Neonatal Hypothyroid Screen—Other Sources of Error, and Their Elimination

To the Editor:

In October 1976 we began research on a method for neonatal thyroxine determination, using whole blood absorbed on a piece of filter paper. This method was developed by our laboratory by modifying the serum thyroxine method of Clinical Assays, Inc.

Davis described several sources of error in the filter-paper method that they are developing (1). The size of the blood spot and the regions of the spot from where the diastase was punched were said to be sources of error in thyroxine concentrations reported. In our procedure, we stress several other factors that we believe help diminish errors.

1. Only one type of filter paper should be used. We use a Sigma Chemical Co. (St. Louis, Mo. 63178) blood-collection card (cat. no. 160-C). This helps to eliminate any irregularities in absorption of blood due to differences in paper density and texture between different manufacturers of blood-collection cards.

2. To keep blood-spot size as uniform as possible, we suggest absorbing only a single drop of blood, which should fill and completely soak through the entire 11.5-cm circle outlined on the collection card. For convenience we absorb a blood spot for a phenylalanine determination at the same time we collect for thyroxine. Multiple applications of blood to one spot are not satisfactory, because this "stacking" of erythrocytes leads to a falsely elevated phenylalanine.

3. After the blood is spotted on the filter paper, Davis (1) assays 1/8-inch samples from the central and peripheral regions of the spot. To eliminate differences in the absorption of sera between these two areas, we punch out a 0.25-inch (7-mm) disk.

4. We found that in order to get adequate elution/extraction of the blood sample into the 8-anilinonaphthalene sulfonic acid—salicylic acid buffer reagent, the tube coated with rabbit antithyroxine, with sample and buffer, must be shaken three times during a 20-min incubation at room temperature before tracer is added.

If specimen collection is adequate, we recommend a serum thyroxine determination only if the neonatal screen shows a thyroxine filter-paper value below 60 μg/liter. We have completed almost 5000 such determinations with a recall of only 1.6%.

References

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Thin-Layer Chromatography of Phenolic Acids with Use of Toluene Instead of Benzene

To the Editor:

Mixtures of benzene, acetic acid, and small amounts of water are frequently used for the chromatographic separation of phenolic acids on both paper and thin-layer plates (1). Compounds separate as diffuse zones, and this type of solvent is commonly more successfully used in the second dimension, following propan-2-ol/ammonia mixtures (1). The substitution of toluene for benzene in this system was investigated for the following reasons:

(a) Although both solvents are skin irritants, produce nausea and other symptoms, and are lethal when inhaled in high concentrations (2), chronic exposure to toluene is considered less toxic because any retained by the body is rapidly oxidized to benzoic acid and excreted as hippuric acid (3).

(b) In contrast, free benzene remains in the tissues much longer, being only slowly converted to phenols and phenolic conjugates of glucuronic acid or ethereal sulfates (3). Prolonged exposure to benzene vapor and its constant absorption through the skin result in hepatic and blood disorders (2).

(c) Toluene, which has a higher boiling point (111 °C) than benzene (80 °C), is more suitable for use in hotter climates.

Chromatography was performed on flexible layers coated with microcrystalline cellulose (No. 5552; E. Merck, Darmstadt, W. Germany) 100 mm thick. Samples were applied as bands 15 mm long and ascending chromatography was performed with solvents containing either benzene or toluene (70 ml), glacial acetic acid (20 ml), and de-ionized water (1 ml). Phenolic acids were located on the air-dried sheets by spraying duplicate layers with either alkaline diazotized sulfanilic acid or p-nitroaniline (1). Table 1 summarizes the results obtained with a representative number of common urinary phenolic acids.

In all cases, Rf values were lower in the faster-moving toluene solvent. The slower-moving compounds, whose migration could be measured with the least accuracy, seemed to be retarded proportionately more than the faster-moving substances. This general reduction in mobility was counteracted by discernibly less diffuse zones, particularly when the toluene solvent was used in the second dimension following propan-2-ol/2-methylpropan-2-ol/25% ammonium hydroxide/water (4/2/2/1) (1).

Toluene is only a little more expensive than benzene and we recommend its substitution for the latter in solvents.

<table>
<thead>
<tr>
<th>Phenolic acid</th>
<th>Rf in solvents containing either Toluene or Benzene</th>
<th>Rf in toluene</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Hydroxymandelic</td>
<td>0.08</td>
<td>0.73</td>
</tr>
<tr>
<td>Caffeic</td>
<td>0.18</td>
<td>0.72</td>
</tr>
<tr>
<td>4-Hydroxy-3-methoxy mandelic</td>
<td>0.17</td>
<td>0.68</td>
</tr>
<tr>
<td>o-Hydroxyhippuric</td>
<td>0.42</td>
<td>0.75</td>
</tr>
<tr>
<td>p-Hydroxyphenylacetic</td>
<td>0.47</td>
<td>0.76</td>
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<tr>
<td>m-Hydroxybenzoic</td>
<td>0.53</td>
<td>0.80</td>
</tr>
<tr>
<td>o-Hydroxybenzolic</td>
<td>0.55</td>
<td>0.82</td>
</tr>
<tr>
<td>o-Hydroxyphenylacetic</td>
<td>0.60</td>
<td>0.80</td>
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<tr>
<td>Homovanillic</td>
<td>0.74</td>
<td>0.86</td>
</tr>
<tr>
<td>Vanillic</td>
<td>0.80</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* Rf = distance moved by compound (mm)/distance moved by solvent front (mm)

Table 1. Mobility of Phenolic Acids on Cellulose Layers