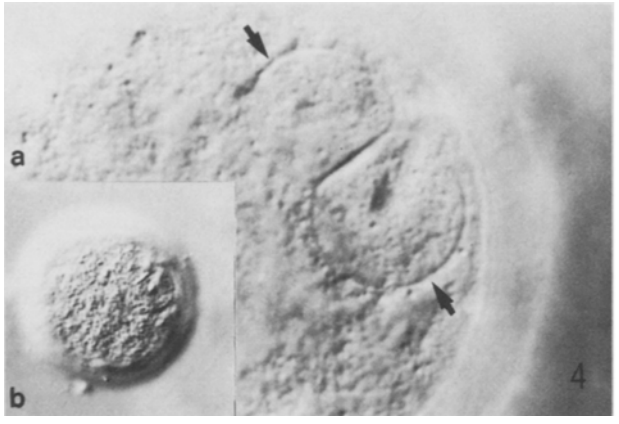

Figure 4. An almost denuded oocyte in nuclear division under a low (b) and the higher (a) magnifying power. Note the eccentricity of the nuclei adjacent to the vitelline membrane (arrows).

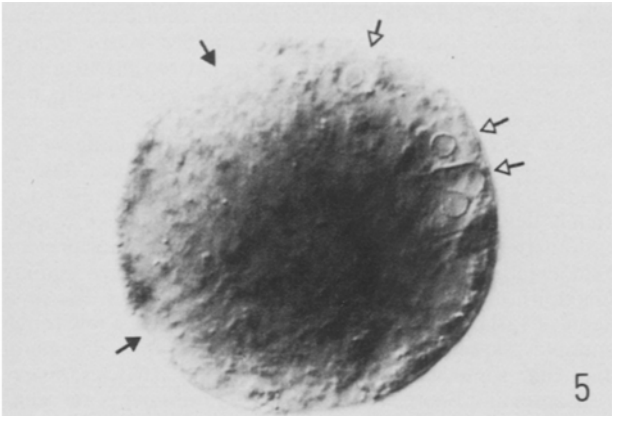

Figure 5. An oocyte which has lost the ZP and had disruptions in the vitelline membrane (closed arrows). Note the multiple nuclei situated very close to the cell membrane (open arrows).

Figures 4 and 5 represent non-fragmented oocytes with multiple nuclei and nucleoli. The oocyte in figure 4 is finishing nuclear division without synchronizing cell division. The oocyte in figure 5 has disruptions of the vitelline membrane and is being eliminated. In both oocytes it is noteworthy that the nuclei are very close to the vitelline membrane.

Success in experimental induction of fragmentation of oocytes in the Fallopian tubes[9] as well as in the ovaries[10] indicates that fragmented oocytes are formed by repeated cell divisions, whether they may be meiotic or mitotic. However, the synchrony between cell and nuclear divisions is completely lost in fragmented oocytes. Moreover, the asynchrony also functions in the process of elimination of non-fragmented oocytes in the ovary.

Presence of follicle cells in the ZP and perivitelline space was reported by Zamboni and Thompson[11]. However, existence of GCLS in the perivitelline space requires consideration of the relationship between GC and GCLS. In the present study GCLS in the perivitelline space almost invariably accompanied the thickened processus (fig. 3). Such a processus was thick enough (up to a few µm in diameter) to allow a cytoplasmic shift from GC to GCLS. It is possible that granulosa cells can change their position from the outside of the ZP to the perivitelline space by amoeboid movement.

1 To whom future correspondence and reprint request should be addressed. The authors are very grateful to Mr T. Horii, Dr K. Takeda, Dr S. Morisawa, Mr K. Takahashi, Mr Y. Matsumoto and Mrs M. Shinohara for their laboratory help.

2 L. Loeb, J. Am. med. Ass. *56*, 1327 (1911).
3 M.C. Chang, Anat. Rec. *108*, 31 (1950).
4 R.G. Edwards, Sci. Am. *215*, 72 (1966).
5 K. Mikamo, Cytogenetics *7*, 212 (1968).
6 R.P. Donahue, J. exp. Zool. *169*, 237 (1968).
7 W.J. Swartz and A.W. Schuetz, Am. J. Anat. *144*, 365 (1975).
8 H. Shinohara, S. Okoyama, K. Akasofu and E. Nishida, Experientia *36*, 1329 (1980).
9 N.W. Fugo and R.L. Butcher, Fert. Steril. *17*, 807 (1966).
10 H. Shinohara, Experientia *37*, 1353 (1981).
11 L. Zamboni and R.S. Thompson, Biol. Reprod. *7*, 425 (1972).

Transendothelial transport of lipids in the absorbing lymphatic vessel

G. Azzali[1]

Department of Anatomy, Medical School, University of Parma, Via Gramsci 14, I-43100 Parma (Italy), 26 June 1981

Summary. Intraendothelial channels have been shown in the lacteal vessels under normal conditions and during experimentally induced stasis, using ultrastructural and tridimensional reconstruction methods. Through the channels of the lymphatic endothelial wall, free lipid drainage from the interstitium into lymph was detected, although the intercellular junctions did not appear to be modified.

In spite of frequent and recent ultrastructural studies the question of transendothelial transport in the absorbing lymphatic vessel is still debated. As far as fluids and plasma proteins are concerned, significant roles are played by vesicular transport, by diffusion mechanisms[2-6] and by normal intercellular channels[7]. On the other hand the 'open junctions' route among contiguous endothelial cells[8-16], and the presence of temporary intraendothelial channels[17], have been suggested in the transport of small and large molecules. In order to identify and characterize the mechanisms intervening during the passage of large sized molecules through lymphatic endothelium, the absorbing lymphatic vessels of the intestinal villi have been studied in normal and experimental conditions.

Materials and methods. A total of 20 Chiroptera *(Vesperugo savi)* were used in the present study. The animals were killed under ether anesthesia, 6 during the lethargic winter fasting period, and 14 during the summer. Of the latter, 8 were sacrificed 2-3 h after feeding and six 5 h after lymphatic stasis had been induced by mesenteric trunk ligature. The duodenum and jejunum were removed from each animal. Fragments of duodenal and jejunal mucosa, 2-4 mm in thickness, were dehydrated in acetone and embedded in Durcupan after 1% osmium tetroxide solution

(pH 7.3) at 4 °C for 2 h fixation. The ultrathin slices, stained with Reynold's lead citrate, were examined with a Philips 300 electron microscope. Tridimensional reconstruction of 7 absorbing lymphatic vessels following Werner's technique was obtained by means of serial sections.

Results and discussion. The absorbing lymphatic vessel from the intestinal villi of bats killed 2–3 h after feeding displays walls with continuous endothelium almost completely devoid of basal lamina. These walls are made up of endothelial cells whose main cytoplasmic expansions are held together by means of interdigitating or overlapping junctions, tightened by zonulae adhaerentes and occludentes (fig. 1). In these cytoplasmic expansions, a few rough endoplasmic reticulum tubules, small vesicles 80–250 nm in diameter, some vacuoles containing lipid particles, sparse lysosomes and bundles of actin-like thin filaments are seen. In the interstitial space close to the external endothelial surface frequent lipid particles are detected, which may be adherent to the surface itself. The lipid particles are often encircled by abluminal cytoplasmic projections. The latter, after delimiting more or less wide interstitial spaces (fig. 1, s), join the external surfaces of the adjacent endothelial cell and tighten to it with zonulae adhaerentes and occludentes. As a result, in the endothelial wall a space is established which, in the three-dimensional reconstruction (fig. 2), appears to be an oblique channel, 8–10 µm in length (fig. 2a). Through a break in the continuity of the main cytoplasmic expansion (fig. 1a and 2a) the channel opens into the lymphatic vessel lumen. As a consequence the lipid content of the interstitial space is drained into the lymph. Through the extension of the lacteal vessel, 18–24 intraendothelial channels are detected after feeding conditions. During the above-mentioned endothelial rearrangements and during the transendothelial passage of the lipid particles the intercellular junctions among contiguous cells were not observed to open.

The same ultrastructural adjustment of the endothelial wall (intraendothelial channels and unmodified junctional complexes) have been detected in bats killed after prolonged lethargic winter fasting or after lymphatic stasis, conditions in which it is known that luminal-interstitial osmotic gradients are different[18].

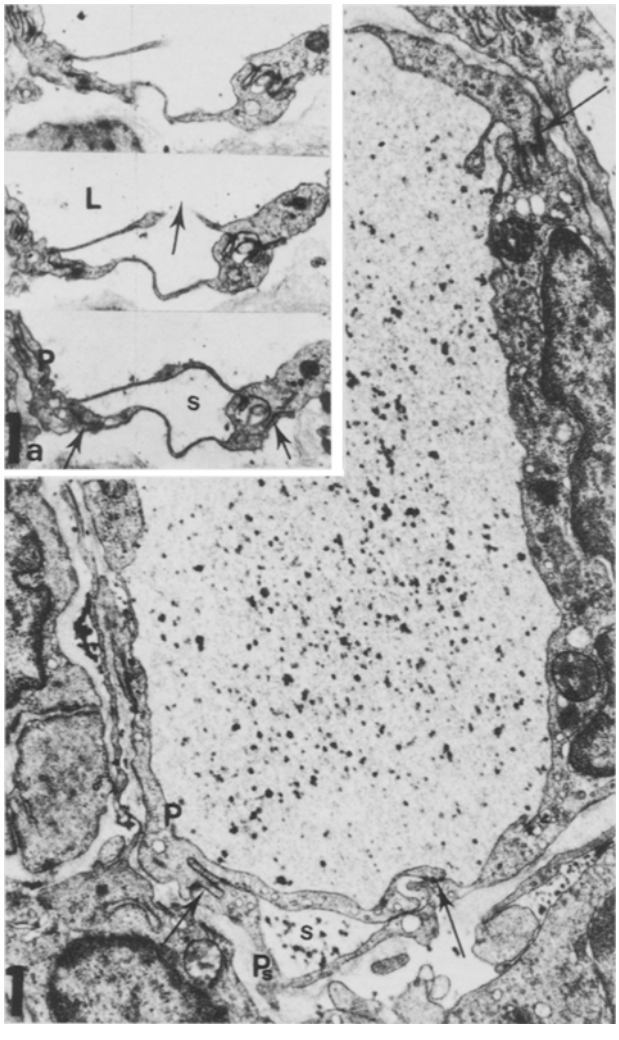

Figure 1. Absorbing lymphatic vessel of intestinal villus 2–3 h after feeding. Main cytoplasmic expansion (P) and abluminal cytoplasmic projections (Ps) are delimiting a discrete area (s) of interstitium containing lipid particles (bottom of the figure). The arrows indicate junctional complexes. × 17,000. Inset 1a: serial sections showing opening of the channels (s) into the lumen (L) by interruption of the main cytoplasmic expansion (P). × 6840.

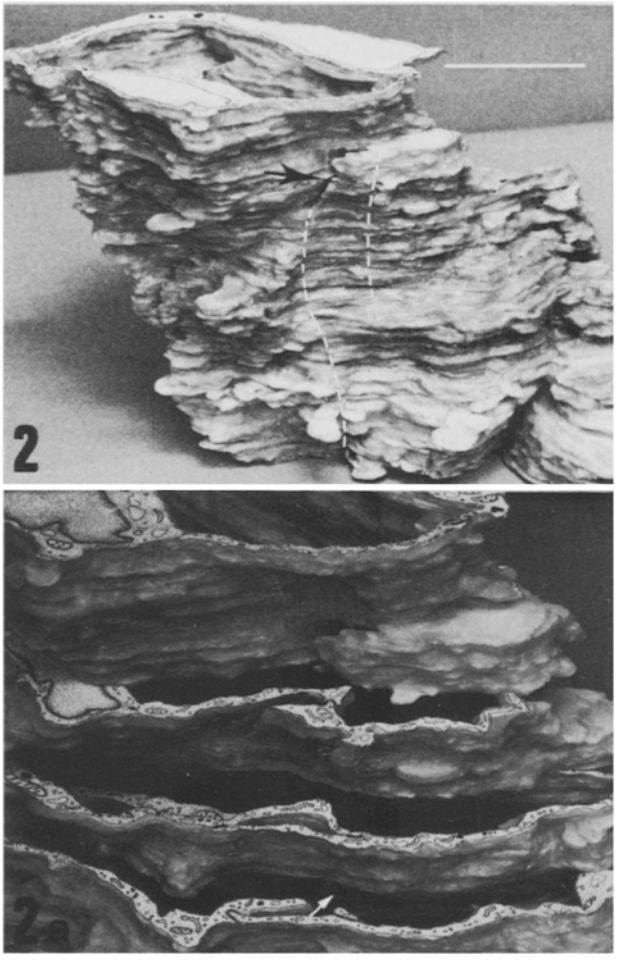

Figure 2. Three-dimensional reconstruction of an absorbing lymphatic vessel. It shows the endothelial surface facing the interstitium, with the abluminal opening of the intraendothelial channel (tube and arrow). The course of intercellular junctions is marked by small white lines. The bar indicates 5 µm. *a* 3 different sections of the three-dimensional reconstruction shown in fig. 2. The abluminal opening (black arrow), the intraendothelial channel (intermediate section) and the luminal opening (white arrow) are demonstrated.

These results demonstrate that intraendothelial channels represent the main transport pathway for the drainage of large molecules (lipids) from the interstitium into the lymphatic vessel lumen. Apart from the mechanism reported above, a small amount of lipid is carried to the lumen through phagocytic vacuoles. Intracytoplasmic vesicles and diffusion mechanisms play a significant role in the transport of fluids and small molecules. It has also been pointed out that specialized junctional complexes were observed to be unmodified during the functional and experimental conditions studied. Therefore the finding of 'open junctions' in endothelial wall must be considered to be a consequence of injury or pathological conditions[19-22].

1 Acknowledgment. The author thanks Dr Angelo Gandolfi for the suggestions during preparation of this manuscript.

2 K.H. Albertine and Ch.C.C. O'Morchoe, Microvasc. Res. *19*, 338 (1980).
3 G. Azzali, Archo ital. Anat. Embriol. *82*, suppl., 220 (1977).
4 J.R. Casley-Smith, Br. J. exp. Path. *46*, 35 (1965).
5 W.O. Dobbins and E.L. Rollins, J. Ultrastruct. Res. *33*, 29 (1970).
6 G. Ottaviani and G. Azzali, Acta anat. *56*, suppl., 325 (1969).
7 V.V. Yang, P.J. O'Morchoe and Ch.C.C. O'Morchoe, Microvasc. Res. *21*, 75 (1981).
8 J.R. Casley-Smith, Lymphology *12*, 59 (1979).
9 S. Elhay and J.R. Casley-Smith, Microvasc. Res. *12*, 121 (1976).
10 J. Collan and T.V. Kalima, Lymphology *7*, 175 (1974).
11 F. Huth and D. Bernhardt, Lymphology *10*, 54 (1977).
12 L.V. Leak, J. Cell Biol. *50*, 300 (1971).
13 L.V. Leak and J.F. Burke, J. Cell Biol. *36*, 169 (1968).
14 M. Ohkuma, Lymphology *6*, 175 (1973).
15 J. Ohshima, Archvm histol. jap. *40*, 153 (1977).
16 S.L. Palay and K.J. Karlin, J. Biophys. Biochem. Cytol. *5*, 373 (1959).
17 G. Azzali, Archo ital. Anat. Embriol. *85*, 391 (1980).
18 I. Rusznyák, M. Földi and S. Szabó, in: Lymphatics and lymph circulation, p. 408. Pergamon Press, London 1967.
19 R.M. Franch, P. Wiedemann and E. Fellinger, Cell Tissue Res. *178*, 299 (1977).
20 L.V. Leak and F. Kato, Lab. Invest. *26*, 572 (1972).
21 I. Carr, J. reticuloendothel. Soc. *21*, 397 (1977).
22 S.Z. Virágh, M. Papp and I. Rusznyák, Acta morph. hung. *19*, 203 (1971).

Existence of an α-chymotrypsin-like immunoreactivity in bovine mast cells

T. Shikimi and T. Kobayashi[1]

Department of Pharmacology, Shimane Medical University, Enya, Izumo, Shimane 693 (Japan), 30 December 1980

Summary. Using an indirect fluorescent antibody technique, we detected an α-chymotrypsin-like immunoreactivity, but no trypsin-like immunoreactivity in bovine mast cells.

An α-chymotrypsin-like and/or trypsin-like enzyme has been detected in mast cells. In the mast cells of humans[2], dogs[2], rabbits[2], rats[2-7] and mice[2], α-chymotrypsin-like activity was demonstrated, while in these cells in humans[8-10], dogs[8-10] and cats[10], a trypsin-like activity was reported. Aprotinin, which inhibits the activities of chymotrypsin and trypsin, is present in bovine mast cells[11-13], but it is unknown whether an α-chymotrypsin-like and/or trypsin-like enzyme is also present in these cells. We now report the existence of an α-chymotrypsin-like immunoreactivity in bovine mast cells.

Materials and methods. The following reagents were used: trypsin (EC 3.4.21.4) and α-chymotrypsin (EC 3.4.21.1) – both crystallized 3 times – (Miles Laboratories, USA); trypsinogen, type 1, and α-chymotrypsinogen-A, type 2, (Sigma, USA); acrylamide, N,N′-methylenebisacrylamide and Alcian blue 8 GX (Wako Pure Chemicals, Japan); Nuclear Fast Red (E. Merk, FRG); tosyl-L-arginine methyl ester hydrochloride (TAME) and benzoyl-L-tyrosine ethyl ester (BTEE) (Protein Research Foundation, Japan); goat antirabbit IgG conjugated fluorescein isothiocyanate (FITC) (Medical and Biological Laboratories Ltd, Japan); complete Freund's adjuvant (Difco Laboratories, USA).

Production of antibody to trypsin or α-chymotrypsin. Polyacrylamide disc-gel electrophoresis was carried out with 7% gel in 0.4 M glycine-HCl buffer (pH 4.0), first at 1 mA per tube for 10 min and then at 3 mA per tube for 30 min. Amido black was used to stain protein in the gel. The small gel segments containing enzyme activity (trypsin activity was measured with TAME as substrate by the method of Simlot and Feeney[14] at 37 °C, and chymotrypsin activity was determined with BTEE as substrate by the method of Hummel[15]) were pooled, and homogenized with 0.9% NaCl. An equal amount of complete Freund's adjuvant was added; the mixture was thoroughly emulsified and injected s.c. into Japanese white rabbits. Similar injections were repeated every 2 weeks. After 6 injections, the serum was harvested. Immunoglobulin was precipitated from the serum at 50% saturation with ammonium sulfate.

Immunocytochemistry. Fresh bovine lung, obtained from a local slaughter house, was fixed in 10% formaldehyde, embedded in paraffin, and sectioned in the usual way. After removel of the paraffin, the location of enzyme in the tissue was determined by the indirect Coons technique[16] with FITC-labeled goat antirabbit IgG. After observation of the fluorescence, the sections were stained with 1% Alcian blue in 3% acetic acid (pH 2.5). The nucleus was stained with Nuclear Fast Red.

Results and discussion. In the immunized rabbits, all antiserum titers were similar. In the double diffusion test, their serum gave a clear precipitin line with a 16-fold diluted solution of 1 mg/ml of trypsin or α-chymotrypsin, whereas serum from nonimmunized rabbits or from immunized rabbits treated with excess antigen did not show any precipitate. Immunological cross-reaction between bovine trypsin and α-chymotrypsin has been reported[17]. In the present study, at a concentration of 1 mg/ml of α-chymotrypsin, a precipitin line was produced with antitrypsin serum, but trypsin even up to 15 mg/ml did not react with anti-α-chymotrypsin serum. Trypsinogen or α-chymotrypsinogen-A also gave an antigen-antibody reaction. The optical concentration in the reaction was the same as that of the original antigen, and the same antigenicity as that of the original antigen was apparent.

The site of the antigen-antibody reaction in bovine lung was determined by the indirect fluorescent antibody techni-