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LYMPHATIC VALVES OF THE RAT PANCREAS

V. Navas, P.J. O'Morchoe, C.C.C. O'Morchoe

Biology Department (VN), University of Puerto Rico, Mayaguez, Puerto Rico, Departments of Cell and Structural Biology (PJO,CCCO), and Pathology (PJO), University of Illinois College of Medicine at Urbana-Champaign, Illinois, USA

ABSTRACT

The detailed structure of pancreatic lymphatic valves in rats was examined in an attempt to identify ultrastructural features that could be correlated with the ability of these delicate structures to withstand retrograde flow. Sprague-Dawley rats were perfusion-fixed and the pancreas processed for light and electron microscopy. Lymphatic vessels were identified by their typical appearance coupled with the presence of valves within their lumen. These valves were consistently formed of cuspid leaflets joined to the lymphatic wall at the bases and sides enclosing valvular pockets or sinuses between cusp and wall. Each cusp or leaflet consisted of two simple squamous endothelial layers separated by a connective tissue core and thus appeared, at first sight, as a simple infolding of the lymphatic vessel lining with its underlying connective tissue. However, certain differences were seen. Frequently the free margins of the cusps, instead of being smooth as might be expected, exhibited endothelial extensions or processes which were arranged in such a way that they could interdigitate with similar extensions on the opposing cusps and thus aid in closure of the valves. A striking difference between the endothelial lining of the vessel and that of the cusp was the presence of a distinct and almost continuous basal lamina underlying the endothelial cells which lined the surface of the cusp facing the valve pocket. The opposing surface of the cusp, which faced the central lumen was similar to the typical lining of lymphatics in showing little or no basal lamina. There also appeared to be a specific orientation to the connective tissue core of the cusps in that the collagen fibers predominated at the base of the cusps facing the valvular pockets, whereas the finer filamentous material was most evident towards the free edge of the cusp and under the endothelium facing the central lumen. The presence of processes and the specific orientation of the basal lamina and underlying connective tissue may play a role in ensuring effective closure of the valve while serving to reinforce that surface of the cusp which lines the valvular pocket and faces the greatest intraluminal pressure.

The valves within lymphatic vessels although delicate in appearance have a remarkable ability to withstand retrograde flow even in the face of the high pressures that are often used in experiments. Comparatively little information on the detailed structure of these valves is presently available, especially as it pertains to the apparent disparity between their strength in resisting differential fluid pressures (1) and their appearance. The majority of studies on the morphology of lymphatic valves have been concerned with their form or their arrangement. These include studies on human lymphatic valves (2), cat mesenteric

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lymphatic vessels (1), canine peripheral collecting vessels (3), rabbit subperitoneal vessels, mice dermal lymphatic vessels (4), rabbit pulmonary lymphatic vessels (5), canine renal lymphatics (6), and rat uterine lymphatics (7).

The purpose of the present study was to examine the ultrastructure of rat lymphatic valves with special emphasis on those features that might reflect the structural basis for their comparative strength. Valves from rat pancreatic vessels were chosen because we are conducting a comprehensive study of the morphology of the pancreatic lymphatic system and thus the material was available, and because there is no evidence from previous studies that the structure of lymphatic valves differ significantly in the different organs of the body.

MATERIALS AND METHODS

Twelve Sprague-Dawley rats were anesthetized with intraperitoneal sodium pentobarbital (50mg/kg body weight). The abdominal aorta was exposed and cannulated through a midline incision and perfused with 20 to 40ml of a physiological saline solution followed by 200-300ml of 4% glutaraldehyde in 0.157M cacodylate buffer (pH 7.4) for 40 to 60 minutes. The pancreas was excised and divided into head, body and tail, which were then cut into smaller slices and immersed in the same fixative. All specimens were postfixed in 1% osmium tetroxide for one hour, rinsed, dehydrated, and embedded in an epoxy mixture of Polybed 812 and Araldite 502, in inverted Beem capsules.

Several thick sections 3-4 microns in thickness were cut and adjacent sections were placed on alternate slides. One set of alternating slides was stained with toluidine blue and coverslipped for light microscopy. In these slides, lymphatic vessels were tentatively identified from their general appearance and especially from the presence of valves in the vessel lumen. The adjacent sections, on the other set of slides, were re-embedded for electron microscopic confirmation and study of these lymphatic vessels. Re-embedding was achieved by applying a small amount of the Polybed/ Araldite medium onto the section and around the rim of an inverted Beem capsule (with tip removed), which was then placed over the section. Several hours later, the glued Beem capsule was filled with epoxy medium and polymerization allowed at 60°C for 24-48 hours. These re-embedded blocks were trimmed for electron microscopic examination of the predetermined lymphatics using the adjacent sections as a guide. Thin sections were cut, stained with uranyl acetate and lead citrate and examined with a Hitachi H600 transmission electron microscope.

Lymphatic vessels, predetermined as such by light microscopy, were confirmed ultrastructurally using now well accepted criteria, of which the most characteristic is the absence of a continuous basal lamina. Twenty five pancreatic lymphatic valves were identified ultrastructurally and examined extensively, both qualitatively and quantitatively. For the quantitative analysis, areas of each valve were photographed at a set magnification. The negatives of the electron micrographs were then transmitted from a light box to a monitor screen through a video camera and used with a Bioquant Image Analysis System and a Hipad digitizer to obtain morphometric values.

RESULTS

Lymphatics within the pancreas were found to be of widely different caliber depending on their position. They were classified as intralobular, which were smaller vessels lying in fine connective tissue septa in the lobules, and larger interlobular vessels present in the broad trabeculae between the lobules. All vessels that were seen to contain valves at the light microscopic level, and therefore provisionally identified as lymphatic vessels, were subsequently confirmed as lymphatics by electron microscopy. Lymphatic valves in the rat pancreas were seen in both interlobular and intralobular lymphatics.

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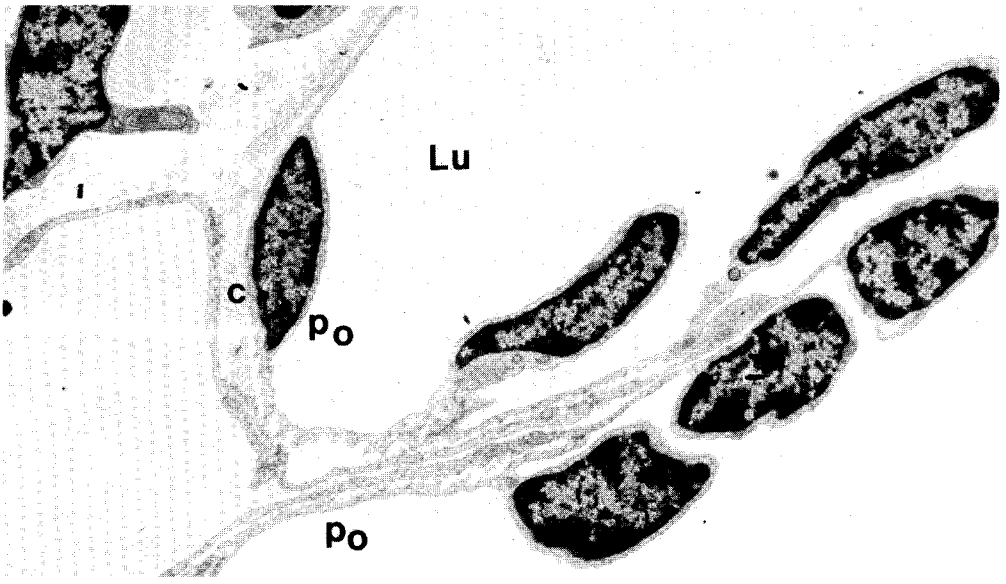

Fig. 1. Electron micrograph showing a closed bicuspid valve. The two walls of each cusp and the connective tissue core (c) in between the walls are continuous with the lymphatic vessel wall and underlying connective tissue. Both cusps have protruding endothelial processes with nuclei located either at the very tip of the cusps or aligned on only that side of the cusp which faces the pocket or sinus (Po). Note the interdigitation between the endothelial processes of the two cusps. Lu=lumen x3,750

The appearance of all valves as seen by both light and electron microscopy was consistent with a cuspid arrangement *(Fig. 1)* although in many instances the exact number of cusps could not be determined because of the plane of section. Each cusp appeared as an extension into the lumen of part of the lymphatic vessel lining and its associated connective tissue. It thus consisted of two layers of squamous endothelial cells separated by a connective tissue core *(Fig. 1)*. These thin cusps or leaflets were attached directly to the vessel wall and to each other at their sides. Over the freer part of their sides opposing cusps were usually fused and were conjointly attached at their sides to the vessel wall by a thickened region, sometimes referred to as a buttress *(Fig. 2)*. In each case, a valvular pocket or sinus was thus formed between the cusp and the wall *(Fig. 1)*. The free margin protruded into the lumen and together with the smooth luminal surface of the leaflet contacted the opposing cusp during closure of the valve *(Fig. 1)*.

The thickness of each cusp, including the two endothelial surfaces and the intervening connective tissue core, ranged from 1,318nm at the base of the valve to 933nm at the free edge (*Table 1*). Although the thickness of each region (base, middle and tip) varied from valve to valve and at different points of the same region, the decrease in size from base to free margin was consistent. The surface lining of each cusp was composed of a single layer of many endothelial cells. The mean thickness of this endothelial surface was 253nm, well within the range found for endothelial cells lining the pancreatic lymphatic vessels (8).

The organelles in the endothelial cells were typical of those in lymphatic endothelium generally and included mitochondria, rough endoplasmic reticulum, cytoplasmic vesicles and nuclei which often bulged into the lumen. The mitochondria seemed to be more numerous in the endothelium of the cusps than in that lining the vessel wall. Cytoplasmic vesicles were slightly more abundant in valves (*Table 2*)

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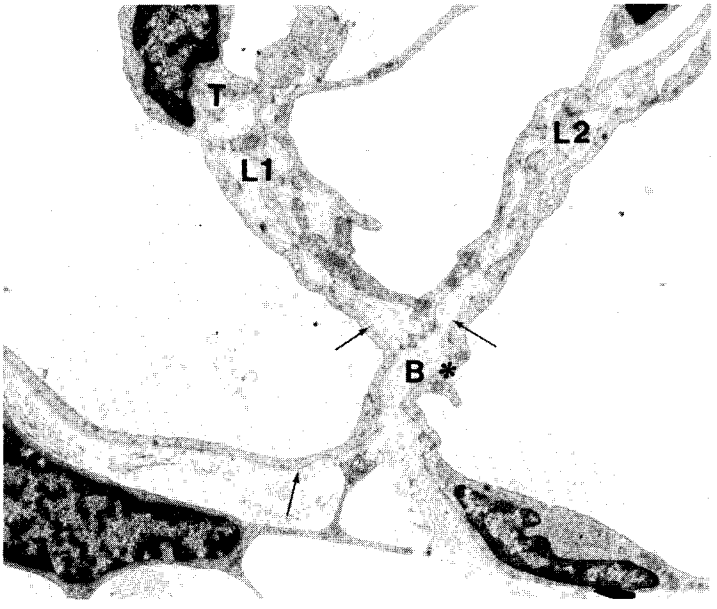

Fig. 2. Electron micrograph of a transverse oblique section of a valve. The edges of both cusps of the valve (L1 and L2) are shown to be anchored to the lymphatic vessel wall by a buttress (B). A distinct basal lamina (arrows) is shown on both sides of the vessel wall and on both walls forming the buttress. From that point on, the basal lamina is found only on the outer walls of the two cusps. At the tip (T) region of the cusps, the basal lamina is present on both walls. Mitochondria (*) are abundant in valves. x8,000.

than elsewhere in the vessel wall (8), but the distribution was similar. Thus, at both sites about half the vesicles were more centrally intracytoplasmic while the rest were almost equally distributed between the abluminal and the luminal areas.

Protruding cytoplasmic extensions, mainly aggregated at the free edge or tip of the cusps were commonly seen (*Figs. 1 and 3A*). These extensions commonly appeared in sections as finger-like processes and were often seen to contain endothelial nuclei surrounded by very little cytoplasm. Mitochondria were also abundant in these endothelial processes. The number of processes per cusp ranged from one to three and they frequently appeared to be connected to the remainder of the cusp by only a thin portion of cytoplasm. On the one closed valve that was seen, the endothelial extensions present at the free margin of the cusp were shown to interdigitate *(Fig. 1)*. Processes that did not appear to be attached to the free edge of the cusp were found only in relationship to the surface that faced the valvular pocket *(Fig. 1)*. Similarly endothelial nuclei, when not found in an extension, were located preferentially on that side of the cusp which faced the pocket *(Fig. 1)*. Thus, the luminal surfaces which oppose one another on closure of the valve consisted of a smooth endothelial surface *(Fig. 1)*.

Intercellular junctions with junctional complexes were more abundant in the valves than elsewhere in the vessel, thus affirming that such structures consisted of many endothelial cells. These junctions were more evident towards the free edge of the valve *(Fig. 4)*. All three types of intercellular junctions common to lymphatic vessels, that is, end to end, overlapping and inter-digitating, were present in valves although the first type appeared to predominate. A fourth type of intercellular junction which consisted of a cell body to cell body contact was also found in the valves *(Fig. 1)*. This junction was mainly present at the tip of the cusp and thus joined the two parallel

TABLE 1
Valve Leaflet Thickness (nm)
Mean (± SEM)

Base	Middle	Tip
1317.5 ± 64.6	1108.5 ± 56.9	932.5 ± 58.0

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Fig. 3. **A.** *Electron micrograph showing a continuous basal lamina (arrows) beginning at the base of the cusp (*) and extending to the tip, mainly on that side of the cusp which faces the sinus (Po). At the tip of the cusp, the basal lamina associates with both walls. x3,750.* **B.** *High power view of the base of the valve seen in* **A.** *Note a distinct basal lamina (arrows) on that side of the vessel and cusp wall (arrows) which face the sinus (Po). Collagen fibers (co) are abundant at the base of the cusp and then aggregate towards the same side of the cusp which contains the basal lamina. x19,200.*

endothelial walls to seal the free edge of the cusp. Dilatations or channels at the intercellular junctions, as noted in our study on the walls of the lymphatic vessels of the pancreas (8) were rarely found in the valves. Open intercellular junctions were never found between the valvular endothelial cells.

The connective tissue core separating the two walls of the cusp contained collagen fibers, filamentous material (elastic-like and anchoring filaments) and basal lamina *(Fig. 5)*. The quantity of filaments and fibers present varied widely from leaflet to leaflet and even within the different regions of the

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TABLE 2
Mean Numerical (N_v) and Volume (V_v) Density of Vesicles in the Nonnuclear Cytoplasm of Valves

Region	Den	OAb	TAb	IC	TLu	OLu	Combined
Inter	N_v	19.6	14.1	68.0	14.9	8.0	*124.6*
	V_v	0.109	0.015	0.061	0.019	0.009	*0.123*
Intra	N_v	12.1	4.2	113.8	3.9	3.8	*137.6*
	V_v	0.010	0.003	0.084	0.0062	0.002	*0.107*

Den=density; Inter=interlobular; Intra=intralobular; O=open; T=touching; Ab-abluminal; IC=intracytoplasmic; Lu=luminal.
N_v=No. vesicles/μm^3
V_v=$\mu m^3/\mu m^3$

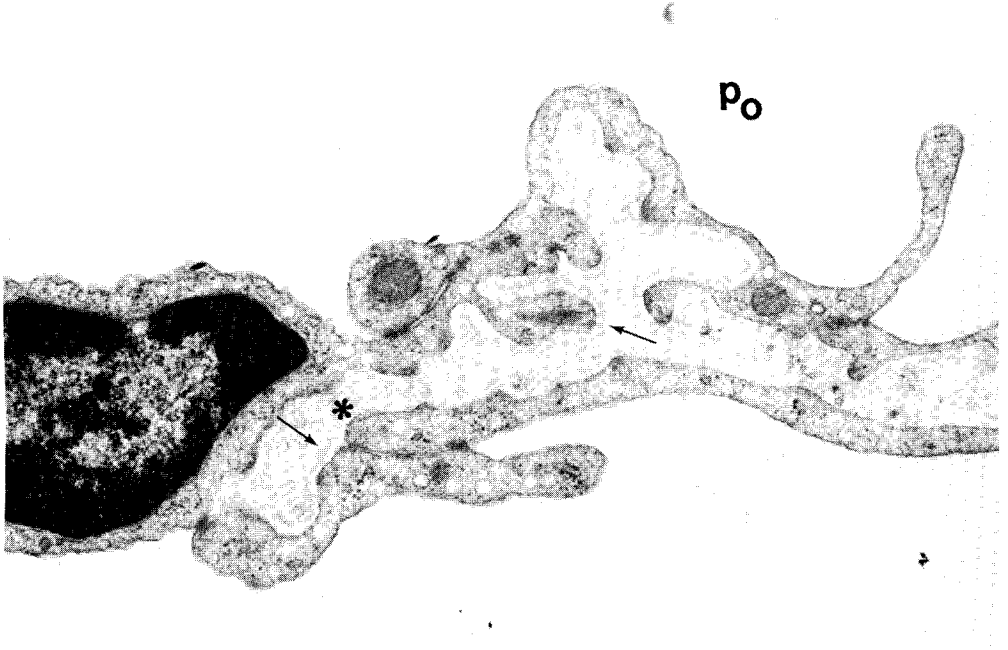

Fig. 4. Electron micrograph of the tip of a cusp showing the basal lamina (arrows) reflected on both walls of the cusp. The continuous basal lamina then ends (*) on that wall not facing the sinus (Po). x19,200.

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same leaflet. Furthermore, they appeared to be specifically oriented within the core. The collagen fibers were more abundant at the base of the cusp *(Fig. 3A)* and beneath the wall of the cusp that faced the pocket *(Fig. 5)*. They decreased in amount as the free edge of the valve was approached to disappear completely at the tip *(Fig. 4)*. On the other hand, the filamentous material was abundant at the tip of the cusp and was often found concentrated along the inner wall of the cusp.

Basal lamina was definitely more abundant in the valves than elsewhere in the lymphatic vessel. A striking finding was the presence of a distinct and almost continuous basal lamina associated with only the wall which faced the pocket of the valve *(Figs. 3A, 3B, and 5)*. The basal lamina began at the site where the cusp wall joined the lymphatic vessel wall and continued towards the tip of the cusp *(Fig. 3A)*. These findings were particularly obvious in those sections through the buttress of the valve *(Fig. 2)*. At the tip of the cusp, the basal lamina was reflected for a short distance onto the inner wall of the cusp *(Fig. 4)* and then gradually disappeared. The presence of the unilaterally distributed continuous basal lamina in the valves was used to distinguish between two apposing branches of a lymphatic vessel and a tangential section through a cusp which appeared to be attached to both sides of a vessel.

The structure of the valves in the interlobular and intralobular lymphatic vessels of the rat pancreas was essentially the same. The frequency of valves in the different regions of the pancreas (head, body and tail) was also similar.

DISCUSSION

The procedure for the identification of lymphatic vessels used in this study was similar to that previously applied by us in other organs such as the kidney, the liver and the thyroid and proved to be as effective for this purpose in the pancreas as elsewhere (9-11). These pancreatic lymph vessels were unusually delicate in appearance having no smooth muscle in their walls and a

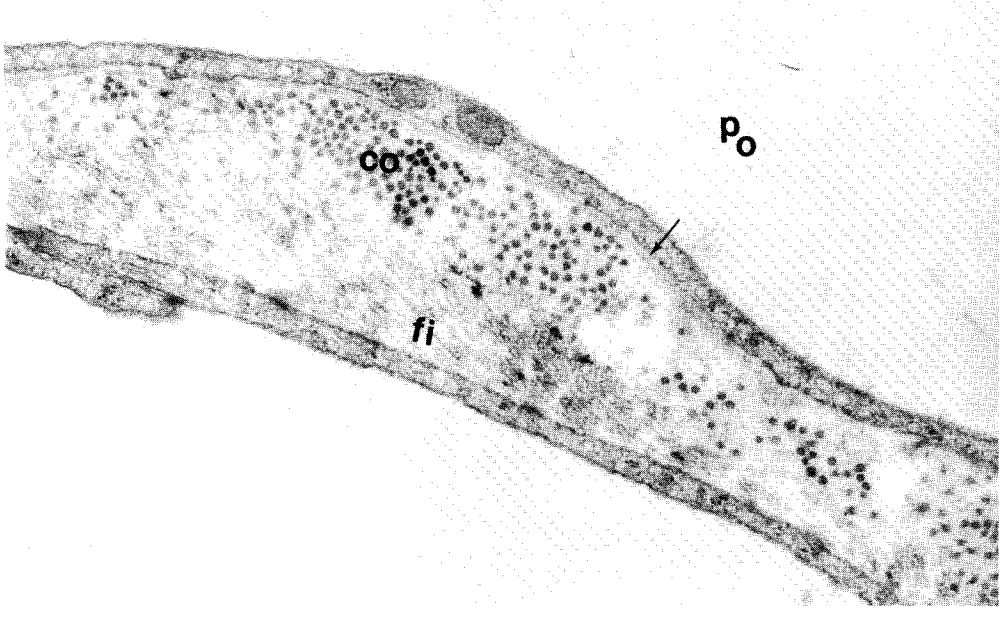

Fig. 5. Electron micrograph of the middle section of a cusp. A distinct basal lamina (arrow) is present on only that side of the cusp which faces the sinus (Po). Collagen fibers (co) are also shown to aggregate on that same side of the cusp while the other side mainly contains filamentous material (fi). x19,200.

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minimum of connective tissue, even when they could be classified as collecting vessels. Thus, they appear to be in contrast with lymphatic collectors in most other regions of the body which contain some elements of smooth muscle mixed with a variable quantity of supporting connective tissue (12). In conformity with this regional difference, the larger lymphatic vessels of the pancreas had very little if any basal lamina between the valves (8) whereas lymphatics of comparable size elsewhere have frequently developed a continuous or almost continuous basal lamina. It may be surmised, from the similarity in structure between the smallest pancreatic lymphatics and the larger interlobular vessels both consisting of little more than a single layer of endothelial cells that their role in lymph formation was similar.

The general structure of the lymphatic valves in the pancreas, as seen in the present study, conform with that described for other organs (1,3-5,7). Although there has been disagreement over the arrangement of the valves (i.e., whether they are cone shaped or have multiple cusps), the pattern seen here is consistent with the now generally accepted cuspid nature of these structures.

Little information for comparative purposes is available on the thickness of lymphatic valvular cusps. Lauweryns and Boussauw (5) reported a range of 500nm to more than 6,000nm but did not specify the region of the valve. The distinction is important since the thickness of the cusp varies significantly from base to tip (see *Table 1*). As expected, the cusps were thickest at their bases. The difference in thickness from base to tip could be attributed to variation in the extent of the connective tissue core because the covering epithelium was not found to vary either within itself (mean thickness of 253nm) or from the endothelium that lined the vessel wall between the valves. The region which attaches the sides of the cusps to the vessel wall has been referred to as an anchoring buttress and has been credited with helping to prevent inversion of the valve (6,13).

Two features having a probable significance in valvular function became evident in the course of the present study. One was the arrangement of the basal lamina in the cusps and the other was the presence of atypical endothelial cells projecting form the tip and the outer surface of the cusps.

The presence of a basal lamina in lymphatic cusps has been reported previously (1,4,5). Indeed, it has been suggested that the basal lamina is more evident in valvular cusps than in the walls of at least the smaller lymphatic vessels. What became evident, however, as we studied the arrangement of this intracuspid basal lamina, is that it existed only beneath the epithelium that faced the valvular pocket and not subjacent to that which faced the luminal surface. Since this uneven disposition was consistent and since it is only the pocket surface that is subject to strain, it seems probable that this particular arrangement contributes to the ability of the valve to withstand retrograde forces. Obviously the basal lamina alone cannot account for valvular competency but when combined with the other elements that form the connective tissue core, the whole can be remarkably effective in withstanding pressure. A similar situation may exist at the tip of the valve where the basal lamina is always present before becoming discontinuous or absent on reflection from the outer surface of the cusp. The tip of the cusp must also be vulnerable during opening and closure of the valve.

The projections of endothelial cells from the outer surfaces of the cusps, seen so prominently in the present study, have been referred to previously by Takada (4), as "tip cells." In that report the cells were considered to be different from those that lined the main wall of the cusp. In the pancreas we found them to be extensions of the endothelial wall of the cusp often containing the nuclei and attached to the surface of the cusp by cytoplasmic extensions. The cytoplasm did not differ in its ultrastructural components from that of the lining epithelial cells elsewhere in the valve. Frequently these cytoplasmic processes were concentrated at the free edge

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or tip of the valve but they were not limited to that position. When not at the tip, they were found only along the outer walls of the cusp facing the sinus an expected finding since projections on the inner surface of the cusp would interfere with the closure of the valve and prevent close apposition of the opposing surfaces. The purpose of these extensions is not clear. Those at the free edge of the cusps may act to reinforce a closed valve by interdigitating with one another as seen occasionally in the present study. Their presence on the outer surface of the cusp is more difficult to explain.

Whether the findings in this study, particularly those pertaining to the organization of the structures within and on the cusps, pertain solely to the lymphatic valves of the rat pancreas seems unlikely. Preliminary examination of valves in the lymphatic vessels of the thyroid show similar patterns to those described here for the pancreas.

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Charles C.C. O'Morchoe, M.D.
Director, University of Illinois
College of Medicine at Urbana-Champaign
190 Medical Sciences Building
506 South Mathews
Urbana, Illinois 61801

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