Study of ion transport across biopsies of ileostomy in vitro: search for evidence of intestinal 'adaptation' after colectomy

P C HAWKER, A I MORRIS, J MCKAY, AND L A TURNBERG

From the Department of Medicine, Hope Hospital (University of Manchester School of Medicine), Salford, Lancashire

SUMMARY To explore the possibility that small intestinal 'adaptation' may occur after colectomy we examined the ability of ileal mucosal biopsies, taken from patients with ileostomies, to transport electrolytes in vitro. Ileostomy mucosal electrical potential difference was higher (5.4 ± 0.5 mV) than in normal mucosa (3.3 ± 0.3, p < 0.001), resistance was higher (98 ± 12, against 40.3 ± 2.8 Ω cm⁻²), while short circuit current was lower (54.8 ± 6.0; against 89.9 ± 6.1 μA cm⁻²). Net sodium absorption, 1.25 ± 0.41 μmol cm⁻² h⁻¹ (n = 6), rose on addition of glucose (15 mM.l⁻¹) to 11.57 ± 0.8 μmol cm⁻² h⁻¹ (n = 4), and these were similar to results from normal ileum. Net chloride transport was also similar to normal. In one subject, with intermittent ileostomy diarrhoea, net sodium absorption was normal, 2.14 μmol cm⁻² h⁻¹, but there was marked active chloride secretion, 14.03 μmol cm⁻² h⁻¹. These studies do not provide any evidence of enhanced electrolyte absorption across ileal mucosa as a response to colectomy. Some cases of ileostomy diarrhoea may be due to active chloride secretion.

After colectomy, the volume of ileostomy effluent decreases over a seven to 10 day period and becomes less fluid, suggesting that there is increased intestinal fluid absorption (Smiddy et al., 1960; LeVeen et al., 1962; Kanaghinis et al., 1963). This change is accompanied by an increased transmucosal electrical potential difference which, if it has been proposed, may reflect enhanced sodium absorption (Isaacs et al., 1976). These findings suggest that, after colectomy, a small intestinal 'adaptation' may occur with the development of an enhanced capacity for electrolyte absorption. However, Prado-p-de Moraes Filho et al. (1974), using the in vivo dialysis technique of Edmonds (1971), could not confirm this.

In order to examine ileal absorptive function more closely we have performed an in vitro study of the electrical and transport characteristics of biopsies of human ileostomy mucosa using a modified flux chamber (Using and Zerahn, 1951) developed in our laboratory.

Methods

PATIENTS Ten patients, two male and eight female, with an age range of 34–66 years, were studied. Panproctocolectomy had been performed for ulcerative colitis in eight cases and for Crohn's colitis in two. All patients had well established ileostomies, the time from operation ranging from 1.5–16 years. None of the patients was taking steroids or electrolyte supplements and nine were well, passing normal volumes of semi-fluid effluent. One patient complained of intermittent ileostomy diarrhoea.

The studies were performed with the informed consent of the subjects, the protocol having been approved by the Ethical Committee of Salford Area Health Authority (Teaching).

TECHNIQUES The potential difference (PD) across ileostomy mucosa in vivo was measured as previously described (Isaacs et al., 1976). An Ag/AgCl skin reference electrode was placed over an intradermal bleb of saline on the forearm, and an exploring Ag/AgCl electrode and a saline in agar bridge was placed in contact with the mucosa 10 cm from the

1Address for correspondence: Professor L A Turnberg, Department of Medicine, Hope Hospital (University of Manchester, School of Medicine), Salford, Lancs M6 8HD, UK.

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ileostomy tip. The PD between these electrodes was
recorded directly onto a calibrated chart recorder
and the 'offset potential' between the skin reference
and exploring electrode (≤5 mV), was subtracted
from the observed transtestinal PD to give the
true PD.

After recording the in vivo potential difference,
two mucosal biopsies, approximately 8 mm in
diameter, were taken from the same area of the
ileum, 10 cm from the ileostomy tip, using a Dick
suction biopsy instrument. The biopsies were
rapidly transported to the laboratory and clamped
together on specially designed miniature half
chambers exposing an area of mucosa 3 mm in
diameter. The mucosal biopsies were bathed on each
side by 2 ml isotonic buffer solution stirred and
oxygenated with 95% O2/5% CO2 mixture, via a
bubble lift mechanism and maintained at 37°C.
Two biopsies were usually mounted at the same
time and histological assessment of specimens revealed
no evidence of the patients' original disease. The
composition of the buffer was Na+ 146, K+ 4·2,
Ca++ 0·6, Mg++ 0·6, Cl− 126, HCO3− 27·6, HPO4−
0·1, H2PO4− 1·2, mM.L−1 (pH 7·55–7·6).

The spontaneous transmucosal PD was measured
via narrow tipped electrode bridges of 3% agar in
3M KC1 connected to a high impedance digital
voltmeter (Solartron A240, Solartron Electronic
Group Limited, Farnborough, Hants, UK) via
matched calomel half cells. The PD was reduced to
zero by passage of a direct current (short circuit
current, Isc) from external batteries via Ag/AgCl
electrodes connected to the bathing solution by 1%
agar in 1 M NaCl bridges. The short circuit current
(Isc) was adjusted manually and a correction for
the drop in potential caused by the solution between
the electrode tips was applied using the method of Field
et al. (1971). Tissue resistance, R, was calculated
from the open circuit PD and Isc using R = PD
Isc , A,

where A = area of tissue exposed (0·0707 cm2).

Isotopic fluxes were measured using 0·5 μCi 22Na
and 2·5 μCi 36Cl (The Radiochemical Centre,
Amersham, Bucks, England) the isotopes being added
to the mucosal reservoir of one and the
serosal reservoir of the other of the pair of chambers.
After a 35 minute period during which isotopic
equilibration occurred, isotopic fluxes were measured
during two 10 minute periods from 35–55 minutes
from mounting by taking successive 0·2 ml samples
from both labelled and unlabelled reservoirs. Steady
state was shown to be achieved by the constancy of
the fluxes. The samples were replaced with warm
unlabelled buffer of the same composition. The
22Na and 36Cl were counted and unidirectional fluxes
calculated as described previously (Isaacs et al
1976). The difference between the oppositely directed
fluxes of the simultaneously incubated tissues gave the
net flux of each ion. The sum of the net fluxes of all
ions is equal to the total current generated across the
tissue calculated in μmol.cm−2.h−1 (short circuit
current). Thus the difference between this current and
the sum of the measured net Na and net Cl fluxes provides a figure for the sum of all other un-
measured ion fluxes and is termed the residual ion
flux (JRnet). This 'flux' is likely to be largely derived
from bicarbonate or hydrogen ion transfer. The effect
of glucose, 15 mM.L−1, on steady state fluxes was
measured in the 20 minute flux period starting 10
minutes after addition of glucose (65–85 minute from
mounting).

The results obtained from ileostomy biopsies
were compared with results previously obtained in
this laboratory from normal mucosal segments
obtained from operative specimens and incubated
in a larger version of the biopsy chambers (Corbett
et al., 1977).

When tissues are clamped between half chambers
some crushing of the edges of the mucosa may occur,
possibly influencing results (Dobson and Kidder,
1968). This 'edge effect' may become significant in
the biopsy chambers where the area of mucosa ex-
posed to the bathing solutions is small (0·0707 cm2)
relative to the circumference. The edge damage was
minimised by clamping the biopsies gently between
two recessed, rubber 'O' rings. In order to assess the
extent to which 'edge effect' influenced the results an
initial series of experiments were performed using
rabbit ileum. The electrical values obtained from
tissue incubated in the biopsy chambers (exposed
mucosal area (0·0707 cm2) were compared with those
from tissues simultaneously incubated in the large
chambers (exposed mucosal area 1·77cm2).

Statistical comparisons were made using Student's
 t test for paired and unpaired values, p<0·05 being
considered significant. Graphs were drawn by the
method of least squares.

Results

Electrical Values

Comparison of biopsy chambers and large chambers
In rabbit tissues incubated in the small 'biopsy'
chamber potential difference and tissue resistance
were slightly lower than in simultaneously incubated
tissue in the large chamber 1·25±0·2 (ISD) com-
pared with 1·74±0·3 mV, and 35±0·8 compared with
47·2±6Ω.cm−2 respectively (n=4). Short circuit
current was slightly higher in the small chambers
(42·0±4 compared with 37·5±4 μA.cm−2). This
27% lower resistance and potential difference
probably reflects a greater ‘edge effect’ in the small chambers.

**Electrical values from human ileostomy mucosa**

After mounting, there was a slow decline in short circuit current, initially 54.8±6 μA.cm⁻² and reaching 40±9 μA.cm⁻² (n=12), after 55 minutes' incubation. The initial stable values of potential difference and tissue resistance (PD=5.4±0.5 mV, R=98±12 Ω.cmμ², n=12) fell after 15 minutes’ incubation and continued a slow decline to 3.9±0.6 mV at 55 minutes, tissue resistance falling slightly to 80±9 Ω.cm⁻². After glucose had been added to the mucosal reservoir potential difference and short circuit current rose (PD=5.6±0.8 mV, Iₑᵥ=101±12 μA.cm⁻²) and tissue resistance fell (70±10 Ω.cm⁻² n=12). Although the mean initial in vitroc potential difference (5.4±0.5 mV) was significantly lower than that measured in vivo (10.8±0.9 mV, n=8) (p<0.05) there was a highly significant correlation (R=0.99, p<0.01) between values obtained from the same subject (Figure).

Comparing the initial stable electrical values from ileostomy mucosa with normal values, Table 1 shows that ileostomy mucosa developed a higher potential difference but had a lower short circuit than the normal mucosa. The most striking observation was the high tissue resistance which was more than double control values even after the slight fall in resistance occurring with prolonged incubation and despite the greater influence of ‘edge effect’ in the biopsy chambers.

**ISOTOPIC FLUXES OF Na⁺ AND Cl⁻**

Isotopic sodium fluxes measured across tissue bathed in glucose free buffer showed lower unidirectional sodium fluxes across ileostomy mucosa when compared with normal, but there was no significant difference in net active sodium absorption (1.25±0.41, n=6, compared with 1.2±0.5 μmol.cm⁻².h⁻¹, n=15) (Table 2a). After glucose had been added to the mucosal reservoir net sodium absorption was

![Graph](http://gut.bmj.com/10.1136/gut.21.2.146)

**Figure**  In vivo electrical potential difference plotted against the in vitro potential difference of mucosal biopsies from the same subject. Where two biopsies were mounted the mean of the values is plotted. The in vitro values are those recorded during the initial stable period 10 minutes from mounting the tissue. There is a significant correlation between these values (r=0.99, p<0.001).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of in vitro electrical values obtained from ileostomy biopsies and from ileal mucosa obtained at operation</th>
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<tbody>
<tr>
<td></td>
<td>Ileostomy mucosa</td>
</tr>
<tr>
<td></td>
<td>Normal ileal mucosa</td>
</tr>
<tr>
<td>PD (mV)</td>
<td>5.4±0.5                                                          54.8±6.0                                                          98±12</td>
</tr>
<tr>
<td>Iₑᵥ (μA.cm⁻²)</td>
<td>&lt;0.01</td>
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The separate studies were unpaired and the results are the mean (±SEM) of all values.

**Table 2**  Comparison of unidirectional fluxes of sodium and chloride across ileostomy mucosa and normal ileal mucosa obtained at operation bathed (a) in glucose free buffer and (b) in buffer containing 15 mm glucose*

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ms</td>
<td>sm</td>
</tr>
<tr>
<td>a. Glucose free buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileostomy mucosa</td>
<td>9.28</td>
<td>8.03</td>
</tr>
<tr>
<td>n=6</td>
<td>±0.84</td>
<td>±0.91</td>
</tr>
<tr>
<td>Normal ileal mucosa</td>
<td>11.3</td>
<td>10.1</td>
</tr>
<tr>
<td>n=15</td>
<td>±0.6</td>
<td>±0.4</td>
</tr>
<tr>
<td>&lt;0.05 &lt;0.05 NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Glucose containing buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileostomy mucosa</td>
<td>20.4</td>
<td>8.83</td>
</tr>
<tr>
<td>n=4</td>
<td>±1.6</td>
<td>±0.8</td>
</tr>
<tr>
<td>Normal ileal mucosa</td>
<td>20.2</td>
<td>8.1</td>
</tr>
<tr>
<td>n=15</td>
<td>±1.5</td>
<td>±0.5</td>
</tr>
<tr>
<td>NS NS NS</td>
<td></td>
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</tbody>
</table>

*Flux values are expressed in mmol cm⁻².h⁻¹.
†Not significantly different from zero.
equally stimulated in ileostomy mucosa (11.57 ± 0.8, n=4) and normal tissue (12.1 ± 1.4 μmol.cm⁻².h⁻¹, n=15), and there were no significant differences in unidirectional sodium fluxes (Table 2b). There were no significant differences between chloride fluxes either in glucose free or glucose containing buffer (Table 2). When glucose was added the calculated residual ion flux became significantly different from the normal values. This response could have been due, for example, to a stimulation of bicarbonate secretion or of hydrogen absorption by glucose in the ileostomy biopsies, but the significance of this observation is unclear.

One patient had had intermittent ileostomy diarrhoea since operation, four years before the study. The in vivo and in vitro electrical measurements observed in this subject (Table 3a) were similar to those from subjects without ileostomy diarrhoea. Sodium was absorbed normally (2.14 μmol.cm⁻².h⁻¹), but there was a marked net active chloride secretion of 14.03 μmol.cm⁻².h⁻¹, due almost entirely to an increase in the serosa to mucosa flux in this patient (Table 3). Because the chloride secretion was not electrogenic, there was a high calculated residual ion flux, possibly representing bicarbonate absorption, to maintain electroneutrality.

**Discussion**

It has been clearly shown here that ileostomy mucosa has a higher tissue resistance than normal ileal mucosa and it is also apparent that ileostomy mucosa is capable of maintaining a higher PD both in vivo and in vitro. In the absence of any increase in short-circuit current this higher potential difference simply reflects the increased tissue resistance. In making these changes in electrical activity and in the reduction in unidirectional fluxes of sodium the ileostomy mucosa begins to superficially resemble colonic mucosa.

Although the PD in vivo was higher than that measured in vitro in ileostomy mucosa, values from the same subject did correlate well with each other and the differences probably reflect the influence of ionic concentration and pH gradients in vivo and the 'edge damage' effect in vitro. In any event it seems likely that the high in vivo PD is also due to increased tissue resistance rather than enhanced active sodium transport as has been proposed (Isaacs et al., 1976). It is likely that 'edge' damage in these small chambers would result in an underestimate of mucosal resistance and thus the increased resistance demonstrated here is even more striking.

The reason why mucosal resistance is altered is unclear. There were no gross histological changes on light microscopy. The possibility exists that changes in the tight junction or lateral intercellular space, occurring in response to exposure of the mucosa to an increase in ileal bacterial flora, trauma, or changes in vascularity may be responsible.

The isotopic fluxes of sodium and chloride did not show evidence of enhanced absorption of sodium or chloride, despite conditions favourable for its demonstration in the biopsies which, as they were mounted more rapidly, are likely to be more viable than the operation specimens. The diminished unidirectional fluxes probably reflected a decreased passive permeability to sodium but this affected movement in both directions equally and did not influence net sodium absorption. The significance of the increased residual ion flux in the ileostomy mucosal response to glucose is uncertain. It could represent enhanced bicarbonate secretion or hydrogen absorption stimulated by glucose but the reason for its occurrence is obscure.

In this study we have measured absorption per square cm of mucosal surface area, and although it is possible that our technique is insufficiently sensitive to detect small changes in absorption, we have found no evidence to support increased electrolyte absorption per unit area of ileostomy mucosa. Any enhanced absorption which might occur must therefore

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**Table 3** (a) In vivo and in vitro electrical recordings obtained from a patient with ileostomy diarrhoea; (b) unidirectional fluxes across mucosal biopsies from same patient

<table>
<thead>
<tr>
<th></th>
<th>In vivo</th>
<th>In vitro</th>
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<tr>
<td></td>
<td>(MV)</td>
<td>(MV)</td>
</tr>
<tr>
<td>a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>12</td>
<td>5.6</td>
</tr>
<tr>
<td>Cl⁻</td>
<td></td>
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<table>
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<tr>
<th>Flux values including Iₑₑ are expressed in mmol cm⁻².h⁻¹.</th>
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<tr>
<td>b. 8-15, 6-11, ± 2-14</td>
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be derived from an alternative mechanism.

The study performed on the subject with ileostomy diarrhoea was of interest. The potential difference measurements in vivo and in vitro were the same as those from well ileostomists and the in vitro tissue resistance was similar to that measured in the other ileostomy biopsies. This suggests that these changes occur in all ileostomists whether they have diarrhoea or not. Although we were unable to repeat the biopsy study in this subject to confirm our results it seems likely that the chloride secretion was the major driving force in producing diarrhoea. As it was an electrically neutral process in these short circuited tissues movement of chloride must have been accompanied by transfer of another ion to maintain electro neutrality. The most likely possibilities are absorption of HCO₃⁻ in exchange for chloride or secretion of H⁺ with chloride. These cannot be distinguished in these studies and in either case diarrhoea would result from accumulation in the lumen of H⁺ and Cl⁻. The fate of H⁺ in the lumen has not been investigated in this study, but it could remain there, lowering pH of ileostomy discharge or it could exchange for sodium across the mucosa. The possibility that another ion such as potassium might have accompanied the chloride seems remote, and indeed the potassium concentration of this patient's ileostomy ejecta varied only between 5·4 and 8·3 mM.1⁻¹.

References


