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ABSTRACT

The glyceride and free fatty acid contents of thoracic, intestinal, and hepatic lymph of rabbits and thoracic lymph of rats were determined before and after intravenous administration of heparin. The principal effects observed were the following:

1. The turbidity that was always observed initially in thoracic or intestinal lymph was caused to diminish or disappear. Hepatic lymph was not originally turbid.

2. In all cases the glyceride content was reduced and the free fatty acids increased.

3. The relative amount of glycerides was highest in the intestinal lymph, and the change in that component was markedly greater than in hepatic lymph.

4. Hepatic lymph had a low initial content of glycerides, and showed a relatively larger change in free fatty acids.

Trielaidin was fed to rats and heparin was given intravenously during the absorptive phase. Elaidic acid was detected in the free fatty acids subsequently appearing in the lymph.

Postheparin rabbit lymph was found to be capable of producing lipolysis of a fatty substrate in vitro.

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Introduction

The discovery by Hahn in 1943[1] that intravenously injected heparin abolished alimentary lipemia in dogs is now well known. Clearing of lipemic serum did not occur in vitro upon addition of heparin itself,[2,3] however, clearing of lipemic serum could be brought about by incubation with post-heparin plasma. Gofman et al.[4] in this laboratory have shown that plasma lipids are closely associated with a "spectrum" of lipoprotein molecules, which are distinguishable in the ultracentrifuge by virtue of their different densities. The effect of heparin in vivo on these molecules was found to be a conversion of the species of high flotation rates to those of lower flotation rates.[5]

Shore, Nichols, and Freeman[6,7] in this laboratory also showed that clearing of a lipemic substrate by postheparin plasma in vitro was accompanied by the release of free fatty acids. Robinson and French also reported the release of fatty acid when lymph was incubated with postheparin plasma.[8] Grossman et al.[9] have subsequently found that free fatty acid concentration in the plasma was increased after the injection of heparin, especially in lipemia. Similar observations have been made in both animals and humans in this laboratory.

It appears from the foregoing that the free fatty acids produced during in vitro or in vivo clearing of lipemia are at least partly the result of glyceryl ester hydrolysis, activated in some way by heparin. It is of interest to know whether such glyceryl ester hydrolysis can be demonstrated in the lymphatic system as well as in the blood. Evidence for lipolysis of triglycerides with release of fatty acids in the lymphatic system is presented in this paper.

Materials and Methods

A. Materials

1. The animals used were male New Zealand White rabbits of body weight between 3 and 3.5 kilograms, and male Long Evans rats, 250 to 350 grams. All animals were fasted for 12 to 18 hours before cannulation, unless otherwise stated.

2. The anesthetics were: Nembutal, 50 to 60 mg/kg of body weight, administered intravenously for rabbits; 30 to 40 mg/kg for rats, intraperitoneally; and ether, used to supplement the nembutal anesthesia.

3. Heparin, aqueous solution, 10,000 units/ml, was used intravenously in doses of 15 mg/kg for rabbits and 5 mg/kg for rats.

4. C.P. ether, methanol, chloroform, and carbon disulfide were used (J.T.Baker Chem. Co.). Commercial grade hexane was redistilled.

5. Trielaidin was prepared in this laboratory by the reaction of elaidoyl chloride and glycerol. The infrared spectrum of the partially purified product used for feeding indicated that small amounts of partial glycerides and free fatty acid were present. These do not interfere with the experiment, since presumably they would be formed in the digestive process.

B. Methods

Collection of lymph. (a) Cannulation of rat lymphatics: the method of collection is essentially the same as that employed by Bollman et al.[10] (b) Cannulation of rabbit lymphatics: In different experiments lymph was collected from three different regions--thoracic duct, hepatic lymph vessel, and intestinal lymphatic vessel. A section of polyethylene tubing was used as a cannula. For the collection of thoracic-duct lymph the tubing was cannulated into the thoracic duct at the junction of the left subclavian and left internal jugular veins. For collection of hepatic lymph the tubing was inserted into the hepatic lymphatic duct at the point approximately 1 cm caudal to the liver near the hepatic artery. The intestinal lymph was collected from the main intestinal lymphatic vessel, along the celiac artery, just above the left adrenal gland. The cannulae were left in place post-operatively and the lymph in each case was collected continuously over a period of 48 hours after the rabbits had recovered from anesthesia. During each study lymph samples were collected at 30-minute intervals for lipid analysis.

Lipid analysis. Lipids of lymph were extracted and separated by a method essentially similar to that of Borgstrom.[11,12] The extracted lipids were separated into three fractions by elution from silicic acid columns. Triglycerides and free fatty acids were determined quantitatively by infrared spectrophotometry as developed in this laboratory.[7]

For the determination of elaidic acid, the free fatty acids were separated from the glycerides by extraction from a petroleum ether solution with dilute alkali in 50% methanol (Borgstrom[11]). The content of elaidic acid was measured both in the glycerides and in the free fatty acids by infrared spectrophotometric measurements of the absorption band at 10.3 μ. The basis of this method has been given by Shreve et al.[13] Since the amounts of material and the percentages of elaidic acid were both small in our experiments, we have used a base line method of calculation instead of the equations developed by those authors. The results by the two procedures agree well when the total sample is large enough. For the smaller samples, in particular the free fatty acids of preheparin lymph, only maximum values have been quoted. As a result of fatty acid exchange in the precesses of digestion and absorption, the glycerides subsequently found in lymph are presumed to contain partially elaidinated fat molecules. The analysis gives the equivalent amount of trielaidin.

Results

Thoracic Duct Lymph (Rabbit and Rat)

All lymph samples collected from the thoracic duct of rabbits before the administration of heparin, 15 mg/kg intravenously, were visibly turbid. The postheparin samples were clear. Table I summarizes the changes in triglycerides and free fatty acids in the thoracic duct lymph of five rabbits. The concentration of triglyceride decreased about 35% within the first half hour after administration of heparin, and the decrease reached its maximum, an average of 74%, in 2 hours. After that time its concentration increased gradually back to the original level. The free fatty acids, on the other hand, increased by about 360% in the first 30 minutes. The increase in free fatty acids after the administration of heparin does not compensate for the decrease in triglycerides. The conditions of the in vivo experiment are not such that an accurate balance of triglyceride and fatty acid changes can be expected; also, incomplete hydrolysis of triglyceride probably occurred.

Similar results obtained in rats are shown in Table II. Smaller doses of heparin were effective in this species of animal, about 5 mg/kg being comparable with 15 mg/kg in the rabbit. The average maximal drop (47%) in triglyceride concentration was at 3 hours postheparin. The maximal increase in free fatty acid concentration (290%) was observed in 2 hours.

Intestinal Lymph (Rabbit)

The fat content of intestinal lymph was much higher than that of thoracic and hepatic lymph. The results of heparin injections are presented in Table III. It is notable that the elevation of the free fatty acid concentration is much less than for thoracic duct lymph. Also the lowering of triglycerides proceeds to a greater extent and for a longer period of time.

Hepatic Lymph (Rabbit)

Hepatic lymph of normal rabbits was watery clear. The fat content of hepatic lymph was lower than that of the lymph collected from either thoracic duct or intestinal lymph. Sixty minutes after administration of heparin, there was a slight change in triglycerides and a relatively large percentage increase in free fatty acids. Table IV shows the triglyceride and free fatty acid contents of hepatic lymph before and after administration of heparin.

Trielaidin Feeding

In order to learn more about the origin of the free fatty acids produced in lymph after heparin administration, some tracer experiments were performed using trielaidin as a labeled fat. Rats cannulated in the thoracic duct as described were fed (by stomach tube) a suspension of 50-100 mgm. of trielaidin in 2 ml. of non-fat milk. Heparin was given 3-3-1/2 hours after this feeding, and lymph samples were collected and analyzed. Results are summarized in Table V.

Table I

The effect of heparin on the concentrations of triglycerides and free fatty acids of thoracic lymph of five rabbits

Time after heparin (hr)	Triglycerides (mg per 100 ml)						Free fatty acids (mg per 100 ml)					
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
-2.0	102	---	104	---	---	103	11	--	10	--	--	11
0.0	100	106	101	114	113	106	13	14	12	10	8	12
0.5	58	59	81	88	72	72	52	61	31	36	21	38
1.0	36	45	32	42	32	37	21	29	29	30	38	29
2.0	28	33	21	32	40	31	18	21	20	20	18	19
2.5	40	39	25	22	55	36	16	20	20	19	21	20
3.5	50	---	---	25	78	51	20	--	--	19	26	22
4.5	---	93	---	---	---	93	--	18	--	--	--	18
6.0	---	---	94	---	---	94	--	--	17	--	--	17
12.0	---	---	---	103	---	103	--	--	--	18	--	18
24.0	---	100	---	---	---	100	--	16	--	--	--	16

Table II

The effect of heparin on the concentrations of triglycerides and free fatty acids on thoracic lymph of five rats

Time after heparin (hr)	Triglycerides (mg per 100 ml)						Free fatty acids (mg per 100 ml)					
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
0.0	207	218	214	220	239	240	25	39	28	35	48	35
1.0	116	112	160	128	221	147	140	90	73	57	160	104
2.0	105	125	135	105	224	138	160	192	56	101	213	144
3.0	---	---	---	---	127	127	---	---	--	---	128	128

Table III

Effect of heparin on the concentrations of triglycerides and free fatty acids in the intestinal lymph of rabbits

Time after heparin (hr)	Triglycerides (mg per 100 ml)				Fatty acids (mg per 100 ml)			
	1	2	3	Mean	1	2	3	Mean
0	316	289	308	304	19	8	17	15
1	287	290	292	289	24	10	16	17
2	150	200	187	179	25	18	18	21
3	61	148	123	111	16	19	30	22
4	34	41	80	52	19	23	30	23

Table IV

The effect of heparin on the concentrations of triglycerides and free fatty acids in the hepatic lymph of rabbits

Time after heparin (hr)	Triglycerides (mg per 100 ml)				Free Fatty Acids (mg per 100 ml)			
	1	2	3	Mean	1	2	3	Mean
0	65	70	60	65	7	10	7	8
1	65	45	25	45	70	31	32	44
2	45	33	20	26	76	54	46	58
3	29	-	16	23	40	-	43	42
4	51	-	18	35	39	-	40	39
5	56	-	-	56	41	-	-	41

Table V

Changes in Elaidin and Free Elaidic Acid in Rat Lymph After Heparin

Expt.	Time with respect to heparin injection	Total glyceride (mg/100 ml)	Elaidin (equivalent trielaidin) (mg/100 ml)	Total free fatty acids (mg/100 ml)	Free elaidic acid (mg/100 ml)
1	-2	299	26	-	-
	0	540	75	51	< 5
	1	314	40	192	12
2	-3	236	51	37	< 5
	0	304	62	61	6
	2	137	40	189	20
3	-3	189	32	57	< 8
	0	207	47	29	< 8
	2	116	35	156	16
4	-3-1/2	264	59	30	< 8
	0	570	175	50	< 8
	2	365	143	136	26

Table VI

Lipolytic activity of postheparin lymph in vitro

Expt. 1. Incubation of rat lymph lipids with rabbit hepatic lymph for 3 hours at 38° C.

Expt. 2. Rabbit thoracic duct lymph kept at 4° C. for three weeks.

	Glycerides		Free Fatty Acids	
	Initial Conc. (mg/100 ml)	Final Conc. (mg/100 ml)	Initial Conc. (mg/100 ml)	Final Conc. (mg/100 ml)
Experiment 1				
Preheparin	260	258	39	40
Postheparin	233	196	75	115
Experiment 2				
Preheparin	116	112	10	14
Postheparin	75	43	25	57

In vitro Effects

If lipolysis occurs in postheparin lymph in vivo it may be inferred that the active factor is present in such lymph and should be demonstrable by an in vitro test. Such confirmation is provided by two observations. (a) Pre- and postheparin rabbit hepatic lymph were incubated with a fatty substrate prepared from rat lymph by centrifuging 3.5 hours at 20,000 rmp. In each case 0.2 ml of the top 1 ml from this centrifugation was mixed with 1 ml of the lymph to be tested for lipolytic activity and 0.5 ml of saline, and the mixture was incubated for 3 hours at 38°C. Glycerides and free fatty acids were determined in the same way as described for the in vivo experiments. (b) Analyses were made of fresh pre- and postheparin rabbit thoracic duct lymph and compared with results obtained on the same samples after they were allowed to remain at 4° C for three weeks. The results for these two experiments are presented in Table VI.

Discussion

The data that have been presented demonstrate an effect in the lymph of both rabbits and rats, following intravenous injection of heparin, that is evidently parallel to the effect in blood. The triglyceride content drops, and the amount of free fatty acids increases. It is further indicated that in the absence of excess fat influx from the intestine (i.e., in fasted animals) a predominant share of the free fatty acids found in thoracic duct lymph has arrived there via the hepatic lymph channel. This suggests implication of the liver as a principal source of the fatty acids under these conditions. However, if trielaidin is fed and heparin is given during the absorptive state, 10% to 20% of the free fatty acids formed consist of elaidic acid. Thus it appears that hydrolysis of some newly absorbed fat has taken place. This hypothesis is further supported by the ability of postheparin lymph to produce lipolysis in vitro. An alternative explanation which has not been ruled out, however, is that the absorption process from the intestine may have been so altered that a larger fraction of fatty acids is passed into the lymph without being reconstituted as glycerides.

In intestinal lymph the disparity between the large decrease in glyceride content and the relatively small rise in free fatty acids after heparin is not readily explained by lipolysis alone. This observation suggests that other processes may be operating either to suppress the absorption of fat from the intestine, or to divert the lipides to other circulatory pathways from the site of absorption. Further experiments are needed to develop evidence on these unsettled questions.

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