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Editorial Review

Intestinal permeability: functional assessment and significance

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PART I

INTRODUCTION

The intestinal epithelium demonstrates differential properties, providing both barrier and transport functions with respect to luminal molecules. Intestinal permeability relates to barrier function and, although it is the 'permeation' of molecular markers that is used to measure 'permeability', the terms are often confused. Permeability is that property of the intestinal epithelium or of a membrane which refers to the facility with which it allows molecules to pass through by non-mediated diffusion [1], whereas the term permeation describes the act of non-mediated diffusion itself. Non-mediated diffusion means the passage of molecules down a concentration (or pressure) gradient without the assistance of a passive or active biochemical carrier system.

Intestinal permeability in a clinical context mainly concerns the permeation of molecules with a molecular mass >150 Da, rather than ions such as sodium or chloride, to which the term membrane permeability is usually applied. In some respects the barrier function shows dynamic properties [2], as shown by the observation that altered permeability induced by hyperosmotic stress is reversible within a few hours [3]. Intestinal barrier function is also affected by disease, drugs, diet, cytokines, hormones or the environment, but does not appear to be much influenced by age, race or hereditary factors. The consequences of impaired barrier function for antigen access [4], which could initiate or perpetuate inflammation, and the potential for non-invasive assessment of disease or therapeutic manipulation, are reasons for the current interest in intestinal permeability.

This review is divided into two parts. The first part outlines the nature of the intestinal epithelial barrier and our still incomplete understanding of the pathways of permeation, as well as mechanisms by which permeability can be altered. The second part discusses methods available for measuring intestinal permeability *in vivo*, the clinical circumstances in which it is altered, and the relevance of clinical tests of permeability. First, a brief history of intestinal permeability is appropriate.

HISTORICAL PERSPECTIVE

The concept of using markers to follow the passage of substances through the intestinal wall is not new. Martin Lister in 1673 and William Musgrave in 1701 introduced milk mixed with indigo, which has a molecular mass of 262 Da, into the small intestine of living dogs, in an attempt to show that dyes pass from the intestinal lumen into the lacteals [5, 6]. Experiments on isolated dog intestine in the mid-19th century demonstrated that increased hydrostatic pressure and low luminal flow rates enhanced the appearance of indigo in the bathing solution [7, 8], although no account was taken of osmotic gradients. Waymouth Reid in Dundee was the first to recognize, in 1892, the importance of avoiding chemical, osmotic and hydrostatic gradients when investigating intestinal transport *in vitro*, by using identical isotonic bathing solutions of the same volume [9]. He used the back flux of 'sodic chloride' into the mucosal bathing solution, which occurs when the mucosa is damaged and permeability increased, as a measure of tissue viability [10].

The view that it was the cell membrane rather than the cytoplasm that regulated the transport of molecules through epithelia was proposed in 1884 by Heidenhain [11], who observed that the intestine of a dog could reabsorb large quantities of its own serum. In 1901, it was suggested that molybdate (molecular mass 180 Da) was absorbed by an intercellular route [12] and as early as 1912 Osterhout [13] wrote that "antagonistic substances affect certain life processes which control permeability" –

Key words: intestinal permeability, permeation.

Abbreviations: ^{99m}Tc-DTPA, ^{99m}Tc-diethylenetriaminopenta-acetate; NSAID, non-steroidal anti-inflammatory drugs; PEG, polyethylene glycol; TNF, tumour necrosis factor.

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in other words that permeability is a dynamic property of membranes. The absorption of test markers (rhamnose, xylose and arabinose) was first studied by McCance & Madders in 1930 [14] and in 1937 the absorption rate of a variety of substances in rats was observed to depend on molecular size as well as lipid solubility [15]. Absorption was restricted above a certain size (molecular mass 180 Da), corresponding to a molecular radius of about 0.4 nm [16]. Although it is now understood that molecular mass alone is an unreliable criterion of non-mediated diffusion through semi-permeable membranes [17], the passage of markers across the intestinal mucosa is nevertheless subject to the laws of simple diffusion [18]. This includes Graham's law, which states that molecular diffusion is inversely proportional to the square root of the molecular mass.

Human gut permeability was first investigated by Fordtran *et al.* [19]. Solutions rendered hyperosmotic by the addition of solutes of differing molecular size [20] were infused into the small intestine and the osmotic water flux was assessed by measuring the dilution of a non-absorbable reference marker, polyethylene glycol (PEG)-4000. Their findings were compatible with the hypothesis that the enterocyte apical membrane was interspersed with water-filled pores which permitted the permeation of non-lipid polar molecules. The average effective size of these membrane pores or channels, calculated from the reflection coefficients obtained, indicated a radius of about 0.8 nm in the jejunum and 0.3 nm in the distal ileum. The reflection coefficient of a solute is a measure of the resistance to permeation presented by a barrier relative to the permeation of a non-absorbable marker. The coefficient is inversely related to the permeability of the barrier (in this case, the intestinal epithelium), from 1.0 to a value of 0.0, which implies infinite permeability to the solute. This method, however, is not capable of differentiating between co-existing pore populations of different size. Fluid influx induced by an osmotic gradient was shown to be ninefold greater in the jejunum than in the ileum, suggesting that the jejunum had a greater porosity, which is the combined influence of available mucosal area, pore incidence and pore size. This difference between the permeability of the proximal and distal intestine has been confirmed using marker fluxes [21]. Water flow through a pore or channel is proportional to the fourth power of the radius, so a twofold difference in pore size could explain the large difference in net water flow observed between the jejunum and ileum.

Also during the 1960s, transport physiologists and electron microscopists defined the properties of epithelial barriers. Farquhar & Palade [22] described rings of adhesion that connect adjacent epithelial cells, which they called zonula occludens or tight junctions. Ussing & Windhager [23], working with microelectrodes on frog skin, then recognized the importance of the paracellular pathway between epithelial cells as a route of trans-epithelial ion transfer, postulating that intercellular junctions were not necessarily occlusive. Subsequently, Fromter & Diamond [24] introduced the terms 'tight' and 'leaky' for epithelia demonstrating different permeabilities, indicated by the electrical resistance of the paracellular pathway. Leaky epithelia, such as the human small intestine, are those with a total tissue resistance <1000 ohm/cm^2, or those with a paracellular resistance $<50\%$ of the total resistance. Tight or moderately tight epithelia, such as the distal colon, were much less permeable to polar, lipid-insoluble molecules and were characterized by a higher potential difference across their surfaces.

The basis of non-invasive tests that could be used for the clinical assessment of intestinal permeability came from observations in patients with coeliac disease, initially suspected of having increased intestinal permeability because of disaccharidiuria [25, 26]. At the same time there was growing interest in the mode of intestinal uptake of potentially toxic protein antigens [27]. Early in the 1970s, it was discovered that ingestion of hyperosmotic solutions could temporarily increase intestinal permeability in normal subjects to oligosaccharides such as lactulose and raffinose and to even larger molecules such as dextran (molecular mass 3000 Da) [3, 28–30]. This emphasized the practical importance of controlling the osmolarity of test solutions. An early attempt to assess intestinal permeability by measuring recovery of ingested lactulose (a non-metabolizable disaccharide, molecular mass 342 Da) in urine failed to demonstrate any difference between coeliac patients and control subjects [31], because undiluted lactulose was administered (J. Walker-Smith, personal communication; D. H. Shmerling, personal communication). Using iso-osmolar solutions of lactulose, the urinary excretion of lactulose was subsequently found to be increased [28, 32, 33]. Fluorescein-labelled dextran, which had been introduced for assessing the permeability of biological membranes in 1972 [34], was employed in combination with lactulose, raffinose and stachyose (molecular mass 342, 504 and 666 Da, respectively) for non-invasive assessment of human intestinal pore profiles [30]. Further differential tests of permeability employing disaccharide/monosaccharide combinations, such as cellobiose/mannitol [35] and lactulose/L-rhamnose [36], were introduced during the late 1970s. Low-molecular-mass PEGs [37] and ^{51}Cr-EDTA [38] were introduced in the late 1970s and early 1980s. Unlike carbohydrates, both these probes resist degradation by bacteria and are therefore theoretically suitable for use in those parts of the intestine that support an active bacterial flora.

Many clinical circumstances associated with abnormal measurements of intestinal permeability were then defined, especially diseases and drugs [39]. Several fields have now evolved. Some relate to clinical questions, such as whether increased permeability is an aetiological factor or simply a consequence of intestinal disease [40], or the value of permeability tests for assessing patients with suspected intestinal disease [41]. Other areas are the evaluation of test markers in relation to intestinal pathways of permeation [42] and mechanisms by which epithelial permeability may become altered [43], which concern interpretation of changes in measured permeation.

NATURE OF THE INTESTINAL BARRIER

There are several good reviews on general aspects [43, 44] and specific components of the intestinal barrier, including tight junctions [2, 45], the unstirred layer [46], transcytosis of larger molecules [47] and plasma clearance of molecules [48]. The description below concentrates on factors that should be considered when interpreting marker permeation in a clinical context.

Permeation pathways

Two routes are available for permeation across the healthy intestinal epithelium: either across cells (transcellular) or between cells (paracellular) (Fig. 1). Unfortunately, we are still by no means certain which route forms the principal pathway for specific marker probes. Current concepts suggest that the intestinal epithelium consists of a heteroporous barrier perforated by a large population of small pores (0.4–0.7 nm radius) and by a small population of large pores (6.5 nm radius). This is derived from observations that the permeation of small molecules (radius <0.4 nm), whilst still restricted, is 20–30-fold greater than that of larger molecules (radius 0.4–0.8 nm) [1], and from the original work by Pappenheimer *et al.* [49], who developed the theory of passive transport through membranes penetrated by cylindrical pores or parallel-walled slits. Consistent with this hypothesis are small (0.4 nm) electroneutral pores, probably located in the apical membrane, that have been described in rabbit ileum [50]. Using probe molecules of selected sizes and osmotic gradients applied to the mucosal or serosal surfaces, both large (6.5 nm) electroneutral and small (0.7 nm) cation-selective pores were also identified, probably located between cells (Fig. 1). If this hypothesis is correct, large molecules will be restricted to large pores, but small molecules are likely to pass through both large and small pores. Large pores may contribute little to the total permeation of small molecular solutes in normal intestine, owing to their relative infrequency. Several physical factors relating to fluid dynamics need to be mentioned, however, which are well described in relation to permeation through capillary endothelium [51]. Diffusion of a solute in aqueous solution is proportional to the square root of the molecular mass (Graham's law), but in a membrane with pores of dimensions comparable with those of the diffusing molecules, diffusion would also be restricted as a function of the ratio of the molecular radius to the radius of the pore (Faxén–Ferry theory). The con-

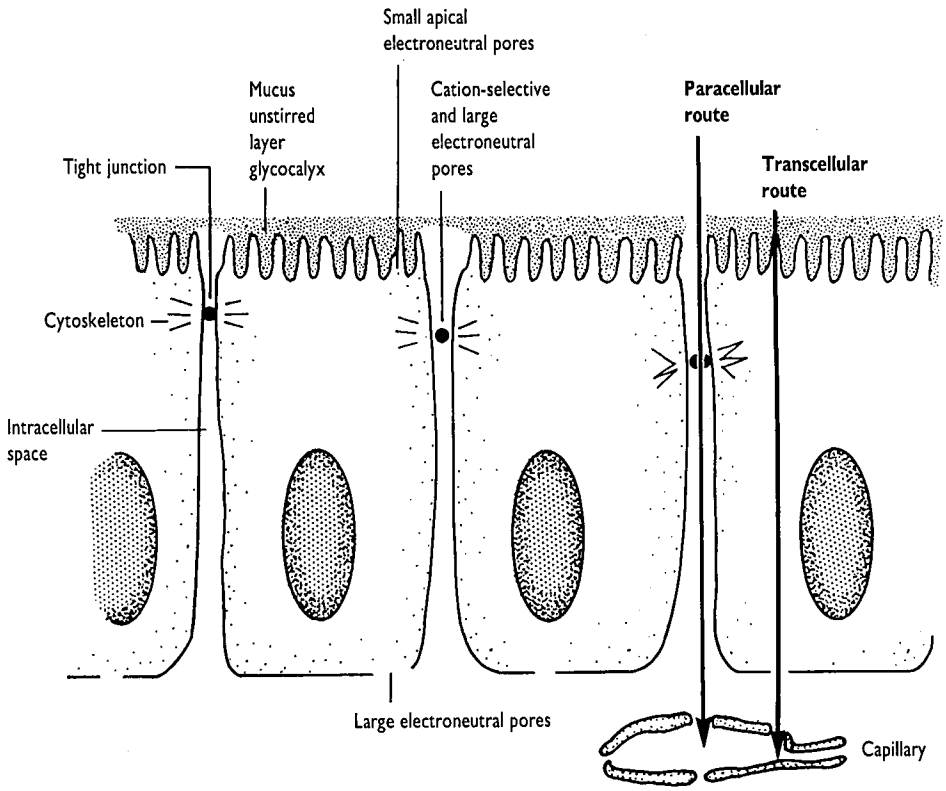

Fig. 1. Intestinal epithelial barrier: routes of non-mediated permeation. Larger molecules, including lactulose, cellobiose, raffinose and ^{51}Cr-EDTA, are thought to pass through the paracellular pathway, whereas smaller molecules (mannitol, L-rhamnose) mainly permeate through the transcellular pathway.

centration gradient for a solute has an influence on permeation across a membrane proportional to the square of the pore radius, but for molecules such as water, which have a radius very much smaller than the pore radius (R), permeation is proportional to R^4. These considerations relate to the diffusion of spherical molecules through membranes with cylindrical pores of uniform size, so the influence of fluid dynamics on marker permeation through the intestinal epithelium is likely to be much more complex.

Paracellular pathway

The two components of the paracellular pathway are the 'tight junction' and the underlying intracellular space, but the potential diffusion of molecules through gaps in the epithelium caused by exfoliation of dead cells or ulceration is also conveniently considered under this heading. There are several reasons why the paracellular pathway is thought to be the route of permeation for molecules larger than monosaccharides (molecular mass > 180 Da), such as lactulose, cellobiose, EDTA, raffinose or dextran. These molecules adopt a strictly extracellular distribution after intravenous injection and are therefore presumed to be unable to pass through cell membranes. Intercellular junctions represent a natural interruption in membrane continuity and thus present a potential pathway for such molecules. Furthermore, junctions constitute only a small proportion (< 5%) of the total surface area of the intestinal epithelium [52], which is compatible with the location of a small population of large pores. An additional population of small cation-selective pores within the junction [50], however, might act as an additional barrier to molecules that carry a small negative charge, such as EDTA. The situation with regard to enterocytes is not proven, because there is as yet no direct evidence that oligosaccharides or EDTA pass through tight junctions.

Tight junctions. The tight junction encircles epithelial cells at the apical pole, being a narrow belt that both connects adjacent cells and maintains cell polarity by separating apical from basolateral membranes [45]. The gap between the epithelial cells at this point is of the order of 20 nm, and is traversed by strands that can be visualized by freeze–fracture electron microscopy. The number of these strands correlates with the tightness of the junction when the surface area of the epithelium is taken into account [49, 53]. Two proteins (cingulin and ZO-1) that constitute these strands have so far been identified [54, 55], but, more significantly, the strands are linked to the cytoskeleton [56], which suggests that tight junctions, far from being filled with 'cellular superglue', actually represent a gating mechanism in the paracellular pathway. Evidence for this includes observations that intracellular mediators both increase (cyclic AMP or Ca^{2+}) [57, 58] and decrease (protein kinase C activation by phorbol esters) [59] tight junction resistance. Cytochalasin D, which affects microfilaments, alters tight-junction structure, charge selectivity and resistance in intestinal absorptive cells [60]. Glucose absorption by enterocytes has also been reported to alter tight-junction structure [61], but the significance of this observation depends on the concentration of glucose that normally occurs in the intestinal lumen after a meal. This may be only 25 mmol/l [62] and much lower than the 50–500 mmol/l previously reported [63]. Tight-junction permeability differs between goblet and villus columnar cell types, and junctions between cells in the crypts appear to be more permeable than those in the villi [64]. This is at odds with the lower permeability seen in the human colon, where crypts predominate, compared with the small intestine [21], and in sheep colon which has a lower permeability and higher crypt density than bovine colon [65].

Intercellular space. The intercellular spaces are sufficiently narrow and tortuous that they may restrict solute diffusion of the larger solutes, but the size of the spaces depends in part on the rate of fluid transport [66]. Although a small osmotic gradient has been proposed to account for paracellular water and solute absorption, which may influence permeation of mannitol [67], direct measurement of the osmolality of the fluid transported into the sub-epithelial layer of the intestine has not been possible. It is too simplistic to imagine a standing osmotic gradient in the intercellular space and the electrical resistance of the paracellular pathway may be varied *in vitro* by changing the ionic composition of the bathing fluid [68]. Because it has also not been possible to estimate the magnitude of transjunctional as opposed to transcellular water flow [69], it is difficult to know how much the intercellular space contributes to the flux barrier for small solutes. Sub-mucosal tissue pressure may influence intercellular diffusion [50], but hydrostatic pressure gradients probably play a minor role in the context of normal bowel physiology.

'Extrusion zones'. It remains possible that large molecules permeate between cells through gaps left by the extrusion of dead cells or 'extrusion zones' [70]. Normal exfoliation of effete cells from the intestine, however, appears to occur without disrupting the barrier, because adjacent cells 'zip-up' from base to apex as the cell is extruded [71]. The effect of exfoliation in disease is not known, but it seems very likely that mucosal erosions or ulcers increase the permeation of molecules because the major permeability barrier of multi-layered epithelia appears to be the first living cell layer [72]. Mucosal ulceration, however, would also decrease the absorptive area and thus modify the permeation of molecules independently of changes in mucosal permeability, especially in the small intestine where ulceration would demolish the villi. Epithelial integrity can be restored within 30–60 min by lateral migration of cells [73, 74] after a breach in the mucosa, so the importance of these mechanisms remains to be established.

Transcellular pathway

Monosaccharides such as L-rhamnose and the smaller PEGs, which are capable of entering erythrocytes, are thought to diffuse through enterocyte cell membranes [1].

The unstirred fluid layer adjacent to the apical membrane and mucin glycoproteins should also contribute to this part of the intestinal barrier.

Cell membranes. The existence of membrane pores was suspected from the reasonable ease with which small, non-lipid solutes permeate through lipid membranes that constitute about 95% of the surface area of the intestine. The work by Naftalin & Tripathi [50] on rabbit ileum that has suggested the presence of small (0.4 nm radius) electroneutral pores in the apical membrane and also large (6.5 nm radius) pores in the basolateral membrane supports this idea, but no similar data are available from human intestine for comparison. The nature of these pores is almost entirely unknown, but it would be misleading to imagine membranes with holes of fixed size, like a collander. Membrane pores could represent water-filled channels, but it is possible that proteins are inserted into the membrane, analogous to water channels [75]. Whether such pores are non-specific conduits, possess a gating mechanism similar to sodium or chloride channels, or even whether specific ion channels double up as transcellular pathways for the diffusion of small molecules, is unknown. The potentially dynamic nature of membrane pores is illustrated by the effect of changes in membrane phospholipid composition on the permeation of small molecules [76], such as occur in ulcerative colitis [77].

Apart from pores, there are at least three other mechanisms by which molecules can cross cell membranes. First, molecules with a degree of lipid solubility may diffuse directly through the membrane. This has been proposed to account for the unexpectedly high permeation of PEGs, a controversy that is discussed in the second part of this review. Secondly, membrane wounds caused by mechanical forces within the gastrointestinal tract, or abrasion by food and faecal particles, can allow water-soluble macromolecules, such as horseradish peroxidase, to enter epithelial cells, but not to penetrate the basolateral membrane [78]. It appears remarkable that a cell with a membrane defect of sufficient severity to allow inward diffusion of macromolecules (and thus presumably a massive loss of ions and regulatory proteins) is yet able to recover [78]. It is, however, plausible that molecules of a size small enough to exit through the 6.5 nm radius pores proposed to exist in the basolateral membrane [50] could enter the cell by this mechanism. Thirdly, transcellular transport of large molecules, including protein antigens, appears to occur by endocytosis into vesicles and subsequent exocytosis. This process of transcytosis may be very rapid and has been reviewed elsewhere [47].

Unstirred fluid layer and mucin glycoproteins. Overlying the intestinal brush-border membrane, within the glycocalyx, is a layer of relatively stationary fluid, known as the unstirred layer [79]. The presence of such a layer, which according to previous estimates was thought to be about 600 μm in depth [80], would present such a major barrier to diffusion that even the presence of an efficient active transport system would contribute little improvement to absorption. It now appears from work with dogs that the maximum thickness of the layer is only 40 μm [46], consistent with a cell depth (and corresponding lateral intercellular space width) of about 30 μm. This reduction is achieved by highly efficient stirring, possibly by contraction of villi and microvilli, and means that alteration in either luminal stirring or epithelial function could influence the absorption of rapidly absorbed compounds. It also implies that the unstirred layer still constitutes an appreciable barrier to compounds that permeate by non-mediated diffusion. In this context it should be remembered that hydrophilic molecules permeating through the unstirred aqueous layer are subject to Graham's law of diffusion [30, 42]. Intestinal mucus glycoproteins which are altered in ulcerative colitis or cancer [81], are also likely to contribute to the intestinal permeability barrier by affecting the viscosity of the aqueous layer [82], but the effect of sialo- or sulphomucins on permeation is unknown. It is also possible that the glycocalyx itself may be dense enough to restrict solute diffusion, as has been proposed in the fibre-matrix theory for capillary endothelium [83], but this probably only applies to molecules larger than the disaccharide probes or EDTA that are used for measuring intestinal permeability *in vivo*.

Common pathways

Passage of molecules through the basement membrane, extracellular matrix and across the capillary or lymphatic endothelium is common to both transcellular and paracellular pathways. Together these appear to present little impediment to diffusion of molecules below the size of proteins [84]. Direct study of the contribution of the intestinal basement membrane has not been possible, but it appears to contain oval gaps 0.5–5.0 μm in diameter, which makes it unlikely to act as a sieve for small solutes [44]. The interstitial volume in disease may be markedly increased by oedema, thus increasing the distance that molecules have to diffuse before entering the circulation, but this is not a problem for molecules the size of ^{51}Cr-EDTA or smaller, which rapidly equilibrate within the interstitial space [85]. Variable clearance of water-soluble molecules by the intestinal microcirculation might theoretically alter measured permeation, but EDTA clearance in the rat intestine is not affected by a 25% reduction or a 200% increase in splanchnic blood flow compared with controls [86]. However, patients with severe falciparum malaria appear to have markedly impaired absorption of sugars associated with reduction in splanchnic blood flow [87].

MECHANISMS OF ALTERED PERMEABILITY IN DISEASE

The central role of tight junctions in regulating paracellular permeability [2] has already been discussed. Intestinal disease is likely to affect the permeability not only of the tight junctions, through the actions of chemical mediators or inflammatory cells, but of the cellular membranes and the total intestinal absorptive area as well.

Drugs used for treatment may introduce an additional (iatrogenic) factor that affects mucosal permeability.

Inflammatory mediators

A variety of isolated observations has shown that mediators of inflammation can alter intestinal as well as capillary permeability, but it is not clear how these are integrated or regulated. Cytokines are substances released by lymphocytes or macrophages that influence the function of other cells. They include γ-interferon, interleukins and tumour necrosis factor (TNF), which appear to promote increased permeability in inflamed tissue by altering tight-junction resistance; however, epithelial and endothelial cells react differently. γ-Interferon decreases transepithelial resistance and increases mannitol flux in cultured intestinal epithelial (T84) monolayers, but neither TNF, interleukin-1, nor interleukin-2 affect the permeability of these cells [88]. On the other hand, TNF reversibly increases mannitol permeation within 60 min in a renal epithelial cell line [89] and in endothelial cells [90]. Mannitol appears to act as a marker of paracellular permeation *in vitro* [88]. Platelet-activating factor causes macroscopic gastrointestinal damage when infused into rats [91] and increases blood to lumen permeation of ^{125}I-albumin and ^{51}Cr-EDTA, possibly by stimulating the release of free oxygen radicals from adherent neutrophils [92], which increase paracellular permeability in cultured epithelial cells [93]. There is also evidence that platelet-activating factor induces a greater increase in permeability in rabbit distal colon compared with caecum *in vitro* [94]. Other mediators are likely to influence permeability, including nitric oxide [95], and much work has yet to be done on how inflammatory mediators influence epithelial cells, especially in relation to inflammatory bowel disease [96].

Inflammatory cells

A chemotactic peptide released by bacteria, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine, has been shown to induce migration of human neutrophils between cultured human intestinal epithelial cells in monolayer culture, with an associated increase in permeation of mannitol and horseradish peroxidase [97]. This led to the suggestion that increased permeability induced by peptides released from luminal bacteria contributes to the pathogenesis of Crohn's disease [4] and it is of interest that *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine perfusion increased the uptake of ^{51}Cr-EDTA only in rat terminal ileum [98]. The increase in permeability after neutrophil transmigration has been reported to return to normal within 18 h and appears to be due to a specific effect on tight junctions, independent of free oxygen radicals [99]. The response varies according to the type of epithelium: for instance, neutrophil transmigration induced by leukotriene B_4 appears to be more rapid across cultured renal than intestinal epithelial cell monolayers [100]. Effects produced by mononuclear inflammatory cells on intestinal permeability, other than those mediated by cytokine production, are at present unknown. In view of the influence of inflammatory cells and their products on permeability, any study that relates intestinal permeability to inflammatory bowel disease should provide some estimate of the density of the inflammatory infiltrate.

Cell membranes

The total small-intestinal surface area is decreased in villous atrophy and this is no doubt the reason for reduced permeation of small molecules, such as L-rhamnose [36] and mannitol [35], in coeliac disease. Transcellular permeability to water, ions and presumably other small solutes may be altered by changes in membrane fluidity [76, 101], which may be related to altered phospholipid composition observed in mucosal biopsies from patients with ulcerative colitis [77, 102]. The situation becomes complex in conditions such as severe villous atrophy or Crohn's disease that are associated with reduction in absorptive area (which will decrease total permeation of any marker), disruption of membrane integrity (which is likely to increase permeation, especially of large molecules), and alterations in membrane lipid composition as well. Evidently several mechanisms operate simultaneously to alter permeability in intestinal disease.

SUMMARY

While permeability is a property of semi-permeable membranes, permeation may be regarded as the act of non-mediated diffusion that can be used, with respect to specified test molecules, to measure permeability. In the intestine it is the epithelial cell layer that constitutes the principal barrier to permeation, and through which molecules pass either by the paracellular or transcellular route. Changes in the paracellular pathway, probably mediated through cytoskeletal links, appear to be a major factor which determines altered permeability to larger molecules. Cytokines, inflammatory cells, drugs or physical factors, such as osmotic stress, may influence tight junctions. Alterations in membrane composition in intestinal disease may also contribute to changes in intestinal permeability. Other factors, including differences in mucosal area, the thickness of the unstirred layer or intestinal transit, may alter the permeation of a marker independently of changes in epithelial permeability.

The next part of this review concerns methods for measuring intestinal permeability *in vivo*, clinical factors that alter permeation and permeability, and the relevance of the available tests.

PART 2

INTRODUCTION

The first part of this review presented the historical background to measurements of intestinal permeability and examined the nature of the intestinal epithelial

barrier, including mechanisms through which this may be altered in disease. In the second part, methods of measuring intestinal permeability are described, as well as the influence of physical factors, disease and drugs on these measurements, and the clinical relevance of the tests.

METHODS OF MEASURING INTESTINAL PERMEABILITY

It is important to realize that the permeation of molecules across the intestine is determined not only by the permeability of its epithelial surface, but also by its area, the rate of passage down the intestine (time of exposure) and the concentration gradient of the molecules across the surface [103, 104]. Although the two last-named factors can affect permeation, they are non-mucosal and should not be confused with permeability, which is a condition of the membrane itself.

At the present stage tests used for the measurement of permeability in clinical practice can be conveniently divided into three groups: those that employ (1) sugars, (2) isotopes and (3) PEGs as test markers. Selection of an appropriate test system should involve a knowledge of the properties of permeation markers available and of ways in which error due to factors other than mucosal permeability on the test result can be avoided or minimized.

Marker properties

Ideal markers of intestinal permeability should be biochemically inert and cross the intestinal epithelium by non-mediated diffusion through defined pathways. Lipid-soluble molecules, such as nicotine, alcohols and quite a number of drugs, are capable of non-mediated permeation through membranes, irrespective of the presence of aqueous pores. Whilst permeation through lipid membranes might be interesting to assess [105], if permeability with respect to aqueous pores is to be measured, then polar, lipid-insoluble probes must be selected. The probe must fit the pathway. Intestinal permeation is usually measured by renal excretion after oral ingestion, in which circumstance it is necessary to demonstrate that the test substance is qualitatively recoverable in urine after intravenous administration and can be reliably measured by a convenient technique.

No test marker yet introduced fulfils all these criteria and the permeation of all test substances will be affected by individual variation in gastric emptying, intestinal transit and dilution by secretions, while altered renal clearance and incomplete collection of urine are inherent sources of error in all single-marker tests based upon recovery in urine. Measurement in urine is used for convenience: probe concentrations are approximately 100 times higher than they are in plasma, which makes analysis easier and more reliable. Suitable test probes should be fully excreted by the kidney without significant tubular reabsorption. Even though impaired renal function will alter excretion of a marker probe, this can be overcome by using two marker probes subject to the same influence (see later).

Intestinal permeability calculated from observed permeation rates of different probes (Table 1) has been related by different authors to either the hydrodynamic

Table 1. Permeation and recovery of different marker probes in normal subjects. Permeation values are means of 5 h urine collections cited in the references. References with an asterisk indicate that these authors employed a 6 h urine collection, for which allowance has been made.

Probe	Molecular mass (Da)	Permeation (% excretion of oral dose/5 h)		Recovery* (% excretion after i.v. dose)	References
		Iso-osmolar (200–300 mosmol/kg)	Hyperosmolar (1350–1500 mosmol/kg)		
L-Arabinose†	150	17.5	—	73	127, I.S. Menzies, unpublished work
L-Rhamnose	164	10.1	11.7	72	36, 42, 107, 125, 178*, 124
D-Mannitol	182	16.8	20.6	79	106, 110–12, 118*, 168, 178
Lactulose	342	0.25	0.41	97	28, 30, 33, 42, 111, 112, 108, 118*, 125, 178*
Cellobiose	342	—	0.38	92	35, 41, 109, 110, 168
^{51}Cr-EDTA	359‡	0.64 1.64 (24 h)	0.70 1.44 (24 h)	96	38*, 42, 118*, 128, 133
Raffinose	504	0.26	—	97	28, 31, 127
PEG-400	194–502	18.2	20.3	41	37, 42, 111*, 142
^{99m}Tc-DTPA	549	2.8	—	—	138, 139
Dextran	3000	0.04	0.12	96	30

*Mean of 12 h urine collections in humans cited in the references.

†May be subject to some mediated transport.

‡Molecular mass of radioligand.

radius or diameter [17] or the volume [106] of the test molecules employed. Most reports have used viscometry to calculate the hydrodynamic (Stokes') molecular radius [16], including those that have calculated the equivalent pore radii of enterocytes [19, 50]. Computerized modelling [17], which takes into account molecular composition, mass, geometry, optical conformation and Van der Waals radii, suggests that most test molecules may have a smaller effective size than is generally thought. Whatever the conformation might be, diffusion of different sized molecules across a permeable membrane must also reflect physical constraints. These include Graham's law, whereby diffusion is inversely proportional to the square root of the molecular mass [18, 30, 42], also the influence of concentration gradient and membrane area, in addition to the incidence and size (diameter) of available pores ('membrane porosity'). Unfortunately, there are as yet no published data describing the calibration of molecular size by rate of diffusion through semi-permeable reference membranes of known pore size. This would seem an appropriate and direct method for expressing effective molecular dimensions of probes intended for the measurement of membrane permeability.

Differential permeability tests

Individual variation due to non-mucosal factors can be circumvented by using two markers that are affected equally by the whole range of factors, except the route of intestinal permeation (Fig. 2). Combining a larger molecule, such as lactulose, cellobiose or EDTA, with a smaller molecule, such as L-rhamnose or mannitol (Table 2), allows a permeation ratio to be calculated (of large/small molecules), which demonstrates greater clinical discrimination than the behaviour of either marker alone [1, 28].

Disaccharide/monosaccharide tests

There is little practical difference between any of the dual-sugar (disaccharide/monosaccharide) differential permeability tests, although the tests and sources of error differ in detail. Some have been extended by combination with other sugars to provide simultaneous assessment of carbohydrate absorption or intestinal disaccharidase activity [107, 108].

Cellobiose/mannitol and lactulose/mannitol. The cellobiose/mannitol [35, 41, 109, 110] and lactulose/mannitol [111–113] combinations are the best-documented dual-sugar tests, but there are potential sources of error arising from the metabolism and analysis of the sugars. Although human disaccharidases have no action

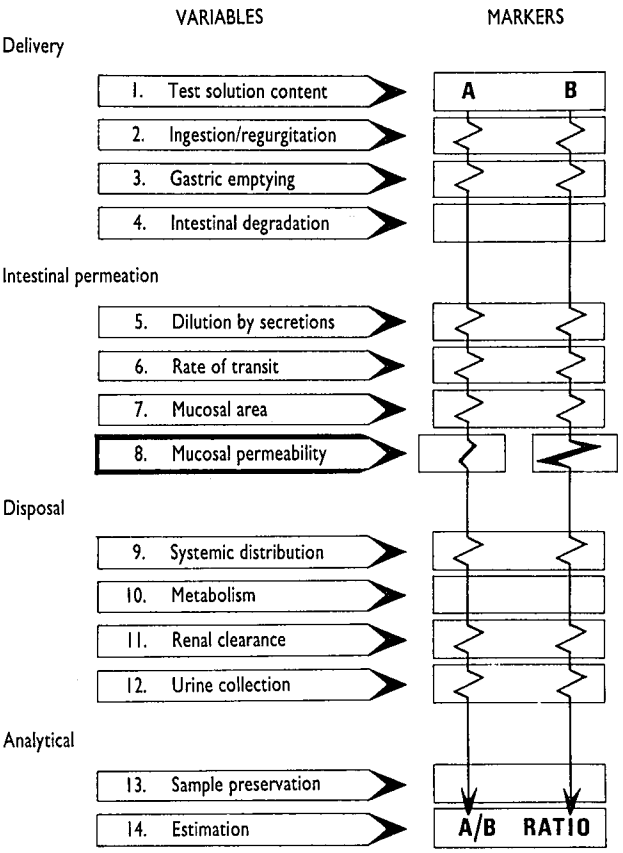

Fig. 2. Principle of differential tests of permeability. The diagram illustrates the many factors that influence the urinary concentration of marker probes. Mucosal permeability is the only point of difference between the handling of two marker probes (A and B). The other influences cancel out when excretion of the markers is expressed as a ratio.

Table 2. Urinary excretion ratios of some differential permeability tests in normal subjects. Ratios are given as the percentage of ingested dose excreted in 5 h, except for ^{51}Cr-EDTA/mannitol (6 h).

Marker probes	Ratio (iso-osmolar) (200–300 mosmol/kg)		Ratio (hyperosmolar) (1350–1500 mosmol/kg)		References
	Mean ± SEM	Range	Mean ± SEM	Range	
Lactulose/L-rhamnose	0.027 ± –	0.008–0.052	0.036 ± —	0.022–0.068	36, 108
Lactulose/mannitol			0.016 ± 0.002	—	112
Cellobiose/L-rhamnose			0.029 ± 0.004	—	124
Cellobiose/mannitol			0.014 ± 0.002	0.004–0.028	41, 112
^{51}Cr-EDTA/L-rhamnose	0.037 ± 0.003	0.022–0.66	—	—	196
^{51}Cr-EDTA/mannitol			0.034 ± 0.005	—	118, 137

on lactulose, cellobiose is hydrolysed by intestinal lactase [114]. However, the affinity of lactase for cellobiose is probably too low to interfere with the use of cellobiose in clinical tests of mucosal integrity.

Particular confusion exists about the route of mannitol permeation. *In vivo*, its behaviour suggests transcellular permeation, with a relatively high recovery in urine after oral administration (Table 1), which declines in the presence of villous atrophy [35, 41]. *In vitro*, mannitol is widely used as a marker of paracellular permeability [88, 115, 116]. It probably permeates by both routes [117]. Up to 15% is degraded after absorption in rats or dogs, probably by hepatic sorbitol dehydrogenase [67], which suggests that mannitol can penetrate some cell membranes. It also means that recovery in urine after intravenous administration to human subjects is more likely to be in the region of 70% [106] than approaching 100% as has also been claimed [118]. A variable natural excretion in human urine may further complicate interpretation of mannitol excretion in clinical tests [119].

A further problem is that published data for both tests relate mainly to the use of hyperosmolar stress (test solution 1500 mosmol/l), which improves discrimination by increasing the permeability of abnormal epithelia to disaccharide [3, 29]. The use of such hyperosmolar stress calls for careful control of fluid intake during the early stages of the test and makes interpretation more difficult (see below).

Despite complications related to baseline excretion of mannitol, cellobiose hydrolysis in human intestine and the control and interpretation of hyperosmolar stress, the cellobiose/mannitol or lactulose/mannitol differential permeability tests provide useful clinical discrimination for detection of small-intestinal pathology.

Lactulose/L-rhamnose and cellobiose/L-rhamnose. L-Rhamnose absorption is decreased in coeliac disease, whereas that of lactulose is increased, which leads to an increase in the lactulose/L-rhamnose excretion ratio that provided complete discrimination between 13 patients with untreated coeliac disease and healthy control subjects [36]. Lactulose appears to be a reliable marker of paracellular permeability, but whilst urinary recovery of lactulose approaches 100% after intravenous injection, recovery of L-rhamnose is about 75% [42, 118]. The fate of the missing fraction of L-rhamnose is unknown, but does not appear to vary widely between subjects. The route of L-rhamnose permeation, like mannitol, remains uncertain, but is presumed to be transcellular *in vivo*. Interestingly, L-rhamnose, but not mannitol, enters erythrocytes [1], implying the ability to cross certain cell membranes. L-Rhamnose was also transported five times more effectively than mannitol across a 1000 Da membrane in aqueous solution and diffused more readily than mannitol across an artificial lipid barrier [120].

The lactulose/L-rhamnose test was first administered in hyperosmolar solution [30], but is now employed most commonly in iso-osmolar form [121–123]. The lactulose/L-rhamnose test is a good test for measuring permeability in intestinal disease, but unfortunately there are no published data to compare with the cellobiose/mannitol test for unselected patients [41]. Although not widely employed, the cellobiose/L-rhamnose test has been used to demonstrate that intestinal permeability in adults varies little with age [124].

Multiple-sugar tests

Assessment of permeability together with intestinal disaccharidase activity or mediated transport are important extensions of the differential marker test principle. Combination of the lactulose/L-rhamnose test with lactose, sucrose and palatinose in an iso-osmolar test solution permits simultaneous assessment of lactase, sucrase and isomaltase activity, as well as permeability [107]. This allows distinction of primary from secondary disaccharidase deficiency [28, 107, 108]. Non-mediated permeation and mediated transport of carbohydrates can be measured simultaneously by combining lactulose and L-rhamnose with D-xylose and 3-*O*-methyl-D-glucose [125], thus providing another measure of intestinal function and much better discrimination than a single-marker test such as the D-xylose test in coeliac disease [126]. Raffinose, L-arabinose and lactose have also been combined to evaluate hypolactasia and intestinal permeability simultaneously. The test described was iso-osmotic and urinary sugars were measured by enzymic assay [127]. L-Arabinose, however, appears to have an affinity for mediated intestinal transport, because absorption from a perfused segment of jejunum was fourfold greater than for L-rhamnose or mannitol (J. Bull & I. S. Menzies, unpublished work). If this is true, then L-arabinose is inappropriate for assessing permeability (non-mediated permeation).

Isotope tests

Isotopic probes are in general more easily measured than other markers of permeability, but suffer from the inherent disadvantages of single-marker tests and the use of radioactivity. The latter makes them less attractive for routine or serial tests, especially in children.

^{51}Cr-EDTA. Previously employed for the measurement of renal clearance, this probe has now become established for the estimation of intestinal permeability [38, 42, 128, 129]. Unlike the sugar probes, it resists bacterial degradation and can therefore be used to assess colonic permeability. For clinical use it has been given orally in combination with lactulose, which provides a correction for the contribution from the small intestine [130]. Although a 24 h urine collection was initially employed [38, 128], a 5 h collection [42] is more appropriate for measuring small-intestinal permeability. The main disadvantages are the rather wide variation in published control values for urinary excretion after oral administration [131–133] and the relatively short half-life of ^{51}Cr (27 days), which limits the shelf-life and the length of time that samples can be kept before analysis. This is inconvenient for tests that are performed infrequently or at distant field sites. ^{51}Cr-EDTA may also be less reliable than dual-sugar tests for detecting coeliac disease [134],

but this is likely to be a disadvantage shared by all single-marker tests. Combination of ^{51}Cr-EDTA with L-rhamnose [135] and [^{14}C]mannitol [136, 137] has been tried in an attempt to improve discrimination, but such ratios tend to be distorted by an unbalanced contribution of ^{51}Cr-EDTA absorption from the colon, which is not completely eliminated by decreasing the urine collection period from 24 to 5 h.

^{99m}Tc-Diethylenetriaminopenta-acetate (^{99m}Tc-DTPA). This marker (disodium complex; molecular mass 549 Da) has been advocated for oral assessment of colonic permeability, since excretion is increased in ulcerative colitis [138]. Pharmacokinetic data is limited. The half-life of 6 h creates considerable problems for presentation and analysis, and ^{99m}Tc-DTPA is also liable to disadvantages common to any single-marker test of intestinal permeability (Fig. 2). Attempts have been made to relate excretion to activity of ulcerative colitis [138], but it is not at all clear that it offers any advantage over ^{51}Cr-EDTA as a measure of intestinal permeability [139], or more widely accepted criteria of disease activity [140].

PEG tests

Range of PEGs. Many different sized PEGs have been used to assess intestinal permeability: PEG-400 [37, 111, 141, 142], PEG-600 [143, 144], PEG-900 [145], PEG-1000 [146, 147] and PEG-4000 [148, 149], which was originally used as a non-absorbable reference marker for intestinal perfusion procedures by Fordtran in 1965 [19]. Commercial formulations consist of polymers of different size: PEG-400, for instance, is composed of some eight different sized molecules ranging from 194 to 502 Da [42], each of which can be separated by gas–liquid or high-pressure liquid chromatography [150]. The apparent advantage of the wide range of molecular size is, however, offset by substantial variation in the proportion of each individual polymer excreted after oral administration [151, 152]. There is also considerable disparity of excretion ranges after intravenous administration [42]. Opinion differs as to whether PEG-400 excretion is increased [37, 142] or decreased [111, 141] in small-intestinal disease and controversy about the route of PEG permeation still persists [151].

Route of permeation. The finding that PEGs of around molecular mass 340 Da permeate the human intestine about 100-fold more efficiently than either lactulose or ^{51}Cr-EDTA [42], which have a similar molecular mass, has lead to some confusion concerning the permeation pathway(s) involved. One explanation has been that PEGs have a linear molecular configuration of narrow diameter, which, when aligned with a pore in a membrane or junction, allows passage through smaller pores than might otherwise be possible [17, 151]. Whilst this may be valid, it seems likely that there is also a degree of lipid solubility which allows PEGs to permeate through cell membrane lipid by a non-aqueous route. PEGs are very water soluble, but several investigators have found that PEG-400 also has appreciably greater solubility in non-polar solvents, such as butanol, than lactulose, mannitol, L-rhamnose or ^{51}Cr-EDTA [111, 152], which is consistent with a degree of lipid solubility. This view is supported by evidence that PEG-400 can enter liposomes, which have no water pores [1], and consistently greater transport of PEG-600 than carbohydrates between aqueous compartments separated by a lipid phase *in vitro* [120]. The techniques used to assess lipid solubility have been disputed on account of possible water contamination of the organic solvents employed [151]. The same group have also shown that PEG-400 permeation in rat intestine is influenced by osmotic gradients [153], which is in keeping with substantial water solubility, and that the larger PEG-900 molecules do not penetrate rabbit enterocyte brush-border membrane vesicles, which suggests that permeation of the larger polymers might be confined to the paracellular route [145]. A ratio of large-PEG permeation to small-PEG permeation would thus be expected to rise in coeliac disease, in the same way that the lactulose/L-rhamnose or cellobiose/mannitol ratios increase, but it appears that the opposite happens because the PEG-1000/PEG-400 ratio is decreased in coeliac disease [147]. Recovery of PEG-400 in urine after intravenous administration is very incomplete [42], which implies that variation in systemic sequestration is likely to affect the reproducibility of results.

Justifiable reservations therefore exist concerning the use of PEGs for measuring intestinal permeability, especially with respect to the smaller (PEG-400) range [154].

Colonic permeability

Interest in colonic, as distinct from small- or whole-intestinal permeability, is developing. Sugars cannot be used alone because they are degraded by colonic bacteria [155]. ^{51}Cr-EDTA is a suitable marker because it is not broken down by bacteria and appreciable colonic absorption has been demonstrated by lower recovery in ileostomists compared with normal subjects [42, 118, 130]. Either oral [130] or rectal [129] administration have been used, but urine collection after oral administration should continue for 24 h. Combination of ^{51}Cr-EDTA with lactulose enables colonic permeation to be calculated by subtraction of the 24 h renal excretion of lactulose, which relates to the small intestine, from that of ^{51}Cr-EDTA, which relates to both small and large intestine, assuming that lactulose is rapidly degraded by bacteria on entering the colon [130].

Analytical techniques

Excreted sugars are at present most reliably measured by quantitative chromatography, either paper [156] or thin-layer [157], with scanning densitometry, or gas–liquid chromatography [158]. Sugars in test samples should be protected from bacterial degradation: they may be kept frozen, but addition of merthiolate (thiomersal; minimum concentration 10 mg/100 ml) provides adequate preservation for most sugars in urine for several months at room temperature, although a higher concen-

tration is needed when protein is present [156]. An advantage of paper or thin-layer, compared with column, chromatography is that samples can be 'batched' and a large number analysed simultaneously. A spectrophotometric assay for mannitol [159] is satisfactory, and it is also possible to analyse mannitol or lactulose by high-pressure liquid chromatography [160]. Enzymic assays for mannitol [161] and lactulose [162] are available, but enzyme-kits of high specificity present a recurring cost and may prove more expensive than chromatographic procedures in the long run. Enzymic methods are generally less sensitive and specific than chromatography, but give acceptable results in clinical practice [112, 127]. Further development and comparison of methods for sugar analysis are needed, but the technique most appropriate for a laboratory in a district general hospital may well depend on the equipment and expertise locally available.

PEGs can be reliably measured by gas–liquid [42] or high-pressure [163] chromatography. Addition of preservative or storage of samples at low temperature is unnecessary as PEGs are stable and resist bacterial degradation. Use of ^{14}C-labelled molecules (mannitol or PEG) requires scintillation counting, and of ^{51}Cr-EDTA, γ-radiometry; in the latter instance, rapid decay of the isotope means that analysis should not be delayed.

CLINICAL FACTORS INFLUENCING INTESTINAL PERMEABILITY

Intestinal permeability is altered in many situations, but the mechanisms (discussed in Part 1 of this review) are only beginning to be understood. Probe molecules permeate through epithelia either by the paracellular or transcellular route. The paracellular route appears to be the main pathway for larger molecules, appropriate test probes at present available being lactulose, cellobiose or ^{51}Cr-EDTA. Permeation by this route is influenced by the effect of intracellular mediators, cytokines, osmotic stress or drugs (among other substances) on links between the tight junction and the cytoskeleton. The transcellular route appears to be the main pathway for smaller probe molecules (including L-rhamnose and mannitol) and this may be affected by diseases that alter cell membrane composition or contiguity. The capacity of both routes would be expected to be altered by changes in absorptive area, for instance as a result of villous atrophy or surgical resection.

Physical factors

Osmolar effects. Hyperosmolar stress. Ingestion of sufficiently hyperosmolar solutions produces a marked increase in permeability of the normal intestine to polar molecules above 0.5 nm radius [1, 3, 28, 29, 30, 42]. A similar phenomenon has been described for isolated frog skin [43, 164], but some epithelia, including the leaky gallbladder epithelium, respond differently [165]. Ingestion of a solution above 1500 mosmol/kg is needed to alter the permeability of the normal intestine, although it is not certain what the effective osmolality of such a solution would be on reaching the intestinal lumen. Although normal subjects vary in sensitivity to this effect, it is clear that much lower levels of osmolality will increase permeability in the presence of villous atrophy. This was the initial basis for employing hyperosmolar stress for intestinal permeability tests [29–36]. Osmotic stresses in a normal diet [62] may be sufficient to induce intestinal hyperpermeability in patients with villous atrophy and it is conceivable that this could enhance the absorption of potentially antigenic molecules such as gluten fractions. The increase in permeability due to hyperosmolar stress is temporary, returning to normal within 2–3 h [30]. More recent experiments suggest that the effect is very transient, recovery taking place much more rapidly (within 30 min; I.S. Menzies, unpublished work).

Effect of osmotic fluid retention. Simultaneous ingestion of a poorly absorbed solute is capable of reducing the absorption of other constituents present in the intestine. This effect is distinct from hyperosmolar stress and appears to be the result of osmotic retention of fluid within the intestine causing dilution, and increasing the rate of transit by stimulating peristalsis, thus reducing concentration gradients and the time available for absorption. A study of the influence of poorly absorbed solutes (lactulose, mannitol, raffinose, magnesium sulphate) demonstrated impaired absorption of L-rhamnose in particular and xylose [103]. The evidence suggests that osmotically induced retention of fluid within the intestine becomes augmented by an associated movement of electrolyte (and further fluid) into the lumen. This movement of electrolyte, often described by the controversial term 'solvent drag' [166], results in much greater dilution of a probe than that anticipated on an osmotic basis alone.

Osmolar effects are thus complex. The advantages of employing hyperosmolar stress to increase clinical discrimination when measuring intestinal permeability are offset by an appreciable incidence of false-positive results and difficulty with interpretation. Examination of the literature suggests that the importance of stating the osmolarity of the test solution is often overlooked when devising test procedures or interpreting results.

Other physical factors. Altered intestinal motility affects the time that the mucosa is exposed to marker probes, and thus affects uptake independently of any changes in mucosal permeability [103]. Other non-mucosal factors have already been described.

Disease

Small-intestine mucosal disease is often associated with altered permeability, usually with increased permeation of larger molecules. In most cases this is no more specific for the type of pathology than is, for example, creatinine clearance for the cause of renal failure.

Coeliac disease. The initial observation that intestinal permeability to lactulose was increased [33] has been con-

firmed using both hyperosmolar [30, 35] and iso-osmolar [113] dual-sugar absorption tests and ^{51}Cr-EDTA [38]. The pathway involved appears to be paracellular, permeation of oligosaccharides (lactulose, raffinose), dextran-3000 and ^{51}Cr-EDTA, but not of L-rhamnose, mannitol or PEG-400, being increased [1, 30, 35, 38, 111]. Consistent with this is a lower transepithelial resistance and tight-junction strand count in jejunal biopsies from patients with coeliac disease [167]. PEG absorption (PEG-400) is, however, decreased in untreated coeliac disease [111, 147], probably because reduction in absorptive area is the main determinant for permeation of this probe. Permeability returned to normal after 5 months on a gluten-free diet in 21 patients [168], but a persistent increase in ^{51}Cr-EDTA excretion was observed in 10 patients with complete clinical and histological remission [38]. This may indicate a primary defect of permeability in patients with coeliac disease [169], but ^{51}Cr-EDTA excretion in 52 healthy first-degree relatives was invariably normal, although 38% had increased intraepithelial lymphocytes on jejunal biopsy [170]. Though dual-sugar intestinal permeability tests have been advocated for screening for suspected coeliac disease [42, 109], it is generally considered that a jejunal biopsy and proof of response to gluten withdrawal and/or challenge remain essential to establish the diagnosis.

Crohn's disease and ulcerative colitis. Intestinal permeability to disaccharide markers and ^{51}Cr-EDTA are increased in a high proportion of patients with small-intestinal Crohn's disease [128, 171, 172]. It has variously been reported that PEG-400 permeation is increased [142] or decreased [141, 173] in established Crohn's disease, and that PEG-1000 excretion is decreased [174]. In Crohn's colitis, increased permeability to ^{51}Cr-EDTA has been reported [38, 129]; whilst this might be the result of undetected small-intestinal disease, it could also be due to increased colonic absorption of ^{51}Cr-EDTA, which resists bacterial degradation in the large bowel. In ulcerative colitis, oral ^{51}Cr-EDTA absorption has been reported to be either normal [128] or higher even than in patients with small-intestinal Crohn's disease [129]. ^{51}Cr-EDTA excretion after rectal administration was increased in patients with colitis, but there was no difference between patients with ulcerative or Crohn's colitis. ^{51}Cr-EDTA permeation appears to be lower in smokers than in non-smokers [175], but whether this has anything to do with the lower incidence of ulcerative colitis in smokers is a matter for speculation.

A most controversial issue is whether permeability is increased in the healthy relatives of patients with Crohn's disease, which would suggest the presence of a primary defect in permeability [176]. Hollander *et al.* [42] reported increased permeability to PEG-400 in 32 healthy first-degree relatives but there are reservations concerning these findings. The range of PEG-400 permeation in their controls was 5- to 15-fold lower than that found in healthy subjects by most other authors, while results obtained from patients with Crohn's disease and their relatives corresponded to the normal values quoted in other studies [177]. Furthermore, increased PEG-400 permeation in first-degree relatives of patients with Crohn's disease has not been confirmed [173], and these subjects show normal intestinal permeability to ^{51}Cr-EDTA [131], lactulose, rhamnose and mannitol [117, 178].

Other diseases. Tropical sprue [125], viral gastroenteritis [108], starvation [121], cow's milk protein or food allergy [148, 179], diabetic diarrhoea [180], human immunodeficiency virus enteropathy [181] and bacterial overgrowth associated with immunodeficiency [182] have all been reported to be associated with an increase in intestinal permeability to disaccharide markers or ^{51}Cr-EDTA. Experimental work in mice suggests that bacterial toxins, including that of *Clostridium difficile*, increase colonic permeability to an electron-dense macromolecule, horse-radish peroxidase [183], before detectable histological changes occur. In some patients with the irritable bowel syndrome decreased absorption of arabinose has been detected [127], but although this may have a bearing on the aetiology [184], it could also be due to hypermotility decreasing the time available for absorption. This should be considered when selecting patients to act as controls for tests of intestinal permeability.

Abnormal intestinal permeability has been described in patients with dermatological disease, including dermatitis herpetiformis (in most of whom a jejunal biopsy demonstrated villous atrophy) [185, 186], and a minority with eczema [187] or psoriasis [188]. Psychiatric disease, other than the effects of gastroenteritis or starvation in anorexia nervosa, does not appear to be associated with increased intestinal permeability [189]. Sub-clinical gut involvement in rheumatoid arthritis or sero-negative arthropathies has been implicated [190] because permeability to ^{51}Cr-ETDA is increased, but the iatrogenic effect of non-steroidal anti-inflammatory drugs (NSAIDs), which increase permeability in both normal subjects and those with arthritis [191], appears to be responsible. Acute illness, such as falciparum malaria, has also been reported to increase intestinal permeability to disaccharides [87] and it is interesting to speculate whether this may be due to the influence of cytokines [192] or mucosal ischaemia.

Drugs

Oral and rectal NSAIDs increase intestinal permeability to ^{51}Cr-EDTA [193, 194] and the effect is related to the potency of cyclo-oxygenase inhibition. Interestingly, there appears to be no increase in lactulose permeation when NSAIDs are taken with food, even though ^{51}Cr-EDTA permeation is increased [130], suggesting an increase in colonic rather than small-intestinal permeability. NSAIDs may have different effects when taken without food, since glucose and citrate together reduce the increase in ^{51}Cr-EDTA permeation induced by NSAIDs [195]. Furthermore, the pro-NSAID nabumetone does not increase differential lactulose/L-rhamnose permeation, which indicates that NSAIDs increase permeability either by direct mucosal contact during absorption or after biliary excretion, rather than through a systemic effect [196]. PEG-400 permeation is

also claimed to be increased by NSAIDs [153, 197]. Prostaglandin analogues [135, 153], but not H_2-receptor antagonists [198], reverse the effects of NSAIDs, but may increase permeation to PEG-400 in patients not taking NSAIDs [197].

Cytotoxic drugs, such as 5-fluorouracil [199] or methotrexate [200], and radiotherapy with regional hyperthermia [136] also appear to increase intestinal permeability, but it is not clear whether this has any effect on drug absorption or efficacy. The anti-diarrhoeal agent loperamide inhibits the increase in permeation of EDTA caused by anthraquinone laxatives in perfused guinea-pig colon [201] and there has been interest in the possibility that bile acid adjuvants may enhance paracellular absorption of poorly absorbed drugs [202]. The detergent cetyltrimethyl-ammonium bromide (Cetrimide) also increases intestinal permeability to larger molecules such as lactulose and EDTA, but not to monosaccharides [42].

Age and race

The neonatal intestine is reported to be more permeable to disaccharide (lactulose) than the adult intestine [203]. Infant control values of lactulose/mannitol permeation [204] are higher than in adults [112], although this may be due to environmental influences, such as lactulose in commercial milk feeds, infection in the Gambian children studied [204] or differences in measurement technique. Differential sugar permeability is unaltered in normal subjects aged 22–96 years [124].

Geographical location appears to be important, probably due to an environmental factor because there is no difference between racial groups resident in Britain (I. S. Menzies, unpublished work). Increased dual-sugar (lactulose/L-rhamnose) permeability has been recorded in apparently healthy members of indigenous populations in Indonesia, Thailand, India, Botswana, Cameroons, The Gambia, Jamaica [205], Uganda [181] and Sudan [206]. Residents of tropical and sub-tropical countries are likely to have gastrointestinal parasitic infections, or sequelae such as tropical enteropathy, that may contribute to the increased permeability. Visitors to such countries may also be susceptible to tropical enteropathy and subsequent changes in intestinal permeability. This means that the interpretation of intestinal permeability tests is likely to be difficult in countries where tropical enteropathy is prevalent, because this condition may mask the changes produced by other intestinal pathology.

CLINICAL RELEVANCE OF INTESTINAL PERMEABILITY TESTS

Apart from research into the underlying mechanisms of disease, intestinal permeability tests are likely to be of value as screening tests to indicate the need for further investigation which may be more invasive, or to assess clinical progress and response to treatment. These non-invasive tests are particularly convenient for investigating infants and young children.

Screening

An effective screening test must have high discrimination, being sensitive (reliable for detecting disease), with a high negative predictive value (reliable for excluding disease when the test result is normal). It need not have high disease specificity when alternative methods for resolving the diagnosis are available. So far only the cellobiose/mannitol version of the disaccharide/monosaccharide ratio test, used in conjunction with hyperosmolar stress, has been fully evaluated in unselected patients [41]. In Britain it reliably detected patients with 96% sensitivity, and had a 99% negative predictive value, for coeliac disease. However, almost 25% of 197 patients with a normal cellobiose/mannitol ratio were found to have some abnormality on jejunal biopsy [41]. Most of these histological abnormalities were 'non-specific', but it is not clear what the final diagnoses were in this group, or whether such minor changes were clinically important. Despite this, a normal test was calculated to have a 97% negative predictive value for excluding small-intestinal disease diagnosed by jejunal biopsy or any other means.

It would be very useful to know whether intestinal permeability tests could help reduce the radiological and endoscopic workload, by selecting patients likely to benefit from diagnostic small-bowel radiology or biopsy. No studies have yet addressed these questions.

Functional assessment

It seems logical that a test which assesses the whole intestine, rather than a point sample (such as a jejunal biopsy) or gross anatomy (small-bowel radiology), would be complementary and clinically valuable. This has yet to be proven. Several studies have attempted to correlate disease severity with results of permeability tests [110, 123, 138, 207]. However, it is still not clear whether this contributes to management, or whether intestinal permeation of markers is simply an elaborate alternative to measuring biochemical variables such as albumin or C-reactive protein. Correlation with disease activity in inflammatory bowel disease is tenuous [138, 208], but intestinal permeability may be of value in predicting recurrence of Crohn's disease [209, 210]. The lactulose/mannitol permeation ratio was fourfold higher in 16/36 patients with inactive Crohn's disease who subsequently relapsed during a year of follow-up, than in 20/36 patients who remained in remission [209]. Furthermore, those who remained in remission had lactulose/mannitol ratios similar to normal control subjects, and there was a negative correlation between the ratio and time to relapse [209, 210].

Response to treatment

From a patient's point of view, any non-invasive test that obviates the need for a repeat jejunal biopsy to confirm response to gluten withdrawal, or deterioration after gluten challenge in children with coeliac disease, would be welcome. Differential sugar permeability tests in

coeliac patients return to normal after gluten withdrawal [35, 111, 211] and deteriorate after gluten challenge [168, 212], but abnormal ^{51}Cr-EDTA absorption appears to persist [169]. Increased differential sugar permeability has been shown to improve during remission of inflammatory bowel disease [213] and when Crohn's disease is treated with an elemental diet [214]. Differential sugar absorption tests (dual or multiple) that provide simultaneous information about intestinal permeability and mediated transport or disaccharidase activity [107, 108, 215] would probably be more widely used to follow the progress of diseases (such as atypical gastroenteritis) or to monitor the response to treatment (such as gluten withdrawal in coeliac disease) if the analytical facilities and expertise were generally available.

CONCLUSIONS

No current test of intestinal permeability is ideal, but a dual-marker test (either lactulose/L-rhamnose or an alternative combination), given in iso-osmolar solution will achieve useful clinical discrimination for small-intestinal disease. ^{51}Cr-EDTA is more easily measured and may also be used for assessing colonic permeability. Single-marker tests, including the use of PEGs are subject to greater individual variation and interference from uncontrollable factors, which are limitations in routine clinical use.

Much work has been directed towards the detection of abnormal permeability in disease, but attention should now be directed to questions that will affect clinical management of patients. Practical questions that need clarification are whether intestinal permeability tests can reduce radiological or endoscopic workload, by allowing selection of those patients who require more invasive investigations; whether measurement of permeability will provide a reliable index of response to treatment; and to what extent such non-invasive tests could complement or replace jejunal biopsy for the purpose of confirming response to gluten withdrawal in coeliac disease. There is a need to improve and simplify the analytical procedures in order to facilitate introduction into routine clinical practice. It should be stressed that pathways of intestinal permeation and the mechanisms by which permeability is altered are at present incompletely understood and deserve further research.

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