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Metronidazole Interference in Hexokinase Glucose Determinations

To the Editor:

Recently, our laboratory happened to measure glucose in a patient's specimen with two different instruments: a Beckman Astra-8, in which glucose oxidase and an oxygen-sensitive electrode are used, and a Technicon SMAC, in which a bound-hexokinase methodology is used. The value obtained with the SMAC was 510 mg/L higher. In our hands, results by these two techniques generally agree within 50 mg/L. For a second specimen, obtained just before the patient's death, the glucose result from the SMAC was 200 mg/L higher.

One of the drugs the patient was receiving was metronidazole (Flagyl, Searle Pharmaceuticals). We added metronidazole to a serum pool to give a concentration of 450 mg/L. The added drug had no effect on results with the Astra methodology, but the SMAC result was higher by 1090 mg/L. Moreover, the 450 mg/L metronidazole addition increased apparent glucose by 590 mg/L in a Du Pont *aca*, in which an endpoint hexokinase procedure with dichromatic photometry at 340/383 nm is used.

The ultraviolet spectrum for metronidazole shows an absorption peak at 325 nm, with a molar absorptivity of $9.5 \times 10^4$ L mol$^{-1}$ cm$^{-1}$. At 340 nm the molar absorptivity is $6.5 \times 10^2$ L mol$^{-1}$ cm$^{-1}$, slightly higher than the value of $6.3 \times 10^2$ for NADH, which is the measured end product of hexokinase methodology. At 383 nm the molar absorptivity is $0.3 \times 10^3$ L mol$^{-1}$ cm$^{-1}$. Interference by metronidazole is probably ascribable to its absorption at 340 nm. This mechanism would account for an increase in apparent glucose concentration of 480 mg/L in a pool containing 450 mg/L of metronidazole per liter. In the case of the *aca* the interference was 590 mg/L, presumably because the interference filters in this instrument have a wide enough bandpass that the effective absorptivity of metronidazole is increased. The much greater interference in the case of SMAC may be the result of metronidazole's passing the dialyzer membrane more quickly than glucose.

Our patient was on a standard dosage of metronidazole, which, according to the package insert provided by Searle, should produce peak concentrations in plasma of about 25 mg/L, a concentration that would cause glucose measurement errors of minor consequence (60 mg/L or less). From our data, we can estimate that one specimen had a drug concentration of about 200 mg/L. This high value may have been because the patient was in hepatic failure, which decreases metronidazole clearance, or because the plasma was contaminated with insufficiently mixed intravenous medication.

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A Linear Standard Curve for Determining Amylase Isoenzymes by Selective Inhibition

To the Editor:

A method of selective inhibition for measurement of amylase isoenzymes originally was published by O'Donnell et al. (1) in combination with the Phadebas method (Pharmacia Diagnostics AB, Uppsala, Sweden) for amylase. In their study a sigmoid standard curve is reported for the calculation of the two (pancreatic and salivary) amylase isoenzymes. Such a nonlinear standard curve has drawbacks for accuracy and ease of handling.

Recently, Huang and Tietz (2) reported use of the same inhibitor in combination with other amylase reagents (BMD Single-Vial Amylase Reagent Kit of Boehringer Mannheim and the Beckman Enzymatic Amylase-DS Reagent Kit). They not only claimed to use about 12% of the inhibitor concentration proposed by O'Donnell et al. (1), but they also showed a linear standard curve for the calculation of pancreatic and salivary amylase from the remaining activity after inhibition. This result is then commented on as follows: "The demonstrated linear relationship contrasts with the results of O'Donnell et al., who found a non-linear relationship when they used the 'Phadebas' method and the same inhibitor. This may have been caused by the difference in affinity of inhibitor to substrate, or the longer reaction period, which would allow a greater degree of dissociation of the enzyme-inhibitor complex, as discussed above."

I would like to comment on both statements.

In the publication of O'Donnell et al. (1) no evidence can be found of an inhibitor concentration about eightfold that used by Huang and Tietz (2), either during the incubation period or after addition of reagent. Admittedly the absolute amount of inhibitor used by Huang and Tietz is only 12% of that added in the original method. But this amount is in a volume of 10 µL added to 50 µL of sample for the preincubation in their case, while O'Donnell et al. perform the incubation with 0.3 mL of buffer, 0.2 mL of sample, and 10 µL of inhibitor solution. Therefore the inhibitor concentration used by Huang and Tietz is in fact 102% of the original concentration during the preincubation. Because the final volume after addition of amylase reagent is 1.06 mL instead of the original 4.1 mL, the inhibitor concentration used by Huang and Tietz is only 46.4% of the original concentration during the reaction with substrate.

A comparison of the standard curve presented by Huang and Tietz (2) and the one presented by O'Donnell et al. (1) is hindered by the fact that different ratios are given on the abscissa, \[ \text{Pancreatic amylase (P)/Total amylase (T)} \times 100 \] in the former and \[ \text{Pancreatic amylase (P)/Salivary amylase (S)} \] on a logarithmic scale in the latter.

Therefore I recalculated the figures obtained from the graphical representation of the standard curve in the O'Donnell publication to the same units as used by Huang and Tietz. The results are shown in Figure 1. Linear regression analysis of the 18 values gives a perfectly straight line with \( y =\)