Lymphology 28 (1995) 4-20

LYMPHATIC SYSTEM OF THE RAT PANCREAS

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

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

ABSTRACT

Light and electron microscopy combined with morphometric analysis were used to investigate the distribution, extent and structure of lymphatic vessels in the head, body and tail of the rat pancreas. Serial sections 3-4μm in thickness were cut from tissue fixed by perfusion. Alternate sections were processed for light microscopy. Intervening sections were left uncovered to be re-embedded and sectioned for electron microscopy as needed. Vessels with valves were tentatively identified as lymphatics using the light microscope, with final identification being made on adjacent sections by electron microscopy. The ultrastructure of the pancreatic lymphatic vessels was typical of lymphatics generally. Interlobular lymphatic vessels were present throughout the pancreas and were found to be associated primarily with blood vessels lying in connective tissue septa. Intralobular lymphatics were also seen but were comparatively rare. Only about 19% of the wall of the lymphatic system of the pancreas was in close relationship to acinar cells—none was closely related to the endocrine islets. The mean volume density of the system was 0.0012μm^3/μm^3 and the profile density of lymphatics was 3.24/mm^2. Special attention was paid to the areas of contact between adjacent endothelial cells. Open gaps of more than 30 nm in width were rare. Dilatations and associated cytoplasmic processes, suggestive of a type of intercellular transport, were seen in addition to the intracellular cytoplasmic vesicular system. The findings are consistent with the view that the lymphatic system of the pancreas does not have a specific role in the transport of pancreatic secretions other than the removal of macromolecules that may escape to the interstitium in small amounts under normal circumstances. The fine structure of the endothelial wall suggests that the mechanism of lymph formation in the pancreas is more comparable to that in other encapsulated organs such as the kidney and liver than to that in the dermis or diaphragm where fluid appears to enter lymphatics primarily by way of gaps between adjacent cells.

Unanswered questions about the lymphatic system of the pancreas include the extent to which pancreatic enzymes and hormones enter the lymph and what role, if any, the system may play in the etiology of pancreatitis. Information on the distribution and fine structure of the lymphatics can help to answer these questions by depicting the interface between the parenchyma and the sites of lymph formation. Detailed information on structure is, however, largely lacking. Lymphatics are notoriously hard to identify with any degree of certainty by light microscopy and sections prepared for electron microscopy are too small to give adequate information on the distribution of vessels within the organ.

Information on the morphology of the pancreatic lymphatic system has been almost entirely derived by light microscopy often with the aid of injection techniques. Pioneer studies are reviewed by Rusznyak, et al (1). More recent reports include those by Godart (2) and Grau and Taher (3). There are several areas of disagreement among these studies including whether lymphatics exist within the lobules (intralobular) or are confined to the regions between the lobules (interlobular). The only published ultrastructural study on the subject is by Kaneko (4), who injected silver nitrate interstitially and concluded, using electron microscopy, that lymphatic vessels were limited to the interlobular connective tissue. They were accompanied by both arteries and veins but were seen more frequently with the former. Kaneko (4) interpreted the apparent absence of lymphatics within the lobules as meaning that they play little role in reabsorption of secretions from the endocrine or exocrine tissue of the pancreas.

The purpose of the study reported here was to define the extent, distribution and fine structure of the intrapancreatic lymphatic vessels. To achieve this, a combination of light and electron microscopy was used. Morphometric data were obtained to serve as a baseline for future studies and to provide comparison with other organs examined previously in this laboratory. Finally, the ultrastructure of the lymphatic endothelium and its intercellular junctions was studied for comparison with other regional lymphatics and to provide structural information on the mechanism of lymph formation in the pancreas.

MATERIALS AND METHODS

Twelve Sprague-Dawley rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg body weight) and then perfused retrogradely through the abdominal aorta. The perfusate consisted of 20 to 40 ml of physiological saline followed by 200-300 ml of 4% glutaraldehyde in 0.157M HCL-Na cacodylate buffer (pH 7.4) for 40 to 60 minutes.

Tissue samples, 2 to 3 mm^3, were cut from the head, body and tail of each pancreas and immersed in the same fixative as the perfusate before being postfixed in 1% osmium tetroxide for one hour. Dehydration was carried out in a graded series of ethanol or acetone dilutions and the tissue was embedded in a mixture of Poly/Bed 812 and Araldite 502. Serial sections (3-4 µm) were cut and mounted in such a way that alternate sections formed a series on two sets of slides. One set of slides was stained and used for light microscopic detection of possible lymphatics that were then recorded by means of sketches and photomicrographs. The other set was left uncovered and later used for ultrastructural confirmation of any suspected lymphatics by preparing, for transmission electron microscopy, the section adjacent to the one containing the presumptive lymphatic. This re-embedding was achieved by placing a Beem capsule over the section on the slide and then filling it with epoxy medium. After polymerization at 60°C for 24-48 hours, the capsule containing the re-embedded section was lifted off and thin sections of the area containing the vessel were cut and stained with uranyl acetate and lead citrate. Whenever possible, both endocrine and exocrine areas of the pancreas were included in the trimmed field. Correlation between the light and electron microscopic images was facilitated by projecting the light microscopic sketch or photograph while viewing the same field through the electron microscope. The thin section was also scanned for additional lymphatics not detected by light microscopy: indeed, most of the smaller lymphatics were found at the ultrastructural level.

Once a lymphatic vessel had been confirmed as such it was followed in serial sections at the light microscopic level to determine its distribution within the gland and its relationship to individual components of the pancreas. Morphometric analysis of each vessel was performed with the Bioquant Image Analysis System on both light and ultrastructural images. Light microscopic sections were analyzed using a Hipad digitizer and a

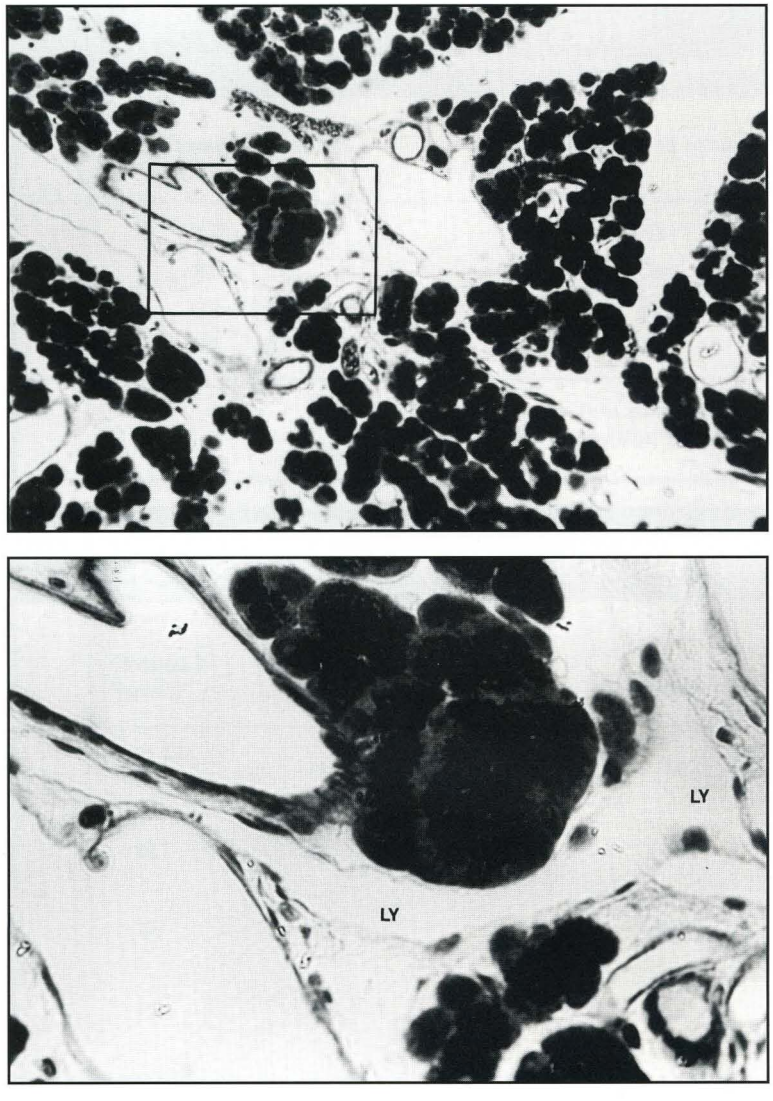


Fig. 1. Light micrograph of rat pancreas showing an interlobular lymphatic vessel (LY). The area outlined is shown in higher magnification in the inset (below). The lymphatic is related to blood vessels and acinar cells — and extends into the connective tissue of a lobule. x200

monitor screen attached to a video camera mounted on the microscope. For ultrastructural analysis randomly selected areas of each lymphatic vessel were photographed at a magnification of 12,000. The negative was then viewed over a light box and analyzed with the same system as that used for light microscopy.

These analyses provided quantitative data on a variety of features of the lymphatic system of the pancreas. Values for the volume density and profile density of lymph vessels in the pancreas as a whole were obtained. Estimates of their primary relationships were made by measuring the areas of close apposition between the lymphatic vessels on the one hand and the blood vessels, connective tissue and acinar tissue on the other. Ultrastructural morphometric analysis of the vessels provided data on their diameters, on the size, volume density and numerical density of the intracytoplasmic vesicles, and on the number and types of contacts between adjacent endothelial cells. Diameters were

TABLE 1
Mean Maximum Diameter of the Interlobular Lymphatic Vessels

Region	Number of Lymphatics	Maximum Diameter[a] (μm ± SEM)
H-B-T	101	32.5 ± 2.1
Head	37	31.2 ± 2.8
Body	25	31.2 ± 5.7
Tail	39	34.5 ± 3.0

H-B-T = combined data for the head, body and tail regions
[a] No significant difference between the head, body and tail regions

TABLE 2
Primary Relationships of the Interlobular Lymphatic Vessels in Percentages

Region (number of lymph vessels)	Artery	Vein[d]	Duct[d]	Acinus[d]	Connective Tissue[d]
H-B-T (101)	26[e]	28[e]	2	19	25
Head (37)	24[a]	34	2	16	24
Body (25)	20[b]	26	1	18	35
Tail (39)	30[c]	26	2	22	20

H-B-T = combined data for the head, body and tail regions
[c] > [b,a] ($p < 0.05$); letters separated by a comma do not differ significantly
[d] No significant difference between the head, body and tail regions
[e] No significant difference between association with artery and vein

measured using the greatest internal diameter of the vessel seen in the sections.

Most of the quantitative data were collected independently from the different regions of the pancreas and statistically compared by a one way analysis of variance (ANOVA). If this test showed a statistically significant difference among the groups, a Duncan Multiple Range Test and a Tukey's Studentized Range Test were performed to determine which groups differed. Differences between the interlobular and intralobular lymphatic vessels were analyzed in a similar manner. The data were also compared with

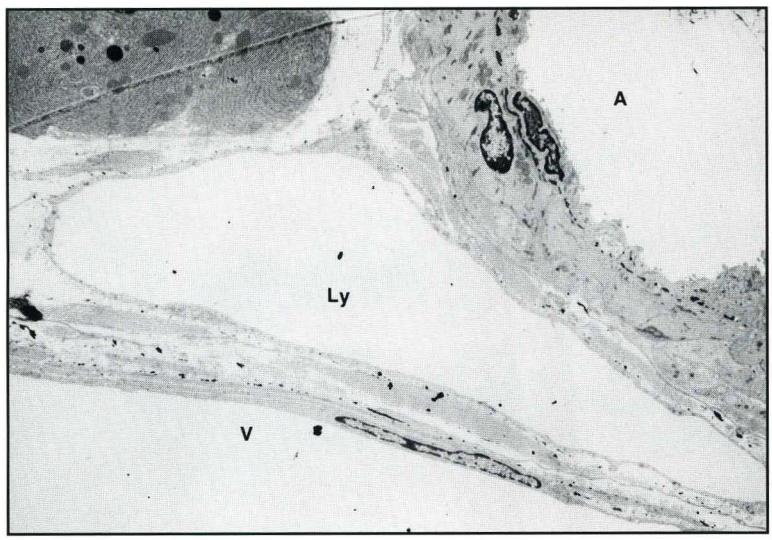


Fig. 2. Transmission electron micrograph showing an interlobular lymphatic vessel (Ly) in the connective tissue that separates an artery (A) and a vein (V). An acinar cell lies nearby. x3,000

those reported earlier for the thyroid, kidney and liver (5-10).

RESULTS

General

A total of 148 lymphatic vessels was seen in this study. Many were tentatively identified as lymphatics by light microscopy because of the presence of valves or because of their general appearance and disposition. All were confirmed as lymphatics by their ultrastructural features, the most important of which were the absence of endothelial fenestrations and the lack of a continuous basal lamina. The blood capillaries of the pancreas, in contrast, exhibited a fenestrated endothelium and a continuous basal lamina.

The distribution of lymphatics appeared to be fairly even throughout the organ. Most were found in the connective tissue septa which separate the gland into lobules and were therefore designated as interlobular *(Fig. 1)*. A few, which we refer to as intralobular, were found in the slender septa that penetrate the substance of lobules providing thin connective tissue sheaths for the acini, intralobular ducts and accompanying vessels. Structurally there appeared to be no difference between interlobular and intralobular lymphatics: a few of the intralobular lymphatics even appeared to contain valves.

Morphometric analysis at the light microscopic level was performed on 101 lymphatics. Of these 37 were from the head, 25 from the body and 39 from the tail. Much of the data for lymphatic vessels from each of these regions are shown separately in the Tables although for the most part there were no significant differences among them. The consistency of the data in the three regions provides support for the overall reliability of the results.

The mean volume density of the lymphatic system in the pancreas was 0.0012 $\mu m^3/\mu m^3$, and the profile density was 3.24/mm^2. These figures apply to the pancreas overall since it was not feasible to estimate values for each of the regions of the organ separately. The size of the vessels appeared to be fairly consistent throughout—the mean maximum diameters for lymphatics in the head, body and tail are shown in *Table 1*—the overall average being about 32 µm.

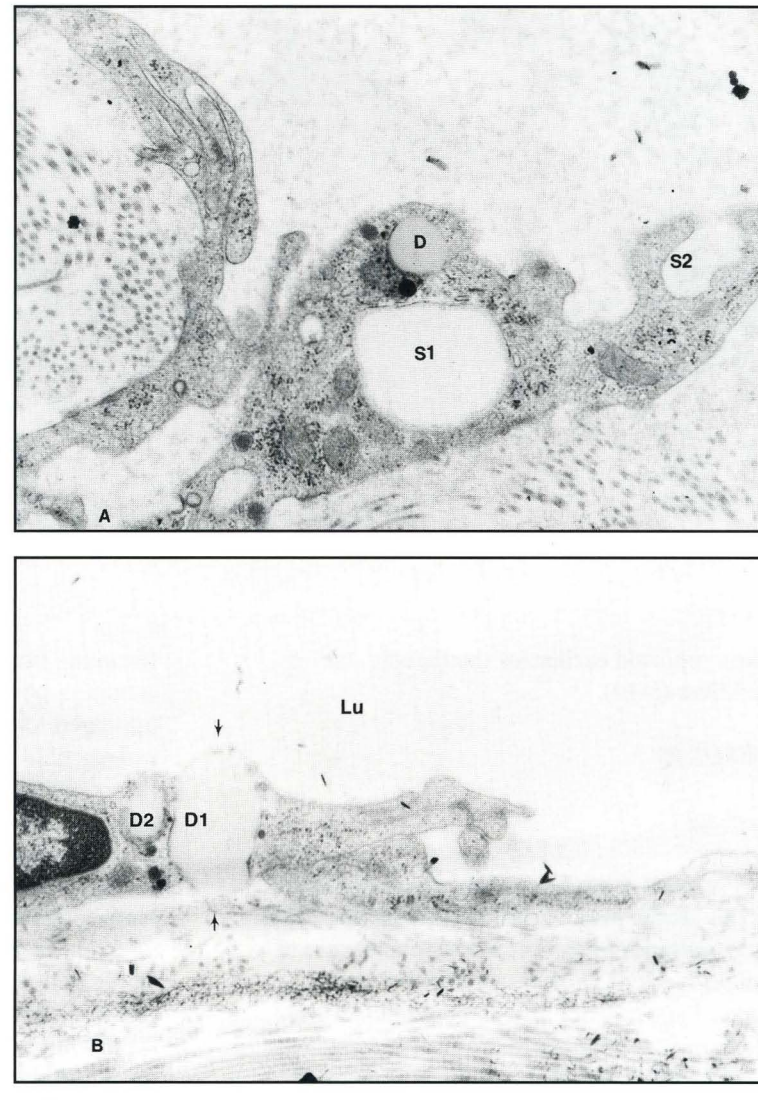


Fig. 3. Electron micrographs. A) Lymphatic endothelium showing a large cytoplasmic sac (s1) next to a zymogenic or lipid droplet (D). A nearby sac (s2) partially enclosing luminal material is shown opening onto the lumen (Lu). x27,600. B) Lymphatic endothelium showing two zymogen or lipid droplets (D1 and D2) next to each other near an intercellular junction. D1 is connected with the luminal and abluminal surfaces (arrows) while D2 is near the luminal surface. Droplets were never found between endothelial cells. Lu=lumen x32,400

Relationships

As expected, most lymphatic vessels within the pancreas were seen to run in close proximity to blood vessels in connective tissue *(Fig. 1)*. Measurements of the primary relationships of the interlobular lymphatics are shown in *Table 2*. Only about 19% of the lymphatic endothelial wall could be considered to be in close contact with the acinar cells *(Fig. 2)*. Intralobular lymphatics appeared, as might be expected, to have a closer relationship with the acini, but the frequency of these vessels was insufficient to provide reliable quantitative data on this point. Lymphatic vessels were not found in relationship to the endocrine tissue in the Islets of Langerhans—37 islets were examined—although blood capillaries were seen in those areas.

Ultrastructure

Ultrastructural morphometric analysis

TABLE 3
Frequency of Lymphatic Endothelial Vesicle Position in Percentages

Type of Vessel	(Number of Vesicles)	OAb	TAb	IC	TLu	OLu
Interlobular	Wall (1,393)	14.1	8.3	49.4	12.6	15.6
H-B-T	Valve (476)	15.1	11.6	54.2	12.6	6.5
	Combined	14.34	9.15	50.62	12.63	13.26
Head	Wall	13.7	8.2	48.9	14.1	15.1
	Valve	20.7	9.8	51.2	13.4	4.9
Body	Wall	13.2	7.8	47.8	13.6	17.6
	Valve	13.2	12.4	49.6	14.9	9.9
Tail	Wall	15.6	9.0	51.9	9.6	13.9
	Valve	11.5	12.6	59.7	10.47	5.8
Intralobular	Wall (67)	13.4	20.9	41.8	16.4	7.5
	Valve (33)	9.1	3.0	81.8	3.0	3.0

O-open; T-touching; Ab-abluminal; IC-intracytoplasmic; Lu-luminal

H-B-T = combined data for head, body and tail

was carried out on 123 interlobular (46 from the head, 37 from the body and 40 from the tail) and on 8 intralobular lymphatic vessels. No obvious ultrastructural differences were found among the lymphatics of the different regions of the pancreas nor between interlobular and intralobular vessels. Most lymphatics had little, if any, basal lamina. When present it was usually fragmented and more obvious at intercellular junctions or at valvular attachments.

Organelles in the endothelial cells included nuclei, mitochondria, rough endoplasmic reticulum, free ribosomes and many cytoplasmic vesicles. The nuclei, which caused the cells to protrude into the lumen, were characterized by occasional cytoplasmic invaginations and a rim of heterochromatin: nucleoli were not prominent. The mitochondria were most evident close to the nuclei.

Lysosomes and lipid-like droplets were also found within the endothelium *(Fig. 3A&B)*. The lipid-containing droplets—35 were seen—resembled zymogen granules and

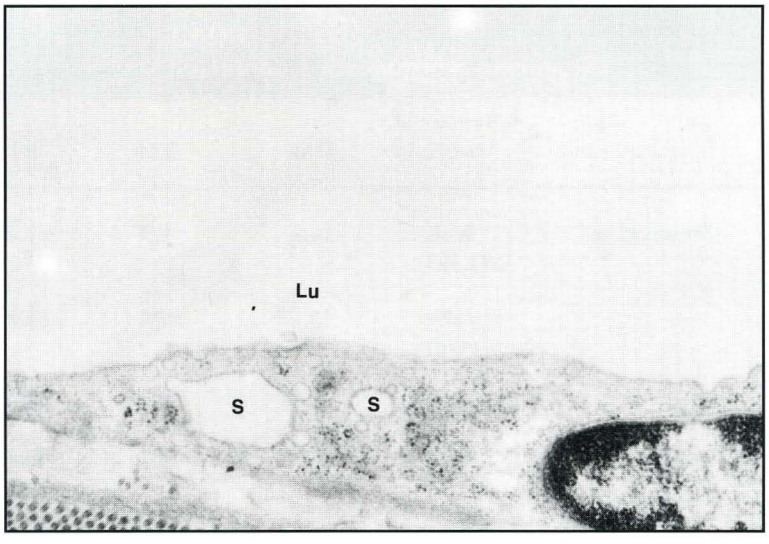


Fig. 4. Electron micrograph showing a large and a small cytoplasmic sac(s). Cytoplasmic vesicles are shown to open onto their surfaces. Lu=lumen x27,600

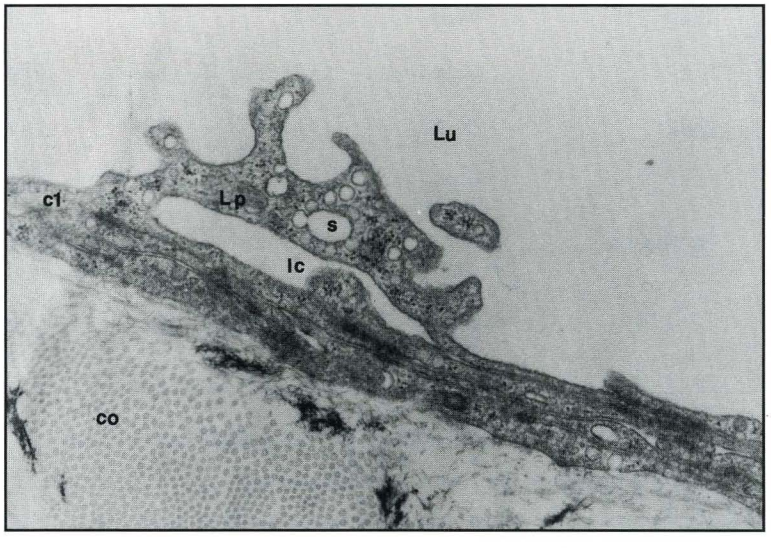


Fig. 5. Electron micrograph. A luminal cytoplasmic process (Lp) from one endothelial cell (C1) loosely interdigitates with the adjacent cell. A large dilated area which appears as an intercellular channel (Ic) is seen. Anchoring filaments and bundles of high electron dense elastic-like filaments border the endothelium. The collagen fibers (co) are usually peripheral to the anchoring and elastic-like fibers. s=cytoplasmic sac x30,000

were, in our experience uncharacteristic of lymphatic endothelium generally, although their appearance was similar to the chylomicra described by Casley-Smith (11). Some were associated with a high density material in the neighboring cytoplasm and some were seen to open onto the endothelial surface where their contents appeared to escape into the surrounding medium. Cytoplasmic vesicles were mostly of the uncoated type—having a simple lining membrane—and averaged 92 to 94 nm in diameter. The mean numerical density of these uncoated vesicles in non-nuclear cytoplasm was 90/um^3 and their volume density was 0.094µm^3/µm^3. Slightly larger coated vesicles were also occasionally seen. The uncoated type was classified into three groups depending on their position in the endothelial cells—abluminal, luminal or apparently free within the cytoplasm. The distribution of these vesicles among the groups is shown in *Table 3*. Vesicles that appeared to open onto the endothelial surface were often

TABLE 4
Intercellular Junctions

Region and Type ICJ	ICJ[1]		Maximum Width[2a,b] (nm)	Open ICJ Number (diam-nm)		Specialized Junctional Complexes[1]	
Interlobular Lymphatics							
H-B-T	396						
EE	38	(10)	NA	1	(230)	55	(9)
OL	204	(51)	64.8±7.8	3	(168)	220	(36)
ID	154	(39)	118.7±12.6	NA		336	(55)
Head	133	(34)					
EE	11	(8)	NA	1	(230)	13	(8)
OL	74	(56)	68.1±17.9	3	(168)	59	(36)
ID	48	(36)	130.3±20.5	NA		91	(56)
Body	148	(37)					
EE	13	(9)	NA	0		18	(7)
OL	67	(45)	73.7±10.1	0		78	(31)
ID	68	(46)	104.2±13.0	NA		153	(61)
Tail	115	(29)					
EE	14	(12)	NA	0		24	(12)
OL	63	(55)	51.6±12.3	0		83	(42)
ID	38	(33)	129.5±37.9	NA		92	(46)
Intralobular Lymphatics	12						
EE	3	(25)	NA	1	(1299.8)	1	(4)
OL	5	(42)	75.8±22.2	0		8	(33)
ID	4	(33)	32.4	NA		15	(63)

Note the average width of the typical nondilated intercellular space was 13.55 nm.
H-B-T = combined data for the head, body and tail regions
ICJ-intercellular junctions; EE-end to end; OL-overlapping; ID-interdigitating; NA-not applicable
[1] Total numbers with percentages in parentheses.
[2] Includes dilatations and intercellular channels
[a] No significant difference between the head, body and tail regions
[b] No significant difference between interlobular (H-B-T) and intralobular lymphatic vessels

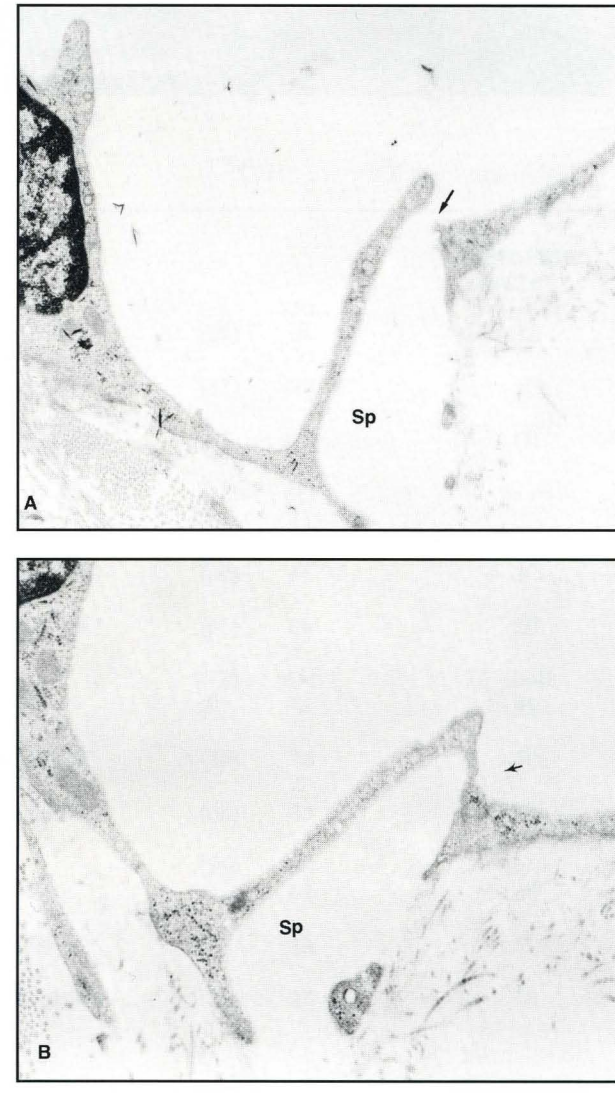


Fig. 6. Electron micrographs. A and B are adjacent serial sections. A shows an open gap (arrow) which is however not present in the adjacent section (B). An abluminal space (Sp) devoid of filaments and fibers is associated with the junction. A) x19,200; B) x24,000

seen to contain a diaphragm at the opening site. Larger vesicles or channels possibly formed by the fusion of several cytoplasmic vesicles were frequently found but were not seen to connect opposing surfaces of the cells. Even larger sacs or channels were sometimes seen in the endothelial cells *(Figs. 3A & 4)*. The latter did not appear to be connected with intercellular spaces but often encroached or touched upon the luminal or abluminal surface. In some sections they opened to the surface and thus resembled invaginations of the endothelial cell.

Intercellular Regions

Four hundred and eight intercellular contacts were examined and classified into the three common types of arrangement found in lymphatic endothelium (end-to-end, overlapping and interdigitating): quantitative data are shown in *Table 4*. Overlapping (51%) and interdigitating (39%) arrangements predominated over the end-to-end type (10%). Typically the distance between adjacent normally opposed cells measured approximately 13.5 nm. The intercellular

TABLE 5
Comparison of Interlobular Lymphatic Vessels in the Kidney, Liver, Thyroid and Pancreas

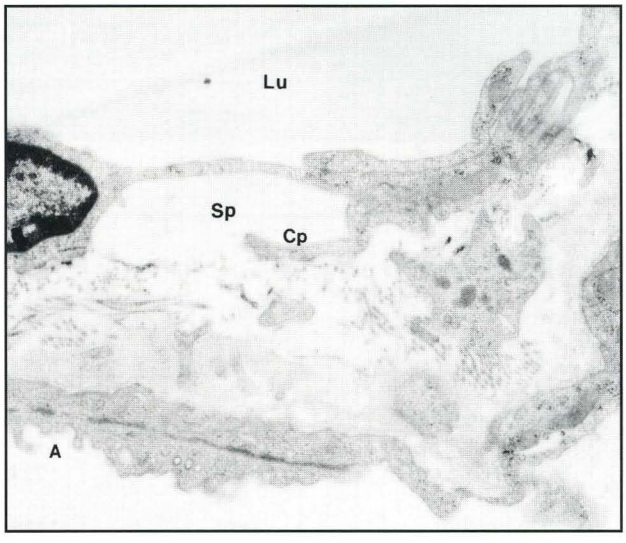
	Kidney[1]	Liver[2]	Thyroid[3]	Pancreas[a]
Lymphatic Vessels				
V_v	0.0011	0.00098	0.007	0.001185
N [a]	5.31	1.76	5.68	3.24
Max Diam (µm)	20.38[a]	20.46[a]	17.87	32.48[a]
Width ICJ (nm)	16-18	22.6	17	14
Open junction	rare	rare	none seen	rare (1%)

V_v = Volume density = $\mu m^3/\mu m^3$
N^a = Profile density = number of lymphatics/mm^2
[1] Niiro et al., 1986
[2] Niiro and O'Morchoe, 1986
[3] O'Morchoe et al., 1987
[a] data from interlobular lymphatic vessels

spaces in overlapping and interdigitating conformations ranged from 4.0 nm at closely opposed areas to as much as 118 nm in areas characterized by what appeared to be large cisterns or dilations between cells. The minimum intercellular width occurred at the periphery of tight junctional complexes and the maximum was seen in the cisterns. As many as 75% of the overlapping or interdigitating forms of intercellular contact revealed these cistern like spaces *(Fig. 5)*.

Specialized junctional complexes including fasciae adherentes and occludentes were seen in all three types of intercellular format *(Table 4)*. The probability of finding junctional complexes appeared to increase directly with the complexity of the area of contact. Intercellular spaces without dilatations often appeared to have more than one specialized junctional complex whereas spaces that included dilatations were often without such complexes. Simple open intercellular gaps that lacked any junctional complex and had a width of no less than 30 nm between the cells were considered to be "open" junctions in keeping with frequently used terminology. Only 1% of the intercellular regions of contact examined in this study fell into this category and in two examples the apparent open junction in one section was not present in the adjacent serial section *(Fig. 6)* suggesting that it may have been artifact or a small circular opening, although junctions described as open may not appear to be open in all sections through it.

Luminal and abluminal cytoplasmic projections were seen adjacent to many overlapping and interdigitating areas of intercellular contact. Some of these projections were not in contact with the adjacent cell, in the plane of section, suggesting that they might be part of the wall of dilatations or



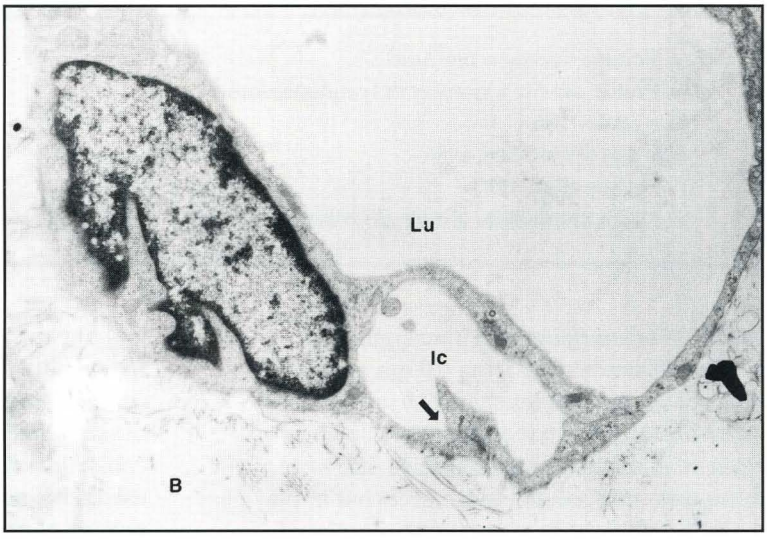
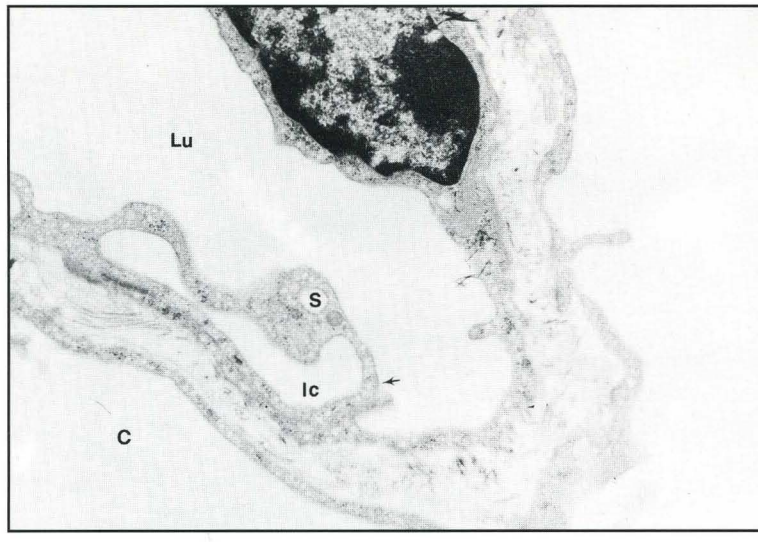
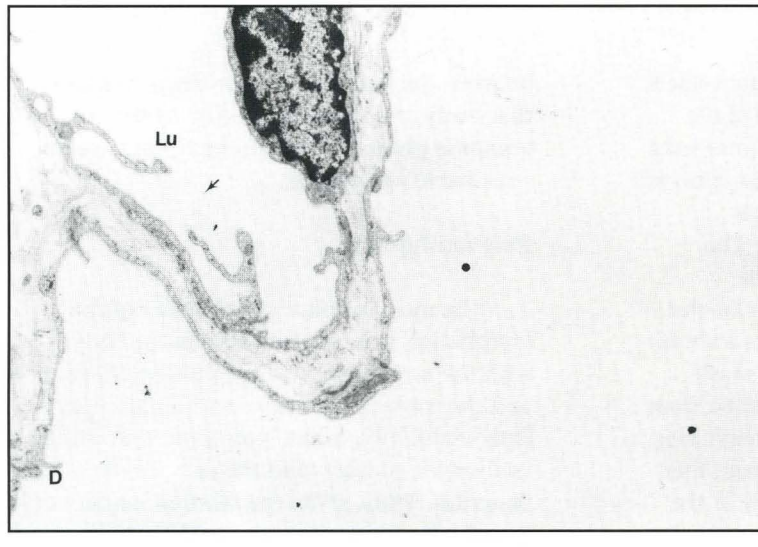


Fig. 7. Electron micrographs. A) An abluminal cytoplasmic process (Cp) partially encloses an abluminal area (Sp). x22,800. B) An intercellular channel (Ic) is shown to have formed when two abluminal processes apparently joined (arrow). x12,250. See next page: C) and D) are adjacent serial sections. C) shows a luminal intercellular channel (Ic) formed as two processes overlapped each other (arrow) x17,400. D) The adjacent serial section shows the channel opening luminally (arrow). x10,200. Lu=lumen, s=cytoplasmic sac.

cisterns that opened onto the luminal or abluminal surface of the endothelium *(Fig. 7)*. No dilatation was seen to open onto both surfaces simultaneously although this might have occurred but not continuously through any one plane of section. It seemed likely that the cytoplasmic projections, which appeared as simple finger like processes in a single plane of section, were really folds of cytoplasm that enclosed the dilatations. The appearance suggests that these folds, by establishing or releasing connections with neighboring cells, could allow the cisterns to fill or empty. Thus the overall impression given by these areas of contact was that the dilatation might open either onto both surfaces of the endothelial wall simultaneously in different planes of section, or, perhaps more likely, at different times—one closing before the other opened. This impression was strengthened by the appearance of the contents of the cisterns which often had the same density of the extracellular fluid as the luminal contents or the lymphatic vessel.

The abluminal surface of the lymphatic endothelial wall was connected to the nearby





connective tissue by an intricate network of fibers and filaments. These included anchoring filaments, collagen fibers and elastic-like filaments *(Fig. 8)*.

DISCUSSION

This study provides information on the extent and distribution of the lymphatic system within the pancreas, on the relationships between the parenchyma and the lymphatics, and on the ultrastructure of the lymphatic vessels. The data provide new information and are valuable in that they serve as a baseline for comparative purposes, provide indirect information on the mechanism of lymph formation, and give clues to the function of the lymphatic system of the pancreas.

Distribution and Density

The general appearance of the lymphatic system of the pancreas seen in this study is comparable to that reported previously (1-3) as well as being analogous to that found in the

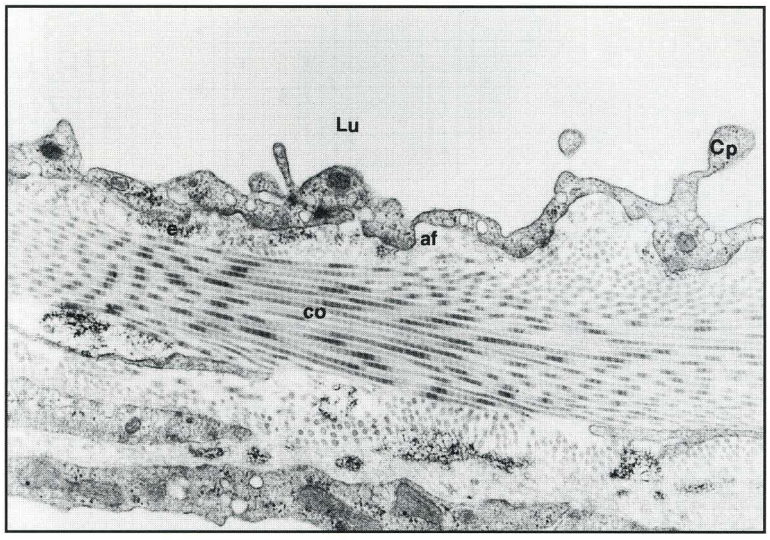


Fig. 8. Electron micrograph of lymphatic endothelium connected to the underlying interstitium by anchoring filaments (af), elastic-like filaments (e) and collagen fibers (Co). Lu=lumen, Cp= Cytoplasmic process x28,800.

kidney by us (8). We found lymphatic vessels both between and inside the lobules of the pancreas although the intralobular ones were comparatively rare. This finding is in contrast to a number of studies (2-4), in which intralobular vessels were not found. The examples that we saw appeared to be occasional simple extensions of interlobular vessels that penetrated the lobule for a short distance. Foldi, et al (12) described small lymphatic vessels which were separated from acinar cells only by the basement membrane that surrounded the acini but we found this intimate relationship rarely and only at the boundaries of some lobules where the acini bordered the connective tissue septa that contained lymphatics.

The extent of the lymphatic system in the pancreas has not been reported on before. Our technique to measure its volume density and numerical density is similar to the one we used previously in other organs (8-10). It suffers from the inherent problem that lymphatic vessels are relatively sparse and irregular in shape, thereby introducing a margin of error into the morphometric technique. Thus the data must be viewed as approximate estimates that are more useful for comparison among organs than as definitive values. A comparison between the data derived for the pancreas in this study and those derived by us for other organs is given in *Table 5*: the figures are in reasonable agreement.

Relationships

The most intimate relationship of the lymphatics, was, as elsewhere in the body, with the accompanying blood vessels *(Table 2)* and the connective tissue in which they lay. Only about 19% of the lymphatic wall was in fairly close contact with the acinar cells of the pancreas. Thus, given the relative sparsity of lymphatic vessels, it seems unlikely that lymph is an important transport system for pancreatic enzymes although the relationship seems adequate to account for the higher than normal concentrations of pancreatic secretions in lymph under conditions of stimulation—as was reported by Dumont, et al (13). This could explain the apparent zymogen granules present in the endothelial wall of the lymphatic vessels. We found no relationship to exist between lymphatics and the Islets of Langerhans. This is contrary to some earlier reports (14,15) but in agreement with Földi, et al (12).

It seems evident from this and other studies that the transport of pancreatic

secretions is not a specific function of lymph. Accordingly, it may be assumed that the major function of lymph in the pancreas, as in other organs of the body, is interstitial fluid balance, especially in the prevention of edema under abnormal conditions such as pancreatic duct obstruction or pancreatitis. It has been suggested (13) that the fibrosis associated with pancreatitis may limit the ability of the lymphatics to drain interstitial fluid.

Structure and Intercellular Arrangements

The lymphatic vessels seen in this study exhibited the typical structure of lymphatics generally (16,17). No consistent differences were found among the three regions of the pancreas or between the interlobular and intralobular vessels.

The three patterns of intercellular contact in the endothelium—end to end, overlapping and interdigitating—are well known (9,18). Not so well recognized or characteristic of lymphatic endothelium generally are the dilatations or cisterns seen in these areas of contact, although they have been seen previously in some specific regions of the body (7,19). A recent study by Azzali, et al (20) on the renal lymphatics of hibernating bats found seasonal differences in the frequency of dilated intercellular channels as well as in the number of cytoplasmic vesicles. The authors suggested that for lymph formation the intercellular transport route prevailed during the summer while vesicular transport predominated in the hibernating period. The function of these intercellular dilatations is still unclear but it has been suggested, in studies involving serial sections and three dimensional models, that they are involved in intercellular transport in the intestine (21,22), kidney (20,23) and heart (18). Certainly their appearance, when combined with the endothelial extensions or processes seen in the present study, is consistent with this interpretation. It is tempting to interpret the sort of pattern seen in *Fig. 7* as reflecting a dynamic process by which cytoplasmic folds can extend outward to enclose an area of fluid which is subsequently released on the opposite side by the opening of another fold-like process. It has been reported (24,25) that fewer processes are present in distended than in collapsed lymphatics, with the suggestion that the cytoplasmic extensions allow for expansion of those vessels most actively engaged in lymph formation. Another possibility is that the cytoplasmic processes merely reflect a transitional stage from an interdigitating to an overlapping area of contact by the release of a junctional complex which then allows the fold to become free at its distal end.

So called open junctions, empirically defined as being larger than 30 nm in width, between adjacent endothelial cells have been a source of controversy. One commonly held theory of lymph formation holds that adjacent cells separate at periodic intervals to allow interstitial fluid to be drawn into the lumen to become lymph. If this happens it seems reasonable to expect that such open junctions will be seen in at least occasional electron micrographs. While they are present in certain regions such as the dermis (17,26), diaphragm (27), heart (18) and parotid gland (28), they are not seen, or only rarely, in encapsulated organs such as the kidney (5-6,8,23), liver (7), thyroid (9), and lung (25). This contrast has led to the suggestion that lymph formation occurs differently in these two types of regions. The scarcity of open junctions in the pancreas *(Table 4)* places it in the same category as other encapsulated organs even though the capsule in the pancreas is not as well developed or as firm as in the other organs. Even when an open junction was seen in the pancreatic lymphatics it was often not present in the adjacent section indicating that it was either an artifact or limited in its extent.

An alternate mode of transport across the lymphatic endothelium is through the cytoplasmic vesicular system (19). Azzali, et al (20,23) report seasonal differences in the density of vesicles in renal lymphatic endothelial cells of hibernating mammals and suggest that this is directly related to

transendothelial movement of protein and interstitial fluid. Uncoated vesicles appear to be ubiquitous in lymphatic endothelium and the pancreas is no exception *(Table 3)*. Thus, it may be assumed that vesicular transport occurs in the lymphatic endothelium of the pancreas. Our study sheds no additional light on the extent to which vesicles are discrete or fuse to form channels or on the precise mechanism by which the system transports molecules.

The nature of the larger intracellular sacs or channels seen in the present study is not clear. One previous study makes reference to similar structures, alluded to as cytoplasmic bodies, in the lymphatic endothelium of the heart (29). It is possible that they are no more than invaginations of the cell membrane that appear to be discrete because of the plane of section. On the other hand, the frequent association of these sacs with cytoplasmic vesicles and their occasional association with the luminal or abluminal surface suggests possible involvement in a transport mechanism. In another study, the endothelium of lacteals, in mice and guinea pigs fed a diet rich in corn oil, contained chylomicrons within large pinocytotic vesicles (24). These vesicles opened onto the luminal or abluminal surface, in a similar manner to that seen in this study, and the investigators concluded that transport of chylomicrons (diameter-200nm), occurred across lacteal endothelium in vesicles rather than through intercellular spaces.

We conclude from these data that the lymphatic system of the pancreas, at least in the rat, contains both interlobular and intralobular vessels although the latter, as in the kidney, are quite rare. The density of the system appears to be comparable to that for the kidney, liver and thyroid. Structurally the vessels resemble other intraorgan lymphatics and show evidence of intercellular and intracellular transport. Dilated cisterns both between and inside the endothelial cells appear to serve as transport pathways, and in this way the vessels are more comparable to those in the intestine than those in the liver and kidney. The almost complete absence of open gaps (>30 nm) between adjacent endothelial cells suggests that the mechanism of lymph formation in the pancreas is more comparable to that in other encapsulated organs than that in areas such as the diaphragm or skin.

REFERENCES

1. Rusznyak, I, M Foldi, G Szabo: *Lymphatics and Lymph Circulation*. 2nd Edition. Pergamon Press, Oxford, 1954.
2. Godart, S: Lymphatic circulation of the pancreas. Bibliotheca Anatomica 7 (1965), 410-413.
3. Grau, VH, E Taher: Histologische untersuchungen uber das innere lymphgefabsystem von pankreas and milz. Berliner and Munchener Tierarztliche Wochenschrift 78 (1965), 147-151.
4. Kaneko, K: Microdistribution of lymphatic vessels in the pancreas. Acta Anatomica Nipponica 47/3 (1972), 205-221.
5. Albertine, KH, CCC O'Morchoe: Renal lymphatic ultrastructure and translymphatic transport. Microvasc. Res. 19/3 (1980), 338-351.
6. Albertine, KH, CCC O'Morchoe: An ultrastructural study of the transport pathways across the arcuate, interlobular, hilar and capsular lymphatics in the dog kidney. Microvasc. Res. 21 (1981), 351-361.
7. Niiro, GK, CCC O'Morchoe: An ultrastructural study of transport pathways across rat hepatic lymph vessels. Lymphology 18 (1985), 98-106.
8. Niiro, GK, HM Jarosz, PJ O'Morchoe, et al: The renal cortical lymphatic system in the rat, hamster and rabbit. Amer. J. Anat. 177 (1986), 21-34.
9. O'Morchoe, PJ. Y Han, MD Doyle, et al: Lymphatic system of the thyroid gland in the rat. Lymphology 20 (1987), 10-19.
10. Niiro, GK, CCC O'Morchoe: Pattern and distribution of intrahepatic lymph vessels in the rat. Anat. Rec. 215 (1986), 351-360.
11. Casley-Smith, JR: The identification of chylomicra and lipoproteins in tissue sections and their passage into jejunal lacteals. J. Cell Biol. 15 (1962), 259-277.
12. Földi, M, J Kepes, G Szabo: Cited by Rusznyak, I, M Földi, G Szabo: In: *Lymphatics and Lymph Circulation*. 2nd Edition. Pergamon Press, Oxford, 1954.
13. Dumont, AE, H Doubilet, JH Mullholland:

Lymphatic pathway of pancreatic secretion in man. Annals of Surgery 152/3 (1960), 403-409.
14. Jdanov, DA: Nouvelles donnes sur la morphologie fonctionnelle du systeme lymphatique des glandes endocrines. Acta Anat. 41 (1960), 240-259.
15. Vorobiyeva, EA: On anatomy of pancreas intraorgan lymphatic system in man. Arkh. Anat. Gistol. i Embriol. 44/3 (1963), 62-67.
16. Leak, LV: Electron microscopic observations on lymphatic capillaries and the structural components of the connective tissue-lymph interface. Microvasc. Res. 2 (1970), 361-391.
17. Leak, LV: The structure of lymphatic capillaries in lymph formation. Fed. Proc. 35 (1976), 1863-1871.
18. Marchetti, C, P Poggi, A Calligaro, et al: Ultrastructural study of the adjoining area between the endothelial cells of the rabbit heart lymph vessels. J. Submicrosc. Cytol. 19/3 (1987), 465-469.
19. O'Morchoe, CCC, WR Jones, HM Jarosz, et al: Temperature dependence of protein transport across lymphatic endothelium *in vitro*. J. Cell Biol. 98 (1984), 629-640.
20. Azzali, G, G Bucci, R Gatti, et al: Topography and ultrastructure of kidney lymphatics in some hibernating bats. Lymphology 21 (1988), 212-223.
21. Azzali, G: The ultrastructural basis of lipid transport in the absorbing lymphatic vessel. J. Submicrosc. Cytol. 14/1 (1982), 45-54.
22. Azzali, G: Ultrastructure of small intestine submucosal and serosal muscular lymphatic vessels. Lymphology 15 (1982), 106-111.
23. Azzali, G, G Bucci, R Gatti, et al: The lymphatic vascular system of some hibernating mammalian kidneys. Z. Mikrosk. Anat. Forsch 102/6 (1988), 945-961.
24. Dobbins, WO, EL Rollins: Intestinal mucosal lymphatic permeability: An electron microscopic study of endothelial vesicles and cell junctions. J. Ultrastruc. Res. 33 (1970), 29-59.
25. Roberge, S, Y Boucher, P-E Roy: Transient ultrastructural variations of pulmonary lymphatic capillaries during the respiratory cycle of the rat lung. Anat. Rec. 213 (1985), 551-559.
26. Leak, LV: The structure of lymphatic capillaries in lymph formation. Fed. Proc. 35 (1976), 1863-1871.
27. Casley-Smith, JR: Endothelial permeability. I. The passage of particles into and out of diaphragmatc lymphatics. Quart. J. Exp. Physiol. 449 (1964), 365-383.
28. Marchetti, C, P Poggi, A Calligaro, et al: Microscopic and ultrastructural study of the lymphatic system in the human parotid gland. Acta Anat. 134 (1989), 106-112.
29. Poggi, P, C Marchetti, A Calligaro, et al: Cytoplasmic bodies in lymphatic endothelial cell. Lymphology 19 (1986), 125-129.

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