patients after a renal transplant operation, isolated samples gave results as much as 40% higher than those by the Jaffé method, because of interference by the drugs used to treat these patients: azathioprine, cyclosporin A, cefotaxime, and prednisolone. Adding a mixture of these drugs to an aqueous creatinine standard increased the peak height and peak width and prolonged the retention time of "creatinine." In addition, at a flow rate of 1 mL/min and a temperature of 40 °C, repeated analysis of serum samples led to poor resolution and a prolonged retention time. To reduce the frequency of column washing and to maintain consistently good separation of creatinine from other serum constituents and interferents, we increased the oven temperature to 50 °C and the flow rate to 1.5 mL/min. We also increased the pH of the sodium acetate to 7.5.

These conditions produced sharp, well-defined peaks, with creatinine eluting at 2.7 min. Precision was good; CVs were 2.83%, 2.44%, and 1.47% ($n = 10$) at low, intermediate, and high concentrations of creatinine. Accuracy and correlation with the Jaffé method (x) were excellent ($n = 33$; $r = 0.9970$; $y = 1.02x - 11.5$). We used serum standards for the calibration and measured serum creatinine in µmol/L. There was no interference from glucose, bilirubin, urea, acetone, ascorbic acid, pyruvate, sodium bicarbonate, thymol, KCl, NaCl, valproate, acetaminophen (paracetamol), phenobarbital, amitriptyline, acetylsalicylic acid, carbamazepine, phenytoin, or urate, and no interference from azathioprine, cyclosporin A, cefotaxime, or prednisolone up to 1000 mg/L.

We conclude that our modified "high-performance" liquid-chromatographic technique provides a simple, rapid, accurate, reproducible, and interference-free method for analysis of serum and urinary creatinine.

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R. F. A. Ginman

Dept. of Pharmacy
Brighton Polytechnic
Moulescoombe, Brighton
E. Sussex, U.K.

J. S. Colliss

Dept. of Clin. Chem.
Worthing Hosp.
Lyndhurst Road, Worthing
W. Sussex, U.K.

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 (Howanitz 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 nonhemolyzed 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 $600 \times g$ for 5 min to produce platelet-rich plasma, while the other was centrifuged at $10\,000 \times g$ 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 3.5×10^9/L (range, 2×10^9/L to 5×10^9/L); respective counts for platelet-rich plasma and platelet-poor plasma were 100 (25–250) $\times 10^9$/L and 2 (1–6) $\times 10^9$/L.

The mean concentration of ammonia in platelet-poor plasma was 21 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 10^9 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 3 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.

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D. M. Cowley
B. A. Nagle
A. H. Chalmers
T. J. Sinton

Dept. of Pathol.
Mater Misericordiae Adult Hosp.
Stanley St.
South Brisbane 4101
Queensland, Australia

The authors of the article cited respond:
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 from 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