

Intestinal permeability to ^{51}Cr -EDTA in rats with experimentally induced enteropathy

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SUMMARY Intestinal permeability has been investigated in the normal rat by measuring the five hour urine excretion of ^{51}Cr -EDTA after intragastric administration. Twelve control animals excreted $2.06\% \pm 0.22$ (mean \pm SE) of the administered dose. Prolonged intestinal transit times with atropine had no significant effect on the apparent permeability with a urine excretion $2.31\% \pm 0.36$. The concomitant administration of a hypertonic, but rapidly absorbed glycerol solution, was accompanied by increased urinary excretion ($3.05\% \pm 0.33$) while the administration of a poorly absorbed sugar, lactulose, significantly decreased the apparent permeability (urine excretion $0.61\% \pm 0.14$) showing that passive intestinal permeability estimations are affected by test dose composition. Enteropathy was induced by ethanol, cetrimide, or methotrexate and each was associated with increased permeability, with urine excretions of $4.19\% \pm 0.47$, $4.20\% \pm 0.66$ and $3.97\% \pm 0.49$ respectively. It is thus suggested that the normal rat mucosa is maximally resistant to the absorption of foreign compounds such as ^{51}Cr -EDTA and intestinal damage will disrupt this barrier.

It has been suggested that altered intestinal permeability may be important in the pathophysiology of various gastrointestinal disease¹⁻⁵ and it seems possible that increased permeability to antigens may account for some of the extraintestinal manifestation seen in these disorders.⁶⁻¹⁰ Moreover it has been suggested that this increased intestinal permeability is important in other disorders, such as rheumatoid arthritis,¹¹ and atopic eczema,^{12,13} which are usually not associated with gastrointestinal symptoms.

We have recently described the use of ^{51}Cr -EDTA as a test substance for detecting alterations in intestinal permeability in man both *in vitro*¹⁴ and *in vivo*.¹⁵ By analysing urine excretion following oral administration of ^{51}Cr -EDTA, we have demonstrated a persistent increase in intestinal permeability in patients with treated coeliac disease.¹⁵ Our results from patients with inflammatory bowel disease indicate that the test is of value in discriminating between small intestinal involvement by Crohn's disease and inflammatory colonic disease.¹⁶

The aim of this study was to examine the effects of hyperosmolar solutions on intestinal permeability and to investigate the importance of small bowel transit time in affecting the apparent permeability to ^{51}Cr -EDTA. The absorption of ^{51}Cr -EDTA was also examined in various models of experimentally induced enteropathies.

Methods

ANIMALS

Seven to nine week old male Sprague-Dawley rats, 220-390 g, were fed on a commercial diet (Standard Rodent Diet LAD1, K K - GREEFF Chemicals Ltd, Croydon). Water was allowed *ad libitum* but the animals were fasted overnight before use.

PROCEDURE

Gastric intubation was carried out under light ether anaesthesia and 1 ml of water containing 2 μCi , approximately 10 nmol, ^{51}Cr -EDTA (Amersham International Ltd) administered, followed by 1 ml of water unless otherwise stated. The animals were placed in individual metabolic cages and killed by cervical dislocation five hours later. The abdomen was opened and the bladder

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emptied by puncture. The urine obtained (<0.2 ml) was mixed with that excreted during the five hour test period. The caecum was tied off with double ligatures. The small intestine was removed gently and double ligatures placed at the oesophago-gastric junction, the pylorus and 20 cm distal to the pylorus. The stomach, proximal, and distal small bowel, caecum and colon were removed. The different parts of intestine, faeces and urine were placed in a 25 ml container and disinfectant (Chlorox^R, Womersley Boome Chemicals Ltd, Essex, UK) added up to 25 ml. Each sample was placed centrally between two detectors, set at minimal separation, in a high resolution bulk sample counter¹⁷ and counted for 100 sec. The minimum detectable activity was 0.01% of the administered dose. The residual carcasses were counted in the same counter at minimal possible detector separation.

HISTOLOGICAL ASSESSMENT

A sample was taken from each animal 2 and 20 cm distal to the pylorus and fixed in 10% formalin, embedded in wax and stained with haematoxylin and eosin for histological assessment (4 μ m sections). All samples were coded and randomly assessed. Morphometric analysis was performed by computer-aided microscopy (Magiscan).¹⁸ Mucosal height was measured as the distance from the muscularis mucosa to the tip of a well-orientated villus and crypt depth as the distance between the basement membrane at the base of the crypts to the open 'mouth' of the crypt. Ten measurements of mucosal height and crypt depth were performed on each sample.

TRANSIT TIME ALTERATION

Intestinal transit was delayed by the subcutaneous administration of atropine 25 mg/kg body weight (Antigen Ltd, Roscrea, Ireland) immediately before administration of the ⁵¹Cr-EDTA.

STUDIES WITH HYPEROSMOLAR SOLUTIONS

Six rats in each group were given either a 1500 mosm/l solution of glycerol or 500 mosm/l of lactulose (Sigma Ltd) with the 1 ml ⁵¹Cr-EDTA and in the subsequent 1 ml wash.

EXPERIMENTAL ENTEROPATHY

Ethanol

Seven week old rats were allowed food from 10 am to 6 pm, six days a week and *ad libitum* on the seventh. Each morning 2 ml of 40% (v/v) ethanol was administered intragastrically. Permeability studies were carried out on the 15th day after the animals had been in this regime for two weeks. No

alcohol was administered on the day of study or the previous day.

Detergent

Six rats received 20 mg alkyltrimethylammonium bromide (cetrimide, Sigma Ltd) in addition to the test solution to investigate the effect of a strong detergent on intestinal permeability.¹⁹

Methotrexate

Intestinal damage was produced in six rats by the subcutaneous administration of methotrexate (Sigma Ltd) 25 mg/kg body weight. ⁵¹Cr-EDTA permeability studies were performed 72 hours later when the small intestinal morphological alterations are at its maximum.^{20 21}

STATISTICS

Wilcoxon's rank sum test was used for statistical analyses.

Results

VALIDATION OF METHOD OF PERMEABILITY MEASUREMENTS

Twelve rats were given the test solution and urine collected hourly for eight and then for 16 hours. Faeces were collected over the same period. The mean hourly urine excretion was 10% of the total 24 hour urine activity for each time period up to five hours. Radioactivity appeared in the faeces seven to eight hours after administration. As the faecal activity is high and contamination of the urine inevitable at this time, a five hour collection was used. Urine passed during the five hour period contained, on average, 50% (range 39–63%) of the total 24 hour urine activity. No detectable faecal activity was found in subsequent experiments during the five hour test period.

Eight rats were given a 10-fold higher dose of ⁵¹Cr-EDTA and killed five hours later. Caecal contents were removed and homogenised by five strokes of pestle A and five strokes of pestle B in 5 ml of 0.15 M NaCl (four rats) or in 15% (w/v) trichloroacetic acid (TCA) (four rats) in a 7 ml Dounce tissue grinder (Kontes Glass Co, Vineland, New Jersey, USA). A 1 ml aliquot was centrifuged at 100 000 g for 30 min in a 10 \times 10 angle rotor in a Superspeed 65 ultracentrifuge (MSE Ltd, Crawley, Sussex, UK). The supernatant, pellet and 1 ml of homogenate were counted. Most (97–98%) of the radioactivity was found in the supernatant with total recoveries of 98–101%. These results show that the ⁵¹Cr-EDTA complex is stable in the gastrointestinal tract as any free Cr³⁺ binds strongly to proteins²² and would be

precipitated by TCA. Thus binding of the test substance to intestinal contents or to mucosal tissue, which might be expected to affect absorption of $^{51}\text{Cr-EDTA}$, does not occur. Urine was collected for five hours during these experiments and the mean ($\pm\text{SE}$) urinary excretion, $2.27\pm 0.18\%$ of the administered dose, did not differ significantly from that of the control group (Table 1). Thus the amount of EDTA used in these studies does not itself change intestinal permeability.

PERMEABILITY STUDIES IN EXPERIMENTAL ANIMALS

Table 1 shows that after five hours virtually all the $^{51}\text{Cr-EDTA}$ has passed from the stomach and proximal small intestine in the 12 control animals. Approximately 10% each is found in the distal intestine and colon with 75% in the caecum. Two per cent of the test dose has been excreted in the urine with less than 0.4% in the residual carcass. Total recoveries of radioactivity in all experiments were close to 100%. Table 1 also shows the mean ($\pm\text{SE}$) percentage urine excretion of $^{51}\text{Cr-EDTA}$ in the various animal groups together with the amount of $^{51}\text{Cr-EDTA}$ in each segment of intestine which reflects gastric emptying and intestinal transit times.

EFFECTS OF ALTERATION IN GASTROINTESTINAL TRANSIT TIME

Atropine both delayed gastric emptying and prolonged intestinal transit time (Table 1). No morphological changes were observed in the proximal small intestine and the five hour urine excretion and residual carcass radioactivity were unaffected.

EFFECTS OF HYPEROSMOLAR SOLUTION

Although the glycerol and lactulose solutions were both hyperosmolar their administration with the $^{51}\text{Cr-EDTA}$ had opposing effects on the apparent permeability (Table 1). Glycerol, a rapidly absorbed substance, increased permeability significantly while the non-absorbable lactulose caused a significant decrease. Neither solution was associated with detectable histological effects on the small intestine and there were only minimal changes in gastric emptying or transit times. It would therefore seem that the absorption of $^{51}\text{Cr-EDTA}$ is in part determined by water fluxes induced by the test substances.

EXPERIMENTAL ENTEROPATHY

Ethanol

During the two week ethanol treatment the rats failed to gain weight, (weight 331 ± 11 and 325 ± 10 g [mean \pm SD], respectively, before and after treatment), although they did not look ill. The small bowel morphological changes consisted of slight broadening of a few villi and the occasional loss of epithelial cells from the duodenal-jejunal villus tips. Table 2 shows that there were no significant morphometric changes associated with the ethanol treatment. Gastric emptying and proximal intestinal transit was slightly delayed but mean urine excretion of $^{51}\text{Cr-EDTA}$ was increased two-fold (Table 1) reflecting increased mucosal permeability.

Detergent

In the duodenum the administration of cetrinide caused loss of epithelial cells from the upper third to half of the villi, exposing the stroma. Only the villus tips were affected in the jejunum. Because of

Table 1 Tissue distribution and urine excretion of $^{51}\text{Cr-EDTA}$

Treatment Group	Urine excretion	Stomach	Proximal intestine	Mid and distal intestine	Caecum	Colon	Carcass	Total recoveries
Controls	2.06 ± 0.22	0.17 ± 0.12	0.04 ± 0.02	11.20 ± 4.00	76.8 ± 4.0	9.25 ± 3.73	0.36 ± 0.08	99.8 ± 2.2
Atropine	2.31 ± 0.36	$3.15\pm 1.36\ddagger$	$0.84\pm 0.35^*$	$35.20\pm 7.30\ddagger$	$56.2\pm 7.2\ddagger$	5.21 ± 1.31	0.17 ± 0.05	103.0 ± 4.0
Glycerol	$3.05\pm 0.33^*$	$0.84\pm 0.44\ddagger$	0.22 ± 0.13	12.00 ± 4.20	77.7 ± 6.2	5.61 ± 3.08	0.41 ± 0.06	99.9 ± 4.4
Lactulose	$0.61\pm 0.14\ddagger$	1.03 ± 0.82	0.23 ± 0.17	7.82 ± 1.80	85.8 ± 5.1	8.21 ± 2.80	0.33 ± 0.15	104.0 ± 1.0
Ethanol	$4.19\pm 0.47\ddagger$	$1.06\pm 0.38\ddagger$	$0.41\pm 0.10\ddagger$	6.20 ± 2.26	82.5 ± 3.4	8.90 ± 3.28	0.39 ± 0.09	103.0 ± 2.0
Cetrinide	$4.20\pm 0.66\ddagger$	$4.38\pm 0.58\ddagger$	$1.21\pm 0.38\ddagger$	17.10 ± 2.90	$57.3\pm 4.7^*$	11.80 ± 2.90	$1.44\pm 0.55^*$	97.4 ± 3.7
Methotrexate	$3.97\pm 0.49\ddagger$	$12.27\pm 4.38\ddagger$	$1.08\pm 0.36\ddagger$	5.72 ± 2.16	71.5 ± 4.4	10.10 ± 3.80	0.81 ± 0.43	105.0 ± 2.0

Mean ($\pm\text{SE}$)% urine excretion, intestinal distribution and total recoveries of $^{51}\text{Cr-EDTA}$ 5 hours after intragastric administration. There were 12 rats in the control group and 6 in each of the others. Dosage and route of administration is described in text. No activity was found in faeces passed during the test.

* differed significantly from controls ($p<0.05$)

† differed significantly from controls ($p<0.01$)

Table 2 Morphometric analysis of jejunum

	Duodenum		Jejunum	
	Mucosal height (μm)	Crypt depth (μm)*	Mucosal height (μm)	Crypt depth (μm)*
Controls (12)	808 \pm 38	219 \pm 12	657 \pm 32	185 \pm 8
Alcohol (6)	891 \pm 28	228 \pm 7	723 \pm 37	194 \pm 9
Cetrimide (6)	—†	246 \pm 9	764 \pm 78	184 \pm 12
Methotrexate (6)	719 \pm 50	365 \pm 45‡	656 \pm 146	298 \pm 28‡

Values are mean (\pm SE) with number of animals shown between parentheses

* Crypt depth measurements were made as described in text except in methotrexate treated rats where longitudinal cuts were not achieved because of proliferative activity. In these animals crypt depth was measured as the distance from the crypt 'mouth' to the basement membrane of the deepest cross cut section of a crypt straight below

† Measurement not possible because of distorted architecture

‡ Significantly greater than controls, $p < 0.01$.

the distorted architecture estimations of mucosal heights in duodenal samples were not possible but crypt depths did not differ from that of control animals (Table 2). Gastric emptying was prolonged and transit times were delayed throughout the small intestine though this was less than that seen after atropine. The increased urine excretion of ^{51}Cr -EDTA (Table 1) was markedly increased in spite of increased radioactivity in the carcass.

Methotrexate

All rats passed loose stools. Table 2 shows that crypt depth was significantly increased in the methotrexate-treated rats. Mucosal height did not differ significantly from controls although four of the six rats treated with methotrexate had the lowest values. The duodenal enterocyte cytoplasm was vacuolated with slight nuclear irregularity. The crypt architecture was most distorted with crowding of crypts. The vast majority were cut in cross-sections suggesting an increased complexity due to crypt cell proliferation. The crypt cells were enlarged with large and prominent nuclei but with loss of nuclear polarity. Occasional mitoses were noted and apoptotic bodies were present in the upper half of the crypts. Polymorphs were prominent in the lamina propria and occasional crypt abscesses were noted. The jejunal mucosae showed similar but less severe change. Gastric emptying and to a lesser extent intestinal transit were significantly delayed (Table 1). Urine excretion of ^{51}Cr -EDTA was significantly greater than that of control animals.

Discussion

Urine excretion of ^{51}Cr -EDTA was most rapid in the first five hours after administration to the normal rat. At this time most of the ^{51}Cr -EDTA had reached the large intestine. Despite low

carcass activity substantial amounts of ^{51}Cr -EDTA appear in the urine over the next 19 hours indicating that the complex is absorbed throughout the gastrointestinal tract. This is in agreement with Lokken²³ who investigated the absorption of ^{51}Cr -EDTA in ligated parts of the rat gastrointestinal tract. While there was negligible absorption from the stomach, significant amounts were absorbed by the duodenal mucosae, declining progressively down the intestinal tract.²³

Disodium EDTA has been shown to alter intestinal permeability in the rat^{24,25} possibly by chelating Ca^{2+} , essential for cell-cell adhesion, or by the direct toxicity of Ca-EDTA .^{26,27} $\text{Ca}^{2+} - \text{Cr}^{3+}$ exchange seems however highly unlikely because the stability constant of $^{51}\text{Cr-EDTA}$ (10^{23}) is 10^{12} times larger than that of Ca-EDTA (10^{11}).²⁸ Furthermore, the amount of EDTA used in our experiments is only 30 nmol compared with concentrations of 250 mM required to consistently alter permeability in the experimental animal.^{24,25} A 10-fold increase in the amount of $^{51}\text{Cr-EDTA}$ had no effect on the percent urine excretion and therefore intestinal permeability was presumably unaltered.

The absorption of $^{51}\text{Cr-EDTA}$ is enhanced by the simultaneous administration of a hyperosmolar solution of glycerol in contrast with the reduced absorption when given in a solution of lactulose. Both substances cause an initial shift of water into the lumen to preserve intraluminal isotonicity. Glycerol is, however, rapidly absorbed with concomitant water reabsorption. Lactulose is essentially non-absorbable maintaining its osmotic effect, and thus reabsorption of water does not occur.²⁹ The altered absorption of $^{51}\text{Cr-EDTA}$ is probably due to a number of factors including changes in the concentration and effective mucosal contract time but it suggests that water and $^{51}\text{Cr-EDTA}$ shares, at least partially, a common

absorption path. It is therefore possible that enhanced serosal to mucosal water flux could account for the decreased absorption of ^{51}Cr -EDTA when given with lactulose. The results are in agreement with those of other workers who have shown that hyperosmolar solutions of absorbable substances cause widening of intercellular junctional spaces³⁰⁻³² and increased permeability in many other epithelia.^{33 34} Furthermore, similar permeability changes have been reported in man and it has been suggested that hyperosmolarity may contribute to increased absorption of antigens and carcinogens which are probably largely excluded from absorption under normal circumstances.^{1 35 36}

Ethanol, cetrimide and methotrexate administration all resulted in loss of gastrointestinal integrity as shown by the increased absorption of ^{51}Cr -EDTA. This implies that the normal mucosa is maximally resistant to the absorption of ^{51}Cr -EDTA. Certainly in man the occurrence of antibodies to dietary antigens is evident in disorders affecting the small bowel³⁷ which could be attributable to increased permeability as well as maldigestion of antigens and changes in immunological surveillance.

Decreases of jejunal villus height has been described in rats fed for four weeks on a liquid ethanol.³⁸ We, however, found no significant morphometric changes in intestinal samples from ethanol-treated rats which is in agreement with the results of Worthington *et al*,³⁹ who likewise administered ethanol by a single daily dose. Electron microscopic changes are however observed and permeability to horseradish peroxidase is increased by ethanol, possibly because of disruption of intercellular junctions.³⁹⁻⁴¹ These changes are reflected in man by defects of permeability to various test substances.⁴²⁻⁴³ It seems possible that such alterations could in part accelerate the development of cirrhosis by allowing increased permeation of noxious compounds and possibly explain the increased incidence of certain malignancies in chronic alcoholics.⁴⁴

Increasing transit times with atropine had no significant effect on the five hour excretion of radioactivity. The alterations found in the cetrimide treated rats are therefore unlikely to be of major importance in the increased urine excretion of ^{51}Cr -EDTA seen in these rats. In comparison, results with 'non absorbable' sugars show that the absorption of disaccharides is increased to the same extent as monosaccharides.^{21 45} Thus, although their urine excretion ratio, the usual index used by these workers, does not change, it is clear that intestinal

permeability is increased. These conclusions further show the difficulties in interpreting alterations in intestinal permeability based on changes in the excretion ratio of various sugars.

The crypt changes after a single injection of methotrexate had histological features of both degeneration and regeneration. These findings contrast with those Cobden *et al*²¹ who used a similar procedure but found villous atrophy with hypoplastic crypts. Jeynes *et al*⁴⁶ have, however, shown that crypt cell regeneration begins approximately 36 hours after methotrexate administration and our findings support this view. Intestinal permeability to ^{51}Cr -EDTA was increased in these animals which differs from the conclusions with the sugar tests; these show a significant reduction in the absorption of both the di- and monosaccharides.^{21 45} The reason for these differences are unclear but would seem to imply that the absorption pathways of ^{51}Cr -EDTA differed from that of the disaccharides. Alternatively methotrexate treatment could allow small bowel bacterial overgrowth which would result in rapid degradation of the sugars and thus in the amount available for absorption. The decreased absorption could therefore erroneously be ascribed to decreased gut permeability.

In summary our results show that ^{51}Cr -EDTA is stable in the alimentary tract and that it is a convenient marker for assessing gastrointestinal permeability in the rat. Permeability estimations are dependent on test dose composition and minor alterations of gastric emptying and intestinal transit do not significantly affect the results. Experimentally-induced enteropathies were associated with increased urine excretion of ^{51}Cr -EDTA which may reflect increased gastrointestinal permeability. Further studies using ^{51}Cr -EDTA in both the experimental animal and man seem indicated to clarify the importance of altered intestinal permeability in the pathogenesis of small bowel disease and the extraintestinal manifestations.

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