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## Comparative Studies of Lymph and Lymphocytes of the Thoracic Duct and Right Lymphatic Duct I. Normal Dogs\*

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### Introduction

The thoracic duct drains principally the abdominal viscera and lower extremities, the left upper limb and left side of the head and neck, while the right lymphatic duct drains most of the lungs, serous cavities, right upper extremities and right side of the head and neck.

A comparison of the two systems was made by the simultaneous drainage of lymph from the thoracic duct and right duct which was collected hourly for 5 hours. The purpose of the experiments was to study the rate of flow, lymphocyte content and output, morphologic types of cells and electrophoretic differences in the supernatant fluid of lymph from the two largest lymph systems in the body.

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### Methods

Healthy dogs were anesthetized by the intravenous injection of 29 mgm/kg body weight of pentobarbital sodium. Respirations were maintained by an intermittent positive pressure respirator with an endotracheal catheter. Evans blue dye (T-1824) was instilled into the right bronchus for visualization of the right lymphatic system. Cannulation of the right lymphatic duct was performed by the insertion of a catheter into the isolated external jugular vein where the multiple channels of the right duct enter (1, 2). Evaporated milk was given orally to facilitate cannulation of the thoracic duct. Both cannulations were done in the neck without opening the thorax. Lymph was collected hourly for 5 hours in separate heparinized tubes. Specimens of venous blood were collected hourly for 5 hours for leucocyte, erythrocyte and hematocrit determinations, and for chemical and immunological evaluations of the serum. Intravenous fluids were not administered.

Electrophoretic studies were done on thin layer agarose gel films (3). Lipoproteins were stained by the method of *Elevitch* (4) and counterstained with Amidoschwarz for the visualization of proteins.

### Results

#### Peripheral blood

The average PCV, RBC and WBC of peripheral blood drawn at the first and fifth hours is shown in Table 1. There were only small differences indicating that the 10 animals which were used for the experiments remained in good condition during the five hours of anesthesia.

Table 1 PCV, RBC and WBC of peripheral blood drawn at last and 5th hour of experiment. Average of 10 experiments.

	1st hour specimen	5th hour specimen
Av. PCV vol./%	43	44
Av. RBC mil./cu.mm	5.4	6.1
Av. WBC cell./cu.mm	8,800	13,580

#### Flow and composition of lymph

The average hourly lymph flow and cell content of thoracic duct (TD) and right lymphatic duct (RD) calculated from 5 hours of flow is recorded in Table 2. The average lymph flow from the TD was 18.5 ml/hr and that of the RD was 3.5 ml/hr. The concentration of cells/hr/mm<sup>3</sup> was 8,000 for the TD and 4,170 for the RD. The total cells in millions/5 hours was 3,668 (TD) and 382 (RD).

Table 2 Average hourly lymph flow and cell content of thoracic duct and right lymphatic duct calculated from 5 hours of flow. 10 experiments.

	Wt. kg	Lymph flow		Cell output	
		Flow ml/hr	Total lymph flow 5 hrs (ml)	Cells/hr/mm <sup>3</sup>	Total cells millions/5 hrs
Thoracic duct	15.0	18.5	89.1	8,000	3,668
Right lymph duct	15.0	3.5	17.7	4,170	382
Ratio RD :TD		1 : 5.3	1 : 5.0	1 : 1.9	1 : 9.6

The average lymph flow and cell content obtained during the first and fifth hours are illustrated in Table 3. The TD flow decreased from 18.6 ml/hr to 14.85 ml/hr while the cells per mm<sup>3</sup> decreased from 9,040 to 7,430. The total cell count in millions/10 kg body weight decreased from 128.2 to 80.85. The RD flow increased from 3.14 ml/hr to 4.1 ml/hr while the cells per mm<sup>3</sup> increased from 4,530 to 4,750. The total cell count in millions/10 kg body weight increased from 9.30 to 14.5.

Table 3 Lymph flow and cell content of thoracic duct and right lymphatic duct. Average values obtained 1st hour and 5th hour, 10 experiments.

	Lymph flow ml/hr		Lymphocytes per cu.mm		Lymphocytes millions/10 kg B.W.	
	1st hour	5th hour	1st hour	5th hour	1st hour	5th hour
Thoracic duct	18.6	14.85	9,040	7,430	128.2	80.85
Right lymphatic duct	3.14	4.1	4,530	4,750	9.30	14.5

#### *Cell morphology of lymph*

The morphologic types of lymphocytes in lymph from TD and RD are depicted in Table 4. The thoracic duct lymph contains a high percentage of pachychromatic small ("mature") lymphocytes (mean 72%; range 64–83%) with a moderate number of medium size (mean 19%; range 12–26%), and a few large basophilic lymphocytes (mean 9%; range 2–13%).

Table 4 Morphologic types of lymphocytes in lymph from TD and RD. Average of 10 experiments.

	Left duct lymph	Right duct lymph
Small lymphocytes	72 ± 10%	52 ± 13%
Medium-size lymphoc.	19 ± 7%	35 ± 8%
Large lymphocytes	9 ± 6%	13 ± 4%

Right duct lymph has a mean count of 52% small (range 41–65%), 35% medium (range 26–42%) and 13% large (range 9–17%) lymphocytes. Occasionally plasmacytoid cells are noted.

The above data have been subjected to statistical analysis with the aid of a 3 × 2 contingency table. The Log-Likelyhood Ratio (G) test was applied and the sample statistic with 2 degrees of freedom was computed to be  $G = 8.78411064$  which is statistically significant at  $p < 0.05$ .

#### *Supernatant fluid electrophoresis*

Electrophoresis studies were made on the supernatant fluid after centrifugation of the hourly samples of lymph from the right duct and thoracic duct. All dogs had received a fatty meal prior to the experiments, therefore a striking difference in the lipid-electrophoretic patterns of right and left duct lymph was observed (Table 5). In lymph from the left duct, chylomicrons at the locus of application were present and lipids (lipoproteins) were observed to migrate into the pre-albumin area. In contrast to these observations, chylomicrons were not found in lymph from the right duct, with a single exception during the first hour (dog No. 3). Subsequent specimens were clear. If the initial appearance of lipids in the right duct in dog No. 3 was due to a communication

Table 5 Comparison of lipoprotein electrophoresis of serum and supernatant fluid from thoracic duct and right duct specimens.

Specimen	Lipoprotein in pre-albumin phase	Alpha-lipoprotein	Beta-lipoprotein	Chylo-microns
Serum	not present	present +++ (distinct line)	present +++ (distinct line)	not present
Thoracic duct supernatant lymph fluid	present	present +-+ (indistinct line)	present +++ slurred	present
Right duct supernatant lymph fluid	not present	present +-+ (distinct line)	present +-+ (distinct line)	not present <sup>1</sup>

<sup>1</sup> Except in one specimen described in text.

with the thoracic duct, one must assume that after the first hour this ceased to function. The electrophoretic appearance of the supernatant fluid from the right duct appeared more like serum than did thoracic duct lymph. The main difference between the pattern of dog serum and right duct supernatant lymph fluid is a smaller amount of beta-lipoproteins in the latter.

### Discussion

The general condition of the dogs during the 5 hour period of the experiments was stable (Table 1).

The average lymph flow from the thoracic duct of 10 anaesthetized dogs measured hourly for 5 hours was 18.5 ml/hr (range 10.4–29.4 ml/hr) (Table 2). This average value agrees with that reported by other observers (5). *Shafroff* and *Kan* (6), confirming the findings of many previous observers, found the thoracic duct flow to be extremely variable (14 to 110 ml/hr) in 22 patients in whom lymph was collected intermittently from 1 to 8 days.

The number of leucocytes in thoracic duct lymph averaged 110.4 million/hour/10 kg body weight, with a wide variation (34.7–300 million). *Yoffey* and *Courtice* (5) reported 21 experiments in dogs in which the average number of cells in lymph from the thoracic duct (the right duct was not cannulated) was 211.6 million cells/hour/10 kg body weight with a wide variation (30.2–875.5 million).

The decrease in thoracic duct lymph flow and cell content measured during the first and fifth hours (Table 3) may be due to dehydration associated with prolonged anesthesia without fluid replacement. Another factor may be a lessened activity, due to anesthesia, of the liver and intestinal tract which are considered to be the main areas drained by the thoracic duct (5).

The average value of the right duct flow calculated from all the hourly specimens was 3.5 ml/hr which agrees with our previously published material (2,7) and that in the literature (5).

The increase in right lymphatic duct flow and cell content (Table 3) was associated with an increase in the average number of RBC's from 0.87 million cells/mm<sup>3</sup> in the first hourly specimen to 1.56 million cells/mm<sup>3</sup> in the fifth hourly specimen. This increase

appears to be related to the operative procedure, prolonged anesthesia, and particularly to the use of an endotracheal tube and positive pressure respiration for five hours.

The thoracic duct and right duct lymph, when collected hourly for five hours, is seen to undergo alterations in both flow and composition, the changes differing on the two sides. Possible explanations for the observed differences have been suggested.

Studies of lymphocytes derived from the left and the right lymph duct systems show a marked difference both in the total output of cells and in the morphologic differential count. If one considers those lymphocytes with a leptochromatic nucleus, i.e., large and medium sized lymphocytes to be a factor in cellular immunity, one must conclude that under normal conditions the right duct lymph has a statistically significantly greater content of these cells compared to the thoracic duct.

It has long been recognized that most of the cells in thoracic duct lymph are pachy-chromatic small lymphocytes (e.g., *Reinhardt* and *Yoffey*, 8; *Zucker-Franklin*, 9). The fact that lymph from the right duct contains an appreciably lower percentage of small lymphocytes poses an obvious question which we cannot as yet answer.

The electrophoretic demonstration that chylomicrons are only found in the supernatant fluid of the thoracic duct suggests strongly that an anastomosis between the two drainage systems was not a factor in these experiments (Table 5). The lipoprotein pattern of the right duct fluid resembled that of serum rather than that of thoracic duct lymph.

The electrophoretic studies suggest that the two lymph streams, in the right and the left ducts, are derived from two distinct areas and do not undergo admixture in our experiments. This view gains support from the morphologic studies on the lymphocytes contained in the two streams.

### Summary

1. The thoracic duct and right lymphatic duct were drained simultaneously for 5 hours.
2. The rate of lymph flow, lymphocyte concentration and total output, morphologic types and electrophoretic differences in the supernatant fluid are reported.
3. The number of morphologic types of lymphocytes from the two largest lymph systems in the body are statistically different.
4. There is a striking difference in the lipid-electrophoretic patterns of right and left duct lymph further suggesting that, in addition to the anatomic distribution, there are important functional differences between the two lymph systems. Communications between the right duct and thoracic duct were not factor in these experiments.

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