

Acetate absorption in the normal and secreting rat jejunum

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Abstract

Acetate absorption was studied in rat jejunum using steady state perfusion *in vivo*. Absorption conformed to apparent saturation kinetics and was similar in magnitude to glucose absorption. When compared with normal saline, acetate perfusion was associated with luminal alkalinisation. There was no difference in total CO₂ secretion when similar rates of acetate and glucose absorption were compared, suggesting that total CO₂ secretion was the result of mucosal metabolism. Absorption of acetate and propionate were mutually inhibitory. Acetate absorption was also inhibited by Tris-Hepes pH 7.0. When the gut was pretreated with cholera toxin to induce a secretory state, acetate absorption was reduced by 41.9%. This effect could be reproduced if similar water secretion was osmotically induced by the addition of mannitol. These data suggest that acetate is absorbed, at least, partially by non-ionic diffusion in the rat jejunum and that its absorption is reduced in the secreting intestine by solvent drag.

The mechanism by which acetate is transported across mammalian epithelia remains controversial. Previous studies in a number of species have suggested that acetate is absorbed by non-ionic diffusion.¹⁻⁸ In the guinea pig proximal colon, however, it has been shown that ionized acetate is absorbed through a paracellular pathway.⁸ Evidence for an acetate/bicarbonate exchange mechanism has been found in human ileal brush border membrane vesicles.⁹ In the guinea pig gall bladder, butyrate has been shown to be absorbed passively into the epithelial cell and then back into the lumen by a chloride/butyrate exchange.¹⁰ Finally, in the proximal renal tubule of the rabbit, short chain fatty acids are absorbed through a sodium coupled short chain fatty acid cotransport mechanism.¹¹ As previous studies in the rat have used *in vitro* methods it is unclear which mechanism predominates *in vivo*. It is also unknown whether acetate absorption is preserved in the secreting intestine. This is of interest as we have previously shown that acetate stimulates sodium absorption in the normal small intestine but not in the secreting small intestine after exposure to cholera toxin.¹²

In this paper we report our studies of acetate absorption in rat jejunum using *in vivo* steady-state perfusion. The concentration dependence of total acetate absorption, its relationship to luminal pH and total CO₂ and its inhibition by propionate and a structurally unrelated buffer were studied in the jejunum. The effect on acetate absorption of comparable intestinal secretion induced by cholera toxin and by

osmotic forces was also studied, to establish the effect of solvent drag.

Methods

STEADY STATE PERFUSION OF RAT JEJUNUM IN VIVO

Male Wistar rats (180–260 g) were fasted for 18 h but allowed free access to water. Anaesthesia was induced and maintained with sodium pentobarbitone (60 mg/kg ip followed by 30 mg/kg im as required). Body temperature was maintained by a heating pad and an overhead lamp. The abdomen was opened with a midline incision and a 15–20 cm segment of jejunum immediately distal to the ligament of Treitz was isolated. This was gently flushed with 0.9% NaCl solution and cannulated at proximal end with 1.5 mm external diameter polyethylene tubing and at the distal end with 4 mm external diameter polyethylene tubing. The segment was returned to the abdomen and closed with adhesive tape. Perfusion solution at 37°C was then infused continuously at a rate of 0.5 ml/minute by a Braun ED2 syringe pump. After an initial equilibration period of 30 minutes, three consecutive 10 minute collections of the effluent from the distal cannula were made into open test tubes. Serial measurements of PEG concentration showed the jejunum to be in a steady state during the experimental time (results not shown). In experiments where pCO₂ was measured, samples were taken through the wall of the distal collection tube using a glass syringe and a 26 gauge needle. The syringe was placed on ice and the contents immediately analysed for pH and pCO₂. Each animal was perfused with one solution only. In experiments in which a secretory state was induced, 75 µg cholera toxin (Sigma Chemical Co, Poole, Dorset) in 2 ml 0.9% NaCl was instilled into the intestinal segment which was clamped off for two hours before perfusion. After this two hour period the perfusion protocol previously described was followed. Acetate absorption was similar in the normal jejunum whether or not the perfusion protocol was preceded by a two hour incubation period. Seventy five micrograms cholera toxin was found to induce a reproducible secretory state which was in steady state during the 30 minute study period.¹³ In separate experiments, osmotic secretion was induced by addition of 90 mmol/l mannitol to perfusion solutions thus making them hypertonic with respect to plasma. Polyethylene glycol recovery during the collection period was found to be 98.8 (1.9)% (n=21) in the absorbing and secreting intestine.

At the end of the experiment the perfused segment was carefully dissected and opened

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TABLE I Composition of perfusion solutions used

Experiments	Na	Acetate	Propionate (mmol/l)	Glucose	Tris-Hepes	Mannitol
(i) Concentration dependence of Acetate	150	5-150	-	-	-	30
Glucose	150-80	-	-	150-80	-	30
(ii) Effect of pH	150	30,80	-	-	-	30
(iii) Effect of propionate and Tris-Hepes	150	30,50,80	30,70	-	10	30
(iv) Effect of intestinal secretion						
Acetate	150	30,100	-	-	-	30
Glucose	100	-	-	100	-	30
Acetate plus mannitol	150	30	-	-	-	90

Na was added as NaCl unless added as the acetate or propionate salt. Polyethylene glycol 400, 2.5 g/l and [¹⁴C] PEG, 5.0 µCi/l was added to all solutions.

along the antimesenteric border, firmly blotted and weighed. Comparison between the blotted wet weight and the dry weight of the segments in 46 secreting and non-secreting intestinal segments showed a correlation coefficient of 0.96 so only the blotted wet weight was used for reporting observations in this paper.

SOLUTIONS PERFUSED

The composition of the perfusion solutions are shown in Table I. All solutions perfused were iso-osmotic to rat plasma (305 (5) mosmol/kg) and were titrated with molar HCl or NaOH as appropriate to pH 7.0. Osmolarity was maintained by the addition of mannitol. Polyethylene glycol (PEG) 4000, 2.5 g/l and [¹⁴C] PEG, 5.0 µCi/l was used as a non-absorbable volume marker. All reagents were of analytical grade.

EXPERIMENTAL DESIGN

(i) The concentration dependence of acetate and glucose absorption in the jejunum was studied using isotonic solutions of acetate and glucose at concentrations between 5 and 150 mmol/l. (ii) The effect of acetate on luminal pH and total CO₂ accumulation was studied. (iii) The effect of propionate on acetate absorption was studied. (iv) As propionate is a buffer and its effects on acetate absorption might be solely the result of its buffering capacity, the effect on acetate absorption of a structurally unrelated buffer (Tris-Hepes) was studied. (v) The effect of intestinal

secretion induced by cholera toxin was studied and compared with the effect secretion induced osmotically by including excess mannitol in the perfusion solution.

ANALYTICAL METHODS AND CALCULATIONS

Bicarbonate was measured as total CO₂ (TCO₂) using a Corning 965 Carbon Dioxide Analyser. pH and pCO₂ were measured with glass electrodes (Radiometer Copenhagen, ABL 1). Acetate and propionate were measured by gas liquid chromatography (Perkin Elmer F30).¹⁴ Glucose was measured by a glucose oxidase method.¹⁵ [¹⁴C]-PEG was measured by liquid scintillation spectroscopy in an LKB 1210 Ultrabeta counter.

Solute concentrations from the three 10 minute collections were averaged. Absorption rates of water and solutes were calculated from the measured concentrations in the perfusate and the mean aspirate concentrations using standard formulae.¹⁶ Net absorption (+) indicates a net transfer of solute from the lumen; net secretion (-) indicates net transfer of solute into the lumen.

STATISTICAL ANALYSIS

Results are expressed as the mean and standard error of the mean (SE). Comparisons were made with one-way analysis of variance using the statistical program STATPAC (Karolinska Institute, version 1981).

Results

CONCENTRATION DEPENDENCE OF ACETATE ABSORPTION

Comparison of total acetate and glucose absorption showed their absorption rates to be similar at the same luminal concentrations (Fig 1). Absorption curves showed apparent saturation for both solutes. Endogenous acetate could not be detected in the jejunum when perfused with 150 mmol/l NaCl.

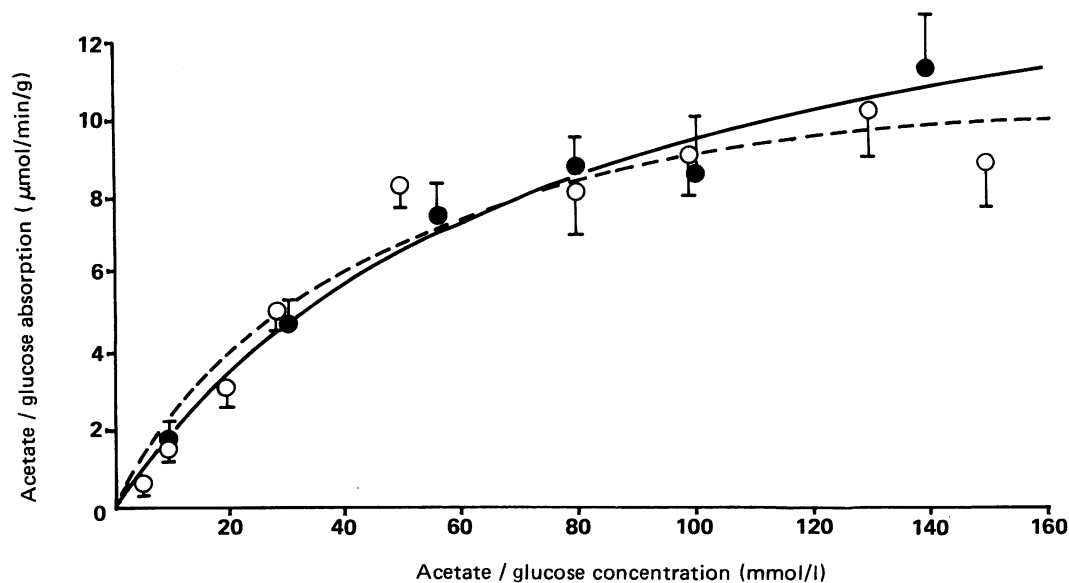


Figure 1: Total acetate (●) and glucose (○) absorption in rat jejunum. Results are expressed mean (SEM). (n=6).

TABLE II Effect of acetate absorption on luminal pH and TCO₂ accumulation

Solution perfused	[pH]	pH of effluent	TCO ₂ accumulation (μmol/min/g)	pCO (mmHg)
Isotonic NaCl (n=6)	[7.0]	5.9 (0.04)	-0.06 (0.36)	2.3 (0.14)
Acetate 30 mmol/l (n=6)	[7.0]	6.46 (0.03)*	-1.37 (0.23)*	1.88 (0.28)
Acetate 80 mmol/l (n=6)	[7.0]	6.82 (0.02)*	-1.32 (0.2)*	2.5 (0.21)

Results are expressed as the mean (SEM). * = $p < 0.01$ compared with isotonic NaCl. n refers to the number of animals studied. Negative values represent luminal accumulation.

EFFECT OF ACETATE ON LUMINAL pH AND TCO₂ ACCUMULATION

Because acetate absorption seemed likely to be influenced by pH, the pH of the effluent from the perfused segment together with the constituents of the major buffer system, pCO₂, and TCO₂, were measured during perfusion with unbuffered saline and 30 and 80 mmol/l acetate solutions. When unbuffered saline was perfused at pH 7.0 the pH of the effluent fell to 5.9 (0.04) and its pCO₂ rose to 2.2 (0.4) mmHg, but there was no significant net accumulation of TCO₂ in the luminal fluid (Table II). (The appearance of TCO₂ in the lumen is referred to as 'accumulation' because it is not possible from these data to distinguish with certainty between HCO₃ secretion and hydrogen ion absorption.) During acetate perfusion, effluent pH was higher than with saline, rising progressively with acetate concentration (Table II). The pCO₂ of the luminal fluid was unchanged but there was an increase in net TCO₂ accumulation in the luminal fluid when compared to perfusion with saline (Table II, Fig 2). No difference could be detected in TCO₂ accumulation, however, between acetate and glucose containing solutions when related to substrate absorption rates (Fig 2).

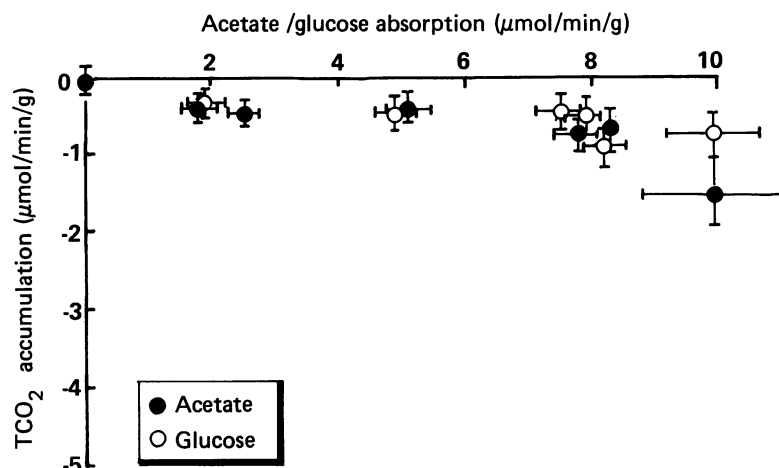


Figure 2: Relationship between total CO₂ accumulation with varying acetate (●) or glucose (○) absorption rates in the rat jejunum. Results are expressed as the mean (SEM). Negative values represent luminal accumulation. (n=6).

TABLE III Effect of propionate (30 mmol/l) and Tris-Hepes (10 mmol/l) on acetate absorption and effluent pH

Perfused acetate concentration (mmol/l)	Acetate absorption (μmol/min/g)	Effluent pH	Acetate absorption with propionate (μmol/min/g)	Effluent pH
30 (n=6)	5.1 (0.3)	6.44 (0.03)	2.9 (0.26)*	6.85 (0.02)§
50 (n=6)	8.2 (0.5)	6.65 (0.02)	4.5 (0.6)†	6.81 (0.01)‡
80 (n=6)	7.1 (1.0)	6.82 (0.02)	5.9 (0.59)	6.91 (0.01)‡
			Acetate absorption with 10 mmol/l Tris-Hepes	
30 (n=6)	5.1 (0.3)	6.44 (0.03)	3.14 (0.38)*	6.89 (0.03)‡

Results are expressed as the mean (SEM). Comparisons are made between acetate absorption rates at a given acetate concentration with and without propionate or Tris-Hepes in the perfused solution. * $p < 0.05$, † $p < 0.01$. Similarly comparisons are made between effluent pH at a given acetate concentration with and without propionate or Tris-Hepes in the perfused solution. ‡ $p < 0.05$, § $p < 0.01$. n refers to the number of animals studied.

EFFECT OF PROPIONATE AND TRIS-HEPES ON ACETATE ABSORPTION

Acetate absorption was inhibited by the addition of 30 mmol/l propionate to perfusion solutions in the jejunum (Table III). For example, 30 mmol/l propionate inhibited acetate absorption from a 50 mmol/l acetate by 45.1%. Conversely, acetate could inhibit propionate absorption; 80 mmol/l acetate inhibited propionate absorption from a 70 mmol/l propionate solution by 43.3% (Table IV). The final luminal pH was higher after perfusion with acetate and propionate rather than acetate alone, suggesting that propionate was providing additional buffering to the bulk phase.

We speculated that propionate could have inhibited acetate absorption either by competing for a rate limiting common pathway in the mucosa or by raising luminal pH, rendering acetate more ionized and thus less lipid soluble.² In order to distinguish between these two possibilities, the jejunum was perfused with 30 mmol/l acetate with the addition of a structurally unrelated buffer, 10 mmol/l Tris-Hepes at pH 7.0. Tris-Hepes reduced acetate absorption in the jejunum by 39% ($p < 0.01$) (Table III). The effluent pH was similar after perfusion with added Tris-Hepes or added 80 mmol/l propionate (pH 6.89 (0.03) v 6.82 (0.02)).

EFFECT OF INTESTINAL SECRETION ON ACETATE ABSORPTION

The physiological condition of the animal may affect absorption rates particularly after cholera toxin. Weight loss, osmolality and arterial pH were measured in 10 rats after instillation of 75 μg cholera toxin (in 2 ml NaCl) for two hours followed by perfusion with 150 mmol/l NaCl with 30 mmol/l mannitol for one hour. Serum values were compared with those of unoperated rats which had received only anaesthesia (Table V). There was a significant rise in osmolality and weight loss suggesting that there was a predominant water loss. There was no fall in mean arterial pH although there was a greater range in pH after instillation of cholera toxin (range pH 6.9–7.6).

To determine the effect of intestinal secretion on acetate absorption a secretory state was induced by cholera toxin (Table VI). During cholera toxin induced secretion acetate absorption was reduced from 30 and 100 mmol/l acetate containing solutions. The functional integrity of the gut was confirmed by the finding that a 100 mmol/l glucose containing solution was able to reverse the cholera toxin induced secretion to absorption (3.1 (1.8) μmol/min/g) and glucose absorption in the secreting intestine (8.7 (0.7) μmol/min/g) was the same as in the normal intestine (8.6 (1.4) μmol/min/g).

To assess whether this reduction in acetate absorption was because of a direct effect on cellular transport processes or because of entrainment of acetate in the fluid stream a secretory state was induced by increasing the osmolality of the perfusion solution to 390 mosmol/l by the addition of 90 mmol/l mannitol. This resulted in water secretion which was similar to that obtained with cholera toxin (Table

TABLE IV Effect of acetate on propionate absorption

Perfused propionate concentration	Propionate absorption ($\mu\text{mol}/\text{min}/\text{g}$)	Propionate absorption +30 mmol/l (ace) ($\mu\text{mol}/\text{min}/\text{g}$)	Propionate absorption +50 mmol/l (ace) ($\mu\text{mol}/\text{min}/\text{g}$)	Propionate absorption +80 mmol/l (ace) ($\mu\text{mol}/\text{min}/\text{g}$)
30 (n=6)	4.1 (0.12)	3.5 (0.23)*	3.3 (0.37)*	3.1 (0.3)†
70 (n=6)	9.9 (1.4)	9.0 (0.8)	6.8 (0.3)*	5.6 (0.4)†

Results are expressed as the mean (SEM). Ace refers to acetate. Comparisons are made between solutions containing propionate alone and propionate plus acetate. * $p < 0.05$, † $p < 0.01$. n refers to the number of animals studied.

TABLE V Effect of two hours instillation of 75 μg cholera toxin on weight, osmolality, and arterial pH

	Normal (n=6)	After cholera toxin (n=6)
Weight loss (%)	—	7.8%
Osmolality (mosmol/l)	297.4 (2.4)	315 (3.2)*
Arterial pH	7.35 (0.05)	7.4 (0.25)†

* $p < 0.01$, † $p < 0.05$. n refers to the number of animals studied.

TABLE VI Effect of intestinal secretion on acetate absorption in rat jejunum

Solution perfused	n	Water secretion ($\mu\text{l}/\text{min}/\text{g}$)	Acetate absorption ($\mu\text{mol}/\text{min}/\text{g}$)
Acetate 30 mmol/l	6	+7.6 (1.4)	5.1 (0.3)
Acetate 30 mmol/l plus cholera toxin	5	-30.4 (4.0)*	3.2 (0.2)†
Acetate 30 mmol/l plus mannitol 90 mmol/l	6	-32.3 (2.4)*	2.9 (0.3)†
Acetate 100 mmol/l	6	+25.3 (2.8)	9.1 (1.0)
Acetate 100 mmol/l	9	-40.3 (3.9)*	5.3 (0.5)†

Results are expressed as the mean (SEM). Comparison in acetate and water absorption rates are made between solutions of appropriate acetate concentration. * $p < 0.001$, † $p < 0.01$. Positive values indicate net absorption and negative values net secretion. n refers to the number of animals studied.

VI). Acetate absorption was reduced to 2.9 (0.43) $\mu\text{mol}/\text{min}/\text{g}$ which was similar to the rate found after cholera toxin (3.2 (0.2) $\mu\text{mol}/\text{min}/\text{g}$) (Table 6).

Discussion

The data presented here show that acetate absorption follows apparent saturation kinetics and is absorbed at a similar rate to glucose. Although one previous study of short chain fatty acid absorption in rat jejunum *in vitro* has shown saturation kinetics⁴ this was not found in another *in vitro* study of rat jejunum possibly because lower acetate concentrations were used.² The present study confirms previous observations that mutual inhibition of short chain fatty acid absorption occurs in the rat jejunum.^{1,2}

Because acetate is a weak acid with a pKa of 4.76, pH would be expected to influence absorption. *In vitro* studies in the rat have shown that the jejunum is more permeable to acetate at low pH suggesting that acetate is absorbed in the undissociated form.^{1,2} Apparent saturation kinetics and reduction of absorption by a structurally related substance are commonly taken to indicate a membrane carrier. Such phenomena, however, merely indicate the presence of a saturable step in the whole absorptive process from lumen to blood. Thus a rate limiting step – for example, consumption of a finite supply of luminal hydrogen ions could account for these observations. In this study it was not possible to address this issue directly as juxtamucosal pH was not measured. Juxtamucosal pH is known to be more acidic than the bulk phase and to some extent independent of bulk phase pH.¹⁷ Nevertheless, the observation that acetate absorption

could be reduced by the structurally unrelated buffer Tris-Hepes suggest that hydrogen ion supply might be rate limiting. Thus when there are insufficient hydrogen ions acetate cannot be converted to the more permeant acetic acid. Although it is not possible to exclude the possibility of a membrane carrier, we suggest this is less likely as it has been shown in the rat jejunum and guinea pig colon that a short chain fatty acid absorption increases with chain length.^{1,8} A stepwise increase in absorption is difficult to explain on the basis of a membrane carrier.¹

TCO₂ AND ACETATE ABSORPTION

There are a number of possible explanations for the enhanced luminal accumulation of TCO₂. First, it could be as a result of mucosal metabolism of acetate and diffusion of CO₂ back into the lumen: acetate is known to be metabolised in the rat small intestine and generate CO₂.² Glucose is also metabolised by the rat small intestine.¹⁸ No difference was found in TCO₂ appearance in response to comparable rates of acetate or glucose absorption. Although a propionate/bicarbonate exchanger has been claimed in the human ileum⁹ this does not seem a likely explanation for these data as a glucose/bicarbonate exchange has not been described. Another possible explanation is that intraluminal CO₂ is hydrated leading to the formation of bicarbonate and hydrogen ions.⁵ The hydrogen ions would then be consumed by acetate which would then be absorbed in the protonated form, leaving bicarbonate in the lumen. This seems less likely as TCO₂ accumulation also occurred with glucose absorption where presumably this mechanism would not apply.

EFFECT OF CHOLERA TOXIN INDUCED SECRETION ON ACETATE ABSORPTION

It is unlikely that an alteration in the physiological state of the rats can account for the reduction in acetate absorption. Fluid and electrolyte absorption in the rat jejunum is not affected by acid base disturbance.¹⁹ Hypovolaemia is known to increase absorption rates and so this factor cannot account for the reduction in acetate absorption.²⁰ Mucosal damage seems equally unlikely as glucose absorption was preserved. In the jejunum both cholera toxin and osmotically induced secretion reduced acetate absorption to a similar extent and this suggests that cholera toxin may have reduced acetate absorption by entrainment of acetate in the fluid stream. Glucose absorption was not affected by solvent drag as the secretory effect of cholera toxin was reversed. Cholera toxin is known to increase intestinal mucin secretion.²¹ It is possible that intestinal mucin could have contributed to the reduction in acetate absorption as mucin is known to act as a significant diffusion barrier to butyrate.²² This is unlikely, however, to have been a major factor as the osmotically induced secretion fully accounted for the reduction in acetate absorption (Table VI).

In conclusion we have found evidence from *in vivo* perfusion of rat jejunum for non-ionic diffusion of acetate. We have previously shown

that acetate stimulates water and sodium absorption in the normal rat small intestine but that this effect is abolished when the gut is pretreated with cholera toxin.¹² The data presented here suggest that cholera toxin might be inhibiting the stimulatory effect of acetate on sodium absorption by reducing the absorption of acetate itself.

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