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Ductus thoracicus Lymph in Mice
1. A technique for cervical approach

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Summary

A method is described for the collection of lymph from the thoracic duct in anaesthetized mice by a silastic catheter inserted into the duct proximal to the jugulo-subclavian junction. The operative procedure takes about 5 min, and there is less operative trauma to the animals than by the previously used abdominal approach. Lymph flow amounts to 0.396 ml/h and cell content to 38.6 x 10^6/ml.

Introduction

The methods of cannulation described for the mouse (1, 2, 3, 4, 5, 6) are adaptations of Bollmann's (7) abdominal approach in the rat to the thoracic duct at the cysterna chyli. In the rat, however, *Reinhardt* (8), *Saldeen* and *Linder* (9) and *Azargoschasb* (10), reported on thoracic duct cannulation at the neck. Whereas *Reinhardt* (8) gained access to the thoracic duct from the cervical duct, both of the other groups (9, 10), chose its junction with the venous system for cannulation. Generally, the proximal route permits a more quantitative view of lymphatic transport of cells or chemical constituents into the circulating blood than does the approach at the site of the cysterna chyli, since there is a risk of excluding hepatic lymph, which contributes nearly half of thoracic duct flow, at least in anaesthetized mammals (11, 12).

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Animals and Methods

Animals: Male BALBcANn/Han inbred mice (Zentralinstitut für Versuchstiere, Hannover-Linden), 8–10 weeks old, weighing 28–31 g were used. They were held under SPF conditions in polycarbonate cages (Makrolon, type II) on sterile wooden granules in an air-conditioned and light controlled environment (22° ± 1 °C; 55 % relative humidity; light period from 19.00 to 07.00; feeding ad libitum with Altromin 1320; tap water from makrolon bottles). The animals were free from all the pathogens specified in the GV-SOLAS list (13).

Lymph collaction: The animals were anaesthetized by intraperitoneal injection of mixed xylazine (Rompun®, diluted with 0.9 % NaCl to 1.6 mg/ml), and ketamine (Ketavet®, diluted to 10 mg/ml) with 16 mg/kg and 100 mg/kg body weight, respectively. Cannulation was performed between 08.00 and 11.00. It was not necessary to administer olive oil by a gastric tube or to give an intraperitoneal injection of dye for facilitating the identification of the thoracic duct, as often proposed (6, 8, 9, 10). All manipulations and cannulations were carried out under a Zeiss IV/b stereomicroscope (Carl Zeiss, Oberkochen) with a , zoom attachment, using a four point incident illuminator fixed under the objective, with fibre conducted cold light from a step down transformer (KL 150 B; Schott, Mainz). The magnification scale varied between 3.2–20 x. The terms right and left are related to the field of vision of the experimenter on an ani-

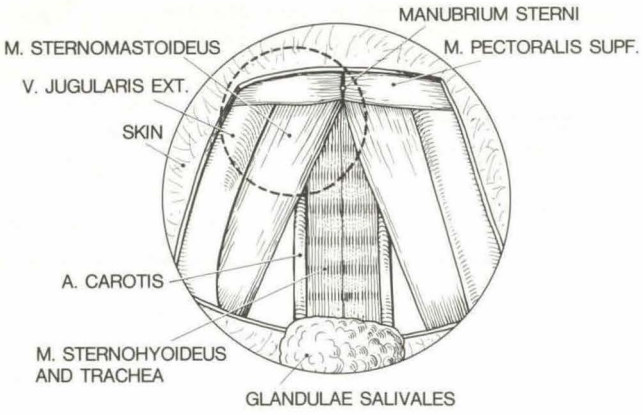

Fig. 1a A schematic representation of the anatomy of the neck of mice (ventral aspect) is shown. Skin over sternum and neck were partly removed. The salivary glands were folded cranially.

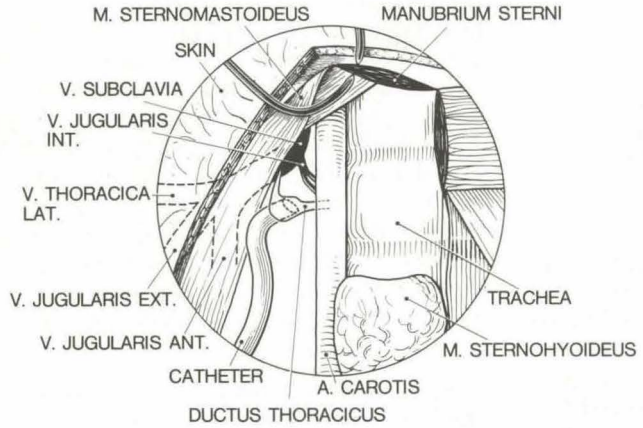

Fig. 1b A part of Fig. 1a is shown. The manibrium sterni was split in the middle and the left part was retracted caudo-laterally by menas of a clamp. The left sternohyoid muscle was bluntly dissected away from its attachments with the manubrium. The topography of the thoracic duct with cannula fixed in place and of the angulus venosus is shown.

mal fixed on the back with the head forced to the experimenter. 1 cm of skin over sternum and neck were removed. The salivary glands were exposed at their caudal attachment to the pectoralis superficialis muscle and folded cranially (Fig. 1). The manubrium sterni was split in the middle with scissors to about 0.5 cm in length as far as the sternal angle. Occassionally bleeding from the manubrium or pectoralis muscles is easily stopped with small pieces of haemostypticum (Tabotamp®, Johnson & Johnson, Norderstedt). The left part of the manubrium was retracted caudo-laterally by means of a clamp and fixed at the operation table. The left sternohyoid muscle is bluntly dissected away from its attachments with the manubrium, and the whitish distended thoracic duct becomes visible. In our experiments the thoracic duct was always undivided. It often forms a small sac between the internal jugular vein and the junction of external jugular and subclavian veins, from which a smaller tributary enters into the venous junction (topography from 14). Beneath the margin of the vein a small transversal incision was made with the tip of a small knife (Yasargil FD5; Aesculap, Tuttlingen). The moderately beveled end of a Liquemin (40 I.U./ml) filled silastic catheter (Dow Corning Corporation, USA, i.d. 0.30 o.d. 0.64 mm) was inserted up to 1 mm under gentle rotation of the cannula between thumb and forefinger and was fixed in place by small droplets of the tissue adhesive isobutyl 2-cyanoacrylate (Ethicon, Norderstedt). Upon a first aspiration, lymph flows spontaneously by suckling gravity forces

of a catheter, 10 cm in length. If lymph did not flow into the cannula, the tip of the cannula was withdrawn by gentle rotating, which leaves the tissue adhesive in place, but forms a channel of cannula width, through which lymph flows spontaneously. Then the cannula was reinserted into this channel and fixed anew. The operation procedure lasted for about 5 min. During lymph collection the animals were placed on a heated table at 37 °C and lymph was sampled for 30 to 40 min in preweighed test tubes with minute amounts of heparin. Lymph flow was determined gravimetrically. Lymph leucocyte counts were done manually on a hemocytometer after staining with Türk's solution. Lymph then was centrifuged at 12000 x g for 2 min and lymph plasma was stored at −70 °C.

Blood sampling: At the end of the lymph sampling period blood was withdrawn from the external jugular vein by a silastic catheter (Down Corning Corporation, USA; i.d. 0.64 mm, o.d. 1.19 mm) into a heparinized syringe by gentle aspiration. Blood leukocyte counts were performed as in lymph.

Results and Discussion

Cannulation of the thoracic duct at the site of entry into the venous system at the neck exhibits several advantages over the abdominal approach.

At this site thoracic duct has centered about 80–95 % of whole body lymph (11, 12), whereas at the cysterna chyli of small laboratory animals fractions of millimeters in length of the inserted cannula involve the risk of excluding hepatic lymph.

There is a minor operative trauma to animals. Despite splitting of the manubrium only blunt dissection of tissue is performed, which seems a less severe injury with minor alteration of lymph flow compared with laparotomy and the necessity to retract and/or remove abdominal contents extracorporealy during surgery. Compared with the nylon or polyethylene cannula used so far, there is less tendency to clotting in the silastic catheters; in addition, owing to their suppleness, they can be handled more easily during insertion than the formerly used rigid cannula.

The use of surgical adhesive is an improvement in that it renders sutures superfluous, as also confirmed by others (3, 6, 9). All the previously published methods of thoracic duct cannulation in mice employed the long term fistula technique in conscious mice (1, 2, 3, 4, 5, 6). The aim of these studies was to obtain lymphocytes and/or to produce lymphocyte depleted animals. In analogy to reports on the rat (8, 9, 10), it should, in principle be possible to establish the method described herein for chronical purposes. For determinations of chemical constituents in lymph and plasma, however, as was our major interest in mice thoracic duct lymph, chronically fistulated animals cannot be used (15).

The wide range of lymph flow and cell output reported in the literature (1.4–19.7 ml/d and 30.8–167.8 x 10^6 cells/ml, respectively) is mainly due to certain pretreatments of mice such as heparin injections and loading with saline or glucose solutions and to the altered colloid osmotic forces during chronic lymph sampling. Thus, the lymph flow and cell count described here bears a greater resemblance to physiological conditions.

Table 1 Lymph flow (ml/h) and leukocyte counts in lymph and blood (x 10^6 cells/ml)

	Lymph	Blood
Lymph flow	0.396 ± 0.01	–
Leukocytes	38.6 ± 5.1	4.4 ± 0.28

n = 14; mean ± standard error of the mean

References

1 *Shrewsbury, M.M.:* Thoracic duct lymph in unanaesthetized mouse. Method of collection, rate of flow and cell content. Proc. Soc. exp. Biol. Med. 101 (1959) 492–494

2 *Gesner, B.M., J.L. Gowans:* The output of lymphocytes from the thoracic duct of unanaesthetized mice. Brit. J. exp. Path. 43 (1962) 424–430

3 *Boak, J.L., M.F.A. Woodruff:* A modified technique for collecting mouse thoracic duct lymph. Nature 205 (1965) 396–397

4 *Morse, S.J., S.K. Riester:* Studies on the leukocytosis and lymphocytosis induced by Bordetella pertussis. II. The effect of pertussis vaccine on the thoracic duct lymph and lymphocytes of mice. J. exp. med.: 125 (1967) 619–631

5 *Mandel, M.A.:* Isolation of mouse lymphocytes for immunologic studies by thoracic duct cannulation. Proc. Soc. exp. Biol. Med. 126 (1967) 521 524
6 *Deaton, J.G.:* Thoracic duct lymph drainage in the mouse: a technique for producing lymphocyte-depleted animals. Lymphology 5 (1972) 115–120
7 *Bollmann, J.L., J.C. Cain, J.H. Grindlay:* Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. J. Lab. Clin. Med. 33 (1948) 1349–1352
8 *Reinhardt, U.O.:* Rate of flow and cell count of rat thoracic duct lymph. Proc. Soc. exp. Biol. Med. 58 (1945) 123–124
9 *Saldeen, T., Linder, F.:* A method for long term collection of lymph from the thoracic duct in rats. Acta path. microbiol. scand. 49 (1960) 433–437
10 *Azargoschasb, K.:* Ductus thoracicus und Ductus thoracicus-Lymphe der Ratte. Ein Beitrag zur experimentellen Untersuchungsmethodik der lymphogenen Krebsmetastasierung. Z. ges. exp. Med. 137 (1963) 541–553
11 *Yoffey, J.M., F.C. Courtice:* Lymphatics, lymph and the lymphomyoloid complex. Academic Press. London – New York 1970
12 *Cain, J.C., J.H. Grindlay, J.L. Bollmann, E.V. Flock, F.C. Mann:* Lymph from liver and thoracic duct: experimental study. Surgery Gynec. Obstet. 85 (1947) 559–562
13 *GV-SOLAS:* Liste von Erregern zur Spezifizierung bei SPF-Versuchstieren. Veröffentlichungen der Gesellschaft für Versuchstierkunde Nr. 2, Zürich 1977
14 *Cook, M.J.:* The anatomy of the laboratory mouse. Academic Press, London 1965
15 *Lindena, J., I. Trautschold:* Ductus thoracicus lymph in mice. II. Enzyme activities and protein content. Lymphology 16 (1983) 245–247

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