fraction of serum lipid extract(7). The lysolecithin fraction contained material from the other 3 fractions; from rechromatography studies, it was estimated to contain about 50% lysolecithin. Hydrolysates of all fractions contained small amounts of ninhydrin-reacting substances which may have represented constituents of as yet unidentified phospholipids. The low molar ester bond-to-phosphorus ratios of the "cephalin" and lecithin fractions, as was observed with serum (7), may be related to a defect in the ester bond method, to alteration of phospholipids during isolation procedure, or to presence in these fractions of other phospholipids, such as plasmalogen, with ester bond-to-phosphorus ratios of less than 2.

No previous investigators reported the presence of lysolecithin, which comprised about 2% of total red blood cell phospholipids. It is unlikely that this represented adsorbed serum lysolecithin, as the red blood cells were washed 3 times with isotonic saline before extraction, or that it was produced as an artifact during the extraction or isolation procedure(6).

It is of interest to note the striking contrast in the relative proportions of individual phospholipids in red blood cells as compared to serum, especially since there is evidence for exchange of phospholipids between these 2 compartments(11,12).

Summary. Human red blood cells were analyzed for individual phospholipids using chromatography on silicic acid. Concentrations of the main components, which appeared to be ethanolamine- and serine-containing phospholipid, lecithin, sphingomyelin, and lysolecithin, were measured in red blood cells of 6 normal fasting subjects.

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Effect of Epinephrine upon Plasma Optical Density, Unesterified Fatty Acids, Lipemia Clearing and Heparin Levels. (24671)

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The role of epinephrine in the physiology of fat mobilization has been known, and experimental evidence has been summarized(1,2). Importance of plasma unesterified fatty acids as the major fat transport mechanism involved in this effect of epinephrine was recently revealed(3,4). Subsequently tissue studies indicated that there was increased triglyceride lipolysis in fat depots following adrenalin injection(5), and that unesterified fatty acids were released(6). In these studies no observations were made of plasma lipemia clearing activity or of heparin levels. Since heparin lipoprotein lipase system probably plays a physiologic role in removal of alimentary lipemia from the bloodstream, and perhaps in tissue triglyceride lipolysis, it seemed desirable to study this mechanism in plasma following injection of epinephrine.

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Procedures and methods. The hormone was administered intravenously during post-absorptive period, in 9 dogs and 4 human volunteers. The former received .1 - .2 ml of 1:1000

TABLE I. Plasma Optical Density, Lipemia Clearing Activity and Unesterified Fatty Acids before and after Intravenous Epinephrine in 9 Dogs.

Measurement	Before	Intrav. epinephrine, .15 ml, 1:1000 2-3′	5′	10′	15-20′
Plasma O.D.	.04	.03	.04	.03	.04
Lipemia clearing*	3	4	4	3	4
Plasma U.F.A.†	1.7	1.7	2.3	2.3	1.8
Plasma O.D.	.02	.04	.02	.03	.02
Lipemia clearing	2	3	3	3	3
Plasma U.F.A.	.9	.9	1.0	.9	.8
Plasma O.D.	.03	.03	.03	.02	.02
Lipemia clearing	1	1	0	1	1
Plasma U.F.A.	1.5	1.3	1.8	1.9	1.3
Plasma O.D.	.03	.04	.02	.03	.02
Lipemia clearing	2	3	1	1	0
Plasma U.F.A.	1.0	1.25	1.45	1.0	.95
Plasma O.D.	.03	.04	.04	.05	.03
Lipemia clearing	3	2	4	3	2
Plasma U.F.A.	1.1	1.15	1.2	1.4	1.0
Plasma O.D.	.03	.03	.03	.03	.05
Lipemia clearing	0	0	1	0	0
Plasma U.F.A.	1.45	1.7	1.9	1.6	1.4
Plasma O.D.	.05	.06	.09	.08	.07
Lipemia clearing	3	3	2	3	3
Plasma U.F.A.	.80	.85	1.05	.95	1.05
Plasma O.D.‡	.07	.21	.19	.16	.12
Lipemia clearing	18	14	20	18	18
Plasma U.F.A.	1.1	1.15	1.15	1.1	.80
Plasma O.D.	.04	.05	.04	.04	.04
Lipemia clearing	8	8	8	9	9
Plasma U.F.A.	1.65	1.9	2.3	1.9	1.8

* Optical density decrease in 1 hr upon incubation of plasma + coconut oil emulsion.
† Values in meq/l.
‡ All blood samples slightly hemolyzed.

adrenalin whereas in humans 1 ml of 1:1000 solution was diluted with 100 ml of physiological saline and given as continuous intravenous infusion for 20 minutes. Venous blood samples were taken prior to and at frequent time intervals after injection, placed in cold citrated tubes, and the plasma immediately separated in refrigerated centrifuge. Analyses were performed soon after plasma was obtained. In all samples plasma optical density, unesterified fatty acids, lipemia clearing activity, and additionally in humans, heparin levels, were determined. Optical density was measured in Coleman Jr. spectrophotometer at 700 mμ. Unesterified fatty acids were extracted and titrated by Borgstrom's method (7). Lipemia clearing activity was determined using 1 ml plasma plus .4 ml of .25% suspension of coconut oil(8). Endogenous plasma heparin was extracted in duplicate and assayed by its anticoagulant activity as previously described(9).

Results. Data obtained in 9 dogs are shown in Table I. Plasma optical density was unchanged in 7 animals, possibly slightly increased in one (#7), and the results were unreliable in #8 because of hemolysis. Lipemia clearing activity, due to serum lipase in the dog and not to lipoprotein lipase, was not altered after epinephrine. In 7 dogs there was a rise in unesterified fatty acids following injection. Results in 4 human subjects are presented in Table II. There was no change in plasma optical density, and no increase (a decrease occurred in one individual) in lipemia clearing activity during infusion for 20 minutes. Unesterfied fatty acids were definitely

TABLE II. Plasma Lipemia Clearing Activity, Heparin Level and Unesterified Fatty Acids after I.V. Adrenalin in 4 Humans.

Sex	Age	Time after adrenalin I.V., min.	Plasma optical density	O.D.* decrease in 1 hr	Plasma heparin	Plasma U.F.A.,† meq/l
♀	27	0	.03	0	13	1.55
		10	"	0	15	1.9
		20	.04	0	16.5	2.5
♀	28	0	.03	6	19	1.45
		10	"	8	18	1.5
		20	.04	6	19	1.95
♂	27	0	.03	18	17.5	1.25
		10	"	7	19	2.05
		20	.04	8	17.5	2.4
♀	20	0	.03	0	17.5	1.3
		10	"	0	20	1.7
		20	"	0	18.2	2.2

* Optical density.
† U.F.A.—Unesterified fatty acids.

elevated in all cases, and there was apparently a slight rise in heparin levels in 3 of the 4 individuals. Although not shown, pulse rate and blood pressure rose moderately in all instances.

Discussion. Plasma optical density changes and those of neutral fat(10) or total lipids and chylomicron counts(11), are usually parallel. Our findings of fairly constant plasma optical density, therefore, indicate that there is no triglyceride rise in plasma after epinephrine during initial period of fat mobilization and plasma unesterfied fatty acid elevation, thus supporting observations(5,6) which showed that fat is released from adipose tissue stores as unesterified fatty acid rather than as triglyceride. Further, failure of plasma lipemia clearing activity to increase after hormone injection, although unesterified fatty acids became elevated, is evidence that the latter did not occur as a result of plasma triglyceride lipolysis. This possible source of increase in plasma unesterified fatty acid was not completely ruled out by previous studies.

The rise in plasma heparin levels, which is certainly not statistically significant but which is borne out by other observations (unpublished) in 2 human volunteers subjected to prolonged stress (72 hours of sleep deprivation) has not previously been noted. It is of interest that there was no concomitant increase of plasma lipemia clearing factor, implying that a dissociation can exist between a stimulus to heparin release and lipoprotein lipase formation. In these experiments it is probable that the rise in heparin was part of a stress reaction, similar to increased fibrinolytic activity in blood after adrenalin(12), rather than a response to increased lipemia (13). Results are at variance with an earlier report(14) of decrease in protamine titre in blood after epinephrine, and illustrate that the protamine titration procedure is not always specific for heparin.

Summary. Immediate effects of intravenous epinephrine in dogs and humans upon plasma optical density, lipemia clearing activity, unesterified fatty acids and heparin levels were determined. There was no change in the first 2 parameters. Unesterified fatty acids were definitely increased in both species. Circulating heparin was increased in 3 of 4 humans studied. These findings indicate that elevation of unesterified fatty acids did not occur as a result of enhanced plasma triglyceride lipolysis, and that the rise in heparin was probably part of a stress reaction unrelated, in this instance, to its role in fat transport.

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