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INCREASE IN HUMAN INTESTINAL PERMEABILITY FOLLOWING INGESTION OF HYPERTONIC SOLUTIONS

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SUMMARY

1. A simple oral loading technique involving the ingestion of solutions containing lactulose is described. Timed urinary excretion of lactulose, which is non-metabolizable, is used as an indicator of intestinal permeability, and measured by quantitative paper chromatography.

2. This technique has been used to investigate the intestinal permeability of apparently healthy adults following the ingestion of solutions made hypertonic by the addition of the solutes sucrose, glucose, mannitol, glycerol, urea and sodium chloride.

3. These experiments show that intestinal permeability to lactulose increases as the solute concentration in the ingested solution is increased. Susceptibility to this effect, though consistent for each individual, shows considerable variation between subjects.

4. Factors thought to be pertinent to the enhancement of intestinal permeability by hypertonic solutions, and some possible implications of this, are discussed.

INTRODUCTION

It is well known that small amounts of intact dietary disaccharide cross the normal intestinal wall and are subsequently excreted in the urine (Folin & Berglund, 1922; Utter, 1927; Bickel, 1961). Abnormally high urinary excretion of dietary sucrose and lactose has been reported in patients suffering from various diseases of the gastrointestinal tract (Gryboski, Thayer, Gabrielson & Spiro, 1963; Weser & Sleisenger, 1965). The explanation of such increased absorption of disaccharide, which seems a paradox in malabsorptive conditions such as coeliac disease, has been postulated as due either to impaired disaccharide hydrolysis or increased permeability of the damaged intestinal wall, or a combination of both mechanisms (Weser & Sleisenger, 1965).

There are also several reported instances where increased absorption of

intact disaccharide seems to have been due to physiological mechanisms rather than to established intestinal pathology. Thus Utter (1927) showed that the degree of sucrosuria induced by oral loading solutions could be increased by raising the concentration of sucrose contained in them without altering the total amount ingested. An unexplained rise in urinary disaccharide excretion has also been reported when disaccharides are ingested together, or in combination with other sugars (Moncrieff & Wilkinson, 1954; Menzies & Seakins, 1969). Unfortunately these results cannot be clearly related to the state of intestinal permeability because the experiments described all involve disaccharides that are actively hydrolysed in the normal small intestine.

The present series of experiments has been designed to discover the part played by intestinal permeability in determining renal excretion of ingested disaccharide in healthy human subjects, and to find if this is altered following ingestion of solutions made hypertonic by the addition of sucrose and other solutes to the oral loading solution. The synthetic disaccharide lactulose (Gal $p\beta$1–4 Fru) has been chosen as an indicator for this purpose. This disaccharide resists hydrolysis by human intestinal disaccharidases (Dahlqvist & Gryboski, 1965; Udupihille, 1974), and urinary excretion after I.V. injection is rapid and approaches 100% (Menzies, 1974). For these reasons the amount reaching the urine after ingestion of a standard load should closely reflect the state of intestinal permeability to disaccharide.

METHODS

A group of twenty-one apparently healthy adult volunteers without evidence of gastrointestinal or systemic disease participated. None of the subjects were receiving treatment with lactulose syrup ('Duphalac') at the time of the experiments. The age of the subjects ranged from 18 to 60 yr.

Composition of the oral loading solutions. Each series of loading solutions contained lactulose in constant amount and concentration as 'indicator', the final molarity being achieved by addition of a second constituent as the major solute. The compositions of loading solutions with sucrose as the major solute are given in Table 1, and of those in which either glucose, mannitol or glycerol served this purpose are given in Table 2. The whole group of subjects participated in the sucrose loading experiments, and a smaller number also took solutions containing glucose, mannitol and glycerol. Two subjects took part in a further series of experiments in which lactulose-containing loading solutions were made hypertonic with urea and sodium chloride (for details see Figs. 3 and 4). Duphalac syrup (Duphar Laboratories, Basingstoke, Hants), which contains 67% (w/v) lactulose, was used as a source of this disaccharide for these experiments. Oral loading solutions were prepared in batches and deep frozen at −17° C until required. Osmolalities were measured with an Advanced Osmometer (Advanced Instruments Inc., 45 Kenneth Street, Newton Highlands, Massachusetts 02161, U.S.A.).

Experimental procedure. Subjects were deprived of all food and drink for a period of 8 hr before and 3 hr after the ingestion of each sugar load. The entire loading solution

TABLE 1. Composition of the sucrose loading solutions; 100 ml. of each was taken. Because lactulose syrup contains fractions of other constituents (e.g. lactose 5%, w/w; galactose 8%, w/w) calculated molarity was invalid. Molality was measured, and at the concentration used, assumed to be approximately equivalent to molarity

		Load			
		1	2	3	4
Lactulose	g/100 ml.	5	5	5	5
	m-mole kg^{-1}	275	275	275	275
Sucrose	g/100 ml.	0	20	40	60
	m-mole $l.^{-1}$	0	585	1170	1754
Total molarity (m-mole $l.^{-1}$)		275	860	1445	2029

TABLE 2. Composition of the glucose, mannitol and glycerol loading solutions, 100 ml. of each of which were taken

		Load		
		5	6	7
Lactulose	g/100 ml.	5	5	5
	m-mole kg^{-1}	275	275	275
Glucose	g/100 ml.	30	0	0
	m-mole $l.^{-1}$	1667	0	0
Mannitol	g/100 ml.	0	22	0
	m-mole $l.^{-1}$	0	1225	0
Glycerol	g/100 ml.	0	0	13
	m-mole $l.^{-1}$	0	0	1225
Total molarity (m-mole $l.^{-1}$)		1942	1500	1500

was ingested undiluted within a period of 4 min, following which a complete and accurately timed urine collection of 5 hr duration was made. The urine volumes were recorded and 30 ml. aliquots stored in universal containers at 4° C, with 0·01 g merthiolate/100 ml. added, for sugar estimations. Under these conditions preservation of lactulose and other sugars has been found to be satisfactory for a period of at least 12 months (Menzies, 1973). The different loading solutions were given to subjects in varied order, and an interval of at least 3 days was allowed between consecutive tests.

Estimation of lactulose in urine. The quantitative paper chromatographic method described by Menzies (1973) was used. This involves desalting the urine sample by shaking with a mixed ion-exchange resin (Zerolit DM-F; B.D.H. Chemicals Ltd, Poole, Dorset) used in the H^+ $acetate^-$ state followed by application of a suitable measured volume to Whatman No. 3 Chromatography Paper. After 20 h descending development using a solvent system consisting of butan-1-ol : ethyl acetate : pyridine : water (6:6:5:4, by volume) the chromatograms are dried and treated with

butanol to remove residual pyridine which interferes with the locating reaction. Chromatograms are then dipped in locating reagent (4-aminobenzoic acid, 700 mg; orthophosphoric acid, 88 % (w/v), 0·4 ml.; and methanol to 100 ml.), dried, and then uniformly heated using a 'rotation' technique in a hot air oven at 110–115° C for 10 min to bring about the colour reaction. Clarification is then performed by dipping in a mixture of liquid paraffin B.P. and light petroleum b.p. 60–80° C (55/45 by volume) and allowed to dry. Quantitation is achieved by comparison of peak areas of test and standard sugar zones which have been run on the same chromatogram using reflectance scanning with a Chromoscan double-beam recording and integrating densitometer (Joyce–Loebl & Co. Ltd, Princes Way, Team Valley, Gateshead 11). When used under ideal conditions this method has a coefficient of variation of ± 2·0 to 3·5 % for the estimation of different types of sugars.

Estimation of lactulose in plasma. Estimation of the low plasma lactulose levels required a more sensitive technique. For this the following gas–liquid chromatographic method was developed by one of us (M.L., to be published in detail). 1 ml. plasma or standard lactulose solution was diluted with 1 ml. of an appropriate concentration of internal standard, turanose (3 (α-D glucosido) D-fructose) supplied by Sigma (London) Chemical Company Ltd, Station Yard, Norbiton, Surrey KT2 7BH. 1 ml. 7 % sulphosalicylic acid was added to deproteinize the plasma samples. The supernatant was desalted using Zerolit DM-F ion-exchange resin in the H^+ $acetate^-$ form, and 1 ml. of the resulting solution was pipetted into a conical centrifuge tube. The samples were blown down under a current of air at 45° C, and further dried *in vacuo* over phosphorus pentoxide overnight. Trimethylsilyl derivatives were prepared by adding 50 μl. of a mixture of anhydrous pyridine (4 vol.); bis trimethylsilyl acetamide (2 vol.); trimethylchlorosilane (1 vol.). Silyl reagents were supplied by Field Instruments, Tetrapak House, Orchard Road, Richmond, Surrey. The tubes were then closed and heated for 30 min at 60° C. 1 μl. was injected on to the chromatograph. Quantitation was by comparison of peak heights.

A Pye 104 heated dual flame ionization chromatograph was used (Pye–Unicam Ltd, York Street, Cambridge) linked to a Hewlett–Packard 3380A Reporting Integrator (Hewlett–Packard Ltd, King Street Lane, Winnersh, Wokingham, Berks RG11 5AR). The column was a 9 ft 10 % OV-17 on 80–100 mesh gaschrom Q (Phase Separations Ltd, Deeside Industrial Estate, Queensferry, Flintshire CH5 2LR) having 4432 theoretical plates measured by a C25 hydrocarbon standard. The injector temperature was 275° C, column temperature 250° C, detector temperature 350° C. Argon was the carrier gas at 50 ml./min. The limit of detection was 40 μg/100 ml. (equivalent to 8 μg injected material), coefficient of variation 2 % and recovery 98·8 % at a level of 2 mg/100 ml. in the original sample.

RESULTS

Effect of added sucrose on lactulose excretion. A scattergram showing the effect on renal excretion of lactulose when sucrose is added to the standard 100 ml. oral lactulose solution as the major solute is shown in Fig. 1. The 5 hr lactulose excretions corresponding to ingestion of solutions containing no added sucrose (isotonic control, 275 m-mole kg^{-1}), were 10–30 mg, median 15 mg; 20 g sucrose (860 m-mole $l.^{-1}$), 11–50 mg, median 17 mg; 40 g added sucrose (1445 m-mole $l.^{-1}$), 8–127 mg, median 19 mg; and for 60 g added sucrose (2029 m-mole $l.^{-1}$), 12–134 mg, median 33 mg. Analysis of variance carried out on this data demonstrated a significant regression

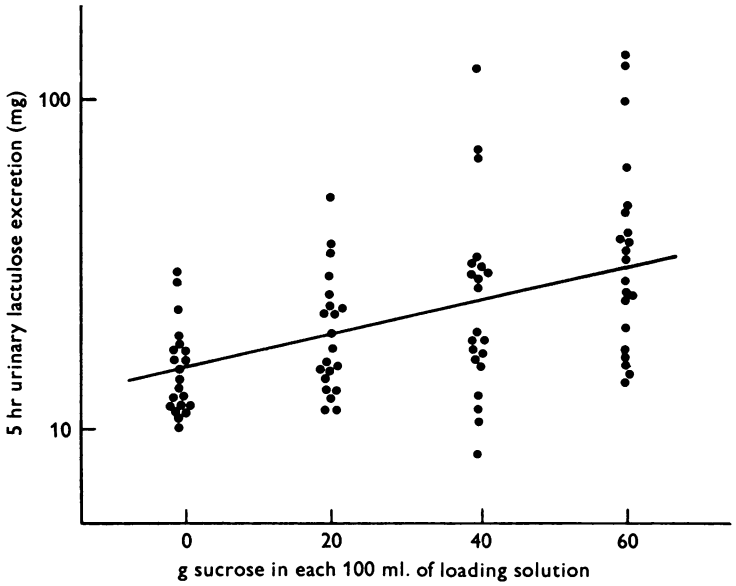

Fig. 1. Lactulose excretion during the first 5 hr after the ingestion of sucrose loading solutions. The scale of lactulose excretion is logarithmic. There is significant regression ($P = < 0{\cdot}01$), and the regression line does not deviate significantly from linear.

($P = < 0{\cdot}01$). The logarithm of lactulose excretion did not significantly deviate from a linear relationship to the molarity of the ingested solution, as shown in Fig. 1.

Effects of added glucose, mannitol, and glycerol on lactulose excretion. Smaller groups of subjects received standard oral lactulose loads made hypertonic by addition of either 30 g glucose (1942 m-mole $l.^{-1}$), 12 g glycerol (1500 m-mole $l.^{-1}$) or 22 g mannitol (1500 m-mole $l.^{-1}$).

Eighteen subjects receiving glucose-containing loads excreted 41 (13–172) mg lactulose, median and range, in 5 hr, which was not significantly different from that which followed the ingestion of the 60 g sucrose-containing load of equivalent molarity (Student's t test). Data from these experiments are compared in Fig. 2.

The 5 hr excretion produced by four subjects following the ingestion of glycerol and mannitol-containing oral loads are compared with responses to the equivalent sucrose load (40 g sucrose, 1455 m-mole $l.^{-1}$) in Table 3. One of these subjects showed consistently high lactulose excretions in response to the sucrose, glycerol and mannitol-containing loads.

Reproducibility of lactulose excretion. Duplicate tests with the lactulose plus 40 g sucrose load (1445 m-mole $l.^{-1}$) were performed on three subjects: one male excreted 22 and 22 mg, a second male 21 and 14 mg, and a

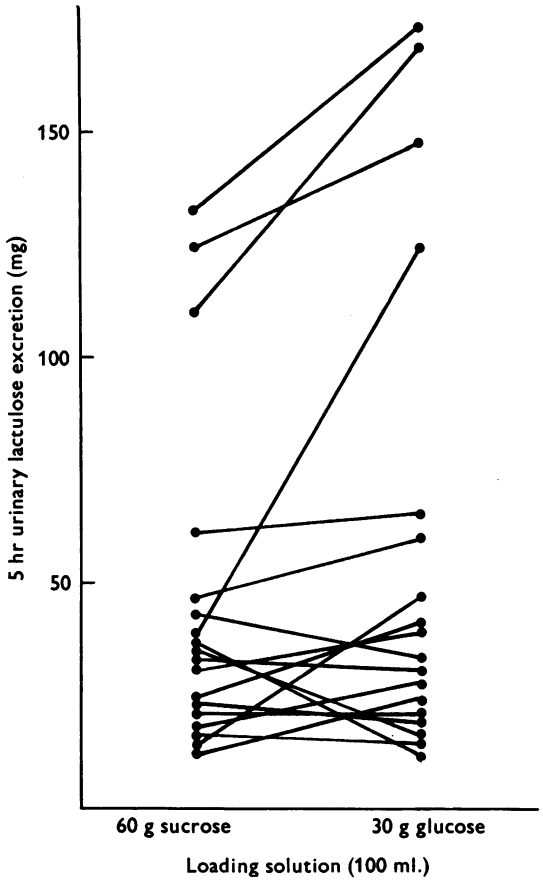

Fig. 2. A comparison between the increased intestinal permeability to lactulose induced by approximately equimolar glucose (1942 m-mole $l.^{-1}$) and sucrose (2029 m-mole $l.^{-1}$). There is no significant difference between the effects of the two solutes.

TABLE 3. A comparison of the effects of different solutes on lactulose excretion

	Lactulose excretion (mg/5 hr)		
Subject	Sucrose load (1445 m-mole $l.^{-1}$)	Mannitol load (1500 m-mole $l.^{-1}$)	Glycerol load (1500 m-mole $l.^{-1}$)
M.L.	14·4	8·9	31·7
I.M.	7·8	9·5	18·8
D.H.	10·8	8·9	17·0
M.S.	69·3	62·5	78·5

female (high excretor) 51 and 60 mg lactulose in 5 hr. The responses of individual subjects were generally found to be reproducible and consistent, thus those with high lactulose excretion following hypertonic sucrose-containing loads responded in a similar fashion following the glucose, mannitol and glycerol-containing loads, and others with low lactulose transfer also behaved in a consistent fashion irrespective of the major solute involved.

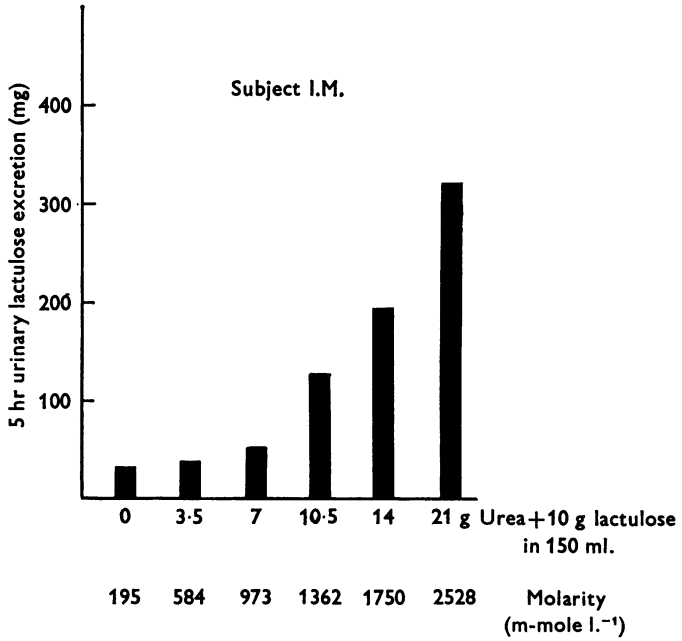

Fig. 3. The effect of increasing the molarity of the loading solution beyond that of the most hypertonic sucrose (2029 m-mole $l.^{-1}$) in an individual who did not demonstrate enhanced permeability to the sucrose loading solutions. The major solute used was urea.

Experiments with urea and sodium chloride as major solutes. The response of a single subject (I.M.) to a series of lactulose-containing oral loads, made up to have a range of different molarities by adding urea, are recorded as a histogram in Fig. 3. There was a very marked progressive increase in excretion of ingested lactulose to the urine when the molarity of the ingested solution was raised to 1362 m-mole $l.^{-1}$ and above.

Urinary lactulose excretion by the subject N.D. following ingestion of two separate oral loads containing 40 g lactulose in 133 ml. water, one of which was made very hypertonic by addition of 6·7 g sodium chloride (2600 m-mole $l.^{-1}$) are recorded in Fig. 4. In this case lactulose excretion

was increased from 85 to 2030 mg (i.e. from 0·2 to 5·1 % of the ingested dose) during 5 hr, as a result of the addition of sodium chloride. A sharp bout of diarrhoea occurred within the first 2½ hr on both occasions, suggesting that the ingested solution had been rapidly distributed through the entire length of the alimentary tract.

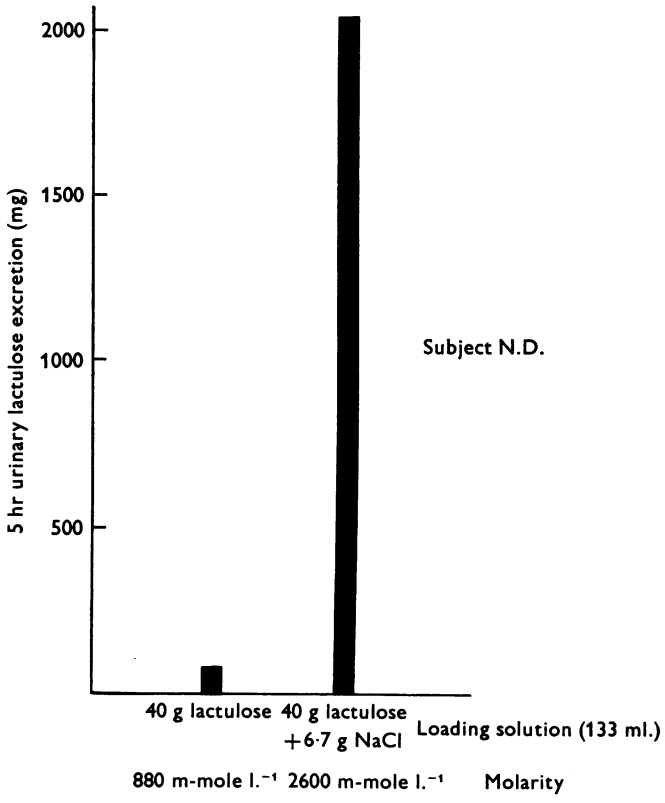

Fig. 4. The effect of an increase in the molarity of a loading solution containing a laxative dose of lactulose. The major solute used is sodium chloride.

Plasma levels of lactulose after isotonic and hypertonic loads. Two subjects ingested isotonic (10 g lactulose in 150 ml. water; 195 m-mole $l.^{-1}$) and hypertonic loads (10 g lactulose and 21 g urea in 150 ml. water; 2528 m-mole $l.^{-1}$)

(*a*) to show the relationship between plasma lactulose levels and the rate of lactulose excretion,

(*b*) to establish the time course of lactulose excretion in the urine.

These solutions were ingested and plasma samples taken from a forearm vein at 30, 60, 90, 150, 210, 270, and 375 min after ingestion of the hypertonic load and 15, 30, 60, 90, 150, 210 and 270 min after ingestion of the isotonic load. Hourly urine collections were made for the first 5 hr followed by two 2½ hr collections. The results of these experiments are shown in Figs. 5 and 6. Blank samples showed no lactulose present in plasma before

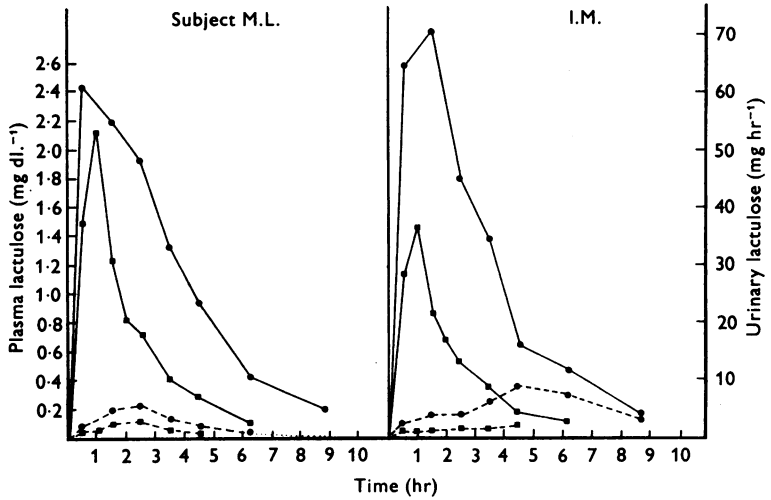

Fig. 5. Plasma levels of lactulose (■) plotted with rates of urinary excretion (●) after ingestion of 195 m-mole (– – –) and 2528 m-mole (——) solutions. 150 ml. of each solution was ingested by the subjects.

the ingestion of a load. Lactulose excretion is shown to be maximal during the first hour in the subject M.L. and between 1 and 2 hr in the subject I.M. Plasma levels closely parallel urinary excretion rates in both subjects.

Recovery of intravenously injected lactulose. 2 g lactulose was injected into a forearm vein of two subjects and blood was collected at 15, 30, 60, 90, 120, 150, 210, 270 and 375 min after injection from the opposite forearm vein. Urine collections were made hourly for 5 hr followed by three 2½ hourly collections. Plasma lactulose levels are plotted with excretion rates of lactulose in Fig. 7. Total recoveries of lactulose were M.L. 2·022 g (101·1%) and I.M. 1·978 g (98·8%). These experiments show that the previous low recoveries of lactulose are due to poor absorption rather than impaired renal excretion.

DISCUSSION

The present study shows that the fraction of lactulose excreted in the urine of healthy subjects following the ingestion of a lactulose-containing solution increases if it is made sufficiently hypertonic by addition of various

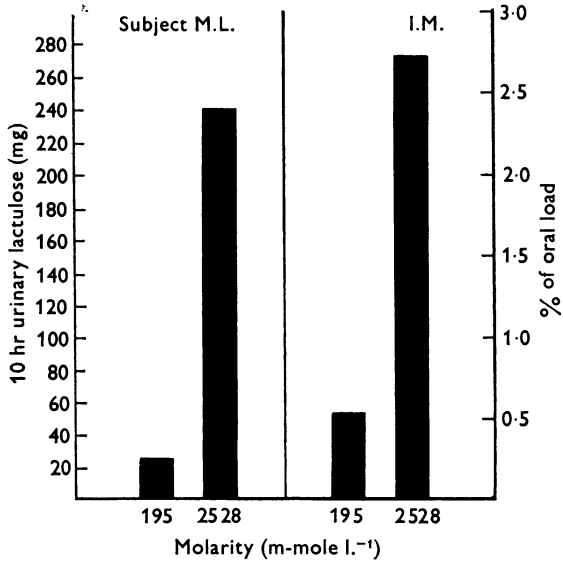

Fig. 6. Histogram showing total 10 hr excretion of lactulose after the ingestion of isotonic (195 m-mole) and hypertonic (2528 m-mole) solutions. This data is obtained from Fig. 5.

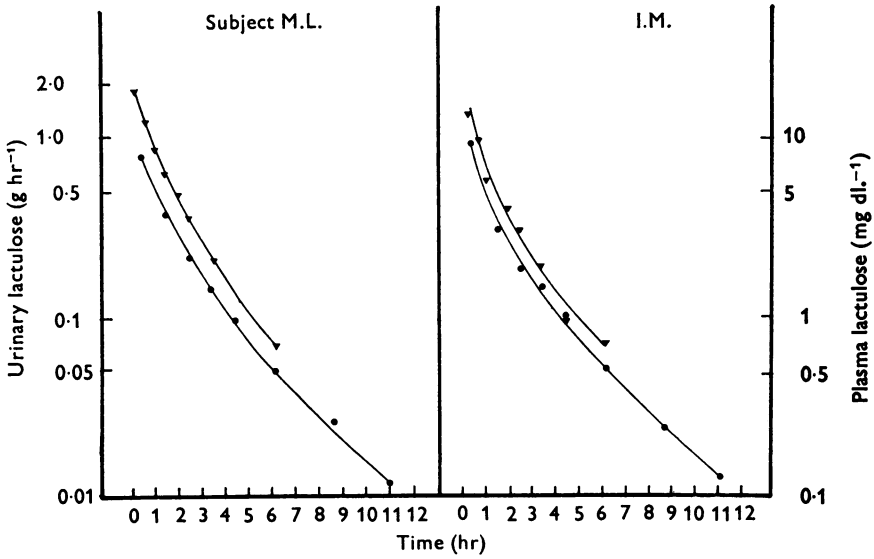

Fig. 7. Plasma levels (▼) and urinary excretion rates (●) of lactulose following intravenous injection of 2·0 g lactulose at time 0. Total recoveries of lactulose were M.L. 1·101 %, I.M. 98·8 %.

solutes. Though several individuals did not show an obvious increase in lactulose excretion in response to the range of hypertonic sucrose-containing solutions studied (see Fig. 1), statistical analysis of the whole group data shows significant regression. Apparently unresponsive individuals would probably show some change if given solutions of greater tonicity than those used in the present study. The subject I.M., who showed no change over the range of hypertonic sucrose loads (maximum molarity 2029 m-mole $l.^{-1}$), demonstrated a marked increase in lactulosuria, shown in Fig. 3, when tested with a series of urea-containing solutions extending to higher levels of molarity (maximum 2528 m-mole $l.^{-1}$), and the subject M.L. demonstrated a similar marked lactulosuria after ingestion of a 2528 m-mole $l.^{-1}$ solution (see Figs. 5 and 6).

The phenomenon demonstrated cannot be due to a change in metabolism because excretion of intravenously injected lactulose in the urine is, like most other disaccharides, rapid and complete (Menzies, 1974). Variations in renal clearance are also excluded as a cause, since the rate of urinary lactulose excretion closely follows the plasma concentration whether this is after I.V. administration (see Fig. 7) or ingestion of both isotonic and hypertonic lactulose-containing solutions (see Fig. 5). It must therefore be attributed to an increase in lactulose absorption from the intestinal lumen, induced in some way by the ingestion of a hypertonic solution. A reaction between specific solutes is not involved because several different solutes (sucrose, glucose, glycerol, mannitol, urea and sodium chloride) are capable of inducing this effect, and intestinal absorption of other non-metabolizable carbohydrates such as raffinose (Menzies, 1974; Udupihille, 1974) stachyose and dextran (Wheeler, Menzies & Creamer, 1976) can also be increased by the ingestion of hypertonic solutions.

Less than 1 % of ingested lactulose is normally absorbed and subsequently excreted in the urine. This indicates a very slow rate of transfer across the intestinal wall, which has been shown to be proportional to the concentration gradient (Menzies, 1974). This, together with the discovery that rates of transfer for lactulose, raffinose and stachyose (di-, tri-, and tetrasaccharide respectively), in relation to increasing molecular weight, comply approximately with the Stokes equation for simple diffusion (Wheeler *et al.* 1976), suggest that transfer of this group of non-metabolizable oligosaccharides across the intestinal wall is a passive, non-mediated process.

Assuming a process of passive diffusion, increased absorption of lactulose from the intestinal lumen could theoretically be produced by an elevated concentration gradient, exposure to the absorptive surface for a longer period of time, distribution to a greater area of the absorptive surface, or increased permeability, per unit area, of the absorptive surface.

Each series of test solutions used in the present study was devised so

that a constant amount of lactulose would be ingested on each occasion at a controlled concentration, and it is difficult to see how the diffusion gradient for lactulose in the intestine could become increased as a result of the addition of a second solute. On the contrary, lactulose concentration should become reduced in the intestinal lumen by osmotic influx induced by the hypertonic solution (Fordtran, Rector, Ewton, Sotor & Kinney, 1965), though to different extents depending on the rate of solute absorption.

There is also the possibility that osmotically induced influx of fluid might hasten transit along the intestine: this would increase the intestinal surface exposed to the ingested solution, but at the same time might also reduce the time available for absorption as lactulose is rapidly fermented by bacterial action on reaching the colon. However, the experiment performed on N.D. suggests that none of these factors are responsible for the observed increase in absorption. This subject demonstrated a very marked (twenty-fourfold) increase in lactulose absorption when the test solution, which contained a purgative dose of lactulose (40 g), was made hypertonic by addition of sodium chloride. A sharp bout of diarrhoea occurred within 2·5 hr of ingesting the control and hypertonic test solutions, which implies that transit through the entire alimentary tract had been equally rapid on both occasions. The only remaining obvious possibility is that intestinal permeability becomes increased following the ingestion of sufficiently hypertonic solutions.

As to the anatomical site of altered permeability, an intubation study undertaken by Udupihille (1974) in this hospital demonstrated that the degree of lactulosuria following ingestion of hypertonic lactulose-containing solutions tends to correlate with duodenal rather than gastric osmolality, suggesting that the effect arose distal to the pylorus. Patients suffering from coeliac disease, in which the lesion is confined to the small intestine, show a characteristically exaggerated response to the ingestion of hypertonic lactulose solutions (Menzies, 1974), which also suggests that the phenomenon is of small intestinal rather than gastric origin. Consistent differences of individual susceptibility to this effect, noted in the present study, could therefore be due to individual patterns affecting the rate of gastric emptying, dilution by gastrointestinal secretion and osmotic influx of fluid into the intestine, rate of transit through the intestine and response of the absorptive surface to contact with hypertonic solutions. The elucidation of all these factors will require a comprehensive series of intubation studies.

The transfer of water soluble substances across the intestinal wall has been postulated to take place by a process of diffusion through water-filled pores present in the lipoidal surface membrane of the mucosal cells (Fordtran *et al.* 1965). Höber & Höber (1937), investigating absorptive mecha-

nisms in rats, correlated transfer rates of non-lipid soluble substances with their molecular size. They found that there was virtually no absorption of compounds with a molecular weight greater than 180, which corresponds to a molecular radius of 0·4 nm. This work was independently confirmed by Lindemann & Soloman (1962). Fordtran *et al.* (1965) investigated intestinal permeability in man by measuring water flow into the intestinal lumen induced by the osmotic action of hypertonic solutes of different molecular size, and calculated that the upper limit of effective pore radius to be between 0·67 and 0·88 nm for jejunum and between 0·30 and 0·38 nm for ileum.

While it is possible that lactulose and raffinose, with molecular radii of about 0·5 and 0·8 nm respectively, might pass very slowly through the jejunal pores described by Fordtran *et al.* (1965), another pore of larger size but lower incidence may also be involved. Thus, Wheeler *et al.* (1976) have demonstrated human intestinal permeability to a dextran of molecular weight 3000, which can be enhanced by ingestion of hypertonic solutions and others have found some permeability of rat intestine to substances up to a molecular weight of 40,000 (Cornell, Walker & Isselbacher, 1971; Warshaw, Walker, Cornell & Isselbacher, 1971). It is therefore possible that the ingestion of hypertonic dietary constituents, such as sucrose and other sugars that are both popular and soluble, could increase the absorption of unrelated compounds, some of which may have potentially harmful effects (e.g. carcinogens and antigens). The characteristically exaggerated response of even mild and well treated patients with coeliac disease to ingestion of hypertonic solutions (Menzies, 1972*a*, *b*), invites the speculation that abnormal permeability induced in this fashion might, by facilitating access of gluten fractions to the intestinal mucous membrane, be one factor of aetiological importance.

Increased intestinal permeability induced by the ingestion of a hypertonic solution is of short duration. This has been shown by using lactulose and raffinose as consecutive markers and demonstrating that increased permeability induced by hypertonic urea returns to normal within 2·5 hr (Menzies, 1972*c*). Ussing (1966, 1969), who reported that isolated frog skin underwent a similar temporary increase in permeability when exposed to hypertonic solutions, has postulated that the mechanism may be due to an alteration in the condition of the intercellular 'tight seals' or ('tight junctions'), which become leaky under these circumstances. The applicability of this hypothesis to the intestinal phenomenon awaits verification.

It is with grateful thanks that we acknowledge the willing co-operation given by Dr Neill Davidson and other members of the staff of the Department of Chemical Pathology, and other colleagues in St Thomas' Hospital, for participating in the sugar loading experiments described, and to Dr J. S. M. Hutchinson for help with the

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