Gluconeogenesis from caecal propionate in the horse

BY E. J. H. FORD AND H. A. SIMMONS

Department of Veterinary Clinical Science, University of Liverpool, Leahurst, Neston, South Wirral L64 7TH

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1. The production of propionate in the caecum of the horse has been measured in two Shetland-type ponies fitted with caecal and colonic cannulas and fed on hay or on hay and wheat bran. A continuous intracaecal infusion of ^{14}C-labelled sodium propionate was used and samples were obtained from a cannula at the origin of the right ventral colon. A simultaneous intravenous infusion of [2-^{3}H]glucose was used to measure total glucose entry.

2. On a hay diet which provided 177 kJ/kg body-weight per d, mean caecal propionate production was 19·6 (range 17·2–21·2) mg/h per kg body-weight and on a hay and wheat bran diet, which provided 187 kJ/kg body-weight per d, mean caecal propionate production was 34·0 (range 28·9–38·3) mg/h per kg body-weight.

3. Mean total glucose production (mg/h per kg body-weight) in one pony was 104 (range 100–110) and in the other 135 (range 123–153). Rates were not influenced by diet.

4. About 7% of total glucose production was derived from propionate produced in the caecum and this percentage was unaffected by diet or by individual animals.

Herbivorous animals convert a variable proportion of dietary carbohydrate to fatty acids by bacterial fermentation. In ruminants the process takes place mainly in the rumen and there is extensive literature on the production and absorption of the short-chain or volatile fatty acids, acetic, butyric and propionic. Propionic acid is an important substrate for gluconeogenesis in ruminants. Estimates of the percentage of glucose arising in this way range from 27 (Bergman *et al.* 1966) and 36–56 (Judson *et al.* 1968) to 28–63 (Ford & Winchester, 1974).

It is generally thought that the caecum and colon of equidae perform a similar function to the rumen and whilst there are reports on the production of volatile fatty acids in the caecum, there is little information on the synthesis of glucose from substrates produced in this way. Therefore, we have used the technique of isotope dilution to measure propionate production and its conversion to glucose in ponies fitted with infusion and sampling cannulas in the caecum and at the origin of the right ventral colon.

MATERIALS AND METHODS

Animals

Two female ponies, weighing between 120 and 170 kg and aged 3–4 years, were bedded on wood shavings in loose boxes and fed on one of two diets with free access to drinking-water. One diet consisted of chopped hay and provided 177 kJ/kg body-weight per d and the other, which provided 187 kJ/kg body weight per d, consisted of chopped hay with 27% of the hay replaced by wheat bran. Under general anaesthesia, screw-capped plastic cannulas were inserted into the caecum and into the right ventral colon, about 100 mm from its origin at the caecum. The cannulas were a modification of those described by Alexander (1970). Animals were not used for experiments until they had completely recovered from surgery and were eating the full diet and maintaining steady body-weight. They were trained to stand in stocks during infusions and had access to food and water throughout these periods. At any one time one pony was fed on hay and wheat bran and the other on hay. Diets were then switched and, after a settling-down period, a second series of measurements was made.

Experimental procedure

Before infusion, a polyethylene cannula (o.d. approximately 2 mm) was inserted into each jugular vein under local anaesthesia. Each experiment commenced with the collection, by gentle suction, of a sample of digesta from the lumen of the gut through a tube inserted in the cannula in the right ventral colon. This was followed by the continuous infusion into the caecum by peristaltic pump of sodium [2-^{14}C]propionate (Amersham International plc, Amersham, Bucks) at a rate of, usually, between 400 and 800 nCi/min for about 8 h. Samples of digesta were collected by gentle suction through the cannula in the right ventral colon at 30-min intervals from the fourth to eighth hour of infusion. Preliminary experiments had shown that, irrespective of diet, a reasonable plateau of propionate specific radioactivity was obtained from 3·5 to 4 h after the commencement of infusion. At about 4 h after the commencement of the infusion, a priming dose of 40 μCi[2-^{3}H]glucose was injected through the indwelling polyethylene cannula which had been previously fitted in the left jugular vein. The injection was followed by the continuous infusion of [2-^{3}H]glucose in buffered sodium chloride (9 g/l) at the rate of approximately 500 nCi/min for 4 h. Blood samples were collected at intervals from the cannula in the right jugular vein.

Chemical methods

The samples of digesta collected from the cannula in the colon were deproteinized, steam-distilled and titrated. The Na salts of the fatty acids were evaporated to dryness and applied to a preparative gas–liquid chromatograph (Pye Model 104). The propionate fraction was collected in toluene as described by Ford & Winchester (1974). The amount of propionate in a portion of the toluene solution was measured with an analytical gas–liquid chromatograph and the rest of the solution was transferred to a vial containing 10 ml scintillation fluid which contained 5 g 2,5-diphenyloxazole (PPO) and 0·4 g 1,4-di-2(5-phenyloxazole)benzene (POPOP)/l toluene. Radioactivity was measured in an automatic liquid-scintillation counter (Packard Model 2450) with external standard.

The jugular blood samples were centrifuged and the plasma separated. The concentration of glucose in plasma was measured by the enzymic method of Huggett & Nixon (1957). After the addition of 100 mg unlabelled glucose to a portion of each sample, glucose penta-acetate was prepared by the method of Jones (1965). After drying to constant weight, the glucose penta-acetate was dissolved in the scintillation fluid previously described and the radioactivities of ^{3}H- and ^{14}C-labelled glucose were measured in the automatic liquid-scintillation spectrometer by the channels-ratio method of Hetenyi & Reynolds (1967).

RESULTS

Propionate production

Table 1 gives the results of eleven infusions in the two ponies which were fed on diets of hay and of hay plus wheat bran. Expts 1, 2, 3, 7 and 8 were done on one pony and Expts 4, 5, 6, 9, 10 and 11 on the other. The production rate of caecal propionate (Table 1) was obtained by the formula: production rate (mg C/min) = rate of infusion of radioactive propionate (nCi/min) divided by mean plateau specific activity of caecal propionate (nCi/mg C). The results given in Table 1 suggest that the production of caecal propionate expressed as mg C/min was greater in the second pony irrespective of the diet fed. However, when the production rate was adjusted relative to the body-weight of the ponies and the mean values were compared (Table 1), there appeared to be no animal effect. The mean rate of production (mg propionate/h per kg body-weight) in both ponies when on the hay diet was 19·6 (range 17·2–21·2) and when on the hay and wheat bran diet was 34·0 (range

Table 1. *The effect of diet on the production of propionate in the caecum and on the percentage of glucose derived from caecal propionate in the pony*

Pony no.	Wt of pony (kg)	Expt no.	[^{14}C]propionate infused (nCi/min)	Mean plateau SA of propionate (nCi/mg carbon)	Propionate production rate		Mean plateau SA of [^{14}C]glucose (nCi/mg carbon)	Percentage of glucose from caecal propionate
					mg C/min	mg/h per kg body-wt*		
				(*a*) Hay				
1	125	1	710	33	21·5	21·2	2·3	7·0
1	125	2	824	40	20·6	20·3	1·7	4·3
1	120	3	459	27	17·0	17·4	2·5	9·3
2	165	4	668	24	27·8	20·8	1·4	5·8
2	165	5	460	20	23·0	17·2	1·4	7·0
2	165	6	578	21	27·5	20·6	1·5	7·1
				(*b*) Hay and wheat bran				
1	125	7	454	12·5	36·3	35·8	0·8	6·4
1	125	8	822	21·2	38·8	38·3	1·7	8·0
2	170	9	675	15·3	44·1	32·0	0·9	5·9
2	170	10	626	15·7	39·9	28·9	1·4	8·9
2	170	11	1254	26·1	48·0	34·8	1·7	6·5

SA, specific activity.

* Mean propionate production rate on hay, 19·6 mg/h per kg body-wt (range 17·2–21·2); mean production rate on hay and wheat bran, 34 mg/h per kg body-wt (range 28·9–38·3).

Table 2. *The effect of diet on total glucose entry rate and on the production of glucose from caecal propionate in the pony*

Pony no.	Wt of pony (kg)	Expt no.	[^{3}H]glucose infused (nCi/min)	Mean plateau SA of [^{3}H]glucose (nCi/mg carbon)	Glucose production rate		Percentage of glucose from caecal propionate	Amount of glucose from propionate		Percentage of propionate converted to glucose
					mg C/min	mg/h per kg body-wt*		mg C/min	mg/h per kg body-wt	
					(*a*) Hay					
1	125	1	458	5·0	92	110	7·0	6·4	7·7	30
1	125	2	352	4·2	84	101	4·3	3·6	4·3	18
1	120	3	365	4·5	81	101	9·3	7·5	9·4	44
2	165	4	589	4·1	144	131	5·8	8·4	7·7	30
2	165	5	538	3·2	168	153	7·0	11·8	10·7	51
2	165	6	500	3·7	135	123	7·1	9·6	8·7	35
					(*b*) Hay and wheat bran					
1	125	7	221	2·65	83	100	6·4	5·3	6·4	21
1	125	8	220	2·5	88	106	8·0	7·0	8·5	23
2	170	9	555	3·8	146	129	5·9	8·4	7·6	20
2	170	10	601	3·7	162	142	8·9	14·4	12·6	36
2	170	11	562	3·7	152	134	6·5	10·0	8·7	21

SA, specific activity.

* Mean glucose production rate for pony 1, 104 mg/h per kg body-wt (range 100–110); mean production rate for pony 2, 135 mg/h per kg body-wt (range 123–153).

28·9–38·3). Comparison of the specific activities of [^{14}C]glucose in plasma (Table 1) with that of [^{14}C]propionate during the plateau period of specific activity gave an indication of the rate of incorporation of C derived from caecal propionate into synthesized glucose and this is given in Table 1.

Glucose production

Table 2 gives information on total glucose production during the eleven infusions that were described in Table 1. Table 2 shows the rate of intravenous infusion of tritiated glucose and the mean plateau specific activity of tritiated glucose isolated from plasma collected during the last hour of infusion. The total glucose production rate (mg glucose-C/min) was obtained by dividing the infusion rate (nCi/min) by the mean plateau specific activity of plasma glucose (nCi/mg glucose-C) (Table 2). The same results adjusted to the body-weight of the ponies and converted from glucose-C to glucose are also given in Table 2 which also gives the mean values. The mean values suggest that there was no effect of diet but that the two ponies produced glucose at different rates, the mean value of 135 (range 123–153) for the second pony being about 23% higher than the mean of 104 (range 100–110) mg/h per kg body-weight for the first pony. As the percentage of glucose derived from caecal propionate of 6·9 (range 4·3–9·3) (Table 1) was unaffected by diet or animal, the mean amount of glucose derived from propionate produced in the caecum (calculated from column 10 of Table 2) of 9·3 (range 5·8–8·9) mg/h per kg body-weight, was greater in the second pony than the value of 7·2 (range 4·3–9·3) mg/h per kg body-weight in the first pony.

DISCUSSION

Production of propionate

Although there are numerous reports on the concentrations of volatile fatty acids in the contents of the gastrointestinal tract of the horse (e.g. Elsden *et al.* 1946; Argenzio *et al.* 1974) there is little information on rates of production of the individual volatile fatty acids. However, Glinsky *et al.* (1976) described experiments on ponies weighing 162 kg fitted with caecal cannulas and fed on two mixtures of hay and concentrates. They used both constant infusion and single injections of mixtures of ^{14}C-labelled acetic, propionic and butyric acids. Although there was considerable variation between production rates in different animals, most of their measurements of propionate production were between 0·4 and 0·6 mmol/min; that is, similar to our results on the hay diet but less than our production rates of 1·0–1·4 mmol/min on hay and wheat bran.

Production of glucose

White *et al.* (1969) have suggested that the intravenous infusion of [2-^{3}H]glucose and measurement of plateau specific activity in plasma facilitates the measurement of total glucose entry or total production because the tritium released by the metabolism of glucose labelled with this isotope is diluted in the body water pool and excreted rather than recycled. Our measurements of total glucose production can therefore be compared with those reported previously (Ford & Evans, 1982). In the present experiments the two ponies produced 1·7 and 2·2 mg glucose/min per kg body-weight, rather more than the value of 1·44 mg glucose/min per kg body-weight previously reported. Our value of about 7% of total glucose production synthesized from propionate produced in the caecum is, to the best of our knowledge, the first reported measurement of this aspect of intermediary metabolism in the horse but the results need confirmation on a larger number of animals.

Since the colon of the horse has a much greater capacity than the caecum, a correspondingly

greater production of propionate in that part of the large intestine would be expected. Measurements are presently being made in ponies with cannulas at both ends of the large colon.

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