Comments on the Spectrophotometric Procedure for Angiotensin-Converting Enzyme

To the Editor:

I have been using the spectrophotometric assay of Cushman and Cheung (1) as modified by Lieberman (2) to assay for serum angiotensin-converting enzyme (ACE) in diagnosis of possible sarcoidosis. However, replicate determinations produced results that varied considerably, creating some doubt about its analytical usefulness. Taylor and Freeman (3) identified a cause of this variability as cloudiness in the cuvet, from extracted lipid material, which develops when the extract ed residue is reconstituted with 1 mol/L sodium chloride. They eliminated the cloudiness by substituting acetonitrile for saline solutions and further improved the method by doubling the extraction volume of ethyl acetate to 3.0 mL, which eliminated troublesome emulsions.

For the past two years, I have been reconstituting the extracts with “spectro-grade” methanol. No cloudiness develops and the odor is not as objectionable as that of acetonitrile. Ethanol will work equally well but is not available to commercial laboratories in a spectro-grade quality. I determined the molar absorptivity of hippuric acid in methanol at 228 nm to be 10.3 L·mmol⁻¹·cm⁻¹ compared with 9.8 L·mmol⁻¹·cm⁻¹ reported by Cushman and Cheung for aqueous solutions (1).

Cushman and Cheung also determined that 91% of hippuric acid was extracted with 1.5 mL of ethyl acetate. Given that the solvent volume was not doubled in relation to the aqueous phase, I rechecked the amount of hippuric acid extracted from standards containing 1.0–4.0 mmol/L (16.7–66.7 U/L). Analytical recovery of hippuric acid was 89% when 1.5 mL of ethyl acetate was used, 99% for 3.0 mL. Therefore, the factor of 0.91 in Lieberman’s formula can be eliminated, changing his final factor from 56.1 to 50.8 to calculate the enzyme activity at 1-h incubation, or to 101.6 for 30-min incubation. The standards are linear over the range listed and can also be used to calculate ACE units. ACE units are defined as nanomoles of hippuric acid formed per minute per milliliter of serum (2).

To compare results with those by Lieberman’s method, I tested 12 samples in duplicate by both methods. To assess cloudiness in the cuvet, I used the “nepheloscope” of a Perkin-Elmer Amylase/Lipase analyzer to measure the cloudiness before taking the spectrophotometer readings. Both saline and methanol solutions gave a reading of 6 to 7 “nephlos,” as did all of the blanks and samples that were reconstituted with methanol. Samples reconstituted with saline, however, produced “nephlo” readings ranging from 12 to 85. There was poor duplication of “nephlo” values between blanks and their respective test samples or between paired test samples, which ultimately caused poor precision in the spectrophotometer readings that were used to calculate the enzyme activity. The enzyme activity for the saline samples had a SD of 13.3 U/L, a CV of 33.1%. The samples reconstituted with methanol had much better precision: SD 2.3 U/L, CV 6.2%.

Incorporating these changes, I re-established a normal range for ACE by analyzing samples from 22 employees and 40 patients, none diagnosed as having sarcoidosis and each assayed in duplicate. The mean was 20.8 (SD = 7.9) U/L and the range of values was 5.5 to 38.6 U/L. ACE in samples from 15 cases of active sarcoidosis exceeded the normal mean by 2 SD.

One apparently healthy laboratory technician had an ACE value of 57 U/L. Her serum was darkly icteric but her bilirubin value was normal. Questioning revealed that she had been taking 40 000 int. units of vitamin A each day. When she stopped this, her ACE values began to decrease and reached 20.7 U/L after four weeks.

References

Letter

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Catecholamine Standards in Radioenzymic Assays

To the Editor:

Ellis and Burns (1), using the “Cat-A-Kit,” advocate a “mean net standard” in calculating catecholamine concentration in plasma instead of individual internal standards. They argue that the dpm value of the internal standard varies markedly, especially for standards with exceptionally high values. The Cat-A-Kit is designed for measuring catecholamines in human plasma, giving normal values for nor-epinephrine of 200–600 ng/L, for epinephrine 20–100 ng/L. A 100 ng/L internal standard, as indicated in the instructions furnished by the supplier, would be equivalent to a concentration of 2000 ng/L in plasma. The dpm values are of the same order of magnitude as the nanogram per liter values (see also Tasseron et al. (2)).

The dpm value for the internal standard is the difference between that of the "sample + standard" and that of the "sample." For samples with high values, this is a difference between two large numbers, and as such would be expected to be variable. Even with a reasonable CV of 5% for the dpm values, at 10 µg/L the CV of the difference would be 40%. Ellis and Burns mention in their figure values as great as 50 µg/L.

We did a study of about 100 apparently healthy individuals, collecting within 3 h six samples from each person. The within-individual CV for the dpm value for the internal standard was about 7%. The between-individual CV was on the same order of magnitude, but about 10% of the individuals gave an aberrant value for the internal standard (sometimes only 50% of the average), with an within-individual CV of about 5%.

References

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Above-Normal Concentrations of Lipid Peroxide in Serum of Patients with Gout

To the Editor:

The damaging effects of lipid peroxide on cellular constituents are well confirmed (1). Mickel and Horbar (2) reported that peroxidized lipids affect platelet aggregation. The concentration of lipid peroxide in the arterial wall and the severity of atherosclerosis are positively correlated (3). These findings suggest a possible role of peroxidized lipids in the pathogenesis of atherosclerosis, but there is little clinical evidence for this view.

We measured the serum lipid perox-