Absorption of Intact Oligosaccharide in Health and Disease
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It is well known that small quantities of intact dietary sucrose and lactose pass across the normal intestinal wall, probably by a process of non-mediated diffusion, and are subsequently excreted in the urine (Folin & Berglund, 1922; Utter, 1927). Except for lactosuria during pregnancy and lactation (Brock & Hubbard, 1935) and very rare instances of endogenous sucrosuria (Elmer et al., 1939), disaccharides appearing in the urine are usually of dietary origin. Excretion of sucrose and lactose in larger amounts, associated with gastroenteritis, coeliac disease, hiatus hernia and other intestinal abnormalities, have been attributed to either disaccharidase deficiency producing elevated intestinal concentrations, or increased permeability of a structurally damaged mucosa (Moncrieff & Wilkinson, 1954; Owen & Lewis, 1956; Santini et al., 1957; Bickel, 1961; Gryboski et al., 1963; Weser & Sleisenger, 1965).

Further analysis of the factors that determine absorption of intact oligosaccharide is presented, and the clinical significance of increased absorption is discussed. Urinary sugar measurements presented were made by quantitative paper chromatography (Menzies, 1973).

Renal excretion of oligosaccharides after intravenous injection

Earlier work suggests that intact sucrose and lactose are very slowly metabolized on reaching the blood stream, being almost quantitatively excreted in the urine in man and other mammals. Both Keith & Power (1937) and Deane & Smith (1955) report recoveries of over 89% after large intravenous doses of sucrose, whereas Koehler et al. (1935) recovered over 86% of 15g of intravenous lactose from the urine of healthy adults. Steinitz (1940) showed that renal clearance of sucrose and inulin were practically identical. Weser & Sleisenger (1967) recovered 87, 63 and 1.1% respectively of lactose, sucrose and maltose from the urine of healthy adults after intravenous doses of 10g, and (by using isotope techniques) showed that whereas systemic metabolism of lactose and sucrose was very slow, that of maltose was rapid.

During the present study urinary excretions of stachyose, raffinose, lactulose, melibiose, lactose, palatinose and sucrose, after intravenous administration of 0.5g quantities, were found to be above 88% and to follow the same pattern in each of five healthy adults, approx. 65, 19, 9, 3 and 1% of the injected amount being excreted in consecutive periods of 2.5h. One anencephalic neonate excreted over 92% of 0.2g of intravenous raffinose, lactulose, lactose, palatinose and sucrose during 24h. Therefore provided situations in which endogenous production (e.g. lactosuria of pregnancy) or marked decrease of renal clearance are avoided, urinary excretion of the above oligosaccharides,
except for maltose, should be closely similar to the quantity entering the blood stream from the alimentary tract.

**Transfer of oligosaccharides resistant to intestinal hydrolase activity**

Human small-intestinal mucosa lacks hydrolytic activity for the disaccharides melibiose (Gal \( pα1 \rightarrow 6 \) Glc) and lactulose (Gal \( pβ1 \rightarrow 4 \) Fru), and the trisaccharide raffinose (Gal \( pα1 \rightarrow 6 \) Glc \( pα1 \rightarrow 2 \) Fru) (Dahlqvist & Gryboski, 1965; Udupihille, 1974). After oral administration as a mixture, individual concentrations of these oligosaccharides in the intestinal lumen should therefore remain proportional to the ingestion ratios, although this may become altered by bacterial activity in the distal intestine. If non-mediated diffusion is the mode of transfer across the intestinal wall, then the rate of entry of these sugars into the blood stream, and hence the urine should, in turn, be directly proportional to concentrations in the intestinal lumen. Under these circumstances, urinary and ingestion ratios of melibiose and lactulose, being of similar molecular size, should be the same. The urinary excretion of melibiose, lactulose and raffinose after oral administration of these sugars as a mixture to two healthy adults is shown in Fig. 1. Whereas excretion and ingestion melibiose/lactulose ratios are closely similar over a wide range of differing proportions, raffinose/lactulose excretion ratios are consistently lower than the ingestion ratios, suggesting that the intestine is less permeable to trisaccharide than disaccharide (molecular radii approx. 0.8 and 0.54–0.88 nm respectively). The very slow rate of absorption (average 0.25 % of ingested disaccharide and 0.16 % of trisaccharide/5h), and its linear relation to concentration gradient, implied by the close similarity between ingestion and excretion ratios for melibiose and lactulose, both suggest that non-mediated diffusion is the transfer mechanism involved. These findings are compatible with the ‘intestinal-pore’ hypothesis that the lipoidal surface membrane of the mucosal cells is interspersed with water-filled pores of molecular radius between 0.3 and 0.88 nm which determine permeability to non-lipid solutes (Fordtran et al., 1965).

Besides intestinal permeability, several other factors influence transfer of ingested oligosaccharide to the urine. Distribution to and concentration at absorptive surfaces, and therefore diffusion rate, are determined by amount and concentration ingested, dilution by gastrointestinal fluid, rates of passage along and removal from the intestine by transit, and hydrolysis by intestinal hydrolases. Normal renal clearance, by which oligosaccharide is concentrated in urine to amounts about 50 times those in plasma, facilitates accurate measurement of the small quantities absorbed from the intestine.

![Fig. 1. Relation of urinary excretion to ingestion ratios of the 'non-hydrolysable' oligosaccharides melibiose, lactulose and raffinose, after administration in varying proportion to two healthy adult subjects](image)

Melibiose (○), Me; lactulose (■), Li; raffinose (□), Ra.
Effects of intestinal disaccharidase activity and permeability

The parts played by intestinal disaccharidase activity and permeability can be distinguished by comparing the behaviour of lactose and sucrose with that of lactulose, which is not hydrolysed (Menzies, 1972a). Urinary excretion of lactulose, lactose and sucrose after an oral load (7, 20 and 20 g respectively, dissolved in 300 ml of water) was measured in two groups of healthy adults, 20 normolactasic and 14 hypolactasic. Fig. 2 shows the excretion ratios of lactose/lactulose differed considerably between the two groups, in contrast with those of sucrose/lactulose (lactose/lactulose = 0.42±0.14 and 1.42±0.42; sucrose/lactulose = 0.34±0.11 and 0.52±0.21 for normo- and hypo-lactasic groups, respectively, values are mean±s.d.). Two patients with asucrasia were also studied (kind permission of Dr. G. Neale, Hammersmith Hospital): both showed sucrose/lactulose excretion ratios identical with the ingestion ratios.

These results indicate that active intestinal hydrolysis decreases the transfer of intact ingested disaccharide to the urine by a factor of between 5 and 10. Thus, when intestinal permeability is normal, about 0.07 and 0.09 % of ingested sucrose and lactose are excreted in the presence of normal disaccharidase activity; 0.2 % of lactose in the presence of constitutional hypolactasia, and 0.5 % of non-hydrolysable disaccharide such as lactulose (or sucrose when there is asucrasia) in the urine during 10 h.

A group of 10 patients with coeliac disease showed, when given the above disaccharide test (kind permission from Dr. B. Creamer, St. Thomas's Hospital), abnormal disaccharide transfer corresponding to clinical severity. Though, with mild disease, lactulose excretion was often within the normal range, severe cases had marked lactulosuria, suggesting intestinal hyperpermeability, rising to above 250 mg (3.6 % of ingested dose). Lactose/lactulose and sucrose/lactulose excretion ratios indicated lactase deficiency in all patients, but sucrase was less affected, being normal in some of the milder cases. Thus disaccharidase deficiency, well known in coeliac disease, and increased intestinal permeability both combine, in varying degrees, to produce the disacchariduria characteristic of this condition.

![Fig. 2. Urinary excretion of lactose and sucrose compared with 'non-hydrolysable' lactulose after ingestion of a standard mixture](image-url)

Isolated hypolactasia, asucrasia and combined disaccharidase deficiency with intestinal hyperpermeability due to coeliac disease each produce characteristic deviations from the normolactasic response pattern. The oral load was: lactulose (口), 7 g; lactose (■), 20 g; sucrose (■), 20 g, dissolved in 300 ml of water. Values are means ± s.d.
Effect of hyperosmolar solutions on intestinal permeability

Utter (1927) demonstrated that the concentration of sucrose in ingested solutions, independent of the amount, influenced the degree of resulting sucrosuria; later Moncrieff & Wilkinson (1954) and Menzies & Seakins (1969) noticed that lactose and sucrose ingested together, or with other sugars as a mixture, produced an unexpected rise in urinary disaccharide excretion. Reappraisal of the relation between osmolality of ingested solutions and disacchariduria indicated the importance of controlling this factor (Menzies, 1972b), and the above findings can be ascribed, in retrospect, to the effect of hyperosmotic solutions on intestinal permeability.

Healthy adult subjects ingested, on separate occasions (minimum interval, 2 days) a standard solution containing 10 g of lactulose in 100 ml of water, to which graded amounts of urea were added to produce an osmolality range between 200 (no urea) and 2800 mosmol/kg (21 g of urea). Resulting urinary lactulose excretions, shown in Fig. 3, were between 30 and 60 mg in 5 h (mean 0.45% of ingested dose) when the osmolality was below 1200 mosmol/kg, but above this value a marked progressive increase in lactulose transfer occurred, reaching 330 mg in 5 h (3.3% of ingested dose) at 2800 mosmol/kg. Sufficiently hyperosmolar solutions of monosaccharides, disaccharides, mannitol and salts (NaCl and KCl) also produced the same effect, though to a variable extent: urea was, for instance, more active than equimolar glucose. Ethanol, even in very hyperosmolar solution, failed to produce this effect. Transfer of lactose, sucrose, melibiose and raffinose were affected in the same way as lactulose; alteration in gastrointestinal permeability by hyperosmolar solutions appears to be the most logical explanation.

Intubation studies indicate that increased lactulose transfer after the ingestion of hyperosmolar solutions is related to an increase in duodenal rather than gastric osmolality (Udupihille, 1974), implying that this effect occurs mainly in the upper small intestine. Further, by using raffinose and lactulose as consecutive 'markers', the increased permeability induced by hyperosmolar urea was shown to be very temporary, returning to normal within 2.5 h (Menzies, 1972c). In several respects this phenomenon is similar to the response of isolated frog skin and toad bladder to hyperosmolar solutions, described by Ussing (1966) and Urakabe et al. (1970), the cause of which has not yet been explained.

Cetrimide (cetyltrimethylammonium bromide, 100 mg/100 ml) also produces a marked temporary increase in intestinal disaccharide permeability, but this effect is not shared by other detergents (ox bile and dioctyl sodium sulphosuccinate). The permeability dif-

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**Fig. 3. Effect of hyperosmolar urea solutions on transfer of ingested lactulose to the urine**

Solutes other than urea produce a similar alteration of intestinal permeability. The oral load was 10 g of lactulose in 150 ml of water. (a), subject I.M.; (b), subject R.H.
ferential between lactulose and raffinose remains unchanged during phases of increased permeability induced by both hyperosmolar solutions and cetrimide, suggesting that pore incidence rather than size becomes increased.

Intestinal permeability of healthy adults to lactulose responds to hyperosmolar solutions in an individually consistent way, some subjects being more predisposed to the effect than others. In coeliac disease, however, this response is greater than normal (Menzies, 1972b,c). Urinary lactulose excretion in 20 healthy adults during 5h after ingestion of 5 g of lactulose in 100 ml of water (23.5 ± 11 mg (mean ± s.d.), and scarcely rose when the same load, made hyperosmolar by the addition of 40 g of sucrose (1500 mosmol/kg), was repeated, becoming 25.7 ± 8.7 mg. In contrast, a group of 19 patients with coeliac disease of varied severity, excreted almost twice as much lactulose after the hyperosmolar compared with the 'isosmolar' load, mean values being 95.33 and 51.14 mg/5h. Most of the milder cases showed normal permeability to the 'isosmolar' load, but of 26 proven coeliacs receiving the hyperosmolar load 24 (92%) showed abnormal lactulosuria, above 43 mg/5h (normal mean ± 2 s.d.), A few apparently healthy female subjects, aged 20–30 years, have shown high responses to the hyperosmolar load. Although high values are sometimes recorded with gastrointestinal pathology other than coeliac disease, sufficient data for a useful analysis is not yet available.

In conclusion, the fraction of ingested oligosaccharide appearing in the urine is of interest in several ways. Measurement of this can provide a practical means for assessing intestinal disaccharide hydrolysis and permeability for clinical purposes; in particular, lactulose transfer after hyperosmolar loading shows a high incidence of abnormality in coeliac disease, even in mild cases, and has value as a screening test. Induction of increased intestinal permeability to oligosaccharides, caused by disease, hyperosmotic dietary constituents, or other factors could be associated with increased access of dietary constituents that have a pathological significance, such as carcinogens or antigens. Conversely, clinically important leakage of plasma constituents into the intestinal lumen may also occur.

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Glycosidase Deficiencies

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Glycosidases capable of hydrolysing all the common linkages in naturally occurring heteropolymers of carbohydrates are known to occur in animal tissues. They are generally considered to be of lysosomal origin and many of them have been demonstrated to be specifically located in that organelle.

With the exception of hyaluronidase they are exo-glycosidases that degrade linear polymers by sequential hydrolysis of the non-reducing terminal sugar. This feature of their specificity accounts for the storage phenomena that arise in the event of a genetic inability to produce a functional enzyme. The metabolic block results in the accumulation of partially degraded polymer in the lysosomes and the continued biosynthesis at normal rates aggravates the process until the familiar inclusion bodies are a marked histochemical feature of the disease.

Where the accumulated product is water-soluble or of relatively small molecular weight an increased excretion of heteropolysaccharide in the urine is symptomatic. The concept of such lysosomal storage diseases was first elaborated by Hers (1965) and is now firmly established and is not confined to man, there being several well documented animal counterparts of these human genetic diseases (Jolly & Blakemore, 1973). The hydrolases are generally thought to be specific for the type of linkage and not for a single substrate so that a single defect may cause the storage of partially degraded mucopolysaccharides, glycolipids and glycoproteins. However, it is frequently found that one of these types of substance predominates and it has been convenient to distinguish the sphingolipidoses in which lipid accumulation is an obvious feature from the mucopolysaccharidoses where skeletal deformities frequently predominate.

Many of the glycosidases occur in multiple forms, but in most cases the physiological significance of the individual isoenzymes is poorly understood and their interrelations still remain to be worked out (Robinson, 1974). In some storage diseases a complete lack of a particular glycohydrolase activity can be demonstrated by the use of synthetic substrates. Partial deficiencies, limited to specific isoenzymic forms may result in less severe symptoms and a more prolonged course of the disease whereas in a few cases the deficiency can only be demonstrated by the use of natural substrates, the amounts being essentially normal when synthetic substrates are used.

Confirmation that the stored product and missing enzyme have a causal relationship has been elegantly provided by correction experiments as pioneered by Neufeld and co-workers (Fratantoni et al., 1969; Neufeld & Cantz, 1971). Cultured cells from affected individuals develop the characteristic storage bodies, but become histologically normal on addition of the missing enzyme to the medium. The phenomenon offers a basis for studies of systems in vitro for the therapeutic replacement of enzyme, but the few attempts that have been made in this enzyme therapy have been of marginal value and probably premature.

Deficiencies in sphingolipid metabolism

Metabolic blocks of each stage of the degradation of the common glycolipids have been described with the exception of neuraminidase deficiency. There is increasing evidence that sialation and desialation is one of the most important cell-surface reactions for cell–cell recognition and for differentiation. It is highly likely therefore that if such a deficiency exists it will be rapidly fatal in foetal life.