What is the "Correct" Activity of Angiotensin-Converting Enzyme?

To the Editor:

I agree with Buttery and Chamberlain (1) that it is essential, when calculating the activity of angiotensin-converting enzyme (ACE; EC 3.4.15.1) with furylacryloyl-L-phenylalanylglutamylglycine (FAPGG) as substrate, to have measured the difference absorption coefficient between FAPGG and its product (furylacryloyl-L-phenylalanine, FAP) at the wavelength and with the same instrument used for performing the assay. They (1) found that the difference absorption coefficient depended both on the instrument used and on whether it was computed by determining separately the absorption coefficient of substrate and product or by measuring the absorbance of mixtures of substrate and product, their combined concentrations being constant. They recommend use of the latter method, as this more accurately mimics the conditions in the assay. This indeed was the method we used (2). They (1) do not attempt to explain their findings. The explanation could be as follows.

The difference spectrum between FAPGG and FAP is extremely sharp (3, 4), the difference being maximum at 328 nm. At 345 nm, the wavelength used by Buttery and Chamberlain, the difference absorption coefficient is 20% of maximum and changes at the rate of approximately 15% per nanometer (calculated from refs. 3 and 4). Thus it would require only a small error in the wavelength calibration to lead to differences between instruments. In addition, possible differences in the bandwidth (by this I mean the range of wavelength around the chosen wavelength) between instruments would lead to further differences, because the difference absorption coefficient is not a linear function of wavelength. This discussion illustrates the advantages of making absorbance (or difference absorbance) measurements at the absorbance (or difference absorbance) maximum, where the change in absorbance (or difference absorbance) coefficient with respect to wavelength is at a minimum.

Differences in blanking procedure might explain the method-dependent differences found by Buttery and Chamberlain (1). They measured the absorbance of FAP vs buffer, whereas they blanked against 0.2 g/L potassium dichromate for FAPGG. Depending on the particular instrument involved, to achieve the same output from the photodetector (the "blanking" process) with dichromate as blank would require either a greater slit width, with consequent increase in bandwidth, or an increase in the gain of the photodetector, with consequent increase in signal noise.

While recognizing the importance of using the appropriate difference absorption coefficient for calculating ACE activity, the choice of measuring wavelength imposes its own limitations on assay design. Thus while measurement at the maximum (328 nm) would minimize instrument-dependent differences in absorption coefficient, the strong absorbance of FAPGG at this wavelength means that the maximum concentration that can be used in the assay (so as not to exceed an initial absorbance of 2.0) is about 0.1 mmol/L. This, in turn, given a $K_m$ of human serum ACE for FAPGG of 0.3 mmol/L (2, 3), means the activity measured will be 25% of $V_{max}$. However, if the absorbance measurement is made at 345 nm, allowing a concentration of 1.0 mmol/L to be used, then the activity would be 77% of $V_{max}$, threefold greater. Despite this, because of the large difference absorption coefficient at 328 nm (fivefold that at 345 nm), the assay at 328 nm is more sensitive than that at 345 nm, because at the same enzyme concentration the absorbance change per minute is greater by 60%. It is therefore not possible to optimize the assay both for maximal sensitivity and for maximal activity.

Thus one must recognize the influence of assay design on the activities measured. We optimized our assay for maximal sensitivity (2), recognizing that the measured activity would be less than maximal. The use of enzyme standards would overcome the problem of assay design on measured activity; however, this would contribute its own costs to an otherwise reliable, simple, convenient, and inexpensive assay.

References


Gerald A. Maguire

Dept. of Clin. Biochem.
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne
NE1 4LP, U.K.

Positive Interference with Immunoassay of Theophylline in Serum of Uremics

To the Editor:

Recently, Compton et al. (1) reported improved results for theophylline obtained for uremic patients with a new TDx (Abbott Laboratories, North Chicago, IL) procedure based on a monoclonal antibody. This may be true for many patients, but we present data showing that there is still the possibility of falsely high values when the improved TDx method is used.

We recently encountered an instance in which the validity of theophylline values of four specimens from a uremic patient, as measured by the TDx (monoclonal assay), were questioned. The patient's serum creatinine and urea nitrogen values ranged from 37 to 61 mg/L and 740 to 1050 mg/L, respectively, at the time the samples were drawn. The theophylline concentrations were redetermined by a liquid-chromatographic method modified from that of Orcutt et al. (2). We found that the values obtained with the TDx were, on the average, 100% higher than the chromatographic results (Table 1). We then determined theophylline values for these specimens by the Emit procedure (Syva) adapted for the Cobas B6 centrifugal analyzer (Roche Analytical Instruments, Inc., Nutley, NJ) and by particle-enhanced turbidi-