Assay of Glucose in Urine by Near-infrared Spectrophotometry

To the Editor:

Hall and Pollard (1) recently reviewed the application of near-infrared spectrometry (NIRS) in clinical chemistry. NIRS has been applied to measuring cholesterol, total protein, albumin, triglycerides, and glucose in serum. Here, preliminary evidence is given that NIRS can also be used for analysis of glucose in urine.

Urinary samples were selected from samples that were submitted for routine glucose analysis, collected without preservative. Urine glucose was determined with an EPOS analyzer (Eppendorf, Hamburg, Germany) by the hexokinase method (Gluc-o-quant; Boehringer, Mannheim, Germany), according to the manufacturer's instructions. Near-infrared (NIR) absorbance of urine samples was determined with a Technicon InfraAlyzer IA-450 (Bran & Luebbe/Technicon Industrial Systems, Maarsen, The Netherlands) as described before (2); the same low-volume cuvette was used as that in the analysis for total protein in serum (2). Software for calibration and multiple regression analysis were also provided by Technicon (2) and manufacturer's instructions from Technicon, Gorinchem, The Netherlands.

The NIR absorbance for 33 urine samples, with glucose concentrations that were equally distributed over the range 0–400 mmol/L, was determined as at 19 different wavelengths (between 1440 and 2350 nm). The relation between the absorbance readings and the glucose content of the individual samples (as determined by the hexokinase method) was analyzed by multiple linear regression analysis, as described previously (2). By stepwise deletion of the wavelength giving the lowest t-test value, the highest t-ratio was associated with a combination of nine wavelengths. The amount of glucose (mmol/L) in a sample was calculated with the following algorithm:

\[ \text{Glucose} = F_0 + F_n \log(1/R_n) \]

for \( n = 2, 4, 8, 9, 11, 13, 14, 16, \) and 20 \((F_0 = \text{bias}, R_n = \text{reflectance with filter } n).\)

The wavelengths of filters 2, 4, 8, 9, 11, 13, 14, 16, and 20 were 2336, 2310, 2190, 2139, 1982, 1778, 2100, 1940, and 1680 nm, respectively. The F-values for bias \( F_0 \) and these nine wave-lengths were 410, 41 692, −57 533, 14 427, −16 522, −57 10, 26 726, 21 808, 323, and −25 041, respectively. The regression F-ratio for the calibration set was 256.7, with \( r = 0.9953, \) residual standard deviation \( = 7.639, \) and an index system error of 170.1.

After calibrating the InfraAlyzer with 33 urine samples, I determined the absorbance of another 19 urines at the nine different wavelengths. A spreadsheet program (Quattro Pro 4.0; Borland International) converted these absorbance values into glucose results, using the algorithm described above. Fig. 1 shows the glucose concentrations of the 19 urine samples plotted against the glucose values derived from the NIR absorbance values of the urine samples at nine different wavelengths. Orthogonal regression analysis of these data gave a correlation coefficient of 0.990 (slope 0.923, intercept 6.020).

These results suggest that urine glucose can be analyzed by pure spectrophotometry.

The experiments described were done in 1987 with the InfraAlyzer, equipped with 20 different filters; it took 45 s to measure the absorbance at these 20 wavelengths. With modern equipment, a complete scan of the NIR spectrum can be done in <1 s (1); calculation of the second-derivative absorbance spectra may further improve the results (1). Hall and Pollard (1) showed that fluoride/oxalate anticoagulant interferes with the measurement of serum glucose by NIRS. Similarly, preservatives (which were absent in the urine samples analyzed in the present study) and drugs may interfere with urine glucose analysis by NIRS, and should be the focus of further studies.

References


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Less Expensive Enzymatic Analysis for Inulin with a Kinetic Assay

To the Editor:

Glomerular filtration rate (GFR), an important indicator of renal function, is widely estimated by determining serum creatinine and creatinine clearance (1, 2). However, the use of creatinine for GFR estimation is inadequate in some patients, e.g., when greater accuracy is required or when endogenous concentrations of creatinine are low. An alternative classic method of measuring GFR is through inulin clearance. Inulin, a polysaccharide composed largely of fructose, is ideal for measuring GFR because it is freely filtered by the glomerulus and is not reabsorbed, secreted, or metabolically altered by the renal tubule. Because inulin is not produced endogenously, however, it must be given intravenously, and accurate assessment of GFR requires several timed measurements in plasma and urine.

We recently reported the development of an automated enzymatic method for inulin (3), in which inulase (EC 3.2.1.7; InterSpex Products, Foster City, CA) hydrolyzes inulin to fructose, and sorbitol dehydrogenase (SDH; EC 1.1.1.14; Boehringer Mannheim, Indianapolis, IN) converts fructose to sorbitol with the consumption of NADH, which is detected by spectrophotometry. This assay is automated, avoids the use of toxic reagents, has little or no interference by
glucose, and correlates well (3) with a procedure based on the anthrone reaction (4), the most widely accepted laboratory method for quantifying inulin in plasma and urine.

Currently, we perform this two-step assay on the Cobas-Fara II (Roche Diagnostics Systems, Montclair, NJ). Despite its advantages, the assay requires large amounts of SDH, a relatively expensive reagent, to convert the fructose to sorbitol completely. In this endpoint method, we use 6.4 U of SDH at a cost of $1.55 per inulin measurement. To reduce consumption of this reagent, we developed a kinetic version of this enzymatic method, which reduced SDH consumption 30-fold and lowered the cost of SDH to $0.052 per inulin measurement.

In initial experiments we sought optimal conditions for recovery of inulin from a solution of 40 g/L bovine serum albumin (BSA) in 0.25 mol/L HEPES buffer, pH 7.6. Given the initial reaction period of 2 to 6 min, we chose a 30-fold dilution (2700 U/L) of our original SDH concentration (80 000 U/L) (3); for a 50 mg/L solution of inulin, this SDH concentration yielded a decrease in NADH absorbance of 0.02 A/min, which we considered the minimally acceptable rate. Despite the small amount of SDH used, the reaction was nonlinear, but the deviation from linearity was slight in the first 20 min. Nonlinearity was somewhat greater when assaying inulin in serum, probably because of an endogenous dehydrogenase activity acting on an endogenous substrate that oxidized NADH. Linearity of the reaction was improved by optimizing the reaction period for the kinetic assay. Use of earlier initial rate periods (2 to 10 min) for calculation of reaction rate overstated the blank correction (performed with inactivated inulinsin), but a reaction period of 10 to 20 min largely eliminated the effects of early side-reactions.

Results of the kinetic procedure (y) correlated well with those of the previous endpoint assay (x) (S_{y/x} = 7.3 mg/L, y = 1.01x - 1.2 mg/L) over the linear range of 10 to 300 mg/L, and the kinetic method appeared suitable for urine, plasma, and infusate samples, provided the urine and infusate samples were diluted in 40 g/L BSA as in the endpoint procedure (Fig. 1) (3). When sets of plasma and urine specimens from 10 clearance studies were analyzed with the new kinetic procedure (y) and the endpoint procedure (x), the calculated inulin clearances correlated well: y = 0.979x + 1.5 mL/min (S_{y/x} = 2.1 mL/min). Day-to-day CVs for assays performed with dilute SDH solutions stored at 2–8°C were 5.2% and 4.0% for urine (47.7 ± 2.5 mg/L) and plasma (180.4 ± 7.2 mg/L) controls (mean ± SD, n = 32 each), respectively. Comparable day-to-day CVs for the endpoint procedure were 3.6% for urine (48.2 ± 1.7 mg/L; n = 44) and 5.2% for plasma (177.9 ± 9.3 mg/L; n = 32) controls. Within-run CVs for the kinetic assay were 4.4% and 2.3% for urine (49.0 ± 1.9 mg/L; n = 26) and plasma (143.6 ± 1.9 mg/L; n = 27) controls, respectively. Similar CVs were seen with SDH stored at -70°C (data not shown).

The stability of diluted SDH in 250 mmol/L HEPES, pH 7.6, was evaluated over a 5-week storage at 2–8°C; ΔA values in assays of controls performed with this material decreased by <9% for urine and <5% for plasma. Dilute SDH stored at -70°C showed remarkable stability, with negligible changes in 10- to 20-min ΔA values over the same time period. Bilirubin (up to 345 μmol/L) and hemoglobin (up to 6 g/L) interferences were negligible. Triglyceride interfered positively, giving an apparent increase of 6.8 mg/L in inulin concentration per 100 mg/dL (1.13 mmol/L) triglyceride. In summary, given the excellent patient comparison, comparable CVs, negligible interferences, wide range of linearity, and the feasibility of storing dilute solutions of SDH for long periods, we believe that this assay will be a widely used, inexpensive method to measure inulin and ultimately GFR.

References

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Poor Reproducibility of Strontium Absorption Test

To the Editor:

We read with interest the report by Sips et al. (1) about the 1-h "area under the curve" strontium chloride test of intestinal calcium absorption but note that no assessment of test reproducibility was carried out. We also investigated the strontium chloride method of estimating intestinal calcium absorption, by measuring the serum concentration of strontium in fasting subjects after they received a 2.5-mmol dose of strontium chloride (from Sigma Ltd.) in a gelatin capsule. In four subjects, the test was repeated after 4 weeks, and in one subject it was repeated every 4 weeks for a total of four tests. Our results (Fig. 1) clearly showed a very wide intraindividual variation in strontium absorption. A similar, wide intraindividual CV was shown by Milsom et al. (2) in their

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**Fig. 1.** Comparison between the endpoint and kinetic inulin assay performed on patients' samples with the Cobas-Fara II.