serum albumin, and 1 g of sodium azide per liter. We diluted $^{125}$I-labeled PG C in working buffer (prepared by mixing diluent buffer containing, per liter, 2.5 g of normal rabbit serum with 0.1 mol of EDTA in a volume ratio of 6:1) to provide about 10 000 counts/min in 100 μL; anti-PG C was diluted in the working buffer 1:100 000. The standard curve consists of serial dilutions of unlabeled PG C standards ranging from 80 to 2.5 μg/L. After incubating 100 μL of standards or test sera with 100 μL of $^{125}$I-labeled PG C for 18-24 h at room temperature, we added 1 mL of the second antibody (goat anti-rabbit gamma globulins in 0.01 mol/L phosphate buffer, pH 7.4, containing polyethylene glycol, 80 g/L) to precipitate the immunocomplex. The samples were then centrifuged at 2500 × g at 4°C for 20 min, the supernates decanted, and the radioactivity in the precipitates counted for 3 min with an automatic gamma counter. Nonspecific binding was about 2%, and binding in the absence of added antigen was about 50%.

Intra-assay CV (n = 15) was 3.4% at 18.3 μg/L and 5.3% at 44.2 μg/L. Interassay CV (n = 11) at the same concentrations was 4.3% and 6.0%, respectively. Analytical recovery of PG C from serum supplemented with PG C standard in concentrations ranging from 5.5 to 62.1 μg/L ranged from 98.3% to 109.4% (mean = 104.3%). Serial dilutions of three samples showed a good linear response to dilution. The detection limit, corresponding to 3 SD minus the mean counts bound in five zero standards in duplicate, is 2.1 μg/L. There was no cross-reactivity with PG A at 500 μg/L.

The mean serum concentration of PG C in 40 healthy subjects was 14.07 (SD 8.7) μg/L. No statistically significant differences were found between values for males and females for females.

In conclusion, the modified assay is accurate and reliable. Its greatest advantage is its shorter incubation time (18-24 h vs 96 h); it also better separates bound from free fractions than the original method.

Further studies are in progress to evaluate the clinical usefulness of the PG A/PG C ratio in gastric cancer and precancerous conditions.

References

Simplified Fluorometric Determination of Salicylate in Plasma, Ram N. Gupta and Mohesullah Zaman; Dept. of Lab. Med., St. Joseph's Hospital, Hamilton, Ontario, Canada L8N 4A6

For the last few years immunoassay reagents (1, 2) and enzyme reagents (3) have been available for the emergency
determination of salicylate in plasma. However, for economy many clinical laboratories still measure salicylate in plasma by Trinder's colorimetric procedure (4), even though Trinder's reagent contains mercuric salt, which is hazardous for the environment. Here we report an alternative for Trinder's reagent for rapid and economical determination of salicylate in plasma.

We mix by hand inversion 10 μL of sample (plasma or aqueous standard) with 10 mL of 0.1 mol/L ammonium hydroxide in glass-distilled water in a 16 × 100 mm glass tube. We then measure the fluorescence of the diluted sample at λex = 306 nm and λem = 400 nm, using a Model 650-15 fluorospectrophotometer (Perkin-Elmer, Norwalk, CT 06859-0012).

The observed fluorescence of aqueous and plasma salicylate standards is similar and is linearly related to salicylate concentrations from 0.1 to 8.0 mmol/L. The presence of increased concentrations of protein (12 g/L), bilirubin (35 μmol/L), hemoglobin (0.5 g/L), and triglycerides (5 mmol/L) did not lead to any decrease in the observed fluorescence in various plasma samples supplemented with 2 mmol of salicylate per liter. Analysis of 25 plasma samples known to be salicylate-free gave fluorescence values corresponding to salicylate concentrations of 0.05 to 0.2 mmol/L. Acidic drugs that, like salicylate, have relatively high therapeutic concentrations (e.g., acetaminophen, ibuprofen, tolmetin, sulindac, and diclofenac) gave insignificant values for salicylate when plasma supplemented with these drugs at a concentration of 1 g/L was analyzed by the described procedure in separate experiments. However, diffusion, an analog of salicylate, gave a fluorescence response similar to that of salicylate. Chlorpromazine, one of the commonly prescribed phenothiazines, does not show any fluorescence response at a concentration of 1 g/L by this procedure. Acetylsalicylate in plasma is, for all practical purposes, present as salicylate and is measured as such.

To check reproducibility, we analyzed Therachem® low and high therapeutic drug controls (Fisher Scientific, Orangeburg, NY 10962). The results show acceptable precision and accuracy, comparing well with the values obtained by the Abbott TDx procedure:

<table>
<thead>
<tr>
<th>Therachem, low</th>
<th>Therachem, high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, mmol/L</td>
<td>Mean, mmol/L</td>
</tr>
<tr>
<td>0.45 (0.51–0.29)*</td>
<td>2.83 (3.29–3.37)</td>
</tr>
<tr>
<td>CV, %</td>
<td>CV, %</td>
</tr>
<tr>
<td>0.80</td>
<td>1.17</td>
</tr>
<tr>
<td>6.48</td>
<td>5.9</td>
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</tbody>
</table>

n = 10 each. *Mean values (and range) obtained by Abbott TDx as reported in Fisher's product insert.

Several fluorometric procedures for the determination of plasma salicylate have been described and applied for pharmacokinetic studies of salicylate because of the high sensitivity and specificity provided by fluorescence procedures. In most cases, samples have been prepared for fluorescence measurement by extraction with organic solvents and back-extraction into the aqueous phase (5); in some cases, proteins have been removed by precipitation with tungstic-sulfuric acid reagent (6). Recently, a direct fluorometric procedure in the presence of EDTA and tribium salts and without the removal of proteins has been described (7). The procedure we describe is economical and compatible with the environment.

References

Screening for Pauclalbuminuria with Frozen Urine Samples
Bernhard K. Krämer, Christiane M. Erley, Inge Lindena, and Teut Risler (Section of Nephrol. and Hypertension, III. Dept. of Internal Med., Univ. of Tübingen, Otfried-Müller-Str. 10, D-7400 Tübingen, F.R.G.)

Pauclalbuminuria ("microalbuminuria"), a strong predictor of future overt nephropathy in type I diabetic patients, can be stopped or even reversed by control of blood pressure and (or) blood glucose, thus probably slowing or preventing development of overt diabetic nephropathy (1). A decrease in albumin concentration in samples stored at −20 °C (2, 3) and uncertain effects of centrifugation before assay (4, 5) have recently been reported. Therefore our aim in this study was to examine what effects storage at −20 °C, centrifugation before assay, or urinary pH might have on urinary albumin concentration.

We studied 43 urine specimens after storage at −20 °C for two weeks and three months. Albumin concentration was measured by enzyme-linked immunosorbent assay as described previously (6). Albumin concentrations decreased significantly (statistical evaluation by analysis of variance, Student–Newman–Keuls test) after three months of storage but not after two weeks (Table 1). Coating the storage tubes with casein to prevent adsorption of albumin to the tube as well as centrifugation before assaying the albumin concentration had no consistent effect on the decrease of albumin concentrations (Table 1). The pH was significantly lower (P < 0.02) in samples in which the albumin concentration decreased compared with that in samples with no decrease (pH 5.75 ± 0.23 vs 6.46 ± 0.13, respectively).

The number of urine samples with visible precipitation increased during storage (44% after three months); those samples also tended to have lower pH values (P > 0.05), but the presence of precipitates was not clearly correlated with the decrease of albumin content in prolonged storage.

The possibility that storage conditions of urine samples may affect albumin measurement must be taken into account when monitoring or detecting paucialbuminuria. Storing urine samples before determination of albumin concentration is not recommended for periods markedly exceeding two weeks.