Lymphology 19 (1986) 125-129

CYTOPLASMIC BODIES IN LYMPHATIC ENDOTHELIAL CELL

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ABSTRACT

Although micropinocytotic vesicles and other large vesicular structures have been observed in lymphatic endothelial cells on electron microscopy, in this study ultrastructure attention was focused on a variety of other membrane-bounded structures in the cytoplasm of these cells in the rabbit heart. The first type was oval or round in transverse section and elongated in longitudinal section, was 100-200 nm in diameter and contained regularly spaced tubules. These features resembled rod-shaped bodies detected thus far only in blood vascular endothelial cells. A second type of cytoplasmic body was also frequently seen in lymphatic endothelium. It contained irregularly spaced small vesicles, 5-7 in number and 45 nm in diameter. A third type of vesiculated body was only occasionally found. This latter structure was greater than the previously described bodies and ranged from 3-5 μm in diameter, and filled with granular, inhomogeneous material and filamentous-like components. The functional significance of these intraendothelial bodies is as yet unexplained.

The ultrastructural morphology of lymphatic endothelial cells in the mammalian heart has previously received some attention (1-4). These studies have documented a variety of anatomic structures, sometimes subtle, in the cytoplasm of these cells. Vesicular, membrane-bounded bodies are frequently present and micropinocytotic vesicles are the most numerous of these elements.

Previously (5,6) we described the ultrastructural characteristics of lymph capillary endothelial cells in the ventricle and atrium of the rabbit heart. Besides micropinocytotic vesicles, we also observed other variously shaped vesicular bodies.

Because little or no attention has been directed to the characteristics of these vesicular bodies we narrowed our focus in this study on these membrane-bounded bodies and specifically their distribution and fine structure.

MATERIALS AND METHODS

Hearts were obtained from adult rabbits of both sexes. After general anesthesia the hearts were removed and fixed by retrograde perfusion through the aorta. The fixative solution was a glutaraldehyde-paraformaldehyde (2.5%-2%) mixture in 0.1 M sodium cacodylate buffer pH 7.4 (Karnovsky modified) (7). Fifteen minutes later, small samples of the ventricular and atrial walls were isolated and reimmersed into the same fixative for three hours at 4°C. They were then post-fixed in OsO_4 in collidine buffer for 1 1/2 hours at 4°C, dehydrated and embedded in epoxy resin.

Semithin sections were taken to identify the lymph vessels and then ultrathin sections, stained with uranyl acetate and lead citrate, were prepared for electron microscopy.

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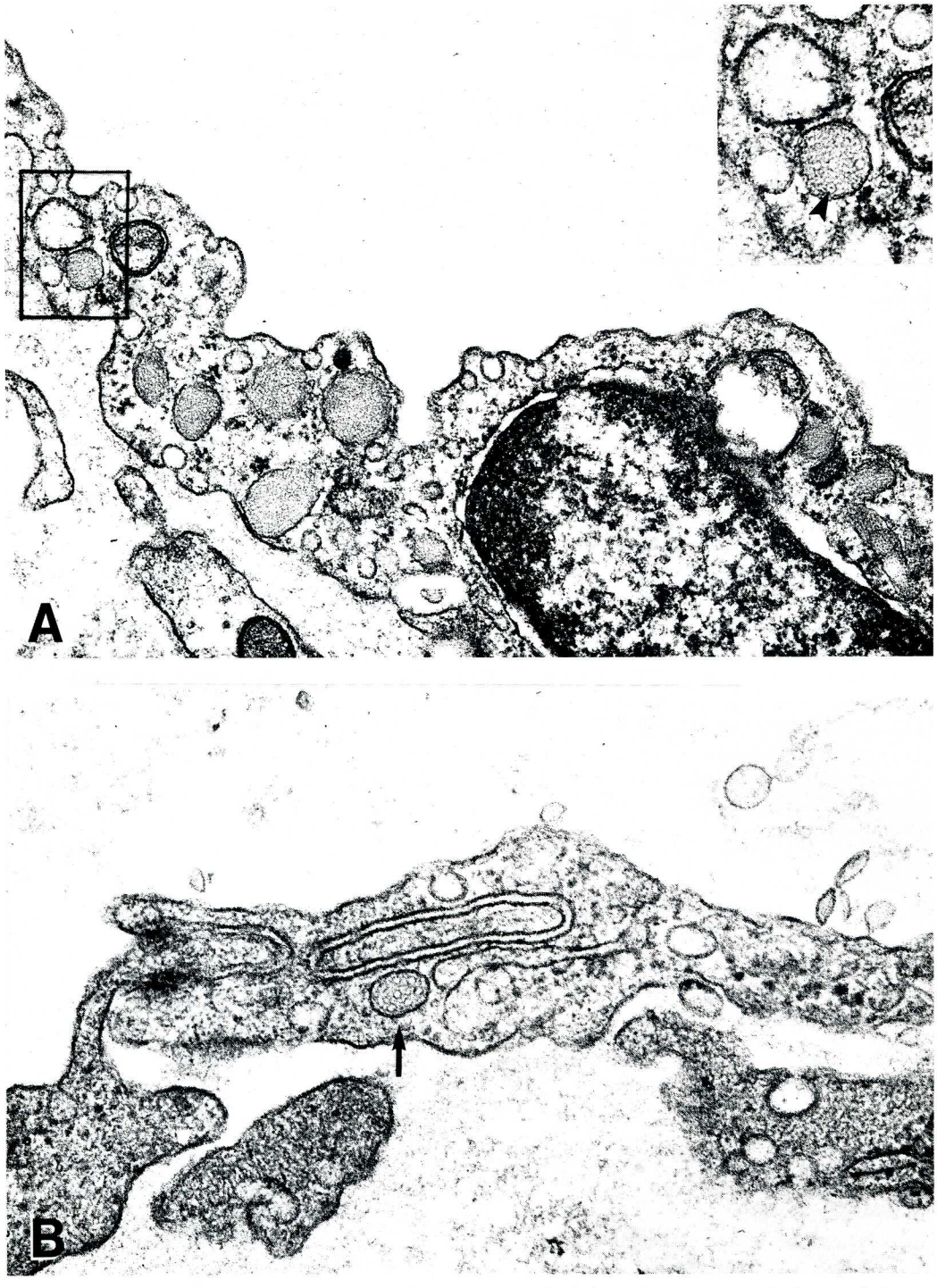

Fig. 1: First type of cytoplasmic bodies in the lymph vascular endothelial cells. A) in the perinuclear region; on transverse section they contain small, regularly spaced tubules with, sometimes, a little central dot (see inset) (x24,000; inset x60,000); B) in junctional region between two contiguous cells (arrow) (x60,000)

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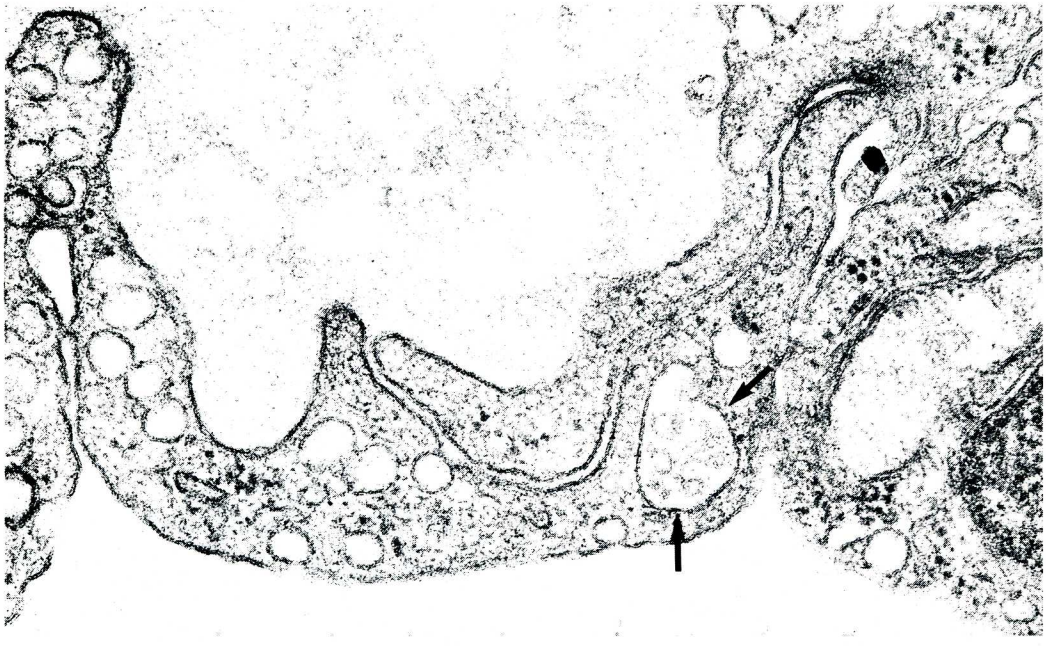

Fig. 2: A second type membrane bounded body containing small irregularly spaced vesicles (arrows) (x60,000)

RESULTS

Ultrastructural studies disclosed three types of vesicular bodies, not as yet described in the endothelial cells of lymph vessels. These components are typically grouped in the perinuclear region but they are also present in other cytoplasmic areas. The first type is oval or round in transverse section and elongated in longitudinal section and delineated by a triple-layered membrane. Their diameter is 100-200 nm but it is difficult to define the length because they are frequently obliquely sectioned; however the length is probably four to eight times the width. The interior of these structures is filled by numerous tubules, 20 nm in diameter, with a clear lumen occasionally containing a little central dot. These tubules are separated from each other by a more dense matrix and in longitudinal sections they appear parallel to the long axis of the structure *(Fig. 1)*.

A second type of cytoplasmic membrane-bounded body is round in section, 300-600 nm in diameter and contains small vesicles, 5-7 in number and 45 nm in diameter. These inner vesicles are more irregularly spaced and in general there is no difference between their lumen and surrounding matrix. The content of the vesicles is sometimes inhomogeneous *(Fig. 2)*.

A third type of vesiculated body is also occasionally found. It is larger than the previous ones (range of 3 to 5 μm in diameter). The interior is filled with a granular, inhomogeneous material and by "filamentous-like" components that, at high magnification, show a lamellar stratified appearance *(Fig. 3)*.

Micropinocytotic vesicles are the most numerous vesicular structures seen in heart lymphatic endothelium. They range from 70 to 95 nm in diameter and appear along both the abluminal and adluminal cytoplasmic membranes. In the thinnest region of the endothelial cell they are the only membrane-bounded structure present.

Larger vesicles (range 200 to 500 nm in diameter) are also present. They are clear, oval or round in shape and probably originate by a confluence of micropinocytotic vesicles. They are usually located next to micropinocytotic vesicles and on occasion

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these vesicles are seen to merge *(Fig. 1A, 3B)*.

DISCUSSION

This study demonstrates that a variety of vesicular bodies that vary in appearance are consistently present in cardiac lymph vascular endothelium. Specifically, they differ in shape, dimension, content and position within the cytoplasm. The first type of membrane-bounded body contains small tubules and although not previously described in lymph vascular endothelium they resemble rod-shaped tubulated bodies commonly seen in blood vascular endothelium (8). Their frequent presence in lymphatic endothelium leads us to believe they are a regular cytoplasmic component of all endothelial cells. Their function, however, remains obscure.

The second type of vesicular body we observed is similar to the so-called "multivesicular body" described in other cells (9,10).

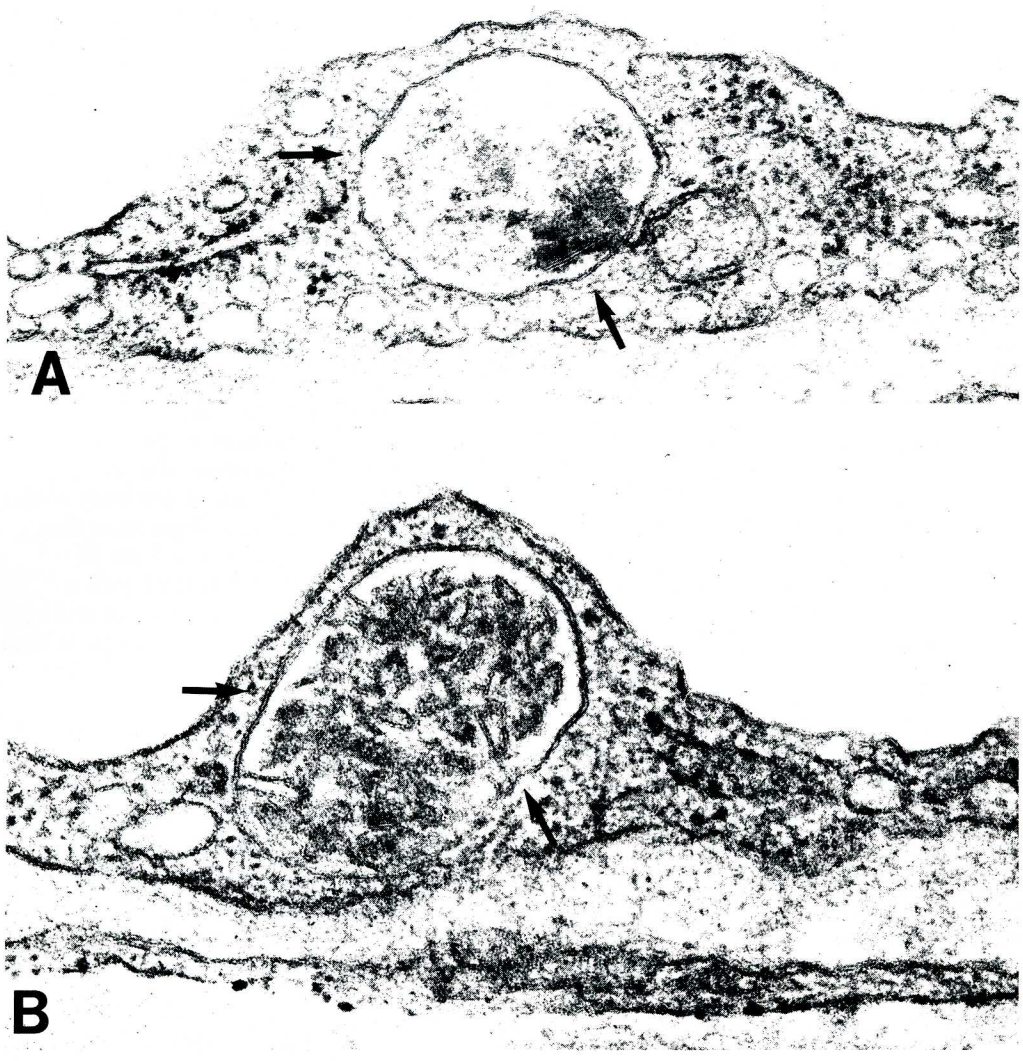

Fig. 3: Third type (A,B) large vesiculated body filled by a granular, inhomogeneous material and by "filamentous-like" components (arrows) (x60,000)

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In lymph vascular endothelial cells of tissues other than the heart, variously shaped inclusion bodies have been described (11) of paracrystalline, myeloid and tubular shape. We also observed vesicular bodies containing a "rod-like" structure but they were infrequent.

As for micropinocytotic vesicles and other large clear vesicles, these are similar to previous reports and their role in transendothelial transport (e.g. of protein) is well recognized.

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