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 hypercalciemia probably is related to the acidosis, which increases calcium resorption and bone and results in hypercalciuria. (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 parathyrin 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 depressing 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 hematoloy primarily to differentiate chronic myelogenous leukemia, a condition that results in a low score because of the neoplastic cells' decreased enzyme content. In other leukemoid 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 (1).

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


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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 Hichrom ODS-2578 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 + 0.34), with aqueous phosphoric acid 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 plasma 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 acetonitrile precipitation procedure of 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
Effects of Platelets on Collection of Specimens for Assay of Ammonia in Plasma

To the Editor:

We have read with interest the report on the collection of specimens for determining plasma ammonia (Howanit et al., Clin Chem 30: 906–908, 1984) and would like to report some of our findings on the effect of platelets on ammonia analyses in non-hemolyzed samples.

We collected samples from 28 randomly selected outpatients and divided each sample into three aliquots. One aliquot was placed in an ammonia-free plain glass tube and allowed to clot, and the serum was separated by centrifugation; the two other aliquots were placed in separate ammonia-free tubes containing lithium heparin. One set of lithium heparin tubes was centrifuged at 600 × g for 5 min to produce platelet-rich plasma, while the other was centrifuged at 10 000 × g for 5 min to produce platelet-poor plasma. Without delay we measured ammonia with a Cobas Bio centrifugal analyzer, using the glutamate dehydrogenase method of the "Monotest" ammonia kit (Boehringer Mannheim). Platelets in all three groups of specimens were counted with a Coulter Counter. The mean platelet count for the serum specimens was 3.5 × 109/L (range, 2 × 109/L to 5 × 109/L); respective counts for platelet-rich plasma and platelet-poor plasma were 100 (25–250) × 109/L and 2 (1–6) × 109/L.

The mean concentration of ammonia in platelet-poor plasma was 21.5 μmol/L (range, 11–35 μmol/L), substantially less than in both serum and platelet-rich plasma: 63 (31–109) and 34 (15–54) μmol/L, respectively.

We also compared the ammonia content (y) and the platelet content (x) in the platelet-rich plasma specimens by regression analysis. The regression line had an intercept (ammonia concentration) of 18.8 μmol/L at zero platelet count and a slope of 0.16 μmol/L per 109 platelets per liter. The correlation coefficient (r) was 0.88 (significant at p < 0.005).

In a separate study, we investigated the stability of ammonia in platelet-poor plasma at room temperature and at -70 °C in 26 of the randomly selected outpatient specimens. The platelet-poor plasma was divided into three aliquots and was assayed immediately after collection, after 5 h at room temperature, and after 3 h at -70 °C. At room temperature, the ammonia increased substantially, from 19 (5–35) to 39 (15–63) μmol/L after 3 h. The ammonia concentrations after storage at -70 °C increased slightly, to 21 (4–37) μmol/L. This mean increase of 10.5% is comparable with the results of Howanitz et al. after storage of heparinized plasma.

We also compared the results of platelet-poor nonhemolyzed plasma specimens collected simultaneously from peripheral veins and fingerprick sites from 10 randomly selected patients. The fingerprick (capillary blood) results were much higher—74 (32–117) μmol/L vs 18 (7–28) μmol/L—presumably because of the release of ammonia from activation of platelets in the fingerprick wound.

In conclusion, therefore, we recommend the following:

- Specimens for plasma ammonia measurements should be collected from peripheral veins into ammonia-free heparinized containers.
- Specimens should be centrifuged immediately with sufficient force to remove platelets.
- Specimens should be stored at -70 °C if they cannot be analyzed promptly.

We thank the John P. Kelly Mater Research Foundation for a grant supporting this project.

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To the Editor:

We are delighted that Dr. Cowley et al. agree with us on the conditions for collection of plasma ammonia. These authors comment on the role of platelets in ammonia generation but it appears that there are at least five possible sources of this ammonia: plasma itself, diffusion from platelets, diffusion from intact erythrocytes, hemolysis, and the coagulation process, which may generate ammonia by diffusion from platelets or by erythrocytes or both.

Our data, as well as those of Cowley et al., show that even when plasma is stored at -70 °C, ammonia content increases. Other studies of the generation of ammonia from platelet-rich and platelet-poor specimens have found similar increases (1). In some elegant studies, Fushimi et al. (1) concluded that the major source of ammonia increases in stored specimens is erythrocytes. Therefore, and because erythrocytes reportedly have about threefold as much ammonia as plasma does, we speculate that most of the increases in