quantitative assay of serum acetaminophen such as immunoassay or HPLC. A commercial immunoassay is the option adopted by this laboratory.

References

Louella Davey
Daya Naidoo
Dept. of Clin. Chem.
The Prince of Wales Hosp.
High and Auoca Sts.
Randwick 2031, Australia

1 Author for correspondence.

Microwave-Induced Rapid Hydrolysis of Acetaminophen and Its Conjugates in Urine for Emergency Toxicological Screen

To the Editor:

In our laboratory, we screen for acetaminophen in urine with a widely used manual colorimetric assay involving o-cresol and ammonium hydroxide after the hydrolysis of acetaminophen and its conjugates with concentrated hydrochloric acid (1, 2). The reaction time of 10 min at 100 °C recommended by Berry and Grove in their original method (1) led to incomplete hydrolysis; for complete hydrolysis, 20–30 min is required. Novotny and Elser recommended a hydrolysis time of 35 min (3). Therefore, hydrolysis with conventional heating is the major limitation to improving the turnaround time for this frequently ordered stat test. Assay for 4-aminophenol was once considered an adequate urine screening test, but is no longer indicated because the phenol is only a minor metabolite of acetaminophen (4). We developed a technique for a rapid hydrolysis of acetaminophen and its metabolites in urine by microwave irradiation, which can be easily implemented in a clinical laboratory.

We analyzed urine samples from 9 patients who screened positive for acetaminophen, from 12 patients who were negative for acetaminophen, and from 3 volunteers, each of whom ingested 1 or 2 Tylenol® tablets, each containing 500 mg of acetaminophen. For colorimetric detection of acetaminophen, we mixed 200 μL of urine with 200 μL of concentrated hydrochloric acid in a 16 × 100 mm thick-walled glass tube and incubated this in a heating block at 100 °C for 10–30 min or irradiated it with microwave radiation for 2 min at power level 2 (120 W). We then added 5 mL of o-cresol reagent and 2 mL of ammonium hydroxide and mixed. Development of a blue color indicated the presence of acetaminophen. We measured with a spectrophotometer the intensity of the blue color at 620 nm 5 min after adding the color reagents to provide the basis for comparing the two methods of hydrolysis.

In additional studies, an aliquot of 800 μL of urine from a volunteer (4 h after ingestion of 1 g of acetaminophen) was mixed with 800 μL of concentrated hydrochloric acid and heated at 100 °C for 20 min. Another 800-μL aliquot was mixed with concentrated hydrochloric acid and subjected to microwave irradiation (120 W) for 2 min. In both cases, the reaction mixtures were neutralized with 10 mol/L sodium hydroxide and the reaction products were extracted twice with 5 mL of ethyl acetate. The organic layer was separated, evaporated to dryness in a Reacti-vial, and reconstituted with 400 μL of acetic anhydride and 50 μL of pyridine. After heating the reaction mixture at 100 °C for 10 min, we removed acetic anhydride and pyridine by evaporation under nitrogen. The residue was reconstituted with ethyl acetate and injected into a gas chromatograph/mass spectrometer.

Our experiments clearly showed (Table 1) that a hydrolysis time of 10 min with conventional heating led to incomplete hydrolysis; 20 min was much more suitable for complete hydrolysis of acetaminophen and its conjugates in urine. On the other hand, under microwave irradiation, hydrolysis was complete in 2 min. Analysis of 12 urine specimens containing acetaminophen (nine patients' samples and three from volunteers) by either the microwave or the conventional technique showed that both methods were comparable. We also mixed an aliquot of three specimens with concentrated hydrochloric acid and added color reagents immediately after mixing. All three final reaction mixtures remained colorless even after 10 min, indicating that 4-aminophenol in the urine specimens was not of measurable quantity. The analysis of 12 urine specimens that screened negative for acetaminophen by the conventional technique did not develop any blue color when the hydrolysis was carried out under microwave irradiation, indicating that microwave-induced reaction in the negative specimens did not lead to any false-positive results.

The mass spectrum of the acetyl derivative of commercially available 4-aminophenol, the acetyl derivative of the hydrolysis product of urine obtained after either microwave irradiation or conventional heating, all showed peaks at m/z 193 (molecular ion), m/z 178, and m/z 151. Analysis of the complete absorbance spectra of chromogens in the visible region after hydrolysis by either microwave irradiation or conventional heating indicated that the chromogens have the same chemical identity. The hypothesis was further confirmed by observing the matching mass spectral fragmentation pattern of the acetylated derivative of the reaction product obtained by microwave-induced hydrolysis with the pattern for the acetylated derivative of 4-aminophenol, the expected hydrolysis product of acetaminophen and its conjugates. Therefore, microwave-induced hydrolysis reactions produce the same product in 2 min as that requiring 20 min of heating at 100 °C. We conclude that commercially available domestic microwave ovens can be used for rapid hydrolysis of acetaminophen and its conjugates in urine for colorimetric screening.

References
2. Hassan MF. Drug screening. In: Kaplan L, Pesc A, eds. Clinical chemistry theory,
Above-Normal Urinary Excretion of Albumin and Retinol-Binding Protein in Patients with Acute Myocardial Infarction

To the Editor:

Concentrations of albumin and retinol-binding protein (RBP) in urine are more sensitive indicators of renal dysfunction in hypertensive patients than the serum creatinine concentration and the Albustix® reaction (Ames, Tarrytown, NY) (1). Many patients with heart failure had above-normal urinary albumin and RBP concentrations (2), and these values decreased as the patients were treated with captopril (3). Patients with diabetes mellitus often have above-normal urinary albumin and RBP concentrations (4, 5), and this might dispose them to cardiovascular events (6). We have now studied the urinary excretion of albumin, RBP, and creatinine in 22 patients admitted for acute myocardial infarction (AMI) (17 men and 4 women). One of these patients had non-insulin-dependent diabetes mellitus, two had hypertension, and two had had AMI earlier. Diagnosis of AMI was based on patients' history, determination of serum creatine kinase subunit MB, serum lactate dehydrogenase isoenzyme 1, and electrocardiograms (7) according to the World Health Organization criteria (8). The study was in accordance with the ethical standards of the Helsinki Declaration of 1975 as revised in 1983.

Of the 22 patients studied, 15 were admitted within 24 h after onset of symptoms and were grouped as early admissions; the other 7 were admitted later in the clinical course and were grouped as late admissions. Twelve were given streptokinase and two were entered into a double-blind study comparing actilyse and placebo. None of the late admissions underwent thrombolyis. All patients were given acetylsalicylic acid regularly (150–300 mg/day). The patients were mobilized gradually according to a seven-step protocol during the first week of admission (7).

Creatinine concentrations in serum and urine were measured with a photometric method (jafe®). All patients had a serum creatinine concentration <120 µmol/L. We determined the concentrations of albumin and RBP in the first voided sample of urine by immunochemical assays on the day of admission (day 0) and in the first voided urine on days 1, 2, and 5 (5, 9) afterwards. The upper limits of the reference ranges for urinary albumin and RBP concentrations were 0.45 µmol/L and 0.21 mg/L, respectively (5, 9).

On the day of admission, 13 of the 22 patients had an above-normal urinary albumin concentration; 8 of the 22 had an above-normal urinary RBP concentration. The urinary albumin and RBP values correlated (Spearman ρ = 0.65, P = 0.001, Spearman rank correlation coefficient test).

The urinary RBP concentrations were higher in the early admission group (median 1.43, range 0.18–80.30) than the late admission group (median 0.06, range 0.04–43.0) (P = 0.04, Mann–Whitney U-test, two-tailed). The urinary albumin concentrations were slightly higher in the early admissions (median 0.82, range 0.07–4.31) than in the late admissions (median 0.20, range 0.02–62.10). The urinary creatinine concentrations were similar (median 8.70 vs 6.06 mmol/L).

The urinary albumin and RBP of the early admissions decreased significantly from day 0 to day 1 and from day 2 to day 5 (P <0.05, Wilcoxon–Pratt test, two-tailed). Urinary creatinine concentrations increased slightly during the first 3 days (Table 1).

Half of the patients with AMI may have had above-normal urinary albumin and RBP concentrations in the initial phase of the cardiac event. We found a lower proportion of above-normal values in patients with hypertension and a higher proportion in patients with heart failure (1, 2). The above-normal albumin and RBP concentrations are due to dysfunction of the glomeruli and the proximal tubuli, respectively (1–3).

Most of the above-normal urinary albumin and RBP excretion in our present study was a temporary phenomenon, reflecting the initial phase of the AMI. The time course for urinary albumin and RBP concentrations after the onset of symptoms did not differ between the groups of patients given streptokinase and those not (10). Thus the above-normal urinary excretion of albumin and RBP in the acute phase of the AMI may mainly reflect transitory hemodynamic and neurohormonal changes. This phase is characterized by above-normal concentrations of norepinephrine, renin, angiotensin-converting enzyme, and aldosterone; the neurohormonal activation begins to subside within the first 72 h (11).

Although mobilization of the patients during the first week of hospitalization might have increased their urinary protein excretion, other effects following the AMI seemed to more greatly decrease their concentrations of urinary albumin and RBP.

Goelting et al. suggested that the microalbuminuria of the acute phase of AMI was due to an inflammatory reaction in the renal vascular system (12). However, this explanation does not fit with the rapid reversibility of the renal dysfunctions. Above-normal urinary albumin excretion was viewed as a cardiovascular risk factor in other studies (6). But the interrelation may be more complex. Our study shows that the acute events of an AMI may cause similar, albeit transitory, renal dysfunctions.

We thank Hanne Braudd for technical assistance.

References

1. Ellekilde G, von Eyben FE, Holm J, Hemmingsen L. Above-normal urinary ex-

---

Table 1. Time Course of Urinary Albumin, RBP, and Creatinine Concentrations in 15 Patients with AMI and Early Admission

<table>
<thead>
<tr>
<th>Day of admission</th>
<th>Albumin, µmol/L</th>
<th>RBP, mg/L</th>
<th>Creatinine, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.82 (0.07–4.31)</td>
<td>1.43 (0.18–80.30)</td>
<td>8.70 (3.22–15.16)</td>
</tr>
<tr>
<td>1</td>
<td>0.30 (0.03–4.0)</td>
<td>0.27 (0.06–50.00)</td>
<td>10.56 (5.30–20.10)</td>
</tr>
<tr>
<td>2</td>
<td>0.22 (0.06–0.98)</td>
<td>0.20 (0.07–5.76)</td>
<td>13.53 (4.18–34.80)</td>
</tr>
<tr>
<td>5</td>
<td>0.09 (0.02–1.55)</td>
<td>0.06 (0.02–0.64)</td>
<td>5.02 (1.82–11.54)</td>
</tr>
</tbody>
</table>