17 Splanchnic carbohydrate and energy metabolism in growing ruminants[1]

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Ruminal fermentation precludes a simple description of nutrient availability based on nutrient intake. Thus, we must strive to understand the nutrient needs of the microflora and gut and then evaluate nutrient availability after these needs have been met. Glucose is extensively metabolized by gut tissues such that the net supply to the liver is often zero or negative. Despite this extensive metabolism, small intestinal digestion can significantly increase glucose availability and metabolism. Lactate is derived from the diet, from ruminal bacterial metabolism and from endogenous metabolism. Because of its ubiquitous nature, lactate production from the gastrointestinal tract and viscera varies widely. However, lactate is a major glucose precursor in ruminants, supplying 9–35% of hepatic glucose carbon. Short-chain fatty acids are the major currency of ruminant energy metabolism, accounting for 45% of digestible energy intake. Significant quantities of short-chain fatty acids are metabolized by ruminal epithelium; however, it appears that in the fed ruminant this epithelial metabolism is limited to butyrate and longer short-chain fatty acids. Estimates indicate that 5% of ruminally supplied propionate is metabolized by the rumen epithelium and 30% of arterially supplied acetate is metabolized by the portal-drained viscera. These findings allow estimates of ruminal short-chain fatty acid production to be obtained from portal appearance of short-chain fatty acids corrected for portal-drained visceral metabolism of arterial short-chain fatty acids and ruminal epithelial metabolism of butyrate.

1. INTRODUCTION

Compared with other mammals, ruminants could seem less efficient in capturing energy in the form of body tissue, fetus, or milk. For example, a young pig on a nutritionally adequate

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diet captures 76% of ME (metabolizable energy) as tissue gain (Dunkin and Campell, 1982) whereas a growing steer on a forage diet captures 46% or less of ME as tissue gain (Varga et al., 1990). Does this indicate that ruminants are energetically inefficient? The answer to this question is not as straightforward as might be indicated by its simplicity. First of all, the relative growth rates of the pig and the steer will affect the energetic efficiency, i.e. lower relative growth rate means that a relatively higher proportion of the total energy is used for maintenance. A quite different aspect is that interchanging the diets would have disastrous consequences for the performance of the young pig whereas the steer might do well though likely to suffer from overfeeding. The pig will not be able to obtain sufficient amounts of nutrients from a forage diet to obtain its potential growth rate.

These findings and the fact that ruminants utilize intravenously infused glucose as efficiently as nonruminants (Reid et al., 1980) indicate that the key to understanding both possibilities and limitations in ruminant nutrition and efficiency is related to the digestive strategy of ruminants. The forestomach fermentation in ruminants implicates that there is only an indirect relationship between the molecular composition of the feed and the actual nutrients available for absorption. The fermentation has a major influence on digestion and metabolism of all organic dietary components, i.e. carbohydrate, protein, fat, and vitamins. Carbohydrates make up the largest fraction of almost any diet for functional ruminants and the utilization of carbohydrate will therefore be of importance to both efficiency and performance. However, a number of controversies still exist connected to the availability of carbohydrate (starch) for postruminal digestion and absorption as well as quantitative relationships between carbohydrate fermentation and end-product (short-chain fatty acid; SCFA) availability to the animal. The purpose of this chapter is to detail some of the unique aspects of ruminant energy metabolism. Primarily, we aim to focus on the supply of glucose, lactate, and SCFA as sources of energy and their availability to body tissues. Only through a thorough understanding of these interrelationships can we hope to predict and explain growth responses based on dietary inputs.

2. GLUCOSE

Because of pregastric fermentation much of the dietary carbohydrate is fermented to SCFA. This fermentation leaves little dietary carbohydrate available for absorption in the small intestine. Only when high-concentrate diets are fed are significant quantities presented to the small intestine for absorption (Huntington, 1997). Thus, pregastric fermentation necessitates a continual need for very high rates of gluconeogenesis (Bergman, 1973) to meet the glucose needs of the ruminant. The fermentation of dietary carbohydrates necessitates unique adaptations in ruminant glucose metabolism and many of these adaptations have been detailed in some excellent reviews (Leng, 1970; Bergman, 1973; Young, 1976); gluconeogenesis is also discussed in a separate chapter within this book (see Chapter 15 by Donkin and Hammon). We shall focus on how dietary influences affect the glucose economy of growing ruminants and on current information on the interorgan metabolism of glucose in ruminants.

3. SOURCES OF GLUCOSE

Blood glucose concentrations are typically 4–6 mM in most mammals; however; ruminant concentrations tend to be lower, at 2–5 mM (Bergman, 1973). Despite low blood glucose concentrations and continual gluconeogenesis, ruminant blood glucose concentrations are very responsive to intestinal carbohydrate digestion and absorption (Larson et al., 1956).

The major carbohydrate that ruminants consume in early life is lactose from milk. However, by 1–3 weeks of age ruminal fermentation is active and only through suckling will animals achieve closure of the esophageal groove and bypass significant quantities of materials to the abomasum for gastric digestion (Orskov et al., 1970). With the increase in ruminal fermentation there is a decline in the ability to digest lactose in the small intestine. Intestinal lactase (St. Jean et al., 1989) and glucose transport (Shirazi-Beechey et al., 1989) activities in the small intestine decline after weaning.

Postweaning dietary carbohydrates contributing directly to glucose supplies include the various forms of α-linked glucose available in plants. Russell and Gahr (2000) described the classification of food carbohydrates as occurring in four forms: (1) free (not associated with the cellular structure), such as lactose in milk or fructose in honey; (2) intracellular, which includes soluble sugars and storage polysaccharides such as starch and fructans; (3) cell wall components including cellulose, hemicellulose, pectins, and gums; and (4) chitin, a component of the exoskeleton. For the functioning ruminant, only the intracellular storage polysaccharide, starch, contributes significantly to absorbed glucose. The remaining forms of food carbohydrate are first fermented to SCFA.

Huntington (1997) summarized numerous digestion experiments with starch intakes ranging from 1.5 to 10.6 kg/d. In these experiments, ruminal starch digestibility ranged from 94% to 50%. The net result is that starch flow to the small intestine ranged from 90 to over 5000 g/d. These data demonstrate that starch intake can make a sizable contribution to the glucose needs of growing ruminants. However, to determine the contribution of starch intake to glucose availability, the efficiency of small intestinal digestion must be known.

4. IMPACT OF INTESTINAL DIGESTION ON GLUCOSE SUPPLY

Several experiments have used animals fitted with hepatic portal vein and hepatic vein catheters to measure the quantity of glucose exiting the portal-drained viscera (PDV) and entering the liver (Huntington et al., 1989). This measurement provides a means of determining the net glucose contributions to the liver or peripheral tissues and measures the sum of glucose absorption and metabolism. Across a wide range of experiments encompassing varied diets, intakes, and physiological states, net glucose absorption is almost always zero or negative (Reynolds et al., 1994). This is not to say that glucose is not being absorbed, but rather that very large amounts of glucose from the arterial supply are being metabolized such that the "net" result from absorption and metabolism is zero or negative. In a study designed to quantitate intestinal contributions to portal glucose supply, Huntington and Reynolds (1986) abomasally infused glucose and corn starch into heifers. Overall, they recovered an average of 65% of the glucose and 35% of the starch as glucose in portal blood. No differences were observed for the amounts of glucose recovered from animals fed alfalfa hay or a high-concentrate diet at two intakes, suggesting little effect of adaptation for carbohydrate assimilation. Kreikemeier et al. (1991) fed steers alfalfa hay to minimize intestinal carbohydrate supply and abomasally infused them with glucose, corn starch, or corn dextrins at 20, 40, and 60 g/h. Infusions all lasted 10 h, with samples taken during the final 6 h. Glucose infusion resulted in 90% recovery of intestinal glucose disappearance in portal blood whereas only 19% and 32% of the dextrin and starch intestinal disappearance were recovered in portal blood, respectively.

Factors such as microbial fermentation and gut tissue metabolism must certainly make a large contribution to small intestinal carbohydrate disappearance and emphasize the need for measures of tissue metabolism and intestinal disappearance to more accurately describe

processes of digestion and absorption. The very high metabolic activity of the PDV tissues has been shown to be a major factor in the apparently low net rates of glucose absorption (Reynolds and Huntington, 1988a). These authors (Reynolds and Huntington, 1988a,b) measured directly the contribution of stomach and intestinal tissues to nutrient absorption in beef steers. When steers were fed a concentrate diet, comparatively large amounts of glucose were absorbed from the intestines; however, the amounts utilized by ruminal and other stomach tissues were so great that the overall net PDV absorption was negative. Attempts were made in previous studies to account for this negative net glucose absorption and thereby obtain a better estimate of net glucose absorption by including control (water) infusions (Kreikemeier et al., 1991). However, more recent work has shown that increasing the peripheral supply of carbohydrate, either through intraduodenal or intrajugular infusion of glucose, increases the metabolism of arterially supplied glucose by the PDV (Balcells et al., 1995), making these corrections tenuous at best.

5. DIET EFFECTS ON GLUCOSE METABOLISM

In previous sections we have attempted to define relationships of intestinal supply and glucose availability. However, it needs to be clearly pointed out that the major determinant of glucose supply is dietary energy intake (Herbein et al., 1978). Experiments assessing whole-body glucose metabolism have clearly shown that glucose irreversible loss, a measure of the flow of glucose through the body pool never to return, and thus, at steady state, an indicator of glucose production, is a function of digestible energy intake. Schmidt and Keith (1983) tested this hypothesis using steers fed 70% corn vs. 70% alfalfa diets fed at equal energy intakes. They demonstrated that when steers were fed at equal energy intakes, glucose irreversible loss was equal. When dry matter intakes were equalized, glucose irreversible loss was greater for the 70% corn diet because of the greater energy intake with the corn. In a related study (Russell et al., 1986) it was demonstrated that glucose irreversible loss was directly related to energy intake independent of body size in steers ranging in weight from 136 to 470 kg.

These relationships depend on the tight control between digestible energy intake and gluconeogenesis. Organic matter fermented in the rumen will supply glucose precursors, primarily propionate, to meet the glucose needs of the host. These relations are borne out in the work of Van Maanen et al. (1978), who determined ruminal propionate production and glucose irreversible loss in steers fed forage and grain-based diets with the propionate-enhancing antibiotic, monensin. Monensin increased ruminal propionate production by 49% on the forage diet and by 76% on the grain diet. Associated with these increases in propionate were increases in glucose irreversible loss of 7% and 16% for the forage and grain diets, respectively. This study shows that increasing propionate supply can increase glucose irreversible loss, but not in direct proportion. These results were similarly borne out by Seal and Parker (1994) using intraruminal infusion of propionate in calves. Only at their highest propionate infusion (1 mol/d) was glucose irreversible loss increased. Interestingly, ruminal propionate infusion decreased PDV glucose use from 28% to 11% of glucose irreversible loss.

The relationships between dietary energy intake and glucose irreversible loss depend upon two related assumptions: (1) ruminants have a very tight control of hepatic glucose production, and (2) digestion and absorption of starch in the small intestine contributes little to glucose irreversible loss in these studies because they are dependent on glucose derived from the products of ruminal fermentation. Bauer et al. (1995) infused phlorizin, a potent inhibitor of SGLT1, into the abomasum of steers and sheep and demonstrated that when glucose active

transport was inhibited, hepatic glucose production increased resulting in no change in total splanchnic glucose output. This demonstrates that glucose production is well coordinated between the PDV and the liver.

A different approach was used in the study by Harmon et al. (2001). They infused a partially hydrolyzed starch solution either ruminally or abomasally in growing steers. Shifting the site of starch digestion from the rumen to the small intestine increased glucose utilization by PDV tissues (132%), PDV glucose flux (310%), and irreversible loss of glucose (59%). Abomasal infusion resulted in greater total energy availability (28%) from the total splanchnic tissues. Thus, shifting starch digestion to the small intestine increases PDV glucose uptake and utilization without a corresponding decrease in hepatic glucose production. This shift results in greater glucose supplies to the periphery. This would seem in contrast to the results of Herbein et al. (1978), who related glucose irreversible loss solely to energy intake. These relationships may not hold if significant quantities of starch are digested and absorbed in the small intestine. Balcells et al. (1995) infused sheep jugularly with glucose and found that glucose irreversible loss increased over 2-fold. Accompanying this increase in systemic glucose availability was an increased utilization of glucose by the PDV. However, in their experiment, the fraction of whole-body glucose used by the PDV remained constant (30% of whole-body glucose irreversible loss) despite the increase in glucose irreversible loss. These results are in agreement with their later work (Cappelli et al., 1997) where sheep received exogenous glucose either intrajugularly or intraduodenally. Supplying glucose by either route increased whole-body glucose irreversible loss and portal glucose utilization, and again, portal glucose utilization was approximately 30% of glucose irreversible loss.

These results suggest that the fraction of whole-body glucose irreversible loss used by the PDV is relatively constant. However, both of these studies were relatively short-term, lasting 6 to 8 h. They do not answer whether or not long-term exposure causes tissues to adapt and use more or less of the available glucose. In the study by Harmon et al. (2001) they infused a partially hydrolyzed starch solution either ruminally or abomasally in growing steers for 7 days. In their study, portal glucose utilization was 23% of whole-body glucose irreversible loss with the ruminal infusion and this increased to 34% when the carbohydrate was infused abomasally. Thus, despite a 58% increase in glucose irreversible loss, there was a concomitant increase in the fraction of glucose metabolized by PDV tissues. It is not known if this increase in metabolism was the result of tissue adaptation or simply differences in cattle and sheep. With the ruminal infusion an increase in metabolism could reflect more energy available as SCFA resulting in less PDV glucose use, as was seen with the ruminal propionate infusions of Seal and Parker (1994) described above. A decrease in net PDV glucose use has also been reported for steers fed 450 g/d sodium propionate (Harmon and Avery, 1987).

McLeod et al. (2001) used the ruminal/postruminal infusion of carbohydrate model described above (Harmon et al., 2001) to study energy balance in growing steers. They reported that abomasal infusion of carbohydrate increased retained energy; however, based on calorimetric data, the energy retained was retained solely as fat. When combined, these results suggest that an increased availability of glucose increases the energetic efficiency and PDV metabolism of glucose, but this may also result in greater fat deposition. One could speculate that increased circulating glucose results in increased insulin and increased fat deposition. Others have suggested that there are specific effects of glucose on lipogenesis in ruminants. Pearce and Piperova (1984) compared duodenal infusions of glucose and dextrins in sheep and found that glucose infusion increased *in vitro* lipogenesis from acetate nearly 7-fold in subcutaneous adipose tissue as compared with control (noninfused) sheep.

6. DIETARY AND DIGESTIVE SOURCES OF LACTATE

Lactate entering portal blood of the gastrointestinal tract of a ruminant can come from the diet, can be a product of rumen fermentation, or can be a product of tissue metabolism. Dietary sources generally include lactate from fermented feeds, e.g., a product of lactobacilli in silages. Lactate is produced in fermented feeds by homo- or hetero-fermenting lactobacilli that vary in substrate (sugar) preferences and isomer of lactic acid produced. Most bacteria can produce either D(−)- or L(+)-lactic acid by virtue of isomer-specific lactate dehydrogenase and lactate racemase enzyme activity (Counotte and Prins, 1981; McDonald et al., 1991).

Lactic acid concentrations in most silages prepared by adequate or competent techniques range from 3 to 12 g/kg DM. Treatments that limit fermentative activity, e.g. treatment with mineral acids, formic acid, or formalin, or wilting before ensiling, can reduce lactic acid concentration by one-half or more. Treatments that induce or enhance fermentative activity in the silo, e.g. inoculation with bacteria, addition of sugars or propionic acid, decreased particle size by precision chopping of herbage before ensiling, in general increase lactic acid concentration 1.5–2.0-fold (McDonald et al., 1991; Sheperd et al., 1995; Kung et al., 2000; Kung and Ranjit, 2001). The isomeric proportions of lactic acid in these feedstuffs have not been studied extensively; available reports indicate that L(+):D(−) ratios range from 0.3:1 to 1:1 (Schaadt, 1968; Hull, 1996; Kung et al., 2000). McDonald et al. (1991) suggested that as time of ensiling increases, the L(+):D(−) ratio approaches 1:1 because of racemase activity of lactobacilli.

Lactate is both produced and used by ruminal microbes. Numbers (and activity) of lactate producers and users respond rapidly to readily fermentable substrate (Counotte and Prins, 1981; Goad et al., 1998), which means that ruminal lactate concentrations usually are very low (1–3 mM) to nondetectable. Calculations of lactate production in the rumen are in a similar range, 1–3 mmol/h (Counotte and Prins, 1981). In cases of abrupt changes in intake of readily available carbohydrates there can be a rapid increase in ruminal lactate concentrations, indicating that production can exceed use or removal from the rumen. For example, Harmon et al. (1985) dosed beef steers intraruminally with 12 g of glucose per kg of body weight and measured peak concentrations of L(+)- lactate and D(−)-lactate of 77 and 40 mM, respectively, 30 h after the dosing. As a result of rapid fermentation of the carbohydrates, the proportion of L(+):D(−)-lactate may change from predominantly L(+) to predominantly D(−). The change in isomeric ratio is more a function of increased production than differences in use rates, because both isomers are used by ruminal microbes at similar rates. The rapid production and accumulation causes a ruminal acidosis that is lethal to many ruminal protozoa, and also causes a systemic acidosis in the host ruminant (Dunlop, 1972; Counotte and Prins, 1981; Goad et al., 1998). Ruminal concentrations and isomeric proportions of lactate are the product of the effects of ruminal production, use, absorption from the rumen, and passage with digesta to more distal portions of the gastrointestinal tract.

7. ABSORPTION OF LACTATE FROM THE GASTROINTESTINAL TRACT

L(+)-lactate (and presumably D(−)-lactate) are transported across cell membranes by a family of monocarboxylate transporters (Price et al., 1998). These transporters also transport ketones, pyruvate, and acetate. Because lactate can be a product of tissue metabolism, a substrate for tissue metabolism, and the subject of transport across the plasma membrane of epithelial cells, it is difficult to discern the relative importance of, or interactions among, these processes on the rate of lactate appearance in portal blood draining the gastrointestinal tract.

Further, dietary and endogenous factors that alter blood flow can negate or amplify *in vivo* changes in concentration differences in blood supplying and draining the PDV. The few data available for absorption of D(−)-lactate suggest that factors that promote production of D(−)-lactate in the rumen also promote its absorption and appearance in hepatic portal blood (Huntington et al., 1980, 1981; Harmon et al., 1985). For the remainder of this discussion of lactate absorption and metabolism, "lactate" and "L(+)-lactate" will be used synonymously unless otherwise indicated.

The studies summarized in table 1 are representative of published literature that quantifies net flux of lactate across splanchnic tissues. The studies show that lactate absorption in sheep and cattle ranges from approximately 2 to 200 mmol/h. Increased intake of a given diet increases net absorption (Reynolds et al., 1991; table 1), as does increased body mass (usually accompanied by increased intake), albeit at a nonlinear rate (Eisemann et al., 1996; table 1). The data from Taniguchi et al. (1995; table 1) exemplify the positive relationship between increased ruminal fermentation and lactate absorption (alfalfa vs. alfalfa and ruminal starch infusion in table 1), and also indicate that increased intestinal appearance of glucose results in increased portal appearance of lactate, ostensibly as a result of postruminal gut tissue metabolism (ruminal vs. abomasal infusion of starch in table 1). The postruminal digestive tract accounted for about one-third of lactate absorption in beef steers fed alfalfa hay or a high-concentrate diet (Reynolds and Huntington, 1988b). The lactating dairy cows in the studies in table 1 had similar daily dry matter intakes (data not shown), but the cows eating the grass diet absorbed less lactate than the cows eating corn silage and supplement (Reynolds et al., 1991; De Visser et al., 1997; table 1). McLeod et al., (1997) (table 1) found that infusion of somatostatin decreased blood flow through PDV of sheep, but increased venoarterial difference of lactate (data not shown), resulting in increased net absorption of lactate. The study of Bauer et al. (1995; table 1) included intragastric infusion of phlorizin, which decreased net absorption of glucose (data not shown) but had no statistically significant effect on lactate flux. Other examples of lack of effects of metabolic regulators include similar net absorption of lactate in control beef steers vs. steers fed a β-adrenergic agonist (Eisemann and Huntington, 1993) or control steers vs. hyperinsulemic, euglycemic beef steers receiving intravenous infusion of insulin and glucose (Eisemann and Huntington, 1994).

Lactate makes a small but measurable contribution to the overall energy supply for ruminants. Lactate accounted for approximately 4.3% of the sum of energy absorbed as SCFA and lactate by lactating dairy cows consuming all-forage diets (De Visser et al., 1997; table 1), 8% by lactating dairy cows consuming a 60:40 corn silage:supplement diet (Reynolds et al., 1991; table 1), 9% by steers consuming all-forage diets (Huntington et al., 1988), and 16% by heifers consuming a diet containing 780 g corn grain/kg of DM (Huntington and Prior, 1983).

8. HEPATIC METABOLISM OF LACTATE

The metabolic importance of lactate for ruminants centers on its role as a glucose precursor in the liver; net lactate removal by the liver often exceeds portal supply (table 1) and can theoretically account for 9–35% of net hepatic glucose release (data not shown) in studies with bovines listed in table 1. Studies with infusions of radiolabeled glucose and lactate into lambs and steers indicate that from 5% to 11% of glucose carbon comes from L(+)-lactate, and less than 1% comes from D(−)-lactate (Huntington et al., 1980, 1981; Harmon et al., 1983). Recycling of carbon through lactate and glucose would cause underestimations from isotope infusions, and calculations from net fluxes likely overestimated the true conversion of lactate to glucose. In the sheep studies of McLeod et al. (1997; table 1) net lactate removal could

Table 1

Selected studies of L(+)-lactate flux[a] across portal-drained viscera (PDV), liver, and total splanchnic (TSP) tissues of sheep and cattle

Species	BW, kg	Diet description	Net flux, mmol/h			Reference
			PDV	Liver	TSP	
Sheep wethers	36	Alfalfa hay, duodenal starch and casein infusion	4.8	−9.8	−5	McLeod et al. (1997)
Sheep wethers	36	Alfalfa hay, duodenal starch and casein infusion, somatostatin injection	6.6	−10.6	−4	McLeod et al. (1997)
Sheep wethers	40	Alfalfa hay, starch infusion	2.2	3.2	5.4	Bauer et al. (1995)
Beef heifers	321	Alfalfa:concentrate, low intake	45	−17	28	Reynolds et al. (1991)
Beef heifers	321	Alfalfa:concentrate, high intake	82	−28	54	Reynolds et al. (1991)
Beef steers	236	Bromegrass hay:concentrate 60:40	47	−81	−34	Eisemann et al. (1996)
Beef steers	438	Bromegrass hay:concentrate 60:40	67	−101	−34	Eisemann et al. (1996)
Beef steers	522	Bromegrass hay:concentrate 60:40	63	−85	−22	Eisemann et al. (1996)
Beef steers	253	Alfalfa hay	39	−63	−24	Taniguchi et al. (1995)
Beef steers	253	Alfalfa hay, ruminal starch infusion	50	−77	−27	Taniguchi et al. (1995)
Beef steers	253	Alfalfa hay, abomasal starch infusion	75	−68	−7	Taniguchi et al. (1995)
Lactating dairy cows	645	Corn silage:supplement 60:40	216	−249	−33	Reynolds and Huntington (1988c)
Lactating dairy cows	500	Fresh ryegrass	121	−144	−23	De Visser et al. (1997)

[a]Positive numbers indicate net absorption or release, negative numbers indicate uptake or removal.

maximally account for 41–62% of net hepatic glucose production. Lactate contribution is not calculated for the data of Bauer et al. (1995; table 1) because in some of their treatments they measured net hepatic output of lactate.

The range of these potential hepatic fluxes and potential contribution to hepatic gluconeogenesis attest to the flexibility and versatility of lactate to participate in postabsorptive metabolism. The complete data of Reynolds et al. (1991; table 1) for beef heifers showed an interaction between intake level and percentage of dietary concentrate; net hepatic lactate removal and potential contribution of lactate to gluconeogenesis increased when the heifers' intake of a high-forage diet increased. However, lactate removal and potential contribution to gluconeogenesis decreased when the heifers' intake of a high-concentrate diet increased. A mesenteric vein infusion of alanine in the same heifers (Reynolds and Tyrrell, 1991) increased net alanine removal and reduced net lactate removal by the liver, but did not affect net hepatic glucose output. These results indicate a replacement of lactate by alanine as a glucose precursor. The complete data of Eisemann et al. (1996; table 1) predict decreased net hepatic removal or extraction of lactate, and increased net hepatic removal of amino acids to support increased hepatic glucose production in beef steers as they grow from 235 to 525 kg of body weight. The somatostatin injection that increased net portal absorption of lactate in sheep also increased net hepatic removal of lactate and increased glucose output by the liver (McLeod et al., 1997; table 1). Steers fed a β-adrenergic agonist had an acute surge in lactate removal by the liver that could account for up to 63% of liver glucose output on the first day of treatment. Hepatic removal and potential contribution to gluconeogensis subsided after 7 days of treatment (Eisemann and Huntington, 1993).

9. PERIPHERAL METABOLISM OF LACTATE

Circulating concentrations of L(+)-lactate range from 0.2 to 1.0 mM, and concentrations of D(−)-lactate are 0.10 to 0.50 of concentrations of L(+)-lactate (Huntington et al., 1980, 1981; Harmon et al., 1983); these studies are cited in table 1. Whole-body lactate turnover in beef cattle and sheep ranges from approximately 5 to 10 times net portal absorption (Huntington et al., 1980, 1981), indicating the importance of the Cori cycle in movement of carbon through lactate and glucose between the liver and peripheral tissues, mostly muscle. Excitement or agitation of animals can cause a rapid rise in blood lactate levels as a result of heightened muscle activity. The major fate of D(−)-lactate is oxidation, which accounted for essentially all D(−)-lactate turnover in steers (Harmon et al., 1983). *In vitro* studies with bovine tissues show significant potential for oxidation of D(−)-lactate, with the greatest activity in kidney cortex followed by heart and liver, the lowest activity being detected in muscle tissue (Harmon et al., 1984).

Net flux of L(+)-lactate across hindlimbs of cattle varies in response to physiological state of the animal and physiological interventions by researchers. As stated previously, lactate interacts with glucose through the Cori cycle, but lactate also is used as a substrate for lipid synthesis. Therefore, depending on the contribution of fat to tissue makeup, the hindlimbs may be net users or net releasers of lactate (Prior et al., 1984; Eisemann et al., 1996). The acute response of beef steers to an orally administered β-adrenergic agonist was a dramatic increase in lactate production by hindlimbs which was not evident after 7 days of treatment (Eisemann and Huntington, 1993). Establishment of hyperinsulemia with euglycemia in steers enhanced glucose uptake by hindquarters, but did not significantly change lactate flux across those tissues (Eisemann and Huntington, 1994).

10. SHORT-CHAIN FATTY ACIDS OVERVIEW

Short-chain fatty acids are simple aliphatic carboxylic acids with straight or methyl-branched hydrocarbon chains of 2 to 5 carbons. The SCFA anions with 2 (acetate), 3 (propionate), and 4 (butyrate) carbons are the most prevalent SCFA in the rumen and colon (Bergman, 1990) and their production is closely related to the energy metabolism of rumen microbes (Russell and Wallace, 1988). The existence of acetate in the rumen was observed more than a hundred years ago; however, not until the 1940s was it discovered that SCFA are absorbed from the forestomachs and make a significant contribution to ruminant metabolism (Barcroft et al., 1944). About 67% of ruminal SCFA are absorbed across the rumen epithelium or taken up by the rumen microbes and about 33% are carried out of the rumen by liquid passage (Peters et al., 1990). Short-chain fatty acids leaving the rumen with liquid outflow are absorbed mainly in the omasum and abomasum (Masson and Phillipson, 1952; Rupp et al., 1994).

In ruminants, as in other animals, a mixture of undigested feed and organic matter of endogenous origin enters the hindgut and is fermented into gasses, SCFA, and microbial organic matter. Fermentation in the hindgut is of little quantitative nutritional importance to the animal compared to the forestomach, mostly because microbial protein and other non-SCFA products of fermentation are not readily absorbed. The SCFA production in the hindgut can be estimated as 6–13% of the total gut production based on the propionate appearance across mesenteric drained tissues compared to the total PDV net appearance (Reynolds and Huntington, 1988b). Studies based on isotopic dilution in the rumen and cecum have yielded similar relative production rates (12%) between forestomach and hindgut (Siciliano-Jones and Murphy, 1989). Therefore, forestomach fermentation is quantitatively the most important fermentation in ruminants, and most focus is given to forestomach physiology. However, it must be kept in mind that total gut production of SCFA does contain a hindgut component.

11. TRANSPORT BY NONIONIC DIFFUSION

The rumen is lined with a keratinized stratified squamous epithelium. The epithelium is a heterogeneous structure with a physical barrier formed by keratinized cells facing the lumen. The chemical barrier of the epithelium is below the keratinized cells. The majority of metabolic activity is located in the basal cells as indicated by their high concentration of mitochondria (Steven and Marshall, 1970; Henrikson and Stacy, 1971).

Weak electrolytes, a group to which SCFA belong, can pass biological membranes via nonionic diffusion; the resulting unidirectional flux is a function of concentration (activity) and solubility in the membrane (Rechkemmer, 1991). In accordance with this theory, it has been shown *in vivo* (Thorlacius and Lodge, 1973) as well as *in vitro* (Sehested et al., 1999b) that the unidirectional flux rate of butyrate across rumen epithelium increases with decreasing pH. However, the lack of proportionality between concentration of protonized acids and acetate and propionate fluxes as well as a relatively high permeability of these acids compared to longer-chain fatty acids has been seen as a challenge for the absorption theory based on nonionic diffusion. Nevertheless, a generally observed phenomenon is that SCFA have a relatively high permeability to biological membranes relative to longer-chain fatty acids (Dietschy, 1978). This means that the membranes behave as rather polar structures toward small solutes such as SCFA. The relative absorption rates of SCFA from experiments with washed reticulorumens show that absorption rates of fatty acids longer than butyrate increase with increased chain length (pH 7), and that methyl-branched SCFA (isobutyrate and isovalerate) have lower absorption rates than their corresponding straight-chain fatty acids (Oshio and Tahata, 1984;

Kristensen et al., 2000a). Although the membranes of the rumen epithelium apparently have a relatively high permeability to acetate, propionate, and butyrate, it still makes sense to describe their absorption as regulated by mass action (as long as we consider unidirectional membrane fluxes of SCFA). Recent work has suggested that anion exchangers may contribute to apical SCFA fluxes in rumen epithelium even though the quantitative importance is unknown (Kramer et al., 1996). So far, the data available on SCFA absorption from the forestomach seem to indicate that absorption of SCFA by diffusion can account for the quantitatively most important SCFA absorption.

12. CARRIER-MEDIATED TRANSPORT IN RUMEN EPITHELIUM

Rumen epithelium mounted in Ussing chambers has consistently shown a remarkable difference in the net transport of butyrate compared with the net transport of acetate and propionate (Stevens and Stettler, 1967; Sehested et al., 1999a). While the rumen epithelium shows a small net secretion (net transport from blood to lumen side of the isolated epithelium) of acetate and propionate when epithelia are incubated without an electrochemical gradient of SCFA, a relatively large net absorption of butyrate carbon usually occurs. The secretion of acetate and propionate by the epithelium at first seems to argue against the concept of nonionic diffusion. However, most estimates of SCFA flux *in vitro* have been based on ^{14}C-labeled acids, implying that release of any substance carrying carbon from SCFA will be interpreted as SCFA flux. A small proportion of acetate and propionate transported across the epithelium will be oxidized under these conditions and the epithelium has been shown to primarily excrete the CO_2 on the luminal side, explaining at least partly the net excretion of these acids (Sehested et al., 1999a).

The rumen epithelium has long been known to be capable of metabolizing SCFA and, in particular, to have high affinity and capacity for metabolism of butyrate (Pennington, 1952). This in fact is the key to explaining the differences in the epithelial transport of butyrate compared with acetate and propionate. The metabolism of butyrate into acetoacetate and 3-hydroxybutyrate and the subsequent release of these compounds across the basolateral membrane would be in agreement with the apparent normal metabolic activity of the epithelium and would also explain why [^{14}C]-butyrate was transported differently from acetate and propionate. It is likely that the products of butyrate metabolism are transported to the serosal (blood side) buffer carrying the label from butyrate. Keto- and hydroxyacids such as acetoacetate, 3-hydroxybutyrate, and lactate are more polar than SCFA because of their hydrophilic, secondary functional group, and consequently these acids have a lower permeability in biological membranes. In skeletal muscle a monocarboxylate transporter which co-transports lactate and protons solves an analogous transport problem for lactate across the cell membrane (Juel, 1997). The missing piece of the puzzle would therefore be to find monocarboxylate transporters in the epithelium that enable polarized transport of acetoacetate and 3-hydroxybutyrate. Recently, this transporter was shown to be present in rumen epithelium which agrees with this sequence of events (Müller et al., 2001). It has also been shown that blocking cellular metabolism abolishes the active component of butyrate absorption *in vitro* (Gäbel et al., 2001), confirming that it is the ketone bodies formed from butyrate that are selectively transported to the serosal side of the epithelium and not butyrate itself.

13. RUMEN EPITHELIAL METABOLISM

One of the central observations on SCFA metabolism in ruminants has been the apparently extensive metabolism of ruminally produced SCFA by the rumen epithelium. However, this

has been among the most difficult features of SCFA metabolism to understand. Numerous reviews are available discussing SCFA metabolism (Bergman, 1990; Britton and Krehbiel, 1993; Seal and Reynolds, 1993; Rémond et al., 1995; Kristensen et al., 1998; Seal and Parker, 2000). Recent studies have challenged the view that the rumen epithelium has a dominant role in the metabolism of acetate and propionate absorbed from the rumen.

The classic attempt to determine the quantitative relationship between SCFA production in the gut and SCFA absorption was the work by Bergman and Wolff (1971). The production of SCFA in the rumen based on isotopic dilution was compared with portal appearance of SCFA corrected for PDV uptake of arterial acetate. It was concluded that large amounts not only of butyrate, but also of acetate and propionate, were metabolized by gut epithelia. In support of this conclusion, rumen epithelium also seemed to metabolize a large fraction of SCFA transported *in vitro* (Stevens, 1970). Nevertheless, these figures have long been doubted when considering the large amounts of SCFA apparently being absorbed in high producing ruminants (Sutton, 1985). These figures also lead to the paradoxical conclusion that the rumen epithelium of a lactating cow should have oxidative needs comparable to the entire fasting heat metabolism of the animal (Kristensen and Danfær, 2001).

Studies on rumen epithelial metabolism of absorbed SCFA may have overestimated the metabolism by the epithelium because the actual estimation is the mixed effect of rumen microbial and rumen epithelial metabolism. Studies on SCFA absorption under washed reticulo-ruminal conditions that minimize bacterial activity have shown that the portal appearance of acetate, propionate, and isobutyrate could account for the entire disappearance of these acids from the rumen when the PDV uptake of arterial acetate is taken into account and 5% of the propionate is assumed metabolized into lactate by the rumen epithelium (Kristensen et al., 2000a). Butyrate was also extensively metabolized by the rumen epithelium under washed reticulo-rumen conditions and no more than 23% of the butyrate disappearance from the rumen could be accounted for by portal appearance of butyrate. It has previously been observed that there is increasing portal recovery of butyrate with increasing disappearance rates of butyrate from the rumen of sheep (Kristensen et al., 1996b, 2000b; Nozière et al., 2000). This effect is in agreement with a saturable metabolic capacity of the epithelium.

To what extent there is interspecies differences in the metabolic capacity of butyrate in the rumen epithelium is not yet clear, but in a study with steers, the portal recovery of butyrate did not increase with increasing ruminal infusion rates of butyrate (Krehbiel et al., 1992). The recovery was relatively high at all infusion levels in the steers (25%), and was equivalent to the highest recovery level obtained in the sheep experiments. In sheep, increasing ruminal butyrate infusion not only leads to increasing portal recovery of butyrate, but also to increasing portal recovery of ruminal valerate (Kristensen et al., 2000b). These results point to a redefinition of the role of the rumen epithelium in SCFA metabolism and suggest that the rumen epithelium is not metabolizing large amounts of acetate and propionate as previously assumed.

14. IS BUTYRATE OXIDIZED TO CARBON DIOXIDE DURING ABSORPTION?

In vitro studies have shown that rumen epithelium is able to oxidize all of the three quantitatively most important SCFA (Baldwin and McLeod, 2000); however, the epithelial production of 3-hydroxybutyrate and acetoacetate imply that butyrate oxidation is far lower than its disappearance across the epithelium. Studies comparing net portal appearance of butyrate and butyrate infusion into the rumen have indicated that major parts of the butyrate were

oxidized (lost), because net portal appearance of butyrate, 3-hydroxybutyrate, and acetoacetate accounted only for 25–45% of ruminal butyrate infusion (Krehbiel et al., 1992; Kristensen et al., 1996b). However, the PDV has been shown to utilize 3-hydroxybutyrate from arterial blood equivalent to 32–42% of the whole-body flux in sheep and thereby mask the true production rate by the gut epithelia (Kristensen et al., 2000c). Intraruminal microbial pathways might also utilize part of the infused butyrate and thereby contribute to what could be interpreted as epithelial oxidation. This latter effect has been indicated by relatively high recoveries (as compared to expected recovery in the fed animal) of butyrate when infused into animals maintained under total intragastric nutrition (Gross et al., 1990a) or temporarily washed reticulo-rumen conditions (Kristensen et al., 2000a). In conclusion, the rumen epithelium has oxidative needs and butyrate is likely the most important carbon source. The majority of the butyrate absorbed is released as butyrate, acetoacetate, and most importantly, 3-hydroxybutyrate to the portal blood.

15. WHY DO EPITHELIA METABOLIZE BUTYRATE?

Butyrate is generally considered a special metabolite for gut epithelial function (Topping and Clifton, 2001). One way to explain the special behavior of gut epithelia toward butyrate compared with acetate and propionate is that butyrate is important as an energy source for epithelial cells (Bugaut, 1987). However, the rumen epithelium has a range of other metabolites available, e.g. acetate and propionate absorbed from the rumen as well as arterially supplied glucose. One might speculate that butyrate's role as an important substrate for epithelial energy metabolism might have evolved secondary to the basic need of having butyrate removed before it enters the blood stream. Butyrate metabolism by rumen and hindgut epithelia could therefore be seen as a protective mechanism that has two disposal pathways, oxidation and ketogenesis. It is obvious that butyrate is handled differently from acetate and propionate by the epithelia (Pennington, 1952), but another question remains to be answered: is butyrate a unique metabolite? Valerate, for example, is also efficiently metabolized by the rumen epithelium (Kristensen et al., 2000a,b) and it has been shown that the epithelium have the capacity to metabolize medium-chain (Hird et al., 1966) as well as long-chain fatty acids (Jesse et al., 1992).

Butyrate is an important substrate for gut epithelia compared with acetate and propionate, but it is apparently not a unique nutrient. Acetate, propionate, and isobutyrate are all metabolites of endogenous pathways in the organism. Acetate has the lowest membrane permeability, is utilized from peripheral arterial blood in major extrahepatic tissues (Pethick and Lindsay, 1982), and is a universal metabolite in the body in the form of acetyl-CoA. Propionate is the main donor of 3-carbon units for gluconeogenesis in the ruminant liver and is efficiently taken up by the liver (Leng and Annison, 1963). The endogenous sources of propionate include degradation of uneven chained fatty acids and some amino acids (methionine, threonine, isoleucine, and valine). Isobutyrate (an intermediate from catabolism of valine) appears in relatively low concentrations in the rumen, but is efficiently taken up by the liver for gluconeogenesis (Stangassinger and Giesecke, 1979). These SCFA are not only well tolerated in hepatic and peripheral tissues, but are key metabolites (especially acetate and propionate) in these tissues, and this agrees with a limited uptake of these SCFA in the gut epithelia.

Butyrate, valerate, and probably longer, medium-chain fatty acids (MCFA) are less polar and will have a relatively high permeability in cell membranes. One way of controlling permeability is partial oxidation of these SCFA into acetoacetate and 3-hydroxybutyrate in the gut epithelia. When butyrate appears in the systemic circulation or is added to cell cultures,

it has been shown to have a number of adverse effects: inhibition of growth and induction of morphological changes in cultured cells of different origins including ruminal epithelial cell lines (Prasad and Sinha, 1976; Gálfi et al., 1991); being an insulin secretagogue (Manns and Boda, 1967); inhibition of gastrointestinal motility by stimulation of epithelial receptors (Crichlow, 1988) and/or via systemic effects (Le Bars et al., 1954); stimulation of rumen epithelial development (Sander et al., 1959); or killing (2.5 mmol butyrate/kg BW in lambs) the animal (Manns and Boda, 1967). The epithelia of the gut have apparently evolved to perform gatekeeping functions by controlling the entry of butyrate and longer-chain fermentation acids into the peripheral circulation. It is tempting to speculate that the side effects of the gatekeeper function are that these metabolites also become quantitatively important oxidative substrates.

16. ACYL-CoA SYNTHETASES

Activation of SCFA by an acyl-CoA synthetase (also named CoA ligase or thiokinase) is the first step in the metabolism of any SCFA in the cells of the gut epithelium, liver, or peripheral tissues (Groot et al., 1976). The acyl-CoA synthetases are therefore believed to be key enzymes in different tissues' selectivity to metabolism of different SCFA. There exist a number of distinct acyl-CoA synthetases: acetyl-CoA, propionyl-CoA, butyryl-CoA, medium-chain fatty acid, and long-chain fatty acid-CoA synthetases.

The acetyl-CoA synthetase (EC 6.2.1.1) has a high affinity for acetate, and some affinity for propionate (Campagnari and Webster, 1963; Groot et al., 1976; Ricks and Cook, 1981b). However, it is noteworthy that the activity of this enzyme has been found to be low in the rumen epithelium and liver of ruminants (Cook et al., 1969; Ash and Baird, 1973). These observations are in line with a limited role of the rumen epithelium and the liver in metabolism of absorbed acetate.

The ruminant liver has a relatively high propionyl-CoA synthetase (EC 6.2.1.17) activity (Ash and Baird, 1973) and there exist a number of indications that propionyl-CoA synthetase is a distinct enzyme (Ricks and Cook, 1981a,c). Among the interesting features of this enzyme is that it is not present in rumen epithelium. This is not the same as denying any possible activation of propionate in rumen epithelium, which obviously can occur (Weekes, 1974), but it has been shown that the propionyl-CoA synthetase activity in the liver is almost insensitive to the presence of butyrate whereas the activity in the rumen epithelium is almost completely inhibited by the presence of butyrate (Ash and Baird, 1973; Harmon et al., 1991). As is the case with acetyl-CoA synthetase in rumen epithelium, the lack of propionyl-CoA synthetase activity is in agreement with *in vivo* observations showing a very limited uptake of propionate by the rumen epithelium.

As described above, one of the most striking features of rumen epithelial metabolism is a high affinity and capacity for metabolism of butyrate. This feature is reflected in the butyryl-CoA synthetase activity of the epithelium (Ash and Baird, 1973). The relative importance of the liver and the rumen epithelium in the metabolism of propionate and butyrate, respectively, is directly reflected in the acyl-CoA synthetase activities. Moreover, as butyrate was found to have an insignificant effect on propionate activation in the liver, propionate had no effect on butyrate activation in the rumen epithelium, but decreased the butyrate activation in the liver (Ash and Baird, 1973). A distinct butyryl-CoA synthetase (EC 6.2.1.2) was first purified from bovine heart mitochondria and this enzyme showed a high affinity for valerate and caproate (Webster et al., 1965). In ruminants, butyrate affinity is also found in xenobiotic/medium-chain fatty acid-CoA synthetases. These acyl-CoA synthetases activate a broad spectrum of

straight-chain fatty acids: butyrate, longer SCFA, branched-chain fatty acids, and a number of xenobiotic (of foreign origin) carboxylic acids; others include benzoate and phenylacetate (Aas, 1971; Vessy et al., 1999). Indirect evidence from PDV flux studies indicates cross-specificity for valerate activation, which agrees with both types of butyrate activating systems. So far, no specific information seems to be available on the interaction of SCFA activation with longer-chain fatty acids or xenobiotic compounds absorbed from the rumen (Cremin et al., 1995); however, the fact that the isolated rumen epithelium or isolated rumen epithelial cells are able to use a wide range of fatty acids from SCFA to palmitate indicates the presence of some activity for activating medium- as well as long-chain fatty acids by the epithelium (Jesse et al., 1992; Hird et al., 1966).

17. HOW IS BUTYRATE METABOLIZED BY GUT EPITHELIA?

Rumen epithelial ketogenesis is remarkable compared to hepatic ketogenesis by virtue of the fact that rumen epithelial ketogenesis is a main pathway in the fed state, and not a pathway turned on at fasting or when the organism is facing a high "metabolic drain". This feature is obviously connected to the constant fueling of rumen epithelial ketogenesis via butyrate absorption in combination with an apparent need for removal of butyrate before entering the blood stream.

The oxidation of butyryl-CoA to acetoacetyl-CoA in rumen epithelium (fig. 1) is, from a chemical point of view, identical to the initial steps of long-chain fatty acid β-oxidation. (For a review on this subject, see Eaton et al., 1996.) The first 3-hydroxybutyrate intermediate of this pathway is the L-(*S*)-isomer, which is not released to the peripheral circulation. Oxidation of L-3-hydroxybutyrate-CoA yields acetoacetyl-CoA. Acetoacetyl-CoA is a branching point between acetyl-CoA formation and ketone release because of the acetoacetyl-CoA thiolase (EC 2.3.1.9) catalyzed equilibrium between acetoacetyl-CoA and acetyl-CoA (fig. 2). The equilibrium constant of the reaction (6×10^{-6}; Williamson et al., 1968) is strongly favoring acetyl-CoA and this means that the concentration of acetoacetyl-CoA probably will be relatively low in the mitochondrion. The production of ketone bodies from acetate (Harmon et al., 1991) or valerate (Weigand et al., 1975) in rumen epithelium confirms that acetoacetyl-CoA thiolase is present in the rumen epithelium, an observation also confirmed by assays on epithelial cell extracts (Baird et al., 1970). The main function of acetoacetyl-CoA thiolase is probably not to mediate ketogenesis from absorbed acetate, although this mediation is possible.

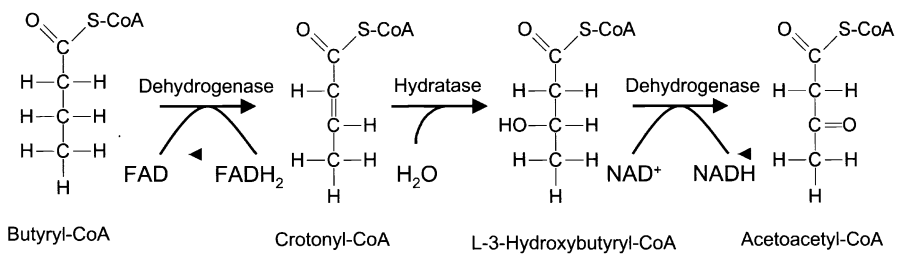

Fig. 1. Initial oxidation of butyryl-CoA to acetoacetyl-CoA in rumen epithelium proceeds via a pathway similar to the initial steps in β-oxidation. A number of isoenzymes are known for both acyl-CoA dehydrogenases (first dehydrogenase of the pathway) and 3-hydroxyacyl-CoA dehydrogenases (Eaton et al., 1996). However, the isoenzymes with specificity for short-chain acyl-CoA are likely to predominate in the rumen epithelium. The hydratase in the pathway is likely crotonase (EC 4.2.1.17), also an enzyme with the highest specificity toward short-chain acyl groups.

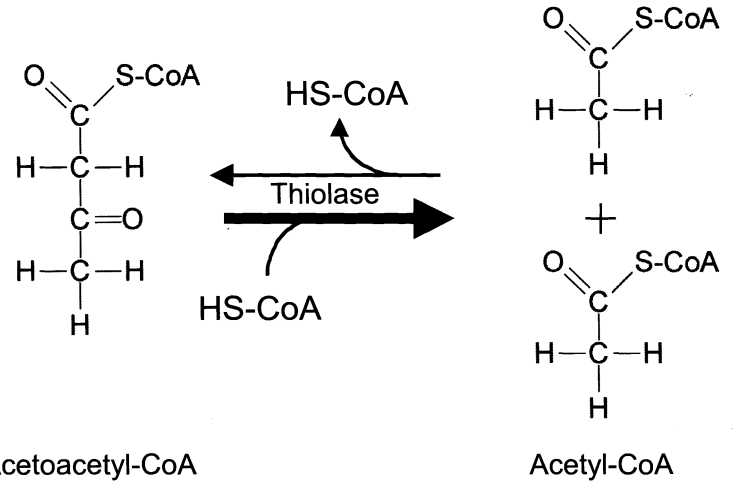

Fig. 2. The acetoacetyl-CoA thiolase (EC 2.3.1.9) is catalyzing the reversible thiolytic cleavage of acetoacetyl-CoA into two acetyl-CoAs. The equilibrium between acetoacetyl-CoA and acetyl-CoA is favoring acetyl-CoA and as a result the acetoacetyl-CoA concentration in the mitochondrion will usually be low.

The fact that the rumen epithelium contains low amounts of acetyl-CoA synthetase, and the low affinity of the butyryl-CoA synthetase for acetate when butyrate is present, points to the conclusion that the main function of the acetoacetyl-CoA thiolase is feeding acetyl-CoA from the acetoacetyl-CoA pool to the TCA cycle. Therefore, even though butyrate metabolism in the epithelium cannot be explained from the point of the energy needs of the epithelium, butyrate metabolism is ensured to be the main oxidative substrate under *in vivo* conditions.

Contrary to the consensus about the initial steps of butyrate metabolism, there has been more discussion of the subsequent metabolism of acetoacetyl-CoA. This compound can be deacylated directly (acetoacetyl-CoA deacylase; EC 3.1.2.11) or deacylated via the 3-hydroxy-3-methylglutaryl-CoA pathway (3-HMG pathway; 3-hydroxy-3-methylglutaryl-CoA synthetase and lyase; EC 4.1.3.5 and EC 4.1.3.4); however, other alternative pathways have been suggested and will be discussed briefly. The presence of the 3-HMG pathway (fig. 3) in rumen epithelium is supported by the fact that the enzymes of the pathway (3-hydroxy-3-methylglutaryl-CoA synthetase, and lyase) have been shown to be present in the epithelium in significant amounts (Baird et al., 1970; Leighton et al., 1983).

However, isotopomer studies have had a dominant role in the arguments about ketogenic pathways in the epithelium. Hird and Symons (1961) investigated isolated ruminal and omasal epithelial metabolism of [1-^{14}C]butyrate and [3-^{14}C]butyrate into acetoacetate. The isotopomers of acetoacetate could be partly identified by measuring the label in position 1 (CO_2 from decarboxylation of acetoacetate) and in the label in the acetone fraction after decarboxylation (interpreted as position 3). When the epithelium was incubated with [1-^{14}C]butyrate, 80% of the label in acetoacetate was found in position 1 and 20% of the label was found in position 3. When the substrate was [3-^{14}C]butyrate, 37% of the label in acetoacetate was found in position 1 and only 63% in position 3. The probable explanation for the 1 to 3 shifts in labeling is the thiolase-catalyzed equilibrium between acetoacetyl-CoA and acetyl-CoA (fig. 2). The labeling pattern also gives an indication of the relative importance of the pathway. The fact that 20% of the label in acetoacetate was found in position 3 could lead

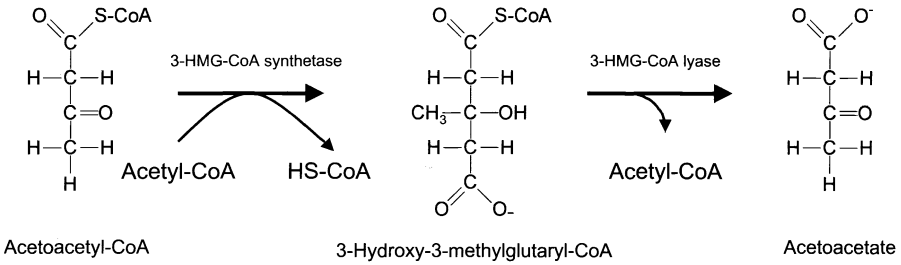

Fig. 3. The 3-hydroxy-3-methylglutaryl-CoA pathway (3-HMG pathway) ensures that acetoacetyl-CoA, despite its low concentration, can be "trapped" and deacylated. These steps of ruminal ketogenesis are similar to hepatic ketogenesis.

to the conclusion that 80% of acetoacetate was generated without degradation to acetyl-CoA. However, that is a false conclusion because if we assume that the labeling does not cause fractionation, then the acetoacetyl-CoA generated from acetyl-CoA (acetyl-CoA will be labeled in position 1) will be evenly distributed among carbon 1 and carbon 3 of acetoacetate. This means that at least 40% of the acetoacetate must have been equilibrating with acetyl-CoA to explain 20% of the total activity in position 3.

The fact that the large majority of label from [1-^{14}C]butyrate ends up in C1 of acetoacetate has been used as an argument against the function of the 3-HMG pathway in the epithelium. However, this argument might not be justified because this pathway will conserve C1 label in position C1, especially if the thiolase activity is relatively low compared to the flux through the 3-HMG pathway. The fact that Hird and Symons (1961) found a larger 3 to 1 shift in labeling of acetoacetate from [3-^{14}C]butyrate is therefore in agreement with the 3-HMG pathway not only working on acetoacetyl-CoA derived from acetyl-CoA, but also on acetoacetyl-CoA from the initial-oxidation steps on butyrate. It seems puzzling that only 37% of the label in acetoacetate generated from [3-^{14}C]butyrate was found in position 1, especially if the majority of the acetoacetate production is through the 3-HMG pathway. However, the relative enrichment of the acetyl-CoA pool and the acetoacetyl-CoA pool will have a major impact on the results. It is likely that the metabolism of [3-^{14}C]butyrate will be accompanied by a lower specific activity of the acetyl-CoA pool compared with the [1-^{14}C]butyrate because the [3-^{14}C]butyrate will be less likely to deliver a labeled acetyl-CoA to the acetyl-CoA pool compared with [1-^{14}C]butyrate as substrate. The only labeling of the acetyl-CoA pool from [3-^{14}C]butyrate will be through the thiolase-catalyzed acetyl-CoA/acetoacetyl-CoA equilibrium. This implies that the 3 to 1 shift observed with the [3-^{14}C] butyrate incubation indicates a far higher importance of the 3-HMG-CoA pathway than that apparently shown by the 37% of [3-^{14}C]butyrate found in position 1 of acetoacetate simply because the specific activity of acetyl-CoA will be lower under these conditions.

Though acetoacetate is the product of rumen epithelial ketogenesis, it is not the primary circulating ketone in plasma. A large proportion of acetoacetate is reduced to D-3-hydroxybutyrate (fig. 4) before leaving the epithelial cells catalyzed by 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30). Data on rumen epithelial enzyme activity and isotopomer distribution in acetoacetate suggest that the 3-HMG pathway is as quantitatively important in this tissue as it is in liver. Earlier denials (Annison et al., 1963) are partly correct in pointing out that butyrate is not completely degraded to acetyl-CoA before incorporation into ketone bodies.

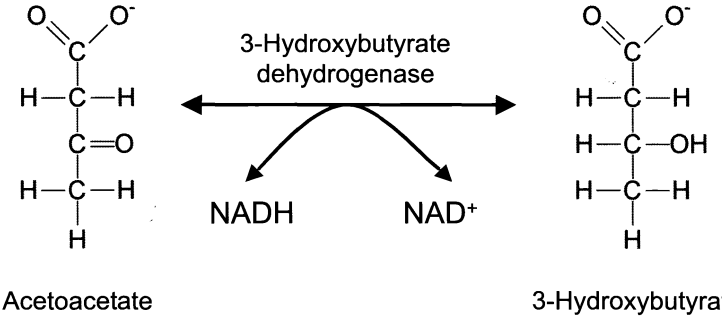

Fig. 4. D-3-Hydroxybutyrate is the dominating "ketone" in plasma due to the 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) catalyzed and NADH-dependent reduction of acetoacetate.

18. ALTERNATIVE KETOGENIC PATHWAYS

Although the 3-HMG pathway is active in rumen epithelium, it might not be the only ketogenic pathway. One obvious alternative would be acetate release by the epithelium. Endogenous acetate production has been observed *in vitro* when the epithelium is incubated without substrate (Sehested et al., 1999a). The hydrolysis of acetyl-CoA into acetate and CoA is catalyzed by acetyl-CoA deacylase (EC 3.1.2.1; Grigat et al., 1979). We might wonder why the ruminant produces ketone bodies at all when it seems much simpler just to use acetate as a carrier of acetyl units. One of the reasons for the production of ketone bodies could be the cost of reactivation in recipient tissues because they would have to pay double the price for activation when acetate is the substrate compared with acetoacetate. Nevertheless, endogenous acetate production can be observed *in vitro* by rumen epithelium and acetate would be an obvious candidate for interorgan acetyl transfer. However, we have only limited and indirect evidence of endogenous acetate production by rumen epithelium *in vivo* (Kristensen et al., 2000a). It is unknown to what extent endogenous acetate from the PDV has a role in interorgan acetyl exchange (i.e. acetyl carbon originally absorbed in fatty acids other than acetate itself).

Not only acetyl-CoA, but also acetoacetyl-CoA, might be directly deacylated (acetoacetyl-CoA deacylase; EC 3.1.2.11), and thereby lead to 3-HMG-CoA-independent acetoacetate synthesis. The acetoacetyl-CoA deacylase has been found in rumen epithelium though only at a low activity (Baird et al., 1970). One of reasons why direct deacylation of acetoacetyl-CoA might be of limited importance is the low acetoacetyl-CoA concentration in the mitochondrion. The low affinity of the acetoacetyl-CoA deacylase present in rat liver was quantitatively not important, although it was functional under *in vitro* conditions with high acetoacetyl-CoA concentrations (Williamson et al., 1968).

A number of alternative pathways have been suggested to explain various parts of ketone body formation in rumen epithelium: succinyl-CoA:3-ketoacid CoA-transferase (Bush and Milligan, 1971); a L-3-hydroxybutyrate pathway not involving acetoacetate formation (Emmanuel et al., 1982); and a butyrate:acetoacetyl-CoA transferase pathway (Emmanuel and Milligan, 1983). These pathways all suggest metabolism of butyrate to 3-hydroxybutyrate as one unbroken C4 unit. The two latter pathways appear to be closely related to cytosolic pathways in tissues utilizing acetoacetate in de novo synthesis of fatty acids (Robinson and Williamson, 1980). However, it is difficult to determine the quantitative importance of non-3-HMG-CoA pathways in the rumen epithelium from the limited data available.

The situation for the succinyl-CoA:3-ketoacid CoA-transferase (EC 2.8.3.5; SCOT) is different because this enzyme is indeed anticipated to be a key enzyme in ketone body metabolism; however, its function is opposite to the function proposed in the rumen epithelium. This enzyme is a key enzyme in the activation of ketone bodies in peripheral tissues and the rare deficiency of this enzyme in human infants leads to severe ketoacidosis (Synderman et al., 1998). Succinyl-CoA:3-ketoacid transferase has been assumed to contribute to the acetoacetyl-CoA hydrolysis in rumen epithelium because addition of succinate was followed by increased disappearance of acetoacetyl-CoA (Bush and Milligan, 1971). If SCOT was important for the hydrolysis of acetoacetyl-CoA, it would suggest that the concentration of either acetoacetyl-CoA or succinate was higher in rumen epithelium compared with other tissues. However, owing to the true reversibility (Stern et al., 1956) of the reaction catalyzed by SCOT (acetoacetyl-CoA + succinate $\leftrightarrow$ acetoacetate + succinyl-CoA), it might be suggested that this activity in the rumen epithelium was connected to the specific incubation conditions *in vitro* and not necessarily the pathway of acetoacetyl-CoA metabolism *in vivo*.

19. METABOLITE INTERACTIONS IN RUMEN EPITHELIAL KETOGENESIS

If the rumen epithelium works in its usual position in a ruminant, or is maintained for a short period under *in vitro* conditions as epithelial slices or isolated cells, it will have an obligate requirement for chemical energy to maintain Na^+, Ca^{2+}, and K^+ ion concentration gradients and other vital cell functions. Considering a situation with a relatively constant workload of the epithelium, it would then be expected that tissue supplied with small amounts of butyrate would oxidize a large fraction to CO_2 simply to fulfill the basic needs of ATP and sustain basic cell functions. This relationship has been confirmed *in vitro* when different butyrate concentrations were compared. Increasing the supply of butyrate was followed by the oxidation of a decreasing fraction and an increasing fraction metabolized into ketone bodies (Beck et al., 1984).

From a whole animal perspective, glucose is antiketogenic (Hamada et al., 1982) and initially it was surprising that glucose had the opposite effect on rumen epithelial ketogenesis, i.e. ketogenesis was stimulated by glucose (Stangassinger et al., 1979). A number of glucogenic substrates have been shown to impose a similar effect on epithelial metabolism. Some variability in the response concerning the uptake of butyrate and the proportion of butyrate oxidized has been observed, but generally a shift toward the more reduced "ketone body", 3-hydroxybutyrate, compared with acetoacetate has been observed with the addition of a glucogenic substrate (Goosen, 1976; Beck et al., 1984; Giesecke et al., 1985; Baldwin and Jesse, 1996). Although the rumen epithelium is able to take up a broad range of metabolites including glucose, glutamine, and glutamate and oxidize them (Harmon, 1986; Baldwin and McLeod, 2000), this does not mean that glucose is the oxidative substrate that caused the shift in ketone body production. In fact, we would surmise from the discussion of butyrate metabolism (see above) that the epithelium had a source of acetyl-CoA from butyrate that would be able to fulfill any oxidative need. The reason might be that epithelium incubated without a glucogenic source will become depleted of TCA cycle intermediates and subsequently have difficulty maintaining ATP, NADH, and NADPH potentials. A very elegant example of this effect is the comparison between metabolite production from butyrate and valerate in rumen epithelium incubated *in vitro* (Weigand et al., 1975). When rumen epithelium was incubated with butyrate, 0.67 of the ketone bodies produced were acetoacetate; however, when incubated with valerate only 3-hydroxybutyrate was produced. This production was accompanied by

lactate produced from the 3-carbon fraction of valerate. Therefore, there seems to be no reason to believe that glucose or any other glucogenic substrates play a particular role as regulators of rumen epithelial ketogenesis, but the results point to the general conclusion that rumen epithelium has a range of nutritional requirements for proper function.

20. THE *IN VIVO/IN VITRO* PROPIONATE ENIGMA

In vitro, rumen epithelium metabolizes propionate into lactate (Weigand et al., 1975). *In vivo*, however, it has not been possible to demonstrate any major propionate metabolism into lactate using ruminal infusion of ^{14}C or ^{13}C labeled propionate (Weigand et al., 1972; Weekes and Webster, 1975; Kristensen et al., 2001). These matters have been even further confused by the fact that *in vivo* experiments on portal recovery of ruminal propionate indicate that a large proportion of propionate was metabolized by the epithelium (Bergman and Wolff, 1971). Because of the large capacity of the liver to metabolize propionate *in vivo* (Berthelot et al., 2002) it is difficult to explain why the rumen epithelium should limit the propionate supply to the liver. The reason for the large activation of propionate under *in vitro* conditions is probably the cross-specificity of the butyryl-CoA synthetase. *In vivo*, propionyl-CoA could be generated by thiolysis of 3-oxo-valeryl-CoA (from valerate). This latter source might be the explanation for the high capacity of propionyl-CoA-utilizing pathways in rumen epithelium. The usual metabolism of propionate via propionate carboxylation to methylmalonic acid followed by the TCA intermediate succinate will lead to the buildup of TCA intermediates. In the liver the main pathway to export surplus TCA intermediates is gluconeogenesis. Other tissues use nonessential amino acids (e.g. alanine and glutamine synthesis in muscles and other tissues) to control excess TCA intermediates. In rumen epithelium, it is apparently the malic enzyme (EC 1.1.1.40) catalyzed decarboxylation of malate into pyruvate (coupled to reduction of NADP) and the subsequent reduction of pyruvate to lactate that removes the surplus of propionyl-CoA from the rumen epithelial cells (Young et al., 1969). By these mechanisms we are able to explain the differing *in vitro* and *in vivo* observations on rumen epithelial metabolism.

21. FITTING THE CARBON BALANCE OF FERMENTATION IN THE GUT

Although there is no doubt that SCFA are important in ruminant metabolism, no feed evaluation system has been able integrate knowledge of SCFA production, absorption, and metabolism in ruminants under production conditions. Simulation models constructed to describe fermentation and SCFA absorption, as well as other nutrients, need to improve in order to predict SCFA proportions in the rumen (Baker and Dijkstra, 1999). The problem has also been what to do with the apparently huge metabolic activity of the rumen epithelium. No model has been able to incorporate this metabolism, and this review attempts a possible explanation.

Simulation models of ruminal fermentation and metabolism developed to date have been constructed and validated mainly against duodenal nutrient flows. The re-evaluation of the role of the gut epithelia in metabolism of SCFA has enabled an alternative method of model comparison. If the rumen and other gut epithelia do not metabolize significant amounts of acetate and utilize only a small percentage of the propionate flux, then the net rumen production of these acids would be predictable from PDV fluxes. Major corrections to be considered are, however, uptake of arterial acetate by PDV tissues and epithelial butyrate metabolism.

The percentage of arterial acetate uptake by PDV tissues has been shown to be stable (about 32% of arterial flux) when evaluated across different rations and with relatively little postprandial variation in meal-fed sheep (Bergman and Wolff, 1971; Kristensen et al., 1996a). As discussed earlier, the portal recovery of butyrate has been shown to be a more complex function of its availability, and an increased portal recovery of butyrate with increasing ruminal production rates might be expected.

In an attempt to compare data from studies on PDV fluxes with model predictions of gastrointestinal fermentation, Kristensen and Danfær (unpublished data) compared portal fluxes of SCFA from 36 studies in which a total of 58 different diets were fed to sheep and cattle under different physiological conditions (growing/maintenance, nonpregnant/pregnant, dry/lactating). The model used to predict fermentation and digestion of the diets was Karoline (Danfær, 1990) (version 8a, a dynamic simulation model of a lactating cow mainly validated against duodenal flow data; Danfær et al., unpublished). In the model, all diets were compared at a fixed dry matter intake of 20 kg/d and predicted carbon output in moles SCFA carbon per kg dry matter intake was compared to the observed/recalculated PDV fluxes in the studies. The experimentally observed PDV fluxes were corrected for acetate uptake in PDV tissues (assumed 32% of the arterial flux), propionate uptake by epithelial tissues (assuming that portal flux was equal to 95% of true absorption), and butyrate recovery [assuming portal recovery of gut butyrate production = $0.35 \times P/(P + 0.05)$ where P = portal net appearance mmol $\times$ h^{-1} $\times$ (kg $BW^{0.75})^{-1}$]. The portal recovery of butyrate is deliberately set to a higher level than those typically found following butyrate infusion into the normally functioning rumen. This recovery agrees with observations with sheep maintained on intragastric nutrition or short-term washed reticulo-rumen (Gross et al., 1990a,b; Kristensen et al., 2000a).

The calculated SCFA production in the 36 experiments using the correction factors above was 11.9 ± 0.4 moles C in SCFA/kg dry matter intake. The simulated value was 12.3 ± 0.2 moles C in SCFA/kg dry matter intake and the mean bias was 0.4 moles C/kg DMI [Σ(predicted − observed)/number of observations; see Kohn et al. (1998)]. However, the root mean square prediction error (RMSPE) was 2.6 moles C in SCFA/kg DMI [(Σ(predicted − observed)^2/number of observations); see Kohn et al. (1998)]. On average, the model and the corrected experimental data are in good agreement. However, there is still a need for better models to predict net SCFA output. The corrected, experimentally determined SCFA production was, on average, 45 ± 2% of the simulated digestible energy. However, estimates based on intragastric tracer dilution, as discussed above, seem to overestimate the SCFA production and indirect evidence also supports these figures. In fact, the SCFA production accounting for 45% of digestible energy implies that 65% of total digested carbon is found in fermentation gases and SCFA. However, if the true relationship between portal absorption and gut production of SCFA is similar to the relationship described by Bergman and Wolff (1971), then the production of SCFA would need 116 ± 5% of the digested carbon to account for SCFA and fermentation gases. This would seem to be impossible. The good news is that values of portal absorption of SCFA actually make sense in terms of animal energy metabolism. It must be emphasized, however, that models of ruminal and hindgut fermentation still have a lot to gain in terms of precision of SCFA production, especially in the prediction of ruminal SCFA composition.

22. CONCLUSIONS

Glucose is a major metabolic fuel for ruminant tissues, similar to most mammals. The pregastric fermentation dictates that gluconeogenesis serves to supply the glucose needs under

most feeding situations. Lactate derived from the diet, ruminal bacterial metabolism and from endogenous metabolism is a major glucose precursor in ruminants, supplying 9–35% of hepatic glucose carbon, and is a key carbon intermediate in growing ruminants. Ruminants have been shown experimentally to be capable of contributing significant quantities of glucose through intestinal digestion and glucose absorption. This additional glucose does impact growth and retention of body tissues.

The gut epithelia have a central function as gatekeepers for butyrate and longer-chain SCFA and MCFA. These acids have also become the main energy substrates of gut epithelia. There is no evidence suggesting that the rumen epithelium should have excessive requirements for energy metabolism, but rather intraruminal (luminal) isotopic dilution techniques overestimate net SCFA production because of microbial metabolism. Data on portal appearance of SCFA corrected for PDV metabolism of arterial metabolites is therefore the best direct measure of SCFA availability in ruminants. The average absorption of SCFA in ruminants is equivalent to about 45% of the digestible energy intake.

23. FUTURE PERSPECTIVES

Current feeding systems often fail to meet today's demand for accurate prediction of the nutrient needs of animals consuming a large menu of feedstuffs under a wide array of environmental conditions. To accomplish this task we need to understand all phases from digestion and nutrient assimilation to the subsequent use of nutrients by various tissues. Thus, successful models in the future will span concepts from commodity to animal product by incorporating the metabolic transformations in between. This chapter has reviewed recent findings on the impact of intestinal digestion on glucose availability, the contributions of lactate to meeting the glucose needs, and how our understanding of SCFA metabolism has been revised from long-accepted concepts. Developing models for predicting animal biological response are dependent on findings such as these to supply quantitative information to describe animal systems. Data are still greatly lacking for concepts as fundamental as SCFA production and glucose absorption, and their metabolism at various stages of growth and production. The near-global de-emphasis on agricultural production research may make these pieces of the puzzle long in coming.

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