

Effects of Excess Dietary Methionine and Niacinamide in the Rat. (23769)

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Excess dl-methionine in the diet inhibits growth and causes a marked loss of body fat, enlargement of the kidneys, increased excretion of creatinine and a small increase in liver fat in the immature rat (1,2,3). Excess dietary niacinamide also inhibits growth in the rat (4). Since these effects were observed in short term studies in rats maintained on basal diets, this study was designed to ascertain the effects of prolonged administration of excess dietary methionine and niacinamide on the growth rate and organ weights of rats maintained on a standard laboratory ration.

Methods. The effect of various dietary supplements on growth in the rat was determined in 2 series of experiments. In the first series, 5 groups of 12 male Holtzman rats, weighing 110-135 grams, were treated with various combinations of dl-methionine, niacinamide, and biotin for a period of 12 weeks. The rats were allowed food and water *ad libitum*. The food a standard rat chow, was finely ground and definite amounts of the nutrients being tested were added. The animals were weighed at weekly intervals and 4 rats of each group were sacrificed at the end of 2, 8 and 12 weeks of treatment. The adrenal glands, liver and kidneys were removed and weighed. In the second series, 4 groups of 10 rats were administered dietary supplements of malic acid, dl-methionine and niacinamide. The procedures used were the same as above except animals were sacrificed only at the end of 8 and 12 weeks of treatment. The results obtained from the 2 series of experiments were analyzed statistically using the Analysis of Variance Method. For this analysis all gland weights were expressed as a function of the body weight.

Results. All diets containing excess methionine inhibited the growth rate during the first 2 weeks of treatment ($P < 0.01$) (Fig. 1). At the end of 8 and 12 weeks of treatment,

only the animals receiving diets containing both 2% methionine and 0.1% niacinamide exhibited a significant retardation of growth ($P < 0.01$). However the addition of 1% malic acid to the diet prevented the decrease in the growth rate. Niacinamide administered at the level of 0.1% of the diet for 8 weeks caused no significant change in the growth rate, but at the 0.2% level, growth was enhanced and at the 0.4% level, growth was inhibited ($P < 0.05$) (Fig. 2). During the next 4 weeks of treatment the body weights of animals receiving 0.2% niacinamide in the diet returned to control levels but inhibition of growth by the 0.4% dosage became more marked ($P < 0.05$). As shown in Table I, there is a transient enlargement of the kidneys during the first few weeks of treatment with all diets containing 2% methionine. In addition, a transient adrenal hypertrophy is observed in rats treated with methionine alone. There is a decrease in the liver size in rats receiving 2% methionine plus 0.1% niacinamide for 12 weeks but this is prevented by the ad-

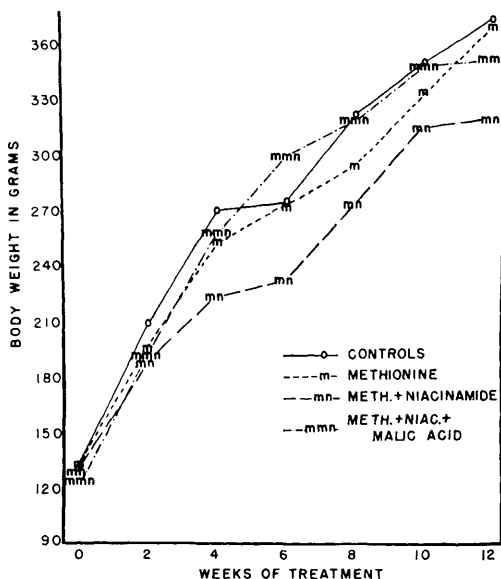


FIG. 1. Effect of excess dietary methionine, niacinamide and malic acid on growth rate of the rat.

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TABLE I. Effect of Excess Dietary Nutrients on Relative Weights of Liver, Kidney and Adrenal Glands of Rats.

Treatment†	Liver (g %)*			Kidney (g %)			Adrenals (mg %)		
				Weeks of treatment					
	2	8	12	2	8	12	2	8	12
Control	4.73	3.92	3.92	.96	.79	.79	17.0	15.6	13.9
2% M	5.04	3.74	4.10	1.17§	.83	.77	20.0‡	15.9	12.9
2% M	5.04	3.77	3.39§	1.12§	.84	.83	18.2	16.9	12.4
.1% N									
2% M	5.29	3.95	3.96	1.13§	.82	.80	17.5	14.6	13.2
.1% N									
.004% B									
2% M		3.89	3.55§		.87	.85		13.7	13.1
.1% N									
1% MA									
2% M		4.02	3.84		.89	.90		15.8	13.6
1% MA									
.1% N	4.84	3.67	3.82	1.03	.79	.69‡	15.2	15.1	12.9
.2% N		4.06	3.63‡		.74	.74		13.9	13.0
.4% N		4.10	3.67		.73	.84		17.4	15.5
.004% B	4.92	3.62	3.77	.99	.77	.81	17.0	12.0§	12.2
1% MA		3.83	4.05		.80	.83		14.7	13.7

* Gland wt/100 g of body wt.

† M = Methionine; N = Niacinamide; B = Biotin;

MA = Malic acid.

‡ Significant at the 5% level.

§ Significant at the 1% level.

dition of 0.004% biotin to the diet. The relative weight of the adrenal glands in rats receiving 0.004% biotin only is significantly decreased after 8 weeks ($P < 0.01$) but not after 12 weeks of treatment.

Discussion. Handler and Perlzweig(5) suggested that excess dietary niacinamide

causes a retardation of growth by inducing a secondary methionine deficiency. Niacinamide undergoes obligatory methylation prior to excretion and methionine appears to be the source of the labile methyl groups. The data presented here are not in agreement with this hypothesis since niacinamide was found to augment the effect of methionine on the growth rate rather than to inhibit it. However, the discrepancies between the data presented here and that obtained by Handler and Perlzweig are probably caused by the difference in the dosages and the basal diets that were used. The prevention of the effect of treatment with methionine and niacinamide on growth by malic acid suggests that growth is retarded *via* an action on carbohydrate metabolism at or preceding the tricarboxylic acid cycle. Since pyridoxine will counteract the effect of moderate amounts of methionine on growth in the rat(6), the addition of malic acid to the diet may compensate for a pyridoxine deficiency. It is interesting to note that malic acid prevents the inhibition of growth but not the decrease in liver size caused by the treatment whereas the reverse is seen when biotin is added to the diet. In general, the various

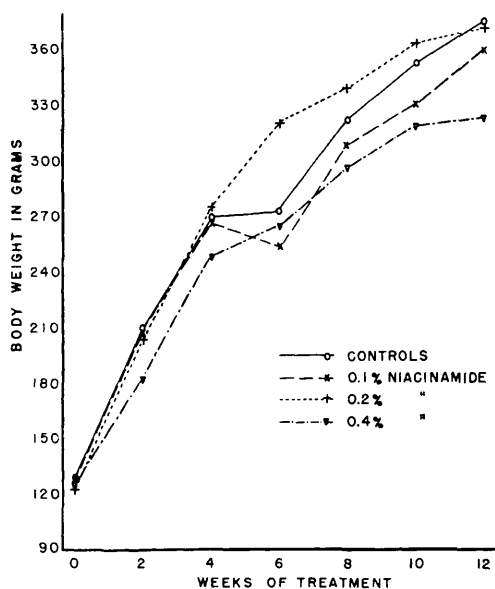


FIG. 2. Effect of various dosages of niacinamide on growth rate of the rat.

treatments are relatively non-toxic. There is a transient hypertrophy of the kidneys during the first few weeks of treatment with methionine but as prolonged treatment did not cause adrenal hypertrophy, it is doubtful that any of the regimens placed a severe stress on the animals.

Summary. The effects of prolonged administration of excess dietary dl-methionine and niacinamide on growth rate and on the relative weights of the liver, kidneys and adrenal glands of rats were determined. Although both methionine and niacinamide caused a decrease in the growth rate, a greater retardation of growth was obtained when both nutrients were administered together. The addition of 1% malic acid to the diet prevented the inhibition of growth by these substances.

Although methionine caused a transient enlargement of the kidneys, none of the treatments appeared to place a severe stress on the animals.

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Prevention of Aortic Atherosclerosis in Rabbit by Intravenous Microcrystallized Estradiol Benzoate and Dextran®. (23770)

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It has been demonstrated that estrogens are able to prevent development of atherosclerosis in several species(1-3) and exert a beneficial effect even in man (see review in 4). Experiments designed to reproduce these results in male rabbits, however, have been consistently negative (personal communication) although contradictory conclusions have been reported in work done with female rabbits(5,6). In the present paper prevention of aortic atherosclerosis in cholesterol-fed male rabbits by a new method, *i.e.*, by the intravenous injection of a water-insoluble estrogen, is reported.

Methods. In this study male rabbits of mixed breed weighing 1.5 to 2.5 kg were maintained on carrots, clover, water and additional vitamins. All individuals, except the controls, also received 6 days a week 750 mg

of crystalline cholesterol in 5 ml of sunflower seed oil (I. N. 130) thoroughly mixed with mashed carrots. One week after starting the cholesterol feeding, half of the animals thus fed were injected intravenously twice a week with 4 ml of saline in 2 divided doses, 2 hours apart; the other half received intravenously twice a week 2 ml of 6% Dextran® and 0.5 mg of microcrystallized estradiol benzoate[†] in 5 ml of saline, also in divided doses given 2 hours apart. Microcrystals less than 1 μ in diameter and suitable for intravenous

TABLE I. Aortic Macroscopic Atherosclerosis in Male Rabbits.

Group	No. of rabbits	Lesion grade				
		0	1	2	3	4
Control	12	12	0	0	0	0
Chol-saline	21	6	4	4	6	1
Chol-dext-estradiol	22	14	1	5	2	0

Chol, cholesterol; dext, Dextran®; estradiol, estradiol benzoate.

[†] Estradiol-3-benzoate (Schering).

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