Metabolism of propionate by pair-fed vitamin B$_{12}$-deficient and vitamin B$_{12}$-treated sheep

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1. Metabolism of intravenously administered propionate was studied after 18 h starvation of pair-fed vitamin B$_{12}$-deficient and vitamin B$_{12}$-treated ewes fed on a cobalt-deficient diet.

2. The slower rate of propionate clearance in the deficient animals was accompanied by a slower initial rate of rise of blood glucose, blood lactate and blood pyruvate, but there was no depression in the rate of clearance of injected lactate or glucose in deficient animals as compared with their treated controls.

3. The increment in oxygen consumption that accompanied infusion of propionate was less pronounced and more delayed in deficient than in treated animals.

4. Injection of biotin had no effect on the rate of clearance of propionate in either a deficient or a treated animal.

5. The rate of acetylation of sulphanilamide after feeding was slightly faster in a deficient animal than in its pair-fed counterpart receiving vitamin B$_{12}$.

6. The results are discussed in relation to previous work with homogenates and it is concluded that the aberrant metabolism of propionate in vitamin B$_{12}$-deficient sheep may be entirely attributed to failure to convert methylmalonyl-CoA into succinyl-CoA in the animal's tissues.

Evidence has been presented that a depression in the rate of clearance of propionate is an early consequence of vitamin B$_{12}$ deficiency in sheep (Marston, Allen & Smith, 1971). The presence of elevated concentrations of propionate in the bloodstream of the deficient animal was shown to lead to a depression in the rate of clearance of acetate, and it was conjectured that such a failure to clear the volatile acids absorbed from the rumen could lead to the depression in appetite which is the major immediate cause of loss of weight.

In the present work a closer study was made of the metabolism of propionate in pair-fed vitamin B$_{12}$-deficient and vitamin B$_{12}$-treated sheep. The results are examined in terms of the known intermediary metabolism of propionate in sheep tissues and indicate a simple and specific failure of the tissues of the vitamin B$_{12}$-deficient animal to convert propionate into phosphoenolpyruvate and pyruvate.

EXPERIMENTAL

Animals. Australian Merino ewes, 2–5 years of age, were made deficient in vitamin B$_{12}$ and pair-fed as described by Smith & Marston (1970). Animals for pair-feeding were selected from a group of fifteen to twenty animals that had been given the cobalt-deficient diet until food intakes had declined. Selection was made on the basis of similarity of age, body-weight and food intake, and one member of each pair was chosen at random for treatment with vitamin B$_{12}$. The treated animal of each pair

received 50 μg cyanocobalamin/d intramuscularly and its daily food intake was restricted to that consumed by the deficient animal on the previous day. The degree of deficiency is indicated in the legends to tables and figures by food intake which fell from a maximum of 1050 g/d to the quantities shown. At food intakes around 200 g/d the deficiency was severe and death was likely to occur within a few weeks unless the animal was treated with vitamin B_{12}.

Respiratory exchange. The open-circuit calorimeter described by Marston (1948) was used. Isotonic saline (0·9 % NaCl, w/v) or M-sodium propionate (pH 7·4) was administered by means of a polyethylene cannula (0·5 mm internal diam.) inserted in the jugular vein and entering the chamber through a rubber stopper. Flow-rate (83 ml/h) was maintained with a crank-operated syringe incorporating a system of ground-glass valves, and driven by an electric motor. Total flow was checked by depletion of the reservoir.

Clearance rates of injected metabolites. Acids were injected as 1·0 M-solutions of the sodium salts (pH 7·4) via a polyethylene cannula inserted into the jugular vein 2 h before the test and containing a dilute solution of heparin in isotonic saline. Solutions were injected from a glass syringe at a uniform rate over 5 min. Blood samples (5 ml) were withdrawn through the same cannula immediately before and 3 min after the injection and then at intervals for up to 4·5 h. After each operation the cannula was washed through by injecting saline, and then filled with dilute heparin in saline. All tests were made with the animals confined in cages. Animals were without food during the tests and for 18 h beforehand, but they had free access to water.

Chemical methods. Protein-free extracts of blood were prepared by centrifugation after treatment with tungstic acid or, for estimation of pyruvate, with trichloroacetic acid. Volatile acid was estimated as described by Marston *et al.* (1971), glucose by the iodometric method of Somogyi (1952), lactate by the method of Barker & Summerson (1941) and pyruvate by the method of Friedemann & Haugen (1943). Sulphanilamide in blood and urine was estimated by the method of Bratton & Marshall (1939).

Materials. D-Biotin was obtained from L. Light and Co., Colnbrook, Bucks. Other materials were as previously described (Smith & Marston, 1970) or were reagent grade chemicals.

Statistics. Because of the way in which animals were selected for pair-feeding, analysis of variance of results was judged to be inappropriate and statistical tests were confined to comparisons of means and their standard errors. Repeated observations on individual pairs were converted into mean values and weighted according to the number of observations in calculating means and variances for treatments. In many instances the variances of the means for treatments to be compared differed significantly by the F test and the significance of the difference in treatment means was determined by the d test (Fisher & Yates, 1948). Where the variances did not differ significantly, these were pooled and the significance of the difference between treatment means was determined by the t test.

RESULTS

Response of blood glucose, lactate and pyruvate to injection of propionate. Propionate clearance rates and associated changes in blood glucose after injecting 3·5 m-mol propionate/kg body-weight were determined in twenty tests with eleven pairs of animals. The relationship between the rate of clearance of propionate and the degree of deficiency as indicated by daily food intake in these tests has been described (Marston *et al.* 1971). The tests were made after 18 h starvation when mean blood
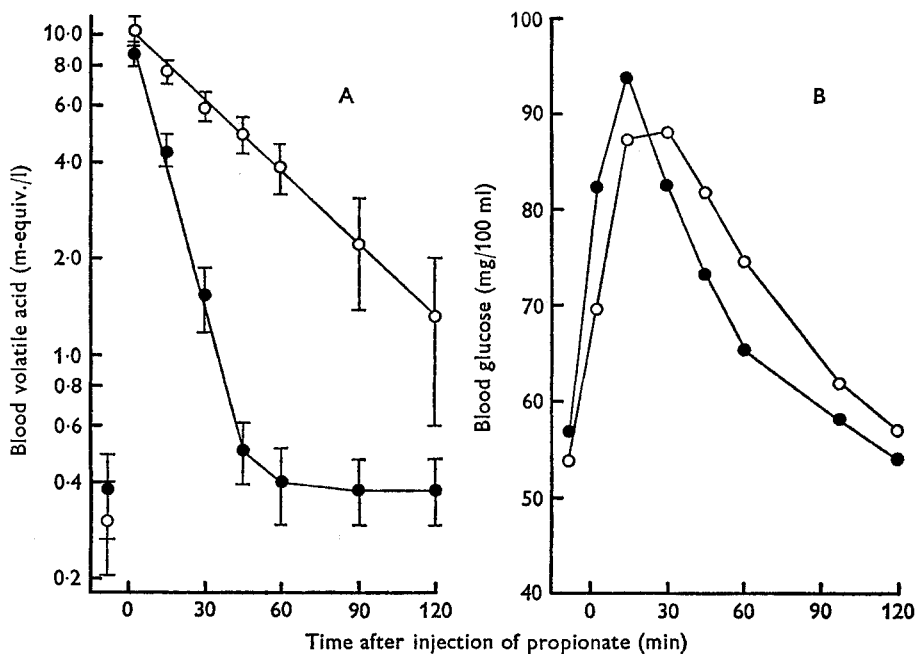

Fig. 1. Volatile acid and glucose concentrations in blood of vitamin B_{12}-deficient and pair-fed vitamin B_{12}-treated sheep after injection of propionate. Results shown are the means of eleven tests on eight pairs of animals whose food intake covered the range 287–555 g/d. Propionate (3·5 m-mol/kg body-weight) was injected intravenously from −5 to 0 min in both deficient (○) and treated (●) animals. A shows the means and 95 % confidence intervals for volatile acid plotted on logarithmic ordinates and B shows mean blood glucose. The blood glucose concentration of deficient and treated animals differed significantly ($P < 0·05$) only in the 3 min samples.

glucose of deficient animals was $57·3 \pm 3·0$ (SEM) mg/100 ml, and of pair-fed animals receiving vitamin B_{12} was $55·8 \pm 1·6$ mg/100 ml. The difference was not significant by the *d* test with 10 degrees of freedom for each treatment. In all tests blood samples were taken immediately before and 3 min after injection of propionate, the injection occupying 5 min. In nine of the tests (five pairs of animals) further blood samples were taken at 30 min intervals for 3·5 h, and in eleven tests (eight pairs) samples were taken at 15 min intervals for 1 h, then at 30 min intervals for a further 1 h. The mean concentrations of blood volatile acid and blood glucose in the latter tests are shown in Fig. 1.

Fig. 1A shows the means and 95 % confidence intervals for volatile acid concentrations in blood. Apart from the 3 min values, volatile acid concentrations in the blood of deficient and treated animals were significantly different over the entire 2 h period. Fig. 1B shows the associated changes in blood glucose. In the deficient animals blood glucose appeared to rise more slowly and to stay high longer than in animals receiving vitamin B_{12}. The results for glucose, however, were more variable than those for volatile acid and, except for the 3 min value, the corresponding points in time for deficient and treated animals did not differ significantly. At 3 min, blood glucose in the treated animals was significantly higher ($P < 0.05$ by the t test with 14 df) than that in deficient animals.
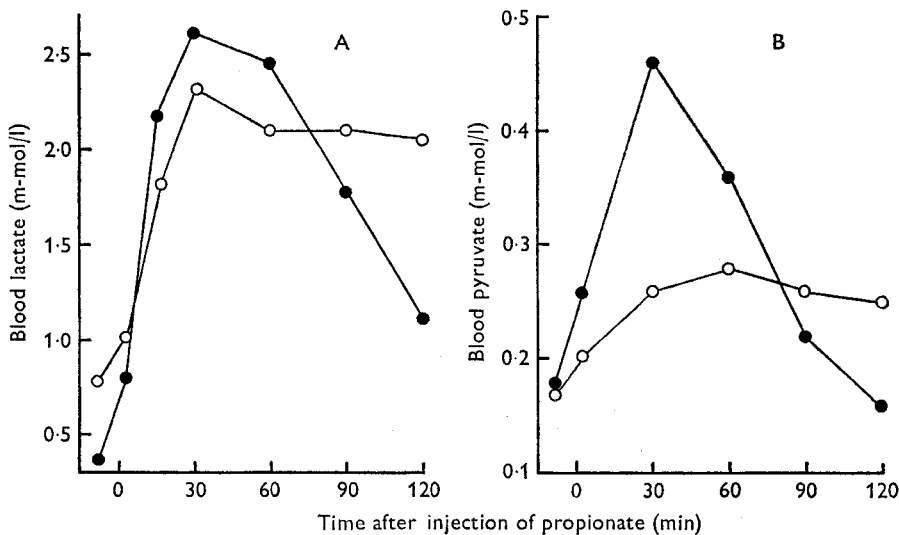

Fig. 2. Blood lactate and pyruvate concentrations after injection of propionate in vitamin B_{12}-deficient and pair-fed vitamin B_{12}-treated sheep. The experiments were performed with different pairs of animals. Both untreated animals were severely deficient and consuming about 210 g food/d. The responses follow intravenous injection of 3·5 m-mol propionate/kg body-weight. O, deficient; ●, pair-fed and treated with 50 µg vitamin B_{12}/d.

The slower rate of rise of blood glucose in deficient animals after propionate had been injected was borne out by the combined results for all twenty tests. In deficient animals the mean rise in blood glucose between the pre-injection sample and the sample taken 3 min after the injection was complete was by 14.4 ± 1.2 (SEM) mg/100 ml, whereas in treated animals it was by 24.4 ± 2.2 mg/100 ml, the difference being significant ($P < 0.01$ by the d test with 10 df for each treatment).

In other tests not shown, smaller amounts of propionate were injected (1·0 or 1·75 m-mol/kg). The rises in blood glucose during the injection were smaller, but the difference between deficient and treated animals was maintained.

In six of the tests either blood lactate or blood pyruvate was also determined and the results for two of these experiments are shown in Fig. 2. Both lactate and pyruvate rose more slowly and remained high longer after propionate injection in deficient animals than in pair-fed animals receiving vitamin B_{12}.

Rates of clearance of injected lactate and glucose. Rates of clearance of injected DL-lactate were measured in five tests with three pairs of animals. All the untreated animals were in an advanced state of vitamin B$_{12}$ deficiency with food intakes ranging from 190 to 420 (mean 250) g/d, after 44–57 weeks on the cobalt-deficient diet. DL-Lactate (6·0 m-mol/kg body-weight) was injected after 18 h starvation and lactate and glucose in blood were determined at 30 min intervals for 4·5 h.
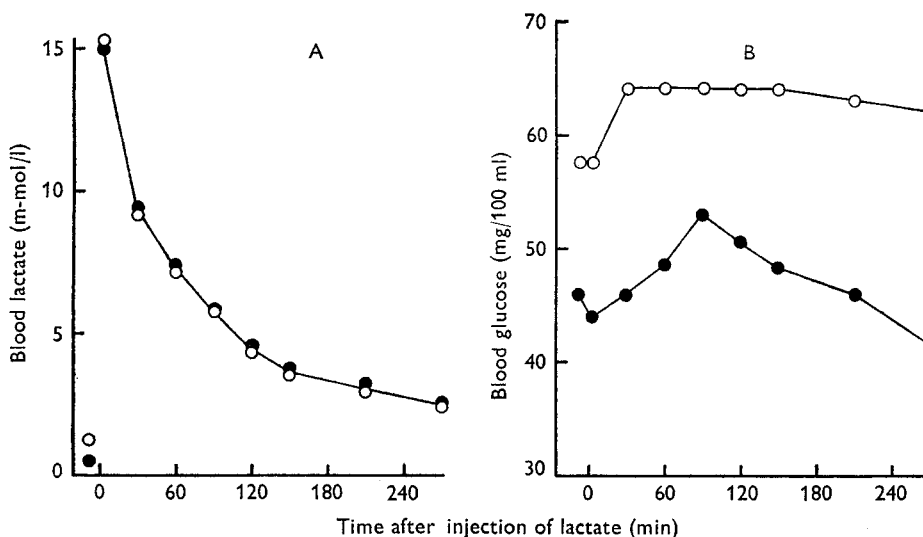

Fig. 3. Rate of clearance of injected lactate by vitamin B$_{12}$-deficient and pair-fed vitamin B$_{12}$-treated sheep. Blood lactate (A) and glucose (B) concentrations were determined over 4·5 h after intravenous injection of 6·0 m-mol DL-lactate/kg body-weight. The untreated animal was severely deficient, with a food intake of about 200 g/d. O, deficient; ●, pair-fed and treated with vitamin B$_{12}$.

In the five observations, initial blood lactate concentrations in deficient animals (0·86 ± 0·15 (SEM) m-mol/l) were higher, but not significantly higher (by the *d* test with 2 df for each treatment) than those in pair-fed animals receiving vitamin B$_{12}$ (0·61 ± 0·03 (SEM) m-mol/l). Injection of lactate raised the blood concentration to more than 15 m-mol/l and this then declined continuously for 4·5 h. Over the first 140 min blood lactate fell exponentially to about 4 m-mol/l, when there was a sharp decline to a slower rate that was assumed also to have been exponential. In the five tests made, the apparent first-order rate-constants for decline of blood lactate in deficient animals averaged $8·1 \times 10^{-3}$ min^{-1}, falling to $4·4 \times 10^{-3}$ min^{-1} below 4 m-mol/l. In treated animals the rate-constants averaged $8·8 \times 10^{-3}$ min^{-1} falling to $3·6 \times 10^{-3}$ min^{-1} below 4 m-mol/l. The differences were not significant.

The results of one of the lactate clearance tests are shown in Fig. 3 which also shows the response in blood glucose. The slow rise and fall in blood glucose after the injection of lactate seen in Fig. 3 did not occur in all experiments and it never exceeded 12 mg/100 ml. In one experiment blood pyruvate was also measured. Following injection of lactate, blood pyruvate rose by about 0·3 m-mol/l in the 3 min sample,

then declined to the pre-injection level in about 150 min in both deficient and treated animals.

In four tests with three pairs of animals the rate of clearance of injected glucose (1·0 or 1·75 m-mol/kg body-weight) was determined. No differences were detected between deficient and treated animals, and in two of the tests (1·0 m-mol glucose/kg) where this was measured there was no response in blood pyruvate in either animal. In the glucose tests the untreated animals were all in an advanced state of deficiency and food intakes were 200–400 g/d.

Oxygen consumption following intravenous injection of sodium propionate. Intra-ruminal infusion of propionic acid in sheep starved for 2–4 d was shown by Armstrong & Blaxter (1957) to lead to increased oxygen consumption, increased evolution of carbon dioxide and an elevated blood glucose. The heat increment of propionic acid under these conditions was about 13 %.

In the present work experiments were conducted with three pairs of deficient and treated animals to discover whether this feature of propionate metabolism was impaired in vitamin B_{12} deficiency. Preliminary tests with an animal receiving vitamin B_{12} showed, however, that after 3 d starvation the response of blood glucose to intra-venous injection of propionate (3·5 m-mol/kg body-weight) differed from that obtained after 18 h starvation (Fig. 1). Although blood glucose rose in 15 min to about 100 mg/100 ml, it then remained at this level until observations ceased after 2 h. For this reason comparative measurements of O_2 consumption after injection of propionate in deficient and treated animals were made after only 20 h starvation.

All animals had consumed the cobalt-deficient diet for between 52 and 57 weeks and treated animals had been pair-fed and had received vitamin B_{12} for between 23 and 39 weeks. Untreated animals were all in an advanced state of deficiency, with food intakes from 330 to 380 g/d. Animals were 3·5 years of age and body-weights had declined from about 40 kg to the values given in Table 1. With two of the pairs (trials 2 and 3 in Table 1), propionate clearance tests were conducted 1 week before the respiratory measurements. Apparent first-order rate-constants for removal of propionate from the blood-stream (3·5 m-mol/kg) were 0·019 and 0·013 min^{-1} for the two deficient animals and 0·069 and 0·058 min^{-1} for the two treated animals respectively.

Animals were fed for 4 h on the day before administration of propionate, then polyethylene cannulas were inserted into the jugular veins and animals were starved in the calorimeters while sterile isotonic saline was administered. After 18 h, four consecutive 2 h samples of respired air were collected and analysed. During the first of the 2 h periods isotonic saline was again administered, followed for 2 h with a sterile solution of sodium propionate (1 M, pH 7·4), then again with saline for the final two 2 h periods. Total propionate given was 166 m-mol.

The results for O_2 consumption and evolution of CO_2 for the four 2 h periods are shown in Table 1. While each of the treated animals showed a sharp response in O_2 consumption that was complete within 2 h of discontinuing the injection of propion-ate, the deficient animals showed a more delayed response. There was no consistent alteration in CO_2 production by either deficient or treated animals on injection of

Table 1. *Response in oxygen consumption and carbon dioxide evolution of pair-fed vitamin B$_{12}$-deficient and vitamin B$_{12}$-treated ewes to infusion of propionate*

(Animals were starved for 20 h before being injected with 166 m-mol propionate intravenously over 2 h. Respiration was measured over 2 h periods before, during and for 4 h after the injection. When propionate was not being injected, the animals received isotonic saline at the same rate (83 ml/h))

Animal no.	Body-wt (kg)	Treatment	Trial no.	Oxygen consumption (l/2 h)				CO$_2$ evolution (l/2 h)			
				Saline 0–2 h	Propionate 2–4 h	Saline 4–6 h	Saline 6–8 h	Saline 0–2 h	Propionate 2–4 h	Saline 4–6 h	Saline 6–8 h
263	26·4	B$_{12}$-deficient	1	16·6	16·3	15·6	17·4	12·2	11·5	11·1	11·7
205	25·8	B$_{12}$-deficient	2	11·9	13·1	13·6	14·6	9·4	10·1	9·9	10·7
433	27·8	B$_{12}$-deficient	3	15·7	16·1	15·4	16·6	11·0	11·8	10·6	11·5
Mean				14·7	15·2	14·9	16·2	10·9	11·1	10·5	11·3
325	28·7	B$_{12}$-treated and pair-fed with animal 263	1	11·7	14·0	14·1	11·3	9·0	9·3	9·8	7·8
341	26·3	B$_{12}$-treated and pair-fed with animal 205	2	13·0	15·3	14·8	12·7	9·6	8·7	8·7	9·2
218	27·7	B$_{12}$-treated and pair-fed with animal 433	3	13·5	17·1	15·2	13·0	9·3	9·7	9·7	9·9
Mean				12·7	15·5	14·7	12·3	9·3	9·2	9·4	9·0

propionate, but this may have been obscured by release of Na^+ on conversion of propionate into non-acidic products.

Although values for respiratory exchange over 2 h periods cannot be regarded as precise, the oxygen increment on injection of sodium propionate into animals receiving vitamin B_{12} (about 40 % of the O_2 required for complete combustion) was substantially greater than that obtained by Armstrong & Blaxter (1957) on infusing propionic acid into the rumen. In deficient animals over the 6 h period studied, the oxygen increment was of the order of 20 % of that required for complete combustion, but this value is based on the increment relative to the first 2 h period only and may be underestimated. The fasting O_2 consumption of deficient animals after this period of starvation was found still to be falling (Smith & Marston, 1970).

The results show that although the respiratory response of deficient animals to injected propionate was qualitatively similar to that of pair-fed animals receiving vitamin B_{12}, the stimulation of O_2 consumption was much less sharp, and was more prolonged.

Lack of effect of biotin on the clearance rate of injected propionate. Biotin is a component of the enzyme propionyl-CoA carboxylase (Halenz & Lane, 1960; Kaziro, Ochoa, Warner & Chen, 1961), and Marchetti & Testoni (1964) have shown that treatment of biotin-deficient rats with vitamin B_{12} increases the biotin content of the liver. The possibility was therefore considered that the reduced rate of propionate clearance in vitamin B_{12}-deficient sheep may have been due in part to a lack of available biotin with a consequent reduction in carboxylating enzyme activity.

Three propionate clearance tests were conducted at weekly intervals on a deficient animal and its pair-fed control that received vitamin B_{12}. Food intake was about 300 g/d, and body-weights had declined from about 46 kg to about 32 kg in 55 weeks. Between the first and second tests both animals received 100 μg biotin/d (intramuscularly), for 6 d, and between the second and third tests the dose was increased to 1 mg/d. In neither animal was there any perceptible effect on the rate of clearance of propionate. In the deficient animal the apparent first-order rate-constant was 0·014 min^{-1} for all three tests, and in the treated animal the rate-constants were 0·050, 0·041 and 0·046 min^{-1} in the three tests respectively.

Rates of acetylation of injected sulphanilamide. The inhibition of acetate metabolism by propionate in slices and homogenates from the livers of rats and sheep was suggested by Pennington & Appleton (1958) to be due to a depressed rate of formation of acetyl-CoA. A very marked depression of acetate clearance by propionate was found in vitamin B_{12}-deficient sheep by Marston et al. (1971) and was suggested to be the cause of the observed accumulation of acetate in the blood of deficient animals after feeding. In the present work direct evidence for impaired acetyl-CoA formation after feeding was sought by measuring the rate of acetylation of injected sulphanilamide in pair-fed deficient and treated animals.

In three experiments with fed sheep, urine samples were collected over 24 h after intravenous injection of 150 mg or 200 mg sulphanilamide, and free and total sulphanilamide were estimated. Both deficient and treated animals within each pair consumed the same amount of food over the 24 h (306–480 g) but there were no

consistent differences in the extent of acetylation of the sulphanilamide in the urine (50–65 % acetylated).

Two experiments were then performed with a severely deficient animal and its pair-fed and treated control, in which the rate of acetylation was studied in blood. Both animals had been fed on the cobalt-deficient diet for 48 weeks, and the treated animal had been pair-fed and had received vitamin B_{12} for 18 weeks. Food intake was about 180 g/d and body-weights were 22 kg (deficient) and 20 kg (treated). The animals were injected with 300 mg sulphanilamide in 50 ml isotonic saline at the time of feeding (185 g) and free and total sulphanilamide in blood were determined at intervals over 7 h. The results are shown in Fig. 4, expressed as a percentage of total blood sulphanilamide acetylated. The rate of acetylation was somewhat faster in the deficient than in the treated animal and similar results were obtained in a second experiment 7 d later.
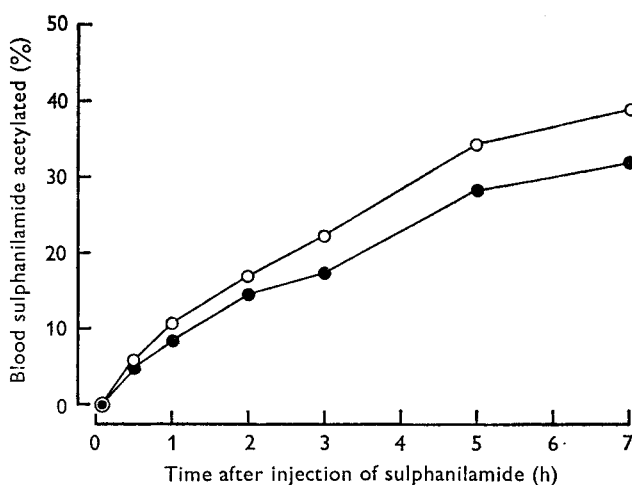

Fig. 4. Rate of acetylation of injected sulphanilamide by vitamin B_{12}-deficient and pair-fed vitamin B_{12}-treated sheep. Concentrations of free and total sulphanilamide in blood were determined at intervals for 7 h after intravenous injection of 300 mg sulphanilamide. The untreated animal was severely deficient with a food intake of about 180 g/d. The animals were allowed access to food during the experiment. O, deficient; ●, pair-fed and treated with vitamin B_{12}.

DISCUSSION

Except in lactating mammary gland (James, Peeters & Lauryssens, 1956; Katz & Kornblatt, 1962), the major pathway of propionate metabolism in animal tissues is believed to be by fixation of carbon dioxide on to propionyl-CoA to form methyl-malonyl-CoA, which is then converted into succinate (Flavin & Ochoa, 1957). After formation of propionyl-CoA, three enzymes are involved specifically in the introduction of propionate carbon into the pool of common metabolic intermediates. These are respectively the biotin-enzyme propionyl-CoA carboxylase (Kaziro, Grossman & Ochoa, 1965), methylmalonyl-CoA racemase (Mazumder, Sasakawa, Kaziro & Ochoa, 1962) and methylmalonyl–CoA mutase (Cannata, Focesi, Mazumder, Warner & Ochoa, 1965). The last enzyme in the sequence converts L-methylmalonyl-CoA into succinyl-CoA and it contains tightly bound 5′deoxyadenosyl-cobalamin as coenzyme

(Cannata *et al.* 1965). Liver homogenates from vitamin B_{12}-deficient sheep fail to metabolize propionate, and this is accompanied by a failure to convert methyl-malonyl-CoA into succinate when propionyl-CoA is the substrate. The latter capacity is restored by addition of 5'deoxyadenosyl cobalamin (Marston & Smith, 1961).

Part of the energy and much of the glucose required by sheep is derived from propionate produced in the rumen (Judson, Anderson, Luick & Leng, 1968). Experiments with infused labelled metabolites indicate that 25–50 % of absorbed propionate is converted into glucose in the sheep's body, while 10–50 % is promptly oxidized (Bergman, Roe & Kon, 1966; Leng, Steel & Luick, 1967; Annison, Brown, Leng, Lindsay & West, 1967). Oxidation of succinate to oxaloacetate and conversion of the latter into phosphoenolpyruvate or pyruvate provides a rational sequence to explain respectively the conversion of propionate into glucose via phosphoenolpyruvate or its oxidation via pyruvate. There is evidence (Leng *et al.* 1967), however, that a substantial fraction of the propionate that is converted into glucose in the fed animal first forms lactate. Conversion of lactate into glucose involves the reformation of oxalo-acetate at the expense of a terminal phosphate bond of ATP (Scrutton & Utter, 1968). Formation of lactate from propionate on the way to forming glucose is therefore energetically wasteful and may account in part for the heat increment of propionate. Lactate is a major end-product of propionate metabolism by rumen epithelium (Pennington & Sutherland, 1956) and some lactate will be formed from propionate during its absorption from the rumen. Substantial amounts of lactate, however, are also produced from propionate by sheep liver slices (Leng & Annison, 1963).

Under normal feeding conditions, most of the absorbed propionate that reaches the portal blood is metabolized during its first passage through the liver, but there appears to be an upper limit in propionate concentration in portal blood beyond which no additional uptake by the liver occurs (Cook & Miller, 1965). In the present experiments, under propionate load, high concentrations of propionate were maintained in peripheral circulation and metabolism by extrahepatic tissues must be considered.

In sucrose homogenates of normal sheep liver, metabolism of propionate occurs at significant rates only in the mitochondria and only by the methylmalonate pathway (Smith, Osborne-White & Russell, 1967). At maximum stimulation the mitochondrial rate is stable (17·5 μmol propionate consumed/mg mitochondrial nitrogen per h at 37°) and appears to be limited by the activity of propionyl-CoA carboxylase (Smith & Russell, 1967 b). From the average yield of mitochondria combined with the efficiency of homogenization in eight preparations, it is estimated that sheep liver contains about 4·8 mg mitochondrial nitrogen/g, suggesting a maximum rate of propionate uptake by liver of about 85 μmol/g per h (R. M. Smith & W. S. Osborne-White, unpublished results). The mean body-weight of the vitamin B_{12}-treated ewes referred to in Fig. 1 was 29·6 kg, and by interpolation from measured values a liver weight of about 380 g may be assigned. This leads to a maximum metabolism in the liver of these animals of about 32 m-mol propionate/h as compared with the observed rate of clearance of 105 m-mol in 45–50 min. Despite the approximate nature of these calculations, it seems safe to conclude that extrahepatic tissues must have been involved.

Pennington (1952) found relatively rapid rates of propionate utilization by sheep

liver and kidney slices and by the epithelium of parts of the alimentary tract, with low or negligible rates in the other tissues examined. Quantitatively, liver and kidneys would be expected to contribute most significantly to propionate removal under propionate load, but it is possible that the epithelium of the alimentary tract would also metabolize propionate from the blood-stream. Liver and kidney cortex are known to be active sites of gluconeogenesis in mammals, and net synthesis of glucose from propionate or lactate has been observed in liver and kidney slices from rodents (Krebs, Notton & Hems, 1966; Ross, Hems & Krebs, 1967). Sheep rumen epithelium produces lactate from propionate and there is evidence that all three tissues from sheep use the methylmalonate pathway (Flavin, Ortiz & Ochoa, 1955; Pennington & Sutherland, 1956; Smith *et al.* 1967). Under the conditions of our experiments, therefore, it is probable that removal of propionate from the blood-stream reflected metabolism to pyruvate, lactate and glucose via the methylmalonate pathway. The rate of propionate clearance in vitamin B_{12}-deficient animals was greatly retarded and this was accompanied by a delayed response in oxygen consumption, and by a slower rise of blood glucose, lactate and pyruvate.

The sharp rise in blood glucose after injection of sodium propionate into normal animals, however, is not generally accepted as representing glucose newly synthesized from propionate (Ash, Pennington & Reid 1964). Equivalent quantities of sodium butyrate when injected cause an even greater rise in blood glucose (Potter, 1952; Ash *et al.* 1964), and this seems to be due to glycogenolysis accompanying a rapid response to butyrate of liver phosphorylase activity (Phillips, Black & Moller, 1965). The rate of clearance of propionate in sheep, however, is much faster than that of butyrate and is accompanied by a 170 % greater rise in blood pyruvate (Jarrett & Potter, 1957). Annison, Leng, Lindsay & White (1963) found that injection of substantial quantities of labelled propionate into anaesthetized sheep led to little labelling of liver or muscle glycogen, but circulating lactate, and particularly glucose, became heavily labelled. The rise in blood lactate and pyruvate that accompanied the disappearance of propionate in vitamin B_{12}-treated sheep can account for no more than one-third of the propionate metabolized in 30 min, and the estimated total consumption of oxygen by the animal in this time for no more than two-thirds. Whether or not a net breakdown of glycogen occurred when propionate was injected, it is difficult to avoid the conclusion that part, at least, of the rise in blood glucose and lactate must be attributed to synthesis from propionate, particularly in the absence of an increment in CO_2 evolution (Table 1). The delayed responses to propionate in the deficient animals together with the normal rates of clearance of glucose and lactate strongly suggest a failure of the deficient tissues to convert propionate into phosphoenolpyruvate and pyruvate.

Propionate and acetate are believed to be activated by a common CoA ligase (Hele, 1954; Smith & Russell, 1967*a*) and this is consistent with the inhibition of acetate clearance by propionate in both deficient and treated animals (Marston *et al.* 1971). Propionate clearance was not inhibited by acetate, however, in either deficient or treated animals; in deficient animals the clearance rate of acetate alone was only slightly impaired (Marston *et al.* 1971). These observations, together with the enhanced rate

of acetylation of injected sulphanilamide in a deficient animal after feeding (Fig. 4) and the elevated CoA concentrations in deficient livers (Dawbarn, Forsyth & Kilpatrick, 1963), make it highly unlikely that activation of propionate was impaired in deficient sheep. Similarly, the failure of injected biotin to modify the rate of propionate clearance in a deficient animal combined with the normal rate of carboxylation of propionyl-CoA in deficient liver homogenate (Marston & Smith, 1961) give no grounds to suspect failure at this point.

The constriction of propionate metabolism in vitamin B_{12}-deficient sheep described in the present paper is in complete accord with the failure of liver homogenates to convert methylmalonyl-CoA into succinate, or with the accumulation after feeding of methylmalonic acid in liver and blood of deficient sheep (Smith, Osborne-White & Russell, 1969). All the effects may be attributed to depletion of 5′deoxyadenosyl cobalamin in the tissues, with a consequent loss of activity of methylmalonyl-CoA mutase.

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