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Intestinal permeability in the ileal pouch

M N Merrett, N Soper, N Mortensen, D P Jewell

Abstract

***Background*—Villous atrophy, mucin changes ('colonic metaplasia'), and chronic inflammation occur to varying degrees in all patients with ileal pouch-anal anastomosis whereas acute inflammation (pouchitis) affects a subgroup of patients with prior ulcerative colitis.**

***Aim*—To measure epithelial barrier function looking for possible functional adaptation in ileal 'pouch' mucosa.**

***Patients*—Patients with an ileal pouch prior to ileostomy closure (n=12), functioning pouch (n=14), pouchitis (n=8), and ulcerative colitis (n=12) were assessed.**

***Methods*—^{51}Cr-EDTA was administered into the 'pouch' or rectum and urinary recovery over 24 hours was taken as an indication of permeability (barrier function). Histological analysis of 'pouch' biopsy specimens was undertaken.**

***Results*—Mucosal permeability is decreased from median 9·4% (range 5·4% to 39·1%) to 1·4% (range 0·38% to 2·2%) after ileostomy closure ($p<0·002$) with levels being negatively correlated with two histological parameters of colonic metaplasia – mucin changes ($p=0·03$) and villous atrophy ($p=0·05$). Pouchitis was associated with increased permeability 5·9% (1·9% to 19·5%) compared with healthy 'pouch' 1·4% (0·35 to 2·2%) ($p<0·006$).**

***Conclusion*—Despite the presence of chronic inflammation in the mature 'pouch' functional adaptation with reduced permeability occurs in conjunction with colonic metaplasia.**

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Keywords: ileal pouch-anal anastomosis (IPAA), ulcerative colitis, permeability, ^{51}Chromium-EDTA, colonic metaplasia.

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Ileal pouch-anal anastomosis (IPAA) is the procedure of choice for most patients with ulcerative colitis (UC) requiring proctocolectomy.[1–5] Despite this IPAA is associated with significant morbidity both in the early postoperative period as well as in the longer term.[1 4–6]

The most significant longterm complication is pouchitis.[1 7–11] The reported incidence varies from 7–45% depending on definition and particularly on whether histological confirmation is required.[10–15] Pouchitis is characterised by an increase in frequency of stools with occasional blood and mucus and general malaise. These symptoms often respond well to antibiotics. However, some patients with pouchitis develop recurrent or chronic symptoms that may require prolonged medical treatment including corticosteroids.[1 7 10] The aetiology of pouchitis is unknown. It occurs almost exclusively in patients who previously had UC, which suggests that it may represent recurrent inflammation similar to the original disease in ileal mucosa that has undergone colonic metaplasia.[9] Other possible causes for pouchitis include bacterial overgrowth[16–18] and changed bile acid metabolism,[8] ischaemia[19–21] and in some cases unrecognised Crohn's disease.

Chronic inflammation and colonic metaplasia occur in all pouches regardless of the original indication for IPAA. The key histological features of colonic metaplasia include loss of villous height with compensatory crypt elongation and a raised crypt cell proliferation rate (CCPR).[11 15] These changes occur soon after ileostomy closure with the resumption of faecal stream (and stasis) within the pouch.[15] The significance of colonic metaplasia is largely unknown. It may represent a useful adaptive response with improved (colon-like) barrier function to the new luminal environment but may also predispose susceptible subjects to pouchitis.[14]

Barrier function may be assessed in vivo by measuring permeation of different molecular weight probes across the intestinal wall. These probes have been used to study the increased permeability of mucosa seen in different disease states. In this study, methodology using ^{51}Chromium-EDTA (^{51}Cr-EDTA) as a probe was adapted to assess barrier function in IPAA.[22–25]

The aim of this study was to assess barrier function (as measured by ^{51}Cr-EDTA permeability) in IPAA before and after ileostomy closure and with pouchitis. Rectal permeability in active and inactive UC was measured as an indication of relative barrier function to IPAA. Finally a pilot study was undertaken to assess the hypothesis that nicotine improves barrier function in IPAA.

Methods

Patients

Twelve patients awaiting ileostomy closure, eight patients with pouchitis, and 14 patients with healthy functioning pouches were assessed. All patients had had prior UC. For the purposes of this study healthy pouches were defined as patients with ≤ 8 stools/day with normal endoscopy and a histology index ≤ 5.[26] Pouchitis was defined as an increase in stool pattern ≥ 8/day with the presence of erythematous, granular mucosa with or without ulceration at endoscopy, and with a histology index ≥ 7. The histology index is calculated by scoring 0–3 for the degree of villous

atrophy, ulceration, acute and chronic inflammation. A further 12 patients with UC and intact colons had rectal permeability tests. Six patients had an acute exacerbation of their colitis requiring corticosteroids – that is, 'active colitis', and six patients had 'quiescent UC' and were taking oral 5-aminosalicylic acid (5-ASA).

Eight patients with IPAA prior to ileostomy closure were assessed on two occasions four weeks apart. Four patients were given transdermal nicotine and four had no pharmacological intervention during the four weeks. The patients taking transdermal nicotine were instructed to increase the dose by 15 mg/week until symptoms of nicotine 'toxicity' such as nausea occurred.

Preparation of patients

Four patients were assessed twice in preliminary experiments. The pouch was 'washed' with 500 ml of isotonic saline after evacuation via a Quill (Southern Syringe Services, Dudley, UK) introduced per anus to ensure clearance of faecal material. The permeability test was then repeated two weeks later after evacuation only. A minor increase in permeability was noted. The 'washed' pouches had a mean permeability of 1·83 compared with 1·28 in pouches without prior washout. To minimise potential discomfort to patients we elected to assess permeability after evacuation only. All patients were asked to abstain from alcohol and not to take medication for 24 hours before the test. Patients were excluded if they were taking non-steroidal anti-inflammatory drugs (NSAIDs), had pouch-anal stenosis as assessed by digital examination or had significant concomitant medical disease, including reduced creatinine clearance. The test was explained and informed consent obtained. The ethics committee of the Oxfordshire Health Authority gave permission for the study. The Medicines Control Agency (London) granted permission for the use of transdermal nicotine (Elan Laboratories, Ireland). The Administration of Radioactive Substances Advisory Committee in London permitted the use of radioisotopes.

Preparation of the dosing solution

Aliquots of 3·7 MBq ^{51}Cr-EDTA (Amersham, UK) were prepared at the time of the test and made up to 4 ml with isotonic saline. A 100% ^{51}Cr-EDTA standard was prepared and counted in the same manner as the urine samples.

Administration of dose

A 10 cm Quill (Southern Syringe Services, Dudley, UK) was introduced per anus into the pouch/rectum with the patient in the left lateral position. Two ml of isotonic saline were administered to ensure correct positioning. The 4 ml radioactive sample was instilled followed by a further 4 ml of isotonic saline to minimise residual radioactivity in the Quill. Patients remained prone for 20 minutes and were encouraged to drink 500 ml of fluid to ensure an adequate urine output. No pouch evacuation was permitted for four hours after administration of ^{51}Cr-EDTA. Urine collection continued for 24 hours.

Sample preparation and measurement

The 24 hour urine volume was recorded and 10×1 ml aliquots taken for assay. The urine aliquots and standards were counted within 24 hours using a well type gammacounter (LKB 1260 Multigamma II). A 20 ml aliquot of urine was stored at −20°C for subsequent creatinine assay.

Patient follow up

Patients were endoscoped immediately after urine collection with pouch or rectal biopsy specimens being taken. Paraffin wax sections were stained routinely with haematoxylin and eosin as well as with high iron diamine-alcian blue as previously described.[27] The sections were scored for mucin (1=sialomucin only, 2=predominant sialomucin, 3=predominant sulphomucin, and 4=sulphomucin only) and for the degree of villous atrophy.[1–4] Eight patients awaiting ileostomy closure were assessed on a second occasion. Of these, four had taken transdermal nicotine for four weeks and a control group had no intervention.

Statistics

Group comparisons were made using the Wilcoxon rank test for non-parametric data. The significance of association was assessed by means of Spearman's rank correlation.

Results

Permeability as assessed by urinary recovery of ^{51}Cr-EDTA showed pronounced differences (Table I, Fig 1). There was lower permeability in the healthy pouches more than 12 months after ileostomy closure compared with healthy pouches in the first 12 months but the difference was not significant (p=0·1) and so healthy pouches were combined as one group.

TABLE I *Urinary ^{51}Cr-EDTA recovery as percentage of per anal administered dose*

	Preclosure (n=12) (%)*	*Healthy pouch*† (<12 months, n=6) (%)*	*Healthy pouch*† (>12 months, n=8) (%)*	*Pouchitis† (n=8) (%)*	*Active UC (n=6) (%)*	*Inactive UC (n=6) (%)*
Mean	14·5	1·29	0·88	5·9	15·1	2·1
SD	11·7	0·66	0·46	5·8	6·9	1·6
Median	9·4	1·30	0·79	5·9	14·5	2·2
Range	5·4–39·1	0·36–2·24	0·32–1·68	1·9–19·5	7·1–24·6	0·3–4·0

*Preclosure versus healthy pouch p≤0·002; †pouchitis versus healthy pouch p≤0·006.

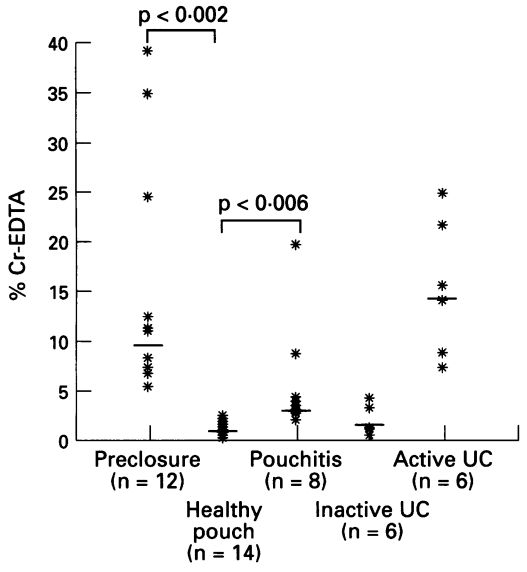

Figure 1: Scattergram showing differing urinary recovery of Cr-EDTA (permeability).

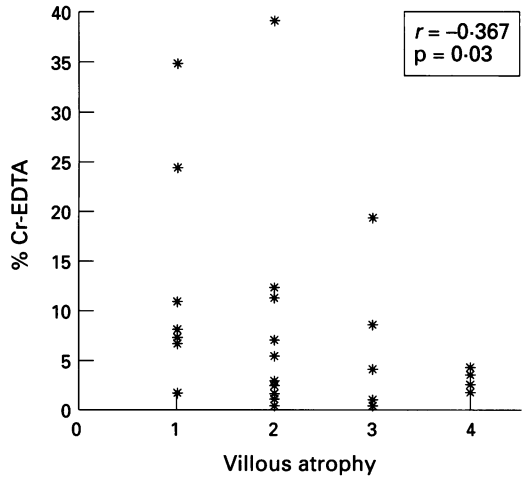

Figure 2: Scattergram showing a negative correlation between urinary ^{51}Cr-EDTA (permeability) and villous atrophy.

Permeability was significantly higher in preclosure pouches compared with healthy pouches ($p \leq 0{\cdot}002$). Pouchitis was associated with higher permeability than for a healthy pouch ($p \leq 0{\cdot}006$) but did not achieve preclosure levels. The permeability of healthy pouch and quiescent UC was similar ($p = 0{\cdot}12$). Active UC produced higher permeability levels than pouchitis ($p \leq 0{\cdot}02$).

Histological scores of villous height and mucin histochemistry for the three IPAA groups are shown (Table II). A significant loss of villous height is seen from preclosure to healthy pouches ($p \leq 0{\cdot}009$) and from healthy pouches to pouchitis ($p \leq 0{\cdot}02$). A partial switch to sulphomucin is seen in healthy pouches ($p < 0{\cdot}05$) compared with preclosure, however, this was less noticeable in pouchitis mucosa. There was a significant correlation between permeability score and histological grades. A scattergram comparing all three groups (preclosure, healthy pouch, and pouchitis) shows a significant negative correlation between ^{51}Cr-EDTA recovery and villous atrophy ($r = -0{\cdot}367$, $p = 0{\cdot}03$) (Fig 2) and mucin score ($r = -0{\cdot}34$, $p = 0{\cdot}05$) (Fig 3). The significance is increased if the pouchitis group is excluded for both villous atrophy ($r = -0{\cdot}65$, $p = 0{\cdot}0003$) and mucin ($r = -0{\cdot}48$, $p = 0{\cdot}01$).

Inflammation as assessed by a histology index[26] is listed in Table III. Pouchitis biopsy specimens were significantly more inflammed than preclosure pouch ($p = 0{\cdot}09$). Preclosure pouch scores were higher for inflammation than healthy pouch, however, the difference was not significant ($p = 0{\cdot}07$).

Discussion

Intestinal epithelium has conflicting functions. It permits absorption of nutrients, ions, and water while acting as a barrier to prevent potentially toxic substances in the intestinal lumen entering the subepithelial space. Aspects of barrier function can be assessed by the use of non-metabolisable probe markers with varying size. High molecular weight (MW) probes, for example, ^{51}Cr-EDTA (MW 340) are thought to traverse epithelium via a paracellular route while the small pore pathway (probes with MW <180) is less defined and probably includes transcellular and paracellular paths.[25] Such tests of barrier function are recognised as measures of intestinal permeability.[22 25 29]

IPAA is a useful model for assessing intestinal adaptation to a new luminal environment. Ileal epithelium before surgery is predominantly absorptive with long villi to increase the total surface area in contact with luminal content. This is reflected in the high values of ^{51}Cr-EDTA permeability in preclosure ileal pouch reported in this study. At the completion of surgery the ileal pouch (neorectum) takes on a reservoir function. The histological changes in pouch epithelium are well described[10–12 14 15 18] and are collectively known as 'colonic metaplasia'. The main features include loss of villous height, crypt elongation with an increased CCPR with an increase in both sulphomucin and 8-0 acetyl

TABLE II *Histological scores for villous atrophy and mucin. Results expressed as mean (SD)*

	Preclosure	*Healthy pouch*	*Pouchitis*
Villous atrophy (1–4)	1·4 (0·5)	2·2 (0·6)	3·4 (0·7)
Mucin score (1–4)	1·3 (0·5)	2·1 (1·0)	1·8 (0·9)

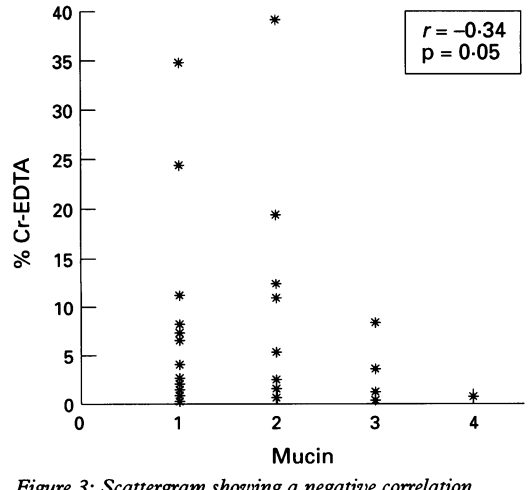

Figure 3: Scattergram showing a negative correlation between urinary ^{51}Cr-EDTA and mucin.

TABLE III *Histology index (26): a score for 'pouchitis'*

	Preclosure (n=12)	*Healthy pouch (n=12)*	*Pouchitis (n=8)*
Mean	4·0	3·1	7·9
SD	2·2	1·3	1·3
Median	4·0	3·0	7·5
Range	1·0–6·0	1·0–5·0	7·0–10·0

sialomucin (colonic mucins).[10–12 14 15 30 31] The significance of such changes and particularly whether functional adaptation occurs with respect to barrier function has not previously been reported.

Before assessing the results of this study several criticisms of the methodology need to be considered. Although patients evacuated before instillation of ^{51}Cr-EDTA it is possible that some of the reduction of urinary recovery of ^{51}Cr-EDTA from functioning pouches is due to dilution of the probe in residual faecal effluent. This is probably not an important factor as preliminary experiments using pouch washouts with isotonic saline versus evacuation alone did not significantly increase urinary recovery of ^{51}Cr-EDTA. Another problem relates to completeness of urine collection. To minimise this a creatinine clearance was measured and if $<1·5$ ml/sec the patient was excluded. At the end of the study only one exclusion (a patient with inactive UC) was necessary. The degree of inflammation as assessed by histology was higher in the preclosure group than healthy pouch ($p=0·07$), which may account for some of the difference between the two groups. Finally, no assumptions regarding macromolecule/antigen absorption can be made from studies using these comparatively small probes. No studies comparing 'antigen' or 'macromolecule' versus probe permeation have yet been performed.

In this study patients with ileal pouches before ileostomy closure have relatively high but variable permeability as assessed by permeation of ^{51}Cr-EDTA. These relatively high values (compared with orally administered isotope) may relate to concentration of isotope in the ileal reservoir.[24 25] A significant fall in permeability is seen in functioning pouches, which suggests that barrier function is increased in mature pouch mucosa. A trend toward further increase in barrier function after one year was not significant but this may indicate a type 2 error. Furthermore permeability is negatively correlated with two parameters of colonic metaplasia in the pouch – that is, villous atrophy and mucin type. The reduction in permeability seen in pouch mucosa is at least partly due to a loss of villous height and hence absorptive area. However, it should be noted that some patients with mature ileal pouches have good preservation of villous height although variability within the pouch needs to be considered. The improved barrier function may also relate to the increased 'colonic mucin' content or to some other factor, for example, 'tightening' of tight junctions. As mentioned before lower scores for inflammation in healthy pouches would also contribute to reduced permeability. The similar levels of permeability for healthy pouch and inactive UC suggests that the functional adaptation in the ileal pouch is almost complete despite variable degrees of histological colonic metaplasia. Although this is the first permeability study of ileal pouch adaptation much work has been published on the adaptation of small bowel mucosa in the blind loop syndrome.[32 33] Using self filling blind loops of rat jejunum Schulzke *et al* were able to show an increase in epithelial resistance from 8 ± 1 to $23\pm3/cm^2$ (Ussing chamber) in mucosa from the 'blind loop'. Ultrastructural study of the adapted mucosa showed an increase in depth of crypt tight junctions.[32]

The increase in permeability in pouchitis compared with healthy pouch (Table I, Fig 1) is not surprising. Increased small intestinal permeation of ^{51}Cr-EDTA has been reported in small bowel inflammation for both Crohn's disease[34–36] and coeliac disease.[37 38] The increase in permeability is probably due to a variety of factors including breaches in the epithelial barrier (ulcers), transepithelial migration of polymorphonuclear cells,[39] and soluble mediators, for example, PAF, γ interferon.[40] It is of interest that permeability to ^{51}Cr-EDTA in pouchitis was significantly lower than for active UC. This suggests that the inflammation in pouchitis is not as severe or that pouchitis may be a fundamentally different process to UC. The two patients with the highest permeability in the pouchitis group (Fig 1) were the only patients with extraintestinal manifestations. One patient had arthritis, pyoderma, and erythema nodosum (19·5% ^{51}Cr-EDTA) and the other patient had arthritis (8·5% ^{51}Cr-EDTA). Although anecdotal, it supports the concept that reduced barrier function to luminal content may be important in the pathogenesis of extraintestinal manifestations of inflammatory bowel disease.[41 42]

A small unblinded control study assessing the effect of transdermal nicotine on preclosure ileal pouch permeability was performed in eight patients. Although these data should be regarded as preliminary a pronounced reduction in permeability was seen in the patients treated with nicotine. In support of this Prytz *et al* have reported a reduced small intestinal permeability in smokers.[43] This effect on epithelial barrier function may account for the reduced incidence of UC[44–46] and pouchitis[47] in smokers. Barrier function is likely to be particularly important in the period immediately after ileostomy closure. At this time the absorptive (highly permeable) ileum is exposed to a large quantity of potential antigens and toxins. Exposure to the mucosal immune system in susceptible patients may initiate pouchitis. This is supported by the finding that pouchitis occurs on average 3·5 months after ileostomy closure.[10] It is in this early period that augmentation of barrier function by smoking/nicotine may be important in the subsequent reduction of pouchitis. This is of course highly speculative and it will be important to confirm the early findings reported in this study. If correct a controlled trial of nicotine for the six months after

ileostomy closure would be worthwhile particularly with regard to early pouch function and in the longer term prevention of pouchitis.

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