Effect of Heparin on Triglyceride and Free Fatty Acid Concentrations in Lymph.* (22066)

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The discovery by Hahn(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 post-heparin 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 post-heparin 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 the presence of 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. In the present communication evidence for lipolysis of triglycerides with release of fatty acids in the lymphatic system is presented.

A. *Materials.* 1. Animals: Male New Zealand white rabbits of body weight between 3 and 3½ kg and male Long Evans rats, 250-350 g were used. All animals were fasted for 12 to 18 hours before cannulation, unless otherwise stated. 2. Anesthetics: Nembutal, 50 to 60 mg/kg body weight administered intravenously for rabbits; 30 to 40 mg/kg for rats intraperitoneally. Ether was used to supplement nembutal anesthesia. 3. Heparin, aqueous solution, 10000 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 reaction of elaidoyl chloride and glycerol. Infrared spectrum of 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.* 1. Collection of lymph: a) Cannulation of rat lymphatics—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 3 different regions, thoracic duct, hepatic lymph vessel and intestinal lymphatic vessel. A section of polyethylene tubing was used as cannula. For collection of thoracic duct lymph, tubing was cannulated into thoracic duct at junction of left subclavian and left internal jugular veins. For collection of hepatic lymph tubing was inserted into hepatic lymphatic duct at point approximately 1 cm caudal to liver near hepatic artery. Intestinal lymph was collected from main intestinal lymphatic vessel, along coeliac artery, just above left adrenal gland. Cannulae were left in place post-operatively and lymph in each case was collected continuously 48 hours after rabbits had recovered from anesthesia. During each study lymph

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TABLE I. Effect of Heparin on Concentrations of Triglycerides and Free Fatty Acids in Thoracic Lymph of 5 Rabbits.

Time after heparin (hr)	Triglycerides (mg/100 ml) 1	2	3	4	5	Mean	Free fatty acids (mg/100 ml) 1	2	3	4	5	Mean
−2.0	102	—	104	—	—	103	11	—	10	—	—	11
.0	100	106	101	114	113	106	13	14	12	10	8	12
.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

samples were collected at 30 minute intervals. 2. Lipids of lymph were extracted and separated by method essentially similar to that of Borgstrom(11,12). Extracted lipids were separated into 3 fractions by elution from silicic acid columns. Triglycerides and free fatty acids were determined quantitatively by infrared spectrophotometry(7). For determination of elaidic acid, free fatty acids were separated from glycerides by extraction from petroleum ether solution with dilute alkali in 50% methanol(11). Content of elaidic acid was measured in both glycerides and free fatty acids by infrared spectrophotometric measurements of absorption band at 10.3 μ. Basis of this method has been given by Shreve *et al.*(13). Since amounts of material and percentages of elaidic acid were both small in our experiments, we used a base line method of calculation instead of equations developed by those authors. Results by the two procedures agree well when total sample is large enough. For smaller samples, in particular free fatty acids of pre-heparin lymph, only maximum values have been quoted. As a result of fatty acid exchange in processes of digestion and absorption, glycerides subsequently found in lymph are presumed to contain partially elaidinated fat molecules. The analysis gives equivalent amount of trielaidin.

Results. 1. Thoracic Duct Lymph (Rabbit and Rat). All lymph samples collected from the thoracic duct of rabbits before administration of heparin, 15 mg/kg intravenously, were visibly turbid. Post-heparin samples were clear. Table I summarizes changes in triglycerides and free fatty acids in thoracic duct lymph of 5 rabbits. Concentration of triglyceride decreased about 35% within first half hour after administration of heparin. The decrease reached its maximum, an average of 74%, in 2 hours. After that time its concentration increased gradually to the original level. Free fatty acids, on the other hand, increased by about 360% in the first 30 minutes. The increase in free fatty acids after 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, and 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, about 5 mg/kg being comparable with 15 mg/kg in the rabbit.

TABLE II. Effect of Heparin on Concentrations of Triglycerides and Free Fatty Acids in Thoracic Lymph of 5 Rats.

Time after heparin (hr)	Triglycerides (mg/100 ml) 1	2	3	4	5	Mean	Free fatty acids (mg/100 ml) 1	2	3	4	5	Mean
0	207	218	214	220	239	220	25	39	28	35	48	35
1	116	112	160	128	221	147	140	90	73	57	160	104
2	105	125	135	105	224	138	160	192	56	101	213	144
3	—	—	—	—	127	127	—	—	—	—	128	128

TABLE III. Effect of Heparin on Concentrations of Triglycerides and Free Fatty Acids in Intestinal Lymph of Rabbits.

Time after heparin (hr)	Triglycerides (mg/100 ml) 1	2	3	Mean	Free fatty acids (mg/100 ml) 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

The average maximal drop (47%) in triglyceride concentration was at 3 hours post-heparin. Maximal increase in free fatty acid concentration (290%) was observed in 2 hours.

2. 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 elevation of 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. 3. Hepatic lymph of normal rabbits was watery clear. The fat content of hepatic lymph was lower than that of 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 triglyceride and free fatty acid contents of hepatic lymph before and after administration of heparin.

4. Trielaidin Feeding. To learn more about the origin of free fatty acids produced in lymph after heparin administration, 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 mg trielaidin in 2 ml of non-fat milk. Heparin was given 3-3½ hours after this feeding, and lymph samples were collected and analyzed. Results are summarized in Table V.

TABLE IV. Effect of Heparin on Concentrations of Triglycerides and Free Fatty Acids in Hepatic Lymph of Rabbits.

Time after heparin (hr)	Triglycerides (mg/100 ml) 1	2	3	Mean	Free fatty acids (mg/100 ml) 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	33	76	54	46	58
3	29	—	16	23	40	—	43	42
4	51	—	18	35	39	—	40	39
5	56	—	—	56	41	—	—	41

5. *In vitro* effects. If lipolysis occurs in post-heparin lymph *in vivo* it may be inferred that the active factor is present in such lymph and should be demonstrable by *in vitro* test. Such confirmation is provided by two observations. (1) Pre- and post-heparin rabbit hepatic lymph were incubated with a fatty substrate prepared from rat lymph by centrifuging 3½ hours at 20000 r.p.m. In each case 0.2 ml of top 1 ml from this centrifugation was mixed with 1 ml of lymph to be tested for lipolytic activity and 0.5 ml of saline, and the mixture incubated 3 hours at 38°C. Glycerides and free fatty acids were determined in the same way as described for the *in vivo* experiments. (2) Analyses were made of fresh pre- and post-heparin rabbit thoracic duct lymph and compared with results obtained on same samples after they remained at 4°C for 3 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 free fatty acids found in thoracic duct lymph has arrived there via the hepatic lymph channel. This suggests an implication of the liver as principal source of fatty acids under these conditions. However, if trielaidin is fed and heparin is given during absorptive state, 10-20% of 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 ability of post-heparin lymph to produce lipolysis *in vitro*. However, an alternative explanation which has not been ruled out is that absorp-

TABLE V. Changes in Elaidin and Free Elaidic Acid in Rat Lymph after Heparin. (Concentrations are in mg/100 ml.)

Exp.	Time with respect to heparin inj.	Total glyceride	Elaidin (equivalent trielaidin)	Total free fatty acids	Free elaidic acid
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	−3½	264	59	30	<8
	0	570	175	50	<8
	2	365	143	136	26

tion process from the intestine may have been so altered that a larger fraction of fatty acids is passed into lymph without being reconstituted as glycerides.

Summary. 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 diminished or disappeared. Hepatic lymph was not originally turbid. 2. In all cases the glyceride content was reduced and free fatty acids increased. 3. Relative amount of glycerides was highest in 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. 5. Trielaidin was fed to rats and heparin given intravenously during absorptive phase. Elaidic acid was detected in free fatty acids subsequently appearing in lymph. Post-heparin rabbit lymph was capable of producing lipolysis of a fatty substrate *in vitro*.

TABLE VI. Lipolytic Activity of Post-Heparin Lymph *In Vitro*. (Concentrations are in mg/100 ml.)

	Glycerides		Free fatty acids	
	Initial conc.	Final conc.	Initial conc.	Final conc.
Exp. 1				
Pre-heparin	260	258	39	40
Post-heparin	233	196	75	115
Exp. 2				
Pre-heparin	116	112	10	14
Post-heparin	75	43	25	57

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

Exp. 2. Rabbit thoracic duct lymph kept at 4°C for 3 wk.

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