A Modified Bollman's Technique for Cannulation of the Rat's Thoracic Duct: Lymph Flow Standardization* (33604)

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The cannulation of the abdominal thoracic duct of the rat was first described by Bollman (1) and has been used for the study of the lymphatic intestinal absorption of a number of substances (2–8), especially lipids and fat-soluble materials. The thoracic duct is a very delicate vessel, which can be easily broken with manipulation. Bollman's original technique presents several difficult maneuvers. One, prior to the introduction of the catheter, an opening is made with a needle into the thoracic duct; often the duct is broken. Second, the catheter is tied in position and damage to the duct is common. Third, the use of a straight cannula rather than a curved one results in tearing of the duct. On the average, Bollman's preparations last only 2–3 days.

Most lymph studies so far have been done in animals in which the fluid intake was uncontrolled owing to their *ad libitum* drinking of water or saline. This has resulted in variable lymph-flow rates and has made it difficult to reproduce results from experiment to experiment or laboratory to laboratory.

Described herein is a modification of Bollman's technique and the investigation of the effect of infusion of isotonic saline on lymph-flow rate.

Materials and Methods. A. Animal preparation. Surgery. The study was carried out on Purina Chow-fed female rats (300 ± 15 g) obtained from the Charles River Breeding Lab. (North Wilmington, Massachusetts). Used as anesthetic, two ml of Penthrane (Methoxyfluorane, Abbott Lab., North Chicago, Illinois) were poured into a 100-ml beaker containing a small gauze. The animal was oriented with the head away from the experimenter. The surgical procedure consists of cannulation of the thoracic duct first, followed immediately by a double catheterization of the common bile duct.

The abdomen is opened by a midline incision below the xyphisternum. This is different from the original technique (1), in which an oblique incision, which requires cutting the abdominal muscles, was used. Gauze swabs (wetted in normal saline) are used to prevent dehydration of the intestine. The animal is then placed on its right side; this causes the whole mass of viscera to fall into the right flank, bringing into view the retroperitoneal surface on the left flank. The thoracic duct is located by referring to nearby vascular landmarks, as shown in Fig. 1.

Taking as reference that part of the aorta proximal to the left kidney, the thoracic duct is easily exposed proximal to the cisterna chyli. The retroperitoneal cavity is opened with small blut-tipped forceps; the aorta is dissected out of its abdominal course distal to the diaphragm. The thoracic duct lies dorsal to the abdominal aorta and slightly to the left. It can be recognized with magnifying glasses and even better yet, by its white coloration if the animal has been administered a fatty meal 1 hr prior to the operation. The thoracic duct (covered by loose connective tissue and fat) is blunt-dissected for a distance of 8–10 mm with small fine forceps. To facilitate this, two threads are passed around the aorta to draw it to one side (Fig. 1). In contrast to Bollman's technique, no ligature is placed around the thoracic duct.

For the catheterization of the duct itself (see Fig. 1), polyethylene tubing PE 50 (Intramedic, Medical Formulation PHF, Clay Adams Inc., New York) is used. The cannula

* Supported in part by grants in aid from the National Institute of Health, Bethesda, Maryland (TIAM-5369, and H-4150) and a grant from Hoffmann-La Roche Incorporated, Nutley, New Jersey.

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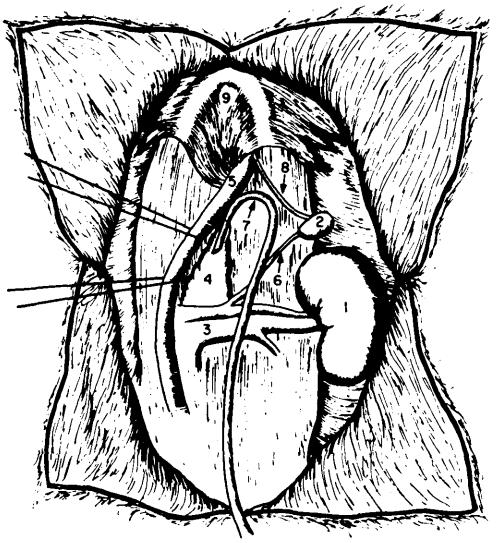

FIG. 1. Anatomical references and catherterization of the abdominal thoracic duct in the rat: 1. left kidney; 2. left adrenal gland; 3. left renal vein; 4. thoracic duct; 5. abdominal aorta, refracted; 6. inferior suprarenal artery; 7. cannula (PE 50) fixed with glue; 8. superior suprarenal artery; and 9. diaphragm.

is 30 cm long and bent in such a way that it forms a hook about 15 mm from one end; the small end is cut with a bevel so that it is actually used for the cannulation. An opening is made in the ventral wall of the exposed thoracic duct by using the small bevelled tip-end of the cannula and the catheter is passed 8–12 mm distally down the duct. When the catheter is in position, lymph flows out immediately. A very small portion of glue (Eastman 910 Adhesive, Armstrong Cork Co., Lancaster, Pennsylvania) is applied with a disposable pipette to the curved part of the cannula to fix it firmly along the axis of the thoracic duct; another small portion of glue is then applied only at the point of the insertion of cannula into the duct. One should be careful not to apply too much glue on the viable part of the thoracic duct, to avoid possible collapse of the duct. A small piece of gauze is used to cover the glue. A puncture is made in the abdominal wall with a 17-gauge needle from the outside; the cannula is inserted into the pointed end of the needle and withdrawn from the abdominal cavity. The viscera, still covered by the gauze compresses, are retracted toward the left of the animal in order to proceed with the other cannulations.

Because the excretory pancreatic ducts drain into the common bile duct, care must be taken to place two cannulas in the upper part of the bile duct. Polyethylene tubing size PE 50 is used. One of the catheters collects pure bile, and the other, placed through the same incision but in opposite direction (9–11), is utilized as a cannula for infusion into the intestine. The latter cannula is used to infuse isotonic saline (0.85% NaCl) at constant rates directly into the duodenum (Fig. 2). Thus, in this preparation, lymph is collected by a cannula in the thoracic duct, bile is collected by a cannula in the upper part of the bile duct, and isotonic saline is infused into the duodenum by a second cannula in the bile duct. The pancreatic secretions are left undisturbed. This operation requires 25–30 min for an experienced operator to make the 3 cannulations described above.

B. Animal postoperative care. After closure of the abdomen, the animal is put in a restraining cage, similar to that described by Bollman (12) (distributed by C. H. Stoelting Company; Chicago, Illinois). Ordinarily, the animal awakes in about 5–15 minutes

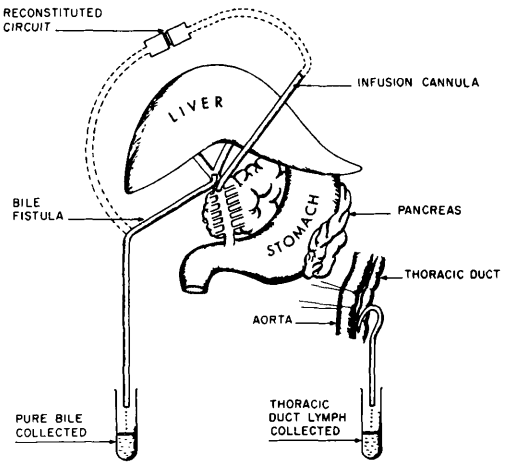

FIG. 2. Double catherterization of the common bile duct in the rat. Summary of the preparation used in the present studies.

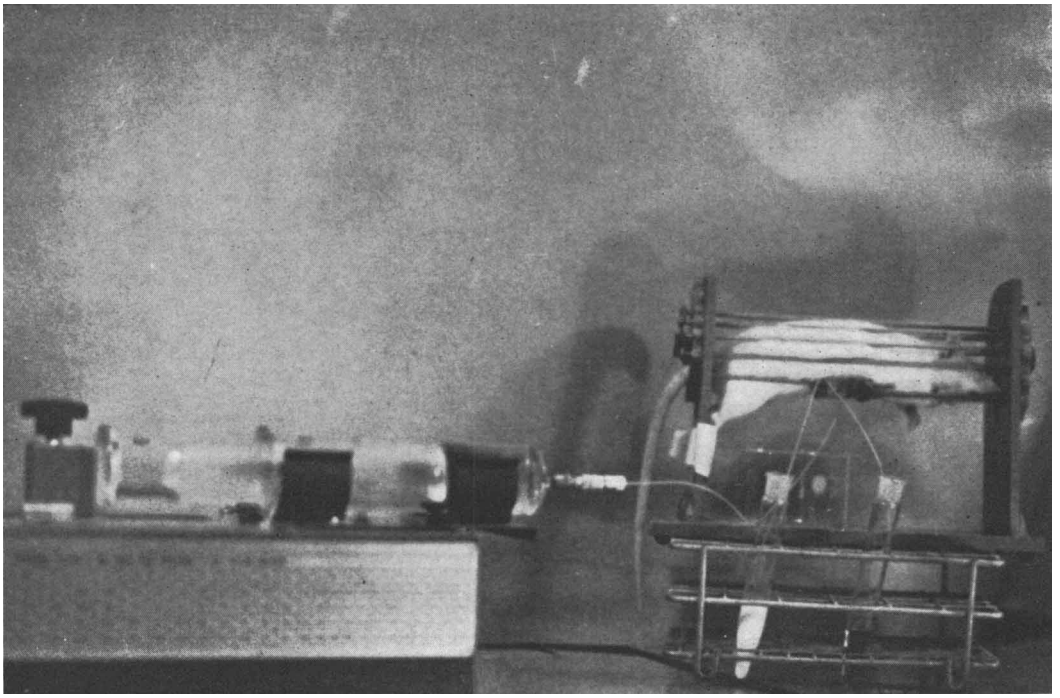

FIG. 3. Infusion pump and restraining cage in use.

(Fig. 3). The infusion cannula is connected to a plastic tubing adapter (size A, Clay Adams, Inc., New York) which in turn is attached to a 100-ml syringe on the infusion pump. Two types of infusion pumps were used, both made by Harvard Apparatus Company, Inc. (Dover, Massachusetts): one was a variable speed Infusion Withdrawal pump, model 600, and the other, a compact Infusion pump, model 975.

Six animals, prepared as described above, received isotonic saline during experimental periods of 6 hr. Lymph was collected continuously in graduated 15-ml centrifuged tubes and the volume was recorded hourly. Lymph volumes are thus expressed in milliliters per-hour.

Results. Experiments were designed to study the relationship of the rate of saline infusion to the rate of lymph production. After an initial experimental period without infusion, saline was infused at the rates indicated in Fig. 4. In the absence of infusion of saline into the gut there is some lymph flow, but it is minimal. If insufficient fluid intake is prolonged for periods longer than 6 hr, there is usually obstruction of the catheter due to clotting of the lymph with the subsequent loss of the preparation. Infusion rates of 1.2–4.6 ml/hr produced lymph in a constant relation to the rate of infusion and this can be expressed by the equation:

$$L = mI + k,$$

in which L is the lymph production rate; I is the infusion rate; m is the slope of the line; and k is the ordinate intercept. As shown in Fig. 4, the approximate slope of the line appears to be 0.73, which means that, under the conditions of these experiments for each ml of saline infused, 0.73 ml of lymph is obtained.

Discussion. The technique of cannulating the thoracic duct in the rat is a difficult task; the improvements reported remove some of the difficulties. This preparation remains functional on the average of 10 days. Our investigations show that a constant relationship is established between the infusion rate of saline into the duodenum and lymph production. Lymph production is quantitatively related to the rate of infusion of isotonic

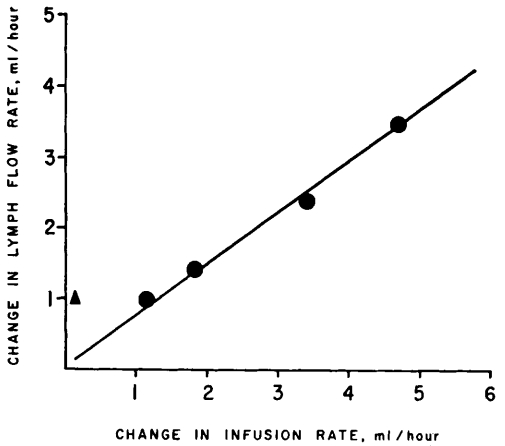

FIG. 4. The relationship between the change in rate of infusion of isotonic saline solution and change in lymph flow rate. Each (●) represents the average found in 6 animals. The pump infusion rates were 1.2, 2.3, 3.0, 4.6, and 5.8 ml/hr. The respective average lymph flow rates were 0.8, 1.8, 2.2., 3.1, and 4.2. Since no infusion (▲) or infusion of 1.2 ml/hr gave the same lymph flow (0.8 ml/hr) the figures on the ordinate were corrected by substracting 0.8 from the observed values. Similarly, the figures on the abscissa were corrected by subtracting 1.2 from the pump infusion rates.

saline for infusion rates greater than 1.2 ml/hr.

The standardized lymph-flow technique reported here makes possible the study of the effect of specific compound or metabolites on the absorption of lipid-soluble materials from the intestine by the lymphatic route. In order to determine small differences in rates of absorption, it was then necessary to develop a preparation in which the rate of lymph production is constant. Experience has shown that nonstandardized lymph-flow conditions produce absorption in proportion to lymph production; thus, a large volume of lymph is accompanied by low absorption. Under these conditions, lymph production is a function of the variable *ad libitum* fluid intake of the animal and hence uncontrolled. An example of a lymph-flow controlled study, which is to be reported elsewhere, is a comparison of the ability of the various bile salts to facilitate the absorption and esterification of cholesterol by the gut. The modified technique has also permitted the evaluation of hormones and dietary components on the lymph-flow rate (paper in preparation). Occasionally, when studying the effect of bile on lipid absorption, control values were obtained with the infusion of bile into the duodenum through the infusion cannula (reconstituted circuit) which prevented the infusion of saline into the common bile duct.

Summary. A modification of Bollman's technique for the cannulation of the abdominal thoracic duct in the rat has been reported. A preparation is described in which three fistulas are made in the same animal, the thoracic duct fistula and two fistulas in the upper part of the common bile duct. One of these catheters in the bile duct drains pure bile and the other is used to infuse isotonic saline at a constant rate into the duodenum. Data are presented which demonstrate that lymph-flow rate is quantitatively related to the infusion rate of isotonic saline. The modified Bollman's technique can be used for assessment of small differences in the rate of absorption of fat-soluble materials.

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Received Oct. 17, 1968. P.S.E.B.M., 1969, Vol. 130.