feces. After administration to dogs, the major portion of the labeled drug was excreted within 3 days but more radioactivity was present in feces than in urine. The electrophoretic patterns of radioactivity obtained for rat and for dog urine were similar but more glucuronide appeared in the urine of dogs.

1. Cass, L. J., Frederik, W. S., J. New Drugs, 1964, v4, 38; Piala, J. J., High, J. P., Burke, J. C., Arch. int. Pharmacodyn., in press.

2. McIntire, F. C., Clements, L. M., Sproull, M., Ann. Chem., 1953, v25, 1757.

3. Kelly, R. G., Peets, E. A., Gordon, S., Buyske, D. A., Anal. Biochem., 1961, v2, 267.

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Effect of Triton Ingestion on Fat Absorption.* (30024)

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In an earlier study there appeared to be some delay in the absorption of fat when Triton† was administered orally along with the fat(1). Variations in fat absorption as measured by the appearance of radioactivity in the blood prompted a further study of this and associated phenomena. Janicki *et al*(2) have reported the rate of absorption of I-131 labeled oleic acid was not influenced by circulating Triton in the guinea pig.

If Triton should inhibit the lipolytic activity of pancreatic lipase, then its presence in the intestinal tract would be expected to decrease fat absorption. A study was undertaken to learn the extent of any effect of Triton on lipolytic activity and also to determine whether the presence of bile salts might alter this effect.

The possibility of a gradual loss of Triton from the intestinal tract when fed with the fat is suggested by a greater early decrease in the rate of appearance of fat in the blood, 39% as compared with only 16% later on(1). However, Cornforth *et al*(3) failed to detect any Triton in the blood of mice fed large amounts of Triton for one month.

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† Triton WR-1339 (oxyethylated tertiary octyl phenol formaldehyde polymer), Winthrop Laboratories, is a detergent which has been used as an aid in studying fat metabolism.

The results obtained in this study support the contention that Triton, given orally, is not absorbed and that it interferes with fat absorption by markedly inhibiting lipolysis of ingested fat.

Experimental. Initially 5 male albino rats, weighing about 250 g, were allowed 10% cerelose solution and water for 2 days. Then they were given orally by tube 1.5 ml of 10% Triton solution (WR-1339) and 0.3 ml of corn oil per sq dm of body surface. A similar amount of Triton solution was administered one hour later. Initially and 1, 2, 3 and 4 hours later, 0.2 ml blood samples were collected from the rat's tail for esterified fatty acid (EFA) determinations by the method of Stern and Shapiro(4). These determinations under similar conditions were repeated on the same rats after intervals of at least 2 weeks with only fat and then with Triton alone.

To determine the effect of Triton on the lipolytic activity of pancreatic lipase, the following procedure was employed. An incubation mixture was prepared with 1 ml of 0.05 M tris buffer (pH 8.5) containing 100 mg of bovine albumin (Armour), 0.4 ml supplement of water or various dilutions of Triton (0.0 to 0.125%) and 0.1 ml Lipomul-IV.‡ Aliquots of the incubation mixture were taken immediately after addition of 1.0

‡ Lipomul-IV, Upjohn Co., Kalamazoo, Mich., is a fat emulsion for intravenous infusion.

TABLE I. Triton Effect on Fat Absorption.

Feeding*	EFA at hours 0	1	2	3	4	Area under curve	Overall change in EFA (%)
F	8.52	8.84	9.34	10.38	11.13	38.4	30.6
F, T	8.55	8.48	8.76	8.72	8.83	34.7	3.3
T	8.78	8.49	8.58	8.49	8.52	34.2	−3.0

* F, FT and T to indicate oral feedings of fat, fat and triton, and triton alone respectively. EFA values are given in mEq per liter.

ml of a solution containing pancreatic lipase. Then the mixture was incubated for 60 minutes at 37°C and another aliquot removed. As a measure of the amount of fat hydrolyzed by the lipase, glycerol determinations, according to the method of Kern *et al*(5), were made on the aliquots. The method was slightly modified by decreasing the tungstate solution concentration from 25% to 10% and by addition to each aliquot of 1 drop of 10% H_2SO_4 containing 0.5% $FeCl_3$ before precipitating the proteins. The latter modification was found to lessen the colorimetric interference of bile salt when added.

The same procedure was followed to determine whether the presence of a bile salt would alter the inhibiting effect of Triton. Changes in lipolytic activity occasioned by the presence of 0.2% cholic acid, 0.03% Triton or the same concentrations of both the bile salt and Triton were determined.

Evidence regarding the possible absorption of Triton was sought by testing for its presence in the blood after the ingestion of large amounts. The 5 rats, again after 2 days on water and cerelose, were given 1.5 ml of 10% Triton solution initially and again 30 minutes later. Sufficient blood was drawn from the tails to provide 1.0 ml of serum from each rat, initially and after a 4-hour interval. Again incubation mixtures were prepared to contain the same concentrations of constituents as in the earlier determinations without Triton, allowing for the addition of 1 ml of serum. Aliquots were withdrawn immediately after the addition of the lipase and again after incubation for 1 hour at 37°C. Glycerols were determined as before on each aliquot as a measure of lipolytic activity.

Results and discussion. Variations obtained by measuring radioactivity in the blood after labeled fat ingestion prompted the use of another method for following the absorption of fat. Although rats do not develop a very marked lipemia after fat ingestion, EFA levels were selected as a means of following the appearance of absorbed fat in the blood.

The EFA levels were consistently higher in the blood of the rats receiving only the fat (Table I). This was true whether measured by the area under the curve obtained or by the overall per cent change in EFA levels. Little change occurred in the levels when Triton and fat, or Triton alone was ingested. It is evident that the presence of Triton does delay fat absorption. The lack of agreement with the finding of Janicki *et al*(2) might be accounted for by his use of a fatty acid instead of a glyceride and no orally ingested Triton.

Preliminary experiments had suggested that Triton might appreciably inhibit the lipolytic activity of pancreatic lipase. This possibility was investigated by measuring the extent of lipolysis when the concentration of Triton in the incubation media was varied from none to 0.125% (Table II). Approximately 50% inhibition of lipolytic action was obtained with only a concentration of 0.03% Triton in the media. This inhibition probably accounts, at least in part, for the marked decrease in rate of fat absorption when Triton is present.

TABLE II. Inhibition of Pancreatic Lipase Activity by Triton.

Exp No.	Triton conc (%)	Glycerol freed (μg)	Inhibition (%)
1	.125	.0	100.0
2	.060	6.9	89.9
3	.050	22.3	67.5
4	.040	26.6	61.2
5	.030	35.1	48.9
6	.015	46.6	32.1
7	.0075	56.2	18.1
8	.00	68.6	.0

TABLE III. Effect of Bile Salt on Triton Inhibition.

Supplement	Change in lipolytic activity with added supplements: None	Bile salt .2%	Triton .03%	Bile and Triton
μg/ml*	53	81	29	28
%	100	153	55	53

* Amount of glycerol freed during incubation without and with the various supplements.

Although this decrease in absorption does occur, some fat is absorbed as evidenced by the appearance of radioactivity in the blood after ingestion of labeled fat. Since the presence of bile acids in the intestine might alter conditions, the possibility that their presence might prevent some of the inhibitory action of Triton on fat absorption was investigated. The bile acid (0.2%) was found to stimulate lipolytic activity while a combination of both bile acid and Triton (0.03%) had the same inhibitory effect as Triton alone (Table III).

If Triton is absorbed after ingestion of massive amounts (1 g per kg), its presence in the blood should inhibit *in vitro* lipolysis since only trace amounts are required for this action. The serums from each of the 5 rats had no inhibitory action on the activity of pancreatic lipase when tested for *in vitro*. This evidence supports both the finding of Cornforth *et al*(3), and our failure to obtain an elevated lipid level after ingestion of Triton alone(6), that such Triton is not absorbed (Table I).

Summary. The effect of oral ingestion of Triton on fat absorption in rats was investigated by feeding either fat or Triton alone, or a combination of fat and Triton. Serum esterified fatty acid levels were elevated after the fat feeding but not in either case after Triton ingestion. An attempt to account for this decreased fat absorption was made by determining the effect on the lipolytic activity of pancreatic lipase of Triton, alone and in the presence of a bile acid. A marked inhibition of lipolysis occurred in both cases when Triton was present. The absence of any inhibitory effect of blood serum on lipase activity after the ingestion of massive amounts of Triton indicated that the Triton was not absorbed.

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1. Moore, W. T., Tidwell, H. C., McPherson, J. C., Am. J. Clin. Nutrition, 1965, v16, 135.

2. Janicki, B. W., Leahy, W. V. C., McNickle, T. F., Aron, S. A., Am. J. Physiol., 1962, v202, 367.

3. Cornforth, J. W., Hart, P. D., Rees, R. J. W., Stock, J. A., Nature, 1951, v168, 150.

4. Stern, I., Shapiro, B., J. Clin. Path., 1953, v6, 158.

5. Kern, F., Jr., Steinmann, L., Sanders, B. B., J. Lip. Res., 1961, v2, 51.

6. Kellner, A., Correll, J. W., Ladd, A. T., J. Exp. Med., 1951, v93, 373.

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Restoration of Tyramine Pressor Responses in Reserpine-Treated Animals by Methyldopa and Its Amine Metabolites. (30025)

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The hypotensive agents, reserpine and methyldopa (L-α-methyldopa, Aldomet®), are known to lower tissue levels of norepinephrine in experimental animals(1,2), thereby suggesting similar mechanisms of action on the sympathetic nervous system. While reduced norepinephrine levels have been found in surgical biopsies of atrial appendages from patients receiving reserpine(3), corresponding data are not available in man for methyldopa. Since pressor responses to tyramine are diminished after depletion of norepinephrine stores by reserpine(4), responsiveness to tyramine has been used as a

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