Light and Electron Microscopic Observations on the Lymph Vascular Systems in the Hamster Testes

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Abstract. The existence and the distribution of the lymph vessels in the hamster testis were studied morphologically in detail. By the perfusion method for fixation, the lymph vessels were observed in the interstitial tissue just adjacent to the seminiferous tubules with the light and electron microscope.

The penetrating of the lymph vessels through the albuginea testis was confirmed and as a result of tracing these lymph vessels, the coarse net works of the lymph capillaries were reconstructed and figured. The lymphatic net works were recognized to anastomose each other and their branches were situated just close to the seminiferous tubules and the interstitial cells. In the electronmicroscopic observation, these lymph capillaries showed the closed system and so their free endings were not opened.

The tissue spaces had no direct communication to the lymph capillaries but they were suggested to form the sinusoidal pathways of the same protein-rich liquid as that in the lymph vessels, and the important role of the interstitial tissue spaces as the passages of extracellular liquid were discussed.

The existence of the lymph system in the testis has long been recognized on the various species of animals. However, as to the problem whether the lymph vessels are observed or not just near the seminiferous tubules, there are many discrepancies in opinion.

Ludwig and Tomsa [16], Frey [6] and Regaud [23] reported that the tissue channels existed among the interstitial cells and opened directly into the lymphatics in the septules. That is, the lymphatics were suggested to form an open system.

Rény-Vámos [20-22], von Brzezinski [27], Staut and Wenzel [26], and Wenzel and Kellermann [31] argued that the lymphatics

were recognized only in the septules, therefore the extracellular fluid in the interstitial tissue was thought to drain rapidly into these lymphatics by way of the endothelial walls.

The lymphatic capillaries, on the other hand, were observed near the seminiferous tubules with the light microscope by von Mihalkovics [28], Gerster [7], Hasumi [8] and Hundeiker [10], and further with the electron microscope by Fawcett et al. [5] and McIntosh and Morris [18].

This study was undertaken to clarify the confirmation of morphological shape, distribution and flow of the both lymphatic capillaries and tissue channels in detail.

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Materials and Methods

The male adult golden hamsters (*Mesocricetus auratus*) were examined.

The testes fixed in Allen-Bouin and Susa solution by perfusion, embedded in paraffin and cut serially in 5–7 μ thick. Some testes, which were injected directly the Chinese ink (0.05–0.1 ml) into the parenchyma after the animals were anesthetized by sodium pentobarbital, were fixed in the same fixative after a few minutes. Moreover, the lymph vessels observed in the paraffin sections and the Epon embedded thicker sections were drawn out on the glass plates and reconstructed by means of placing them in layers.

For the electron microscopic observations, the animals were anesthetized by sodium pentobarbital and then Hanks' or Ringer solution was perfused through the thoracic aorta with the catheter at 37°C, 100 mmHg. After about 5 minutes, cacodylate-buffered 4% paraformaldehyde-5% glutaraldehyde, according to the method of Karnovsky [11], was perfused continuously at 4°C for 20 to 45 minutes. The testes were then cut into small pieces and immersed in the same fixative for an additional 90 minutes. After repeated rinsing in the same buffer, the tissues were fixed again in s-collidine or phosphate-buffered 1–2% osmium tetroxide solution for 60–90 minutes at 4°C. Then they were rapidly dehydrated through alcohol series and embedded in Epon. The ultra-thin sections were stained with uranyl acetate and lead citrate. The thicker sections (1–2 μ) cut with the glass knives were stained with toluidine blue for the light microscopic observations.

Results

When the injection with Chinese ink into the parenchyma of the testis under anesthesia was done, within 3–5 minutes the lymph vessels continuing through the albuginea testis were filled with ink.

It was demonstrated in the paraffin sections that the lymph vessels in the testis passed through the "small-holes" of tunica albuginea and connected with the another lymph vessels outside the testis. These "small-holes" were observed sparsely on the upper halves of the albuginea testis with the serial sectioning method. Their shapes were

very irregular and their sizes were about 50–150 μ in diameter (Fig. 1). On account of this character of the morphological location of these small-holes, it was indicated to be very useful for the investigation of the whole lymphatic system by tracing along these vessels.

The lymph vessels penetrating into the testis ran at first parallel to the rather large blood vessels just under tunica albuginea. Then the lymph vessels branched off the lymph capillaries into the deeper areas in the testis, but they were observed only in the depth of 3–5 times of a diameter of seminiferous tubules (Fig. 2).

The anastomoses between the larger lymph vessels and the smaller ones were persued by the method of reconstruction. The result of this examination clearly showed that the lymph vessels formed the loose irregular networks in the connective tissue spaces on the testis.

The distal ends of the lymph vessels, which were sometimes observed in the networks of the lymphatics, were always closed by the extension of the endothelial cytoplasm and further tapered slender or ended as the very small sac.

With the electron microscope, the similar configuration of lymph vessels was observed as ones in the light microscopic observations. The shapes of these lymph vessels were very irregular and embraced by a few layers of fibroblasts and collagen fibers. Their endothelial cells were generally very thin but very ampullar only at the places where the nucleus was included. In their cytoplasm, the mitochondria, ER and fine filaments such as myofilaments lied sparsely, especially a great number of vesicles were observed in the whole of the cytoplasm. And these vesicles often adhered to cell membranes. Sometimes, the overlapping terminal margins of the adjacent endothelial cells were

observed but generally most of them interdigitated each other. In this way, the adjacent endothelial cells apposed closely without separation to show the wide intercellular clefts. The anchoring filaments, which were studied in detail by Leak and Burke [14, 15], were sometimes observed at the outer surfaces of the endothelial cells.

In the deeper areas of the interstitial tissues in the testis, there were the same lymph vessels as those of the light microscope. Their configurations were similar to the lymph vessels under the albuginea, however, the number of them decreased strictly and the references of them to the another tissue were very characteristic because of the figure that the loose connective tissue enclosed the lymph vessels.

Around the seminiferous tubules, a layer of tunica propria consisting of a basement membrane and a contractile myoid cell layer was there and outside of a tunica propria, one or more layers of fibroblasts were observed. The areas between those fibroblast layers and other interstitial components were filled by the many slender fibroblasts. Almost of collagen fibers, especially, had a tendency to be in only one side of fibroblasts. In the intercellular spaces between the two parallel fibroblasts, whose one accompanied with the seminiferous tubules and the other opposite interstitial components, there were few collagen fibers but the fine flocculent precipitates. These precipitates were interpreted to be a products of coagulation of protein-rich tissue liquid. The intercellular junctions of this fibroblasts were very loose and often formed large spaces without neighbour cells. These spaces were suggested to form the sinusoidal pathways of the extracellular liquid, because the liquid only in this spaces but other ones was adulterated with the Chinese ink particles with the injection method and fur-

ther the morphological characteristics of intercellular spaces incompletely bordered by the fibroblasts were thought to be very easy for extracellular liquid to flow into these spaces.

Discussion

On the occasion of investigation on the testicular lymph systems, the difficulties of preparing the testis were certified as follows. (1) On account that the interstitial tissue of the testis is a very loose connective tissue, it tends to result in making the shrinkage or other artefacts in the course of preparing. (2) The lymphatic vessels are not easily distinguishable from the interstitial cells even by the various stainings. In addition, since the lymphatic endothelial cells are very thin and their lumens in normal tissues are usually very irregular, it is difficult to distinguish them from the cleft in the specimen without misleading. (3) The injection techniques are not sufficiently useful to demonstrate the lymph vessels, because most pigments translate rapidly into the lumen through the endothelial layer of the lymphatics, so it is difficult to distinguish the lumen from the interstitial spaces.

The use of the perfusional fixations contributed to a solution of these problems with satisfactory results. By means of this method, some configurations of the lymph vessels were showed in the hamster testes. That is, the lymph vessels entering into the parenchyma of the testis flew parallel to the blood vessels at first and then branched off the capillaries into the deeper area in the interstitial tissue. It was observed that those capillaries composed the coarse networks and sometimes formed the blind-sac at the distal portion.

By the way, the relations between the lymphatic capillaries and the surrounding tissue have been the subjects of much dis-

cussion since about 1860. Von Recklinghausen [29] maintained that the fine channels (Saftkanälchen), running among the interstitial cells, opened directly into the open system of the lymphatics. His [9], Clark and Clark [2], and Webb and Nicoll [30], however, demonstrated that the lymph vessels were limited throughout by a definite membrane, so that in this way the terminal ends of the lymphatics were thought to be closed. This view was confined by our observations using the reconstruction method.

In the injection technique, after 1–3 minutes of injection into the parenchyma of testis, the lymph vessels were traced with the particles of the ink, but the particles mainly flew at first through the tissue spaces lined by the fibroblasts. This phenomenon was suggested that the lymphatic system might function physiologically as the open system regardless of the morphologically closed system.

Concerning this phenomenon Leak [12, 13] studied morphologically on the permeability of various kinds of substances into the lymphatic capillaries from the surrounding interstitial tissue. According to this report, the points of close approximation ($30\text{--}40\ \mu$) were occasionally observed, but in many cases, it was demonstrated in the electron micrographs that the widths of up to $0.5\ \mu$ may extend the total length of the intercellular cleft to form patent junctions (open junctions).

Although in our observations by the perfusion method of fixatives there were usually the points of the close approximation, the open junctions were not observed in any cases. This difference was supposed to be caused by the artefacts of preparation processes, and the fact that the large particles of the Chinese ink could pass rapidly through the endothelial cells, may agree with the view of the presence of the open

junction. But another transporting process such as the active transport, that is, the stimulus of the alien substances which makes the endothelial junctions open, may possibly indicate the existence of the fine filaments like the myofilaments in the smooth muscle cells.

Ludwig and Tomsa [16], Frey [6] and Regaud [23] reported that the tissue channels among the interstitial cells in the lobules of the testis connect directly into the open lymph vessels in the septula. According to these authors, the lymphatic system seems to be open in the testis and the tissue channels are also a part of the lymphatic system. But our investigation denied the both opinions distinctly. Namely, the lymphatics are closed as stated before and the tissue spaces are not a part of the lymphatic system morphologically. The tissue spaces in the testis, however, are conjectured to be the very short-circuit-like and sinusoidal pathways for the liquid because of the phenomenon of the ink particles injected.

Concerning the tissue spaces in the testis, Mancini et al. [17] studied on the extravascular diffusion of fluorescein-labeled serum protein in the rat testis and demonstrated a rapid passage of intact protein through the blood vessel walls. In earlier quantitative studies using iodinated albumen, Everett and Simons [4] found that a marked increase of labeled albumen content with time after injection was characterized by having a loose areolar connective tissue like that in the intertubular spaces of the testis. It was later confirmed by Setchell [24, 25] and Cowie et al. [3] using the ram's testes. These studies showed that the tissue spaces were functionally significant in spite of not connecting directly with the lymph vessels. As described in the observations, the tissue spaces presented the sinusoidal distribution between the both sides,

whose each was formed with the fibroblast cells. Hence it suggests that the liquid in the extracellular spaces flows rapidly through them as the pathways.

As mentioned above, the lymphatic system was closed distinctly at the distal portions or anastomosed each other. The tissue spaces, on the other hand, were thought to form the sinusoidal liquid pathways. Therefore there was no morphological communication between the lymph vessels and the tissue spaces. The "sinusoidal lymphatic capillaries" noted by Fawcett et al. [5] were not observed in our investigations, but they seemed to be probably the tissue spaces forming the walls bordered by fibroblasts.

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Explanation of Figures

- Fig. 1. Lymphatic vessels entering into the parenchyma of the testis through the pin-hole of albuginea testis. $\times 200$.
- Fig. 2. Lymphatic vessels distributing in the interstitial tissue are showed (arrow) in the section stained with toluidine blue. $\times 500$.
- Fig. 2-a, b, c, d. Higher magnification of the lymphatic vessels demonstrated in Fig. 2. $\times 800$.
- Fig. 3. Lymphatic vessels just under albuginea testis are surrounded by the many fibroblasts. $\times 3,000$.
Ly: Lymphatic vessel, Al: Albuginea testis, A: Testicular artery.
- Fig. 4. Lymphatic capillary and intertubular capillary in interstitial tissue. Lymphatic endothelial cells are very thin and irregular and blood vessel perfused sufficiently is spread roundly. $\times 4,000$.
Ca: Capillary.
- Fig. 5. Higher magnification of a part of Fig. 4.
- Lymphatic capillary among interstitial cells, blood capillary and tissue space. Endothelial junctions are two types of over-lapping and interdigitation. $\times 6,000$.
- Fig. 6. Lymphatic endothelial cell junction. Note the close apposition of the endothelial cells (arrow). $\times 24,000$.
- Fig. 7. Close apposition of lymphatic endothelium in higher magnification. $\times 40,000$.
- Fig. 8. Typical tissue spaces bordered by the fibroblasts. Fibroblasts show always no throughout continuity. Blood cells happen to appear by artefact. $\times 3,000$.
F: Fibroblast.
- Fig. 9. A part of network of lymphatic vessels in the testis illustrated by reconstruction method. Distal end (arrow) and anastomosis (double arrow) was demonstrated in this method. Outside lymphatic vessel enters through the pin hole. $\times 400$.