Gut, 1984, **25**, 145–150

Alimentary tract and pancreas

In vitro determination of small intestinal permeability: demonstration of a persistent defect in patients with coeliac disease

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SUMMARY Previous methods for measuring intestinal permeability involve the urinary measurements of various poorly digested sugar or inert poly(ethylene glycol) polymers after their oral administration. The results reflect a variety of gastrointestinal parameters including transit time, mucosal surface area and transfer, mesenteric blood and/or lymphatic flow and renal function, as well as mucosal permeability. A new *in vitro* method for direct measurements of mucosal permeability to three probes is described and permeability is shown to be inversely related to the molecular weight of the probe molecule. Using this technique, a persistent increase in mucosal permeability to certain probes (molecular weight less than 1500 daltons) has been shown in patients with coeliac disease in whom treatment by strict gluten withdrawal has been accompanied by restoration of intestinal histological normality. It is suggested that this finding represents a primary defect in this syndrome.

There is considerable interest in the question of altered intestinal mucosal permeability in various gastrointestinal disorders.[1–6] Investigation of this phenomenum has normally involved urine collections after oral administration of poly(ethylene glycol) polyers[1] or of various sugars.[2 3] The results are influenced by the rate of gastric emptying, luminal dilution, intestinal transit time, mucosal surface area, mucosal transfer, blood and/or lymphatic flow, renal excretion as well as mucosal permeability. Not surprisingly, conflicting results have been obtained and this has led to the general view that intestinal permeability is paradoxically increased to high but decreased to low molecular weight compounds in coeliac disease. These changes are claimed to be transient, returning to normal after treatment by gluten withdrawal.[7 8]

We have developed an *in vitro* method for determining intestinal permeability which overcomes many of the aforementioned problems. Using this technique, we have shown that permeability is inversely related to molecular size of the probes and have shown increased jejunal permeability in untreated coeliac patients. Histologically normal mucosa from patients treated for long periods by gluten withdrawal showed a persistent increase in permeability. It is suggested that this defect may be a primary abnormality, allowing gluten peptides access to the mucosa and thus initiating pathological reactions in susceptible individuals.

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Received for publication 14 April 1983

Methods

CONTROL SUBJECTS

Thirteen patients undergoing routine gastrointestinal investigation, but in whom no significant intestinal pathology was subsequently found, served as a control group. Their mean age was 47 years (range 18–81 years). The jejunal biopsy in each case was histologically entirely normal.

PATIENTS WITH COELIAC DISEASE

Patients with coeliac disease were divided into two groups depending on the severity of the histological findings in their jejunal biopsies, malabsorption indices and their symptomatic status. One group of eight patients showed severe partial or subtotal villous atrophy with histological findings of marked inflammatory cell infiltration of the lamina propria and epithelium, and degeneration of the enterocytes. These patients were either recently diagnosed or were symptomatic and had admitted to

recent gluten ingestion. Their mean age was 57 years (range 38–76 years). The other group of seven patients had made excellent recovery after gluten withdrawal. All were asymptomatic on questioning. Histological changes ranged from normal to minimal partial villous atrophy. None had increased numbers of inflammatory cells in the lamina propria and all had healthy looking surface epithelium with a well defined brush border, and all but two had normal intra-epithelial lymphocyte counts. Their mean age was 38 years (range 17–64 years) and mean duration of treatment was 11 years (range 6 months to 23 years, Table).

Intestinal biopsies were obtained with an adult Watson biopsy capsule, under radiological guidance, just distal to the ligament of Trietz. A portion of the biopsy was sent for histological examination and the remaining tissue was divided into 2–6 mg pieces and incubated at 37°C for up to 15 minutes in an oxygenated physiological medium.[9] No more than two minutes elapsed from retrieving the sample until the start of incubation. Three extracellular fluid markers were added to the medium, selected on the basis of their different molecular weight and their relative inability to cross the normal jejunal surface mucosa. The probes were (^{14}C)-hydroxy methyl inulin (mol wt 5200) (0·2 nmol/l), (^{57}Co)-cyanocobalamin, (mol wt 1240) (5 nmol/l) and ^{51}Cr-EDTA (mol wt 340) (5–10 nmol/l) (Amersham International, Amersham, Bucks). Duplicate tissue samples from each patient were removed after three, five, 10 and 15 minutes incubation except in three of the patients with untreated coeliac disease where the 15 minute incubation was not possible because of lack of tissue. After incubation the samples were washed in 1 ml of the physiological medium for three seconds on a Vortex mixer, blotted and weighed. The tissue radioactivity (^{51}Cr, ^{57}Co) was counted directly in a Wallac 1280 gamma counter. The sample was then combusted in a Packard Tri-Carb sample oxidiser and the trapped $^{14}CO_2$ counted in a Wallac 8100 liquid scintillation counter. The amounts of each marker entering the tissue was calculated:

$$\frac{\text{cpm/mg tissue}}{\text{cpm/}\mu\text{l medium}}$$

– that is, μl medium/mg wet weight of tissue

Wilcoxon's rank sum test was used for statistical analysis. These studies were approved by the Harrow Health Authority Ethical Committee.

Results

The Table shows the results of morphometric analysis from both groups of patients with coeliac disease and with control subjects. Mean mucosal height and crypt depth was measured as described by Slavin *et al*[10] and intra-epithelial lymphocyte counts were as described by Ferguson and Murray.[11] The relapsed patients had markedly raised intra-epithelial lymphocyte counts while all the treated patients had normal counts except for two who had

Table *Clinical details, intra-epithelial lymphocytes and morphometric analysis of jejunal mucosa from treated and relapsed patients with coeliac disease*

	Sex	*Age*	*Duration of treatment*	*Mean mucosal height (μm)*	*Mean crypt depth (μm)*	*Mucosal crypt ratio*	*Lymphocytes/ 100 enterocyte nuclei*
Normal range				323–553	78–181	2·87–6·11	<40
Control subjects				292–614	89–180	2·94–4·42	<38
Patients with	M	38		396	174	2·28	108
coeliac disease	M	42		329	276	1·19	65
in relapse	F	48		469	370	1·27	78
	F	51		367	322	1·14	57
	F	62		496	317	1·57	66
	M	66		351	289	1·22	56
	F	75		445	319	1·40	62
	F	76		344	250	1·38	55
Patients with	F	64	6 months	388	159	2·44	49
coeliac disease	F	52	3 yrs	440	182	2·42	52
in remission	F	32	7 yrs	453	118	3·84	35
	F	38	8 yrs	503	170	2·96	34
	M	17	15 yrs	364	114	3·17	35
	F	41	20 yrs	319	92	3·47	34
	M	24	23 yrs	408	98	4·12	24

mild elevation. The mucosal height/crypt depth ratio reflects the degree of villous atrophy. Two of the treated patients had a slightly abnormal ratio and the other five were normal.

Figs. 1, 2, and 3 show the mean ± SEM uptake for (^{14}C)-inulin, (^{57}Co)-cyanocobalamin and ^{51}Cr-EDTA by the control subjects and the two groups of patients with coelic disease. There was rapid equilibration of the probes by the tissue within three minutes, followed by a slower entry phase. Tissue uptake was significantly increased for all markers in the relapsed patients. Patients in remission had a significant increase in tissue uptake of (^{57}Co)-cyanocobalamin and ^{51}Cr-EDTA but the uptake of (^{14}C)-inulin did not differ significantly at any time point from control patients.

The tissue uptake tends to be greater in samples from untreated patients compared with treated patients but there is variable overlap of the values. Discrimination between controls and patients with coeliac disease is shown in Fig. 4 where tissue uptake from each subject is shown after 10 minutes incubation.

Figure 5 shows the relationship between tissue uptake and the molecular weight of the probes on a semi-logarithmic scale. There is an inverse linear relationship in controls between the tissue uptake and the molecular size of the probe. This also holds true for the patients with coeliac disease in remission. Moreover the slope of the line is increased suggesting that there is a proportionally greater increase in tissue permeability to ^{51}Cr-EDTA compared with that of ^{14}C-inulin, in samples from the coeliacs.

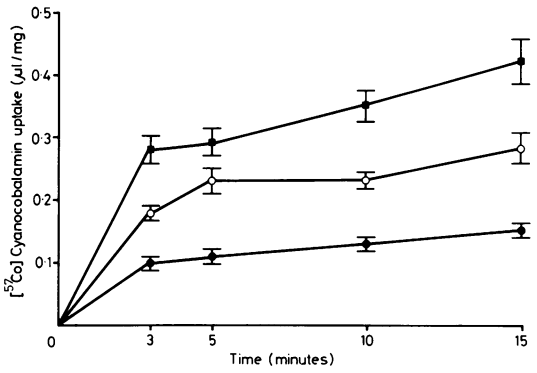

Fig. 2 *Tissue uptake (mean ± SE) of (^{57}Co)-cyanocobalamin. Symbols as in Fig. 1. Treated and relapsed patients differed significantly from controls ($p<0·01$) at all times. Patients in relapse differed significantly from patients in remission ($p<0·05$) at all times except at 5 minutes.*

Analysis of the data from individual patients with coeliac disease in relapse and remission showed no significant correlation between the number of intra-epithelial lymphocytes and permeability changes. Neither was there a correlation between the morphometric results and permeability.

Discussion

The studies reported in this paper describes a new *in vitro* technique for measuring intestinal permeability which overcomes many of the promblems of *in vivo*

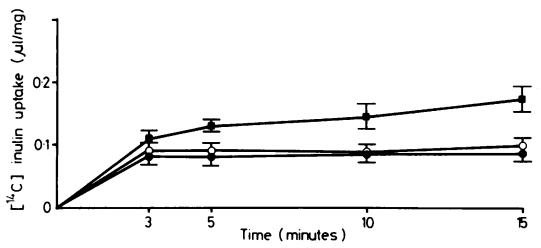

Fig. 1 *Tissue uptake (mean ± SE) of (^{14}C)-inulin from 13 control subjects (●), seven patients in remission (○) and eight relapsed patients (■) with coeliac disease. Tissue uptake was significantly greater in samples from relapsed patients compared with controls at all times ($p<0·05$). There was no significant difference between treated patients and controls.*

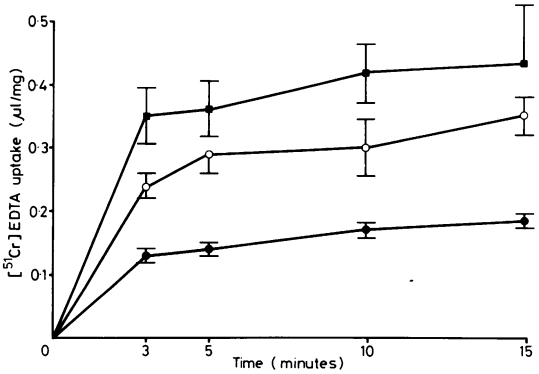

Fig. 3 *Tissue uptake (mean ± SE) of ^{51}Cr-EDTA. Symbols as in Fig. 1. Treated and relapsed patients differed significantly from controls ($p<0·01$) at all times. Patients in relapse did not differ significantly from patients in remission except at 3 minutes ($p<0·05$).*

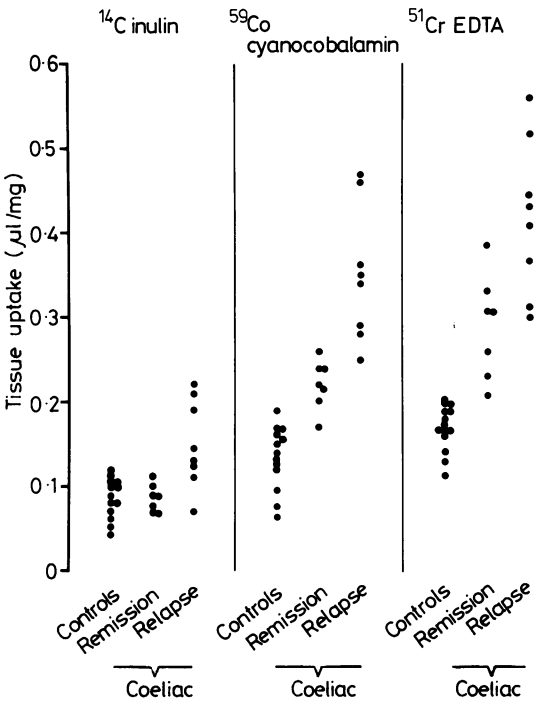

Fig. 4 *Tissue uptake of (^{14}C)-inulin, (^{57}Co)-cyanocobalamin and ^{51}Cr-EDTA after 10 minutes incubation. Each point represents the mean of duplicate samples from each subject.*

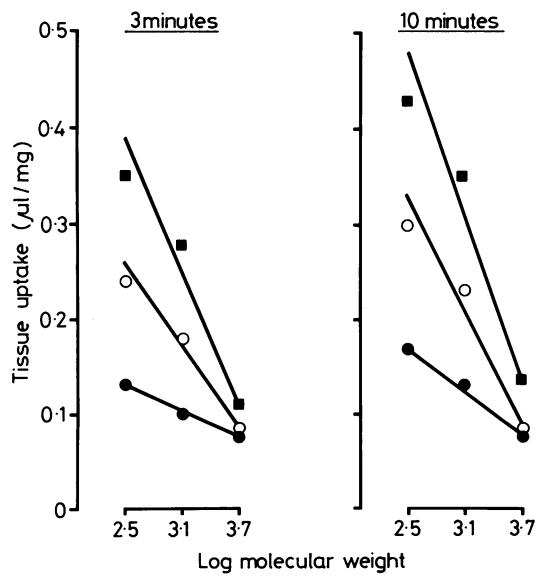

Fig. 5 *Tissue uptake (mean) plotted against the log molecular weights of the three probes. Symbols as in Fig. 1.*

methods in current use. The technique has been used to show enhanced jejunal permeability in patients with coeliac disease and has shown that this abnormality persists even when the mucosa has returned to histological normality after gluten withdrawal.

The probes used in these experiments are not absorbed to a significant extent by the normal jejunal mucosa. The rate of uptake in control tissue will therefore be determined largely by the cut non-mucosal surface area. Jejunum from patients with untreated coeliac disease are in part characterised by broadening and fusion of the villae, without a change in the thickness of the lamina propria. On a weight basis, the cut non-mucosal surface area is relatively reduced. If enterocyte integrity was preserved one would expect a slight decrease in the rate of uptake of the probes by coeliac mucosa. Because of the inflammatory cell infiltration of the lamina propria which could conceivably change the permeability properties of the cut non-mucosal surface, tissue from untreated patients may not be directly comparable with normal.

Tissue from treated patients was, however, histologically normal and showed a striking and a selective increase in the rate of uptake for cyanocobalamin and Cr-EDTA. The most likely explanation for this observation is that there is enhanced uptake through the epithelial layer by coeliac patients. This has been confirmed using ^{51}Cr-EDTA *in vivo*.[12]

Present methods for measuring intestinal permeability in man involves the oral administration of a variety of test substances with subsequent urine collection and assay of the excreted probes. There are two main types of compounds in general use. Chadwick[1] and colleagues introduced the use of poly(ethylene glycol) polymers. These have molecular weights ranging from approximately 250 to over 20 000 daltons and have been extensively studied in a variety of diseases.[1 4 5] One particular problem with the use of poly(ethylene glycol) polymers is that there is a wide variation in absorption rate between polymers of differing molecular weights. Thus normal subjects excrete in six hours approximately 25% of a test dose of poly(ethylene glycol), mol wt 400[1] but less than 2·7% of poly(ethylene glycol), mol wt 4000 in 24 hours.[4] For polymers with high transfer rates, mucosal surface area becomes a rate limiting factor for absorption whereas with poorly absorbed probes permeability changes obtained in absorption studies with poly(ethylene glycol) mol wt 400 in coeliac patients is difficult.

In order to overcome many of the problems inherent in the use of poly(ethylene glycol) 400

Menzies *et al*[2] and Cobden *et al*[3 7] introduced the use of administering simultaneously a poorly absorbable oligosaccharide with a readily absorbable monosaccharide. Urine collections are made over the subsequent five hours and the urine excretion ratio of these saccharides gives excellent discrimination between untreated coeliac patients and controls. These studies clearly show increased permeability to the oligosaccharide. The reduced absorption of the monosaccharide (60%) is as difficult to interpret as the results with poly(ethylene glycol) polymers (mol wt 400) as there may be an up to four fold reduction in the small intestinal surface area in untreated coeliac patients. Clearly absorption is not synonomous with permeability under these circumstances. Failure to recognise this problem undoubtedly accounts for the paradox in which 'permeability' to small molecular weight probes is decreased whereas that to large probes is increased in patients with untreated coeliac disease. In addition, Hamilton[8] and colleagues could not show any abnormalities in treated patients after as a short a time as three months of gluten withdrawal.

A problem with these *in vivo* studies is the importance of several other parameters. Thus gastric emptying, intestinal transit, dilution of the probe solution, particularly if given as a hypertonic solution, mucosal transfer, mesenteric blood and/or lymphatic flow, renal excretion as well as intestinal permeability will determine the urinary excretion of the probes. Other difficulties in interpretation of *in vivo* studies are that the site of altered permeability cannot be accurately determined and that adaptive or compensatory alterations in uninvolved areas of the intestine may affect the overall absorption of the probes.

The *in vitro* method described in this paper overcomes many of the above difficulties. Tissue samples are collected from the area of the gut under study and initial rates of mucosal uptake are simply determined with routine radioactive counting procedures. The studies in biopsies from relapsed patients with coeliac disease clearly show that the increased permeability applies to all three probes although the increase is less for the larger as compared with the smaller molecules. The results do not support the claim of *in vivo* workers that in coeliac disease there is increased permeability to high molecular weight probes while at the same time there is decreased permeability to low molecular weight probes. As discussed above this *in vivo* finding probably stems from the use of inappropriate probe molecules.

The consistent finding that the mucosal permeability for (^{57}Co)-cyanocobalamin and ^{51}Cr-EDTA is enhanced in biopsies from patients with coeliac disease who have histologically normal mucosae is of particular interest as it may represent a primary lesion. This is consistent with previous subcellular studies describing brush border changes in coeliac disease.[13] It suggests an abnormality of this organelle which would permit enhanced entry of toxic material into the enterocyte thus initiating organelle damage – for example, by lysosomal disruption.[14] Alternatively the increased permeability may reflect alterations in the intercellular junctional complexes allowing enhanced entry into the intercellular and submucosal spaces. This would be consistent with the finding of secretory diarrhoea in coeliac disease[15 16] and with the suggestions that immunological mechanisms are implicated in the pathogenesis of the disease.[17 18] Of particular interest is the report by Bronstein *et al*[19] that shows the molecular weight of the toxic gluten peptide to be about 1000 daltons, a size well within the permeability range that we have shown to be increased. Thus increased permeability to gluten fractions would permit, in susceptible subjects, direct or indirect cytotoxic effects on the intestinal epithelial cells.

The technique described here was developed for use with jejunal biopsy samples but is applicable to biopsy material from other tissue sites, such as stomach and colon. Furthermore by combining it with sequential subcellular fractionation studies[20] it may be possible to localise and characterise this permeability defect further and thus increase our understanding of the pathophysiological processes that are involved in coeliac disease.

We are grateful to Dr N Veall for many helpful suggestions and advice on the isotope counting, to Drs G Slavin and A Price for jejunal histology reports, Dr A J Levi for permission to study his patients and Ms Rosamund Greensted for typing the manuscript. IB gratefully acknowledges financial support from Nordisk Insulinfond and The Wellcome Trust.

References

1 Chadwick VS, Phillips SF, Hofmann AF. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man and animals. *Gastroenterology* 1977; **73:** 247–51.

2 Menzies IS, Pounder R, Heyer S *et al.* Abnormal intestinal permeability to sugars in villous atrophy. *Lancet* 1979; **2:** 1107–9.

3 Cobden I, Rothwell J, Axon ATR. Intestinal permeability and screening tests for coeliac disease. *Gut* 1980; **21:** 212–8.
4 Jackson PG, Baker RWR, Lessof MH, Ferrett J, MacDonald DM. Intestinal permeability in patients with eczema and food allergy. *Lancet* 1981; **1:** 1285–6.
5 Robinson GM, Orrego H, Israel Y, Devenyi P, Kapur BM. Low-molecular weights polyethylene glycol as a probe of gastrointestinal permeability after alcohol ingestion. *Dig Dis Sci* 1981; **26:** 971–7.
6 Ukabam SO, Clamp JR, Cooper BT. Abnormal small intestinal permeability to sugars in patients with Crohn's disease of the ileum and colon. *Clin Sci* 1982; **61:** 21–2P.
7 Cobden I, Dickinson RJ, Rothwell J, Axon ATR. Intestinal permeability assessed by excretion ratios of two molecules. Results in coeliac disease. *Br Med J* 1978; **2:** 1060.
8 Hamilton I, Cobden I, Rothwell J, Axon ATR. Intestinal permeability in coeliac disease. The response to gluten withdrawal and single dose gluten challenge. *Gut* 1982; **23:** 202–10.
9 Cox TM, Peters TJ. The kinetics of iron uptake *in vitro* by human duodenal mucosa. Studies in normal subjects. *J Physiol* 1979; **289:** 469–78.
10 Slavin G, Sowter C, Robertson K, MacDermott S, Paton K. Measurement in jejunal biopsies by computer-aided microscopy. *J Clin Pathol* 1980; **33:** 254–61.
11 Ferguson A, Murray D. Quantitation of intra-epithelial lymphocytes in human jejunum. *Gut* 1971; **12:** 988–94.
12 Bjarnason I, Peters TJ, Veall N. A persistent defect in intestinal permeability in coeliac disease demonstrated by a ^{51}Cr-labelled EDTA absorption test. *Lancet* 1983; **1:** 323–5.
13 Peters TJ, Jones PE, Wells G. Analytical subcellular fractionation of jejunal biopsy specimen. Enzyme activities, organelle pathology and response to gluten withdrawal in patients with coeliac disease. *Clin Sci Mol Med* 1978; **55:** 285–92.
14 Peters TJ, Heath JR, Wansbrough-Jones M, Doe WF. Enzyme activities and properties of lysosomes and brush borders in jejunal biopsies from control subjects and patients with coeliac disease. *Clin Sci Mol Med* 1975; **48:** 259–67.
15 Fordtran JS, Rector FC, Lockyear TW, Ewton MF. Water and solute movement in small intestine of patients with sprue. *J Clin Invest* 1967; **46:** 287–98.
16 Schmid WG, Phillips SF, Summerskill WHJ. Jejunal secretion of electrolyses and water in non tropical sprue. *J Lab Clin Med* 1969; **73:** 772–83.
17 Booth CC, Peters TJ, Doe WF. Immunopathology of coeliac disease. In: *Immunology of the gut.* Ciba Foundation Symposia No. 46. North Holland, Amsterdam: Elsevier, 1977: 329–46.
18 Marsh MN. The small intestine: mechanisms of local immunity and gluten sensitivity. *Clin Sci* 1981; **61:** 497–503.
19 Bronstein HD, Haeffner LJ, Howlessar OD. Enzymatic digestion of gliadin. The affect of the resultant peptides in adult coeliac disease. *Clin Chim Acta* 1966; **14:** 141–55.
20 Peters TJ. Investigation of tissue organelles by a combination of analytical subcellular fractionation and enzymic microanalysis. A new approach to pathology. *J Clin Pathol* 1981; **34:** 1–12.