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ACETATE, BUTYRATE AND PROLINE UPTAKE IN THE CAECUM AND COLON OF PRAIRIE VOLES (*MICROTUS OCHROGASTER*)

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Summary

We have measured unidirectional uptake (not transmural flux) of acetate, butyrate and proline by everted sleeves of intact tissue from the jejunum, caecum, proximal colon and distal colon of prairie voles (*Microtus ochrogaster*).

There was active (i.e. Na^+-dependent) transport of L-proline in the jejunum, but we found no evidence for it in any region of the hindgut (i.e. the caecum, proximal colon and distal colon). Uptake of acetate was carrier-mediated in all three regions of the hindgut, but the J_{max} and apparent K_m ($\leqslant$1.5mmol l^{-1}) were low, and uptake was primarily passive over the concentration range 10–50mmol l^{-1}, which spanned measured acetate levels in the caecum and proximal colon. At 100mmol l^{-1}, acetate uptake (nmol min^{-1} cm^{-2}) was higher (P<0.001) in distal colon (359±33) than in the proximal colon (225±17) and caecum (150±5) (mean ± S.E., N=8). Uptakes summed over the length of each region were also higher (P<0.001) in the distal colon at 100mmol l^{-1}, but not at low concentrations (0.1mmol l^{-1}).

Uptakes normalized to diffusion coefficents were higher for butyrate than acetate and were lowest for L-glucose (which is absorbed passively *via* an aqueous pathway) in all regions, indicating that uptake of the short-chain fatty acids involves solubilization in the lipid bilayer of the apical membrane. The short-chain fatty acids absorbed from the hindgut of the vole were equivalent to 22% of standard metabolic rate or 15% of resting (but fed) metabolic rate.

Introduction

Within the genus *Microtus*, the caecum is among the largest and the proximal colon the most elaborate of all myomorph rodents (Vorontsov, 1962; Behmann, 1973). Although the significance of microbial fermentation in the caecum to the energy economy of the animal has not been quantified for any species of *Microtus*, from the size of the organ and the concentration of short-chain fatty acids (SCFAs) in caecal contents (see below), it is assumed to be considerable.

The SCFAs are produced mainly in the caecum of small hindgut fermenters or ‘caecum

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fermenters' (Hume and Warner, 1980) and are absorbed directly from that organ and from the colon (Engelhardt and Rechkemmer, 1983*a*). Recently these same workers (Rechkemmer and Engelhardt, 1988) reported that in the guinea pig the proximal colon was more permeable to acetate (the principal SCFA usually produced) than was the distal colon. Morphological differences along the colon are more marked in voles than in the guinea pig. We were therefore interested in relative rates of uptake of acetate in the caecum, proximal colon and distal colon of prairie voles (*Microtus ochrogaster*), one of the most herbivorous of the microtine rodents (Batzli and Cole, 1979).

Many reports indicate that in mammals SCFAs are absorbed by a linear, concentration-dependent passive process in which uptake rate is directly proportional to SCFA concentrations in the incubation medium or perfusate (Bugaut, 1987; Stevens, 1988). However, several studies have reported at least some of the characteristics of carrier-mediated transport (e.g. Vernay, 1986; Sellin and De Soignie, 1990; Titus and Ahearn, 1988, 1991). We therefore fitted our data to two kinetic models, one based on simple diffusion, the other on diffusion plus one mediated system, over a wide range of acetate concentrations ($0.05–100 mmol\,l^{-1}$) in order to test for evidence of carrier-mediated uptake of acetate in *M. ochrogaster*. We also tested the effects of chain length of SCFAs by comparing uptakes of acetate and butyrate at $100 mmol\,l^{-1}$.

Recent reports of significant uptake of amino acids from at least part of the caeca of some avian species (Obst and Diamond, 1989) have resulted in renewed interest in the possibility of active transport of these nutrient molecules across the wall of the mammalian hindgut. Small hindgut fermenters have relatively high mass-specific nutrient requirements (Hume and Warner, 1980). Microbial protein synthesised in the caecum is partially hydrolysed in the colon (Stevens, 1988). Active transport across the hindgut wall could be an important source of amino acids to the animal, even though the concentration of free amino acids in the hindgut is kept low by the action of microbial deaminases. This paper includes the results of experiments to assess the probable importance of L-proline uptake in the caecum and colon of *M. ochrogaster*.

Materials and methods

Animals

A breeding colony of prairie voles (*Microtus ochrogaster*) was maintained on a mixture of Purina Lab Rabbit Chow (16% crude protein) and Purina Formulab Chow (23.5% crude protein), both foods available *ad libitum*. The animals used in the experiments were all adults or sub-adults (32–68g body mass) housed individually in plastic mouse boxes under a 12h/12h light/dark cycle and at 20°C.

Morphometric measurements

The dry masses of contents and the lengths of the stomach, small intestine, caecum, proximal colon and distal colon were measured in all animals used in the uptake experiments. Nominal surface areas of the three regions of the hindgut were calculated from their lengths and circumferences, the latter being derived from the diameter of the

widest rod to slide easily into individual everted sleeves. Eight additional voles were used for measurements of the proportion of the hindgut wall that was mucosa. This was done by scraping off the mucosa of a 2-cm length of hindgut with a glass microscope slide. This mucosa and the remaining tissue were separately dried and weighed, and the dry mucosa was expressed as a fraction of the total dry wall. Uptake rates (J) as $nmol min^{-1}$ are expressed per cm^2 of nominal surface area.

Measurement of uptake rates

The method used was based on that described by Karasov and Diamond (1983) for the small intestine. The preparations used for measuring uni-directional solute uptake by intact tissue (not transmural flux) were excised, everted sleeves of small intestine, caecum, proximal colon or distal colon, each 1cm long, secured to a stainless-steel rod of 2, 3 or 4mm diameter with the mucosa facing outwards. Typically, there was enough tissue for three sleeves from the caecum, six from the proximal colon and six from the distal colon.

Animals were anaesthetized with Nembutal (0.25ml per 100 g bodymass), and the small intestine or hindgut was excised and immediately perfused with ice-cold Krebs Ringer mammalian buffer solution, containing (in $mmol l^{-1}$) 128 NaCl, 20 $NaHCO_3$, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4 and 1.2 $MgSO_4$, osmolarity $290 mosmol l^{-1}$, and gassed with 5% CO_2/95% O_2 to yield a pH of 7.3–7.4. Everted sleeves were kept in ice-cold buffer bubbled with 5% CO_2/95% O_2 until used for uptake measurements (usually 60–180min after dissection). After preincubation for 5min in buffer at 37˚C, the sleeve was incubated for 1–4min at 37˚C in a bubbled and stirred ($1200 revs min^{-1}$) solution containing radioactive tracers. Tissue uptake of [1-^{14}C]acetic acid, sodium salt (ICN Biochemicals; 1.5–2.2GBq$mmol^{-1}$), or [1-^{14}C]-*n*-butyric acid, sodium salt (ICN Biochemicals; 1.44GBq$mmol^{-1}$), was corrected for SCFAs in adherent fluid by reference to [1,2-^{3}H]polyethylene glycol (PEG, relative molecular mass 4000) (Dupont NEN; 19–74MBq g^{-1}) in the same tissue. A 5-min preincubation was used on the basis of results of Westergaard and Dietschy (1974) using a similar technique. These workers found no difference in SCFA uptake by the brush border of intact small intestine between 5-min and 30-min preincubation periods.

After incubation, the flat end of the stainless-steel rod was touched to a filter paper in order to remove excess fluid adhering to the sleeve. The sleeve was then cut from the rod, weighed in a scintillation vial, solubilized and counted for radioactivity (Karasov and Diamond, 1983).

To select the most appropriate incubation period, preliminary experiments with four voles and a uniform $10 mmol l^{-1}$ acetate concentration in the incubation solution included incubation times of 1, 2 and 4min. When normalized to the uptake at 2min measured in an adjacent sleeve from the same animal, uptakes at 1 and 4min did not differ significantly (t-test) from those expected by assuming linear uptake between 0 and 4 min. In the same experiments, tissue PEG either increased to 2min but between 2 and 4 min incubation did not change, or showed no significant change between 1, 2 and 4min. Hence for all subsequent experiments on uptake kinetics we used a 2-min incubation period.

Kinetics of acetate uptake

In each region of each vole, uptake was measured at 1.0mmol l^{-1} plus three of six other concentrations: 0.05, 0.1, 0.5, 10, 50 and 100mmol l^{-1}. Each absolute uptake value (in units of nmol min^{-1} cm^{-2}) was expressed relative to absolute uptake at 1.0mmol l^{-1} in the same region of the same vole. This mode of expression minimizes the effect of inter-vole variation. We used the procedures recommended by Motulsky and Ransnas (1987) for fitting these relative uptake rates to kinetic models to derive kinetic variables. The kinetic models included:

(1) simple diffusion, in which $J=P[S]$, where J is uptake, $[S]$ is substrate concentration and P is the permeability coefficient; and (2) diffusion plus one mediated system, in which $J=P[S]+J_{max}[S]/(K_m+[S])$, where J_{max} is maximal influx, K_m is the transport constant such that $K_m=[S]$ when $J=J_{max}/2$.

We used nonlinear curve-fitting routines (Gauss–Newton algorithm, Systat) to derive estimates of the variables in the models and to derive the sums of squares for each fit. The sums of squares (SS) of residuals were used in an *F*-test to select the model that provided the best fit. The derived variables P and J_{max} were reconverted to absolute uptakes (i.e. nmol min^{-1} cm^{-2}) by multiplying them by the mean uptake at 1.0mmol l^{-1}.

Uptake of proline

Proline uptake was studied in the jejunum, caecum, proximal colon and distal colon of eight voles. Total uptake (i.e. sum of carrier-mediated and passive uptake) of L-[2, 3-^{3}H]proline (American Radiolabel; 0.7–1.5GBq mmol $^{-1}$), was corrected for proline in adherent fluid using [carboxyl-^{14}C]inulin (relative molecular mass 5000) (ICN; 37–111GBq mg^{-1}). The incubation time selected was 2min (Karasov *et al.* 1992). After touching to a filter paper to remove excess fluid, 1cm lengths of tissue were removed from the rods for weighing, solubilization and scintillation counting (Karasov and Diamond, 1983). Uptake of proline in the presence or absence of Na^+ (Na^+ replaced by choline) was measured at 0.01mmol l^{-1} because we sought primarily to test for Na^+-dependent (i.e. active) uptake of proline and because the proportion of total uptake that is Na^+-dependent increases as concentration decreases (Karasov, 1988).

Effects of chain length and lipophilicity on SCFA uptake

The kinetic analysis indicated that at high concentration (100mmol l^{-1}) virtually all acetate uptake was *via* a nonmediated pathway, but it did not identify what that pathway was. At the pH of the bulk solution (7.4) most of the acetate (pK_a 4.75) was in the ionized form and thus one pathway might be a pathway for water-soluble compounds (paracellular or through membrane pores). Alternatively, ionic acetate may be protonized in an acidic microenvironment immediately adjacent to the mucosal surface (Engelhardt and Rechkemmer, 1983*b*), becoming more lipid-soluble and then diffusing across the membrane. To delineate further the nonmediated pathway of SCFAs in voles, we incubated adjacent sleeves from four voles in 100mmol l^{-1} butyrate, 100mmol l^{-1} acetate and tracer L-glucose (0.8–1.6GBq mmol $^{-1}$, Amersham) (whose uptake is nonmediated). If all three solutes moved by the same aqueous pathway, their uptake rates

normalized to their diffusion coefficients (Sallee and Dietschy, 1973) should be equal within each region of the gut. Alternatively, if the transported SCFAs were lipophilic, uptakes of the three solutes normalized to their diffusion coefficients should fall in the order butyrate>acetate>L-glucose, because fatty acid solubility in membrane lipid increases with increasing chain length (Danielli *et al*. 1945) and the membrane's resistance to solutes decreases with increasing lipophilicity.

Results

Gut morphometrics

In the hindgut, the caecum was the shortest region but had the greatest nominal surface area and total mass (Table 1). The proximal and distal colons had similar nominal surface areas but total mass was lower in the distal than in the proximal colon. Also, the proportion of total mass that was mucosa was lower in the distal colon (0.29) than in either the proximal colon (0.59) ($F_{1,20}$=72; P<0.001) or the caecum (0.40) (F=9.7; P<0.01) (two-way analysis of variance). Caecal length was not significantly correlated with body mass (P=0.64), but total colon length (proximal plus distal) was (colon length 14.83+0.117bodymass; P=0.008) (linear regressions on untransformed data).

Mean acetate concentrations in the caecum and proximal colon were 35–39mmoll^{-1} and on a molar basis acetate was 66% and 67% of total SCFAs in the caecum and proximal colon, respectively.

Kinetics of acetate uptake

Uptakes at the six concentrations used below 100mmoll^{-1} (i.e. 0.05–50mmoll^{-1}) were used for determining kinetic variables (Fig. 1) because the passive route proved to dominate uptake at 100mmoll^{-1} (see below), making derivation of the carrier-mediated component of uptake impossible. The model of diffusion plus one mediated system fitted the data better than the model of passive diffusion alone in the caecum ($F_{2,3}$=106; P<0.005), proximal colon (F=10.3; P<0.05) and distal colon (F=72; P<0.025).

There was no way to compare statistically our kinetic variables among the regions

Table 1. *Morphometrics of the hindgut of prairie voles (*Microtus ochrogaster*)*

	Caecum	Proximal colon	Distal colon
Length (cm)	7.6±0.3	9.3±0.4	11.1±0.2
Nominal surface area (cm^2)	9.6	6.8	7.0
Wet mass gut wall ($mgcm^{-1}$)	43.7±3.2	33.0±1.4	20.0±1.0
Mucosa (proportion of wet mass gut wall)	0.40±0.03	0.59±0.04	0.29±0.01
Dry matter content of mucosa (%)	18.3	19.1	19.7
Acetate concentration ($mmol l^{-1}$)	34.7±2.4	38.5±4.9	–
Total short-chain fatty acid concentration ($mmol l^{-1}$)	53.1±4.0	57.4±7.7	–

Values are mean ± S.E. (N=8).
Acetate concentration was not measured in the distal colon.
Mean body mass of voles 48±3g.

(Motulsky and Ransnas, 1987; but see below), but the data indicate that the apparent K_m (K_m^*; apparent because it is not corrected for the effects of unstirred layers) was less than 1.5mmol l^{-1} in all three hindgut regions (Table 2). Thus, the kinetic analysis indicated that at acetate concentrations measured in the lumen (35–39mmol l^{-1}; Table 1) at least 85–90% of uptake is *via* a nonmediated pathway.

Acetate uptake along the hindgut

We first compared the caecum, proximal colon and distal colon for uptake per cm^2. Then we compared uptakes summed over each region, calculated as uptake per cm×cm

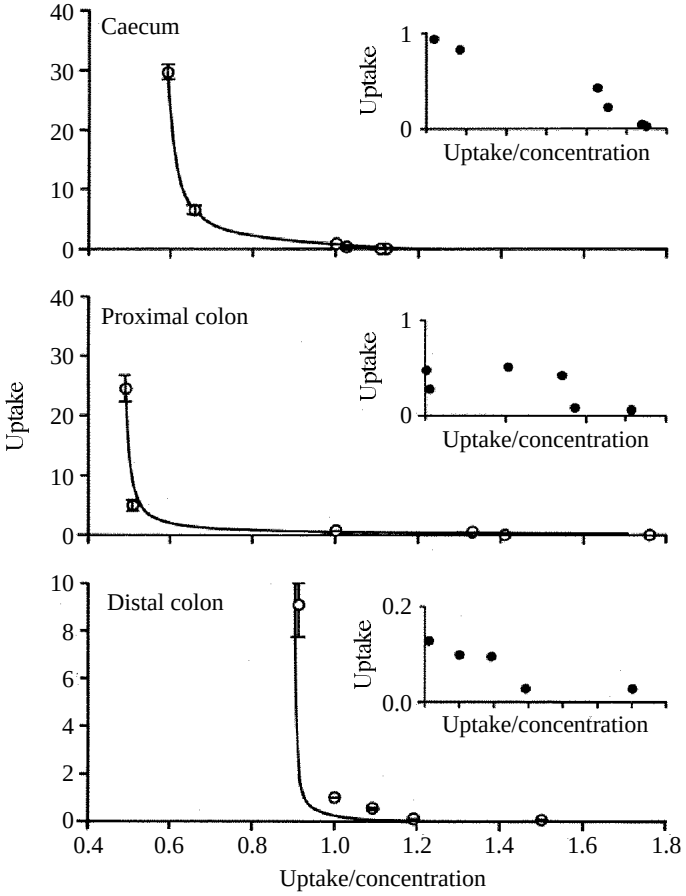

Fig. 1. Concentration-dependent uptake of acetate in the caecum, proximal colon and distal colon of prairie voles. The large figures are Eadie–Hofstee plots of total uptake (○). Values are means ± S.E. for 4–8 tissues from different voles. The curves are least-square non-linear fits of total uptake to the equation: $J=P[S]+J_{max}[S]/(K_m+[S])$, where J is uptake and S is concentration. Derived values for P, J_{max} and K_m are given in Table 2. The insets are plots of the same data after subtraction of calculated passive uptake (●). Uptake values were normalized to total uptake at 1mmol l^{-1} in an adjacent sleeve from the same vole, and so lack units. Absolute uptakes, calculated by multiplying relative uptakes by the corresponding mean uptake at 1mmol l^{-1}, are listed in Table 2.

length. The comparisons were performed at low, moderate and high concentrations (0.1, 10 and 100mmol l^{-1}) (Figs 2 and 3).

Acetate uptake varied significantly among hindgut regions ($P<0.05$ for every solute concentration by repeated-measures ANOVA). At high concentration, where the passive route was dominant (100mmol l^{-1}), total uptake in the distal colon exceeded that in the proximal colon ($P<0.001$). At low concentrations (e.g. 0.1mmol l^{-1}), where the passive route was not dominant, uptake did not differ significantly between the proximal and distal colon. Uptake in the caecum was significantly less than in the distal colon at all

Table 2. *Kinetic constants for relative uptake of acetate at the luminal membrane* in vitro

Region	P [nmol min^{-1} cm^{-2} (mmol l^{-1})]	J_{max} (nmol min^{-1} cm^{-2})	K_m* (mmol l^{-1})
Caecum	1.30±0.00	12.15±0.09	1.4±0.2
Proximal colon	1.52±0.01	1.42±0.39	0.2±0.1
Distal colon	2.66±0.01	0.39±0.07	0.2±0.1

K_m*, apparent K_m; P, permeability coefficient; J_{max}, maximal rate of influx.
Values are mean ± S.E. (S.E. values are from the non-linear regression program).

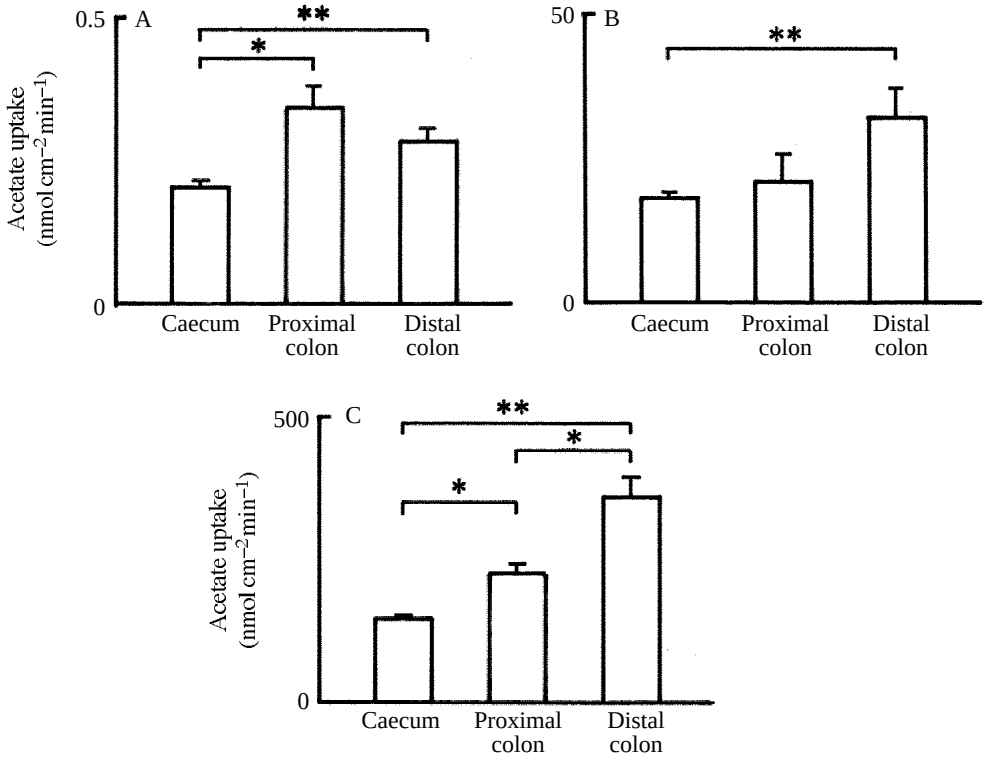

Fig. 2. Acetate uptakes at (A) 0.1mmol l^{-1} acetate (B) 10mmol l^{-1} acetate and (C) 100mmol l^{-1} acetate in the caecum, proximal colon and distal colon of prairie voles. Asterisks indicate significant differences: *$P<0.05$; **$P<0.01$.

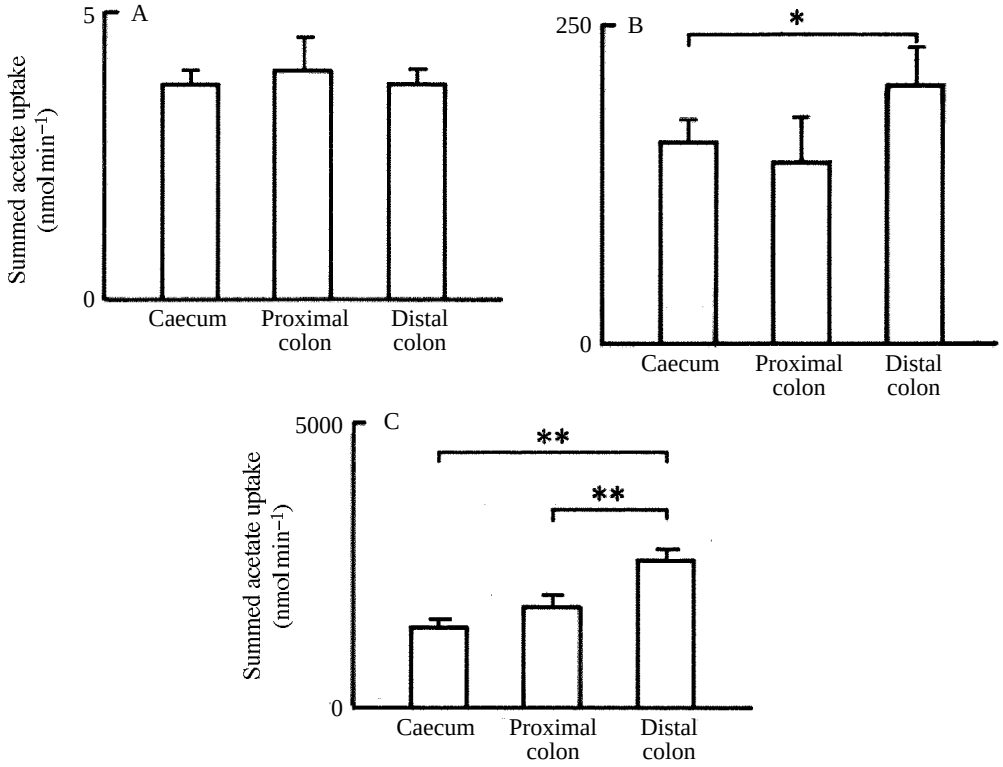

Fig. 3. Summed acetate uptake at (A) 0.1mmol l^{-1} acetate, (B) 10mmol l^{-1} acetate and (C) 100mmol l^{-1} acetate in the caecum, proximal colon and distal colon of prairie voles. Asterisks indicate significant differences: *P<0.05; **P<0.01.

concentrations tested (P<0.012 in every case) (Fig. 2). Thus, acetate uptake was highest in the distal colon. These differences are due largely to a significantly higher passive permeability coefficient (P^*) per unit tissue in the distal colon.

Uptakes summed over each region, calculated as uptake per cm×cm length, were also compared at the three concentrations (Fig. 3). At low concentrations (0.1mmol l^{-1}) there were no significant differences among the regions (P>0.5 by repeated-measures ANOVA). However, at 100mmol l^{-1} regional differences were significant (P=0.001), with uptake in the distal colon exceeding that in the other two regions by about 70 % (P<0.006). At the intermediate concentration of 10mmol l^{-1}, uptake in the distal colon exceeded that in the caecum (P<0.05) and tended (P<0.14) to be greater than that in the proximal colon.

Uptake of proline

Adjacent tissues from the four gut regions were incubated in 0.01mmol l^{-1} proline in the presence and absence of Na^+ (Table 3). Uptake in the presence of Na^+ was significantly higher than in the absence of Na^+ only in the small intestine (jejunum) and possibly the caecum but not the colon. Ratios of Na^+-independent uptake per cm^2 to total uptake (along with corresponding P-values for paired t-tests) were: small intestine, 0.54±

0.07 (N=6, t_5=6.62, P<0.005; caecum, 0.73±0.10 (N=8; P<0.05); proximal colon, 1.01±0.18 (N=8; P>0.5); distal colon, 0.81±0.15 (N=8; P>0.5). The data were similar when normalized to tissue wet mass, except in the caecum where the ratio calculated on this basis was 0.86±0.10 (P>0.1). Within the eight voles, total proline uptake per cm^2 was significantly higher in the jejunum (P=0.001 by repeated-measures ANOVA) as was Na^+-independent uptake (P<0.001), but in the hindgut there was no significant regional variation in Na^+-dependent or Na^+-independent uptake (P>0.5 and 0.05 respectively).

Table 3. *Uptake of L-proline at the luminal membrane in the gastrointestinal tract of voles*

	Uptake of 0.01mmol l^{-1} proline ($pmol\,cm^{-2}\,min^{-1}$)	
Intestinal position	With Na^+	Without Na^+
Jejunum	200±24	112±6
Caecum	57±4	43±6
Proximal colon	52±5	53±9
Distal colon	53±6	38±5

Values are means ± S.E. in eight voles.
See text for statistical comparisons.

Table 4. *Uptake of butyrate, acetate and L-glucose at the luminal membrane in the gastrointestinal tract of four voles*

	Butyrate	Acetate	L-Glucose
D ($cm^2\,s^{-1}\times10^6$)*	12.1	16.7	9.3
Jejunum	(8)	(8)	(8)
J†	2.57±0.48	2.90±0.50	1.32±0.08
J/D‡	3541±664	2894±494	2374±143
Caecum	(8)	(8)	(3)
J	1.24±0.19	1.17±0.17	0.26±0.07
J/D	1708±260	1169±170	460±125
Proximal colon	(8)	(8)	(4)
J	2.24±0.22	1.85±0.26	0.80±0.06
J/D	3080±308	1843±258	1427±115
Distal colon	(8)	(8)	(4)
J	4.22±0.38	3.79±0.11	1.13±0.07
J/D	5814±528	3785±107	2019±131

Values are mean ± S.E. (N).

*Diffusion coefficients (D) at 37°C from Sallee and Dietschy (1973). D for L-glucose is taken to be the same as that for mannitol because their molecular weights differ by only 1%.

†Uptake ($nmol\,min^{-1}\,cm^{-2}$) determined at 100mmol l^{-1} for butyrate and 100mmol l^{-1} acetate and at tracer concentration for L-glucose, and then normalized to a concentration of 1mmol l^{-1}.

‡Uptake normalized to the solute's diffusion coefficient, $nmol\,cm^{-4}$.

Note that (a) in each region, J/D decreases in the order butyrate>acetate>L-glucose; (b) across hindgut regions, J increases for the SCFAs in the order caecum<proximal colon<distal colon.

J, rate of uptake.

Thus, overall we found little evidence for significant active transport of L-proline in the hindgut of voles.

Effects of chain length and lipophilicity on SCFA uptake

Uptake rates normalized to diffusion coefficents were highest for butyrate and lowest for L-glucose (Table 4). Using adjacent sleeves, we calculated ratios of butyrate uptake to acetate uptake in each region and compared them to unity by paired *t*-tests. The ratios did not differ significantly between regions, and the mean for all regions was 1.56±0.12 (t_{23}=4.75, P<0.001). The ratio of acetate uptake to L-glucose uptake was 1.88±0.26 (t_{13}=3.37, P=0.005). These results are consistent with the idea that nonmediated uptake of SCFAs is not *via* the same aqueous pathway as for L-glucose, but instead involves solubilization in the lipid bilayer of the apical membrane.

Discussion

The finding of negligible active transport of L-proline in the caecum and colon of prairie voles confirms the generally held view that Na^+-dependent (i.e. active) transport of amino acids is not significant in the hindgut of adult mammals. Higher rates of Na^+-independent uptake per cm^2 nominal surface area in the small intestine could be related to surface area elaboration by villi and microvilli or to a Na^+-independent carrier for proline (Karasov, 1988). Proline not absorbed in the small intestine could potentially be taken up in the hindgut by a passive process. However, this is unlikely ever to be of quantitative significance to the animal, because the concentrations of amino acids are kept low by microbial action.

The major site of this microbial activity in the vole is the large haustrated caecum, and a major component of the short-chain fatty acids produced is acetate. The proximal colon, although complex in structure (Björnhag, 1987), contains much less digesta than the caecum, and is thus less likely to be as important a site of SCFA production. However, the high concentration of SCFAs in the proximal colon, together with high uptake rates of acetate and of butyrate implies that not all the SCFAs produced in the caecum are absorbed there, and that there is considerable total absorption in the proximal (and distal) colon (Fig. 3).

Studies with ruminal epithelia from sheep and goats and with colonic and caecal mucosa from pigs and horses have suggested that SCFAs are absorbed by passive diffusion in a association with a proton–sodium exchange and chloride–bicarbonate exchange in the apical membrane (Stevens, 1988). In our study on the caecum and colon of prairie voles, the model of diffusion plus a mediated system fitted the data better than the model based on passive diffusion alone. This suggests the presence of a carrier for acetate. However, as the apparent K_m for this process was 1.5mmoll^{-1} at most, it is likely that at least 85% of acetate uptake in our animals, in which luminal acetate concentrations averaged 35–39mmoll^{-1}, was by a nonmediated pathway. The presence of a mediated pathway may have survival value when luminal concentrations of acetate fall to lower levels, such as during periods of seasonal fasting or starvation. Recently Titus and Ahearn (1988) demonstrated the presence of a carrier for acetate with an

apparent K_m of 6.4mmol l^{-1} in the intestine of the herbivorous fish tilapia (*Oreochromis mossambicus*). Acetate levels in tilapia intestine averaged only 15–20mmol l^{-1}, lower than in the hindgut of our voles. Carrier-mediated uptake of acetate may well be of significance to the energy economy of tilapia under a wide range of nutritional conditions.

Our finding that SCFA uptake normalized to diffusion coefficient exceeded that of L-glucose uptake is consistent with the idea that a substantial portion of SCFA uptake is as undissociated SCFAs *via* a nonaqueous pathway. The greater rate of uptake of butyrate compared with that of acetate also suggests lipid solubilization in the luminal membrane. Since 98% of the SCFAs would have been ionized at the pH of the incubation solution (7.4), there must be an acidic unstirred layer or microclimate at the mucosal surface, in which protonation of the SCFAs takes place (Engelhardt and Rechkemmer, 1983*b*).

When acetate uptakes were compared between the caecum, proximal colon and distal colon, rates of uptake were highest in the latter region (Figs 2, 3). This is notably different from the report by Rechkemmer and Engelhardt (1988) that the absorption rate of acetate in the guinea pig was approximately two times higher in the proximal colon than in the distal colon. They attributed this to additional passive absorption of acetate in the ionized form *via* paracellular pathways in the proximal colon. Support for this contention is found in evidence of 'leaky spots' in the tight junctions of guinea pig mucosal cells of the proximal colon but not in those of the distal colon (Luciano *et al.* 1984). Regional differences in hindgut epithelial transport have also been reported by Clauss *et al*. (1985) in rabbits and by Fromm and Hegel (1978) in rats. However, we cannot rule out the possibility that there was paracellular uptake (by solvent drag) of acetate and butyrate in the distal colon of our voles because uptake of L-glucose was also higher there. Even though we observed that uptake of tracer PEG did not increase with increasing incubation time beyond 2min, one cannot conclude that there was no paracellular uptake, as Pappenheimer and Reiss (1987) proposed that paracellular pores open only in the presence of high nutrient concentrations. Characterization of the pathways involved in uptake of SCFAs and proline in the colon would require further studies at the membrane level (e.g. density and type of aqueous pores, lipid concentrations and composition, and proportion of SCFAs absorbed in the ionic *versus* undissociated state).

The effect of concentration on comparisons of proximal and distal colon can be understood in the light of the kinetic constants in each region (Table 2). The trend was for P^* in the distal colon to be greater than that in the proximal colon, whereas the trend for J_{max} was in the reverse direction. Comparisons with the caecum are not so straightforward because of the possibly higher K_m^*, and further studies would be needed to clarify the relationship between caecum and colon.

Results from our study can be used to estimate the probable importance of SCFAs to the energy economy of *M. ochrogaster*. By using the simple diffusion kinetic model of $J=P[S]$, values for P in Table 2 and concentrations of acetate, propionate, butyrate and valerate measured in the caecum and proximal colon, uptake of SCFAs is calculated to be 1.38, 1.11 and 2.07mmol per day in the caecum, proximal colon and distal colon, respectively. This assumes that SCFA concentrations in the distal colon (not measured) are the same as in the proximal colon, that SCFA concentrations measured are representative of the whole 24-h cycle, and that the permeability coefficient (P) is the

same for all SCFAs. The first two assumptions are probably valid, based on published values of SCFA concentrations in rats and guinea pigs (Engelhardt and Rechkemmer, 1983*a*,*b*), and on activity and feeding patterns measured in our study and in that of Ouellette and Heisinger (1980). However, the third assumption is likely to underestimate total absorption, since, in guinea pigs (Rechkemmer and Engelhardt, 1988) and in our voles, SCFA absorption increased with increasing chain length.

By using the measured energetic equivalents of the SCFAs of Blaxter (1962), total SCFA absorption can be calculated to be 1.75, 1.38 and 2.59kJ per day in the caecum, proximal colon and distal colon, respectively. This is equivalent to 22.3% of the standard metabolic rate of a 50 g *M. ochrogaster*, or 15.2% of the resting (but fed) metabolic rate of a 50g *M. ochrogaster* fresh from the field in summer (Wunder, 1985). These values are similar to estimates made on the basis of SCFA production rates in several other small hindgut fermenters (Bergman, 1990).

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