Human intestinal ion transport *in vitro*

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SUMMARY The transport of sodium and chloride across human jejunal and ileal mucosa was studied using an *in vitro* technique. Specimens of mucosa removed at operation were stripped of muscle coats, mounted in specially designed Perspex flux chambers and bathed in warmed oxygenated and stirred buffer solutions.

Evidence was obtained for the active transport of sodium in both jejunum and ileum and of chloride in the ileum. Sodium absorption was enhanced by glucose in both regions of the gut but net chloride transport was unaffected. Glucose had a greater effect on sodium transport in the ileum than the jejunum.

The electrical potential difference and resistance was greater and unidirectional ion fluxes smaller in jejunal than ileal mucosa.

Many of these results with human intestine are similar to results reported with *in vitro* animal intestine. Apparent discrepancies between the behaviour *in vivo* of human intestine and *in vitro* of animal intestine are thus likely to be due predominantly to technical rather than species differences.

Much current information about mechanisms of intestinal electrolyte transport rests on the results of *in vitro* investigations using animal intestine. Human intestinal ion transport on the other hand has been studied mainly by *in vivo* techniques and relatively few *in vitro* investigations have been undertaken. Such discrepant observations as the failure of glucose to enhance sodium absorption *in vivo* in human ileum (Fordtran *et al.*, 1968) but not *in vitro* in the rabbit ileum (Schultz and Zalusky, 1964b) and the failure to demonstrate bicarbonate absorption *in vitro* in rabbit jejunum (Fromm, 1973), while *in vivo* in man it clearly occurs (Fordtran *et al.*, 1968), could be due to species differences or to technical variations. The present human *in vitro* studies were undertaken for comparison with *in vitro* animal and *in vivo* human intestinal behaviour.

Methods

Human intestine was obtained from patients undergoing small bowel resection for a variety of gastrointestinal disorders. Ileum was mainly from

patients with colonic carcinoma, ulcerative colitis, and Crohn's disease, and jejunum from patients undergoing reconstructive biliary or pancreatic surgery, and upper gastrointestinal anastomoses. A variety of drugs had been given to the patients preoperatively but none had received corticosteroids or immunosuppressive therapy for at least two months before operation. The main anaesthetic was usually halothane but premedication varied. Atropine, omnopon with scopolamine, and mepyramine with pethidine were most commonly given. The cooperation of the surgeon was enlisted to ensure that the time between devascularisation and removal of the bowel was as short as possible. A segment of the resected bowel was opened along its mesenteric border and any intestinal contents washed away with buffer solution. The bowel was then pinned out onto a cork board and the mucosa carefully dissected off the muscle and serosal coats, being moistened throughout this time with oxygenated buffer solution. The mucosa, immersed in chilled oxygenated buffer was transported to the laboratory for mounting in the flux chambers. The buffer solution used before mounting was identical with that employed in the subsequent experiment. The time between removal of the bowel from the patient and mounting seldom exceeded 20 minutes.

The stripped mucosal preparation was similar to that described by Field *et al.* (1971) and Nellans

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et al. (1974) and consisted of mucosa, lamina propria, muscularis mucosae, and occasional tags of submucosal tissue. Only mucosa which was normal on naked-eye examination was accepted, and normality was subsequently confirmed by histological examination.

The design of the flux chambers was based on that of Schultz and Zalusky (1964b), the mucosa being clamped between two Perspex half-chambers, exposing an area of 1.77 cm² to separate 10 ml volumes of warmed (37°) buffer solution. The chambers and reservoirs were waterjacketed to maintain a constant temperature, and oxygenation and stirring were accomplished by a gas-lift circulating system. The gas employed, a 95% O₂/5% CO₂ mixture, was bubbled through water to minimise evaporative loss. The composition of the buffer solution was Na 146, K 4.2, Ca 0.6, Mg 0.6, Cl 126, HCO₃ 27.6, HPO₄ 0.1, H₂PO₄ 1.2 mmol.l⁻¹ (pH 7.55-7.60). In some studies D-glucose 15 mmol.l⁻¹ was present in both the mucosal and serosal solutions.

Histological examination of mucosal preparations incubated for up to 3½ hours in the flux chambers showed only slight oedema of the lamina propria at the villous tips. Electron microscopic examination of mucosa incubated in glucose-containing solutions showed dilated lateral intercellular spaces with intact tight junctional complexes. The viability of the mucosa over long periods was demonstrated by its ability to maintain a significant potential difference (PD) and short circuit current (SCC) for at least three hours, the PD increasing with the addition of glucose at this time, and declining rapidly on cessation of oxygenation or the addition of metabolic inhibitors. Electron microscopy showed a normal subcellular structure and intact organelles after three hours' incubation.

ELECTRICAL MEASUREMENTS

The PD across the mucosa was measured with matched calomel electrodes (EIL) immersed in saturated KCl, and connected to a high-impedance digital voltmeter (Solartron A240). The calomel electrodes were connected to the chambers by two narrow-tipped saturated KCl-in-agar bridges placed close to the mucosa. The diameter of the bridge tips was less than 0.5 mm to minimise diffusion of KCl into the bathing solutions. Electrolyte concentrations in the bathing solutions varied by less than 2% during the course of an experiment. The combined electrode and bridge junction potentials were determined before tissue mounting and rarely exceeded 0.5 mV. The mucosa was short-circuited by a current passed from an external dry battery via Ag/AgCl electrodes immersed in saturated KCl to narrow-tipped bridges containing NaCl

(1 mol.l⁻¹) positioned at opposite ends of the conical half-chambers. The current passed was measured by a microammeter (Scalamp) and adjusted manually at least every five minutes and usually more frequently. In all experiments corrections were made for the resistance to the fluid gap between the tips of the potential measuring electrodes as described by Field *et al.* (1971). Transepithelial resistance (R) was calculated from open circuit PD and SCC. Although this does not give an unequivocal estimate of resistance, it provides a reasonable approximation.

Rapid changes in PD, SCC, and R, similar to those described in rabbit ileum (Glew *et al.*, 1973), occurred with both jejunal and ileal mucosa on mounting in the flux chambers. After a rapid initial rise, a fall in PD was seen in nearly all tissues followed by a second rise of variable magnitude. By 30 to 40 minutes after mounting, PD, SCC, and R had reached stable values and in the absence of stimulation declined slowly and linearly over the next two to three hours. Because of these early variations, electrical values quoted were taken at 50 minutes after mounting unless otherwise specified.

RADIO-ISOTOPE FLUXES

1.5 µCi ²²Na and 2.5 µCi ³⁶Cl (Radiochemical Centre, Amersham, Bucks, England) were added to either the mucosal or serosal solutions and, after a 20 or 40 minute equilibration period, both mucosal and serosal reservoirs were serially sampled at 20 minute intervals, the volume removed being replaced with unlabelled buffer. Tissue pairs in which the electrical resistance differed by less than 25% were used to obtain net flux results, and their electrical parameters were averaged to yield single values for each tissue pair.

Samples were counted for ²²Na in an automatic well-type gamma counter (Wallac) together with a standard for ²²Na. An aliquot of the sample was then transferred into 5 ml Bray's solution for liquid scintillation counting (Nuclear Chicago Mk. II). The ²²Na gamma counts, multiplied by a factor for the relative efficiency of the two counters for ²²Na, were then subtracted from the total beta counts to give the ³⁶Cl counts. All samples were counted to 10 000 counts and the output of the gamma and beta counters was recorded on punched tape. The calculations were performed on a PDP 12 digital computer.

CALCULATIONS

Unidirectional fluxes of Na and Cl were calculated using an equation similar to that of Schultz and Zalusky (1964a). In the present studies both the mucosal and serosal reservoirs were serially sampled,

hence the specific activity of the initially labelled solutions was recalculated for each sample. The equation used was as follows:

$$J = \left(\frac{C_2}{P_2^*} - \frac{C_1}{P_1^*} \right) \cdot \frac{V}{A \cdot t}$$

where J is the unidirectional flux of an ion, C_1 and C_2 are the activities (cpm.ml⁻¹) of the unlabelled solution in consecutive samples, P_1^* and P_2^* are the specific activities (cpm.ml⁻¹. μ Eq⁻¹) of the labelled solution in consecutive samples, V is the volume of the labelled reservoir (10 ml), A is the area of exposed mucosa (1.77 cm²), and t is the interval between samples (hours).

Tissue resistance was calculated as $\frac{PD \times A}{SCC}$.

Short circuit current in μ amps was converted to μ mol.cm⁻².h⁻¹ by multiplying by a factor of 0.02108. $\left(\frac{3.6 \times 10^3}{AF} \right)$ where F is the Faraday constant). This formula relates electrical current to total net ion flux.

Residual ion flux (JRnet), which is that part of the SCC (in μ mol.cm⁻².h⁻¹) not accounted for by net movement of Na and Cl, was calculated as SCC—(JNa net + JCl net).

All values are expressed as the mean $\pm$ 1 standard error.

Statistical comparisons were performed by Student's t test. Paired and unpaired analysis were performed where appropriate.

Results

The electrical characteristics of jejunal and ileal

mucosa in glucose-free solutions are shown in Table 1. PD was significantly higher in the jejunum, while SCC was higher in the ileum. The calculated tissue resistance in jejunum was greater than that in the ileum. In both jejunum and ileum there was a significant net absorption of sodium in glucose-free solutions (Table 2). There was a small net secretion of chloride in the jejunum and ileum, neither value being significantly different from zero. The calculated residual ion flux was compatible with a small, insignificant absorption of an anion in the jejunum and with a significant secretion of an anion in the ileum. This is usually taken to indicate bicarbonate ion movement but could equally be due to movement of hydrogen ions in the opposite direction. It is unlikely, however, to be due to movement of other ions which are present only in small concentrations. The smaller unidirectional fluxes in the jejunum reflect the greater electrical resistance of jejunal tissues.

Table 1 Influence of glucose on potential difference, short circuit current, and tissue resistance in human jejunal and ileal mucosa

	PD (mV)	SCC (μ A. cm ⁻²)	R (ohm. cm ⁻²)
<i>No glucose</i>			
Jejunum n = 22	5.6 $\pm$ 0.4	70.6 $\pm$ 4.3	82.6 $\pm$ 5.8
Ileum n = 48	3.3 $\pm$ 0.3	89.9 $\pm$ 6.1	40.3 $\pm$ 2.8
P	<0.001	<0.05	<0.001
<i>Glucose</i>			
Jejunum n = 49	7.7 $\pm$ 0.3	181.9 $\pm$ 12.2	52.7 $\pm$ 4.3
Ileum n = 50	8.4 $\pm$ 0.4	252.4 $\pm$ 15.8	36.5 $\pm$ 1.6
P	NS	<0.001	<0.001

n refers to number of pieces of mucosa. Usually four pieces were obtained from each operative specimen.

Table 2 Ion transport across human jejunal and ileal mucosa and influence of glucose on transport

	JNa ms	JNa sm	JNa net	JCl ms	JCl sm	JCl net	JR net	SCC
<i>No glucose</i>								
Jejunum n = 7	6.5 $\pm$ 1.2	5.2 $\pm$ 1.1	+ 1.3 $\pm$ 0.4*	4.8 $\pm$ 1.0	6.6 $\pm$ 0.7	- 1.8 $\pm$ 0.9†	- 0.7 $\pm$ 0.7†	2.4 $\pm$ 0.4
Ileum n = 15	11.3 $\pm$ 0.6	10.1 $\pm$ 0.4	+ 1.2 $\pm$ 0.5*	9.7 $\pm$ 0.6	10.1 $\pm$ 0.3	- 0.4 $\pm$ 0.6†	+ 1.3 $\pm$ 0.5*	2.9 $\pm$ 0.3
P	<0.001	<0.001	NS	<0.001	<0.001	NS	<0.05	NS
<i>Glucose</i>								
Jejunum n = 15	12.9 $\pm$ 1.5	7.1 $\pm$ 0.6	+ 5.9 $\pm$ 1.2	7.9 $\pm$ 0.9	9.1 $\pm$ 0.8	- 1.3 $\pm$ 0.8†	- 0.3 $\pm$ 0.8†	6.8 $\pm$ 0.7
Ileum n = 15	20.2 $\pm$ 1.5	8.1 $\pm$ 0.5	+ 12.1 $\pm$ 1.4	11.1 $\pm$ 0.6	9.5 $\pm$ 0.7	+ 1.6 $\pm$ 0.7*	+ 0.1 $\pm$ 0.7†	10.6 $\pm$ 1.0
P	<0.005	NS	<0.005	<0.01	NS	<0.02	NS	0.01

Jms indicates unidirectional flux from the mucosal to the serosal side of the tissue, Jsm the flux in the opposite direction, and Jnet the difference between these unidirectional fluxes. +: absorption. -: secretion. All fluxes and SCC are given in μ mol.h⁻¹.cm⁻².

n = number of tissue pairs. Results from two or three consecutive 20 minute flux periods were averaged for each pair of tissues.

*significantly different from zero $P < 0.05$.

†not significantly different from zero.

EFFECT OF GLUCOSE

In both jejunum and ileum glucose enhanced PD and SCC ($P < 0.001$) (Table 1). Ileal resistance was unaffected, but in the jejunum the resistance was lowered by glucose ($P < 0.001$). This is reflected in greater unidirectional fluxes in the jejunum in the presence of glucose compared with those in glucose-free solutions. PD was similar in jejunum and ileum in the presence of glucose, while SCC was higher in ileum. Resistance was again greater in the jejunum than the ileum.

Glucose increased net sodium absorption in jejunum ($P < 0.02$) and ileum ($P < 0.001$) mainly because of an increase in the mucosa to serosa unidirectional flux (Jms Na) (Table 2). Ileal unidirectional chloride fluxes were not significantly altered by glucose but in this group of experiments net Cl absorption was significantly greater than zero. In the jejunum, unidirectional chloride fluxes were greater in the presence of glucose, but the net value was unchanged. Residual ion flux was not significantly affected by glucose in the ileum or jejunum. The increase in net sodium absorption and SCC produced by glucose was greater in the ileum than the jejunum.

Discussion

These results are broadly similar to those reported with *in vitro* animal intestine. They differ, however, in several important respects from those obtained *in vivo* in man and animals and it is clear that technical rather than species differences are responsible for many of the discrepancies between *in vivo* human and *in vitro* animal intestinal behaviour.

JEJUNUM

There have been difficulties in demonstrating transport of sodium against electrochemical gradients in the human jejunum *in vivo* (Fordtran *et al.*, 1968; Turnberg *et al.*, 1970b), probably because of the high mucosal permeability to sodium which prevents the development of concentration gradients. The present studies and those of Binder (1974), however, do demonstrate that human jejunal mucosa can transport sodium actively *in vitro* in glucose-free solutions. Glucose enhanced sodium absorption, as *in vivo*, and the rise in SCC was entirely attributable to this effect on sodium transport.

The absence of a significant net chloride movement in short-circuited jejunal mucosa is compatible with similar observations *in vivo* where all chloride movement appears to be a passive consequence of electrochemical gradients (Fordtran *et al.*, 1968). Glucose had no effect on net chloride transport,

although both unidirectional fluxes were increased, presumably associated with the lower tissue resistance in the presence of glucose.

Significant bicarbonate movement as judged by the residual ion flux, was not seen, although *in vivo* studies in man have demonstrated jejunal absorption of bicarbonate against a considerable electrochemical gradient (Turnberg *et al.*, 1970b). It is likely that factors such as pH, PCO_2 , and HCO_3 gradients, which are present *in vivo* but usually excluded *in vitro*, may explain these differences.

Fromm (1973) demonstrated that short-circuited rabbit jejunum *in vitro* secreted sodium and bicarbonate, while chloride movement was negligible. These differences in sodium and bicarbonate movement between human and rabbit jejunum presumably reflect species differences.

ILEUM

We have confirmed the experimental observation in human and animal tissue that sodium absorption occurs against an electrochemical gradient in the ileum *in vitro* (Grady *et al.*, 1967; Field *et al.*, 1971; Al-Awqati *et al.*, 1973) and *in vivo* (Fordtran *et al.*, 1968; Turnberg *et al.*, 1970a).

However, net absorption of chloride by ileal mucosa was demonstrated in only some of the present studies. For example, it was not demonstrated in the control investigations in the absence of glucose but in the presence of glucose net chloride absorption became significant (Table 2). This variability could conceivably be due to variations in preoperative drug therapy and anaesthesia but it is of interest that a similar variability has been remarked on by several workers in animal experiments where this possible source of variation is obviated (Schultz *et al.*, 1964; Binder *et al.*, 1973; Al-Awqati *et al.*, 1973; Nellans *et al.*, 1975). It has been suggested that some of this variability might be due to variations in mucosal adenyl cyclase activity or cyclic 3', 5', adenosine monophosphate concentrations but there is no firm evidence in support of this (Nellans *et al.*, 1974).

This demonstration of active sodium and chloride absorption *in vitro* confirms the *in vivo* behaviour of human ileum (Fordtran *et al.*, 1968; Turnberg *et al.*, 1970a). Ileal residual ion fluxes suggested bicarbonate secretion (or hydrogen absorption) in glucose-free solutions, as has been demonstrated in man *in vivo* (Turnberg *et al.*, 1970a) and in animals *in vitro* (Field *et al.*, 1971; Dietz and Field, 1973).

Glucose enhanced net sodium absorption as in animal ileum (Schultz and Zalusky, 1964b; Field *et al.*, 1971; Fromm, 1975), and the increase in

sodium absorption was greater in ileum than jejunum. This observation is apparently dissimilar from the *in vivo* ileal response (Fordtran *et al.*, 1968) where significant stimulation of sodium absorption is not observed.

COMPARISONS BETWEEN JEJUNUM AND ILEUM

It is clear that *in vivo* (Fordtran *et al.*, 1968; Turnberg *et al.*, 1970a, b), human jejunum and ileum behave differently towards water and electrolytes. Thus, *in vivo* jejunal mucosa is much more permeable to water and small ions than ileal mucosa (Fordtran *et al.*, 1965). Thus, it might have been predicted that *in vitro* unidirectional fluxes, especially of sodium, would be higher in jejunum than ileum, and electrical PD and tissue resistance higher in ileum than jejunum. However, the reverse situation was observed and unidirectional fluxes of Na and Cl were lower in jejunal than ileal mucosa.

This paradoxical finding of a higher ileal permeability is difficult to explain but it seems likely that it is due to differences in experimental conditions between *in vivo* and *in vitro* studies. Conceivably mucosa from one region of the intestine changes its function relative to the other on transfer to the *in vitro* situation. In support of this is the observation here that the presence of glucose in the bathing media was associated with a significant fall in jejunal resistance over a 50 minute period suggesting an effect on permeability not observed in ileal mucosa and indicating differing responsiveness to *in vitro* experimental conditions. Whatever the cause for these discrepancies between *in vitro* and *in vivo* studies, they do suggest the need for caution in relating results obtained *in vitro* to the *in vivo* situation.

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