SLING FILAMENTS IN THE VESSEL WALLS OF THE LYMPHATIC SYSTEM

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The ultrastructure of lymphatic capillaries of intestinal villi, the diaphragm, and the thoracic duct of rabbits was investigated. A system of sling filaments 40-110 Å in diameter, attached at one end to the plasmalemma of the endothelial cells and at the other end to smooth-muscle cells or to fibrous elements of the connective tissue, was found in the walls of all the lymphatics studied. It is postulated that these sling filaments are identical with the microfibrils of the extracellular space. The filaments discovered perform a special function by securing the endothelium of the lymphatic vessels to surrounding structures.

Connections between the walls of the lymphatic capillaries and reticulin and collagen fibers have been described [1-3, 21]. Several investigators have demonstrated connections between thin filaments given off by reticulin and collagen fibers and the plasmalemma of the endothelial cells of the lymphatic capillaries [6, 15-17]. They have been studied in detail in the lymphatic capillaries of the diaphragm of rabbits and have been called "anchoring" [18] and "sling" filaments [4]. More recently sling filaments have been found in the lymphatic capillaries of the rabbit conjuctiva [9], in the lymphatic capillaries of the rabbit, monkey, and human lung [14], in the lacteal sinuses of rat intestinal villi, and in the wall of the rabbit thoracic duct [5].

The object of the present investigation was to demonstrate filaments in the wall of the lymphatic capillaries of the diaphragm, in the lacteal sinuses of the villi of the small intestine, and in the wall of the thoracic duct of rabbits in order to deepen our understanding of the functions of these structural components of the lymphatic vessel walls.

EXPERIMENTAL METHOD

The tendinous center of the diaphragm, an intestinal villus, and the thoracic duct were taken from sexually mature chinchilla rabbits weighing 3-3.5 kg. The material was fixed in 3% glutaraldehyde solution in phosphate buffer, pH 7.4, for 2 h, after which the tissue was washed for 3-5 min in phosphate buffer and postfixed in 1% osmium tetroxide solution for 1 h. After dehydration in alcohols of increasing strength in the usual manner the material was embedded in Vestopal "A" or Araldite with Epon 812 [19]. Ultrathin sections were stained with 2% aqueous solution of uranyl acetate and lead citrate [22] and examined in the IEM-6C electron microscope.

EXPERIMENTAL RESULTS

The sling filaments found in the lymphatic capillaries of the rabbit diaphragm lay directly beneath the epithelium as clusters of dense filamentous structures (Figs. 1 and 2). The filaments were attached at one end to the outer layer of the elementary membrane of the plasmalemma of the lymphatic capillary endothelial cells, and at the other end they penetrated into the underlying connective tissue between the

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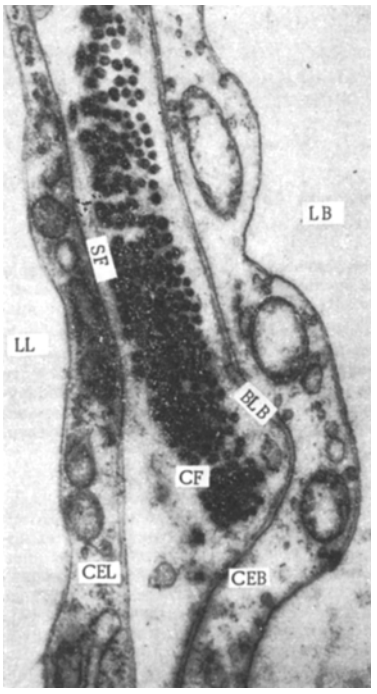


Fig. 1. Wall of lymphatic and blood capillaries of the rabbit diaphragm (longitudinal section): CEL) cytoplasm of endothelial cell of lymphatic capillary; CEB) Cytoplasm of endothelial cell of blood capillary; LL) lumen of lymphatic capillary; LB) lumen of blood capillary; BLB) basal layer of blood capillary; SF) sling filaments; CF) collagen fibrils, 32 000 ×.

collagen fibers and connective-tissue cells. The sling filaments were usually from 40 to 110 Å in thickness. Thin filaments 40-60 Å in diameter (Fig. 1) and thick filaments 100-110 Å in diameter (Fig. 2) could be distinguished among them. The highest concentration of filaments was observed in the boundary zones between two endothelial cells (Fig. 2). The sling filaments can be presumed to be a "tying" mechanism firmly attaching the wall of the lymphatic capillary to the adjacent collagen fibrils and connective-tissue cells like the slings of a parachute. Thin filaments are similar to the microfibrils of the intercellular substance of connective tissue first described by Jakus [12], and also to the filaments observed in the peripheral layer of elastic fibers.

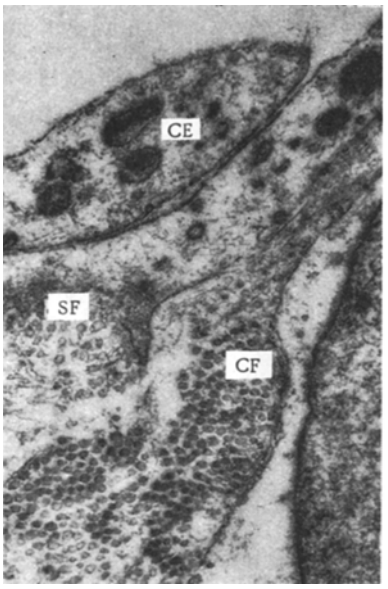
Sling filaments in the lymphatic capillaries of the rabbit intestinal villus also were 40-110 Å in diameter, and in longitudinal sections the thick filaments consisted of thread-like structures with a hollow cross-section (Fig. 3), On the side of the endothelial cells facing the connective tissue small projections of cytoplasm were frequently observed. The impression was obtained that these were the origin of the sling filaments, the other end of which was attached to corresponding projections on the smooth-muscle cells covered by the basement membrane; these filaments ran perpendicularly to the plasmalemma of the endothelial cells. The collagen fibers in the wall of the lymphatic capillaries were always separated from the endothelial cells by sling filaments.

Investigation of the ultrastructure of the thoracic duct* also revealed sling filaments attached at one end to the endothelial cells and at the other end to the basement membrane of the smooth-muscle cells in the duct wall. At the same time similar filaments were found around the elastic elements and between the smooth-muscle cells.

Leak and Burke [18] put forward 3 suggestions regarding the origin of the sling ("anchoring") filaments. First, their precursors may be synthesized by the endothelial cells themselves; second, they may be peripheral fibrils of elastic fibers; third, the possibility cannot be ruled out that they are microfibrils of the extracellular space. Connections between these microfibrils and elastic elements have been described [10, 13, 23]; the opinion has been expressed that the microfibrils are precursors of collagen and elastic fibers [20], while Haust [11] defined them as the "common denominator" of the connective-tissue fibers. The present writers are inclined to regard the sling filaments as identical with the microfibrils and they consider that both the endothelial cells and the ground substance of the surrounding connective tissue take part in their formation.

Casley-Smith [7] found rupture of the filaments attached to the wall of the lymphatic capillaries after treatment of the material with hyaluronidase, thereby proving that they contain acid mucopolysaccharides; the collapse of the lymphatic capillaries observed under these conditions proves that these filaments have a fixing function. Another fact of great importance to the function of the lymphatic capillary walls is that the sling filaments are attached as a rule to the boundary region between adjacent endothelial cells or to their cytoplasmic processes. This feature plays an important role in regulating the size of the gap between the two neighboring cells, so that if necessary it can be increased, and, if edema is present, the excess of fluid can be quickly moved from the connective tissue into the lumen of the lymphatic capillaries. Interesting results from this standpoint have been obtained by Collin [9], who studied the ultrastructure of the rabbit conjunctiva under normal conditions and in edema. He found that whereas under normal conditions the sling filaments weave into the plasmalemma of the endothelial cells of the lymphatic capillaries at an acute angle, during edema this angle becomes a right angle. Collin concludes from this fact that the sling filaments somehow prevent collapse of the lymphatic capillaries in tissue edema.

*For a detailed description see Vestnik Akad. Med. Nauk SSSR, No. 10 (1971).



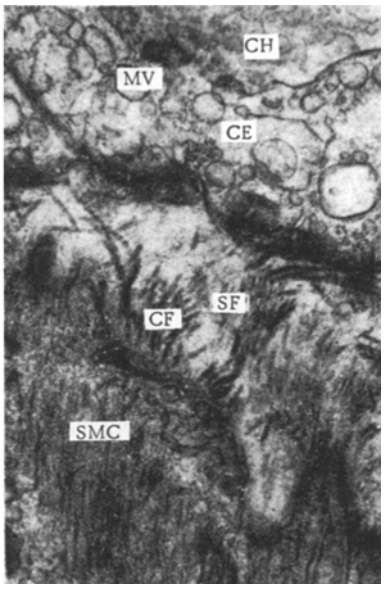


Fig. 2 Fig. 3

Fig. 2. Wall of lymphatic capillary from villus of the rabbit small intestine: CE) cytoplasm of endothelial cell; MV) micropinocytotic vesicles; SF) sling filaments; CF) collagen fibrils; SMC) smooth-muscle cell; CH) cylomicrons in capillary lumen, 40 000 ×.

Fig. 3. Boundaries between 2 endothelial cells from lymphatic capillary of the rabbit diaphragm: CE cytoplasm of endothelial cell; SF) sling filaments in transverse and longitudinal sections; CF) collagen fibrils in transverse section, 30 000 ×.

Because of the absence of a basal layer in the lymphatic capillaries and because of the weak development of that layer in the wall of the lymphatic vessels (up to and including the thoracic duct), the filaments discovered near the lymphatic capillaries and vessels thus perform a special function, supporting the walls of the lymphatic vessels like the slings of a parachute.

The authors regard sling filaments as constant structures characteristic of the vessel walls of the lymphatic system and playing an important role in their function.

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