Influences of Specimen Processing and Storage Conditions on Results for Plasma Ammonia

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Ammonia concentrations in plasma may increase because of contamination and deterioration of blood components during specimen handling and storage. Using replicate specimens from healthy volunteers, we studied influences of specimen processing and storage procedures on ammonia measurements made with a self-contained reagent system. Under some conditions, ammonia concentrations more than doubled. The use of nonhemolized plasma specimens and prompt centrifugation, separation of plasma, and ammonia determination apparently were important in avoiding such increases, the duration of contact between plasma and cells being the most important factor. Lower temperatures had minimal effect on whole-blood storage and centrifugation, but retarded increases in ammonia in stored plasma. We conclude that procedures for collection and storage of specimens for ammonia determinations should be standardized and strictly observed.

Additional Keyphrases: variation, source of hemolysis, discrete analysis, enzymic methods

Accurate measurement of plasma ammonia is difficult because concentrations in blood are low compared with values from potential contaminants from the laboratory environment or from endogenous sources released during specimen handling and storage (1). A variety of conditions, including pollution of laboratory atmosphere and glassware by ammonia-containing detergents, adversely affect reproducibility of plasma ammonia measurement (2). Although it is recognized that ammonia increases in a specimen with storage, there is disagreement under which conditions the increase is most pronounced. Conditions reported as important include the temperature at which the whole-blood specimen is stored, promptness of centrifugation and plasma separation, and duration and temperature of storage of plasma specimens (3–7).

Using replicate blood specimens drawn from healthy volunteers, we examined the effects of processing and storage conditions on plasma ammonia as determined by an enzymic method involving a self-contained reagent system that obviates external contamination with ammonia during analysis. Our objective was to identify procedures that would minimize artifactual increases in ammonia.

Materials and Methods

Specimens. Blood specimens were obtained by venipuncture from resting healthy volunteers who gave their informed consent. Blood was drawn into evacuated glass tubes, both without anticoagulant and containing sodium heparin (Vacutainer Tubes; Becton Dickinson, Rutherford, NJ 07070). All specimens were randomized as to treatment group.

Ammonia method, standards, and controls. Ammonia was measured with a discrete analyzer, the acu, with ammonia reagent packs (both from Du Pont Instruments, Wilmington, DE 19899). Aqueous solutions of ammonium chloride (Du Pont Ammonia Calibrators) were used for calibration and to determine within-day precision. We prepared 40 and 80 μmol/L controls by diluting a 400 μmol/L solution of ammonium chloride (Mallinckrodt Inc., St. Louis, MO 63147) with Enzyme Diluent (Du Pont) and used them to determine day-to-day (n = 30) precision. We also placed deionized water in each of the two types of blood-collection tube and determined its ammonia content.

Procedures. In the following experiments, unless otherwise indicated, specimens were centrifuged (4 °C, 10 min, at 1500 × g), ammonia was measured without delay, and the results were analyzed by a paired t-test (8). Precision of duplicate determinations was determined from data on 10 specimen pairs (8). Using 10 specimen pairs, we compared results for heparinized plasma specimens with those for serum specimens, and compared the effect of centrifugation of specimens at 4 and 22 °C for 10 more specimen pairs.

We used 30 triplicated whole-blood specimens to study the effect of storage conditions. The triplicates were assigned to control, 4 °C, and 22 °C treatment groups. Fifteen specimens assigned to each of the 4 and 22 °C treatment groups were stored uncentrifuged for 1 h and 15 were stored uncentrifuged for 2 h. We then centrifuged (22 °C, 10 min, 1500 × g) the specimens and analyzed the results by two-way analysis of variance (9). Fifteen paired heparinized whole-blood specimens were randomized into two groups: one group was stored uncentrifuged at 4 °C for 2 h; the other group was centrifuged without delay and the plasma was stored at 4 °C for 2 h after separation from cells.

To study the effect of temperature and duration of storage of heparinized plasma, we used 20 specimens for each condition. Ammonia was measured before and after storage at 22 °C and 4 °C for 0.5 h; 22 °C and 4 °C for 1 h; 4 °C, −20 °C, and −70 °C for 24 h; and −70 °C for 72 h.

Results

Analytical variation. The within-day CV was 2.3% and 2.8% for 250 and 500 μmol/L concentrations of controls, respectively. For the 40 and 80 μmol/L controls, the day-to-day CV was 3.2% and 6.8%, respectively.

Specimen variation. The CV for the difference between results on duplicate specimen pairs was 4.7%. Ammonia was undetectable in distilled, de-ionized water left in contact for 1 h with blood-collection tubes, both those with no anticoagulant and those containing sodium heparin. There was a significant difference (p < 0.001) between results for heparinized plasma and serum specimens: the mean ammonia concentration in plasma was 30 μmol/L, in serum 44 μmol/L. There was no statistically significant difference (p > 0.40)
in the whole-blood group had a higher ammonia concentration than did the corresponding plasma. As Table 1 shows, there were statistically significant increases in plasma ammonia under each of these storage conditions, being greater after storage for 24 h at 4 °C than for specimens stored at −20 °C or −70 °C.

**Discussion**

Information on plasma ammonia concentration is potentially useful in numerous clinical situations, but methodological problems have led to doubts about its reliability. Intra-individual CVs as great as 47% are reported, apparently because ammonia is so ubiquitous, e.g., in ammonia-containing detergents and tobacco smoke (2). Our precision data are consistent with previous reports (10, 11) that indicate such technical difficulties are minimized by use of a self-contained enzymatic reagent system.

There are conflicting reports as to the appropriate methods for processing specimens for ammonia, perhaps due, at least in part, to problems with specimen contamination. With minimization of contamination, problems with specimen handling became the major source of spurious increases in ammonia. Factors related to specimen collection that reportedly (3, 6, 12, 13) affect ammonia concentrations include anticoagulants and duration and temperature of storage. Plasma specimens anticoagulated with heparin or EDTA are recommended for ammonia determinations. That the increase of ammonia in plasma specimens anticoagulated with heparin is minimal may be due to the inhibitory effect of heparin on adenylic acid deaminase (12). Specific lots of heparin vary greatly as to ammonia content (14): Doumas et al. (15) prefer to use heparinized Vacutainer Tubes because their ammonia content is essentially negligible. We have confirmed that there is significantly less ammonia in heparinized plasma than in the corresponding serum (14).

Despite reports that there is no significant change in the ammonia content of stored whole-blood specimens (12, 13), and despite the practice of some investigators, presumably to avoid spurious increases in ammonia, of processing or storing whole-blood specimens in the cold (1, 7, 15), our data indicate that concentrations of ammonia markedly increase in plasma from specimens stored as whole blood. Duration, rather than the temperature of handling and storage, is an important influence on subsequent measurement of plasma ammonia. However, with blood stored at 4 °C for 2 h, hemolysis was frequent; this could potentially increase the concentrations of plasma ammonia because erythrocytes contain about threefold as much ammonia as plasma.

This also is controversy regarding plasma storage. Plasma has been stored without appreciable increases in amo-

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**Table 1. Effects of Duration and Temperature of Storage on Plasma Ammonia**

<table>
<thead>
<tr>
<th>Duration, h</th>
<th>Temp, °C</th>
<th>Mean increase from baseline</th>
<th>%</th>
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<td>4</td>
<td>10.3</td>
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<tr>
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<td>22</td>
<td>2</td>
<td>5.5</td>
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<td>22</td>
<td>6</td>
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<td>20</td>
<td>2</td>
<td>5.6</td>
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<td>10</td>
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<td>−70</td>
<td>4</td>
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</tr>
<tr>
<td>72</td>
<td>−70</td>
<td>7</td>
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</table>

* p < 0.01 for this condition, p < 0.001 for all others, as compared with value for specimen analyzed before storage.
nia for 1 h at room temperature (13), 2 h at 4 °C (7), as long as 3.5 h at 0 °C (14), three days at −20 °C (7), or 40 h at −70 °C or below (4). Others find that ammonia concentrations rapidly increase in plasma, even when the plasma is stored at 0 °C for 1 h or frozen at −20 °C (3, 16). We found a mean increase in ammonia of 5.6% when plasma specimens were stored for 1 h at 4 °C, as compared with a 12.8% increase after storage at 22 °C. In contrast, storage of whole blood for 1 h at 4 °C and 22 °C led to increases in subsequent measurements of plasma ammonia of 31.7% and 39.9%, respectively.

We recommend use of nonhemolyzed heparinized plasma specimens, and separating the plasma from the blood cells immediately after venipuncture. As compared with increases that occur when plasma is in contact with cells, the increases in ammonia in plasma stored at 4 °C for as long as 1 h are relatively small. However, if plasma samples cannot be analyzed promptly, we recommend freezing them at −70 °C.

References

Simultaneous Determination of Penicillin and Cephalosporin Antibiotics in Serum by Gradient Liquid Chromatography

Thomas Annesley, Karen Wilkerson, Keith Matz, and Donald Glarchiro

We describe a "high-performance" liquid-chromatographic method for simultaneously measuring various penicillin and cephalosporin antibiotics. After extraction from serum, which in general is quantitative, the drugs are separated by use of a "Bondapak phenyl" column and a gradient mobile phase. For these drugs retardation times depend on the pH of the mobile phase; we present retardation times under selected pH conditions.

Additional Keyphrases: antibiotics ° multiple-drug assay

Penicillin and cephalosporin compounds, members of the beta-lactam family of antibiotics, currently represent a most widely prescribed group of drugs. These natural and semisynthetic compounds possess broad antimicrobial activity against both Gram-positive and Gram-negative organisms. Although toxic side effects from these drugs are generally minimal, substantial nephrotoxicity is possible in patients with impaired renal function (1, 2) or in patients concurrently being treated with aminoglycosides (3). Because antibiot-

ics are frequently administered in large doses, it is sometimes important to be able to monitor their concentrations in serum, especially in patients with impaired renal function.

"High-performance" liquid chromatography is superior to the previously used microbiological assays because, with it, one can measure only the specific antibiotic of interest, independent of other drugs that may be present. Of the analytical methods reported for certain penicillin (4, 5) and cephalosporin (6–13) antibiotics, nearly all measure a single drug, so that several columns and mobile phases are needed if more than one antibiotic is to be measured. Although some liquid-chromatographic methods have been reported for the assay of several cephalosporins (14, 15) or penicillins (6), none has involved simultaneously measuring both groups of antibiotics in biological samples. The procedure reported here can be used to determine multiple beta-lactam antibiotics, including the "third-generation" antibiotics moxolactam, cefotaxime, and cefoperazone. The use of a gradient mobile phase allows rapid measurement of drugs having various polarities.

Materials and Methods

Reagents: Chloroform and methanol were "HPLC-grade"