

## A MODIFIED RAT MODEL FOR CANNULATION AND COLLECTION OF THORACIC DUCT LYMPH

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### ABSTRACT

*Difficulty in collecting lymph samples in small animals has impeded studies on lymphatic function and lymph composition. Here we report a simple and effective modified rat model for thoracic duct lymph drainage where animals remain in full consciousness and have free movement and access to water and food over 12 hours. The operative procedure required approximately 30 minutes to perform. Mean lymph drainage was  $0.71 \pm 0.33$  ml/h, and protein concentration did not change significantly (mean  $37 \pm 2.59$  mg/ml) over the 12 hours. However, the number of lymphocytes fluctuated widely between  $0.08 \pm 0.03 \times 10^6$ /ml and  $12.17 \pm 6.58 \times 10^6$ /ml. This modified animal model of thoracic duct lymph collection avoids influences of lipid intake, general anesthesia, or limited activity of animals on experimental outcomes, and therefore more accurately reflects lymph flow and composition under normal physiological conditions.*

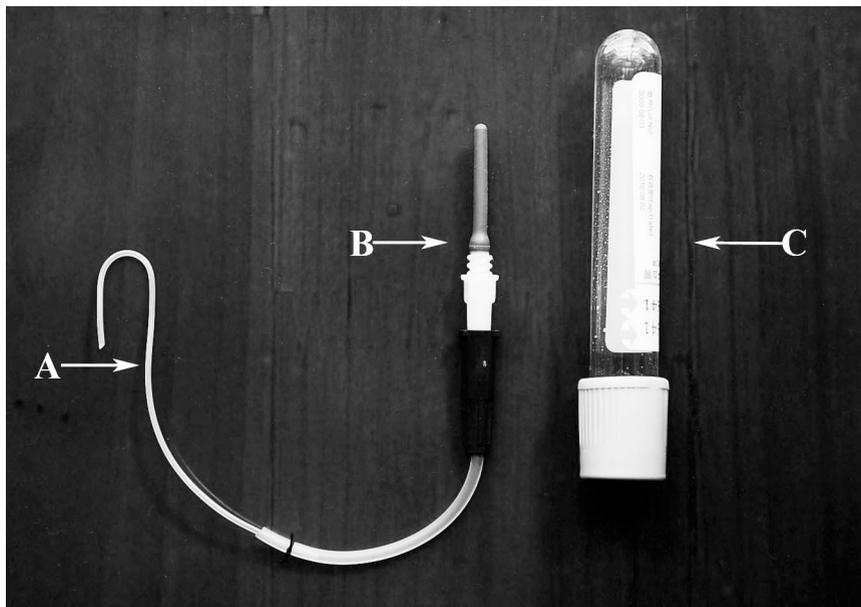
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The lymphatic system plays important roles in body fluid homeostasis, lipid absorption, metastasis, and immune function as well as a key component in maintaining normal interstitial fluid volume and protein

concentration (1). Disturbances from severe injury, burns, and peritonitis may cause systemic inflammatory response and multiple organ dysfunction (2), and these are reflected in lymph flow and composition as are a variety of other disorders.

Various animal models of lymph drainage sampling the gastrointestinal lymphatic route have been used for evaluating drug absorption and distribution. Many of these studies have been initiated in rats because experiments in large animals are now more restricted by bioethical and economic considerations. Advantages of these models include that drugs absorbed directly into the lymphatic system can avoid the first-pass effect through the liver, allowing absorption and distribution of lipid-soluble drugs to be studied more effectively. In addition, immunoregulatory factors or chemicals used for treatment of metastatic cancers could also achieve optimal therapeutic effects if they are delivered directly through the lymphatic system and not the blood vascular route.

In the present study, we modified a previous lymph drainage method (3) for a simpler and more effective thoracic duct lymph collection in which the rats are fully conscious and have free movement and access to water and food providing a method for long-term study of the lymphatic circulation with minimal interferences. It is not necessary to feed fat to the rats in advance,



*Fig. 1. The lymph collecting system consists of the PE collection tube with the beveled edge on the U curve (A) which is securely connected to a blood collection needle (B). The needle is then inserted into a standard blood collection tube (C).*

restrain them by means of general anesthesia, or use intravenous fluid replacement or gastric gavage. Thus, this model minimizes factors that may influence formation and composition of thoracic duct lymph and ensures examination, intervention, and treatment of the animals in a situation closer to the physiological condition.

#### **MATERIALS AND METHODS**

##### **Animals**

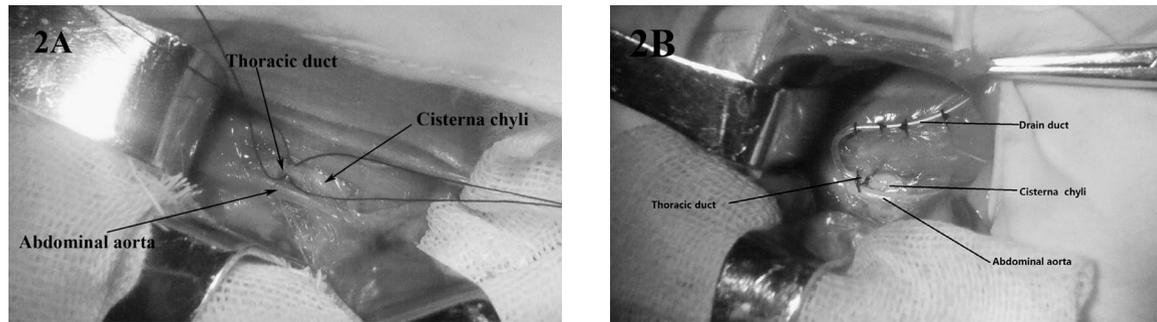
Twenty clean adult male SD rats weighing 180 -200g (Shanghai Animal Center of the Chinese Academy of Sciences) were acclimated in our laboratory for one week and fed with normal diet in a 12-h dark/light cycle. The protocol of the animal experiment was approved by the Ethics Committee of the Second Military Medical University (Shanghai, China). All procedures were carried out under aseptic conditions in accordance with the "Principles of Laboratory

Animal Care" (NIH publication No. 85-23 revised 1985). The protocols for anesthesia, postoperative care, and sacrifice were identical for all animals. Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate at 0.35mL/100g body weight and sacrificed at 24 h by an intravenous overdose of potassium chloride.

##### **Procedures**

One end of a 10-cm PE tube (ID 0.58mm, OD 0.99mm) was immersed in hot water to form a "U" shape with an angle of 180°. The "U"-shaped end was approximately 1.5cm long, and a beveled face (~30°) was made on the open end. The other end was securely connected to a disposable vacuum blood collection needle (*Fig. 1*). This prepared tube system was sterilized with 60% ethylene oxide for use.

The skin around the surgical site was prepared and sterilized with iodophor two times. A 4-cm median incision was made on



*Fig. 2. Anatomy of abdominal thoracic duct and cisterna chyli in rat as seen from the left lateral approach. Before cannulation (A), the cisterna chyli, thoracic duct, and aorta have been isolated and cleared of crossing vessels. Sutures have been placed below the thoracic duct in preparation for ligation. After cannulation (B), the collection tube can be seen as it enters the thoracic duct and passes the ligature into the cisterna chyli. The suturing of the collection tube to the muscle provides stability.*

the upper 2/3 abdomen between the xiphoid process and the pubic symphysis to open the abdominal cavity and reach the retroperitoneal space by separating the perirenal fascia from the left kidney laterally. The left kidney and adrenal gland were isolated medially, and the soft tissue was separated along the surface of the psoas major muscle. The left kidney, adrenal gland, small intestine, and spleen were wrapped with warm saline-soaked gauze to prevent dehydration. Fascia and small vessels running across the surface of the abdominal aorta were dissected, ligated, and cut away from the surface of the abdominal aorta. The cream-colored cisterna chyli and thoracic duct were exposed by pushing the abdominal aorta rightward. The abdominal aorta and the thoracic duct were separated bluntly, and the thoracic duct was separated from the muscle. Two 5-0 Vicryl silk sutures were passed through under the thoracic duct, with one placed near the crura of the diaphragm and the other near the cisterna chyli (Fig. 2A).

Avoiding the major vessels and nerves, a 20-gauge needle was passed through the left abdominal wall to introduce the PE catheter into the abdominal cavity. The catheter was sutured intermittently by 3-4 stitches with a 5-0 silk thread and fixed on the psoas major muscle to prevent displacement or dislodgement

during cannulation. The length and angle of the catheter were adjusted properly to avoid excessive tension after placement of the catheter in the thoracic duct. The thoracic duct was pricked by the tip of a 1ml syringe needle, and the beveled end of the cannula was placed carefully through the puncture site into the thoracic duct and then into the cisterna chyli. Following insertion, one of the silk sutures was tied, and the vacuum blood tube connected. When the cream-colored lymph was seen flowing quickly into the catheter, the second suture was tied to prevent lymph reflux (Fig. 2B).

After assuring no significant active bleeding, the abdominal cavity was irrigated with warm normal saline. The left renal fascia was sutured intermittently and fixed on the retroperitoneal wall, and the peritoneum and the abdominal wall was closed intermittently with two layers of 5-0 silk thread. The rat was then placed in a prone position and rubber loops to hold the vacuum blood collection tube were made and sutured onto the midline of the cervical back of the animal. The blood collection needle was sutured and fixed on the rat back, and the position between the blood collection tube and the needle adjusted properly (Fig. 3). Lymph was collected for 12 hours with a change of the collection tube at every 2 hour time point.



*Fig. 3. The exteriorized collection tube and insertion of collection needle into the attached collection tube is shown in this fully awake rat.*

#### *Precautions during the procedure*

Aseptic technique is required and all materials sterilized before use. Surgical procedures should be performed gently, maintaining distance from the cisterna chyli when separating the thoracic duct to prevent possible injury to its wall. If lymph leakage occurs due to injury, it can be stopped by applying pressure with a cotton swab for several minutes. There are usually small vessels such as the lumbar artery running across the thoracic duct, which may interfere with the procedure. These small vessels can be ligated and cut off to facilitate exposure of the thoracic duct.

After successful cannulation, lymph can be seen quickly flowing into the catheter as soon as the collection tube is connected, and the cisterna chyli empties quickly. If the cisterna chyli is dilated or there is slow flow of lymph into the catheter, this finding may indicate that the catheter is placed improperly. In this case, it is necessary to adjust the catheter until the cisterna chyli can be seen

to empty. The lymph collection system should be fixed properly to the rat. If it is not, it may become crimped and affect lymph drainage. Long-term obstruction may cause lymph coagulation and loss of flow.

#### *RESULTS*

The model has proven successful in collecting thoracic duct lymph over 12 hours in awake animals. Nineteen of the 20 animals in this study were cannulated and the flow was consistent over the 12 hours with an average collection of  $0.71 \pm 0.33$  ml/h (mean  $\pm$  SD) (Fig. 4). The protein concentration did not change significantly over the 12 hours at  $37 \pm 2.59$  mg/ml (mean  $\pm$  SD) (Fig. 5). However, the number of lymphocytes did fluctuate significantly between  $0.08 \pm 0.03 \times 10^6$  and  $12.17 \pm 6.58 \times 10^6$ /ml over the 12 hours of the experiment (Fig. 6).

#### *DISCUSSION*

There are some differences between the

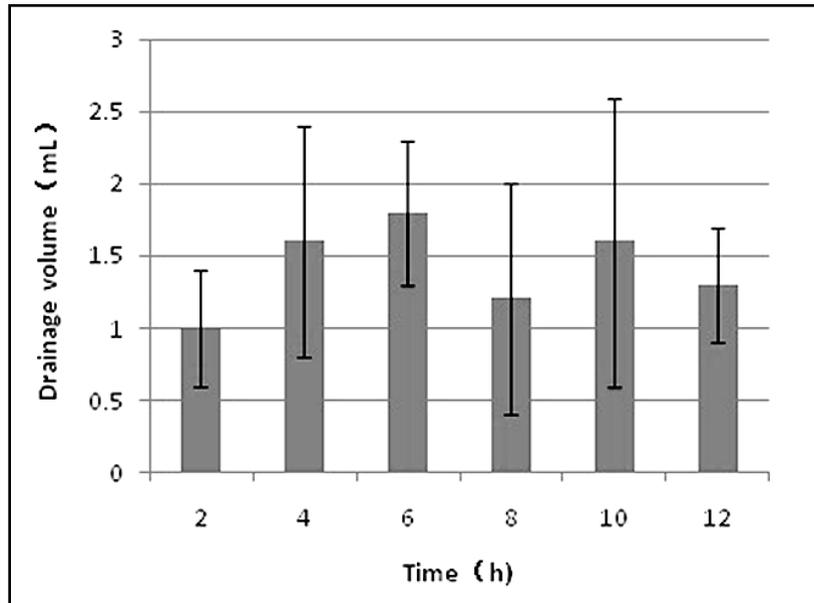


Fig. 4. Mean±SD lymph collection (ml) rates at time points 2-12 hours.

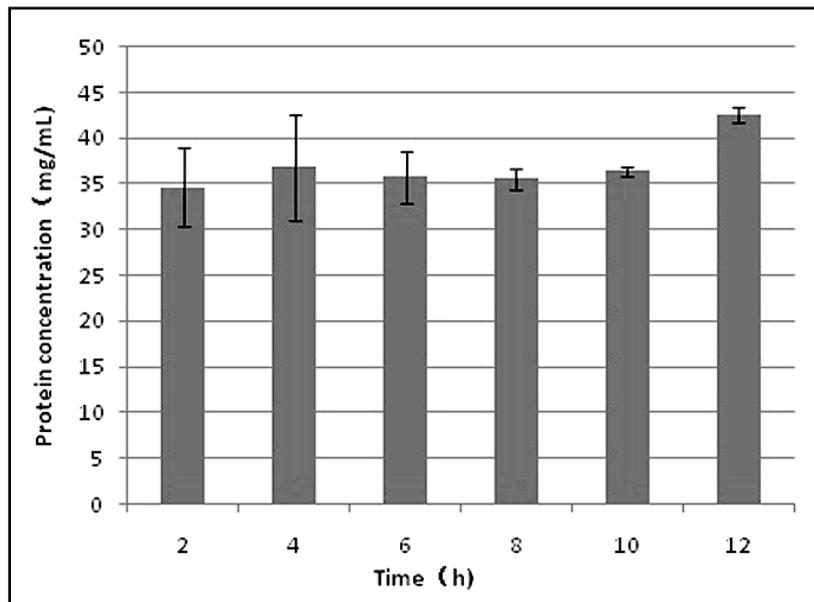


Fig. 5. Mean±SD of protein concentration in lymph at time points 2-12 hours.

flow of lymph drainage found in our study and other studies previously reported in the literature (3,4). The reason may be that the SD rats used in our study were relatively small (180-220g), while other studies usually

used rats weighing 300-350g. Rat size does have a direct influence on the total production of lymph. Boyd et al (4) reported that the amount of lymph drainage from the thoracic duct was  $12.5 \pm 2.5$  ml/h. The difference may

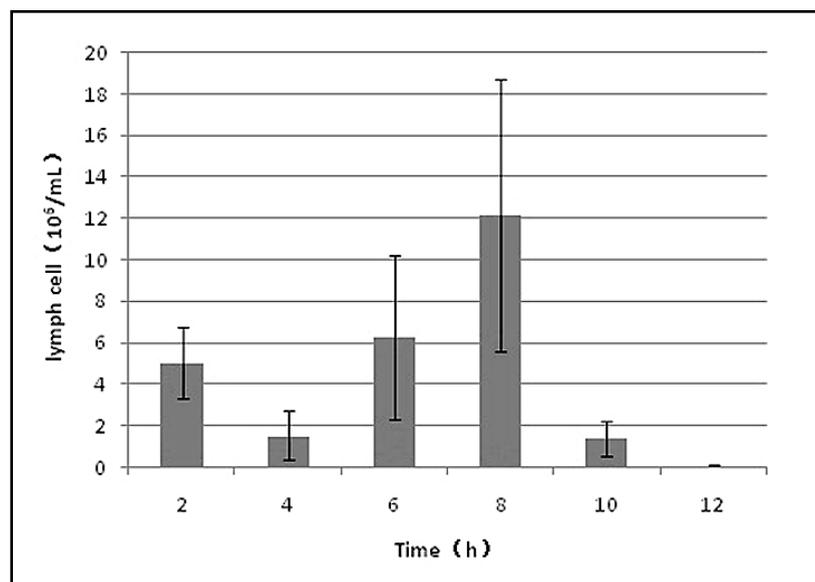


Fig. 6. Mean $\pm$ SD number of lymphocytes ( $10^6/\text{ml}$ ) in lymph at time points 2-12 hours.

relate to their use of olive oil 1 hour before operation and the fluid replacement given during operation. Feeding fat in the initial stage of experiments helps to identify the location and anatomic structure of the thoracic duct, and increased lymph flow by fluid replacement creates a favorable condition for placement of the thoracic duct. However, large amounts of fat intake may interfere with later research measurements on lymph, and the model may not truly reflect lymph under normal physiologic conditions. We feel it is preferable not to feed fat before operation as long as the anatomic structure of the thoracic duct is clearly understood, and cannulation is skillfully applied. In addition, the amount of lymph drainage is closely related to food/water intake. Recognizing that our animals would resume consciousness in about 1 hour after operation and that their free access to food and water would increase or influence the flow, we did not give any fluid replacement to the animals in our study.

Lymph protein concentration is lower than that in the blood. In our study, lymph protein concentration remained relatively stable within the 12 hours of the experiment,

and it did not decrease with the increased amount of lymph drainage. These data support the stability of the model for research purposes.

One might think that since the number of lymphocytes in the blood of normal rats is relatively stable that the number of lymphocytes in lymph should also be fairly stable. In our study, the number of lymph lymphocytes was slightly lower in the early stage of lymph drainage with the lowest level of  $1.5\pm 1.2\times 10^6/\text{ml}$  at the 4-hour point on average. The number of lymphocytes quickly rose again to a mean of  $12.2\pm 6.6\times 10^6/\text{ml}$  at 6-8 hours possibly related to a feedback response with decreased number of lymphocytes in the circulating blood mobilizing lymphocytes from the thymus, spleen, and other central lymphatic organs to enter the lymphatic system and causing the number of lymphocytes in lymph to rise markedly. After a transient rise, the number of lymphocytes began decreasing sharply at 10 hours. The mechanism of this great fluctuation of lymphocytes in lymph is unclear and needs further investigation.

Preparation of the rat thoracic-duct cannulation model is relatively difficult due to its deep anatomic position and limited space for operation. In our study, we prepared the model by entering the retroperitoneal space lateral to the left kidney, which facilitates finding the thoracic duct on the left side of the abdominal aorta. According to the literature, the thoracic duct has three different locations: at the left posterior, right posterior, or behind abdominal aorta (5), and the latter two are anatomic variations. In our study, the thoracic duct was located in left and posterior to the abdominal aorta in 16 rats; posterior in 3; and right and posterior in 1. The length of the thoracic duct from the cisterna chyli to the diaphragm crura averaged 0.8cm (0.6-1.0cm), and anatomic variations rendered operation more difficult. The one failed case was mainly due to the unexpected structure of the cisterna chyli and thinness of the thoracic duct. A second cannulation was attempted but was not successful.

Anticoagulants were used before operation in most previous similar studies. In our study, we did not give the animals any anticoagulants, and only used ethylene diamine tetraacetic acid (EDTA) to treat the collection tube since lymph in the collection tube would coagulate without anticoagulation treatment. Knowing that injection of heparin and other anticoagulants would to some extent cause intraoperative and postoperative hemorrhage and alter the normal physiologic status of the rats, we tried to avoid possible influencing factors during collection of lymph. As observation of our model lasted for only 12 hours, it is not known whether anticoagulation is necessary for longer time periods of drainage.

The lymph collection system used in the present study is original in that it was done without general anesthesia or restraint of the animals, which are stressful maneuvers and may interfere with normal physiologic conditions. Our system makes use of negative pressure of the vacuum blood collection tube to collect lymph in a closed circuit when the

animals are fully conscious and freely movable with free access to water and food, thus minimizing possible interferences with the physiologic activities of the animals. The slight negative pressure in the collection tube could be thought to increase flow, but our flow rates were consistent over time and lower than others reported.

This thoracic duct lymph drainage model can be useful for the study of immunoregulation, drug absorption and distribution, and lipid transport and metabolism. As the intestinal tract plays an important role in the pathogenesis of sepsis, this modified model may also facilitate exploration of the pathogenesis of sepsis through the lymphatic system.

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