

Summary. Guinea pigs which have been sensitized to tuberculin by the injection of heat-killed tubercle bacilli in paraffin oil show an increased *in vitro* tuberculin leucocyte

cytolysis within 48 hours after a positive intracutaneous tuberculin reaction.

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Appearance of Protein Tagged with Radioactive Iodine in Thoracic Duct Lymph.* (17599)

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When plasma protein or albumin is tagged with radioactive iodine (I-131) and the combination is injected intravenously into dogs or humans to determine the plasma volume, there is a progressive decrease in the measurable radioactivity of blood samples withdrawn periodically.(1,2) This suggests the passage of the iodinated protein into extravascular spaces. It is known that protein circulating in the blood stream passes with considerable facility into the thoracic duct lymph. The concentration of protein in lymph varies in different parts of the body. Field *et al.*(3) reported the plasma protein concentration in 13 dogs to be 6.25 g per 100 cc whereas the concentration in thoracic duct lymph was 4.00 g per 100 cc. The average concentration of albumin in the plasma and thoracic duct lymph of the same animals was 3.61 and 2.45 g per 100 cc respectively. The purpose of the present experiments was to determine the rapidity with which the intravenously injected radioactive protein appeared in thoracic duct lymph. The amount of radioactivity in

lymph 10 minutes following the intravenous injection was of special interest, since this period is the commonly accepted interval between the injection of either radioactive protein or T 1824 and withdrawal of blood for the determination of this plasma volume.

Method. Eleven healthy adult fasting dogs, weighing 8 to 16 kilos, were used. Each dog was anesthetized with intravenous sodium pentobarbital .030 g per kilo of body weight. Through a left cervical approach the junction of the thoracic duct and the left subclavian vein was exposed. The duct was ligated close to the subclavian vein and, depending upon its size, a number 20 or 18 gauge needle was inserted into the lumen of the duct and fixed with a No. 000 black silk ligature. The needle was irrigated with a few drops of heparin to prevent clotting. A femoral vein and artery were exposed and isolated to be used for injection of the iodinated protein and collection of blood samples. Twenty cc of iodinated protein containing less than 1 g of albumin and approximately 40 microcuries of radioactivity were injected into the femoral vein. Simultaneous samples of blood and lymph were collected from the femoral artery and thoracic duct 10, 30, 60, and 120 minutes following the injection. The animals were divided into two groups. Group I consisted of 6 dogs into which radioactive iodinated human albumin was injected. Group II consisted of 5 dogs into which was injected canine plasma protein activated with the iodine. Technical difficulties prevented the collection of some of the lymph samples in the first

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TABLE I.
Percent Radioactivity of the Thoracic Duct Lymph in Animals Injected with Human Albumin Tagged with Radioactive Iodine.

Dog No.	Radioactivity $\frac{\text{cc/chyle}}{\text{cc/plasma}} \times 100$ Time after injection			
	10 min.	30 min.	60 min.	120 min.
6	9.6	33.9		
8	4.6	20.2		
9	3.2	36.8	53.9	
10	10.6	49.1		53.8
11	4.0	10.1		44.6
12	8.9	34.1		59.6
Avg	6.8	30.7		52.7

TABLE II.
Percent Radioactivity of the Thoracic Duct Lymph in Animals Injected with Canine Plasma Protein Tagged with Radioactive Iodine.

Dog No.	Radioactivity $\frac{\text{cc/chyle}}{\text{cc/plasma}} \times 100$ Time after injection			
	10 min.	30 min.	60 min.	120 min.
13	1.5	6.2	19.9	35.5
14	2.5	4.8	15.0	29.7
15	1.7	12.0	31.6	43.0
16	0.7	15.5	38.6	54.4
17	0.3	9.6	24.1	36.8
Avg	1.3	9.6	25.8	39.9

group. Lymph samples were collected by allowing the lymph to drop into a test tube containing a few crystals of powdered heparin. Blood samples were collected from the femoral artery. The blood was centrifuged for 15 minutes at 3,500 rpm and the supernatant plasma decanted. One cc samples of lymph and plasma were pipetted into small porcelain dishes and dried at room temperature for 24 hours. The radioactivity of the samples was then determined by means of a Geiger-Mueller counter. The radioactivity of a sample of lymph was compared to that of a corresponding plasma sample as follows:

$$\frac{\text{Counts per min. per cc of lymph}}{\text{Counts per min. per cc of plasma}} \times 100 = \frac{\text{radioactivity of lymph}}{\text{radioactivity of plasma}} \%$$

Results. Table I shows the per cent radioactivity of the lymph samples of each dog in Group I. There is a progressive increase with the time the sample was obtained. There is some variation in the values ranging from

3.2% to 10.6% for the 10-minute specimens and from 10.1% to 49.1% for the 30-minute specimens. Insufficient 60- and 120-minute samples were obtained in this group to warrant a comparison of results.

The per cent radioactivity of the lymph samples of Group II are shown in Table II. This series demonstrates some variation in values between the animals but of a lesser degree than was noted in the previous group. Values ranging from 0.3% to 2.5% were found in the 10-minute specimens and values ranging from 4.8% to 15.5% were encountered in the 30-minute specimens. It is noted that the average value for the 10-minute specimen in Group I was 6.8% whereas in Group II it was 1.3%. Similarly the average percentage for the 30-minute specimen in Group I was 30.7% compared to 9.6% in the second group. There is little doubt that the human albumin diffused from capillaries into lymphatic spaces much more rapidly than did the canine

serum protein. It is possible that the use of a foreign protein (human albumin) produced an alteration in capillary permeability and allowed the more rapid passage of the protein into the thoracic lymph. Also, in the first group of animals, manual pressure was applied to the abdomen in order to increase the flow of lymph; this was not true in the second group. The trauma associated with this procedure may have produced some increase in capillary permeability.

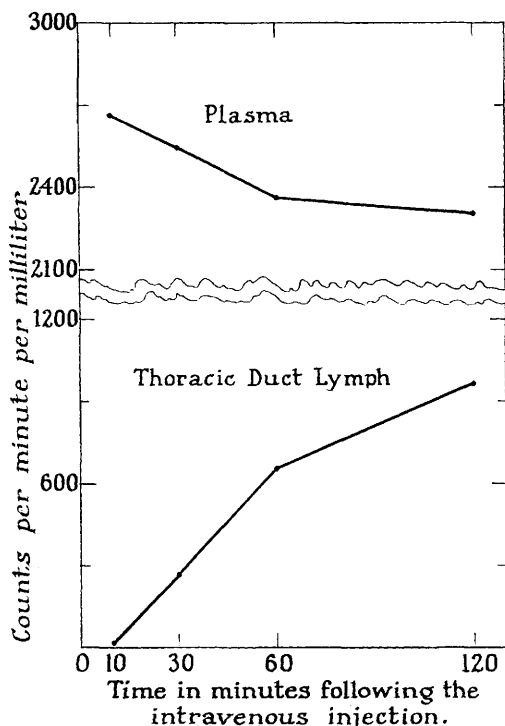


FIG. 1.

Illustration of the increase and decrease of radioactivity of the thoracic duct lymph and plasma respectively, during the 2-hour interval following the intravenous injection of radioactive iodinated protein. The curves represent averages from the 5 dogs in Group II.

Fig. 1 demonstrates the changes in concentration of radioactive iodinated protein in the thoracic duct lymph and the plasma plotted against time. During the first hour, there is a rapid increase in the concentration of the protein in the lymph followed by a more gradual increase. There is a simultaneous decrease in the concentration of the protein in the plasma. Cope and Moore demonstrated that the radioactivity of the serum and thoracic duct lymph reach an equilibrium between six and seven hours following the intravenous injection of radioactive protein.(4) Amino acids are present in thoracic duct lymph. Petersen and Hughes found an average of 23.9 mg per 100 cc in 28 dogs.(5) Lymph samples from two dogs in Group II were dialyzed in a cellophane bag for 48 hours in order to remove any free amino acids or electrolytes which were present. There was no change in the activity of this lymph, indicating that all the radioactivity was due to albumin-bound I-131.

Conclusions. 1. The total lymph volume is not measurable, so that the exact amount of activated protein that is present in the body lymph, following intravenous injection of iodinated protein, cannot be calculated. However, it appears from this experiment that a negligible amount is present in the thoracic duct lymph 10 minutes after injection.

2. During the first hour there is a rapid increase in the lymph content of radioactive protein followed by a more gradual rise.

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