possible exception of the non-spayed group of females. The ages of the donor grafts were not significant in relation to growth of fragments at the ages investigated. There was no apparent influence of the tissue transplants upon the weight gain of the recipient rabbits.

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Parenteral Nutrition. XI. Studies with Stable and Unstable Fat Emulsions Administered Intravenously.* (18953)

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It has been clearly demonstrated that suitably prepared emulsions of fat can be given safely in large quantities to various species of experimental animals(1,2), and to man(3). Furthermore, studies concerning nitrogen and potassium balances(3,4), rates of growth (1,4), and analyses of body fat(1,4), have demonstrated the utilization of the fat in such emulsions. Experiments with emulsified C^{14}-labeled trilaurin(5) and tripalmitin(6) have shown the metabolism of these lipids to be rapid and extensive. *In vitro* studies with emulsified C^{14}-labeled trilaurin have indicated that various rat tissues have a high capacity to metabolize this and other lipid substrates(7). Rat blood, moreover, has little effect on these substrates directly or in affecting the metabolism of these substances by tissue slices. Thus, the evidence supports the assumption that the blood plays a minor direct role, if any, in the utilization of intravenously administered emulsified fats.

The question arises, however, as to the ability of the body to remove extracellular fat which, for example, has become lodged in capillaries. This problem is of *no* concern when finely dispersed, stable emulsions of fat are employed. It *is* of great concern when an emulsion is administered intravenously which (a) is not finely dispersed, but contains large particles of fat (3 to 15 μ), (b) is finely dispersed, but breaks down when in contact with plasma, or (c) is finely dispersed, but breaks down because of concurrent infusions of materials not compatible with the emulsified fat. Studies with iodinated fat emulsions of both a high and low degree of dispersion have been reported(8). The data presented in this paper deal with both stable and unstable emulsions which were studied from two standpoints: (1) the increase in the fat content of various tissues following the administration of such emulsions and (2) the rate of removal from the tissues of the excess fat which resulted when these emulsions were given.

Experimental. A number of emulsions of

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1. Mann, G. V., Geyer, R. P., Watkins, D. M., Smythe, R. L., Dju, D., Zamcheck, N., Stare, F. J., *J. Lab. and Clin. Med.*, 1948, v33, 1503.

2. Woerner, C. A., *Anat. Rec.*, 1949, v104, 61.

3. Gorens, W. G., Geyer, R. P., Matthews, L. W., and Stare, F. J., *J. Lab. and Clin. Med.*, 1949, v34, 1627.

4. McKibbin, J. M., Ferry, R. M., Jr., and Stare, F. J., *J. Clin. Invest.*, 1946, v25, 679.

5. Geyer, R. P., Chipman, J., and Stare, F. J., *J. Biol. Chem.*, 1948, v176, 1469.

6. Lerner, S. R., Chaikoff, I. L., and Entenman, C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 384.

7. Geyer, R. P., Matthews, L. W., and Stare, F. J., *J. Biol. Chem.*, 1949, v180, 1037.

8. Sato, G., *Tohoke J. Exp. Med.*, 1931, v13, 120.

TABLE I. Composition of Emulsions.*

Emulsion No.	Corn oil,† %	Coconut oil,‡ %	Stabilizer system B(F²)§	Stabilizer system B(F¹)§	Stability *in vitro*
1	—	30	3	—	Stable
2	30	—	3	—	"
3	30	—	—	3	Unstable
4	30	—	—	1	".

* All emulsions were made with 5% dextrose solution as the aqueous phase. They were all stable to autoclaving.

† Refined corn oil. Corn Products Refining Co., Argo, Ill.

‡ Edible coconut oil. E. F. Drew and Co., Boonton, N. J.

§ Prepared from purified soybean phosphatides (11).

coconut and corn oils were prepared by the high-pressure homogenization procedure previously described (9). Sterilization of all emulsions was accomplished by autoclaving at 15 lb per square inch for 15 minutes. Pertinent data including the composition of these emulsions are given in Table I. It should be emphasized that all of the emulsions used in these studies contained 30% fat, not 15% as is usual in the emulsions used clinically by this laboratory. The stability of the emulsions in the presence of plasma was tested as follows: 0.5 ml of emulsion was mixed with 2 ml of heparinized dog plasma and the mixture was incubated at 37.5°C for 4 to 6 hours. Samples of the mixture were removed at various time intervals and examined under the microscope for evidence of a change in the size of the particles of fat. Other samples were simultaneously removed, diluted to 2 ml with 5% dextrose and the turbidity of the mixture was determined by means of the Klett-Summerson photoelectric colorimeter as described previously (10). Emulsions No. 1 and 2 were found to remain stable; whereas No. 3 and 4 slowly broke down to larger fat particles.

Non-fasted male rats of the Wistar strain, weighing approximately 200 g, were used in all experiments and were maintained on a commercial stock feed. Injection of the emulsions was done through the tail vein while the animal was under light ether anesthesia. The quantity of emulsified fat administered varied between 2.5 and 10 g of fat per kilo body weight. Some animals were sacrificed when the level of infused fat remaining in the blood was negligible as determined by turbidimetric analysis. The remaining animals were sacrificed at various intervals after the blood fat level had returned to normal. In several instances the animals died and their tissues were used since the time of death was fairly well known. Animals which had received injections of 5% dextrose solution were also sacrificed and their organs were analyzed for the normal fat content. Histological sections were made to confirm the location of the fat in the tissues.

Fat analysis of the lung, liver, brain, spleen, and kidney was done as follows: the fresh tissue was weighed, a suitable sample of it was ground in a small mortar with powdered anhydrous Na_2SO_4, and the mixture was extracted with hot $CHCl_3$ in a continuous extractor for 8 hours. The chloroform solution of lipids was then evaporated to dryness; traces of solvent being removed by means of a stream of nitrogen. The residue was repeatedly extracted with small portions of hot petroleum ether (B.P. 65°C) and all of the extracts were filtered through a sintered glass funnel into a tared 50 ml conical flask. The funnel was washed with small quantities of petroleum ether and the washes were also collected in the flasks. After complete removal of the ether, the flasks were placed in a desiccator for several hours and then weighed. The percent of fat was calculated from the weight of the extracted ether-soluble lipids.

Results and discussion. As judged by gross

9. Geyer, R. P., Mann, G. V., and Stare, F. J., *J. Lab. and Clin. Med.*, 1948, v33, 153.

10. Geyer, R. P., Mann, G. V., and Stare, F. J., *J. Lab. and Clin. Med.*, 1948, v33, 175.

11. Geyer, R. P., Mann, G. V., Young, J., Kinney, T. D., and Stare, F. J., *J. Lab. and Clin. Med.*, 1948, v33, 163.

TABLE II. Fat Content of Various Tissues After Injection of Emulsions.*

Group No.	No. of animals	Body wt	Emulsion No.	g fat/kg body wt	% fat (wet basis) Spleen	Liver	Lung	Brain	Kidney
I	3	236 (219–257)	5% dextrose	0	2.5 (2.3– 2.6)	4.5 (4.5– 4.5)	3.9 (3.8– 4.1)	7.8 (7.3– 8.3)	4.2 (3.8–4.5)
II	3	162 (160–167)	1	5	3.2 (2.4– 4.5)	6.5 (6.2– 6.7)	4.7 (4.4– 5.1)	8.8 (8.6– 8.9)	4.4 (4.2–4.5)
III	3	228 (208–245)	2	5	5.7 (4 – 7.2)	6.9 (6.7– 6.9)	3.8 (3.1– 4)	9.4 (9.1– 9.6)	3.4 (3.2–3.6)
IV	3	228 (208–245)	3	5	30.5 (28.2–32.1)	6.4 (6 – 6.5)	6.1 (4.6– 9)	9.8 (8.6– 9.9)	4.6 (4.3–4.9)
V	3	231 (205–246)	3	10	26.3 (21.4–33.1)	8.1 (6.6– 8.8)	10 (8 –13.1)	9.8 (9.6–10)	5.2 (5 –5.4)
VI	3	259 (241–286)	4	10	34.5 (24 –42.8)	11.5 (9.2–14.9)	20.3 (14.1–25.2)	10.5 (8.4–13.1)	5.6 (4.1–7.1)

* Animals sacrificed when blood level had returned to normal.

TABLE III. Effect of Dosage of Fat Emulsions on the Fat Content of Various Tissues.*

Group No.	No. of animals	Body wt	Emulsion No.	g fat/kg body wt	% fat (wet basis) Spleen	Liver	Lung	Brain	Kidney
VII	3	171 (166–177)	1	2.5	2.9 (2.5– 3.3)	5.9 (4.7–6.7)	4.7 (4.3– 5.1)	8.8 (8.1– 9.2)	4.3 (4.1–4.3)
II	3	162 (160–167)	1	5	3.2 (2.4– 4.6)	6.5 (6.2–6.7)	4.7 (4.4– 5.1)	8.8 (8.6– 8.9)	4.4 (4.2–4.5)
VIII	3	216 (205–222)	3	2.5	15.9 (14.8–17)	5.6 (5 –6.5)	4.2 (3.9– 4.9)	9 (8.9– 9.1)	4.9 (4.6–5.2)
IV	3	193 (190–200)	3	5	30.5 (28.2–32.1)	6.4 (5.9–6.6)	6.1 (4.6– 9)	9.8 (8.6– 9.9)	4.7 (4.3–4.9)
V	3	231 (205–246)	3	10	26.3 (24.4–33.1)	8.1 (6.6–8.9)	10 (8 –13.1)	9.8 (9.6–10)	5.2 (4.9–5.4)

* Animals sacrificed when blood fat level had returned to normal.

fat analysis, the fat content of various rat organs increased moderately following the intravenous injection of stable, finely dispersed emulsions of fat. On the other hand, a fairly great increase in fat content occurred when the emulsions used were finely dispersed, but unstable *in vivo*. These phenomena are demonstrated by the data presented in Table II. All of the emulsions (see Table I) were equivalent from the standpoint of size of the fat particles as determined by visual microscopy employing an ocular micrometer. Emulsions No. 1 and 2 were stable *in vivo* in the presence of blood; whereas, Emulsions No. 3 and 4 were unstable *in vivo* presumably because of the effect of the electrolytes contained in the plasma. The latter emulsions were also unstable *in vitro* when mixed with heparinized rat plasma at 38°C. The breakdown of these emulsions either *in vivo* or *in vitro* was not immediate and complete, but rather progressive over a period of several hours. This type of instability would enable fat to accumulate extracellularly in many tissues instead of lodging mainly in the first tissue through which it passed, which might be the case if immediate, complete breaking occurred. Histological sections of lung, liver, brain, spleen, and kidney showed so much extracellular fat present after the injection of unstable emulsions that it was impossible to discern the tissue structure.

The data in Table II show that the highest *relative* increase in fat content occurred in the spleen with both stable and unstable emulsions. The liver showed the next highest increase when stable emulsions were used, but the lung was second highest when the unstable emulsions were used. Thus, it would appear that an appreciable increase in fat content of the lungs following intravenous administration of fat would be one indication that the emul-

sion was unstable. Little increase in fat content of either brain or kidney tissue resulted after the administration of stable emulsions. A greater increase in the total lipid of these two tissues occurred after injection of the unstable emulsions, especially Emulsion No. 4.

As shown by the data in Table III, increasing the dose of the stable fat emulsion (Emulsion No. 1) from 2.5 g fat per kilo of body weight to 5.0 g did not alter very much the total content of the organs. In the case of the unstable emulsion (Emulsion No. 3) this increase in dose raised the fat content of the spleen from approximately 16% up to 30%. The slight increase in the case of the liver and lung is interesting. When the dose of Emulsion No. 3 was raised to 10 g of fat per kilo of body weight, an increased fat content resulted in all organs analyzed. It should be pointed out that at the lowest dose used, the total-fat analysis method used here is not delicate enough to indicate very small changes in fat content. A more exact approach to this problem is available through the use of emulsions of radioactive triglycerides.

Data on the rate of removal of fat from the various organs after administration of fat emulsions are presented in Table IV. The rats in Groups IX, X, and XI which received a stable emulsion removed the excess fat from the various organs in fairly short time, for an almost normal level was reached in 24 hours. The amount of fat which these rats were given (5 g/kg body weight) is a much greater dose than would be given to patients requiring about 1500 to 2000 calories from a fat infusion. The animals which received Emulsion No. 4 were given an even higher dose per kg of body weight than those which received the stable emulsion (Emulsion No. 2). It is apparent that even under such drastic conditions the animals survived and the increased fat content of the organs slowly returned to normal. Some of this increase in fat content would be due to lipid which had gained access to the various cells prior to the complete breakdown of the emulsion. However, most of it was extracellular and was probably removed by phagocytic and lipolytic action. Whether a rise in serum lipase results after

TABLE IV. Rate of Removal of Fat from Various Organs After Administration of Stable and Unstable Emulsions.

Group No.	No. of animals	Body wt	Emulsion No.	g fat/kg body wt	Time sacrificed (min)	% fat (wet basis) Spleen	Liver	Lung	Brain	Kidney
IX	3	291 (285–290)	2	5	290 (285–295)	9.3 (6.6–11.1)	7.4 (7.3– 7.4)	5.5 (5.5– 5.6)	9.9 (9.7–10.1)	5.6 (4.4–8.3)
X	3	206 (195–219)	2	5	1307 (1025–1460)	3.4 (3.2– 3.6)	6.5 (6.3– 6.8)	4.3 (3.4– 5)	8.8 (8.3– 9.2)	4.3 (3.5–5.4)
XI	3	208 (200–214)	2	5	4341 (4340–4345)	2.4 (2.2– 2.5)	5 (3.1– 6.1)	3.1 (2.1– 4.1)	9 (8.8– 9.1)	4 (3.5–4.5)
VI	3	259 (241–286)	4	10	165	34.5 (24 –42.8)	11.8 (9.2–14.9)	20.3 (14.1–25.2)	10.4 (8.4–13.1)	5.6 (4.1–7.1)
XII	3	236 (213–260)	4	10	710 (690–720)	22.6 (15.5–31.1)	9.8 (9 –11.1)	13.3 (11.6–17.6)	10 (8.9–11.5)	5.7 (4 –6.9)
XIII	3	234 (220–257)	4	10	5726 (5024–6160)	23.4 (20.2–34.7)	4.6 (3.5– 5.8)	9.6 (7.1–11.3)	8.8 (8.4– 9.2)	4 (3.5–4.4)
XIV	3	257 (255–260)	4	10	11463 (11160–11650)	13.6 (8.8–21.3)	5.3 (3.8– 7.9)	5 (4.7– 5.4)	9.4 (8.7– 9.9)	4.6 (3.7–5.5)

unstable emulsions are introduced into the blood stream remains to be determined.

Thus, it can be seen that from the standpoint of safety in the intravenous administration of fat emulsion there is little danger of embolic phenomena occurring, especially when stable, finely dispersed emulsions of fat are used.

Summary. Both stable and unstable emulsions of fat have been administered intravenously to rats. The stable emulsions caused a moderate increase in total lipids of the spleen, liver, and lungs; whereas, a very marked increase was observed when unstable emulsions were given. Much of the increase due to the latter type of emulsion was caused by large extracellular fat globules. The removal of the excess fat in the tissues has been studied and found to be rapid in the case of stable emulsions, and slower but definite in the case of the unstable emulsions.

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