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13. ABSTRACT Red blood cell mobility and whole blood viscosity were measured in 15 baboons after the intravenous administration of heparin or Intralipid or both. Red cell mobility and whole blood viscosity did not change in the group given heparin. The animals given fat or fat and heparin showed a decrease in red cell mobility. The whole blood viscosity, however, decreased in the group given just fat and heparin. The implications of these findings to flow through the microcirculation and to the clinical use of Intralipid are discussed.			

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Red blood cell mobility and whole blood viscosity changes following the administration of intravenous fat*

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The use of fat emulsions for intravenous feeding has been widely adopted in recent years. Since many of the patients receiving these solutions may have significant abnormalities in the microcirculation, secondary to the underlying disease process (e.g., septic shock, congestive heart failure, or other conditions etc.), the effect of the intravenous administration of fat on the microcirculation directly or on factors that significantly influence the microcirculation is of major importance. Merrill^{1,2} states that the rheological properties of blood are not significantly altered by hyperlipemia; however, Kroeger³ feels that hyperlipemia does result in decreased blood flow through the capillaries. The present study was undertaken to evaluate the effect of Intralipid,‡ a commercial fat emulsion, on two rheological properties of blood.

MATERIALS AND METHODS

Fifteen adult male baboons (*Papio dogueta*), weighing between 21 and 28 kilo-

grams, were studied by dividing them into three groups of five animals. The baboons were fasted for eight to 12 hours prior to the study. On the day of the experiment, the animals were anesthetized with 1-(1-phenyl-cyclohexyl) piperidine hydrochloride (Sernylan), 1.0 mg. per kilogram of body weight intramuscularly, for insertion of catheters into one femoral artery and vein. Each animal was then placed in a primate chair and allowed to awaken. Four hours after the Sernylan injection, when the baboon was fully awake, the experiment was begun. One group received Intralipid alone, one group received heparin alone, and the third group received both. The fat emulsion was given intravenously over 20 seconds in a dose of 0.4 Gm. per kilogram. The dose of Intralipid given to the first group was 98.8 ± 4.6 ml. and the dose given to the third group was 99.4 ± 4.4 ml. Likewise, heparin was given the same way in a dose of 100 I.U. per kilogram of body weight. When Intralipid and heparin were both given, the heparin was administered a few seconds before the fat was administered.

Blood was withdrawn from the arterial catheter prior to the injection of the fat or heparin or both and then at 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 120 minutes after the fat injection. The blood was immediately centrifuged after it was allowed to clot for five minutes, and the serum was removed and frozen for subsequent analysis of free fatty acids and total lipids. Free fatty acids were analyzed by the colorimetric micro-

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The experiments reported herein were conducted according to the principles set forth in *Guide for Laboratory Animals: Facilities and Care* prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council. Accepted for publication Sept. 14, 1971.

*The opinions or assertions contained herein are those of the authors and not to be construed as official or reflecting the views of the Navy Department or of the Naval Service at large.

†Research Task No. 21R005.20.01-0287A211R.

‡Vitrum Co., Stockholm, Sweden.

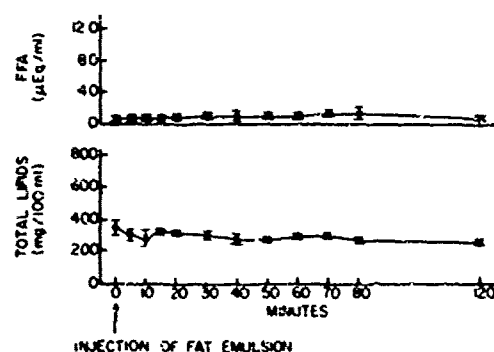


Fig. 1. The effect of intravenous heparin at a dose of 100 I.U. per kilogram of body weight on serum levels of free fatty acids and total lipids. The values are expressed as the mean $\pm$ the standard error of the mean.

method of MacKenzie and colleagues.⁶ Total lipids were measured by the colorimetric method based on the sulfo-phospho-vanillin reaction.⁷

In addition, blood was withdrawn from the arterial catheter into heparinized tubes at zero, 15, and 70 minutes after the fat injection for measurement of red blood cell mobility and whole blood viscosity. Separate plasma viscosities were not measured. Electrophoretic mobility was determined in a commercially available Northrup-Kunitz cataphoresis apparatus⁸ similar to that described by Abramson and co-workers.¹ The time for red cells to migrate 0.01 mm. in an electrical potential gradient of 2.86 v. per centimeter was determined for ten cells from each sample. All measurements were made in a room maintained at $25 \pm 1^\circ$ C. The heparinized blood used for the red cell mobility measurements was diluted 1:100 in isotonic phosphate-buffered saline at pH 7.4. Previous studies in this laboratory have shown that the heparin concentrations used for anticoagulation (10 I.U. per milliliter) do not effect electrophoretic mobility.¹ Whole blood viscosities were measured in a cone-plate microviscometer⁹ at shear rates of 11.5 to 115 sec.⁻¹

Erythrocyte surface charge is related to

Table I. Serum free fatty acids

Time (min.)	Group		
	Heparin (μ Eq/ml.)	Intralipid (μ Eq/ml.)	Intralipid and heparin (μ Eq/ml.)
0	$0.61 \pm 0.26^*$	0.71 ± 0.31	0.59 ± 0.16
5	0.71 ± 0.17	2.82 ± 1.73	11.30 ± 3.58
10	0.75 ± 0.23	2.20 ± 0.93	8.67 ± 2.38
15	0.66 ± 0.23	4.58 ± 1.88	9.62 ± 2.00
20	0.99 ± 0.19	4.22 ± 1.78	10.49 ± 2.62
30	1.11 ± 0.40	3.89 ± 1.99	11.46 ± 3.14
40	1.11 ± 0.39	3.31 ± 1.36	8.63 ± 1.71
50	0.96 ± 0.39	3.41 ± 2.39	5.56 ± 1.49
60	1.05 ± 0.45	3.32 ± 1.88	4.16 ± 1.16
70	1.44 ± 0.38	2.84 ± 2.06	3.20 ± 1.27
80	1.28 ± 0.73	4.93 ± 3.48	2.77 ± 1.24
120	0.76 ± 0.15	4.02 ± 2.08	2.53 ± 1.13

*Mean $\pm$ SEM.

electrophoretic mobility by the equation

$$Q = 6\pi rn (v/Ex) \times 10^{-7}$$

where Q is the charge in coulombs, 6π is a proportionality factor from Stokes' law, r is the radius of the red cell, n is the viscosity of plasma, and (v/Ex) is the electrophoretic mobility (velocity/potential gradient). The factor 10^{-7} is needed to convert the final result to coulombs. The formula must be considered an approximation as the factor 6π is exact only for a sphere.

The mean, standard deviation, and standard error were calculated for all data, and statistical significance was determined with Student's t test and analysis of variance.

RESULTS

The group treated with heparin showed no significant changes in free fatty acids or total lipids during the experiment (Fig. 1 and Tables I and II). The animals given Intralipid alone showed a rise in total lipids within five minutes after injection of the fat (from a mean $\pm$ SEM [standard error of the mean] of 351 ± 32 mg. per 100 ml. to 880 ± 69 mg. per 100 ml., $p < 0.001$) (Fig. 2). This peak value began to decrease over the next hour and returned to a base-line level of 554 ± 112 ($p > 0.05$) 70 minutes after the injection. The fatty acids immediately rose five minutes after the injection, reaching a peak value of 4.58 ± 1.88 μ Eq per milliliter ($p < 0.025$) 15 minutes after administration

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⁹Brookfield Engineering Labs., Inc., Stoughton, Mass

Table II. Serum total lipids

Time (min.)	Group		
	Heparin (mg./ 100 ml.)	Intralipid (mg./ 100 ml.)	Intralipid and heparin (mg./ 100 ml.)
0	330 ± 40*	331 ± 32	332 ± 30
5	304 ± 28	880 ± 86	1,095 ± 93
10	280 ± 48	824 ± 80	914 ± 74
15	321 ± 16	880 ± 69	1,005 ± 75
20	312 ± 20	806 ± 78	915 ± 65
30	302 ± 26	734 ± 81	839 ± 44
40	275 ± 23	711 ± 97	710 ± 31
50	271 ± 15	666 ± 56	484 ± 72
60	287 ± 20	604 ± 90	495 ± 83
70	296 ± 15	554 ± 112	471 ± 64
80	274 ± 12	540 ± 107	450 ± 62
120	266 ± 16	450 ± 90	356 ± 51

*Mean ± standard error of the mean.

of the fat. This elevated level returned to base-line values at the end of two hours. The injection of heparin along with Intralipid resulted in a significant initial rise in total lipids from 332 ± 30 mg. per 100 ml. to $1,095 \pm 93$ mg. per 100 ml. (Fig. 3). The lipids returned to base-line level 50 minutes after the injection. This initial rise in total lipids was higher in the group given Intralipid and heparin than in the group given Intralipid alone ($1,095 \pm 93$ versus 880 ± 86), but this was not statistically significant ($0.05 < p < 0.1$). The fatty acids, likewise, rose immediately after injection of the heparin and fat from 0.59 ± 0.16 μ Eq per milliliter to 11.30 ± 3.58 μ Eq ($p < 0.02$) and then began to decline, reaching a base-line value of 2.55 ± 1.13 μ Eq per milliliter at the end of two hours ($p > 0.1$). Once again, the fatty acid elevation appeared greater in this group than in the animals given only Intralipid (11.30 ± 3.58 versus 4.58 ± 1.88); however, these differences were not statistically significant ($0.05 < p < 0.1$).

Throughout the experiment, no changes in hematocrit occurred in any of the groups, and there were no significant differences in hematocrit among the three groups of animals. The preinjection red cell mobilities for all three groups were not statistically different (0.85 ± 0.09) μ per second per volt per centimeter for the heparin-treated animals,

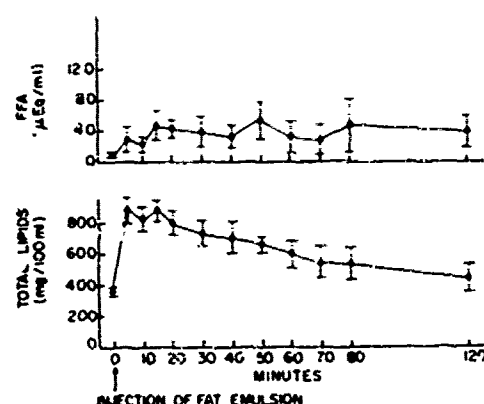


Fig. 2. The effect of intravenously administered fat (Intralipid) at a dose of 0.4 Gm. per kilogram on serum levels of free fatty acids and total lipids. The values are expressed as the mean ± the standard error of the mean.

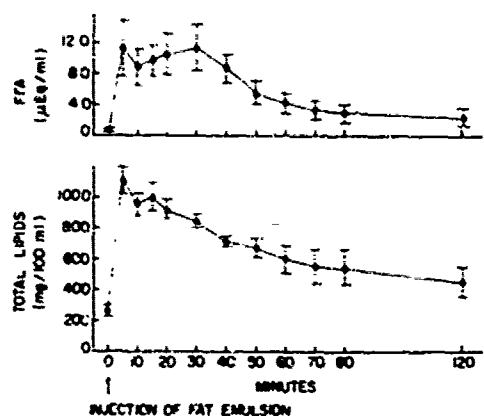


Fig. 3. The effect of intravenously administered fat (Intralipid) and heparin on serum levels of free fatty acids and total lipids. The values are expressed as the mean ± the standard error of the mean.

0.76 ± 0.09 for the group given Intralipid, and 0.83 ± 0.12 for the animals injected with both heparin and fat (Table III). The animals given heparin alone showed no change in red cell mobility at 15 or 70 minutes after the heparin injection (Fig. 4). In the Intralipid group, the red cell mobility decreased from 0.76 ± 0.09 to 0.47 ± 0.09 ($p < 0.05$) 15 minutes after the fat injection and then returned to a base-line value of 0.81 ± 0.16 ($p > 0.7$) after 70 minutes. The same pattern was seen in the animals

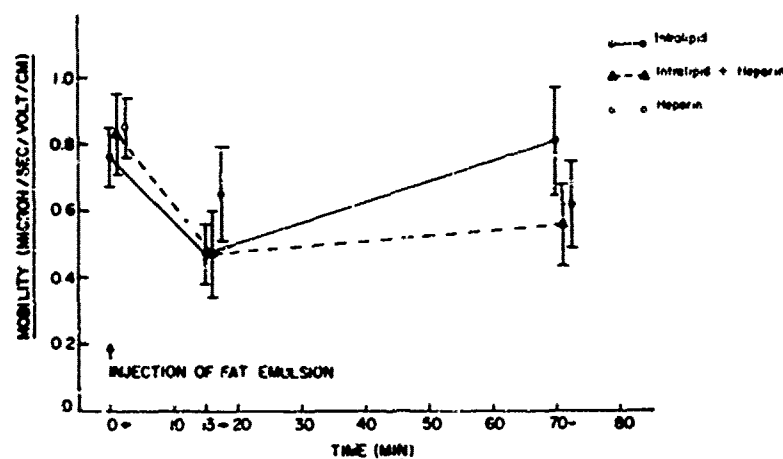


Fig. 4. Changes in red cell mobility (mean $\pm$ SEM) following the injection of Intralipid.

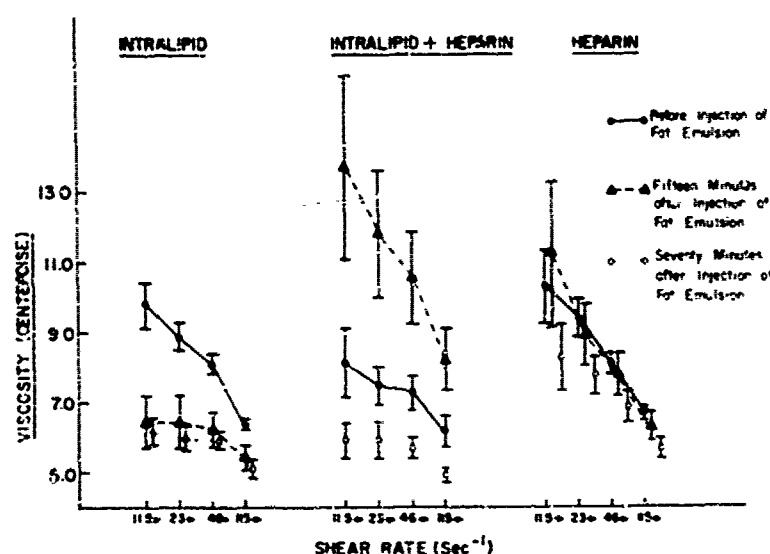


Fig. 5. Changes in whole blood viscosity (mean $\pm$ SEM) following the injection of Intralipid.

treated with both fat and heparin. Fifteen minutes after the injection, the mobility decreased to 0.47 ± 0.13 ($p < 0.05$) and then returned to a base-line value of 0.56 ± 0.12 ($p > 0.1$) after 70 minutes. The whole blood viscosity measurements were evaluated statistically by analysis of variance. Groups of data shown to contain significant differences were further examined by Duncan's multiple range test to find those values significantly different from base-line values.² Prior to injection of fat or heparin or both,

the whole blood viscosity values of all three groups, at shear rates varying from 11.5 to 115 sec^{-1} , were not significantly different (Fig. 5 and Table IV). Likewise, the animals given only heparin showed no changes in viscosity, at the varying shear rates, 15 and 70 minutes after injection. In the Intralipid group, a significant decrease in viscosity was seen 15 minutes after the fat injection at a shear rate of 11.5 sec^{-1} . The value decreased from 9.78 ± 0.66 to 6.46 ± 0.77 centipoise ($p < 0.05$). At 70 minutes, a

Table III. Red blood cell mobility

Time (min.)	Group		
	Heparin (μ /sec./ r./cm.)	Intralipid (μ /sec./ r./cm.)	Intralipid and heparin (μ /sec./ r./cm.)
0	0.85 $\pm$ 0.09*	0.76 $\pm$ 0.09	0.83 $\pm$ 0.12
15	0.65 $\pm$ 0.14	0.47 $\pm$ 0.09	0.47 $\pm$ 0.13
70	0.62 $\pm$ 0.13	0.81 $\pm$ 0.16	0.56 $\pm$ 0.12

*Mean $\pm$ standard error of the mean.

significant decrease ($p < 0.05$) was seen at shear rates of 11.5 and 23. On the other hand, the animals given fat and heparin showed a significant rise in viscosity 15 minutes after injection and then a return to base-line viscosity values at 70 minutes. The viscosity increased from 8.12 ± 0.96 to 13.72 ± 2.63 centepoise at a shear rate of 11.5 sec.⁻¹ ($p < 0.01$), from 7.44 ± 0.57 to 11.82 ± 1.83 at a shear rate of 23 ($p < 0.01$), and from 7.26 ± 0.52 to 10.54 ± 1.30 at a shear rate of 46 ($p < 0.05$) 15 minutes after the injection of Intralipid and heparin.

DISCUSSION

The decrease in red cell mobility 15 minutes after the injection of fat alone or of fat and heparin paralleled the peak rise in free fatty acids and total lipids. At 70 minutes, when the red cell mobility had returned to a preinjection value, the free fatty acids and total lipids had returned to base-line levels. The high levels of fatty acids, occurring 15 minutes after the fat injection, may in some way alter the surface charge of the red cell and thereby lead to decreased red cell mobility. Red cell mobility is related to red cell surface charge by the equation given in the section on materials and methods; therefore, a decrease in red cell mobility would correspond to a decrease in surface charge. A fall in red cell surface charge would favor aggregation of red blood cells with subsequent stasis and decreased flow through capillaries.^{4, 5, 6} Horwitz⁴ observed a decrease in red cell surface charge in septic shock but not in hemorrhagic shock. Therefore, the use of intravenous fat during septic shock might lead to further decrease in capillary blood flow.

Table IV. Whole blood viscosity

Shear rate (sec. ⁻¹)	Group		
	Heparin (cente- poise)	Intralipid (cente- poise)	Intralipid and heparin (cente- poise)
Time = 0 min.			
11.5	10.33 $\pm$ 1.05*	9.78 $\pm$ 0.66	8.12 $\pm$ 0.96
23	9.38 $\pm$ 0.54	8.89 $\pm$ 0.38	7.44 $\pm$ 0.57
46	8.08 $\pm$ 0.32	8.09 $\pm$ 0.23	7.26 $\pm$ 0.52
115	6.65 $\pm$ 0.18	6.35 $\pm$ 0.16	6.16 $\pm$ 0.44
Time = 15 min.			
11.5	11.25 $\pm$ 2.09	6.46 $\pm$ 0.77	13.72 $\pm$ 2.63
23	8.90 $\pm$ 0.89	6.44 $\pm$ 0.74	11.82 $\pm$ 1.83
46	7.78 $\pm$ 0.62	6.20 $\pm$ 0.49	10.54 $\pm$ 1.30
115	6.25 $\pm$ 0.39	5.40 $\pm$ 0.37	8.22 $\pm$ 0.89
Time = 70 min.			
11.5	8.25 $\pm$ 0.95	6.44 $\pm$ 0.40	5.96 $\pm$ 0.21
23	7.73 $\pm$ 0.54	5.94 $\pm$ 0.33	5.96 $\pm$ 0.31
46	6.35 $\pm$ 0.43	5.90 $\pm$ 0.24	5.64 $\pm$ 0.47
115	5.65 $\pm$ 0.29	5.08 $\pm$ 0.24	4.90 $\pm$ 0.47

*Mean $\pm$ SEM.

The injection of fat alone led to a significant decrease in whole blood viscosity at 15 and 70 minutes after the injection, whereas the injection of Intralipid and heparin resulted in a rise in viscosity 15 minutes later with a return to baseline values at 70 minutes. These two opposite findings are difficult to explain. The total lipid values in both groups were essentially the same; whereas at 15 minutes, the free fatty acids in the animals given fat and heparin were higher than in those given just fat. At 70 minutes, both groups had similar levels of free fatty acids. Whether the higher level of fatty acids in the group given Intralipid and heparin could account for the viscosity differences is pure conjecture. Merrill and colleagues⁷ found that the yield shear stress rose three hours after a fatty meal but returned to normal six hours after the fat ingestion, at a time when the serum triglyceride level was at a maximum. They also showed that the yield shear stress in these subjects increased markedly as the temperature was lowered. In an earlier article, Merrill⁷ commented that there were no clear-cut data showing that hyperlipemia affected the rheology of

blood. Kroeger,⁵ utilizing the technique of intravital microscopy, showed that the administration of the fat emulsion, Lipofundin, led to decreased flow through the capillaries of the pancreas and mesenteric adipose tissue. Lipofundin, however, differs significantly from Intralipid in that it is a cottonseed oil emulsion with a soybean-phosphatide as the emulsifier, whereas Intralipid is a soybean oil emulsion with an egg yolk phosphatide as the emulsifier.

In conclusion, on the one hand, red cell mobility decreased, which would appear to favor red cell aggregation and result in decreased capillary flow; on the other hand, whole blood viscosity appeared to decrease after heparin injection but increased when heparin was given in addition to the fat. The clinical implications of these data are purely speculative, and no assertion can be made as to whether Intralipid is beneficial or harmful in patients with a compromised microcirculation.

SUMMARY

Red blood cell mobility and whole blood viscosity were measured in 15 baboons after the intravenous administration of heparin or Intralipid or both. Red cell mobility and whole blood viscosity did not change in the group given heparin. The animals given fat or fat and heparin showed a decrease in red cell mobility. The whole blood viscosity, however, decreased in the group given just fat but increased in the animals given both fat and heparin. The implications of these

findings to flow through the microcirculation and to the clinical use of Intralipid are discussed.

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