Spectrophotometric Assay for Urinary N-Acetyl-β-D-Glucosaminidase Activity

Eva Horak, Sidney M. Hopper, and F. William Sunderman, Jr.

An improved assay for N-acetyl-β-D-glucosaminidase activity in urine is described that involves (a) gel filtration to separate the enzyme from inhibitors in urine, (b) enzymic hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucosaminide at pH 4.4, and (c) spectrophotometry of the liberated p-nitrophenylate. Measurements of activity of the enzyme in 58 urine specimens correlated closely (r = 0.9998) with results by an established procedure. The within-run coefficient of variation (CV) was 3.7%; the between-run CV averaged 6.8%. Reference values for the activity were established by assays of urine specimens from 135 healthy persons, age two weeks to 52 years. Efficacy of the assay for detection of nephrotoxicity was demonstrated in rats after experimental induction of reversible renal insufficiency by intraperitoneal injection of nickel chloride. Clinical application of the assay in approximately 1000 patients corroborated its utility for detection and monitoring of renal disorders.

Additional Keyphrases: kidney disease detection - nickel nephrotoxicity - renal function test - urinary enzyme - reference intervals

N-Acetyl-β-D-glucosaminidase (2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30, here abbreviated NAG), is an enzyme (M, 140 000) that occurs in high concentrations in lysosomes of renal proximal tubules (1–4). Urinary NAG activity is an extremely sensitive index of renal parenchymal damage (5–10), a reliable test to use in monitoring the progression of kidney diseases (11-16), and an early indicator of immune rejection in patients with renal allografts (17–21). Patients with normal and abnormal renal function can be discriminated by analysis of NAG activity in untimed specimens of urine, because (a) there is little diurnal fluctuation in the rate of NAG excretion (10, 16, 22), (b) variations in urine flow can reliably be compensated by relating urine NAG activity to urine creatinine concentration (6, 10, 16), (c) NAG activity is not increased by bacterial colonization of urine (6), and (d) urine NAG activity is usually normal in subjects with postural proteinuria (15). Urine specimens can be stored for several days at 4–6 °C, and for months at −20 °C, without change in NAG activity (14, 23–25). Assay techniques for urine NAG activity are relatively simple, convenient, and inexpensive. Accordingly, urine NAG assay is being introduced as a routine test for detection and monitoring of renal disorders (6, 9, 10, 26–28).

Three substrates are used for NAG assays: p-phenyl-N-acetyl-β-D-glucosaminide, p-nitrophenyl-N-acetyl-β-D-glucosaminide, and methylumbelliferyl-N-acetyl-β-D-glucosaminide. When the p-phenyl derivative serves as substrate, liberated phenylate ion is measured by the Folini-Cioclateau reaction (1, 29–31). Kato et al. (32) recently adapted this approach for use in a diagnostic reagent kit (Maruko Pharmaceutical Co., Tokyo, Japan). When the p-nitrophenyl derivative serves as substrate, the yellow color of the p-nitrophenylate ion is measured directly by spectrophotometry (23–25, 33–37). When the methylumbelliferyl derivative serves as substrate, the liberated methylumbelliferone is determined fluorometrically (38–40). The fluorometric NAG assay has been adapted for automation with continuous-flow and discrete-sample analyzers (41–43).

When we decided to adopt urinary NAG assays as a routine diagnostic test, we evaluated the procedures described by Knoll et al. (23) and Lockwood and Boismann (24). The former includes three steps: (a) gel-filtration chromatography on Sephadex G-25 to separate NAG from urine inhibitors, (b) enzymic hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucosaminide in sodium citrate buffer for 15 min at pH 4.4, and (c) spectrophotometry of p-nitrophenylate ion at 405 nm. The latter procedure (24) does not involve preliminary gel filtration; the enzymic hydrolysis and spectrophotometric steps resemble those of Knoll et al. (23), except that the reaction period is 2 h, and the spectrophotometry is at 420 nm. We decided not to use the technique of Lockwood and Boismann (24), because we encountered substantial interference by inhibitors in urine. The technique of Knoll et al. (23) proved to be satisfactory in most respects, but we found it desirable to introduce several modifications, to improve analytical precision, reliability, and convenience. The sample volume was reduced; the reaction volume was increased; the dimensions of the chromatographic column were altered; the mesh of the Sephadex G-25 beads was reduced; urine “blank” samples were prepared to contain the same ingredients as the “test” samples, and p-nitrophenol was used as a spectrophotometric standard, in lieu of computations based on an extinction coefficient. We used our modified method of Knoll et al. (23) to assay NAG activity in some 1000 urine specimens from patients at our hospital, and also to investigate nickel-induced nephrotoxicity in rats. From these studies we conclude that the modified NAG assay is practicable and reliable for use in clinical laboratories.

Materials and Methods

Principle of the Method

NAG is separated from urine inhibitors by gel filtration on chromatographic columns of Sephadex G-25. The NAG-containing eluate is added to an enzyme reaction mixture that consists of the substrate (p-nitrophenyl-N-acetyl-β-D-glucosaminide) dissolved in sodium citrate buffer (pH 4.4). During incubation at 37 °C for 15 min, enzymic hydrolysis of the substrate liberates p-nitrophenylate ion. The reaction is stopped by adding 2-amino-2-methyl-1-propanol (AMP) buffer (pH 10.25), and the reaction product is measured by spectrophotometry at 405 nm. Urine NAG activity is proportional to the absorbance of the liberated p-nitrophenylate ion, after correction for absorbance of a urine “blank” sample.

Special Equipment

Chromatographic columns, 250 mm long, 10 mm in diam-
eter, containing fine mesh (20–80 μm) Sephadex G-25 (bed volume, 5.6 mL), suspended in NaCl solution (0.15 mol/L) (cat. no. QS-2C; Isolab Inc., Akron, OH 44321).

**Spectrophotometer (Model 25; Beckman Instruments Inc., Fullerton, CA 92634).**

**Reagents**

Sodium chloride solution, 0.15 mol/L.

Sodium chloride–sodium azide solution: NaCl, 0.15 mol/L; NaN₃, 3.1 mmol/L.

Citric acid solution, 0.2 mol/L. In a 200-mL volumetric flask, dissolve 8.4 g of citric acid monohydrate (M, 210.1) in distilled water, dilute to the calibration mark, and store at 4 °C.

Sodium citrate solution, 0.2 mol/L. In a 200-mL volumetric flask, dissolve 10.3 g of anhydrous trisodium citrate (M, 258.1) in distilled water, dilute to the calibration mark, and store at 4 °C.

Citrato buffer, pH 4.4, 0.1 mol/L. Put 56 mL of citric acid solution and 44 mL of sodium citrate solution in a beaker and allow the solution to equilibrate at 37 °C in a water bath. Check the pH with a pH meter and adjust to pH 4.40 (±0.05) by adding citric acid solution or sodium citrate solution. Transfer the solution quantitatively to a 200-mL volumetric flask, dilute to the calibration mark with distilled water, and store at 4 °C.

NAG–substrate solution, 10 mmol/L. In a 100-mL volumetric flask, dissolve 342 mg of p-nitrophenyl-N-acetyl-β-D-glucosaminide (p-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside; cat. no. N-9376; Sigma Chemical Co., St. Louis, MO 63179) in citrate buffer and dilute to the mark. Dispense this solution in 10-mL aliquots into screw-capped polystyrene test tubes and store the tubes at −20 °C until the day of use. Under these conditions, the NAG–substrate solution is stable for months. Before use, thaw the NAG substrate solution and equilibrate it in a water bath at 37 °C.

AMP buffer, pH 10.25, 0.75 mol/L. In a 200-mL volumetric flask dissolve 18.84 g of 2-amino-2-methyl-1-propanol hydrochloride (cat. no. 5888, Sigma Chemical Co.) in 50 mL of distilled water. With a pH meter, adjust the pH to 10.25 at 25 °C by adding NaOH, 6 mol/L solution. Dilute to the calibration mark with distilled water, and store at 4 °C.

p-Nitrophenol working standard solution, 100 μmol/L. With a calibrated volumetric pipet, transfer 1 mL of 10 mmol/L p-nitrophenol stock standard solution (cat. no. 104-1, Sigma Chemical Co.) into a 100-mL volumetric flask, and dilute to the calibration mark with (0.15 mol/L) NaCl solution. Prepare this solution immediately before use.

**Procedures**

**Treatment of urine samples.** Place 10-mL of fresh urine in a conical tube and centrifuge at 900 x g for 10 min. Decant the clear supernatant urine and take aliquots for assay for NAG activity by the present technique and for analysis of creatinine concentration by the picric acid reaction.

**Gel chromatography.** Remove the upper and lower caps from a Sephadex G-25 column, and wash the column with 10 mL of NaCl solution, and allow it to drain completely. Apply 1 mL of centrifuged urine to the column. When the urine has entered the column, add 0.5 mL of the NaCl solution as a wash solution. Allow the column to drain completely, and discard the eluate (1.5 mL). Place a collection tube under the column, add 0.5 mL of NaCl solution to the column, and allow the NAG-containing eluate (2 mL) to drain completely into the collection tube. Then regenerate the Sephadex G-25 column by passing 25 mL of NaCl–NaN₃ solution through the column. Fill the column with NaCl–NaN₃ solution, replace the upper and lower caps, and store the column at room temperature until it is to be used again.

**Enzymic reaction.** Label four spectrophotometer cuvets (1-cm path light) “RB” (reagent blank), “S” (standard), “U” (urine), and “UB” (urine blank). Into these respective cuvets pipet the following solutions: cuvet RB, 0.5 mL of NaCl solution; cuvet S, 0.5 mL of p-nitrophenol working standard solution; and cuvets U and UB, 0.5 mL of urine eluate. Equilibrate the cuvets in a water bath at 37.0 °C (±0.2 °C). Pipet 0.5 mL of NAG–substrate solution into cuvets RB, S, and U, and incubate the samples for precisely 15 min at 37.0 °C. Add 0.5 mL of AMP buffer to all of the cuvets, and add 0.5 mL of NAG–substrate to cuvet UB. Measure the absorbance of the cuvets at 405 nm. (Note: if the absorbance of cuvet U exceeds 1.8, dilute the urine fivefold with NaCl solution and repeat the assay.

**Computations**

NAG activity, U/L

\[
A/[U - B] \times (100 \, \mu\text{mol/L}) \times \text{diln. factor} \times 2
\]

\[
A = [S - UB] \times \text{incubation time} \times 15.3
\]

NAG activity in untimed specimens of urine is expressed as U/g of creatinine.

**Other Materials and Methods**

Tests for chemical interference in NAG assays involved the following diagnostic products: (a) “Renografin-60” (Squibb; a radiopaque contrast medium supplied as an aqueous solution containing 520 g of meglumine diatrizoate and 80 g of sodium diatrizoate per liter, with 3.2 g of sodium citrate per liter as buffer and 0.4 g of disodium edetate as sequestrant); (b) “Bromsulphalein Sodium,” a 50 g/L aqueous solution of disodium phenolotetrabromophthalate sulfonate (Hynson, Wescott and Dunning); and (c) “Bilirubin Control—Dade,” a lyophilized preparation that, when dissolved in water according to the manufacturer’s directions, contains 0.2 g of bilirubin per liter and 55 g of crystalline human albumin per liter (Dade Diagnostics Inc., Aguada, PR 00602).

NAG activity in selected urine samples was assayed by the methods of Lockwood and Bosmann (24) and Knoll et al. (23). In the latter procedure are used 80 × 15 mm (i.d.) chromatographic columns, containing medium mesh (50–150 μm) Sephadex G-25 in a bed volume of 9 mL, suspended in a 0.15 mol/L NaCl solution (cat. no. PD-10; Pharmacia Fine Chemicals, Inc., Piscataway, NJ 08854). Urinary creatinine concentrations were measured by the alkaline picrate reaction (44). Urinary protein concentrations were measured by a microadaptation (45) of the biuret procedure of Savory et al. (46).

**Test subjects** included 135 ostensibly healthy humans (66 males, 69 females) ages two weeks to 62 years. Untimed urine specimens were collected from asymptomatic infants and children during periodic health examinations by their pediatrician. Twenty-four urine specimens were obtained from healthy adults (physicians, chemists, technologists, and clerical personnel) who had no present illness or medications, and who gave no history of renal–genital–urinary-tract disorders.

**Experimental animals** were nine female albino rats of the Fischer-344 strain [mean body weight = 155 (SD 7) g, range = 146–167 g; Charles River Breeding Laboratories Inc., North Wilmington, MA 01886], kept in metabolism cages. Urine was collected on the day before and on the 1st, 2nd, 3rd, 4th, and 9th days after intraperitoneal injection of nickel chloride (6)}
The measure of nickel (mg of Ni per kg of body weight) as previously described (45, 47). The 24-h collections of rat urine were diluted to uniform volumes of 10 mL.

Statistical procedures included linear regression analysis (48), Student's t-test (paired and non-paired) (48), Mann and Whitney's U-test (48), Barnett's technique to calculate standard deviations of differences between duplicates (49), and Hohnadel's "H" statistic to test the significance of differences between coefficients of variation (50).

Results

Gel Filtration Chromatography

Figure 1 illustrates typical elution profiles of NAG activity from chromatographic columns of Sephadex G-25. Panel A of Figure 1 shows the profile obtained by the present method when 1-mL samples of six urine specimens were applied to the Isolab columns (fine-mesh Sephadex G-25). The elution volume from 1.5 to 3.5 mL contained 96.6% (SD 1.1%) of the total NAG activity recovered from the columns. As shown in Figure 1B, a broader elution profile was obtained by the method of Knoll et al. (23). Two-milliliter samples of each of four urine specimens were applied to Pharmacia columns (medium-mesh Sephadex G-25). The elution volume from 2.5 to 6.5 mL contained 97.6% (SD 2.2%) of the total NAG activity recovered from the columns.

Variations of Enzyme Reaction Interval

To assess the effect of the duration of the enzymic reaction upon the release of p-nitrophenylate ion, we assayed six urine specimens with low NAG activities (0.4, 0.6, 1.6, 2.2, 4.3, and 4.9 U/L) by the present method, with incubation periods that ranged from 15 to 120 min. As shown in Figure 2A, the duration of the enzymatic reaction and the absorbance of liberated p-nitrophenolate ion were linearly related.

Study of Urine Dilutions

To assess the effect of urine dilutions upon the assay of NAG activity, we prepared dilutions of seven urine specimens from patients with renal diseases with (0.15 mol/L) NaCl solution as indicated in Figure 2B. The NAG activities of the undiluted urine specimens were 10, 17, 28, 38, 47, 51, and 58 U/L, respectively. When the diluted samples were analyzed by the present method, the fractional volumes (mL of urine per mL of final solution) and the absorbances of liberated p-nitrophenolate ion were seen to be linearly related (Figure 2B).

Method Comparisons

We assayed 58 urine specimens from hospital patients and healthy adult subjects for NAG activity by the present method and by the method of Knoll et al. (23) (Figure 3). Results by the present method averaged 3% higher (mean NAG activity = 7.1 U/L by the present method vs. 6.9 U/L by the method of Knoll et al; mean difference, 0.21 U/L). This difference was not statistically significant by Student's paired-sample t-test. Linear regression analysis of the data yielded regression coefficients (for the equation y = a + bx) as follows: a = −0.17 (SEM, 0.07); b = 1.066 (SEM 0.007). The correlation coefficient (r) was 0.9998 and the standard error of y on x was 0.36.

We assayed 117 urine specimens from hospital patients and healthy subjects for NAG activity by the present method and by the direct spectrophotometric technique of Lockwood and Bosmann (24) (Figure 4). Results by the present method averaged 63% higher (mean NAG activity was 5.7 U/L by the present method, 3.5 U/L by the method of Lockwood and Bosmann; mean difference = +2.26 U/L, p <0.001 by Student's paired-sample t-test). Linear regression analysis of the data yielded regression coefficients (for the equation y = a + bx) as follows: a = 0.66 (SEM, 0.25); b = 1.46 (SEM, 0.05). The correlation coefficient (r) was 0.9430, and the standard error of y on x was 2.1.
Table 2. Between-Run Precision of Urinary NAG Assays

<table>
<thead>
<tr>
<th>No. analytical runs</th>
<th>Urine pool A</th>
<th>Urine pool B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/L</td>
<td>U/L</td>
</tr>
<tr>
<td>NAG activity, mean (and SD)</td>
<td>4.5 (0.3)</td>
<td>23.9 (1.9)</td>
</tr>
<tr>
<td>CV, %</td>
<td>5.8</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* Assays done in successive analytical runs, on aliquots of pooled urine specimens (stored frozen).  
  b Pooled urine from healthy subjects.  
  c Pooled urine from patients with urinary protein concentrations > 1 g/L.  
  d Not significantly different from the CV obtained with urine pool A.

Reference Values for Urine NAG Activity

Table 4 lists measurements of NAG activity in urine specimens from 135 healthy subjects (age two weeks to 52 years). We saw no significant sex-related differences in urinary NAG activities, either in any of the age groups or in the combined group of 36 adults (18 men, 18 women, ages 18–54 years). No significant differences in mean or median values for urinary NAG activities were observed in any of the age groups as evaluated with Student’s t-test or the Mann-Whitney U-test. When urine NAG activities were expressed as U/g of creatinine, the mean and median values were significantly increased (p < 0.01) in the three groups of children under three years of age, as compared with the combined group of adults.

Nickel-Induced Nephrotoxicity in Rats

We tested the efficacy of urine NAG assay for detection of renal damage in an established experimental model (45, 47) for induction of mild, reversible nephrotoxicity. As indicated in Table 5, urinary NAG excretion averaged about twofold the baseline value during four successive days after intraperitoneal injection of NiCl₂ (6 mg of Ni per kilogram body weight); urinary NAG excretion returned to the pre-injection value by the 9th day. In contrast, urinary protein excretion was twice the baseline value on the day after the intraperitoneal injection of NiCl₂, increased to almost fourfold on the 2nd day, and returned to the pre-injection value by the 4th day.

Table 3. Effects of Drugs and Pigments on Results of Urinary NAG Assays

<table>
<thead>
<tr>
<th>Added drug or pigment</th>
<th>Urine 1</th>
<th>Urine 2</th>
<th>Urine 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>5.7</td>
<td>10.3</td>
<td>21.3</td>
</tr>
<tr>
<td>Bilirubin, 0.2 g/L</td>
<td>(−18%)</td>
<td>(−14%)</td>
<td>(−15%)</td>
</tr>
<tr>
<td>Hemoglobin, 3 mg/l</td>
<td>5.1</td>
<td>9.3</td>
<td>19.4</td>
</tr>
<tr>
<td>Methylen blue, 5 mg/l</td>
<td>(−12%)</td>
<td>(−4%)</td>
<td>(−10%)</td>
</tr>
<tr>
<td>Salicylic acid, 3 g/l</td>
<td>(−9%)</td>
<td>(−8%)</td>
<td></td>
</tr>
<tr>
<td>Bromsulphalein, 5.7 g/l</td>
<td>10.7</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid, 0.5 g/l</td>
<td>(+4%)</td>
<td>(+1%)</td>
<td></td>
</tr>
<tr>
<td>Renograin, 25 ml/L</td>
<td>(−5%)</td>
<td>(+1%)</td>
<td>(&lt;1%)</td>
</tr>
</tbody>
</table>

* Percentage change from NAG activity in untreated urine is given in parentheses.
Table 4. Measurements of NAG Activity in Urine Specimens from Ostensibly Healthy Subjects

<table>
<thead>
<tr>
<th>Age</th>
<th>No. and sex *</th>
<th>Urine NAG activity, U/L</th>
<th>NAG activity, U/g of creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>of subjects</td>
<td></td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>2–11 weeks</td>
<td>14 (8 M, 6 F)</td>
<td>3.4 (0.9–9.2)</td>
<td>3.3 (2.0)</td>
</tr>
<tr>
<td>3–12 months</td>
<td>23 (12 M, 11 F)</td>
<td>2.5 (0.7–17.2)</td>
<td>3.1 (3.4)</td>
</tr>
<tr>
<td>1–3 years</td>
<td>20 (8 M, 12 F)</td>
<td>2.8 (0.3–13.3)</td>
<td>4.1 (4.4)</td>
</tr>
<tr>
<td>4–8 years</td>
<td>24 (12 M, 12 F)</td>
<td>1.8 (0.3–9.9)</td>
<td>3.6 (3.1)</td>
</tr>
<tr>
<td>9–17 years</td>
<td>18 (6 M, 10 F)</td>
<td>3.2 (0.6–7.9)</td>
<td>3.3 (2.0)</td>
</tr>
<tr>
<td>18–28 years</td>
<td>19 (7 M, 12 F)</td>
<td>2.5 (0.3–7.4)</td>
<td>3.7 (2.3)</td>
</tr>
<tr>
<td>29–52 years</td>
<td>17 (11 M, 6 F)</td>
<td>3.1 (1.3–9.0)</td>
<td>4.1 (2.3)</td>
</tr>
</tbody>
</table>

* M = male, F = female. p < 0.01 vs combined group of adults (ages 18–54 years), computed by the Mann–Whitney U-test and Student’s t-test (49). In the combined group of adults (ages 18–54 years), urinary NAG activity averaged 3.8 ± 2.3 U/L (median value, 2.9); urinary NAG activity per gram of creatinine averaged 3.2 ± 1.3 U/g (median value, 3.3), and urinary NAG averaged 4.3 ± 2.2 U/day (median 3.7, range = 0.7–9.6).

Table 5. Urinary Excretion of NAG and Protein by Rats Injected Intraperitoneally with NiCl₂

<table>
<thead>
<tr>
<th>Day before or after injection *</th>
<th>Urinary NAG excretion, mU/day</th>
<th>Urinary protein, mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>–1</td>
<td>100 (87–130)</td>
<td>106 (16)</td>
</tr>
<tr>
<td>+1</td>
<td>181 (105–271)</td>
<td>187 (46)</td>
</tr>
<tr>
<td>+2</td>
<td>213 (138–274)</td>
<td>204 (57)</td>
</tr>
<tr>
<td>+3</td>
<td>190 (139–268)</td>
<td>190 (41)</td>
</tr>
<tr>
<td>+4</td>
<td>176 (138–210)</td>
<td>174 (23)</td>
</tr>
<tr>
<td>+9</td>
<td>110 (87–119)</td>
<td>106 (11)</td>
</tr>
</tbody>
</table>

* NiCl₂ was injected i.p. in nine female Fischer rats at a dosage of 6 mg of Ni per kg body wt. p < 0.001 vs paired pre-injection value in each rat, computed by the Mann–Whitney U-test and Student’s t-test (49). Urine samples were collected from only five rats on days 4 and 9 after the injection.

Discussion

Reported values for NAG activity in untimed urine specimens from healthy adults are compiled in Table 6. Our reference intervals are higher than those reported by some others (19, 22, 34), lower than those reported by others (35), and agree closely with those of Whiting et al. (21). Kunin et al. (6) assayed NAG in untimed urine specimens from 815 healthy subjects (ages <1 year to 83 years). Numerical values for urine NAG activity cannot be accurately discerned from the graphical presentation of these data, but their findings for infants and children younger than three years appear to correlate closely with our results. They (6) noted increased NAG activity in urine specimens from healthy adults of age 56 to 83 years, in comparison to adults of age 21 to 55 years. Similarly, Price (10) found that urinary NAG activity progressively increases in healthy middle-aged and elderly adults (6th, 7th, and 8th decades) as compared with younger healthy adults.

Okada et al. (51) reported that ascorbic acid causes nonenzymic hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucosaminidase and methylumbelliferyl-N-acetyl-β-D-glucosaminidase under the reaction conditions of serum NAG assays, resulting in factitious increases in NAG activity. In the present method, ascorbic acid is separated from urine NAG activity during the preliminary gel-filtration step; its interference is thereby circumvented. In the present study, however, partial inhibition of NAG activity was observed after in vitro addition of bilirubin, hemoglobin, salicylic acid, or methylene blue to urines.

During the past year, NAG was assayed by the present method in about 1000 urine specimens from patients at our hospital. Our clinical experience corroborates the findings of previous workers that urine NAG assays contribute to the diagnosis and clinical management of patients with renal diseases. Urine NAG assays have been particularly useful to us in the following situations: (a) screening for renal damage induced by gentamicin, gold thiomalate, cis-platinum compounds, 5-fluorouracil, and other nephrotoxic drugs; (b) gauging severity of renal involvement in patients with lupus erythematosus; and (c) monitoring renal functional impairment in patients after surgery, accidental trauma, or burns. Clinicians in our hospital find the assay a valuable addition to the offerings of the clinical laboratory.

We thank Robert H. McLean, M.D., Thomas L. Kennedy, III, M.D., Martin T. Randolph, M.D., and Peter Feig, M.D., for cooperation in obtaining urine specimens from their patients and for valuable clinical advice, and Mrs. Esther King and Mr. John Chikah for skillful technical assistance.

Supported by Grant No. EY-03140 from the U.S. Department of Energy, and Grant No. ES-01337 from the National Institute of Environmental Health Sciences.

References


