Effect of volatile fatty acids on water and ion absorption from the goat colon

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ARGENZIO, R. A., N. MILLER, AND W. VON ENGELHARDT. Effect of volatile fatty acids on water and ion absorption from the goat colon. Am. J. Physiol. 229(4): 997-1002. 1975.—The absorption of volatile fatty acids (VFA) and the influence of VFA on the net transport of inorganic electrolytes and water were examined in the temporarily isolated colon of the conscious goat. Perfusion of the colon with a solution similar in content to that normally present in the cecal outflow showed that the net absorption of VFA was more rapid than that of any other ion present in the solution. When the VFA were replaced with Cl, the net absorption of Na and water was reduced nearly twofold. Increasing the pH of the solution from 6.0 to 7.4 in the presence of VFA also resulted in a twofold decrease in the net transport of Na and water. Perfusion of the colon with a hypertonic solution resulted in approximately zero net water transport; however, the net absorption of Na and VFA continued at similar rates as before. These results support the concept that the colon primarily conserves solute followed by the passive movement of water. They also demonstrate that VFA are rapidly absorbed from the goat colon and indicate a striking influence of VFA on the net transport of Na and water. The presence of VFA in the large intestine may be important for normal absorptive function.

colonic perfusion; Na and VFA absorption; inorganic ion absorption; organic anion absorption; organic acid absorption

THE IMPORTANCE of the mammalian large intestine in recovering electrolytes and water secreted into the upper digestive tract is well recognized. However, the fact that it also serves as a site for microbial digestion is less appreciated. This latter function results in the production of volatile fatty acids (VFA) from soluble and insoluble carbohydrate digestion (17). These organic acids constitute the major anions in the large intestinal contents of most mammals studied (10) and in the feces of man (23).

Ruminants rely heavily on VFA as an energy source, since little glucose is available for absorption (23). Although large quantities of VFA are produced and absorbed from the forestomach (3), significant amounts also appear to be produced in the large intestine (13). Thus, large intestinal VFA may contribute significantly to the overall energy metabolism, providing that they can be rapidly and efficiently absorbed. At the pH of colonic contents, these acids exist primarily in their ionized form. For this reason it has been suggested that they may be poorly absorbed and even retain Na and water in the large intestine of man (15, 20). However, both in vivo and in vitro studies of the horse colon (1) and in vitro studies of the pig colon (2) have shown that VFA can be rapidly absorbed even at a neutral pH. Absorption of VFA from the large intestine of ruminants and other mammalian species has received little attention. Furthermore, the relationship between the absorption of Na and VFA has not been examined.

The present study, utilizing the temporarily isolated colon of the conscious goat, was conducted in an attempt to determine the rate of fatty acid absorption from the colon, as well as the influence of VFA on the net transport of inorganic electrolytes and water.

METHODS

Experimental animals consisted of one castrated, adult male and two adult female goats weighing from 50 to 70 kg. Animals were fed a concentrate mixture and hay. Experiments were always conducted about 2 h following the morning feeding, but feed and water were also available to the animals during the experiment.

The goats were surgically prepared with two intestinal cannulas. The proximal cannula was positioned in the cecum just distal to the cecal colonic junction, and the second was placed ca. 20 cm distal to the cecal colonic junction. At least 2 wk were allowed for recovery before experiments began. The first goat (goat O) used in the series of preliminary experiments developed an anastomosis between the cecum and rectum 6 mo following surgery. The remaining experiments were conducted with only two animals (goats A and F).

The composition of the test solutions together with the number of experiments conducted with each solution are shown in Table 1. In each experiment, six samples were collected over a 2-h period. The composition of the control solution (soln I) was selected to approximate the colonic contents analyzed from samples obtained from the distal cannula in goat O. Concentrations of Na, K, Cl, PO₄, and VFA in these samples were 111 ± 9, 21 ± 6, 28 ± 3, 24 ± 4, and 55 ± 6 mM, respectively. The osmolality was 309 ± 5 mosmol/kg. The pH of the control solution (pH 6.0) was chosen to approximate that recorded anaerobically in the large intestine of the horse and pig (1, 2). All solutions were adjusted to approximately 300 mosmol/kg (with mannitol, except the hypertonic solutions which were adjusted to 400 mosmol/kg. Polyethylene glycol (PEG) was added to the solutions at a concentration of 1 g/liter.

At the beginning of an experiment, a balloon was posi-
tioned immediately proximal to the second cannula. This was accomplished by the previous passing of a thread from the proximal to distal cannula, which allowed the balloon to be brought into place through the proximal cannula and secured into position with the thread. Upon inflation of the balloon, drainage of ileal and cecal content occurred spontaneously from the proximal cannula, and the test solution was then infused into the distal cannula. Injection of markers into either of the cannulas indicated the absence of flow past the balloon, once it was inflated with ca. 200 ml of air. The balloon was connected to Hg manometer to assure that a constant pressure was maintained throughout the experimental period.

Once the balloon was positioned, the colon was cleansed until the rectal fluids became clear. This generally required a 1.5- to 2-h infusion with 3–4 liters of the test solution heated to 39°C. If the rectal fluid failed to become clear by this time, the balloon was further inflated until the pigmentation disappeared. A continuous infusion of the test solution was then initiated with a calibrated infusion pump. At least 1–1.5 h were found necessary for a steady state to develop. The steady state was defined by maintenance of a constant concentration of PEG, pH, and electrolytes in rectal samples. In later experiments a bolus of phenol red was injected at the time the constant infusion was started (7), and it was found that a steady state was attained by the time most of the phenol red had passed the rectum (Fig. 1). This could easily be determined at the time of the experiment. Following the equilibration period, six samples were taken at 20-min intervals for an additional 2 h via a rectal tube.

In experiments in which bicarbonate was measured, it was found necessary to perfuse through a closed system as the HCO₃⁻ concentration would increase as much as 20% with time. Samples taken at intervals from the closed perfusion solution reservoir indicated a slight increase of 2.6 ± 1.7% in HCO₃⁻ during a 3-h period. Samples were also collected anaerobically from the rectum. The pH was measured immediately, and the samples were stored in sealed glass vials and analyzed for HCO₃⁻ the same day.

The transit time was determined in several experiments by the methods described by Dillard et al. (9). A dose of 9 mg of phenol red was injected into the infusion catheter in the presence of steady-state conditions. Samples were then collected every 5 min from the rectum, and a dye dilution curve was constructed. Recovery of phenol red in these experiments was 88.7 ± 5.2% (n = 14).

All chemical analyses were performed in duplicate and VFA in triplicate. PEG was analyzed on the day of the experiment by the method of Hyden (19). The osmolality was also measured within 24 h on the fresh sample by freezing-point depression (Advanced Instruments osmometer). Two-milliliter samples were acidified with formic acid and stored in sealed glass vials immediately after the experiments for VFA analysis by gas chromatography (11). Sodium and potassium were determined by flame photometry, Cl by coulometric titration (Chlor-O-Counter, Marius, Utrecht), and phosphate by the method of Zilver-smit and Davis (29). Total CO₂ was determined by the method described by Fisher (14), and the HCO₃⁻ concentration was calculated from the sample pH. Phenol red was determined colorimetrically at a wavelength of 546 nm after alkalinization.

Calculations of net water or ion absorption (positive) or secretion (negative) were made from the PEG concentrations with the following formulas: net water absorption = \( V_1 - (V_1(PEG_i/PEG_o)) \), and net electrolyte absorption = \( E_1V_1 - (E_1V_i(PEG_i/PEG_o)) \), where \( V_1 \) is the perfusion rate and \( PEG_i, PEG_o, E_i, \) and \( E_o\) are the concentrations of PEG and electrolytes in the perfusion fluid and rectal outflow, respectively. Results are reported as means ± standard deviations of values obtained during 20-min collection periods.

RESULTS

Influence of perfusion rate. Results of the experiments conducted with three different infusion rates in goat 0 are shown in Fig. 2. These are expressed as the net percentage of water or solute presented to the colon which was absorbed from the colonic lumen. In each case except for phosphate, an increasing flow rate was associated with a significant decrease in the percentage net absorption of ions and water. The fatty acids and Cl were absorbed in greater percentages than water while Na and K were absorbed at a rate similar to H₂O. There was little or no net absorption of phosphate. Figure 2 also shows that the fatty acids were absorbed more rapidly than any other ion present in the solution. At the lowest rate of perfusion, 95 ± 2.5% of the acetate was absorbed. Propionate was similarly absorbed, even though its initial concentration in the perfusate was only 10 mM.
COLONIC ABSORPTION OF IONS AND H₂O

Fig. 2. Effect of perfusion rate on net absorption of water and ions. Net absorption is expressed in percentage of total load presented to colon. Experiments were conducted with perfusion rates of 10, 20, and 31.5 ml/min with control solution in goat 0. Values are means ± SD. Final pH values were 7.59 ± 0.1, 7.49 ± 0.1, and 7.29 ± 0.1 for perfusion rates 1, 2, and 3, respectively. Corresponding osmolalities were 262 ± 6, 263 ± 4, and 256 ± 5 mosmol/kg.

Further examination of these data revealed that as the perfusion rate increased, the absorbate became increasingly hypertonic. Calculation of net osmolar transport from the osmolality and PEG concentrations indicated an absorbate osmolality of 321 ± 22, 371 ± 10, and 450 ± 34 mosmol/kg for perfusion rates of 10, 20, and 31.5 ml/min, respectively. Since this was presumably due to differences in the mean transit time, perfusion rates were selected for the other two animals that would give comparable mean transit times as well as the slowest perfusion rate which would also allow a relatively constant rectal outflow. The volume capacity of the large bowel (determined from the dye dilution curves) of these two animals differed by a factor of 2. Therefore, a flow rate of 12 ml/min was found to result in a mean transit time of 51 ± 7 min in goat A, whereas a flow rate of 20 ml/min resulted in a mean transit time of 57 ± 5 min in goat F. These transit times, in turn, resulted in a calculated absorbate osmolality of 333 ± 23 and 379 ± 50 mosmol/kg for goats A and F, respectively. At slower rates of perfusion, development of a relatively constant rectal outflow proved difficult. These conditions were comparable to the flow rate of 20 ml/min in goat O with a mean transit time of 65 ± 6 min.

Influence of VFA. Due to the rapid absorption of the fatty acids shown in the previous experiments, even though they were present primarily in their dissociated form at the pH of these solutions, a second series of experiments with goats A and F were conducted to determine the effect of removing these acids on the net absorption of water and ions. Six experiments were conducted with the control solution and six with a solution identical to this except for the replacement of acetate and propionate with their equivalent of NaCl (Table 1). The pH of solution 2 was adjusted with HCl to equal that of solution 1. The results of this study, shown in Fig. 3, demonstrate that in the absence of fatty acid, the net absorption of Na and water in both animals was markedly and significantly reduced (P < 0.001). Potassium transport remained unaffected, and the presence or absence of VFA had no significant effect on the net appearance of HCO₃⁻. The osmolalities of rectal effluent recorded for these experiments were identical for both solutions (259 ± 3 and 260 ± 7 mosmol/kg for goat A; 264 ± 8 and 261 ± 5 mosmol/kg for goat F).

Influence of pH. To further examine Na, fatty acid, and water transport as a function of pH, the pH of the perfusate was increased by the addition of NaHCO₃ (solution 3–8, Table 1). Figure 4 shows that an increase in the pH of the rectal outflow by 1 U was associated with a 50% reduction in the net absorption of water. There was a similar reduction in the net absorption of Na in spite of its higher concentration in these solutions.

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<th>Table 1. Composition of solutions</th>
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*Acetate was included as the Na salt and propionate as the acid. †Fifteen of the experiments with solution 1 and two with solution 9 were conducted with goat O. The remainder were conducted with goats A and F.

Fig. 3. Effect of VFA on net absorption or secretion of water and ions. Values are means ± SD of 20-min collection periods. Bicarbonate was not measured in all experiments, and number of 20-min collection periods for HCO₃⁻ are shown in parentheses. Final pH values were 6.94 ± 0.2 and 6.7 ± 0.1 for solutions 1 and 2, respectively.
from 34 ± 3 to 21 ± 2 mmol/h in goat A and from 84 ± 12 to 53 ± 5 mmol/h in goat F when the pH of the perfusate was increased from 6.0 to 7.4. Examination of the relationship between Na and acetate absorption, over the ranges in pH studied with solutions 3–8 in the two animals, indicated a close correlation in the net absorption of these two ions (Fig. 5).

The variation in net water absorption over the twofold range in the studies of solutions 3–8 also allowed examination of the relationship between solute and water absorption with a constant perfusion rate and an isotonic solution (Fig. 6). The net absorption of Na was isotonic and the ordinate intercept was not significantly different from zero. However, the net absorption of acetate was in excess of net Na absorption, and the extrapolated intercept on the Y axis was significantly different from zero ($P < 0.001$). Chloride appeared to be absorbed in proportion to its concentration in the perfusate, and the net movement of Cl was zero when net water transport was zero. The net movement of K was poorly related to water movement and was similar to values obtained in control studies.

Influence of solvent drag. In order to examine the influence of bulk water movement on the net transport of solute, experiments were conducted in which the control solution was made hypertonic with mannitol (solution 9, Table 1). These results are compared with those obtained with the control solution (Table 2). A solution with an initial osmolality of 400 mosmol/kg effectively prevented net absorp-
tion or secretion of water during transit through the colon. However, the net transport of all solutes, although slightly reduced from control values, continued in the absence of net water flow. Furthermore, net Na absorption from this hypertonic solution was still greater \((P < 0.01)\) than that noted in VFA-free solutions, even though the Na concentration of rectal fluid was reduced to a greater degree \((101 \pm 6\) and \(62 \pm 6\) mM from solutions 2 and 9, respectively).

**DISCUSSION**

Studies of the colonic absorption of inorganic ions and water in various mammalian species such as the rat \((6, 21)\), dog \((5)\), and man \((8, 16)\) have described relationships between Na and water transport similar to those of the present study. The net transport of Cl from open-circuited bowel is usually more rapid than net Na absorption and appears to be related to net HCO\(_3\) secretion \((8, 28)\). Chloride was also absorbed quite rapidly from the goat colon; however, an increase in the Cl concentration appeared to have little or no effect on net HCO\(_3\) appearance. The net secretion of K, which is usually observed in the mammalian colon \((8, 16)\), was also absent in the present experiments. However, both K and HCO\(_3\) are absorbed from the colon of the guinea pig \((22)\).

The most significant findings of the present study were the rapid absorption of the volatile fatty acids and the stimulatory effect of the VFA on net Na and water absorption. The finding that the VFA were rapidly absorbed at a pH well above their \(pK_a\) raises the question as to the mechanism of this transport. While VFA are rapidly transported across forestomach epithelium \((25)\), their rate of absorption from the isolated rumen pouch of the goat \((11)\) appears to be considerably less than that from the colon at a similar pH. However, this may be due to differences in the volume-to-surface area ratio, and in vitro studies of colonic mucosa from the pony \((1)\) and pig \((2)\) have shown that VFA are transported at a rate as great as or greater than that of bovine forestomach epithelium \((24)\) under identical experimental conditions.

The marked stimulation of Na and water transport in the presence of these fatty acids suggests that VFA may be important for Na and water conservation in this species and also suggests a linkage in the transport of Na and VFA. This point is further supported by the close relationship in the absorption of Na and VFA over a 1-U pH range and when net water movement was held to zero with a hypertonic solution. A pH dependence of a similar magnitude has been shown for VFA absorption from the rumen \((25)\) and for weak organic acid absorption from the jejunum of the rat \((18)\). The present results suggest that net Na and water absorption from the goat colon are also influenced by pH, at least in the presence of fatty acids. While the control solution was carefully selected to approximate the normal composition of the proximal large bowel, under normal conditions the continuous microbial production of VFA along the length of the large intestine would tend to maintain a higher VFA concentration and lower pH than that actually obtained in rectal fluid \((26, 27)\). Therefore, under normal conditions, the rate of Na, VFA, and water absorption might be considerably greater.

Neither the mechanism for rapid VFA absorption nor the interrelationship between VFA absorption and the absorption of Na by the large intestine of this species can be elucidated from the present experiments. However, it is interesting to examine results of the present study in light of proposed models for epithelial transport of organic acids. The rat jejunum also transports weak organic acids at a rate which cannot be explained by conventional ion trapping formulas, and it was proposed that a thin layer of fluid, maintained at a pH lower than that of the bulk digesta, existed at the luminal surface of the intestinal mucosa cell \((18)\). This layer of low pH appeared to be relatively resistant to changes in the pH of the bulk solution and would explain why the effect of pH on absorption was only qualitatively consistent with the hypothesis that the un-ionized species was selectively absorbed. This layer of low pH could be maintained by secretion of lactic acid or H ion. An active secretion of H ion in exchange for Na has been proposed for the bovine rumen epithelium, which also rapidly absorbs VFA from solutions of neutral pH \((25)\). It proposes that if the tissue contained a compartment separated from its opposing bathing solutions by two membranes which differed in their relative permeability to dissociated and undissociated forms of the weak organic electrolyte, the transport of weak acid could be driven by an electrochemical gradient of II ion between compartment and bathing solutions. The present results of VFA absorption appear compatible with either of these proposed mechanisms.

However, as noted above, the present study also suggests a linkage in the absorption of Na and VFA from the goat colon. While this relationship has not been previously described in the colon of other species, Binder and Rawlins \((4)\) have shown that active Na-H exchange in the isolated rat colon was markedly stimulated in the presence of glucose. They concluded that the role of glucose appeared to be one of an energy source. These experiments, together with the demonstration that fatty acids are extensively metabolized by the colon of the pony \((1)\) and pig \((2)\), suggest that VFA may supply an energy source for the active transport of Na.

A critical examination of the interrelationship between these strong and weak electrolytes therefore appears necessary for a better understanding of colonic function in this species. Certain aspects of absorption by the goat colon appear to differ from those reported in other species. However, the presence of VFA in large intestinal contents of all mammalian species studied suggests that this interrelationship may be of general importance and deserving of further study.

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