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**Anatomy**

# Morphology and Ultrastructure of the Collecting Lymph Vessel, Lumbar Trunk and Thoracic Duct in the Rat

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**Abstract** **Original Research Article**

***Objective:*** To determine morphological and ultrastructural details of the collecting lymph vessel, lumbar trunk and thoracic duct of the rat. ***Methods:*** Sixteen adult SD rats were utilized for the study. A small amount of 6% hydrogen peroxide was applied to find the lymph vessel in the dorsodistal hind-limb and sides of the abdominal aorta in the rat. Under a surgical microscope, the vessel was injected by Indian ink or a radiopaque mixture via a fine needle to locate sites of the collecting lymphatic vessel, lumbar trunk and thoracic duct. Vessels were then harvested for the histological and transmission electron microscopic examinations. ***Results:*** Diameters of the collecting lymph vessel, lumbar trunk and thoracic duct in the rat were diverse, small in the former and large in the latter. Containing multiple valves in the lumen, their walls were very thin and composed of three layers of tissue. Ultrastructures of the tunica intima and externa of these vessels were similar. A single layer of endothelium cells with a discontinuous basement membrane formed the tunica intima of the vessel. One or two discontinuous layers of smooth muscle cells was found in the tunica media of the collecting lymph vessel, one to three layers of smooth muscle cells in the lumbar trunk and three to five layers of smooth muscle cells in the thoracic duct. The tunica externa of the vessel was comprised by connective tissue (collagen fibrils) and fibroblasts. ***Conclusion:*** Morphological and ultrastructural details of the collecting lymph vessel, lymphatic trunk and duct in the rat have been demonstrated that may help for the purpose of further lymphatic vessels studies.

**Keywords:** Morphology; ultrastructure; collecting lymph vessel; lumbar trunk; thoracic duct; rat.



## INTRODUCTION

As a part of the circulation system, the lymphatic system plays an important role in human health and disease [1-3]. It is also involved in many diseases including tumor metastasis, lymphedema, inflammation, cardiovascular diseases and so on. Detailed basic knowledge of the lymphatic system will provide great significance for the prevention and treatment of related diseases. Although the morphology of lymphatic vessels in human and animals have been mentioned previously [4-9], the ultrastructure of lymphatic vessels needs to be understood in certainty. Due to the thin, colorless and transparent characteristics of the lymphatic vessel wall, it is difficult to identify using traditional anatomical methods. Since the establishment of new lymphatic micro-perfusion technology, the research of the lymphatic system has made great progress [10]. Therefore, further studies should be carried to meet the needs of precise clinical management, modern education and advanced scientific research.

In this study, morphological details of the collecting lymph vessel, lumbar trunk and thoracic duct in the rat are described and demonstrated.

## MATERIALS AND METHODS

Experiments in this study were approved by the Animal Ethics Committee of Xuzhou Medical University (L20210226466, Jiangsu, China) and performed under the guideline for Care and Use of Laboratory Animals of Xuzhou Medical University.

Sixteen adult SD rats were utilized for the study. Each rat received an intraperitoneal injection with 10% chloral hydrate (0.35ml/100gm) (CAS# 302-17-0, BBI Life Sciences, Shanghai, China) for anesthesia prior to the experiment. For finding the lymph vessel, a small amount of 6% hydrogen peroxide (Zhonglian Chemical Co., Ltd, Suzhou, China) was drawn by a 1 ml syringe with a needle and injected into the dermis and subcutaneous tissue of the dorsodistal hind-limb and sides of the abdominal aorta in the rat. Under a surgical microscope (Leica Microsystems Ltd, Heerbrugg, Switerland), the collecting lymphatic vessel, lumbar trunk and thoracic duct were found. A fine needle was inserted and an Indian ink mixture (Indian ink: Anhui Red Star Ink Industry Co. Ltd., China; 4% paraformaldehyde solution; Ratio 1:20) or a radio-opaque mixture (Barium Sulphate 15g: Shanghai Silian Indusry Co. Ltd., China; Milk powder 5g: Heinz Ltd., Qingdao, China; Concentrated poster color - dark green 3g: Liaoyuan arts and stationery Ltd., Hunang, China; Water 20 ml). Vessels were harvested and fixed in 4% paraformaldehyde (PFA) solution for histological section and hematoxylin-eosin (HE) stain, and in a solution of 4% PFA mixed with 2% glutaraldehyde (GA) for transmission electron microscopic (TEM) examination.

## RESULTS

Detailed morphological information of the collecting lymph vessel, lumbar trunk and thoracic duct in the rat were obtained and presented as follows:

**1. Collecting lymph vessel**

Collecting lymph vessels with their tributaries were found in the subcutaneous tissue of the dorsolataral hind-limb of the rat. They travelled concentrically along with the major vein. They merged and then drained into the popliteal lymph node (Fig. 1A). Diameters of the vessels were 0.1 to 0.4 mm (average 0.2 mm). Vessels had a very thin wall and contained multiple valves in the lumen (Figs. 1B, 1C). A single layer of endothelium cells with a discontinuous basement membrane formed the tunica intima of the vessel. Various types of cellular junctions including tight, adherens and desmosome junctions were observed between the endothelial cells. Containing a larger spindle shape of nucleus, endothelial cells were thicker in the perinuclear zone and thinner in the rest. A higher electron density zone was detected in the peripheral area of the nucleus, while a lower electron density zone in the centre. Numerous vesicles of the plasma membrane were observed in the endothelial cytoplasm. At the plasma membrane, vesicles opened into the lumen or intercellular substance. Some protrusions of the plasma membrane protruded into the lumen were noticed. The tunica media of the vessel was constituted by one or two discontinuous layers of smooth muscle cells and connective tissue (collagen fibrils). The tunica externa of the vessel was comprised by connective tissue (collagen fibrils) and fibroblasts (Fig. 2).

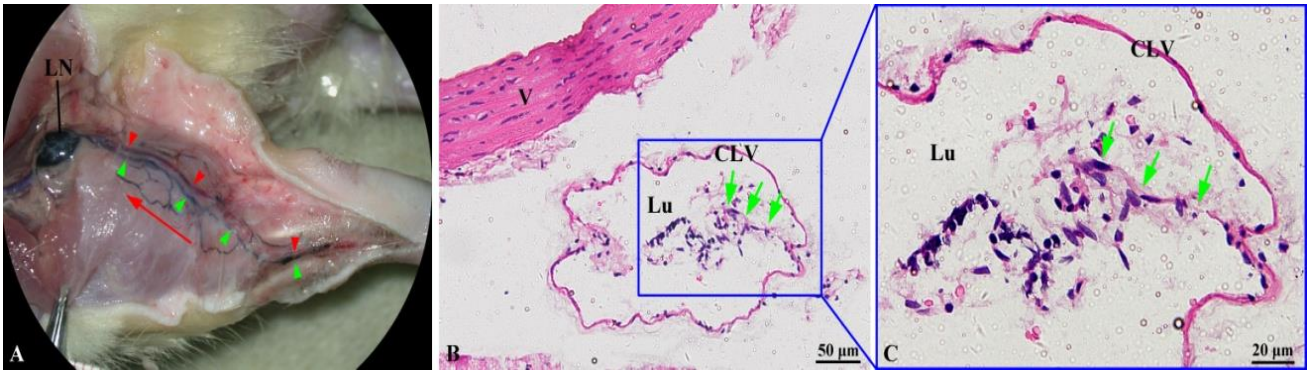


**Fig-1: The collecting lymph vessel in the hind-limb of the rat**

A. The collecting lymph vessel (green arrowheads) with tributaries travel with the major vein (red arrowheads) and drain into the popliteal lymph node (LN). B. The histological result shows that the collecting lymph vessel (CLV) has a thin wall comparing with the venous (V). C. Magnified image from blue boxed area of the image B shows the thin wall of the lymphatic vessel. Red arrow indicates the flow direction of the lymph. Green arrows indicate the valves in the lumen of the lymphatic vessel. Lu = lumen.

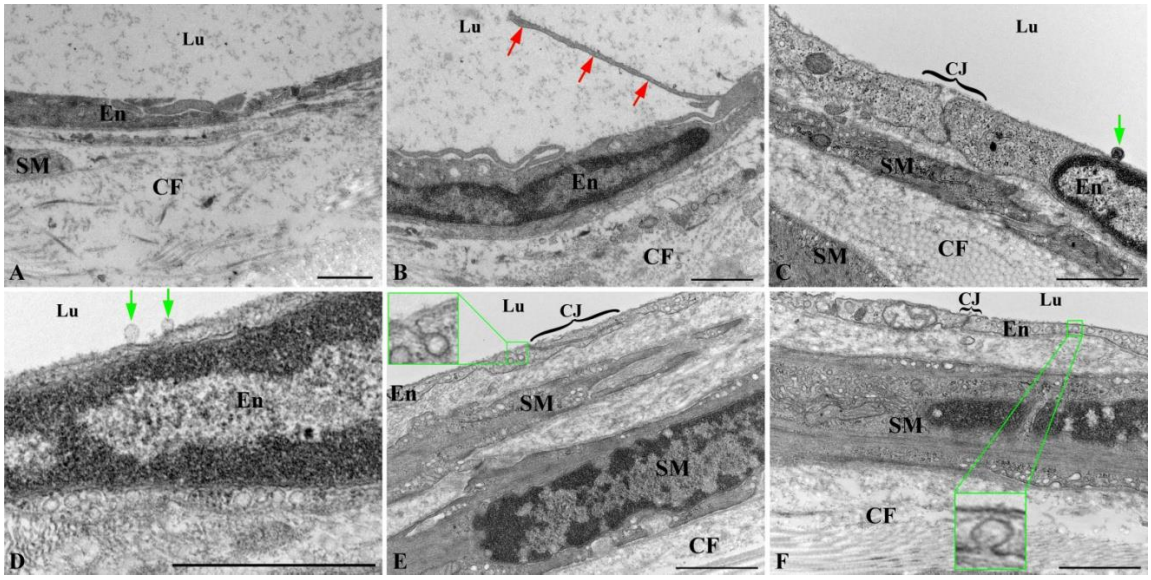


**Fig-2: Ultrastructure of the collecting lymph vessel in the hind-limb of the rat**

A. A discontinuous layer of smooth muscle cell (SM) lies beneath the endothelial cell (En) with a discontinuous basement membrane. B. The endothelial cell with the valve (red arrows) of the vessel. C. Two layers of smooth muscle cell (SM) situate beneath the endothelial layer. D. Protrusions of the plasma membrane (green arrows) protrude into the lumen of the vessel. E & F. Green boxed areas indicate that vesicles of the plasma membrane open into the lumen (in image E) or intercellular substance (in image F). En = endothelial cell; SM = smooth muscle cell; CJ = cellular junction; Lu = lumen; CF = collagen fibrils; Bars = 1μm.

**2. Lumbar trunk**

A pair of lumbar trunks was found on both sides of the abdominal aorta (Fig. 3 A). They originated from iliac lymph vessels or efferent lymph vessels of iliolumbar lymph nodes, and drained into the chylous cistern. Diameters of vessels were 0.3 to 0.9 mm (average 0.6 mm). Lumbar trunk had a thin wall and contained multiple valves in the lumen (Figs. 3B, 3C). A single layer of endothelium cells with a discontinuous basement membrane formed the tunica intima of the vessel. Various types of cellular junctions including tight, adherens and desmosome junctions were observed between endothelial cells. Numerous vesicles of the plasma membrane were observed in the endothelial cytoplasm. At the plasma membrane, vesicles opened into the lumen or intercellular substance. It was noticed that some protrusions of the plasma membrane protruded into the lumen. The tunica media of the lumbar trunk was constituted by one to three layers of smooth muscle cells and connective tissue (collagen fibrils). The tunica externa of the vessel was comprised by connective tissue (collagen fibrils) and fibroblasts (Figs. 3D to 3F).

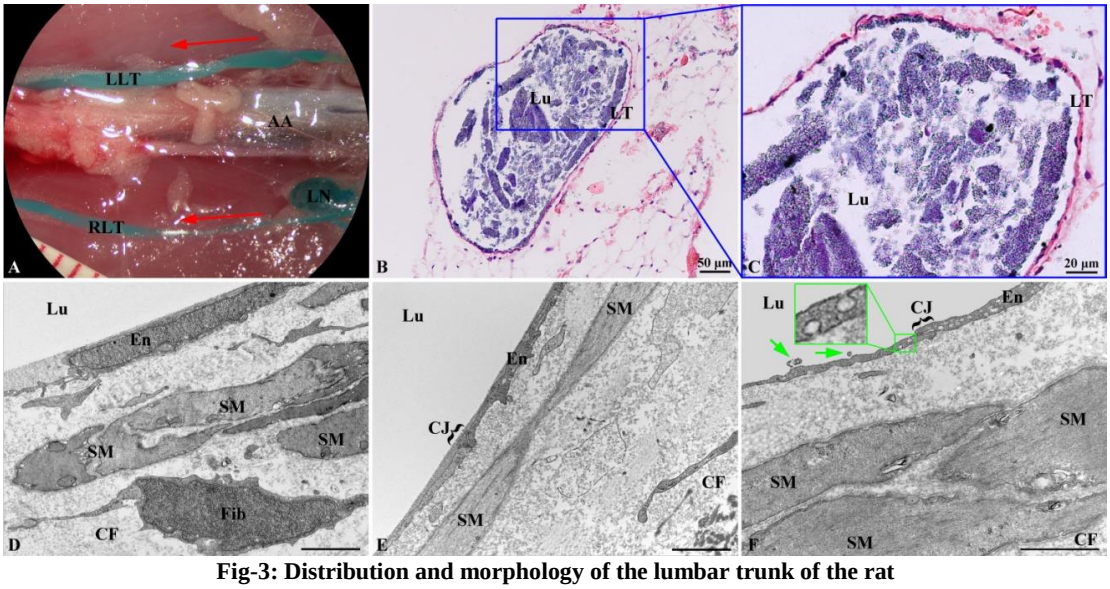


**Fig-3: Distribution and morphology of the lumbar trunk of the rat**

A. A pair of lumbar trunks travels on both sides of the abdominal aorta (AA). B. The histological result shows that the lumbar trunk (LT) has a thin wall compared with the venous in Figure 1B. The lumen of the vessel is filled with injectant. C. The image is magnified from the blue boxed area in image B. D. Double layers of smooth muscle cells (SM) form the tunica media. E. A single layer of smooth muscle cells (SM) situate beneath the endothelial layer. F. Triple layers of smooth muscle cells (SM) form the tunica media. Red arrows indicate the flow direction of the lymph. Green arrows indicate protrusions of the plasma membrane. Green boxed areas indicate that vesicles of the plasma membrane open into the lumen or intercellular substance. LLT = left lumbar trunk; RLT = right lumbar trunk; LN = iliolumbar lymph node; Lu = lumen; En = endothelial cell; Fib = fibroblast; CJ = cellular junction; CF = collagen fibrils; Bars in images D to F = 1 μm.

**3. Thoracic duct**

Originating from the chylous cistern, the thoracic duct ascended on the right posterior side of the thoracic aorta and terminated at the left jugular angle (Fig. 4A).

Diameters of vessels were 0.6 to 1.0 mm (average 0.8 mm). The thoracic duct had a thin wall and contained multiple valves in the lumen (Figs. 4B, 4C). A single layer of endothelium cells with a discontinuous basement membrane formed the tunica intima of the vessel. Various types of cellular junctions including tight, adherens and desmosome junctions were observed between endothelial cells. Numerous vesicles of the plasma membrane were observed in the endothelial cytoplasm. At the plasma membrane, vesicles opened into the lumen or intercellular substance. It was noticed that some protrusions of the plasma membrane protruded into the lumen. The tunica media of the thoracic duct was constituted by three to five layers of smooth muscle cells and connective tissue (collagen fibrils). The tunica externa of the vessel was comprised by connective tissue (collagen fibrils) and fibroblasts (Figs. 4D to 4G).

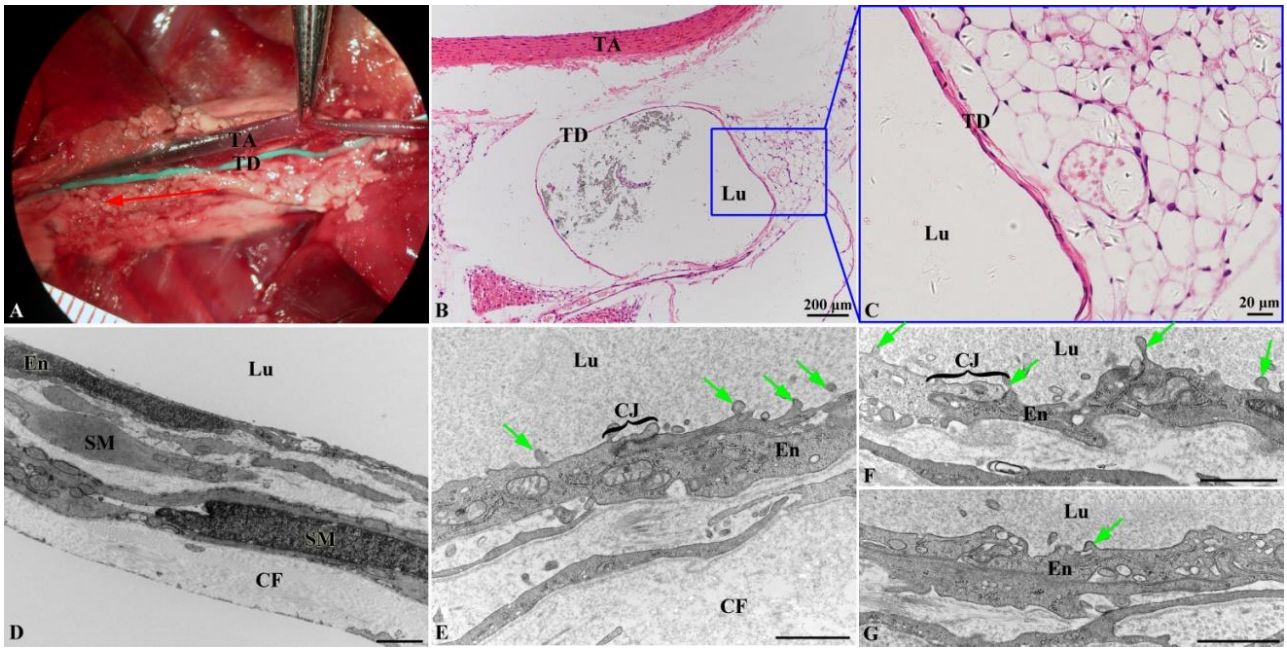


**Fig-4: Distribution and morphology of the thoracic duct of the rat**

A. The thoracic duct travels on the right posterior side of the thoracic aorta (TA). B. The histological result shows that the thoracic duct (TD) has a thin wall compared with the thoracic aorta (TA). C. The image is magnified from the blue boxed area in image B. D. Triple layers of smooth muscle cells (SM) form the tunica media. E to G. Protrusions (green arrows) of the plasma membrane protruded into the lumen. Lu = lumen; En = endothelial cell; CJ = cellular junction; CF = collagen fibrils; Bars in images D to G = 1 μm.

## DISCUSSION

The morphology of lymphatic vessels in human and animals have been mentioned in the past [4-13], however the ultrastructure of lymphatic vessels needs to be understood in certainty. In this study, the detailed morphological structure of the collecting lymph vessel, lumbar trunk and thoracic duct of the rat have been presented. It should be noted that the most distinctive difference of the ultrastructural feature among them was the quantitative change in the number of smooth muscle layers of the tunica media (Figs 2, 3D to 3F, 4D). As can be seen from figures 1B and 4B, the artery and vein have many layers of smooth muscle

cells in the tunica media [11] comparing with lymphatic vessels of the rat. Besides, it has been shown that the collecting lymph vessel of the human [12] and the thoracic duct of the monkey [13] have more layers of smooth muscle cells in the tunica media than those in lymphatic vessels of the rat.

The experiment of lymphatic tissue engineering in vitro has been conducted for creating human tissues to meet clinical needs for viable tissue grafts [14-16]. However, experiments mostly focused on tissue engineering of lymphatic capillaries. The information from this study may provide a morphological and ultrastructural basis for tissue engineering of the collecting lymph vessel, lymphatic trunk and duct.

## CONCLUSION

Morphological and ultrastructural details of the collecting lymph vessel, lymphatic trunk and duct in the rat have been demonstrated that may help for purpose of further lymphatic vessels study.

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**Author Disclosure Statement**

No competing financial interests exist.

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