et al. (3), who reported significant amounts of 5-HT in supposedly platelet-poor plasma.

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Inhibitory Effect of Ascorbic Acid in Oxalate Assays Involving Oxalate Decarboxylase

To the Editor:

Determination of urinary and serum oxalate is important for the clinical diagnosis of and research into calcu-

lous disease. Oxalate decarboxylase (EC 4.1.1.2) has a rigid substrate specificity, which makes it ideally suited for use in the assay of oxalate. Moreover, the enzyme has only one known requirement, namely oxygen, and its activity is affected by very few compounds (1). The enzyme is commercial-

ly available and methods involving its use have been developed based on the detection of CO2 by means of mano-

metric (2), spectrophotometric (3, 4), conductometric (5), potentiometric (3), and radioisotopic techniques (6, 7). We (8) have described a simplified modification of the radiometric assay in which 14CO2 liberated from 14COxalate by the action of oxalate decarboxylase is trapped in Hymamine hydroxide and its radioactivity determined.

Ascorbic acid is considered to be a major source of endogenously produced oxalate, but there are few reliable data to support this contention. "Mega-dose" intakes of ascorbic acid are not gener-

ally associated with hyperoxaluria and, as we discussed recently, the limited intestinal uptake, the rapid renal clearance, and the post-micturition conversion of ascorbic acid to oxalate all raise considerable doubts as to the role, if any, of ascorbic acid in urinary calculous disease (9, 10). In investigations of the metabolism of ascorbic acid by isolated rat hepatocytes and of the nonenzymatic conversion of ascorbic acid to oxalate in urine, we noticed that, in our assay, oxalate decarboxylase was inhibited by ascorbic acid.

Figure 1 illustrates the effect of an increasing ascorbic acid concentration on the oxalate decarboxylase reaction at 0.5 and 5.0 mmol of oxalate per liter. Each reaction mixture contained, in a total volume of 1.0 mL of potassium citrate buffer (0.5 mol/L, pH 3.0), 20 mU of oxalate decarboxylase, the ap-

propriate oxalate concentration, and 0.1 mCi of [14C]oxalate. The 14CO2 liberated was trapped and counted as described previously (8). The amount of enzyme is such that each point repre-

sents the rate of CO2 production from oxalate after a 30-min incubation. As others have observed (1), the reaction was stimulated by low ascorbic acid concentrations. This stimulation does not present a problem in the routine determination of oxalate. It may be due to an actual stimulatory effect on the oxalate decarboxylase or a more efficient carboyer of the 14CO2 into the Hymamine hydroxide, but we have not investigated the effect further. Howev-

er, when the ascorbic acid/oxalate ratio exceeds 0.5, the reaction is inhibited (Figure 1). The inhibition is competitive; it can be overcome by increasing the oxalate concentration. Ascorbic acid is known to give rise to oxalate in aqueous solution, but this does not appear to be important here, because 1.34Clascorbic acid did not significant-

ly contribute to the amount of 14CO2 obtained in this assay.

This inhibition by ascorbic acid cannot be overcome by increasing the en-

zyme concentration or the time of incubation, but it can be corrected for by including (14C)oxalate (1.25 nmol, 0.1 mCi) as an internal standard in the assay. The calculated oxalate concentra-

tions obtained in this way are very close to the expected oxalate values.

We conclude that this inhibition is probably a major source of interference in assays in which oxalate decarboxylase is used to determine the oxalate content of biological specimens that also contain ascorbic acid. In such cases appropriate corrections are needed, e.g., by use of the internal standard as suggested here.

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lism of oxalate precursors in isolated per-

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-phenylalanylglycylglycine (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 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


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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 determined by a liquid-chromatographic method modified from that of Orecutt 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 Bio centrifugal analyzer (Roche Analytical Instruments, Inc., Nutley, NJ) and by particle-enhanced turbidi-