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Effect of Pyridoxal 5-Phosphate on the Stability of Alanine Aminotransferase

To the Editor:
We (1) recently confirmed earlier reports (2, 3) of significant decreases in alanine aminotransferase (ALT, EC 2.6.1.2) activity in serum specimens stored frozen (−20 °C). Aspartate aminotransferase (AST, EC 2.6.1.1) activity in these specimens was not affected. ALT and AST activities were assayed in Tris buffer without added pyridoxal 5-phosphate (P-5-P), according to a protocol of the International Federation of Clinical Chemistry (IFCC) (4). When assayed by an older (pre-IFCC) method involving phosphate buffer without exogenous P-5-P, no significant loss of ALT activity was observed. A conformational difference between ALT molecules in Tris vs phosphate buffer was proposed, but Rej (5) recently reported no differences in the circular dichroic spectra for purified ALT in these two buffers. We have performed further experiments to determine the effect of added P-5-P on ALT (and AST) activity in frozen serum specimens.

We assayed 21 different fresh serum samples (day 0) in Tris buffer in the absence of P-5-P according to the IFCC method (4). The serum samples were then stored in aliquots at −20 °C in the presence (0.4 mmol/L) or absence of P-5-P. Samples were thawed at room temperature on day 2 and assayed without delay. After enzyme activities were measured, the specimens were refrozen at −20 °C. On day 3, the samples were again thawed at room temperature and assayed. Thus, the ALT and AST activities were measured under the same conditions after each of two freeze–thaw cycles. Repeated freeze–thaw cycles were used to determine the extent of the cofactor’s ability to stabilize preferred conformation(s) of ALT during the stress of freezing and thawing. The samples were at ambient temperature no longer than 2–3 h during each testing procedure.

The results for ALT and AST activities in these specimens under these conditions are shown in Table 1. Changes in the ALT and AST activities were tested for statistical significance with a paired t-test.

The results indicated the following:
• The decrease in ALT activity for samples frozen in the absence of P-5-P was statistically significant during both the first (P < 0.001) and second (P < 0.001) freeze–thaw cycles.
• The decrease in ALT activity between the first and second freeze–thaw cycles for samples frozen in the absence of P-5-P was similar.
• Samples frozen in the presence of P-5-P showed a greater decrease in ALT activity than did samples frozen without this cofactor (P = 0.006), with the decrease in enzyme activity persisting through the second freeze–thaw cycle.
• Statistically significant but minor decreases in ALT activity occurred during the first (P = 0.003) and second (P = 0.009) freeze–thaw cycles in the absence of P-5-P, but even these small decreases in AST activity were prevented by the addition of P-5-P.

These observations suggest that the cofactor P-5-P does not stabilize ALT activity during freezing. Our data, along with Rej’s circular dichroic spectra (5), indicate that the decrease in ALT activity upon freezing is probably not due to major conformational changes. The situation for AST is different, as the minor loss in enzyme activity observed during freezing is prevented by P-5-P.

References
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Urinary Glycosaminoglycans in Active Graves Ophthalmopathy

To the Editor:

Increased excretion of glycosaminoglycans (GAG) in 24-h urine samples (P <0.05) was reported by Kahaly et al. (1) in patients with Graves ophthalmopathy (mean 19.2 mg/24 h, range 12.2–28.7; control group 15.8 mg/24 h, range 10.4–21.8). According to these authors, GAG values can reach twice the normal value in active, untreated Graves ophthalmopathy (36.7 mg/24 h, range 28.1–48.4).

Because of these data, measurement of GAG in 24-h urine samples was included in the Graves ophthalmopathy protocol in our hospital. From September 1990 to March 1992, 59 individuals, ages 18–81 years, were included in the protocol (inclusion criteria 2c, 3b, 3c, 4b, 4c, 5b, 5c, 6a, 6b, and 6c, according to the American Thyroid Association classification modified by Werner in 1977 (2)). These patients underwent endocrinological, ophthalmological, and neuroradiological examinations to determine who had clinically active Graves ophthalmopathy (according to the clinical classification of Mourits et al. (3) and the ophthalmological index of Bartalena et al. (4)). Diagnosis of Graves ophthalmopathy was based on both endocrinological and ophthalmological examination. Mean (SD) values for free thyroxine (FT<sub>4</sub>) and thyrotrophin (TSH) were as follows: 67.3 (59.3) pmol/L (range 12.0–200.0, reference interval 9–24 pmol/L) and 75 (53) μIU/L (range 10–200, reference interval 300–4000 μIU/L), respectively. Measurements of proptosis (axial computerized tomography) were:

Table 1. Age and GAG Values in Control Subjects and Patients with Active Graves Ophthalmopathy

<table>
<thead>
<tr>
<th>Age, y</th>
<th>GAG, mg/24 h</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Mean</td>
<td>48.5</td>
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<tr>
<td>SD</td>
<td>18.9</td>
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<tr>
<td>Minimum</td>
<td>19.0</td>
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<tr>
<td>Maximum</td>
<td>85.0</td>
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right eye, 21.7 (2.35) mm (range 18.0–25.0); left eye 21.6 (2.26) mm (range 18.0–26.0). Values for two patients who developed hypothyroidism as a result of antithyroid treatment are excluded. Urinary GAG was also measured in a control group, ages 19–85 years (n = 37).

Urine specimens were collected for 24 h and kept refrigerated without preservative throughout the collection period. Aliquots were separated and frozen at −20 °C until assay. For 41 of the patients included in the protocol, urine for measurement of GAG was obtained at the time of inclusion in the protocol and before any antiinflammatory therapy.

Precipitation with CPC and ethanol (5) was used to isolate urinary GAG, which were quantified spectrophotometrically as uronic acid with the carbazole reaction (6). Analytical interassay imprecision (CV) was 4.83% (mean 14.92 mg/24 h, SD 0.72). GAG distributions were almost normal in both groups (controls and patients), and differences between variances (Snedecor test) were not significant. One-way analysis of variance was therefore used.

Once we had established that there were no significant differences for age between groups, we compared the GAG values and found no significant differences for GAG excretion (P <0.05) between control individuals and Graves ophthalmopathy patients. Ages and GAG values for both groups are shown in Table 1.

These results do not support the hypothesis that measurement of urinary GAG is useful for the follow-up of Graves ophthalmopathy. Thus, we no longer include measurement of urinary GAG in 24-h urine samples in the Graves ophthalmopathy protocol in our hospital.

References

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Between-Lot/Between-instrument Variations of the Abbott IMx Method for Prostate-Specific Antigen

To the Editor:

Prostate-specific antigen (PSA) measurements are used primarily for long-term monitoring of patients with prostate cancer; thus, long-term stability is an important characteristic for any assay of PSA. When we performed independent evaluations of the IMx (Abbott Diagnostics, North Chicago, IL 60064) method for PSA at Peninsula Regional Medical Center (PRMC) and Maryland Medical Laboratory, Inc. (MML) in late 1991, using different reagent lots and patients' samples, we found marked differences in the correlations of the two evaluations to results obtained with the Tandem (Hybritech, San Diego, CA 92126) PSA methods. Because we were concerned that variations in PSA measurements between instruments and reagent lots could be reflected as long-term instability, we collaborated to study the between-lot/between-instrument variations of the IMx method for PSA in February 1992. Testing the same patients' specimens with reagents from several lots on several instruments should give a reasonable estimate of the analytical variation that would be observed over an extended time for a single patient. We also studied the overall correlation between the IMx method for