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Isolation of Mouse Lymphocytes for Immunologic Studies by Thoracic Duct Cannulation.* (32494)

MARK A. MANDEL (Introduced by K. Habel)
Laboratory of Germfree Animal Research, National Institute of Allergy and Infectious Diseasees, National Institutes of Health, Bethesda, Md. 20014

The lymphocyte has been shown to participate in transfer of immunologic memory (1), graft versus host reactions(2), homograft reactions(3), and antibody synthesis(4). Cannulation of the thoracic duct of mice provides the investigator with a pure population of lymphocytes from a highly inbred animal. The technique for collection of lymph from small laboratory animals was originally described by Bollman, Cain and Grindlay (5) in 1948. Gesner and Gowans(6) adapted the method for use in mice; Boak and Woodruff(7) changed the technique by using a surgical adhesive, methyl 2-cyanoacrylate, for cannula fixation. Using these techniques considerable difficulty, in terms of cannula blockade and animal survival, was encountered. This paper will describe several modifications, including the use of a new surgical adhesive, isobutyl cyanoacrylate monomer, that have led to an improved operative success rate.

Materials. The animals used were BALB/cAnN male mice, approximately 25-30 g, obtained from the Rodent and Rabbit Production Section, National Institutes of Health. Sodium pentobarbital* at a dosage of 0.06 mg/g body weight provided satisfactory anesthesia for one hour. The cannula was a 20 cm segment of PE10 polyethylene tubing† (inner diameter 0.028 cm, outer diameter 0.061 cm). One end of the cannula was shaped into an inverted U by placing a fine metal wire inside, bending to the desired form, and then plunging the cannula into boiling water for 5 seconds. After a 2-minute cooling period, the wire stylet was removed leaving the cannula properly molded. The cannula tip was beveled with a scalpel. Crystalline heparin‡ was used for anticoagulation. Methyl, butyl, and isobutyl cyanoacrylate monomers§ were the surgical adhesives used for cannula fixation.

Technique and results. One hour prior to the induction of anesthesia the mouse was given 0.1 ml of corn oil, orally. Following an intraperitoneal injection of pentobarbital the mouse was tied into a modified left lateral position and 1.5 ml of saline was given subcutaneously in the scapular regions. A left subcostal incision was made and the thoracic duct was approached transperitoneally. Abdominal contents and the left kidney were retracted, providing access to the duct, seen as a glistening white structure lying posterior to the aorta (Fig. 1a). The duct, from its origin at the cysterna chyla to its entry into the chest through the aortic hiatus of the diaphragm, was bluntly dissected. A 6-0 silk suture was positioned in the left posterior abdominal musculature, adjacent to the duct at a level several millimeters below the diaphragm (Fig. 1a, 1b). The cannula, brought into the operative field by passage through a #19 needle positioned in the left flank several centimeters caudad to the incision, was flushed with heparinized saline (5 mg/ml); it was positioned with the curved portion lying beneath the left diaphragmatic crus and the beveled tip, parallel to the duct, pointing in a caudad direction. The duct was put on traction and the beveled cannula tip passed through the lateral duct wall. The silk suture was tied around the cannula, where it en-

* Abbott Laboratories, North Chicago, Ill.

† Clay-Adams, Inc., New York.

‡ Hynson, Westcott, and Dunning, Inc., Baltimore, Md.

§ Ethicon, Inc., Somerville, N. J.

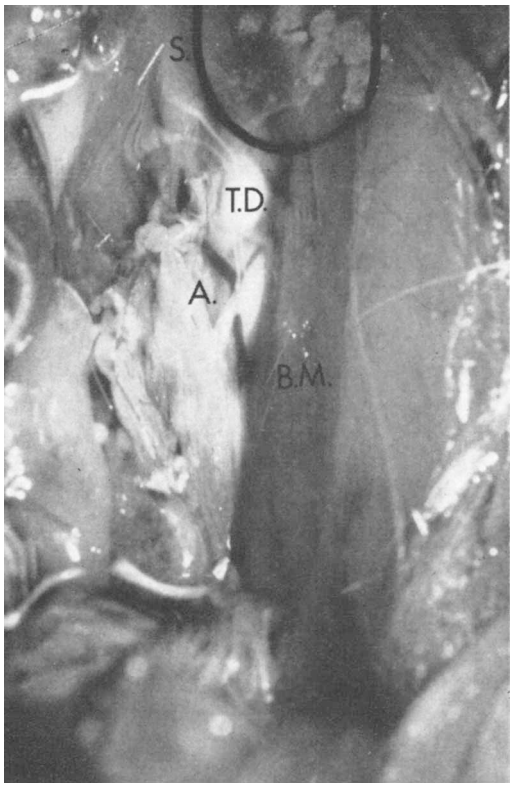

FIG. 1a. Intra-abdominal portion of the thoracic duct (T.D.) is seen lying next to the aorta (A.) Abdominal contents are retraced. A 6-0 silk suture (S.) is positioned, adjacent to the duct, in the musculature of the back (B.M.).

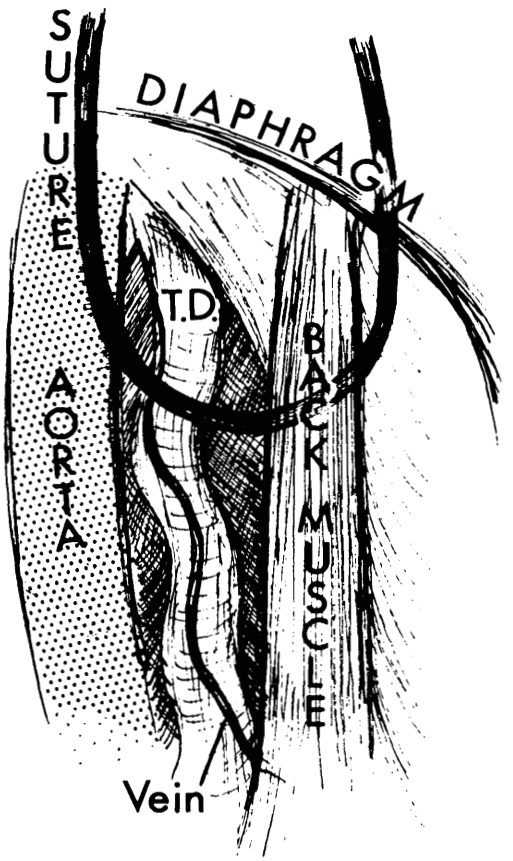

FIG. 1b. Line drawing of Fig. 1a.

tered the duct, fixing it to the posterior abdominal musculature (Fig. 2). A drop of isobutyl cyanoacrylate surgical adhesive was placed at the site of entry of the cannula into the duct to prevent lymph leakage. The incision was closed in 2 layers using a continuous stitch of 6-0 silk and a collodion dressing applied. Postoperatively, the animals were released from their ties and warmed under a heating lamp. Heparin, 0.1 mg, was injected subcutaneously. The lymph was collected in an iced 25 ml flask which contained 1 ml of phosphate buffered saline (pH 7.2). No problem with cell clumping was noted using this buffered saline. When the animal began to awaken, its thoracoabdominal region was encircled with tape and the mouse was positioned on a Bollman cage (Fig. 3). Repeat subcutaneous injections of heparin were given every twelve hours during the collection period. Animals drank 5% dextrose in normal saline for the first day; they were fed a low fat solid food diet after this time (#356 with one-fourth normal fat content) (8). Clots occasionally formed in the cannula during the first days of drainage; these could sometimes be removed by applying gentle suction with a tuberculin syringe and a #27 needle.

Thoracic duct drainage was continued for 4 days. Every 24 hours the lymph was harvested and the collection receptacle changed. In the cold (4°C) the cells were separated by centrifugation at 1000 rpm and then washed twice in phosphate buffered saline; total and differential cell counts were done. The first day of drainage yielded both the largest absolute number of cells (100×10^6) and the highest percentage of "small" lymphocytes (>98%). Lymph volume was approximately 5 ml the first day; continued drainage resulted in a slightly larger daily volume of lymph

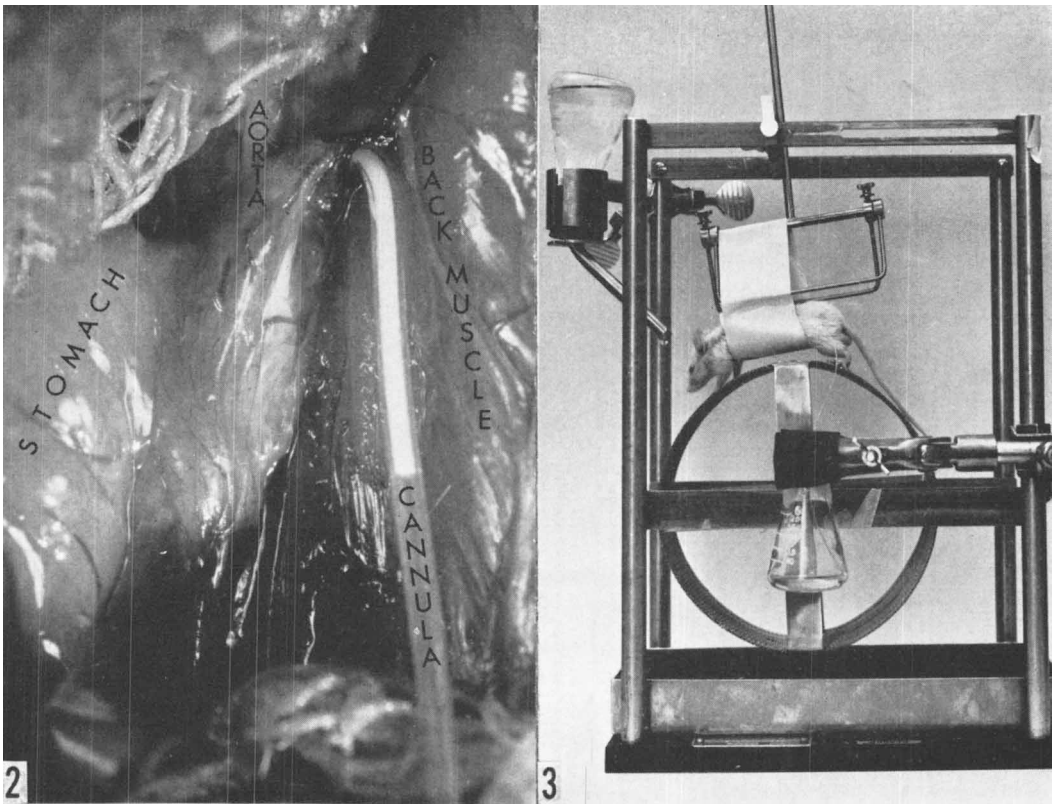

FIG. 2. The polyethylene cannula is fixed in the thoracic duct by a suture and surgical adhesive. Lymph is seen starting to flow through the cannula.

FIG. 3. A cannulated mouse is positioned on a Bollman cage. This method of restraint allows the animal to exercise without dislodging the cannula.

which contained a progressively smaller number of total cells and percentage of small lymphocytes.

Discussion. Cannulation of the mouse thoracic duct provides one with cells that all appear, on light microscopy, to be lymphocytes. The mouse is well characterized in terms of immunoglobulin classes(9) and histocompatibility loci(10). Using thoracic duct cells the investigator can work with a relatively homogenous (as opposed to the heterologous cell population obtained from the various lymphoid organs) cell population and perform immunologic experiments not possible in other animal species. The cannulation procedure used is a modification of Gesner's; in this study a surgical adhesive, isobutyl cyanoacrylate, was used to prevent lymph leakage at the site of cannula entry into the duct. In agreement with Bhaskar *et al*(11) it was found that the butyl family of the cyanoacrylate monomers caused less tissue reaction than the methyl derivatives. The isobutyl cyanoacrylate monomers seem to provide the most satisfactory results in terms of lymph production and animal survival.

The cannulated mice did well for periods up to 5 days when restrained, in an unanesthetized state, on a Bollman cage. Attempts to use small restrictive-type cages uniformly resulted in animal death within one day.

The number of cells obtained during the first day of drainage was approximately 100 million; this subsequently fell to only 10 million by the fourth day. These values are intermediate between those reported by Boak and Woodruff(7) and by Gesner and Gowans (6). Volume output increased slightly with length of drainage despite the diminished cellular numbers. By heparinizing the animals

and feeding them a low fat diet the problems of cannula blockade by clotting and chylomicra occlusion were minimized. Using this technique, over 50% of the animals operated upon can be expected to drain for prolonged periods of time.

Summary. A simple method for cannulating the mouse thoracic duct is described that enables the collection of lymph for periods of up to five days. A pure lymphocyte population is obtained that is useful for immunologic and antibody synthesis studies.

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Development of Tachyphylaxis to the Antilipolytic, Hypoglycemic Agent, 5-Methylpyrazole-3-carboxylic Acid, U-19425.* (32495)

GEORGE C. GERRITSEN AND WILLIAM E. DULIN
Metabolic Diseases Research, The Upjohn Co., Kalamazoo, Mich.

Acute hypoglycemic and antilipolytic effects of 5-methylpyrazole-3-carboxylic acid (U-19425) in rats have been described(1). U-19425 decreased blood sugar acutely in alloxan-diabetic rats, but tachyphylaxis was suspected since U-19425 did not have a sustained effect on glucosuria of these animals.

This report describes development of tachyphylaxis to U-19425. U-19425 pretreatment results in a more rapid return of plasma FFA to control levels after a challenge dose of the pyrazole. The data suggest that lipolytic enzyme systems of pretreated rats escape faster from the inhibitory effect of U-19425. This faster return of plasma FFA to normal is related to adrenalcortical secretion.

Methods. Studies on development of tachyphylaxis to U-19425 were done in intact male Spartan Sprague-Dawley rats weighing 150-160 g. Since hormones influence lipid metabolism(2), studies were also done in adrenalectomized and hypophysectomized rats. Rats were adrenalectomized at a weight of 150-170 g two days prior to the start of pretreatment. Adrenalectomized animals were maintained on 0.9% saline drink from time of operation until termination of the experiment. Studies on the effect of hydrocortisone replacement on tachyphylaxis were also done in adrenalectomized rats. Half of the adrenalectomized animals received a single daily subcutaneous injection of 0.5 mg of hydrocortisone while the other half received saline during the 4-day pretreatment period.

Rats were hypophysectomized by the transauricular approach of Koyama(3) as modified by Falconi and Rossi(4). Rats were injected with 150 μg of prednisolone per day for the first 2 days and maintained on 5% sucrose drink for the first 10 days after hypophysectomy. Rats which did not gain weight were used for tachyphylaxis studies 2 weeks after hypophysectomy.

* Cyclopal is The Upjohn Co., registered trademark for 5-allyl-5-(2-cyclopenten-1-yl) barbituric acid.