In vitro Inhibition of the Fat-Mobilizing Action of Norepinephrine. (28742)

R. PHILIP EATON (Introduced by D. Steinberg)
Laboratory of Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Md.

Carlson and Orö have reported that pretreatment of dogs with nicotinic acid prevents the rise of plasma FFA seen with norepinephrine infusion(1). The present studies were undertaken to determine whether this inhibition was an indirect or direct action by studying the effect of nicotinic acid on adipose tissue *in vitro*.

Methods. Epididymal fat pads (150-350 g) were removed from fed Sprague-Dawley rats after decapitation. Each was incubated in 2 ml of Krebs' bicarbonate medium, pH 7.4, containing 3% bovine serum albumin (Fraction V, Armour Pharmaceutical Co.). The flasks were shaken for one hour at 37° C in a Dubnoff incubator, with a gas phase of 95% O_2 and 5% CO_2. Medium FFA were determined at the end of incubation by the method of Dole(2). Glycerol in the medium was determined by Korn's modification(3) of the method of Lambert and Neish(4). Norepinephrine solutions were prepared from the bitartrate with concentrations expressed in terms of the free base. Nicotinic acid solutions were prepared by neutralization with NaOH.

Results. Nicotinic acid in the absence of norepinephrine had no effect on the basal release of glycerol from adipose tissue. The mean value and standard deviation of the rate of glycerol production by 4 fat pads incubated with 10^{-3} M nicotinic acid was 1.40 ± 0.3 μM/g/hr while that of the controls was 1.36 ± 0.5 μM/g/hr.

Norepinephrine alone increased the net release of glycerol by 3.01 ± 0.27 μM/g/hr over that seen in the paired controls (4 pairs).

As shown in Table I, nicotinic acid at concentrations of 10^{-3} M and 10^{-5} M produced a significant inhibition of the total release of glycerol and FFA from adipose tissue in the presence of norepinephrine. Nicotinamide at the same concentrations failed to inhibit the norepinephrine fat-mobilizing effect.

Discussion. The present data show that nicotinic acid at low concentrations can counteract the FFA-mobilizing effect of norepinephrine in isolated adipose tissue. Presumably it interferes somehow with catecholamine activation of the hormone sensitive lipase system in adipose tissue(5,6). This direct inhibition may be an adequate explanation for the *in vivo* effects observed by Carlson and Orö, although additional indirect action cannot be ruled out. For example, nicotinic acid *in vivo* might alter blood flow patterns or modify the rate of breakdown of norepinephrine.

Catecholamines have been shown to elevate plasma lipoprotein levels, and it has been suggested that these changes may stem from

TABLE I. Effect of Nicotinic Acid and Nicotinamide on Norepinephrine-Stimulated Release of Glycerol and FFA.*

Drug added	No. of pairs	Glycerol released, μM/g/hr		FFA released, μeq/g/hr	
		No drug	Change due to drug†	No drug‡	Change due to drug†
Nicotinic acid					
10^{-3} M	11	4.8 ± .5	−1.5 ± .3§	7.9 ± 1.2	−2.9 ± .6§
10^{-5} M	3	7.0 ± .4	−3.2 ± .3‖	6.3 ± .9	−3.4 ± .7‖
Nicotinamide					
10^{-3} M	3	3.5 ± .3	− .2 ± .07		
10^{-5} M	3	6.2 ±1.1	− .4 ± .1		

* All pairs of tissues were incubated in the presence of 0.5 μg/ml of norepinephrine.
† Mean of differences between paired tissues ± standard error of mean.
‡ In the absence of nicotinic acid, norepinephrine increased the rate of FFA release from 3.47 ± .3 to 7.62 ± .4 μeq/g/hr.
§ $p < .05$. ‖ $p < .005$.

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.

1. Carlson, L. A., Orö, L., *Acta Med. Scand.*, 1962, v172, 641.
2. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.
3. Korn, E. D., *J. Biol. Chem.*, 1955, v215, 1.
4. Lambert, M., Neish, A. C., *Canad. J. Res.*, 1950, v28B, 83.
5. Rizak, M. A., *J. Biol. Chem.*, 1961, v236, 657.
6. Vaughan, M., *J. Lipid Res.*, 1961, v2, 293.
7. Shafir, E., Steinberg, D., *J. Clin. Invest.*, 1960, v39, 310.
8. Atschul, R., Hoffer, A., Stephen, J. D., *Arch. Biochem.*, 1955, v54, 558.
9. Parson, W. B., Jr., Achor, R. W. P., Berge, K. G., McKenzie, B. F., Barker, M. W., *Proc. Staff Meet., Mayo Clin.*, 1956, v31, 377.
10. Miller, O. N., Hamilton, J. G., Goldsmith, G. A., *Am. J. Clin. Nutrition*, 1962, v10, 285.

Received July 15, 1963. P.S.E.B.M., 1963, v114.

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

GEORGE D. GAMMON AND BERNARD W. AGRANOFF (Introduced by R. W. Gerard)
Veterans Administration Hospital and Mental Health Research Institute, Ann Arbor, Mich.

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-