Use of Isolated Ruminal Epithelial Cells in the Study of Rumen Metabolism

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ABSTRACT A comprehensive understanding of ruminal development and metabolism has not yet been achieved. The study of rumen epithelial metabolism during development can facilitate the development of feeding strategies for developing pre-ruminant animals and mature animals. Understanding the effect of the physical form and nutrient composition of the diet on the ruminal epithelium will lead to changes in dietary regimens that exploit beneficial tissue responses. Characterization of the ontogenic shifts in ruminal metabolism, in association with the description of physical changes, has established more discrete periods during the development of the ruminal epithelium for future studies to be conducted. Isolated ruminal epithelial cells, specifically cells of the strata basale and spinosum, have been used for metabolic studies of rumen epithelial energy metabolism. Because the ruminal epithelium is a major producer of ketone bodies in the fed ruminant animal, it is integral to the energy metabolism of the whole animal. Arguably, whole tissue slices may provide better estimations of actual tissue performance; however, the benefits gained by maintaining tissue integrity are offset because of the high variability in tissue composition due to dietary influences. Use of enriched cell populations is ideal for short-term incubations and provides high cell yields with limited delay following removal of the tissue from the animal. Although the ruminal cell isolation system is continuously undergoing refinement, enriched cell cultures have provided realistic results with respect to known responses in vivo. J. Nutr. 128: 293S–296S, 1998.

KEY WORDS: • rumen • metabolism • epithelium • isolated cells

METABOLISM OF THE RUMINAL EPITHELIUM

The rumen and reticulum account for more than 70% of the total digestive tract volume in ruminants (Stobo et al. 1966). Vast numbers of papillae protrude from the ruminal surface into the lumen, greatly increasing the surface area available for absorption of volatile fatty acids (VFA) (Harfoot 1978), and thus a large majority (>75%; Church 1975) of the VFA are absorbed through the epithelial lining of the rumen and reticulum, with less than 10% of the VFA passing into the small intestine (Harfoot 1978). In fact, most of the host ruminant animal's energy requirements are met by the VFA absorbed through the epithelial lining of the rumen (Annison and Armstrong 1969). Ruminal epithelium is not simply a barrier to nutrient diffusion, but through the metabolism of VFA, primarily butyrate, it acts to maintain metabolite concentration gradients and facilitate nutrient absorption.

The primary ruminal volatile fatty acids (acetate, propionate, and butyrate) are metabolized to different extents by the ruminal epithelium. Experiments in vitro demonstrated that although acetate is absorbed readily by the ruminal epithelium, it fails to induce an increase in oxygen uptake by ruminal epithelial tissue slices (Goosen 1976), thus indicating that the tissue does not metabolize acetate to a great extent. Propionate is metabolized to lactate and pyruvate by the ruminal epithelium, with estimates ranging from 3% to 15% (Emmanuel 1981, Nocek et al. 1980, Weekes 1974a, Weigand et al. 1975), whereas addition of propionate in vitro to ruminal slices resulted in no change in oxygen uptake by the ruminal pieces (Goosen 1976). Despite the apparently low use of propionate by the ruminal epithelium, disappearance of propionate carbon from the rumen cannot be completely accounted for by lactate, pyruvate and propionate appearance in the portal blood.

In the fed ruminant animal, the rumen and the liver are the primary producers of ketone bodies (Heitmann et al. 1987). Ruminally derived acetoacetate (AcAc) is quantitatively removed from portal blood by the liver, following metabolism to β-hydroxybutyrate (β-HBA). Thus, in the fed state, the ruminal epithelium is the primary source of circulating ketone bodies in the ruminant animal (Heitmann et al. 1987). Most (85% to 90%) of ruminally absorbed butyrate carbon appearing in portal blood is in the form of β-HBA and AcAc (Beck et al. 1984). The balance of the absorbed butyrate carbon can be accounted for as CO₂ produced as a result of butyrate oxidation (Goosen 1976).

Ketogenesis in the ruminal epithelium is performed exclusively in the mitochondria (Leighton et al. 1983) due to the
compartmentalization of the ketogenic enzymes. Two pathways of ketogenesis are available to the ruminal epithelium. Ketogenesis can progress through 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase and HMG-CoA lyase, as in the liver, or via deacetyl of AcAc-CoA catalyzed by succinyl-CoA transferase. Both of these pathways result in the production of AcAc. The final step in renal ketogenesis is the production of βHBA catalyzed by βHBA dehydrogenase. In the fed ruminant, this pathway is favored because of the NADH:NAD ratio within the mitochondria (Heitmann et al. 1987). There are sufficient activities of these ketogenic enzymes present within the ruminal epithelium to account for the ketogenic capacity of the rumen (Baird et al. 1970, Bush and Milligan 1971, Leighton et al. 1983).

**STRUCTURE OF THE RUMINAL EPITHELIUM**

The rumen epithelium is responsible for physiologically important functions, such as absorption, transport, VFA metabolism and protection (Gálfi et al. 1991, Stevens 1969). The ruminal epithelium is a stratified squamous epithelium consisting of four strata: stratum basale, stratum spinosum, stratum granulosum, and stratum corneum (Steven and Marshall 1969). The stratum basale is the layer of cells immediately adjacent to the basal lamina, and these cells contain fully functional mitochondria and other organelles. The intermediate cell layers are the stratum spinosum and stratum granulosum, which are not separated by a distinct division (Stevens 1969). As cells migrate through these intermediate layers, they contain progressively fewer numbers of mitochondria and take on a less uniform appearance. Thus, the cells of the stratum basale and spinosum, which have significant numbers of mitochondria, contribute most to the metabolic properties of the tissue, i.e., ketogenesis. Consequently, these cells are the most important cells of the rumen with regard to whole-animal energy metabolism. In the stratum granulosum, the cells have established tight gap junctions (desmosomes) that act as a barrier to diffusion across the rumen wall (Steven and Marshall 1969). Cells in the stratum corneum, the most external cell strata, are highly cornified, and presumably function as a defensive barrier against the physical environment of the rumen. The desmosomal integrity of the stratum corneum has degenerated, and large gaps exist between individual cells (Steven and Marshall 1969). The number of cell layers present in the stratum corneum is highly related to the dietary composition (Gaëbel et al. 1987). High concentrate diets, decreased ruminal pH, increased propionate:acetate ratios, and increased molar proportions of butyrate all result in a stratum corneum of up to 15 cell layers in thickness (Gaëbel et al. 1987). Conversely, in an animal maintained on a high roughage diet the stratum corneum may consist of as few as four cell layers (Gaëbel et al. 1987). Feeding regimen (i.e., how and when the feed is presented) has also been implicated as affecting the stratum corneum (Sakata and Tamate 1974, Tamate et al. 1974), which is most likely attributable to the subsequent alterations in the ruminal environment (Gálfi et al. 1991). Because of the unique and variable physical composition of the ruminal epithelium, it has been difficult to investigate the effects of differing ruminal environments on all aspects of ruminal metabolism. Use of isolated ruminal epithelial cells affords several advantages in the study of ruminal metabolism and development. Isolation of the metabolically active cells of the strata basale and spinosum minimizes the effects of increased strata corneum and granulosum components when evaluating metabolism of ruminal epithelium isolated from animals fed diets differing in energy density (i.e., forages vs. concentrates) and ruminal characteristics. Furthermore, use of isolated cells is necessary in order to compare data from the undeveloped ruminal epithelium, consisting primarily of strata basale and spinosum cell types, and fully differentiated ruminal epithelium, consisting of four strata with highly variable cell content. Thus, comparisons between mature and neonatal ruminal epithelial metabolic activities are difficult to interpret when results can be expressed only upon a total tissue dry weight basis or tissue protein basis. Only through investigation of cells actively metabolizing VFA, i.e., cells of the strata basale and strata spinosa, from the neonatal and mature rumens can valid comparisons of cellular capabilities be made. Significant limitations exist with cell isolation techniques, such as the disruption of cell to cell interactions, and need to be addressed when results of isolated cell preparations are to be extended to the whole tissue or whole-animal level.

**DEVELOPMENT OF ISOLATED CELL SYSTEMS**

Ruminal epithelial cell isolation systems have been developed by a number of groups for various purposes. Weekes (1974b) reported that epithelial propionate metabolism to lactate and pyruvate was severely compromised when bovine ruminal papillae were pre-treated and incubated with papain to disrupt the tissue. Despite the isolation of apparently healthy cells, Weekes (1974b) demonstrated that the activities of lactate dehydrogenase, glutamate dehydrogenase, βHBA dehydrogenase, and NADP-malate dehydrogenase were reduced as a result of papain treatment. Gálfi et al. (1980) demonstrated through the use of a serial trypsinization procedure that bovine ruminal cells, primarily from the strata basale and spinosum, could be isolated and maintained in primary culture. Using this isolation and primary culture system, these researchers were able to demonstrate keratinization of ruminal cells in culture (Gálfi and Néogrady 1989), an inhibitory role of butyrate (10 mmol/L) on DNA synthesis (Gálfi et al. 1981, Néogrady et al. 1989) and stimulatory effects of insulin on ruminal cell proliferation (Néogrady et al. 1989). However, this procedure was severely limited for use in metabolic evaluations in that it required approximately 8 h to isolate the cells from the strata basale and spinosum. Inooka et al. (1984) were able to successfully isolate and culture ruminal cells using a pretrypsinisation procedure that facilitated isolation of both epithelial and fibroblastic cell types by subsequent trypsinization in approximately 2 h. Inooka et al. (1984) were further able to maintain and subculture isolated ruminal cells that had fibroblast characteristics. To facilitate the study of ruminal epithelial metabolism during development and under conditions where ruminal morphology was affected, Baldwin and Jesse (1991) developed an isolation procedure that also used serial trypsinization and resulted in a yield of viable cells sufficient to conduct short-term (2-h) incubations with multiple treatments within 2 h following removal from the animal. Baldwin and Jesse (1991) reported linear rates of βHBA production through 2-h incubations, demonstrated that chilling the ruminal epithelium before isolating cells depressed subsequent ketogenic capacity, and established that cells from the initial incubation fractions were primarily of stratum corneum origin through histological analysis of the digested tissue.

**METABOLISM STUDIES USING ISOLATED RUMINAL EPITHELIAL CELLS**

The rumen is incompletely developed both physically and metabolically at birth, representing only 30% of the total gastrointestinal capacity (Warner et al. 1956). Rumens of neo-
nates do not exhibit the high degree of keratinization characteristic of the mature organ. Metabolically, the rumen of neonates is essentially nonfunctional with respect to ketogenic capacity (Warner et al. 1956). It has been assumed that glucose is the primary energy substrate of the immature tissue, as is the case with other neonatal tissues (White and Leng 1980). However, Baldwin and Jesse (1992) demonstrated, using isolated ruminal epithelial cells from normally reared lambs ranging from 0 to 56 d of age, that both butyrate and glucose were used by the pre-ruminant preparations in vitro. The pattern of glucose use by ruminal epithelial cells reported by Baldwin and Jesse (1992) is consistent with previous experiments in vitro using oxygen uptake by rumen slices from 14-d-old calf ruminal epithelium (undeveloped) or mature ruminal papillae (Giesecke et al. 1979). Oxygen uptake by the neonatal rumen was greatest when glucose was present as the oxidizable substrate. Although oxygen consumption by mature ruminal papillae increased above basal oxygen uptake when glucose was added, the response was not as dramatic as observed with neonatal ruminal slices (Giesecke et al. 1979). However, both glucose and butyrate were oxidized to carbon dioxide by isolated ruminal cells as early as 7 d of age (Baldwin and Jesse 1992). The rate of metabolism, on a cellular basis, declined for butyrate as the animals matured, whereas glucose remained elevated through 42 d and thereafter was markedly reduced (Baldwin and Jesse 1992). The presence of butyrate in the medium decreased the rate of glucose use by the isolated cells, and butyrate oxidation was similarly affected by glucose (Baldwin and Jesse 1992). Thus, the neonatal ruminal epithelium can apparently use either butyrate or glucose, depending upon supply of nutrients and cellular requirements.

A 10-fold increase in $\beta$HBA production by isolated cells occurred between d 42 and 56, with no change in $\beta$HBA dehydrogenase activity (Baldwin and Jesse 1992). Lane (1996) conducted experiments that demonstrated that the rate of $\beta$HBA production observed in conventionally reared sheep was similar to that observed in lambs maintained solely on milk replacer through 84 d of age. Furthermore, Lane (1996) demonstrated that ketogenic enzyme mRNA concentrations, specifically 3-hydroxy-3-methylglutaryl-CoA synthase and acetacetyl-CoA thiolase, increased with increasing age in both conventionally reared and milk replacer-fed lambs. Volatile fatty acid infusions into the rumen of milk replacer-fed lambs resulted in increased AcAc production and decreased glucose oxidation by isolated ruminal epithelial cells (Lane and Jesse 1997). Thus, metabolic adaptations in the developing ruminal epithelium seem to be related to age and the presence of luminal VFA.

Metabolite interactions have also been investigated using isolated ruminal epithelial cells. Baldwin and Jesse (1991) reported increased $\beta$HBA production from butyrate (10 mmol) by isolated ruminal epithelial cells when physiological (25 mmol) concentrations of propionate were included in the incubation medium, whereas acetate (50 mmol) inhibited $\beta$HBA production. In further experiments, propionate stimulation of butyrate oxidation to $\beta$HBA was found to be highly correlated with lactate production ($r^2 = 0.78$; Baldwin and Jesse 1996). Production of AcAc was decreased in these experiments such that total ketone body production was unaffected by propionate, whereas the ratio of $\beta$HBA to AcAc increased from 1.2 to 6.22 with propionate concentrations from 0 to 50 mmols/L. Addition of succinate resulted in comparable lactate production, yet inhibited $\beta$HBA production and none of the additions (succinate or propionate) affected butyrate oxidation to carbon dioxide. Thus, Baldwin and Jesse (1996) concluded that the mitochondrial redox status of the ruminal epithelial cell is affected by the presence of propionate such that complete conversion of AcAc to $\beta$HBA is favored. This is consistent with previous work with ruminal papillae in short-term culture, which demonstrated that changes in redox state affect the ratio of ketones produced (Emmanuel 1981, Goosen 1976). Additional evidence that substrate level interactions influence ruminal metabolism was reported by Jesse et al. (1992), who showed that palmitate metabolism was more sensitive to ruminal derived factors than to blood-borne factors. Jesse et al. (1992) also demonstrated that rates of palmitate oxidation to carbon dioxide by isolated ruminal cells were lower in neonatal lambs (3–4 d) than in 24- to 30-wk-old lambs with mature ruminal epithelium.

Very few of the studies conducted to evaluate changes in ruminal epithelial metabolism in response to alterations in dietary regimen (i.e., forage vs. concentrate) have been able to demonstrate metabolic changes (Harmon 1986). Harmon (1986) reported that ruminal papillae from animals fed concentrate at 6.3 MJ ME/d oxidized both glucose (5 mmol/L) and glutamine (1 mmol/L) to CO2 at higher rates than papillae from steers fed forage at 3.4 MJ ME/d. Baldwin and McLeod (1997) incubated ruminal epithelial cells isolated from lambs fed either forage- or concentrate-based diets at two levels of energy intake in the presence of multiple concentrations of oxidizable substrates (acetate, propionate, butyrate, glucose, glutamine and glutamate) in order to facilitate the development of estimates of maximal oxidative capacity ($V_{max}$) as well as the dose at which half-maximal oxidative rates were achieved ($K_{m}$). They found that oxidation rates of butyrate and propionate were greater in epithelial cells isolated from concentrate-fed lambs as compared with those isolated from forage-fed lambs at some concentrations, whereas rates of acetate oxidation were generally enhanced with increasing ME intake. However, these changes in VFA oxidation rates did not affect the overall predicted Michaelis-Menten parameter estimates, $K_{m}$ and $V_{max}$. The observed rates of oxidation reported by Baldwin and McLeod (1997) were generally consistent with the literature derived from research on ruminal tissue pieces. Furthermore, the estimated $K_{m}$ values reported are consistent with the contention that VFA are the primary oxidizable fuels used by ruminal epithelium (0.4, 0.9 and 9.9 mmol/L for butyrate, propionate and acetate, respectively). In fact, at ruminal concentrations, all of these metabolites would be oxidized by the ruminal cells at maximal rates. This raises the question of whether the concentration of the VFA surrounding the strata basal and spinosum ever reaches such high concentrations in vivo. Possibly, through the protection of the outer strata, rapid diffusion into the blood, and metabolism by the ruminal epithelial cells themselves, VFA may be maintained at lower concentrations. Clearly, more research needs to be conducted to further refine these estimates and facilitate the accurate prediction of the effect of dietary manipulation on ruminal metabolism in vivo.

FUTURE RESEARCH

Isolation of cells from ruminal papillae biopsies has been reported (Waldron et al. 1995) and represents a significant improvement upon previously developed models. Harvesting of tissue in this manner will facilitate research into the effects of physiological status, as well as allow for the definition of temporal responses of ruminal metabolism during transitions between diets. The effect of alterations in ruminal pH and VFA concentration also needs to be studied in more detail using both in vivo and in vitro perturbations to refine our understanding of the role of the ruminal epithelial metabolism in the energy metabolism of the whole animal.
LITERATURE CITED


