The Permeability of Large Lymphatics to Ions, Studied with the Electron Microscope

Unlike the small 'initial' lymphatics[1,2], which have many open endothelial intercellular junctions, the large lymphatics have only closed ones[1-3]. The open junctions of the small lymphatics, especially numerous during motion or oedema of the tissues, allow many large molecules etc. to enter them; they become temporarily closed during transient compression of the tissues, thus preventing the escape of these large molecules. The permanently closed junctions of the large, collecting lymphatics prevent most of the large molecules from escaping before the lymph is discharged into the blood stream, although a small number still escape by traversing the endothelium via its vesicles. The closure of the junctions is caused by their numerous zonulae occludentes and adhaerentes, the well-developed basement membranes, and by the collagenous, elastic and muscular elements in the walls of the large lymphatics. All these support the endothelial junctions; their absence in the small lymphatics allows their junctions to open.

While it is well known that very few of the large molecules escape from the lymphatics[4-6], it has also been amply demonstrated that many small ones do indeed pass out of the system[4,5]. Thus the lymph becomes more concentrated as it passes centrally from the peripheral vessels. This has been demonstrated in a particularly elegant fashion using variously sized, fluorescent molecules (p. 426 in [5]). It is evident that the mechanism underlying this selective permeability is of interest.

In the small, initial lymphatics, it has been shown[7] that the small molecules (in this case, ions) can pass through the endothelial junctions even when they are to closed by tissue compression that they are sealed to the large molecules; by this means there is some concentration of the lymph even in the periphery, as the transient compressions of the tissues both concentrate the lymph and force it into the larger vessels. Similarly, it has been shown that ions can pass through closed junctions, even those possessing zonulae occludentes, in blood capillaries in muscle, in the brain and the retina, and through mesothelial junctions[7-9]. Although some of the ions also traverse the endothelium via the vesicles, it has been shown that these would be far too slow to contribute significantly to the passage of the small molecules[10-12], although they appear to be very important for the passage of large molecules in those sites where there are no open junctions or fenestrae[10-13]. It therefore seemed likely that the small molecules leave the collecting lymphatics by passing through their closed junctions.

To examine this possibility, observations were made on the collecting lymphatics of anaesthetized mice. The lymphatics studied were those in the diaphragm, the internal mammary chain and the thoracic duct. 5 ml of an isotonic solution of sodium ferrocyanide or of sodium sulphate was injected into the peritoneal cavity and the animals were suspended, tail upwards, for 10 min. Then 5 ml of glutaraldehyde was injected into the pleural cavities. This also contained ferric chloride or barium chloride so that insoluble precipitates of ferri-ferrocyanide or barium sulphate were formed in and about the collecting lymphatics. Specimens were excised after 15 min, given a further fixation in the solutions for 12 h, post-fixed in osmium tetroxide and embedded in araldite. (The details, concentrations, etc. are given elsewhere[7].) The sections were usually examined unstained, to avoid possible confusion with staining artefacts.

The Prussian blue reaction allowed one to observe many large and small lymphatics in the diaphragm, beside the internal mammary arteries, and the thoracic duct. The electron microscope showed that these precipitates, as well as those of barium sulphate, occurred in the lumens of collecting lymphatics, including the thoracic duct, in their walls, and in the surrounding connective tissue. In particular, the precipitates were frequent and dense in the endothelial intercellular junctions (Figures 1 and 2). The density of the deposits,

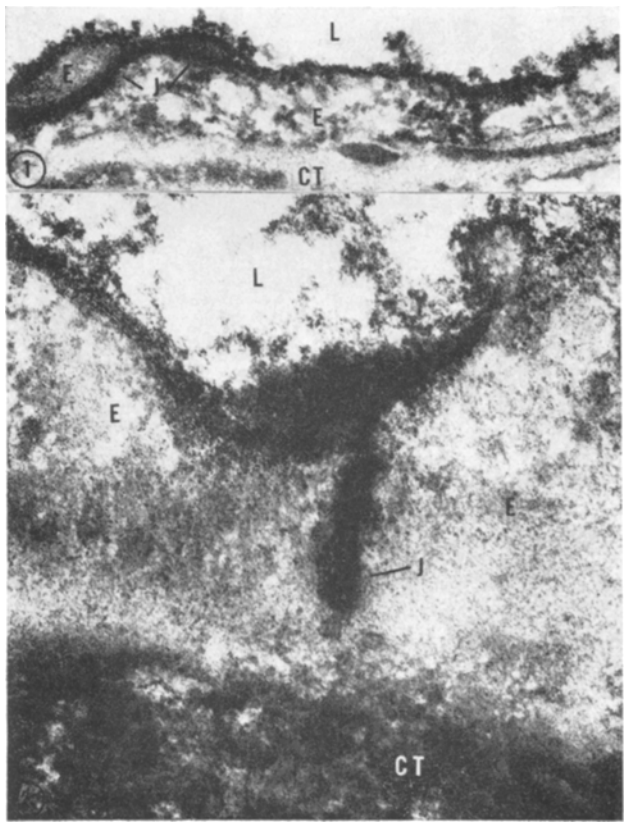

Fig. 1. A large lymphatic accompanying the internal mammary artery. Deposits of ferri-ferrocyanide may be seen in the lumen (L), in intercellular junctions (J) between the endothelial cells (E), and in the connective tissue (CT). Unstained, × 55,000.

Fig. 2. The thoracic duct. Ferri-ferrocyanide is present in the lumen, in a junction, and in the connective tissue. The deposit in the junction is much wider than the gap which is found between these cells; this is probably caused by this gap lying obliquely with respect to the plane of the section. Unstained, × 115,000.

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coupled with the lack of normal electron staining, made it difficult to locate zonulae. However one could sometimes make out the typical narrowing of the gap between the cells, etc. The deposits were continuous through these regions. In addition, it is known that the zonulae (including z. occludentes) are almost universal in the larger lymphatics, especially the thoracic duct[3]. The fact that the deposits often ran continuously from the lumens to the adventitia showed that the zonulae must be permeable to ions. (This permeability of the z. occludentes and adhaerentes has been established in other sites, including the brain and retina[7-9].)

As might have been expected, the endothelial vesicles also often contained precipitates. No doubt, since they periodically open to the exterior, they must take in ions and transport them just as they do the large molecules[7,9-13]. However, as mentioned earlier, it is most unlikely that they contribute significantly to the passage of the small molecules.

It is evident, therefore, that the endothelial intercellular junctions of the large lymphatics, although closed to large molecules, are freely permeable to small ones. There seems little doubt that these correspond to the 'pores' of PAPPENHEIMER[14]. There also seems little doubt that it is via these junctions that the small molecules escape from the lymphatic system. The high hydrostatic pressures generated by the contractions of the walls of the collecting lymphatics[15,16], to which may be added the effects of gravity[1,2], must force considerable amounts of the small molecules out of the vessels. No doubt they rapidly pass out of the tissues into neighbouring blood vessels. If the lymphatics are carrying off large volumes of fluid from active muscles or oedematous regions, it is evident that the local high tissue pressures will prevent most of the fluid from leaving the vessels until they pass through a normal, inactive region. Then the escape will commence. Hence the lymphatic system can be considered to carry large molecules, almost without loss, to the various lymphatico-venous anastemoses; small molecules are carried out of active or oedematous regions, then they tend to pass into the blood system via the lymphatic walls and the tissues[17].

Résumé. Les petites molécules s'échappent des grands vaisseaux lymphatiques grâce aux jonctions endothéliales intercellulaires, qui ne laissent pas passer les grandes molécules.

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Effect of Calcitonin on Glycosaminoglycan Synthesis by Embryo Calf Bone Cells in vitro

Calcitonin lowers serum calcium by inhibiting bone resorption[1-3]. Crude calcitonin preparations have been shown to stimulate the incorporation of ^{14}C-glucose into glycosaminoglycan (GAG) synthesis by embryo calf bone cells in culture[4]. Increases ranged from 25–40% with hormone preparations of activities 0.2–2.5 MRC U/mg. The present work consists of further experiments to demonstrate this effect, and to study the effect of high specific-activity calcitonin on GAG synthesis.

The methods of establishing the cultures and measuring ^{14}C-GAG production by cells have been described previously[4]. In essence, cells were grown in medium 199 containing 20% human serum (HS), 10% foetal calf serum (FCS) and ^{14}C-glucose in polystyrene flasks half of which contained calcitonin. After 18–22 h culture the ^{14}C-GAG's were precipitated, washed, dissolved and counted. Calcitonin prepared by phenolic extraction and TCA precipitation[5] (TCA-CT, 0.45 MRC U/mg) consistently caused an increased ^{14}C-GAG synthesis (Table I). However no effect was obtained with preparations of high specific activity (Lilly-CT, 50 U/mg; Armour-CT, 58 U/mg; CIBA-CT, 55 U/mg), even at concentrations of 1 U/ml. One explanation of this failure was that the more purified preparations lost biological activity under the conditions of culture. This was investigated by assaying Armour-CT after incubation at 37 °C for 24 h in medium 199 with and without serum. TCA-CT in medium 199 and serum was assayed at the same time. Armour-CT lost all activity while the crude preparation retained some hypocalcaemic activity (Table II). The results in Table III indicate that the rate of inactivation of hormone is very rapid, since Lilly-CT had no detectable biological activity after 90 min.

Further experiments were aimed at growing the embryo calf bone cells in media containing considerably less serum, with a view to avoiding hormone inactivation.

Table I. Effect of TCA-CT (0.45 U/mg) on ^{14}C-glucose incorporation into GAG synthesis

Cell density (cells/ml)	^{14}C-glucose (μc/ml)	Calcitonin (mU/ml)	^{14}C-GAG synthesis (cpm) control	treated	p
120,000	2.0	22.5	251 ± 17 (3)	382 ± 38 (3)	< 0.05
70,000	8.0	40	2295 ± 88 (5)	2662 ± 90 (5)	< 0.02

Replicate cultures were grown in medium 199 with 20% HS, 10% FCS for 20 h; mean ± S.E.M. of cpm above background; No. of flasks in parenthesis.

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