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AN ULTRASTRUCTURAL STUDY OF TRANSPORT PATHWAYS ACROSS RAT HEPATIC LYMPH VESSELS

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ABSTRACT

Hepatic lymph vessels in the rat were examined by qualitative and quantitative analyses in order to obtain data pertinent to the mechanism of lymph formation. The ultrastructually visible transport pathways across these vessels appeared to be by way of intracytoplasmic vesicles (89.6 μm mean diameter) and normal channels (22.6 μm wide) between endothelial cells. Three types of intercellular contacts were seen, end-to-end, overlapping, and interdigitating. Only one open junction (>30 nm) was seen in 226 contacts examined. Specialized junctional complexes, either fasciae occludentes or fasciae adherentes, were seen in 65% of the contacts. Approximately one-third of the contacts had a dilatation along part of their length separating the opposing endothelial cells. Vesicles occupied 3.5% of the endothelial cytoplasmic volume and were distributed as follows: 40% opening onto or touching the luminal membrane, 34% without visible connection to either surface, 23% opening onto or touching the abluminal membrane, and less than 3% associated with membranes forming intercellular contacts. It was concluded that the mechanism of lymph formation in the liver is similar to that in the kidney and different from that in the dermis or diaphragm.

The movement of macromolecules from interstitium to lymph can occur, according to morphological studies, by one or both of two transendothelial routes. One is the potential pathway provided by the intracytoplasmic vesicular system (1-3); the other comprises the space that intervenes between adjacent endothelial cells. This space may appear in electron micrographs either as normal intercellular channels of about 15 to 20 nm wide (4-6) or as larger gaps of more than 30 nm, commonly referred to as open junctions (7-9). The relative preponderance of these two forms of intercellular space has implications for the way that lymph is formed. The presence of open junctions, as a major pathway favors the view that lymph formation is primarily an intercellular phenomenon (9). Such is apparently the case in lymph vessels of the dermis (7,10) and the diaphragm (11). In contrast, these open junctions are rarely seen in renal cortical lymphatics (4-6) suggesting that the intercellular route is of less importance in the kidney. In an attempt to reconcile these disparate observations, Casley-Smith (12) postulated that initial lymphatics functioned in two modes depending upon the local tissue hydrostatic pressure. He suggested that in encapsulated organs, like the kidney where tissue hydrostatic pressure is considered to be higher than atmospheric, fluid enters the lymphatics continuously through intercellular channels down a pressure gradient. In contrast, in regions where interstitial pressure is subatmospheric, fluid enters lymphatics intermittently (force pumps) and so may require potentially

wider spaces between cells. Studies by Albertine and O'Morchoe (4,5), Yang et al (6) and O'Morchoe et al (13) indicate that the vesicular system plays an important role in lymph formation, especially where open junctions are not present.

The primary purpose of the present study was to examine the transport pathways across hepatic lymph vessels in the rat by qualitative and quantitative analyses. The liver was chosen because it, as Casley-Smith (12) suggests, is similar to the kidney in that both organs are enclosed by a connective tissue capsule and so are thought to have a positive tissue hydrostatic pressure. Thus, hepatic vessels might be expected to bear a structural similarity to renal lymphatics rather than to dermal or diaphragmatic vessels, at least as far as intercellular junctions are concerned. The endothelial vesicular system was also studied quantitatively; According to the most widely accepted theory of lymph formation in the liver (14,15), hepatic lymph is principally derived from protein-rich interstitial fluid from the space of Disse. If protein stimulates an increase in the apparent number of vesicles as suggested by Yang et al (16), hepatic vessels might be expected to have a prominent vesicular system.

MATERIALS AND METHODS

Livers of eight male Sprague-Dawley rats (Harlan), weighing 225-330 g (mean wt. = 273 g) and anesthesized with intraperitoneal sodium pentobarbital (50 mg/kg body weight), were fixed by vascular perfusion through the portal vein. The portal vein was cannulated (PE 90) through a ventral midline incision. A one-liter bottle containing heparinized physiologic saline and another one-liter bottle containing 2% glutaraldehyde in .16 M sodium cacodylate buffer, pH 7.4 were connected to the cannula with a three-way stopcock. Tributaries to the portal vein were ligated. The perfusion was initiated with saline for 30 seconds or until the livers blanched and then switched to fixative. Immediately after the perfusion was started, the vena cava caudalis was cut and the celiac artery clamped. Perfusion continued for approximately 15 minutes, during which, 100-150 ml of fixative were used. A perfusion pressure of 40 mm Hg was maintained throughout the procedure. At the end of the perfusion, the livers were light brown in color and firm to the touch.

The perfused livers were excised and 2x2 mm blocks of well-fixed tissue were immersed for an additional hour in 2% glutaraldehyde, post-fixed for one hour in 1% osmium tetroxide, dehydrated in acetone and embedded in epoxy resin (17). Thin sections were cut, stained with uranyl acetate and lead citrate and examined with a Hitachi H600 electron microscope.

The frequency of three types of intercellular contacts — end-to-end, overlapping and interdigitating, as well as evidence of the so-called "open" (>30 nm wide) junction was manually tabulated from the micrographs. The presence of specialized junctional complexes and dilatations within the intercellular contacts were also recorded. For quantification of the vesicular system, forty lymphatics, five from each of the eight livers, were examined. For each lymphatic, 10 random micrographs were taken and printed at a final magnification of 58,700X. The following parameters were measured from the micrographs using a Zeiss Videoplan image analyzer: 1) maximum and minimum non-nuclear endothelial thickness, and 2) mean maximum diameter, volume and numerical densities of small uncoated endocytotic vesicles. The distribution of the vesicles were also determined by assigning each one to a category on the basis of whether it touched the luminal, abluminal or junctional membrane or whether it appeared to lie free within the cytoplasm.

RESULTS

Lymphatics showing a typical ultrastructural appearance were found within the connective tissue of portal canals. The endothelial lining was thin,

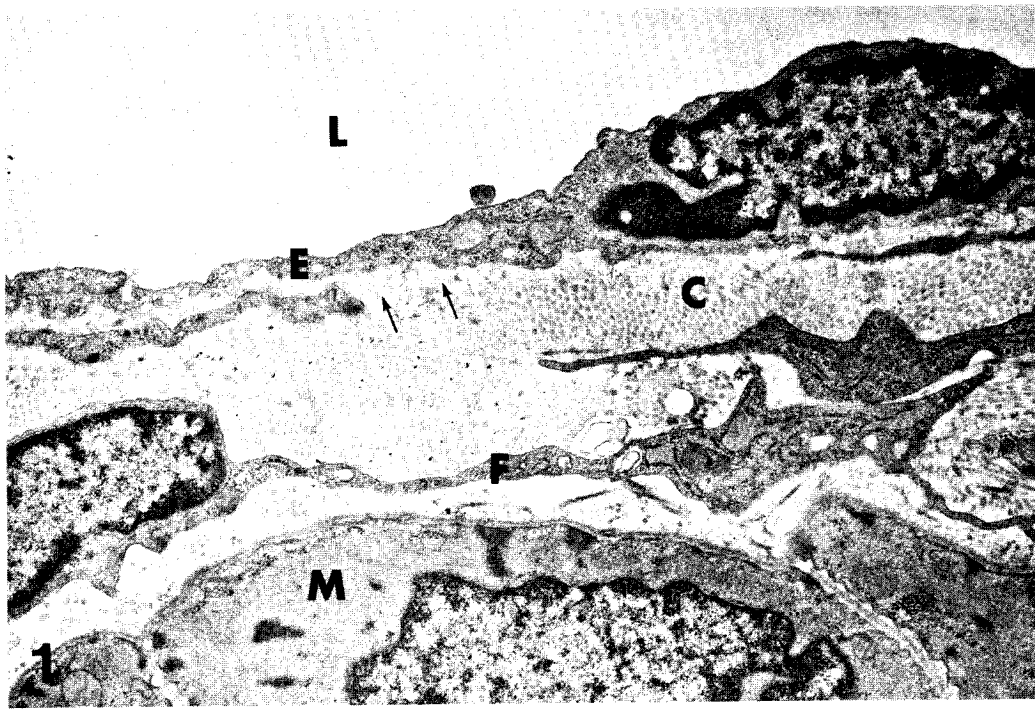

Fig. 1: Electron micrograph of part of a hepatic lymph vessel. The endothelium (E) lacks a basal lamina, however, collagen fibrils (C) and fine anchoring filaments (arrows) are intimately related to the abluminal surface. The lymphatic lumen (L), a fibroblast (F), and smooth muscle (M) are also seen. X14,400.

non-fenestrated and lacked a continuous basal lamina (Fig. 1). Fine anchoring filaments and collagen fibrils connected the endothelial wall to the interstitium. The cytoplasmic organelles included mitochondria, rough endoplasmic reticulum, lysosomes and vesicles, being generally comparable to those in lymphatic endothelium elsewhere in the body.

Three morphological types of intercellular contacts were seen, end-to-end, overlapping and interdigitating (Fig. 2). Table I demonstrates the relative frequency of each type of contact. Only one "open" type junction having a gap greater than 30 nm was seen. Specialized junctional complexes, either fasciae occludentes (tight junctions) or fasciae adherentes (intermediate junctions), were associated with 147 (65.0%) intercellular contacts (Fig. 3). In 65 (28.8%) contacts it was not possible to determine whether or not a junctional complex was present due to the plane of section. Junctional complexes were absent in 14 (6.2%) contacts. The average width of the intercellular channel was 22.6 nm ± 0.4 (SEM) although dilatations (Fig. 4) were present in 74 (32.7%) contacts.

Table 1:
Intercellular Contacts

No. Ly	No. Contacts	E-E	O.L.	I.D.	OPEN (<30nm)
40	226	12 (5.3%)	115 (50.9%)	98 (43.4%)	1 (0.4%)

Abbreviations: Ly = lymphatic; E-E = end-to-end; O.L. = overlapping; I.D. = interdigitating.

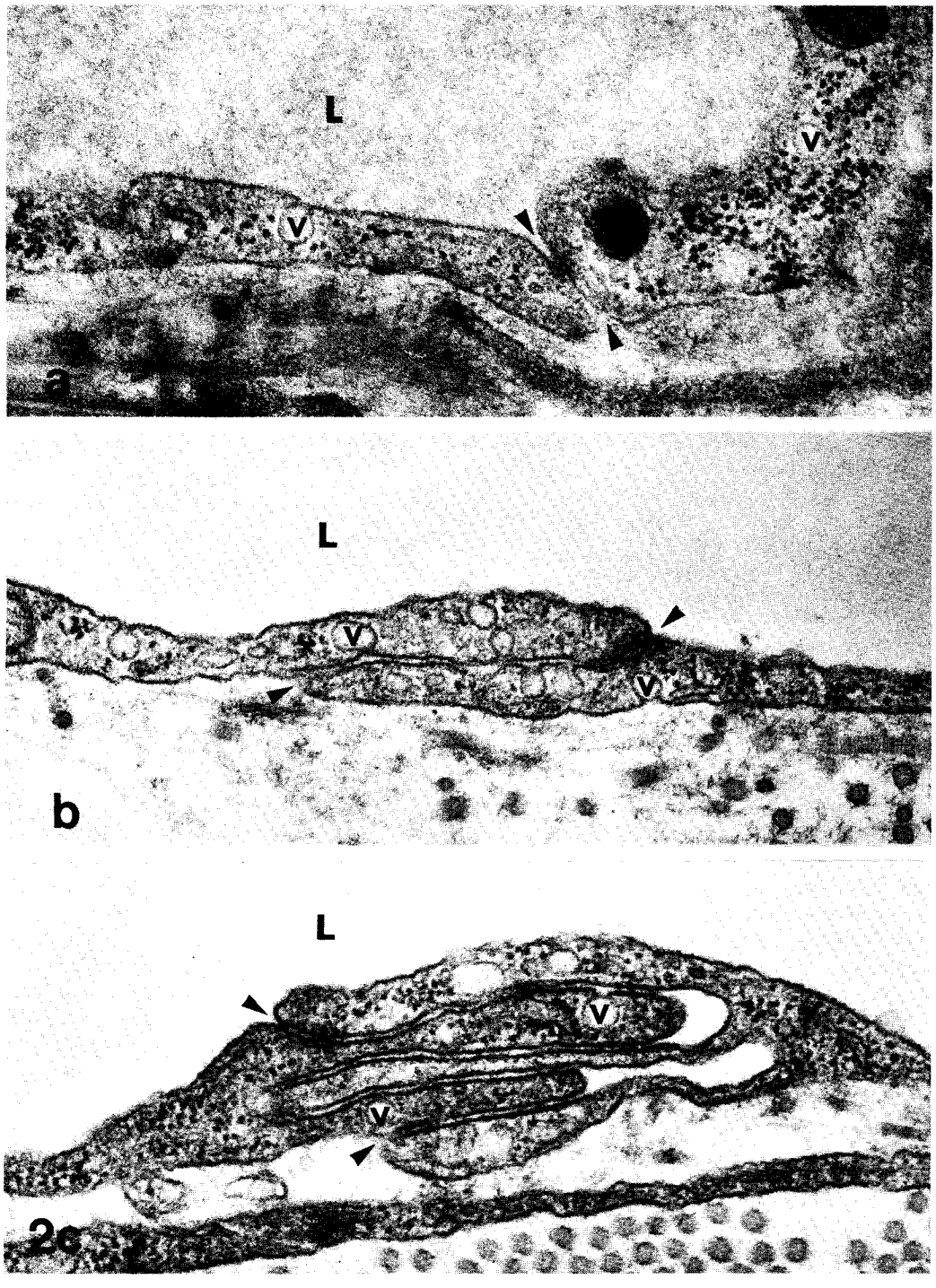

Fig. 2: The three types of intercellular contacts (arrowheads): (a) end-to-end; (b) overlapping; and (c) interdigitating. The lymphatic lumen (L) and small uncoated vesicles (v) are also seen. X56,000.

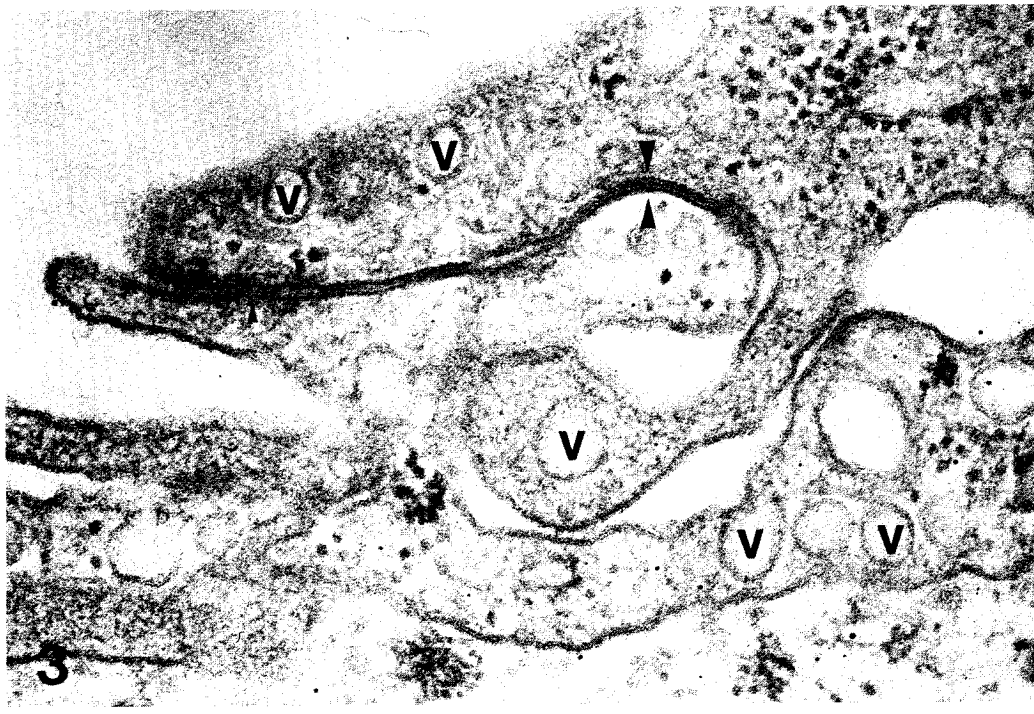

Fig. 3: Electron micrograph of a specialized junctional complex within an interdigitating type of intercellular contact. A fascia occludens (large arrowsheads) and a fascia adherens (small arrowheads) are seen between two contiguous endothelial cells. Numerous small uncoated vesicles (v) are present within the cytoplasma. X86,000.

The endothelium measured at non-nuclear areas was 0.1 μm ± 0.004 (SEM) at its minimum thickness and 0.5 ± 0.02 μm at its maximum. Within the cytoplasm, coated and uncoated endocytotic vesicles were observed. The larger vesicles (20-400 nm in diameter) had a fuzzy coating and were infrequently seen: they were not analyzed further. The distribution, volume density, numerical density and mean diameter of the small uncoated vesicles are given in Table II. A greater number of uncoated vesicles were associated with the luminal surface of the endothelium than the abluminal surface. Approximately one third of the vesicles appeared to lie free within the endothelial cytoplasm. A small percentage of vesicles were distributed along intercellular contact surfaces of the cell. The volume and numerical density data also reflect a higher proportion of vesicles associated with the luminal surface.

DISCUSSION

The major visible routes of translymphatic transport in renal lymphatics of the dog (4,5) and rat (6) are provided by normal intercellular channels and intracytoplasmic vesicles. In the present study, qualitatively and quantitatively similar pathways were observed in hepatic lymph vessels of the rat. Like renal lymphatics, hepatic vessels possessed three principal types of intercellular contacts that are held together by specialized junctional complexes. In contrast, the incidence of open junctions, a major thoroughfare across lymphatics of the dermis (7,8) and diaphragm (9) was found to be extremely low as it is in renal vessels (4-6). Also, the average width of intercellular channels (22.6 nm) measured in the present study resembled that found in renal vessels (5-30 nm). Although some of the contacts possessed dilatations, these

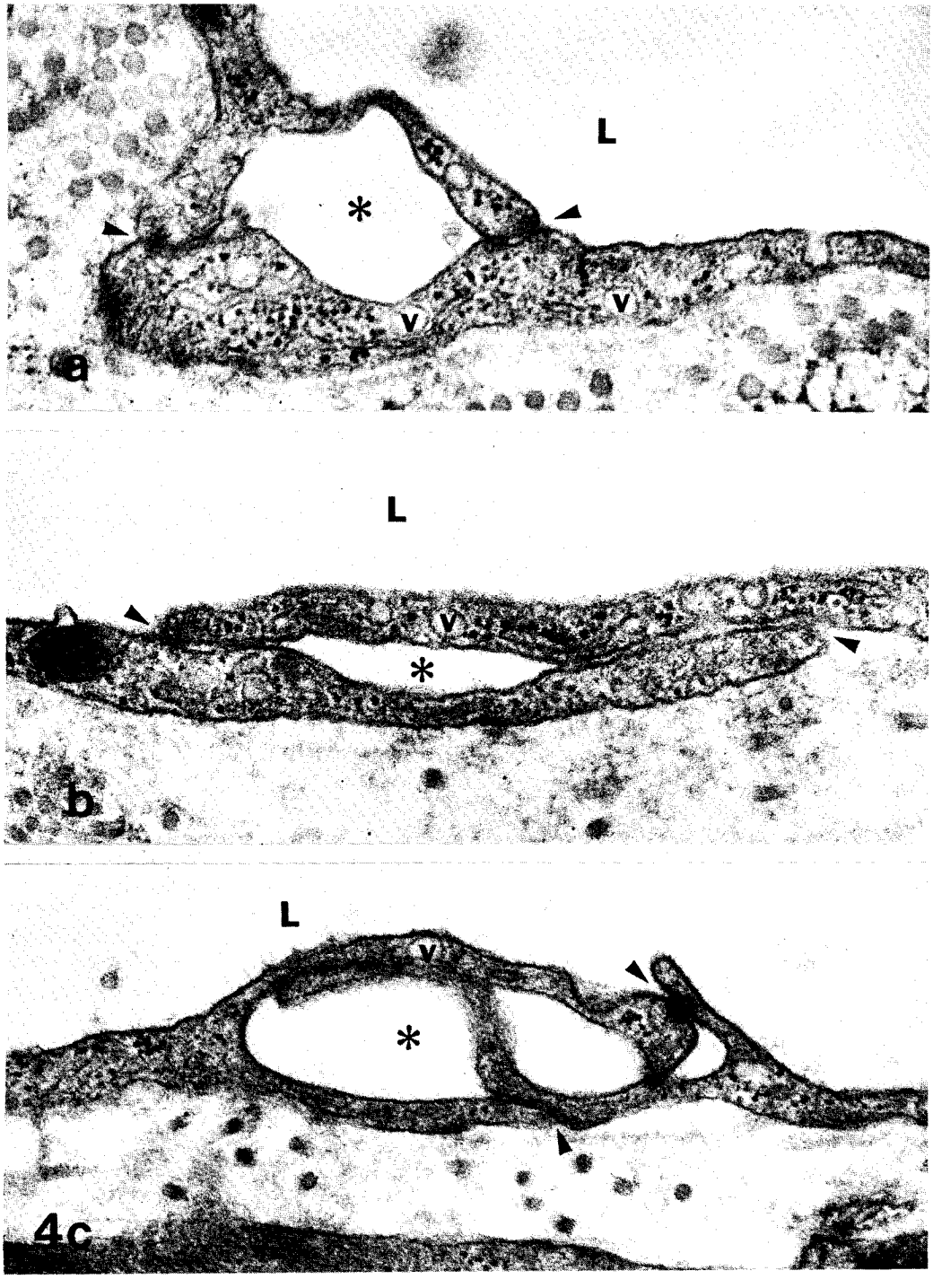

Fig. 4: Dilatations (*) within the three types of intercellular contacts (arrowheads): (a) end-to-end; (b) overlapping; and (c) interdigitating. The lymphatic lumens (L) and small uncoated vesicles (v) are also seen. X65,000.

Table 2
Distribution and Morphometry of Endocytotic Vesicles

	Distribution[1] (%)	V_V ($\mu m^3/\mu m^3$)	N_V ($\#/\mu m^3$)	D (nm)
Abluminal	22.5 ±1.3	0.009 ±0.001	17.9 ±2.0	90.6 ±0.2
Cytoplasmic	34.0 ±2.2	0.013 ±0.001	23.2 ±2.0	93.6 ±0.2
Luminal	40.9 ±2.0	0.014 ±0.001	29.9 ±3.0	91.3 ±0.2
Junctional	2.8 ±0.4	0.001 ±0.0002	2.3 ±0.4	88.0 ±0.2
TOTAL	100% (3028 vesicles)	0.035 ±0.003	74.6 ±6.2	89.6 ±0.1

Abbreviations: V_V = volume density; N_V = numerical density; D = mean diameter.

Data is expressed as mean ± SEM of 40 lymphatics.

[1]Abluminal, luminal and junctional vesicles touched or opened onto the abluminal, luminal or junctional surface, respectively. Cytoplasmic vesicles had no obvious connection to either surface.

spaces never completely separated adjacent endothelial cells. Whether these dilatations represent patent clefts for transport like those in intestinal lymphatics (18-20) is unclear from the present study.

The other major pathway in renal lymphatics, the vesicular system, was a prominent feature of hepatic vessels. Their volume density in hepatic lymph vessels (3.5%) is similar to that in rat renal cortical (2.8%, (6)) and canine renal cortical lymphatics (3.0% — 4.9%, (4)). The distribution of these vesicles is also like that of some renal vessels. For example, the percentage of vesicles without a visible connection to either luminal or abluminal surface was 34% in hepatic vessels in comparison to 43% in arcuate, 31% in interlobar, 38% in hilar and 36% in capsular lymphatics of the canine kidney (5). A higher percentage of apparently free vesicles was seen in cortical lymphatics of the dog (62%, (4)) and rat (64%, (6)).

Two of the three principal theories of lymph formation require the presence of large intercellular clefts which are open at least part of the time. The hydrostatic and oncotic pressure theory postulate that lymph is formed on a cyclical basis through these large openings (9,10). On the other hand, the third theory holds that transport by the vesicular system represents a major pathway across lymphatics and does not require the existence of large gaps between endothelial cells (4-6, 13). The vesicular theory was advanced to account for a component of the macromolecular transport in the kidney where open junctions are rare. By the same reasoning and evidence presented here, vesicular transport may be largely responsible for lymph formation in the liver as well. Vesicular movement of molecules is an especially attractive concept in the liver for two reasons. First, the transport of protein by vesicles, at least in blood capillaries, has been suggested by many (e.g., see review by Wagner and Casley-Smith (21)) and hepatic lymph contains a relatively high percentage of protein (22,23). Secondly, if hepatic lymph is indeed formed by vesicles, this finding would support Casley-Smith's theory (12) that the mechanism of lymph formation in the liver and kidney is comparable, yet different from certain other regions in the body.

An obvious question which arises from

this work is whether or not perfusion fixation had either a qualitative or quantitative effect on the observed transport pathways. If perfusion had caused edema or other trauma (no morphological evidence of edema was seen), an increase in the frequency of open junctions might have been expected (24,25). However, since only one was seen, it appears the intercellular contacts were not affected by perfusion. Likewise, the presence of dilatations, although unexplained, has been previously reported in immersion-fixed tissue (3,18). The effects of perfusion fixation on the vesicular system are less clear. Not only is the discreteness of the plasmalemmal vesicles themselves questioned (2,26), but Mazzone and Kornblau (27) detected considerably more vesicles in blood capillaries of rabbit lungs fixed by vascular perfusion than after rapid freezing. Therefore, the volume density of endothelial vesicles may, in fact, be lower than the 3.5% found in the present study.

In conclusion, the present study provides morphological evidence that the mechanism of lymph formation in the liver is similar to that in the kidney but unlike that found in the dermis or diaphragm. If lymph can be formed in more than one way, the conflicting theories of lymph formation, presently advanced, may be equally valid depending on local factors. These factors include tissue hydrostatic pressure (12), interstitial protein (13), and edema induced by local trauma (24, 28).

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REFERENCES

1. Dobbins, WO, EL Rollings: Intestinal mucosal lymphatic permeability: an electron microscopic study of endothelial vesicles and cell junctions. J. Ultrastruc. Res. 33 (1970), 25-59.
2. Jones, WR, PJ O'Morchoe, CCC O"Morchoe: The organization of endocytotic vesicles in lymphatic endothelium. Microvasc. Res. 25 (1983), 286-299.
3. O"Morchoe, CCC: The anatomy of the lymphatic system. In: Blood Vessels and Lymphatics in Organ System. DI Abramson, R.B. Dobrin, eds. Academic Press, Inc., New York, (1984), 126-133.
4. Albertine, KH, CCC O'Morchoe: Renal lymphatic ultrastructure and translymphatic transport. Microvasc. Res. 19 (1980), 338-351.
5. Albertine, KH, CCC O'Morchoe: An ultrastructural study of the transport pathways across the arcuate, interlobar, hilar and capsular lymphatics in the dog kidney. Microvasc. Res. 21 (1981), 351-361.
6. Yang, VV, PJ O'Morchoe, CCC O'Morchoe: Transport of protein across lymphatic endothelium in the rat kidney. Microvasc. Res. 21 (1981), 75-91.
7. Leak, LV, JF Burke: Fine structure of the lymphatic capillary and the adjoining connective tissue area. Am. J. Anat. 118 (1966), 785-810.
8. Leak,LV, JF Burke: Electron microscopic study of lymphatic capillaries in the removal of connective tissue fluids and particulate substances. Lymphology 1 (1968), 39-52.
9. Casley-Smith, JR:Lymph and lymphatics. In: *Microcirculation*, G. Kaley, BM Altura, eds. University Park Press, Baltimore, (1977), 423-502.
10. Leak, LV: The structure of lymphatic capillaries in lymph formation. Fed. Proc. 35 (1976), 1863-1871.
11. Casley-Smith, JR: Endothelial permeability. I. The passage of particles into and out of diaphragmatic lymphatics. Quart. J. Exp. Physiol. 49 (1964), 355-383.
12. Casley-Smith, JR: The fine structure and fine functioning of initial lymphatics. In: *Advances in Lymphology: Proceedings of the 8th International Congress of Lymphology*. V Bartos, JW Davidson, eds. Avicenum, Czechoslovak Medical Press, Prague, (1982), 6-13.
13. 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.
14. Rusznyak, I, M Foldi, G Szabo: *Lymphatics and Lymph Circulation*, Pergamon Press, New York, (1967).
15. Yoffey, JM, FC Courtice:*Lymphatics, Lymph and the Lymphomyeloid Complex*. Academic Press, London, (1970).
16. Yang, VV, PJ O'Morchoe, CCC O'Morchoe: Influence of exogenous albumin on intraendothelial vesicles of lymphatic capillaries. Proc. Electron Mic. Soc. of Am. G.W. Bailey, ed., (1980), 524-525.
17. Kushida, H: A new method of embedding with Epon 812. J. Elec. Microsc. 20 (1971), 206-207.

18. Azzali, G: Ter, RC, JR Casley-Smith: Endothelial vesicles. Microvasc. Res. 21 (1981), 267-298.
22. Barrowman, JA: *Physiology of the Gastrointestinal Lymphatic System.* Cambridge University Press, Cambridge, (1978), 229-255.
23. Lee, JS:Hepatic lymph flow and protein concentration during intravenous saline infusion and intestinal fluid absorption. Microvasc. Res. 27 (1984), 370-378.
24. O'Morchoe, PJ, VV Yang, CCC O'Morchoe: Lymphatic transport pathways during volume expansion. Microvasc. Res. 20 (1980), 275-294.
25. Leak, LV: Physiology of the lymphatic system. In: *Blood Vessels and Lymphatics in Organ Systems*, DI Abramson and PB Dobrin, eds. Academic Press, New York (1984), 1934-1964.
26. Bundgaard, M, P Hagman, C Crone:The three-dimensional organization of plasmalemmal vesicular profiles in the endothelium of rat heart capillaries. Microvasc. Res. 25 (1983), 358-368.
27. Mazzone, RW, SM Kornblau: Pinocytotic vesicles in the endothelium of rapidly frozen rabbit lung. Microvasc. Res. 21 (1981), 193-211.
28. Casley-Smith, JR: Endothelial permeability. II. The passage of particles through the lymphatic endothelium of normal and injured ears. Brit. J. Exp. Pathol. 46 (1965), 35-49.