the initial catecholamine-induced mobilization of FFA(7). Nicotinic acid has been shown to lower plasma lipoprotein levels(8,9), and Carlson and Orö have suggested that these effects may be related to the inhibition of the FFA-mobilizing action of catecholamines. Nicotinamide, which is without effect on serum lipoprotein levels(10), failed to produce any inhibition of the lipolytic action of norepinephrine *in vivo*(1), or as shown here, *in vitro*. The extent to which basal lipoprotein levels are related to catecholamine stimulation of FFA release is not known but this interesting hypothesis deserves further exploration.

Summary. Nicotinic acid at concentrations of 10^{-3} M and 10^{-5} M markedly reduced the norepinephrine induced release of glycerol and FFA in isolated adipose tissue. Nicotinamide at equal concentrations had no effect.

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Toxicity of Tripropionin in Rats Fed a Vitamin B_{12}-Deficient Diet. (28743)

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It has been demonstrated that vitamin B_{12} deficiency in the mammal is characterized by a defect in the metabolism of propionate (1,2). This acid is normally carboxylated as its coenzyme A derivative to form methylmalonyl coenzyme A, which is subsequently isomerized to succinyl coenzyme A in a Vit. B_{12}-catalyzed reaction(3). It has long been known that rats may excrete methylmalonate in their urine(4), and it has been learned more recently that the amount of methylmalonate excreted on various diets is decreased by Vit. B_{12} administration(5). It has also been observed that excretion of methylmalonate in the urine in the human is a sensitive index of Vit. B_{12} deficiency(6,7).

Correlation of this metabolic defect with various observed clinical symptoms of Vit. B_{12} deficiency has not been reported. It has been shown that the inability of sheep to metabolize the large amount of propionate formed by the rumen bacteria is responsible for the illness observed in cobalt deficiency (8). Propionate is not ordinarily considered to be toxic. Mice have been maintained on a diet containing as much as 40% by weight of tripropionin(9).

In the present experiment, an attempt was made to intensify neurological defects of Vit. B_{12} deficiency by tripropionin loading in the diet. The diet used was commercially available Vit. B_{12}-deficient diet for rats (Nutritional Biochemicals Corp., Cleveland, Ohio). It was supplemented with 0.4 g of nicotinamide per kg of diet. A control diet contained in addition 100 μg of cyanocobalamin per kg of diet (Rubramin, Squibb, New Brunswick, N. J.) A third group was maintained on Rockland Chow. Each group contained 32 female weanling albino rats weighing 75 to 100 g (Holtzman derived, Rawley Farms, Plymouth, Mich.). Rats were housed in neighboring cages containing originally 8 and later 5 rats per cage. Food was presented *ad lib* and all drinking water was distilled. After 37 days, the diets were augmented with 10% tri-

TABLE I. Mortality of Rats on Vitamin B_{12}-Deficient Diets Supplemented with Triacetin or Tripropionin.

Days	1-37	38-52		53-59	
Group	A + B	A	B	A	B
Supplement	None	Tripropionin	Triacetin	Triacetin	Tripropionin
B_{12}-deficient diet	7/32	11/12	1/13	0/1	7/12
Idem + B_{12}	12/31	3/9	0/10	0/6	0/10
Rockland Chow	1/32	0/31		0/31	

At day 60, all rats were placed on Rockland Chow. No further fatalities occurred during the subsequent 2 weeks. Coats improved, nasal lesions cleared and weight gain approached that of the original chow group.

propionin or triacetin (Eastman Organic Chemicals) as shown in Table I and fed to half of each experimental group for 14 days. The chow-fed group received no triglyceride supplement. The diet was then altered so that rats receiving tripropionin were given triacetin and *vice-versa*.

Results. Supplementation of the Vit. B_{12}-deficient diet with Vit. B_{12} did not restore a normal growth rate. There was no significant difference in the weight of rats on the Vit. B_{12}-supplemented and unsupplemented Vit. B_{12}-deficient diets. This observation has been reported with a somewhat similar Vit. B_{12}-deficient diet(10). It would appear that there were other deficiencies in the experimental diets.

The experiment was further complicated by an infection of the ordinary type involving the upper and lower respiratory tracts. Bloody nasal discharge, often associated with snout lesions were present in the animals throughout the experimental diets. There were deaths in other rats housed in the same room, but many more in both experimental diet groups. Addition of 10% tripropionin or 10% triacetin to the diet was nevertheless instituted. All rats showed an immediate improvement in appetite, activity, and coat. Weight loss was reversed, and rats gained 1-2 g over the next several days. One death only, on the fifth day, occurred in 13 rats on triacetin. In rats on tripropionin, deaths began on the fourth day. By the sixth day, 11 of 12 Vit. B_{12}-deficient rats on the tripropionin diet, but only 3 of 9 rats on the same diet with Vit. B_{12} died. This was the first observable difference in the Vit. B_{12}-deficient and Vit. B_{12}-supplemented rats. When the triglyceride supplement was reversed, deaths were again first observed on the fourth day. Seven of 12 rats on tripropionin died within a week. None in the other groups died. There was no obvious flare-up of infection during this period. Deaths often were sudden, and active animals were found dead an hour subsequent to the previous observation.

No evidence of neural disability was encountered. Coordination, grasping, and placing reactions, standing on hind legs, leaping onto and crawling along the edge of a cage, and fending-off gestures all appeared normal.

An animal that died early in the experiment showed purulent bronchitis and pneumonitis and acute congestion of the liver and kidney. The animals that died after the propionate addition showed intense congestion and hemorrhage of the lungs without pneumonitis and had clear bronchi. The kidney, liver, and spleen showed marked acute congestion with considerable siderosis of the spleen.

In evaluating these observations, two questions arose. Did the added Vit. B_{12} preparation actually increase the Vit. B_{12} level of the diets; and was the tripropionin toxic *per se*? The first question was answered by Vit. B_{12} bioassays on the deficient and supplemented experimental diets (kindly furnished by Dr. R. Bishop, Univ. of Michigan). The question of tripropionin toxicity was tested by feeding 5 rats previously on Rockland Chow, a 10% tripropionin supplement for 10 days. No weight loss or other evidence of toxicity was noted. The correlation of mortality with the switch in diet in the 2 groups is also convincing evidence that Vit. B_{12} deficiency and tripropionin loading result in death. It is possible that a somewhat lower tripropionin content in the diet

would produce chronic defects such as neurological changes.

Summary. When tripropionin is added to the diet of rats on a diet deficient in Vit. B_{12} and possibly other factors, there is a fatal toxicity which is not as marked when the diet is supplemented with Vit. B_{12}.

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Occurrence of C^{14}-Oxalate in Rat Urine after Administration of C^{14}-Tryptophan.* (28744)

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Studies from this laboratory have indicated that there is an increase in endogenous oxalate excretion in Vit. B_6 deficiency(1,2). Following administration of tryptophan load tests to human subjects, Gershoff and Prien (3), Farvar *et al*(4), and Faber *et al*(5), have reported increased excretion of oxalate. In the present study the effect of tryptophan on excretion of oxalate by Vit. B_6-deficient and control rats has been investigated.

Methods. Two groups of 18 male weanling Charles River CD rats were fed a purified diet *ad libitum* with or without Vit. B_6 consisting of: casein, 15%; sucrose, 75.7%; corn oil, 4%; salts IV(6), 4%; cod liver oil, 1%; and choline, 0.3%. Four mg of thiamine hydrochloride, 8 mg of riboflavin, 40 mg of niacin, 20 mg of calcium pantothenate, 1 mg of folic acid, 1 mg of menadione, 0.2 mg of biotin, 0.05 mg of Vit. B_{12} and, when used, 4 mg of pyridoxine hydrochloride, were added to each kilo of diet. After 3 weeks the rats were injected intraperitoneally with 5 μc of either DL tryptophan 2-C^{14} or DL tryptophan 3-C^{14} per 100 g of body weight.

The rats were placed in metabolic cages in groups of 3 and 48-hour urine collections were made. Oxalates were extracted and precipitated from the urines as described by Powers and Levatin(7). The calcium oxalate precipitate was dissolved in 1 ml of 20% sulfuric acid and 0.5 ml of 1% manganous sulfate and transferred to a Warburg flask. One ml of 0.03 N potassium permanganate was added from the side arm and the flasks were shaken for 30 minutes. The CO_2 evolved was trapped in the center well on filter paper wet with KOH and the radioactivity determined in a liquid scintillation counter as described by Buhler(8). The total oxalate in the sample was determined by titrating the excess permanganate in the Warburg flask (7). Total urine radioactivity was determined in a liquid scintillation counter after adding 0.2 ml of urine to a mixture of 5 ml of absolute alcohol and 10 ml of a solution of 0.4% PPO and 0.005% POPOP in toluene.

Results and discussion. The data presented in the Table show that tryptophan is a precursor of oxalate in rats and that approxi-

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