Automated Enzymatic Assay for the Determination of Sucrose in Serum and Urine and Its Use as a Marker of Gastric Damage

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Sucrose has been used as probes for intestinal damage because healthy gastrointestinal tissue, unlike damaged mucosa, is almost totally impermeable to disaccharides. Sutherland et al. proposed the use of sucrose, which is degraded in the small intestine by a specific disaccharidase, for the detection of mild upper gastrointestinal damages. Therefore, even with intestinal disorders, the presence of sucrose in urine or blood after oral ingestion is indicative of gastric permeability. At a cutoff of 180 mg sucrose/5 h urine, the test has a specificity of 84% for the detection of gastric ulcers and a sensitivity of 96% for normal endoscopy. The presence of Helicobacter pylori does not substantially affect the permeability to sucrose.

In children, the specificity and sensitivity for gastric damage were 90% and 83%, respectively. This assay was also applied to patients suffering from malaria and used in various animal studies.

The practicability of the test is limited by the precision of urine collection and the analytical method used for the detection of sucrose in urine. Sucrose measurement in serum instead of urine could improve the test and allow a faster, more accurate diagnostic tool; however, its concentration in this biological fluid is low.

Sucrose can be measured in biological fluids by HPLC with electrochemical/aperometric detection, gas-liquid chromatography, and colorimetric assay; these methods, however, are slow and/or insensitive. Two enzymatic assays have been described, but they were not used for sucrose in serum. In 1984, Birnberg and Brenner described the measurement of sucrose in plant extracts with the following sequence of reactions:

\[
\begin{align*}
\text{Sucrose} & \quad \xrightarrow{\text{phosphorylase}} \quad \text{Glucose-1-phosphate + fructose} \\
\text{Glucose-1-phosphate} & \quad \xrightarrow{\text{Phosphoglucomutase}} \quad \text{Glucose-6-phosphate} \\
\text{Glucose-6-phosphate} & \quad + \text{NAD}^+ \quad \xrightarrow{\text{Glucose-6-dehydrogenase}} \quad 6\text{-phosphogluconate} + \text{NADH} + \text{H}^+ \quad (340 \text{ nm})
\end{align*}
\]

We describe here a modification of this method and its adaptation for the determination of sucrose in serum and urine.

We obtained sucrose phosphorylase (from Leuconostoc mesenteroides; sucrose:orthophosphate \(\alpha\)-d-glucoosyltransferase; EC 2.4.1.7; cat. no. S7760), phosphoglucomutase (from chicken muscle; \(\alpha\)-b-glucose 1,6-phosphomutase; EC 5.4.2.2; cat. no. P6156), glucose-6-phosphate dehydrogenase (from Leuconostoc mesenteroides; \(\alpha\)-b-glucose 6-phosphate:NAD(P)\(^+\) 1-oxidoreductase; EC 1.1.1.49; cat. no. G8404), NAD\(^+\) (\(\beta\)-nicotinamide adenine dinucleotide from yeast; cat. no. N1511), fructose, maltose, bilirubin, caffeine, and other drugs from Sigma Chemical Co. All other reagents were from Fisher Scientific.

To prepare calibrators at 1400 \(\mu\)mol/L, 20 \(\mu\)L of a 2.40 g/L sucrose solution in deionized water was added to 1.0 mL of a serum pool made from leftover specimens from fasting subjects or to 1.0 mL of freshly voided urine. Calibrators were prepared at 700, 350, or 175 \(\mu\)mol/L by serial dilution with the serum pool or urine. The phosphate-magnesium buffer was prepared by dissolving 3.5 g of \(K_2HPO_4\) and 664 mg of \(MgSO_4\cdot7H_2O\) in deionized water, and then bringing the final volume to 1 L with deionized water to obtain final concentrations of 20 mmol/L \(K_2HPO_4\) and 2.7 mmol/L \(MgSO_4\). The pH was adjusted to 7.0 with phosphoric acid. For the NAD-buffer solution, 66 mg of NAD was dissolved in 100 mL of phosphate-magnesium buffer to obtain a 1 mmol/L solution. For the enzyme reagent, 80 \(\mu\)L of the phosphoglucomutase suspension and 6.5 \(\mu\)L of the glucose-6-phosphate dehydrogenase suspension were added to 10 mL of the NAD-buffer solution. This reagent was stable for at least 9 weeks at 4 °C. For the sucrose phosphorylase solution, 1 mL of deionized water was added to the lyophilized powder. This solution was diluted 1/10 with the phosphate-magnesium buffer to obtain a final enzyme activity of 10000 U/L. The enzyme is stable for at least 3 days at 4 °C or can be kept at −20 °C for several months.

We used an automated centrifugal analyzer, the Cobas Fara II (Roche Analytical Instruments, Inc.) at 30 °C. The enzyme reagent is used as the main reagent (125 \(\mu\)L) and

References
the sucrose phosphorylase final solution as the starting reagent (10 μL). Sample volume was 5 μL. The absorbance at 340 nm (A_340) was measured 10 times at 60-s intervals starting at 0.5 s. The 6th and 10th readings are used by the instrument for calculation of the change in absorbance (kinetic mode). The detailed instrument settings are available from the author (B.V.).

The final concentrations in the reaction media were 0.5 mmol/L NAD^+, 10 mmol/L KH₂PO₄, pH 7.0, 1.35 mmol/L MgSO₄, 4000 U/L phosphoglucomutase, 1250 U/L glucose-6-phosphate dehydrogenase, 400 U/L sucrose phosphorylase, and 20 mL/L sample.

Volunteers were subjected to the sucrose test before and after gastric irritation. The eligibility criteria for volunteers were that they be 18–70 years of age, without digestive symptoms or known past pathology, and free of alcohol consumption and antiinflammatory drugs for the last 2 weeks. The research protocol was approved by our local ethics committee. In the sucrose permeability test (administered to the same subjects on 2 consecutive days, before and after gastric irritation), fasting (>4 h) subjects ingested 100 g of sucrose in 450 mL of water within 15 min. Blood samples were collected from a venous catheter every 15 min from 0 to 75 min after the beginning of the test. Urine was collected for 5 h. Gastric irritation was produced by ingestion of 600 mg of acetylsalicylic acid, followed 20 min later by 50 mL of vodka [400 mL/L (69 mmol/L) ethanol]. The sucrose solution was then administered immediately. Serum and urine samples were kept at ~20 °C before analysis.

Phosphate in the range of 5 to 15 mmol/L had little effect on enzyme activity. A final concentration of 10 mmol/L was selected to provide enough phosphate for the phosphorylation of sucrose and to represent a plateau where phosphate from the sample should have minimal effect. A final Mg^{2+} concentration between 1 and 20 mmol/L had no effect on enzymatic activity.

The presence of a biological fluid (serum or urine) in the reaction mixture increased the reaction rate when compared with the reaction in the presence of water (Fig. 1A). This may represent the effect of a co-factor that is not fully optimized when water alone is present. In the absence of sucrose phosphorylase, there was essentially no increase in the absorbance at 340 nm (not shown), indicating that the increase of the rate with urine or serum was not produced by a secondary reaction producing NADH. In all cases, a lag phase was observed, which occurs for ~3 min. Therefore, to be in the linear portion of the reaction kinetics, a delay time of 5 min was selected before starting the measurement period, which occurs over a 4-min period (between the 6th and 10th readings).

The calibration curves in various media were linear, with r values of 0.9998 and 0.9980 for urine and serum, respectively. The linearity was to at least 1400 μmol/L. Because of differences in the response of the enzymatic system with the different types of biological samples, we recommend using calibrators prepared in the corresponding media (serum or urine). Because some sucrose may be present endogenously in some serum specimens, we recommend measuring sucrose in the individual specimens used to prepare the pool.

For serum, the intrarun imprecision (CV) was 2.9%
Table 1. Interference study.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Final concentration</th>
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<tbody>
<tr>
<td>Urea</td>
<td>100 mmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>50 mmol/L</td>
</tr>
<tr>
<td>Fructose</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>Lactose</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>Maltose</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>0.1 mmol/L</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.1 mmol/L</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>2 mmol/L</td>
</tr>
<tr>
<td>Phenobarbital (Na)</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1 mmol/L</td>
</tr>
<tr>
<td>Salicylate (Na)</td>
<td>20 mmol/L</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.1 mmol/L</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>0.5 g/L (Hb; serum only)</td>
</tr>
<tr>
<td>Lactescene</td>
<td></td>
</tr>
</tbody>
</table>

* These substances, added at the stated concentrations in serum and urine, were shown not to interfere with the assay. The sucrose concentration in serum and urine was 350 μmol/L for this study.

(132 ± 3.82 μmol/L, n = 12); for urine the CV was 5.9% (96 ± 5.66 μmol/L, n = 12). The lower limit of detection (3 SD) was 12 μmol/L for serum and 18 μmol/L for urine. The day-to-day precision was determined at three concentrations on seven different days with the same samples kept at 4 °C. The between-days CVs for serum were 8.3% (146 μmol/L), 6.8% (394 μmol/L), and 7.6% (801 μmol/L) for each 1 mmol/L ethanol. During the study, volunteers ingested alcohol to produce gastric irritation. Blood ethanol was measured by gas chromatography and never exceeded 5 mmol/L; this concentration of sucrose: 0.3 ± 24 μmol/L, n = 25. Endogenous sucrose detected in serum specimens is of unknown origin, and at this time it is unclear whether it is an unknown interference or true endogenous sucrose. Because the method was shown to be free of interference from numerous drugs and serum constituents, the latter possibility should be preferred.

With sucrose added to urine specimens, correlation analysis yielded a slope of 0.937 (95% confidence limits, 0.890–0.984), an intercept of 8.8 μmol/L, and r = 0.9904 (n = 33). Sucrose was stable in serum or urine without preservatives for at least 13 days at 4 °C.

Fig. 1B shows the appearance of sucrose in the serum of volunteers after mild gastric irritation produced with alcohol and aspirin and after the ingestion of 100 g of sucrose. Serum sucrose concentrations were stable in the sucrose test the day before gastric irritation. Peak concentrations are reached 15–45 min after ingestion of sucrose. The peaks are at least twice the baseline values, and in all cases the peak serum concentration is >100 μmol/L. All of these subjects presented high concentrations of sucrose in urine (500–800 μmol/L) after ingestion of the sucrose test solution.

We conclude that this automated method in serum can lead to a simpler test for the detection of gastric damage. The determination of sucrose in serum 15–45 min after ingestion shortens the sucrose test and eliminates the imprecision usually encountered with urine collection over a 5-h period. The test must be investigated to determine the best cutoff value and sampling time for the detection of gastric damage in patient suffering of gastric disease.

References