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Assessing the site of increased intestinal permeability in coeliac and inflammatory bowel disease

K Teahon, S Somasundaram, T Smith, I Menzies, I Bjarnason

Abstract

***Background*—The precise site of intestinal permeability changes in patients with coeliac and inflammatory bowel disease is unknown.**

***Aims*—To design a non-invasive technique for the localisation of altered gastrointestinal permeability to ^{51}chromium labelled EDTA (^{51}CrEDTA). The method depends on comparing and defining concentration/time profiles in serum of a series of simultaneously ingested indicators with a well defined absorption site (3-0-methyl-D-glucose (jejunal indicator), ^{57}cobalt labelled vitamin B_{12} (ileal indicator), and sulphasalazine (caecal-colonic indicator)) in relation to simultaneously ingested ^{51}CrEDTA.**

***Subjects*—Five normal controls, six patients with untreated coeliac disease, five with Crohn's ileitis, and five with pan-ulcerative colitis underwent study, which entailed the simultaneous ingestion of the above four test substances followed, during the next 24 hours, by timed serial collection of urine and serum for marker analysis.**

***Results*—Urinary excretion of ^{51}CrEDTA was significantly increased in all patient groups. Analysis of serum appearances and profiles of the markers suggested that the increased intestinal permeation of ^{51}CrEDTA took place in the diseased jejunum in patients with coeliac disease, predominantly in the ileum in Crohn's disease and in the colon in the patients with pan-ulcerative colitis.**

***Conclusion*—A new non-invasive technique has been assessed that permits the localisation of the site of permeability changes with the gastrointestinal tract.**

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Keywords: intestinal permeability, intestinal function, coeliac disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis.

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Non-invasive tests of intestinal permeability are widely used to screen for small intestinal disease and assess the importance of the intestinal barrier function in the aetiology and pathogenesis of intestinal and systemic disease.[1 2] Choice of test procedure for assessing intestinal permeability depends on clinical and experimental circumstances. In general there is good agreement between results obtained with the five hour differential urinary excretion of lactulose/L-rhamnose (or lactulose/mannitol) and the 24 hour urinary excretion of ^{51}chromium labelled EDTA (^{51}CrEDTA).[2–4] After ingestion the uptake of test sugars, which are subjected to rapid bacterial degradation after reaching the caecum, being largely from the small intestine is not affected by colonic pathology. In the case of ^{51}CrEDTA given by itself, distinction between small intestine and colonic contributions to the 24 hour urinary excretion is uncertain.

In an attempt to discriminate between changes in small intestinal and colonic permeability Jenkins *et al*[5] administered lactulose, L-rhamnose, and ^{51}CrEDTA together and measured timed excretions of these markers in urine. The ratio of urinary lactulose/L-rhamnose during the first five hours provided an index of small intestinal permeability, and the total 24 hour urinary excretion of ^{51}CrEDTA, less that of lactulose (as % of doses) served as an index of colonic permeability.[5] The results showed that the colon was likely to be the site of increased intestinal permeation of ^{51}CrEDTA in patients with active pan-ulcerative colitis,[5] and others have found similar changes after abdominal radiation.[6] Nevertheless the technique does not permit the additional differentiation between an upper and lower small intestinal site of altered intestinal permeability that is possible using the technique we now report, based on serum concentration/time profiles after ingestion of ^{51}CrEDTA together with 3-0-methyl-D-glucose, ^{57}Cobalt labelled vitamin B_{12} (^{57}CoVitB_{12}), and sulphasalazine, which serve as indicators of absorption site.

Methods

Basis of test procedure

The principle of the test was to compare the serum concentration/time profiles of three absorption indicators, selected because each is specifically absorbed from a defined region within the intestinal tract, with that of simultaneously ingested ^{51}CrEDTA, which should, in theory, permit assessment of the main intestinal site of ^{51}CrEDTA permeation. The indicators used were 3-0-methyl-D-glucose, absorbed from the jejunum by an active carrier mediated transport system shared with D-glucose and D-galactose,[7] vitamin B_{12}, specifically absorbed as a complex with intrinsic factor by carriers in the ileum,[8] and sulphasalazine, which is metabolised by the azoreductase of bacteria in the caecum and colon

to yield 5-aminosalicylic acid and sulphapyridine.[9] The first appearance of 3-0-methyl-D-glucose, $^{57}CoVitB_{12}$ and sulphapyridine in serum should therefore indicate the arrival of the 'head' of the test solution after oral administration in the jejunum, ileum, and caecum, respectively, provided that the rate of mucosal uptake is similar for each of the indicators.[9] The subsequent serum concentrations/time profiles are determined by the same factors that influence the pharmacokinetic profile of an ingested drug, namely the site and relative efficiency of mucosal uptake, the rate of intestinal transit, metabolic degradation (for sulphapyridine), volume of systemic distribution and rate of clearance. Because of the nature of the test most of these factors apply equally to all the test substances. However, as renal function is an important determinator of the permeation profiles glomerular filtration rates were assessed during each test.

Subjects

Five healthy volunteers acted as controls (four males, one female, mean age 37 years, range 22–58). Six symptomatic, newly diagnosed patients with coeliac disease (four males, two females, mean age 42 years, range 32–56), five patients with Crohn's ileitis of activity index over 150[10] (four males, one female, mean age 29 years, range 20–42), and five patients (all males, mean age 44 years, range 26–59) with active pan-ulcerative colitis as defined by Truelove *et al*[11] were studied.

All the patients with inflammatory bowel disease had sufficiently severe disease activity to warrant hospital admission. Three were receiving 5-aminosalicylic acid. The studies were carried out before treatment was changed and no patient was receiving corticosteroids or immunosuppressants. Disease location in patients with inflammatory bowel disease had been established but was confirmed by colonoscopy, radiology or ^{111}indium leucocyte scintigrams, or all three, within a week of these studies.

The reliability of 'first appearance in serum' to indicate arrival at a particular level in the intestine requires that mucosal uptake of the markers used is not unduly delayed and takes place at a similar rate. The rates of mucosal permeation for $^{57}CoVitB_{12}$ and sulphapyridine have been assessed previously.[8 9 12] To assess the relative mucosal permeation rates of 3-0-methyl-D-glucose and ^{51}CrEDTA in the same intestine four patients (males, mean age 38 years, range 32–40) with the irritable bowel syndrome, undergoing routine gastroduodenoscopy, had a test solution containing 3-0-methyl-D-glucose (2·5 g) and ^{51}CrEDTA (1 mCi) in 50 ml water instilled directly into the duodenum. Serum samples taken before, and at five minute intervals for a period of 30 minutes after instillation, were assayed for these markers.

No subject had recently had alcohol (within seven days), non-steroidal anti-inflammatory drugs or other drugs (within six months) known to affect intestinal integrity.[2]

These studies were approved by the Harrow and Camberwell Health Authority Ethical Committees and all subjects gave informed consent.

Procedure

All subjects were admitted to a metabolic research ward for the study. They fasted from 12 hours before the test until the study was complete. Throughout the studies they lay supine except for toilet purposes. At 7 am on the day of the test subjects received an intramuscular injection of cyanocobalamine (1 mg in 1 ml, Glaxo, UK) to saturate vitamin B_{12} receptors and hence to facilitate the detection of $^{57}CoVitB_{12}$ in serum. An indwelling intravenous cannula was placed into the antecubital veins of both arms, one being used for intravenous injection and fluid replacement (started at 11 am; 3 litres of glucose-saline with 60 meq potassium given over 18 hours) and the other for obtaining blood samples for analyses.

At 7.45 am subjects swallowed two capsules containing human intrinsic factor (Amersham International, Amersham, Buckinghamshire, UK and Frost Laboratories, Quebec, Canada) with 20 ml of water. At exactly 8 am patients received 10 ml of sterile saline intravenously containing 100 μCi ^{99m}technetium diethylenetriaminopenta acetate (^{99m}TcDTPA) (3·7 MBq, Amersham International) over a period of 10 seconds while drinking the 100 ml test solution (within one minute), which contained: 3-0-methyl-D-Glucose (2·5 g, Sigma, Poole, Dorset, UK), $^{57}CoVitB_{12}$ (5 μCi, 19 kBq, Amersham International), sulphasalazine (2·0 g, Syrup, Pharmacia, Milton Keynes, UK), ^{51}CrEDTA (1 mCi, 37 MBq, Amersham International).

Ten ml of blood was obtained before the test, at five minute intervals for the first 30 minutes, at one hour, hourly until 8 pm and two hourly until completion of the test at 8 am the next morning. A complete 24 hour urinary collection was made.

The radiation dose received during the test is less than 1·5 mSieverts.

Marker analyses

To ensure precision the ^{99m}TcDTPA, $^{57}CoVitB_{12}$, and ^{51}CrEDTA doses for ingestion were all weighed. The blood samples were allowed to clot, spun down, and the serum collected. Exactly 1·0 ml of each serum sample and 5 ml of urine was counted along with standards on a LKB 1280 or 1282 gamma-counter along with appropriate standards. The ^{99m}Tc was counted on the day of the test. One week later, when all ^{99m}Tc activity had decayed, the samples and standards were counted for ^{57}Co and ^{51}Cr, appropriate crossover corrections being made.

To calculate glomerular filtration rates the ^{99m}Tc disappearance data in serum was plotted (% dose/litre) on a logarithmic scale and the linear part of the slope extrapolated to the y axis. The intercept value divided into 100%

Time of first appearance of test markers in serum and glomerular filtration rates

	Patient numbers																				
	Normal controls					Coeliac disease						Crohn's disease					Ulcerative colitis				
	1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	5
3-0-m-D-glucose	20	10	15	10	5	10	30	10	5	60	20	5	10	10	10	10	5	15	10	10	10
51CrEDTA	30	60	60	60	25	5	30	20	5	60	10	10	15	10	60	15	60	60	10	15	20
57CoVitB12	240	300	300	180	300	120	240	360	180	300	180	300	240	120	240	300	300	240	180	240	360
Sulphapyridine	180	180	240	300	300	180	240	360	300	420	240	180	240	120	240	420	360	240	240	120	300
GFR (ml/min)	121	135	112	113	123	131	105	108	97	110	119	127	111	101	116	134	102	118	94	115	129

and multiplied by 1000 ml gives the extracellular volume distribution of ^{99m}TcDTPA (in ml). This is then multiplied by λ, which is the slope of the linear part of the ^{99m}TcDTPA disappearance plot, and this gives the glomerular filtration rate (ml/min).[13]

Serum 3-0-methyl-D-glucose was measured by thin layer chromatography and densitometry as previously described[14] and sulphapyridine by high pressure liquid chromatography with ultraviolet detection[15] both of which have satisfactory accuracy and precision, coefficients of variation ranges being 3·5–8% and 4–11%, respectively.

Statistics

Statistical significance between patient groups was assessed by Wilcoxon's rank sum test.

Results

The rest was well tolerated by all except one normal subject who had severe nausea and vomiting, starting six hours after start of the test and coinciding with the rise in the serum sulphapyridine value.

Appearance of markers in serum and renal function

Detectable values of both 3-0-methyl-D-glucose and ^{51}CrEDTA appeared in serum at the same time after instillation into the duodenum, at five minutes in three patients with irritable bowel syndrome, and at 10 minutes in the fourth.

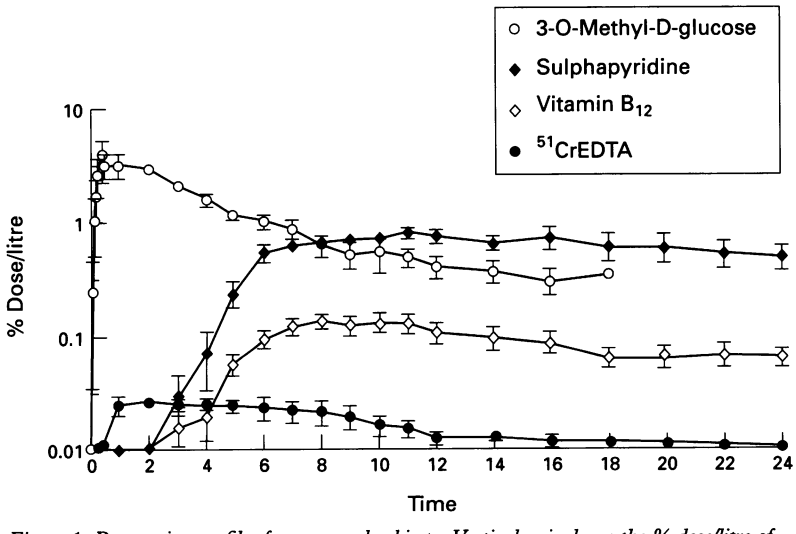

Figure 1: Permeation profiles from normal subjects. Vertical axis shows the % dose/litre of the test substances in serum (on a logarithmic scale) over 24 hours after their simultaneous ingestion.

In the main study serum creatinine was normal in all subjects. The Table shows that glomerular filtration rates varies from 94 ml/min to 138 ml/min, which is within the reported normal physiological range for these age groups, and there were no significant difference between the study groups.

The Table shows the time of first appearance of 3-0-methyl-D-glucose, ^{51}CrEDTA, ^{57}CoVitB$_{12}$ and sulphapyridine in serum after oral administration. While there is some variation between different people in the time of first appearance of each marker, there was no significant ($p>0·3$) difference between patient groups except for the appearance of ^{51}CrEDTA. Appearances of 3-0-methyl-D-glucose precedes that of ^{51}CrEDTA in both the normal and ulcerative colitis groups ($p<0·05$), but no significant difference was found in the time of appearance of these two markers in the serum of patients with coeliac and ileal Crohn's disease.

The appearance of ^{57}CoVitB$_{12}$ preceded that of sulphapyridine in eight subjects, but in seven subjects these markers appeared at the same time, and in the remaining six the 'caecal' marker preceded the 'ileal' marker. The gastric to caecal transit time was usually in the range of three to six hours and did not differ significantly between the groups.

Permeation profiles

The summation permeation profiles (mean (SEM)) from the four different groups are shown in Figures 1–4.

Figure 1 shows the permeation profile for the normal group. Peak levels of ^{51}CrEDTA (less than 0·046% of the dose/litre in each case) are achieved one to two hours (in one case ^{51}CrEDTA peaked at six hours) after the appearance of 3-0-methyl-D-glucose, but before the appearance of the 'ileal' and 'caecal' markers. Insignificant serum values of ^{51}CrEDTA were found between eight and 14 hours after starting the test, and only one normal subject had detectable values throughout the 24 hour period. The mean 24 hour urinary excretion of ^{51}CrEDTA was 2·13 (0·31)%, range 1·46 to 3·15%.

Figure 2 shows the permeation profile from the patients with untreated coeliac disease. Comparison with normal subject response (Fig 1) shows that there are no significant differences in the profiles (or serum peak values) of the 'jejunal', 'ileal', or 'caecal' markers except that the 3-0-methyl-D-glucose peak at 120 minutes instead of 30 minutes. ^{51}CrEDTA appears earlier, and reaches a

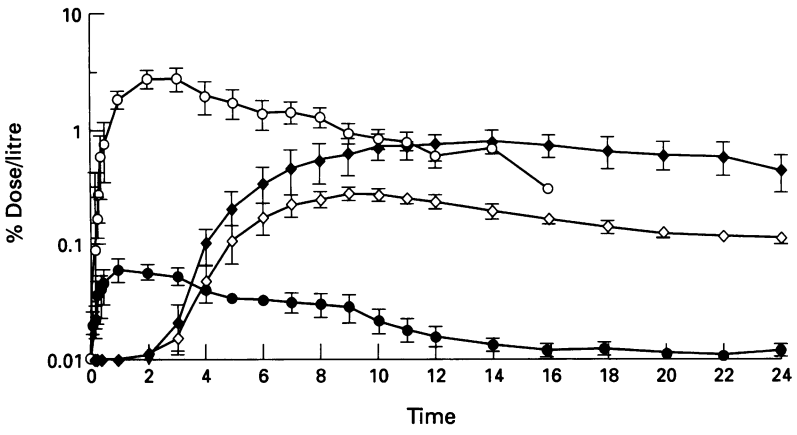

Figure 2: Permeation profiles from patients with untreated coeliac disease. Symbols as in Figure 1.

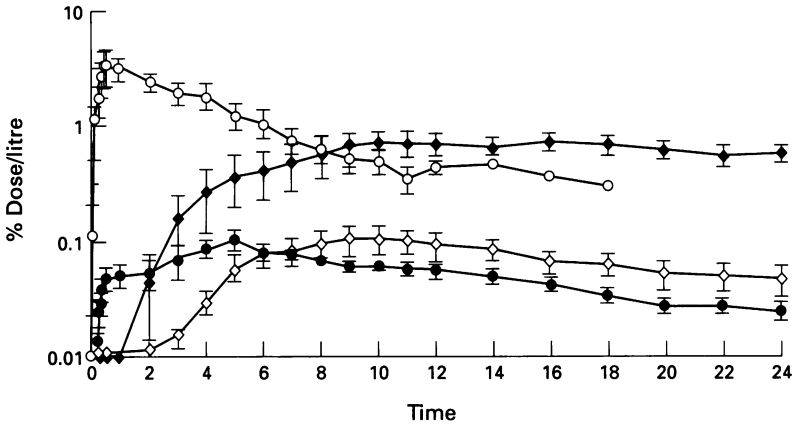

Figure 3: Permeation profiles from patients with Crohn's ileitis. Symbols as in Figure 1.

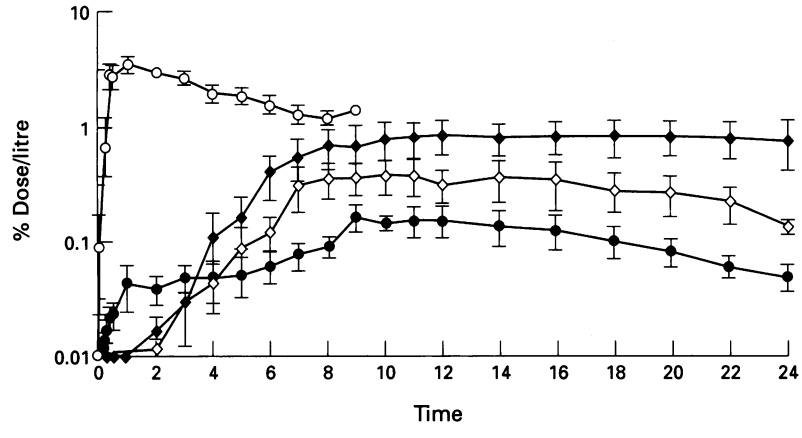

Figure 4: Permeation profiles from patients with pan-ulcerative colitis. Symbols as in Figure 1.

significantly higher peak value than normal in coeliac disease (>0·08% dose/litre in each case, $p<0·01$) and at a similar time as 3-0-methyl-D-glucose (in five of six cases). However, as in normal subjects only one patient had measurable serum ^{51}CrEDTA at 14 hours. The urinary excretion of ^{51}CrEDTA (3·61 (0·10)%, range 3·29 to 4·02%) was significantly greater than in normal subjects.

Figure 3 shows the permeation profile from the patients with Crohn's ileitis. There is an early rise of ^{51}CrEDTA (in two cases indistinguishable from that seen in the patients with coeliac disease), but a second increase ranging from 0·06 to 0·21% dose/litre and coinciding with the appearance of the 'ileal' and 'caecal' markers in the serum, was also evident in four of five patients. Unlike the normal and coeliac disease groups, significant levels of ^{51}CrEDTA were detected in serum of the Crohn's ileitis group throughout the test. The 24 hour urinary ^{51}CrEDTA excretion, 5·32 (0·98)%, range 2·94 to 8·31%, was significantly greater ($p<0·01$) than in normal subjects.

Figure 4 shows the permeation profile from patients with pan-ulcerative colitis. The early peak values of ^{51}CrEDTA in serum at one hour did not differ significantly from the normal group (one patient had increased early serum values of ^{51}CrEDTA similar to that in coeliac disease). There was, however, a clear increase in ^{51}CrEDTA values two to five hours after the appearance of the 'ileal' and 'caecal' markers, which was evident in all the patients. The 24 hour urinary ^{51}CrEDTA excretion, 5·97 (1·05)%, range 3·06 to 8·85%, was significantly greater than in normal subjects ($p<0·01$).

Presenting the mean permeation profiles can be slightly misleading because of the variation in transit times, etc. In particular the difference between individual cases of Crohn's disease and ulcerative colitis were sharper than Figures 3 and 4 would imply. Figure 5 shows representative traces from a patient with Crohn's ileitis and ulcerative colitis where the increase in serum values of ^{51}CrEDTA clearly coincides with the appearance of the 'ileal' and 'caecal' markers in Crohn's disease and comes distinctive later in ulcerative colitis.

Discussion

This paper describes a new technique that was designed to assess the site of permeability changes within the gastrointestinal tract. The results suggest that the site of increased intestinal permeability in coeliac disease, Crohn's disease, and pan-ulcerative colitis is the diseased intestine itself. In the context of this study there are, however, a number of factors that need to be considered when interpreting the data. In particular the use of the time of appearance of indicator substances in serum and the permeation profiles is determined by a number of variables that could affect the results. These variables include the site, rate, and amount of marker permeating across the intestine, gastric emptying, intestinal dilution and transit times, metabolism in the case of sulphapyridine, distribution volume, and renal function. However, because the test substances were given at the same time the influence of some of these factors apply equally to all the test substances. Thus gastrointestinal dilution affects all the markers to a similar extent, and gastric emptying (time to appearance of 3-0-methyl-D-glucose) and small intestinal transit times (time difference between 3-0-D-glucose and sulphapyridine) can be calculated and were not found to be significantly different between the patient groups. Also the mode and rate of renal excretion is similar for all the markers apart from sulphapyridine, which is

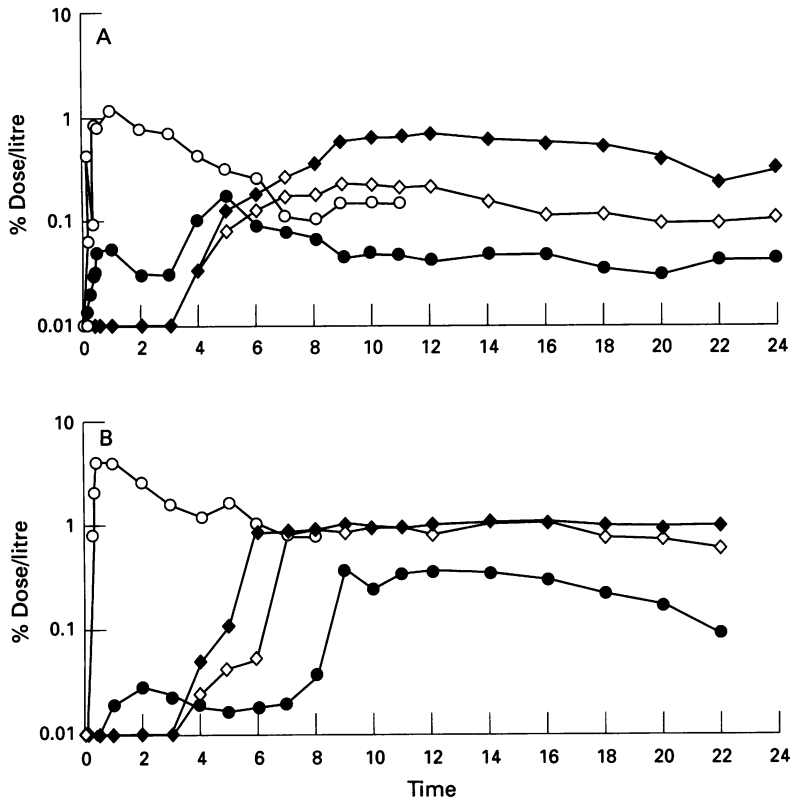

Figure 5: Comparison of profiles from an individual patient with Crohn's ileitis (A) and ulcerative colitis (B). The increase in serum values of ^{51}CrEDTA coincides with and follows the appearance of 'ileal' and 'caecal' markers in Crohn's disease and ulcerative colitis, respectively. Symbols as in Figure 1.

subjected to hepatic metabolism. It is the rate of permeation across the intestine that determines how accurately the 'first appearance' of the markers in serum represents the 'head' of the test solution in each part of the intestine. There was no discrepancy between the rate of appearance of 3-0-methyl-D-glucose and ^{51}CrEDTA after duodenal instillation in normal subjects and previous studies have also shown that sulphapyridine appears within six minutes of caecal instillation.[9] Our results, however, show an inconsistency between the time of appearance of the 'ileal' and 'caecal' markers in serum and in six cases sulphapyridine appeared before the 'ileal' marker. These findings are consistent with reports of a 30–240 minute delay for the appearance of vitamin B_{12} after ileal instillation,[8 12] which is probably due to intracellular processing of the vitamin B_{12} intrinsic factor complex.[16 17] The 'first appearance' of ^{57}CoVitB_{12} in serum is therefore likely to overestimate the time it takes for the 'head' of the test solution to reach the ileum.

In both normal subjects and patients with ulcerative colitis there is a significant lag time between the serum detection of 3-0-methyl-D-glucose and ^{51}CrEDTA whereas the appearance of both markers in the serum after direct instillation into the duodenum is both rapid and simultaneous. This might suggest that the main site of permeation of ^{51}CrEDTA after ingestion is normally somewhat distal to that of 3-0-methyl-D-glucose, but it might also be that the uptake of ^{51}CrEDTA on reaching the duodenum in the 'head' of the test solution gives serum values that are initially below the detection limits of the gamma counting. Reliable detection of ^{51}CrEDTA in serum might therefore be delayed until the bulk of the test solution had entered the jejunum.

In patients with untreated coeliac disease the appearance of ^{51}CrEDTA in serum coincided with and peaked at the same time as 3-0-methyl-D-glucose suggesting that there was increased intestinal permeability in the upper small intestine. These findings are consistent with results of studies in coeliac disease, which indicate increased in vitro permeation of various markers[18 19] and with freeze fracture studies,[20] which demonstrate decreased strand number and depth in the intercellular junctions. The results in Crohn's disease were not as clear cut. A similar pattern of increased permeation of ^{51}CrEDTA coinciding with that of 3-0-methyl-D-glucose was found in two patients. One of these and the remaining three patients had peak values of ^{51}CrEDTA coinciding with the appearance of the 'ileal' and 'caecal' markers. As this rise (seen in Fig 5) in the serum values of ^{51}CrEDTA occurred much earlier than in the patients with ulcerative colitis it seems likely that it represents increased ileal permeability to ^{51}CrEDTA. Increased 'jejunal' permeability in some patients with Crohn's ileitis is consistent with in vitro findings of increased permeability in apparently unaffected jejunal mucosa of patients with Crohn's disease.[19] The initial serum profiles of ^{51}CrEDTA in pan-ulcerative colitis were indistinguishable from normal subjects in four of five cases. The increase in serum ^{51}CrEDTA in the four patients occurred somewhat later than in the patients with Crohn's ileitis, occurring after the appearance of the 'ileal' and 'caecal' indicators. This suggests that the inflamed colonic mucosa is the site of increased permeation of ^{51}CrEDTA in ulcerative colitis as suggested by Jenkins *et al.*[5] This is also in keeping with studies showing increased permeation of ^{51}CrEDTA after rectal administration in ulcerative colitis.[21 22] Indeed, the permeation of ^{51}CrEDTA correlated significantly with histopathological assessment of disease activity in these patients.[21]

In summary a new non-invasive technique has been assessed that permits the localisation of the site of altered permeability with the gastrointestinal tract. The principle of the method seems to be generally applicable to other test substances than permeability probes, but there is a need to identify an equally site specific 'ileal indicator' to that of vitamin B_{12}, which does not have the delayed rate of absorption.

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