Correspondence

Molecular radii of probes used in studies of intestinal permeability

SIR,—Many comments have been made about the usefulness of findings from studies of intestinal permeability in health and disease. The relationship of the observed urinary excretion of orally administered probes to molecular dimension, charge, and permeation pathway also has evoked much discussion.[1 2] It has been suggested that, because of molecular size, smaller probes can cross the epithelial barrier *via* a transcellular pathway, whereas larger probes can only cross *via* an intercellular pathway.[2] L-rhamnose (MW 164 Da) and mannitol (MW 182 Da) belong to the former category and lactulose (MW 342 Da) and ^{51}Cr-EDTA.H_2O (MW 358 Da) to the latter. All of these molecules are hydrophilic, and uptake is by unmediated diffusion. The generally quoted molecular radii for these probes are: 0·4 nm[3]; 0·4 nm[2]; 0·5 nm[3]; and 0·63 nm[4], respectively. With the exception of the value given for the chromium-EDTA complex, effective hydrodynamic radii for uncharged molecules in dilute aqueous solution have been determined by viscometry through use of the Stokes-Einstein equation.[5] As the use of ^{51}Cr-EDTA in studies of intestinal permeability both in our unit and in others has increased demonstrably, we thought it necessary to take a closer look at the structure of this molecule and to compare it with other probes.

We have conducted theoretical studies using CHEMGRAPH (April 1985 Update, Chemical Design Ltd, Oxford, England) of the relationship between molecular structure and hydrodynamic radius and solvated volume for the four probe molecules mentioned. Measurements of the solvated volume and theoretical maximum hydrodynamic radius of these molecules have been made through computer simulation of the molecular structure as elucidated by x-ray crystallography.[6–9] The results are shown in the Table.

The radii shown above are taken from the centre of the longest axis with the assumption that gyrational motion about that point describes a sphere of maximum volume for the particular molecule. These theoretical maximum radii are larger than existing values and are consistent with current knowledge of molecular structure and solvation processes. We have not listed a radius for mannitol, a polyhydric alcohol, as this molecule, because of its structure, could easily assume a more globular form in aqueous solution. Passage across the epithelium would involve minimal energy as found in unmediated diffusion, and presentation of the probe to the epithelium would be in the manner of least resistance. Thus, the radius of mannitol would probably be about 0·4 nm, described along the short axis. The volume of each molecule is taken as the volume described by the van der Walls surface. These show rough equivalence between the lower molecular weight pair, L-rhamnose and mannitol, and the higher molecular weight pair, lactulose and Cr-EDTA.H_2O. From this, it would seem possible that the 'different inverse relationship' discussed by Hamilton *et al*[1] might be affected by factors other than molecular size, unless the suggested cutoff (0·23 nm^3) is very sharp indeed.

Table

	Short axis nm	*Long axis nm*	*Radius (maximum) nm*	*Volume nm^3*
L-rhamnose	0·85	0·98	0·49	0·139
D-mannitol	0·75	1·12	(0·38)*	0·154
Lactulose	0·87	1·24	0·62	0·262
Cr-EDTA.H_2O	1·30	1·36	0·68	0·253

*See discussion.

Clinical measurements of intestinal permeability have shown that similar profiles of urinary excretion are found with L-rhamnose and mannitol and with lactulose and ^{51}Cr-EDTA. The similarities in the respective excretion patterns are explained by the physicochemical nature of the probes used in the studies.

The question of whether measuring the urinary excretion of probes such as these will yield inferences about antigen uptake by the gut and its possible role in gastrointestinal inflammation is yet open. Nevertheless, the technique is valid. Investigations of intestinal permeation have shown that there is an inverse relationship between permeation and the size of hydrophilic molecules, with permeation decreasing as molecular size increases.[10 11] The probes mentioned are smaller than likely antigens – that is, MW>1000 Da, r>1 nm. It cannot be expected that the currently used probes are taken up by the same pathway as antigen. Useful information is still derived about changes in intestinal permeability, however, as one can expect to find these alterations in uptake occurring in parallel.

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Peptic ulcer and non-steroidal anti-inflammatory agents

SIR,—I was most interested in the recent case control study by Duggan *et al* (*Gut* 1986; **27:** 929–33) confirming an association of non-steroidal anti-inflammatory agents (NSAIAs) with gastric ulcer in Australia. I note that no less than 42% of the cases presented with haemorrhage, and wonder whether comparison of this group with the remainder would add anything to the conclusions. British studies have shown a significant association of NSAIAs with haemorrhage from gastric ulcer in the elderly[1–3] and also with ulcer perforations.[4] Although it might seem logical that if an agent causes ulcers to bleed and perforate it could also cause an ulcer in the first place, this problem is far from simple – especially as we do not know how gastric ulcers begin. If, for example, they can arise from gastric erosions, why are they usually single and why do they not follow mucosal biopsies?

If the authors found that NSAIA ingestion was related to symptomatic non-bleeding ulcers, this would be valuable information. Unfortunately it still would not solve the question of cause, as so many ulcers are symptomless and/or undiagnosed,[5 6] especially in the elderly[7] who are the chief users of these agents.

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Reply

SIR,—We thank Dr Montgomery for his comments about a possible difference between bleeding and non-bleeding ulcer. For bleeding ulcers there were nine pairs in which one member took NSAIAs regularly and the other none at all; seven were patients and two controls giving an odds ratio of 3·5. In the nine non-bleeding such pairs, eight were patients and one was a control (OR=8).

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