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Metabolic Effects of Propionate in Normal and Vitamin B_{12}-Deficient Rats

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1. Administration of propionate caused a twofold increase in the concentrations of lactate and pyruvate in the blood of vitamin B_{12}-deficient rats, whereas there was a slight decrease in lactate and a 50% increase in pyruvate in normal rats. 2. Concentrations of total ketone bodies in the blood of normal rats were not significantly altered by propionate administration but the [3-hydroxybutyrate]/[acetoacetate] ratio decreased from 3.0 to 2.0. In the vitamin B_{12}-deficient rats there was a 40% decrease in total ketone bodies and a change in the ratio from 3.4 to 1.2. 3. The changes in the concentration of ketone bodies in freeze-clamped liver preparations were similar in pattern to those observed in blood. 4. Propionate administration caused a decrease in the concentration of acetyl-CoA in the livers of both groups of animals, but the absolute decrease was greater in the vitamin B_{12}-deficient group. The decrease in the concentration of CoA was similar in both groups. 5. As in blood, there were threefold increases in the concentrations of lactate and pyruvate in the livers of the vitamin B_{12}-deficient rats after propionate administration, whereas there was no significant change in the concentrations of these metabolites in the normal rats. 6. There was a 50% inhibition of glucose synthesis in perfused livers from vitamin B_{12}-deficient rats when lactate and propionate were substrates as compared with lactate alone. 7. It is concluded that the conversion of lactate into glucose is inhibited in vitamin B_{12}-deficient rats after propionate administration, and that this effect is due to inhibition of the pyruvate carboxylase step resulting from a decrease in acetyl-CoA concentration and a postulated increase in methylmalonyl-CoA concentration.

Propionate metabolism is known to be abnormal in vitamin B_{12}-deficiency. Thus the raised urinary excretion of methylmalonate by vitamin B_{12}-deficient rats is increased further after the administration of propionate (Barness, Young, Nocho & Kahn, 1963; Williams, Spray, Newman & O'Brien, 1969). Also, livers and kidneys from vitamin B_{12}-deficient rats utilize propionate for gluconeogenesis *in vitro* less effectively than do the tissues of rats fed on a diet containing vitamin B_{12} (Weidemann, Hems, Williams, Spray & Krebs, 1970). These effects are not unexpected, because vitamin B_{12} is an essential cofactor for the interconversion of methylmalonyl-CoA (derived from propionyl-CoA) and succinyl-CoA that is catalysed by methylmalonyl-CoA mutase (EC 5.4.99.2). In the present paper we compare the effects of the administration of propionate on some aspects of intermediary metabolism in normal and vitamin B_{12}-deficient rats.

EXPERIMENTAL

Animals. Male rats of the Wistar strain weighing 180–250g were used for the experiments *in vivo*. Vitamin B_{12}-deficient rats were bred, reared and selected for further study on the basis of a high urinary excretion of methylmalonate, as described by Williams *et al.* (1969). The normal rats were fed on a standard breeding diet for rats and mice (Herbert Styles Ltd., Bewdley, Worcs., U.K.).

Experiments in vivo. The rats were starved for 48h and 1ml of 1M-sodium propionate or 1ml of 0.9% NaCl solution was injected intraperitoneally. The rats were killed by cervical dislocation 30min after injection. The

livers were rapidly removed and freeze-clamped (Wollenberger, Ristau & Schoffa, 1959). The frozen livers were treated as described by Williamson, Lund & Krebs (1967). Blood (0.2 ml) collected from the aorta was deproteinized with 2 ml of 3% (w/v) $HClO_4$ and then neutralized with KOH. In separate experiments to determine free fatty acid concentrations in plasma, rats were decapitated 30 min after injection and blood was collected into tubes containing heparin. The plasma was separated by centrifugation. In the experiments where the concentrations of propionate in blood were measured, blood was collected from the tail vein at 30, 60 and 120 min after the injection. Only one sample was taken from each animal because at least 3 ml of blood was required for the propionate determination. Livers from these rats were not used.

Perfusion experiments. Liver perfusions were carried out by the method of Hems, Ross, Berry & Krebs (1966). The rats were starved for 48 h.

Determination of propionate in blood. Blood (3 ml) was mixed with 1 ml of 4 mM-butyric acid as an internal standard. The mixture was diluted to 13.5 ml with water and after complete haemolysis 1.5 ml of 20% (w/v) $HClO_4$ was added. The protein precipitate was removed by centrifugation and the entire supernatant solution was steam-distilled in a Markham still. About 125 ml of distillate was collected into a flask containing 0.1 ml of 4 M-NaOH. The distillate was evaporated to dryness under reduced pressure in a rotary evaporator and the residue was dissolved in 1 ml of 5% (v/v) H_3PO_4. A sample (1 μl) of the solution was applied to a column of neopentyl glycol (2,2-dimethylpropane-1,3-diol) succinate operating at 135°C in a Perkin–Elmer model F11 gas chromatograph with a flame ionization detector, with oxygen-free nitrogen as the carrier gas.

When 1 ml of 3 mM-propionate was added to portions (3 ml) of three different samples of pooled rat blood, the mean recovery under these conditions was 98.2 ± 3.3% (mean ± S.E.M. of 14 observations).

Determination of metabolites. The concentrations of the following metabolites were determined in neutral deproteinized extracts of blood or liver by standard enzymic methods: glucose (Slein, 1963); pyruvate, L(+)-lactate, L(−)-malate and L(−)-glycerol 3-phosphate (Hohorst, Kreutz & Bücher 1959); phosphoenolpyruvate and 2- and 3-phosphoglycerate (Czok & Eckert, 1963); acetoacetate and D(−)-3-hydroxybutyrate (Williamson, Mellanby & Krebs, 1962); acetyl-CoA (Pearson, 1965); CoA (Garland, 1964); L-glutamate (Bernt & Bergmeyer, 1963); 2-oxoglutarate (Bergmeyer & Bernt, 1963); L-aspartate (Pfleiderer, 1963).

The free fatty acid concentration in plasma was determined by the colorimetric method of Itaya & Ui (1965).

RESULTS

Effects of administration of propionate on concentrations of metabolites in blood

Propionate. Little or no propionate (less than 0.01 μmol/ml) was detected in the blood taken from rats before the intraperitoneal injection of propionate (Table 1). At 30 min after injection the mean propionate concentration in vitamin B_{12}-deficient rats was about eight times that of the normal animals, and the difference increased to 70 times after 60 min. After 120 min the concentration of propionate was hardly measurable in the blood of normal rats, but the mean value for the vitamin B_{12}-deficient rats was only slightly less than that at 60 min.

Table 1. *Concentration of propionate in blood of normal and vitamin B_{12}-deficient rats after intraperitoneal injection of sodium propionate*

Rats fed on either a standard diet or a vitamin B_{12}-deficient diet were starved for 48 h and then given 1 ml of 1 M-sodium propionate by intraperitoneal injection. Blood for the determination of propionate was collected from the tail vein. The results are expressed as means ± S.E.M., with the numbers of observations in parentheses.

Time after injection (min)	Concn. of propionate in blood (μmol/ml)	
	Normal rats	Vitamin B_{12}-deficient rats
0	<0.01 (5)	<0.01 (5)
30	0.22 ± 0.11 (5)	1.71 ± 1.2 (4)
60	0.04 ± 0.02 (5)	2.76 ± 0.27 (4)
120	<0.01 (4)	2.10 ± 0.18 (5)

Other metabolites. Administration of propionate caused an increase in blood glucose concentration of about 1 μmol/ml in both vitamin B_{12}-deficient and normal rats (Table 2). In normal rats propionate had no marked effect on the blood lactate concentration, but in the vitamin B_{12}-deficient animals it caused a twofold increase. The blood pyruvate concentration increased by about 50% after propionate was administered to the normal animals, but this increase was less than that observed in the vitamin B_{12}-deficient group. The acetoacetate concentration increased slightly in both groups after propionate administration; the concentration of 3-hydroxybutyrate did not alter in the normal animals but it was halved in the vitamin B_{12}-deficient group. The mean [3-hydroxybutyrate]/[acetoacetate] ratio decreased from 3.0 to 2.0 after propionate was injected into normal rats, whereas in the vitamin B_{12}-deficient group the ratio decreased to 1.2.

Plasma free fatty acids. The decrease in the concentrations of total ketone bodies in the blood of vitamin B_{12}-deficient rats treated with propionate was not due to a decrease in the concentrations of plasma free fatty acids. There was no significant difference between the concentrations in vitamin B_{12}-deficient rats treated with 0.9% sodium chloride (0.98 ± 0.11 μequiv/ml; mean ± S.E.M. for five rats) and those in vitamin B_{12}-deficient rats treated with propionate (0.99 ± 0.08 μequiv/ml; mean ± S.E.M. for six rats).

Table 2. *Effects of propionate on the concentration of metabolites in blood of normal and vitamin B_{12}-deficient rats*

Rats fed on either a standard diet or a vitamin B_{12}-deficient diet were starved for 48h and either 1ml of 1M-sodium propionate or 1ml of 0.9% NaCl solution (saline) was injected intraperitoneally. After 30min the rats were killed and blood was collected from the aorta. Other experimental details are given in the text. The results are expressed as means±S.E.M., with the numbers of observations in parentheses. Values for propionate-treated rats that are statistically different from those for saline-treated rats are indicated by: * $P<0.05$; ** $P = 0.01$–0.001.

	Concn. of metabolite (μmol/ml) or concentration ratio of metabolites in blood			
	Normal rats		Vitamin B_{12}-deficient rats	
Metabolite or ratio of metabolites	Saline-treated (6)	Propionate-treated (7)	Saline-treated (9)	Propionate-treated (10)
Glucose	4.6 ±0.2	5.6 ±0.3*	3.7 ±0.2	4.5 ±0.2**
Pyruvate	0.093±0.008	0.14±0.01**	0.11±0.02	0.30±0.08*
Lactate	1.8 ±0.3	1.4 ±0.1	1.3 ±0.2	3.0 ±0.6*
Acetoacetate	0.64 ±0.06	0.92±0.09*	0.83±0.07	1.1 ±0.1*
3-Hydroxybutyrate	1.9 ±0.1	1.8 ±0.2	2.8 ±0.3	1.4 ±0.2**
[Lactate]/[pyruvate]	20 ±3	9.8 ±0.5*	14 ±2	12 ±2
[3-Hydroxybutyrate]/[acetoacetate]	3.0 ±0.1	2.0 ±0.2**	3.4 ±0.3	1.2 ±0.1**

Effects of administration of propionate on concentrations of metabolites in liver

Glycolytic intermediates and glucose precursors. The concentration of lactate was the same in the normal and vitamin B_{12}-deficient rats injected with 0.9% sodium chloride. In the normal group propionate injection caused only a very slight increase in the concentration of lactate, but in the vitamin B_{12}-deficient animals there was a threefold increase (Table 3). The changes in pyruvate concentration paralleled those of lactate, and thus the [lactate]/[pyruvate] ratios were not significantly altered by propionate treatment.

The concentration of 2-oxoglutarate did not change significantly after propionate administration in either group, but there was a statistically significant ($P<0.05$) decrease in glutamate content in both normal and vitamin B_{12}-deficient animals. There was an increase of 0.1μmol/g in the concentration of malate in the livers of the normal rats treated with propionate; in the vitamin B_{12}-deficient group there was a slight decrease in the malate concentration after propionate injection. The concentration of aspartate almost doubled after administration of propionate to the normal group, but it did not change significantly in the vitamin B_{12}-deficient rats.

The concentrations of phosphoenolpyruvate and of 2-phosphoglycerate plus 3-phosphoglycerate increased twofold and threefold respectively in the livers of the vitamin B_{12}-deficient rats after propionate treatment, whereas the concentration of glycerol 3-phosphate was nearly halved. In the normal rats the changes in the concentrations of these metabolites were similar but much less marked. The concentrations of glucose in both groups was slightly increased after the administration of propionate.

Acetyl-CoA and CoA. After propionate administration the concentrations of acetyl-CoA and CoA decreased in both the normal and deficient groups. However, in the vitamin B_{12}-deficient group the decrease in acetyl-CoA concentration was sevenfold, compared with a less than twofold decrease in the normal animals. The concentration of CoA decreased by about the same amount in the two groups. Thus the decrease in the concentration of acetyl-CoA plus CoA was 0.18μmol in the normal rats and 0.23μmol in the vitamin B_{12}-deficient rats; presumably a considerable proportion of this decrease is due to the formation of propionyl-CoA.

Ketone bodies. The concentrations of acetoacetate were similar in all groups of rats. However, in the vitamin B_{12}-deficient group the concentration of 3-hydroxybutyrate was decreased nearly threefold after propionate injection, whereas in normal rats the concentration decreased only from 1.6 to 1.2μmol/g. Consequently the [3-hydroxybutyrate]/[acetoacetate] ratio decreased threefold in the deficient group, whereas there was little change in the control group.

Infusion experiments. It was considered that some of the effects of propionate injection in vitamin B_{12}-deficient rats might be due simply to the higher circulating concentrations of propionate (see Table 1). To test this possibility, propionate was infused into the femoral vein of conscious rats (for technique see Hawkins, Williamson & Krebs, 1971) to obtain

Table 3. *Effects of propionate administration on the concentrations of metabolites in livers of normal and vitamin B_{12}-deficient rats*

The rats were treated as described in Table 2. The results are expressed as means±S.E.M., with the numbers of observations in parentheses. Values for propionate-treated rats that are statistically different from those for saline-treated rats are indicated by: * $P<0.05$ ** $P = 0.01$–0.001.

Metabolite or ratio of metabolites	Concn. of metabolite (μmol/g wet wt. of tissue) or concentration ratio of metabolites in liver			
	Normal rats		Vitamin B_{12}-deficient rats	
	Saline-treated	Propionate-treated	Saline-treated	Propionate-treated
Lactate	0.39 ±0.03 (7)	0.41 ±0.05 (7)	0.39 ±0.07 (10)	1.20 ±0.20 (12)**
Pyruvate	0.030±0.003 (7)	0.042±0.002 (7)*	0.040±0.005 (11)	0.13 ±0.02 (12)**
2-Oxoglutarate	0.11 ±0.02 (7)	0.13 ±0.01 (7)	0.10 ±0.01 (11)	0.11 ±0.01 (11)
Glutamate	3.0 ±0.2 (7)	2.2 ±0.1 (7)**	3.8 ±0.2 (10)	3.1 ±0.3 (10)*
Malate	0.18 ±0.03 (7)	0.28 ±0.04 (7)	0.26 ±0.01 (10)	0.22 ±0.01 (11)*
Aspartate	0.49 ±0.04 (4)	0.97 ±0.1 (4)*	0.80 ±0.18 (7)	0.96 ±0.08 (8)
Phosphoenolpyruvate	0.071±0.007 (7)	0.11 ±0.01 (7)*	0.066±0.009 (10)	0.20 ±0.02 (9)**
2-Phosphoglycerate	0.036±0.004 (7)	0.043±0.003 (7)	0.025±0.004 (10)	0.052±0.007 (9)**
3-Phosphoglycerate	0.19 ±0.02 (7)	0.26 ±0.02 (7)*	0.16 ±0.02 (10)	0.44 ±0.03 (9)**
Glycerol 3-phosphate	0.15 ±0.02 (7)	0.13 ±0.01 (7)	0.21 ±0.02 (10)	0.11 ±0.01 (11)**
Glucose	4.9 ±0.3 (7)	5.6 ±0.2 (7)	4.4 ±0.5 (10)	5.2 ±0.4 (12)
Acetyl-CoA	0.086±0.005 (7)	0.053±0.011 (7)*	0.109±0.005 (11)	0.016±0.001 (12)**
Acetoacetate	0.86 ±0.07 (7)	0.82 ±0.06 (7)	0.80 ±0.06 (11)	0.86 ±0.04 (12)
3-Hydroxybutyrate	1.6 ±0.1 (7)	1.2 ±0.1 (7)**	1.7 ±0.2 (10)	0.60 ±0.08 (12)**
CoA	0.34 ±0.01 (3)	0.19 ±0.02 (3)**	0.29 ±0.02 (5)	0.15 ±0.01 (6)**
[3-Hydroxybutyrate]/[acetoacetate]	1.9 ±0.2 (7)	1.5 ±0.1 (7)	2.1 ±0.2 (10)	0.70 ±0.07 (12)**
[Lactate]/[pyruvate]	14 ±1.5 (7)	9.7 ±0.9 (7)	9.7 ±1.3 (10)	10.2 ±1.5 (12)

concentrations of propionate in the blood of 2–3 mM. Control rats were infused with similar amounts of sodium chloride. The rats were killed after 30 min and the livers freeze-clamped. When expressed as a percentage change from the control values the results were similar in pattern to those for the normal rats injected with propionate, but the changes were in general more marked, especially the decrease in acetyl-CoA concentration (Fig. 1). However, there was no increase in lactate concentration nor any marked change in the [3-hydroxybutyrate]/[acetoacetate] ratio.

Effects of propionate on glucose synthesis from lactate in isolated perfused liver

The increases in the concentrations of lactate and pyruvate in the livers of vitamin B_{12}-deficient rats after administration of propionate suggested that the conversion of these compounds into glucose was partially inhibited. To investigate this possibility, livers from starved normal and vitamin B_{12}-deficient rats were perfused with lactate, propionate or a mixture of both. The rate of formation of glucose from lactate by the livers of vitamin B_{12}-deficient rats was 38% less than that for normal rats, but in both instances nearly all the lactate removed could be accounted for as glucose. As expected, the amount of glucose formed from propionate in the livers of vitamin B_{12}-deficient rats was decreased by 75%. With livers from normal rats the results when propionate plus lactate were perfused were similar to those when lactate alone was perfused, except that some of the glucose must have been formed from propionate. However, with livers from vitamin B_{12}-deficient rats the combination of substrates gave rates of gluconeogenesis that were 50% lower than those obtained with lactate alone. When vitamin B_{12}-deficient rats were given cyanocobalamin and fed on the standard diet for 3 weeks, this inhibition was completely abolished (Table 4).

DISCUSSION

The higher concentrations of propionate found in the blood of vitamin B_{12}-deficient rats after intraperitoneal injection of propionate indicate that in the vitamin B_{12}-deficient animal propionate utilization is impaired in a similar way to that reported for isolated perfused livers and kidney-cortex slices from vitamin B_{12}-deficient rats (Weidemann *et al.* 1970). The changes in the concentrations of various metabolites in the livers of normal and vitamin

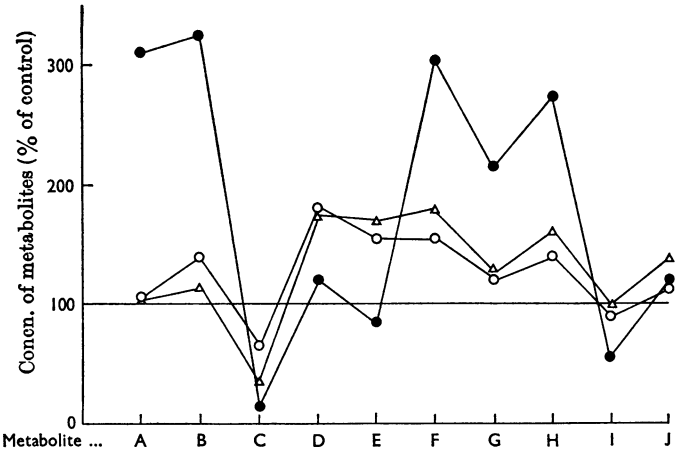

Fig. 1. Patterns of metabolites in rat liver after the administration of propionate. The values shown are the percentage changes in concentration in the liver, compared with NaCl-injected controls, when normal rats (○) or vitamin B_{12}-deficient rats (●) were injected with propionate or normal rats were infused with propionate (△). For other details see the Experimental section. Key for metabolites: A, lactate; B, pyruvate; C, acetyl-CoA; D, aspartate; E, malate; F, phosphoenolpyruvate; G, 2-phosphoglycerate; H, 3-phosphoglycerate; I, glycerol 3-phosphate; J, glucose.

B_{12}-deficient rats after administration of sodium propionate are compared in Fig. 1.

Inhibition of synthesis of glucose from lactate. A notable effect of vitamin B_{12} deficiency is the accumulation of lactate and pyruvate, suggesting that it inhibits conversion of these precursors into glucose. This was confirmed for lactate by the liver perfusion experiments (Table 4). A possible reason for this inhibition might be the decrease in the concentration of acetyl-CoA, an activator of pyruvate carboxylase (EC 6.4.1.1) (Utter, Keech & Scrutton, 1964). A direct relationship between the acetyl-CoA concentration and the rate of gluconeogenesis over a limited range in perfused livers has been reported by Toews, Lowy & Ruderman (1970). There was no change in the lactate or the pyruvate concentration in the two groups of normal rats in which the acetyl-CoA concentration was observed to decrease, although not to the same extent as in the liver of the vitamin B_{12}-deficient rats. Thus in the normal rats a decrease in acetyl-CoA concentration did not lead to an inhibition of pyruvate carboxylase. Possible reasons for this are that the decrease in acetyl-CoA is probably compensated by an increase in propionyl-CoA, also an activator of pyruvate carboxylase (Utter *et al.* 1964), or that the acetyl-CoA may not have decreased below the concentration that may be critical for the activation of pyruvate carboxylase. In the vitamin B_{12}-deficient animals the decrease in the concentration of acetyl-CoA and the increase in the concentration of propionyl-CoA are likely to be accompanied by an increase in the concentration of methylmalonyl-CoA, a powerful inhibitor of pyruvate carboxylase (Utter *et al.* 1964). Although no direct evidence for an increase in the concentration of methylmalonyl-CoA is available at present, the increased methylmalonate excretion in the deficient animals after propionate administration (Williams *et al.* 1969) is strong indirect evidence because methylmalonate can originate from deacylation of methylmalonyl-CoA. It is concluded that the inhibition of gluconeogenesis from lactate in the vitamin B_{12}-deficient animals is probably due to the decrease in acetyl-CoA concentration and an increase in methylmalonyl-CoA concentration. The inhibition would provide an explanation for the hyperlacticacidaemia that occurred in a patient with methylmalonicacidaemia (Linblad, Linblad, Olin, Svanberg & Zetterström, 1968).

Decrease in concentration of acetyl-CoA. A decrease in the concentration of acetyl-CoA in rat hearts perfused with acetate, after propionate or pentanoate was added to the medium, has been reported by Pearson & Tubbs (1967). Propionyl-CoA formed from propionate can take part in two reversible reactions, namely those catalysed by carnitine acetyltransferase (EC 2.3.1.7) and those catalysed by thiolase (EC 2.3.1.9), for which acetyl-CoA and CoA are also substrates. Therefore an increase in the concentration of propionyl-CoA would be expected to cause a decrease in the concentration of acetyl-CoA if the reactions are at equilibrium within the liver. The reciprocal changes in propionylcarnitine and acetylcarnitine concentrations on transition from the fed to the

Table 4. *Effects of propionate on synthesis of glucose from lactate by the perfused livers of normal, vitamin B_{12}-deficient and vitamin B_{12}-repleted rats*

All animals were starved for 48h before they were perfused. Substrates were added 40min after perfusion was begun and the perfusion was continued for a further 90min. The results are expressed as means±S.E.M., with the numbers of experiments in parentheses. The vitamin B_{12}-repleted rats were animals that, after being reared on the vitamin B_{12}-deficient diet, received 100μg of cyanocobalamin by intramuscular injection and were then put on to the standard diet 3 weeks before perfusion. The endogenous rate of gluconeogenesis without substrate in livers from normal rats is 8.4±0.18 (5) μmol/h per g fresh wt. The *t* test of the effect of propionate in vitamin B_{12}-deficient rats compared with corresponding normal controls gave a value of $P = 0.01$–0.001.

Dietary state	Substrates added	Glucose formed (μmol/h per g fresh wt.)	Glucose formed in 90min (μmol)	Lactate removed in 90min (μmol)
Normal	10mM-Lactate	63.6±5.4 (12)	595±34 (12)	1094±86 (12)
Normal	4mM-Propionate	30.3±1.4 (4)		
Normal	4mM-Propionate+ 10mM-lactate	59.5±6.2 (5)	468±67 (5)	553±83 (5)
Vitamin B_{12}-deficient	10mM-Lactate	39.1±2.9 (4)	358±24 (4)	951±28 (4)
Vitamin B_{12}-deficient	4mM-Propionate	7.8±0.1 (4)		
Vitamin B_{12}-deficient	4mM-Propionate+ 10mM-lactate	18.4±3.2 (4)	158±47 (4)	494±91 (4)
Vitamin B_{12}-repleted	4mM-Propionate+ 10mM-lactate	38.3±4.3 (4)	335±46 (4)	536±87 (4)

starved state (Bøhmer & Bremer, 1968) indicate that this may occur *in vivo*.

Other effects of propionate. The concentration of malate and aspartate in liver increased after the administration of propionate to normal rats (Fig. 1). These results are consistent with the known pathway of propionate metabolism, in which propionate is metabolized to succinate and then to malate. The absence of an increase in the concentration of these two metabolites in the livers of the vitamin B_{12}-deficient rats is further confirmation of the partial inhibition of this pathway in these rats. In view of this, and the observed accumulation of lactate, it is somewhat surprising to find marked increases in the concentrations of phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate in the livers of the vitamin B_{12}-deficient group (Fig. 1). Possible clues to the reason for this are the threefold decrease in the [3-hydroxybutyrate]/[acetoacetate] ratio and the decrease in total ketone bodies in the livers of the vitamin B_{12}-deficient group, reflecting an increase in the mitochondrial [free NAD^+]/[free NADH] ratio (Williamson *et al.* 1967) and a decrease in fatty acid oxidation. An important role of free fatty acid oxidation in the starved rat is to provide reducing equivalents required for glucose synthesis at the triose phosphate dehydrogenase step (Lardy, Paetkau & Walter, 1965). Recent evidence for this role obtained *in vivo* is the decrease in the mitochondrial [free NAD^+]/[free NADH] ratio when glucose synthesis is inhibited at the phosphopyruvate carboxykinase step in the starved rat (Williamson, Mayor & Veloso, 1970). Thus the increase in the concentration of 3-phosphoglycerate (and the two glycolytic intermediates linked to it via equilibrium reactions) may be a consequence of the more oxidized state of the mitochondrial NAD^+–NADH system and decreased transport of reducing equivalents to the cytoplasm. Additional evidence for this suggestion is the lower concentration of glycerol 3-phosphate found in the vitamin B_{12}-deficient group. The cause of the inhibition of fatty acid oxidation in this situation is not certain, but it may be due to the redistribution of CoA between its acylated forms.

Although propionate is not an important fuel in the rat, the experiments reported here may have more significance for vitamin B_{12} deficiency in sheep, because propionate is a major source of glucose in ruminants.

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