Effect of Cortisone on Metabolism of Certain "B-Vitamins" in the Rat.* (20028)

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In addition to the well-known influence of cortisone on protein metabolism and gluconeogenesis, several components of animal tissues, including blood lipids, cholesterol, and uric acid, have been shown to be increased by administration of this hormone, possibly as a consequence of its action on proteins and carbohydrates. Recently, Kochakian (see Dorfman and Goldsmith)(1) has reported that cortisone causes an increase in liver and kidney arginase in the rat, and Umbreit (see Dorfman and Goldsmith)(1) observed that the decreased concentrations of D-amino acid oxidase in the liver and of proline oxidase in the kidney of adrenalectomized rats could be restored to normal by the administration of cortisone. Microbiological activity of cortisone acetate was reported by Gaines, Broquist, and Williams(2) who found that folic acid in the medium of *Streptococcus faecalis* and citrovorum factor in the medium of *Leuconostoc citrovorum* could be replaced by cortisone. In addition, this steroid was found capable of reversing the inhibition of aminopterin for the latter organism. Mild acid treatment was reported to increase the activity of clinical cortisone (Merck's "Cortone") for *L. citrovorum*.

In view of the effect of cortisone on protein and carbohydrate metabolism and on certain enzyme systems, consideration of a possible influence on the metabolism of certain vitamins and on dietary requirements for these factors appeared to be indicated. Meites(3,4) has indicated that cortisone administration increased the vit. B_{12} requirement of the young rat. The present study was undertaken to determine the effect of cortisone on the over-all metabolism of 2 other B vitamins, pyridoxine and riboflavin, as indicated by their urinary excretion by rats, and, in the case of the former, by its influence on survival of rats on a pyridoxine-deficient diet. Additional observations were made on the effect of acid-treated cortisone on aminopterin toxicity, and data were also obtained on blood cell distribution and prothrombin times of rats receiving cortisone.

TABLE I. Composition of Experimental Diet.

Ingredient	Concentration, %
Cerelose	66.05
Casein ("vitamin-free")	18
Mineral mixture	4
Corn oil	5
Sulfathalidine	2
Methocel	4
Choline dry-mix (25%)	.40
Vitamin pre-mix*	.50
Vit A and D concentrate (100000 A, 10000 D)	.05
Alpha-tocopherol acetate	12 mg

* To provide the following quantities of vitamins (μg/g of diet): thiamine 2.5, riboflavin 2.5, nicotinic acid 5, pyridoxine 2.5, calcium pantothenate 10, biotin .05, folic acid .50 and 2-methyl-1, 4-naphtho-quinone .50.

Methods. Two groups (1 and 2) of 8 male Sprague-Dawley rats each, averaging 54 g were pair-fed through a preliminary period of 3 days and a test period of 7 days on a diet whose composition is given in Table I. This diet contained 2.5 μg of riboflavin and pyridoxine per g, levels calculated to provide adequate, but not excessive, amounts of these vitamins. Prior to being placed on experiment, the rats were fed the diet *ad libitum* for a period of 5 days, following which they were placed in metabolism cages for purposes of urine collection. After the 3-day preliminary period, group 1 was given 2.5 mg cortisone (Merck's "Cortone") per day by intraperitoneal injection and group 2 was given

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TABLE II. Excretion of Nitrogen, Pyridoxine and Riboflavin by Cortisone-Treated Rats and Pair-Fed Controls.

Group	Avg wt (g) 1	Avg wt (g) 2	Avg food consumed (g) 1 & 2	24 hr excretion of: Nitrogen (mg) 1	Nitrogen (mg) 2	Pyridoxine (μg) 1	Pyridoxine (μg) 2	Riboflavin (μg) 1	Riboflavin (μg) 2
Cortisone treatment	+	—		+	—	+	—	+	—
Preliminary period (days):									
1	53.8	54	6	61.4	53	.51	.46		
2			7	54.4	48.2	.54	.62		
3	70.8	72	8	52.6	48.9	.63	.66		
Experimental period (days):									
1	70.8	72	9	91.4	51.1	.52	.59	4.7	5.1
2			9.1	123.3	81.4	.57	.56	6.7	6.4
3			9.1	117.3	74.9	.69	.76	5.1	4.9
4			8.6	120	83.7	.83	.64		
5			7.7	137.8	86.7	.71	.59		
6			7	125.4	83.3	.68	.71	4.6	4.5
7	75.1	87.8	2.5	106.2	76.6	.71	.76		
Avg (exp. period)				117.3	76.8	.67	.66	5.3	5.2

one ml injection of saline. Urine samples, diluted to 100 ml, were stored under refrigeration at pH 2.0 until assayed. Pyridoxine was determined according to the yeast assay first proposed by Atkin, Schultz, Williams, and Frey(5) using *Saccharomyces carlsbergensis,* after hydrolysis of the samples for 2 hours at 20 lb pressure (pH 2.0). Riboflavin was estimated by the *Lactobacillus casei* method of Snell and Strong(6) and nitrogen was determined by the micro-Kjeldahl method. *Two additional groups* (3 and 4) of 5 male weanling rats each were fed *ad libitum* the diet shown in Table I with pyridoxine omitted. After a depletion period of 49 days, group 3 was given cortisone (2.5 mg per day intraperitoneally) and group 4, injections of saline. Observations on the survival times of the rats in the respective groups were recorded. A *fifth and sixth group* of 8 pair-fed weanling rats were fed the complete diet with daily injections of aminopterin (20 μg). In addition, group 5 received injections of acid-treated cortisone at a level of 3 mg per day. The acid treatment was the same as that described by Gaines *et al.*(2) which resulted in increased citrovorum factor replacement activity for *L. citrovorum.*

Results and discussion. From the data given in Table II for Groups 1 and 2 it is evident that the administration of cortisone had no appreciable effect upon the urinary excretion of either pyridoxine or riboflavin. A characteristic increase in the excretion of nitrogen was observed, along with an inhibition of growth and food consumption. The increase in nitrogen excretion persisted throughout the 7-day period in the case of 5 rats, apparently disappeared after 3 to 4 days in 2 others, and was evident for only one day in the last. Food consumption was limited by the appetite of the cortisone-treated member in 7 of the 8 pairs.

Group 3, given daily injections of cortisone after 7 weeks on a pyridoxine-deficient diet, survived an average of 33.5 additional days on the deficient regimen. Control group 4, receiving saline, averaged 38.7 days survival. Standard deviations were 7.4 days and 9.5 days, respectively. Average daily food consumption was 3.8 g for Group 3 and 4.4 for Group 4. These results are compatible with those on excretion of pyridoxine by cortisone-treated rats, both indicating that the hormone has little, if any, effect on the pyridoxine requirement.

The method of Huff and Perlzweig(7) was employed in an attempt to determine 4-pyridoxic acid in the urine, but this procedure was found to be too insensitive to provide reliable data on the content of this metabolite in the diluted urine samples.

Eight male weanling rats (Group 5) receiving the diet shown in Table I, plus 20 μg of aminopterin per day by injection, survived an average of 7.7 days. Comparable animals (Group 6) receiving, in addition to the same amount of aminopterin, 3 mg per day of acid-treated cortisone, exhibited a mean survival time of 7.3 days. Thus, the reported activity of acid-treated cortisone in reversing the inhibition of aminopterin for *L. citrovorum* does not extend to the rat, at least at the levels used in this experiment.

Eosinopenia was observed in the blood of 7 of the 8 rats of Group 1 after they had received cortisone injections for 7 days. A thromboplastin preparation obtained fresh from acetone-extracted rabbit brain was used in prothrombin time determinations on the animals of Groups 1 and 2. No appreciable differences were noted in prothrombin times between those animals which had received cortisone for 7 days and their controls, the averages being 22 seconds and 21 seconds, respectively.

In view of the observations recorded in the literature, for example, Czaczkes and Guggenheim(8) and Riesen, Schweigert, and Elvehjem(9) that the riboflavin excretion is associated with the level of protein intake, it was surprising that the increased nitrogen excretion by the cortisone-treated rats was not accompanied by a concomitant increase in riboflavin excretion. As a further check, 2 additional groups of 8 rats each were pair-fed the diet shown in Table I for one week, during which time one group received injections of 2.5 mg of cortisone daily and the other received a mock injection of saline. The livers of these animals were then removed and examined for their riboflavin content by the *L. casei* method. In terms of μg per g dry matter, the average riboflavin concentrations in the livers of cortisone-treated and control rats were 72.5 and 66.2, respectively, with standard deviations of 14.8 and 15.5. These results confirm the data on riboflavin excretion and indicate that cortisone had no appreciable influence on the metabolism of this vitamin.

Summary and conclusions. 1. The effect of cortisone administration on the metabolism of certain "B-vitamins" by the growing rat has been studied. This hormone did not influence the excretion of pyridoxine in the urine or the survival time of rats on a pyridoxine-deficient diet. Likewise, no effect was observed on the excretion of riboflavin or on the riboflavin content of the liver, although a marked increase in nitrogen excretion was recorded. Acid-treated cortisone exhibited no activity in counteracting the toxicity of aminopterin. 2. Eosinopenia was observed in rats receiving cortisone, but no differences in prothrombin times were found after 7 days of cortisone administration.

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