function. In this study of 23 subjects, analytical problems were encountered in seven of 23 tests. Three of six control samples and four of the 17 patients' samples showed either rapid fading of color or unexpected colors (usually green) in the chemical determination of urinary PABA.

We have been using the PABA test for the past 18 months and so far have investigated 70 subjects. The test is performed according to the protocol of Braganza et al. (2) except that we use acid hydrolysis rather than alkaline hydrolysis. We have had no instances of fading color or abnormal color development. As Braganza et al. recommend, we obtain a pretest urine sample, which is analyzed in parallel with the post-test sample.

In only six of the 70 cases did the pre-test urine have an absorbance equivalent to an aromatic amine concentration of 10 mg/L or more, and no case has been recorded in this laboratory of a pre-test sample of urine containing 30 mg/L or more of aromatic amines, which would invalidate the test procedure by the criteria of Braganza et al. A note of caution must be made, however, with regard to concentrations of aromatic amine less than 30 mg/L. If the absorbance of the 6-h post-dose urine sample at 550 nm is not corrected for background absorbance as measured in the pre-test sample, significant errors may be introduced into the procedure.

For the 70 investigations carried out so far at this center, the mean volume of urine excreted over the 6-h test period was 660 mL (range 124-2880 mL). We found that the absorbance of samples with an aromatic amine content of 25 mg/L in the pre-test urine would be equivalent to a value of 17 mg in a 6-h volume of 660 mL. Thus if post-dose amine content was not corrected for pre-dose amine content, the percentage excretion rate would be falsely increased by at least 10% for a 0.5-g dose of benzoyl-tyrosyl-PABA (corresponding to 0.17 g of PABA).

The only apparent difference in procedure between that of Faulder and Strange and ourselves is our preference for acid hydrolysis. Braganza et al. claim that acid hydrolysis, but not alkaline hydrolysis, leads to losses of PABA due to decarboxylation. We compared their methods of acid and alkaline hydrolysis with the acid hydrolysis procedure given by Yamato and Kinoshita (3). Briefly, the three techniques are:

Alkaline hydrolysis—Braganza. Dilute urine samples 100-fold with distilled water and incubate 2.5 mL with 0.5 mL of 5 mol/L sodium hydroxide at 100 °C for 90 min. Then acidify the hydrolysate with 6 mol/L hydrochloric acid.

Acid hydrolysis—Braganza. To 1.0 mL of urine sample, add 2.0 mL of 1.2 mol/L hydrochloric acid and incubate at 100 °C for 60 min.

Acid hydrolysis—Yamato. To 1.0 mL of urine, add 2.0 mL of 1.5 mol/L hydrochloric acid and incubate at 100 °C for 15 min.

We hydrolyzed urine samples from 14 subjects by all three methods and then analyzed for PABA in the usual manner. We corrected for any evaporative losses by bringing total volumes back to 3.0 mL in each case. Mean PABA concentrations for the 14 samples were 180.2 mg/L after alkaline hydrolysis, 200.0 mg/L for 60-min acid hydrolysis, and 213.9 mg/L for 15-min acid hydrolysis. These findings support those of Foster et al. (4) that prolonged acid hydrolysis results in a loss of as much as 20% of the PABA in the urine. We therefore remain unconvinced that alkaline hydrolysis over such long incubation periods offers any real advantage over 15-min acid hydrolysis. Indeed, such long incubation periods at high temperatures may be a contributory factor in the high incidence of interference encountered by Faulder and Strange, as may the use of alkaline rather than acid hydrolysis. Foster et al. also postulate that alkaline hydrolysis may increase the frequency of drug interference and Braganza (5) quotes a high (12%) technical failure rate on using alkaline hydrolysis. This assumes, of course, that a correct patient preparation protocol is used and interference from sulfonamides, duretics, nitrazepam, phenacetin, paracetamol, and chloramphenicol can be ruled out.

Our own reservations about the PABA test concern its clinical utility. In this center we have found considerable overlap between our control group and patients with known pancreatic disease (6).

References


I. Tarbit

Dept. of Clin. Biochem.
Freeman Hospital
Newcastle upon Tyne
NE7 6DN U.K.

Low Neutrophil Alkaline Phosphatase in Renal Tubular Acidosis with Hypophosphatemia after Toluene Sniffing

To the Editor:

An unanticipated low score of 4 for neutrophil alkaline phosphatase (NAP) (normal range, 13 to 130) was found for a specimen being tested as a normal control in a series of stains being done on formol/methanol-fixed peripheral blood smears. Scoring is based on a combination of number, size, and staining intensity of neutrophil granules (1). The test was found that the blood was from a patient with multiple metabolic abnormalities related to paint- and glue-sniffing. The 31-year-old Oriental man had been admitted for investigation of several weeks' motor incoordination and increasing fatigue. His past history included surgical removal of two urinary calculi within a year. Physical examination revealed a strong odor of glue on the patient's breath, and he admitted to the abuse of hydrocarbon inhalants since high school.

Laboratory values (and normal reference intervals) were as follows:

Leukocytes 21 400/μL (4.8 to 10.8)

Neutrophils 82%

Hemoglobin 159 g/L (128-180)

Arterial blood gases

pH 7.14 (7.35-7.45)

Pco₂ 20 mmHg (34-46)

Po₂ 113 mmHg (80-100)

Base excess −21.0 (±2)

Bicarbonate 6.4 mmol/L (21-27)

Electrolytes

Sodium 141 mmol/L (136-146)

Potassium 1.3 mmol/L (3.5-5.0)

Chloride 126 mmol/L (98-109)

Total CO₂ 10 mmol/L (24-34)

Blood urea nitrogen normal

Creatinine normal

Calcium 110 g/L (86-108)

Phosphorus 13 g/L (25-49)

Magnesium 29 g/L (18-24)

Blood toluene concentrations were 6 μg/L by gas chromatography.

Results of liver-function tests were within normal limits. Urine pH was 6.25 by pH meter.

This hyperchloremic, hypokalemic metabolic acidosis without an above-
normal anion gap and with respiratory compensation was thought to represent distal renal tubular acidosis, an opinion supported by the kidney's apparent inability to acidify the urine further in the absence of azotemia. The hypercalcemia probably is related to the acidosis, which increases calcium renal tubular reabsorption and results in hypercalcuria. (This was probably the etiology of the patient's prior urinary calculi.) The hypophosphatemia might be secondary to increased renal excretion of phosphorus, with or without coexisting secondary hyperparathyroidism. Concentrations of urinary phosphorus and serum parathyrim were not measured because the patient discharged himself against medical advice.

Toluene is used as a solvent in paint thinners, glue, and transmission fluid. It is abused, usually by teenagers, through inhalation because of its initial euphoric and subsequent depressant effect on the nervous system (2). The principal toxic effect is a reversible inability of the distal tubule to excrete hydrogen ion and reabsorb bicarbonate, which results in a hypokalemic, hyperchloremic nonanion gap metabolic acidosis that usually resolves (3-5).

The NAP assay is used in hematology primarily to differentiate chronic myelogenous leukemia, a condition that results in a low score because of the neoplastic cells' decreased enzyme content. In other leukemia conditions, the score is usually above normal. Low NAP scores may be seen in acute myelogenous leukemia, paroxysmal nocturnal hemoglobinuria, hereditary hypophosphatasia, and some viral infections. The test is performed on fixed blood smears at pH 9.1, with naphthol phosphate as substrate. The product, aryl naphthalamide, is coupled to a diazonium salt, causing a localized blue color in leukocytes where alkaline phosphatase (EC 3.1.3.1) is present (J).

We initially believed the patient's decreased NAP activity to be related to the effect of acidic serum on some enzymes with an alkaline pH optimum, but samples from patients with acidosis from other causes did not give low NAP scores. Depressed leukocyte function with decreased chemotactic, phagocytic, and bactericidal properties described in hypophosphatemia (6) is thought to be related to decreased availability of organic phosphate compounds for ATP synthesis and consequent impairment of all ATP-dependent processes; this is most often seen in patients with low phosphate who are receiving parenteral hyperalimentation or in alcoholics. In this patient, however, ATP depletion secondary to decreased phosphorus cannot explain our finding because alkaline phosphatase is not an ATP-dependent enzyme system nor do any known ATP-dependent coenzymes participate in the reaction. Substrate (phosphate) depletion, though present in vivo, would not account for the decreased activity in vitro, where excess substrate is added. Thus, the explanation may lie in either decreased endogenous NAP synthesis or in an intrinsic structural defect such as in denaturation by a known agent. Whether the toxic effects of either toluene or any of the coexisting chemical disturbances in this patient can account for this remains subject to further investigation.

References

Stephen Weinstein
A G. Scottolini
N V. Bhagavan

Kaiser Permanente Med. Center
1697 Ala Moana Blvd.
Honolulu, HI 96815

Modified Liquid-Chromatographic Method for Creatinine Determinations

To the Editor:
In a recent paper Okuda et al. (1) described the quantitative determination of creatinine in serum and urine samples by the application of reversed-phase liquid chromatography. Samples were prepared by adding 0.4 mL of acetonitrile to 0.2 mL of sample, then centrifuging. Achari et al. (2) described an alternative procedure in which the protein was removed from serum samples by use of Amicon filtration units.

We found the method of Achari et al. excellent for measurement of creatinine in urine; samples were injected directly onto a Highrom ODS-2678 column after a 50-fold predilution. Our mobile phase consisted of sodium acetate buffer (50 mmol/L, pH 6.5)/acetonitrile (85/15 by vol) and we found that sensitivity was doubled by monitoring the effluent at 245 nm instead of 254 nm. The retention time for creatinine was 3.2 min, and results (y) correlated well with those by the Technicon N11B continuous-flow Jaffé method (x) (n = 25, r = 0.9912, y = 0.962x + 0.344), where aqueous standards were used for the calibration and urinary creatinine concentration was expressed in mmol/L.

However, we encountered problems when we tried to use the method of Achari et al. to determine creatinine in serum. Although they reported that the ultrafiltration method of serum "clean-up" was rapid and reproducible, with complete recovery, and that precipitating the proteins with acetonitrile was unsuitable (because of a high blank value and up to 16% higher results for aqueous creatinine samples than for serum samples of the same concentration), we found that the protein precipitation procedure was more reliable than the ultrafiltration method for serum samples. We used and washed the Amicon membrane cones according to the manufacturer's instructions but, after such use, the concentrations of creatinine in the ultrafiltrates differed from those in the original samples. We detected this initially by a loss in linearity of the serum standard curve. Also, some quality-control samples (e.g., Ortho) gave results about as expected, but others (e.g., Beckman Decision) gave considerably lower volumes of ultrafiltrates, in which the concentrations of creatinine (determined by the Jaffé method) were 1.5- to sixfold higher than the manufacturer's stated means.

For serum, the sample preparation method of Okuda was more rapid than the ultrafiltration procedure, requiring 3 min rather than 10 to 15 min of centrifugation. The analytical recovery of creatinine from serum by this method was 96-103% in our hands; the blank value was negligible and linearity was obtained at least up to 1000 μmol/L. The creatinine peak was poorly resolved from other nearby peaks if the volume ratio of acetonitrile to serum was much greater than 2/1. We attribute this effect to the decrease of the polarity of the sample solution as acetonitrile is added.

For measuring serum creatinine, separation of creatinine from other serum constituents is improved by increasing the volume of aqueous sodium acetate in the mobile phase to 98%, as used by Achari et al. Thus, by using their chromatographic conditions and the protein precipitation procedure used by Okuda et al., we found good agreement between the results obtained with this modified method and the Jaffé method.

However, when we used the method to determine creatinine in the serum of