Metabolism of propionate in the vitamin B_{12}-deficient rat

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1. The blood of vitamin B_{12}-deficient or control rats, either fed or fasted for 48 h, contained only a trace of propionate. After the intraperitoneal injection of 1 mmol sodium propionate, concentrations of propionate in the blood of deficient rats were markedly raised even 2 h after injection.

2. Vitamin B_{12}-deficient rats excreted seven times more propionate, but less acetate, in their urine as compared with their supplemented litter-mates. The administration of propionate increased propionate excretion in deficient rats, but this amounted to only a small proportion of the dose and much more could be accounted for as methylmalonic acid. Valine caused a slight increase in the excretion of propionate whereas isoleucine did not affect it.

3. The urinary excretion of propionate and methylmalonate increased with increasing doses of propionate. However, when the excretion was expressed as a percentage of the dose, there was no increased excretion of methylmalonate with increasing doses of propionate.

The metabolism of propionate is known to be abnormal in vitamin B_{12} deficiency. Thus the administration of propionate to vitamin B_{12}-deficient rats increases their abnormally high excretion of methylmalonic acid yet having no effect in supplemented, control animals (Barness, Young, Nocho & Kahn, 1963; Williams, Spray, Newman & O'Brien, 1969). Furthermore, mitochondrial preparations from the livers of vitamin B_{12}-deficient rats convert $^{14}CO_2$ and propionyl-CoA into succinic acid less effectively than those from control rats (Gurnani, Mistry & Johnson, 1960). We have recently demonstrated a marked depression of gluconeogenesis from propionate by perfused livers or kidney cortex slices of vitamin B_{12}-deficient rats (Weidemann, Hems, Williams, Spray & Krebs, 1970). Increased urinary excretion of administered [^{3}H]-propionate by deficient rats as compared with the controls has been demonstrated (Stokstad, Webb & Shah, 1966). We therefore decided to study some aspects of propionate metabolism in the intact vitamin B_{12}-deficient rat, in particular the concentration of propionate in the blood and urine, and the effects on this propionate of starvation and the injection of various substances.

EXPERIMENTAL

Animals and their management. Rats were reared, housed and handled as described previously (Williams *et al.* 1969). Rats on the deficient diet were selected for further study on the basis of a high urinary excretion of methylmalonic acid.

Propionate in blood. Blood (3 ml) was collected from the tail artery of stock, vitamin B_{12}-supplemented or vitamin B_{12}-deficient rats before or 30, 60 or 120 min after the intraperitoneal administration of 1 mmol sodium propionate.

Urinary excretion of organic acids. Urine was collected from rats housed in individual

glass metabolic cages. To study the excretion of endogenous propionate and acetate, urine was collected from vitamin B_{12}-deficient and vitamin B_{12}-supplemented rats for two successive periods of 24 h while the rats were starved.

To measure the effects of the injection of various substances on the excretion of propionate and methylmalonate, vitamin B_{12}-deficient male rats were starved for 24 h and were then injected intraperitoneally with saline, or 1 mmol sodium propionate, 1 mmol L-valine, or 2 mmol DL-isoleucine per 150 g body-weight. The collections of urine were continued for a further 24 h.

To study the effects of various doses of propionate, vitamin B_{12}-deficient male rats were starved for 24 h and were then injected with 0·5, 1·0 or 2·0 mmol sodium propionate per 150 g body-weight.

Determination of fatty acids. Urine (10 ml) was adjusted to pH 2 with 20% (v/v) perchloric acid, 1 ml of a solution containing 4 μmol *n*-butyric acid was added to act as an internal standard and the solution was steam-distilled in a Markham still. The distillate (125 ml) was collected into a flask containing 0·1 ml 4 M-NaOH. Blood (3 ml) was diluted to 10 ml with water, 1 ml of a solution containing 4 μmol *n*-butyric acid was added to act as an internal standard, and after haemolysis 1·5 ml 20% (v/v) perchloric acid was added and the mixture was made up to 15 ml with water. The protein precipitate was removed by centrifugation and the supernatant fraction was steam-distilled. The distillates were evaporated to dryness under reduced pressure in a rotary film evaporator and the residue was dissolved in 1 ml 5% (v/v) H_3PO_4. Portions of the solution (1 μl) were applied to a column of neopentyl-glycol-succinate operating at 135° in a Perkin Elmer model F11 gas–liquid chromatograph with a flame-ionization detector, using oxygen-free nitrogen as the carrier gas. The heights of the peaks were compared with those produced by known amounts of acetic or propionic acid and were corrected for the internal standard.

Determination of methylmalonate. Methylmalonate was determined in urine by the method described previously (Williams *et al.* 1969).

RESULTS

The concentration of propionic acid in the blood of vitamin B_{12}-deficient, vitamin B_{12}-supplemented and stock rats. Only a trace of propionate was detected in the blood of six fed or starved, deficient rats ($0·003 \pm 0·006$ (SD)) and $< 0·01$ μmol/ml blood respectively). Similarly the concentration in fed vitamin B_{12}-supplemented rats was very low ($0·02 \pm 0·01$ (SD)).

The concentration of propionate in the blood of deficient rats 30 min after injection was six times as high as that in stock rats (Table 1). After 60 min the difference was eighty times and after 120 min the deficient rats still had appreciable concentrations of propionate in their blood, whereas the values in stock rats had returned to pre-injection concentrations.

The urinary excretion of acetic, propionic and methylmalonic acids by fasted vitamin B_{12}-deficient and vitamin B_{12}-supplemented rats. As we have reported previously for methylmalonate (Williams *et al.* 1969), there were wide variations in the individual

Table 1. *Concentration of propionic acid in the blood (μmol/ml) of vitamin B_{12}-deficient and stock rats starved for 48 h before and after the intraperitoneal administration of 1 mmol sodium propionate*

(Mean values with their standard errors; no. of observations in parentheses)

Time after administration of propionate (min)	Vitamin B_{12}-deficient rats	Stock rats
0	< 0·01 (8)	< 0·01 (8)
30	1·85 ± 0·39 (14)	0·29 ± 0·07 (11)
60	2·47 ± 0·33 (14)	0·03 ± 0·01 (11)
120	1·41 ± 0·29 (12)	< 0·01 (4)

Table 2. *Urinary excretion of acetic, propionic and methylmalonic acids by male vitamin B_{12}-deficient and vitamin B_{12}-supplemented rats*

(Mean values with their standard errors; no. of observations in parentheses)

Period of starvation (h)	Urinary excretion of organic acids (μmol/d)		
	Acetic	Propionic	Methylmalonic
	Vitamin B_{12}-deficient rats		
0–24	28·1 ± 4·5 (12)	9·2 ± 1·3 (12)	291 ± 50 (11)
24–48	49·1 ± 11·5 (12)	2·6 ± 0·3 (12)	64 ± 19 (11)
P values*	< 0·2, > 0·1	< 0·001	< 0·001
	Vitamin B_{12}-supplemented rats		
0–24	57·6 ± 10·4 (12)	1·3 ± 0·5 (12)	38 ± 6 (12)
24–48	72·6 ± 9·2 (11)	1·5 ± 0·5 (11)	9 ± 2 (11)
P values*	< 0·3, > 0·2	> 0·9	< 0·001

* Student's *t* test.

values for the excretion of both methylmalonate and acetate and propionate, as shown by the magnitude of the standard errors (see Tables 2, 3 and 4). Deficient rats excreted over seven times as much propionic acid as the controls during the 1st day of starvation (Table 2). However, when urine was collected for a further period of 24 h the urinary excretion of propionate had decreased significantly to less than twice the level in the controls; the mean value in the control animals showed a slight increase. In both the deficient and the supplemented rats there were significant decreases in the excretion of methylmalonate during the second 24 h of starvation The deficient rats excreted less acetic acid than the controls and in both instances the acetate excretion increased during starvation for the second period of 24 h.

Deficient rats showed the expected fall in the excretion of methylmalonate in the second 24 h of starvation after the injection of saline (Williams *et al.* 1969). In contrast, following the intraperitoneal administration of 1 mmol sodium propionate to the rats after 24 h of starvation, there were increased excretions of both propionate and methylmalonate (Table 3). However, most of the injected propionate was either excreted as methylmalonate or was converted into some other metabolite and only a relatively insignificant proportion was excreted as propionate. After the administration of valine, the excretion of methylmalonate increased markedly. There

Table 3. *Urinary excretion of methylmalonic and propionic acids by vitamin B_{12}-deficient male rats*

(Rats were starved for the first 24 h and were then injected with saline, 1 mmol sodium propionate, 1 mmol L-valine, or 2 mmol DL-isoleucine per 150 g body-weight. Urine was collected for a second period of 24 h. Mean values with their standard errors; no. of observations in parentheses)

	Urinary excretion (μmol/d)			
	Methylmalonic acid		Propionic acid	
	Period of starvation			
Treatment	0–24 h	24–48 h	0–24 h	24–48 h
Saline	341 ± 100 (7)	37 ± 9·3 (7)	—	—
Sodium propionate	383 ± 4·2 (7)	532 ± 5·3 (7)	7·1 ± 0·8 (7)	26·9 ± 7·9 (7)
L-valine	202 ± 31 (7)	238 ± 4·5 (7)	6·6 ± 1·3 (5)	7·6 ± 1·4 (6)
DL-isoleucine	213 ± 46 (7)	228 ± 82 (7)	4·5 ± 2·3 (5)	2·9 ± 1·0 (6)

Table 4. *Excretion of methylmalonic and propionic acids by vitamin B_{12}-deficient rats after the intraperitoneal injection of various doses of propionate*

(Mean values with their standard errors for a group of six rats; excretion as percentage of dose in parentheses)

Dose of propionate (mmol/150 g body-wt)	Urinary excretion (μmol/d)	
	Methylmalonic acid	Propionic acid
0·5	374 ± 75 (47 ± 11)	12 ± 6·3 (0·7 ± 0·2)
1·0	429 ± 43 (25 ± 3)	31 ± 4·8* (1·7 ± 0·2)
2·0	888 ± 245 (27 ± 8)	1260 ± 750* (36 ± 18)

* Result for five rats.

was a small increase in the excretion of propionate, compared with the marked fall after simple starvation in another group of rats (Table 2). The propionate excretion of the rats in Table 3 after injection of saline was unfortunately not determined. With isoleucine very little propionate was excreted although there was a large increase in methylmalonate excretion.

The excretion of methylmalonate tended to increase with increasing doses of propionate; a similar trend was observed with prioponate excretion (Table 4). When the results are expressed as percentages of the dose of propionate, there is no consistent increase in the methylmalonate excretion but a consistent increase in the excretion of propionate.

DISCUSSION

The concentration of propionate in the blood of deficient rats was higher and remained high even 2 h after the intraperitoneal administration of 1 mmol sodium propionate, as compared with control rats. This is presumably due to a reduced rate of utilization of propionate by the liver as a result of the effects of vitamin B_{12} deficiency on the methylmalonyl-CoA isomerase reaction.

Additional evidence for a reduced metabolic utilization of propionate in the

deficient rats is the increased urinary excretion of endogenous propionate (Table 2). In this respect they resemble vitamin B_{12}-deficient human subjects, who also excrete excessive amounts of propionate (Cox, Robertson-Smith, Small & White, 1968). Unlike our rats, however, human subjects excreted increased amounts of acetate, which was thought to be related to neurological involvement. Starvation of the rats for 24 h reduced the urinary excretion in the following 24 h to levels nearer to those found in control rats. This was possibly due to the reduction in the concentration of propionate in the caecum after starvation (Williams & Spray, 1970). Thus, in general terms, the excretion of propionate in deficient rats is affected similarly to that of methylmalonate. The excretion of acetate increased during the 2nd day's starvation in both deficient and control rats. In the deficient rats the mean increased by 21 μmol/d and that in the supplemented rats by 15 μmol/d; the increases were not statistically significant. Under conditions of ketosis, the activity of the citric acid cycle may be depressed (Wieland, 1968), leading to a fall in the rate of oxidation of acetyl-CoA. It is possible, therefore, that some of this unoxidized acetyl-CoA may be de-acylated and excreted as acetate. This would explain the increased excretion of acetate during the second 24 h of starvation.

Since the administration of valine or isoleucine can increase the urinary excretion of methylmalonate in deficient rats, it was of interest to see whether the urinary excretion of propionate would be enhanced under these conditions. The excretion of both propionate (Table 2) and methylmalonate (Tables 2 and 3) dropped markedly in the 2nd 24 h of starvation. The increases in the excretion of methylmalonate above those found in the rats injected with saline (Table 3) must therefore have been due to the propionate, the isoleucine or the valine. Similarly, the increase in the excretion of propionate after the injection of valine (Table 3), compared with the fall after simple starvation (Table 2), suggests that propionate could be a metabolite of valine, though this possibility does not appear in present-day charts of metabolic pathways.

The experiments to study the effect of increasing doses of propionate on the excretion of propionate and methylmalonate by vitamin B_{12}-deficient rats showed that, though the amount of methylmalonate excreted increases with increasing doses, when the results are expressed as percentages of the dose there is little or no increase in the mean excretions. This presumably indicates that the efficiency of the methylmalonyl-CoA isomerase enzyme pathway remains constant with increasing doses up to 2 mmol/150 g body-weight.

The results provide further evidence that the metabolism of propionate is abnormal in the vitamin B_{12}-deficient rat.

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