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LYMPHATIC SYSTEM OF THE THYROID GLAND IN THE RAT

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ABSTRACT

Intraglandular thyroid lymph vessels in the rat were studied by qualitative and quantitative analyses in order to obtain information regarding their structure, distribution, relationships, and possible mechanisms of lymph formation. Ultrastructurally, the lymphatic vessels were similar to those described in other organs. The volume density of the lymphatic vessels was 0.007, the profile density 5.68mm^2 and the maximum diameter 17.87nm. Ultrastructurally, visible transport pathways across the vessels appear to be represented by intracytoplasmic vesicles and channels between endothelial cells. The mean maximum diameter of the vesicles was 96nm and they occupied 6.9% of the cytoplasm. They were equally distributed between luminal, abluminal, and intracytoplasmic positions. Open junctions (>30nm) were not seen between endothelial cells, but dilations along part of the length of interdigitating and overlapping contacts were frequent. It was concluded that the mechanism of lymph formation in the thyroid is similar to that in the kidney and liver, but differs from that in the dermis or diaphragm. However, the volume density of the vesicles of the thyroid was twice that of the liver and more than twice that of the kidney. This finding is consistent with an increase in transendothelial vesicular transport of macromolecules.

INTRODUCTION

The concentration of T3 and T4 in lymph from the thyroid gland has been shown to be 100 times greater than in thyroid venous blood (1). Furthermore, numerous investigators have concluded that the contents of the lymphatic capillaries are the same as the colloid of the follicles, although there are conflicting reports as to the mechanism by which colloid passes from the follicular lumen into the lymphatic capillary (2). Whether these findings indicate an important role for the lymphatic system in the transport of hormones is a controversial question since physiologically the flow rates are likely to be more important than the concentration. The high level of hormones do, however, imply a relatively extensive lymphatic system within the organ. Thus although there appears to be some species variation, there is general agreement that a rich, delicate network of lymphatic capillaries exists around the follicles (2). These vessels apparently unite to form larger ones which eventually empty into a superficial subcapsular network that covers the entire surface of the gland, and thence into collecting ducts (3). It must be emphasized, however, that these morphological descriptions can be accepted only with caution since the techniques previously employed in the identification of intraorgan lymphatic vessels are highly questionable. Such vessels are, in most instances, impossible to identify and distinguish by light microscopy, in routine histological preparations, from empty blood capillaries or venules, and even from tissue spaces. Retrograde injection of lymphatics with dye has been used

in an attempt to outline the vessels and thereby overcome this difficulty, but the high pressure required to fill lymphatics retrogradely against the valves commonly causes tracer to leak from the vessels and erroneously label interstitial spaces. It is now generally accepted that ultrastructural observation is the only certain method for the identification of intraorgan lymphatics (4,5). Unfortunately these vessels are usually sparse and the electron microscope alone does not reveal their pattern and distribution because of the smallness of the tissue blocks.

In view of uncertainties on the extent, pattern, and distribution of intrathyroid lymphatics, it was the purpose of this study to carry out a detailed qualitative and quantitative morphometric analysis of the internal lymphatic vessels of the normal thyroid gland in the rat using a combined light and electron microscopic approach with serial sections.

MATERIALS AND METHODS

Fifteen Sprague-Dawley rats of either sex weighing between 303 and 630gm were anesthetized with intraperitoneal sodium pentobarbital (50mg/kg body weight). Fixation was achieved by perfusion, from a height of 120cm, either retrogradely through the aorta or via the left ventricle with 20 to 40ml of saline for 1 to 2 minutes, followed by 150 to 200ml of 4% glutaraldehyde in cacodylate buffer for 30 to 60 minutes. The thyroid gland was then exposed, separated from the larynx, trachea, and surrounding tissues, immersed in 4% glutaraldehyde for 2 hours at 4°C. Each lobe, with the associated parathyroid gland, was then cut transversely into 2 to 3mm thick blocks with a razor blade. The blocks were postfixed in 1% OsO_4, dehydrated in a graded series of acetone, and embedded in a mixture of Poly/Bed 812 and Araldite 502. Serial sections, 3μm-thick, were cut and stained with toluidine blue. Alternate sections were used for light microscopy to pick out potential lymphatic vessels. The adjacent uncovered sections were saved for examination at the ultrastructural level using a re-embedded technique. This was done by sealing an inverted Beem capsule over the uncovered section with epoxy glue and filling it with embedding medium. The medium was polymerized for 71 hours at 60°C and the capsule containing the section removed from the glass slide with a razor blade. The re-embedded sections were trimmed, sectioned, and stained with uranyl acetate and lead citrate and examined in a JEOL 100C electron microscope. Lymphatic vessels were identified by their ultrastructural characteristics. The confirmed lymphatics were then traced in the serial sections at the light microscopic level, in both directions, in order to determine their distribution and patterns and their relationship with the follicles, blood vessels, and surrounding tissues. The quantitative analysis was performed with the aid of the Bioquant Image Analysis System. The sections for light microscopy were transmitted on to the monitor screen through a video camera mounted on the microscope. Light microscopic images were observed and digitized with a computer-based video digitizer and stored for future reference *(Fig. 1)*. By means of the Hipad digitizer, the perimeter, cross-sectional area, and relationship to adjacent structures of the lymphatics were calculated. To determine the volume density of lymphatic capillaries, 100 fields from 100 sections from 10 animals were randomly selected. The cross-sectional area of the

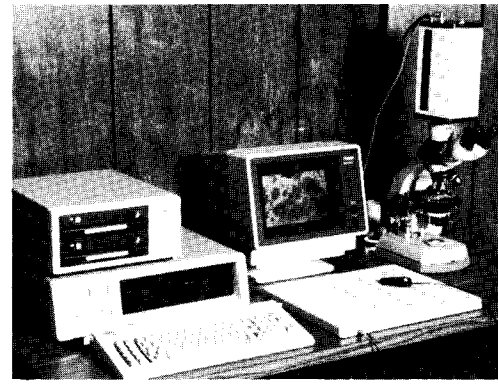

Fig. 1. Photograph of the morphometric analysis system. The video camera is mounted on a standard microscope and the image of the section transmitted to the monitor screen. Measurements are made by means of the Hipad digitizer.

lymphatics and the area of the field were measured and the areal density calculated by dividing the total cross-sectional area of lymphatics by the total area of the fields. The volume density is directly related to the areal density. The profile density of the lymphatic vessels was calculated by dividing the total number of lymphatics by the total area of the fields.

A detailed analysis of the endothelium of the lymphatic vessels was performed at the ultrastructural level with the aid of the Bioquant Image Analysis System. Electron micrographic negatives were transmitted from a light box to the monitor screen through a video camera and a variety of morphometric values were obtained using the Hipad digitizer. Thus the maximum and minimum thickness of the non-nuclear and perinuclear endothelium was measured, the mean thickness of the valve leaflets determined, the different types of intercellular contacts counted and their widths measured, the percentage of abluminal endothelial wall associated with basal lamina estimated, and the percentage of uncoated and coated vesicles determined. Uncoated vesicles were categorized according to their position in the endothelium, i.e., opening on to the abluminal or luminal surface, touching the abluminal or luminal surface, or apparently lying free within the cytoplasm. It is recognized that many such "cytoplasmic" vesicles could communicate with the cell surface out of the plane of section. The mean diameter, volume density, and numerical density of vesicles in valvular and non-valvular endothelium were determined.

RESULTS

A total of 291 lymphatic vessels was identified. The mean maximum diameter was 17.87μm $\pm$ 0.33 (S.E.), the profile density was 5.68/mm^2, and the volume density was about 0.007. The vessels were classified into four categories: 1) interfollicular (intralobular), 2) interlobular, 3) capsular, and 4) interglandular.

The interfollicular vessels (*Fig. 2*) were sparse in number, ran in the delicate inter-

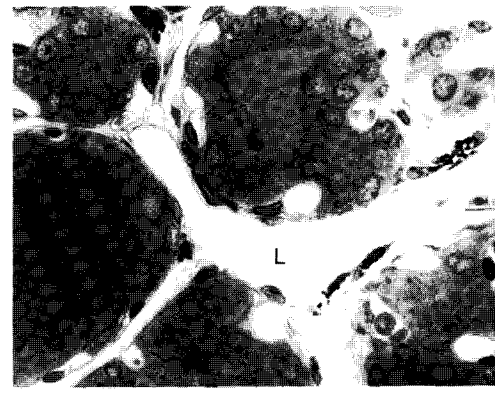

Fig. 2. Light micrograph of rat thyroid. An interfollicular lymphatic vessel (L) is seen coursing between follicles (x942).

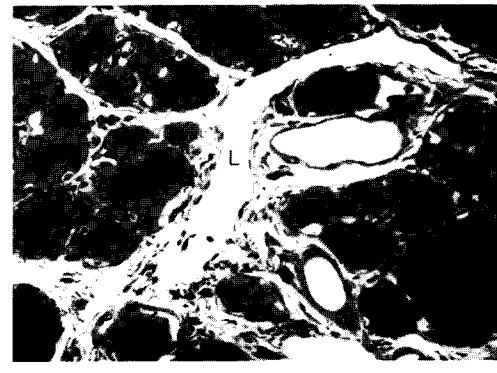

Fig. 3. Light micrograph of rat thyroid. An interlobular lymphatic (L) is seen running in connective tissues between lobules (x480).

Fig. 4. Light micrograph of rat thyroid. A capsular lymphatic (L) is running in the connective tissue of the capsule. (V) valve (x496).

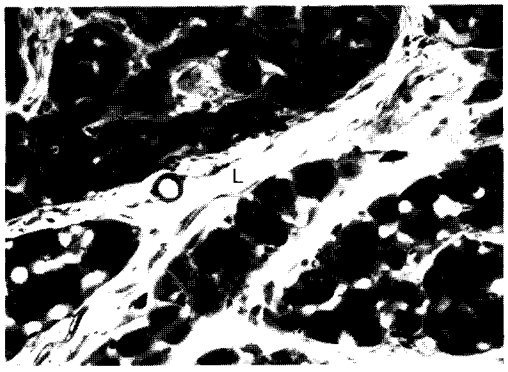

Fig. 5. Light micrograph of rat thyroid. An interglandular lymphatic (L) runs between the thyroid (T) and parathyroid (P) glands (x672).

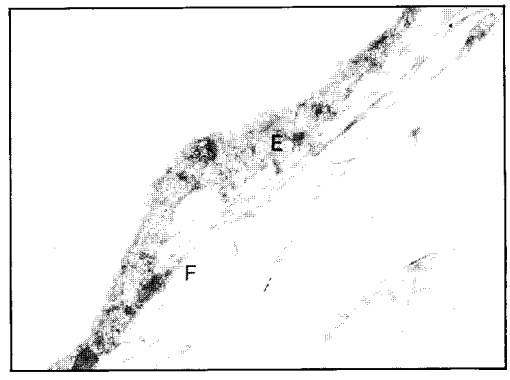

Fig. 7. Electron micrograph of lymphatic endothelial cell (E). Note anchoring filaments (F) and numerous pinocytotic vesicles (x21,500).

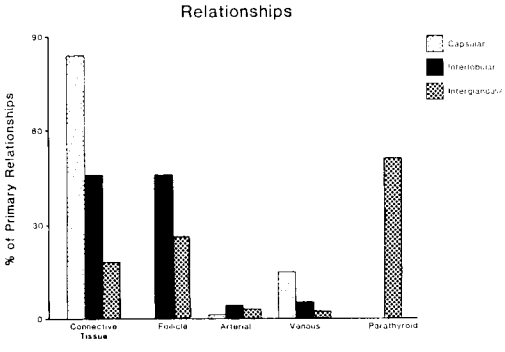

Fig. 6. This graph shows the primary relationships of the different classes of lymphatic vessels.

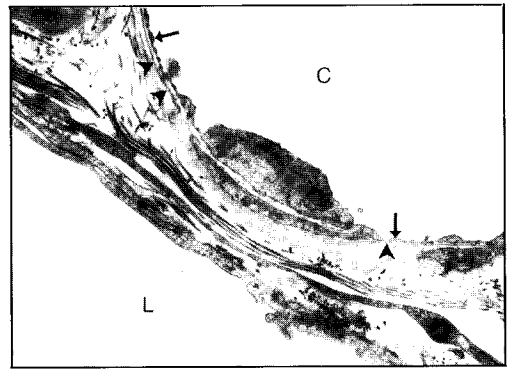

Fig. 8. Electron micrograph of lymphatic capillary (L) and blood capillary (C). Note the basal lamina (arrowheads) and fenestrated endothelium (arrows) of the blood capillary (x15,270).

follicular connective tissue, and drained into interlobular vessels. There were insufficient interfollicular lymphatics to obtain meaningful quantitative data on them.

The interlobular lymphatics *(Fig. 3)* comprised the majority of the lymph vessels. They ran in the interlobular connective tissue accompanying the corresponding blood vessels. From the orientation of the valves, some vessels appeared to drain to the periphery of the thyroid where they entered capsular or interglandular vessels, whereas others drained toward the center of the gland. In both arrangements, the vessels increased in size as they approached their termination.

Capsular lymphatic vessels *(Fig. 4)* coursed in the connective tissue of the capsule.

Interglandular vessels *(Fig. 5)* ran in the connective tissue between the thyroid and parathyroid glands.

Figure 6 shows the primary relationships, other than the delicate investing connective tissue elements associated with lymphatic capillaries, of the different classes of vessels. These relationships were to connective tissue, follicular cells, blood vessels, and parathyroid tissue. 46% of the interlobular and 26% of the interglandular lymphatics were related to follicular cells. In many instances the lymphatics adjacent to follicles were particularly close to the juxtaepithelial capillaries. In all instances

only a small percentage of lymphatic vessels were primarily associated with arteries or veins.

Ultrastructurally the lymphatic vessels *(Figs. 7 and 8)* were similar to those described in other organs (6-13). The endothelial cells rested upon a sparse and discontinuous basal lamina and anchoring filaments extended from the cell into the surrounding tissue. Only 0.14% of the abluminal endothelial cell surface was in contact with basal lamina. In the non-nuclear area, the mean endothelial cell thickness ranged from 104nm to 852nm nuclear area and the range was from 769 to 1,698nm *(Table 1)*. 180 intercellular contacts were examined. Of these, 5.5% were of the end-to-end types, 80% were overlapping, and 14.5% were of the interdigitating variety *(Fig. 9)*. *Table 2* shows the mean maximum and minimum intercellular distances at these contacts. The greatest distances measured were at interdigitating and overlapping contacts where dilatations were present but nowhere were "open junctions" (>30nm) noted. 54.8% of the contacts measured were seen to be associated with junctional complexes but these were often not identified because of the plane of the section.

Table 1
Mean Lymphatic Endothelial Cell Thickness (nm)

Region	*Maximum*	*Minimum*
Non-nuclear	852	104
Perinuclear	1,698	769

Typical bicuspid valves *(Fig. 10)*, with a mean leaflet thickness of 1,196nm, were identified in 82 of the interlobular, capsular, and interglandular vessels.

From randomly chosen fields, 172 endothelial vesicles were noted. Only two of these were coated and of the 170 uncoated, 24 were within valve leaflets. *Table 3* indicates the relative percentages of the vesicles which opened on to or touched either the abluminal or luminal cell surface or were apparently free within the cytoplasm. When all vesicles were considered together, approximately one-third of them opened on to or touched the abluminal surface, one-third were similarly related to the adluminal surface, and one-third were "cytoplasmic." However, the valve leaflet vesicles were distributed somewhat differently with approximately 42% associated with the inner surface, 42% intracytoplasmic, and 16% associated with the outer surface. The mean maximum diameter of the different categories of vesicles is shown in *Table 4*. *Table 5* shows the mean volume densities and numerical densities for the different categories of vesicles.

DISCUSSION

A comprehensive review of the literature on the arrangement of lymphatic vessels within the thyroid was provided by Rusznyak and his co-authors in 1967 (2). They concluded that the follicles were surrounded by a rich, delicate network of lymphatic capillaries which united and eventually emptied into a subcapsular network that covered the entire surface of the gland. However, the techniques employed to identify intraorgan lymphatic vessels in these earlier studies are highly questionable because in most instances, lymphatics are impossible to identify with certainty in routine light microscopic histological sections. Ultrastructural observation is now generally accepted as the only sure method of identification of intraorgan lymphatics (4,5). As these vessels are usually sparse and the tissue blocks used for ultrastructural analysis are very small, it is necessary to combine light and electron microscopy analysis in order to obtain data on the pattern and distribution of the vessels.

In our study we identified four categories of intrathyroid lymphatic vessels and designated them as interfollicular (intralobular), interlobular, capsular, and interglandular. We found the interfollicular vessels to be infrequent in contradistinction to the results of Lietz and Böcker who, in 1974, described a complex system of communicating channels around the follicles in the thyroid of the golden hamster. An explana-

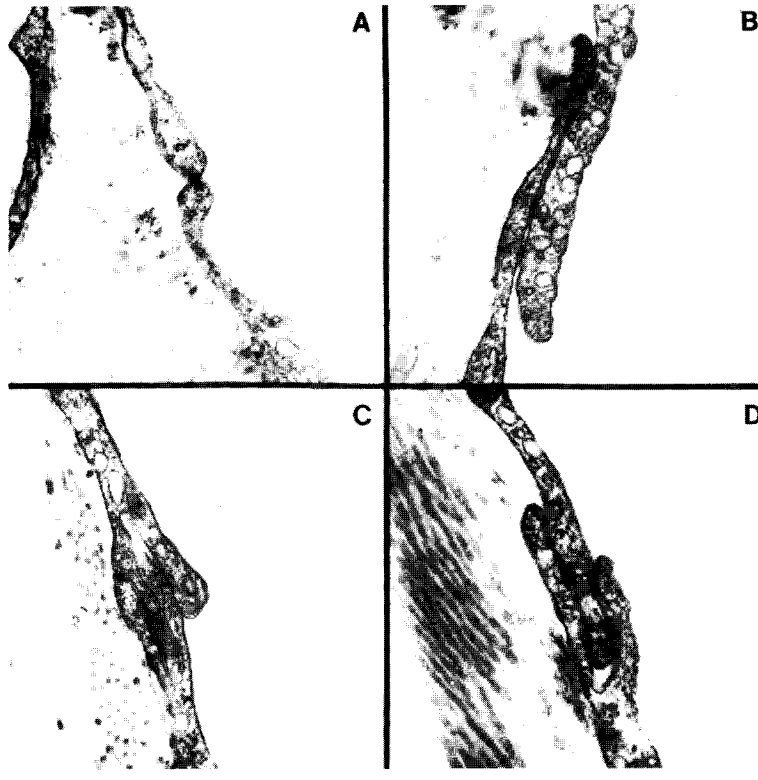

Fig. 9. Electron micrographs of lymphatic endothelial intercellular contacts. A) End to end (x48,000); B) overlapping (x48,100); C) complex interdigitating (x47,200); D) simple interdigitating (x47,500).

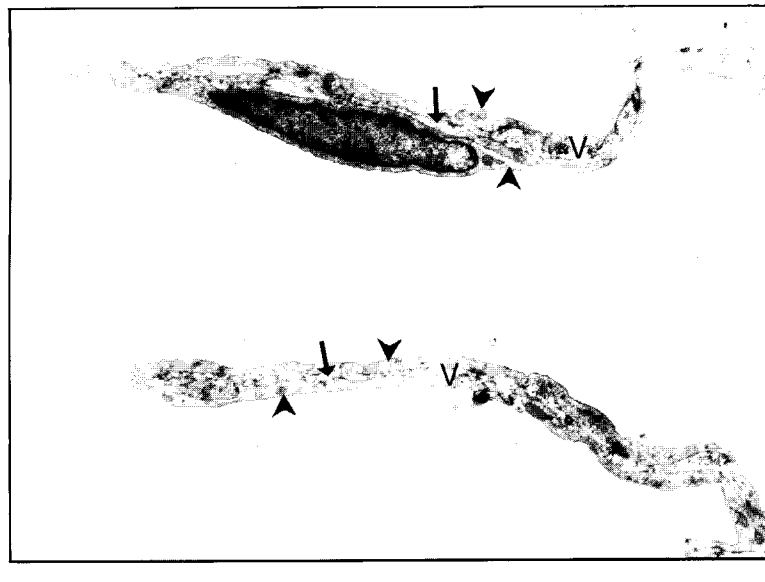

Fig. 10. Electron micrograph of lymphatic vessel valve leaflets (V). Note endothelial cells (arrowheads); connective tissue (arrows) (x8,740).

Table 2
Endothelial Cell Contacts

Type	*Number*	*Maximum Width (nm)*	*Minimum Width (nm)*	*# of Contacts with Junctional Complexes*
End to end	7 (4.6%)	25	7	1 (14.3%)
Overlapping	116 (76.8%)	164*	36	18 (15.5%)
Interdigitating	28 (18.5%)	258*	7	7 (25%)
Total	151			26 (54.8%)

*Width of dilated areas

Table 3
Frequency of Vesicle Position (%)

Group	*Ab-O*	*Ab-T*	*IC*	*L-T*	*L-O*
Endothelial wall	30.8	8.2	29.5	6.8	24.7
Valve leaflet	4.2	12.5	41.7	20.8	20.8
Combined data	27.1	8.8	31.2	8.8	24.1

Ab-O = opening onto abluminal surface, Ab-T = touching abluminal surface, IC = intracytoplasmic, L-T = touching luminal surface, L-O = opening onto luminal surface.

Table 4
Mean Maximum Diameter of Vesicles (nm)

Group	*Ab-O*	*Ab-T*	*IC*	*L-T*	*L-O*	*Mean*
Endothelial wall	96	99	91	97	93	94
Valve leaflet	71	73	143	80	92	108
Combined data	95	94	101	91	108	96

Ab-O = opening onto abluminal surface, Ab-T = touching abluminal surface, IC = intracytoplasmic, L-T = touching luminal surface, L-O = opening onto luminal surface.

tion for this discrepancy could be one of species variation.

Although the majority of the lymphatic capillaries that we saw were of the interlobular types, 46% of their wall was intimately related to the follicles. In addition, 26% of the wall of the lymphatics which ran between the thyroid and parathyroid glands were related to thyroid follicular cells. In the areas where there was a primary relationship between lymphatics and follicles, there was often a particularly close association between the lymph vessels and the follicular capillaries. The intimate relationship between intrathyroid lymphatics and follicles provides a morphological explanation for the high levels of T3 and T4 in thyroid lymph. This correla-

Table 5
Mean Volume Density and Numerical Density of Vesicles

Group		*Ab-O*	*Ab-T*	*IC*	*L-O*	*L-T*	*All*
Wall	V_v	0.023	0.007	0.022	0.005	0.017	0.074
	N_v	20.1	5.3	19.8	4.4	16.4	66.1
Leaflet	V_v	0.001	0.003	0.039	0.005	0.007	0.056
	N_v	1.6	4.8	10.5	7.6	6.9	30.4

Ab-O = opening onto abluminal surface, Ab-T = touching abluminal surface, L-O = opening onto luminal surface, L-T = touching luminal surface, V_v = volume density, N_v = numerical density.

Table 6
Comparison of Vesicles in the Kidney, Liver, and Thyroid

	Position (%)						*Max. Diam.*
Organ	*Ab*	*IC*	*L*	*J*	V_v	N_v	*(nm)*
Kidney	5	64	31		0.028	16	80-100
Liver	22.5	34	40.9	2.8	0.035	75	89.6
Thyroid	35.9	31.2	32.9		0.069	57.19	96

Ab = abluminal, IC = intracytoplasmic, L = luminal, J = cell junctions, V_v = volume density, N_v = numerical density.

tion between morphologic distribution and lymph composition is strongly reminiscent of that in the kidney where the concentration of renin in lymph is high and where some tubular functions are reflected in the lymph (5). It should be emphasized, however, that those lymphatics which were in close association with the follicles and juxtaepithelial blood capillaries were always separated from the follicular cells by small amounts of collagen and occasional fibroblasts. No part of the wall of the capsular lymphatics studied was related to follicular cells.

The two other intraorgan lymphatic systems of the rat which have been extensively studied in our laboratory are those of the kidney and liver (14-16). *Tables 6 and 7* show a comparison of the results obtained for the liver, kidney, and thyroid.

The width of intercellular channels was similar in all three organs. Dilatations, measuring up to 258nm, at the overlapping and interdigitating contacts, were seen in the thyroid and were similar to those previously noted in the liver. However, open junctions were never seen in the thyroid gland.

Uncoated vesicles in lymphatic endothelial cells of the thyroid were distributed such that one-third were associated with each surface and one-third "cytoplasmic." However, we have shown in a previous study that the majority of "cytoplasmic" vesicles are in fact connected to or open on to one or the other but not both of the endothelial surfaces (15). When all the thyroid vesicles were considered, there was little variation in their mean diameter which was similar to that seen in both the renal cortical and hepatic lymphatics. However, if the valve leaflets alone were considered, the abluminal vesicles were smaller than the others and the "cytoplasmic" considerably larger. The vesicular volume density in the three organs differed considerably, that of the thy-

Table 7
Comparison of the Renal, Hepatic, and Thyroid Lymph System

	Kidney	*Liver*	*Thyroid*
Lymphatic vessels			
V_v	0.0011	0.00098	0.007
N_v	5.31	1.76	5.68
Max. diam. (nm)	20.38[1] 12.17[2]	20.46[1]	17.87
Width I-C channels (nm)	16-18	22.6	17
Open junctions	Rare	Rare	None seen
Endothelial vesicles			
V_v	0.028	0.035	0.069
N_v	16	75	57.19
Max. diam. (nm)	80-100	89.6	96
Rate of lymph formation (ul/min/cm² endo)	0.29-0.37	0.055-0.085	

[1]interlobular
[2]intralobular

V_v = volume density
N_v = numerical density

roid being twice that of the liver and more than twice that of the renal cortex. It must be appreciated, however, that the method of fixation may affect the number, size, and distribution of the vesicles. Wagner and Andrews (17) reported that in rapidly frozen blood capillary endothelium, the values obtained for vesicle volume density, diameter, and numerical density associated with cell surface membranes were significantly less than in comparable tissue fixed with glutaraldehyde. Despite this, the bulk of the available data for volume densities in endothelium is from glutaraldehyde-fixed tissues. Therefore we believe that the values presented here are valid for comparative purposes.

The results from this study indicate that the fine structure of the intrathyroid lymphatic vessels is similar to that of the rat renal and hepatic vessels as well as those of other species. It is of considerable interest that open junctions were not obvious in the thyroid vessels even though it has been suggested that colloid itself enters the lymphatic lumen by some intercellular pathway (2). In this respect, lymphatic vessels of the thyroid, kidney, and liver differ from those of the dermis (18,19), the diaphragm (11), and the lung (10) in which open junctions are a prominent feature and form a major thoroughfare for transport. However, in the overlapping and interdigitating types of intercellular contacts, dilatations of considerable width were noted but never seen to extend the full length of the channel. The fact that the volume density of the endocytotic vesicles of the thyroid is twice that of the liver and more than twice that of the kidney is consistent with an increase in transendothelial vesicular transport of macromolecules.

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