basic chemical principles. Reviewing uric acid methodology, we discovered one incorrect and two unbalanced reaction equations for uric acid analysis by the uricase/peroxidase-phenol-4-aminopyrpyline method. Figure 1 illustrates the overall reaction.

An investigator has incorrectly proposed the formation of a dichloro- 
quinoneimine dye from the reaction between 2,4-dichlorophenol and 4-
aminopyrpyline (1). Formation of this sterically hindered product would require the unlikely liberation of a hydride ion. Another investigator has suggested that the predominant product is a chloro-p-quinoneimine dye (2). The correct reaction is illustrated in Figure 1, reaction 2(a).

Two unbalanced reaction equations have also appeared (3, 4). Balanced equations for the reaction of tribromophenol or 4-hydroxybenzoic acid with 4-aminopyrpyline are given in Figure 1, reaction 2(b,c). A major difference in these reactions is the amount of hydrogen peroxide consumed. If one assumes that the products have equivalent molar absorptivities, the stoichiometry suggests that the tribromophenol method (3) (Figure 1, reaction 2b) should be twice as sensitive as the 4-
hydroxybenzoic acid method (4) (Figure 1, reaction 2c). This important point was not obvious from the unbalanced equations.

The choice between similar methods that are being developed or applied should be based on their chemical principles. Authors should ensure that the chemistry involved is correctly represented, to facilitate such choices. Others who are interested in developing a more sensitive or selective phenol-4-
aminopyrpyline method should consult previous work (2) in order fully to understand the chemistry of this colorimetric method.

References
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Rapid Determination of the p-
Aminobenzoic Acid Excretion Index
in Urine without Use of Radioactivity

To the Editor:

The p-aminobenzoic acid (PABA) test is a simple, noninvasive method to assess exocrine pancreatic function. However, it shares a lack of specificity with other indirect tests of function. To compensate for abnormalities in PABA resorption, metabolism, and excretion, Mitchell et al. (1) introduced the "PABA Excretion Index," in which the analytical recovery of PABA released from administered N-benzoyl-
tyrolyl-PABA is compared with the recovery of an equimolar dose of free PABA given on a second occasion (1) or with the recovery of (14)C-PABA given concomitantly (2, 3). The use of radioactivity, however, excluded children, pregnant women, and patients with renal insufficiency from the test.

In a recent Letter, Lawson et al. (4) described a liquid-chromatographic method for measuring PABA in plasma. One of the suggested advantages of their technique is the possibility of incorporating a nonradiochemical marker into the PABA test, to correct for general malabsorption. We propose p-aminosalicylic acid (PAS) for this purpose. Its molecular structure is very similar to that of PABA and its pharmacokinetic properties are also similar (5, 6). Moreover, PAS is readily separated from PABA by liquid chromatography and is nontoxic in the applied dose.

Liquid chromatography was performed after alkaline hydrolysis (1 h at 120 °C) of equal volumes of 6-h specimens of urine and 10 ml/L NaOH containing m-hydroxybenzoic acid as an internal standard. After cooling the samples, we diluted them 25-fold with 1.05 mol/L acetic acid and injected 20-
µL aliquots onto a 25 cm × 4.6 mm (i.d.) stainless-steel column filled with 10-µm "Lichrosorb RP 18" (Chrompack, Middelburg, The Netherlands). The column was isocratically eluted with a 3:1 (by vol) mixture of sodium acetate buffer (10 mmol/L, pH 4.0) and methanol at a pressure of 6 MPa. The column effluent was monitored at 280 nm with a Model LC-75 detector (Perkin-Elmer Corp., Gouda, The Netherlands) equipped with an 8-µL flow cell. PABA and PAS metabolites were quantified by peak-height analysis. The time required for a run was 7 min. Linear response curves were obtained for both PABA and PAS in concentrations ranging from 0.2 to 400 µg/ml, and within-day and day-to-day CVs for both ranged between 1.2 and 3.1% (Table 1). We tested some commonly used drugs for interference: cimetidine, procainamide, tolbutamide, chloramphenicol, 5-aminosalicylic acid, acetylsalicylic acid, trimethoprim, phenacitin, and acetaminophen. Except for slight interference from procainamide, none of these drugs in 100 µg/ml concentration produced peaks in the chromatogram.

To compare the pharmacokinetics of free PABA and PAS, we administered 340 mg of PABA, 360 mg of PAS (as 500 mg of Na PAS·2 H2O), and 150 mL of Lundh test meal to five healthy volunteers. Urine was collected at 1-h intervals and PABA and PAS were measured.

Table 1. CVs of HPLC Analysis for Urinary PABA and PAS

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<tr>
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<th>Within-day</th>
<th>Day-to-day</th>
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<tr>
<td>Mean, mg/L</td>
<td>CV, %</td>
<td>Mean, mg/L</td>
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<tr>
<td>PABA</td>
<td>256.7</td>
<td>1.2</td>
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<tr>
<td>PAS</td>
<td>252.5</td>
<td>1.6</td>
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* n = 10.  † n = 5.
measured therein. In 6 h 69.5% (SD 4.2%) of the PABA and 65.6% (SD 9.2%) of the PAS was excreted. During this 6 h PABA and PAS excretion were very comparable. Identical PABA and PAS excretion were observed after administration of 900 mg of PABA alone with 150 mL of Lundh meal.

PABA and PAS recoveries were evaluated in 12 healthy volunteers in the normal PABA test. After administration of 1 g of N-benzoyl-L-tyrosyl-PABA and 500 mg of Na PAS, 2 H2O with a test meal, PABA excretion in the 6-h urine was 65.3% (SD 10.0%) and PAS excretion was 74.2% (SD 9.1%) of that ingested. Mean PABA/PAS ratio was 0.89 (range 0.62–1.15). On the basis of these results we arbitrarily chose 0.75 as the lower limit of normal for this ratio. A strong positive relationship between % PABA recovery (x) and the PABA/PAS ratio (y) was demonstrated in healthy volunteers and patients with pancreatic disease or various forms of gastrointestinal disorder: y = 0.011x + 0.24 (r = 0.793, n = 64).

In all cases where % PABA excretion was >50%, the PABA/PAS ratio was >0.75. However, in six patients with falsely low PABA recoveries and without clinical evidence for pancreatic disease, normal PABA/PAS ratios were observed.

These preliminary data support our opinion that the nonradioactive marker PAS can be used instead of [14C]-PABA to obtain a fast and reliable PABA excretion index. More extensive studies of patients are in progress.

References

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Affinity Electrophoresis of Alkaline Phosphatase Isoenzymes

To the Editor:

Electrophoresis is commonly used to identify qualitatively the isoenzyme patterns of an increased value in alkaline phosphatase (ALP; EC 3.1.3.1). Most clinical requests for isoenzyme evaluation arise from the need to differentiate enzyme from liver and bone sources, but there is little difference in electrophoretic mobility between these two isoenzymes making their identification difficult.

Rosalki and Foo (1) have noted that the ability of plant lectins to bind specifically to carbohydrate-containing proteins provides an alternative procedure for separating ALP isoenzymes. We have subsequently incorporated lectin from wheat germ (Triticum vulgare) in the pre-cast agarose gels from Corning Medical, Medfield, MA 02052, and we confirm that this modification greatly enhances the resolution of the bone and liver ALP fractions.

In this procedure, the buffer present in the agarose gel was replaced by one containing lectin by placing a single sheet of "stamoon" paper (Corning cat. no. 47015800H) onto the gel surface (Corning "Special Gel," cat. no. 47014000F) for 15 s, then removing it and applying 4 mL of Tri-Bicine electrophoresis buffer (Corning cat. no. 470187000) containing 50 mg of wheat-germ lectin (Sigma Chemical Co., Poole, U.K.) per liter. The lectin–Tri-Bicine buffer was freshly prepared by suitably diluting a stock 500 mg/L solution of lectin, which was stored at 4°C in sodium barbital buffer (50 mmol/L, pH 8.6; Corning cat. no. 47018000L). The buffer was left in contact with the gel for 15 min, then excess buffer was drained from the surface, the sample wells were gently blotted with lint-free tissue, and 0.6 mL of serum sample was applied to each well in the usual way. Electrophoresis was performed for 20 min at 170 V with use of unmodified Tri-Bicine buffer. The separated fractions were subsequently located with 4-methylumbelliferyl phosphate substrate (Corning cat. no. 470073000) and scanned with a Corning Model 720 densitometer.

Resolution of bone and liver ALP was enhanced as shown in Figure 1, permitting densitometry of the fractions. In the presence of lectin, ALP isoenzymes separate in order of decreasing electrophoretic mobility as liver, placental, bone, and intestinal. We find that a lectin concentration of 50 mg/L in the Tri-Bicine buffer best resolves the liver and bone fraction (1).

Fig. 1. Fluorometric scans of patients' samples: 1–3 display the presence of liver and bone fractions, 4–6 show the additional presence of intestinal isoenzyme.