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Thoracic Duct Lymph Drainage in the Mouse: A Technique for Producing Lymphocyte-Depleted Animals*

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Summary

A modified method of cannulating the thoracic duct of the mouse is described. Postoperatively, mice are encircled with a strip of waistband elastic and returned to their cages to drain lymph. Correlative studies with mice kept on an exercise wheel indicate lymphocyte depletion does occur when mice are returned to their cage during lymph drainage. Moreover, the mice survive much better than when restrained, and can be used in experiments as lymphocyte-depleted animals.

Previous reports of techniques for thoracic duct cannulation in the rat (1, 2) and mouse (3–7) emphasized the collection of lymph. This can only be accomplished when the animal is restrained, which leads to a high postoperative mortality rate. During the course of experiments requiring lymphocyte-depleted mice, a method has been developed wherein the thoracic duct is cannulated and the mice are returned to their cages, where they drain lymph for periods up to 3 days, and can then be used in grafting or other experiments. A different method of thoracic duct cannulation from the one in general usage (5–7) is described.

Materials and Methods

Equipment. The cannulas were 10-inch segments of Intramedic, PE 20 (Clay-Adams, Inc., N.Y.) polyethylene tubing with an inner diameter of 0.043 inches. The distal two inches of one end of the cannula was roughened with a small file. This facilitated the adhesiveness of the tissue adhesive, isobutyl cyanoacrylate monomer (Ethicon, Inc., Somerville, N.J.), to the cannula and obviated the need for a suture to fix it in place. The roughened tip of the cannula was beveled with scissors.

Mice. Male C57BL (H-2^b) mice (Jackson Laboratories, Bar Harbor, Maine), 8–10 weeks old, weighing 18–24 g, were used. They were force-fed 0.5–1.0 cc of olive oil 1–4 hours before surgery. For anesthesia, an intraperitoneal injection of sodium pentobarbital, 5 mg/100 g body weight (8) was given.

Thoracic Duct Cannulation. The mice were tied to a dissection board in the left lateral position (Fig. 1a), and the hair over the left abdomen and chest was removed by an electric clipper. The skin and surgical equipment were washed with 70% ethanol, and a 2 cm left subcostal incision was made.

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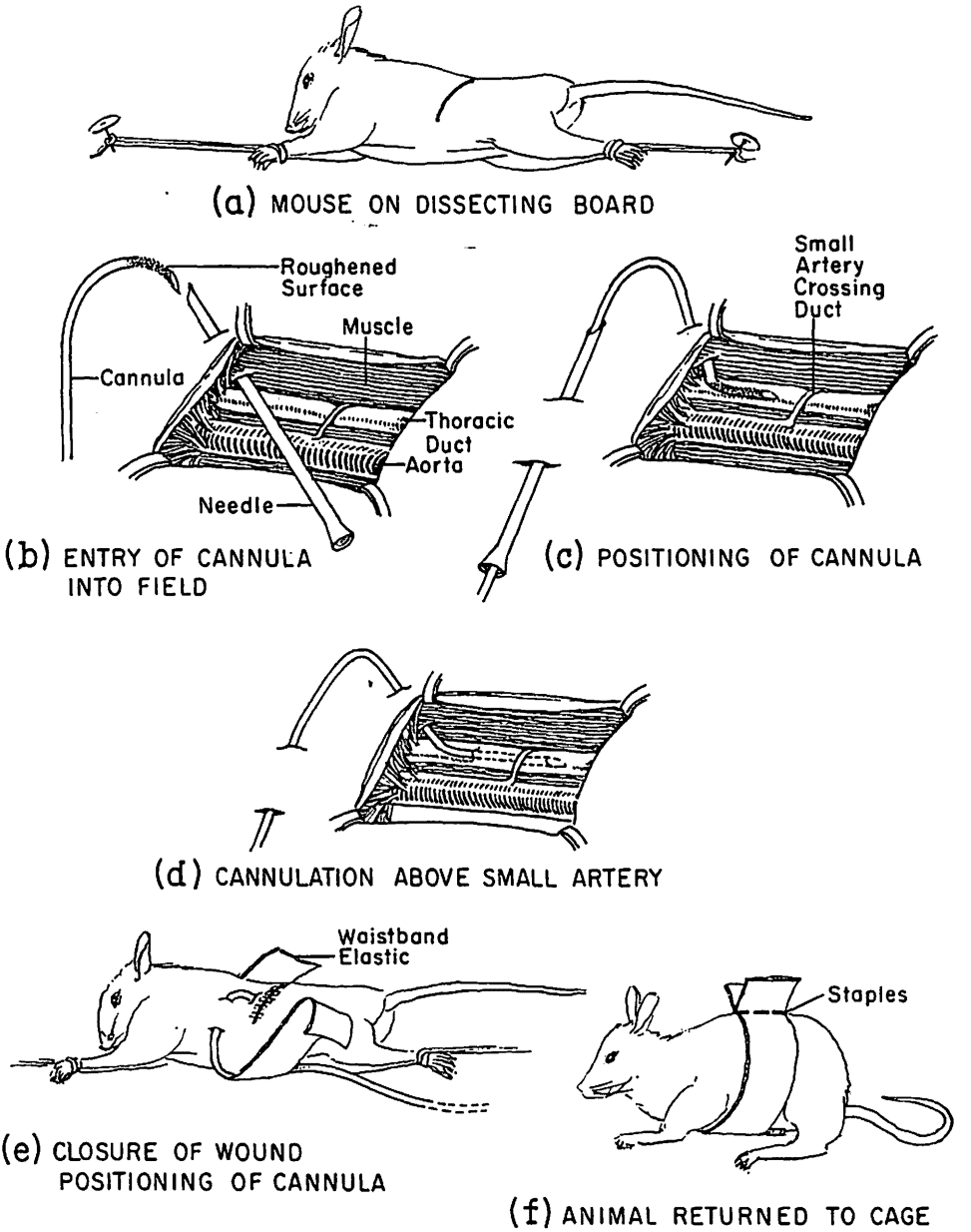

Fig. 1 Diagram of cannulation procedure.

The peritoneal reflections just dorsal to the left kidney were divided by traction with two cotton-tipped applicators. The thoracic duct (Fig. 1b), a glistening white structure, was exposed from the level of the renal artery, which overlies the cysterna chyli, up to its entrance into the diaphragm, and a site for cannulation was selected. One or two small arteries course over or through the duct in its exposed part (Fig. 1c). The cannulation

was best done just above one of these small arteries, as the duct is bound to them and less likely to tear during insertion of the cannula (4). In general, the most cephaled site for cannulation was chosen, as cannulation close to the cysterna chyli, while technically easier, was less apt to drain successfully.

From inside the abdominal cavity, at a point just above the intended spot of cannulation, a 15-guage needle was stabbed upward through the posterior abdominal muscles and out through the skin of the subcostal area (Fig. 1 b). The tip of the cannula, which had been roughened to facilitate the adhesiveness of the surgical glue, was threaded through this needle, beveled tip first. The needle was then withdrawn, leaving the cannula in place. Again using the 15-guage needle as trochar, the non-beveled end of the cannula was tunneled under a loop of skin parallel to the incision. The free end of the cannula was then withdrawn through this passageway until the beveled end, which had been bent so that if would parallel the duct (Fig. 1 c), was adjacent to the site of cannulation.

Gentle tension on the duct was produced by caudad traction with a cotton-tipped applicator, and the cannula tip was grasped by forceps and carefully stabbed into the duct a distance of a few millimeters (Fig. 1d). Alternatively, a tiny incision in the duct itself was made with a 27-guage needle, and the cannula was introduced into the duct through this opening. The latter maneuver gave a smoother cannulation, with less chance of avulsing the duct. Its disadvantage was that lymph escaped into the area after the incision was made; the lymph often obscured the duct, which tended to collapse with the loss of lymph. If lymph did not flow into the cannula upon aspiration through it or gentle massage of the abdominal contents, the tip of the cannula was withdrawn and reinserted through the same puncture site until lymph reflux did occur.

When it was certain that the cannula was in the thoracic duct, a small droplet of tissue adhesive was applied to fix it in place, and the peritoneal reflections were pulled over the cannulation site to leave a smooth surface. Another droplet of surgical adhesive was used to fix the loop of cannula at its exit from the skin. The peritoneum was closed with continuous 5–0 chromic intestinal gut and the skin was reapproximated with skin clips and a collodion dressing (Fig. 1e). The distal end of the cannula was looped posteriorly and inferiorly, and placed in the animal's left groin. The cannula was held in place by a dressing of waistband elastic, which encircled the mouse's trunk and was stapled in place above the spine (Fig. 1f).

Postoperative care. If collection of lymph was desired, the animal was restrained by means of the elastic bandage on an exercise wheel (4). For lymphocyte depletion studies, the cannula was snipped off at the level of the groin and the mouse was returned to his cage. A regular chip diet and water ad libitum were supplied. Cannulated mice required little more than routine daily attention, yet drained nicely, perhaps due to their fairly normal activities within the cage. Occasionally the cannula tip became plugged by material from the cage: this was corrected by severing the distal few mms of the cannula just above the obstruction, or by removing the obstructing debris with the tip of a small needle.

After the desired period of drainage the elastic bandage was removed, the cannula was trimmed up close to its exit from the skin and its orifice was occluded by a drop of tissue glue.

Leukocyte counting. Blood and lymph leukocyte counts were done manually on a hemocytometer, using 2% glacial acetic acid as diluent-hemolysin. Lymph samples for counting were obtained directly from the cannula. Samples of peripheral blood were obtained from the tail vein.

Results

Table 1 gives the output of lymph and lymphocytes in 14 mice who were placed on an exercise wheel for the collection of lymph. The blood lymphocyte counts of an additional 12 mice who were returned to their cages after cannulation are shown in Table 2. The fall in the blood lymphocyte count of these mice correlates very closely to that of the animals in Table 1, indicating that lymphocyte depletion did occur even when the mice were returned to their cage during lymph drainage.

Table 1 Output of lymph and lymphocytes in C57BL (H-2^b) mice that were restrained on an exercise wheel after cannulation. The lymph was collected in separate 24-hour periods*.

	No. of Animals	Mean Lymph Output in cc	Mean Lymphocyte Counts (x 10^6 cells/ml) Lymph	Blood	Mean lymphoc. Output (x 10^6 cells/ml)
Day of Cannulation	14		18.7 (6.0–59.0)	4.7 (4.0–6.0)	–
Post-op Day 1	14	2.0 (1.0–3.0)	15.8 (3.0–35.0)	2.0 (0.75–3.3)	35 (10–94)
Post-op Day 2	14	2.3 (0.5–15.0)	4.0 (1.2—6.0)	1.1 (0.5–2.0)	23 (5–46)
Post-op Day 3	6	4.5 (1.0–15.0)	2.0 (1.0–3.0)	0.8 (0.5–1.5)	13.5 (5–20)

* Numbers in parentheses are ranges of values given in this and subsequent tables.

Table 2 Tail vein blood lymphocyte levels of C57BL (H-2^b) mice that were returned to their cage after cannulation and whose lymph drainage was not collected.

	No. of Animals	Blood lymphocyte count (x 10^6 cells/ml)
Day of Cannulation	12	5.0 (4.0–6.0)
Post-op Day 1	12	2.5 (1.5–4.0)
Post-op Day 2	12	1.3 (0.5–2.3)
Post-op Day 3	12	0.8 (0.3–1.3)

Moreover, the 12 mice in Table 2 were used as lymphocyte-depleted recipients for allogeneic bone marrow transplants (*Deaton, J. G.*, submitted for publication), and they survived the several months of the study. By contrast, only 2 of the 14 mice shown in Table 1 lived longer than a week after surgery.

Discussion

The method of cannulation described here is a modification of that used by *Bollman* et al. (2), in the rat. The Intramedic, PE 20 polyethylene tubing is larger than any so far used in cannulating the thoracic duct of a mouse. Once in the duct, there is very little space for lymph to flow around it, so that a ligature at the cannulation site is unnecessary. Direct flow of lymph into this cannula, rather than through an initial 180° bend (4–7), in part accounts for the very low incidence of clotting in this system. Compared to the nylon cannulas (Portex Limited, Hythe, Kent, England) used by *Boak* and *Woodruff* (5), there is less propensity to clotting in the polyethylene catheters, regardless

of the method of cannulation. The use of waistband elastic as a postoperative bandage is an improvement in that, unlike tape, it does not depilate the animal when removed, and it permits more respiratory freedom as well as general mobility when the mouse is returned to the cage.

The lack of mobility while lymph is being collected is no doubt responsible for the high mortality rate of mice left on a exercise wheel for lymph collection. By returning the cannulated mice to their cage, the survival rate is much better, and the lymphocyte-depleted mice can subsequently be used in experiments. A drawback is that the exact number of lymphocytes depleted from the mouse isn't known, but the fall in tail vein lymphocyte levels is a reasonable way of monitoring this.

Finally, a note on the comparative results of the cannulation method described herein and those previously published. Table 3 gives the *first day* output of lymph and lymphocytes from 5 other studies in mice. The mean first day lymph volume of 2.0 ml and lymphcyte output of 35 x 10^6 cells/ml in the present study are values which agree closest with *Shrewsbury* (3). The wide range of lymph and lymphocyte outputs summarized in Table 3 may be due to several things. These include inate variations in these values from one mouse strain to another, certain pretreatments of the mice, such as the use of heparin, which causes lymphocytosis in the rat (9) and increases the number of thoracic duct lymphocytes in calves (10), and the selection of which cannulated animals – and how many – are included in the report.

Table 3 Output of lymph and lymphocytes in mice during the first day after cannulation: results of the present study are compared to those in the literature.

Authors	No. of mice	Strain	Treatment of mice	Mean Lymph Volume	Mean Lymphocyte Output (x 10^6 cells/ml)
Shrewsbury (3)	17	NIH Webster	None	1.4 ml	30.8
Gesner a. Gowans (4)	10	random-bred albino	10% glucose and[1] saline to drink	19.7 ml	167.8
Boak a. Woodruff (5)	not stated	CBA	Heparin injections. Pre-immunization with mammary carcinoma cells	19.0 ml	84.0
Mandel (6)	not stated	Balb/cAnN	Heparin injections	5.0 ml	100.0
Morse a. Riester (7)	16[2]	NCS Swiss	None	15.1 ml	123.6
Present Study	14	C57BL	None	2.0 ml	35.0

[1] Dextrose and saline were given in all the subsequent studies.
[2] Only this study's control mice are included here.

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Studies on the Lymph Node-Venous Communications

II. The Passage of Labeled Exogenous Erythrocytes*

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Summary

The passage of ^{51}Cr labeled avian erythrocytes has been followed through the medial retropharyngeal lymph node of the dog by injecting labeled avian erythrocytes into one afferent lymphatic channel and assaying for radioactivity in samples recovered from the efferent lymphatic channel, the adjacent internal jugular vein, and distally from the femoral vein. Profiles of recovery were consistent with those found when using radioactive iodinated serum albumen. A secondary peak of recovery from the efferent lymphatic channel and adjacent internal jugular vein coincided with timed nodal palpation. The amounts of nodal retention and transnodal passage were quantitated and discussed in terms of passage rates and possible clinical relevance. The transnodal passage of avian erythrocytes suggests the size of the lymph node-venous communication to be on the order of a capillary or larger.

Introduction

Evidence consistent with a pressure dependent lymph node-venous communication has been described and was based upon the rate of recovery of ^{131}I labeled serum albumen injected at elevated, manually induced pressures (1). One afferent lymphatic channel served as the site of injection in these studies which could hence be considered as utilizing the "normal" flow patterns and anatomical pathways through the lymph node. Whereas the lymph node-venous communications may or may not be nonfunctional in the "normal" animal, there is experimental evidence to support their becoming operative when lymphatic pressures are elevated (1, 2, 3).

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