layer (open, sometimes in the closed). The substance FeLEK satisfies the demands made on markers and it can be used to study the permeability of the vascular wall.

The ways by which various substances pass through the endothelial barrier is being closely studied at the present time. A number of different contrast substances with a particle size ranging from 80 to 3000 Å can be used for the electron-microscopic study of permeability of the wall of lymphatics and blood vessels.

Investigations have shown that most small molecules pass through the intercellular junctions of the endothelial cells, while some of them are transferred with the aid of vesicles [2, 3, 7, 8, 11-14, 18]. The "closed" junctions (i.e., junctions between adjacent endothelial cells possessing attachment belts of the obliteration plaque and zone type) are known to be impermeable to small molecules and particles, although the opposite view is held [13] on the basis of the discovery of horseradish peroxidase in the "closed" junctions. There is evidence to show that the "closed" junctions are readily permeable to ions [9]. Possibly, therefore, markers with a diameter less than that of peroxidase particles but greater than that of ions can be used to investigate the permeability of the endothelium in the wall of lymphatics, blood vessels, and capillaries. Such a marker must be nontoxic and must have a high mass density or must react in the tissues with some other substance to form a product with high mass density [5].

The substance FeLEK suggested by the writer as marker is a stabilized complex of iron oxide and polyisomaltose, a product obtained by depolymerization of glucose. On entering the blood stream FeLEK is broken down in the blood and the iron is deposited in cells of the reticulo-endothelial system, from which it can again pass into the blood (from the leaflet accompanying the preparation, manufactured in Ljubljana, Yugoslavia).

The view is also held that precipitation of iron by phosphate ions can take place during fixation by Millonig's method [17] and that the precipitate thus formed can be deposited in the tissues, as occurs with ferrous gluconate [6].

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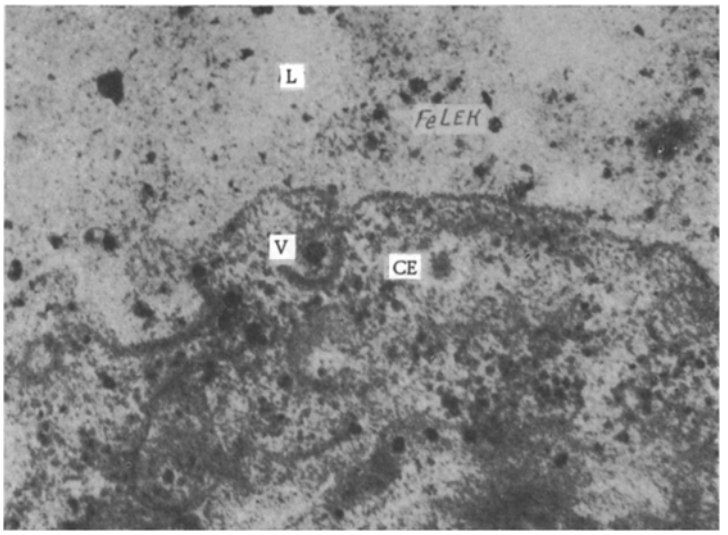

Fig. 1. Fragment of endothelial cell of lymphatic vessel. A vesicle in the stage of taking up an FeLEK particle is demonstrated. L) lumen of lymphatic vessel with FeLEK particles; V) vesicle; CE) cytoplasm of endothelial cell. 80,000×.

EXPERIMENTAL METHOD

Sexually mature chinchilla rabbits were used. By means of a type DP-2 artificial respiration apparatus intubation anesthesia was applied to the rabbits with a mixture of ether and air.* An injection of 5–8 ml of FeLEK solution was given into the plantar pad of the hind limb of the anesthetized animal, after which the limb was moved vigorously for 5–10 min. A wide incision was then made along the anterior surface of the leg and thigh of the animal and the lymphatics, which contained FeLEK in their lumen and were thus stained yellowish-brown, were carefully dissected after preliminary application of a few drops fixing solution to the vessels. The vessels were taken at the level of the upper third of the leg in the region where they accompanied the saphenous vein. Next, ligatures having been applied for a distance of 1–2 cm, the vessels were excised above and below the ligatures and immersed in 3% glutaraldehyde solution in phosphate buffer at 4°C (pH 7.4) for 1–2 h. Additional fixation was carried out with 1% osmium tetroxide solution in phosphate buffer and the tissue was dehydrated in alcohols of increasing strength. The material was embedded in a mixture of Epon 812 with Araldite. Ultrathin sections were investigated as a rule unstained, but sometimes they were negatively stained with uranyl acetate and lead citrate. Material was examined with the JEM-6C electron microscope.

EXPERIMENTAL RESULTS

In the ultrastructure of their endothelium the small lymphatics are very similar to lymphatic capillaries. They have "open" junctions, and the attachment belts between adjacent endothelial cells in their wall are composed mainly of obliteration plaques. The basement layer is either ill-defined or absent over wide areas. A few scattered smooth-muscle cells are found in the vessel wall.

High-contrast particles of FeLEK were found both in vesicles of the cytoplasm of the endothelial cells and in the different types of intercellular junctions; FeLEK particles were found both in small vesicles connected with the plasmalemma of the endothelium and in vesicles lying freely in the cytoplasm of the endothelial cells.

Vesicles in the stage of taking up FeLEK particles were observed (Fig. 1). Many particles of the marker were seen in large vesicles 0.1–1 μ in diameter, formed evidently as the result of the union of many

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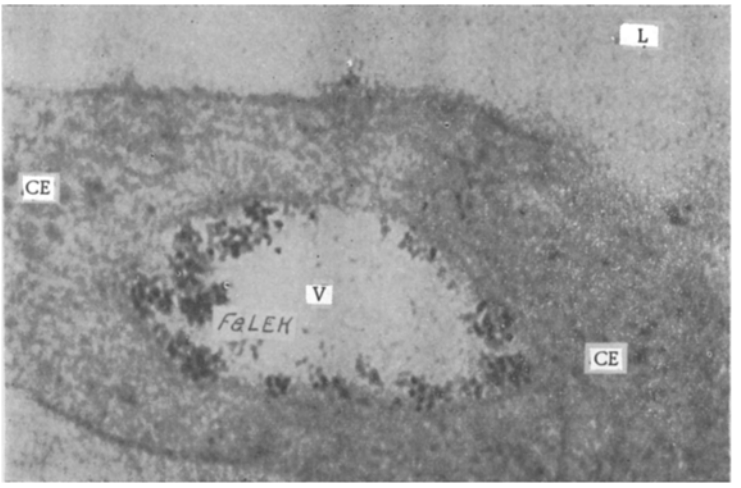

Fig. 2. Fragment of endothelial cell of lymphatic vessel with vesicle containing high-contrast FeLEK particles. L) Lumen of lymphatic vessel; V) vesicle with high-contrast FeLEK particles; CE) cytoplasm of endothelial cell. 78,000×.

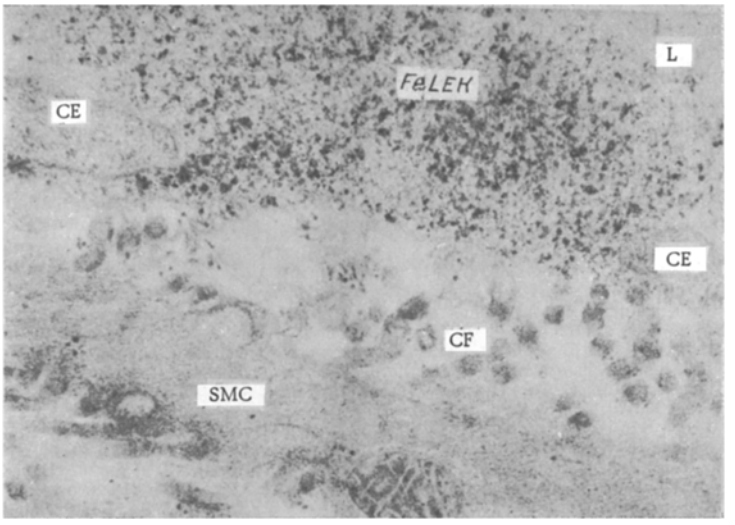

Fig. 3. "Open" junction in wall of lymphatic vessel with numerous FeLEK particles. CE) Cytoplasm of endothelial cell; L) lumen of lymphatic vessel with FeLEK particles; SMC) smooth-muscle cell; CF) collagen fibrils. 58,000×.

vesicles and merging of their contents (Fig. 2). The diameter of the FeLEK particles in these vesicles varied from 50 to 250 Å. High-contrast particles of the marker were constantly found between adjacent endothelial cells in junctions of both "open" and "closed" types. In the "open" junctions FeLEK particles were arranged in large groups (Fig. 3). The "closed" junctions, with attachment belts consisting of obliteration plaques, were impermeable to large molecules, but FeLEK particles 150 Å in diameter were found in them; in these junctions they were distributed as single units between the plasmalemmas of the adjacent endothelial cells (Fig. 4).

Penetration of the FeLEK particles through the endothelium of the small lymphatics was thus identical to penetration through the endothelium of the wall of the lymphatic and blood capillaries, i.e., it took place by means of vesicles and through intercellular junctions; movement of the particles of marker in this experiment took place in the direction from lymph to tissue, in agreement with results obtained by other workers [1, 16]. Zhdanov [1] explained this fact by the phagocytic properties of the endothelium.

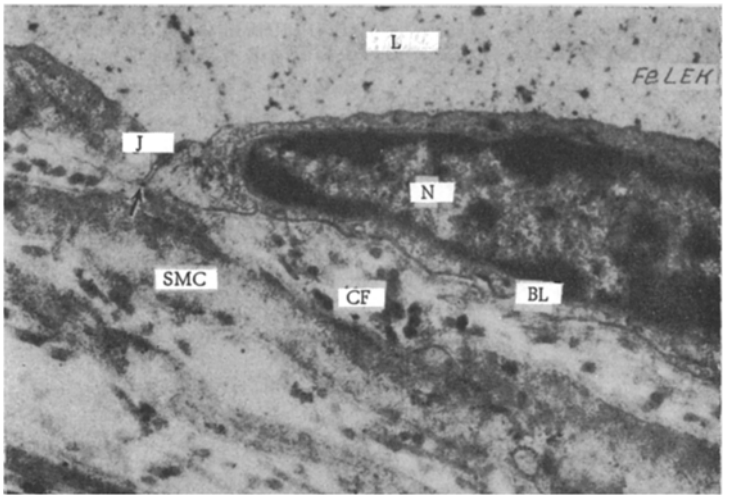

Fig. 4. Fragment of wall of lymphatic vessel. N) Nucleus of endothelial cell; L) lumen of lymphatic vessel with high-contrast FeLEK particles; J) intercellular junction with FeLEK particle (indicated by arrow); BL) basement layer; SMC) smooth-muscle cell; CF) collagen fibrils. 32,000×.

Calculations have shown [18] that vesicles cannot transport all the water and ions which pass through the endothelial barrier. There is no general agreement in the literature regarding the mechanism of movement of the vesicles. Some workers [10, 19] consider that movement of the vesicles in the endothelium of lymphatic and blood capillaries takes place on account of Brownian movement, and that it is very slow when compared with the transport of substances through the intercellular junctions. Other investigations [3, 15] have yielded evidence more in favor of active vesicular transport in the endothelial cells of the blood capillaries. However, further special investigations are necessary for a final solution of this problem to be obtained.

The results of the present investigation using FeLEK showed that under experimental conditions particles of the marker pass through the endothelial barrier of the small lymphatic vessels mainly through "open" intercellular junctions between adjacent endothelial cells. The "open" junctions have been shown to be completely absent in the thoracic duct, where "closed" intercellular junctions containing obliteration plaques and zones are mainly found [4]. The results of the present investigation are basically in agreement with those obtained by Casley-Smith [10], who studied the permeability of the small lymphatics of rats, mice, and guinea pigs.

In can be concluded from the results of this investigation that FeLEK satisfies the demands made on markers and that it can be used to study the permeability of the vessel wall.

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