Influence of Nicotinic Acid and Nicotinamide on Insulin Hypoglycemia.* (26815)

H. D. KLEBER AND R. W. MANTHEI (Introduced by J. M. Coon)
Department of Pharmacology, Jefferson Medical College, Philadelphia, Pa.

Blood sugar concentration can be altered by administration of either nicotinic acid or nicotinamide, but the direction of the response induced apparently depends primarily on the species, agent and dosage used. Both nicotinic acid and nicotinamide have been found to increase blood sugar concentration in the rat and rabbit(1,2). Mirsky *et al.*, however, obtained a marked hypoglycemia with nicotinic acid in the rat and the mouse(3). Hypoglycemia has also been produced in man with nicotinamide(4), although most investigators have not found it to cause any significant change in blood sugar concentration(5,6). The present study delineates the action of various doses of these compounds in normal and insulin-treated mice.

Materials and methods. Male Swiss-Webster mice weighing from 18 to 24 g were maintained in an air-conditioned room at 26°C. Crystalline zinc insulin (40 units/ml) was diluted with physiological saline and sufficient hydrochloric acid added to adjust the final pH to 2.5-3.0. It was administered intraperitoneally in a dose of 5 units/kg. Nicotinic acid or nicotinamide (500 mg/kg) was administered subcutaneously. The nicotinic acid was adjusted to pH 7 with $NaHCO_3$. All solutions were administered in a volume of 0.1 ml per 10 grams of body weight. Physiological saline was used as control.

Animals previously maintained on normal mouse diet with water *ad libitum* were fasted 22 to 24 hours and then divided by random selection into the various groups. Onset of hypoglycemic convulsions was determined by a modification of the sloped screen technic of Thompson(7). Blood sugar was initially determined by the method of Nelson(8) and later by an enzymatic method(9) without any significant change in the values obtained. At approximately half-hour intervals, 0.2 ml of blood was drawn from the orbital sinus of individual mice in each group(10). No animal had blood taken more than once.

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TABLE I. Hypoglycemic Falls in Insulin-Treated Mice.

Drugs	No. of mice	% falls within 150 min.
Insulin + .9% NaCl	50	54%
" + nicotinic acid	50	90
" + nicotinamide	50	14
" + nicotinamide and nicotinic acid	20	15
Nicotinamide + .9% NaCl	15	0
Nicotinic acid + " "	15	0

Insulin (5 units/kg, I.P.); nicotinic acid and nicotinamide (500 mg/kg, subcut.).

P (x^2) Insulin *vs* insulin + nicotinic acid = <.001.

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Results. The percentage of animals in each group falling from the inclined screen within 150 minutes after injection is given in Table I. A cut-off time of 150 minutes was chosen because approximately 75% of exogenous insulin is known to be metabolized in mice by that time(11). Neither nicotinic acid nor nicotinamide alone caused any mice to fall. Of the animals showing hypoglycemic falls with insulin alone, the majority fell within the first 90 minutes. Administration of both nicotinic acid and insulin resulted in a significant increase in the number of animals falling. Nicotinamide on the other hand caused a significant decrease in the number of insulin-treated animals falling. Earlier falls were noted (the majority within 60 minutes) in mice treated with insulin plus either nicotinic acid or nicotinamide.

Saline-injected controls had an average blood sugar level of 70.9% (Table II). Insulin lowered blood sugar by 78% within from 1.5 to 2 hours. Nicotinamide (500 mg/kg) elevated the level to a maximum of 32% over control values at 2 to 2.5 hours after

TABLE II. Blood Sugar Concentration* in Insulin-Treated Mice.

Group	Drug	Dose, mg/kg	Minutes after administration					
			0-30	30-60	60-90	90-120	120-150	150-180
1	.9% NaCl	—	70.4 ± 3.3 (10)	67.1 ± 2.4 (10)	71.8 ± 2.3 (10)	74.5 ± 2.8 (10)	72.6 ± 2.5 (10)	69.1 ± 2.7 (10)
2	Nicotinic acid + .9% NaCl	500	—	65.2 ± 3.4 (5)	60.0 ± 3.7 (5)	56.1 ± 3.1 (5)	59.2 ± 3.5 (5)	66.2 ± 3.3 (5)
3	Nicotinamide + .9% NaCl	500	—	76.4 ± 3.8 (5)	83.1 ± 4.1 (5)	89.3 ± 3.6 (5)	96.1 ± 3.8 (5)	85.3 ± 3.2 (5)
4	Insulin + .9% NaCl	—	32.2 ± 2.9 (10)	27.6 ± 2.2 (10)	22.3 ± 2.5 (10)	16.2 ± 3.2 (10)	29.7 ± 2.3 (10)	— — —
5	" + nicotinic acid	500	24.3 ± 2.4 (10)	19.1 ± 2.3 (10)	14.0 ± 2.5 (10)	9.4 ± 2.5 (10)	21.1 ± 2.8 (10)	
6	" + nicotinamide	500	38.1 ± 2.6 (10)	40.1 ± 2.8 (10)	36.3 ± 2.3 (10)	38.8 ± 3.3 (10)	43.7 ± 3.2 (10)	
7	*Idem*	250	28.2 ± 3.1 (5)	30.2 ± 3.2 (5)	24.9 ± 3.4 (5)	21.9 ± 3.3 (5)	28.5 ± 3.5 (5)	
8	"	125	25.2 ± 3.4 (5)	27.7 ± 3.1 (5)	15.8 ± 3.6 (5)	12.2 ± 3.6 (5)	24.2 ± 3.1 (5)	

* Blood sugar concentration in mg % ± stand. dev. (No. of mice/group).
Insulin = 5 units/kg, i.p.; other agents, subcut.

administration. This dosage also caused a significant elevation in blood sugar concentration in insulin-treated mice. When the dose of nicotinamide was decreased to 250 mg/kg, the level was not significantly different from that of the insulin-treated group. With a further decrease in dosage to 125 mg/kg the blood sugar concentration tended to be lower than that of the insulin-treated controls. By contrast, nicotinic acid (500 mg/kg) produced a significant decrease in blood sugar concentration in both control and insulin-treated mice.

Discussion. It is known that administration of 500 mg/kg of nicotinamide can significantly increase the level of active DPN in mice(12). However, since maximum changes in DPN levels do not occur until 8-12 hours after administration of the vitamin, it does not seem likely that the rapid effect demonstrated in the present study depends on the same action. Mirsky *et al.* have found that nicotinic acid at this dosage inhibits insulinase activity while nicotinamide increases insulinase activity(13). Since nicotinamide produced the same increase in blood sugar level over both control and insulin-treated animals, the nicotinamide effect was apparently independent of the level of circulating insulin. This suggests that the action was on other than on an insulin dependent system. Although the exact mechanism of action by which the vitamin influences blood sugar concentration is unknown, the present study demonstrates that in mice, high doses of nicotinic acid and nicotinamide have opposite effects on blood sugar whether given alone or in combination with insulin. At a lower dose nicotinamide mimics nicotinic acid in its effect on blood sugar concentration.

Summary. Nicotinamide (500 mg/kg) elevates blood sugar in both normal and insulin-treated mice and decreases the incidence of hypoglycemic convulsions in the latter. At 125 mg/kg, however, nicotinamide increases the depth of insulin hypoglycemia. Nicotinic acid (500 mg/kg) lowers blood sugar concentration in both control and insulin-treated mice and increases the incidence of hypoglycemic convulsions.

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Effect of Dietary Vitamin B_{12} on Methionine Biosynthesis by Chick Liver Homogenates.* (26816)

JAMES S. DINNING
Department of Biochemistry, University of Arkansas School of Medicine, Little Rock

It was observed several years ago that dietary vitamin B_{12} would spare the methyl requirements for animals(1). More recently, considerable evidence has accumulated to implicate Vit. B_{12} in methionine biosynthesis in bacterial systems(2,3). Arnstein(4) has demonstrated in *in vivo* experiments with rats that Vit. B_{12} stimulates the conversion of formate to the methyl groups of choline and methionine. We have reported(5,6) that Vit. B_{12} stimulates the conversion of formate to the 5-methyl group of thymine by chick bone marrow cells but does not stimulate conversion of formaldehyde to the 5-methyl group of thymine. These results suggest that Vit. B_{12} functions in reduction of one-carbon compounds between the formate and formaldehyde levels of oxidation. In the present experiments we have studied the effect of Vit. B_{12} deficiency in chicks on formation of the methyl group of methionine by liver homogenates and the results suggest that B_{12} stimulates the conversion of formate to methionine methyl but does not increase conversion of formaldehyde to methionine methyl, a situation analogous to our previously reported results in thymine formation by chick bone marrow cells.

Experimental. Day-old White Leghorn chicks were given the Vit. B_{12} and methionine-deficient basal diet described by Spivey-Fox *et al.*(7). When present, methionine was added at a concentration of 6% of the diet and Vit. B_{12} was given by weekly injections at a dosage of 3 μg per chick the first week and 9 μg per chick thereafter. After 3 to 4 weeks on the various diets, the chicks were taken for experiment. Peripheral blood counts were made using standard hematological procedures.

Methionine formation from homocysteine and formate-C^{14} or formaldehyde-C^{14} was determined in liver homogenates. The livers were homogenized in an all-glass homogenizer with 2 volumes of Robinson's buffer(8). The incubation mixture consisted of 2 ml of homogenate, 3 mg of DL-homocysteine, and either 0.27 μmole of formate-C^{14} (142,000 c.p.m.) or 2 μmoles of formaldehyde-C^{14} (140,000 c.p.m.). The final volume was 2.5 ml. After an hour of incubation at 37° under air in the Dubnoff shaker, the reaction was stopped by addition of 0.5 ml of 50% trichloroacetic acid and the contents of the incubation beakers were transferred to 12 ml conical centrifuge tubes and centrifuged. 1.5

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