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Effect of Acetoacetate and β-Hydroxy Butyrate on Riboflavin and Nicotinic Acid. (20003)

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It has recently been reported(1) that sodium acetoacetate on prolonged injection in rabbits brings about several diabetic symptoms associated with increase of blood sugar, decrease of glucose tolerance and depletion of glycogen storage in liver and muscle. This substance has also been found to cause depletion of plasma ascorbic acid and increase of blood lactic acid(2), disturbance in the balance of hepatic glycogenesis and glycogenolysis(3) and increase in blood urea and inorganic phosphate(4). It has also been shown(5) that acetoacetate and β-hydroxy butyrate, when injected intraperitoneally for a considerable period cause enormous depletion of vit. B_1 in blood and urine thus accounting for the observed neuritic conditions in the old cases of diabetic patients. Besides development of neuritic conditions through deficiency of vit. B_1, other symptoms such as glossitis and stomatitis are also frequently noticed in diabetics suffering for a long period, which have been found by Sydenstricker *et al.* (6) to be cured by nicotinic acid. Several investigators showed how members of the vit. B_2 complex have a beneficial role in carbohydrate metabolism. Gordon(7) noticed a drop of blood sugar by oral administration of nicotinic acid amide in diabetic patients and Ricciute(8) reported its importance in raising the low glycogen content of muscle and liver. Schroeder(9) has shown that riboflavin is helpful in relieving hyperglycemia and according to the findings of Collazo and Bayo(10) the biochemical functions of riboflavin consist in promoting the accumulation of liver and muscle glycogen from administered glucose or lactic acid produced in the organism.

Since carbohydrate metabolism is disturbed by the ketone bodies and since nicotinic acid amide and riboflavin are the constituent parts of coenzymes I and II and the yellow enzyme, the presence of which is essential for proper utilization of carbohydrate, it was thought worthwhile to ascertain whether the substances like acetoacetate and β-hydroxy butyrate have any destructive influence on these vitamins *in vivo*. The present paper will show how both the substances on injection to rabbits cause tremendous depletion of nicotinic acid as well as riboflavin in blood and urine.

Experimental. Twenty healthy rabbits each weighing approximately 2 kg were selected. They were kept on a diet of germinated gram (*Cicer arietinum*). They were divided into 3 groups of 8, 8, and 4. Group 1 was given daily injection of sodium acetoacetate. Group 2 was injected with sodium β-hydroxy butyrate intraperitoneally in doses shown in Table I. The third group was not given any injection (control). For riboflavin estimation in blood, the blood sample was first deproteinized by trichloroacetic acid and kept overnight in the incubator. After incubation, the riboflavin contents of the blood were estimated according to the method of Burch *et al.*(11). Urine riboflavin was estimated according to

TABLE I. Blood and Urinary Riboflavin and Nicotinic Acid Contents of Rabbits Receiving Repeated Daily Injection of Sodium Acetoacetate and β-hydroxy Butyrate.

No. of rabbits	Substance inj.	Days of inj.	Blood content of — Riboflavin (γ/100 cc)	Blood content of — Nicotinic acid (mg/100 cc)	Daily urinary excretion of — Riboflavin (γ)	Daily urinary excretion of — Nicotinic acid (mg)
8	Acetoacetate*	0	39.5 ± 6.55	.71 ± .05	40.1 ± 4.80	1.9 ± .33
8		15	31.8 ± 4.42	.61 ± .04	38.5 ± 4.66	—
7		40	20.6 ± 1.97	.47 ± .06	16 ± 3.91	.36 ± .05
5		90	14.3 ± 2.77	.13 ± .04	9.2 ± 1.99	.05 ± .03
8	β-hydroxy butyrate†	0	41 ± 2.74	.72 ± .09	40.7 ± 3.69	2.1 ± .18
8		15	20.4 ± 5.31	.63 ± .06	39.6 ± 3.57	—
8		40	21.5 ± 6.84	.48 ± .06	19.7 ± 5.25	.44 ± .08
8		90	9 ± 1.88	.18 ± .04	10.9 ± 4.26	.04 ± .03
4	Control	0	44.2 ± 3.05	.71 ± .02	45.4 ± 2.90	2.60 ± .33
4		15	41.9 ± 2.78	.71 ± .05	48.9 ± 4.11	—
4		40	43.8 ± 3.08	.70 ± .07	43.5 ± 2.17	2.62 ± .22
4		90	45 ± 3.91	.73 ± .03	41.6 ± .93	2.63 ± .26

* Daily dose of acetoacetate inj.: 1st to 15th day—100 mg/kg; 16th to 30th day—150 mg/kg; 31st to 45th day—200 mg/kg; 46th to 60th day—250 mg/kg; 61st to 75th day—300 mg/kg; 76th to 90th day—350 mg/kg.

† Daily dose of β-hydroxy butyrate: 1st to 15th day—100 mg/kg; 16th to 30th day—125 mg/kg; 31st to 45th day—150 mg/kg; 46th to 60th day—175 mg/kg; 61st to 75th day—200 mg/kg; 76th to 90th day—250 mg/kg.

the method of Ferrebee(12). For the estimation of nicotinic acid in blood the method of Swaminathan(13) was used, while the method of Wang and Kodick(14) was used for determination of nicotinic acid in urine.

Results. It is evident from Table I that rabbits receiving sodium acetoacetate and β-hydroxy butyrate injection for 90 days show a more marked depletion of riboflavin and nicotinic acid in blood than in control animals. The daily excretion of these vitamins in urine is lowered considerably during the experimental period. About 75% of the rabbits injected with sodium β-hydroxy butyrate show severe lesions over the nose, face, legs and paws. and 50% of the animals injected with sodium acetoacetate show this type of skin lesion.

Discussions. Animals injected with sodium salts of acetoacetic acid and β-hydroxybutyric acid show depletion of riboflavin and nicotinic acid in blood. The daily excretion of these vitamins in urine is also lowered. The depletion may be either due to the destruction of these vitamins due to excess accumulation in the system of the ketone bodies or due to the excess demand for the coenzymes containing riboflavin and nicotinic acid as the prosthetic groups required for the metabolism of the accumulated acetone bodies. It is believed that the mechanism of hydrogen transport to oxygen in the Krebs' cycle is helped by di- and tri-phosphopyridine nucleotides (coenzymes I and II) and flavoproteins and cytochromes. That acetoacetate is metabolized through the Krebs' cycle was postulated by Breusch(15), and Wieland and Rosenthal(16) and confirmed by Buchman *et al.*(17). Unless these vitamins are given sufficiently along with the food, it is likely that the system will soon exhaust all these vitamin reserves or those little amounts served with the diets and will ultimately suffer from their deficiencies. It has actually been observed by Mannering, Lipton, and Elvehjem(18) that symptoms of riboflavin deficiency in rats increase in severity upon administration of increased amounts of fats. They have further observed that when increased doses of riboflavin are given together with increased amounts of fats, no deleterious effect is observed. Our present finding will also serve to explain how proper utilization of lactic acid (which requires the help of coenzyme I and yellow enzyme) does not take place in animals treated with acetoacetate and accumulates in the system, as has been reported earlier(2).

Summary. 1. Rabbits receiving continuous injection of sodium acetoacetate and β-hydroxy butyrate for 3 months show a state of riboflavin and nicotinic acid deficiency. 2. Both nicotinic acid and riboflavin levels in

blood of these animals are lowered considerably after 90 days of injection. 3. Marked decrease in daily excretion of these vitamins in urine has also been observed. 4. Animals show severe skin lesions over the nose, face, paws and legs.

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Effects of Proteins on Body and Organ Weights in Thyroid-Fed Rats. (20004)

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We recently reported the effects of defatted liver residue, Liver Concentrate N.F. and Vit. B_{12} on body and organ weights of thyroid fed rats(1). This confirmed the data of Ershoff (2,3), Betheil *et al.*(4,5), and Emerson and Folkers(6,7) that liver residue contains an "anti-thyrotoxic factor."

We have extended this study to include soybean alpha protein and the partially purified animal and vegetable proteins distributed by the Rutgers University Bureau of Biological Research.

Procedure. The basal diet and animals employed were identical to those previously reported(1). The protein supplements were added in isonitrogenous amounts. The following proteins were studied: *Casein (1.12; 13.66),* beef muscle (4.68; 14.92), egg albumin (7.03; 12.59), wheat gluten (0.67; 13.38), peanut flour (4.18; 9.61) and whole egg (5.1; 11.85).* These proteins were obtained from the Rutgers University Bureau of Biological Research. They have been the subject of many studies, and methods of their preparation have been published(8). *Alpha protein (1.52; 13.8),* obtained from the Glidden Co., Chicago, Ill. This was supplemented with 2% d,l-methionine prior to use. *Defatted liver residue (2.61; 13.92)* similar to that used in our previous publication(1).

* First figure in parenthesis is % ash. Second figure % total nitrogen.

Results and discussion. Our data are reported in Table I. As previously shown, defatted liver residue counteracts the growth retardation due to thyroid and permits maintenance of normal adrenal and thymus weights.

Since defatted liver residue is largely protein, it was of interest to examine other proteins as a source of "anti-thyrotoxic factor". Our results show that when tested by their