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Gas chromatographic method for detection of urinary sucralose: application to the assessment of intestinal permeability

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Abstract

We developed a capillary column gas chromatography (CCGC) method for the measurement of urinary sucralose (S) and three other sugar probes including, sucrose, lactulose (L) and mannitol (M) for use in in vivo studies of intestinal permeability. We compared the capillary method with a packed column gas chromatography (PCGC) method. We also investigated a possible role for sucralose as a probe for the measurement of whole gut permeability. Sample preparation was rapid and simple. The above four sugars were detected precisely, without interference. We measured intestinal permeability using 5- and 24-h urine collections in 14 healthy volunteers. The metabolism of sugars was evaluated by incubating the intestinal bacteria with an iso-osmolar mixture of mannitol, lactulose and sucralose at 37 °C for 19 h. Sugar concentrations and the pH of the mixture were monitored. The use of the CCGC method improved the detection of sucralose as compared to PCGC. The average coefficient of variation decreased from 15% to 4%. It also increased the sensitivity of detection by 200–2000-fold. The GC assay was linear between sucralose concentrations of 0.2 and 40 g/l (r=1.000). Intestinal bacteria metabolized lactulose and acidified the media but did not metabolize sucralose or mannitol. The new method for the measurement of urinary sucralose permits the simultaneous quantitation of sucrose, mannitol and lactulose, and is rapid, simple, sensitive, accurate and reproducible. Because neither S nor M is metabolized by intestinal bacteria, and because only a tiny fraction of either sugar is absorbed, this pair of sugar probes appears to be available for absorption throughout the GI tract. Thus, the 24-h urinary concentrations of S and M, or the urinary S/M ratio following an oral dose of a sugar mixture, might be good markers for whole gut permeability.

Keywords: Intestinal permeability; Intestinal bacteria; Sucralose; Mannitol; Lactulose; Sucrose

1. Introduction

The most direct and accurate method for the evaluation of intestinal barrier integrity is the measurement of intestinal permeability. This can be done

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by measuring the excretion of orally ingested macromolecular probes [1,2]. The most-widely used probes are sugars like sucrose, mannitol (M), cellubiose, and lactulose (L), which are absorbed from different regions of the gastrointestinal (GI) tract in trace amounts and are excreted unchanged in the urine [3–5]. Following an oral test dose of sugars, the urinary level of sucrose selectively reflects permeability of the gastroduodenal mucosa since sucrase degrades this sugar in more distal regions of the GI tract [4]. Because the small bowel brush border digests neither lactulose nor mannitol, these two sugars are used in combination to assess small intestinal permeation [6–10]. Mannitol and lactulose excretion are thought to be good markers of permeation via transcellular and paracellular pathways, respectively. The lactulose to mannitol (L/M) ratio is considered to be a sensitive and accurate marker of small bowel permeability [11]. Sucralose (S), a chlorinated sugar that is used as a sweetener, has recently been introduced as a probe for assessment of intestinal permeability [12]. Like lactulose, sucralose is passively absorbed across the intestinal mucosa, possibly through the paracellular pathways, and is excreted unchanged in the urine [13–17]. Since sucralose, which has a molecular mass comparable to that of lactulose, has a limited ability to passively diffuse across normal intestinal mucosa, and is not metabolized by bacteria in the colon, the urinary excretion of sucralose might be used to estimate whole gut (small bowel+colon) permeability [12].

Urinary lactulose and mannitol have been quantitated by several methods including paper and thin layer chromatography [18,19], enzymatic assays [20–23], gas chromatography [7,8,24–26] and HPLC [3,27–32]. Previous reports indicate that sucralose can be quantitated by HPLC [12,31,33]. Despite the availability of these methods, the high degree of technical expertise required to perform them has precluded the widespread clinical use of sugar permeability tests for the assessment of gut barrier function.

To overcome this obstacle, we have developed two simple gas–liquid chromatographic methods for the simultaneous quantitation of urinary concentrations of sucralose, mannitol, lactulose and sucrose. The first method involves gas chromatography of trimethyl silyl derivatives using packed column gas chromatography (PCGC), which has previously been used by us for measurement of urinary mannitol and lactulose in humans [34,35] and rats [36]. The second method is a new technique in which the silyl derivatives are analyzed by gas chromatography on a capillary column (CCGC). In this report, we have compared the sensitivity and precision of these two methods. In addition, we have used the CCGC method to measure the urinary concentrations of sucralose, mannitol and lactulose and to evaluate gut barrier function in a group of healthy volunteers that consumed a test dose of the four sugar probes.

2. Experimental

2.1. Materials

Lactulose (4-*o*-β-D-galactopyranosyl-D-fructofuranose) was obtained from Bertek Pharmaceuticals (Morgantown, WV, USA), as Kristalose. Mannitol (D-mannitol) and sucrose (α-D-glucopyranosyl-β-D-fructofuranoside) were obtained from Sigma (St. Louis, MO, USA). Sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-glucopyranoside) was supplied by Johnson & Johnson (New Brunswick, NJ, USA) under the brand name of Splenda. Phenyl β-D-glucoside, myo-inositol (meso-inositol), hydroxylamine hydrochloride and pyridine were purchased from Sigma. *N*-Trimethylsilylimidazole was supplied by Pierce (Rockford, IL, USA).

2.2. Equipment

Gas chromatography was performed using a Hewlett-Packard instrument HP5890A (Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The packed column was a 6-foot×2 mm I.D. 1/4 in. glass column packed with 3% SE-30 on 80/100 chromosorb WHP (Supelco, Bellefonte, PA, USA). The capillary column was a 15 m×530 μm I.D. fused-silica capillary column with a 1.5-μm film thickness of the chemically bonded phase DB-1 (J&W, Folsom, CA, USA).

2.3. Subjects

Fourteen healthy individuals were enrolled in the study. The study was approved by the Institutional

Review Board of Rush Medical College. Informed consent was obtained from each subject. The colonic bacteria for the sugar metabolism studies were obtained from a stool sample from a healthy volunteer.

2.4. Sugar ingestion and urine collection

The sugar permeation test was begun at 8:00 AM, following an 8-h fast. Each subject emptied her/his bladder completely and the collection from this voiding was used as a baseline sample (blank). The subject drank 150 ml of water that contained 7.5 g lactulose, 2.0 g mannitol and 40 g of sucrose and swallowed four capsules each containing 250 mg of sucralose. Thereafter, all of the urine passed for the next 5 h was collected into one container (5-h sample), and the urine passed for the next 19 h was collected into the second container (19-h sample). During the collection period, the baseline sample, the 5-h collection and the 19-h collections were preserved by the addition to the collection vessels of 10, 100 or 500 mg, respectively, of sodium fluoride. After collection, urine volumes were recorded. Taking a 10-ml sample of the 5-h urine collection, the 5-h urine collection was added to the 19-h urine collection to make-up the 24 h (24-h) urine collection and 10-ml aliquots of baseline, 5-h and 24-h urine collections were stored at −20 °C for subsequent analysis.

2.5. Sample preparation

2.5.1. PCGC samples

Urine samples were thawed and mixed using a vortex. One ml urine was transferred to an Ultrafilter-CL centrifugal filter vial (NMW 30,000; Millipore Corporation, Bedford, MA, USA) and centrifuged at 2250 rev./min for 20 min. The filtrate was mixed in a glass test tube with 40 μl of an internal standard containing 20 mg/ml of phenyl beta-D-glucoside, and 20 mg/ml of myo-inositol, and the mixture was evaporated to dryness at 70 °C under a stream of nitrogen. Standards consisting of human urines containing known amounts of the four sugars were prepared and analyzed in parallel. The dried residues were taken up in 200 μl of anhydrous pyridine containing 25 mg/ml of hydroxylamine, mixed, heated at 70 °C for 1 h, and centrifuged at 2250 rev./min for 5 min. An aliquot (100 μl) of the supernatant was transferred to a small conical tube and the sugar oximes were silylated with 100 μl of *N*-trimethylsilylimidazole for 30 min at 70 °C. An aliquot (100 μl) of the silylated derivatives was sealed in an autosampler vial for testing.

2.5.2. CCGC samples

An aliquot, 200 μl, of an unfiltered urine sample was mixed with 40 μl of internal standard in a glass test tube, and the mixture was evaporated to dryness. The rest of the sample preparation was identical to that used for the packed column method.

2.6. Chromatography

2.6.1. PCGC method

During chromatography, the detector temperature was 280 °C and the injector temperature was 250 °C. The initial column temperature of 220 °C was held for 2 min and then raised initially 10 °C/min for 2 min, 5 °C/min for 4 min and 3.5 °C/min for 4 min to a final temperature of 274 °C, which was held for 2 min. The total run time was 14 min. Hydrogen and air were used for flame ionization detection. The carrier gas was nitrogen at a flow-rate of 30 ml/min. The injection volume was 4 μl. The location of each sugar was identified by the retention time of the corresponding standard and the amount of each sugar in the sample was calculated from the ratio of its peak height to that of the internal standard. Under these conditions, mannitol, sucrose, sucralose and lactulose had retention times, respectively, of 2.3, 9.0, 9.3 and 10.8 min (Fig. 1a).

2.6.2. CCGC method

The detector and injector temperatures were the same as for the packed column method. The initial temperature of 220 °C was held for 5 min and then increased at 10 °C/min for 2 min, 5 °C/min for 4 min and 3.5 °C/min for 4 min to a final temperature of 274 °C, which was held for 7 min. The total run time was 22 min. Hydrogen and air were used for flame ionization detection. The carrier gas was helium at a flow-rate of 10 ml/min. The injection volume was 1 μl. The amount of each sugar in the samples was calculated from the ratio of its peak area to that of the internal standard. Under these conditions, mannitol, sucrose, sucralose and lactulose

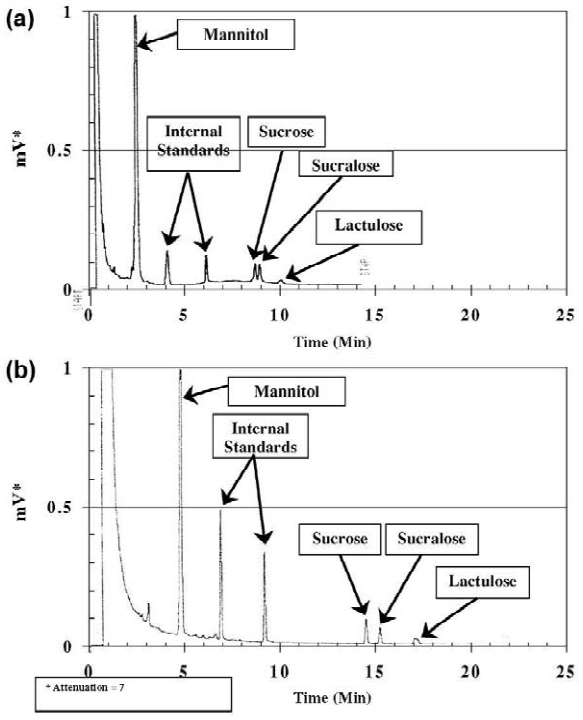

Fig. 1. Representative gas chromatograms of urinary sugars using a packed column (a) and a capillary column (b).

had retention times of 4.7, 14.5, 15.2 and 17.1 min, respectively (Fig. 1b).

2.7. Calculations

Concentrations of sucrose and lactulose were determined for the 5-h urine samples. Mannitol and sucralose were quantitated in both 5-h and 24-h urine samples. Because there was no restriction on the consumption of dietary sugar during the permeability test, urinary sucrose values could not be used as a marker for gastroduodenal permeability. However, we did use sucrose in the test sugar mixture to insure that it could be detected and to insure that the sucrose peak would not coelute with other peaks of interest. In each batch of analyses, we included eight different mixtures of urine-based standards to obtain calibration curves for each sugar. We calculated sugar concentrations in patients' urine samples based on these 8-point standard curves. The total amounts of each sugar in the 5-h and 24-h urine samples were expressed as percentages of the amounts of sugar that were ingested in the oral dose.

2.8. Comparisons

The PCGC and CCGC methods were compared according to several parameters.

2.8.1. Linearity

The linearity of each assay was evaluated by adding known amounts of each sugar to the urine and comparing the recoveries relative to internal standards. The concentrations of the standards were plotted against the detector response and analyzed by linear regression.

2.8.2. Analytical sensitivity and minimum detection limit

The detection limit was the smallest detectable amount of spiked sugar in a urine sample based on a signal-to-noise (S/N) ratio of 3.

2.8.3. Interference

Both methods were compared for resolution of four sugars and the two internal standard peaks.

2.8.4. Precision

The mean run-to-run and day-to-day variability was estimated using the coefficient of variation (C.V.) by dividing the standard deviation by the mean of the value for concentrations obtained after five runs (C.V. = SD/mean). The C.V. for each sugar was calculated using the average C.V. from various concentrations of that sugar in the calibration range.

2.9. Sugar degradation by intestinal flora

An iso-osmolar mixture of lactulose, mannitol and sucralose was prepared by dissolving 7.5 g lactulose, 1.66 g mannitol, and 1 g sucralose in 33.5 ml of distilled water. The amounts of these sugars were chosen to approximate the amount of a test sugar that would reach the cecum after an oral dose, assuming that 15% of the administered oral dose of mannitol, 0.1% of the lactulose and 1% of the sucralose would have been absorbed during passage through the upper GI tract and the small intestine. A solution of colonic bacteria containing $\sim 10^{10}$ microorganisms

per cubic millimeter was prepared by mixing 3.35 ml of human feces with 30.15 ml of buffered saline solution. A mixture of bacterial suspension and sugar solution (initial pH, 7.2) was incubated at 37 °C for 19.5 h (the approximate time of exposure of sugars to the colonic environment during an in vivo permeability test) with continuous gentle stirring. Samples were withdrawn at 0.5, 1, 1.5, 2, 3, 4.5, 6, 7.5, 10 and 19.5 h for measurement of pH (glass electrode) and sugar concentrations.

3. Results

3.1. Gas chromatography analysis

3.1.1. Linearity

The average r^2 of 0.989 was observed for the PCGC method. The average r^2 for the CCGC method approached 1.000 even though the calibration range was lower (Table 1).

3.1.2. Minimum detection limit

The detection limits for the CCGC method were much lower than those observed for the PCGC method. Thus, CCGC was estimated to be 2000-fold more sensitive to mannitol, 1000-fold more sensitive to sucralose and sucrose and 200-fold more sensitive to lactulose (Table 1).

3.1.3. Interference

All four sugars and the two internal standard peaks were resolved by PCGC (Fig. 1a). The resolution of sucrose and sucralose was improved by using CCGC (Fig. 1b). We found that the peak for the disaccharide lactose (a common dietary constituent) partially overlapped with that of lactulose (data not shown). Thus, it might be advisable for subjects to avoid consumption of dairy products on the day of the permeability test.

3.1.4. Precision

Within-run variability was small (<1%). The overall average C.V. for all sugars was 15% for the PCGC method but only 4% for the CCGC method (Table 1). The average C.V. for sucrose, lactulose and sucralose was improved by the CCGC method. The average C.V. for mannitol was lower using the PCGC method. However, the C.V. for mannitol in the PCGC method was based on values obtained in the calibration range of 0.5–40 g/l, while the C.V. for mannitol in the CCGC method was based on the values obtained in the calibration range of 0.01–40 g/l. Nevertheless, the C.V. for mannitol in the CCGC method remained in an acceptable range (3.45%).

3.2. Specimen collection and preparation

3.2.1. Specimen collection conditions

Because sugar permeability testing requires a 24-h urine collection, the urine containers are typically stored at room temperature during the collection period. Although the containers contained sodium fluoride as a preservative, it was possible that bacteria in the samples might metabolize the sugar probes and decrease the amount of sugar recovered. We evaluated this possibility by measuring the recovery of sugars in samples that were stored with preservative at room temperature for 1 or 4 days. We found that the concentrations of sugars recovered decreased minimally and linearly at a rate of 2.2% per day for mannitol, 1.3% per day for sucrose, 0.7% per day for sucralose and 1.6% per day for lactulose. These results showed that the ex-vivo changes in fluoride-preserved urine were minimal and should not be a significant source of variability in the assay.

3.2.2. Ultrafiltration of samples prior to PCGC

Ultrafiltration of the urine samples was required as a pretreatment step in the PCGC method in order to decrease the frequency of syringe clogging during injection. The mean (±SEM) recoveries of the sugars following ultrafiltration were 99±1.4% for mannitol, 95.7±6.79% for sucrose, 96.8±7.47% for sucralose and 94.6±4.56% for lactulose, with an overall average for the four sugars of 96.8±2.41%. One advantage of the CCGC method of analysis is that there is no need to do ultrafiltration on urine specimens as part of sample preparation.

3.2.3. Stability of TMSI derivatives

Overnight refrigeration or freezing of the TMSI sugar derivatives in their autosampler vials had no significant effect on sugar recovery. The mean (±SEM) absolute recoveries following overnight refrigeration were 99.0±1.9% for mannitol,

Table 1
Detection properties of packed column gas chromatography (PCGC) and capillary column gas chromatography (CCGC)

	Mannitol		Sucrose		Sucralose		Lactulose	
	PCGC	CCGC	PCGC	CCGC	PCGC	CCGC	PCGC	CCGC
Calibration range (g/l)	0.5–40	0.01–40	0.05–4	0.001–4	0.05–4	0.001–4	0.025–2	0.0005–2
Linearity (r^2)	0.999	1.000	0.996	1.000	0.982	1.000	0.980	1.000
Slope of regression line	0.089 (±0.001)	0.089 (±0.001)	0.0057 (±0.0001)	0.0071 (±0.0001)	0.0041 (±0.0001)	0.0048 (±0.0001)	0.00074 (±0.00001)	0.00040 (±0.00001)
Regression line intercept	0.082 (±0.081)	0.116 (±0.019)	−0.005 (±0.015)	0.0049 (±0.002)	−0.0009 (±0.014)	0.0024 (±0.001)	−0.027 (±0.009)	0.005 (±0.001)
Limit of detection*	2 μg	1 ng	0.2 μg	0.2 ng	0.2 μg	0.2 ng	0.1 μg	0.5 ng
Detection limit (mg/l)	500	1	50	0.2	50	0.2	25	0.5
Coefficient of variation (%)	0.65	3.45	18.1	4.04	14.6	4.24	26.6	4.06

*Amount per injection

Table 2
Excretion fraction (mean percent±SEM) of sugar probes in 5- and 24-h urine collections in 14 healthy volunteers

Mannitol 5 h	Lactulose 5 h	Sucralose 5 h	L/M ratio 5 h	S/M ratio 5 h	Mannitol 24 h	Sucralose 24 h	S/M ratio 24 h
14.2% (±1.32%)	0.16% (±0.02%)	1.03% (±0.15%)	0.013 (±0.002)	0.072 (±0.007)	26.4% (±2.79%)	2.32% (±0.33%)	0.090 (±0.010)

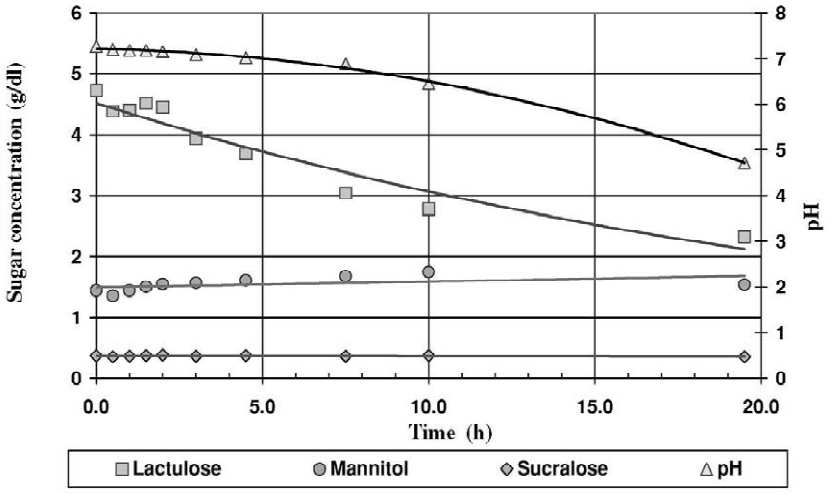

Fig. 2. Degradation of sugar probes by fecal flora.

91.4±1.28% for sucrose, 96.0±3.56% for sucralose and 92.0±3.39% for lactulose, with an overall average of 94.6±2.5%. The mean (±SEM) absolute recoveries of the sugars following freezing of the prepared samples in their autosampler vials were 99.3±1.84% for mannitol, 91.2±6.69% for sucrose, 97.1±10.87% for sucralose and 91.9±4.36% for lactulose, with an overall average of 94.9±5.94%.

3.3. Incubation of sugar probes with intestinal flora

Incubation of a mixture of sugars with colonic bacteria resulted in a 51% decrease in the lactulose concentration, which was accompanied by a gradual decrease in the pH of the mixture (Fig. 2). This shows that the bacteria were viable and metabolically active during the incubation. In contrast to lactulose, the concentrations of mannitol and sucralose did not change significantly during the incubation. Because metabolically active colonic bacteria do not metabolize mannitol or sucralose, these probes are probably available throughout the small and large bowel for passive permeation.

3.4. Intestinal permeability in healthy controls

We studied 14 healthy subjects (10 female, and four male). Their average age was 49. The excretion fractions of mannitol, sucrose, sucralose and lactulose were calculated in 5- and 24-h urine collections using CCGC (Table 2). The lactulose to mannitol (L/M) ratio in 5-h urines (the most widely used marker of small intestinal permeability) and the S/M ratio in 24-h urines (a potential marker of whole gut permeability) are presented in Table 2.

4. Discussion

The assessment of small intestinal permeability using L/M ratios has been an important tool in many

research and clinical studies. This has been particularly true in recent years as the importance of GI barrier function in GI and systemic disorders has become more widely appreciated [37,38]. The use of minimally absorbed, non-metabolized sugars as probes for the assessment of barrier function is convenient and non-hazardous [9,39]. Thus the establishment of an accurate and convenient method of analysis for urinary sugars is both feasible and a matter of great importance. Gas chromatography of silyl derivatives of sugars on packed columns has been one method that provides for the analysis of low concentrations of carbohydrates in many biological samples [7,8]. We have shown that the adaptation of this approach to capillary column-based separation provides even greater sensitivity for sucralose and other sugars and permits the use of smaller volumes of urine in the analysis. Also improved is the resolution of sucralose from any sucrose that may be present in clinical samples of subjects consuming a regular diet during urine collection.

Meddings and co-workers have used HPLC for quantitation of sucralose for the assessment of intestinal permeability [12,31,33]. Although the technical aspects of this method were not described in detail, sucralose could not be measured under the same conditions used for the other sugars [31]. Sensitivity for detecting lactulose by HPLC has been reported and it is comparable to the sensitivity for lactulose in our method (0.5 mg/l) [27,30]. More important, our method has even greater sensitivity (0.2 mg/l) for detection of sucralose and we were able to easily detect sucralose both in vitro and in vivo in urine samples from healthy subjects with intact intestinal integrity. In addition, all sugars could be detected under the same conditions in a single run. Other advantages of the CCGC method include simple and rapid sample preparation and ability to store samples for later analysis.

Our in vitro data show that the CCGC method improves the sensitivity and accuracy of sugar detection as compared with the PCGC method. For example, CCGC is 200–2000 times more sensitive than PCGC for the detection of urinary sugars. The CCGC method could detect as little as 0.2 ng sucralose per injection, which corresponds to a urinary sucralose concentration of 0.2 mg/l. The precision of the CCGC method was also significantly better than that obtained with PCGC. Moreover, the CCGC technique did not require pretreatment of urine samples with ion-exchange resin or ultrafiltration prior to derivatization, because significantly smaller urine volumes were taken for analysis.

We also found that the new method was resistant to the effects of several confounding factors that might influence the results of intestinal permeability measurement. For example, collection and storage of fluoride-preserved urine samples at room temperature did not significantly affect the urine sugar concentrations. The storage of the urine specimens at room temperature during collection makes the procedure more practical and convenient. Furthermore, TMSI derivatives prepared as described above were stable overnight at refrigerator temperature and/or for days when frozen, making it possible to prepare larger batches of samples and store them for later analysis.

Sucralose is a synthetic disaccharide with a molecular mass similar to that of lactulose. It is claimed that sucralose, unlike lactulose, could not be metabolized by intestinal bacteria. Indeed, in a rat model, sucralose was not metabolized by colonic bacteria, and was therefore a suitable probe for measurement of whole gut permeability [12]. We were unable to find published data to support this claim in man. Our experiment showed that similar to rat, human colonic bacteria do not metabolize sucralose, suggesting that an oral dose of sucralose would be available for absorption throughout the whole gut in man. Our data also showed that mannitol is not metabolized to any significant extent by human colonic bacteria and could therefore serve as a second marker for assessment of whole gut permeability. In addition, parallel to sucralose, almost half of the 24-h excreted mannitol appeared in the 5-h urine while the other half appeared in the 19-h urine, suggesting that the absorption of mannitol was not limited to the small bowel and was continued throughout the colon.

Several investigators have described the limitation of using a single probe for the measurement of intestinal permeability. This is mainly due to other factors that could affect the concentration of sugar probes in the urine, including: the intestinal transit time, volume of distribution of the sugars, glomerular filtration rate, and the completeness of urine

collection. In the past, these factors have been addressed by the administration of two sugar probes of different molecular sizes and the use of the ratio of the urinary levels of the two probes as an index of increased permeation. We now suggest that sucralose and mannitol probes can be used in a similar fashion (sucralose/mannitol or S/M ratio) to provide a more accurate measure of whole gut permeability.

Our results for the excretion of sugars in 5-h and 24-h urine collections from 14 healthy volunteers showed that our data of 5-h L/M ratio was similar to that reported in other published studies [11,34,35]. We could find no reports for comparison of 5-h and 24-h urinary sucralose values. However, Meddings et al. measured urinary sucralose in an overnight urine collection after the ingestion of 2 g sucralose by 19 healthy volunteers and showed that a total of 41.1 ± 14.4 mg sucralose (2% of the ingested dose) was excreted [31]. Although, not fully equivalent, our data suggests that 2.32% of an ingested dose of sucralose is excreted in the 24-h urine collection.

Additional studies are needed to further establish the validity of the S/M ratio as a marker of whole gut permeability, and to determine if this ratio is helpful in the diagnosis/prognosis of various GI disorders, especially those that are associated with intestinal hyperpermeability. If the 5-h L/M ratio can be shown to correlate with the 5-h S/M ratio, it might make unnecessary the need to use lactulose as a test sugar, and in turn, decrease unpleasant lactulose-related side effects (GI upset, bloating and loose stools). However, the relative utility of the two measures (L/M ratio versus S/M ratio) would need to be tested in each different intestinal disorder since the alteration of colonic pH that is normally induced by lactulose might modify the nature of the colonic microflora and thereby modulate intestinal permeability [40].

5. Summary

We developed a new method for the measurement of urinary sucralose. Our method is practical, reproducible, accurate and highly sensitive. The method detects urinary sucralose, a promising marker of whole gut permeability, and also improves the measurement of urinary concentrations of mannitol, lactulose and sucrose—the sugar probes that are currently used for the evaluation of gastrointestinal permeability in humans and animals.

Since colonic bacteria metabolize neither sucralose nor mannitol, this pair of sugar probes might be used for the assessment of whole gut permeability either by calculation of the excretion fraction of each probe or by expressing the results in terms of a sucralose to mannitol (S/M) ratio.

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