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THE FINE STRUCTURE OF THE AMPHIBIAN LYMPH SAC

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ABSTRACT

The fine structure of lymph sacs in the amphibian was observed by transmission electron microscopy. The wall was lined by endothelial cells which were similar to those of the endothelium of lymph capillaries in mammals, but with many more abluminal projections and a much more prominent basement membrane--especially centrally, near the lymph hearts. There were four types of intercellular junctions: open, overlapping, end-to-end, and interdigitating or complex. Specialized complexes were seen in 50% of the junctions. The cytoplasm frequently projected from the lumen and abluminal surfaces. Many thin filaments anchored the abluminal endothelial wall to the surrounding connective tissue. There were numerous small vesicles in the cells and opening onto both surfaces.

Within recent years, much research has been devoted to the fine structure and function of the lymphatic system in mammals (1-5), but considerably less to that of the lower vertebrates or their microcirculation (6-8). The lymphatic system began in the amphibia and not in the elasmobranchs and teleosts, as is frequently stated (6-8). The general anatomy and physiology of the amphibian lymph sac has been studied by many workers (9). However, the ultrastructure of the amphibian lymph sac has not been previously examined. It seemed worthwhile studying this structure since it represents the most primitive lymphatic uptake region which is available.

MATERIALS AND METHODS

Adult Queensland cane toads (*Bufo fowleri*) were pithed. To aid in dissection, injections into the dorsal lymph sac and the interdigital web of the hindlimbs were made, using 0.5ml and 1.0ml of China (Indian) ink (Pelikan, Günther Wagner, Hannover, Batch No. C11/1431a). They were killed 40 seconds after the injection. Specimens of the lymph sacs of the hind leg, hind thigh, and dorsal and ventral surfaces were removed and fixed with 3% glutaraldehyde, buffered with 0.1M phosphate at pH 7.4 for 30 minutes. Using a dissecting microscope, the specimens were divided into 1mm^2 pieces and then fixed for a further 4 hours. After washing in 0.2M phosphate buffer, they were post-fixed in 1% osmium tetroxide in phosphate buffer for 60 minutes, dehydrated in ascending alcohols and embedded in araldite by the usual methods. Semi-thin sections were used to find suitable regions. Eight blocks were used and 3 to 5 grids were examined from each block. Sections were stained with uranyl acetate and lead citrate (pH 10).

RESULTS

The fine structure of the lymph sacs in different areas were similar. The basement membrane was of variable thickness

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Fig. 1. An overlapping endothelial intercellular junction which possesses a number of zonulae adherentes. x15,000.

and was often discontinuous *(Figs. 1,4)*; at other times it was prominent *(Figs. 2,3,5)*. Frequently, there were obvious endothelial projections from the abluminal surface *(Figs. 2,3,5)*. The endothelium and the adjacent interstitium were often thrown into folds, extending into the lumen.

The endothelium possessed the usual organelles. Golgi apparatus and rough endoplasmic reticulum were only occasionally found near the nuclei of endothelial cells. There were many small (~50nm) smooth vesicles, which often opened to the surface, and some dense coated ones.

Anchoring filaments and some fibrils were seen connected to the cytoplasmic processes of endothelial cells which projected into the interstitium. The interstitial tissues were often quite thin between the endothelial lining and the striated muscle deep to the sac *(Fig. 3)*. Sometimes anchoring filaments extended between endothelial projections to similar ones of the striated muscle cells *(Fig. 3)*.

Four endothelial intercellular junctional types were found: overlapping *(Figs.*

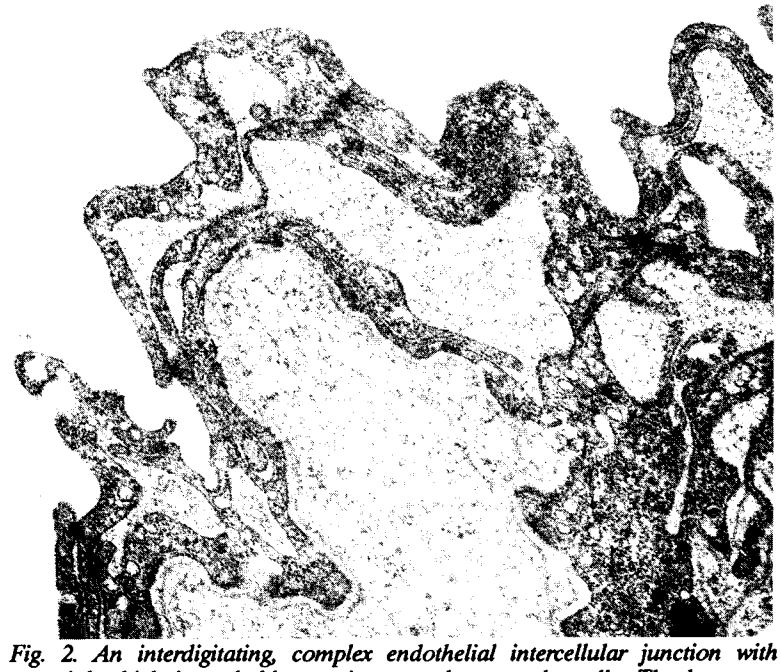

Fig. 2. An interdigitating, complex endothelial intercellular junction with material which is probably proteinaceous between the cells. The basement membrane is prominent. x25,000.

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Fig. 3. A junction (J) which is partly opened along its sectional length. There is much proteinaceous material between the cells. Anchoring filaments (arrows) extend from the endothelium to the striated muscle which lies just deep to them. A basement membrane is quite prominent between the two cell types. There are a number of projections form the endothelium into the interstitial tissue. x20,000.

1,3), end-to-end, interdigitating or complex *(Fig. 2)*, and open *(Fig. 4)*. In all, 299 intercellular junctions were examined. There were 19 (6%) open junctions, with gaps measuring from 30nm to 8,000nm (usually 30-120nm); 28 (9%) had end-to-end contacts; 122 (41%) overlapped; 130 (43%) were complex. Some junctions were formed by more than two endothelial cells. Most closed junctions possessed zonulae adherentes and occludentes.

Sometimes *(Fig. 3)* a junction appeared to be open over part of its sectioned length, containing dense material which resembled plasma protein. Open junctions *(Fig. 4)* were usually filled with

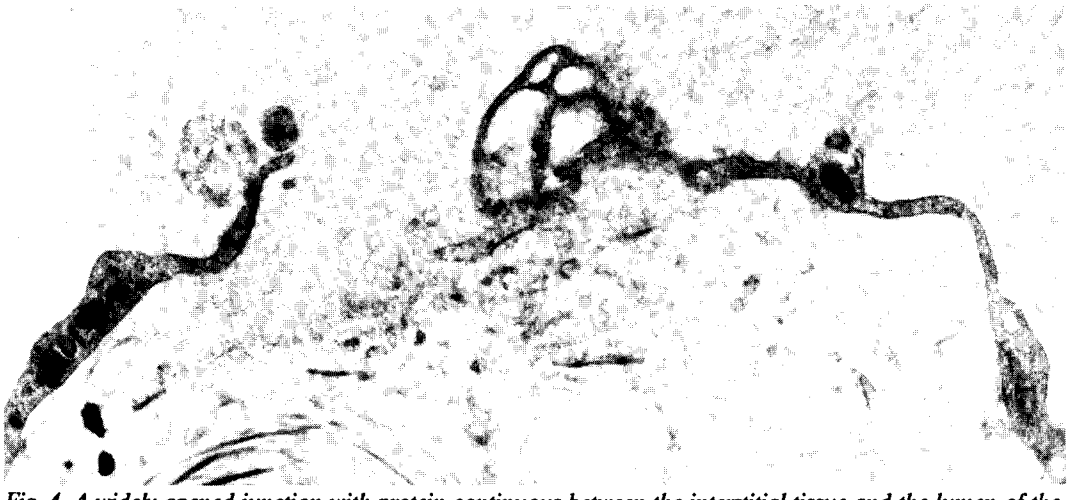

Fig. 4. A widely opened junction with protein continuous between the interstitial tissue and the lumen of the sac. x15,000.

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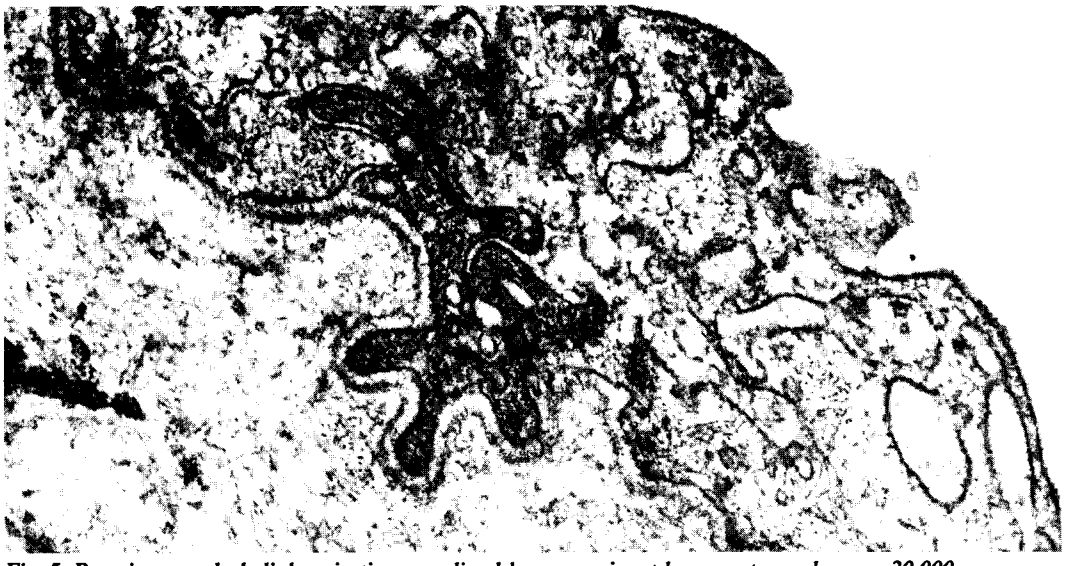

Fig. 5. Prominent endothelial projections are lined by a prominent basement membrane. x30,000.

this material, extending from the tissues to the lumen of the sac.

DISCUSSION

It is evident that the endothelium of the lymph sacs is similar to that of the initial lymphatics of mammals (1-5). The contents of the endothelial cells of the sacs also resemble those of endothelium in mammals. They usually exhibit an irregular shape and a thin cytoplasm. The endothelium often has processes on the luminal surface. There are many abluminal projections, and these are more pronounced than in mammals.

The intercellular junctional types are also similar to mammalian lymphatics. There are perhaps more open junctions in the lymph sacs than in quiescent mammalian initial lymphatics, but fewer than in active mammalian lymphatic regions (1,2). Clearly, there are open junctions, usually filled with what appeared to be plasma proteins. Thus, these amphibian structures probably also form inlet valves. They are often linked to the interstitial tissue and even the adjacent skeletal muscle by anchoring filaments. These findings, and the frequent and pronounced endothelial projections into the interstitial tissue, suggest that these junctions can open and close with muscular activity, just as do those of mammalian initial lymphatics adjacent to muscles (e.g., in the diaphragm and intestinal villi).

It thus seems likely that the endothelial intercellular junctions of the amphibian lymph sac act in a similar manner to those of a mammalian initial lymphatic. It probably closes during muscular contraction, when the lymphatic endothelial cells are telescoped over one another and muscular compression raises the hydrostatic pressure within the lymph sac. During muscular relaxation, the endothelial cells "stretch", become less overlapping and with the concomitant reduction in lymph sac hydrostatic pressure, junctions open apart, permitting fluid and protein to enter from adjacent interstitial tissue.

ACKNOWLEDGEMENTS

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