Enzyme Immunoassay, with Use of a Centrifugal Analyzer, of Phenyo tin, Phenobarbital, and Theophylline

To the Editor:

Recently, Haven (1) and London et al. (2) described adaptation of the "Enzyme Multiplied Immunoasssay Technique" (EMIT; Syva, Palo Alto, Calif. 94304) for phenytoin and phenobarbital to the Centrifichem (Union Carbide, Rye, N. Y. 10580). We describe here a modified method for the assay of phenytoin and phenobarbital, which also is applicable for theophylline determinations.

This modification deletes the manual pipetting of Reagent A and avoids the addition of water during the sample flush. The Sample Diluent Pump on the Centrifichem is emptied, dried, and then filled with diluted Reagent A from the Diluent Reservoir. The remaining Reagent A in the reservoir is then returned to the reagent bottle. In this manner Reagent A is added automatically as the diluent flush following the sample.

During pipetting the Diluent Reservoir filled with distilled water acts to rinse the pipette tip between sample aspirations. Upon completion of pipetting, residual Reagent A is aspirated from the pump and returned to the reagent bottle. These modifications eliminate the need for prior dilution of standards or samples as previously reported by London et al. (2). The sample size meets instrument specifications and avoids sample volumes of less than 5 μl as suggested in a previous method (1).

All reagents, samples, and buffers are in the same proportion in this method as in the recommended EMIT procedure. Reagents are prepared as per kit instructions except as follows:

Reagent A—reconstitute with 6.0 ml of distilled water.

Working Reagent A—to one volume of stock Reagent A add 1.3 volumes of EMIT Buffer Solution. This reagent is used to fill the Sample-Diluent Pump.

Working Reagent B—to one volume of Reagent B add 6.0 ml of distilled water.

Results are calculated by plotting the ΔA from prints one and six. Lot-specific graph paper provided with each kit is used.

The within-run CV averages 2.5% or less. The inter-run CV ranges from 4.0 to 5.0% within the therapeutic range.

References

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More on Use of Hair in Trace-Metal Analysis

To the Editor:

I wish to comment upon the interesting and informative paper of Assarian and Oberleas (1).

Obviously it is impossible, from analysis of untreated hair, to state categorically which proportion is "true" metal content and which is present as a contaminant. However, it is usually possible to make an intelligent assessment of the probability of contamination.

In these authors' paper, the marked loss of magnesium can be simply explained. Because this element is present in water and readily forms insoluble soaps, it is very probable that magnesium was lost simply by removal of the magnesium soaps on the hair during the washing procedure. Furthermore, this particular example is not a practical problem, because it seems unlikely that hair analysis would be used in the case of this element.

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Test mode = Terminal
Printout = Absorbance
Number of prints = 9

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In the case of copper and zinc, the authors are to be congratulated on the excellent reproducibility of the techniques used. The fact that different wash procedures gave somewhat different results need not be surprising if one assumes that the efficiency in removing contaminating metal varied with the procedure.

A point of criticism is their use of an ionic detergent, sodium lauryl sulfate, because this is more likely to leach out bound metal from the hair than is a nonionic detergent. Furthermore, if the authors suspected that bound metal was being leached out by their washing procedure, surely the experiments should have been performed for various periods of time.

The authors' contention that "hair analysis is sensitive to the preparation technique used" has been confirmed by others (2), but this does not invalidate the comparison of values obtained by the same technique.

I think that their conclusions are unnecessarily negative, particularly as regards hair analysis in epidemiological surveys, because the alternatives—urine or blood analysis—are, without stringent precautions to exclude contamination, much less reliable than the authors' zinc and copper analyses.

As a final point, in at least one of the references cited by the authors (3), hair analysis gave clinically useful information for diagnosis and treatment, and that, after all, is the name of the game.

References

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Dr. Oberleas comments that "most, if not all, [shampoo] preparations contain detergents which do not form insoluble complexes, rather than soaps. It is doubtful that water contains enough magnesium to account for 50% of [the found in] hair. Even the method in which detergent was not used removed considerable quantities of both copper and magnesium. Though the paper may have seemed a bit negative, it should serve as a warning that the washing procedures and hair analyses [must be used with due caution]."

**Table 1. Effect of Bilirubin on Results by the Kinetic Jaffé Method**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. Bilirubin mg/liter</th>
<th>Conc. Creat. mg/liter</th>
<th>Apparent Error, mg creat/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho(^c) Normal Cont.(^b)</td>
<td>2.0</td>
<td>9.2 ± 0.4</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>51.0</td>
<td>6.8 ± 0.3</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>69.0</td>
<td>6.8 ± 0.3</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>101.0</td>
<td>5.8 ± 0.5</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>149.0</td>
<td>4.2 ± 0.8</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>183.0</td>
<td>2.8 ± 0.6</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>240.0</td>
<td>1.2 ± 0.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Pathrol(^c)</td>
<td>0.0</td>
<td>11.8 ± 1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Pathrol</td>
<td>101.0</td>
<td>9.4 ± 1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Control Prod., Inc. Range I(^c)</td>
<td>&lt;2.0</td>
<td>32.8 ± 0.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Control Prod., Inc. Range II(^c)</td>
<td>101.0</td>
<td>27.6 ± 0.9</td>
<td>5.2</td>
</tr>
<tr>
<td>Control Prod., Inc. Range II(^c)</td>
<td>40.0</td>
<td>63.2 ± 1.3</td>
<td>4.4</td>
</tr>
</tbody>
</table>

\(^a\)Results are the mean of at least five determinations.

\(^b\)SD

\(^c\)No bilirubin added.

**Bilirubin Interference with the Kinetic Jaffé Method for Serum Creatinine**

To the Editor:

The kinetic Jaffé method (1) is used with various clinical laboratory instruments to determine serum creatinine. Unlike end-point Jaffé methods (2), the kinetic method does not include protein precipitation or separation, which also removes bilirubin. Thus when protein separation is omitted, bilirubin is present during the assay for creatinine. We, and others (3, 4), have observed that bilirubin interferes with the kinetic Jaffé method, resulting in falsely low creatinine values. We evaluated this effect of bilirubin on the kinetic Jaffé method by comparing the results of creatinine determinations on pooled sera with and without added bilirubin.

Samples of pooled sera containing known amounts of bilirubin were prepared by adding small amounts of a stock bilirubin solution to an aliquot of the pooled serum. Stock bilirubin solutions were freshly prepared by dissolving 5-6 mg of bilirubin in 250 µl of 0.10 mol/liter NaOH and diluting to 2 ml with bovine serum albumin solution (50 g/liter). The maximum dilution of pooled sera by the added bilirubin was less than 10%. Creatinine assays were performed on a Gemeni Centrifugal Analyzer (Electro-Nucleonics, Inc.) with reagents supplied by the manufacturer.

We followed the procedure specified by the manufacturer, except that in experiments on pooled sera a 10 mg/liter creatinine standard was used and entered as 10.0 (rather than 1.0); this was done to increase sensitivity. In all other experiments with the Gemeni, the manufacturer's procedure was followed exactly. Results of these experiments are summarized in Table 1.

Some icteric samples obtained from patients were assayed for creatinine by both the kinetic and the end-point methods (Table 2). The data in Tables 1 and 2 clearly indicate that bilirubin present in the 100–200 mg/liter range interferes with the kinetic Jaffé method in both control sera supplemented with bilirubin and in samples from patients. Whether or not both unconjugated and conjugated bilirubin interfere with the kinetic method is of interest but of little practical importance. The concentrations of unconjugated and conjugated bilirubin may not be known for many of the samples submitted for a creatinine assay. It would be inconvenient, and probably uninformative, to perform an additional assay on these samples. When dealing with pediatric samples, the amount of sample available may even preclude an additional assay. The simplest treatment of icteric samples would be to avoid the bilirubin interference by using an end-point method for creatinine. Accordingly, we did not attempt to determine the relative effects of unconjugated and conjugated bilirubin.

Other substances likely to be present in serum from patients have been shown to interfere with the end-point Jaffé method (5). The discrepancies shown in Table 2 may in part be due to such substances.

To determine whether the interference was attributable to some peculiarity associated with centrifugal analyzers, we did some experiments with use of a recording spectrophotometer. In a typical experiment, 500 µl of alkaline pic