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A Fat Emulsion for Intravenous Feeding.*

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Attempts to utilize fat for intravenous feeding, because of its high caloric content, have led to the development of many types of fat emulsions.[1-9] The special problems encountered in such usage involve the dispersion of the fat into suitable particle sizes (0.5 to 1 μ) and the stabilization of that dispersion. The former has been achieved by high pressure homogenization of such fats as coconut, olive, corn, and butter oils. Egg or soya phosphatides have, in most cases, been used as stabilizers.

McKibbin *et al.* devised an emulsion of coconut oil which was stabilized with soya phosphatides.[6] It was well tolerated when injected slowly, but foreign body responses were observed following chronic injections. The investigators reported, however, that impurities in the soya phosphatides caused the foreign body reactions and that simple purification freed the emulsion of toxicity.[10] These findings have been confirmed by Ng in this laboratory.[11] He found that daily intravenous injections of a commercially prepared soya phosphatide, for 15-25 days, produced a mild hemolysis and a lipid-storage reaction in the reticulo-endothelial system. Use of the fraction of this commercial preparation which was soluble in 95% ethyl alcohol at 60° did not prevent the untoward reactions.

Meng and Freeman devised an emulsion of butter oil stabilized by the presence of a polyalcohol derivative and soya phosphatides, in an oil phase, and sodium cholate in an aqueous phase.[7] This emulsion was tolerated during its intravenous administration and, as shown by postmortem examination, resulted in only minimal changes in lungs and kidneys of dogs that had received daily injections for several weeks.

Shafiroff and Frank prepared an emulsion of coconut oil in a menstruum composed of 6% gelatin, 5% protein hydrolysate, and 5% glucose.[8,9] Apparently it was well tolerated for they observed no evidence of toxicity.

In the present investigation, a fat emulsion not hitherto used for intravenous feeding is described. This consisted of an olive oil dispersion stabilized by means of glycerol monostearate. The daily intravenous injection of this emulsion for as long as 2 to 4.5 weeks produced only minimal reactions in dogs as judged by microscopic examination of their tissues.

Experimental. Preparation of emulsion. Two parts of olive oil and one part glycerol monostearate† were heated, with stirring, until

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4 Myers, R. J., and Blumberg, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 79.

5 Dunham, L. J., and Brunschwig, A., *Arch. Surg.*, 1944, **48**, 395.

6 McKibbin, J. M., Pope, A., Thayer, S., Ferry, R. M., Jr., and Stare, F. J., *J. Lab. Clin. Med.*, 1945, **30**, 488.

7 Meng, H. C., and Freeman, S., *J. Lab. Clin. Med.*, 1948, **33**, 689.

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9 Shafiroff, B. G. P., Baron, H., and Roth, E., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 387.

10 Geyer, R. D., Mann, G. V., Young, J., Kinney, T. D., and Stare, F. J., *L. Lab. Clin. Med.*, 1948, **33**, 163.

11 Ng, E., unpublished observations.

† A commercial preparation manufactured by Glyco Products Co., Inc., Brooklyn, New York, and designated Aldo 28, was used. This product is glycerol monostearate modified by the inclusion of a small amount of a sodium soap. Emulsions prepared with unmodified glycerol monostearate gelled a few hours after autoclaving.

a clear solution was obtained. This melt was then added to a sufficient volume of 5% glucose to yield a final concentration of 10% fat, and the mixture was stirred in a Waring blender for 3-5 minutes. Three gallons of this preliminary emulsion were passed through a dairy homogenizer 5 times at 2500 lb. per sq. inch. The diameters of the fat particles of the final emulsion were determined by visual measurement with an ocular micrometer and, as a rule, found to be one micron or less. Particles as large as 2 μ were only occasionally observed. The pH of this emulsion was 7.0. Such emulsions contained about 10% fatty acids of which 2/3 were derived from olive oil and 1/3 from glycerol monostearate. Bottling in cotton-stoppered 500 cc Erlenmeyer flasks, autoclaving for 30 minutes at 5 lb., and cooling in an ice bath produced a sterile emulsion that creamed only slightly after 2 months. The emulsion was stored at room temperature.

Treatment of dogs. For several days before injection and throughout the injection period, the dogs were fed once daily a mixture composed of 40 g of lean horse meat per kg body weight, 20 g of sucrose, 20 g of bone ash, one cc of fish oil,‡ and 10 g of yeast. Before the start of the injection, samples of blood were withdrawn for determination of red cell counts and hemoglobin concentration. Liver function also was measured by means of the Rose Bengal test as described by Hough and Freeman.[12]

The dogs were injected with 200 cc of the fat emulsion, either daily or 6 days per week. The dogs received, in all, 16 to 31 intravenous injections. A few days before the last injection, evidence of toxicity was sought by determination of red cell counts, hemoglobin concentrations of blood, and of liver function.

Methods. The method used for the determination of fatty acid contents of plasma and

TABLE I.
Effect of Intravenous Injections of a Fat Emulsion on Dogs.
(Each dog received 200 cc of 10% fat emulsion at each injection.)

Dog	No. of inj.	Wt		Hemoglobin		Red blood cell count		Rose Bengal clearance		Liver fatty acid, % wet wt
		Before inj. started, kg	At end of inj. period, kg	Before inj. started, g per 100 ml	On indicated day of inj. period, g per 100 ml	Before inj. started, $\times 10^6$	On indicated day of inj. period, $\times 10^6$	Before inj. started	On indicated day of inj. period	
1	13	12.7	12.5	16.0	13.0 (13)*	8.1	7.1 (13)*	136†	114 (13)*	3.1
2	24	8.7	8.5	13.2	10.8 (20)	7.1	4.7 (20)	130	82 (20)	2.9
3	16	15.1	15.4	15.6	12.5 (15)	7.6	6.6 (15)	114	110 (15)	3.2
4	16	10.2	10.3	14.0	12.6 (15)	7.7	6.4 (15)	114	82 (15)	4.1
5	31	13.2	12.5	16.0	9.5 (31)	8.7	5.2 (31)	104	108 (31)	—

* Numbers in parentheses indicate the day of the injection when test made.
† Values lower than 100 indicate impaired excretion.[12]

‡ Each cubic centimeter of the fish oil (sardilene) contained not less than 100 A.O.A.C. chick units of vitamin D and 600 U.S.P. units of vitamin A.

[12] Hough, V. H., and Freeman, S., *Am. J. Physiol.*, 1942, **138**, 184.

liver has been recorded elsewhere.[13,17] Red cell counts and hemoglobin concentrations of blood were determined as described by Gradwohl.[14]

Results. The 200 cc of the emulsion which each dog received daily by intravenous drip contained 13.3 g of olive oil and 6.7 g of glycerol monostearate, or a total of 20 g of fatty acids. It was administered at a rate of about 1 cc per minute per kg of body weight. No evidence of distress other than a slight restlessness was observed during the injections which occupied, as a rule, from 20 to 30 minutes. Immediately after completion of the injection, the dogs were fed their daily ration which was readily consumed.

Five dogs were used in this investigation (Table I). They received 16 to 31 daily injections. Red cell counts, hemoglobin concentrations of the blood, and liver function were measured on the day before the injections were begun and at designated days near the end of the injection period.

Before the injections were begun, the hemoglobin content of the blood of the 5 dogs ranged from 13.2 to 16.0 g per 100 cc. These values are well within the range reported by other investigators.[6,7,15] The concentration of hemoglobin in all 5 dogs had decreased by the time the injections had continued for 13 to 31 days. This is in keeping with the observed fall of the red cell counts. Similar hematological findings were reported by McKibbin *et al.*[6] and by Meng and Freeman[7] in their dogs that received injections of fat emulsions. Despite the slight anemia a weight loss was not observed in 4 dogs that received as many as 31 daily infusions. Dogs 1, 3, and 5 showed no evidence of impaired liver function as judged by the rate at which injected Rose Bengal was lost from the blood stream.

TABLE II.

Rate of Disappearance of Injected Fat from the Blood Stream of the Dog.
(All lipid values expressed as mg per 100 cc plasma).

Dog 1*			Dog 2†			Dog 3†		Dog 4†		Dog 5‡			
Interval after inj., min.	Total fatty acids	Total cholesterol	Interval after inj., min.	Total fatty acids	Total cholesterol	Interval after inj., min.	Total fatty acids	Interval after inj., min.	Total fatty acids	Interval after inj., min.	Total fatty acids	Total cholesterol	Phospholipid
—12§	356	166	—6§	338	167	—1§	317	—2§	194	—21§	456	213	364
9	1510	169	2	1310	134	5	1170	4	1940	9	1110	163	282
94	665	172	38	819	159	44	710	23	2000	49	766	198	321
246	412	167	72	582	142	99	410	69	1150	136	526	199	328
535	405	172	132	465	155	160	317	152	402	202	431	196	328
			189	472	157			217	293				

* This dog received a total of 13 injections and the data shown above are for the eighth injection.
† The measurements recorded above were made on the day of the last injection.
‡ This dog received a total of 31 injections and the data shown above are for the 26th injection.
§ Minus values indicate the time in minutes before the injections were started.

[13] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1937, **119**, 423.

[14] Gradwohl, R. B. H., Clinical Laboratory Methods and Diagnosis, 3rd ed., 1943.

[15] Allison, J. B., *Trans. N. Y. Acad. Sci.*, Ser. II, 1946, **8**, 260.

[16] Entenman, C., Chaikoff, I. L., and Zilversmit, D. B., *J. Biol. Chem.*, 1946, **166**, 15.

[17] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

The excretion of the dye by dogs 2 and 4 was 82% of normal.

The rate of disappearance of the injected fat from the blood stream was measured for each dog and the results are shown in Table II. The first examination of plasma fatty acids was made from 2 to 9 minutes after the completion of the injection, and at these times fatty acid concentrations were between 1110 and 1940 mg %. Utilization of the injected fat, as judged by its disappearance from the blood stream, was rapid in all 5 dogs, and by the time 4 hours had elapsed, the concentration of plasma fatty acids approached the preinjection levels.

The dogs were sacrificed by an overdose of nembutal 24 hours after the last injection. All organs were examined grossly and lungs, liver, kidney, spleen, small intestine, and heart studied microscopically. The lungs of all 5 dogs were free of lipoid granulomatous lesions and the lungs of only 2 dogs showed a few inflammatory foci. The reticulo-endothelial elements of the spleens and livers contained moderate amounts of blood pigments, a finding that indicates that some hemolysis had occurred. In dog 4, lipid-like material was found in the reticulo-endothelial cells of the liver and in the capillary loops of occasional glomerular tufts. Dog 2 had a few shrunken and scarred glomeruli adherent to the capsule. Protein containing fluid was found in the glomerular and tubular spaces. Dog 5 was pregnant and 2 of its 6 fetuses were dead. The fatty acid contents of the livers of dogs 1-4 were normal (Table I). The liver of dog 5, as judged by microscopic examination, was free of abnormal amounts of fat.

Comment. It is shown here that by the use of glycerol monostearate as stabilizer, an olive oil emulsion in which the fat remains dispersed for several months can be easily prepared. This emulsion is well tolerated by dogs when injected intravenously at rates of 1 cc per minute per kg, and is rapidly removed from the blood stream. Although dogs that had received as many as 31 injections were not free of tissue responses, the reactions observed were quite mild in character.

In confirmation of earlier findings,[1,2,4,7] it was observed here that intravenously administered fat rapidly disappears from the blood stream. The rate at which this occurs suggests that the administered fat is taken up by the liver for it has been shown that lipids disappear slowly, if at all, from the plasma of hepatectomized dogs.[16]

Summary. 1. The preparation of a stable olive oil emulsion in which glycerol monostearate was employed as the emulsifying agent is described. 2. Observations on its use for intravenous feeding in dogs are recorded.

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Excretion of 17-Ketosteroids by Patients Suffering from Poliomyelitis.*

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It has long been recognized that the incidence of poliomyelitis is greater for children than for adults (Fanconi[1]). Since the excretion of 17-ketosteroids increases rapidly during adolescence it was thought possible that a low androgen excretion may be directly or indirectly associated with an increased incidence to poliomyelitis. The excretion of 17-ketosteroids by poliomyelitis patients was, therefore, investigated.

The urine specimens from a total of 39

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1 Fanconi, G., *Die Poliomyelitis und Ihre Grenzgebiete*, Benno Schwabe and Co., Basel, Switzerland.