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Some Observations of Effects of Intravenous Fat Emulsions on Erythrocyte Fragility. (20031)

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After the administration of intravenous fat emulsions, a moderate but self-limited anemia has frequently been reported. Mann *et al.* (1,2) noted the occurrence of moderate anemia in dogs which were given corn and coconut oil emulsions. Anemia also occurred in rats given both coconut and corn oil emulsion, and in 2 human subjects who received coconut oil emulsion. In the latter report, increased osmotic fragility of the red cells was noted. The authors found that the phosphatide used as stabilizer could alone produce changes of the same magnitude. Meng and Freeman(3) observed a slight anemia in a group of dogs given stabilizing agents, but in only one of 5 dogs given complete emulsion. Meng(4) observed no anemia in infused rabbits. Van Itallie *et al.*(5) stated that preliminary studies of red cell fragility failed to show changes in the integrity of the red cell membrane, but no details were given. Lerner, Chaikoff, and Entenman(6) likewise reported the occurrence of anemia in a group of dogs given intravenous fat for prolonged periods as did Dunham and Brunschwig(7) and Collins (8). The latter believed that it was probably not hemolytic since reticulocytosis did not occur. In this laboratory, significant decreases in hemoglobin, red blood cell count and hematocrit were observed in a group of normal dogs after one to 2 weeks of daily intravenous infusion of fat(9). In contrast, Shafiroff *et al.*(10,11) found no hematologic changes after single infusions of fat emulsion in humans and stated that in no case did infusion result in a positive test for urine urobilinogen.

The mechanism of the observed anemia remains unexplained. It could be inferred from the nature of the infused substances that this is a hemolytic anemia. Surface active stabilizers are used in many of the emulsions and these materials might well alter the red cell membrane. The emulsified fat itself might conceivably alter the interfacial relationships between the erythrocyte and its environment. In addition, minute amounts of other surface active agents such as free fatty acids may be present in the emulsion or be liberated after infusion. Some of the hematologic effects of intravenous fat emulsions given to dog and man and the possible significance of these effects are reported here.

Procedure. The fat emulsions used were from 2 sources: Emulsion I is a 10% emulsion of synthetic fats stabilized with Span 20, sodium cholate, soybean phosphatides and Demal—14.* Emulsion II is a 15% olive oil emulsion stabilized with 0.6% soybean phosphatide and 1.0% Demal—14†. The subjects in these experiments were 2 burn patients, one convalescent and the other in the final stages of skin grafting. The dog used was a normal male weighing 22 kg. All subjects were studied in the fasting state. The patients received 500 ml of emulsion and the dogs 300 ml at a rate of approximately 4 ml per minute.

* Generously supplied by Dr. H. C. Meng.
† Generously supplied by Dr. F. J. Stare.

Heparin was used as an anticoagulant. Blood samples were drawn before infusion, immediately at the end of the infusion and at appropriate intervals thereafter. All blood was drawn with maximum precaution to prevent artificial hemolysis. Venapuncture was accomplished with a 15 gauge needle and after flow of blood was established the initial sample was discarded and the sample for analysis was drawn into a mineral oil coated syringe and delivered into a similarly oiled test tube. The specimens were immediately centrifuged and the plasma separated. Twenty-four hour stool specimens were collected for urobilinogen determination. Separation of the daily samples was accomplished by the use of carmine markers.

Methods. 1. Plasma hemoglobin was determined by a benzidine procedure to be reported elsewhere(12). 2. The mechanical fragility of erythrocytes in whole blood was quantitated as follows: 4 ml of heparinized whole blood was pipetted into each of two 13 by 100 mm glass stoppered test tubes containing 2 glass beads. The tubes were shaken for 15 and 30 minutes in a Fisher Clinical Shaker at a rate of 275 oscillations per minute after which they were centrifuged. The hemoglobin concentration of the supernatant plasma was then determined. The 15 and 30 minute values in mg % are averaged and the average is referred to as the fragility index. 3. Plasma "total lipid" was measured by a macromodification of the turbidimetric technic of Geyer *et al.*(13). Using the preinfusion blood as a blank, this method measures the increment in total lipid resulting from the infusion. 4. Urobilinogen: 24-hour excretion

TABLE I. Dog Experiments.

Exp.	Min. after infusion	Plasma total lipid, mg %	Plasma Hgb, mg %	Fragility index
A	P*	0	1.6	218
	0	3647	18.8	5050
	130	1703	14.2	5835
	305	368	3.9	3550
B	P*	0	0	54
	0	575	6.5	5690
	120	75	4.1	1094
	240	11	0	60

* P = Preinfusion.

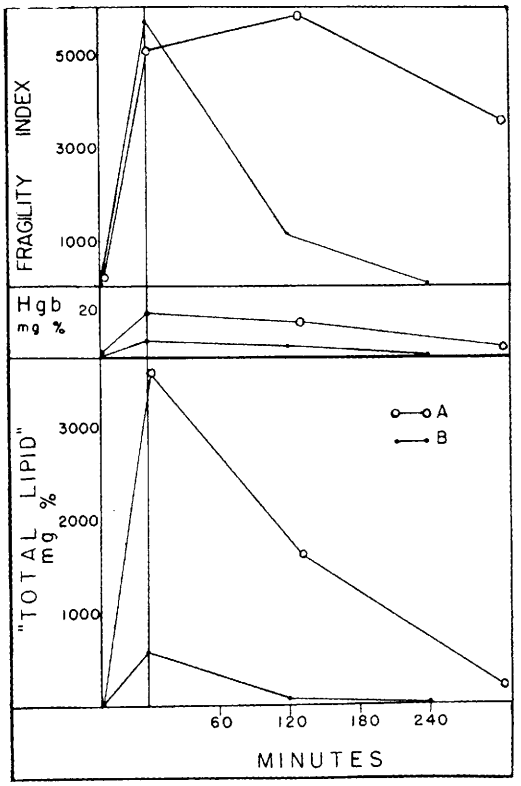

FIG. 1. Dog experiments.
Emulsion II used in Exp. A (open circles).
" I " " " B (closed ").

of fecal urobilinogen was measured by the technic of Schwartz, Sborov, and Watson(14).

Results. Dog experiments. Two dog experiments are reported (Table I, Fig. 1). In Exp. A, emulsion II was used and in Exp. B emulsion I was infused. In one case (Exp. A) the fat emulsion was inadvertently given at a very rapid rate which resulted in the extremely high plasma "total lipid" level of 3700 mg %.

There was an immediate, very marked rise in the fragility index to above 5,000 in both experiments. The average control fragility index determined on 18 samples of blood from the same dog given glucose infusions in place of fat was 72.6 with a range of 10 to 276. The *fragility index* gradually returned toward normal as the plasma "total lipid" decreased, but was still considerably elevated as long as visible lipemia was present ("total lipid" above 50 mg %). In both experiments a small degree of spontaneous hemolysis oc-

TABLE II. Human Experiments.

Exp.	Min. after infusion	Plasma total lipid, mg %	Plasma Hgb, mg %	Fragility index
A	P*	0	0	31
	0	233	49.5	1870
	60	96	6.2	2140
	120	17	5.1	108
	240	0	8.9	87
B	P*	0	0	27
	0	913	23.4	14500
	60		9.6	12700
	120	484	3.1	10000
	240	352	6.8	5500
C	P*	0	0	37
	0	478	1.7	2625
	60	396	1.7	1925
	120	230	10.9	268
	180	90	.5	297

* P = Preinfusion.

curred. Maximum hemoglobin values of 19 mg % and 6.5 mg % were found in the plasma. Plasma hemoglobin fell with clearing of the fat from the plasma. There was no change in daily fecal excretion of urobilinogen in either experiment.

Human experiments. Three experiments were carried out on 2 patients. The results are plotted in Table II, Fig. 2. In A and B fat emulsion II was used and in Exp. C emulsion I was infused. The fragility rose to 14,500 in one experiment indicating hemolysis of almost the entire red cell mass during shaking. The average of 5 control indices was 58 with a range of 27 to 108. Only minimal spontaneous hemolysis occurred in Exp. C, but in A a plasma hemoglobin level of 50 mg % was reached. In this experiment there was increase in body temperature to 100° associated with symptoms of nausea and apprehension. In neither of the other experiments did fever occur although in Exp. B a plasma hemoglobin level of 22 mg % was reached.

Table III illustrates the 24-hour fecal excretion of urobilinogen. In Exp. A there was a rise in urobilinogen excretion. In Exp. B urobilinogen excretion also increased to a lesser degree, proportionate to the observed differences in spontaneous hemolysis. The change in urobilinogen excretion in Exp. C is not significant.

Discussion. Decreased *in vitro* resistance of the erythrocyte to mechanical stress after infusion of fat emulsions has been demonstrated. In earlier experiments in this laboratory in which intravenous fat was given to

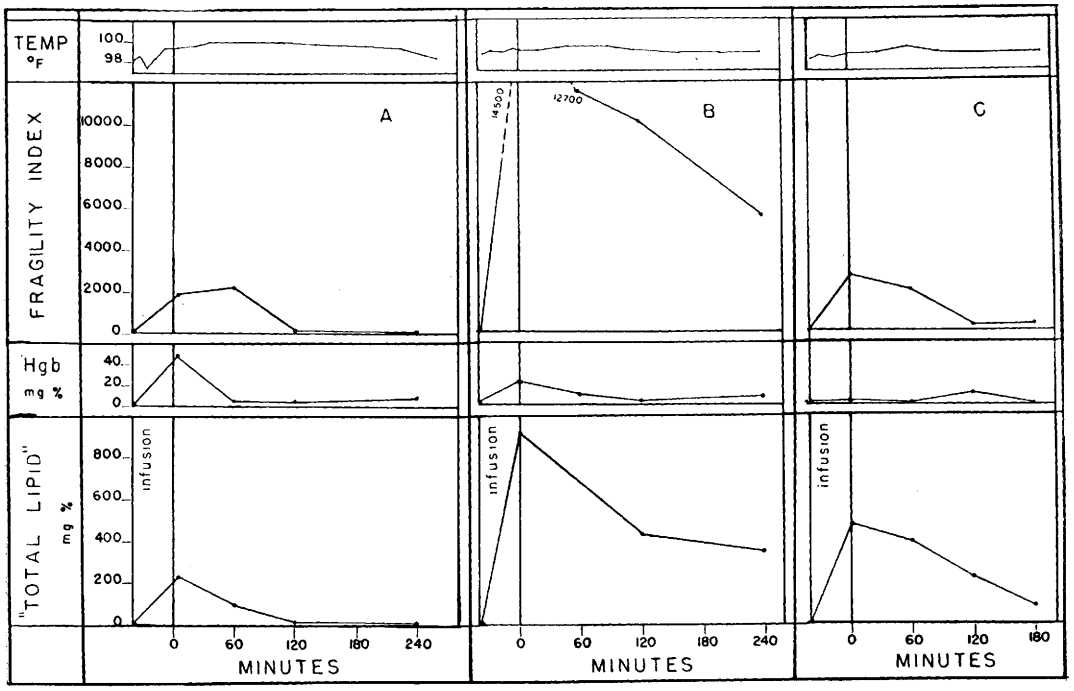

FIG. 2. Human experiments. Emulsion II used in Exp. A and B. Emulsion I used in Exp. C.

TABLE III. Daily Urobilinogen Excretion in Three Human Subjects.

Day	Fat infusion	24-hr urobilinogen excretion, mg/day
1	—	152
2	—	132
3	A	224
1	—	85
2	—	84
3	B	128
4	—	77
1	—	30
2	—	30
3	C	43
4	—	23

Day of fat infusion is indicated by letter corresponding to exp. in Fig. 2.

dogs, mechanical and osmotic fragility was determined in the classical manner using washed red cells in very dilute suspension. In none of these trials was any change in fragility observed. The decreased resistance of the erythrocyte exists only while the cell is in lipemic plasma. Van Itallie *et al.*(5) give no details of technic when they state that no change in the integrity of the red cell membrane can be shown. It appears quite likely that differences in technic might explain the divergent results.

Since increased fragility can be easily reversed by washing *in vitro* and since the fragility index rapidly falls with *in vivo* clearing of the plasma fat, there is no evidence for an irreversible change in the red cell itself. Although this increased fragility could certainly predispose to intravascular hemolysis, the actual occurrence of such hemolysis would remain an inconstant phenomenon. The inconsistency of the occurrence of anemia in published studies lends support to this hypothesis. Of the experiments reported here, only one showed a great degree of intravascular hemolysis.

In view of the extreme fragility of the cells in lipemic plasma, measurable elevation of plasma hemoglobin may be produced in the handling of the blood specimens. However, in our experiments the parallel increases in fecal urobilinogen excretion in the 2 cases where plasma hemoglobin rose above 10 mg % rule against this possibility.

Is the observed increased red cell fragility a function of hyperlipemia *per se*, or is it a phenomenon associated only with parenterally administered fat emulsions? Increased bile pigment excretion has been reported to occur as a result of high fat diets(15,16). In addition, it has been demonstrated that lipemic lymph and plasma cause increased erythrocyte fragility and hemolysis(17-19).

The physical state of hyperlipemia itself is common to all of the situations described here and in the literature. The highly emulsified fat might well distribute itself around the red cell and alter membrane relationships in such a way that fragility is increased.

Shafiroff *et al.*(20) have demonstrated the occurrence of intravascular hemolysis and severe reactions after the infusion of relatively soluble short chain fats. These may be easily saponified and contribute surface active products of hydrolysis. Others(21) have correlated the hemolytic action of fatty chyle with its free fatty acid and soap content.

Fever is the most common reaction to intravenous fat administration(1,5,10,22) and has been reported from every laboratory investigating the use of intravenous fat emulsions. Current estimates put the rate of reaction at 15%(23) to 33%(22) of infusions. The reaction is erratic in occurrence and emulsions may cause fever in one individual but not in another. Most diseases associated with acute intravascular hemolysis are characterized by the occurrence of fever. It also appears reasonable to infer that the febrile response to intravenous fat is a reaction to intravascular hemolysis. In the report of Shafiroff *et al.* (20) the intravascular hemolysis observed was held responsible for the accompanying symptoms, including the temperature response. In our experiments, the only febrile reaction was associated with the greatest degree of hemolysis observed. Further correlations of this type should be sought. If the occurrence of intravascular hemolysis is a chance phenomenon as suggested, it would explain the erratic, unpredictable occurrence of the febrile response to intravenous fat emulsions.

Summary. 1. Intravenous infusion of fat emulsions causes increased mechanical fragility of dog and human red blood cells. 2. Spontaneous intravascular hemolysis has been

seen in humans who have received these emulsions. 3. The possible causes of this phenomenon and its possible relationship to the "pyrogenic" effects of intravenous fat are discussed.

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Production of Group A Streptococcal Cervical Lymphadenitis in Mice.*† (20032)

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The fact that hemolytic streptococcal infections are implicated in the causation of rheumatic fever is well documented(1-3). Further, there is much evidence to suggest that rheumatic fever occurs as a result of an inappropriate immune response on the part of the host to the streptococcus or its products(4,5). Nevertheless, the pathogenesis of rheumatic fever remains obscure, its elucidation being seriously handicapped by the lack of a suitable experimental analog. The development, therefore, of an affection in experimental animals, bearing sufficient resemblance to rheumatic fever in man to satisfy the most stringent criteria, might well constitute a significant advance toward definition of the mechanism by which the human disease arises. One avenue of attack on this problem is represented by the attempt to simulate in experimental animals a sequence of events comparable to that which is assigned importance in human rheumatic fever; namely, repeated infections of the upper respiratory tract with group A streptococci(6). In order to pursue this par-

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