

CHRONIC PERIPHERAL LYMPHATIC CANNULATION IN THE DOG

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ABSTRACT

Peripheral lymph collected acutely has been commonly sampled as representative of non-visceral interstitial fluid. By developing a prenodal lymphatic-lymphatic (L-L) shunt, we were able to collect peripheral lymph for 3-5 days in unanesthetized dogs.

The L-L shunt was constructed entirely of medical grade silicone rubber tubing designed with a slip of coupling which allowed the shunt to be disconnected for lymph collection and reconnected at night. Average peripheral lymph flow (4.9ml/hr leg) in unanesthetized dogs was almost twice the flow rate previously observed in anesthetized dogs. The average lymph/plasma total protein concentration ratio (0.16), however, was similar to that previously found in anesthetized dogs. Lymph protein concentration fell with the collection during the day and became more concentrated at night. Lymph flow did not change greatly during daytime collection. Average peripheral lymph collection volume was greater than 200ml/dog.

The L-L shunt allows collection of prenodal lymph in experiments where unanesthetized dogs are required (e.g., feeding studies). They also are useful when multiple protocols are conducted on the same dog or when large volumes of peripheral lymph are required.

Acute collection of prenodal popliteal lymph in dogs has been commonly used to study the properties of peripheral capillaries and the

composition of peripheral interstitial fluid (1-4). Although popular, acute techniques have a number of disadvantages. Anesthesia may affect microvascular hemodynamics and thus, the composition of interstitial fluid (5-7). Lack of spontaneous movement decreases lymph transport and requires passive movement of the hindlimb to maintain lymph flow (8-10). Lymph collection time is limited by surgical procedures making it impractical to conduct several different protocols in the same animal. The presence of anesthesia obviously precludes experiments, such as feeding and exercise protocols, which require conscious acts by the subject.

Many of these disadvantages can be overcome through the use of a peripheral lymphatic shunt as described here. The shunt allows collection of peripheral lymph (average 200ml) in unanesthetized dogs over periods of at least three to five days.

MATERIALS AND METHODS

Preparation of the Cannulas

The general form of the cannulas is shown in Fig. 1. Two sizes of each cannula pair (OD=0.9 or 1.2mm) were constructed and used to match the size of the lymphatics encountered during surgery. Both inlet and outlet cannulas are constructed entirely from medical grade silicone rubber tubing (Dow Corning Corp., Midland, MI). The external

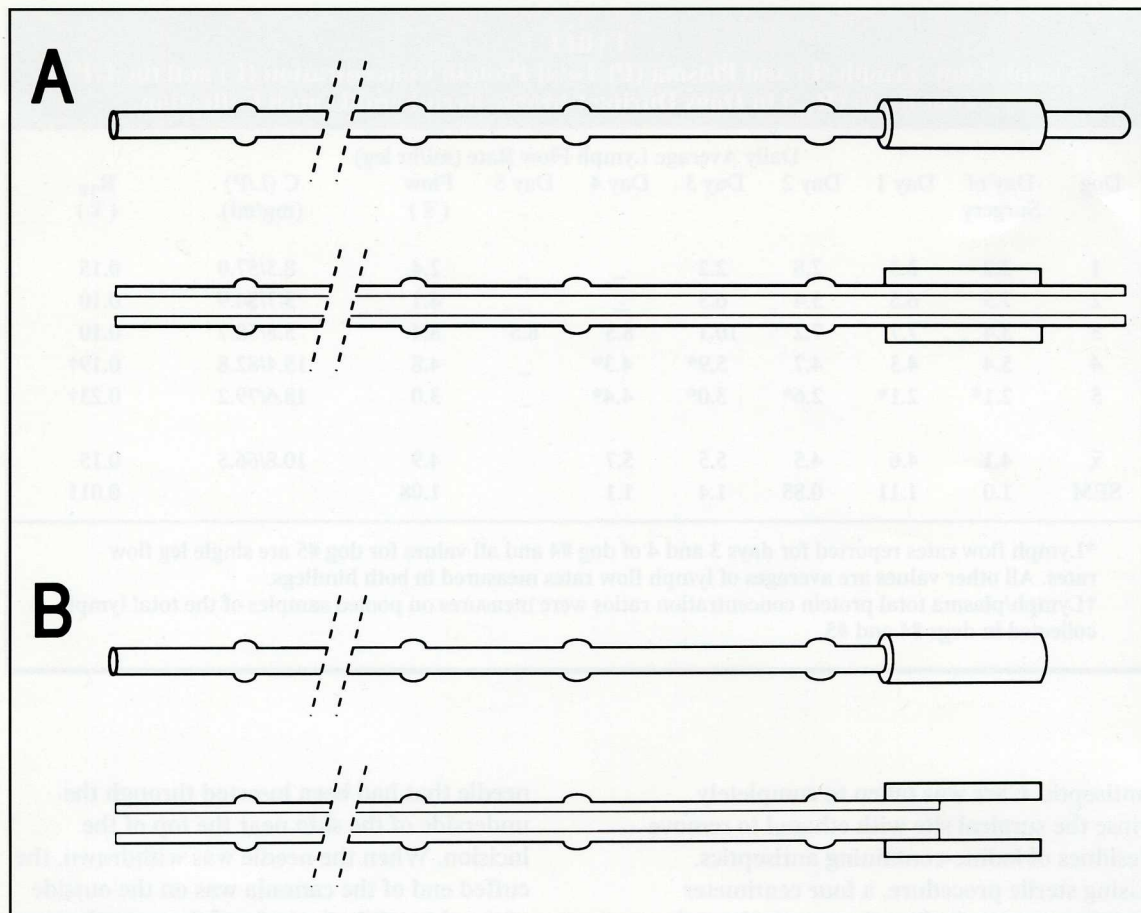


Fig. 1. Chronic peripheral lymph collection cannulas. Plain and cross-sectioned views of the outlet (A) and inlet (B) cannulas which, when connected, form a lymphatic-lymphatic shunt in the prepopliteal area. Both cannulas are constructed entirely of medical grade silicone rubber. Original cannula lengths (100mm) are cut to match individual lymphatic anatomy and to minimize total shunt length.

cuffs (OD's of 1.7 and 2.2mm) were swollen in xylene to allow them to be placed over the cannula tubing in the locations shown in *Fig. 1*. After the xylene evaporated, the cuffs were fixed to the tubing with silicone adhesive (Dow Corning, Type A). Six very small blebs of silicone adhesive (exaggerated in *Fig. 1*) were applied to the non-sleeved ends of the cannulas at 1cm intervals along the tubing. The finished cannulas were treated with a 2% solution of TDMAC-heparin complex (Polysciences, Inc., Warrington, PA). Ethylene oxide was used to sterilize the cannulas after the coatings were dry.

Surgical Preparation

All procedures of this protocol were approved by our institution's Animal Care and Use Committee (Protocol 044-692D). Heartworm-free mongrel dogs were anesthetized with methohexital, sodium (10mg/Kg; Lilly), intubated and placed on halothane (1-2%) and nitrous oxide (60/40 V/V N₂O/O₂) anesthesia. The dogs were then placed in the prone position with hindlegs supported by adjustable fixtures (11). The skin over the lateral saphenous vein was then shaved with an electric clipper and scrubbed with

TABLE 1
Lymph Flow, Lymph (L) and Plasma (P) Total Protein Concentration (C) and the L/P Protein Ratio (R_{TP}) in Dogs During Chronic Peripheral Lymph Collection

Dog	Day of Surgery	Daily Average Lymph Flow Rate (ml/hr leg)					Flow (\bar{x})	C (L/P) (mg/ml)	R _{TP} (\bar{x})
		Day 1	Day 2	Day 3	Day 4	Day 5			
1	2.2	2.3	2.8	2.2	—	—	2.4	8.3/57.0	0.15
2	7.3	6.5	5.4	6.3	—	—	6.1	5.7/54.9	0.10
3	3.4	7.7	7.2	10.1	8.5	8.5	8.4	5.8/58.7	0.10
4	5.4	4.3	4.7	5.9*	4.3*	—	4.8	15.4/82.8	0.19†
5	2.1*	2.1*	2.6*	3.0*	4.4*	—	3.0	18.6/79.2	0.23†
\bar{x}	4.1	4.6	4.5	5.5	5.7	—	4.9	10.8/66.5	0.15
SEM	1.0	1.11	0.85	1.4	1.1	—	1.08	—	0.011

*Lymph flow rates reported for days 3 and 4 of dog #4 and all values for dog #5 are single leg flow rates. All other values are averages of lymph flow rates measured in both hindlegs.
 †Lymph/plasma total protein concentration ratios were measures on pooled samples of the total lymph collected in dogs #4 and #5.

antiseptic. Care was taken to completely rinse the surgical site with ethanol to remove residues of iodine-containing antiseptics. Using sterile procedure, a four centimeter incision was made directly over the lateral saphenous vein beginning just below the popliteal lymph node and extending toward the paw. Loops of silicone rubber (602-155, Dow Corning) were placed under the subcutaneous connective tissue on either side of the vein near the proximal end of the incision. These loops were tied with moderate pressure to occlude the connective tissue and its accompanying lymphatics. The distended lymphatics appeared as colorless lines within the connective tissue. It was often possible to discern branching and valve patterns of the lymphatics with a minimum of further dissection.

The largest lymphatic was normally cannulated with the lymph collection cannula (outlet, Fig. 1A). The site chosen for cannulation was the longest intervalvular segment visible on the lymphatic. The blebbed end of the outlet cannula was placed through a 15ga

needle that had been inserted through the underside of the skin near the top of the incision. When the needle was withdrawn, the cuffed end of the cannula was on the outside of the skin while the body of the cannula was within the incision lying on the subcutaneous connective tissue parallel to the lymphatic. The position of the cannula was adjusted so that no more than 3-4cm of the cuffed end lay outside the skin. This length was necessary to allow subsequent collection of lymph.

The distal end of the cannula was cut with a slight bevel and positioned so that the tip of the cannula lay halfway between the valves of the chosen lymphatic segment. This was necessary to prevent obstruction of the end of the cannula by the distal valve leaflets. Two silk ligatures (5-0) were placed on either side of the more proximal valve. One ligature was tied just proximal to the valve to obstruct the lymphatic further and provide a counter force to prevent movement of the lymphatic during cannulation. One fourth of the lymphatic's diameter was then cut just distal to the more

TABLE 2
Within Experimental Day Lymph Flow (ml/hr/leg)

Dog	Hours After the Start of Daily Collection						
	1	2	3	4	5	6	7
1 [3]	2.8	1.7	2.4	2.0	3.0	2.8	2.5
2 [3]	4.4	6.3	5.9	6.6	6.1	5.6	7.5
3 [5]	5.6	8.2	9.2	7.7	9.4	8.5	9.9
4 [4]	3.4	3.8	3.4	3.3	4.3	4.7	4.1
5 [4]	3.8	4.2	3.6	3.0	2.9	1.8	2.2
\bar{x}	4.0	4.8	4.9	4.5	5.1	4.7	5.2
SEM	0.48	1.11	1.22	1.11	1.21	1.17	1.50

Bracket=Number of days averaged per row

TABLE 3
Lymph (L), Plasma (P) Total Protein Concentration (C) and L/P Protein Ratio (R_{TP}) (Dogs 1-3 see Table 2 above)

	Hours After the Start of Daily Lymph Collection						
	1	2	3	4	5	6	7
C (L/P) (mg/ml)	10.5/56.8	8.6/55.3	6.3/56.3	5.4/57.2	5.3/57.2	4.2/56.6	4.8/57.9
R _{TP}	0.18	0.16	0.11	0.09	0.09	0.07	0.08

proximal valve. With the opening stabilized by a fine spring stainless steel wire (California Fine Wire, Grover City, CA), the tip of the cannula was inserted into the lymphatic. The first cannula bleb entered the lymphatic allowing ligation of the lymphatic behind it. The ligatures were then tied, blotted dry, and glued with ordinary cyanoacrylate adhesive. The temporary atraumatic ties in the connective tissue on the cannula's side of the vein were then replaced with permanent 2-0 silk ligatures. The inlet cannula (*Fig. 1B*) was placed on the other side of the vein in a similar manner to that of the outlet cannula except that its tip was directed toward the popliteal node. Only about 2cm of the inlet cannula's

sleeved end projected from the outer skin surface. The positions of both cannulas were adjusted to minimize the length of the lymphatic shunt as much as possible. The inflow cannula was filled with sterile saline and plugged before cannulation to prevent air embolism of the popliteal node. Infusion of sterile saline was begun into the inlet cannula at a rate of 1-2ml/hr. The atraumatic ties proximal to the inlet cannula were removed and permanent subcutaneous ties placed distal to the inlet cannula. Both sets of permanent ties (proximal to the outlet cannula, distal to the inlet cannula) prevent collateral lymphatic drainage under the lateral saphenous vein and served to direct the total lymphatic outflow of

the dorsum of the paw to the lymphatic shunt. Antibacterial ointment (Burroughs Wellcome Co., Research Triangle Park, NC) was placed into the incision which was closed with 4-0 Vetafil™ suture. It should be noted that the area of operative trauma near the popliteal node was widely separated from the area of lymph formation (dorsum of the paw). This precaution minimized the effects of operative trauma on the microvascular bed responsible for lymph formation. Each dog was treated with antibiotics on the day before surgery and on each experimental day (1-2ml Cambiotic™ IM, Pfizer, Inc., New York, NY).

Both hindlegs were operated upon at the same time to encourage the dog to place its weight on both legs. The inlet and outlet cannulas were connected to form a loop and administration of inhalation anesthesia discontinued. A nylon mesh jacket was used to support stretch stockinette leggings (x Span™, Baxter, McGraw Park, IL) which covered both hindlegs. The dogs were allowed to recover from anesthesia while placed in a mesh support (Alice King Chatham Medical Arts, Hawthorne, CA) attached to adjustable rails (X-95, Klinger Scientific, Richmond Hill, NY). They usually were fully awake and standing in the sling within 30 minutes. At that time, nodal saline perfusion and lymph collection was begun.

Lymph Collection

The L-L shunt was externalized through a small hole cut in the stretch stockinette covering both rear legs. The shunt was opened and an infusion of sterile saline (1ml/hr) was begun into the inlet cannula. The outlet cannula tip was directed through a central hole in the cap of a sterile 2 ml screwtop vial (Sarstedt, Princeton, NJ). Each vial contained 20μL of an anticoagulant preservative mixture (10% w/v disodium EDTA, 10% w/v sodium azide). The vials were then snapped securely into cable clamps (Small Parts, Inc., Miami Lakes, FL), which were glued to surface strips

of "hook" Velcro™ fabric. The "hook" surface of the strip adhered tightly to the stockinette leg covering and moved with the dog's leg. Lymph volume was estimated by comparing the meniscus height of collected lymph to graduated marks on identical collection vials which had been calibrated volumetrically. Total protein in lymph and blood plasma was measured using Folin phenol reagent (12). Dogs were offered drinking water throughout the day. The cannulas were rinsed in sterile saline and reconnected at the end of the day. It was important to cut the inlet cannula's outer cuff to a length which allowed the ends of the cannulas to fit tightly together. A slight gap between the ends of the connection greatly promoted clotting. A very small piece of surgical tape was wrapped once around the outer cuffs to strengthen the coupling. At the end of each collection day, the dogs were fed and a muzzle and foam cervical collar placed on them to prevent destruction of the cannulas during the night. A commercial dog muzzle did not afford sufficient protection to the cannulas. Our muzzles were constructed of 2" wide Velcro™ strips sewn in the form of an "X" with the "loop" side out. This device was placed over the dog's muzzle with two strips aligned with the top and bottom of the muzzle and one on either side. A one inch wide "hook" Velcro™ strip was spiraled around the dog's muzzle starting near the nose. When the spiral strip neared the eyes, it was carried under the jaw and behind the ears. All four strips were secured to the mesh jacket. A foam surgical collar was placed over the muzzle's strips. It was important to bandage each front paw's hallux (dewclaw) to prevent tearing of the muzzle. The dogs had continuous access to water and could easily drink with the muzzle in place. They rapidly adapted to the muzzle and usually did not attempt to remove it after the first night. Acclimated dogs sometimes fell asleep in the lymph collection stand, requiring gentle stroking of the lateral leg surfaces to promote lymph flow. Young, barely adult dogs, with relatively long legs were the most satisfactory experimental subjects.

RESULTS

Three of nine preparations (legs) failed due to clotting. Cannulas consistently clotted overnight. Whereas it was occasionally possible to restart a clotted cannula, the collected lymph was usually tinged with blood and unsuited for analysis.

Table 1 shows average lymph flow rates and total protein lymph/plasma concentration ratios for five chronic dogs. Flow rates for the day of surgery were averaged over the entire day of lymph collection. They represent a composite of several non-steady-state conditions (acute collection under anesthesia, collection during recovery, and collection standing in the sling) (*Table 2*). Lymph/plasma concentration ratios (R_{TP}) of total plasma proteins are also presented in *Table 3*. There were no clear trends with respect to the day after surgery in either the lymph flow (5 dogs) or R_{TP} data (3 dogs.)

DISCUSSION

The average chronic peripheral lymph flow rate shown in *Table 1* (4.9ml/hr leg) is almost twice that we previously reported for pentobarbital anesthetized dogs (2.6ml/hr leg) (13). The more natural movements of the unanesthetized dog apparently account for the increase in lymph formation and transport. The average total plasma protein lymph/plasma concentration ratio in the chronic dog (0.16) is similar to that in anesthetized dogs (13).

The lymph protein concentration ratio consistently fell during daily lymph collection and reached approximately one half the initial value. Most of this change occurred in the first four hours of collection. This decline probably was related to the transition between prone and upright posture. Similar changes in lymph composition were observed during chronic peripheral lymph collection in humans (14). In that earlier study, assumption of an upright position initially increased lymph flow. Later, lymph flow stabilized and lymph protein concentrations fell throughout the day. At the

end of the collection day, lymph protein concentrations were 45% of those collected in the horizontal position. According to Olszewski et al, steady state values of lymph flow and protein composition were observed in normal men after 3-6 hours in the horizontal position (14).

It seems clear that changes in body position and activity exert dramatic effects on the relative rates of peripheral lymph formation and transport. This in turn, affects lymph composition and interstitial fluid volume. Steady-state conditions require stable body position and activity during lymph collection.

We are not aware of previously published techniques which allow chronic collection of prenodal peripheral lymph in dogs. Chronic peripheral lymph studies have been performed on humans (14). Continuously dripping, lymphatic fistulas (pre- and postnodal) have been reported in sheep (15,16). A variety of chronic thoracic duct shunts have been reported in humans (17) and other animals (18-20).

The non-valved lymphatic shunt undoubtedly reduced the efficiency of lymph transport. However, rapid flow of very small trapped air bubbles was observed when the shunt was connected. The normally valved lymphatic segments proximal and distal to the shunt appeared to maintain adequate flow across the shunt. There was minimal peripheral edema even when the shunt became clotted. Collateral deep lymphatic drainage of the paw apparently limited the extent of edema.

The length of time a cannula flowed seemed related to: 1) long intravalvular distances on the lymphatics; 2) short shunt lengths; 3) careful approximation of the coupling ends; and 4) constant stirring of lymph and anti-coagulant during collection.

The cannulation technique reported here allows chronic collection of prenodal peripheral lymph in unanesthetized dogs. It is particularly useful in lymph studies that would be affected by anesthesia, where large volumes of peripheral lymph are needed or when multiple protocols must be conducted in a single animal.

The major disadvantages of this technique are the requirement for sterile surgery and the need for constant supervision of unanesthetized animals during lymph collection.

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