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Intravenous Infusions of a Combined Fat Emulsion into Dogs.*

B. G. P. SHAFIROFF, H. BARON, AND E. ROTH.

From the Laboratory of Experimental Surgery, Department of Surgery, New York University College of Medicine.

The availability of an intravenous emulsion of fat, protein and glucose for experimental and clinical investigation has opened new fields of study in parenteral nutrition.[1] A combined emulsion such as developed in our laboratory was administered intravenously with equal facility to human patients[2] as well as to animals with a favorably low incidence of infusion reactions. The present report is devoted to observations made on a series of dog recipients of the combined emulsion.

Method of study. Nineteen dogs were divided into 3 groups: Group I, seven dogs received single infusions of the combined emulsion in a variety of acute experiments. Group II, six dogs were maintained on daily infusions of the emulsion and a standard kennel diet. Group III, six dogs were carried exclusively on the intravenous emulsion as the sole nutritive source.

Two types of combined emulsion were used. The first type contained 5% coconut oil, 5% amigen and 5% glucose solution emulsified with a 6% special intravenous gelatin solution. The second type of combined emulsion contained 10% coconut oil with the remaining ingredients at the original concentrations. The caloric value of the 5% emulsion was 0.85 calories per ml, that of the 10% emulsion was 1.3 calories per ml. The emulsion was prepared in a special homogenizer constructed to our specifications so that the entire process of blending and homogenization could be carried out under sterile conditions and the resultant yield collected in sterile bottles. The use of the autoclave as a final step in the procedure was obviated. The process of manufacture and a description of the homogenizing machine were described elsewhere in greater detail.[1,2]

The following laboratory tests were made, the number of tests performed depending on the experiment; hemoglobin (Sahli), red blood cell count, white blood cell count, refractive index of plasma (Fischer refractometer), specific gravity of blood and plasma (Scudder apparatus) chylomicra count,[3] total serum lipids and serum lipase.[4] The weight of the animals was recorded at regular intervals. After the completion of an experiment the dog was sacrificed and selected tissues were stained with special fat dyes for microscopic study.

Results. Group I: Three dogs were anesthetized with sodium pentobarbital, the femoral artery was cannulized and connected to a recording manometer. The blood pressure was continuously recorded throughout the infusion and for 2 hours thereafter. In all three of the dogs there was an elevation of 15 to 18% in blood pressure above the initial level. This occurred after ¾ of the total amount of emulsion had been infused. In 2 dogs the thoracic duct was intubated with a Lindemann needle and samples of lymph were tapped at regular intervals. The flow of lymph increased about 22% during the infusion period. The specific gravity of the lymph and refractometric examinations showed little change indicative of the presence or appearance of the emulsion into the lymph circulation. In each of 2 dogs the effect of rapid and slow infusion of the emulsion was tested.

* This project was made possible by grants from the Ben Lewis Fund in Experimental Surgery.

1 Shafiroff, B. G. P., and Frank, C., *Science*, 1947, **106**, 474.

2 Unpublished.

3 Frazer, A. C., and Stewart, H. C., *J. Physiol.*, 1939, **95**, 21.

4 Lagerlof, H., *Acta med. scandinav.*, 1945, **102**, 407.

TABLE I.
Effect of Infusions of Combined Emulsion.

Dog No.	Group	Days	Total g fat infused	Wt (kg)	Hemoglobin (g %)	Red blood cell count (million)	Hematocrit (Wintrobe)	Plasma protein (g %)
9	II—5% combined emulsion 250 ml + diet	1	12.5	14.3	12.9	5.73	45	5.62
		7	87.5	14.3	12.9	6.62	45	5.80
		15	187.5	15.0	13.1	5.62	43	5.78
		21	262.5	15.6	13.9	6.50	45	6.21
		30	375	15.8	13.9	6.28	49	6.17
11	II—5% combined emulsion 500 ml + diet	1	25	11.0	10.2	4.52	51	4.96
		7	175	11.0	12.9	5.81	49	5.48
		15	375	11.2	12.2	6.26	44	5.23
		21	525	11.3	13.2	6.08	48	5.44
		30	750	11.8	12.9	6.00	49	5.35
12	II—5% combined emulsion 500 ml + diet	1	50	16.8	15.3	5.90	51	6.26
		8	400	16.3	15.0	5.80	51	6.00
		15	750	16.6	15.0	5.84	50	6.31
14	III—10% combined emulsion no food	1	50	16.5	14.5	6.18	49	5.92
		8	400	16.0	14.9	6.20	50	5.92
		15	750	15.5	15.2	6.10	48	6.00
19	III—10% combined emulsion no food	1	50	17.0	15.5	6.62	52	6.78
		8	400	17.2	16.0	6.58	51	6.90
		15	750	17.0	15.2	6.25	53	6.62

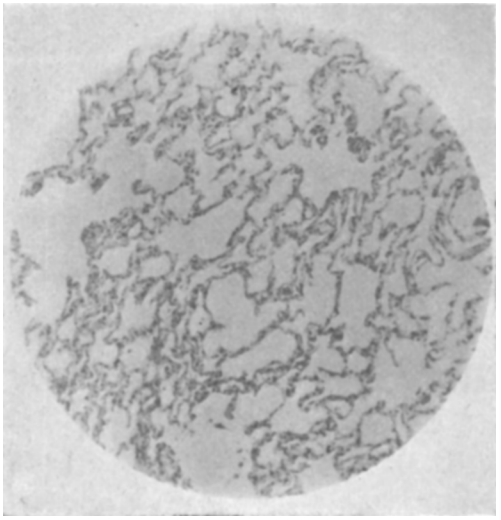


FIG. 1.
Section through the lung of a dog from Group III maintained exclusively on the 10% combined emulsion for a period of 14 days. The lung is microscopically normal.

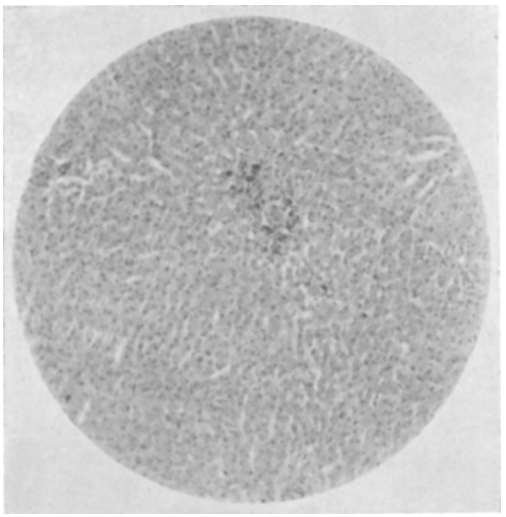
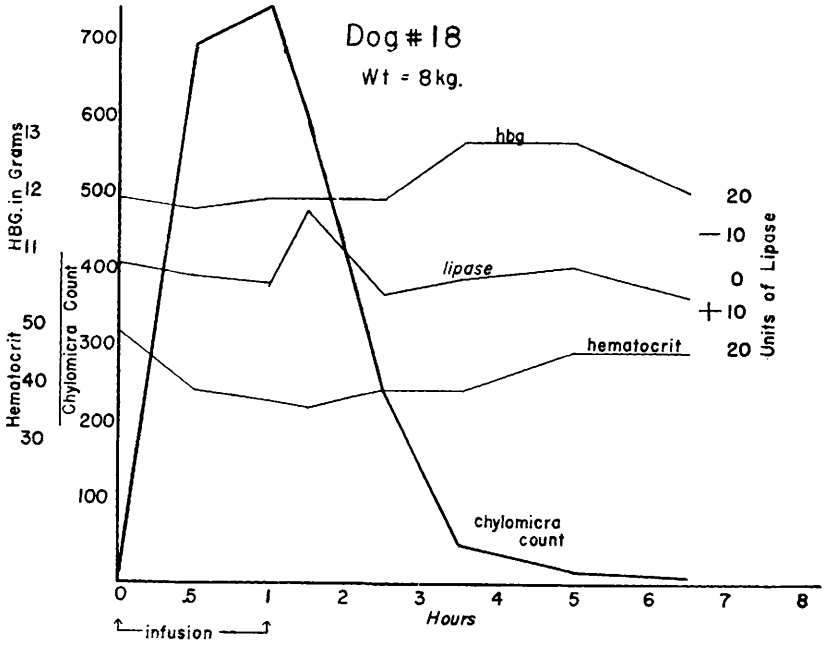


FIG. 2.
Section through the liver of the same dog. Liver shows areas of focal accumulations of fat still to be metabolized. The liver architecture is normal.

One dog received 500 ml of the emulsion within 90 minutes, the other received an equal volume over a period of 300 minutes. Both dogs tolerated the emulsion well. Plasma samples obtained during the rapid infusion were milky white in color and the emulsion could be readily detected. On nephelometric examination there was a marked increase in the suspension concentration of such samples over that of the initial plasma sample. In the former samples a "cream layer" was obtained on standing in a narrow tube or after high speed centrifugation. All plasma samples obtained during the slow infusion were clear in color and showed little turbidity as determined by the nephelometer. The rate of disappearance of the emulsion from the blood as determined by the total serum lipids and the chylomicra count differed from that of the physical disappearance of the emulsion from the plasma. Depending on the rate of infusion there was a progressive rise in the chylomicra count and the total serum lipids and within 4 hours after completion of the injection both latter values returned to the level of their fasting state in the blood.

Group II. Four dogs in this group received daily infusions of the 5% combined emulsion for a 30 day period. They were allowed in addition a standard kennel diet. Dog No. 8 was injected with 150 ml of the emulsion, dog No. 9 with 250 ml, dog No. 10 with 400 ml and dog No. 11 with 500 ml of the emulsion. The number of injected calories ranged from 127 to 425 calories daily. Dog No. 8 received a minimum of 225 g of injected fat while dog No. 11 received the maximum of 750 g of fat during the study period. Two other dogs in this group No. 12 and No. 13 were infused daily with 500 ml of the 10% combined emulsion for a period of 14 days receiving also a total of 750 g of fat. In the dogs infused with large amounts of fat there was a corresponding decrease in appetite for the kennel diet. On many occasions the dogs refused the kennel diet entirely and accepted water only which was taken in large quantities. The dogs were always alert and energetic and never offered resistance throughout the infusion phase. During the injection there was a marked elevation in the chylomicra count in the blood followed by a fall to the pre-infusion level within four hours of completion of the infusion. Lipase activity determinations run concurrently with the latter revealed a significant increase in enzymatic activity in the blood. Red blood counts and



GRAPH I.

Typical graph of the chylomicra count, hemoglobin, lipase activity and hematocrit obtained in Dog No. 18 (Group III) on the 10th study day during the infusion and post-infusion phases of an injection of 500 ml of the 10% combined emulsion.

hemoglobin determinations made at regular intervals during the 30 day period showed no signs of hemolytic anemia. The weight gain in the animals varied from 0.8 kg to 1.6 kg. The largest weight gain occurred in dogs No. 8 and No. 9 who received the smallest volume of emulsion and ate more of the kennel diet than the others of the group. Examination of the tissues revealed the liver cells to contain intra-cellular fat with an occasional focal aggregation of lymphocytes. No true granulomata were observed in the lung tissue. The architecture of the kidney and spleen was normal.

Group III. All the dogs of this group were maintained exclusively on daily infusions of 500 ml of the 10% combined emulsion for a period of 14 days. The total amount of fat injected was 750 g. The dogs were allowed water ad libitum, received orally multiple high potency vitamins and were rewarded after each infusion with 2 rye-krisp biscuits. Chylomicra counts showed the characteristic tendency to increase during the infusion and for a short period thereafter. Lipase activity increased in the blood during the infusion. The specific gravity of the blood and the plasma proteins stayed approximately stationary during the experimental period. Hemoglobin determinations and the red cell counts showed little variations from the original and secondary anemia did not develop. In this group dogs No. 14, 17 and 18 lost one-half to one kilogram in weight while dogs No. 15, 16 and 19 maintained their original weight with slight variations. Tissue studies in this group were also negative.

Comments. The utilization of intravenous fat emulsion for growth and energy has been effectively proved in dogs by many workers.[5,6] The present experiments have demonstrated that the combined emulsion containing 5 or 10% fat may be used intravenously both as a supplement to diet or as the sole source of

[5] McKibbin, J. M., Ferry, R. M., and Stare, F. J., *J. Clin. Invest.*, 1946, **25**, 679.

[6] McKibbin, J. M., Hegsted, D. M., and Stare, F. J., *Fed. Proc.*, 143, **2**, 9.

provision of the minimal daily requirements of the animal. Although mild to serious anemias have been reported to develop as a result of the infusions of the fat emulsions[7,8] the latter complication failed to occur in the present investigation. This is believed to be due to the fact that phosphatide stabilizers were not used in the preparation of our emulsion. The disappearance of fat from the circulating blood determined either by the chylomicra count or by total serum lipids was interpreted to indicate that lipolysis commenced in the blood, the process being initiated by serum pancreatic lipase. Evidence to support the latter view was based on the increase of lipase activity of the blood and substantiated further by the report of Meng and Freeman[9] that the fatty acids increased during the infusion of a fat emulsion. The assimilation of preformed fat infused intravenously may therefore be conceived as a possible biochemical function whereby the neutral fat ultimately reached the liver for catabolism. An analysis of the slides revealed that in the acute experiments after an infusion all the organs were richly laden with fat. In the lung the fat did not permeate into the alveolar spaces but was contained in the capillaries. In the chronic experiments of Group II and Group III, the liver and the spleen were the only organs which contained evidences of fat.

Summary. (1) A combined emulsion of fat protein and glucose was infused intravenously in a series of 19 dogs.

(2). Toxic reactions such as secondary anemia and degenerative pathology in vital organs failed to develop after prolonged injections of the combined emulsion.

(3). An attempt was made to correlate the associated laboratory tests with the biochemical functions of hydrolysis and assimilation of fat.

The authors wish to acknowledge the helpful advice of Professors Mulholland and Co Tui. The authors are indebted to the E. F. Drew Co., to the Mead Johnson Co., and to the C. F. Knox Co. for generous supplies of material used for the preparation of the emulsion. The homogenizer was made by the C. W. Logeman Co., New York City.

7 Johnson, V., Longini, J., and Freeman, L. W., *Science*, 1943, **97**, 400.

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

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Effect of Alloxan Diabetes on Hyaluronidase Level of the Rat Testis.*

MAXINE MOORE. (Introduced by S. L. Leonard.)

From the Department of Zoology, Cornell University, Ithaca, N. Y.

Although hyaluronidase, an enzyme obtained from mammalian sperm and testes, can disperse follicle cells of ovulated mammalian ova, a normal role of this enzyme in reproduction remains to be established.[1,2] In line with the main problem of determining this role it was desired to learn if a physiological disturbance of the metabolism of the animal, without the destruction of the germinal elements, could alter the level of hyaluronidase within the testis. Experimental diabetes, therefore, was induced in male rats and after one month the amount of hyaluronidase was measured in the control and diabetic rat testes.

* Aided by a grant from the Sage Fund of the Cornell University Trustee-Faculty Committee on Research, administered by Dr. S. L. Leonard.

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2 Leonard, S. L., Perlman, P. L., and Kurzrok, R., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 517.