Nucleosides Are Efficiently Absorbed by Na⁺-Dependent Transport Across the Intestinal Brush Border Membrane in Veal Calves

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ABSTRACT

In previous work, a comparatively high capacity for Na⁺-dependent transport of nucleosides across the intestinal brush border membrane (BBM) was observed in dairy cows, which might be related to digestion of the large amount of nucleic acids present in ruminal microorganisms in the ruminant small intestine. If this were the case, the capacity for Na⁺-dependent intestinal nucleoside transport should be much lower in veal calves, in which only small amounts of nucleic acids, nucleotides, and nucleosides reach the small intestine via the milk replacer. To test this hypothesis, we investigated Na⁺-dependent transport of ³H-labeled thymidine and guanosine across the BBM using BBM vesicles (BBMV) isolated from the small intestine of veal calves. In the presence of a transmembrane Na⁺ gradient both substrates were transported against a concentration gradient. Inhibitory studies showed that thymidine and guanosine are transported by two different transporters with overlapping substrate specificity, one accepting predominantly pyrimidine nucleosides (N2) and one accepting particularly purine nucleosides (N1). Nucleoside transport was inhibited by glucose along the whole small intestine. Maximal transport rates similar to those in dairy cows were obtained for the proximal, mid-, and distal small intestine. These findings suggest that the high absorptive capacity for nucleosides is a genetically fixed property in the bovine small intestine, which is already present in the preruminant state of veal calves. It may contribute to the high digestibility of nucleic acids observed by others in veal calves receiving milk replacer supplemented with RNA. Its main function may be the efficient absorption of nucleosides resulting from the digestion of nucleic acids associated with desquamated enterocytes. Due to the limited de novo synthesis of nucleotides in enterocytes intracellular uptake of nucleosides across the BBM may contrib-

ute to nucleic acid synthesis in enterocytes and thus may have a trophic effect on the intestinal epithelium. (**Key words:** absorption of nucleosides, brush border membrane, Na⁺-dependent nucleoside transport, inhibition of nucleoside transport by glucose)

Abbreviation key: BBM = brush border membrane, BBMV = brush border membrane vesicles, N1 = Na⁺-dependent transporter for purine nucleosides, N2 = Na⁺-dependent transporter for pyrimidine nucleosides.

INTRODUCTION

Large amounts of nucleic acids associated with ruminal microorganisms enter the duodenum of ruminants (Smith and McAllan, 1971; Stangassinger et al., 1995). They appear to be efficiently digested and absorbed, because 80 to 90% disappeared between the abomasum and the terminal ileum (McAllan, 1980). Nucleic acid degradation in the upper small intestine of steers was accompanied by a transient appearance of nucleosides, which generally completely disappeared by the terminal ileum. No pyrimidine bases and scarcely any purine bases appeared in the intestinal lumen during nucleic acid digestion (McAllan, 1980).

In former work, we showed by using isolated intestinal brush border membrane (BBM) vesicles (BBMV) that nucleosides are absorbed from the small intestine of cows by active Na⁺-dependent transport (Scharrer and Grenacher, 2001, 2002). Two Na⁺-dependent intestinal nucleoside transporters with overlapping substrate specificity were found, one transporting purine nucleosides (N1) and one transporting pyrimidine nucleosides (N2). The maximal transport capacity (V_{max}) of both transporters was at least 10-fold higher than reported for BBMV isolated from rabbit and human small intestine. The affinity constants (K_m-value) were also higher.

In experiments with isolated, perfused rat jejunum, nucleosides were split to a large extent to nucleobases and ribose phosphate by phosphorolysis after absorption. Purine bases were subsequently transformed to uric acid, while pyrimidine bases remained intact. Cytidine was not split (Stow and Bronk, 1993). With regard

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to absorption of purine nucleosides, the situation appears to be similar in cattle (Balcells et al., 1992). Since, as already noted, large amounts of microbial nucleic acids associated predominantly with rumen bacteria (about 100 to 200 g/d in dairy cows) are digested in the small intestine of ruminants (McAllan, 1980; Storm et al., 1983; Johnson et al., 1998, Scharrer and Grenacher, 2001), it is possible that the high maximal velocity of intestinal nucleoside transport by N1 and N2 in cows might be the result of an adaptation to the large amount of microbial nucleic acids digested in the small intestine (Scharrer and Grenacher, 2001). In that case, nucleoside transport across the intestinal BBM of veal calves should exhibit a lower activity, because microbial growth in the rumen is minimal due to the esophageal groove reflex in veal calves channeling the ingested milk or liquid milk replacer directly into the abomasum from where it is transferred into the duodenum (Titchen and Newhook, 1975). Moreover, milk or milk replacer only contains traces of nucleic acids, nucleotides, and nucleosides (Kobata et al., 1962; Gil Hernandez and Sanchez-Medina, 1981; Tiemeyer et al., 1984; Raezke et al., 1988). We therefore investigated intestinal nucleoside transport in veal calves using isolated BBMVs. ^3H -labeled thymidine and guanosine were used as transport substrates, because they appear to be selectively transported either by N1 (guanosine) or by N2 (thymidine) (Griffith and Jarvis, 1996; Patil and Unadkat, 1997; Scharrer and Grenacher, 2001). To clarify whether this also applies to veal calves the inhibitory potency of various nucleosides regarding thymidine and guanosine transport was first tested using BBMVs isolated from the proximal and distal small intestine. Glucose was also included as an inhibitory substrate, because it inhibited Na^+ -dependent nucleoside transport across the BBM of cows especially in the proximal small intestine (Scharrer and Grenacher, 2002). Due to the higher intestinal glucose transport capacity in calves in comparison to cows (Wood et al., 2000), it was of interest to find out how glucose affects Na^+ -dependent nucleoside transport in veal calves. Finally we determined the substrate affinity and transport capacity of Na^+ -dependent thymidine and guanosine transport across the BBM in veal calves in order to compare them with the pertinent kinetic constants (K_m -value, $V_{\max}$) in dairy cows obtained in former studies (Scharrer and Grenacher, 2001, 2002).

MATERIALS AND METHODS

Preparation of BBMVs

The proximal jejunum (first 2 m of small intestine immediately caudal to the ligamentum duodenocolicum) and distal jejunum (first 2 m of intestine immedi-

ately proximal to the ligamentum ileocaecale) were obtained from freshly killed veal calves (age: 2 to 3 mo) at the local slaughterhouse. The intestine was removed about 10 min after death. The two segments of small intestine were opened along the mesenteric border, washed twice with chilled saline and transferred to the laboratory in ice-cold saline within 15 min after removal. BBMVs were immediately prepared from mucosal scrapings according to the method of Kessler et al. (1978a). BBMVs were prepared (per liter) with 300 mmol of D-mannitol, 100 mmol of KCl, 1 mmol of MgSO_4 , 35 mmol of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)/Tris, pH 7.4. Aliquots of 400 μl were stored under liquid nitrogen until use (Stevens et al., 1982). Purification of the BBM was tested by determining the activity of the BBM enzyme alkaline phosphatase (AP, EC 3.1.3.1) (test kit Unimate 3, Roche Diagnostics, 6343 Rotkreuz, Switzerland). The final BBM-fraction exhibited a 22.0 ± 3.2 (proximal small intestine) and 22.5 ± 1.9 -fold (distal small intestine) enrichment in AP activity with respect to the original homogenate. Cross-contamination of BBM with basolateral membranes was negligible (Hopfer, 1977; Wolfram et al., 1990), as shown by the 0.9 ± 0.1 (proximal small intestine) and 1.3 ± 0.2 -fold (distal small intestine) enrichment of the basolateral membrane marker enzyme Na^+ , K^+ -ATPase (EC 3.6.1.3). Protein content was measured applying the Bio-Rad protein assay kit with bovine albumin as standard (Bio-Rad Laboratories, Glattbrugg, Switzerland).

Measurement of Substrate Uptake into BBMVs

Uptake of ^3H -labeled thymidine ([methyl- ^3H] thymidine, Amersham Life Science, Little Chalford, UK) and guanosine ([8- ^3H] guanosine, American Radiolabeled Chemicals Inc., St. Louis, MO) was determined by a rapid filtration technique (Kessler et al., 1978b; Wolfram et al., 1989). Short-term incubations (1 to 5 s) were performed using a semi-automatic incubation device first described by Kessler et al. (1978b). Uptake was started by adding 10 μl of membrane suspension to 40 μl of the incubation medium and quenched by adding 3 ml of ice-cold stop solution of the same composition, as the final reaction mixture without the addition of substrate. The dilution mixture was immediately drawn through a nitrocellulose filter (0.45- μm pore size, Schleicher & Schüll, Feldbach, Switzerland) and rinsed twice with the stop solution. The composition of the reaction media is indicated in the legend of Figure 1. The transmembrane potential difference was clamped at zero by adding valinomycin from an ethanolic stock to the thawed vesicle suspension to achieve a concentration of 20 μg of valinomycin per liter and 0.1% ethanol

in the final reaction mixture (Wolffram et al., 1989). The ^3H -activity remaining on the filters was determined by liquid scintillation counting in a beta-counter (Packard Scintillation Analyzer, 1600 TR).

Statistical and Kinetic Analysis

Values are presented as means with the standard error of the mean. To verify the effects on nucleoside uptake of various inhibitors, data were subjected to ANOVA with subsequent Dunnett test. Differences between two means were statistically evaluated using the unpaired or when appropriate the paired t -test (Sachs, 1992). Statistical evaluations were performed on a personal computer using the program Graph Pad Instat V 3.00 (Graph Pad Software Inc., San Diego, CA). The apparent kinetic parameters K_m (Michaelis constant, half-saturating substrate concentration) and $V_{\max}$ (maximal transport capacity) of transport of nucleosides across the BBM were calculated from the values of uptake rates obtained in the presence of a transmembrane Na^+ gradient corrected by uptake rates obtained in the presence of a choline gradient by nonlinear curve fitting based on the Michaelis-Menten equation (Preston et al., 1974). Kinetic analysis was performed on a personal computer using the program Graph Pad Prism V 3.00 (Graph Pad Software).

RESULTS

Time Course of Uptake of Nucleosides

The time course of uptake of thymidine and guanosine (concentration: $2 \mu\text{mol/L}$) into BBMV isolated from the proximal and distal small intestine in the presence of an inwardly directed initial Na^+ or choline $^+$ gradient (Na^+_{out} or choline $^+_{\text{out}}$ = 100 mmol/L , Na^+_{in} or choline $^+_{\text{in}}$ = 0 mmol/L) is shown in Figures 1 and 2. Initial uptake under Na^+ -gradient conditions significantly exceeded uptake under choline $^+$ gradient conditions. A marked initial overshoot of Na^+ gradient-dependent intravesicular thymidine and guanosine uptake in comparison to the 60 min uptake value occurred in BBMV isolated from the proximal small intestine, whereas this does not apply to the distal small intestine (Figures 1 and 2), where Na^+ gradient-dependent uptake of both substrates was significantly smaller than in the proximal small intestine.

Inhibition of Uptake of Thymidine and Guanosine by Various Nucleosides and by Glucose

The inhibitory effect of various nucleosides and of glucose (concentration: 0.1 or 1 mmol/L) on uptake of ^3H -labeled thymidine and guanosine (concentration: 2

Figure 1. Time course of uptake of ^3H -labeled thymidine by brush border membrane vesicles isolated from proximal (■, ●) and distal (□, ○) small intestine of veal calves. Vesicles were preloaded with 300 mmol/L D-mannitol, 100 mmol/L KCl, 1 mmol/L MgSO_4 , 35 mmol/L N -2-hydroxyethylpiperazine- N' -2-ethanesulfonic acid (HEPES)/Tris, pH 7.4 and incubated in reaction media containing (final concentrations, mmol/L : 100 NaCl (■, □) or choline chloride (●, ○), 100 D-mannitol , 100 KCl , 1 MgSO_4 , 0.002 ^3H -labeled thymidine respectively guanosine and 35 HEPES/Tris , pH 7.4. The values are means (M) $\pm$ SEM from four different vesicles preparations each determined in duplicate. * and $^+P < 0.05$, ** and $^{++}P < 0.01$, significantly higher than in the absence of Na^+ . # $P < 0.05$, ## $P < 0.01$, values are significantly higher compared to distal small intestine.

$\mu\text{mol/L}$) in the presence of a Na^+ gradient is given in Table 1. Thymidine, cytidine, uridine, and adenosine inhibited ^3H -thymidine uptake concentration depen-

Figure 2. Time course of uptake of ^3H -labeled guanosine by brush border membrane vesicles isolated from proximal (■, ●) and distal (□, ○) small intestine of veal calves. Further conditions and statistical evaluation as described in the legend of Figure 1. The values are means (M) $\pm$ SEM from five different vesicles preparations each determined in duplicate.

Table 1. Effects of various purine and pyrimidine nucleosides and glucose on uptake of thymidine and guanosine (concentration: 2 $\mu\text{mol/L}$, incubation time: 5 s) into bovine intestinal brush border membrane vesicles.¹

Inhibitory substrates	0.1 mmol/L		1.0 mmol/L	
	Thymidine	Guanosine	Thymidine	Guanosine
Proximal				
Mannitol (control)	17.5 $\pm$ 2.4	21.4 $\pm$ 4.0	13.7 $\pm$ 1.3	17.5 $\pm$ 2.4
Cytidine	4.6 $\pm$ 0.7**	21.8 $\pm$ 4.6	0.2 $\pm$ 0.1**	13.0 $\pm$ 1.7
Thymidine	5.2 $\pm$ 0.9**	21.4 $\pm$ 4.1	0.1 $\pm$ 0.0**	12.2 $\pm$ 1.5*
Uridine	7.1 $\pm$ 1.2**	7.1 $\pm$ 1.4**	0.1 $\pm$ 0.1**	0.2 $\pm$ 0.1**
Adenosine	8.4 $\pm$ 0.9**	3.7 $\pm$ 0.7**	0.2 $\pm$ 0.2**	0.3 $\pm$ 0.2**
Guanosine	16.7 $\pm$ 1.9	4.3 $\pm$ 1.0**	9.9 $\pm$ 1.0**	0.2 $\pm$ 0.1**
Inosine	17.0 $\pm$ 1.6	2.2 $\pm$ 0.7**	10.5 $\pm$ 1.5*	0.0 $\pm$ 0.0**
Glucose	9.3 $\pm$ 0.8**	11.5 $\pm$ 0.8**	6.1 $\pm$ 1.2**	10.0 $\pm$ 1.5**
Distal				
Mannitol (control)	5.2 $\pm$ 0.9	4.7 $\pm$ 1.5	3.6 $\pm$ 0.6	5.6 $\pm$ 1.2
Cytidine	2.5 $\pm$ 0.4**	4.0 $\pm$ 1.5	0.3 $\pm$ 0.2**	4.6 $\pm$ 1.3
Thymidine	3.0 $\pm$ 0.5**	4.2 $\pm$ 1.4	0.5 $\pm$ 0.2**	3.2 $\pm$ 0.4*
Uridine	2.6 $\pm$ 0.6**	2.6 $\pm$ 1.0*	0.2 $\pm$ 0.1**	0.1 $\pm$ 0.1**
Adenosine	2.7 $\pm$ 0.3**	1.7 $\pm$ 0.6**	0.3 $\pm$ 0.2**	0.0 $\pm$ 0.0**
Guanosine	5.1 $\pm$ 0.8	1.5 $\pm$ 0.4**	2.6 $\pm$ 0.6	0.2 $\pm$ 0.1**
Inosine	4.5 $\pm$ 0.8	1.7 $\pm$ 0.6**	2.2 $\pm$ 0.7*	0.1 $\pm$ 0.1**
Glucose	4.2 $\pm$ 0.6	3.2 $\pm$ 1.0	1.7 $\pm$ 0.4**	2.6 $\pm$ 0.3*

¹Values are means (M) $\pm$ SEM [pmol/mg of protein] of six (inhibitory substrates: 0.1 mmol/L) or four (three for guanosine uptake in the distal small intestine) (inhibitory substrates: 1 mmol/L) different vesicle preparations each determined in triplicate.

* $P < 0.05$, ** $P < 0.01$ compared to pertinent control value.

dently at both intestinal sites, whereas guanosine, inosine, adenosine, and uridine inhibited ³H-guanosine uptake in a concentration-dependent mode. At the high concentration, guanosine and inosine inhibited ³H-thymidine uptake about 30%, whereas thymidine inhibited ³H-guanosine uptake 30 to 40%. Glucose at both concentrations inhibited ³H-thymidine and ³H-guanosine uptake about 50% in the proximal small intestine. In the distal small intestine at the low concentration, glucose inhibited the nucleoside uptake only about 20 to 30% but 50% at the high concentration.

Similar to uptake of thymidine and guanosine (Table 1), Na⁺-dependent uptake of glucose into BBMV from the proximal small intestine exceeded that from the distal small intestine (Table 2).

Kinetics of Na⁺-Dependent Transport of Nucleosides

Uptake of thymidine and guanosine as a function of substrate concentrations (2 to 64 $\mu\text{mol/L}$) was deter-

mined. Uptake was corrected for Na⁺-independent uptake. Na⁺-dependent transport of both nucleosides increased in a curvilinear manner with mounting substrate concentration and reflected saturation kinetics (Figures 3 and 4). Apparent K_m values were similar for the proximal (thymidine: 38 $\mu\text{mol/L}$, guanosine: 24 $\mu\text{mol/L}$) and distal (thymidine: 41 $\mu\text{mol/L}$, guanosine: 34 $\mu\text{mol/L}$) small intestine, whereas V_{max} values for both nucleosides in proximal small intestine (thymidine: 104 pmol [mg protein]⁻¹s⁻¹, guanosine: 120 pmol [mg protein]⁻¹s⁻¹) exceeded those in the distal small intestine (thymidine: 50 pmol [mg protein]⁻¹s⁻¹, guanosine: 31 pmol [mg protein]⁻¹s⁻¹). The V_{max} value of guanosine uptake was significantly ($P < 0.01$) higher in the proximal part of the small intestine. In an additional series of experiments (four calves) the kinetics of Na⁺-dependent guanosine transport was determined for the middle part of the small intestine (K_m value: 20 $\mu\text{mol/L}$, V_{max}: 74 pmol [mg protein]⁻¹s⁻¹). In those investigations the V_{max} value was in between that of the proximal and

Table 2. Uptake of glucose in the proximal and distal small intestine of calves.¹

	Glucose concentration 0.1 mmol/L	Glucose concentration 1.0 mmol/L
Proximal	2275 $\pm$ 426*	4634 $\pm$ 823*
Distal	911 $\pm$ 143	2244 $\pm$ 358

¹Uptake is given as pmol/mg protein (incubation: 5s); Values are presented as means (M) $\pm$ SEM of four different vesicle preparations determined in triplicate. * $P < 0.05$, values are significantly higher compared to distal small intestine.

Figure 3. Kinetics of Na^+ -dependent thymidine uptake by jejunal brush border membrane vesicles. Values (proximal small intestine (■), distal small intestine (□)) are means $\pm$ SEM of 12 determinations from four different vesicle preparations each determined in triplicate. BBMV were prepared and incubated (incubation time: 1 s) as described in the legend of Figure 1. The lines were calculated by nonlinear curve fitting based on the Michaelis-Menten equation. Kinetic constants proximal (distal): $K_m = 38 \mu\text{mol/L}$ ($41 \mu\text{mol/L}$) and $V_{\max} = 104 \text{ pmol} [\text{mg protein}]^{-1}\text{s}^{-1}$ ($50 \text{ pmol} [\text{mg protein}]^{-1}\text{s}^{-1}$).

distal small intestine, while the K_m values for the three intestinal sites were similar.

In Table 3, the values of the kinetic constants of Na^+ -dependent intestinal thymidine and guanosine transport in veal calves are compared with the pertinent values in dairy cows obtained in a previous investiga-

Figure 4. Kinetics of Na^+ -dependent guanosine uptake by jejunal brush border membrane vesicles. Values are means $\pm$ of 18 determinations from six different vesicle preparations each determined in triplicate. Further conditions as described in legend of Figure 3. Kinetic constants proximal, (distal): $K_m = 24 \mu\text{mol/L}$ ($34 \mu\text{mol/L}$) and $V_{\max} = 120 \text{ pmol} [\text{mg protein}]^{-1}\text{s}^{-1}$, ($31 \text{ pmol} [\text{mg protein}]^{-1}\text{s}^{-1}$).

Table 3. Kinetic constants of Na^+ -dependent transport of thymidine and guanosine across the brush border membrane of the proximal, mid-, and distal small intestine of veal calves and dairy cows.

		Calves		Cows ³	
		Thymidine	Guanosine	Thymidine	Guanosine
Proximal	K_m ¹	38	24	49	28
	$V_{\max}$ ²	104	120	99	86
Mid	K_m	—	20	29	24
	$V_{\max}$	—	74	78	51
Distal	K_m	41	34	52	23
	$V_{\max}$	50	31	37	40

¹ $\mu\text{mol/L}$.

² $\text{pmol} (\text{mg of protein})^{-1}\text{s}^{-1}$.

³Published values (Scharrer and Grenacher, 2001; 2002).

tion (Scharrer and Grenacher, 2001; 2002). Evidently K_m and $V_{\max}$ values in veal calves were similar to those in dairy cows.

DISCUSSION

The main finding of this study is the high capacity ($V_{\max}$) of Na^+ -dependent nucleoside transport across the small intestinal BBM in veal calves (Table 3), which was at least as high as in dairy cows (Scharrer and Grenacher, 2001, 2002). This observation is not in accordance with the hypothesis that the high transport capacity of N1 and N2 in cows results from an adaptation to the large amount of ruminal microbial nucleic acids (100 to 200 g/d in dairy cows) digested in the cow small intestine. The high transport capacity of N1 and N2 of the BBM rather seems to be a genetically fixed property of bovine small intestine, which is already expressed in the preruminant state of veal calves ingesting only very little roughage due to feeding of liquid milk replacer, which bypasses the rumen and therefore impedes microbial growth including nucleic-acid synthesis in the forestomach (Roy et al., 1971, Titchen and Newhook, 1975). However, it remains to be investigated at which developmental stage Na^+ -dependent nucleoside carriers are integrated in the bovine intestinal BBM. It has recently been shown that the nucleoside transporters N1 and N2 are already present in the human fetal intestinal BBM (Ngo et al., 2001).

Possibly the predominant function of N1 and N2 in the intestinal BBM of veal calves is cellular absorption of nucleosides released by degradation of endogenous nucleic acids associated with desquamated enterocytes (Parsons and Shaw, 1983). Due to the rapid turnover of enterocytes in combination with the large tissue mass of the epithelium of the small intestine a substantial amount of endogenous nucleic acids enters the intestinal lumen with desquamated enterocytes. For instance, in the adult rat, about 30 mg of endogenous nucleic

acids enter the small intestine per day (Parsons and Shaw, 1983). Since de novo synthesis of nucleotides is limited in enterocytes (Le Leiko and Walsh, 1996), uptake of nucleosides deriving from endogenous nucleic acids across the intestinal BBM probably supports nucleic acid synthesis in enterocytes of preruminant calves and thus may have trophic effects on the intestinal epithelium (Nunez et al., 1990; Uany et al., 1990, Adjei et al., 1996; Le Leiko and Walsh, 1996). It should be noted in this context that due to these and further properties of nucleosides the European Commission has allowed supplementation of infant formula with nucleotides (Schlimme et al., 2000).

The inhibitory pattern of various nucleosides in regard to thymidine and guanosine transport on the whole is in accordance with the findings in cows (Scharer and Grenacher, 2001, 2002) and also suggests that in veal calves nucleoside transport across the intestinal BBM occurs by N1 and N2. Deviating from the findings in cows, glucose markedly inhibited transport of thymidine and guanosine across the BBM not only in the proximal small intestine (Scharer and Grenacher, 2002), but also in the distal small intestine. The greater inhibitory potency of glucose in veal calves may be related to their higher activity of Na⁺-dependent glucose transport across the BBM (Wood et al., 2000), which seems to compete with Na⁺-dependent nucleoside transport for the electrochemical transmembrane Na⁺-gradient (Scharer and Grenacher, 2002). A close association of the Na⁺-dependent transporters of glucose and of nucleosides in the BBM may favor this competition (Roden et al., 1991). Similar interactions between Na⁺-dependent monosaccharide and amino acid transport across the intestinal BBM have also been described (Alvarado, 1970; Murer et al., 1975; Munck, 1980).

Interestingly, the proximal-to-distal gradient in nucleoside transport activity in the small intestine of veal calves was similar to that of cows (Scharer and Grenacher, 2002) suggesting that this gradient also is not a direct consequence of digestion of large amounts of ruminal microbial nucleic acids in the proximal part of the small intestine of mature ruminants (McAllan, 1980). However, the high nucleoside transport activity in the proximal small intestine probably facilitates nucleic acid digestion in ruminants due to efficient removal of nucleic acid breakdown products.

Intracellular metabolism of nucleosides following uptake across the BBM may further enhance nucleoside removal from the intestinal lumen (Verbic et al., 1990; Barcells et al., 1992; Stow and Bronk, 1993).

The high V_{max} values for Na⁺-dependent intestinal transport of nucleosides in veal calves may be related to the high digestibility of nucleic acids (98%) in veal calves observed in an investigation by Roth and Kirch-

gessner (1979). In this study, the addition of 3.75% RNA (related to DM) to the milk replacer did not increase nucleic acid or N elimination via the feces, whereas urinary excretion of N and allantoin increased markedly.

Collectively, the findings presented show that in the preruminant state, bovine small intestine already possesses a very high activity of Na⁺-dependent nucleoside transport across the brush border membrane. Apparently this transport activity does not increase further in the maturing ruminant and, therefore, seems to be a genetically fixed property in the bovine species, which is already fully expressed in the preruminant state of veal calves.

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