Percentage of non-agglutinable cells before transfusion 1%

$$100\% \text{ survival} = 1 + \frac{13800}{2602} = 5.3\% \text{ non-agglutinable cells.}$$

As seen from Fig. 5, the survival was that which might be expected from fresh blood with a full life expectancy.

Summary. Sucrose and lactose exert a preserving effect upon erythrocytes when stored at 0°C to −3°C. Sixty-four transfusions of blood thus stored for periods of time up to 47 days have shown good survival of the transfused cells. In the experiments *in vivo* the concentration of sugars varied from 3 to 5%. The experimental conditions were not always necessarily optimal for red cell preservation, and therefore the limits of storage are not maximal and will most likely be extended in future work. No attempt has been made to study the mechanism of action of the sugars on the preservation of red cells.

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Fat Emulsions for Oral Nutrition. II. Failure of Phosphatide, Tween 80, or Choline to Influence Fat Absorption.* (18304)

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This laboratory has studied the absorption of fat from fat emulsions given orally, and has investigated the effect of emulsification of fat on its intestinal absorption, using the rat as the experimental animal(1). It was felt that studies of this type would be of value as preliminary observations toward the use of orally administered fat emulsions in humans with normal and abnormal digestive systems. It was found that gelatine-stabilized emulsions of oil in water were unstable in the rat stomach, but were stable when instilled in the ligated rat intestine. The oil in such emulsions was absorbed more rapidly than when present in the same unemulsified systems. This increased rate of absorption was related to the particle size of the emulsions. Other workers(3) have reported that relatively large amounts of lecithin (16.7% in relation to the fat) increased the absorption of fat given orally to the rat in experiments using approximately twice the amount of fat this laboratory used. Tidwell(4), using 20% lecithin in relation to physiological amounts of orally fed fat, found an increase in the chylomicronemia of rats over controls. It has also been reported(5) that lecithin increased the blood fat levels in normal human subjects and in a small series of idiopathic sprue patients. Tween 80 (polyoxyethylene sorbitan monooleate), a synthetic emulsifier, has been stated to reduce the fecal fat excretion in patients with steatorrhea(6). Irwin *et al.*(7) have investigated the effect of the addition of various substances to fat meals upon fat absorption in the rat and found that they had little or no positive effect. It was also reported(8) that the physical condition of the animal determined

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whether orally administered fat would be normally absorbed. Frazer(9) has noted that choline added to a fat meal caused a finer subdivision of the intracellular fat particles in the intestinal epithelial cells and increased the concentration of fat in the areolar tissue of the villus of the rat. Tidwell(4) found that choline deficiency diminished the blood chylomicrons after fat meals.

The present study was undertaken to investigate the absorption of fat in the presence of amounts of emulsifiers greatly in excess of that needed to give good emulsions by mechanical means, both after oral administration of the fat and emulsifiers and after their instillation in the intestinal loop ligated below the pancreas. The role played by choline in fat absorption from the intestine was also studied.

Method. Oral feeding experiments. Albino male rats of the Charles River strain weighing 150 to 250 g were used. They were maintained on a stock diet of Krunchon† and were fasted 48 hours before all experimental procedures. All oral preparations were administered from a calibrated syringe via a stomach tube after light ether anesthesia of 30 to 60 seconds duration. Four hours after intubation, the rats were sacrificed with ether and the total nonsaponifiable and saponifiable lipids remaining in the gastro-intestinal tract were recovered in a manner previously described(1)‡. The preparations fed were as follows: a. 0.45 cc corn oil with 1.5 cc of 6% purified soybean phosphatide(2) in water (0.090 g of phosphatide), b. 0.45 cc corn oil and 1.5 cc of 6% Tween 80 in water (0.090 g of Tween 80). The choline deficient rats were produced by placing the normal animals for one month on a diet(10) consisting of 10% casein, 20% lard, 64% dextrose, 2% cod liver oil, 4% salt mixture(11), plus supplements of l-cystine, thiamine, riboflavin, pyridoxine, and calcium pantothenate, and were fasted for 48 hours before use. They were fed 0.45 cc of corn oil and treated in similar fashion. Liver sections were taken from each at the time of sacrifice and were studied histologically for the presence of fatty infiltration.

Intestinal instillation experiments. Charles River rats fasted for 48 hours were anesthetized with ether and the proximal jejunum was ligated below all pancreatic tissue and a ligature was also placed at the ileocecal junction. The various preparations used were instilled into the intestinal lumen through a small incision below the first ligature via a calibrated syringe and an 8 cm length of ureteral catheter. Another ligature was tied immediately below the incision and the abdomen was closed. This entire procedure took approximately 10 minutes, and the rats recovered from the anesthesia within 15 minutes of the induction of anesthesia. The rats were sacrificed with ether 4 hours after the instillation, and the contents of the ligated intestine were analyzed for total lipids. The preparations instilled were as follows: a. 0.31 cc corn oil with 1.0 cc of 6% purified soybean phosphatide in water (0.060 g of phosphatide), b. 0.31 cc corn oil with 1.0 cc of 6% Tween 80 in water (0.060 g of Tween 80), c. 0.31 cc of corn oil with 1.0 cc of 6% choline

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† Generously supplied by Gaines Division, General Foods, Kankakee, Ill.

‡ It has been recently suggested to the authors that the alcohol-ether solution used to flush the gastrointestinal tract of its contents would extract out interacellular fat as well. Water flushes had been used in our preliminary work but discarded as unsatisfactory because of the very poor recovery obtained (only 60 to 80% from rats intubated with known amounts of oil and killed immediately afterward). Sudan stained sections of water flushed intestines of rats sacrificed 4 hours after the stomach intubation of 0.45 cc of corn oil demonstrated that free intralumenal oil was left behind on the mucosal surface. Similar examinations of alcohol-ether washed sections demonstrated no free oil, with only slight loss of intracellular fat from the epithelial cells apparent.

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TABLE I. Recovery of Oil Preparations in Rats Sacrificed Four Hours After Oral Feeding. (6 rats in each series).

Preparation	State of animal	g lipid admin.	Mean g lipid recovered: Stomach	Intestine	Cecum	Mean total g recovered	Mean g absorbed	Mean % absorbed ± S.D. of M	Stand. error of mean in %
Corn oil*	Normal	.350	.043	.057	.012	.112	.267	70 ± 10	4
Corn oil + .09 g phosphatide	"	.445	.052	.098	.006	.156	.318	66 ± 13	5
Corn oil + .09 g Tween 80	"	.415	.066	.085	.010	.161	.283	64 ± 14	6
Corn oil	Choline deficient	.400	.039	.048	.020	.107	.322	74 ± 4	2

A correction factor of 0.029 g, the mean number of g of total lipid recovered from fasted control rats, has been used in calculating the lipid absorbed.

* Data for corn oil as reported in Reference 1.

TABLE II. Recovery of Oil Preparations in Rats Sacrificed Four Hours After Intestinal Instillation.

Preparation	g lipid admin.	No. of rats	Mean g of lipid recovered	Mean g absorbed	Mean % absorbed ± S.D. of M	Stand. error of mean in %
Corn oil*	.257	8	.233	.042	16 ± 5	2
Corn oil + .06 g phosphatide	.287	6	.255	.050	16 ± 6	2
Corn oil + .06 g choline chloride	.257	6	.223	.052	19 ± 4	2
Corn oil + .06 Tween 80	.267	5	.228	.057	20 ± 5	2

A correction factor of 0.018 g, the mean No. of g of total lipid recovered from the intestines of fasted control rats, has been used in calculating the lipid absorbed.

* Data for corn oil as reported in Reference 1.

chloride in water (0.060 g of choline chloride).

Results. Oral feeding experiments. The results of the oral feeding experiments are summarized in Table I. There was no significant change in the absorption of corn oil from the gastro-intestinal tract of the rat after the addition of large amounts of purified soybean phosphatide or Tween 80 (20% of emulsifier for the amount of oil used). Choline-deficient rats absorbed corn oil at the same rate as normal rats. Frozen sections of liver tissue of all of the choline deficient rats taken at the time of sacrifice showed enormous quantities of sudanophilic lipid deposited in the pre-cirrhotic pattern, demonstrating a severe choline deficiency.

Intestinal instillation experiments. The results of the intestinal instillation experiments are summarized in Table II. There was no significant change in the absorption of corn oil in the ligated intestinal segment with the addition of large amounts of purified soybean phosphatide, choline, or Tween 80 (each 20% in respect to the amount of oil used).

Discussion. It is of interest that the purified soybean phosphatide and Tween 80 used in this present work were of no assistance in increasing the absorption of unemulsified fat either after oral feeding or after instillation into the ligated intestine from which gastric, biliary, and pancreatic secretions were excluded, despite the great increase in the intralumenal emulsification potential. The amounts of emulsifiers used were greatly in excess over that needed to make excellent emulsions by mechanical means. Although the intestinal motility of the ligated intestine must certainly have been diminished when compared to the normal, the absorption of 16% of the corn oil instilled into the loop demon-

strated that a significant amount of fat absorption had occurred. Since it was found in this laboratory that finely emulsified oil particles of approximately 0.5 μ in diameter can be directly absorbed from the rat intestine(1), it seems apparent that the rats did not make effective use of these exogenous emulsifiers to disperse the ingested oil in their digestive fluids more efficiently than they can do without these emulsifiers, nor did the rats make effective use of these exogenous emulsifiers for oil emulsification when their own biliary and pancreatic juices were excluded from the digestive mixtures in their intestines.

The necessity of choline for the turnover of plasma phospholipids has been adequately demonstrated(12-14) and it has also been reported that choline may represent a limiting factor for the formation of phospholipids in the intestine during the absorption of fat from the intestine(15). However, in the work reported in this paper, fat absorption from the choline deficient rat was normal. Furthermore, an adequate choline supplement did not aid the absorption of fat from the ligated intestine in the normal rat from which most of the digestive secretions which might contain choline were excluded. This suggests that under the conditions used here choline had little function in the absorption of fat from the intestinal lumen and that its participation in the metabolism of ingested fat begins after the absorption of fat from the intestine.

Summary. The absorption of orally fed corn oil in approximately physiological amounts by the rat was unaffected by the presence of amounts of purified soybean phosphatide or Tween 80, greatly in excess over that needed to make excellent emulsions by mechanical means. The absorption of approximately physiological amounts of orally fed corn oil by the severely choline deficient rat was similar to that found in the normal rat. The absorption of corn oil after instillation in the rat intestine ligated below the pancreas and at the ileocecal junction was unaffected by the presence of large amounts of purified soybean phosphatide, Tween 80, or choline chloride.

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Lack of Direct Biological Conversion of Glycine to Ethanolamine. (18305)

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Stetten(1) obtained N^{15}-labeled ethanolamine upon feeding the corresponding isotopic glycine and suggested that it was formed by the direct reduction of glycine. This hypothesis has found its way into many textbooks and reviews(2). Subsequently Stetten(3) obtained labeled ethanolamine from N^{15}-labeled serine and proposed instead that serine is decarboxylated to yield ethanolamine. The formation of ethanolamine from serine-β-C^{14} has been demonstrated by Levine and Tarver (4) and also by the authors (unpublished experiments). Since glycine can be readily transformed to serine, it appears quite plausible, as has been proposed by Levine and Tarver(4), that ethanolamine is formed from glycine via serine and not directly. However,

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