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Lymphatic Pathways and Rate of Absorption of ^{131}I-Albumin from Pericardium of Dogs*

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Summary

Labeled albumin diluted to 4.0 ml with normal saline solution was instilled into the pericardium of 14 dogs. Lymph from the right lymphatic duct (RD) and thoracic duct (TD) was collected at intervals as well as blood samples, and radioactivity measured. Radioactivity was found in both RD and TD lymph, indicating a multiple efferent lymphatic system for drainage of the heart and pericardium. The level of ^{131}I-albumin absorbed from the pericardium reached a peak in 6–22½ hours in RD and TD lymph, and in 6–22½ in blood stream.

Introduction

Knowledge is limited regarding the pathways of drainage to the venous system of the cardiac lymphatics which drain the pericardium and heart. The current view is that cardiac lymph drains principally to the right lymphatic duct (1), based primarily on studies of lymph drainage of the peritoneal cavity.

The purpose of this study was to determine the pathways of lymphatic drainage from the pericardium by measuring the amount of radioactivity in lymph from the thoracic duct (TD) and right lymphatic duct (RD) after instillation of ^{131}I-albumin into the pericardial sac. The rates of absorption by the pericardial and cardiac lymphatics and transport into TD and RD lymph and into the blood circulation were also determined.

Methods

Healthy mongrel dogs weighing 12–25 kg were anesthetized with sodium pentobarbital (29 mg/kg) given intravenously. Respirations were maintained with an endotracheal tube and an intermittent positive pressure respirator.

Lymph from the right lymphatic duct (RD) was collected by a method with was devised by Leeds and Uhley in this laboratory more than 15 years ago (2, 3). In brief, the method consists of isolation of that area of the right external jugular-subclavian vein junction into which the multiple fine lymphatics of the right duct empty. After all the venous tributaries are ligated, this portion of the vein receives right duct lymph which is collected into test tubes by a polyethylene catheter. The thoracic duct (TD) was cannulated with a polyethylene catheter through an incision in the neck. Samples of lymph from the right and left duct were collected simultaneously in all but one experiment. Blood samples were drawn from the left external jugular vein.

After control samples of lymph and blood were collected for comparison against background radiation in the scintillation counter well, the right chest was entered through the 5th intercostal space and 15 microcuries of ^{131}I-human serum albumin, obtained from standard commercial suppliers, were instilled into the pericardium. The labeled albumin was diluted with sterile physiological saline solution to give a standard injected volume of 4.0 ml. This was injected into the pericardial sac through a fine gauge hypodermic needle. The pericardium at the stie of the needle hole was clamped with a curved hemostat and ligated with 3–0 silk. The chest was then closed.

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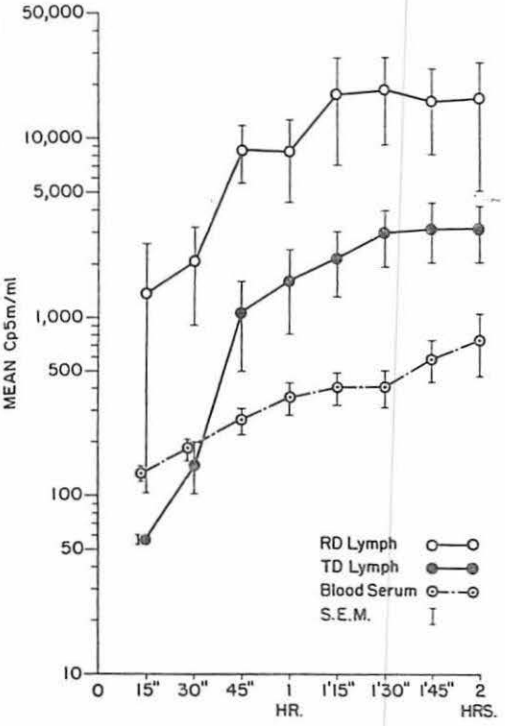

Fig. 1 Mean radioactivity/ml (cp 5 m/ml) in lymph from RD and TD and in blood serum in 5 experiments in which specimens were collected every 15 min for 2 hrs after instillation of ^{131}I-albumin into the pericardial sac. Low levels of radioactivity in TD lymph in 15 and 30 minute samples relative to blood serum and RD lymph is probably related to dilution by the larger flow of lymph in the TD (see Fig. 3 and text).

Specimens of RD and TD lymph and blood serum were collected at varying intervals after the injection. One ml samples of lymph and blood serum were pipetted for measurement of radioactivity in a well scintillation counter. All samples from each study were counted at the same time in order to correct for the decay factor.

The *concentration* of radioactivity of TD and RD lymph (counts per 5 minutes/ml) and the *total* radioactivity or content of lymph (counts per 5 minutes/ml x volume flow of lymph ml/hr), and radioactivity of blood serum (cp 5 m/ml) collected every 15 minutes (Table I, Group I), and each hour (Table I, Group II and III) have been plotted against time on a semilogarithmic scale (Fig. 1–3, 5).

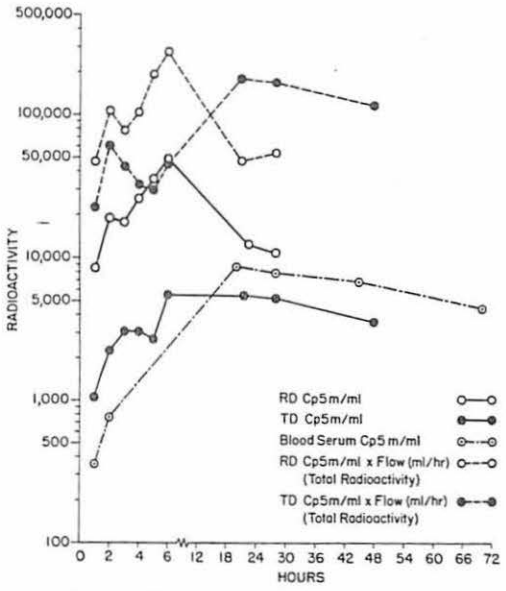

Fig. 2 Graph of 14 experiments showing levels of total radioactivity in RD and TD lymph and in blood serum (lower 3 lines). Radioactivity plotted hourly for 6 hrs and at intervals up to 72 hrs.

Results

The results of 14 experiments are given in Table I.

A. Radioactivity per ml in lymph and blood serum (concentration).

The mean radioactivity *per ml* was greater in RD lymph than in TD lymph in all 13 experiments where both RD and TD lymph was collected (Table I, Fig. 1). The mean radioactivity/ml was greater in RD lymph and in all but the first two samples of TD lymph than in blood serum, 15 min to 2 hrs after instillation of ^{131}I-albumin (Fig. 1). In experiments 11 to 14, in which samples were obtained more than $22^1/_2$ hrs after instillation of ^{131}I-albumin, we observed a steep rise in radioactivity/ml in blood serum, reaching a peak between 2 and $22^1/_2$ hrs and then gradually declining (Fig. 2, lower 3 curves). Blood serum radioactivity/ml was slightly greater than that in TD lymph after $22^1/_2$ hrs but lower than levels in RD lymphs.

Peak values of radioactivity/ml in RD and TD lymph were reached between 6 and $22^1/_2$ hrs, and in blood serum between 6 and $22^1/_2$ hrs, after which the levels fell gradually.

Table 1 Summary of 14 experiments in which ^{131}I-albumin was instilled into the pericardial sac. Lymph flow and radioactivity measured in lymph from RD and TD immediately after instillation (I, II) and beginning $22^1/_2 - 46^1/_2$ hours after instillation (III). Mean values.

Group & # of exp.	Duration of experiment	Frequency of collection	Average lymph flow ml/hr		Average radioactivity/ml cp 5 m/ml			Average total radioactivity/ml/hr cp 5 m/ml x lymph flow ml/hr	
	Hours	Minutes	TD	RD	TD	RD	Blood serum	TD	RD
I/5	2	15	21.6	7.2	1.768	10.635	388	53.377	66.023
Range			9.6-	4.5-	570-	1.756-	206-	8.649-	9.806-
			36.4	14.5	3.751	30.963	455	124.864	145.786
S.E.M.			± 4.8	± 1.9	± 688	± 5.191	± 89	± 26.726	± 25.405
II/5	2–6	60	12.8	3.4	1.967	21.579	–	23.788	96.768
Range			12.0-	1.5-	188-	5.761-	–	2.008-	8.641-
			15.7	6.0	4.163	54.122		47.798	324.735
S.E.M.			± 0.9	± 0.8	± 739	± 8.818	–	± 8.412	± 58.819
III/4	5–6	60	30.3	4.3	5.032	12.115	5.012	158.630	52.767
Range			21.5-	2.9-	3.277-	10.351-	4.337-	70.195-	41.788-
			38.2	5.3	6.896	13.155	5.507	261.685	66.772
S.E.M.			± 3.5	± 0.7	± 930	± 887	± 255	± 41.796	± 7.369

B. Total radioactivity in lymph/ml/hr (content).

The mean levels of *total radioactivity* in RD and TD lymph (recorded every 15 minutes for 2 hours after instillation of ^{131}I-albumin into the pericardium) rose rapidly during the first 90 minutes, followed by a slower rise for the next 30 minutes (Fig. 3). The mean total radioactivity in RD lymph was higher than that in TD lymph for the first 90 minutes. At the end of 2 hours radioactivity levels in RD and TD lymph was approaching equality in the 5 animals in Group I.

Hourly determinations of total radioactivity for 6 hours showed an initial sharp rise. Between 6 and $22^1/_2$ hours the level of total radioactivity in the RD declined but that in the TD continued to rise to a level above that in the RD, the highest level being recorded at $22^1/_2$ hours.

Fig. 3 Mean total radioactivity (cp 5 m/ml x lymph flow/ml/hr) of lymph in RD and TD in 5 experiments. Specimens were collected every 15 min for 2 hrs after instillation of ^{131}I-albumin into the pericardial sac. This graph demonstrates that although the RD lymph on the average contains more ^{131}I-albumin, the TD receives appreciable amounts of ^{131}I-albumin from the cardiac efferent lymphatics.

There was a gradual decline of total radioactivity in TD lymph after 22½ hours. The average total radioactivity/ml/hr, after approximately 24 hours, was higher in TD lymph than in RD lymph in all 3 animals in which this was measured (Fig. 2).

The average total radioactivity/ml/hr (cp 5 m/ml x lymph flow ml/hr) was greater in RD lymph than TD lymph in 6 of the 10 dogs, in the first 2 hours after instillation of ^{131}I-albumin into the pericardial sac. Conversely, total radioactivity was greater in TD lymph than that in RD lymph in 4 of the 10 dogs, in the first 2 hours after instillation of ^{131}I-albumin (Fig. 4).

However, the content of ^{131}I-albumin in RD lymph was generally much higher than that in the TD lymph in 5 of the 6 animals in which the RD was the predominant pathway for lymphatic drainage from the heart and pericardium compared to the content in the four TD dominant dogs (Fig. 4).

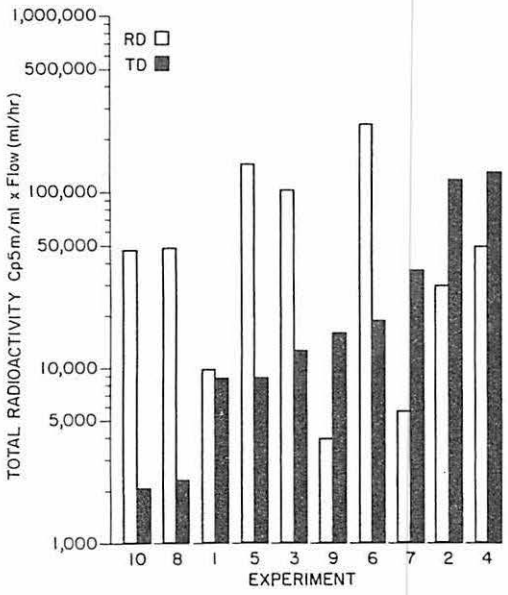

Fig. 4 Bar graph comparing mean total radioactivity in RD and TD in 10 experiments during the first 2 hours of lymph collection after instillation of ^{131}I-albumin into the pericardial sac. The experiments are arranged according to the level of total radioactivity in the TD. It is noted that TD lymph contained more total radioactivity than RD lymph in 4 of 10 experiments.

C. Possible direct absorption into blood vessels.

Fig. 5 shows radioactivity/ml of RD and TD lymph and of blood serum in samples collected every 15 minutes for 2 hours in a single experiment (# 2). Blood serum levels at 15 and 30 minutes are high relative to RD and TD lymph, suggesting the possibility of direct absorption of ^{131}I-albumin by blood capillaries or by lymphovenous communications.

Discussion

A. *Concentration* versus *content* of radioactivity in lymph.

The volume flow of lymph in the TD was 2 to almost 7 times the flow in the RD in our series of experiments, therefore there is a large dilution of radioactivity in TD lymph compared to RD lymph. In order to correct

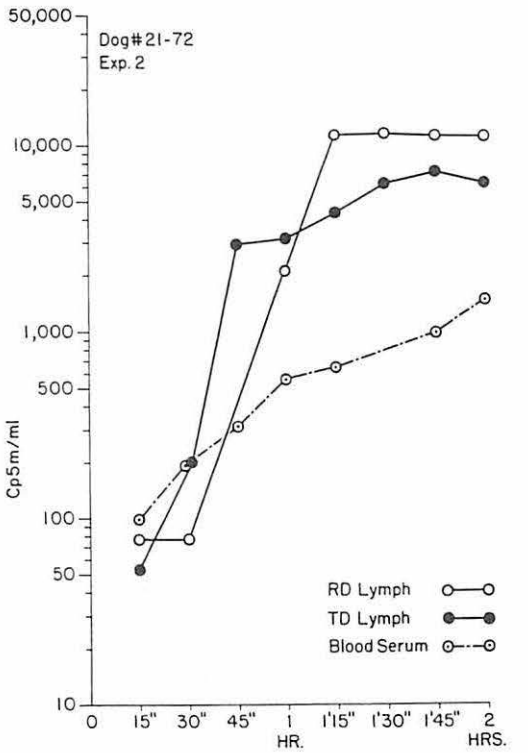

Fig. 5 This graph shows radioactivity/ml in TD and RD lymph and in blood serum in experiment # 2. The higher levels in blood serum in the first 30 minutes compared to RD and TD lymph suggest that absorption by blood capillaries or by lymphovenous communications may have occurred.

for dilution, *concentration* (cp 5 m/ml) was multiplied by volume flow of lymph in ml/hr to give total radioactivity/ml/hr or *content* of radioactivity. Total radioactivity appears to be a more accurate value for comparing the amount of labeled albumin in RD and TD lymph and for assessing absorption of ^{131}I-albumin from the pericardium.

B. Effects of re-circulation of ^{131}I-albumin.

Wasserman and Mayerson (5) state that ^{131}I-albumin appears in TD lymph within 10 minutes after *intravenous* injection and accrues steadily. We have noted that ^{131}I-albumin is present in TD and RD lymph in the first 15 minute sample after *intrapericardial* injection. Since the TD drains a larger area of the albumin pool than the RD (1) it would be surmised that the TD level of total radioactivity is enhanced more than the RD level by re-circulation and intra- and extravascular mixing, with the passage of time.

In order to evaluate such enhancement, samples were drawn every 15 minutes for 2 hours in five experiments, and every hour in 5 additional experiments. When comparison of TD and RD content of ^{131}I-albumin at the earlier time intervals was made, the results were similar to those in Fig. 4, where 2 hour collections are illustrated, i.e., levels of ^{131}I-albumin were higher in TD than in RD lymph in 2 of 5 dogs in group I and in 2 of 5 dogs in group II. Furthermore Fig. 3 shows that TD lymph receives an appreciable amount of ^{131}I-albumin 15 minutes after the instillation of ^{131}I-albumin into the pericardium, presumably before re-circulation and intra- and extravascular mixing have become a critical factor.

These observations are supported by the demonstration by *Rothschild* et al. (4) that ^{131}I-albumin equilibrates with the interstitial tissue of the human body 4–7 days following its intravenous administration. Furthermore, *Wasserman and Mayerson* (5) found that it requires about 7–13 hours for albumin to equilibrate between plasma and thoracic duct lymph after the intravenous administration of ^{131}I-albumin.

C. Pathways of drainage of cardiac efferent lymphatics.

Our observations differ from those of *Courtice and Simmonds* (6) on the route of lymphatic absorption from the serous cavities in that we have demonstrated that *both* the RD and TD receive and transport considerable amounts of ^{131}I-albumin after pericardial instillation, and that the content of labeled albumin was greater in the TD than in the RD in 4 of 10 experiments (Fig. 4). We also differ from *Szabo and Magyar* (7) who measured the absorption of plasma protein, marked with ^{131}I-albumin, from the pericardium of dogs. They collected cardiac lymph by cannulation of the left cardiac efferent lymphatic (LCE) exposed through a right thoracotomy incision. Lymph was simultaneously collected from the TD. They conclude, "the contribution of the thoracic duct to the protein transport from the pericardial cavity was negligible". However, there is evidence, particularly from lymphangiography of the left and right cardiac efferent lymphatics (8), that lymph which drains from left cardiac efferent lymphatic passes to both the RD and TD. By diverting all the lymph from the LCE, the thoracic duct content of radioactivity may have been reduced, giving a falsely low figure.

In our animals in which RD lymph contained more ^{131}I-albumin compared to TD lymph, the levels were much greater in the RD, suggesting that in many animals the cardiac efferent lymphatics transport to the RD a large proportion of lymph from the pericardium and heart. However, even in those animals with high levels of radioactivity in RD lymph the TD received appreciable amounts of ^{131}I-albumin.

Our observations are contrary to the current concept that the pericardial cavity drains almost esclusively to the RD (1.6), and suggest that there is a multiple lymphatic drainage system from the pericardial sac in which both the RD and TD participate, although one or the other may predominate. A number of lymphatics are commonly seen in the mediastinum of dogs after the subepicardial injection of Evans' blue dye. These appear to interconnect and to ascend toward the TD as well as toward the RD.

The predominant amount of total radioactivity measured in TD lymph in the first 2 hours in 4 of the 10 experiments, compared to RD lymph, appears to be due to variations in the anatomic arrangement of the cardiac efferent lymphatics, by which more lymph from the pericardium and heart was transported to the TD in certain animals, and not due to re-circulation and intra- and extravascular mixing.

D. Rate of absorption of labeled albumin

The slope of the curves in Fig. 1 (RD, TD, and blood serum) indicates that ^{131}I-albumin is absorbed relatively rapidly from the pericardium in the first 90 minutes after instillation, followed by a slower rise up to 2 hours. Peak concentration of radioactivity/ml in RD and TD lymph were reached between 6 and $22^1/_2$ hours and in blood serum between 6 and $22^1/_2$ hours (Fig. 2).

Total radioactivity/ml/hr reached its peak in RD lymph in about 6 hours and then fell rather rapidly. After 6 hours total radioactivity in TD lymph continued to rise, presumably due to re-circulation and intra- and extravascular mixing, to reach its peak between 6 and $22^1/_2$ hours, after which the level gradually fell (Fig. 2).

There was a relatively rapid early rise in blood serum levels in the first 30 minutes, compared to levels in lymph, in some of our experiments (e.g., Fig. 5), suggesting that protein is absorbed at the venous end of blood capillaries (9, 10) or that lymphovenous communications participate in the absorption.

In a case of recurrent idiopathic pericarditis reported by *Hollenberg and Dougherty* (11), the curve of appearance of radioactivity in the blood after instillation of 50–100 microcuries of ^{131}I-albumin into the pericardium had not reached a peak after 3 days. The slower absorption, compared to that of our studies, may have been due to the pathologic process in the pericardium of the patient.

Wilson et al. (12) charted the rate of absorption of red blood cells tagged with radioactive sodium chromate from the pericardium of normal dogs. The curves are similar to those we plotted for the absorption of ^{131}I-albumin, except the peak in blood is reached in about 22 hours with ^{131}I-albumin compared to 48 hours or longer for tagged red cells.

E. Clinical implications

Rouviere (13) has provided us with an anatomic description of the efferent cardiac lymphatics in man which is similar to that in the dog.

Servelle et al. (14) have demonstrated in man by lymphangiography during open chest operations that the TD is frequently obstructed in cases of constrictive pericarditis (7 of 10 cases), secondary to posterior mediastinitis. It is reasonable to assume that obstruction of other lymphatics in the mediastinum may also occur from mediastinitis or tumor. We have demonstrated that labeled albumin is transported to the RD and TD presumably by multiple lymphatic channels. This complex system may play an important role in the prevention or amelioration of deleterious effects from obstruction of lymphatic drainage from the heart and epicardium.

The collection of right duct lymph during open heart surgery in patients for study of albumin, electrolyte and enzyme content by *Hansson* (15) points up the need for accurate knowledge of the sources of lymph (i.e., cardiac, pulmonary, etc.) which drain to the right duct as well as to the thoracic duct.

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