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## Preparation of a Chronic Thoracic Duct-Venous Shunt in Calves.*† (28188)

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Good methods for collecting thoracic duct lymph are important for many studies concerned with fluid balance, absorption and transport of nutrients, and kinetics of lymphopoiesis. Cannulation of the thoracic duct with polyethylene or polyvinyl tubing has greatly facilitated the collection of lymph for prolonged periods in unanesthetized animals(1-6). However, inasmuch as these preparations do not provide for the conservation of lymph, the physiological state of the animal beomes greatly altered. The technique described below affords a preparation in calves whereby lymph is readily accessible, and an essentially normal physiological state is maintained by arranging for the return of lymph to the venous system. Calves are excellent animals for experimental work of this nature for they are easily restrained and their size permits removal of relatively large volumes of blood and lymph without physical deterioration. Similar preparations have been described in dogs(7,8).

*Materials and methods.* The polyethylene cannulas were prepared as shown in Fig. 1. Reinforcement of the thoracic duct cannula prevents kinking of the smaller tubing and also permits secure coupling with the venous cannula. Polyethylene tubing was easily molded into the desired shape by bringing it near a small flame. The ends were flared in a similar manner.

Female, Holstein calves, weighing 50 to 60 kg, were purchased on the open market and maintained until no clinical or hematological abnormalities were observed (generally 2 to 3 weeks). Prior to surgery, solid feed was withheld for 48 hours and liquid for 12 hours. One liter of cream fed slowly 1 hour before operation facilitated the location of the thoracic duct. Five mg of methyl atropine nitrate and 200,000 units of procaine penicillin were injected intramuscularly one-half hour preceding anesthesia. After rapid intravenous administration of 12 mg of pentobarbital sodium per kg of body weight, the calf was secured in a supine position with the aid of a trough-shaped table. Surgical anesthesia was obtained and maintained by supplementary intravenous injections of pentobarbital sodium or 5% chloral hydrate. Chloral hydrate was preferred as it resulted in a more rapid recovery from anesthesia. During the operation approximately 500 ml of normal saline were given intravenously.

The area over the left external jugular vein, immediately cranial to the first rib, was prepared for surgical intervention. An incision, 6 to 8 cm long, was made in the skin beginning at the first rib and extending anteriorly along the lateral edge of the left jugular vein. The inferior cervical artery and corresponding vein were then located 2 to 4 cm anterior to the first rib in the tissue between the ster-

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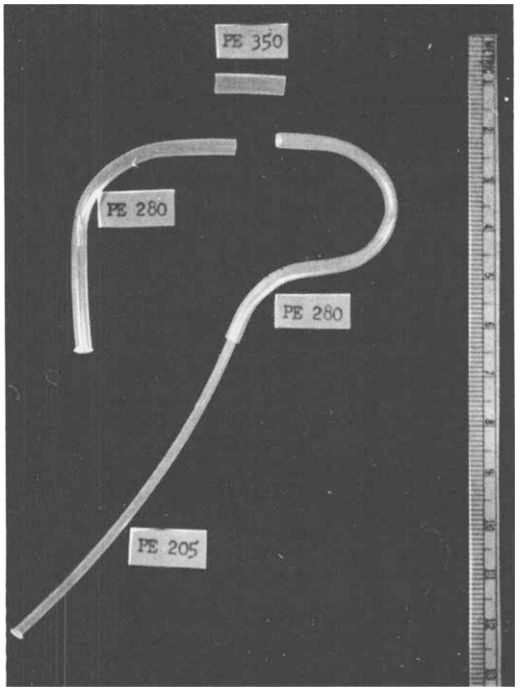


FIG. 1. Thoracic duct cannula on right is composed of PE 205 tubing (Clay-Adams, i.d. 0.062" × o.d. 0.082") reinforced by PE 280 tubing (i.d. 0.085" × o.d. 0.128"). Lower tip is flared. Venous cannula on left is PE 280 tubing with lower end flared, while connecting piece is PE 350 tubing (i.d. 0.125" × o.d. 0.157").

nocephalicus and brachiocephalicus muscles (Fig. 2). These vessels served as useful landmarks. The extra-thoracic portion of the thoracic duct is found near the inferior cervical artery as the artery proceeds dorsally between the scalenus ventralis muscle and the external jugular vein. The thin walled duct is partially embedded in the connective tissue attached to the medial surface of the scalenus ventralis muscle. By blunt dissection, approximately 2 cm of the duct (including the ampulate entrance into the venous system) were carefully freed of the surrounding fascia. The thoracic duct generally entered the left external jugular vein near the junction of the jugular and brachial veins. It most cases the extra-thoracic portion of the duct consisted of a single major vessel which was frequently joined near its termination by smaller lymph vessels some of which appeared to originate from the right side of the body. A ligature was then placed about 1 cm below the ampulla so as to permit continued circulation of lymph through the smaller vessels joining the thoracic duct near its termination. After the duct had dilated slightly, a small slit was made through the wall and the flared end of the reinforced cannula was threaded into the lumen for a distance of 6 to 8 cm. The cannula was secured in position by ligatures, care being taken to prevent contact of the flared end with the lymphatic valves.

The humeral branch of the cephalic vein was isolated and ligated approximately 4 cm from its entrance into the jugular vein. A small slit was made in the vessel wall and the venous cannula was threaded into the lumen until the flared end was flush with the wall of the jugular vein. The cannula was secured by ligatures, one of which was placed immediately behind the flared tip. By securing the cannula in this position, the suction created from the flow of venous blood past the tip aids in keeping the shunt functional until the calf fully recovers from anesthesia. A short piece of PE 350 tubing (see Fig. 1) served to connect the two portions of the shunt.

Two rows of sutures were used to close the incision; one approximated the edges of muscle. The connecting portion of the shunt was exteriorized as shown in Fig. 3.

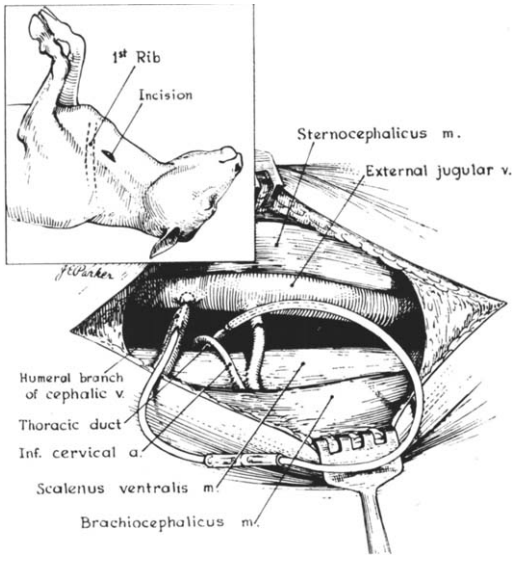


FIG. 2. Schematic illustration of surgical field with thoracic duct-venous shunt secured in position.

FIG. 3. Exteriorized loop in unanesthetized calf (standing and facing left).

*Results and discussion.* No special postoperative care was required other than frequent checks for patency. The calves were housed in open pens without restraint.

In 15 such preparations the shunts remained functional for as long as 18 days, usually 7 to 10 days. Intermittent administration of heparin did not significantly increase this time. The principal cause of obstruction was the formation of thrombi near the shunt outlet; only seldom did the primary cause of blockage occur in the lymph duct. When obstruction occurred early, as was evidenced by back pressure in the lymph duct, patency could frequently be reestablished by forced flushing of the venous cannula with sterile saline. In one calf, in which the venous outlet was beginning to occlude after 8 days, additional 7 days of functional circulation were obtained by inserting a short length of polyethylene tubing (PE 205) into the left jugular vein through a 12 g needle, and coupling the exteriorized portion to the thoracic duct cannula. This was easily accomplished under local anesthesia. The artificial, plugged venous cannula was pulled out. It is possible that the right jugular vein could be used in addition to the left jugular vein, and preparations which function for 20 to 30 days might well be achieved.‡

No apparent physical deterioration of the animals was observed. They gained weight and maintained rectal temperatures within the normal range. Peripheral blood and thoracic lymph cell counts reached stable levels approximately 72 hours following surgery.

In 5 animals (weighing 60 to 80 kg) the lymph flow rate was measured, following a recovery interval of at least 72 hours, by intermittent collection and reinfusion of 250 ml volumes of lymph over a 24 hour period. The calves had been trained to being kept in a stanchion and lymph was collected by attaching a polyvinyl extension to the thoracic duct cannula. During collection the venous cannula was either clamped off or used as a route for intravenous infusion. The range of flow rates was 6.5 to 8.3 ml/kg/hour. The lymph cell counts varied between 11,600 and 32,500/mm$^3$. From these data, along with the white blood cell counts, differential counts, and an estimated blood volume of 78.6 ml/kg(9), it was calculated that the daily influx of lymphocytes from the thoracic duct was 6.1 to 9.9 (average 8.3) times the number of lymphocytes in the blood at any one time.

In several calves branching of the extrathoracic portion of the thoracic duct precluded establishment of a satisfactory cannula. A technique for visualizing the duct before surgery would be helpful and is now being explored.

*Summary.* A technique is described for the preparation of a chronic thoracic duct-venous shunt in calves. These shunts remained functional up to 18 days, usually 7 to 10 days. Calves were found to be excellent animals for experiments involving thoracic duct lymph.

The authors are indebted to Drs. V. Perman, M. O.

‡ In a recent experiment a 12 cm piece of PE 190 tubing (i.d. 0.047″ x o.d. 0.067″) was threaded through the venous cannula into the jugular vein 7 days following surgery. Every second day thereafter the tubing was replaced. In two such preparations the shunts remained functional in excess of 6 weeks.

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## Alterations in Gastric Secretion of Rats Induced by a Potassium Deficient Diet.* (28189)

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The importance of potassium in maintenance of functional and structural integrity of the kidney in rats, dogs, and men, has been recently emphasized by numerous observers (1). Other organs of secretion have received less attention in this regard, although 2 previous studies have been carried out on the effect of potassium deficiency on gastric secretion in the rat(2) and in the dog(3). The present report confirms certain findings in these studies and extends the observations.

*Materials and methods.* Forty-eight female Holtzman rats with a starting weight of 160-200 g served as the experimental animals. One half of the rats were fed a potassium deficient diet of the following composition:

| | % |
|---|---|
| Corn starch | 64.2 |
| Casein | 30.0 |
| Butterfat | 3.5 |
| Calcium carbonate | 1.3 |
| Sodium chloride | 1.0 |
| Complete vitamin fortification (including trace elements) | |

This diet contains 0.23 meq of potassium per 100 g of diet by actual analysis. The other half of the animals were fed the identical diet but with 2.0 g of $KH_2PO_4$ added to each 100 g of diet. The rats were housed as triplets in metabolic cages, given demineralized drinking water, and each experimental triplet pair-fed with a control triplet.

Two weeks after the diet was begun, the rats were fasted for 48 hours and had pyloric ligation under ether anesthesia. Four hours later the rats were again anesthetized, blood was drawn from the inferior vena cava, and the gastric juice was harvested by opening the stomachs over a funnel and insuring a total collection. Sodium, potassium, magnesium, calcium, chloride, and phosphorus were measured on plasma and gastric juice, and free HCl and pepsin were additionally measured on the gastric juice. Sodium and potassium measurements were performed on a flame photometer with an internal lithium standard. Magnesium was determined by the technic of Simonsen, Westover and Wertman (4); calcium by an EDTA chelation method (5); chlorides by the Aminco-Cotlove chloride tritrator; phosphorus by the autoanalyzer; and pepsin by a modification of the method of Anson and Mirsky(6).

*Results* in the test animals were compared to those in the control animals in terms of both concentration and total 4-hour secretion of the various substances.

Table I lists values for some plasma electrolytes. The only statistically significant difference is in plasma potassium concentra-

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