

# Effect of Propionate on the Induction of Vitamin B<sub>12</sub> Deficiency in Chicks and Rats<sup>1</sup>

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**ABSTRACT** A study was made to devise dietary conditions for the induction of simple vitamin B<sub>12</sub> deficiency in experimental animals without the delay and complications involved in methods routinely used. Chicks maintained with a purified vitamin B<sub>12</sub>-deficient diet showed a marked decrease in liver methylmalonyl-CoA mutase activity. Because of the role of vitamin B<sub>12</sub> co-enzyme in the metabolism of propionate, branched-chain fatty acids, lysine and isoleucine, a study was carried out to examine the growth of chicks with diets containing these metabolites. The compounds tested included formate, lysine, propionate, butyrate and isoleucine. Vitamin B<sub>12</sub> deficiency in terms of growth depression was markedly accentuated by the presence of sodium formate and sodium propionate at 5% levels. None of the other additions had a similar effect when incorporated in the vitamin B<sub>12</sub>-deficient rations. In rats, vitamin B<sub>12</sub> prevented growth depression caused by 2% sodium propionate. In addition, propionate markedly reduced liver methylmalonyl-CoA mutase activity both in the presence and absence of the vitamin in the diet.

Although the role of vitamin B<sub>12</sub> in a wide variety of metabolic pathways in both animal and microbial systems has been reported, all the sites of function have probably not been discovered. The pivotal role of vitamin B<sub>12</sub> in propionate metabolism has, however, been well-established as a result of several reports (1-5). Using the rat liver in vitro system, Gurnani et al. (3) have shown the requirement for vitamin B<sub>12</sub> coenzyme in the enzymatic conversion of methylmalonyl-CoA to succinyl-CoA. Hartman and Dryden (6) have reported that dietary propionate intensified weight loss in vitamin B<sub>12</sub>-deficient rats. In light of these observations and because of the significance of the methylmalonyl-CoA mutase reaction in the metabolism of propionate, branched-chain fatty acids, and isoleucine (7), and the report of a vitamin B<sub>12</sub> coenzyme function in lysine metabolism in microorganisms (8), we have examined the effect of these compounds on the growth of chicks and the effect of propionate on the growth of rats in relation to their vitamin B<sub>12</sub> status. The difficulties involved in inducing vitamin B<sub>12</sub> deficiency in experimental animals have warranted the determination of a fast and uncomplicated method of achieving this goal; thus,

the effect of various metabolites in enhancing the vitamin B<sub>12</sub> deficiency condition in laboratory animals was studied.

## EXPERIMENTAL PROCEDURE

Chicks hatched from eggs from hens maintained with a vitamin B<sub>12</sub>-deficient diet were used (4). The diet used for the hens was based on the diet of Lillie et al. (9) and consisted of: (in %) yellow corn, 55.47; soybean oil meal, 30; alfalfa meal, 5; ground limestone, 5.5; dicalcium phosphate, 3.5; manganous sulfate, 0.03; iodized salt, 0.5; vitamin A and D oil (2000 USP units of vitamin A and 400 USP units of vitamin D), 0.2; and nicotinic acid, 1 mg/100 g diet.<sup>4</sup> The basal ration for the chicks was prepared according to Kokatnur et al. (10), omitting vitamin B<sub>12</sub> and *p*-aminobenzoic acid and including vitamin E; it consisted of: (g/100 g basal diet) glucose, 68.18; 35.3 g isolated soybean protein; DL-

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methionine, 0.75; glycine, 0.3; choline chloride, 0.2; calcium carbonate, 2.166; potassium phosphate (monobasic), 1.05; calcium phosphate (dibasic), 0.94; sodium chloride, 0.8; magnesium sulfate, 0.25; ferrous sulfate, 0.03; manganous sulfate, 0.02; zinc carbonate, 0.01; cupric sulfate, 0.002; potassium iodide, 0.001; sodium molybdate, 0.001; (in milligrams) thiamine-HCl, 10; niacin, 10; riboflavin, 1.6; calcium pantothenate, 2.0; pyridoxine-HCl, 0.6; biotin, 0.006; folic acid, 0.4;  $\alpha$ -tocopheryl acetate, 2.0; menadione, 0.5; and vitamin A acetate, 1000 IU and vitamin D<sub>3</sub>, 60 IU. The normal chicks were given 10 g of vitamin B<sub>12</sub> intraperitoneally every week. The compounds tested included sodium formate, L-lysine, sodium propionate, sodium butyrate and L-isoleucine and were incorporated in the diet at the levels specified at the expense of the total diet. The chicks were weighed periodically and their weight gains over a period of 3 weeks were recorded.

Rats of the Sprague-Dawley strain, weighing 45 to 50 g, were maintained with a soy-lactose vitamin B<sub>12</sub>-free diet patterned after that of Cuthbertson and Thornton (11) consisting of: (in %) full-fat soy flour, 72; lactose, 22; salts 446 (12), 3; choline chloride, 0.5; DL-methionine, 0.1; and vitamins (1000 g diet): (in milligrams) thiamine-HCl, 2.5; riboflavin, 1; calcium pantothenate, 4; nicotinic acid, 10; pyridoxine-HCl, 0.6; biotin, 0.06; folic acid, 0.4; menadione, 0.1; vitamin A, 2000 IU; vitamin D, 200 IU; and  $\alpha$ -tocopheryl succinate, 0.5. The control animals received vitamin B<sub>12</sub> supplementation at a level of 50 g/kg diet.

In other rat experiments a purified diet of the following composition was used: isolated soy protein,<sup>3</sup> 26.8; cornstarch, 30.0; sucrose, 30.0; salts 446, (12) 4; corn oil, 8.1; methionine, 0.1; sodium propionate, 10; plus vitamins as in the previous diet. Propionate was tested at a level of 1 and 2% of the diet and was incorporated as the sodium salt. The animals were killed at the end of 6 weeks and the methylmalonyl-CoA mutase activities of their livers were assayed by measuring the incorporation of <sup>14</sup>CH<sub>3</sub>-labeled methylmalonyl-CoA into the permanganate-stable reaction product, succinic acid (3). Protein was esti-

mated by the biuret method (13). The <sup>14</sup>CH<sub>3</sub>-labeled methylmalonyl-CoA was prepared by the two-step process starting with <sup>14</sup>C-methylmalonic acid (14-16).

#### RESULTS AND DISCUSSION

Data on weight gain for 3 weeks of chicks maintained with different rations are presented in table 1. As the metabolites were not tested simultaneously, a separate control group for each was used. Of the compounds tested, sodium formate and sodium propionate at 5% levels intensified growth depression in deficient animals while having no effect on the vitamin-supplemented animals. The other compounds had either no effect or some effect probably unrelated to the vitamin. Sodium propionate at 2% of the diet showed a similar effect in the case of rats (table 2). These observations are in general agreement with those of Hartman and Dryden (6) and of Hogue and Elliot (17). The latter workers had observed only a partial growth response to vitamin B<sub>12</sub> when included in propionate-containing diets, whereas our data show a more complete reversal of the growth-depressing effect due to propionate by vitamin B<sub>12</sub>. Stokstad et al. (17, 18) have recently reported no ap-

TABLE 1  
Effect of certain metabolites on induction of vitamin B<sub>12</sub> deficiency in the chick

Metabolite added to basal diet	Wt gain, 3 weeks	
	Without vitamin B <sub>12</sub>	With vitamin B <sub>12</sub>
—	152.0 ± 13.00 <sup>1</sup>	219.0 ± 16.74
Formate, 1%	109.7 ± 12.40	172.4 ± 19.23
—	158.0 ± 10.75	212.0 ± 16.50
Formate, 5%	113.5 ± 8.63	210.0 ± 10.28
—	120.0 ± 16.28	184.0 ± 18.94
Lysine, 1%	125.0 ± 8.99	186.0 ± 14.00
—	79.0 ± 9.40	128.0 ± 16.17
Sodium propionate, 5% <sup>2</sup>	43.0 ± 3.60	122.0 ± 9.70
—	122.0 ± 8.43	238.8 ± 11.22
Butyrate, 1%	117.4 ± 10.25	179.9 ± 7.05
—	96.0 ± 9.09	169.0 ± 7.10
Isoleucine, 1%	95.0 ± 7.27	210.0 ± 10.20

<sup>1</sup> Averages ± SEM for not less than 6 chicks.

<sup>2</sup> 17-day growth.

<sup>3</sup> Alpha Protein, Nutritional Biochemicals Corporation, Cleveland.

preciable protection by vitamin B<sub>12</sub> against growth depression caused by 2 or 4% calcium propionate. In addition, using formiminoglutamic acid (FIGlu) excretion as the criteria of vitamin B<sub>12</sub> deficiency, these authors could find no evidence for an enhancement of vitamin B<sub>12</sub> deficiency by dietary calcium propionate in the rat. On the contrary, FIGlu excretion was decreased in the presence of propionate in the diet. However, vitamin B<sub>12</sub> influences FIGlu excretion only indirectly by altering folate metabolism (19). We, therefore, studied liver methylmalonyl-CoA mutase activity as a specific criteria of vitamin B<sub>12</sub> deficiency. The results are shown in table 3. The mutase activity was found to be sig-

nificantly reduced as a result of the deficiency condition at the end of 6 weeks. The inclusion of 2% sodium propionate in the diet markedly aggravated the severity of the deficiency, as shown by the mutase levels. However, the enzyme activity was still reduced by propionate even when the diet contained more than a normally adequate amount of vitamin B<sub>12</sub>. Although the vitamin counteracted the growth-depressing effect of propionate almost completely, at the level used it only partially restored the mutase activity.

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TABLE 2  
Effect of propionate on induction of vitamin B<sub>12</sub> deficiency in the rat

Diet	No. of rats	Weekly body wt gain for 5 weeks	Liver wt/body wt
g			
Series 1, soy flour lactose diet (11) <sup>1</sup>			
Without vitamin B <sub>12</sub>	9	21.75 ± 3.38 <sup>2</sup>	0.043
Without vitamin B <sub>12</sub> + propionate 2%	4	13.10 ± 3.45	0.045
With vitamin B <sub>12</sub>	8	33.30 ± 1.91	0.040
With vitamin B <sub>12</sub> + propionate 2%	5	34.30 ± 3.35	0.040
Series 2, soy protein, starch, sucrose, 1% propionate purified diet <sup>3</sup>			
Without vitamin B <sub>12</sub>	22	11.7 ± 3.5	
With vitamin B <sub>12</sub>	13	30.1 ± 2.4	

<sup>1</sup> Rats fed diet at mean weight of 43 g.  
<sup>2</sup> Averages ± SEM.  
<sup>3</sup> Rats fed diet at mean weight of 95 g.

TABLE 3  
Effect of propionate (2%) on rat liver methylmalonyl-CoA mutase activity<sup>1</sup>

Diet	Methylmalonyl-CoA mutase activity mmoles/mg protein
Without vitamin B <sub>12</sub>	45.62 ± 4.94 <sup>2</sup>
Without vitamin B <sub>12</sub> + propionate, 2%	8.03 ± 4.33
With vitamin B <sub>12</sub>	88.37 ± 5.30
With vitamin B <sub>12</sub> + propionate, 2%	35.2 ± 5.83

<sup>1</sup> Mutase assay was carried out by incubating <sup>14</sup>C-methyl-labeled methylmalonyl-CoA (0.8 mole, sp. act. 2.15 × 10<sup>4</sup> cpm/mole) for 1 hour at 37° in a medium containing Tris-buffer, 0.05 M, pH 7.4; MgCl<sub>2</sub>, 0.003 M; ATP, 0.003 M; glutathione, 0.005 M; and 0.2 ml of 10% liver homogenate in a total volume of 2 ml.  
<sup>2</sup> Averages of 3 rats/group ± SEM.

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