thin-layer chromatography (TLC) provides a true, accurate reflection of porphyrin content is not justified. Problems with the TLC method include the incomplete esterification of porphyrins, as evidenced by porphyrin material at the origin of the TLC plates.

MCD is proposed as a semiquantitative technique for porphyrin determination. Using as a limit of the normal range of fecal porphyrin <150 nmol/g dry wt for MCD and <200 nmol/g dry wt for TLC, the classification of patients as normal or abnormal porphyrin excretors agrees in 85% of patients studied. In approximately 25% of the cases the error between the fecal porphyrin concentrations as determined by the two methods is less than 10%.

Of course when one switches x and y values, one obtains different slopes and intercepts.

The variation in the molar ratio of fecal protoporphyrin to coproporphyrin was so large (see text) that there is no reason to prefer the 8:1 ratio.

The porphyrin determination by MCD in urine or wet feces is rapid. Drying of the feces to obtain a wet/dry weight is a slower procedure.

The fourfold difference in ellipticities of protoporphyrin and coproporphyrin is not an advantage. It is the basis for the large error in determining fecal porphyrin by MCD, as the ratio of protoporphyrin to coproporphyrin varies.

References

Kathryn M. Ivanetich
Colin Mowsowitz
Michael R. Moore

Univ. of Cape Town
Cape Town 7925, R.S.A.

Nitrate Appears to Be the Urinary Component Inhibiting Oxalate Oxidase

To the Editor:

Potezny et al. (1) reported the presence of an inhibitor of oxalate oxidase (EC 1.2.3.4) in urine. They could not identify it, but they concluded that its molecular mass is less than 500 daltons. Kohlbecker et al. (2), in their study of oxalate determination by use of oxalate oxidase, reported that it was necessary to add more enzyme to urinary samples than to aqueous solutions. They attributed this to the presence of inhibitory cations and anions. We suggest that this urinary inhibitor is nitrate.

Inhibition of oxalate oxidase by nitrate was first reported by Meeuse and Campbell (3) in a study on the enzyme in beef extracts. They showed that nitrate concentrations as low as 50 μmol/L gave severe (93%) inhibition. Sugiura et al. (4) demonstrated a 33% inhibition of barley oxalate oxidase in the presence of 10 μmol of nitrate per liter. We have confirmed this inhibition by showing that 50 μmol/L nitrate gives 20% inhibition of a barley enzyme preparation with an activity concentration of 15 U/L and that 300 μmol of nitrate per liter gives 90% inhibition. We find that urinary nitrate concentrations range from 0 to 2.5 mmol/L. This means that either a 50-fold increase in the amount of enzyme or a similar dilution of urine is needed to be sure that nitrate would not seriously inhibit the enzyme if it is used to determine urinary oxalate. We found it necessary to dilute various randomly selected urine samples by 16- to 64-fold before oxalate oxidase enzymic activity could be demonstrated.

Using aqueous solutions, we have shown that nitrate acts as a competitive inhibitor of barley oxalate oxidase, with an inhibitor constant of 360 μmol/L.

References

K. L. Goldsack
Chem. Pathol. Dept.
Worthing Hospital
Lyndhurst Road
Worthing BN11 2DH, U.K.

R. F. A. Ginman
J. M. Wright
Pharmacy Dept.
Brighton Polytechnic
Moulscoomb
Brighton BN2 4GJ, U.K.

Convenient Automated Method for Liquid-Chromatographic Measurement of Glycated Hemoglobin

To the Editor:

Recognition of the importance of measuring glycated (glycosylated) hemoglobin in the management of diabetes mellitus has led to a proliferation of clinical assays for it. As experience with the commercially available ion-exchange chromatographic (I, 2), electrophoretic (3), and colorimetric assays (4) has accumulated, various method-specific problems have become apparent. Removal of the labile fraction of glycated hemoglobin is now a well-recognized requirement if the electrophoretic and ion-exchange methods are accurately to reflect the degree of chronic glucose control (5–7). The most widely used "mini-column" ion-exchange methods have the advantage of being inexpensive and requiring little equipment, but are extremely sensitive to minor changes in buffer pH or temperature (8). Electrophoretic methods are inexpensive and are not sensitive to pH or temperature effects, but lot-to-lot variations in the commercially available agar gels make this assay somewhat imprecise (3). The colorimetric assay is not interfered with by labile glycated hemoglobin or hemoglobinopathies, but is a multistep assay that is complicated, time consuming, and difficult to standardize (4).

Ion-exchange chromatography with "high-performance" liquid chromatography (HPLC) is recognized as the most precise of the methods available, the CV being <3% as compared with 8% for the minicolumn and 6% for the electrophoretic methods (9). HPLC assay, however, requires expensive equipment and is time consuming. Preparation of samples takes about 1 h, exclusive of the removal of labile glycated hemoglobin.

We have tested a new automated HPLC system in which samples are hemolyzed, injected, and assayed, and the results are reported automatically. This system preserves the precision of previously described HPLC assays and is accurate, but requires much less technician time than previously described HPLC assays. We compared two HPLC methods. One of them is a semi-automated method that has been well described (7). It includes two programmable isocratic pumps (Model 110 A Liquid Chromatography System; Beckman Instruments, Inc., Fullerton, CA 92634) and a single-column injector, a flow-through spectrophotometer, and an integrating recorder. We prepared whole-blood samples by washing the erythrocytes twice, then incubating them in 10 vol-
umes of isotonic saline for 14 h at 22 °C. They then are hemolyzed by adding three volumes of de-ionized distilled water and vortex-mixing. Lipids are extracted and separated by adding three volumes of toluene and centrifuging (1200 × g, 30 min). The hemolysate is decanted and diluted with phosphate buffer (80 mmol/L, pH 6.8), then 20 μL (10 μg of hemoglobin) is injected onto the HPLC column. An integrating recorder (Model C-R1A; Shimadzu Corp., Kyoto, Japan) prints a chromatogram and calculates the concentrations of HbA0, HbA1a, HbA1b, and HbA1c. Preparation of 20 samples, exclusive of the 14-h incubation time, requires 2 h of technician time. Assay of each sample requires 35 min.

The totally automated system is the "Auto A1c" developed and manufactured by the Kyoto Daichi Kagaku Co., Ltd., Kyoto, Japan. For this assay, we prepared whole-blood samples as described above. A 3-μL sample of washed whole blood is diluted 150-fold in hemolysis reagent with an automatic diluter into a 0.5-mL sample cup. The cup is then placed into a snakechain sample-delivery system, where it is carried to the separation column and automatically sampled. The sample-delivery system holds a total of 50 samples. Ion-exchange reversed-phase partition chromatography effects the separation. A dual-wavelength (415/500a) bichromatic colorimetric measurement system, including a special microcell, is used to detect the fractions. A microcomputer and printer provide a chromatogram and the date, test number, and HbA1c and HbA1 values in relative percents. Preparation of 20 samples, exclusive of the 14-h incubation, requires 10 min of technician time. Assay of each sample requires 13 min.

Using EDTA-containing tubes, we obtained two specimens of blood from each of 43 patients with Type I and Type II diabetes at the time of their routine Diabetes Clinic (Dallas, TX) appointment. One sample was then shipped in an ice slurry to Boston via an overnight-delivery service. Samples were processed and analyzed within five days of collection.

The semi-automated method was extremely precise; CVs based on eight repeated assays were 1.5% and 2.8% for high (HbA1c 10.6%) and low (HbA1c 4.86%) standards, respectively.

The new automated assay was also extremely precise. The CV based on 10 repeated assays of a nondiabetic blood sample (HbA1c 5.0%) was 1.4%. The precision of the assay with moderately (HbA1c 8.2%) and markedly (HbA1c 12.2%) above-normal concentrations of glycated hemoglobins was also excellent (CVs of 1.1% and 2.1%, respectively).

We assayed 42 patients' samples by both methods. Results from the two different methods correlated well for both hemoglobin A1c (r = 0.970, p < 0.001) and hemoglobin A1c (r = 0.976, p < 0.001, Figure 1).

As glycated hemoglobin assays have taken an increasingly important role in diabetes management, faster and more-precise assays are needed. Use of commercially available disposable mini-columns is rapid and inexpensive, but lack of precision remains a significant problem. Because clinical decisions such as changes in insulin dosages are based on these results, the highest accuracy and precision are mandatory. We show here that with the development of the Auto A1c, an automated device specifically designed for the measurement of hemoglobin A1c, the assay can be performed rapidly with very little technician input. The results remain accurate and precise.

References


David M. Nathan
Massachusetts General Hosp.
Harvard Medical School
Boston, MA 02114

Philip Raskin
Univ. of Texas Health Sci. Center at Dallas
5323 Harry Hines Blvd.
Dallas, TX 75235

1 Address correspondence to this author.

Performance Characteristics of a Commercial Kit for Assay of Factor VIII-Related Antigen

To the Editor:

Measurements of Factor VIII-related antigen (FVIII:Ag) are increasingly used in the detection of carriers of hemophilia A, diagnosis and treatment of von Willebrand's disease, characterization of Factor VIII concentrates designed for therapeutic use, and in research. Thus, a technically simple and accurate test for this analyte has been sought, to replace the rather tedious and insufficiently accurate electroimmunoassay used in previous studies. Immunoradiometric assays and enzyme immunoassays, among others, have been developed for this purpose. They provide better accuracy and faster analysis, and are designed for screening of many samples in a single run. Technical problems associated with the preparation of antisera and their labeling can be overcome by using commercially available reagents or kits. In this report I describe the performance of the first enzyme immunoassay kit for the quantification of the antigen ("Asserachrom VIIIIR:Ag"; Diagnostica Stago, 92600 Asnières, France) and compare results with those from the enzyme assay developed in our laboratory (1).

The kit method is based on double-antibody "sandwich"-type enzyme-linked immunosorbent assay (ELISA).