Manipulation of the Functional Activity of the Gut by Dietary and Other Means (Antibiotics/Probiotics) in Ruminants[1,2]

DAVID S. PARKER

Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, United Kingdom

ABSTRACT The mucosa of the gut is some of the most metabolically active tissue in the body. This paper discusses the methodology used to assess enterocyte cell metabolism and nutrient uptake in the reticulorumen and small intestine of ruminant species. Metabolism of volatile fatty acids and glucose by this tissue may limit the availability of essential nutrients to peripheral tissues, and the extent to which this may vary between concentrate-based and forage-based diets is discussed. Factors that affect the development and expression of metabolite uptake by the enterocyte are considered in addition to the influence that manipulation of the microbial flora of the gut by the use of antibiotic growth promoters or probiotics may have upon this process. Data are presented to show that the use of antibiotic compounds in ruminant feeds can influence the rate of cell turnover in the small intestine and the rate of glucose uptake by isolated brush border vesicles. *J. Nutr.* 120:639–648, 1990.

INDEXING KEY WORDS:

- *ruminant* • *mucosa* • *metabolism*
- *antibiotics* • *probiotics*

The objective of this paper is to review the extent to which metabolism by the mucosa of the reticulorumen and small intestine of ruminant species may influence the supply of nutrients to body tissues. In view of the breadth of the topic, the discussion will be restricted to consideration of the methods used to determine mucosal cell metabolism, the influence this activity has upon volatile fatty acid (VFA) and glucose metabolism and the effect manipulation of the microbial flora of the intestine may have upon tissue function and, therefore, nutrient supply. The ability of the cells lining the luminal surface of the gastrointestinal tract to adapt to changes in both the nutritional and physiological status of the animal has been recognized for many years. In ruminant species, the development of the reticulorumen system itself depends on the presence of the end products of microbial fermentation, the VFA, which stimulate blood flow to the tissues and the muscular contractions required for the mixing of ruminal contents (1). Physiological changes such as refeeding after starvation (2), pregnancy (3) and lactation (4, 5) cause hypertrophy of the gut associated with increased feed intake. These changes occur in both the small intestine and the ruminal mucosa although it has been reported that hypertrophy in the small intestine of sheep precedes that in the reticulorumen (4). Recent data from Rompala et al. (6) identified an increase in tissue weight of the large intestine associated with dietary bulk whereas that of the small intestine was not affected. The mass of the gut tissues is a relatively small proportion of body weight [3.75% in nonlactating and 4.85% in lactating cows (7)]. However, in view of those tissues' high rate of metabolic activity, the liver and gut contributing some 40% of total heat production in resting sheep (8), it is apparent that changes in nutrient metabolism or requirements of the gut will have a major influence on the pattern of nutrient availability to the tissues of the animal.

EXPERIMENTAL APPROACH

In view of the complexity of studying the metabolism of individual tissues within the animal, many different

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approaches have been used, ranging from in vitro tissue incubation systems through whole-animal chronic catheterization preparations to mathematical modeling of the whole range of metabolic activity present in the tissue/animal under study. All of these methods have their inherent disadvantages; the in vitro system lacks the sophisticated feedback control mechanisms present in the whole animal whereas the mathematical model relies on data from animal experiments if it is to provide a sound basis for testing the biological system.

In the chronically catheterized animal, it is possible to study metabolite uptake and output by tissues and to quantify these changes by reference to blood flow and, if possible, isotope exchange. This approach has been used very successfully in work on the mammary gland of goats (9) and sheep (10) where discrete input/output relationships can be measured. Extending this approach to the gut results in further areas of uncertainty associated with the dual source of nutrients for cell metabolism, the arterial supply and the intestinal lumen, and also the passage of metabolites from the luminal to serosal surface of the tissue and vice versa. Arteriovenous difference studies, therefore, can only provide a measure of net metabolite exchange across the tissue by reference to concentrations in the portal vein relative to those in the arterial supply. Improved understanding of metabolism in the various sections of the tract requires the siting of venous sampling catheters in the arterior mesenteric vein, to obtain blood leaving the small intestine, and in the portal vein to obtain samples that reflect, by difference, the metabolism in the tissues of the reticulorumen, although even this approach does not allow for the separate determination of nutrients derived from the cecum and large intestine.

Ideally, studies concerned with the metabolism of intestinal tissue should include not only arteriovenous difference and blood flow data but also a measure of metabolite turnover and flow in the intestine. Few experiments to date have provided this level of information with which to evaluate gut tissue metabolism. Responses to changes in diet or physiological state are more often evaluated either within the gut or, in a minority of cases, by reference to changes in nutrient flux in the portal vein. Integration of data from these two approaches provides some insight into the extent of modulation of nutrient supply by tissues of the intestine although our understanding of the whole process is still limited.

METABOLISM IN THE MUCOSA OF THE RETICULORUMEN

Volatile fatty acids. The relationship between feed intake and the development and adaptation of the ruminal mucosa has been extensively reviewed by Fell and Weekes (5). These authors identified the effect of VFA production on the proliferation of the ruminal epithelium, and further studies involving infusions of VFA mixtures into the rumen by Sakata and Tamata (11, 12) also showed that elevation of ruminal butyrate resulted in an increase in mitotic indices and in blood flow to the tissue. The relationship between changes in mucosal structure and metabolism of VFA by the tissue is less well established. Metabolism of all three VFA has been shown in a number of in vitro studies (for reviews, see ref. 5, 13) with the general agreement that propionate and butyrate are more extensively metabolized than acetate. There are, however, only limited studies in vivo from which rates of production of ruminal VFA with various diets can be compared with rates of absorption into the portal vein. Thus, work by Huntington (14), in which rates of total VFA absorption in steers fed either silage or high concentrate diets were compared with ruminal VFA production rates from separate experiments, showed that there was extensive metabolism of all three VFA in the mucosal tissues of animals fed various diets. It also has been suggested that there is a difference in the extent of metabolism of propionate by the mucosa of sheep when compared to that of cattle (15). It is possible to calculate the rate of propionate production in the rumen of concentrate-fed animals from the data of Sharp et al. (16) (as used by Huntington for total VFA production), and for the silage diet the results of Gill and Beever (17) are pertinent. These two studies indicate ruminal propionate production rates of 579 mmol/h and 329 mmol/h, respectively, for intakes of concentrate and silage diets similar to those reported by Huntington (14). These may be compared with net absorption rates of 339 mmol/h for the concentrate diet and 135 mmol/h for the silage diet (14), suggesting that with the concentrate diet 142 mmol/h of propionate was metabolized within the ruminal mucosa whereas 81 mmol/h was utilized by animals fed the silage diet. Although the ruminal wall may have a limited capacity to metabolize propionate to lactate, oxidation of this substrate may account for the observation that 40–60% of propionate produced in the rumen can be metabolized by this tissue and may not, therefore, be absorbed into portal blood (5, 13). Manipulation of propionate production rate in the rumen by the use of ionophores in both roughage and concentrate diets by Harmon et al. (18) showed that increased availability did not alter the proportion of propionate used by the gut tissues, although metabolism of the substrate would be quantitatively higher when ionophores were included in the diet. This observation supports that of Veenhuizen et al. (19), who showed that increased availability of propionate in the rumen increased the contribution of propionate carbon to the blood CO_2 pool. In these latter experiments, the investigators sampled peripheral blood that contained CO_2 derived from propionate carbon following metabolism in the liver, underlining the desirability of sampling from ruminal fluid and portal and peripheral

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TABLE 1

Net glucose utilization by tissues of the gastrointestinal tract of sheep and steers

Animal	Diet	Site of venous blood sample		Reference
		Portal vein	Mesenteric vein	
		$\mu mol/(min \cdot kg^{0.75})$		
Sheep	Hay	-3.7	—	21
	Fasted	-7.4	—	21
Sheep, pregnant	Hay	-5.5	—	21
	Fasted	-2.7	—	21
Sheep	Fasted + rumen propionate infusion	-9.12	—	22
	Low[1]	-6.94	—	
	High[1]	-9.72	—	
Sheep	Hay	-3.6	—	23
	Grain	+10.5	—	
Sheep	Dried grass	—	-1.67	24
	Pelleted corn	—	+15.90	
Sheep	Silage conc.			
	60:40	—	-12.4	25
	55:45	—	-3.56	
	50:50	—	-1.23	
Steer	Hay	-11.5	—	26
	Concentrates	-7.0	—	
Steer	Silage	-11.33	—	14
	Concentrates	-6.66	—	
Steer	Concentrates	-1.18	—	27
	+ Intraruminal glucose	+7.8	—	
Steer	Concentrates	-6.8	—	28
	+ Monensin	-1.14	—	
	+ Intraruminal propionate	+1.65	—	
Steer	Time fed lucerne	-7.22	-4.18	29
	Meal fed lucerne	-5.95	-4.18	
	Meal fed concentrates	-0.18	+5.32	
Steer	Grass pellets	+0.54	—	30
	Grass + corn	+5.3	—	
Steer	Grass + corn	+1.75	—	Wilton, personal communication
	+ Portal NH_4Cl infusion			
	(1) Low	-4.40	—	
	(2) High	-10.70	—	

[1]Low and high concentrate diets.

blood if a true measure of the influence of mucosal metabolism upon VFA availability is to be achieved.

Glucose. The influence of the metabolism of gastrointestinal tissues upon the glucose economy of the ruminant is well established. The recent analysis of the relationship between energy intake and gut and hepatic glucose metabolism by Wieghart et al. (20) focuses attention upon the demand the splanchnic tissues place upon glucose supply to the animal. Data for net glucose utilization by the gut of sheep, steers, heifers and lactating cows are shown in **Tables 1** and **2.** It is apparent from these studies that certain dietary situations can result in a net uptake of glucose from the digestive tract and that this effect may be mediated by overall energy intake (above 125 MJ/d in lactating cows) and by the composition of the diet, with hay and silage diets resulting in a

TABLE 2

Net glucose utilization by tissues of the gastrointestinal tract of heifers and dairy cows

Animal	Diet	Net glucose utilization[1]	Reference
		μmol/(min·kg$^{0.75}$)	
Heifer	High concentrates	−5.53	18
	+ Salinomycin	−2.0	
	+ Monensin	−1.36	
Heifer	75% Lucerne		
	(low)[2]	−2.08	31
	(high)[2]	−7.55	
	75% Corn		
	(low)	+9.63	
	(high)	−0.26	
Cow (L)[3]	Hay/concentrates	−5.11	32
		−13.5	
		+9.50	
Cow (L)	Hay/concentrates	−2.77	33
(D)[3]		+0.99	
Cow (L)	Hay/concentrates	−5.74	34
(D)		−2.87	
Cow (L)	Silage	−6.33	35
Cow (L)	Ad libitum concentrates	+25.1	20
	Restricted concentrates	+10.9	
	Restricted forage	+12.0	
Cow (D)	Hay/concentrates		
	7.1 Mcal/d	−4.95	20
	14.2 Mcal/d	−2.91	
	28.5 Mcal/d	+2.29	
Cow (L)	Silage/concentrates	−6.9	36
		−8.4	

[1]Venous blood was sampled from the portal vein.
[2]Low and high concentrate diets.
[3]L and D in parentheses indicate lactating and dry cows, respectively.

greater net utilization of glucose by the mucosal tissues. In the studies of Harmon and Avery (28) and Harmon et al. (18), the inclusion of ionophore antimicrobial agents in the diet reduced net glucose utilization by gut tissues in steers and heifers fed concentrate-based diets, although these changes were not statistically significant. The potential for manipulating glucose utilization by the gut tissues via the luminal surface of the mucosal cell is apparent from these studies and recent data from Wilton (personal communication). In the Wilton studies, NH_4Cl infusion into the portal vein increased net glucose utilization by gut tissues of steers fed a grass/corn diet; this suggests that conditions at the serosal surface also influence tissue metabolism. Net glucose utilization rates for portal-drained tissues average 2.65 μmol/(min·kg$^{0.75}$) for concentrate and 5.97 μmol/(min·kg$^{0.75}$) for forage diets (Tables 1, 2). On the basis of whole-body glucose turnover rates of between 24 and 36 μmol/(min·kg$^{0.75}$) for sheep and cattle, this represents 0.11–0.24 of total glucose turnover being utilized by portal-drained tissues. For sheep fed hay-based diets, calculation of actual portal glucose utilization rates from changes in glucose specific radioactivity across the gut during continuous isotope infusion (21) showed that actual glucose utilization rates were similar to net utilization values in nonpregnant animals. In pregnant sheep, however, the net rates determined from arteriovenous difference concentrations were lower than the actual values measured using isotope dilution. A further study (23) in which lambs were fed either hay- or grain-based diets showed that despite a positive portal-arterial difference with the grain diet compared to a negative value for the forage diet, actual glucose utilization by the intestinal tissues, as determined by isotope exchange, was higher for the concentrate than for the hay diet. This suggests that the apparent difference between concentrate and forage diets in terms of net glucose utilization may be misleading and that high concentrate diets could impose an increased glucose requirement on the mucosal tissue of the reticulorumen. Animals maintained on cereal-based diets show proliferation of the ruminal mucosa as a result of stimulation by increased absorption of VFA and subsequent release of gastrointestinal hormones (37). This stimulation of mitotic activity could result in an increased requirement for glucose by mucosal tissues. Concentrate feeding to ruminants has been shown to disrupt the structure of the ruminal wall, resulting in the loss of the cornified layer of the ruminal papillae (12), although other studies have shown significant hyperkeratosis of mucosal tissue as a result of grain feeding (5, 38). One further aspect of apparent glucose utilization by mucosal tissues identified by Bergman et al. (21), but as yet not investigated, is the possibility of glucose flux from the blood into the lumen of the digestive tract. Recent studies in our laboratory (Seal, unpublished data), however, in which ruminal fluid has been sampled during and after intravenous infusion of 6-[^{3}H]glucose, have shown no evidence that radioactivity was transferred from the blood glucose pool into the rumen fluid.

Although the majority of experiments to date have relied upon sampling of portal vein blood for measuring net tissue utilization of metabolites, placing catheters in the anterior mesenteric vein can provide additional information relating to metabolism in the tissues of the small intestine. This approach has been used in sheep by our group at Newcastle (24) and provided evidence that sheep fed a corn-based diet absorbed significant amounts of glucose from the small intestine. Isotopic

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analysis of glucose utilization by the intestinal tissues (39), however, showed that there was no alteration in glucose utilization by the tissues of the small intestine as a result of changing from a grass-based to a corn-based diet. Extending this approach to steers in which catheters were placed in both the mesenteric and portal veins (29) clearly identified the absorption of glucose in the small intestine as a key determinant of the apparent net utilization of glucose as measured in the portal vein. Further studies using this type of preparation are required to clarify the extent to which the influence dietary change has upon metabolism of the mucosa of the reticulorumen dominates any changes taking place in the mucosa of the small and large intestine.

METABOLISM IN THE MUCOSA OF THE SMALL INTESTINE

The mucosal cells of the small intestine are also responsive to physiological, nutritional and environmental stimuli; consequently, this tissue has a high rate of protein synthesis and energy demand. Work by Combe et al. (40) with developing lambs showed that total amount of protein synthesized in the small intestine increased rapidly after weaning, with the digestive tract representing 25% of total protein synthesized in the body at 16 wk of age. Fractional rates of protein synthesis are greater in the intestinal tissues of rats (8), cattle (41), pigs (42) and lambs (43) than in other tissues and are sensitive to dietary energy intake (44). Increased energy intake by sheep also has been shown to increase Na^+/K^+-ATPase–dependent respiration in duodenal and jejunal mucosa (45, 46), underlining the importance of mucosal cell activity as a major component of overall metabolism, contributing some 15% of total heat production in the fed sheep (8).

Apart from changes in overall metabolism associated with energy intake, the intestinal cells also show modification of functional activity with respect to changes in brush border hydrolase enzymes. Dauncey and Ingram (47) demonstrated in the pig that lactase activity was modified as a result of changes in environmental temperature although there was no effect on aminopeptidase-N. With rats, however, there have been numerous reports of alterations in protein or carbohydrate content of the diet influencing brush border peptidase (48, 49) and carbohydrase (50) activities; at a more fundamental level, the rate of leucine transport across isolated brush border membrane vesicles is influenced by dietary protein content (51). In experiments of this type, animals are adapted to diets over a period of weeks, and the rapidity of tissue adaptation is not known. In the case of starvation of sheep, however, it is apparent that mucosal respiratory activity has been significantly reduced after 48 h (44).

INFLUENCE OF THE MICROFLORA

In addition to dietary manipulation of intestinal activity, modifications of the intestinal microflora have a marked effect on mucosal function. Studies with germfree animals show that there are specific changes in the histology of the gut, and it has been known for many years that adding antibiotics to the diet reduces the mass of the tissues of the small intestine (52, 53). The potentially toxic effects on mucosal cell metabolism of NH_3 and amines produced as a result of amino acid degradation by endogenous flora have been identified by Visek (54) as a cause of increased tissue mass in conventional animals. Savage (55) has reviewed the influence of the gut flora upon digesta transit time, modification of bile acid function and reduced brush border hydrolase activity. The microflora of the small intestine also have been implicated as the cause of increased rates of protein synthesis in the rat (56) and chick (57) intestinal mucosa, although whether this is a direct effect of one elicited by the products of bacterial activity is still unclear.

Manipulating the normal microbial flora of the small intestine with feed additive antibiotics has been used effectively in the pig and poultry industries for a number of years (58, 59) although the mode of action is still unclear (60). In ruminant species, apart from effects within the rumen, it is expected that compounds that remain active in the gut also would have an effect in the small intestine. This may be particularly relevant when feeding diets containing ionophores such as monensin and lasolocid, which influence metabolism in both eukaryotic and prokaryotic cells. The role of these compounds in the manipulation of Na^+/K^+-ATPase activity and Na^+-mediated active transport has been reviewed by Bergen and Bates (61), and their potential for the disruption of divalent cation absorption by the small intestine also has been identified (62). Both the ionophore monensin and the macrolide antibiotic avoparcin, which are excreted in the active form of the feces, provide a means of modulating the microflora of the small and large intestine during the passage of digesta. In view of the fact, however, that the predominant bacteria in the small intestine are gram positive and sensitive to antimicrobial compounds, whereas those in the large intestine are gram negative, it is expected that the former is the more likely site for antimicrobial effects. Recent data from Newcastle showing that low levels of avoparcin (10 mg/kg) added to the diet of dairy cows increases milk yield despite there being no change in ruminal VFA metabolism supports this hypothesis (63).

MUCOSAL CELL TURNOVER

Many of the changes in enterocyte function noted as a result of dietary manipulation or the alteration in the microbial flora could be anticipated as a result of an

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alteration in the rate of cell division in the intestinal crypts with a consequent reduction in cell migration rate on the villus. Such a change has been demonstrated in germ-free when compared to conventional chickens (64, 65), and in both rats and pigs, cell turnover on the villus increases markedly during weaning (66, 67). In both of these studies, changes in the activities of carbohydrase enzymes were associated with alteration in cell turnover rates in the small intestine. Further studies in rats (68) indicated that although the tissue retains the ability to express lactase activity after weaning, it is the reduced time span of the enterocyte on the villus surface—from 7–10 d in the young rat to 2–3 d in the adult—that results in loss of expression of the mature enzyme at the cell surface. This was in contrast to sucrase activity, which increased along with the change in cell turnover rate. More recently, histochemical techniques and radiolabeled amino acid uptake studies showed that in the pig small intestine a reduced cell migration rate increases the functional capacity of the villus surface for nutrient transport and hydrolase activity (67, 69).

To test the hypothesis that antimicrobial feed additives affect the microflora of the small intestine and the tissue metabolism of ruminants, we undertook a trial in which weaned lambs (10 per treatment) were fed for 6 wk a pelleted diet containing either 0, 19 or 28 mg avoparcin/kg. Weight gain and feed conversion efficiency were similar for all three groups over this period, and there was no change in the molar proportions of VFA in rumen fluid because of avoparcin treatment. At the end of the trial, five sheep from each group were anesthetized and injected with the stathmokinetic drug vincristine sulphate (1 mg/kg body wt), which acts upon dividing cells by preventing the formation of the mitotic spindle such that any cell entering into mitosis following treatment is arrested at metaphase. The results in **Table 3** show the numbers of arrested cells per 1,000 crypt cells 30 and 90 min after administration of vincristine. It is apparent that the rate of cell division in the crypts of duodenal tissue after 90 min is significantly lower ($p < 0.05$) with avoparcin treatment, providing evidence for a possible site of the nutrient-sparing effect in the small intestine resulting from inclusion of the feed additive.

One consequence of a reduction in cell migration rate on the villus is the increased length of the brush border membrane microvilli (65, 70). A recent review (71) has identified the factors that regulate intestinal transport systems at this site on the enterocyte cell. The authors distinguish between rapid upregulation of certain transport processes within a few hours of the alteration of substrate availability and the dietary regulation of glucose and amino acid transport systems requiring 1–3 d. This latter change in intestinal transport processes would be in line with a modification in pattern of differentiation of cells at the villus/crypt junction.

TABLE 3

***Number of divided cells per 1000 crypt cells in sheep duodenal tissue 30 min and 90 min after treatment with vincristine*[1]**

Treatment	Time after vincristine administration	
	30 min	90 min
Control	15.6 ± 2.5	46.2 ± 7.4^{a}
19 mg Avoparcin/kg	16.8 ± 7.6	33.6 ± 9.9^{b}
28 mg Avoparcin/kg	13.2 ± 7.5	29.4 ± 6.1^{b}

[1]Values are means ± SD for five sheep. Values in a column with different superscript letters are significantly different ($p < 0.05$) as assessed by Duncan's Multiple Range Test.

Wolffram and Sharer (51) isolated brush border membrane vesicles from animals fed either a high carbohydrate (11% protein) or high protein (77%) protein diet and demonstrated that both Na^+-dependent and Na^+-independent transport of L-leucine increased with increasing dietary protein level. We have undertaken preliminary studies at Newcastle investigating the effect of a nontherapeutic dietary antibiotic on Na^+-dependent transport of glucose in sheep. Sheep were fed ad libitum a concentrate-based diet containing the antibiotic plus hay. At the end of the trial, the animals were killed by an overdose of barbiturate to prevent mucosal cell shedding (72), and brush border membrane vesicles were prepared by the method of Shirazi et al. (73). Glucose uptake in the presence of either Na^+ or K^+ was measured by the rapid filtration method of Murer et al. (74). Na^+-dependent glucose transport in brush border membrane vesicles isolated from tissue from control and treatment sheep is shown in **Figure 1.** The data show the characteristic "overshoot" between 45 s and 60 s, which was significantly greater ($p < 0.001$) in the tissue isolated from the sheep treated with the antibiotic. There was an increase with treatment in the number or activity of glucose receptors per unit of brush border protein. Although this study only investigated the effects on uptake by an in vitro vesicle preparation, the treatment may also have influenced intestinal structure or sodium gradients, which could also influence glucose uptake (75).

PROBIOTICS

Using antibiotic or antimicrobial compounds to manipulate the intestinal microflora to improve the efficiency of animal production has a number of disadvantages. Using compounds that are effective against a broad spectrum of bacterial species inhibits

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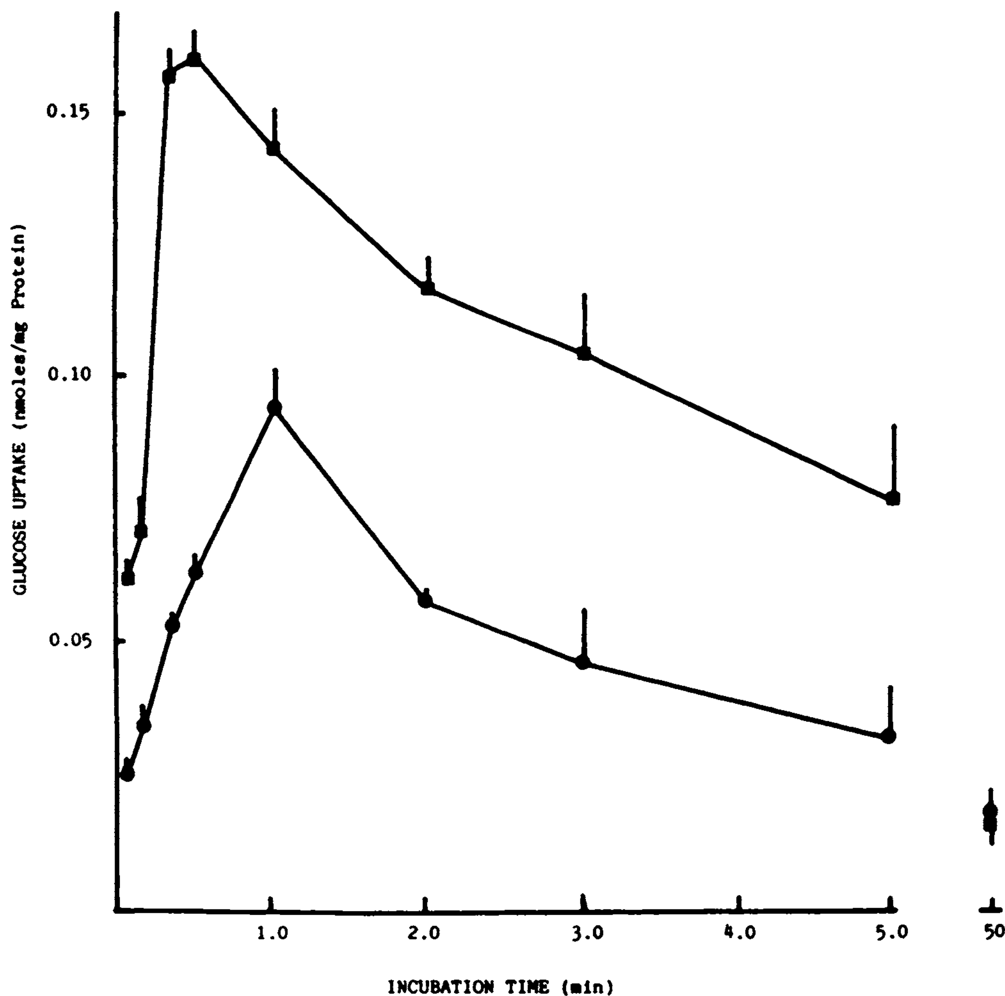

FIGURE 1 Sodium-mediated glucose uptake (nmol/mg protein) by brush border membrane vesicles from intestinal mucosa of sheep fed diets with (■) or without (●) an antibiotic feed additive. Values are means for six incubations; bars represent SEM.

strains that are beneficial and can increase the potential for colonization of the gut by pathogenic species (76). In addition, consumer concern over the residues in animal products and about the use of antibiotics and the potential for development of bacterial resistance in the wider environment has resulted in a reassessment of methods whereby the intestinal flora can be manipulated. Thus, the use of "probiotic" preparations containing selected strains of bacteria, primarily lactobacilli or streptococci, are currently advocated for stabilizing the intestinal microflora to the benefit of the host animal. Their use is considered to be particularly beneficial during periods of stress, such as weaning, when dietary and environmental changes can result in a significant reduction in animal performance associated with marked alterations in the balance of microbial species in the small intestine (77–79). Surveys of the effectiveness of probiotics (80, 81) show that the most consistent response is that achieved when probiotics are fed to neonatal and young pigs and that the overall response is similar to that achieved with conventional low-dosage antibiotic administration. This observation was confirmed by our own studies (82) comparing growth rates in pigs fed diets containing either an antibiotic, a probiotic preparation or no added growth promoter (including copper). Growth rates in the two treatment groups were significantly better than those of the control animals, and examination of enzyme activity in the intestinal mucosa showed that at the critical period around weaning, the antibiotic and probiotic groups has significantly higher levels of lactase and sucrase than did controls. In addition, there were changes in the distribution of dipeptidase and tripeptidase enzymes in the gut with age (83), providing some evidence that the site of probiotic action also may be linked to expression of mucosal cell function.

Selection of probiotic organisms is based upon a range of in vitro tests related to ability to survive at low pH, tolerance to bile, rapid growth rate and adhesion to isolated epithelial cells (84). This latter criterion, however, is sensitive both to changes in source of cultured cells used and to bacterial strain (85). An improvement

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in our understanding of the mode of action of probiotics in the gastrointestinal tract will depend on the development of methods that allow for the identification of the site or sites at which the introduced strains become established. At present, for example, it is not clear whether colonization of the intestinal wall is essential for probiotic function or whether it is the presence of the bacteria or the products of microbial metabolism that are of primary importance. The potential for low numbers of bacteria administered as an oral dose to establish colonies within the digestive tract has been demonstrated in an elegant experiment by Miller et al. (86). In this work, 1000 bacterial cells of a specific pathogenic *Escherichia coli* strain were administered to piglets at 5 d of age, and their presence in feces was determined over time. No bacteria of the selected strain were detected until 5 d after weaning, at which time they were associated with diarrhea and were shed in the feces. This observation suggests that at periods of rapid change in the structure of the intestinal wall, such as those seen at weaning, pathogenic bacteria are able to proliferate and dominate the intestinal microflora. To improve our understanding of how probiotics may be able to mitigate against this process, it will be necessary to develop methods that allow for the identification of the site or sites at which introduced strains become established and to determine whether certain areas of the tract are critical to the effectiveness of the treatment. Therefore, although it is apparent that metabolism of the intestinal mucosa is extensively modified by the microbial population of the gut, it is still far from clear as to how the relationship can best be manipulated to the benefit of the host animal.

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