mesters of pregnancy was added.

TSH values during pregnancy are lower when measured by immunoradiometric assay (3); that being the case, a TSH value of 0.6 milli-int. unit/L in such a system is not suppressed to an abnormally low value for TSH when 300,000 int. units of hCG are added per liter. In fact, only at concentrations exceeding $1.1 \times 10^6$ int. units/L did we find suppression to abnormally low values in this study. At hCG concentrations of $2.2 \times 10^6$ int. units/L the range in decrease of TSH was 71 to 85%.

We think it unwise for clinical chemists to reject TSH assays after assessing interference from hCG by adding such massive concentrations as $2 \times 10^6$ int. units of hCG per liter. This approach subjects what is potentially a useful tool in thyroid-status studies to situations rarely encountered physiologically. Instead, we would direct attention to a recent study by Klee and Hay (4), who, in an extensive study based on a survey of published studies on sensitive assays for TSH and their own patients, reported that the Boots Celltech IRMA-TSH reagents met all the necessary performance criteria.

References


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The “Chromogen Oxidase” Activity of Bilirubin Oxidase

To the Editor:

Bilirubin oxidase (EC 1.3.3.5) can be used to remove bilirubin interference from hydrogen peroxide/peroxidase (EC 1.11.1.7) detecting systems (1). One drawback to its use is its inherent “chromogen oxidase” activity, which can lead to large reagent blank absorbances. This “chromogen oxidase” activity can be inhibited with azide or cyanide (2), but this requires incubation of the sample with bilirubin oxidase before assay. This would not be necessary if a chromogen that was not a substrate for bilirubin oxidase could be found. We investigated several phenol derivatives in an attempt to find one that is a substrate for peroxidase but not for bilirubin oxidase.

We added bilirubin oxidase (10 µL of 55 U/L solution in Tris/HCl, 0.2 mol/L, pH 8.4) at 37 °C to 180 µL of various substituted phenols (3 mmol/L in the Tris/HCl buffer also containing 4-aminophenazone, 1.0 mmol/L). Absorbance at 510 nm was measured every 20 s for 10 min in a Cobas Bio (Hoffmann-La Roche) centrifugal analyzer. To determine the relative absorptivities of the quinonimine dyes, we used them in the measurement of glucose with glucose oxidase and peroxidase and compared the results obtained with each.

All of the substituted phenols we examined were substrates for bilirubin oxidase (Table 1). The differences between the phenols could largely be explained by the differences in the absorptivities of the quinonimine dyes produced (Table 1). Those phenol derivatives produced a more highly colored dye also produced more color with bilirubin oxidase. Thus, use of bilirubin oxidase to remove bilirubin interference in hydrogen peroxide/peroxidase detecting systems, irrespective of which phenol derivative is used, evidently will require prior incubation of sample with enzyme and addition of azide to the assay reagent.

Correlation between Calcium and Magnesium in Plasma or Serum?

To the Editor:

Brown (1) and Speich (2) have questioned the possible correlation between calcium and magnesium, whose physiopathological and chemical similarities are so important.

We have studied hypocalcemia and hypomagnesemia in surgical patients, measuring magnesium by the method of Gindler and Heth (3) as modified by Cohen and Daza (4), and calcium by the method of Connerty and Briggs (5) with reagents from Electronucleonics Inc., Fairfield, NJ 07006. The good

<table>
<thead>
<tr>
<th>Phenol derivative</th>
<th>Relative absorptivity of quinonimine dye</th>
<th>Absorbance change in 10 min with oxidase</th>
<th>Inhibition, %</th>
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</thead>
<tbody>
<tr>
<td>2,4,6-Tri-OH BA</td>
<td>&lt;1</td>
<td>0.06</td>
<td>90</td>
</tr>
<tr>
<td>2,5-Di-OH BA</td>
<td>8</td>
<td>0.25</td>
<td>73</td>
</tr>
<tr>
<td>2,3-Di-OH BA</td>
<td>16</td>
<td>1.16</td>
<td>69</td>
</tr>
<tr>
<td>4-OH BA</td>
<td>40</td>
<td>1.08</td>
<td>78</td>
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<tr>
<td>2,3,4-Tri-OH BA</td>
<td>44</td>
<td>0.65</td>
<td>14</td>
</tr>
<tr>
<td>3,5-Di-Cl-4-OH BA</td>
<td>45</td>
<td>2.14</td>
<td>78</td>
</tr>
<tr>
<td>3-Cl-4-OH BA</td>
<td>55</td>
<td>1.75</td>
<td>78</td>
</tr>
<tr>
<td>3,5-Di-Cl-2-OH BA</td>
<td>100</td>
<td>3.01</td>
<td>77</td>
</tr>
</tbody>
</table>

*BA = Benzoic acid. *Rate of absorbance changes was linear for 3 min only. *Large reagent blank (0.37).

References


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