False-Positive Ketostix in a Diabetic on Antihypertensive Therapy

To the Editor:

A diabetic patient who recently presented at our diabetic clinic reported frequent positive results for ketones when she tested her urine with Ames' Keto-Diastix, and that results varied even within-day.

At the clinic, the patient's blood sugar concentration was 10.0 mmol/L (fasting range 3.0–5.8 mmol/L). Her urine was positive for ketones ("small" = 2.5 mmol/L) with Keto-Diastix—and also with the Boehringer Combertest test strip. With an enzymatic β-hydroxybutyrate kit (Sigma Diagnostics) the β-hydroxybutyrate concentration in serum was 0.08 mmol/L (range 0–0.42 mmol/L) and <0.02 mmol/L in the urine.

Drug interference was suspected, and the patient was found to be taking several prescribed medications. Aqueous solutions of these drugs, tested with Keto-Diastix, revealed that the antihypertensive drug captopril gives a positive reading ("trace" = 0.5 mmol/L) for ketones at a concentration of 25 mg/L (0.12 mmol/L). By comparison, β-hydroxybutyrate at a concentration of 0.5 mmol/L gives an equivalent result; i.e., Keto-Diastix is four times as sensitive to captopril as to β-hydroxybutyrate.

Captopril (5-3-mercaptop-2-methylpropanoyl-a-proline; Capoten, Squibb) (Figure 1) is an orally active inhibitor of angiotensin-converting enzyme. After oral administration, captopril is rapidly absorbed, with peak concentrations in blood reached in 30 to 90 min. The drug’s half-life is about 2 h, and renal excretion is rapid, with about 40% appearing in the urine unchanged.

The patient was on a captopril dosage of 50 mg three times daily. On further enquiry she reported that her first morning urine was usually negative for ketones; that a mid-morning specimen, collected about 2 h after taking her medication, was often positive ("trace," "small"); and that a specimen tested much later (just before the next dose) usually yielded a negative result. If we assume that 40% of her 50-mg dose is excreted unchanged during 3–4 h in a urine volume of 200–300 mL, the concentration of captopril in her urine would be about 65–100 mg/L. Thus the pharmacokinetics of captopril and her dosage regimen together account for the results she observed.

As to the mechanism of interference with the nitroprusside reagent in the test strip, it is probable that the keto group of captopril may be reacting. We did not test metabolites of captopril for a reaction. We are not aware of a previous report of this interference and are surprised that false-positives with captopril are not encountered more often, because this drug is a popular antihypertensive.

Reference

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Endogenous Digoxin-like Immunoreactive Substances Eliminated from Serum Samples from Patients with Liver Disease by the EMIT Column Digoxin Assay

To the Editor:

Skogen et al. (1) evaluated the new EMIT Digoxin Column Assay (Syva Co., Palo Alto, CA), as adapted to the Bas-Bio centrifugal analyzer (Roche Diagnostics, Nutley, NJ). They compared the sensitivity of this assay to interference by digoxin-like immunoreactive substances (DLIS) with four other methodologically distinct digoxin immunoassays. They looked at DLIS interference with measurement of serum digoxin in patients with renal failure, pregnant women, and newborns and found that the EMIT assay eliminated or markedly decreased the incidence of false-positive digoxin results in these three groups of patients.

Previous studies have also demonstrated increased concentrations of DLIS in the serum of patients with liver disease (2). We therefore have investigated such patients for DLIS interference in this assay.

We compared results by the EMIT assay, as previously described (1), with the "NML Digi-Tab RIA" method (Organon Teknika Corp., Irving, TX). In the latter we used two different lots of digoxin antiserum: NML antiserum lot DB-157, which has been described previously (3) as having high cross-reactivity with DLIS, and NML antiserum lot 04-058, which has lower cross-reactivity. For this study, we investigated 10 patients with liver disease, for all of whom we had data on total-bilirubin concentrations (mean 80 μmol/L, range 84–226 μmol/L). These patients had normal renal function and were not receiving digoxin therapy.

DLIS concentrations in these patients are presented in Figure 1. The DLIS
concentrations (x ± SD) as measured by the EMIT assay and by the NML Digi-Tab RIA method with antisera lots 04-058 and DB-157 were 0.03 ± 0.04, 0.14 ± 0.16, and 0.80 ± 0.44 μg digoxin equivalents per liter, respectively.

We conclude that the EMIT assay essentially eliminates false-positive digoxin results in patients with liver disease. This accords with the findings of Skogen et al. (1) for the types of patients they studied.

References

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Why Are There Different Reference Intervals for the Kinetic Angiotensin-Converting Enzyme Assay?

To the Editor:

In the kinetic assay of angiotensin-converting enzyme (ACE; dipeptidyl carboxypeptidase I, EC 3.4.15.1) proposed by Holmquist et al. (1) and adapted for serum by Ronca-Testoni (2), the substrate N-[3-(2-furyl)acryloyl]-L-phenylalanylglucyclicine (FAPGG) is cleaved by ACE to furylacryloylphenylalanine and glycyclicine. The resulting absorbance change, monitored at 340 nm or 345 nm, is converted into enzyme activity by a factor derived from the absorbance change (ΔA) produced by hydrolysis of the substrate in a concentration of 1 mmol/L (2). Several versions of this kinetic method, adapted to various automated instruments and measured at different wavelengths, have been published. Disturbingly, the reference intervals for healthy people determined by the various authors using essentially the same method are very different. Table 1 summarizes the wavelengths, ΔA values, reference intervals, and instruments used.

The diversity of reference intervals could be ascribed to differences in the population examined or in the methodology or instrumentation used. Methodology does not appear to influence ACE results significantly, as I have found on using different buffers and various substrate concentrations. For instance, there was no difference in ACE activity when measured manually in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) buffer, 50 mmol/L, pH 8.2 (5) vs borate buffer pH 8.2 (2) or vs 25 mmol/L Hepes buffer (7). Also, it is highly improbable that the different reference intervals reported are the result of variation in the healthy population studied. However, two very diverse reference intervals have been reported from a laboratory using the same method with different instruments (Table 1, 5, 6). This observation favors the theory that different instruments give different results and hence different reference intervals.

This situation is unlike that for other clinical enzymes, where, for a given method, results are similar irrespective of the instrumentation used. Differences in the absorbance changes and molar absorbptivity values self-correct to produce the same results. This is not so for the kinetic ACE method, suggesting that the ΔA values may be incorrectly determined. In a recent publication on the kinetic ACE method (7), the authors apparently used the same ΔA value to quantify ACE measured on two instruments, a Monospec 105 spectrophotometer with a 345-nm filter and an Olli C + D Compact Analyzer with a 340-nm filter. This ΔA is unlikely to be the same for the two instruments and its indiscriminate use for both instruments is the probable cause for the large reference interval obtained, 30–170 U/L. Likewise, two groups of workers using the Multistat III centrifugal analyzer reported reference intervals of 33–147 U/L and 20–95 U/L (5, 8). Could an incorrect ΔA value be the cause of this?

There is now an urgent need to standardize the kinetic ACE method. Having different normal reference intervals reported for it is confusing. The correctness of the determination for ΔA used in a given study should be critically evaluated. Also, manufacturers can assist in standardization by developing appropriate enzyme reference material. Unless the kinetic assay can have a common reference interval for assays done with essentially the same reagents, irrespective of how ACE is measured, it cannot be claimed to be a simple, inexpensive, and practical test.

References
2. Ronca-Testoni S. Direct spectrophotometric assay for angiotensin-converting en-

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*Absorbance change from the hydrolysis of FAPGG, 1 mmol/L, corrected for 1-cm lightpath.

*No. of subjects for establishing the reference interval (mean ± 2 SD).