Occurrence in Plasma of an Extractable Lipid Mobilizer (LM)* (22164)

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Plasma from rats treated with cortisone, exposed to cold or subjected to the nephrotic syndrome prevented the delactescent action of heparin clearing factor *in vitro*(1). Hypercholesterolemia which was independent of adrenals or renal lesions was observed in rabbits prepared for the Shwartzman reaction and represented another example of the effect on lipids by non-specific stimuli(2). These observations suggested that concentration of a lipid mobilizer (LM) in the plasma could be increased either by cortisone or stress. In this paper we are reporting separation of such a substance from plasma of untreated and cortisone treated animals, also data which demonstrate the effect of LM on plasma lipids, food intake and body weight.

Materials and methods. Blood was collected in 0.1 M sodium citrate (1:9 whole blood) and centrifuged. The decanted plasma (2 ml/rat; 5 ml/human; 150 ml/dog) was dialyzed through Viscose Nu-Jax® casing against an equal volume of demineralized water for 6 hours at 25°C with constant agitation. Bulk lots were prepared from several liters of horse plasma under conditions that minimized bacterial and pyrogen contamination. The horse blood was collected aseptically and stored at 5°C for 72 hours to permit settling of the cells. The viscose casing was washed with 0.5% phenol and sterile, pyrogen-free demineralized water. The dialysis was at 25°C for 72 hours against sterile, pyrogen-free demineralized water in closed sterile containers. The dialyzate was concentrated at 75°C and 0.2 mm Hg pressure for 72 hours and freeze-dried for a similar time. The final product was sterile and did not produce fever in the animals used in the lipemia experiments. All animals were fasted 24 hours before blood samples were taken or before cortisone was administered. In the latter group, blood samples were obtained 4 hours after the administration of the cortisone. The LD_{50} of the dry extract was determined in Manor Farm All-Purpose mice, Wistar rats, guinea pigs, albino rabbits and mongrel dogs. A dose response curve was determined in normal Wistar rats and hypophysectomized albino rats. The effect of chronic administration of large

TABLE I. LM Activity of Plasma Dialyzates Prepared from Untreated and Cortisone-Injected Animals.

Species	Treatment†	No. of animals	No. of dialyzates showing LM activity	Hyperlipemic dose of dialyzate, mg/kg, I.V. in rats*
Human	None	10	10	285
Horse	None	4	4	300
	Cortisone	6	6	1
Dog	None	8	3	325
	Cortisone	5	5	1.85
Rat	None	10	10	300
	Cortisone	10	10	0.95
	Hypophysectomy Cortisone	10	0	>500

* Log spaced doses (10 rats/dose for each dialyzate; at least 100 rats per dialyzate).
† 5 mg/kg intramuscular.

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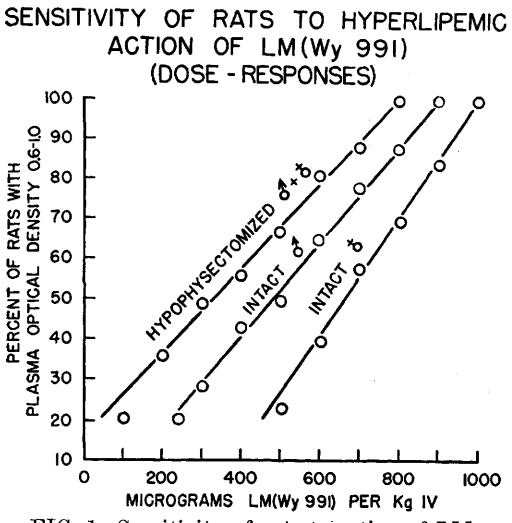

FIG. 1. Sensitivity of rats to action of LM.

doses of LM was studied in rabbits and dogs. At weekly intervals venous blood was analyzed for lipid phosphorus by the method of Fiske and SubbaRow(3), total fatty acids by the method of Stoddard and Dury(4) and total cholesterols by the Sperry-Schoenheimer technic(5). Optical density of the plasma was measured in a model B Beckman spectrophotometer.

Results. The LM activity of various plasma dialyzates when injected into rats is shown in Table I. Of the 63 dialyzates injected 15 failed to exhibit LM activity; these included 5 from untreated dogs and 10 from hypophysectomized rats. On the basis of mg of dialyzate/kg of body weight cortisone treatment caused an increase of approximately 300-fold in LM activity but on the basis of volume of original plasma the increase was approximately 1000-fold. LM activity was considered to be present when the optical density of the plasma of the injected rats increased from the initial 0.01 to at least 0.60. Plasmas exhibiting lipemia by this method also had at least a 50% elevation of total and esterified cholesterol, total fatty acids and lipid phosphorus. The dose necessary to elicit LM activity was 1/50 the LD_{50} or less. The intravenous LD_{50} of the dialyzates prepared from cortisonized animals calculated by the method of Bliss was as follows: (mg/kg) rats 48; guinea pigs 45; rabbits greater than 100; and dogs greater than 75. The injection of cortisone into hypophysectomized rats failed to cause the release of significant amounts of LM.

The dose response curves illustrated in Fig. 1 show that female rats were more resistant than males to the hyperlipemic action of LM and that intact rats were more resistant than hypophysectomized ones. In the hypophysectomized group females were as sensitive as the males. Each point on the curves represents 10 rats. The curves are qualitatively similar (no deviation from parallelism: $X^2 = 0.3$; $P>0.5$) but differ quantitatively ($F = 13.4$; $P<0.05$). The hyperlipemia resulting from injection of the ED_{100} (1 mg/kg) persisted for at least 6 hours and that from 60 mg/kg for at least 7 days. The degree of hyperlipemia following injection of a single dose of LM is shown in Table II. The error in the methods employed was smaller than the standard deviations. There was a prompt 2-fold increase of all plasma lipid components in the species tested. The hyperlipemia was invariably associated with increased optical density except in humans where there was no correlation. The ED_{100} in rats by intramuscular injection was 5 mg/kg. Intragastric administration of 100 mg/kg failed to produce hyperlipemia in rats. In conjunction

TABLE II. Mean Concentration (mg %) of Lipids in Plasma of Animals 1 Hr after Injection of LM.

Animal	No.	mg/kg	Total Ch		FA		LP		OD	
			Initial	1 hr	Initial	1 hr	Initial	1 hr	Initial	1 hr
Mice	100	.5	53	174	148	450	5	12	.05	.90
Rats	475	.10	72	190	155	480	5	13	.04	.86
Guinea pigs	100	.50	66	185	160	440	5	12	.06	.80
Rabbits	40	.50	50	172	200	420	5	11	.05	.86
Dogs	20	1.	86	168	209	430	6	11	.05	.92

Ch = Cholesterol; FA = Fatty acids; LP = Lipid phosphorus; OD = Optical density.

TABLE III. Effect of LM (Mean Values mg %) on Plasma Levels of Lipids in 6 Dogs (60 mg Dialyzate/kg I.V. Once Weekly).

	Week of treatment									
	0	1	2	3	4	5	6	7*	8	9
Total Ch	83	165	190	220	245	240	210	240	202	189
Ester Ch	58	110	118	134	150	142	135	142	122	110
Total FA	212	440	520	680	590	540	606	630	420	250
Lipid P	7	12	12	14	13	13	12	13	10	8
Food taken daily (g)	265	370	540	550	550	552	550	550	500	450
Body wt (kg)	6	6	5	4	4	4	4	5	6	9

* LM discontinued.
Ch = Cholesterol; FA = Fatty acids.

with Dr. William Steiger, Dept. of Medicine, Temple University, 5 human subjects were injected intravenously with 0.25 to 1 mg dialyzate/kg. All responded with elevated plasma concentrations of cholesterol, fatty acids and lipid P of the same magnitude as the other species tested. None of the humans developed chills, fever, or other signs of toxic reactions.

Repeated single weekly injections of large doses of dialyzate containing LM in dogs and rabbits resulted in sustained hyperlipemia in which all elements of the plasma lipids were still elevated at least 2-fold 7 days following injection (Tables III and IV). The hyperlipemic animals developed marked hyperphagia in which food intake was 2-3 times that of control animals. The hyperphagic animals did not gain in body weight as rapidly as the controls and in some instances there was loss of body weight amounting to 33% of the initial. Discontinuing the administration of LM resulted in subsidance of the hyperlipemia and hyperphagia with an accompanying gain in body weight. Gross examination of dogs autopsied after 4 and 5 weeks of LM administration showed almost complete atrophy of the perirenal, omental, mesenteric and spermatic cord fat depots and marked depletion of subcutaneous abdominal fat. The livers were not fatty nor was there evidence of lipid deposition in other viscera or in the blood vessels.

TABLE IV. Effect of LM (Mean Values mg %) on Plasma Levels of Lipids in 20 Rabbits (60 mg Dialyzate/kg I.V. Once Weekly).

	Week of treatment					
	0	1	2	4	5	6
Total Ch	72	150	200	290	350	350
Total fatty acid	200	325	400	490	580	575
Lipid P	6	9	13	13	14	13
Food taken daily (g)	190	300	575	800	950	1000
Body wt (kg)	2.4	2.7	2.5	2.6	2.8	2.7

Ch = Cholesterol; FA = Fatty acid.

The anti-clearing (CFI) action of a dialyzate prepared from horses injected with cortisone is illustrated in Fig. 2. The *in vitro* method(1) utilized lipemic plasma and plasma from heparinized animals.

Discussion. The LM activity of the plasma dialyzate prepared from cortisonized animals was probably due to a mediator released by the injected cortisone. The dialyzate contained no cortisone or other steroids when it was subjected to infra-red analysis or to the Porter Silber and Liebermann-Burchard reactions. Cortisone requires conditions for dialysis which differ from those we employed(9). Assuming that all of the injected cortisone was absorbed and remained concentrated in the plasma and then entered the dialyzate, the amount injected was less than 10 μg/kg of body weight. Only 20% of rats receiving 5 mg of cortisone/kg of body weight developed hyperlipemia in contrast to the 100% receiving 1 mg of dialyzate/kg of body weight. Finally, plasma dialyzate from hypophysectomized rats injected with cortisone had no LM activity.

LM differed from the preparation of protamine available to us in being dialyzable and heat stable. Although LM and protamine(6) are CFIs *in vitro* and produce hyperlipemia when injected into animals other inhibitors such as diisopropylfluorophosphate (DFP)

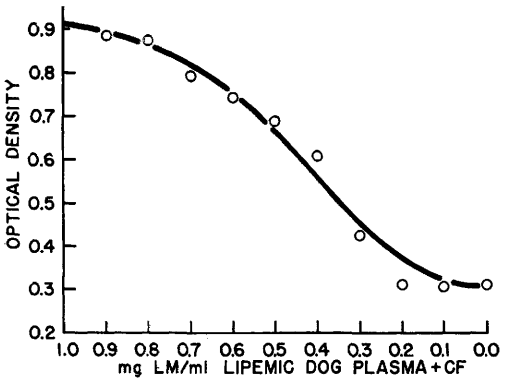

FIG. 2. CFI activity *in vitro* of LM plasma dialyzate vs. heparin clearing factor.

(7), and sodium cholate(8) failed to do so when administered in non-convulsant doses. It appears therefore that the hyperlipemic action of the dialyzate may not be related to its CFI action. The injection of LM into hypophysectomized rats produced hyperlipemia, whereas the injection of protamine failed to do so. Injection of 5 mg arginine/kg did not produce hyperlipemia in rats; larger doses resulted in fatality.

Growth hormone prepared from pituitary glands has been reported to be an active lipid mobilizer(10). LM differs from such preparations in being dialyzable, heat stable and in failing to promote growth in the presence of hyperphagia.

LM appears to resemble the factor extractable from the anterior pituitary which is free from growth-promoting or ACTH properties but is capable of mobilizing lipids(11). Several peptide fragments derived from the decomposition of the adipokinetic hormone are also capable of causing deposition of lipids in the liver(12). Our material, although dialyzable, is not a fragment produced by extraction procedures since the dialyzate without further treatment was capable of producing intense hyperlipemia.

Summary. 1. Freeze dried dialyzates of plasma prepared from dogs, rats and horses receiving cortisone contained a lipid mobilizer (LM) which markedly elevated plasma levels of free and esterified cholesterol, lipid P and total fatty acids when administered to fasted mice, rats, rabbits, guinea pigs, dogs and humans by intramuscular or intravenous injection. LM was not active by the oral route. The dialyzate also inhibited *in vitro* the delactescing action of heparin clearing factor. Plasma dialyzates prepared from untreated humans, horses, dogs and rats were 1/1000 as potent. 2. Cortisone injection failed to release LM in hypophysectomized rats. 3. LM activity of the dialyzate is not due to the same constituent responsible for CFI activity. 4. Hyperlipemia resulting from a single large dose persisted for at least one week in rabbits and dogs. 5. Repeated large doses of LM resulted in sustained and progressive hyperlipemia, hyperphagia and either loss in body weight or failure to gain. 6. Marked depletion of perirenal, mesenteric, omental, spermatic cord and subcutaneous fat were observed in dogs receiving weekly injections of LM.

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