Institut für Klin. Chemie und Laboratoriumsmedizin
Katharinenhospital, Kriegsbergstr. 60
D-7000 Stuttgart 1, F.R.G.

R. Marbach
H. M. Heise

Institut für Spektrochemie und angewandte Spektroskopie
Bunsen-Kirchhoff-Str. 11
D-4600 Dortmund 1, F.R.G.

Discrepancies among Commercial Kits for Assaying Growth Hormone

To the Editor:

Strasburger et al. (1), by proposing a novel nonisotopic method for growth hormone (GH), described some difficulties in the measurement of this analyte. Discrepancies could, in fact, exist among various kits (2), especially between immunoradiometric assays (IRMAs) and radioimmunoassays (RIAs). These differences, which can generate considerable uncertainties in making clinical decisions, can be attributed to various causes: antibodies produced against different epitopes of the protein (1), stochastic production of the antibodies (3), different standardization of commercial kits (2), and “sandwich” vs “competitive” methodology (4).

Discrepancies between radioisotopic methods have already been outlined by Reiter et al. (2), but some commercial kits for GH are nonisotopic. We found discrepancies among radioisotopic and immunoenzymatic methods, comparing two kits involving isotopic label (the RIA kit routinely used in our laboratory (Radioassay Systems Laboratories, Carson, CA) and an IRMA kit (Nichols Institute, San Juan Capistrano, CA)) with two immunoenzymometric assays (IEMA), from Medix Biotech, Foster City, CA, and Eurogenetics, Tessonderlo, Belgium. These kits are standardized vs WHO 1st IRP 66/217 (RIA RSL, and IEMA Medix), 1st IRP 80/505 [IEMA Eurogenetics and NIAMDD-hGH-RP-1 (IRMA Nichols)]. One kit involves use of polyclonal antibodies (RSL); monoclonal antibodies are used in all the others.

We compared the four kits for assays of 85 sera (range of GH 0–50 µg/L) obtained from provocative tests (arginine) in children with impaired growth. Results by the two IEMA methods agreed well (r = 0.93, y = 1.0x + 0.6, where x = Eurogenetics, y = Medix), but they consistently gave lower results than RIA (slopes of 0.4 for Eurogenetics and 0.5 for Medix) and the IRMA methods (slopes of 0.4 and 0.5, respectively). Conversely, the two radioisotopic methods were well correlated (r = 0.91, y = 1.1x + 0.8, where x = IRMA, y = RIA), the RIA results (polyclonal antibodies) being consistently slightly higher than the IRMA ones (monoclonal antibodies). Recalculating data with conversion factors that changed the µg/L units of the kits’ standards to U/mL did not modify the discrepancies.

Clinically, the relevance of the different results obtained in provocative screening tests—for which a cutoff of 10 µg/L (4) is universally accepted as indicating which children need therapy—is evident. We confirmed the reliability of this cutoff value by studying 286 GH curves obtained by provocative tests (clonidine or arginine) in children, measuring GH by RIA. However, if nonisotopic methods are used, the cutoff should be lowered to avoid misclassifications.

It is difficult to specify the origin of the discrepancies we found. In addition to the possible causes mentioned above, we believe another source of interassay GH variability is apparently linked to isotopic/nonisotopic labels and to their different specific activities. The good results obtained by Strasburger et al. (1) when they compared their two-site time-resolved fluorometric assay and an RIA method in some groups of patients suggest that the use of a carefully selected mix of monoclonal antibodies directed against different GH epitopes could be a reliable and elegant solution. Additional comparisons among commercial assays, especially nonisotopic ones, are needed.

References


Giuseppe Banni
Ermelina Casari
Michelangelo Murone
Pierangelo Bonini

Lab. Analisi
Istituto Scientifico
S. Raffaele, via Olgettina
60, 20132, Milan, Italy

Effect of Gel Filtration on Urinary N-Acetyl-β-D-glucosaminidase Activity Measured by the m-Cresolsulfophthaleinyl Procedure

To the Editor:

It is well-known that endogenous low-molecular-mass activators or inhibitors of N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30) are present in human urine. This fact compels some workers to carry out their NAG assays after dialysis (1) or gel filtration of the urine (2), or to use highly diluted urine samples (3).

Noto et al. (4) described a manual colorimetric assay of urinary NAG involving m-cresolsulfophthaleinyl-(MCP)-N-acetyl-β-D-glucosaminide as the substrate. Because of its high solubility, the concentration of the substrate in the test is seven times the Michaelis–Menten constant, favoring the saturation of NAG and minimizing the effect of interferences in the assay. With their adaptation of the MCP technique to the Hitachi 705, Goren et al. (5) examined 50 clinical urine specimens, both undialyzed (y) and dialyzed (x), for NAG activity. These authors used a much higher sample/substrate ratio (1/6) in their procedure than that (1/21) laid down in the manual test (4). The linear-regression equation was found to be: y = x.1 0.04(±0.03) + 1.14(±0.14) U/L (r = 0.98), and these authors concluded that dialysis is unnecessary. Figure 1 in their paper, however, showing the relationship between undialyzed and dialyzed urine, suggests an appreciable decrease in NAG activity after dialysis in three urines (25%, 20%, and 30%, respectively, at 46, 38, and 29 U of NAG per liter).

We wanted to know the effect of gel filtration, which we consider a more precise, less cumbersome, and less time-consuming procedure. Urine specimens were collected from 33 pa-
tients, comprising 17 patients on cancer chemotherapy, nine with acute tubular nephropathy, and seven postrenal-transplantation. NAG activity ranged from 5 to 49 U/L. According to the technique of Knoll et al. (6), we filtered 2 mL of urine over Sephadex G-25 medium (Pharmacia, Uppsala, Sweden). However, we prepared the columns ourselves (dimensions 9 × 1.5 cm) and measured NAG activity in the 2.5- to 7.5-mL eluate. For the NAG assay we used the Hitachi 705 as described (6) and a commercial reagent kit (Boehringer, Mannheim, F.R.G.). The volumes of sample, substrate, and stopping solution were, however, 15, 150, and 300 µL, respectively (sample/reagent ratio = 1:11). Inter-assay CVs were, respectively, 2.5%, 0.6%, and 0.6% at 7, 19, and 50 U/L of NAG per liter. Gel filtrates were analyzed within 2 h after preparation, in the same run with their respective urines.

Deming regression of NAG results obtained with unfiltered (y) and filtered urine (x) showed a slope of 0.99 (±0.02) U/L and an intercept of 1.08 (±0.14) U/L (r = 0.99). Mean NAG activity in urine before (19.1 ± 11.8 U/L) and after gel filtration (18.2 ± 12.0 U/L) differed significantly (paired Student’s t-test, P < 0.01). NAG activity in gel-filtered urine was lower in 25 and higher in eight urine specimens. Further to assess the effect of gel filtration, we calculated for each specimen the difference (d) and %d in NAG activity of the unfiltered and filtered urine. For 32 of 33 urines (%d < 20%), values for d fell within the 95% confidence limits of agreement (7), as represented by the mean ± 2 SD. This was not the case for the remaining urine (20 U/L NAG before gel filtration), which showed a decrease of 7 U/L NAG after gel filtration.

In conclusion, we agree with Goren et al. (5) that the MCP procedure for urinary NAG can be performed without pretreatment of the urine to remove endogenous interfering substances. However, one must be aware of outliers. In our opinion it is better to choose a sample/substrate ratio such as was used in the present study.

References

C. H. Konings C. Mulder
Dept. of Clin Chem.
Free University Hospital
Amsterdam, The Netherlands

Dr. Goren responds:

To the Editor:

We agree with Konings and Mulder that neither gel filtration nor dialysis is required if one is to estimate urinary NAG activity correctly in many clinical studies. Decreased NAG activity in occasional samples after treatment may stem from loss of the enzyme during the procedure, as well as from removal of cryptic enzyme activators. They note that we used a 1:6 sample/substrate ratio; this was done only in our dialysis experiment (to increase the possibility of detecting enzymatic inhibition). A 1:21 sample:substrate ratio was used in other studies. A 10-fold dilution of urine generally suffices to suppress more than 90% of inhibition by urea, the only recognized endogenous inhibitor of human urinary NAG activity (1, 2). The optimal sample dilution depends upon urea concentration and other assay variables, including substrate concentration relative to the K_m of NAG. When the differences between study and control groups are so subtle as to be apparent only by use of statistical analyses, bias ascribable to urea concentration should be considered. However, many nephrotoxins induce increases in NAG excretion far in excess of analytical and nonanalytical (e.g., circadian rhythm) variation.

We reported no appreciable effect of dialysis on NAG activity determined by use of p-nitrophenol-conjugated substrate (PNP) at a 1:21 sample:substrate dilution, as well as with the MCP procedure. The enzyme-saturating substrate concentration attainable with MCP may afford little advantage over the less-soluble PNP after dilution of the urine sample. For low-activity samples, we and others (2) have reported a within-run CV as high as 10% for the MCP procedure. The greater molar absorptivity of the MCP chromatophore appears to be countered by a lesser activity with human NAG. We measure low NAG concentrations (1–5 U/L) by using a 12-min incubation with PNP at a 1:11 sample:substrate dilution. Although an extra channel on the analyzer (Perspective; American Monitor, Indianapolis, IN) must be calibrated with p-nitrophenol to provide a urine and reagent blank, concentrations of NAG and other urinary analytes can be assayed in as many as 500 specimens per day.

Marshall P. Goren
Pathol. and Lab. Med.
P.O. Box 318, Memphis, TN 38101

Unexplained Osmolal Gap in Diabetic Ketoacidosis (Not due to Acetone)

To the Editor:

We previously reported an improved formula for calculating plasma osmolality (1). Certain substances, particularly uncharged molecules such as ethanol (2) and mannitol (3) that might be present in plasma, are not taken into account by the formula. These substances, when present, increase the "osmolal gap" (i.e., the difference between measured and calculated osmolality).

Acetone added to pooled human serum increases osmolality, the activity coefficient being approximately equal to 1, if the osmolality is measured by freezing-point depression rather than by vapor pressure (4). In diabetic ke-