Comparison of Lipoprotein Lipase and Clotting Activity in Lymph and Plasma after Heparin.* (26344)

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A high concentration of lipoprotein lipase or "clearing factor" characteristically appears in blood following parenteral administration of heparin. The activity of this enzyme in other extracellular fluids has not been definitely established. Spitzer stated that clearing factor did not occur in thoracic duct lymph following intravenous heparin (1). Young and Freeman found that post-heparin lymph from 2 rabbits had lipolytic activity(2). Recently Dumont demonstrated that no clearing factor activity was present in the post-heparin lymph from 5 humans despite its presence in blood(3).

In the studies to be reported lipoprotein lipase was measured in thoracic duct lymph and blood plasma before and after heparin administration in 7 dogs and in one patient. In every experiment this enzyme was found in moderate to high concentrations in post-heparin lymph. Such lymph also had greatly prolonged clotting times.

Materials and methods. In the 7 dogs the thoracic duct was isolated in the neck and cannulated with polyethylene tubing.† Each dog had been given a meal of lard (300 g) and commercial dog food pellets 1-2 hours before the procedure. Anesthesia was maintained with sodium pentothal. Usually each animal received an intravenous infusion of 500 ml of 5% glucose in water during the experiment. Blood and lymph were collected into chilled glass tubes containing 1.85% potassium oxalate solution before and at intervals up to 280 minutes after injection of heparin. Heparin‡ was given intravenously in a dose of 150 units/kilo of body weight. In some experiments protamine§ in an equivalent dose (1.5 mg/kilo of body weight) was injected intravenously 60 minutes after administration of heparin. Enzymatic activity of the blood-plasma and lymph specimens was determined after incubation with coconut oil for 2 hours by methods described previously(4). Of the 2 methods used for quantitation of lipoprotein lipase in lymph, measurement of glycerol proved to be more reliable than reduction in optical density of the lymph-coconut oil mixture.

The one human studied was a young woman who developed chylothorax following repair of an interventricular septal defect. The lymph or chyle was obtained by thoracentesis‖ with as complete an aspiration as possible before and 5 hours after 5,000 units of heparin intravenously and 10,000 units subcutaneously.

Clotting times of blood and lymph were determined by the method of Lee and White (5). Lymph triglyceride was measured by the method of Van Handel and Zilversmit (6).

Results. Pre-heparin lymph had virtually no lipoprotein lipase. Lymph collected at intervals from 10 to 260 minutes after intravenous heparin always had lipoprotein lipase activity greatly increased over the zero or trace amounts found in pre-heparin samples. Fifty-two separate lymph specimens from 7 dogs were so tested. Table I shows typical results for lymph and blood before and after

TABLE I. Lipoprotein Lipase in Lymph and Blood before and after Heparin (Expressed in Terms of Glycerol Production μM/ml).

	Pre-heparin		Post-heparin (20-30 min.)	
Dog	Lymph	Blood	Lymph	Blood
1	.06	.08	.32	.82
2	.04	.02	.48	.86
3	0		.18	.70
4	.02	.04	.36	.60
5	.01		.40	.80
6	0	0	.47	.70
7	.02		.26	

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† PE Clay-Adams.

‡ Sodium heparin (Abbott).

§ Protamine sulfate (Eli Lilly).

‖ Courtesy of Dr. J. L. Ehrenhaft.

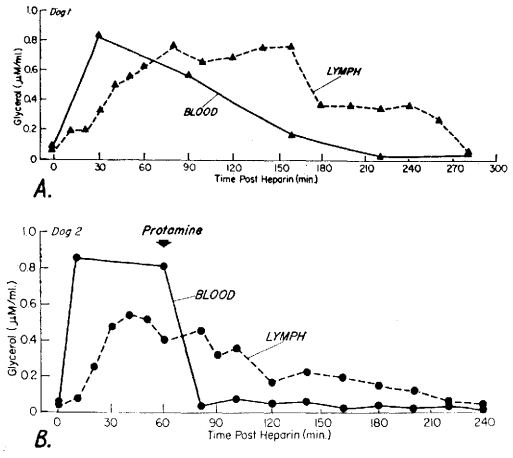

FIG. 1. A. Lipoprotein lipase in blood and lymph before and at intervals of time after intrav. heparin (Dog 1). B. Effect of protamine upon lipoprotein lipase in blood and lymph (Dog 2). Protamine was inj. at 70 min. post-heparin.

heparin. By the 20-30 minute period each dog had a large rise in enzymatic activity in lymph which was considerably lower than blood levels. In Fig. 1-A sequential data for both lymph and blood are given for Dog. 1. Initial values of lipoprotein lipase after heparin were much higher in blood than in lymph. At 80 minutes postheparin, however, concentration of the enzyme was greater in lymph than in blood. In fact the peak value of lipoprotein lipase in lymph (0.76 μM/ml of glycerol) was almost as high as the 30 minute post-heparin value for blood (0.82 μM/ml of glycerol). Lipoprotein lipase was elevated in lymph for 270 minutes. In blood, it was elevated for only 180 minutes. Intravenous injection of protamine 60 minutes after giving heparin seemed to accentuate this disparity between lymph and blood (Fig. 1-B).

The pre-heparin lymph obtained in the patient with chylothorax had little lipoprotein lipase (glycerol release of 0.02 μM/ml). Five hours after heparin, the lymph contained 5 times more lipoprotein lipase (glycerol 0.10 μM/ml). At this same time blood plasma produced 0.54 μM/ml of glycerol.

Clotting activity before and after heparin. Clotting times of lymph in 5 dogs varied from 3 to 15 minutes before heparin administration and increased to clotting times of over 60 minutes within 10 to 30 minutes after heparin. Blood clotting times in these same animals were 2-4 minutes before heparin and were elevated to over 60 minutes when measured 10-20 minutes after heparin. Lymph clotting time remained elevated after the whole blood clotting time had returned to pre-heparin values. In Dog 1, for example, lymph clotting time remained at 60+ minutes for 150 minutes post-heparin, whereas whole blood clotting time had declined to 15 minutes at 90 minutes post-heparin and was 3 minutes long at 150 minutes post-heparin.

Validity of methods to determine lipoprotein lipase. The data in Table II indicate that lipoprotein lipase cannot be measured by a change in optical density of the incubation mixture when the lymph sample is itself lipemic and has a high initial optical density. Little or no clearing occurred despite evidence of the enzyme as indicated by glycerol production. Table III further documents the inadequacy of the clearing method when the incubation mixture has an initial optical density above 1.0.

Discussion. These experiments provide evidence that lymph contained high concentrations of heparin and the enzyme lipopro-

TABLE II. Comparison of Two Methods for Lipoprotein Lipase Determination.

Dog	Post-heparin specimen	Optical density (units) of: Blood plasma or lymph	Incubation mixture Initial	Final	Change	Glycerol production (μM/ml)
1	Blood —40 min.	.02	.59	.20	.29	.82
	Lymph—30 "	1.80	1.90	1.90	0	.32
	" —80 "	1.25	1.25	1.17	.08	.66
4	" —40 "	1.20	1.25	1.20	.05	.40
	" —80 "	.69	.92	.76	.16	.46
6	Blood —30 "	.04	.57	.04	.53	.70
	Lymph—30 "	1.70	1.70	1.60	.10	.47

TABLE III. Analysis of Lipoprotein Lipase Determination.

Substrate*	O.D. of incubation mixture (units) Initial	Final	Change	Glycerol production (μM/ml)
Exp. 1				
Coconut oil 2.5%	.50	.15	.35	.94
" " 15.0%	1.29	1.26	.03	.98
Exp. 2				
Dog 1 chyle, diluted ⅓ with water	.65	.40	.25	.66
Dog 1 chyle,† undiluted	1.05	.96	.09	.68

* Incubation mixture for each experiment consisted of .12 ml of substrate and 2.5 ml of post-heparin plasma with an optical density of .06.
† Triglyceride content = 4220 mg %.

tein lipase after administration of intravenous heparin. Furthermore, lymph retained both anticoagulant and lipolytic activities for a considerably longer period of time than they could be demonstrated in the blood. Heparin is known to appear in the urine following intravenous injection(7). Its occurrence in lymph as indicated by a biological test of its presence (prolongation of clotting time) further supports the idea that it rapidly diffuses through the capillary endothelium. Thus the rapid decline in blood concentration of heparin given intravenously (7) is probably caused as much by its diffusion into the interstitial fluid compartment as by its inactivation or excretion. It is not known that this greater duration of heparin activity in the interstitial tissue fluid has clinical implications. Conceivably heparin may exert its anticoagulant or antilipemic effects for a longer period than might be indicated from tests of its presence in blood.

Lipoprotein lipase reached a maximal concentration much later in lymph than in blood. The slow transit time of lymph enters into the collection problem. In Dumont's study no lipoprotein lipase was demonstrated in human post-heparin lymph from the thoracic duct collected only 20 and 30 minutes after heparin(3). Since the transit time of lymph through the thoracic duct may take longer in man than in the dog(8), perhaps later samplings might have revealed the enzyme.

Summary. Lipoprotein lipase was found in the post-heparin thoracic duct lymph of the 7 dogs studied and in the post-heparin lymph aspirated from the chest in a patient with chylothorax. This enzyme reached a maximal concentration later and remained longer in lymph than in the blood. Post-heparin lymph also reflected the presence of heparin by greatly prolonged coagulation times. Evidence was given that lipoprotein lipase should be determined by measuring its lipolytic activity and not by "clearing" technics alone.

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