**Lactulose \(^{51}\text{Cr}\)-labelled ethylenediaminetetra-acetate, L-rhamnose and polyethyleneglycol 500 as probe markers for assessment *in vivo* of human intestinal permeability**

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**Summary**

1. The urinary excretion of lactulose, \(^{51}\text{Cr}\)-labelled ethylenediaminetetra-acetate (\(^{51}\text{Cr}-\text{EDTA}\)), L-rhamnose and polyethyleneglycol 400 (PEG-400) has been measured after intravenous and oral administration in healthy volunteers.
2. Intestinal permeation of the probes was compared after their ingestion in iso-osmolar, hyperosmolar and cetrimide-containing test solutions.
3. Urinary recovery of lactulose and \(^{51}\text{Cr}-\text{EDTA}\) after intravenous administration reached 75% by 5 h, and exceeded 90% at 24 h, and these values were 62 and 72%, respectively, for L-rhamnose. Recovery of PEG-400, however, varied with the relative molecular mass \((M_r)\) of each polymer from 25.9 to 68.5% in 24 h.
4. Intestinal permeation of ingested lactulose and \(^{51}\text{Cr}-\text{EDTA}\) was low, but that of L-rhamnose was 45-fold, and that of PEG-400 100-fold, greater. Permeation of lactulose and \(^{51}\text{Cr}-\text{EDTA}\) was markedly increased by cetrimide and hyperosmolar stress, whereas that of L-rhamnose showed little change. PEG-400 permeation was not affected by cetrimide, but was slightly increased by hyperosmolar stress.
5. The 5 h permeation of lactulose, but not of L-rhamnose or PEG-400, correlated with that of \(^{51}\text{Cr}-\text{EDTA}\) \((r = 0.98, P < 0.001)\).
6. These findings are compatible with three distinct pathways of unmediated mucosal permeation, L-rhamnose (radius <0.4 nm) passing mainly through small aqueous 'pores' of high incidence, lactulose and \(^{51}\text{Cr}-\text{EDTA}\) (radius >0.5 nm) through larger aqueous 'channels' of low incidence susceptible to cetrimide and hyperosmolar stress, and PEG-400, which has appreciable lipid solubility, by partition through cell membrane lipid as well as the aqueous 'pores'.

**Key words:** \(^{51}\text{Cr}-\text{EDTA}\), human intestinal permeability, lactulose, L-rhamnose, polyethyleneglycol 400.

**Abbreviations:** \(^{51}\text{Cr}-\text{EDTA}\), \(^{51}\text{Cr}\)-labelled ethylenediaminetetra-acetate; \(M_r\), relative molecular mass; PEG-400, polyethyleneglycol 400.

**Introduction**

Initial investigations of human small-intestinal permeability, based upon measurements of osmotic reflection \([1]\) and unmediated permeation of various probe-markers \([2, 3]\), clearly differentiate between the substantial diffusion of small molecules and restricted transmucosal passage of larger polar molecules of radius 0.5 nm and above. The development of less invasive methods in which renal excretion of an ingested marker serves as a measure of unmediated intestinal permeation made the procedure more practical and therefore widely used \([4-16]\), but this has been accompanied by the introduction of probe-molecules with confusing differences in behaviour. For instance, low molecular weight ethyleneglycol polymers [PEG-400, relative molecu-
ular mass ($M_r$) 194–402] evidently penetrate the intestinal mucosa about 100 times more efficiently than lactulose and $^{51}$Cr-labelled ethylenediaminetetra-acetate ($^{51}$Cr-EDTA) ($M_r$ approx. 340) even though they are of similar molecular size [4, 5, 11, 13]. In this respect, and the response to vil-
lous atrophy (when absorption is reduced [5]), PEG-400 behaves more like smaller molecules such as $\alpha$-mannitol and $\iota$-rhamnose ($M_r$, 182 and 164; radius $<0.4$ nm) than lactulose and $^{51}$Cr-
EDTA [17]. Though this accords with the presence of distinct pathways of unmediated permeation [18], it has produced controversy over choice of probe and interpretation of results. There remains a need to verify and expand previous findings and obtain some explanation for outstanding discrepancies.

The renal excretion of lactulose, $^{51}$Cr-EDTA, $\iota$-
rhamnose and PEG-400 after oral and intravenous administration has therefore been directly com-
pared in a group of healthy adult volunteers. Changes in the permeation of these markers pro-
based by hyperosmolar stress [6, 17, 19] and the detergent cetrimide [20, 17] have been investigated in order to differentiate various pathways of mucosal entry.

Methods

Recovery of probe-markers in urine after intra-
venous administration

Six healthy adult volunteers (aged 27–53 years) received lactulose (pure crystalline: gift from Philips-Duphar B.V., Weesp, Holland) 1.46 mmol, PEG-400 (BDH Chemicals Ltd, Poole, U.K.) 1.0 g, and $^{51}$Cr-EDTA (Radiochemical Centre, Amer-
sham, Bucks, U.K.) 20 $\mu$Ci, dissolved together in 10 ml of water as a sterile, pyrogen- and particle-free solution, by bolus injection administered intravenously over a period of about 2 min. A complete urine collection, divided into consecutive periods of 0–2.5, 2.5–5, 5–7.5, 7.5–10, 10–12.5 and 12.5–24 h, was then made into clean sample bottles containing merthiolate 1 ml (10%, w/v) as preserva-
tive; the volumes were recorded and 30 ml aliquots were stored at 4°C for analysis. Fifteen healthy adult volunteers (aged 19–47 years) supplied similar urine samples after they had received 3.05 mmol of $\iota$-rhamnose (pure crystalline, Koch-Light Laboratories, Colnbrook, Essex, U.K.) in 5 ml of water as a sterile, pyrogen- and particle-free intra-
venous injection. All solutions for intravenous administration were prepared and checked to exclude pyrogenic and other reactions by high-dose rabbit injection by the Pharmacy Departments of St Thomas’s or Northwick Park Hospital. Injection solutions were passed through a sterile Millipore filter immediately before use.

Recovery of probe-markers in urine after oral administration

Ten healthy volunteers (aged 21–53 years), without evidence of gastrointestinal or systemic disease, participated. After an overnight fast a test solution, containing lactulose 13.7 mmol (7 ml of Duphalac 67%, w/v, lactulose syrup; Duphar Laboratories Ltd, West End, Southampton, U.K.), $\iota$-rhamnose 6.1 mmol, PEG-400 5 g, and $^{51}$Cr-EDTA 100 $\mu$Ci, dissolved in water to 150 ml (233 mmol/l), was ingested within a period of 4 min. A complete urine collection was then made in consecutive periods of 0–2.5, 2.5–5, 5–10 and 10–24 h, and samples were stored for analysis as above.

To compare the effect of hyperosmolar stress and cetrimide on intestinal marker permeation each subject repeated the above procedure, at minimum intervals of a week, on three further occasions. The same test solution was ingested with the following additions: (1) glycerol 14.2 ml (193.5 mmol) to make test solution 1500 mmol/l; (2) glycerol 22 ml (300 mmol/l) to make test solution 2300 mmol/l; (3) cetrimide 150 mg. Subjects avoided lactulose, drugs known to influence intestinal permeability, and alcohol, for 48 h before and throughout each study.

The Ethical Committees at St Thomas’s Hospital and Harrow Health Authority have given approval for the experimental procedures described, and informed consent was obtained from each subject.

Analysis of sugar markers

Urinary lactulose and $\iota$-rhamnose were esti-
mated by modifications of quantitative paper [21, 22] and thin-layer [23] chromatography. For lactu-
lose analysis urine samples were desalted by shaking with Duolite MB 5113 mixed anion–cation exchange resin (BDH Chemicals Ltd, Poole, Dorset, U.K.) in the H$^+$, acetate form and run, in parallel with appropriate standard applications, on 46 cm × 56 cm sheets of Whatman no. 3 Chroma-
paper (Whatman Laboratory Products Ltd, Springfield Mill, Maidstone, Kent, U.K.) with butan-1-ol/ethyl acetate/pyridine/water (30:30:30: 15, by vol.; 20 h descending). After a 4-aminobenzoic/phosphoric acid colour reaction [21, 22], the separated zones were measured with a recording and integrating densitometer (Chromosean 200, Joyce-Loebel and Co. Ltd, Team Valley, Gates-
head, Co. Durham, U.K.). Sugar concentrations were calculated by comparison of test with standard peak areas assuming a linear relationship below 20 $\mu$g/zone [21, 22].

A modified thin-layer technique [23] was pre-
furred for estimating $\iota$-rhamnose. This involved measurement of peak heights by scanning densi-
backed silica gel (Schleicher and Schuell, Dassel, FRG) using three consecutive ascending runs (8.5 cm each) with butan-1-ol/ethyl acetate/pyridine/acidic acid/water (5:70:15:10:10, by vol.). The layers were dried for at least 30 min between each run, and then for 4 h (preferably overnight) to remove pyridine before performing a 4-aminobenzoic/phosphoric acid colour reaction at 120-130°C for 10 min [23]. After location chromatograms were kept refrigerated in polythene envelopes, and exposure was minimized during scanning. Peak heights were measured and corrected to a constant internal standard value. Test concentrations were then derived by interpolation from a standard rhamnose concentration curve from the same chromatogram.

Both chromatographic procedures employ refinements of technique that improve precision described elsewhere [21-23]. They are accurate and sensitive, recovery being above 90% and minimum level of detection below 0.1 mmol/l for most sugars. The precision of both methods lies between 3 and 8% (coefficient of variation without replication) over the test range of sugar concentration.

**Analysis of polyethyleneglycol 400**

Estimation of individual polymers and total PEG-400 content in urine samples was by gas-liquid chromatography. Pentaerythritol (Sigma Chemical Co., St Louis, MO, U.S.A.), 0.5 ml of aqueous 100 mg/100 ml solution, was added, as internal standard, to 3 ml of a 1 in 3 aqueous dilution of each urine sample, and also to 3 ml of appropriately diluted PEG-400 standard from the original stock. The aliquots were desalted with Duolite MB 5133 in the H⁺, acetate form and, after centrifugation, 1 ml of each supernatant was freeze-dried overnight in a 2 ml ampoule. Each sample was acetylated by heating for 60 min at 70°C with 0.1 ml of acetic anhydride, and then dried in air followed by vacuum desiccation for at least 2 h. Each vial was then shaken rapidly with 0.1 ml of acetone on a vortex mixer, and 5 µl of the product injected on to a Perkin-Elmer F33 liquid chromatograph.

Complete separation of all eight ethyleneglycol polymers (M, 194-502) and pentaerythritol was obtained within 30 min on a column (length 1 m, internal diameter 2 mm) containing Gas-Chrom Q (100-200 mesh) coated with 1% Poly S-179. After sample injection the column was kept at 150°C for 2 min, then programmed to rise to 350°C at 8°C per min. Peak height measurements corrected to a constant internal standard value had a linear relationship over the range 0-400 mg/100 ml, and were used to calculate sample PEG content. Estimations of total PEG-400 had 'within batch' and 'between batch' precisions of 5.5 and 6.3%, respectively, and for individual polymers precision varied between 5.1 and 10.4%, coefficient of variation. Average recovery was 96.9% (range 91-102%).

**Analysis of ⁵¹Cr-EDTA**

Aliquots (5 ml) of urine were counted for radioactivity for 5 min in an LKB Wallac 1280 gamma counter with a 7.5 cm x 7.5 cm sodium iodide well-type crystal, together with 5 ml of a 1 in 500 dilution of the appropriate oral test solution. Sensitivity was 0.03% of the administered dose per litre of urine, and the precision was between 1.0 and 6.0%, coefficient of variation, depending on the level of activity.

**Statistical analysis**

Comparisons were by paired Student's t-test, and intestinal permeation of lactulose and ⁵¹Cr-EDTA was subjected to least squares linear regression analysis.

**Results**

**Recovery of probe-markers in urine after intravenous administration**

The time sequences of renal excretion and total urinary recovery after intravenous injection of lactulose, ⁵¹Cr-EDTA, L-rhamnose and PEG-400 (individual polymers and total) are given in Table 1. Percentage recoveries of lactulose and ⁵¹Cr-EDTA are almost complete, 92.7 ± 1.2 and 97.4 ± 0.5 respectively (mean ± SEM in 24 h), and that of L-rhamnose is 71.5 ± 1.4%. The recovery of PEG-400 is considerably lower, being 40.7 ± 2.9% for M, 194 and 68.5 ± 3.1% for M, 502.

**Urinary excretion of probe-markers after ingestion**

Percentages of each molecular probe excreted in the urine of 10 healthy adult subjects during consecutive periods after ingestion of iso-osmolar, hyperosmolar (1500 and 2300 mmol/l) and cetrimide-containing test solutions are recorded in Table 2.

**Intestinal permeation of probe-markers**

Intestinal permeation, derived by correcting urinary excretion for the systemic loss indicated by
### Table 1. Recovery of probe-markers in urine: time sequence after intravenous administration

Mean percentage of dose ± SEM. M, values shown in parentheses. Six healthy adults received $^{51}$Cr-EDTA, lactulose and PEG-400 simultaneously; 13 healthy adults received L-rhamnose.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$^{51}$Cr-EDTA</th>
<th>Lactulose</th>
<th>PEG-400 polymers</th>
<th>L-rhamnose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(340)</td>
<td>(342)</td>
<td>1 (194)</td>
<td>2 (238)</td>
</tr>
<tr>
<td>0-2.5</td>
<td>64.6 ± 1.6</td>
<td>60.5 ± 1.4</td>
<td>21.1 ± 2.5</td>
<td>25.1 ± 1.6</td>
</tr>
<tr>
<td>2.5-5.0</td>
<td>20.3 ± 0.7</td>
<td>19.1 ± 1.8</td>
<td>3.4 ± 1.2</td>
<td>6.0 ± 1.2</td>
</tr>
<tr>
<td>5.0-7.5</td>
<td>6.56 ± 0.47</td>
<td>6.97 ± 0.42</td>
<td>1.3 ± 0.4</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>7.5-10.0</td>
<td>3.04 ± 0.47</td>
<td>3.07 ± 0.54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.0-12.5</td>
<td>1.46 ± 0.24</td>
<td>1.60 ± 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.5-24.0</td>
<td>1.40 ± 1.24</td>
<td>1.56 ± 0.36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0-24.0</td>
<td>97.4 ± 0.69</td>
<td>92.7 ± 1.51</td>
<td>25.9 ± 3.7</td>
<td>33.7 ± 1.5</td>
</tr>
</tbody>
</table>

### Table 2. Recovery of probe-markers in urine: time sequence after oral administration

Mean percentage of dose ± SEM, not corrected for systemic loss. Ten healthy adults received all four probe-markers simultaneously in iso-osmolar (1500 and 2300 mmol/l), and cetrimide-containing test solutions.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$^{51}$Cr-EDTA</th>
<th>Lactulose</th>
<th>PEG-400: combined polymers</th>
<th>L-rhamnose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1500 mmol/l</td>
<td>2300 mmol/l</td>
<td>1500 mmol/l</td>
<td>2300 mmol/l</td>
</tr>
<tr>
<td>0-2.5</td>
<td>0.192</td>
<td>0.674</td>
<td>1.246</td>
<td>3.381</td>
</tr>
<tr>
<td></td>
<td>±0.052</td>
<td>±0.137</td>
<td>±0.226</td>
<td>±0.498</td>
</tr>
<tr>
<td>2.5-5.0</td>
<td>0.247</td>
<td>0.339</td>
<td>0.543</td>
<td>1.435</td>
</tr>
<tr>
<td></td>
<td>±0.041</td>
<td>±0.060</td>
<td>±0.083</td>
<td>±0.385</td>
</tr>
<tr>
<td>5.0-10.0</td>
<td>0.292</td>
<td>0.323</td>
<td>0.432</td>
<td>0.863</td>
</tr>
<tr>
<td></td>
<td>±0.038</td>
<td>±0.047</td>
<td>±0.053</td>
<td>±0.104</td>
</tr>
<tr>
<td>10.0-24.0</td>
<td>0.413</td>
<td>0.338</td>
<td>0.497</td>
<td>0.516</td>
</tr>
<tr>
<td>0-24.0</td>
<td>1.163</td>
<td>1.674</td>
<td>2.723</td>
<td>6.195</td>
</tr>
</tbody>
</table>
recovery after intravenous injection during appropriate time intervals, is as follows.

Permeation after ingestion of iso-osmolar test solutions. As shown in Fig. 1, the intestinal permeation of each probe-marker is greatest during the first 5 h period, excretion of lactulose, L-rhamnose and PEG-400 falling to negligible levels after 10 h, while that of \(^{51}\)Cr-EDTA continues for a somewhat longer period. The percentages of lactulose and \(^{51}\)Cr-EDTA permeating the intestine during the first 5 h period are similar (0.415 ± 0.050 and 0.518 ± 0.089, mean ± SEM, respectively). However, permeation of L-rhamnose (18.9 ± 1.5) is 45-fold, and that of PEG-400 (42.5 ± 2.4, combined fractions) is 103-fold, greater than that of lactulose.

Effect of hyperosmolar and cetrimide-containing test solutions. Hyperosmolar stress and cetrimide enhance the intestinal permeation of both lactulose and \(^{51}\)Cr-EDTA, as shown in Fig. 2. Thus, permeations of lactulose and \(^{51}\)Cr-EDTA rise, respectively, by factors of 1.5 (from 0.415 ± 0.050 to 0.614 ± 0.076, mean percentage of dose ± SEM; \(P = \text{NS} \)) and 2.3 (from 0.518 ± 0.089 to 1.193 ± 0.195; \(P < 0.02 \)) in response to the 1500 mmol/l test solution; 2.8 (to 1.16 ± 0.21; \(P < 0.001 \)) and 4.1 (to 2.124 ± 0.339; \(P < 0.001 \)) in response to the 2300 mmol/l solution; and 7.7 (to 3.20 ± 0.47; \(P < 0.001 \)) and 11.5 (to 5.961 ± 0.932; \(P < 0.001 \)) in response to the cetrimide-containing test solution. Though the susceptibility of \(^{51}\)Cr-EDTA to these experimental stresses is greater than that of lactulose, a highly significant correlation between the permeation of these two probe-molecules is evident during the first 5 h period \((r = 0.98, P < 0.001, \text{see Fig. 3})\). In contrast, L-rhamnose permeation fell by factors of 0.79 (from 18.86 ± 1.52 to 14.92 ± 1.08, mean percentage of dose ± SEM; \(P < 0.05 \)) and 0.86 (to 16.14 ± 1.81; \(P = \text{NS} \)) in response to the 1500 and 2300 mmol/l hyperosmolar stresses, but altered very little (to 20.2 ± 2.96; \(P = \text{NS} \)) when cetrimide was given.

The average 5 h intestinal permeation of PEG-400 increased slightly, by factors of 1.22 and 1.52 (from 42.54 ± 2.36 to 51.88 ± 2.74 and 64.71 ± 5.2, mean percentage of dose ± SEM; \(P < 0.01 \) for both) in response to the 1500 and 2300 mmol/l hyperosmolar stresses respectively, but there was no significant change (to 49.37 ± 2.50; \(P = \text{NS} \)) after cetrimide. As shown in Fig. 4, there were some differences between the responses of individual polymers, but taken overall the behaviour of PEG-400 did not correlate with that of L-rhamnose, or lactulose and \(^{51}\)Cr-EDTA.

Relative permeability of the human intestine to different probe-markers

Relative permeation of probe-markers, expressed as percentage of ingested dose recovered in urine during 5 h corrected for systemic loss \((P^{5h}) \), and also, according to Graham's Law, for the effect of \(M_f \) upon diffusion through unstirred aqueous media by a factor \(K/M_f \) \((K = \text{a constant})\), is presented in Fig. 5. Approximate values relating to D-mannitol, raffinose and D-xylose, calculated from previously published data [4, 6, 7, 13, 17, 24], have
Fig. 2. Comparison of the effect of cetrimide and hyperosmolar stress on intestinal permeation of $^{51}$Cr-EDTA, lactulose, PEG-400 and L-rhamnose. Recovery in the urine of 10 healthy volunteers after simultaneous ingestion in iso-osmolar, hyperosmolar and cetrimide-containing test solutions, corrected for systemic loss, expressed as mean percentages of oral dose in 5 h ± SEM. Factors of difference from the iso-osmolar (control) responses are given above each column. See Fig. 1 for key to probe-markers, and text for details of the test solutions.

Fig. 3. Comparison of intestinal permeation by lactulose and $^{51}$Cr-EDTA, expressed as percentage of ingested dose recovered during 5 h in the urine of 10 healthy volunteers, corrected for systemic loss. Four different test solutions containing both markers were given to each subject on different occasions. , Iso-osmolar; ○, hyperosmolar 1500 mmol/l; □, hyperosmolar 2300 mmol/l; ■, cetrimide-containing. See text for details of test solutions.

been included for comparison. The lack of correspondence between intestinal permeability to ethylene glycol polymers and other inert probe-markers of similar molecular mass (L-rhamnose, D-mannitol, $^{51}$Cr-EDTA, lactulose and raffinose) is clearly demonstrated. The efficiency of PEG-400 permeation is equalled only by that of D-xylose, a pentose with mediated intestinal transport.

Discussion

The excretion of a probe-marker in the urine after ingestion provides a convenient non-invasive basis for the assessment of intestinal absorption [25, 26]. Though questions of cost, toxicity and ease of analysis are important, the absorption characteristics of selected markers should relate to specified pathways of mucosal diffusion and, as interpretation depends upon recovery from the urine, a consistent relationship should also exist between absorption from the intestine and renal excretion.

The four markers selected for this investigation are considered to resist metabolic degradation in the human [25, 4, 5, 8, 11]. Present results indicate that, though recovery of L-rhamnose after intravenous administration is rather less than that of lactulose and $^{51}$Cr-EDTA, excretion of these markers in human urine after ingestion should closely reflect absorption from the intestine. With regard to ethylene glycol polymers, though Chadwick et al. report...
Probe-markers for human intestinal permeability

Fig. 4. Effect of cetrimide and hyperosmolar stress on intestinal permeation by ethylene-glycol polymers. Recovery in the urine of 10 healthy volunteers after ingestion of PEG-400 in iso-osmolar, hyperosmolar and cetrimide-containing test solutions, corrected for systemic loss, expressed as mean percentages of oral dose of 5 h $\pm$ SEM. Factors of difference from the iso-osmolar (control) responses are given above each column. Relative molecular masses ($M_r$): polymer 1, 194; 2, 238; 3, 282; 4, 326; 5, 370, 6, 414; 7, 458; 8, 502.

Fig. 5. Relative human intestinal permeability to different probe-molecules. Recovery in urine as a percentage of ingested dose in 5 h, corrected for systemic loss ($= P^{98}$), and for effect of molecular mass on diffusion through aqueous media by the factor $K/M_r$ ($K$ = constant). Xyl, $d$-Xylose; Rh, $l$-rhamnose; Mn, $d$-mannitol; LL, lactulose; Cr, $^{51}$Cr-EDTA; Ra, raffinose; polymers of PEG-400 = 1, 2, 3 etc. (see legend to Fig. 4 for $M_r$ values).

that the recovery of total PEG-400 in the urine of a dog was 65% after intravenous infusion [27], Tagesson & Sjodahl [28] found that recovery in the urine of pigs fell from 75% for polymers above $M_r$ 722, to 21% for the smallest they investigated ($M_r$ 282). Our findings in healthy human volunteers agree closely with the latter and suggest that excretion of PEG-400 polymers ($M_r$ 194–502) in urine will underestimate intestinal absorption by between 31 and 79%. As human renal clearance of PEG-400 is similar to that of creatinine [29], the loss is probably due to sequestration into tissues or excretion into the intestinal tract.

Unmediated permeation of small molecules such as $d$-mannitol [7, 9, 12, 13, 24] and $l$-rhamnose [25, 8, 10, 14–17] ($M_r$ 182 and 164, radii <0.4 nm) across the healthy human gastrointestinal mucosa is known to be at least 30 times more efficient than that of even slightly larger inert molecules such as lactulose [4, 6, 8–10, 13–17], $^{51}$Cr-EDTA [11], raffinose [4, 6], stachyose and FITC-dextran [6]: $M_r$ 340 (approximate value for lactulose and $^{51}$Cr-EDTA), 504, 666 and 3000, respectively, radii all <0.5 nm. During the present investigation combined ingestion of probe-markers ensured that experimental variations affecting absorptive area, transit time, luminal concentration (i.e. 'exposure factors') and renal clearance, were the same for each marker, and the results obtained agree well with previously reported findings. Intestinal permeation of lactulose and $^{51}$Cr-EDTA, calculated by correcting urinary excretion for systemic loss as
originally advocated by McCance & Madders [25] is, as might be expected for molecules of corresponding size, closely similar, whereas the absorption of L-rhamnose is about 40 times greater (see Fig. 1).

These findings suggest the presence of two distinct pathways of unmediated mucosal permeation, one consisting of small 'pores' of high incidence, the other of larger 'channels' of low incidence. The small 'pores' are usually thought to be aqueous channels, whether defined or labile [30, 31], in the enterocyte cell-membrane and therefore part of a transcellular pathway [1], and the larger 'channels' to be an aqueous route related to the tight junctions and/or cell extrusion zones [6, 17, 32]. It is possible, however, that both pathways might be paracellular [13], and their true disposition remains to be proven. The 'dual pathway' hypothesis gains further support from the contrasting way in which permeation of large and small molecular probes respond to experimental stresses and mucosal pathology. As shown in Fig. 2 ingestion of hyperosmolar and cetrimide-containing test solutions induce a considerable increase in the permeation of lactulose and $^{51}$Cr-EDTA, but no significant change in that of L-rhamnose. Furthermore, villous atrophy produces an increase in the permeation of large molecules like lactulose and $^{51}$Cr-EDTA, but a coincidental decrease in that of smaller molecules such as L-rhamnose and d-mannitol [8, 9, 13, 17]. The polymers of PEG-400, however, do not conform to this pattern of behaviour.

PEG-400 consists of a series of inert polymers that resist degradation by bacteria [27]. The eight constituents of the product used for the present study ($M_r$, 194-502, $n$ = 4-11 ethylene oxide units) represent a range of molecular mass that coincides with L-rhamnose and d-mannitol at the lower end, lactulose and $^{51}$Cr-EDTA in the middle, and raffinose at the upper end. Nevertheless, intestinal permeation by these polymers is many times greater than that of the other probe molecules mentioned. Renal excretion of ingested PEG-400, reported by both Chadwick et al. [5] and Ukabam & Cooper [13], suggests that permeation of the normal human intestine by these polymers is almost 200 times greater than lactulose and about twice that of d-mannitol. The present comparison of renal excretion corrected for systemic loss demonstrates that human intestinal permeation by PEG-400 polymer 1 ($M_r$, 194) is three times greater than L-rhamnose ($M_r$, 164), and that of polymer 4 ($M_r$, 326) about 120 times greater than either lactulose or $^{51}$Cr-EDTA ($M_r$, about 340). d-Mannitol and raffinose also permeate much more slowly than ethyleneglycol polymers of similar molecular mass: indeed, calculations from previously published data [4, 6, 7, 12, 24, 25] indicate that only those sugars with affinity for mediated transport, like d-xylose, can penetrate the intestine as efficiently as these polymers do (see Fig. 5). The average excretion of lactulose recorded by Ukabam & Cooper (0.15%/6 h) [13] is lower than ours (0.39%/5 h), probably due to osmogenic retention of fluid within the intestine [33], induced by the larger dose of lactulose employed.

Intestinal permeation of PEG-400 also responds to experimental stresses and pathology in a way that differs from that of other probe molecules of corresponding mass. Thus cetrimide produces no change, and response to hyperosmolar stress is much less than that of lactulose and $^{51}$Cr-EDTA. In this, and in respect to the impairment reported in coeliac disease [5], intestinal permeation by PEG-400 behaves more like that of the smaller probe molecules L-rhamnose and d-mannitol.

Various reasons are put forward to explain the inordinate permeation of PEG-400. Degradation by intestinal bacteria could reduce the urinary excretion of ingested sugar markers relative to PEG-400, but recovery of lactulose is very similar to that of $^{51}$Cr-EDTA which resists bacterial degradation, with minimal fall-off during the first 5 h. Furthermore, as colonic permeation accounts for about 20% of the total absorption of ingested PEG-400 [27], resistance to degradation in the lower intestine is unlikely to explain more than a very minor part of the 120-fold difference between recovery of PEG-400 and lactulose in the urine. Unlike lactulose and $^{51}$Cr-EDTA, PEG-400 penetrates erythrocytes [17] and liposomes (which have no water pores) with considerable efficiency [34]: these polymers also show appreciable lipid solubility [13, 17, 35, 36] and are therefore likely to cross cell walls by partition through membrane lipid [6, 13, 17].

Even when due allowance has been made for the effect of molecular mass on diffusion through the unstirred aqueous media (according to Graham's Law [35]) intestinal permeation shows a marked increase in restriction as the size of the permanent molecule becomes greater. For sugar probe molecules there is a 40-fold decrease in permeation between $M_r$, 180 and 340, but for the ethyleneglycol polymers the 'cut off' appears to be displaced upwards, represented by the eightfold decrease coming between $M_r$, 320 and 500 (see Fig. 5). Ethyleneglycol polymers have elongated molecules that might be able to pass through aqueous channels which are unable to accommodate more compact molecules of the same mass. As there is no corresponding change in lipid partition, the position of this 'cut off' might be explained by assuming that a proportion of the polymers up to $M_r$, 370 were, due to molecular configuration, able to penetrate 'small
pore' aqueous channels otherwise available to compact molecules below $M_w$ 200.

The markers discussed therefore appear to penetrate the intestinal mucosa by three different routes, in agreement with the concept discussed by Erlij & Martinez-Palomo [18]. Although L-rhamnose must diffuse mainly through a 'small pore' route not available to larger polar molecules, the passage of lactulose and $^{51}$Cr-EDTA is restricted to larger aqueous 'channels' of much lower incidence which are susceptible to hyperosmolar stress and cetrimide. The route taken by PEG-400 is more controversial, but it seems likely to involve partition across the lipid bilayers of mucosal cell membranes in addition to both aqueous pathways.

For practical purposes there is good agreement between L-rhamnose and D-mannitol for assessment of the 'small pore', and between lactulose and $^{51}$Cr-EDTA for the 'large pore', aqueous pathways of intestinal permeation. The reproducibility of results and close agreement between lactulose and $^{51}$Cr-EDTA permeation suggest that errors due to bacterial degradation of sugar markers are of minor importance, at least in normal subjects and provided the urine collection period is short. Persistence of $^{51}$Cr-EDTA excretion beyond 10 h underlines the resistance of this marker to bacterial degradation in the lower intestine and its suitability for assessing permeability in colonized regions of the gut. The reliability of smaller ethyleneglycol polymers for test procedures involving measurement of urinary excretion must be affected by the magnitude of systemic loss, and it is evident that intestinal absorption of PEG-400 does not relate specifically to those pathways of unmediated mucosal diffusion demonstrated by the permeation of lactulose, $^{51}$Cr-EDTA or L-rhamnose and D-mannitol.

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