Portal recovery of short-chain fatty acids infused into the temporarily-isolated and washed reticulo-rumen of sheep

N. B. Kristensen[1]*, G. Gäbel[2], S. G. Pierzynowski[3], and A. Danfær[1]

[1]*Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology, Box 50, DK-8830 Tjele, Denmark*

[2]*University of Leipzig, Department of Veterinary Physiology, An den Tierkliniken 07, D-04103 Leipzig, Germany*

[3]*Lund University, Department of Animal Physiology, S-223 62 Lund, Sweden*

(Received 3 November 1999 – Revised 29 February 2000 – Accepted 3 May 2000)

The present study was undertaken to study the metabolism of short-chain fatty acids (SCFA) by the reticulo-ruminal epithelium and the portal-drained viscera (PDV) under *in vivo* conditions with no interference from the metabolism of the rumen microbes. The technique of temporary isolation of the reticulo-rumen was applied to wethers implanted with catheters in a mesenteric artery, the hepatic portal vein and the right ruminal vein. Portal blood flow was measured by downstream dilution of *p*-aminohippuric acid; the PDV uptake of arterial acetate, as well as the whole-body irreversible loss rate (ILR) of acetate, was estimated by [2-^{13}C]acetate infusion into the right ruminal vein. The sheep were maintained with a bicarbonate-buffered solution of SCFA in the reticulo-rumen along with continuous intraruminal infusion of SCFA for 4 h. The portal appearance of SCFA of non-reticulo-ruminal origin was estimated before and after the infusion protocol. Of the acetate absorbed by the sheep, 89 (SE 5), 109 (SE 7) and 101 (SE 7) % was recovered as portal net appearance of acetate, portal net appearance of acetate corrected for PDV uptake of arterial acetate and increase in the ILR of acetate respectively. Of the propionate, isobutyrate, butyrate, isovalerate and valerate absorbed by the sheep, 95 (SE 7), 102 (SE 9), 23 (SE 3), 48 (SE 5) and 32 (SE 4) % respectively was recovered as portal net appearance. In contrast to current concepts, the present study showed that the reticulo-ruminal epithelium metabolizes none (or only a small proportion) of the acetate and propionate absorbed from the rumen. This observation could lead to the more efficient use of results obtained with multi-catheterized animals to quantify the net metabolite output of the rumen microbes.

Short-chain fatty acids: Absorption: Metabolism: Portal-drained viscera: Sheep

The metabolic pathway of ruminally-produced or infused short-chain fatty acids (SCFA) is disputed although it has been investigated a number of times during the past 30 years. Bergman & Wolff (1971) showed that 70, 50 and 10 % of the intraruminal turnover of acetate, proponate and butyrate respectively could be accounted for by portal absorption. These values were in good agreement with *in vitro* data on the loss of SCFA transported across rumen epithelium (Stevens & Stettler, 1966*a*). Since this coincidence was noticed, it has been widely accepted that the SCFA are extensively metabolized in the rumen epithelium during absorption (Bergman, 1990; Rémond *et al.* 1995) and few researchers have questioned this assumption (Sutton, 1985). Nevertheless, the fate of 25–50 % of the rumen acetate and propionate has not yet been elucidated. No metabolites of any quantitative importance are known to originate from the postulated epithelial metabolism of these SCFA. We have recently observed that the portal recovery of intraruminally-infused propionate was relatively high (85 %) with a low feed intake (Kristensen *et al.* 2000) and lower (62 %) with an increased feed intake (NB Kristensen, SG Pierzynowski and A Danfær, unpublished results). This observation, in combination with the non-appearance of metabolites from acetate and propionate in the portal blood, led to the hypothesis that acetate and propionate are sequestrated in the microbial biomass to a greater extent than has generally been expected. The objective of the present study was to investigate the portal recovery of SCFA infused into the temporarily-isolated washed reticulo-rumen of sheep.

Abbreviations: ILR, irreversible loss rate; PDB, PeeDee Belemnite; PDV, portal-drained viscera; SCFA, short-chain fatty acids.

* **Corresponding author:** Dr Niels Bastian Kristensen, fax +45 8999 1166, email NielsB.Kristensen@Agrsci.DK

Materials and methods

Four 4-year-old Leicester wethers (body weight 98 (SE 3) kg) were implanted with permanent catheters (Medical Grade Silicone Tubing; Opitek Aps, Søborg, Denmark) in a mesenteric artery, the hepatic portal vein and the right ruminal vein as well as a rumen cannula (75 mm i.d.; Bar Diamond, Parma, ID, USA). A catheter was implanted in a hepatic vein for use in other experiments. Surgery was conducted under general anaesthesia induced with acepromazine (0·1 mg/kg body weight) followed by thiopentone (8 mg/kg body weight), and maintained with N_2O and halothane (O_2–N_2O 60:40, v/v). The sheep were individually penned and had free access to grass hay (crude protein (N × 6·25) 150 g/kg, *in vitro* soluble organic matter 650 g/kg), a complete mineral–vitamin mix for sheep (V.M. Får; Vitfoss, Gråsten, Denmark) and water. The present experiment complied with the Danish Ministry of Justice, Law no. 382 (10 June 1987), Act no. 726 (9 September 1993) concerning experiments with animals and care of experimental animals.

Experimental protocol

Experiments were conducted on one sheep per d and at least 1 week passed between two experiments on the same sheep. At 07.45 hours on the day of the experiment the sheep was moved from its pen to a grating mounted on a plate ensuring free access to both sides of the sheep. Continuous infusion into the ruminal vein of *p*-aminohippuric acid (8 mmol/h) and [2-^{13}C]acetate (0·8 mmol/h; 99 % ^{13}C; Mass Trace Inc., Woburn, MA, USA) was initiated as soon as the sheep was fixed. Both infusates were mixed in the same bottle, adjusted to pH 7·4 with NaOH and sterile filtered (GP Express, 0·22 μm; Millipore Bedford, MA, USA). The rumen was emptied and washed repeatedly with 1–2 litres isotonic NaCl (9 g/l) until the wash water was free of rumen contents; in total, about 10 litres isotonic NaCl were used. All solutions were heated to 39°C before administration. A saliva collector was mounted in the oesophagus, as described by Care *et al.* (1984), with the modification that the collected fluid was accumulated in a flask during the whole experiment and not re-introduced into the rumen until the end of the experiment. The first control period (control 1) was initiated by administering 3 litres control buffer (Table 1) to the rumen. All buffers were bicarbonate-buffered and gassed before and during incubation with a gas mixture containing 70 % N_2 and 30 % CO_2 (400 ml/min). Blood samples (10 ml) were collected from arterial and portal catheters 10 and 20 min after incubation of the control buffer. The control buffer was removed and 3 litres SCFA buffer (Table 1) were introduced quantitatively into the rumen. The rumen was immediately sealed and continuous intraruminal infusion (200 ml/h) of an SCFA solution (Table 1) was initiated. Blood and rumen samples were collected 10, 30, 60, 90, 120, 150, 180, 210 and 240 min after the start of the intraruminal infusion. The rumen was emptied immediately after the last sampling and washed with 3 litres isotonic NaCl. The mixed rumen buffer and wash water was weighed and a sample was stored for analysis of SCFA content. The rumen was filled with 3 litres control buffer (control 2), a rumen sample was obtained after 15 min and blood samples were collected after 15, 30 and 45 min.

Table 1. Composition of buffers and solutions (mmol/l) infused into the temporarily-isolated and washed reticulo-rumen of sheep

	Control buffer*	SCFA buffer*	Infusate
NaCl	117·5	30·0	0·0
$NaHCO_3$	20·0	20·0	0·0
NaOH	0·0	30·0	0·0
$KHCO_3$	15·0	15·0	90·0
K_2HPO_4	2·0	2·0	0·0
$CaCl_2$	1·5	1·5	0·0
$MgCl_2$	1·5	1·5	0·0
Acetic acid	0·0	0·0	140·0
Sodium acetate	0·0	50·0	260·0
Propionic acid	0·0	20·0	200·0
Isobutyric acid	0·0	2·0	20·0
Butyric acid	0·0	5·0	40·0
Isovaleric acid	0·0	1·5	20·0
Valeric acid	0·0	1·5	20·0

SCFA, short-chain fatty acids.
* Gassed with N_2–CO_2 (70:30, v/v) before and during incubation in the rumen.

The collected saliva with traces of rumen buffer was weighed and sampled before re-introduction of the saliva and the temperated originally-removed rumen contents into the rumen. All blood and buffer samples were placed on ice immediately after collection.

Analytical methods and calculations

The packed cell volume was determined for all samples of arterial blood; blood samples from 50 μl capillaries was centrifuged at 12 000 ***g*** (max) for 6 min at room temperature. The rest of the blood was centrifuged at 2500 ***g*** at 4°C for 30 min and the plasma stored at −20°C until analysis. The *p*-aminohippuric acid concentration in plasma was determined according to the procedure of Bratton & Marshall (1939) after a deacetylation procedure in which the TCA-deproteinized samples were incubated at 90°C for 60 min. Since *p*-aminohippuric acid is diluted only in the extracellular fraction of the blood, the whole-blood concentration was estimated as plasma concentration × (1 − packed cell volume). The whole-blood SCFA concentration and the isotopic composition of acetate were determined as described by Kristensen (2000).

The portal recovery of arterial acetate was calculated as:

$$\{[(AF_P - AF_B) \times C_P \times 2 \times BF]$$

$$- [\text{infusion rate of } [2\text{-}^{13}\text{C}]\text{acetate (mmol/h)}$$

$$\times (0.995 - AF_B)]\}/[(AF_A - AF_B) \times C_A \times 2 \times BF],$$

where AF_P, AF_B, AF_A, are $^{13}C/(^{13}C + ^{12}C)$ for portal, background and arterial samples respectively, BF is blood flow (litre/h), C_P, C_A are concentrations for portal and arterial samples respectively (mmol/l), and 0·995 is the AF of ^{13}C on C-2 in the acetate infused into the ruminal vein. The irreversible loss rate (ILR) of [2-^{13}C]acetate was

calculated as:

infusion rate of [2-^{13}C]acetate (mmol/h) × (0·995 − AF_B)/[(AF_A − AF_B) × 2].

The portal net appearance was calculated as:

blood flow × (portal concentration − arterial concentration).

The corrected portal appearance of acetate was calculated as:

blood flow × [portal concentration − (arterial concentration × portal recovery of arterial acetate)].

The mean of the second sample in control period 1 and the third sample in control period 2 was used as reference for portal appearance of non-reticulo-ruminal origin. The portal appearance of SCFA during the experimental period was calculated as the area under the portal appearance *v.* time curve. The absorption of SCFA during the experiment was calculated as:

(SCFA administered with SCFA buffer + continuously infused SCFA) − (SCFA removed when emptying the rumen + SCFA in fluid collected by saliva collector + SCFA dissolved in the control buffer 2).

The portal recovery of SCFA was calculated as:

100 × (portal appearance during the experimental period − reference level)/absorbed SCFA.

The portal appearance rate of SCFA in the control periods 1 and 2, the blood concentrations and the portal appearance in the control and experimental period were compared by the paired *t* test using the means procedure of SAS (SAS/STAT™ Users Guide, Release 6.03; SAS Institute Inc., Cary, NC, USA). There was no evidence of any animal effects on the variables tested; the data were therefore treated as nine independent observations.

Results

The pH of the SCFA buffer introduced into the rumen was 6·80 before infusion. The rumen pH was stable during the experiments and averaged 7·11 (SE 0·05).

Fig. 1 shows the concentration profile of the acetate in arterial and portal blood. There was a tendency to a decreasing arterial concentration and a decreasing portal–arterial concentration difference between sample 1 and sample 2 in control period 1 and between sample 1 and sample 3 in control period 2. The arterial concentrations were stable during the experimental period. The portal concentration as well as the portal–arterial concentration difference increased during the experimental period.

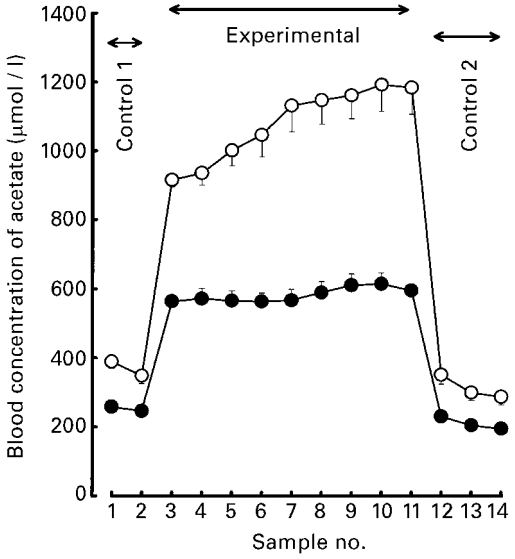

Fig. 1. Concentrations of acetate in arterial (●) and portal (○) blood in sheep where the natural rumen contents were replaced by a bicarbonate buffer without short-chain fatty acids (SCFA) (control 1), a bicarbonate buffer containing (mmol/l): acetate (50), propionate (20), isobutyrate (2), butyrate (5), isovalerate (1·5), valerate (1·5), in combination with continuous intraruminal infusion of SCFA (experimental) and a bicarbonate buffer without SCFA (control 2). All data points are means with their standard errors represented by vertical bars for nine experiments. For details of procedures, see p. 478.

The amounts of SCFA administered to, removed from and absorbed by the sheep are shown in Table 2. The percentage of absorbed relative to administered SCFA was lowest for isobutyrate (41 (SE 2) %) and highest for valerate (72 (SE 2) %).

The arterial concentrations of all detected SCFA increased during the experimental period, compared with the control values ($P < 0·001$; Table 3). The portal–arterial concentration difference increased ($P < 0·001$), the portal blood flow tended to increase ($P < 0.1$), the ^{13}C-enrichment of arterial acetate decreased ($P < 0·001$), and the portal–arterial difference in ^{13}C-enrichment of acetate decreased ($P < 0·05$) during the experimental period compared with the control period. The portal recovery of arterial acetate was not altered during the experimental period compared with the control period ($P > 0·10$).

The portal net appearance of SCFA was five to ten times higher during the experimental period than in the control period (Table 4). The portal net appearance of acetate, the increased ILR of acetate and the portal appearance of acetate corrected for portal-drained viscera (PDV) uptake of arterial acetate accounted for 89 (SE 5), 101 (SE 7) and 109 (SE 7) % respectively of the acetate absorbed by the sheep. The portal net appearance of propionate and isobutyrate could account for 95 (SE 7) and 102 (SE 9) % respectively of the propionate and isobutyrate absorbed by the sheep. The portal net appearance of butyrate, isovalerate and valerate could account for 23 (SE 3), 48 (SE 5) and 32 (SE 4) % respectively of the butyrate, isovalerate and valerate absorbed by the sheep.

Table 2. Amounts and percentage of short-chain fatty acids (SCFA) and buffer solutions administered to, removed from and retained by sheep with isolated and washed reticulo-rumen*

(Values are means with their standard errors for nine experiments)

	Administered				Removed				Retained					
	Rumen buffer		Infusion		Rumen buffer		Saliva†		Dissolved‡		Absorbed		%§	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Acetate (mmol)	150·0	2·0	283·0	15·0	140·0	13·0	26·0	7·0	5·0	2·0	262·0	14·0	60	2
Propionate (mmol)	62·0	1·0	144·0	8·0	68·0	7·0	11·0	3·0	2·0	1·0	125·0	8·0	60	2
Isobutyrate (mmol)	6·8	0·2	16·0	1·0	11·0	1·0	1·6	0·4	0·4	0·1	9·0	1·0	41	2
Butyrate (mmol)	16·0	1·0	30·0	2·0	14·0	1·0	2·8	0·7	0·4	0·1	29·0	2·0	63	2
Isovalerate (mmol)	4·6	0·2	14·0	1·0	6·6	0·5	1·1	0·3	0·3	0·1	11·0	1·0	57	2
Valerate (mmol)	5·1	0·2	15·0	1·0	4·7	0·5	0·7	0·2	0·1	0·0	14·0	1·0	72	2
Liquid (litres)	3·0	0·0	0·8	0·0	2·0	0·2	2·3	0·3			−0·8	0·6		

* For details of animals and procedures, see p. 478.
† Contamination of collected saliva by rumen content.
‡ Estimated from the initial SCFA concentration in the control buffer in control period 2.
§ Absorbed SCFA as a percentage of administered SCFA.

Discussion

The combination of the temporarily-isolated reticulo-rumen and the multi-catheterization technique is, to the best of our knowledge, used here for the first time to investigate the metabolism of SCFA by PDV tissues. Earlier studies using a similar technique (Weigand *et al.* 1972*a*,*b*) did not include blood flow measurements. Other studies applying the long-term intragastric infusion technique (Gross *et al.* 1990*a*,*b*) are less conclusive in this respect, as the rumen was not emptied of organic material before the infusion period.

The present technique has a number of advantages compared with a simple infusion protocol: (1) a considerable difference in portal absorption between control and treatment could be obtained without hyperphysiological SCFA concentrations in the rumen; (2) the abrupt decrease in portal appearance of SCFA after removal of the SCFA-containing buffer from the rumen (Fig. 1) indicated a low activity of the reticulo-ruminal orifice or rapid absorption of SCFA entering the omasum; (3) the technique efficiently abolished microbial metabolism of SCFA, because only microbes attached to the reticulo-ruminal wall were present; (4) the technique allowed precise determination of the SCFA absorbed by the animal during the experiments; (5) absorption from the rumen was not affected by changes in rumen motility because gassing the buffer ensured continuous mixing of the rumen content.

Table 3. Arterial and portal–arterial blood concentrations of short-chain fatty acids (SCFA), isotopic composition of acetate and portal blood flow in sheep with the rumen contents replaced by bicarbonate buffer without SCFA (control) and a bicarbonate buffer containing SCFA (experimental)‡

(Values are means with their standard errors for nine experiments)

	Control		Experimental	
	Mean	SE	Mean	SE
Arterial concentration (μmol/l)				
Acetate	222·0	8·0	584·0***	24·0
Propionate	6·0	1·0	21·0***	2·0
Isobutyrate	1·7	0·1	5·9***	0·5
Butyrate	1·6	0·1	4·4***	0·3
Isovalerate	1·3	0·1	4·8***	0·2
Valerate	nd		nd	
Portal–arterial concentration difference (μmol/l)				
Acetate	97·0	21·0	499·0***	42·0
Propionate	23·0	3·0	227·0***	19·0
Isobutyrate	3·1	0·4	19·0***	1·0
Butyrate	2·0	0·7	13·0***	1·0
Isovalerate	0·9	0·1	9·6***	0·8
Valerate	1·0	0·4	8·3***	0·7
Portal blood flow (l/h)	137·0	14·0	151·0*	14·0
Arterial acetate ($\delta^{13}C_{PDB}$‰)†	572·0	54·0	256·0***	14·0
Portal–arterial difference acetate ($\delta^{13}C_{PDB}$‰)	880·0	310·0	91·0**	30·0
Portal recovery of arterial acetate (%)	72·0	11·0	74·0 NS	6·0

nd, not detectable.
Mean values were significantly different from those for the control group (paired *t* test): NS $P > 0{\cdot}10$, *$P < 0{\cdot}10$, **$P < 0{\cdot}05$, ***$P < 0{\cdot}001$.
† $\delta^{13}C_{PDB}‰ = ((R_{sample} - R_{standard})/R_{standard}) \times 1000$, where R is $^{13}C/^{12}C$ and R of the PeeDee Belemnite standard = 0·0112372.
‡ For details of procedure, see p. 478.

Table 4. Portal net appearance of short-chain fatty acids (SCFA), portal appearance of acetate corrected for portal-drained viscera (PDV) uptake of arterial acetate and irreversible loss rate of acetate, together with portal recovery of the SCFA absorbed from the temporarily-isolated and washed reticulo-rumen of sheep*

(Values are means with their standard errors for nine experiments)

	Appearance of SCFA (mmol/h)				Portal recovery (%)	
	Control		Experimental			
	Mean	SE	Mean	SE	Mean	SE
Portal net appearance						
Acetate	14·0	4·0	72·0	7·0	89·0	5·0
Propionate	3·0	0·5	33·0	2·0	95·0	7·0
Isobutyrate	0·4	0·1	2·7	0·2	102·0	9·0
Butyrate	0·3	0·1	2·0	0·2	23·0	3·0
Isovalerate	0·1	0·1	1·4	0·1	48·0	5·0
Valerate	0·2	0·1	1·3	0·2	32·0	4·0
Portal appearance corrected for PDV uptake of arterial [2-^{13}C]acetate						
Acetate	25·0	6·0	96·0	10·0	109·0	7·0
Irreversible loss rate of [2-^{13}C]acetate						
Acetate	71·0	11·0	136·0	10·0	101·0	7·0

* For details of procedures, see p. 478.

In contrast to a number of previously-published studies, therefore, the results of the present experiment reflect the animal effect on portal recovery of intraruminally-infused SCFA and not the combined microbial and animal effects.

The present experimental design will probably give rise to debate about the possible effect of the technique on rumen epithelial metabolism. It is difficult to assess this point adequately; however, major changes in the epithelial metabolism would appear to be unlikely, as it is exposed to a buffer that, although less complex than rumen fluid, contains a similar buffer system, a similar electrolyte content and similar SCFA concentrations.

The data presented here on acetate and propionate seem to be in conflict with 30 years of experimental evidence. A number of studies have come to the conclusion that 25–50 % of the intraruminally-infused or produced acetate and propionate are metabolized during absorption from the gastrointestinal tract (for a review, see Kristensen *et al.* 1998). We have no reason to question the experimental results of the earlier studies, which together comprise a substantial amount of data. However, on the basis of the present study, we have to question the generally-accepted interpretation of these experiments. Ruminally-produced or infused acetate and propionate, not recovered in the portal vein, were probably sequestrated within the gastrointestinal tract and not metabolized by the reticulo-ruminal epithelium when the rumen contained a natural microbial environment. This interpretation is supported by results relating to microbial utilization of SCFA for biosynthesis (Emmanuel, 1974; Emmanuel, *et al.* 1974) and indirectly by the missing link between the acetate and propionate that are apparently taken up by the rumen epithelium and the absence of metabolites of these SCFA in the portal blood. The amount of acetate and propionate not accounted for would far exceed the oxidative needs of the epithelium.

Isobutyrate was also completely recovered in the portal blood and this finding is in good agreement with the apparently lower epithelial metabolism of isobutyrate than that of other SCFA *in vitro* (Weigand *et al.* 1975). We know of only one study of the portal recovery of isobutyrate *in vivo*, in which hay-fed animals showed a portal recovery of 60 % of intraruminally-infused isobutyrate (Kristensen *et al.* 2000).

The recovery of acetate seems to be overestimated when corrected for PDV uptake of arterial [2-^{13}C]acetate (109 (SE 7) %). Endogenous acetate production by rumen epithelium has been shown *in vitro* (Stevens & Stettler, 1966*b*; Sehested *et al.* 1999). It is possible that part of the butyrate, isovalerate and valerate metabolized by the epithelium was released into the portal blood as acetate. On the other hand, the increase in the ILR of acetate was in good agreement with the absorbed acetate (101 (SE 7) %). In view of the uncertainty of these estimates, we can conclude only that the absorbed acetate could be accounted for by the increased ILR of acetate and the portal appearance corrected for PDV uptake of arterial [2-^{13}C]acetate.

The portal recovery of arterial [2-^{13}C]acetate (72–74 %) was comparable with earlier estimates of 66–68 % (Bergman & Wolff, 1971), 67–79 % (Kristensen *et al.* 1996*b*) and 68 % (Kristensen *et al.* 1996*a*).

The estimated portal recovery of the retained propionate (95 (SE 7) %) is in good agreement with those of other *in vivo* studies which showed that only a small percentage of the absorbed propionate is metabolized to lactate by the rumen epithelium (Weigand *et al.* 1972*a*; Weekes & Webster, 1975).

In the present experiment the portal recoveries of butyrate and valerate were comparable with the values obtained with intraruminal infusion of a mixture of SCFA in sheep maintained on a low feed intake (21–32 and 14–31 % respectively; Kristensen *et al.* 2000). Both SCFA are probably degraded in the reticulo-ruminal epithelium by complete or partial β-oxidation. The presence of similar pathways for butyrate and valerate is in good agreement with their interaction on epithelial metabolism *in vivo* (Kristensen *et al.* 2000) and the simultaneous glucogenic and ketogenic effects of valerate *in vitro* (Weigand *et al.* 1975). Under *in vivo* conditions, 40 % of intraruminally infused butyrate could be accounted for by PDV release of 3-hydroxybutyrate (NB Kristensen, SE Pierzynowski and A Danfær, unpublished results). To what extent valerate-C is recovered in the portal blood as 2-methyl-3-hydroxybutyrate or 2-methylacetoacetate is not known.

A higher proportion of the retained isovalerate was recovered in the portal blood compared with butyrate and valerate. This finding could be a reflection of the relatively low activity of the 3-hydroxy-3-methylglutaryl-CoA pathway in rumen epithelium (Bush & Milligan, 1971). Isovalerate is assumed to be metabolized by the leucine degradation pathway (Bender, 1985). We are not aware that any intermediate metabolites of isovalerate degradation have been isolated in the portal blood.

The results of the present study indicate a solution to the enigma of the metabolic fate of rumen SCFA, especially acetate and propionate. We are aware, however, that the experiment should be repeated, that the postulated sequestration of SCFA-C in the microbial biomass should be studied quantitatively *in vivo* and that the diverging metabolic behaviour of rumen epithelium *in vivo* and *in vitro* requires explanation.

Perspectives

The present study points of a redefinition of the role of the PDV tissues in general and of the reticulo-ruminal epithelium in particular, regarding the metabolic pathways of SCFA in ruminants. Until now, the majority of studies have been carried out with complete confounding between animal and microbial pathways, an aspect that has hitherto received little attention. The present study shows that, when corrected for the PDV uptake of arterial acetate, intraruminally-infused acetate, propionate and isobutyrate could be completely accounted for in the portal vein if a small percentage of propionate is assumed to be metabolized into lactate. The SCFA known to be metabolized by β-oxidation and other ketogenic pathways in the rumen epithelium (butyrate, isovalerate and valerate) were shown to be metabolized extensively. It appears that portal fluxes of SCFA represent a valuable contribution to the quantitative understanding of rumen metabolism because the portal absorption of acetate, propionate and isobutyrate probably reflects the rumen microbial net output.

Acknowledgements

We wish to thank Lene Nygaard, Mette B. Petersen and Tine H. Steensen as well as the personnel of the animal stables for skilful technical assistance. The Danish Agricultural and Veterinary Research Council supported this study (grant 9801555).

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