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Metabolism of propionate in the tissues of the sheep gut

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1. The extent of propionate metabolism during absorption from the gut and the amounts of L-lactate formed and glucose utilized by the portal-drained viscera were determined in conscious sheep from measurements of portal venous blood flow and portal venous and aortic metabolite concentrations. The sheep were fasted overnight and given primed continuous intraruminal infusions of volatile fatty acids (VFA) at two rates, supplying propionate at 40·0 and 79·9 mmol/h. Measurements were made during the 5th and 6th hours of the infusion, when rumen liquor VFA concentrations were constant.

2. The rate of L-lactate formation by the portal-drained viscera was not affected by the VFA infusions and accounted for approximately 15 % of the probable total lactate entry rate.

3. Considerable amounts of glucose were taken up by the portal-drained viscera, amounting to approximately 35 % of the probable glucose entry rate. If this glucose was metabolized through the glycolytic pathway, this would at all times have accounted for the amounts of L-lactate formed.

4. Portal venous blood flow was positively correlated with VFA infusion rates and with the net amount of propionate appearing in the portal blood.

5. It is concluded that although propionate may be metabolized by the rumen epithelium, the unique pathway of L-lactate formation from propionate is of limited quantitative significance to the animal, although it may be of importance to the rumen epithelium itself.

The ability of the rumen epithelium to metabolize a proportion of the propionate absorbed from the rumen is now well established as the result of in vitro incubation studies (Pennington & Sutherland, 1956; Weigand, Young & Jacobson, 1967; Weekes, 1971). The major product of this propionate metabolism in vitro is L(+)-lactate, together with small amounts of pyruvate (Weekes, 1972). The biochemical pathway of lactate formation has been established (Young, Thorp & de Lumen, 1969) and aspects of metabolic control have been investigated in vitro (Pennington & Pfander, 1957; Ash & Baird, 1973; Weekes, 1974). However, the extent and significance of this metabolic pathway in the living animal remain uncertain. Bergman & Wolff (1971) measured the net appearance of volatile fatty acids (VFA) in the portal blood of sheep and concluded that 50% of the propionate produced in the reticulo-rumen was metabolized during absorption, while Leng, Steel & Luick (1967) calculated that in sheep up to 70% of the propionate incorporated into glucose was first converted into lactate. The rumen epithelium was the most likely site for this conversion. However, Weigand, Young & McGilliard (1972*a*) concluded that in calves the true conversion of propionate into lactate during absorption averaged only 2·3%, a value in excellent agreement with an indirect calculation based on in vitro incubations using sheep rumen epithelium (Weekes, 1972).

We therefore attempted to resolve this controversy by measuring the amounts of lactate formed and glucose utilized by the portal-drained viscera of conscious sheep during constant intraruminal infusion of VFA. Our findings suggest that very little

Table 1. *Composition of volatile fatty acid (VFA) priming solution* added to the rumen of sheep and rates of VFA infusion*

	Low-propionate	High-propionate
Priming solution		
Acetate (mmol)	54	54
Propionate (mmol)	162	200
Butyrate (mmol)	69	87·5
Polyethylene glycol (molecular wt 4000) (g)	17·5	17·5
VFA infusion rates (mmol/h)		
Acetate	69·2	69·2
Propionate	40·0	79·9
Butyrate	30·0	36·0
No. of experiments	6	11

* Priming solution (500 ml, pH 5·5, at 39°) was placed in the rumen at the start of each experiment and VFA solutions were infused for 6 h at 107 ml/h.

lactate was formed from propionate under these conditions. A preliminary account of this work has been published elsewhere (Weekes & Webster, 1974).

EXPERIMENTAL

Animals and surgical preparation

Five mature Greyface sheep were used, one animal being a non-breeding female, the others being castrate males. Their live weights ranged from 51 to 97 kg (mean 65 kg). All animals were thoroughly accustomed to handling. A rumen fistula was established at least 8 weeks before the first experiment with any animal. At a subsequent operation polyvinyl sampling catheters were inserted into the anterior mesenteric vein and the aorta through appropriate branches in the mesenteric circulation, and a double-bored catheter consisting of a sampling tube and a thermocouple was placed in the portal vein, as described by Webster & White (1973). The post-operative care of the animals was also similar to that described by Webster & White (1973). None of the sheep were used for experiments until they had completely recovered from surgery. The sheep were housed in metabolism cages between and during experiments and were given chopped dried grass (1·0 kg/d).

Experimental procedure

Animals were fasted overnight and not allowed access to food or water during the course of an experiment. An initial sample of rumen contents was obtained, together with blood samples from the aorta and portal vein. Portal venous blood flow was estimated by the thermal dilution method of Webster & White (1973). Continuous intraruminal infusions of VFA were then given for 6 h, using two levels of infusion that followed priming doses (Table 1). All solutions were warmed to 39°, adjusted to pH 5·5 and then infused at 107 ml/h using a peristaltic pump (LKB Instruments Ltd, Selsdon, Croydon, Surrey). This infusion procedure was intended to achieve constant rumen liquor VFA concentrations between 4 and 6 h after the start of the infusion

(plateau period). The low-propionate infusion (Table 1) was designed to produce rates of absorption of individual VFA comparable to those found in sheep given a maintenance ration of dried grass (Bergman, Reid, Murray, Brockway & Whitelaw, 1965). The high-propionate infusion delivered propionate at twice this rate, an amount calculated to produce rumen liquor VFA concentrations similar to those found when sheep are fed *ad lib.* on rolled, pelleted barley (Ørskov, 1973; Weekes, 1973). Because of the nature of the animal preparation, it was not possible to follow any predetermined randomized or systematic infusion procedure.

Samples of rumen contents were taken every 30 min for 6 h and placed on ice. In some experiments rumen fluid pH was determined immediately after withdrawal of the samples, using a portable pH meter (W. G. Pye & Co. Ltd, Cambridge). During the plateau period, i.e. after 4 h, 10 ml samples of aortic and portal venous blood were withdrawn every 30 min for 2 h. Whole blood (3 ml) was immediately added to 3·0 ml ice-cold perchloric acid (60 g/l) in a tared centrifuge tube and stored on ice for subsequent estimations of lactate, pyruvate and glucose contents. The remaining blood was immediately chilled and subsequently frozen for the estimation of propionate content. Measurements of portal blood flow were made within 2 min of each collection of blood samples.

Because of intermittent blockage of sampling catheters, five pairs of portal venous and aortic samples could not be obtained over the plateau period in six of the seventeen experiments. Experiments in which fewer than three pairs of blood samples were obtained were discarded. In one experiment with the low-propionate infusion and in two with the high-propionate infusion insufficient blood was obtained for propionate estimations.

Chemical analyses

The deproteinized blood samples were centrifuged at 3000 *g* for 20 min, filtered and the supernatant fractions were neutralized with 5 M-K_2CO_3, using methyl orange as the indicator. The concentration of L-lactate was determined by the method of Hohorst (1963), pyruvate by the method of Bücher, Czok, Lamprecht & Latzko (1963) and glucose by the method of Slein (1963). These analyses were done on the day of the experiment. Results were calculated as μmol/ml whole blood, assuming a density of 1060 kg/m^3 for sheep blood (Baird, Hibbitt, Hunter, Lund, Stubbs & Krebs, 1968). Rumen liquor VFA concentrations were determined as described by Weekes (1972). The amount of polyethylene glycol (PEG) in the rumen liquor was estimated by the method of Weigand *et al.* (1972*a*), and the L-lactate content of the rumen liquor was estimated as described for blood lactate, following deproteinization with an equal volume of ice-cold perchloric acid (60 g/l). Blood propionate concentrations were determined by freeze-transfer and gas–liquid chromatography (P. J. Barker & D. B. Lindsay, unpublished results) using [2-^{14}C]propionate (The Radiochemical Centre, Amersham, Bucks.) to estimate recovery during the freeze-transfer procedure.

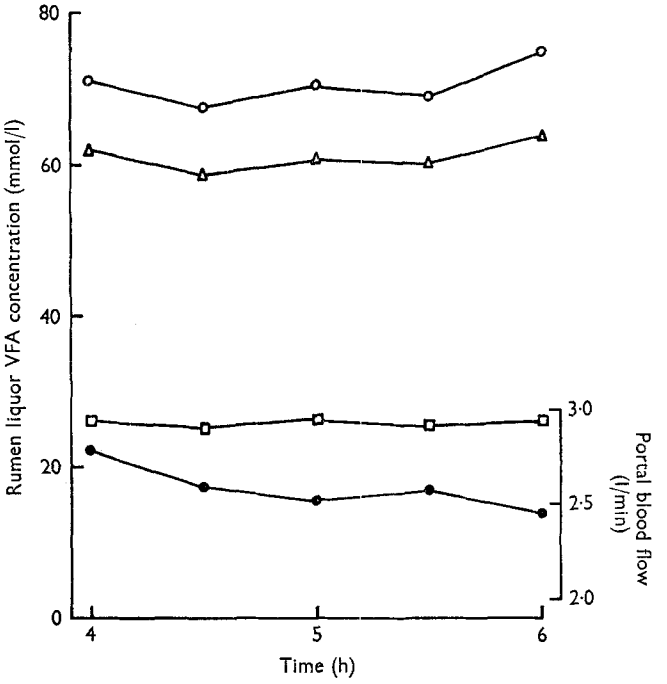

Fig. 1. Effect of a continuous intraruminal infusion of volatile fatty acids (VFA) on rumen liquor VFA concentrations (mmol/l) and portal blood flow (l/min) in sheep. VFA were infused at the high-propionate infusion rate (Table 1); ○, acetate; △, propionate; □, butyrate; ●, portal blood flow.

Calculation of results

The net appearance of lactate, glucose and propionate in portal blood were taken as the product of portal blood flow and the difference in concentration of these metabolites between the portal vein and the aorta. Since the reticulo-omasal orifice was not occluded in these experiments, some VFA may have been absorbed posterior to the reticulo-rumen. The rates of fluid and VFA outflow from the reticulo-rumen were therefore calculated from PEG concentrations by the method of Smith (1959), assuming that the rumen contents were homogenous and that the rumen volume remained constant.

In order to compare results from different experiments all results were expressed as μmol/min per kg body-weight$^{0\cdot75}$.

RESULTS

General remarks

The variation in rumen liquor VFA concentrations and in portal blood flow over the plateau period in a typical experiment are shown in Fig. 1, while Fig. 2 shows the variation in portal venous and aortic metabolite concentrations in the same experiment. The coefficients of variation within an experiment (Table 2) indicate that this procedure resulted in a very uniform rumen liquor composition but differences between portal venous and aortic concentrations of metabolites were more variable.

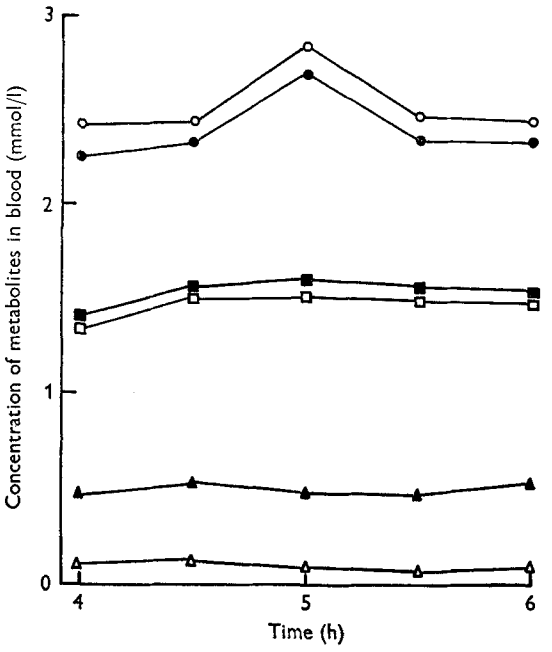

Fig. 2. Effect of a continuous intraruminal infusion of volatile fatty acids (VFA) on portal venous and aortic concentrations of glucose, L-lactate and propionate (mmol/l) in sheep. VFA were infused at the high-propionate infusion rate (Table 1). ●, ○, Glucose; ■, □, lactate; ▲, △, propionate; ●, ■, ▲, portal venous concentrations; ○, □, △, aortic concentrations.

Table 2. *Coefficients of variation (CV)* within an experiment for rumen liquor volatile fatty acid (VFA) composition, portal venous blood flow and differences in concentration of metabolites between portal venous and aortic blood in sheep*

	No. of experiments	CV within an experiment
Rumen liquor composition		
Total VFA concentration (mmol/l)	17	8·0
VFA molar proportions (mmol/mol)		
Acetate	17	2·3
Propionate	17	2·8
Butyrate	17	4·1
Propionate concentration (mmol/l)	17	9·2
Portal blood flow (l/min)	17	8·3
Portal venous–aortic concentration differences (mmol/l)		
Propionate	14	32·6
L-lactate	17	34·5
Glucose	17	32·5

* CV were calculated within an experiment from individual estimations made at 30 min intervals over the plateau period from 4 to 6 h after the start of the intraruminal VFA infusions. Results for experiments using low- and high-propionate infusion rates were combined as values for CV did not differ significantly.

Table 3. *Rumen liquor volatile fatty acid (VFA) composition at 0 h and 4–6 h (plateau period) after the start of intraruminal VFA infusions, at low- and high-propionate infusion rates, in sheep*

(Mean values with their standard errors for six experiments for the low-propionate infusion rate and eleven experiments for the high-propionate infusion rate)

	Total VFA concentration (mmol/l)		VFA molar proportions (mmol/mol)						Propionate concentration (mmol/l)	
			Acetate		Propionate		Butyrate			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Low-propionate infusion†										
0 h	68·6	12·1	641	20	223	19	67	6	13·9	2·4
4–6 h plateau	146·9	18·7	518	12	294	11	146	8	42·6	5·0
High propionate infusion†										
0 h	76·6	6·6	668	12	198	9	80	9	15·4	1·7
4–6 h plateau	167·9	8·2	441	10***	378	7***	153	5	63·4	2·9**

Values for the high-propionate infusion rate differed significantly from those for the low-propionate infusion rate: ** $P < 0.01$; *** $P < 0.001$.

† For details, see Table 1.

However, no consistent trends in these concentration differences were noted, so that mean values over the plateau period from 4 to 6 h after the start of the infusion were used in all subsequent calculations. Blood lactate concentrations are notoriously liable to be affected by disturbances to the experimental animal (Narasimhalu, 1970). The present procedure was designed to cause minimal disturbance and the sheep did not show any signs of stress during experiments, although food intake tended to be reduced on the day following an experiment.

Rumen liquor composition

Table 3 shows that both rates of propionate infusion significantly increased total rumen VFA concentration, propionate concentration and the molar proportions of propionate and butyrate, but significantly reduced the molar proportion of acetate. The plateau (4–6 h after the start of infusion) propionate concentration was significantly higher ($P < 0.01$) for the high-propionate infusion rate, as was the molar proportion of propionate ($P < 0.01$), while the molar proportion of acetate was lower ($P < 0.001$) (Table 3).

Rumen fluid pH was determined in four experiments using the low-propionate infusion rate and in six experiments using the high-propionate infusion rate. Values for samples taken before the start of infusions were (mean, SE) 6.6 ± 0.2 and 6.4 ± 0.1 for the low- and high-propionate infusion rates respectively, while for samples taken at the plateau, the corresponding values were 6.5 ± 0.2 and 6.3 ± 0.1. L-lactate could not be detected in rumen liquor in these experiments.

Portal venous blood flow

Mean values for portal blood flow before infusion of VFA did not differ significantly (Table 4). During infusion portal blood flow was increased. Mean values, given in

Table 4. *Rates of intraruminal propionate infusion, portal venous blood flow and blood concentrations and net portal appearance of propionate, L-lactate and glucose in sheep*

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

	Low-propionate infusion rate†				High-propionate infusion rate†			
	0 h		4-6 h (plateau)		0 h		4–6 h (plateau)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Propionate infusion rate (μmol/min per kg$^{0.75}$)	—	—	27·3	2·1 (6)	—	—	61·4	3·1 (11)***
Portal blood flow (ml/min per kg$^{0.75}$)	83·4	9·6 (4)	91·2	8·1 (6)	81·3	8·8 (11)	123·6	10·7 (11)
Aortic concentration (μmol/l)								
Propionate	104	78 (3)	128	30 (5)	57	9 (9)	165	17 (9)
L-lactate	324	47 (4)	506	56 (6)	473	43 (11)	671	97 (11)
Glucose	2040	135 (4)	2161	61 (6)	1836	94 (11)	2041	157 (11)
Portal venous–aortic concentration difference (μmol/l)								
Propionate	130	60 (3)	346	33 (5)	255	43 (9)	414	59 (9)
L-lactate	66	17 (4)	87	7 (6)	75	11 (11)	59	5 (11)
Glucose	−105	40 (4)	−81	16 (6)	−107	21 (11)	−81	9 (11)
Net portal metabolite appearance (μmol/min per kg$^{0.75}$)								
Propionate	11·3	8·3 (3)	32·3	5·3 (5)	20·7	4·9 (9)	54·4	6·7 (9)*
L-lactate	5·48	1·41 (4)	7·69	0·43 (6)	6·07	1·12 (11)	6·95	0·87 (11)
Glucose	−9·51	2·49 (4)	−6·94	1·11 (6)	−8·74	2·03 (11)	−9·72	1·37 (11)

Values for the high-propionate infusion rate differed significantly from those for the low-propionate infusion rate: * $P < 0.05$, *** $P < 0.001$.

† For details, see Table 1.

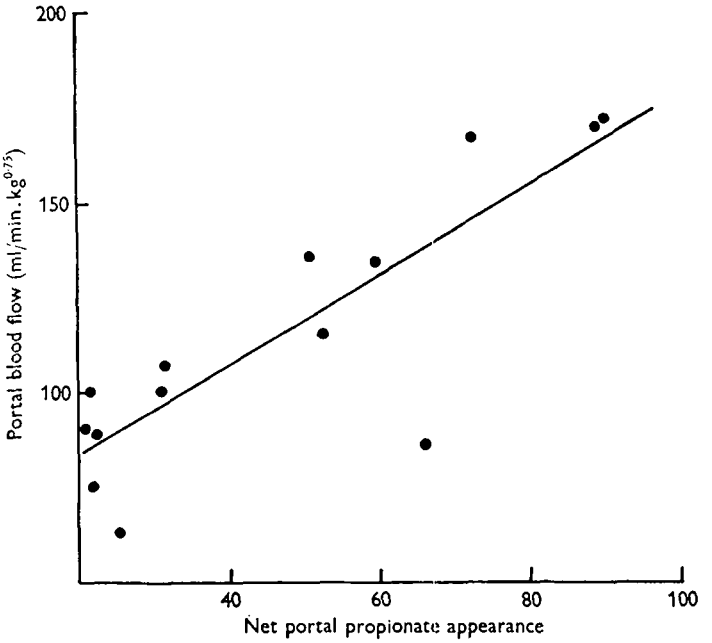

Fig. 3. Relationship between portal venous blood flow (ml/min per kg$^{0.75}$) and the net amount of propionate appearing in the portal blood (μmol/min per kg$^{0.75}$) of sheep receiving intraruminal infusions of volatile fatty acids. The regression equation (2) is given below.

Table 4, did not differ significantly ($0.1 > P > 0.05$) but regression analysis showed that values for portal blood flow (PF, ml/min per kg$^{0.75}$) were positively correlated with the net appearance of lactate (LP) and propionate (PP) (Fig. 3) in portal blood, with the propionate (PI) butyrate (BI) and total VFA (TI) infusion rates and with the calculated total propionate production rate (TP; sum of propionate infusion rates and endogenous rate of propionate production (calculated from equation 7, p. 435)) when all these values were expressed as μmol/min per kg$^{0.75}$. The regression equations, each based on fourteen observations, were:

$$PF = 37.4 + 10.6LP \quad (SE\ 3.4,\ RSD\ 27.6,\ R^2\ 0.44,\ P < 0.01), \tag{1}$$

$$PF = 59.7 + 1.19PP \quad (SE\ 0.22,\ RSD\ 19.7,\ R^2\ 0.72,\ P < 0.001), \tag{2}$$

$$PF = 53.1 + 1.23PI \quad (SE\ 0.41,\ RSD\ 28.0,\ R^2\ 0.43,\ P < 0.05), \tag{3}$$

$$PF = -5.7 + 4.7BI \quad (SE\ 1.3,\ RSD\ 25.7,\ R^2\ 0.52,\ P < 0.01), \tag{4}$$

$$PF = 10.8 + 0.82TI \quad (SE\ 0.23,\ RSD\ 26.0,\ R^2\ 0.51,\ P < 0.01), \tag{5}$$

$$PF = 55.5 + 0.77TP \quad (SE\ 0.32,\ RSD\ 30.4,\ R^2\ 0.33,\ P < 0.05), \tag{6}$$

where SE is the SE for the regression coefficient and RSD is the residual SD.

Values for the net appearance of lactate and propionate in portal blood are, of course, derived in part from measurements of portal blood flow. The relationships between blood flow and infusion rates were, however, free of any element of autocorrelation. Although these experiments were not designed to study this point, the

results did suggest that portal blood flow was more sensitive to butyrate than to propionate. A study specifically designed to measure the influence of VFA on portal blood flow is now in progress.

Net portal appearance of propionate, lactate and glucose

The net appearance of propionate in the portal blood was, on average, almost twice as great at the high rate of infusion than at the low infusion rate (Table 4). Although the results were rather variable, the difference was significant ($P < 0{\cdot}05$). There were, however, no significant differences between treatments in portal lactate appearance or glucose uptake. Glucose uptake was at all times sufficient to account for the amounts of lactate formed by glycolysis but, because the amounts of glucose taken up and lactate formed varied little, the correlation between glucose uptake and lactate appearance was not significant ($0{\cdot}1 > P > 0{\cdot}05$). There was also no correlation between either portal lactate appearance or glucose uptake and the propionate infusion rate or the net portal appearance of propionate. Differences between sheep were not significant.

Differences in pyruvate concentration between portal venous and aortic blood were very small and variable, and these results have not been included in Table 4.

DISCUSSION

The intraruminal infusions of VFA resulted in higher rumen liquor VFA concentrations, although VFA levels remained within the physiological range (Hungate, 1966; Weekes, 1973). The low-propionate infusion rate was similar to the ruminal propionate production rate obtained by Bergman *et al.* (1965) for sheep fed at the maintenance level on dried grass and by Steel & Leng (1973) for sheep fed *ad lib.* on lucerne hay. On a metabolic body-weight basis the high-propionate infusion rate was less than the propionate production rate in cows fed on a high-grain ration (Bauman, Davis & Bucholtz, 1971). Thus the rates of propionate absorption produced in the present study are within the normal range of ruminal propionate production in sheep.

It is clear that the tissues of the gut wall are a major site for lactate formation, the mean plateau values (Table 4) representing 13·5% of the lactate entry rate (54 μmol/min per $kg^{0{\cdot}75}$) in fed sheep (Annison, Lindsay & White, 1963). Other workers have also reported a net lactate production by the portal-drained viscera (Bensadoun, Cushman & Reid, 1966; Roe, Bergman & Kon, 1966). The portal venous–aortic concentration differences (Fig. 2) were similar to those observed in cattle (Weigand *et al.* 1972*a*).

The glucose entry rate in non-breeding ewes fed at the maintenance level on roughages has been estimated to be approximately 24 μmol/min per $kg^{0{\cdot}75}$ (Leng, 1970*a*). The mean glucose utilization by the portal-drained viscera in the present study (Table 4) was 8·7 μmol/min per $kg^{0{\cdot}75}$, indicating that about 35% of the total glucose utilization may occur in the portal-drained viscera.

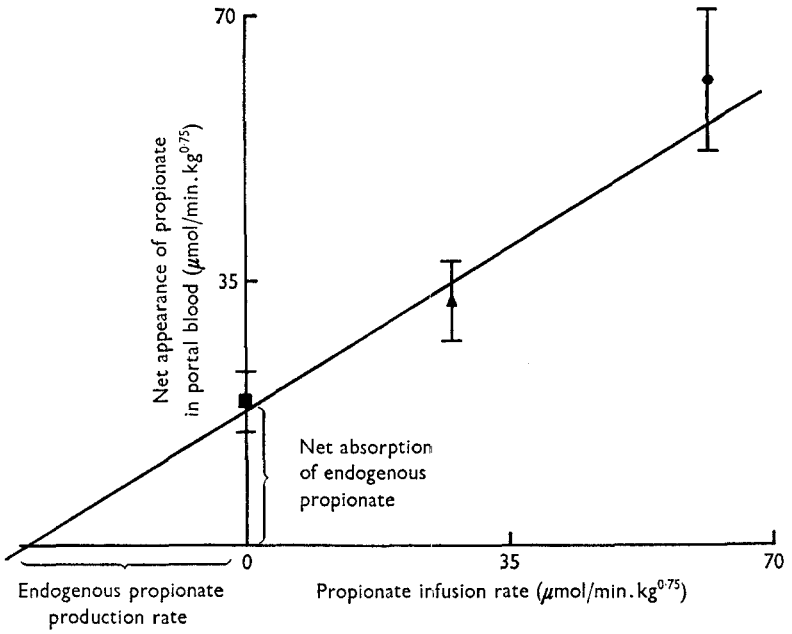

Fig. 4. Relationship between the net amount of propionate appearing in the portal blood (μmol/min per $kg^{0.75}$) and propionate infusion rate (μmol/min per $kg^{0.75}$) in sheep receiving intraruminal infusions of volatile fatty acids. Mean values for the high (●) and low (▲) rates of propionate infusion, and for the o h values (■), with their standard errors represented by vertical bars. The regression equation (7) is given on p. 435.

Others workers have also observed a net portal glucose utilization in sheep (Roe *et al.* 1966; Bergman, Katz & Kaufman, 1970) and cattle (Weigand *et al.* 1972*a*; Baird, Symonds & Ash, 1974). The magnitude of this net uptake varied and could not always account for the net amounts of lactate formed (Bensadoun *et al.* 1966). However, as only net glucose uptake is measured, this ignores the contribution of glucose absorbed from the small intestine, which would have been considerable in the work of Bensadoun *et al.* (1966) when maize was fed to sheep, but would have been very small in our experiments (see Lindsay, 1970). Bergman *et al.* (1970) reported that the portal-drained viscera of sheep utilized 20 % of the total glucose turnover; our higher value of 35 % may reflect our higher estimate of portal blood flow. The results of other studies indicate that the portal-drained viscera contribute about 23 % to the total heat production of fasted sheep (Webster, Osuji, White & Ingram, 1974). This evidence, as suggested by Lindsay (1971), indicates that tissues drained by the portal bed are metabolically very active, with a high demand for glucose, which is probably related to their high rate of cell turnover.

The present studies do not provide direct evidence concerning the extent of propionate metabolism during absorption, as no direct measurements were made either of the endogenous rate of propionate production in the rumen, or of how this rate was affected by the VFA infusion. The extent of propionate metabolism during absorption can be estimated by plotting PP (μmol/min per $kg^{0.75}$) *v.* PI (μmol/

Table 5. *Extent of propionate metabolism during absorption from the gastrointestinal tract of sheep given intraruminal infusions of volatile fatty acids*

(Mean values with their standard errors for results obtained for the plateau period (4–6 h after the start of the infusion), except where stated; no. of experiments in parentheses)

	Low-propionate infusion rate		High-propionate infusion rate	
	Mean	SE	Mean	SE
Propionate infusion rate (μmol/min per kg$^{0.75}$)	27·3	2·1 (6)	61·4	3·1 (11)***
Endogenous propionate production rate† (μmol/min per kg$^{0.75}$)	29·2 (12)			
Total propionate production rate‡ (μmol/min per kg$^{0.75}$)	56·5	2·2 (6)	90·6	3·1 (11)***
Propionate absorption from reticulo-rumen§ (μmol/min per kg$^{0.75}$)	46·8	1·4 (6)	73·7	2·2 (11)***
Net portal propionate appearance				
Propionate infusion rate	1·15	0·12 (5)	0·86	0·14 (9)
Endogenous propionate production rate (0 h values)	0·45	0·23 (3)	0·71	0·17 (9)
Total propionate production rate	0·56	0·07 (5)	0·59	0·09 (9)

Values for the high-propionate infusion rate differed significantly from those for the low-propionate infusion rate: *** $P < 0·001$.

† Calculated from equation 7, see below.

‡ The sum of the propionate infusion rates and the endogenous rate of propionate production.

§ The total propionate production rate corrected for the rate of fluid outflow from the reticulo-rumen.

min per kg$^{0·75}$) (Fig. 4). The regression equation describing this relationship, based on twenty-six observations, was:

$$PP = 17·5 + 0·60\,PI \quad (SE \text{ of regression coefficient } 0·13, \text{ residual SD } 18·3, R^2\ 0·48, P < 0·001), \tag{7}$$

which indicates that 60% of infused propionate was recovered in the portal blood. The endogenous rate of propionate production would therefore be $17·5 \div 0·60 = 29·2$ μmol/min per kg$^{0·75}$ (Fig. 4), although the uncertainty involved in this extrapolation is considerable (fiducial limits ($P < 0·05$) for a single observation are 9·2–75·9 μmol/min per kg$^{0·75}$). Leng (1970*b*) has related propionate production rates to rumen liquor propionate concentrations. Using this relationship the endogenous propionate production rates before the start of the low- and high-propionate infusions would have been (mean, SE) $23·3 \pm 6·0$ and $25·9 \pm 4·0$ μmol/min per kg$^{0·75}$ respectively. These calculations ignore both the contribution of caecal fermentation and possible metabolism of infused propionate within the rumen, but the magnitude of these processes are unlikely to be large relative to the propionate infusion rates (Leng, 1970*b*; Wiltrout & Satter, 1972). Judson & Leng (1973) reported that intraruminal propionate infusions reduced the endogenous rate of propionate production. Our results (Fig. 4) provide

no support for this contention for mixtures of all three principal VFA. Total propionate production rates in our experiments, when calculated from the regression equation of Judson & Leng (1973) relating propionate production rates to the rate of propionate infusion, would have been (mean, SE) $47{\cdot}9 \pm 6{\cdot}2$ and $82{\cdot}4 \pm 5{\cdot}7$ μmol/min per $kg^{0{\cdot}75}$ for the low- and high-propionate infusion rates respectively. These values agree well with our estimates (Table 5) of $56{\cdot}5 \pm 2{\cdot}2$ and $90{\cdot}6 \pm 3{\cdot}1$ μmol/min per $kg^{0{\cdot}75}$ derived from equation 7 (p. 435). We conclude therefore that any overestimate of the 'total propionate production rate' (Table 5) will be less than 15%.

The probable amounts of propionate absorbed from the reticulo-rumen, given in Table 5, are derived from the calculated total propionate production rates by subtraction of the rate of propionate outflow through the reticulo-omasal orifice as estimated from the PEG concentrations. Between 45 and 71% of the calculated total propionate produced was recovered in the portal blood (Table 5), again indicating extensive metabolism of propionate during absorption. The extent of this metabolism was not significantly influenced by the propionate infusion rate.

These calculations are similar to the estimate of Bergman & Wolff (1971) that 50% of the propionate produced in the rumen is metabolized by the tissues of the portal bed. Although both calculations were partly indirect, they do suggest a fairly extensive metabolism of propionate. Much of this propionate may be oxidized, since the results of in vitro incubation studies suggest that propionate oxidation can take place (Pennington & Sutherland, 1956; Weigand *et al.* 1967; Weekes, 1973). These findings contrast with the recent report (Ash & Baird, 1973) that the presence of butyrate strongly inhibits propionate metabolism in rumen epithelial homogenates, by inhibition of propionyl-CoA synthetase activity (acid-CoA ligase (AMP); *EC* 6.2.1.2). It is possible that in vivo the relatively rapid absorption and extensive metabolism of butyrate by the rumen epithelium (Weigand, Young & McGilliard, 1972*b*) lowers the intracellular concentration of butyrate below inhibitory levels.

Therefore, a fairly extensive metabolism of propionate probably occurs in the portal-drained viscera, but lactate is not likely to be a major product. Lactate formation did not respond to variations in the rate of propionate infusion, and could at all times have been formed from glucose by the glycolytic pathway, in agreement with the findings of Weigand *et al.* (1972*a*).

Even if the unlikely assumption were made that all the lactate formed was derived direct from propionate, then during low and high rates of propionate infusion, lactate production would only have accounted for 32 and 24% respectively of the propionate metabolism in the tissues of the gut wall.

These studies do not establish the exact site of the lactate formation, since portal venous blood was used. Catheterization of the ruminal veins of sheep has not proved satisfactory (Webster & White, 1973), but in a few experiments blood samples were obtained from the anterior mesenteric vein. Assuming blood flow in this vessel represents 50% of portal venous flow, then approximately 60% of the total lactate formation by the portal-drained viscera could have occurred in tissues drained by the anterior mesenteric vein. Calculations from the results of in vitro incubations (Weekes, 1972) suggest that the rumen epithelium of non-breeding ewes fed at the maintenance level

may be able to form lactate from propionate at a rate of 1·8 μmol/min per $kg^{0·75}$, while the in vitro rate of glycolysis in the rumen epithelium (Weekes, 1974) was 1·7 μmol/min per $kg^{0·75}$. The intestinal mucosa of adult sheep is, however, able to form lactate from endogenous sources at a rate of 14 g/d (Wahle, Weekes & Sherratt, 1972), equivalent to 6·8 μmol/min per $kg^{0·75}$, close to the values shown in Table 4.

It appears, therefore, that the ability of the rumen epithelium to form lactate from propionate is limited. Most of the lactate formed by the portal-drained viscera is probably derived from glucose by glycolysis. This conclusion is in agreement with the findings of Weigand *et al.* (1972*a*), but contrasts with the earlier study of Leng *et al.* (1967). The calculations of these workers are questionable since they did not take account of the extensive recycling of carbon which can occur in the tricarboxylic acid cycle (Thompson, 1971; Wiltrout & Satter, 1972). This does not explain the higher specific radioactivity in jugular blood lactate than in plasma glucose in some of the [^{14}C]propionate infusion experiments of Leng *et al.* (1967). However, in the studies of Weigand *et al.* (1972*a*) the specific radioactivity of blood glucose was 2- to 12-fold greater than that of lactate when [2-^{14}C]propionate was absorbed from the reticulo-rumen.

The extent of lactate and pyruvate formation (per unit tissue weight) from propionate in vitro was also relatively constant for breeding ewes (Weekes, 1972). This finding, together with the relatively small extent of conversion in vivo found in the present study, suggests that this pathway is unlikely to make any significant contribution to the conservation of substrates for hepatic gluconeogenesis. However, even a small conversion of propionate to lactate would be of considerable importance to the actively dividing cells of the rumen epithelium, as it provides a unique pathway for the extramitochondrial generation of NADPH required for synthetic processes.

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