Simple Method for Improving the Precision of Electrolyte Measurements in Vitreous Humor, Alan R. McNeil,1,2 Andrea Gardner,2 and Simon Stables3 (Departments of 1 Molecular Medicine and 2 Clinical Chemistry, and 3 Section of Forensic Pathology, Department of Pathology, University of Auckland, Auckland, New Zealand; * author for correspondence: fax 64-9-3074939, e-mail Alanmcn@ahsl.co.nz)

Vitreous humor is widely used for postmortem biochemical analysis because it is easier to collect than cerebrospinal fluid and its composition changes more slowly after death than the composition of blood [reviewed by Coe (1, 2)]. The concentrations of sodium, urea, and creatinine in the vitreous humor change little over time, which means that it is possible to use these specimens to make postmortem diagnoses of renal failure, severe dehydration, salt intoxication, or excessive water intake (2–5). Many drugs, including ethanol, can also be measured conveniently after death in vitreous humor (2).

Unfortunately, the biochemical analysis of vitreous humor is not always straightforward, and several authors have reported problems with the reproducibility and accuracy of these measurements. These problems include unexplained differences in results obtained from left and right eyes (6,7) and different results when specimens have been analyzed using different instruments (8). We encountered a problem with the reproducibility of vitreous humor electrolyte measurements made with two Hitachi random access analyzers and found that this could be avoided by heating the specimens at 100 °C for 5 min before they were analyzed. Specifically, vitreous humor specimens were heated in capped glass tubes in a heating block, cooled at room temperature for 30 min, and then centrifuged at 1800g for 5 min. Most of the specimens were obviously less viscous on pipetting after the treatment, and some contained a visible precipitate.

The effect of heat treatment was assessed using specimens collected from either the left or right eyes of 42 different patients during routine postmortem examinations. Each specimen was divided into two aliquots, one of which was treated, and the other acted as a control. Duplicate measurements of each aliquot were performed on Hitachi 737 and Hitachi 911 random access analyzers with precision assessed by calculating the SD of duplicates (9) (Table 1). The reproducibility of sodium and potassium measurements on untreated vitreous humor specimens was significantly worse on the Hitachi 911 (SD of duplicates, 20.8 and 1.97 mmol/L, respectively) compared with the Hitachi 737 (SD of duplicates, 2.3 and 0.23 mmol/L, respectively). Heat treatment improved the precision of sodium and potassium measurements on the Hitachi 911 and of sodium and chloride measurements on the Hitachi 737 (Table 1). The heat treatment increased the mean results for sodium and potassium measured on the Hitachi 911 but produced no change in the precision of

| Table 1. Precision of vitreous humor measurements. |
|----------------|----------------|----------------|----------------|
|                | Control*       | Treated*       | Control*       | Treated*       |
| Sodium         | 40             | 150 (2.3)      | 148 (1.1)*     | 132 (20.8)     | 147 (1.9)*     |
| Potassium      | 42             | 11.8 (0.23)    | 11.8 (0.44)    | 10.6 (1.97)    | 11.7 (0.22)*   |
| Chloride       | 39             | 125 (2.3)      | 122 (0.7)*     | 9.94 (0.25)    | 9.79 (0.20)    |
| Urea           | 35             | 10.55 (0.27)   | 10.21 (0.32)   | 0.10 (0.004)   | 0.10 (0.007)   |
| Creatinine     | 35             | 0.11 (0.004)   | 0.11 (0.004)   | 0.10 (0.004)   | 0.10 (0.007)   |

* Mean (SD of duplicates), mmol/L.
* SD of duplicates different from control specimens on the same analyzer, P < 0.05.
* Mean concentration different from control specimens on the same analyzer, P < 0.05.
mean values of urea or creatinine measurements on either analyzer (Table 1).

To determine whether the heat treatment degraded glucose in vitreous humor, glucose was added to four different specimens that had been heated once to reduce their viscosity and allow reproducible pipetting. Glucose was added because it is usually detectable only in vitreous humor from patients with marked hyperglycemia. The glucose concentrations of these specimens before and after repeating the heat treatment and centrifugation were 12.5 and 13.1 mmol/L, 11.8 and 12.0 mmol/L, 11.7 and 11.6 mmol/L, and 11.9 and 12.1 mmol/L, respectively. These results indicated that glucose, like urea and creatinine, was not degraded in vitreous humor subjected to these conditions. It is even possible that the heat treatment could slow the rate of change of vitreous humor glucose concentrations by denaturing key glycolytic enzymes.

Heating vitreous humor to 100 °C for 5 min was a simple and safe method for improving the precision of electrolyte measurements on the Hitachi 911 and 737 analyzers without any adverse effects on the measurement of urea, glucose, or creatinine. We found that centrifuging vitreous humor specimens alone, as suggested by others (2), did not have the same effect. We postulate that the beneficial effects of heating derived from the reduced viscosity of the treated specimens. Like previous workers (8), we found large differences in the reliability of vitreous humor measurements on different analyzers, presumably caused by differences in the mechanics of pipetting, pumping, and dilution. Heat treatment obviously would not be suitable before the measurement of volatile or labile substances. We are examining whether treatment with enzymes that break down mucopolysaccharides might be a suitable alternative in these situations (10).

It is interesting to speculate that some of the unusual results of biochemical testing of vitreous humor reported in the past may be explained by variable specimen viscosity and poor analytical precision. Coe and Apple (8) showed significant variations in sodium, urea, and glucose results for some specimens between different analyzers and commented on the diagnostic confusion that this could produce. They reported good precision (CV < 4.3%) for all of their tests on a single pooled vitreous control. Their instruments may not have been as susceptible to the precision problems that we encountered, although it is also possible that their single pooled control may not have reflected the wide variation in viscosity that can occur between individual specimens. Balasooriya et al. (6) and Madaea et al. (7) showed large differences in potassium concentrations between the left and right eyes of some patients and used this to argue that potassium measurements are not useful for calculating the postmortem interval. Neither of these groups performed repeat measurements on individual specimens; therefore, it is possible that some of the apparent eye-to-eye variation was the result of analytical variation.

References


Bone Alkaline Phosphatase Isoenzyme and Carboxy-Terminal Propeptide of Type-I Procollagen in Healthy Chinese Girls and Boys, Keh-Sung Tsai,1† Men-Hoang Jang,2 Sandy Huey-Jen Hsu,1 Wern-Cherng Cheng,1 and Mei-Hwei Chang2 (Departments of 1 Laboratory Medicine and 2 Pediatrics, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China; 3 Department of Laboratory Medicine, Taipei City Psychiatric Center, Taipei, Taiwan, Republic of China; * address correspondence to this author at: Department of Laboratory Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, Taipei, Taiwan, Republic of China; fax 886-2-23224263, e-mail kstsaimd@pcmail.com.tw)

The bones of children grow at a faster rate during the first few years of childhood and puberty. Recently, advances in assays for biochemical markers of bone formation have provided noninvasive means to study bone growth and metabolism in children (1–14). Because of the variations in the rate of bone growth in different age groups and possible ethnic differences, age-specific reference ranges for bone formation markers must be established in a particular pediatric population.

Conventional bone formation markers such as osteocalcin (1) have been shown to correlate with serum concentrations of insulin-like growth factor-I and testosterone in children and adolescents. In pathological conditions, osteocalcin (2–4) is lower in growth hormone-deficient children and increases with replacement therapy. Osteocalcin, however, is labile and possesses problems in sample processing and storage (15). Recently, two other bone formation markers in serum, bone alkaline phosphatase isoenzyme (BAP) (16) and the carboxy-terminal