Effects of acetate on solute and water absorption in the pig small intestine

by

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INTRODUCTION

Gastrointestinal function involves a complex interrelationship between the extrinsic autonomic nervous system, the intrinsic neural system, hormones produced both by the gut and other tissue sites in the body, and finally by the contents of the lumen itself. These luminal contents would include the components in the ingested diet, enzymatic products, microbes, microbial byproducts, and certain endogenous secretions. Most notable of these luminal factors are sugars (84,113) and amino acids (1) which have been shown to promote the uptake of water, sodium, and chloride. Short chain fatty acids (SCFA, plural SCFAs) might also be expected to influence fluid and electrolyte movement.

SCFAs are present throughout the gastrointestinal tract of most species with the highest concentrations in the large intestine. A number of researchers have studied the role of SCFAs in the gut and have demonstrated an important potential role for these compounds in gut physiology. However, most investigations have been done in the hindgut or in the ruminant forestomach. No prior studies have been conducted to examine SCFA influence on water and electrolyte absorption in the small intestine of a species, such as the pig, which has a relatively high SCFA concentration in the small intestine.

The main objective of this project was to establish if SCFAs (represented by acetate) and pH influence water and electrolyte absorption from the small intestine of the pig.

LITERATURE REVIEW

General Background

SCFAs are defined as aliphatic monocarboxylic fatty acids with chains of two to six carbons (75). They are weak electrolytes with pKs ranging from 4.75 to 4.86 (89). As water soluble compounds, they are believed to be absorbed via the portal system, unlike the long chain fatty acids with sixteen or more carbons which enter the blood via the lymphatic system. Medium chain fatty acids with seven to fifteen carbons are absorbed by both routes. The exact mechanism for absorption has not been defined.

SCFAs are found throughout the gut of most species. They are derived from dietary ingesta, endogenous secretion, and as the end-products of anaerobic fermentation of carbohydrates and other organic substrates by the microflora in the gut lumen (96,97). The luminal concentration of SCFAs varies between species but generally increases in the most distal segments of the gastrointestinal tract and, in the case of the ruminant, in the rumen. Herbivorous animals utilize SCFAs as a major source of transferable energy (111).

Sources of SCFAs in the Gut

While the concentrations of SCFAs found in the gut may be derived from ingesta, endogenous secretion, and microbial fermentation, the first two sources contribute a very small portion of the total concentrations. Dietary levels are very low since few natural foods contain significant amounts of SCFAs. Endogenous secretion is also not

significant since the amounts secreted are extremely small and vary greatly with each species. For example, Schmitt et al. (105) have observed the secretion of SCFA in the human jejunum in only three out of fifteen control subjects. Acetic acid secretion occurred at the rate of 0.011-0.037 mmoles/hr/cm of jejunum. Butyric acid also was secreted but at a rate of 0.002-0.009 mmoles/hr/cm. However, SCFA secretion in the human jejunum was not constant but flowed intermittently for some unknown reason. Schmitt et al. (106) did not observe any SCFA secretion in the human ileum.

Microbial fermentation of carbohydrates and other organic substrates accounts for the majority of the SCFAs found in the gastrointestinal tract. This fermentation is common to many species of microorganisms which are present in the gut in high numbers. Normally, the various species in the gut coexist in a stable ecosystem (81,103) which is greatly influenced by conditions in the gut lumen, host diet, and the general condition of the host animal. Availability of carbohydrate substrate for fermentation is dependent on such factors as the digestibility of the individual substrates by the host, transit times, relative rates of absorption in the upper portion of the digestive tract, and endogenous secretions. While most of the substrate is dietary carbohydrate, endogenous sugars, as reported in germ-free rats (82), or products of protein degradation may also serve as substrates.

Concentrations of SCFA in the Gut

SCFAs are found throughout the gastrointestinal tract of both ruminant and monogastric species. The SCFA concentrations have been shown to vary with age (60,81), diet (5,18), and time after ingestion of food (5,31). McBee (86) and Bauchop (17) have reported concentrations in the hindgut and foregut, respectively, for many species. Data for all the species will not be presented in this literature review. Only those species which will be subsequently mentioned in the review of SCFA effects on water and electrolyte absorption will be emphasized.

Concentrations in the pig

Friend et al. (60) have determined the organic acid concentrations in the digestive tract of young pigs at 1, 3, 5, 7, and 9 weeks of age. A concentration of 9.2 $\pm$ 1.3 meq/dl in gastric fluid was reported at one week of age but progressively declined to 3.1 $\pm$ 1.0 meq/dl at 9 weeks. The small intestine had the lowest concentrations with values progressively declining from 6.8 $\pm$ 2.5 (SD) to 3.8 $\pm$ 0.03 meq/dl by week 7. At week 9 the concentration had increased to 4.9 $\pm$ 1.0 meq/dl. Differences between concentrations in the upper and lower intestines increased with age. Cecal and colonic concentrations at week 1 were 11.0 $\pm$ 3.7 and 16.2 $\pm$ 8.0 meq/dl, respectively, but these had increased to 21.4 $\pm$ 2.8 and 20.4 $\pm$ 2.2 meq/dl by week 9. Acetate was the predominant SCFA present at all ages, representing, respectively, 5.4% 15.3%, 52.4%, and 45.8% of the organic acids in the stomach, small intestine, cecum, and colon at week 1. The proportion of acetic acid in the stomach contents

increased with age to 30% of the total acids present after 5 weeks. In the small intestine the proportion of acetate increased to about 20% at weeks 3 and 5 before declining to 8.4% at week 9. No other SCFAs, except acetate, were measured at weeks 7 and 9. The combined proportion of butyric and propionic acids in all segments at all ages was less than the proportion of acetate.

Friend et al. (59) also determined the organic acid concentration in the digesta of adult pigs. Concentrations of organic acids in the digesta of the stomach, small intestine, cecum, proximal colon, and distal colon were, respectively, 15.8, 7.5, 22.2, 22.7, and 16.7 meq/dl of liquid digesta. Butyric acid represented 2.4% of the total organic acids in the stomach and 9.3% in the large intestine. Propionic acid represented 17.1% of the SCFA in the stomach, 4.3% in the small intestine, and over 30% in the hindgut. Acetic acid was the predominant SCFA with 24.4% in the stomach, 10% in the small intestine, and 47-52% in the hindgut.

Clemons et al. (31) have also reported organic acid levels in the alimentary tract of pigs weighing 176 $\pm$ 3.3 kg (SE). Mean concentrations of approximately 20 mmoles SCFA/liter were found in the stomach. Concentrations were generally less than 10 mmoles SCFA/liter in the proximal small intestine but increased to between 10 and 40 mmoles/liter in the distal small intestine. Volatile acid concentrations exceeded 170 mmoles/liter throughout the remainder of the tract. Digesta passage through the small intestine was rapid and no retention was apparent.

Argenzio and Southworth (5) also examined organic acid production and absorption in the gastrointestinal tract of young pigs (9-15 kg). Concentrations of 20-40 mmoles SCFA/liter were found in the contents of

the stomach. Less than 10 mmoles SCFA/liter were measured in the proximal small intestine, but the concentration increased to 20-30 mmoles/liter in the distal small intestine. Concentrations ranged from 150-250 mmoles SCFA/liter in the contents of the large intestine. Acetate represented about 55% of the total organic acid in the stomach and large intestine, but approximately 88% in the distal small intestine. Propionate concentrations changed inversely with acetate. Butyrate represented about 10% of the total organic acids throughout the tract.

Concentrations in other species

Elsden et al. (49) have reported volatile acid concentrations in the digesta of other species. The rat stomach and small intestine have concentrations less than 0.5 grams/100 grams DM (expressed as acetic acid). Cecal concentrations were 4.6 grams/100 grams DM but then declined to about 2.0 grams/100 grams DM in the rectum. Volatile acid concentrations in the rabbit anterior tract were similar to those reported in the rat anterior tract but were only 2 grams/100 grams DM in the decum and 1.5 grams/100 grams DM in the rectum. The stomachs of 8-10 week old pigs contained about 1.5 grams/100 grams DM. Concentrations increased from approximately 0.2 grams/100 grams DM in the anterior small intestine to 0.5 grams/100 grams DM in the posterior portion. Cecal concentrations reached 4.0 grams/100 grams DM. Levels in the colon ranged from about 2.0-2.6 grams/100 grams DM. Rumen concentrations ranged from 5 grams/100 grams DM for the cow to 7.6 grams/100 grams DM in the sheep. Less than 1.0 gram/100 grams DM were found in the contents of the ruminant small intestine. In general, the large gut and rumen were the major sites of

high volatile fatty acid levels. Species, such as the rat and rabbit, with a smaller colon relative to the cecum have the largest quantity of SCFAs in the cecum. The highest amounts of SCFAs are found in the colon in species, such as the pig, where the size of the colon is large relative to the cecum.

Organic acid concentrations in the equine gastrointestinal tract have been reported by Argenzio et al. (7). Concentrations of SCFA in stomach contents varied with the phase of the digestive cycle. Concentrations generally decreased from 20-40 mmoles SCFA/liter in the contents of the cranial stomach to 10-30 mmoles SCFA/liter in the caudal stomach. A relatively constant concentration of less than 5 mmoles SCFA/liter was measured in the small intestine. Concentrations in the cecum ranged from 60-120 mmoles SCFA/liter. The highest concentrations were in the contents of the ventral colon where levels ranged from 60-150 mmoles SCFA/liter. Levels in the digesta declined throughout the remainder of the tract to between 30 and 90 mmoles SCFA/liter.

A luminal concentration of 7.7 (2-11.3) mmoles SCFA/liter, of which 80% was acetate, has been reported for the fasted human upper jejunum by Dawson et al. (43). Although the authors suggested that this concentration may have been due to endogenous secretion, this would seem unlikely, as Mahadevan and Zieve (83) have reported human plasma concentrations of acetate and butyrate at approximately 0.27 and 0.003 mmoles/liter, respectively. Fewer numbers of microbes are present in the small intestine than in the large intestine (36). However, sufficient numbers of SCFA-producing anaerobes are probably present to account for the measured concentration.

Often when conditions within the gut are modified or the substrates presented to a particular segment are changed, the microflora within the gut lumen proliferate. A clinical condition known as the stagnant loop syndrome (SLS) may be induced by changes in either gastric secretion or altered small intestinal motility. The SLS is characterized by microbial proliferation or a change in the predominant microflora which is associated with an increase in SCFA production (47).

Chernov et al. (29) have reported concentrations of SCFAs in the human jejunum from patients with the SLS and normal subjects. Acetate, propionate, and butyrate concentrations in the normal jejunum were 0.0-0.5 mM, 0.0-0.05 mM, and 0-15 μM, respectively. Acetate levels in the SLS increased to 0.4-2.3 mM, propionate ranged from 0.04-0.4 mM, and butyrate was reported at 0-150 μM. Acetate represented 85-95% of the SCFA present when the bacterial proliferation occurred. Antibiotics decreased the concentration of acetate and propionate in the SLS to normal levels.

Bustos-Fernandez et al. (25) measured organic anion concentrations in the feces of 20 healthy human subjects. The organic acid anions were the most abundant ions in fecal water at a concentration of 174.2 $\pm$ 8.4 meq/liter. Fecal output of organic anions averaged 13.1 $\pm$ 1.2 meq/24 hours. Fecal weight was correlated with daily organic anion output. When similar measurements were made in 29 patients with malabsorption hyperacidorrhea, fecal organic acid output increased two to five times normal. The high levels of organic anions, as the major contributors of osmotic pressure in fecal water, may produce an osmotic diarrhea. The excess production of SCFA associated with this fecal

acidorrhea is due to malabsorption of carbohydrates in the upper tract and subsequent overload of the colon with excessive substrate.

Nutritional Importance of SCFA

Bergman et al. (18), from studies on the sheep stomach, has estimated that 70-80% of the energy requirement is derived from volatile fatty acids. Most of the acetate in the rumen is transported to the blood while butyrate is largely metabolized by the epithelium (3) to form ketone bodies (68,93,94).

Henning and Hird (66) have found that ketogenesis of SCFA in the mucosa can occur at equal rates from the ileum to the rectum in rabbits, but in normal animals little activity occurs in the ileum and distal colon where SCFA concentrations are low.

The metabolites of butyrate or other SCFAs may function as a constant energy source for the mucosa as suggested by Argenzio et al. (5,8) and Henning and Hird (66). This potential as an energy source for the mucosa is important because the SCFAs might supply the energy for the metabolic dependent uptake of other materials from the lumen, especially in portions of the intestinal tract where glucose and other sugars are in low concentrations (50,53,90,101,115). In contrast, minimal metabolism of SCFA by evented rat jejunal sacs has been reported (13). However, more SCFA was metabolized in the absence of glucose. The mucosa of the large intestine in the horse also does not appear to utilize acetate as an energy source for active sodium transport (9). The amount of epithelial metabolism of the SCFAs probably varies depending on the species and specific segment of the gut.

SCFA Absorption

Factors affecting SCFA absorption

While the exact mechanism for SCFA absorption has not been defined, a number of determinants of weak electrolyte influx into the intestinal cell have been studied. Schanker et al. (104), from studies of drug absorption in the rat small intestine, concluded that the degree of ionization of an organic electrolyte in solution is a major factor in determining the rate of absorption across lipoidal membranes. Cell permeability to the nonionized form of the electrolyte also must be considered.

It is known that preferential movement of certain anions occurs in various segments of the intestinal tract (67,130). This is partially due to changes in the length, number, and radius of the pores in the respective segments (57). The so called "tight junctions" of the intestine are important in that they may influence the movement of strong electrolytes which may be linked with weak electrolyte transport.

Jackson and Morgan (76) have investigated the relationship of weak electrolyte transport and acid-base metabolism in the rat small intestine. They were able to confirm findings in earlier studies (27, 70,77) that the mechanism for transport of weak electrolytes was related to differences in acid-base metabolism of the tissue. For example, a serosally directed alkalinization takes place in the jejunum while luminal alkalinization occurs in the ileum.

Weak electrolytes may be absorbed by simple diffusion in either the ionized or unionized forms. At the pH of the intestines under

physiological conditions and considering the low pK of the SCFAs, most of the SCFAs exist in the lumen in the ionized form. The SCFAs are members of a group of monovalent anions, described by Clarkson et al. (30), which are preferentially absorbed in the unionized form. This group has a large ionic radius relative to the pore size in the intestinal epithelium. These unionized forms diffuse through the lipoidal membranes. Other smaller anions move through the aqueous pores or channels.

Smyth and Taylor (116) conducted a series of experiments utilizing inverted sacs of rat jejunum in which they demonstrated that SCFAs could be transferred against a concentration gradient. When acetate, propionate, or butyrate were added to the solutions bathing the mucosal and serosal surfaces at an initial concentration of 20 μmoles SCFA/ml, the mucosal concentration after 60 minutes had decreased about 2 μmoles/ml. The serosal fluid had gained 6-18 μmoles/ml. Their results were best explained by an active transport mechanism for SCFAs. This transport was inhibited by anaerobic conditions, addition of metabolic inhibitors, and the absence of glucose from the mucosal bathing fluid.

The apparent active transport was confirmed later (15,16). Jackson (74) showed that the basolateral wall of the cell represents the rate limiting diffusion barrier. Entry of the SCFA into the cell was proportional to the concentration in the mucosal fluid suggesting passive diffusion across the mucosal membrane. Only the proximal small intestine exhibits this active uptake.

Ash and Dobson (10), from studies on rumen absorption, suggested that the higher concentration of acetate in the serosal fluid, described

by Smyth et al., could be the result of both absorbed acetate from the lumen and acetate derived from intracellular hydrolysis of glycerides. The process by which acetate moves across the serosal membrane would have the potential to transport more acetate to the serosa than was absorbed from the lumen.

Barry and Smyth (13), in one of a series of experiments, demonstrated that 161 μmoles of acetate and 2.5 ml of water/hr were transferred from the mucosal fluid by everted sacs of rat jejunum. The initial concentration of acetate was 20.3 mM. Uptake of acetate was proportionately greater than fluid uptake. From the total 161 μmoles absorbed, 89 μmoles were taken up by the tissue and 46.2 μmoles were transferred to the serosal fluid. Acetate transfer, when measured from initial concentrations ranging from 5-80 mM, increased with increasing concentrations up to 60 mM. The transfer rate decreased progressively at concentrations in excess of 60 mM. Acetate and water transfer were both inhibited at the higher concentrations. An average rate of disappearance from the intestinal lumen of 440 μmoles of acetate/20 minutes for the whole rat jejunum and ileum was observed in a series of in vivo experiments.

All of the above factors must be considered when attempting to explain the mechanism of SCFA absorption. A number of models have been proposed to explain SCFA absorption. It is likely that several different mechanisms may exist and are specific for each species and each specific segment of the gut.

Models for SCFA absorption

Hogben et al. (71) have proposed a model to explain the absorption of weak organic acids in the rat jejunum at rates which exceed those based on theoretical calculations. The model is based on the unstirred layer which is formed by the trapping of fluid between the microvilli and glycocalyx. The model consists of three compartments: mucosal fluid bulk phase, the unstirred layer of fluid at the cell surface, and the cellular compartment (Figure 1). The undissociated form of the weak acid is preferentially absorbed by the cell membrane. Therefore, influx is determined by the ratio of ionized to unionized forms at the cell surface. A decrease in surface pH will enhance absorption. A low pH can be maintained by hydrogen secretion. Three sources have been proposed for the hydrogen ion. First, intracellular CO_2 may diffuse from the epithelial cell into the luminal fluid where it is converted to HCO_3^- and H^+. The H^+ associates with the organic acid anion and the undissociated molecule is absorbed. Second, there could be an active secretion of hydrogen into the lumen with the resulting undissociated form of the acid absorbed. Finally, a Na-H ionic exchange (20) could provide the necessary hydrogen to complete the absorption process. Transport across the mucosal and serosal membranes is by simple passive diffusion.

Jackson et al. (78) have examined the determinants of weak electrolyte influx into the rat intestinal epithelia cell. They concluded that the unstirred layer is not the rate limiting factor for the compounds considered in their study. A pH equal to that of the mucosal fluid bulk phase determined weak electrolyte uptake into the epithelial cell.

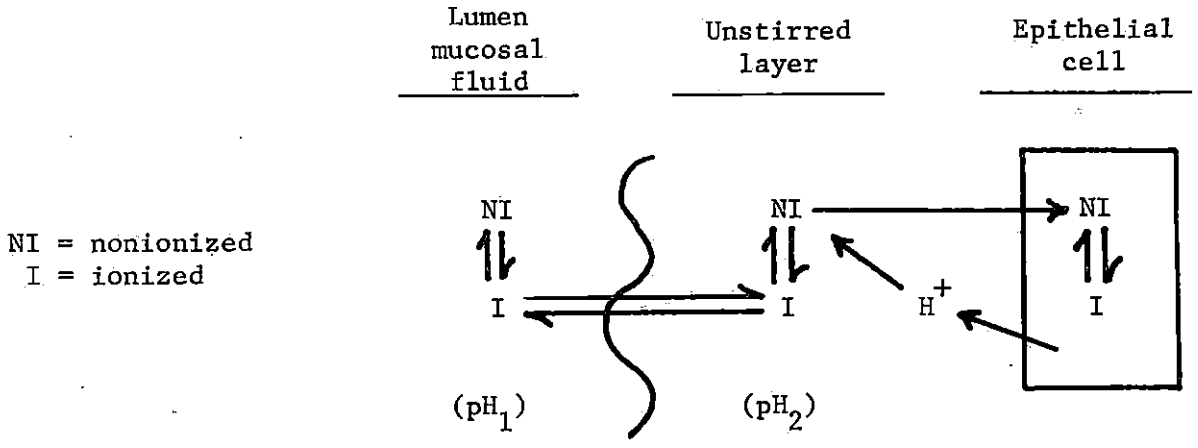

Figure 1. The unstirred layer model proposed by Hogben et al. (71)

Jackson et al. have proposed an alternate three compartment model in which the subepithelial extracellular space is the intermediate compartment. The pH gradient across the compartment determines weak electrolyte absorption. Their model is supported by studies on concurrently transported weak electrolytes (77) and the observed differences in acid-base metabolism and weak electrolyte transport in the various segments (76).

Stevens (119) has proposed a model for the rumen epithelia that is based on an epithelial cellular compartment bathed by the luminal solution on one side and the blood on the serosal side (Figure 2). It is assumed that the cell membrane on the luminal surface is permeable only to the ionized form of the organic acid. The membrane at the serosal surface is permeable only to the unionized form. The driving

force for SCFA absorption is a hydrogen gradient from the cellular compartment to the blood.

Argenzio et al. (9) have proposed two models, which are similar to the model of Stevens, to explain acetate absorption in the equine large intestine. Their first model requires continuous production of H^+ from CO_2 hydration in the lumen (Figure 3). The H^+ and acetate anion associate and the undissociated form then crosses the selective barrier into the cell. Concomitant luminal alkalinization and intracellular acidification takes place. Acetate moves down an electrochemical gradient into the blood either in the dissociated or undissociated form.

The second model entails an intracellular hydration of CO_2 via the action of carbonic anhydrase (Figure 4). The intracellular HCO_3^- is then exchanged for the luminal acetate anion in a manner similar to the HCO_3-Cl exchange reported by Turnberg et al. (128) in the human ileum. Argenzio et al. have postulated that the interaction between Na^+ and acetate transport observed in their studies on the equine colon is explained by a Na-H exchange mechanism. Acidification of the cell contents by HAc entry would inhibit the absorption of Na^+ across the serosal membrane into the blood via a Na-H exchange.

SCFA absorption in specific species

SCFA absorption in the rumen is considered to take place by simple diffusion (92) and is related to concentration gradients between rumen fluid and blood. Barcroft et al. (11) have shown that most of the SCFA produced in the forestomach is absorbed in the forestomach. A significant portion of the absorbed SCFA is metabolized by the ruminal epithelium

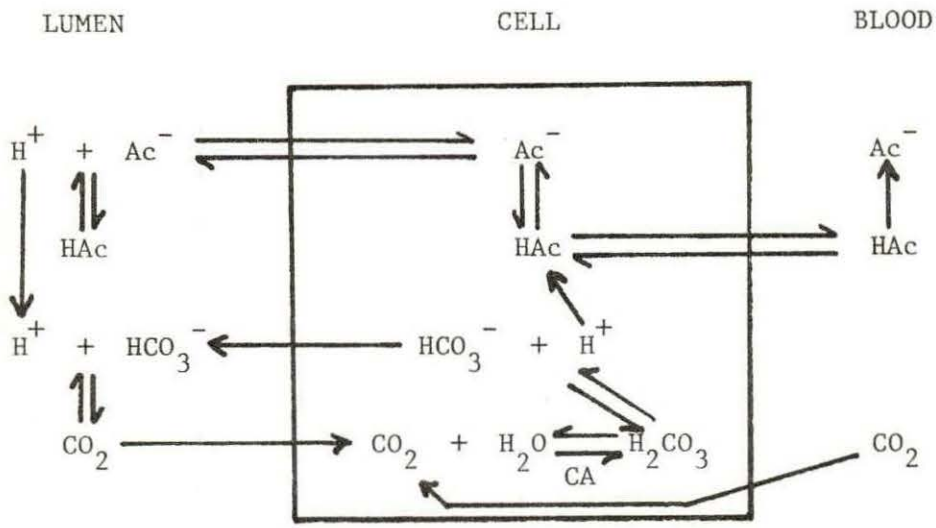

Figure 2. Stevens' model for SCFA absorption across the rumen epithelium

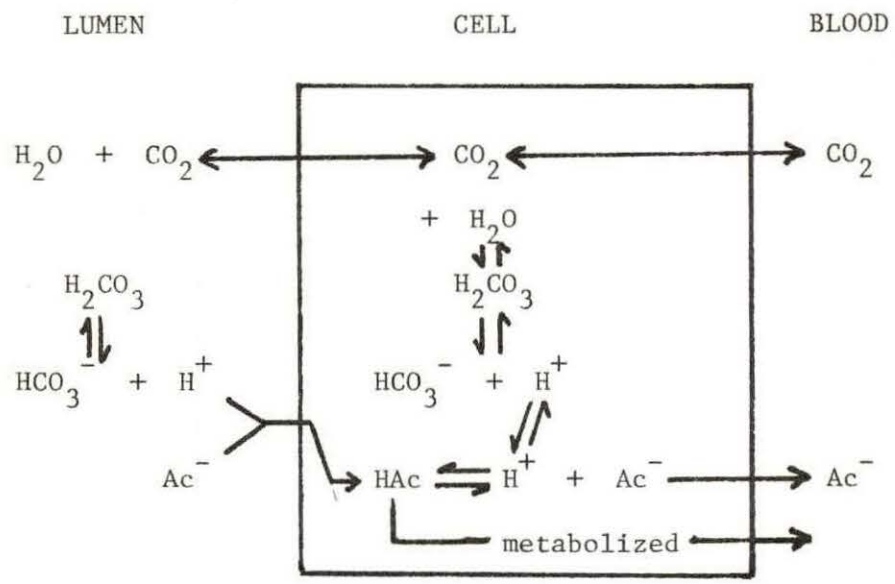

Figure 3. Model B proposed by Argenzio et al. (9) for acetate absorption in the equine large intestine

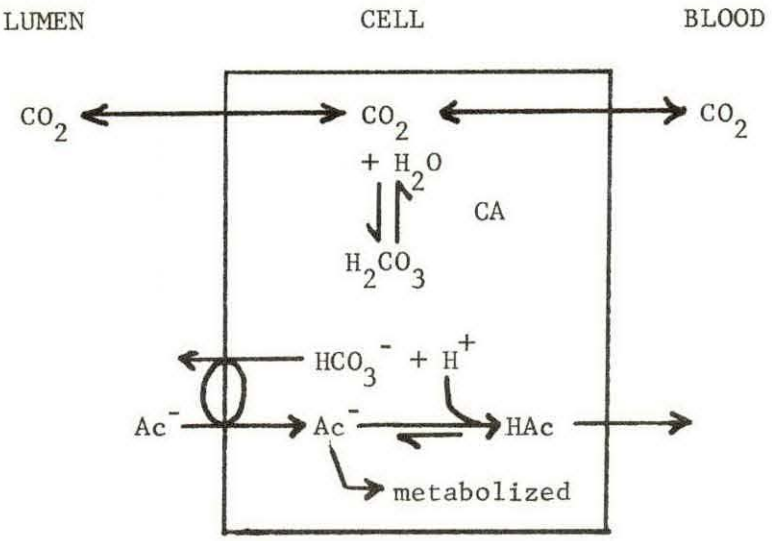

Figure 4. Model A proposed by Argenzio et al. (9) for acetate absorption in the equine large intestine

(120). A decrease in the lumen pH from 7.4 to 6.4 will increase the rate of absorption; although acetate, propionate, and butyrate are all absorbed at relatively rapid rates at both a low and a neutral pH. An increase in chain length also results in increased rates of absorption (12). Stevens (119) has published a comprehensive review of fatty acid absorption by the rumen.

Jackson et al. (78) have calculated the rate of influx for several SCFAs in the rat jejunum and ileum. The influx was directly proportional to the concentration of the unionized SCFA. The rate of influx of one SCFA was not altered in the presence of a tenfold increased concentration of another SCFA. The influx of acetate in the jejunum was observed at 0 $\pm$ 2 (SE) nmol·100 mg dry weight^{-1}·min^{-1}. Propionate and butyrate

were absorbed at 6 ± 1 and 12 ± 1 nmol·100 mg dry weight^{-1}·min^{-1}.

Significant uptake for all three SCFAs was observed in the ileum. The rates were acetate, 3 ± 1; propionate, 9 ± 1; and butyrate, 11 ± 1 nmol·100 mg dry weight^{-1}·min^{-1}. It appeared that influx increased with increasing chain length.

Remesy and Demigne (97) also determined SCFA absorption in the gastrointestinal tract of the rat by measuring arterial-venous differences. High concentrations were measured in the portal vein. This suggests that SCFAs may be nutritionally important to the rat. Production and absorption were predominantly in the distal tract. Absorption in the small intestine appeared negligible (ileal vein and artery contained 0.13 and 0.15 mM, respectively), although the normal concentration in the ileum was a relatively low 6.1 ± 0.8 mM (SE). Any SCFA which was absorbed in the small intestine was probably metabolized. The cecum absorbed substantial SCFA despite a pH of approximately 7.5. In fasted rats, in which the concentration of SCFA in the cecum would be expected to decrease, the relative rates of absorption were more dependent on the concentration gradient than the accompanying increase in pH.

Sallee and Dietschy (100) obtained data on short and medium chain fatty acid uptake and the apparent permeability coefficients (*P) using rat everted small intestine. They concluded that their data supported simple diffusion with the diffusion barrier being the unstirred water layer. No evidence was obtained of saturation kinetics suggestive of a carrier mediated system. Competition between various chain lengths

did not affect the rates of tissue uptake. The apparent permeability coefficients were calculated for pH 7.4 and 6.0. The following points were made: (a) *P increased with increasing chain length except for those SCFAs with less than six carbons; (b) when the pH was lowered to pH 6.0, *P increased; (c) the effect of stirring the unstirred layer had little effect on the SCFA absorption; (d) SCFAs with 2-5 carbons have higher *P than theoretically predicted. Since the *P for SCFAs of less than six carbons exceeds theoretical calculation, uptake via a more polar diffusion pathway was suggested. Apparent permeability coefficients for acetate at pH 7.4 and 6.0 were 22 $\pm$ 1.4 (SE) and 112 $\pm$ 13.4 nmoles/min/100 mg/mM.

Dawson et al. (43) measured the rate of absorption of SCFA in human subjects from an isotonic perfusion solution containing seven SCFAs at a total concentration between 110 and 145 mM at pH 7.0. Only 0.89 (range 0.2-1.1) μmoles SCFA/min/30 cm of jejunum were absorbed. He also measured absorption by the colon of one patient. A total of 4 nmoles/10 minutes was absorbed by the colon. Little difference between the rate of absorption of the respective SCFAs was observed.

Schmitt et al. (105,106) have examined SCFA absorption in 30 cm segments of human jejunum and ileum. Jejunal absorption was linear for concentrations up to approximately 40-50 mM at pH 8.2-8.4. A saturation phenomena was observed in excess of 40-50 mM of SCFA. Maximum rates of uptake for acetate, propionate, and butyrate at this saturable concentration were 12.9 $\pm$ 3.4 (SE), 14.0 $\pm$ 1.0, and 12.6 $\pm$ 1.9 mmoles/hour. In the ileum, all three SCFAs were also absorbed rapidly

from solutions containing 5-100 mM of SCFA at pH 7.4-7.8. The following values for the maximum velocity (V_m) and half-saturation constant (K'_m) were calculated: acetate, V_m = 0.538 mmole/h/cm, K_m = 22.7 mM; propionate, V_m = 0.659 mmole/h/cm, K_m = 26.8 mM; butyrate, V_m = 0.820 mmole/h/cm, K_m = 25.6 mM.

Absorption in the human ileum and jejunum, as reported by Schmitt et al., was rate limited and was accompanied by alkalinization of the intestinal contents. Schmitt et al. proposed two mechanisms to explain the luminal alkalinization. First, CO_2 and H_2O in the lumen are converted to form hydrogen and bicarbonate. The hydrogen associates with the SCFA anion and the unionized SCFA is absorbed leaving the residual bicarbonate with an accompanying decrease in pCO_2 and an increase in pH. A second mechanism involves a carrier mediated exchange between bicarbonate and SCFA anions. The exchange results in an increased pH and pCO_2 of the lumen.

McNeil et al. (87) measured SCFA absorption from the rectum of 46 human subjects utilizing a dialysis bag technique. Both acetate and a mixture of acetate, propionate, and butyrate were absorbed from 100 mmole/liter solutions at pH 7.2 and 5.5. At pH 7.2, 32.8 mmoles/liter/hr of acetate were absorbed while 41.8 mmoles/liter/hr of the SCFA mixture were absorbed. There was no significant difference between values obtained at pH 7.2 and those from similar experiments at pH 5.5. However, the initial pH of 5.5 increased to pH 7.45 in only 30 minutes. Such an increase would be expected to decrease the ratio of unionized to ionized SCFA and inhibit nonionic uptake. Whether absorption occurred in the ionic or nonionic form was not determined.

Calculation of the net rate of movement through the dialysis membrane at pH 7.2 showed acetate loss at the rate of 8.1 ± 0.8 (SE) μmoles/cm^2/hr compared to 4.7 ± 0.8 μmoles/cm^2/hr for sodium. The rates for the combined mixture of acetate, propionate, and butyrate were 5.2, 1.8, and 1.9 μmoles/cm^2/hr, respectively. When the pH was decreased to pH 5.5, acetate movement was 8.5 ± 0.6 μmoles/cm^2/hr. The rates of absorption from the mixture were acetate, 4.3 ± 0.7; propionate, 1.4 ± 0.2; and butyrate 1.6 ± 0.5 μmoles/cm^2/hr.

In vivo and in vitro studies were conducted by Argenzio et al. (7) on the horse colon. The results showed significant acetate absorption in the hindgut. Acetate absorption in the ventral colon from two liters of a control solution containing 100 mM of acetate at pH 6.1 was at 103 ± 6 (SE) mmoles/30 min. Acetate moved down an electrochemical gradient, however, it was also suggested that the high rates could also involve some process other than simple diffusion. The luminal bicarbonate increased as the unionized SCFA was absorbed.

Argenzio et al. (8) have reported acetate absorption from the colons of two goats at rates ranging from approximately 3-75 mmoles/hr from a test solution which included 60 mM of acetate at pH 6.0.

SCFA absorption by the gastrointestinal tract of young pigs weighing 12.5 ± 3.1 (SD) kg has been studied by Argenzio and Southworth (5). Transport across mucosal strips of various gut segments initially bathed on the mucosal side with an equimolar mixture of acetate, propionate, and butyrate totaling 90 mM in a Ringer's solution at pH 7.4 was measured. Absorption in the stomach was greatest in the cardiac portion with 2.8 ±

0.7 (SE) μmoles/cm^2 net absorption. Maximum absorption occurred in the cecum at 10.7 ± 1.6 μmoles/cm^2 but then decreased to 7.7 ± 0.6 μmoles/cm^2 in the lower colon.

In vivo SCFA absorption was determined for various segments of the tract of pigs fed a control diet. The results were as follows: cranial stomach, 0.05-0.2 mmoles SCFA/kg body weight (BW); caudal stomach and proximal small intestine, 0.05 mmoles SCFA/kg BW; distal small intestine, 0.01-0.02 mmoles SCFA/kg BW; cecum, 0.6-1.1 mmoles SCFA/kg BW; terminal colon, 1.0-2.0 mmoles SCFA/kg BW; and rectum, 1.2-2.4 mmoles SCFA/kg BW.

Crump et al. (37) have reported acetate absorption in the pig colon at the rate of 156 ± 4.7 meq/hr at pH 6.4 and at the rate of 105 ± 5.5 meq/hr at pH 7.4. Accompanying acetate absorption was the appearance of HCO_3^- in the lumen. The absorption of acetate in the undissociated form was suggested.

Argenzio and Whipp (6) have reported acetate absorption from temporarily isolated colonic segments of conscious pigs, initially weighing 46 ± 8 (SD) kg. The rates in the proximal and distal colon were respectively 1.9 ± 0.1 (SD) and 1.4 ± 0.1 meq/min. Acetate appeared to be absorbed in the undissociated state and was driven by CO_2 hydration and a Na-H exchange.

Effects of SCFAs on Parameters Which May Influence Water and Electrolyte Absorption

SCFAs have been shown to affect several parameters which could have an influence on intestinal absorption. Injection of SCFA into the sheep rumen produces a rapid and specific stimulus for the release

of insulin (133). Insulin has been shown to enhance the absorption of sodium and water (28). However, the reported lack of a sustained increase in insulin levels probably means that any in vivo effect on absorption would be minimal.

A rapid and transient increase in the transmural electrical potential difference in the rat small intestine produced by infusing SCFA into the lumen has been reported by Wall et al. (132). Alterations in the transmural electrical potential difference would be expected to influence water and electrolyte movement. However, both the transient nature of the reported response and the refractory state of the intestine to additional SCFA make this effect also questionable under physiological conditions.

The effects of SCFA on motility have been examined in several species. A 10 mM concentration of undissociated SCFA has been shown to markedly inhibit sheep cecal motility (122) and rabbit duodenal motility (121). Motility could be enhanced by 0.1 mM concentrations of undissociated SCFA. Rumen (123) and abomasal (21) motility is also greatly reduced by intraluminal infusion of SCFA. There is no evidence that changes in motility caused by SCFAs affect absorptive function. However, when motility is decreased by the drug probanthine, a decrease in the rate of sodium absorption by the human jejunum has been observed (62).

Effects of SCFA on Water and Electrolyte Absorption

Summary of fluid and electrolyte absorption

Excellent reviews of water and electrolyte absorption and secretion have been written by Binder (19), Edmonds (48), Fordtran and Dietschy (54), Fordtran et al. (58), Schultz and Curran (107,108), Schultz and Frizzell (109), Sladen (112), Turnberg (126), and many others. Therefore, a detailed review of electrolyte and water absorption will not be undertaken. Only the general features of absorption will be cited.

Absorption can be defined as the net result of two unidirectional fluxes, from lumen to blood and from blood to lumen. The process is complex and many of the specific mechanisms are unresolved. Electrolyte absorption occurs by either passive diffusion, solvent drag, or via a carrier mediated transport system. Water absorption is considered to occur secondary to solute transfer (38,40).

Absorption may occur either through the cell membrane (transcellular) or through the tight junctions between adjacent cells (paracellular). Movement across the cell membrane may be through water-filled pores (aqueous route) in the cell membrane or through the membrane structure itself (lipoidal route). Water moves primarily through the tight junctions. The degree of tightness of these junctions in the intestine increases in the aboral direction.

Passive diffusion through the pores or channels (7-15 Å) in the mucosal membrane is in response to electrochemical, osmotic, or hydrostatic pressure gradients (38,40,56,61,79). This diffusion may involve movement of single ions (single-file ionic diffusion) (69) or of

undissociated ion pairs (nonionic diffusion) (71).

Solvent drag involves solute transfer secondary to water movement in which the solute is swept through the pores (32,38). The movement of water is in response to osmotic or hydrostatic pressure gradients.

Carrier mediated systems are not completely defined but seem to involve specific binding of the transported substance by a component of the cell membrane. The systems exhibit saturation kinetics and may be competitively inhibited. Curran and Schultz (41) have described three types of carrier transport systems. Active transport involves electrolyte movement against an electrochemical gradient, requires metabolic energy, and can be inhibited by metabolic inhibitors and low temperature. Facilitated transport involves a transport system, as in active transport, except no energy is expended and concentration against an electrochemical gradient does not occur. Finally, there is an exchange diffusion that functions with similar properties as facilitated transfer. The difference involves an exchange of ions in opposite directions by the carrier that results in a unidirectional flux of specific ions but not net absorption of solute.

Special carrier mechanisms for Na^+, H^+, HCO_3^-, and Cl^- have been postulated. Active transport has been reported for Na^+ (34,39,40), H^+ (128), and Cl^- (40,80). A double exchange carrier system, which involves an exchange of Na^+ for H^+ and Cl^- for HCO_3^-, has been described by Turnberg et al. (128) for the human ileum. Only a Na^+-H^+ exchange apparently exists in the jejunum (128) while a Cl^--HCO_3^- exchange has been reported in the colon (23,45). Crane (35) and Adibi (1) have described facilitated transfer involving absorption of glucose or amino

acids and Na^+ in the small intestine.

The small intestine in most species is the primary site of water and nutrient absorption. The colon functions as a final means to maintain a positive balance of water and sodium (24). Accordingly, the small intestine has carriers for glucose and other nonelectrolytes, while the colon has no such carriers. Passive transport decreases in importance in the aboral direction; whereas, active transfer increases in importance in the distal tract.

Segmental differences in rates and mechanisms and types of materials absorbed in the various segments of the gastrointestinal tract are well-known (67,130). The jejunum and ileum appear to have marked functional differences. Characteristics of the jejunum include low luminal bicarbonate, high chloride, and relatively poor absorption of an isotonic sodium chloride solution. The ileum has a low luminal chloride and high bicarbonate concentration. The jejunum shows greater permeability than the ileum to osmotically induced water transfer because of a larger pore radius (54). There is a net absorption of sodium in the ileum (40,131), although similar results for both ileum and jejunum have been reported (118).

Fluid and Electrolyte Movement in the Pig Small Intestine

Hamilton and Roe (64) have studied the ionic concentrations and net changes in the volume and electrolytes during digestion along the entire intestinal tract of four-week-old pigs. Polyethylene glycol, added to the animal's diet, was used as a dilution marker. Contents of the lumen at various segments of the intestinal tract were then measured.

The duodenum and proximal jejunum demonstrated large variation in the osmolality of the lumen contents. This was attributed to equilibration of the lumen contents by secretion of water and electrolytes. More constant osmolality was observed throughout the remainder of the tract. However, the osmolality in the contents of the small intestinal segments was much higher than observed previously in the human and pig (55,65).

Sodium concentration decreased from 99 meq/liter in the duodenum contents to 83, 81, 86, and 78 meq/liter in successive segments of the jejunum. Levels increased to a mean concentration of 95 meq/liter in the ileum. Potassium also decreased from 38 meq/liter in the duodenum to 14 meq/liter in the ileum. Water absorption by the jejunum, ileum, and the more distal tract paralleled and was attributed to Na^+ absorption. Although Cl^- was secreted in the duodenum, Cl^- was absorbed in the remainder of the tract. Chloride absorption by the jejunum and more distal segments occurred at a faster rate than water. This was attributed to a $Cl\text{-}HCO_3$ exchange.

Only 30% of the total fluid entering the proximal jejunum was absorbed by the jejunum, ileum, and cecum. The observations of Hamilton and Roe in the pig are in contrast to the observations in man (55) and the bovine (73,88). In those species most of the fluid and electrolytes are absorbed prior to entering the cecum. Therefore, the pig large intestine must absorb a greater load of fluid and electrolytes. Hamilton and Roe demonstrated that, when the absorption of fluid and electrolytes by the small intestine is impaired, the result may be an overloading of the fluid delivered to the large intestine.

Influence of SCFAs on fluid and electrolyte movement

SCFAs have been observed to influence the absorptive process by affecting both the unidirectional fluxes from lumen to blood and blood to lumen. The influences of SCFAs which have been reported are discussed in the following section of this review. It should be noted that most of the studies were conducted with quite varied techniques, species, concentrations, and gut conditions.

Fordtran (53) has proposed a number of mechanisms for the production of diarrhea. These mechanisms included the presence of excess osmotically active or poorly absorbed material in the lumen (osmotic diarrhea); intestinal secretion; deletion or inhibition of a normal active ion absorptive mechanism; abnormal mucosal permeability; or altered intestinal motility. Clinical diarrheas involve one or more of these mechanisms. The role of SCFAs in the production of diarrhea especially via the first mechanism has been established, although the other mechanisms often can be implicated as the primary mechanism involved.

Impaired carbohydrate absorption by the upper gastrointestinal tract and the resulting production of SCFAs has been implicated in the nonspecific diarrhea in infants reported by Torres-Pinedo et al. (125). Bacterial fermentation of the carbohydrate results in an increase in the osmotically active SCFAs and a reduction in the lumen pH. The increased osmolarity promotes water retention and the decreased luminal pH inhibits electrolyte absorption.

Donaldson (47) has reviewed the clinical condition known as blind-loop or stagnant loop syndrome (SLS) in which a bacterial overgrowth

of the small intestine is produced. This overgrowth is produced when either gastrointestinal secretions or motility are decreased and conditions in the lumen favor bacterial proliferation. Dramatic effects on metabolism are associated with the overgrowth. Chernov et al. (29) have reported a marked increase in the lumen concentration of SCFAs and suggested that the SCFAs may be responsible for the watery diarrhea associated with this condition.

Dawson et al. (43) have observed diarrhea in 4 of 7 human jejunal perfusions using an isotonic solution containing seven SCFAs at a total concentration of 100 or 145 mM. They attributed the diarrhea, observed in the studies, to the high concentration of SCFAs which acted as a bulk purge (osmotic diarrhea).

Schmitt et al. (105) have observed an increase in water absorption by the human jejunum when acetate concentrations there increased up to 50 mM. However, the net absorption was only significant with a solution containing 10 mM acetate at pH 8.2-8.4. Butyrate in excess of 4 mM also increased absorption. Concurrent sodium absorption was attributed to solvent drag. Results of a similar study in the human ileum (106) showed that SCFA absorption does not alter sodium, potassium, chloride, or water movement. Luminal pH and bicarbonate both increased.

A positive correlation between fecal weight and daily organic acid production in the human colon has been reported by Bustos-Fernandez et al. (25). These organic acids, at concentrations of about 175 mM or more (98,100) in healthy individuals, are a major contributor to the osmotic pressure of fecal water. More than 50% of the organic acid

was acetate, propionate, and butyrate. In 20 patients with malabsorption hyperacidorrhea, fecal organic acid output was increased 2-5 times. Osmotic diarrhea may be the end result of excessive organic acid production. The possible role of SCFAs in influencing fecal water excretion or diarrhea was confirmed by Bustos-Fernandez et al. (26) in later studies on the rat colon.

McNeil et al. (87) demonstrated sodium, chloride, and water absorption concurrent with the absorption of acetate or an acetate-propionate-butyrate mixture from dialysis bags in the human rectum. Acetate was absorbed from the solution in the bag with an initial pH of 7.4 at the rate of 8.1 ± 0.8 μmole/cm^2/hr. The other electrolytes were absorbed at the following rates: sodium, 4.7 ± 0.8 (SE) μmole/cm^2/hr; potassium, 0.2 ± 0.4 μmole/cm^2/hr; chloride, 1.9 ± 0.4 μmole/cm^2/hr. Water was absorbed at the rate of 23.8 ± 4.4 mg/cm^2/hr. Bicarbonate was secreted at the rate of 4.5 ± 0.7 μmole/cm^2/hr into the bag. This represented a fivefold increase in the concentration of bicarbonate. Similar results for electrolyte and water movement were observed when a combined mixture of SCFAs was absorbed from the dialysis bag. When the pH was decreased to 5.5, bicarbonate was again secreted at a rate of 4.6 ± 0.6 μmole/cm^2/hr for the acetate solution and at 3.5 ± 0.7 μmole/cm^2/hr for the mixed solution. This secretion of bicarbonate exceeded absorption of chloride from the bag. Chloride was absorbed at 3.5 ± 0.5 μmole/cm^2/hr and 3.6 ± 1.2 μmole/cm^2/hr for the acetate and mixed solutions.

Colonic bacteria, via SCFA production, appear to function to conserve undigested and unabsorbed carbohydrate from the upper gut. The nutrition significance of SCFAs has previously been discussed. Bond

and Levitt (22) have suggested that colonic absorption of the SCFAs may be a mechanism for formation of the normal stool in addition to providing energy to the host. When the bacteria are unable to convert carbohydrate to SCFAs or where the colon cannot absorb SCFA, a diarrhea may result from osmotic overload.

Argenzio et al. (8) observed a significant increase in water and sodium absorption in the goat colon in response to the presence of acetate. Water absorption in two goats increased from mean rates of 120 and 340 ml/hr to 250 and 540 ml/hr. Sodium uptake increased from mean rates of about 15 and 50 mmoles/hr to 35 and 80 mmoles/hr. Decreasing the luminal pH also influenced sodium and electrolyte absorption in the presence of acetate. The results could be explained by the models of either Hogben et al. or Stevens, as previously described. A role for SCFAs in sodium and water conservation in the goat colon was supported by the data.

Although SCFAs are absorbed rapidly in both the goat and horse colon, the effects of SCFAs on fluid and electrolyte absorption in the horse were shown by Argenzio et al. (9) to be different. Acetate, at a concentration of 100 mM in a Ringer solution which was bathing the luminal surface of isolated mucosal sections at pH 6.4, caused a decrease in net sodium transport in the cecum and abolished uptake in the small colon. Net sodium absorption in the cecum ranged from 1.2 ± 0.1 (SE) $\mu eq/hr/cm^2$ with the normal Ringer solution at pH 6.4 to 0.4 ± 0.1 $\mu eq/hr/cm^2$ from the Ringer solution with acetate. In the small colon, 1.2 ± 0.3 $\mu eq/hr/cm^2$ of sodium were absorbed without acetate present, but 0.3 ± 0.2 $\mu eq/hr/cm^2$ of sodium was secreted when acetate was added

to the solution. Removal of glucose from the solution bathing the luminal surface of the tissue resulted in a more pronounced inhibition of net sodium transport by acetate. Complete removal of glucose, but not acetate, from the luminal and serosal bathing fluids resulted in a net sodium secretion of 1.5 ± 0.3 μeq/hr/cm^2.

Results of separate in vivo experiments by Argenzio et al. in the isolated horse ventral colon showed sodium absorption from two liters of a control solution containing 100 mM of acetate at pH 6.1 at the rate of 24 ± 8 (SE) mmoles/30 min. Water was absorbed at a rate of 60 ± 30 ml/30 min. In the absence of acetate, the concentration of bicarbonate in the lumen was significantly decreased.

Argenzio and Whipp (6) have reported a fourfold increase in sodium absorption when acetate was added to a solution perfused through temporarily isolated colons of conscious pigs. While sodium absorption was less than acetate absorption, the difference was accounted for by bicarbonate secretion. Sodium appeared to be exchanged for hydrogen and did not depend on the presence of chloride for absorption.

A similar study in the pig colon by Crump et al. (37) also demonstrated at an increase in net solute transport from 118 ± 5.5 (SE) mOsmol/hr to 175 ± 6.8 (SE) mOsmol/hr when acetate was added to a perfusion solution at pH 6.5. Water absorption doubled from 0.257 ± 0.01 (SE) liters/hr to 0.402 ± 0.03 (SE) liters/hr with acetate added. Bicarbonate was secreted into the lumen at 96 ± 7.2 meq (SE)/hr compared to acetate absorption at 156 ± 4.7 (SE) meq/hr.

PART I. INFLUENCE OF ACETATE AND pH ON WATER AND ELECTROLYTE MOVEMENT IN CHRONIC PREPARATIONS OF PORCINE ILEUM

INTRODUCTION

SCFA influence on water and electrolyte movement in the pig ileum has not been investigated. The lumen of the pig ileum normally contains 20-40 meq SCFA/liter of digesta of which 90% is acetate (5,31). Acetate was, therefore, selected as the representative SCFA for all studies.

A series of perfusion experiments utilizing chronic preparations of ileum were conducted to determine if acetate at a concentration approximating that reported in the pig ileum could affect water and electrolyte absorption in the conscious pig. From the literature reviewed, it was obvious that pH may be important in acetate absorption. Therefore, an acetate and an acetate-free solution were tested at pH 6.5, which approximates the pH of the ileum (5), and at pH 7.4, which approximates plasma pH.

The objectives of these experiments were (a) to determine the relative rate of acetate absorption in the ileum at a high pH (7.4) and a low pH (6.5), and (b) to determine the net absorption of sodium and water in response to the presence of acetate at a high and low pH in the lumen of the pig ileum.

MATERIALS AND METHODS

Animals

Four National Animal Disease Center (NADC) pigs, initially weighing 15-45 kg, were utilized for a series of single-pass perfusion experiments in which cannulae were surgically implanted in the ileum. All pigs received the same commercial diet and water. Food, but not water, was withheld overnight prior to a perfusion experiment.

Surgery[1,2]

Anesthesia was induced and maintained with halothane via an anesthesia cone. A left ventral incision, 8-9 cm in length, was made parallel and immediately posterior to the last rib. The muscles were parted in the direction of the fibers and the peritoneum was incised. After locating the cecum, a purse-string suture was placed in the serosa of the ileum at a location 20 cm anterior to the ileocecal junction. The ileum was then incised and a small outlet catheter was inserted into the lumen and secured with the purse-string suture.

The catheter, which was made from Silastic^{R} (Dow Corning Corp., Midland, MI 48640) tubing, was 4 inches long, 1/4 inch i.d., and 7/16 inch o.d. A nylong mesh skirt was attached immediately above a flange located at the tip of the catheter. This flange was made by gluing a circular piece of Silastic^{R} (Dow Corning Corp., Midland, MI 48460) sheeting, approximately 1 inch in diameter and with a hole punched in the

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[1] [2] Surgery performed by Dr. S. C. Whipp, N.A.D.C., Ames, Iowa.

[2] Portions of the description of the surgical technique, as presented in this section, were provided by Dr. S. C. Whipp.

middle through which the catheter was inserted, to the catheter tip. The flange was inserted into the lumen of the intestine to prevent expulsion of the catheter while, at the same time, the nylon mesh was incorporated in the adhesion. The catheter was exteriorized via a stab wound in the ventral posterior abdominal wall.

A second purse-string suture was placed at a point on the antimesenteric border 60 cm anterior to the small catheter and one-half of a bypass catheter was inserted and secured. This catheter was made of molded Silastic^{R} (Dow Corning Corp., Midland, MI 48640) with a 5/8 inch i.d. and 7/8 inch o.d. It also had a flange for insertion into the intestinal lumen and a nylon mesh to secure the catheter to the serosa. The flange was molded in such a manner that the catheter was directed caudad. A 7/8 inch diameter hole was made in the skin anterior to the initial skin incision. The muscles were then separated and the catheter was pulled through the opening. Before closure of the abdominal incision, 5 cc of Cleocin^{R} (Upjohn Co., 7000 Portage Rd., Kalamazoo, MI 49001)(150 mg/ml) was instilled into the peritoneal cavity.

The peritoneum and three muscle layers were individually closed with Chromic 0 gut suture. Vetafil^{R} (Bengen and Co., Hanover, West Germany) (0.40 mm) was used to close the skin. All animals were prophylactically treated with Combiotic^{R} (Pfizer Inc., 235 East 42nd St., New York, NY 10017) for 5 days. A minimum of 10 days for recovery was allowed prior to any perfusion studies.

The gain in weight of the individual pigs, which was measured periodically during the duration of the experiments, is listed in Appendix A. All four pigs demonstrated a similar progressive weight

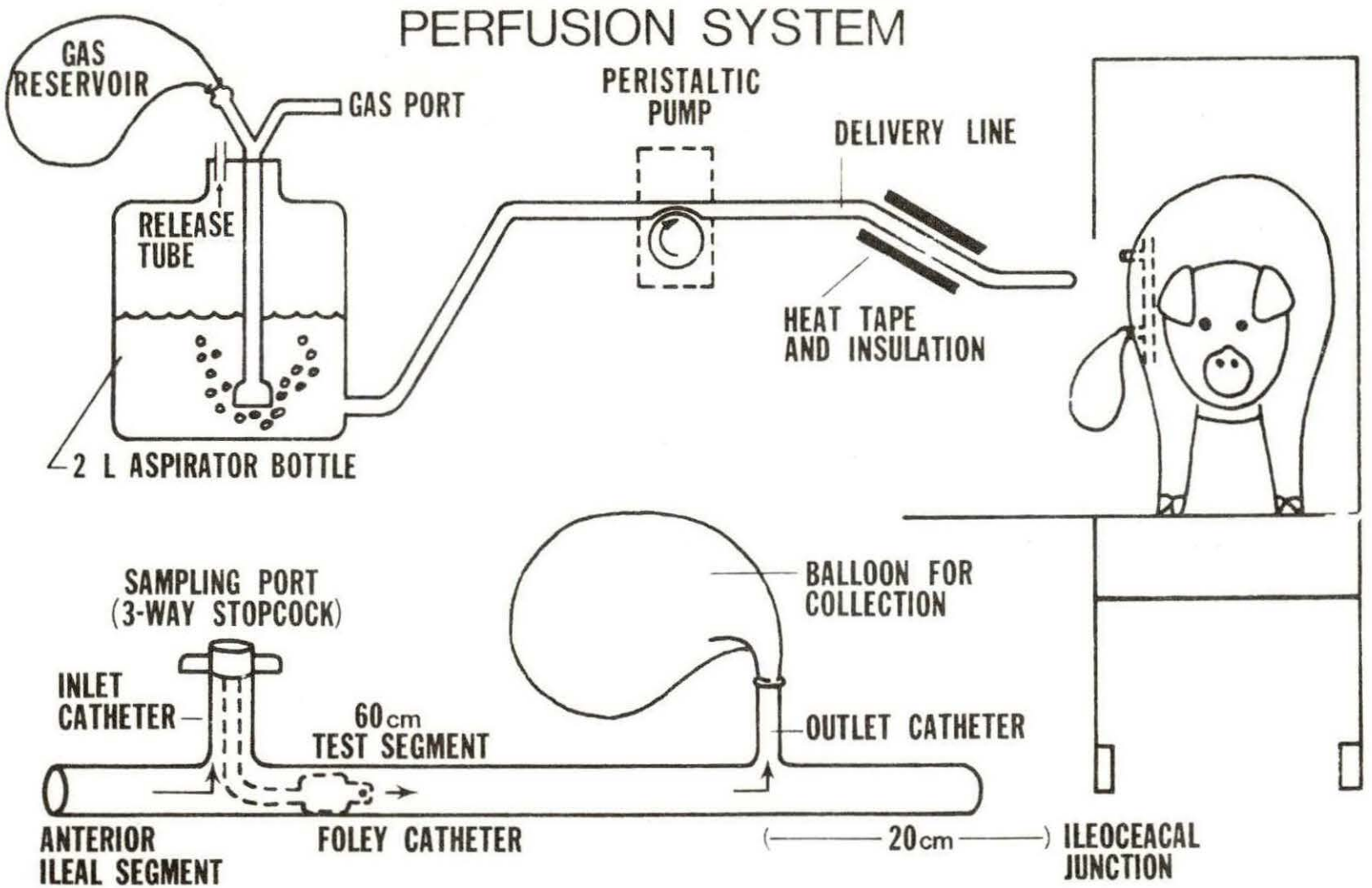
gain of 0.40 $\pm$ 0.02 (SE) kg/day. A summary of the postmortem data can be found in Appendix B. No unusual conditions were noted which would appear to invalidate the experimental data.

Description of the Perfusion System

The perfusion system used in Part I consisted of a delivery system and collection system. The arrangement of the apparatus is shown schematically in Figure 5. Solutions were prepared and placed in 2 liter aspirator bottles with the outlets closed. A stopper was then placed in each bottle opening. Each stopper had been bored with two holes and a Y-tube had been inserted into one. The end of the Y-tube in the bottle was connected to a gas dispersion rod. One of the other ends of the Y-tube was connected to a 3 liter air bladder (gas reservoir) with a stopcock located between the tube and bladder. A second straight tube was inserted into the other hole which was bored and used as an outlet for gases (release tube) during gassing of the solution.

The procedure for preparing the solution for perfusion was as follows. The solution was gassed with a steady flow of CO_2 and N_2 gas through the dispersion rod and out the release tube until the desired pH was obtained. Samples for pH were taken from the bottle outlet. After 10-15 minutes of equilibration at the correct pH, the air bladder was evaculated and then refilled with the gas mixture. All outlets from the bottle were then closed except for the connection between the bladder and bottle. The outlet from the bottle was then connected to the delivery line and opened. As the volume in the sealed bottle decreased, gas from the bladder was drawn into the bottle and the pH maintained.

Figure 5. Schematic diagram of the perfusion system


PERFUSION SYSTEM
GAS RESERVOIR
GAS PORT
PERISTALTIC PUMP
DELIVERY LINE
RELEASE TUBE
HEAT TAPE AND INSULATION
2 L ASPIRATOR BOTTLE
SAMPLING PORT (3-WAY STOPCOCK)
BALLOON FOR COLLECTION
INLET CATHETER
60 cm TEST SEGMENT
OUTLET CATHETER
ANTERIOR ILEAL SEGMENT
FOLEY CATHETER
20 cm
ILEOCEACAL JUNCTION

Fluid from the bottle was moved through the delivery line by a peristaltic pump (Harvard Apparatus Co., Inc., 150 Dover Road, Millis, MA 02054) which delivered fluid to the test segment at 7.0 ml/minute. The delivery line was connected to a threeway stopcock (sampling port) which, in turn, was connected to a Foley catheter placed in the ileal segment. The delivery line between the pump and the Foley catheter was wrapped with a heat tape and insulation and was adjusted to deliver the solutions to the intestinal lumen at 39 C. Addition of the threeway stopcock permitted sampling of the solution just before entrance into the gut segment. The Foley catheter was inserted into the inlet cannula so that when the balloon was inflated, the intestinal lumen was sealed just posterior to the lumen of the cannula but the flow of liquid from the anterior portion was not impeded. From the delivery system, the fluid passed through the test segment and out the outlet cannula.

During collection, a thick-walled large balloon (approximate unstretched volume = 250 ml) was connected to the cannula. This balloon had been modified by insertion of the barrel of a 1 cc tuberculin syringe into the end of the balloon and then sealed by a strapping band. Each balloon was daily checked for structural patency by excessive inflation. The end was sealed and the balloon then squeezed and checked for leaks while submerged in water. In addition, an initial and subsequent checks of each balloon were made by filling the balloon with a gassed solution and checking the pH and pCO_2 over a one-hour period. The pCO_2 rarely shifted more than 5-8 mm Hg after one hour. A fluctuation in excess of 10 mm Hg could be attributed either to error in

sampling or structural flaw.

Experimental Technique

On the day of an experiment, the pig was weighed and placed in a metabolism cage. The cannulae were then opened and a cuffed Foley catheter was pushed distally in the large (anterior) cannula and advanced into the intestinal lumen. The balloon was inflated to seal the lumen but not the cannula. This was done to prevent passage of ingesta into the isolated segment as well as reflux from the experimental segment. Upper gut fluid could still pass out of the cannula around the Foley catheter. The effectiveness of the balloon was periodically checked by instilling phenol red into the intestinal lumen anterior to the balloon to see if any signs of dye appeared in the distal cannula effluent. The Foley catheter was then connected to the perfusion system described previously.

During each experiment, the pig was perfused with both a control solution and an acetate solution at the same pH. The first solution selected for use on a given day was used to flush the test segment until the effluent was clear. Solutions were perfused at a rate of 7.0 ml/minute via a peristaltic pump. Prior experiments had shown that this rate produced essentially no differences in water and electrolyte flux and a more constant efflux of the perfusate from the segment than slower rates.

Collection of anaerobic samples was begun after the segment had equilibrated at least for 60 minutes after the segment was clear of digesta. An initial sample of the solution entering the test segment

was collected in a syringe via a threeway stopcock attached to the Foley catheter. At the same time, a heavy rubber balloon was evacuated and connected to the outlet catheter and the effluent collected over a fifteen minute period. The balloon collection system was previously described. At the end of the collection, the balloon was removed and a sample of the contents taken from the balloon outlet with a syringe. Four or more consecutive collections were made.

The second solution was then perfused and an initial bolus of phenol red was injected into the Foley via the stopcock to indicate clearance of the first solution. After a 60-minute equilibration period, four or more consecutive samples were again collected.

At the conclusion of an experiment, the Foley catheter was removed, the catheters were stoppered, and the animal was returned to the animal room and fed. The interval between experiments on the same animal ranged from alternate days to as much as two weeks.

A sequence was originally devised to vary the order for administering the different solutions and adjusting the pH to minimize any bias. However, this sequence had to be altered due to the loss of one animal, inability to use an animal on a given day when loose stools were observed, administration of the wrong solution first, lack of CO_2 for gassing solutions, and improper preparation of a solution. Some experiments were conducted using only one solution per day when time did not permit testing both or when the animal became extremely irritable.

The following sequence was actually used for each pig:

Pig #982--CA7, A7, CA6

Pig #005--CA6, CA7, C7, A7, CA7, C7, AC7, AC6, A7, AC6

Pig #932--A6, C6, AC6, C7, AC7, A6, C7, AC7, CA7, C6

Pig #47--AC6, CA7, AC7, CA6, A6, CA7, AC6, CA6

(Note: A = acetate solution, C = control solution, 6 = pH 6.5, 7 = pH 7.4. For example, CA7 represents perfusion of the control solution first, followed by the acetate solution. The initial pH of both solutions was pH 7.4.)

The actual composition of the test solution is shown in Table 1. These solutions were selected to approximate the plasma concentrations of the electrolytes with the exception of acetate. Acetate was added at a concentration approximating that reported for the pig small intestine (5,31). Additional chloride substituted for acetate in the acetate-free control solutions. The respective pHs were chosen to approximate the pH of the plasma and the pH reported for the distal small intestine of the pig (5). Polyethylene glycol (PEG 4000), a nonabsorbable marker, was added to all the solutions at a concentration of 1 g/liter.

Upon the completion of all experiments on a pig, the animal was euthanized and the condition of the ileal segment was examined. The segment length was considered to be the distance between the tips of the cannulae in the lumen. The mucosa was also stripped, dried, and the dry mucosal weight was determined. These data can be found in Appendix B.

Analysis

Analysis of the anaerobic samples for pH, pCO_2, and HCO_3^- was done with a Corning Model 165 pH/Blood Gas Instrument (Corning Scientific Instruments, Medfield, MA 02052). Osmolarity was measured by freezing

Table 1. Composition of test solutions in the perfusion experiments

	Solutions[a]			
	pH 6.5		pH 7.4	
	Acetate	Control	Acetate	Control
Na^+	145.0 ± 0.4	144.0 ± 0.5	145.7 ± 0.6	144.8 ± 0.9
K^+ (mM/liter)	5.1 ± 0	5.1 ± 0	5.0 ± 0	5.0 ± 0.1
Cl^-	100.6 ± 0.4	140.0 ± 0.9	100.7 ± 0.4	140.6 ± 0.8
HCO_3^-	9.2 ± 0.3	8.4 ± 0.2	7.1 ± 0.1	6.6 ± 0.1
CH_3COO^-	40.1 ± 0.4	---	40.0 ± 0.3	---
Osmolarity (mOsm/liter)	289.6 ± 1.1	287.2 ± 0.8	286.8 ± 1.6	286.1 ± 1.1
PEG	- - - - - - - - - - 1 gram/liter - - - - - - - - - -			
pH	6.53 ± 0	6.53 ± 0.01	7.42 ± 0.02	7.41 ± 0.01
Perfusion rate (ml/min)	6.6 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.7 ± 0

[a]Concentrations expressed as mean ± standard error.

point depression with a Fiske osmometer (Fiske Associates, Quaker Highway, Uxbridge, MA 01569). Polyethylene glycol was analyzed in duplicate by the method of Hyden (72) in which the absorption was measured with a Beckman Model DB-G Grating Spectrophotometer (Beckman Instruments, 2500 Harber Blvd., Fullerton, CA 92634). Sodium, K^+, and Cl^- were measured on either a Technicon Autoanalyzer (Technicon Instruments, 511 Benedict Ave., Tarrytown, NY 10591) or a Perkin-Elmer Model 146 Flame Photometer (Perkin-Elmer, 800 Main Ave., Norwalk, CT 06856). Acetate samples were frozen at the conclusion of an experiment, later prepared by the method described by Salanitro and Muirhead (99), and then measured on a Hewlett-Packard Model HP 5830A Gas Chromatograph (Hewlett-Packard, Route 41, Avondale, PA 19311).

Dillard et al.'s method (46) was employed to determine transit time for three of the pigs. Phenol red (1 mg/ml) was injected into the inlet catheter during apparent steady state conditions. Samples were collected at 1-3 minute intervals from the distal cannula. The samples were alkalinized with NaOH and absorption read on a Beckman Model DB-G Grating Spectrophotometer at 558 nm. Dye dilution curves were constructed and transit times determined. The mean transit time for the three pigs was 8.4 ± 0.1 (SE) minutes.

Absorption or secretion of water or solute was calculated from the following formulae:

$$\text{Net solute transfer} = (\text{Solute}_{in}) \times (\text{Flow rate}) - (\text{Solute}_{out}) \times (\text{PEG}_{in}/\text{PEG}_{out}) \times (\text{Flow rate})$$

$$\text{Net water transfer} = (\text{Flow rate}) - (\text{Flow rate}) \times (PEG_{in}/PEG_{out})$$

(Note: Solute and PEG = concentration.).

A factorial treatment design was employed to prepare the data for analysis. In this design, the treatments were grouped as combinations of two factors (acetate and control solutions), each at two levels (pH 6.5 and 7.4). The data was then statistically evaluated by analysis of variance (102,117). The total sums of squares was partitioned to provide individual comparisons between the four combinations.

The data for each measured parameter was prepared for statistical analysis in the following manner. A mean net transfer of electrolyte or water for each experiment was calculated from the measurement of the four (sometimes 4-7) samples collected. Each of these means for a specific treatment was used to determine the mean change per treatment for each individual pig. The mean changes for each of the four pigs for a given treatment were then used to determine the mean transfer for a given treatment.

RESULTS

Preliminary Comments

Summaries of the raw data used to derive the results presented in this section may be found in Appendices C through F. The mean changes per experiment have been reduced to mean changes per animal per treatment perfused as either the first or second solution and presented in Appendix D. The order in which a given treatment was administered, i.e., first or second, did not affect the results as no significant differences were observed. The mean changes in the various parameters for each pig receiving a respective treatment may be found in Appendix E. A summary of the treatment means for all parameters is given in Appendix F. The mean changes of the combined treatment for each pig are summarized in Appendix G.

The rates of absorption of acetate and other electrolytes were not expressed either as change per gram of dry mucosa or as change per centimeter of test segment. Segment length and dry weight were obtained at postmortem examination (Appendix B). Since the experiments with a given pig were conducted over lengthy and varying periods of time, the results may not reflect length and weight during the initial experiments. All values in the results, discussion, or appendix are expressed as mean $\pm$ SE.

Great variability in results not only between pigs receiving the same treatment but also between data for an individual pig for a given treatment is evident in Appendices D, E, and G. Although every effort to standardize experimental conditions was made, animal variation and distractions, such as laboratory noise, may not have been uniform.

Whether the variability is due to those factors, error in analysis, or a different physiological response of unknown etiology cannot be determined. Substantial, yet normal, variation has been reported in other studies (58,127).

Movement of Electrolytes and Water

The pattern of water, total solute, and the individual electrolyte movements observed for the respective treatments is shown in Figures 6, 7, and 8. A mean net absorption of total solute, water (Figure 6), Na^+ (Figure 7), Cl^- and acetate (Figure 8) was observed for all treatments. Despite the mean net absorption, net secretion was observed in a few of the 49 experiments conducted. Sodium secretion was observed in 6 experiments; K^+ secretion in 19, total solute in 11, and water secretion in 9. Although the observed secretions were equally distributed over the treatments, they were predominant in two pigs (#47 and #932). Bicarbonate (Figure 8) was secreted in all but one experiment. Acetate and Cl^- were absorbed in all experiments.

There was a highly significant correlation ($p < 0.0001$) between the movement of water and total solute in all treatments. This correlation is anticipated, as it is generally believed that water movement is secondary to solute absorption. The high level of significance may imply the absence of other factors which may influence water movement, such as increased hydrostatic pressure.

Solute absorption was predominantly due to Na^+, Cl^-, and acetate absorption; therefore, correlation coefficients for the movement of these ions were computed to determine any relationship between their

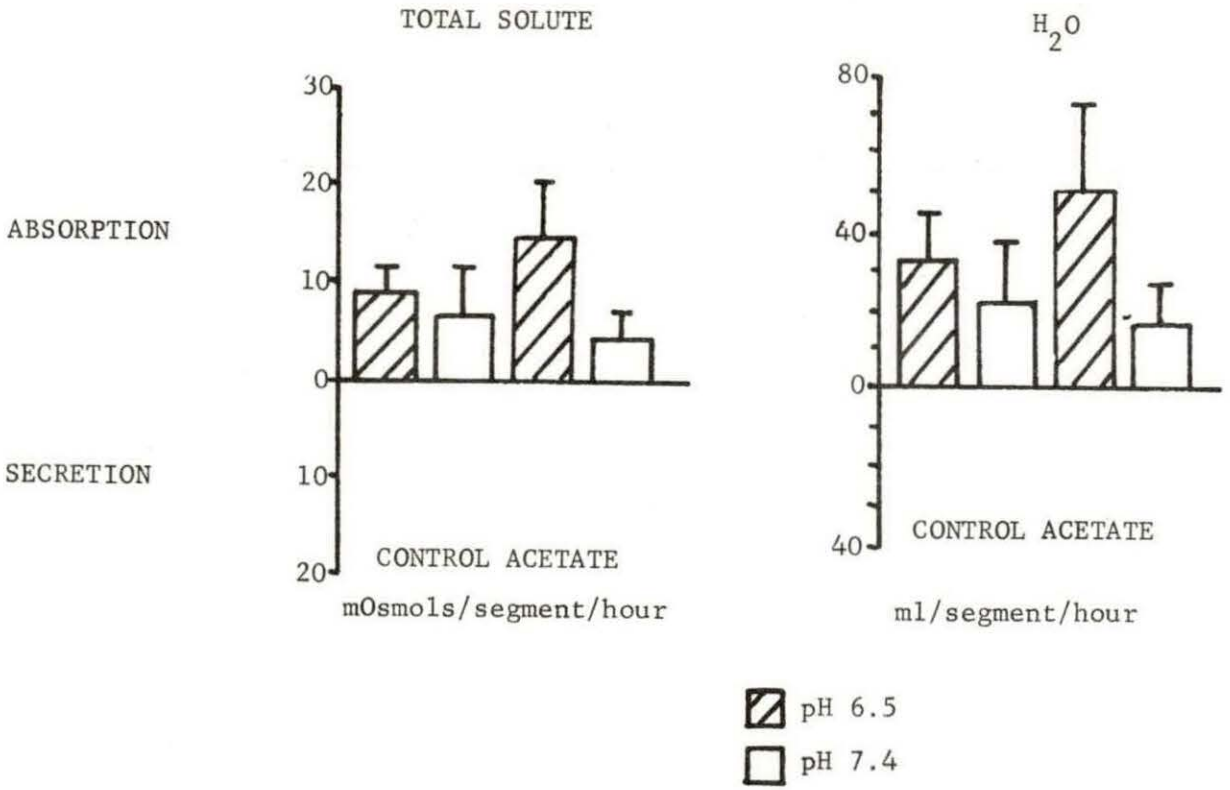


Figure 6. Movement of total solute and water in the perfusion experiments (mean ± SE)

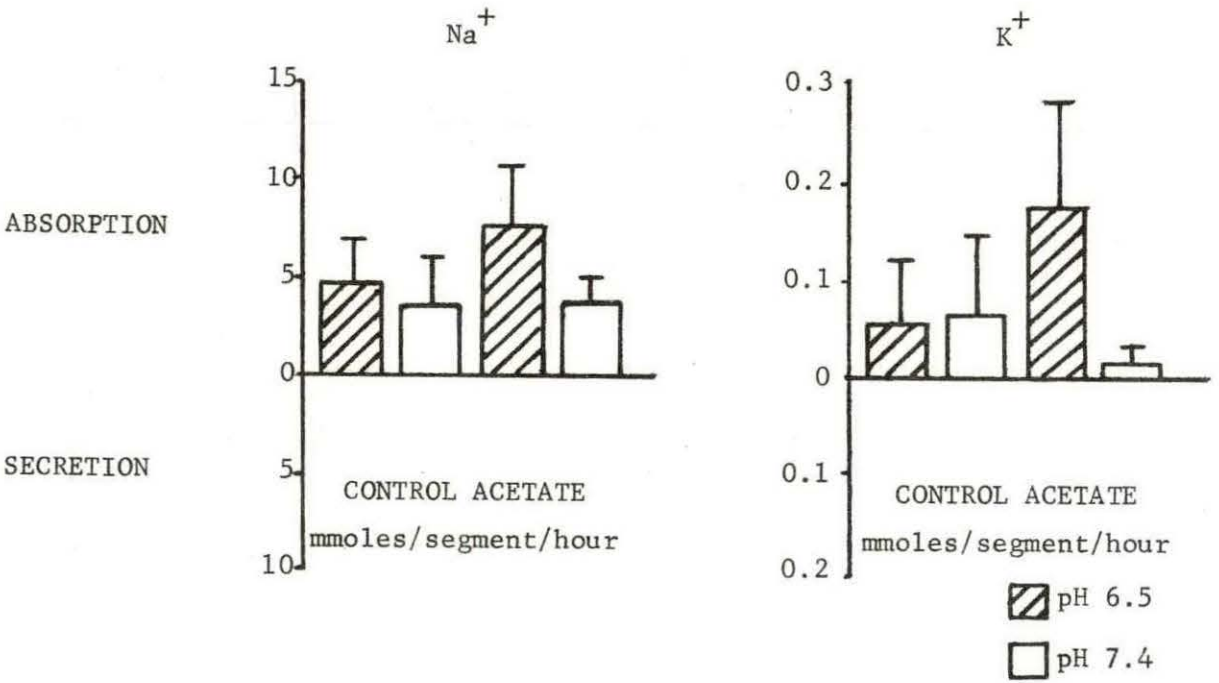


Figure 7. Movement of Na^+ and K^+ in the perfusion experiments (mean $\pm$ SE)

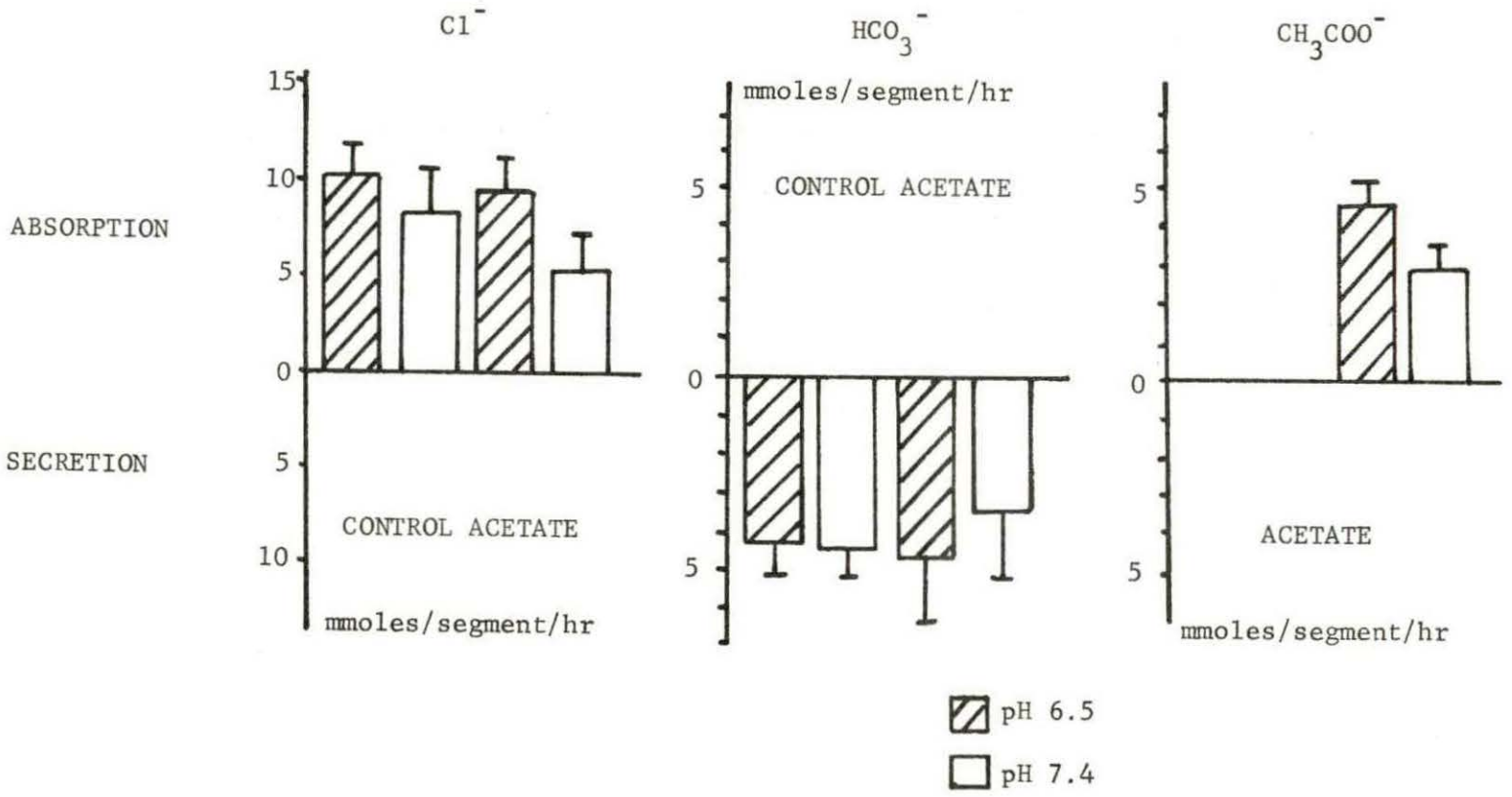


Figure 8. Movement of Cl^-, HCO_3^-, and acetate in the perfusion experiments (mean $\pm$ SE)

respective movements. Sodium and Cl^- absorption were very significantly correlated in both control treatments and significantly correlated in the acetate treatment at pH 6.5 ($0.004 \leq p \leq 0.02$). The less significant correlation in the presence of acetate probably reflects the lower concentration of Cl^- present in the perfusion solution. Sodium and acetate absorption were also highly correlated at pH 6.5 ($p < 0.002$) but not at pH 7.4 ($p < 0.06$). This observation might be attributed to an inhibition of the nonionic absorption of acetate by increasing the pH. Chloride absorption was also correlated with acetate at both low ($p < 0.004$) and high pH ($p < 0.02$). This correlation would be expected not to involve a direct relationship between Cl^- and acetate but probably involves a coupling mechanism in which Na^+ is the common ion.

The reciprocal movement of Cl^- and HCO_3^- (Figure 8) is in agreement with the ileal exchange of Cl^- for HCO_3^-, as described by Turnberg et al. (128). However, these anions were negatively correlated only for the acetate ($p < 0.01$) and control ($p < 0.0009$) perfusions at pH 7.4. Correlation at low pH may not have been apparent due to the high CO_2 tensions which were used to maintain the pH. No relationship was observed between acetate and HCO_3^-. A complete listing of the correlation coefficients for the respective treatment can be found in Appendices H, I, J, and K.

Acetate Absorption

The rates of absorption of acetate by the ileum at both pH 6.5 and 7.4 were not significantly different (Figure 8). Although not

significant, the rate of acetate absorption of 4.75 ± 0.52 mmoles/segment/hr at pH 6.5 did exceed the rate of 3.11 ± 0.59 mmoles/segment/hr at pH 7.4 by more than 50%. Acetate absorption at the low pH probably would have been greater but a rapid neutralization of the gut lumen was observed with the low pH perfusions.

Changes in the pH of the test solutions have been summarized in Table 2. The initial mean pH of the acetate solution entering the test segment was 6.53 ± 0.01, but the effluent collected over fifteen minutes had a mean pH of 7.26 ± 0.16. When the same solution at pH 7.4 was perfused, the initial mean pH of 7.42 ± 0.02 increased nonsignificantly to 7.60 ± 0.04 during the same fifteen minute collection interval. However, the mean transit time, calculated for three of the four pigs, was 8.4 ± 0.1 ml/min. This would indicate a more rapid shift in the pH than the final measurements at fifteen minutes would indicate (Table 2).

It would appear from the observed shifts in CO_2 tensions (Table 2) that much of the increase in pH of the effluent in the experiments conducted at low pH could be attributed to rapid diffusion of CO_2 from a tension varying from 90-120 mm Hg (as the solutions entered the segment) down to a tension of 45-60 mm Hg (as measured in the effluent). Carbon dioxide tensions appeared to be equilibrating with levels in the plasma. Such a diffusion could explain the observed increase in pH, decrease in pCO_2, and HCO_3^- concentrations comparable to those observed at pH 7.4 for both test solutions. Schmitt et al. (105,106) have reported a similar luminal alkalinization associated with SCFA

Table 2. Initial and final measurements of pCO_2, HCO_3, and pH in the perfusion experiments[a]

pH 7.4				pH 6.5			
Control		Acetate		Control		Acetate	
Initial	Final[b]	Initial	Final	Initial	Final	Initial	Final
			pCO_2 (mm Hg)				
10.5 +0.6	19.1[c**] +2.4	10.7 +0.4	17.1[c*] +1.1	102.8 +1.8	46.5[c***] +2.6	109.3 +2.2	51.5[c***] +8.4
			HCO_3^- (mmoles/liter)				
6.7 +0.1	11.1[c] +1.0	7.0 +0.01	11.1[c] +1.0	8.5 +0.2	12.7[c***] +0.9	9.2[d] +0.1	13.9[c***] +1.7
			pH[e]				
7.41 +0.01	7.60 +0.02	7.42 +0.02	7.60 +0.04	6.53 +0.01	7.38 +0.10	6.53 +0.01	7.26 +0.16

[a]Measurements expressed as mean $\pm$ S.E., N = 4 pigs.

[b]Final measurement at end of fifteen minute continuous collection of effluent.

[c]Significantly different than initial value.

[d]Significantly different than control at same pH ($p < 0.05$)

[e]All final values significantly greater than initial value ($p < 0.0001$)

*$p < 0.01$.

**$p < 0.025$.

***$p < 0.001$.

absorption. However, the shifts in pH in this study were similar for both control and acetate solutions. The rapid shift in pH due to CO_2 diffusion out of the lumen appears to have been the major influence on pH. Any change in pH due to SCFA absorption was apparently obscured by the diffusion of CO_2.

Trace amounts of propionic acid were recorded in the effluent during two experiments with one pig (#0005). In the first experiment, 0.06 and 0.09 mmoles/liter were measured in consecutive samples during perfusion of the acetate solution at pH 7.4. Propionic acid was detected in a single sample in another experiment during perfusion of the acetate solution at pH 6.5 at a concentration of 0.16 mmoles/liter. The source of the propionate may have been either contamination of the segment due to leakage, reflux, or endogenous secretion. The small quantities detected would be expected to have negligible influence on this study.

Effects of Acetate on Water and Electrolyte Movement

A comparison of the rates of absorption of water and the various electrolytes from the control and acetate solutions at pH 7.4 has been made (Table 3). No significant differences were noted between the respective solutions for any of the parameters measured. Despite acetate absorption at approximately the same rate as Na^+, acetate absorption neither significantly correlated ($p < 0.06$) with Na^+ absorption nor influenced Na^+ movement.

Absorption of either Cl^- or Cl^- plus acetate from the respective solutions was more than twice the magnitude of Na^+ absorbed. Bicarbonate

Table 3. Effects of acetate on water and electrolyte absorption at pH 7.4

Parameter[a]	Control solution (mean ± SE[b], N = 3[c])	Acetate solution (mean ± SE, N = 4)	Effect of acetate	F-value	PR > F
Na	3.58 ± 2.11	3.56 ± 1.36	No change	0.60	.461
K	0.07 ± 0.08	0.02 ± 0.02	No change	1.31	.285
Cl	8.23 ± 2.83	5.22 ± 1.80	No change	2.86	.129
HCO_3	-4.45 ± 0.66[d]	-3.99 ± 1.00	No change	0.74	.414
Acetate	---	3.11 ± 0.59	---	--	--
H_2O	23.09 ± 15.14	17.75 ± 9.39	No change	0.86	.381
Total solute	6.30 ± 4.51	4.76 ± 2.71	No change	0.81	.394

[a]Absorption of all parameters, except water, expressed in mmoles/test segment of intestine/hour. Water absorption expressed as ml/segment/hr. Total solute expressed as mOsmol/liter/hr.

[b]SE = standard error of mean.

[c]N represents the number of pig means used to derive a parameter mean. Each pig mean is derived from varying numbers of experiments and samples.

[d]Negative values indicate secretion into the lumen.

secretion accounted for the difference between the quantities of anion and Na^+ absorbed. This is consistent with the $Cl\text{-}HCO_3$ coupling or anion exchange described by Turnberg et al. (128) in the human ileum. Increases in lumen HCO_3^- concentration and pH of the effluent would support such an exchange (Table 2).

The net changes in water and electrolytes from the same control and acetate solutions at an initial pH of 6.5 were compared (Table 4). While no statistically significant differences between solutions were apparent, Na^+, total solute, and water absorption were 60% greater in the presence of acetate. Although the rate of Na^+ absorption exceeded the rate of acetate absorption, the rates were not significantly different. However, the absorption of acetate and Na^+ were highly correlated ($p < 0.002$). Chloride and HCO_3^- movements were unaffected by acetate at the low pH. The increased net solute absorption in the presence of acetate is accounted for by the increase in Na^+ and acetate absorption. As with the solutions at pH 7.4, the difference between anion and Na^+ absorption was balanced by HCO_3^- secretion.

If acetate absorption occurs by nonionic diffusion of acetic acid, then the previously described upward shift in the pH of the solutions would dampen the rate of acetate absorption. If acetate absorption influences Na^+ absorption, as shown in other species (6,8,37), then the results may have been significant had it not been for the changes in pH. It should be assumed that the results do not represent absorption throughout the intestinal segment at pH 6.5; and, therefore, the effects of acetate at low pH on solute and water movement cannot be accurately

Table 4. Effect of acetate on water and electrolyte absorption at pH 6.5

Parameter[a]	Control solution (mean ± SE[b], N = 4[c])	Acetate solution (mean ± SE, N = 4)	Effect of acetate	F-value	PR > F
Na	4.76 ± 1.81	7.57 ± 3.11	No change	2.31	.167
K	0.05 ± 0.08	0.18 ± 0.14	No change	1.72	.226
Cl	10.06 ± 1.62	9.54 ± 1.43	No change	0.08	.784
HCO_3	-4.30 ± 0.91[d]	-4.74 ± 1.72	No change	0.33	.581
Acetate	---	4.75 ± 0.52	---	--	--
H_2O	33.25 ± 13.35	52.99 ± 22.03	No change	1.65	.235
Total solute	9.25 ± 3.80	15.00 ± 5.96	No change	1.74	.224

[a]Absorption of all parameters, except water, expressed in millimoles/test segment of intestine/hour. Water absorption expressed as ml/segment/hr. Total solute expressed as mOsmol/liter/hr.

[b]SE = standard error of mean.

[c]N represents the number of pig means used to derive a parameter mean. Each pig mean is derived from varying numbers of experiments and samples.

[d]Negative values indicate secretion into the lumen.

assessed.

Effects of pH on Water and Electrolyte Movement

The responses of the control solutions, perfused at both pH 6.5 and 7.4, were compared to determine if pH in the absence of acetate in the perfusion solutions had an influence on electrolyte movement (Table 5). No significant differences were observed. However, Na^+, Cl^-, water, and total solute movement were slightly greater at the lower pH. Because of the pH shift, previously described, it is not possible to conclude what influence pH had on the movement of solute and water in these experiments.

In contrast to the effects of pH on the control solution and despite the rapid increase in the pH, decreasing the initial pH of the acetate solution appeared to have produced a substantial change in the net absorption of all measured parameters (Table 6). Mean absorption of Na^+ ($p < 0.06$), K^+, Cl^- ($p < 0.043$), acetate, total solute ($p < 0.047$), and water ($p < 0.051$) all increased. The absorption of Na^+ and Cl^- increased nearly twofold, while the rates of water and total solute increased three- and fourfold. Increased acetate absorption at the lower pH would support uptake of that ion, as the acid, by nonionic diffusion. The observed increase in absorption of acetate may not have been significant because of the rapid change in pH. Unfortunately, this could not be determined.

Table 5. Effects of pH in the absence of acetate in the perfusion solution on absorption of water and electrolytes

Parameter[a]	pH 6.5 mean ± SE[b], N = 4[c]	pH 7.4 mean ± SE, N = 3	Effect of decreasing pH	F-value	PR > F
Na	4.76 ± 1.81	3.58 ± 2.11	No change	0.02	.891
K	0.05 ± 0.08	0.07 ± 0.08	No change	0.63	.450
Cl	10.06 ± 1.62	8.23 ± 2.83	No change	0.99	.349
HCO_3	-4.3 ± 0.91[d]	-4.45 ± 0.66	No change	1.58	.244
H_2O	33.25 ± 13.35	23.09 ± 15.14	No change	0.01	.923
Total solute	9.25 ± 3.80	6.30 ± 4.51	No change	0.02	.891

[a]Absorption of all parameters, except H_2O, expressed in millimoles/test segment of intestine/hr. Water is expressed in ml/segment/hr. Total solute expressed as mOsmol/liter/hr.

[b]SE = standard error of mean.

[c]N represents the number of pig means used to derive a parameter mean. Each pig mean is derived from varying numbers of experiments and samples.

[d]Negative values indicate secretion into the lumen.

Table 6. Effects of pH on the absorption of water and electrolytes from the acetate solutions in the perfusion experiments

Parameter[a]	pH 6.5 mean ± SE[b], N = 4[c]	pH 7.4 mean ± SE, N = 3	Effect of decreasing pH	F-value	PR > F
Na^+	7.57 ± 3.11	3.56 ± 1.36	Increase	4.698	0.062
K^+	0.18 ± 0.14	0.02 ± 0.02	No change	2.766	0.135
Cl^-	9.54 ± 1.43	5.22 ± 1.80	Increase	5.746	0.043
HCO_3^-	-4.74 ± 1.72[d]	-3.99 ± 1.00	No change	0.942	0.360
CH_3COO^-	4.75 ± 0.52	3.11 ± 0.59	No change	4.000	0.084
H_2O	52.99 ± 22.03	17.75 ± 9.39	Increase	5.528	0.047
Total solute	15.00 ± 5.96	4.76 ± 2.71	Increase	5.260	0.051

[a]Absorption of all parameters, except H_2O, expressed in mmoles/segment/hr. Water is expressed in ml/segment/hr. Total solute expressed as mOsmol/liter/hr.

[b]SE = standard error of mean.

[c]N represents the number of pig means used to derive a parameter mean. Each pig mean is derived from varying numbers of experiments and samples.

[d]Negative value indicates secretion into the lumen.

Summary of Results--Part I

Several conclusions can be made from the results of the studies just described. First, acetate is absorbed by the pig ileum at an initial concentration in the perfusion solution which approximates a concentration previously reported in the normal pig ileum. It can be inferred from the results that this absorption increases at lower pH. Second, the interaction of pH and acetate were shown to increase the absorption of water and total solute. Increased total solute absorption was due to increases in the rates of Na^+ and Cl^- absorption and the absorption of acetate. Finally, because of the marked change in the pH of the low pH solutions, the actual significance of the influence of acetate and pH on water and electrolyte absorption could not be determined.

PART II. COMPARISON OF THE INFLUENCE OF ACETATE ON WATER AND ELECTROLYTE MOVEMENT BETWEEN THE PORCINE JEJUNUM AND ILEUM

INTRODUCTION

The great variability in results between and within experiments in Part I, the inconclusive results, and an attempt to decrease the duration of the experiments resulted in a second series of experiments with a slightly different protocol. The single-pass steady-state perfusion of the test segment was replaced by a recirculation technique. This recirculation method employed acute preparations of small intestinal segments and maintenance of anesthesia throughout the experiment. Argenzio and Lebo (4) have previously demonstrated in studies of the pig colon that results obtained using a recirculation technique and anesthesia were not significantly different from data obtained with a perfusion method. A pH of 7.4 in the perfusion studies had little effect on water and electrolyte movement. Therefore, it was decided to further examine the influence of acetate only at pH 6.5. The recirculation method was also a means of correcting the shift in pH observed with the low pH solutions in Part I. In these experiments, either an acetate or acetate-free solution was recirculated through the test segment in which the pH of the solution was maintained at pH 6.5 by the constant addition of carbon dioxide.

Differences in function within the various regions of the small intestine are well-known and were briefly discussed in the literature review. The pig ileum normally has a higher concentration of SCFA than the jejunum. Schmitt et al. (105) demonstrated rapid SCFA absorption but little effect on water and electrolyte movement in the human jejunum. Therefore, a jejunal segment was also prepared and tested to provide comparative data. Nine segments of both jejunum and ileum were

examined in these recirculation experiments.

The objectives of these experiments were (a) to determine the relative rates of acetate absorption in the jejunum and ileum at a constant pH (6.5), (b) to determine if acetate enhances water and electrolyte absorption in the jejunum or ileum, and (c) to compare the responses of the jejunum and ileum to the influences of acetate on water and electrolyte movement.

MATERIALS AND METHODS

Animals

Fourteen NADC pigs weighing approximately 15-25 kg were surgically prepared for acute experiments. Food, but not water, was withheld 36 hours prior to the experiment. Halothane was used to induce and maintain anesthesia. Once the animals were well-anesthetized, an endotracheal tube was inserted. On several occasions, intubation was difficult; consequently, intubation was delayed until well into the experiment or the pig was maintained with an anesthesia cone throughout the experiment.

Surgery[1]

A 10-15 cm incision was made on the right flank parallel to the ribs and approximately midway between the last rib and pelvis. A pair of cannulae were placed either in a segment of jejunum or ileum or both. All cannulae used in a given pig were the same diameter and made from Silastic[R] tubing with either a 3/8 inch i.d. and 1/2 inch o.d. or 1/4 inch i.d. and 3/8 inch o.d. The luminal end of the cannula had a sleeve which was made by slipping a 1 cm piece of Silastic[R] tubing of a larger bore over the end of the cannula and gluing it in place with medical grade Silastic[R] adhesive. Approximately 1 cm of cannula protruded from the sleeve.

Jejunum

The jejunum was ligated at point approximately 20 cm from the ligament of Trietz. A longitudinal incision in the antimesenteric wall,

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[1]Surgery performed by Dr. R. A. Argenzio, N.A.D.C., Ames, Iowa.

about 1 cm long, was made just distal to this ligature and the cannula was inserted into the lumen. Once in place, the cannula was secured with a silk ligature just distal to the sleeve. Chromic 2-0 gut suture material was used to close the incision around the cannula. The second cannula was inserted by the same technique 60 cm posterior to the initial cannula. Both cannulae were exteriorized via stab wounds in the body wall anterior to the skin incision. Care was taken not to twist or otherwise occlude any portion of the test segment.

Ileum

The cecum was located and the ileum was ligated 20 cm anterior to the ileocecal junction. The same technique, as described for the jejunum, was used to insert a cannula anterior to the ligature. Likewise, a second cannula was inserted 60 cm anterior to the first. Both cannulae were exteriorized through stab wounds posterior to the skin incision.

The peritoneum, three muscle layer, and skin were then closed with Chromic 0 gut suture. All surgical techniques were done aseptically.

The right femoral artery was then exposed through a 10 cm incision and cannulated with a 15 cm catheter. This catheter was used to record arterial pressure and obtain blood samples. The incision was closed with Chromic 0 gut suture. The patency of the catheter was maintained by periodic flushing with heparinized saline (20 U/ml).

Description, Construction, and Operation of the Recirculation System

The recirculation system is schematically presented in Figure 9. Construction of the various component and general operation of the apparatus is described in the following sections.

Perfusion reservoir

The reservoir was made from a 500 ml Nalgene^{R} (Nalge Company, P.O. Box 365, Rochester, NY 14602) bottle. The bottle top was capped with a #6 rubber stopper through which a glass tube was inserted. Tygon tubing was connected to the glass tube. This tubing then served as a return line from the pig. The bottom of the bottle was then removed. The bottle was inverted and secured in a Napco (National Appliance Company, Box 23008, Portland, OR 97223) Model 220 water bath. The fluid level in the chamber was 6-10 cm above the loop end of the cannulae. The water bath was adjusted to maintain the solution at 39 C.

Reservoir top

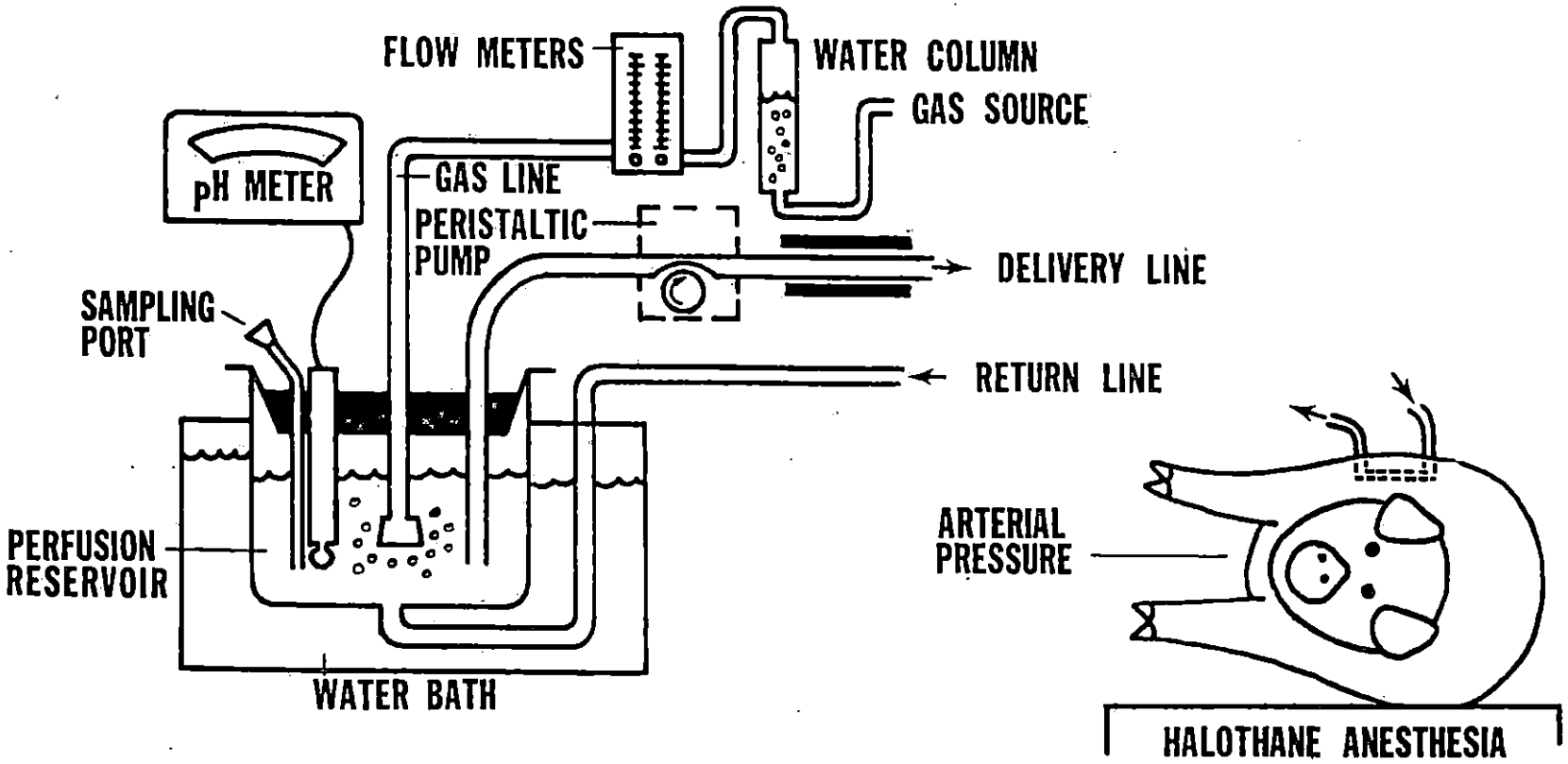
The reservoir top was made by removing the bottom 2/3 of a 250 ml Nalgene^{R} beaker so that the top portion with the flanges would tightly fit inside the reservoir. Three holes were bored in a #13 rubber stopper to accommodate a delivery line, gas line, and pH electrode/sampling port. The rubber stopper snugly fit in the beaker.

Gas line

Carbon dioxide and nitrogen gases were delivered to flowmeters after passing through a column of water to insure saturation. The

Figure 9. Schematic diagram of the recirculation system

RECIRCULATION SYSTEM



calibrated gases were mixed and bubbled into the chamber through a gas dispersion rod. The respective gas ratios were altered throughout the experiment to maintain the solution at a relatively constant pH 6.5.

pH meter

A pH probe was inserted into the chamber through one hole in the chamber top. The pH could then be constantly monitored on a Beckman (Beckman Instruments, 2500 Harber Blvd., Fullerton, CA 92634) Model 76 Expanded Scale pH meter. The gas mixture was adjusted accordingly.

Sampling port

A short length of plastic tubing attached to an 18 gauge needle was used to connect the chamber to the sampling syringe.

Delivery system

A glass rod was inserted in the chamber top until the rod almost touched the bottom of the chamber. Tygon^{R} tubing was used to connect the rod and the anterior intestinal cannula. The solution in the chamber was pumped back to the pig by a peristaltic pump (Hewlett Packard, 1776 Minute Man Rd., Andover, MA 01810) which was located between the chamber and the anterior or inlet cannula. A heat tape was also wrapped around the tubing to maintain the solution at 39 C.

Experimental Procedure

Immediately upon conclusion of surgery, the pig was repositioned on the left flank. A heating pad and surgical drapes were used to maintain body temperature. The arterial catheter was connected to a

Sanborn Model 267 BC pressure transducer (Hewlett Packard, 1776 Minute Man Rd., Andover, MA 01810) which was then connected to a Sanborn 350 Polygraph (Hewlett Packard, 1776 Minute Man Rd., Andover, MA 01810). A stopcock, located between the transducer and catheter, was used to collect arterial blood samples for blood gas determination.

The inlet or upper intestinal cannula was connected to the recirculation system. The segment was then flushed with normal saline at 40 ml/min until the effluent was clear. The segment was then rinsed with 400 ml of the test solution less the nonabsorbable marker (^{14}C-PEG) at 40 ml/min. After flushing the segment with air, the posterior cannula was connected to the return line which completed the recirculation circuit. Exactly 300 (exception: 200 or 250 ml were used in four initial experiments) ml of the test solution containing the nonabsorbable marker was added to the chamber. The solution was gassed until the pH was approximately 6.5. The segment was then perfused at 40 ml/min. When the solution returned to the chamber via the return line, a 10-minute equilibration period was begun. The rate was reduced to 20 ml/min after 10 minutes and zero time samples of the solution and blood were collected. Samples were also taken at 30 and 60 minutes. After the last sample was obtained, the chamber and segment were flushed with air and saline. This procedure was then repeated for the second test solution.

At the conclusion of the experiment, the animal was euthanized with pentobarbital. The abdomen was opened and the segment was examined for any abnormalities, such as twisting or obstruction. Both cannulae

were pulled back through the abdominal wall and the test segment was measured by transecting the segment at the tip of one cannula, clamping the end with a hemostat, and measuring the distance between the tips of the cannulae while the segment was gently suspended by the weight of the hemostat. Jejunal segments in nine pigs measured 65.8 $\pm$ 4.7 cm. The mean segment length in nine ileal preparations was 58.6 $\pm$ 2.5 cm. These means were not significantly different.

The composition of the test solutions was initially the same as described in Part I (Table 1). Polyethylene glycol labeled with ^{14}carbon was used as a nonabsorbable marker. All solutions were gassed with carbon dioxide and nitrogen to pH 6.5. Once the solutions were introduced into the intestinal segment from the recirculation system, the solutions were mixed and diluted by the residual fluid remaining in the gut lumen. The composition of the test solutions is, therefore, expressed as the concentration in the system at the end of the equilibration period. These concentrations are listed in Table 7. The sequence for administration of the acetate and control solutions was alternated with successive experiments to minimize bias.

Analysis

Blood samples and samples of the perfusate were measured for pH, pCO_2, and HCO_3^- with a Corning (Corning Scientific Instruments, Medfield, MA 02052) Model 165 pH/Blood Gas Instrument. Osmolarity was measured by freezing point depression with a Fiske (Fiske Associates, Quaker Highway, Uxbridge, MA 01569) osmometer. Polyethylene glycol, labeled with ^{14}C, was analyzed by liquid scintillation spectroscopy. Samples

Table 7. Composition of test solutions in the recirculation experiments at the time of initial sample[a]

	Jejunum		Ileum	
	Acetate	Control	Acetate	Control
Na^+	133.2 ± 3.8	129.1 ± 2.5	139.7 ± 2.4	139.4 ± 3.0
K^+	4.7 ± 0.1	4.8 ± 0.1	5.0 ± 0.1	5.1 ± 0.1
Cl^- (mmoles/liter)	95.3 ± 2.7	126.5 ± 2.5	94.8 ± 1.5	130.1 ± 2.3
HCO_3^-	9.8 ± 0.3	8.3 ± 0.4	11.3 ± 0.9	11.5 ± 0.3
CH_3COO^-	36.2 ± 0.5	---	35.7 ± 0.8	---
Osmolarity (mOsmol/liter)	293.8 ± 1.9	287.8 ± 1.8	292.7 ± 2.0	288.6 ± 1.3
Number of experiments	9	9	9	9

[a]Concentrations expressed as mean ± standard error.

of 0.5 ml were added to 15 ml of Bray's solution and counted on a Hewlett Packard (Hewlett-Packard, Route 41, Avondale, PA 19311) HP Model 3380 Tri-carb counter. Sodium and K^+ were measured on a Technicon (Technicon Instruments, 511 Benedict Ave., Tarrytown, NY 10591) Auto-analyzer. Chloride was measured with a Corning (Corning Scientific Instruments, Medfield, MA 02052) Chloride Meter 920M. Acetate samples were frozen at the conclusion of an experiment, later prepared by the method described by Salanitro and Muirhead (99), and then measured on a Hewlett-Packard (Hewlett-Packard, Route 41, Avondale, PA 19311) Model HP 5830A Gas Chromatograph.

Calculation of water and solute necessitated correction for removal of the sample volume and contents. The following formulae were used to calculate absorption or secretion.

Water: ml absorbed/30 min $= \left[\frac{PEG_{solu} \times V_{in}}{PEG_0} - S\right] \times \left[\frac{PEG_0}{PEG_{30}} - 1\right]$

ml absorbed/60 min $= \left[\frac{PEG_{solu} \times V_{in}}{PEG_0} - 2S\right] \times \left[\frac{PEG_0}{PEG_{60}} - 1\right]$

Solute: mmoles solute absorbed/30 min $= (V_0 - S)(E_0) - (V_{30})(E_{30})$

mmoles solute absorbed/60 min $= (V_0E_0) - S(E_0 + E_{30}) - (V_{60})(E_{60})$

Note that PEG = polyethylene glycol concentration, V = volume, E = electrolyte concentration, S = sample size in milliliters (same for a given experiment). The samples for the original test solution and at zero time, 30 minutes, and 60 minutes are represented by the subscripts

solu, 0, 30, and 60.

The calculated values were statistically analyzed by the use of t-statistics. Comparison between the acetate and chloride solutions within the same test segment of a specific pig were made with a paired t-test. Comparisons between different segments were made employing an unpaired t-test. Significance levels of the respective t-values were calculated from Equation 26.7.4 in the Handbook of Mathematical Functions (129).

RESULTS

The data for water and electrolyte movement in each of the recirculation experiments have been listed in Appendix L. A summary of heart rate, blood pressure, arterial blood gas analysis, and segment length corresponding to each recirculation experiment listed in Appendix L is given in Appendix M. High pCO_2 and low pH measurements were recorded for pigs #2, 5, 7, 15, and 16. The blood gas samples for pig #5 were from venous blood; therefore, it may be assumed that the arterial blood would not have been as acidic and probably would have been within the range for the other samples. Only the initial blood sample collected for pigs #7, 15, and 16 demonstrated marked acidosis. Pig #2 demonstrated marked acidosis throughout the experiment, although heart rate and blood pressure were maintained. The acidosis was probably the result of anesthesia and respiratory difficulty due to atelectasis or use of an inadequate size endotracheal tube. Despite the acidotic condition of these animals, the experimental data was not rejected. The experimental data for these animals was within the range of values for electrolyte and water movement observed for the other animals, except for Na^+ absorption from the acetate solution with pig #2. The potential influences of anesthesia and acidosis on the results of the study will be presented in the discussion.

Changes in pH, pCO_2, and HCO_3^-

A summary of the pH, pCO_2, and HCO_3^- measurements for the initial and final samples of the control and acetate solutions recirculated in

the jejunum and ileum is given in Table 8. No significant differences between the initial and final pH of any of the test solutions, as measured in the reservoir, was noted. Neither were differences between the initial pH of the respective treatments. The shift in pH observed in the perfusion experiments was eliminated by utilizing the recirculation technique. Consequently, the pH could be maintained relatively constant at pH 6.5.

A higher CO_2 tension in the ileum was necessary to maintain the pH of the solutions at approximately pH 6.5. In addition, the CO_2 tensions in the final samples were significantly greater for both solutions. The CO_2 tensions in the final control and acetate solutions in the ileum were not significantly different, despite higher levels in the acetate solution. Gassing of the ileal solutions with a higher pCO_2 should decrease the pH. However, an increase in pH was observed and can be explained by either the significant HCO_3^- secretion ($p < 0.01$) or H^+ absorption with the ileal solutions. No significant increase in the HCO_3^- concentration in the jejunal solutions after 60 minutes was measured.

Acetate Absorption

Acetate was absorbed in both the jejunum and ileum, as shown in Figure 10. The difference in rates in the ileum (2.44 ± 0.50 mmoles/segment/hr) and in the jejunum (1.34 ± 0.53 mmoles/segment/hr) was not significant. However, the interesting observation is that, while absorption of acetate in the ileum occurred concurrently with the absorption of water and total solute, the absorption of acetate in the jejunum

Table 8. Initial and final measurements of pH, HCO_3^-, and pCO_2 in the recirculation experiments[a]

Jejunum				Ileum			
Control		Acetate		Ileum		Acetate	
Initial	Final[b]	Initial	Final	Initial	Final	Initial	Final
			pCO_2 (mm Hg)				
103.2 ±5.5	121.7 ±12.2	103.4 ±9.7	158.6[c***] ±11.7	126.6 ±3.7	195.9[c****] ±8.2	143.0 ±23.5	217.5[c*] ±2.7
			HCO_3^- (mmoles/liter)				
8.70 ±0.4	9.33 ±0.4	9.84[d] ±0.3	10.47 ±1.2	11.48 ±0.3	14.54[c****] ±0.4	11.28 ±0.9	14.26[c**] ±0.6
			pH				
6.50 ±0.05	6.49 ±0.04	6.64 ±0.06	6.57 ±0.07	6.56 ±0.01	6.57 ±0.02	6.44 ±0.10	6.60 ±0.02

[a]Measurements expressed as mean ± SE; jejunum, N = 7, 8; ileum, N = 3, 4.

[b]Final measurements taken after one hour of recirculation.

[c]Significantly different than initial sample: *$p < .05$, **$p < .025$, ***$p < .005$, ****$p < .001$.

[d]Significantly different than initial control, $p < .05$.

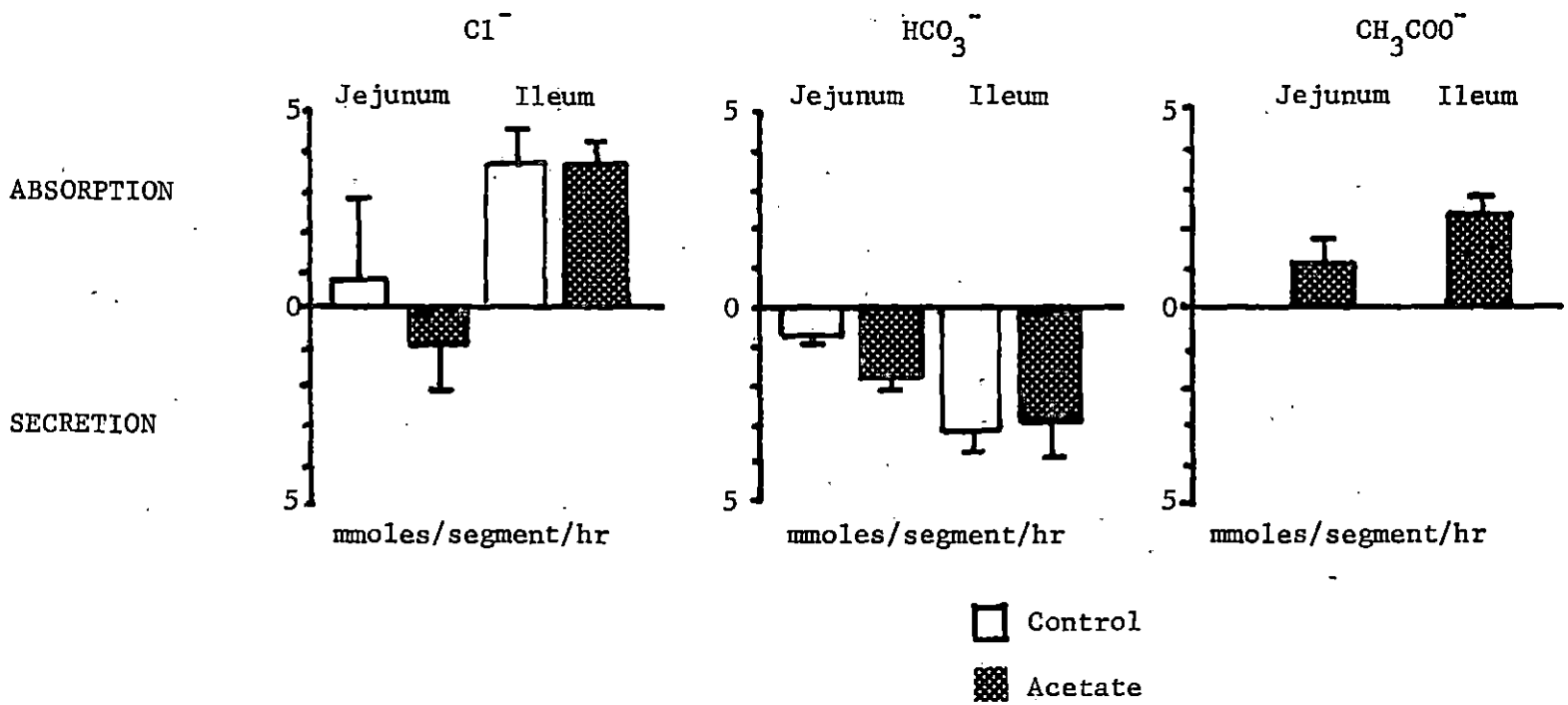


Figure 10. Movement of Cl^-, HCO_3^-, and acetate in the recirculation experiments (mean ± SE)

occurred despite a net secretion of water and total solute (Figure 11).

The pH in the recirculation experiments was held constant at pH 6.5 and would be expected to maintain a relatively greater ratio of unionized to ionized acetate ions than those observed in the perfusion experiments. If the concentration of SCFA in the lumen was the same, then a greater rate of absorption would then be expected in the recirculation experiments. However, the rate of absorption was less than in the perfusion studies, but conditions were different in the respective experiments. These differences will be subsequently discussed in the discussion.

Water and Electrolyte Absorption

The patterns of total solute, water, and the individual electrolyte movement for the recirculation experiments are shown in Figures 10, 11, and 12. The ileum generally exhibits a pattern of water, Na^+, Cl^-, and total solute absorption from both the control and acetate solutions. In contrast, the jejunal pattern is typically either secretion or a lower magnitude of absorption than observed in the ileum for water, Na^+, Cl^-, and total solute. Bicarbonate secretion in the ileum exceeded secretion in the jejunum. Mean K^+ secretion was noticeably less in the ileum. However, K^+ movement was small and variable and, therefore, will receive little mention in the subsequent results and discussion.

The relationship between total solute and water movement for each of the test solutions in both segments is depicted in Figure 13. The relationships are all positively correlated ($0.01 \leq p \leq 0.05$). All solutions were approximately isotonic with plasma and had a mean

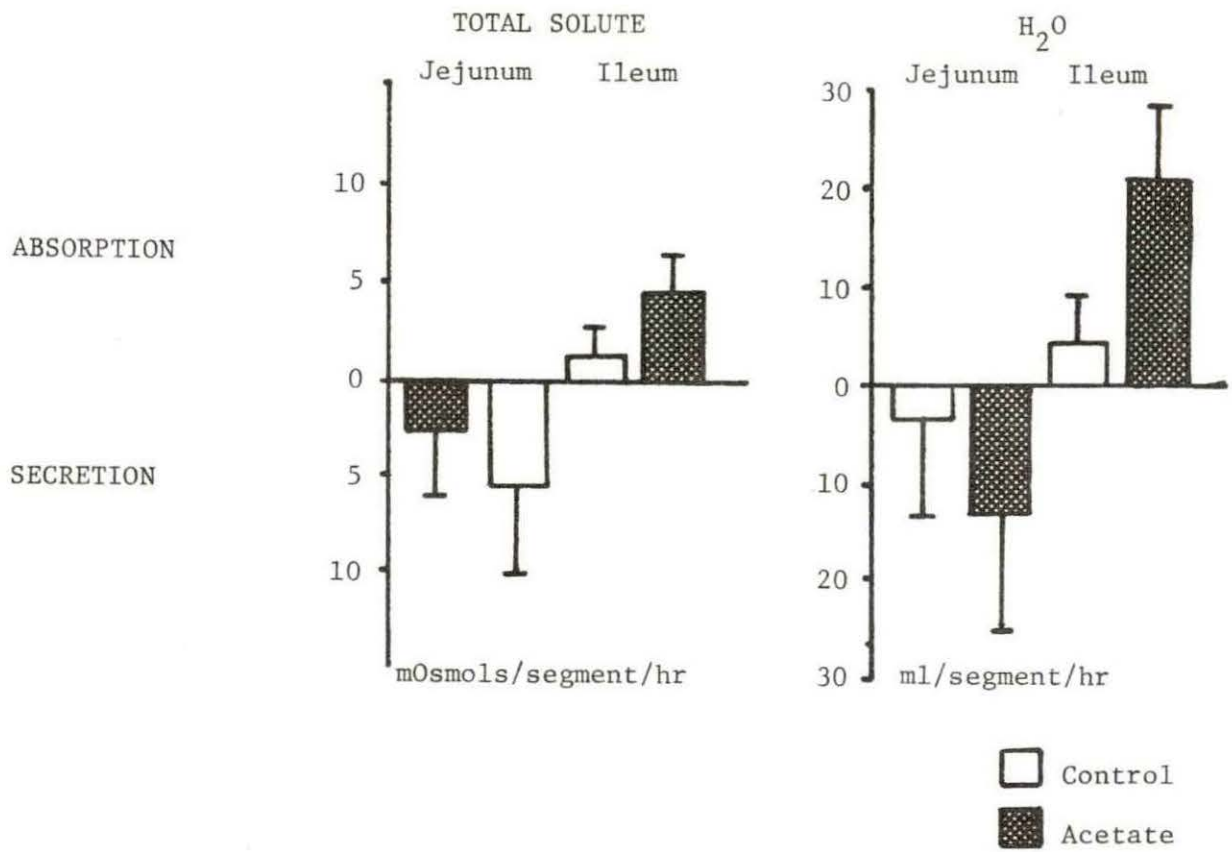


Figure 11. Movement of total solute and water in the recirculation experiments (mean ± SE)

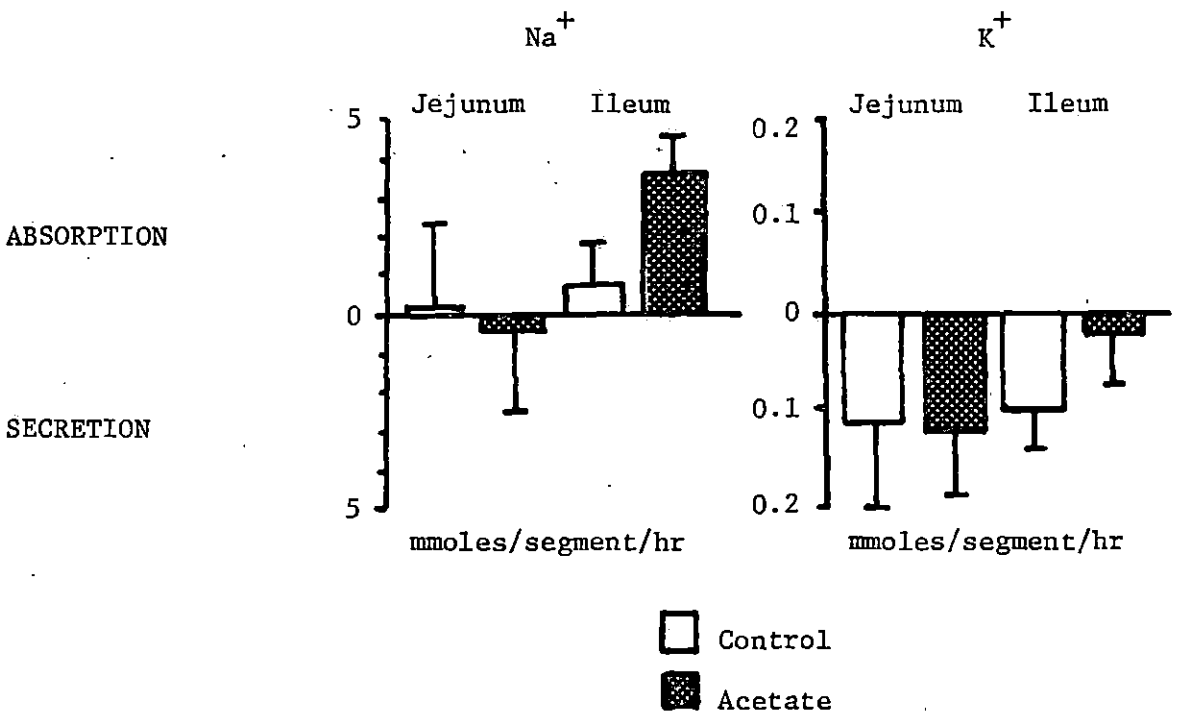


Figure 12. Movement of Na^+ and K^+ in the recirculation experiments (mean $\pm$ SE)

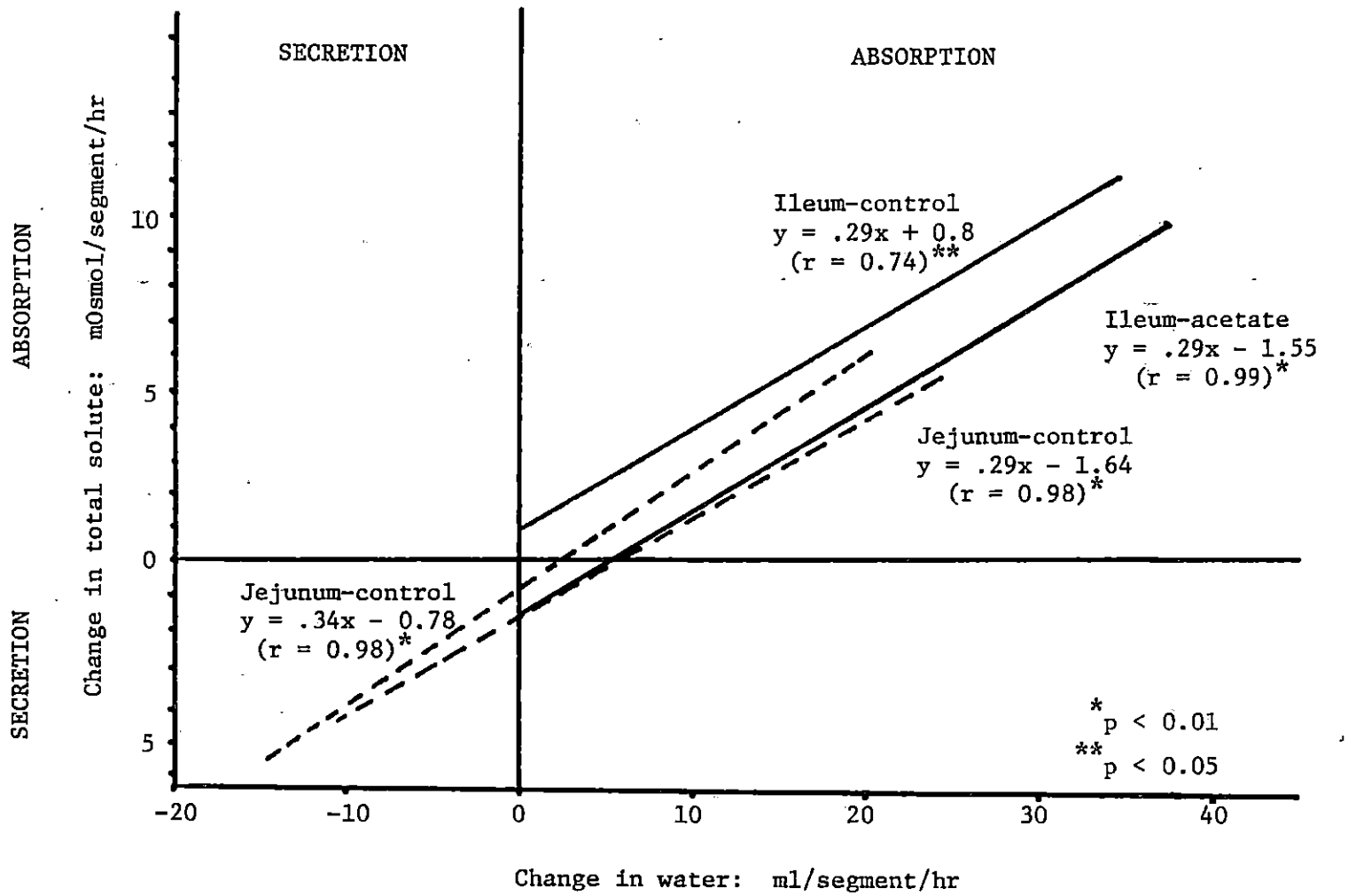


Figure 13. Relationship between total solute and water movement in the recirculation experiments

tonicity of 283 ± 1.3 mOsmol/liter. The linear relationships, shown in Figure 13, support the passive movement of water secondary to solute movement. Such movement was also observed in the perfusion experiments and has been well-documented in the literature (38,40). Although the test solutions were moved through the gut segments by an external pump at 20 ml/min, any influence of increased hydrostatic pressure on water movement would not be anticipated (51,52,63) nor does any effect appear discernible.

Effect of Acetate on Water and Electrolyte Absorption

A comparison of the net changes in water and electrolyte movement from each of the test solutions in the jejunum, as shown in Table 9, does not support an influence of acetate on electrolyte movement in the pig jejunum. Only HCO_3^- accumulation in the lumen in response to the presence of acetate was significant ($p < 0.048$). Although this was significantly different from the control treatment, the HCO_3^- concentration at 60 minutes was not significantly different from the HCO_3^- concentration in the initial samples of the acetate solution.

A comparison of the results for the control and acetate solutions in the ileum is shown in Table 10. Acetate absorption in the ileum resulted in a fourfold increase in Na^+ absorption ($p < 0.013$). While the absorption of water and total solute were not significantly increased ($p < 0.073$ and $p < 0.081$, respectively), 3.6 and fourfold increases in the mean absorption of these parameters in the presence of acetate were observed. The increase in net solute absorption from the acetate solution is quantitatively accounted for by the increased

Table 9. Effect of acetate on water and electrolyte absorption at pH 6.5 in the pig jejunum

Parameter[a]	Control mean ± SE[b]	Acetate (36.2 ± 0.5 mM/liter) mean ± SE	t-value (df = 8)[c]	PR > t
Na^+	0.21 ± 1.98	-0.29 ± 2.04[d]	0.20	.844
K^+	-0.11 ± 0.09	-0.12 ± 0.07	0.10	.922
Cl^-	0.84 ± 1.99	-0.85 ± 1.34	0.83	.419
HCO_3^-	-0.63 ± 0.25	-1.73 ± 0.34	2.14	.048
Acetate	---	1.34 ± 0.53	--	--
H_2O	-3.41 ± 10.93	-13.16 ± 12.41	0.93	.366
Total solute	-2.62 ± 3.22	-5.28 ± 4.31	0.71	.422

[a]Absorption of all parameters, except water, expressed in millimoles/test segment of intestine/hr. Water absorption expressed as ml/segment/hr. Total solute expressed as mOsmol/liter/hr. N = 9 for all parameters.

[b]SE = standard error of mean.

[c]t-value derived from calculations of paired data.

[d]Negative values indicate secretion into the lumen.

Table 10. Effect of acetate on water and electrolyte absorption at pH 6.5 in the pig ileum

Parameter[a]	Control mean ± SE[b]	Acetate (35.7 ± 0.8 mM/liter) mean ± SE	t-value (df = 8)[c]	PR > t
Na^+	0.81 ± 1.15	3.70 ± 0.93	2.81	.013
K^+	-0.10 ± 0.04[d]	-0.02 ± 0.05	1.43	.172
Cl^-	3.78 ± 0.94	3.79 ± 0.65	0.02	.984
HCO_3^-	-3.07 ± 0.67	-2.97 ± 1.03	0.07	.945
Acetate	---	2.44 ± 0.50	--	--
H_2O	5.11 ± 5.08	21.23 ± 7.12	1.92	.073
Total solute	1.24 ± 1.15	4.55 ± 2.07	1.80	.091

[a]Absorption of all parameters, except water, expressed in millimoles/test segment of intestine/hr. Water absorption expressed as ml/segment/hr. Total solute expressed as mOsmol/liter/hr. N = 9 for all parameters except HCO_3^-, where N = 4.

[b]SE = standard error of mean.

[c]t-value derived from calculations of paired data.

[d]Negative values indicate secretion into the lumen.

Na^+ and acetate absorption. Therefore, it is primarily the effect of acetate on Na^+ uptake that accounts for the increased solute absorption and concurrent water movement.

Comparison of the Ileum and Jejunum

A comparison of the responses to the control solution in the ileum and jejunum is shown in Table 11. Only a significantly greater secretion of HCO_3^- in the ileum ($p < 0.004$) was observed. Alkalinization of the ileal lumen is consistent with the pattern in most mammalian species. Chloride absorption in the jejunum (0.84 ± 1.99 mmoles/segment/hr) was considerably less than in the ileum (3.78 ± 0.94 mmoles/segment/hr). However, no statistical correlation was observed between HCO_3^- and Cl^- in either segment, although a reciprocal movement of HCO_3^- and Cl^- was observed (Figure 10). The patterns of electrolyte movement in the respective segments reflects the marked functional differences characteristic of the respective segments (30,40,54,67,130,131).

The jejunal and ileal responses to the presence of acetate are compared in Table 12. The jejunal pattern in the presence of acetate was previously shown in Table 9 to be not significantly different from the response to the acetate-free solution. In contrast, the ileal pattern, as shown in Table 10, demonstrated increased Na^+, water, and total solute absorption. The marked differences in Na^+, Cl^-, water, and total solute movement, as shown in Table 12, relative to the responses of the respective segments to the control solution, as shown in Table 11, suggest a greater influence of acetate on electrolyte

Table 11. Comparison of the response to the control solution between the jejunum and the ileum

Parameter[a]	Jejunum mean ± SE[b]	Ileum mean ± SE	t-value (df = 16)[c]	PR > t
Na^+	0.21 ± 1.98	0.81 ± 1.15	0.26	.798
K^+	-0.11 ± 0.09[d]	-0.10 ± 0.04	0.05	.961
Cl^-	0.84 ± 1.99	3.78 ± 0.94	1.57	.136
HCO_3^-	-0.63 ± 0.25	-3.07 ± 0.67	3.40	.004
H_2O	-3.41 ± 10.93	5.11 ± 5.08	0.71	.448
Total solute	-2.62 ± 3.22	1.24 ± 1.15	0.93	.366

[a]Absorption of all parameters, except water, expressed in millimoles/test segment of intestine/hr. Water absorption expressed as ml/segment/hr. Total solute expressed as mOsmol/liter/hr. N = 9 for all parameters except HCO_3^- in the ileum, where N = 4.

[b]SE = standard error of mean.

[c]t-values derived from calculations of unpaired data.

[d]Negative values indicate secretion into the lumen.

Table 12. Comparison of the effects of the acetate solution on water and electrolyte absorption between the jejunum and ileum

Parameter[a]	Jejunum mean ± SE[b]	Ileum mean ± SE	t-value (df = 16)[c]	PR > t
Na^+	-.021 ± 2.04[d]	3.70 ± 0.93	1.78	.094
K^+	-0.12 ± 0.07	-0.02 ± 0.05	1.13	.275
Cl^-	-0.85 ± 1.34	3.79 ± 0.65	2.47	.025
HCO_3^-	-1.73 ± 0.34	-2.97 ± 1.03	1.73	.109
Acetate	1.34 ± 0.53	2.44 ± 0.50	1.51	.151
H_2O	-13.16 ± 12.41	21.23 ± 7.12	2.60	.019
Total solute	-5.28 ± 4.31	4.55 ± 2.07	2.36	.031

[a]Absorption of all parameters, except water, expressed in millimoles/test segment of intestine/hr. Water absorption expressed as ml/segment/hr. Total solute expressed as mOsmol/liter/hr. N = 9 for all parameters except HCO_3^- in the ileum, where N = 4.

[b]SE = standard error of mean.

[c]t-value derived from calculations of unpaired data.

[d]Negative values indicate secretion into the lumen.

and water absorption in the ileum. The pig jejunum normally does not contain SCFAs at a concentration used in these experiments. Thus the ileum appears more adapted to the absorption of acetate.

Summary of Results--Part II

Several conclusions can be made from the results of the recirculation experiments. First, acetate was absorbed by both the ileum and jejunum. The rate of absorption in the ileum was lower than observed at the same pH in Part I. Second, Na^+ absorption by the ileum was significantly increased when acetate was present in the recirculated solution. Concurrent movement of water was also observed. Finally, despite acetate absorption by the jejunum, no effect of acetate on fluid and electrolyte movement was observed in that segment. Further, the jejunum demonstrated either no net movement or even a slight secretion with the control solution. This is in contrast to the ileum which demonstrated an absorptive pattern with both control and acetate solutions.

The results of these experiments in the ileum confirm the findings in the perfusion experiments in Part I. They further suggest functional differences between the ileum and jejunum with respect to the influence of SCFAs on water and electrolyte movement.

DISCUSSION

Assessment of Errors

Considerable variation in the results both between pigs and between the experiments conducted on the same pig was observed in this study. For example, absorption of sodium and H_2O between pigs ranged from 0.94 to 15.96 mmoles/segment/hr and 6.07 to 112.4 ml/segment/hr, respectively (Appendix E). Na absorption varied from -0.98 to 10.55 and -1.34 to 4.57 mmoles/segment/hr for pigs #5 and #47. Such variation was observed in both parts of this study. A number of possible influences could have been responsible for this variation.

First, the use of the balloon on the Foley catheter to occlude the lumen in the perfusion experiments may have affected intestinal motility and/or mucosal blood flow and altered absorption (95). Such balloons are often poorly tolerated by human subjects (114). In the present study, the animals frequently became quite irritable. Therefore, either a local or a cephalic mediated influence on gut function can not be excluded. Shifting of the Foley catheter due to aboral propulsion of the balloon also occurred and might be expected to decrease segment length. The net result would be to underestimate absorption. No balloon was used in the recirculation experiments.

Reflux and contamination of the test segments might also alter absorption rates. Neither factor could influence the results in the recirculation experiments since the gut was ligated proximally and distally to the segment. It is unlikely that reflux caused any significant variation in the perfusion experiments; however, error due to

reflux cannot be eliminated. Reflux from the cecum might result in measurable quantities of other SCFAs in the effluent. Very low concentrations were recorded in only three collections. Contamination from the upper segment also seems unlikely. Even when phenol red, used to detect leakage, was observed in the effluent, the analytical determinations were in the range for other collections. Such contaminated collections were usually analyzed but not used to compile the results.

Analytical technique is always suspect when variation occurs. Although Na^+, K^+, and Cl^- were not run in duplicate, the small standard errors in the mean concentration of electrolytes in the test solutions (Table 1) would seem to reflect consistency and accuracy.

The ends of the test segments in the recirculation experiments were ligated and the cannulae inserted and again ligated. Such manipulation or local trauma to the gut itself would be expected to result in an intestinal reflex that would inhibit motility (85). Whether via inhibition of motility or another mechanism which would inhibit absorption, such a reflex could partially explain the decreased absorption observed in the recirculation experiments.

The use of anesthesia in the recirculation experiments was a major difference in technique from the perfusion experiments. While the effects of halothane anesthesia on the rates of absorption in the recirculation experiments are unknown, they could partially account for the reduced rates of absorption. Some of the effects of halothane anesthesia generally include cardiovascular depression (hypotension), respiratory depression (increased pCO_2), and a reduction in the basal

metabolic rate (44). Inhibition of intestinal motility has also been reported (85). Altered motility, metabolism, and blood flow may all affect absorption. Acidosis might inhibit acetate absorption by lowering the gradient of undissociated acid from lumen to plasma. Both an increased pCO_2 and acidosis were observed in the recirculation experiments (Appendix M). No signs of hypotension were obvious, although this could be masked by reflex stimulation of the cardiovascular system in response to the acidosis and hypercapnia. The absence of cardiovascular depression may indicate that more attention to proper ventilation might have been useful in eliminating CO_2 retention.

The high CO_2 tensions used to adjust pH in this study might have been an added variable which may have masked or exaggerated the findings. Marked differences between the CO_2 tensions in the perfusion experiments (Table 2) and the recirculation experiments (Table 8) might imply an inhibitory influence of the higher tensions on absorption and explain the lower rate of electrolyte absorption in the recirculation experiments. However, no differences were observed with either technique between the acetate and control treatments. The pCO_2 did significantly increase in the final samples.

Concentrations of CO_2 in this study probably ranged from 8-35%. However, concentrations above 11% have general anesthetic effects (33) and may produce smooth muscle relaxation. Such relaxation could involve decreased motility of the gut itself and dilation of the vasculature supplying the respective small intestinal segment. How altered gut motility itself might affect the results of this study is unknown.

Vasodilation would be expected to increase blood flow (33) and create a larger lumen to blood gradient which would then facilitate absorption (2). Vasodilation, therefore, would not seem to be a mechanism producing the inhibition in the recirculation experiments. The increased acidity generated both by the high CO_2 tensions used to gas the solutions and the high plasma CO_2 tensions produced by anesthetic induced respiratory depression might inhibit absorption by inhibiting cellular function. Alternately, absorption might be inhibited by the change in the electrochemical gradient for H^+ ions between the tissue compartment and the bathing solution (119).

An additional explanation for the sizable variation observed in this study is spontaneous variation. Such spontaneous variation has been previously reported during in vivo studies with human subjects (58,128). It was suggested that this variation was the result of changes in blood supply, motility, and other unspecified factors.

Acetate Absorption

Two fundamental differences in the two techniques used in this study involved maintenance of a concentration gradient (perfusion experiments) and maintenance of pH (recirculation experiments). Assuming acetate was absorbed in the protonated form, then less absorption should take place in the perfusion experiments where the pH rapidly increased. However, the rate in the perfusion experiments exceeded that observed in the recirculation experiments. This would seem to be partially explained by a higher mean concentration of acetate in the ileal segments of the perfusion experiments. In the perfusion

experiments, a concentration of 40 mmoles/liter was continuously introduced to the test segment. In contrast, the initial acetate concentration in the recirculation experiments at zero time was not 40 mmoles/liter but 36.2 $\pm$ 0.5 mmoles/liter in the jejunum and 35.7 $\pm$ 0.8 mmoles/liter in the ileum (Table 7). Unlike the concentration in the perfusion experiments, this concentration decreased with each recirculation through the test segment. In addition, shorter segment lengths in the perfusion experiments also contributed to a higher mean acetate concentration in the segment. However, it can be roughly estimated that 16.8 mmoles (40 mmoles/liter x 0.42 liters/minute) of acetate passed through the ileal segment during the one-hour experiment in the perfusion experiments. If it is assumed that no acetate was absorbed, then approximately 42 mmoles would pass through the test segment during the same one-hour period in the recirculation experiments. Only 2.44 $\pm$ 0.5 mmoles of acetate were actually absorbed by the ileum in the recirculation experiments (Figure 12). More acetate should have been absorbed from the larger quantity presented to the segment in the recirculation experiments. Therefore, it seems more likely that the greater absorption in the perfusion experiments might be attributed to other factors. The acidosis associated with anesthesia would seem to be one logical explanation.

For comparison purposes, the rates of absorption in the perfusion experiments were recomputed by dividing the mean rate of absorption for each pig (Appendix E) by the respective segment length (Appendix B) and then expressing the results as rate/60 cm. In this manner, the

ileal segments perfused at pH 6.5 can be compared to comparable length segments (58.6 ± 2.5 cm) in the recirculation experiments and other results in the literature.

Acetate absorption occurred in the perfusion experiments at the rate of 7.35 ± 0.7 mmoles/60 cm/hr compared with the rate of 2.44 ± 0.50 mmoles/58.6 ± 2.5 cm/hr in the ileum in the recirculation experiments. This difference is substantial and indicates that the single pass steady state perfusion technique and the recirculation technique, as utilized in this study, yield varying results. The effects of anesthesia, previously described, are probably responsible for the inhibition of absorption.

The rate of acetate absorption in the perfusion experiments probably is a better estimate of SCFA absorption in the normal pig ileum where SCFAs are continuously being produced. Unfortunately, the inability to maintain the pH and the use of high CO_2 tensions to adjust pH make an accurate extrapolation of the results to the normal pig ileum difficult.

Crump et al. (37; Crump et al., I.S.U., Ames, Iowa, unpublished observations, 1979), in steady state perfusion studies on the entire pig colon at pH 6.4, observed acetate absorption at the rate of 156 ± 4.7 (SE) mmoles/hr. The pig large intestine, excluding the cecum, has been reported to be 3.1-5.7 meters long (91). From these values, an estimated absorption rate for acetate of 16.4-30.2 mmoles/60 cm/hr for the pig colon may be calculated. Argenzio and Whipp (6) have reported acetate absorption by the entire colon of conscious pigs from a solution comparable to the normal contents at the rate of 2.0 ± 0.1 meq/min. At their observed rate

and assuming the previous estimates for the length of the large intestine, it can be estimated that 12.7-23.2 mmoles/60 cm/hr would be absorbed. This compares to the rate of 7.35 $\pm$ 0.7 mmoles/60 cm/hr in this study. Other studies have shown that rates for the absorption of acetate in the human rectum (7.7-8.9 umol/cm^2/hr) (87), rumen (10.3 umol/cm^2/hr) (120), and the equine large intestine (8.6 umol/cm^2/hr) (7) are comparable to those observed in the pig colon (8.9 umol/cm^2/hr) (5).

The dog colon absorbs acetate at pH 6.4 at the rate of 0.77 $\pm$ 0.7 meq/gram dry mucosa/hr (D. Herschel et al., New York State College of Vet. Med., Cornell University, Dept. Physiology, Ithaca, N.Y., unpublished observation, 1979). Calculated acetate absorption for three of the pigs in the perfusion experiments occurred at the rate of 0.56 $\pm$ 0.09 mmoles/gram of dry mucosa/hr.

Acetate absorption in the large intestine in these other species and in the pig colon appears to exceed the rate in the pig ileum by two to fourfold. Obviously the total quantity of acetate absorbed in the large intestine is large relative to the ileum. This is due to the greater surface area of the entire large intestine. However, the concentration reported in the normal large intestine exceeds the concentration in the pig ileum by four to fivefold. The results of this study seem to indicate that SCFAs are rapidly absorbed in the pig ileum at physiological pH. It would be interesting to determine whether the ileum is capable of absorbing SCFAs from the higher concentrations and at the same rate observed in the pig colon.

At the rate of 7.35 $\pm$ 0.7 mmoles/60 cm/hr and assuming the pig ileum is 0.7-1.0 meters long (91), it can be estimated that 205-280 mmoles/day

are absorbed. This may be an overestimation as the concentration in the normal ileum is not maintained at 40 mmoles/liter throughout the day. Total absorption per day calculated from the data of Argenzio and Whipp (6) for the pig colon would be 2900 mmoles. The nutritional contribution of SCFA absorption by the pig ileum would be only about 7% of the contribution of SCFA absorption by the large intestine. Although SCFA absorption in the pig ileum would appear to contribute to the animal's energy resources, the nutritional role seems minor.

The pig jejunum normally does not contain SCFAs at a concentration utilized in these experiments. However, this study also demonstrated the potential of the proximal small intestine to absorb SCFAs. If the rate of 1.34 $\pm$ 0.53 mmoles/segment/hr, observed in the jejunum, is extrapolated to the entire length of the jejunum, i.e., 14.1-19.6 meters (91), then the pig jejunum is capable of absorbing 750-1050 mmoles/day. This would represent substantial total absorption by the small intestine.

The rates of acetate absorption in the ileum and jejunum were not significantly different (Table 12). However, if it is assumed that the mucosal surface area/cm of ileum is less than in the jejunum (118), then the ileum may be capable of a greater rate of absorption per cm of gut.

Schmitt et al. (105) have reported acetate absorption from a solution with a mean concentration of 40 mmoles/liter in the human jejunum at the rate of 12.0 $\pm$ 1.5 (SE) mmoles/30 cm/hr. This corresponds to a rate of 24 mmoles/60 cm/hr. Acetate was absorbed in the human ileum at the rate of 10.2 mmoles/60 cm/hr (106). While the rate in the human ileum is comparable to the rate in this study (7.35 $\pm$ 0.7 mmoles/60 cm/hr), Schmitt's studies were done at a pH exceeding pH 7.4. Therefore, it

appears that the human ileum and especially the jejunum may be capable of greater SCFA absorption rates.

Effects of pH

From the results of this study, it is not possible to conclude the effects of pH on acetate absorption. However, while decreasing the pH of the perfused solution had no significant effect on the rate of acetate absorption, the rate of absorption observed was 60% greater. Decreasing the pH has essentially no effect on the movement of the various electrolytes during control perfusions in the perfusion experiments (Table 5), but relatively greater differences between high and low pH with respect to electrolyte absorption were observed when acetate was present (Table 6). This observation and the shifts in the pH of the low pH solutions suggest that decreasing the pH does increase acetate absorption. This effect of pH on SCFA absorption has been previously reported in the human small intestine (105,106), rat small intestine (76), and the pig (37) and goat (8) large intestine. Because of the observed shifts in pH in this study, it is likely that the absorption rates for acetate and the other electrolytes, determined at pH 6.5 in the perfusion experiments, may be underestimated.

Mechanism of Acetate Absorption

This study was not designed to examine the mechanism of acetate absorption. Within the limits of this study, it is important to attempt to fit the results to one of the models which have been proposed. In general, the results of both the perfusion and recirculation experiments

must fit a model which accounts for acetate absorption at both pH 6.5 and 7.4 concurrent with an increase in pH and HCO_3^- in the lumen and with the stimulation of Na^+ absorption in the ileum but not in the jejunum.

More than 98% of the acetate was in the ionized form at the pHs used in this study. Unionized acid would be present at such a low concentration that nonionic absorption would not be favored, yet acetate was absorbed. Such absorption has been observed in many other studies and has previously been explained by the microclimate or unstirred layer model proposed by Hogben et al. (71)(Figure 1). However, studies by Jackson et al. (78) with the rat intestine have demonstrated that the pH of the mucosal fluid bulk phase would determine acetate uptake.

An increase in lumen pH and HCO_3^- concentration, such as observed in this study, is consistent with either H^+ ion absorption or HCO_3^- secretion. At the various concentrations of HCO_3^- measured in the lumen in this study (i.e., approximately 6-27 mmoles/liter) and assuming a transepithelial potential difference of 5 mv (serosa positive), a transmembrane potential difference of -40 mv, and a plasma HCO_3^- concentration of 25 mmoles/liter; HCO_3^- movement into the lumen would be generally favored. The two models proposed by Argenzio et al. (9) could be used to explain an increase in lumen HCO_3 but they differ in the form of acetate crossing the luminal membrane and the source of the HCO_3^-.

In order to have an exchange of acetate for HCO_3^- as proposed by Argenzio et al. (9) in their Model B (Figure 3), there must be HCO_3^- secreted from the intracellular compartment into the lumen. In this study, the magnitude of HCO_3^- secretion was the same at both pH 6.5 and 7.4 and between the acetate and control solutions. If an anion exchange

occurred, then a greater accumulation of HCO_3^- in the lumen would be expected when acetate is absorbed. This was not observed (Table 4). However, the control solutions contained a higher concentration of chloride. An anion exchange between HCO_3^- and Cl^- in the ileum, as described by Turnberg et al. (128), might have resulted in an equivalent magnitude of HCO_3^- secretion into the lumen.

According to Model B, the titration of HCO_3^- to CO_2 and subsequent diffusion from the lumen would prevent HCO_3^- accumulation in the lumen. These events would be reflected in an increased lumen pCO_2. Secretion of H^+ ions into the lumen, such as in a Na-H exchange, could account for the increased Na^+ absorption observed when acetate was present.

Results of the perfusion experiments at pH 6.5 would seem inconsistent due to the marked decrease in pCO_2 observed. This may be explained by rapid CO_2 diffusion down a gradient which may have masked an increase in pCO_2 due to the generated CO_2.

This model may not be appropriate to explain acetate absorption in the jejunum. There is evidence that the direction of the anion coupling between Cl^- and HCO_3^- in the ileum may be reversed in the jejunum (112). Preferential absorption of HCO_3^- in the jejunum and secretion in the ileum would appear to be inconsistent with acetate absorption in both segments by the same mechanisms. It might be possible for acetate to exchange for Cl^-. The tendency for a mean secretion of Cl^- (Figure 10) in the presence of acetate might support such an exchange; however, the difference between the respective solutions was small and not significant for Cl^- but was significant for HCO_3^- (Table 9).

Model A (Figure 4) proposed by Argenzio et al. (9) involves acetate absorption as the unionized acid. Lumen H^+ ion concentration is decreased as the undissociated acid is transported out of the lumen and the lumen HCO_3^- concentration then increases. Lumen pCO_2 would be expected to decrease. Accumulation of HCO_3^- would then be proportional to the quantity of acetate absorbed. Intracellular acidification could be compensated by recycling of H^+ ions back into the lumen in exchange for Na^+. This Na-H exchange could be the mechanism for acetate stimulated Na^+ absorption.

The results of the perfusion experiments in the ileum are consistent with this model. Lumen HCO_3^- increased at both pH 6.5 and 7.4, but a decrease in pCO_2 was observed only at pH 6.5. This decrease had previously been attributed to CO_2 diffusion down a gradient. However, the increase in the pCO_2 at pH 7.4 (Table 2) could be due to a tendency for CO_2 to diffuse from the blood (estimated pCO_2 = 40 mm Hg) to the lumen (pCO_2 ≃ 10 mm Hg). The results at pH 7.4 might also be consistent with nonionic absorption.

An increase in the lumen HCO_3^- concentration was also observed in the ileal recirculation experiments. Although the magnitude of HCO_3 changes were the same for both control and acetate solutions (Table 2), it should be noted that higher CO_2 tensions were required to maintain the pH when acetate was present. This would reflect either greater H^+ ion absorption (as the undissociated acid) or HCO_3^- secretion. As in the previous model, HCO_3^- accumulation would have been dissipated by titration to CO_2.

The pH in both the ileum and jejunum in the recirculation experiments was maintained at pH 6.5 by adjusting the CO_2 tension. A smaller magnitude of change in the HCO_3^- concentration in the lumen of the jejunum was observed (Table 8). This could reflect the greater permeability of the jejunum to HCO_3^- (30). The significant increase in HCO_3^- with the acetate solution would certainly support Model A. The failure of acetate absorption to affect Na^+ absorption in the jejunum must be reconciled with this mechanism. No intracellular acidification would occur if the undissociated acid crossed both mucosal and serosal membranes. The preferential absorption of HCO_3^- might then create a pH gradient which might be inhibitory to the H^+ ion secretion in exchange for Na^+. Alternately, the jejunum may lack a Na-H exchange. Therefore, Model A is also consistent with the results of this study on the jejunum.

Effect of Acetate on Electrolyte Absorption

An important observation on this study was the stimulation of Na^+ absorption in the ileum by the addition of acetate to the test solutions. If the rates of Na^+ absorption in the perfusion experiments are expressed as change per 60 cm, then the rates of absorption at low pH from the acetate and control solutions were 10.9 ± 3.7 and 6.7 ± 2.2 mmoles/hr, respectively. The presence of acetate in both the perfusion and recirculation experiments caused Na^+ absorption to increase by about 4 mmoles/60 cm/hr. This represents an estimated net Na^+ absorption of 250 mmoles/day by the ileum. This may be overestimated since the SCFA concentration in the normal pig ileum is not maintained at 40 mmoles/liter over 24 hours. An increase in water absorption, secondary to the movement

of Na^+ and acetate in the perfusion experiments, occurred at about 750 ml/day. Therefore, SCFAs in the pig ileum appear to function as in the large intestine of the pig (37) and other species (8,87) to conserve Na^+ and water.

The pig jejunum also demonstrated SCFA absorption. However, no effect on Na^+ absorption was observed. The human jejunum normally does not absorb Na^+ from isotonic solutions lacking glucose (113). Also, the addition of acetate to perfusion solution in either the human jejunum or ileum does not affect Na^+ absorption, despite acetate uptake (105,106). However, the studies on SCFA absorption in the human small intestine were conducted at a pH in excess of pH 7.4. An effect of acetate at a lower pH in the human cannot be excluded. Both the human small intestine and the pig jejunum have a low SCFA concentration, generally less than 10 mmoles/liter. Therefore, these segments seem to neither require nor have developed a mechanism to facilitate Na^+ absorption similar to the mechanism found in the ileum. The ileum appears to function more like the large intestine.

Implications of the Results

The findings of this study have potential clinical implications. At a concentration used in this study, the pig small intestine is capable of absorbing substantial quantities of SCFA without the production of diarrhea. This would suggest the potential usefulness of SCFAs in providing a peroral supplemental energy source to the pig without affecting electrolyte balance. In addition, supplemental SCFAs might be useful in treating fluid and electrolyte losses in certain conditions

by increasing Na^+ and fluid absorption in the ileum. However, oral supplements at a concentration, such as used in this study, would probably be absorbed prior to reaching the ileum.

In studies by Hamilton and Roe (64), the important role of the pig small intestine in absorbing fluid and electrolytes and in preventing an overload of the absorptive capacity of the large intestine was demonstrated. Unlike most other species, the pig large intestine must absorb the greater portion of fluid and electrolytes presented to the intestines. Therefore, in those conditions in which SCFA concentrations in the pig ileum are diminished, such as by the administration of antibiotics or altered motility, a potentially significant effect on body homeostasis may result.

Future Studies

While this study demonstrated acetate absorption in both the pig jejunum and ileum and a concurrent influence on Na^+ and water absorption in the ileum, additional studies should be conducted. It would seem logical to determine the capacity of the ileum to absorb higher concentrations of SCFA and the corresponding influence on Na^+ and water absorption. Likewise, it would seem useful to determine the capacity of the jejunum to absorb higher concentrations of SCFA without producing an osmotic diarrhea. Such studies might utilize either technique employed in the present study but with progressively higher concentrations of acetate. Such information in both segments would be important in evaluating gut function when abnormally high lumen concentrations of SCFA are present, as with carbohydrate overload or a stagnant loop

syndrome.

Additional studies would also be necessary to elucidate the mechanism for SCFA absorption and increased Na^+ absorption in the pig small intestine. Both in vitro and in vivo studies are usually indicated when studying mechanisms. Use of an ion replacement technique could provide insight into the dependence of acetate absorption on the presence of some other ion, such as Na^+, Cl^-, or HCO_3^-. Carbonic anhydrase inhibitors could be used in evaluating the interrelationship between SCFAs and HCO_3^-.

The potential of SCFAs to function as a constant energy source for the mucosa has been suggested by Argenzio et al. (5,8) and Henning and Hird (66). Mucosal metabolism of SCFAs might be evaluated by an in vitro study, such as conducted on other segments of the pig gastrointestinal tract (5).

Although a potential role for SCFAs in the pig ileum has been established, additional studies are required to define the role of SCFAs in normal and pathological situations. The findings of this study and their application to the delicate fluid balance observed in the pig small intestine by Hamilton and Roe (64) enhance the importance of future studies.

CONCLUSIONS

The influence of a physiologic concentration of acetate in a perfusion solution and the effects of pH in the ileum of conscious pigs were evaluated in the first part of this study. In a second series of experiments, the absorption and influence of acetate in the ileum and jejunum of anesthetized pigs at pH 6.5 was examined by utilizing a recirculation technique.

The importance of a physiological concentration of a SCFA at approximately normal pH for maximum absorptive function in the pig ileum was confirmed. Acetate was rapidly absorbed in both the ileum and jejunum. In addition, the pig ileum demonstrated significant absorption of Na^+ and water when acetate was present. This observation has not been previously reported in the small intestine of the pig or other species.

The pig ileum appears to function like the large intestine of the pig and other species with respect to the effects of SCFAs on water and ion absorption. Those mechanisms proposed for acetate absorption and acetate influenced Na^+ absorption in the large intestine are generally consistent with the results of this study.

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APPENDIX A. WEIGHT GAIN OF INDIVIDUAL PIGS--PART I

	Date (1978)	Weight (kg)
Pig 982	4-13	27.27
	4-20	29.55
	4-25	31.82
Pig 0005	5-09	32.73
	5-11	32.73
	5-19	36.36
	5-26	39.55
	6-01	43.64
	6-12	46.36
	6-14	49.09
	6-16	---
	6-19	49.55
	6-22	48.64
Pig 932	6-02	48.18
	6-06	48.64
	6-13	51.82
	6-23	54.09
	6-26	55.91
	6-28	57.27
	6-30	60.45
	7-03	61.36
	7-05	62.27
	7-13	65.00
	7-18	66.82

	Date (1978)	Weight (kg)
Pig 471	7-10	19.55
	7-12	20.91
	7-14	---
	7-17	---
	7-19	---
	7-21	---
	7-24	25.91
	7-28	---

APPENDIX B. SUMMARY OF POSTMORTEM DATA

Pig 982: Inlet cannula found out 5-4-78. Animal was posted 5-4-78. No signs of abnormalities within the loop were observed. The distance between cannulae was 49 cm. The outlet cannula was located 22 cm from the cecum. The mucosa was stripped from the segment and dried. It weighed 8.03 grams.

Pig 0005: This animal was posted after completion of all experiments on 7-11-78. It weighed 66.4 kg at the time of posting. Both cannula were firmly in place. The ileum 5 cm in either direction from the inlet cannula was adhered to a 10 cm segment of ileum located approximately 60 cm anterior to the inlet cannula. Approximately the middle one-fourth of the test segment was loosely adhered to the peritoneal wall. No twists or excessive bends which may have obstructed the lumen were observed. The distance between cannulae was 43 cm. The outlet cannula was located 27 cm from the cecum. Mucosal dry weight was 9.57 grams.

Pig 932: The outlet cannula had come out 7-14-78 and had been reinserted. The inlet cannula was found out 7-24-78 and the pig posted. The segment was in excellent condition with no adhesions present. Cannulae were 30 cm apart. The outlet cannula was 30 cm from the cecum. When the mucosa was being stripped, a band of scar tissue was noted located 8 cm posterior from the inlet cannula. This scar measured 3-4 cm long and 2-4 mm wide. It probably was the result of excess pressure in the Foley catheter. Mucosa dry weight was 11.278 grams.

Pig 47: The outlet cannula was found dislodged on 7-31-78 and had evidently been out several days. At the time of posting on 8-1-78, the pig weighed 25.9 kg. The sleeve around the inlet cannula was poorly adhered. Only half of the sleeve was secured. An adhesion between the area adjacent to the outlet cannula and a point approximately one-third the length of the test segment anterior to the outlet cannula was noted. At the same point but on the opposite side of the intestine, an adhesion to the kidney was present. The segment was also adhered to the abdominal wall for 10 cm posterior to the inlet cannula. The test segment measured 35 cm. The outlet cannula was located 30 cm anterior to the cecum.

APPENDIX C. SUMMARY OF MEAN CHANGES PER EXPERIMENT

Table C1. Summary of mean changes per experiment[a]

Pig #	Date	# Samples	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
					Acetate, pH 6.5					
5	5-9	5	40.7	5.9	.10	-6.9	9.1	4.3	.945	12.5
	5-11	4	71.5	10.5	.30	-8.8	14.5	6.2	1.174	20.7
	6-16	4	45.2	6.8	.03	-5.9	10.0	4.3	.906	13.3
	6-22	5	37.0	5.4	.04	-7.0	10.0	5.0	.931	10.3
47	7-10	5	34.7	4.6	.20	-6.4	10.3	4.9	.870	11.8
	7-17	5	-5.8	-1.0	-.19	-3.6	2.1	1.9	.569	-2.1
	7-21	4	-13.4	-1.3	-.26	-4.9	2.7	2.5	.630	-3.5
	7-24	5	8.8	1.5	-.17	-5.7	6.2	4.4	.748	3.6
923	6-2	5	40.0	5.0	.16	-6.1	8.6	3.2	.836	9.3
	6-13	4	46.3	6.1	.20	-6.5	10.2	5.0	.900	13.1
	6-28	6	1.3	0.2	-.07	-6.4	5.0	1.7	.774	-0.5
	7-18	4	91.7	13.4	.35	-8.7	17.7	8.9	1.203	27.2
982	4-25	4	112.5	15.9	.56	0.2	11.6	6.0	.302	31.1

Acetate, pH 7.4

5	5-26	4	47.5	6.2	.18	-5.2	10.4	4.1	.387	11.8
	6-1	4	26.8	3.0	.04	-5.4	6.7	3.3	.264	7.0
	6-14	4	49.1	7.6	.27	-6.7	10.7	4.8	.372	14.7
	6-19	5	57.3	10.0	-.20	-6.6	12.7	5.7	.335	16.8
47	7-12	5	31.8	4.9	.12	-5.8	8.4	5.0	.234	10.6
	7-14	5	6.4	1.3	.01	-4.2	4.7	3.1	.096	1.5
	7-19	4	-8.0	-1.5	-.09	-4.9	3.9	2.1	.177	-1.0
923	6-26	7	-3.7	-0.8	-.09	-4.4	2.0	1.5	.234	-2.0
	7-3	4	0.2	1.5	.01	-3.4	2.7	1.8	.221	-0.7
	7-5	6	11.7	2.3	.03	-3.0	3.4	1.4	.173	3.5
982	4-13	4	15.4	1.9	.05	-0.8	2.6	2.5	.142	3.2
	4-20	4	10.7	7.9	-.03	-2.0	2.2	3.4	.140	1.8

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[a] H_2O measured in ml/segment/hr; Na^+, K^+, HCO_3^-, Cl^-, and CH_3COO^- measured in mmoles/segment/hr; Osm measured in mOsm/segment/hr.

Table C1. (Continued)

Pig #	Date	# Samples	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
					Control, pH 6.5					
5	5-9	6	35.9	4.6	.04	-5.7	12.0	---	.916	1.0
	5-11	4	78.6	11.1	.30	-6.1	16.5	---	.972	22.8
	5-16	4	60.2	9.1	.12	-3.5	13.4	---	.741	18.0
	6-22	4	35.4	7.6	-.02	-5.7	14.0	---	.859	8.1
47	7-10	5	8.8	1.8	-.11	-5.0	8.0	---	.743	2.9
	7-17	4	-6.0	0.1	-.16	-3.5	4.0	---	.599	-1.1
	7-21	4	9.6	1.9	-.26	-4.3	6.9	---	2.385	3.3
	7-24	5	2.7	1.0	-.09	-5.0	6.8	---	.701	1.4
923	6-6	5	43.8	5.3	.16	-9.0	15.0	---	1.040	10.6
	6-13	4	24.2	3.2	.04	-5.5	9.0	---	.847	6.0
	7-13	6	-14.7	-2.2	-.11	-2.8	2.3	---	.498	-4.9
982	4-25	4	58.9	7.7	.23	-1.7	11.1	---	.606	16.7

Control, pH 7.4

5	5-19	4	39.4	6.2	.18	-6.2	9.9	---	.228	12.1
	6-1	4	55.6	7.8	.21	-4.3	13.4	---	.132	15.0
	6-12	4	57.0	8.3	.22	-6.3	15.6	---	.260	16.4
	6-14	5	56.9	8.1	.24	-6.3	15.1	---	.205	16.2
47	7-12	5	31.1	4.1	.09	-4.6	10.7	---	.191	9.2
	7-14	4	10.0	2.1	.01	-3.5	6.0	---	.120	2.3
	7-19	5	5.8	1.6	.03	-4.1	5.4	---	.143	1.1
923	6-23	4	6.0	1.1	-.04	-3.9	6.7	---	.226	1.1
	6-23	5	6.0	1.4	-.01	-5.1	7.6	---	.340	1.1
	6-30	5	-1.2	-0.2	-.03	-3.1	1.7	---	.170	-1.2
	7-3	4	-1.1	0.3	-.06	-2.7	1.2	---	.155	-0.8
	7-5	4	-2.7	0.0	-.08	-3.0	1.9	---	.192	-1.4

APPENDIX D. SUMMARY OF MEAN CHANGES PER TREATMENT PER PIG PER SEQUENCE OF ADMINISTRATION

Table D1. Summary of mean changes per treatment per pig per sequence of administration[a]

Pig #	Order of administration	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
				Acetate, pH 6.5					
5	1	41.1	6.1	.03	-6.4	10.0	4.6	0.919	11.8
	2	56.1	8.2	.20	-7.9	11.8	5.3	1.060	16.6
47	1	10.7	1.6	-.03	-5.6	6.5	3.7	0.750	4.1
	2	1.5	0.3	-.18	-4.6	4.2	3.1	0.659	0.7
923	1	44.8	6.2	.16	-6.9	10.4	4.7	0.928	12.3
982	2	112.5	15.9	.56	0.2	11.6	6.0	0.302	31.2
				Acetate, pH 7.4					
5	1	51.3	7.9	.08	-6.2	11.3	4.9	0.365	14.5
	2	26.8	3.0	.04	-5.4	6.7	3.3	0.264	7.0
47	1	6.4	1.3	.01	-4.2	4.7	3.1	0.096	1.5
	2	11.9	1.7	.01	-5.4	6.1	3.5	0.206	4.8
923	1	-1.8	0.4	-.04	-3.8	2.3	1.7	0.227	-1.4
	2	11.7	2.3	.03	-2.0	2.2	3.4	0.140	1.8
982	1	10.7	7.9	-.03	-2.0	2.2	3.4	0.140	1.8
	2	15.4	1.9	.05	-0.8	2.6	2.5	0.142	3.2

				Control, pH 6.5					
5	1	57.2	7.8	.17	-5.9	14.2	---	0.944	16.4
	2	47.8	8.4	.05	-4.6	13.7	---	0.800	13.1
47	1	-1.7	0.5	-.12	-4.3	5.4	---	0.650	0.1
	2	9.2	1.9	-.19	-4.7	7.4	---	1.564	3.1
923	1	14.6	1.6	.02	-5.9	8.6	---	0.769	2.9
	2	24.2	3.2	.04	-5.5	9.0	---	0.847	6.0
982	1	59.0	7.7	.23	-1.7	11.1	---	0.606	16.7
				Control, pH 7.4					
5	1	50.7	7.4	.20	-5.6	13.0	---	0.207	14.5
	2	56.9	8.1	.24	-6.3	15.1	---	0.205	16.2
47	1	18.5	2.9	.06	-4.3	8.0	---	0.167	5.1
	2	10.0	2.1	.01	-3.5	6.0	---	0.120	2.3
923	1	0.7	0.3	-.05	-3.3	3.4	---	0.196	-0.5
	2	2.5	0.9	-.04	-3.9	4.6	---	0.247	0.1

[a] H_2O measured in ml/segment/hr; Na^+, K^+, HCO_3^-, Cl^-, and CH_3COO^- measured in mmoles/segment/hr; Osm measured in mOsm/segment/hr.

APPENDIX E. CHANGES IN PARAMETERS FOR EACH PIG PER TREATMENT

Table E1. Changes in parameters for each pig per treatment[a]

Pig #	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
				Acetate, pH 6.5				
5	48.6	7.2	0.12	-7.2	10.9	4.9	0.989	14.2
47	6.1	0.9	-0.10	-5.1	5.3	3.4	0.704	2.4
923	44.8	6.2	0.16	-6.9	10.4	4.7	0.928	12.3
982	112.5	16.0	0.56	0.2	11.6	6.0	0.302	31.1
				Acetate, pH 7.4				
5	45.1	6.7	0.07	-6.0	10.1	4.5	0.339	12.6
47	10.1	1.6	0.01	-5.0	5.7	3.4	0.169	3.7
923	2.7	1.0	-0.01	-3.6	2.7	1.6	0.209	0.2
982	13.1	4.9	0.01	-1.4	2.4	3.0	0.141	2.5
				Control, pH 6.5				
5	52.5	8.1	0.10	-5.2	14.0	---	0.872	14.8
47	3.8	1.2	-0.16	-4.5	6.4	---	1.107	1.6
923	17.8	2.1	0.03	-5.8	8.8	---	0.795	3.9
982	58.9	7.7	0.23	-1.7	11.1	---	0.606	16.7
				Control, pH 7.4				
5	52.2	7.6	0.21	-5.7	13.5	---	0.206	14.9
47	15.6	2.6	0.04	-4.1	7.4	---	0.151	4.2
923	1.4	0.5	-0.04	-3.5	3.8	---	0.216	-0.2

[a] H_2O measured in ml/segment/hr; Na^+, K^+, HCO_3^-, Cl^-, and CH_3COO^- measured in mmoles/segment/hr; Osm measured in mOsm/segment/hr.

APPENDIX F. SUMMARY OF TREATMENT MEANS

Table F1. Summary of treatment means[a]

Treatment	H_2O	Na^+	K^+	HCO_3^-	Cl^-	pH	Osm
Acetate, pH 6.5	52.99	7.57	0.18	-4.74	9.54	0.731	15.00
Acetate, pH 7.4	17.75	3.56	0.02	-3.99	5.22	0.215	4.76
Control, pH 6.5	33.25	4.76	0.05	-4.30	10.06	0.845	9.25
Control, pH 7.4	23.08	3.58	0.07	-4.45	8.23	0.191	6.30

[a] H_2O measured in ml/segment/hr; Na^+, K^+, HCO_3^-, and Cl^- measured in mmoles/segment/hr; Osm measured in mOsm/segment/hr.

APPENDIX G. SUMMARY OF MEAN CHANGES OF THE COMBINED TREATMENTS FOR EACH PIG

Table G1. Summary of mean changes of the combined treatments for each pig[a]

Pig #	H_2O	Na^+	K^+	HCO_3^-	Cl^-	pH	Osm
5	49.63	7.40	0.13	-6.04	12.12	0.602	14.13
47	8.89	1.58	-0.05	-4.67	6.19	0.533	2.98
923	16.68	2.47	0.03	-4.95	6.40	0.537	4.05
982	61.48	9.51	0.27	-0.95	8.36	0.350	16.78

[a] H_2O measured in ml/segment/hr; Na^+, K^+, HCO_3^-, and Cl^- measured in mmoles/segment/hr; Osm measured in mOsm/segment/hr.

APPENDIX H. CORRELATION COEFFICIENTS FOR CONTROL PERFUSIONS AT pH 6.5

Table H1. Correlation coefficients for control perfusions at pH 6.5

	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
H_2O	1.00000[a]	0.97399	0.89470	-0.21967	0.91793		-0.00200	0.99332
	0.0000[b]	0.0001	0.0001	0.4927	0.0001		0.9951	0.0001
	12[c]	12	12	12	12	0	12	12
Na^+	0.97399	1.00000	0.81456	-0.20311	0.92509		0.00744	0.96630
	0.0001	0.0000	0.0013	0.5267	0.0001		0.9817	0.0001
	12	12	12	12	12	0	12	12
K^+	0.89470	0.81456	1.00000	-0.20591	0.78480		-0.33340	0.87436
	0.0001	0.0013	0.0000	0.5208	0.0025		0.2896	0.0002
	12	12	12	12	12	0	12	12
HCO_3^-	-0.21967	-0.20311	-0.20591	1.00000	-0.53650		-0.21341	-0.16063
	0.4927	0.5267	0.5208	0.0000	0.0721		0.5054	0.6180
	12	12	12	12	12	0	12	12
Cl^-	0.91793	0.92509	0.78480	-0.53650	1.00000		0.06128	0.88449
	0.0001	0.0001	0.0025	0.0721	0.0000		0.8500	0.0001
	12	12	12	12	12	0	12	12
CH_3COO^-								
	0	0	0	0	0	0	0	0
pH	-0.00200	0.00744	-0.33340	-0.21341	0.06128		1.00000	0.00937
	0.9951	0.9817	0.2896	0.5054	0.8500		0.0000	0.9769
	12	12	12	12	12	0	12	12
Osm	0.99332	0.06630	0.87436	-0.16063	0.88449		0.00937	1.00000
	0.0001	0.0001	0.0002	0.6180	0.0001		0.9769	0.0000
	12	12	12	12	12	0	12	12

[a]Correlation coefficient.

[b]Prob > |r| under HO:RHO = 0.

[c]Number of observations.

APPENDIX I. CORRELATION COEFFICIENTS FOR ACETATE PERFUSIONS AT pH 6.5

Table I1. Correlation coefficients for acetate perfusions at pH 6.5

	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
H_2O	1.00000[a]	0.99763	0.95730	0.05965	0.88105	0.83698	0.18412	0.99517
	0.0000[b]	0.0001	0.0001	0.8465	0.0001	0.0004	0.5471	0.0001
Na^+	0.99763	1.00000	0.94037	0.04600	0.88643	0.85121	0.19788	0.99562
	0.0001	0.0000	0.0001	0.8814	0.0001	0.0002	0.5170	0.0001
K^+	0.95730	0.94037	1.00000	0.07177	0.83385	0.74772	0.14706	0.94818
	0.0001	0.0001	0.0000	0.8158	0.0004	0.0033	0.6316	0.0001
HCO_3^-	0.05965	0.04600	0.07177	1.00000	-0.39817	-0.29637	-0.96245	0.02268
	0.8465	0.8814	0.8158	0.0000	0.1778	0.3255	0.0001	0.9414
Cl^-	0.88105	0.88643	0.83385	-0.39817	1.00000	0.93758	0.60485	0.90176
	0.0001	0.0001	0.0004	0.1778	0.0000	0.0001	0.0285	0.0001
CH_3COO^-	0.83698	0.85121	0.74772	-0.29637	0.93758	1.00000	0.49485	0.87258
	0.0004	0.0002	0.0033	0.3255	0.0001	0.0000	0.0856	0.0001
pH	0.18412	0.19788	0.14706	-0.96245	0.60485	0.49485	1.00000	0.22168
	0.5471	0.5170	0.6316	0.0001	0.0285	0.0856	0.0000	0.4667
Osm	0.99517	0.99562	0.94818	0.02268	0.90176	0.87258	0.22168	1.00000
	0.0001	0.0001	0.0001	0.9414	0.0001	0.0001	0.4667	0.0000

[a]Correlation coefficients, n = 13.

[b]Prob > |r| under HO:RHC = 0.

APPENDIX J. CORRELATION COEFFICIENTS FOR CONTROL PERFUSIONS AT pH 7.4

Table J1. Correlation coefficients for control perfusions at pH 7.4

	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
H_2O	1.00000[a]	0.99595	0.98127	-0.79333	0.95259		0.09564	0.99816
	0.0000[b]	0.0001	0.0001	0.0021	0.0001		0.7675	0.0001
	12[c]	12	12	12	12	0	12	12
Na^+	0.99595	1.00000	0.98667	-0.81459	0.94954		0.10032	0.99577
	0.0001	0.0000	0.0001	0.0013	0.0001		0.7564	0.0001
	12	12	12	12	12	0	12	12
K^+	0.98127	0.98667	1.00000	-0.83225	0.93030		0.06931	0.98280
	0.0001	0.0001	0.0000	0.0008	0.0001		0.8305	0.0001
	12	12	12	12	12	0	12	12
HCO_3^-	-0.79333	-0.81459	-0.83225	1.00000	-0.85576		-0.54191	-0.81204
	0.0021	0.0013	0.0008	0.0000	0.0004		0.0688	0.0013
	12	12	12	12	12	0	12	12
Cl^-	0.95259	0.94954	0.93030	-0.85576	1.00000		0.26494	0.95025
	0.0001	0.0001	0.0001	0.0004	0.0000		0.4053	0.0001
	12	12	12	12	12	0	12	12
CH_3COO^-								
	0	0	0	0	0	0	0	0
pH	0.09564	0.10032	0.06931	-0.54191	0.26494		1.00000	0.11121
	0.7675	0.7564	0.8305	0.0688	0.4053		0.0000	0.7308
	12	12	12	12	12	0	12	12
Osm	0.99816	0.99577	0.98280	-0.81204	0.95025		0.11121	1.00000
	0.0001	0.0001	0.0001	0.0013	0.0001		0.7308	0.0000
	12	12	12	12	12	0	12	12

[a]Correlation coefficient.

[b]Prob > |r| under HO:RHO = 0.

[c]Number of observations.

APPENDIX K. CORRELATION COEFFICIENTS FOR ACETATE PERFUSIONS AT pH 7.4

Table K1. Correlation coefficients for acetate perfusions at pH 7.4

	H_2O	Na^+	K^+	HCO_3^-	Cl^-	CH_3COO^-	pH	Osm
H_2O	1.00000[a]	0.84318	0.36170	-0.58617	0.93144	0.87578	0.78428	0.98706
	0.0000[b]	0.0006	0.2480	0.0452	0.0001	0.0002	0.0025	0.0001
Na^+	0.84318	1.00000	0.17588	-0.33372	0.70732	0.83613	0.54826	0.81935
	0.0006	0.0000	0.5845	0.2891	0.0101	0.0007	0.0649	0.0011
K^+	0.36170	0.17588	1.00000	-0.14278	0.27416	0.22435	0.36344	0.34320
	0.2480	0.5845	0.0000	0.6580	0.3885	0.4833	0.2457	0.2748
HCO_3^-	-0.58617	-0.33372	-0.14278	1.00000	-0.80804	-0.62660	-0.72692	-0.65751
	0.0452	0.2891	0.6580	0.0000	0.0015	0.0292	0.0074	0.0201
Cl^-	0.93144	0.70732	0.27416	-0.80804	1.00000	0.88018	0.81311	0.95627
	0.0001	0.0101	0.3885	0.0015	0.0000	0.0002	0.0013	0.0001
CH_3COO^-	0.87578	0.83613	0.22435	-0.62660	0.88018	1.00000	0.58202	0.89805
	0.0002	0.0007	0.4833	0.0292	0.0002	0.0000	0.0471	0.0001
pH	0.78428	0.54826	0.36334	-0.72692	0.81311	0.58202	1.00000	0.77017
	0.0025	0.0649	0.2457	0.0074	0.0013	0.0471	0.0000	0.0034
Osm	0.98706	0.81935	0.34320	-0.65751	0.95627	0.89805	0.77017	1.00000
	0.0001	0.0011	0.2748	0.0201	0.0001	0.0001	0.0034	0.0000

[a] Correlation coefficients, n = 12.

[b] Prob > |r| under HO:RHO = 0.

APPENDIX L. SUMMARY OF RAW DATA FOR RECIRCULATION EXPERIMENTS[1]

Pig #	H_2O	Na^+	K^+	Cl^-	CH_3COO^-	HCO_3^-	Solute	Segment length
				Ileum (acetate solution)				
1	32.42	4.17	-0.03	4.61	1.46		7.83	55
2	17.31	-0.11	-0.19	5.04	2.83		2.14	62
3	12.68	1.28	-0.16	4.16	1.61		2.06	60
4	9.14	1.09	-0.04	1.47	1.47		1.00	60
5	40.86	6.03	-0.11	7.18	2.65		8.44	62
7	53.94	5.95	0.06	3.98	4.36	-3.40	14.52	40
8	37.42	8.53	0.22	4.62	4.99	-2.49	11.01	60
9	-1.19	3.79	0.12	1.84	2.35	-5.47	-1.70	64
10	-11.54	2.61	-0.01	1.23	0.27	-0.51	-4.35	64
X	21.23	3.70	-0.02	3.79	2.44	-2.97	4.55	
SE	7.12	0.93	0.05	0.65	0.50	1.03	2.07	
				Ileum (control solution)				
1	17.10	2.10	-0.04	4.47			3.08	55
2	7.66	-0.27	-0.13	4.10			0.57	62
3	20.72	2.81	0.01	5.83			4.38	60
4	-12.91	-1.41	-0.10	-0.51			-5.29	60
5	21.00	3.45	-0.17	5.56			5.43	62
7	8.29	3.60	-0.07	6.37		-2.83	0.33	40
8	-24.24	4.90	0.10	6.72		-1.41	4.39	60
9	6.47	-5.76	-0.37	-0.50		-3.36	-1.57	64
10	1.90	-2.15	-0.15	2.01		-4.67	-0.18	64
X	5.11	0.81	-0.10	3.78		-3.07	1.24	
SE	5.08	1.15	0.04	0.94		0.67	1.15	

[1] H_2O measured in ml/segment/hr; Na^+, K^+, Cl^-, CH_3COO^-, and HCO_3^- measured in mmoles/segment/hr; solute measured in mOsm/segment/hr; segment length measured in cm.

Pig #	H_2O	Na^+	K^+	Cl^-	HCO_3^-	CH_3COO^-	Solute	Segment length
				Jejunum (acetate solution)				
7	3.39	-0.97	-0.20	-1.10	-1.54	2.66	-2.70	57
8	-11.09	1.94	-0.02	-0.16	-2.47	3.69	-3.68	70
9	9.60	5.06	0.05	2.78	-2.30	2.58	-1.69	87
12	-39.40	-8.48	-0.33	-6.72	-2.48	0.53	-13.94	61
13	-53.11	-4.21	-0.20	-3.34	-3.10	0.04	-17.95	64
14	62.45	11.46	0.26	6.38	-1.20		22.85	90
15	-49.75	0.10	-0.07	0.05	-1.05		-17.37	54
16	-40.95	-6.97	-0.44	-5.37	-1.83	0.61	-14.94	59
17	0.40	-0.54	-0.11	-0.15	0.38	1.20	1.87	50
X	-13.16	-0.29	-0.12	-0.85	-1.73	1.34	-5.28	
SE	12.41	2.04	0.07	1.34	0.34	0.53	4.31	
				Jejunum (control solution)				
7	2.76	3.63	-0.02	3.62	0.20		-0.98	
8	25.43	0.21	-0.08	1.93	0.11		2.36	
9	11.14	-1.60	-0.13	1.50	-1.15		-0.71	
12	-10.80	-4.27	-0.25	-4.47	-0.16		-1.37	
13	-40.21	-6.55	-0.35	-6.05	-1.08		-13.28	
14	24.21	7.21	0.17	7.74	-0.63		6.41	
15	-69.98	-9.47	-0.58	-8.52	-1.19		-22.41	
16	26.56	8.43	0.28	8.64	0.12		8.66	
17	0.20	3.31	0.00	3.16	-1.92		-2.28	
X	-3.41	0.21	-0.11	0.84	-0.63		-2.62	
SE	10.93	1.98	0.09	1.99	0.25		3.22	

APPENDIX M. SUMMARY OF HEART RATE, BLOOD PRESSURE, BLOOD GAS ANALYSIS, AND SEGMENT LENGTH FOR RECIRCULATION EXPERIMENTS

Pig	Time	HR	BP	pH	pCO_2	HCO_3^-	PCV	Segment length ileum	Segment length jejunum
1	A 0	114	90/57						
	30	120	87/55	7.308	52.9	27.4		55	
	60	114	85/55	7.309	54.5	28.1			
	C 0	114	83/55	7.303	61.7	31.1			
	30	114	82.53	7.283	63.0	30.6			
	60	120	78.50	7.276	63.9	30.3			
2	C 0	84	83/58	7.196	66.9	26.2		62	
	30	84	86/55	7.211	68.8	27.7			
	60	96	100/60	7.116	85.4	27.9			
	A 0	120	105/60	7.066	87.2	25.3			
	30	114	110/65	7.064	96.6	27.8			
	60	144	107/62	7.068	92.2	27.1			
3	A 0			7.330	35.1	19.3		60	
	30			7.334	38.1	20.4			
	60			7.318	43.2	22.1			
	C 0			7.317	36.0	38.8			
	30			7.319	48.5	24.3			
	60			7.197	39.8	15.7			
4	C 0	78	78/58	7.406	45.6	27.9		60	
	30	84	78/57	7.362	40.6	23.2			
	60	78	77/57	7.370	47.2	27.0			
	A 0	78	65/50	7.348	54.5	29.3			
	30	72	68/50	7.361	50.2	27.9			
	60	78	73/55	7.283	49.0	23.4			
5	C 0	120		7.166	97.8	34.7		62	
	30	112							
	60	112		7.155	97.6	34.0			
	A 0	108							
	30	104		7.269	78.3	34.3			
	60	96		7.185	71.6	27.2			

Pig	Time	HR	BP	pH	pCO_2	HCO_3^-	PCV	Segment length ileum	Segment length jejunum
7	A 0	125	83/52	7.000				40	57
	30	145	84/54	7.216	65.1	26.8			
	60	144	76/47	7.248	60.9	27.4			
	C 0	132	75/45						
	30	126	74/43	7.175	73.9	27.5			
	60	126	75/43	7.237	63.8	27.5			
8	C 0	138	83/53	7.295	71.4	35.2	35.0	60	70
	30	132	71/46	7.311	59.5	30.5	34.5		
	60	126	76/50	7.322	57.5	30.2	34.5		
	A 0	156							
	30	150	78/52	7.323	52.5	27.7	34.5		
	60	144	75/48	7.326	52.7	27.8	34.6		
9	A 0	114	75/45					64	87
	30	138	83/55	7.312	47.2	24.2	31.0		
	60	108	75/50	7.302	54.3	27.1	38.0		
	C 0	120	78/52						
	30	108	76/50	7.305	50.8	25.6	31.0		
	60	120	86/55	7.340	46.7	25.5	31.0		
10	C 0	96						64	
	30	114	78/45	7.317	57.8	29.6	31.0		
	60	114	74/50	7.312	52.1	26.5			
	A 0	114							
	30	114	75/46	7.299	55.9	27.7			
	60	126	78/47	7.292	53.4	26.1			
12	C 0	126	88/65	7.244	76.1	33.0	32.0		61
	30	138	98/72						
	60	174	98/72	7.377	48.9	29.0	32.0		
	A 0	180	95/68						
	30	174	95/73	7/425	49.5	32.7	32.0		
	60	126	82/55	7.453	48.0	33.8	32.5		
13	A 0	126	95/58						64
	30	102	65/43	7.341	56.2	30.6	38.5		
	60	126	81/57	7.325	65.3	34.3	30.0		
	C 0	120	81/50						
	30	120	85/53	7.324	67.3	35.0	30.0		
	60	126	87/57	7.322	66.0	34.4	29.0		

Pig	Time	HR	BP	pH	pCO_2	HCO_3^-	PCV	Segment length ileum	jejunum
14	C 0	114	82/60	7.280	56.7	26.3	35.5	90	
	30	114	83/62	7.322	52.8	27.5	35.0		
	60	108	85/64	7.292	60.3	29.4	35.5		
	A 0	108	85/63						
	30	114	103/70	7.343	58.3	31.8	37.0		
	60		100/68	7.333	59.5	31.7	38.5		
15	A 0	120	83/41	7.096	82.3	25.6	36.0	54	
	30	114	77/43	7.255	63.1	28.6	36.0		
	60	108	83/48						
	C 0	120	83/47	7.282	56.2	26.9			
	30	120	83/45	7.321	56.5	29.4	33.0		
	60	114	75/43	7.282	62.7	29.7	34.0		
16	C 0	156	108/75	7.173	70.4	26.0	33.0	59	
	30	162	110/78	7.314	53.7	27.6	33.0		
	60	96	103/70	7.383	49.4	29.9	32.5		
	A 0	132	81/53						
	30		75/50	7.304	69.7	35.1	32.5		
	60	126	73.47	7.340	50.4	27.6			
17			Unavailable					50	
			Summary						
		119.3 ±5.1	82±2.3 54.6±2.1	7.286 ±.010	60.03 ±1.8	28.2 ±0.5	33.0 ±0.6	58.6 ±2.5	65.7 ±4.7