ally, the normal introduction time of 2 s leads to the good precision and recovery as found with the use of larger sample volumes, as discussed in the original letter.

Corning has completed a rewording of their instruction manual to clarify the statement "slowly inject the sample until the 'Sample in Place' indicator lights," so that it is obvious to the user that optimum results are obtained with introduction times of between 2 and 3 s.

The authors of the original letter believe that instrument performance should be improved by regular six monthly service checks of the hydraulic system status. This issue is routinely addressed at operator training courses and will be included in the instruction manual.

The revision of the instruction manual as described will overcome the problem encountered during the experiments at IMVS.

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Ed. note: When the original letter was received, Corning was inadvertently not allowed an opportunity to respond.

Cross Reactivity of Dihydropdigoxin with a Commercial Digoxin Kit

To the Editor:
Kramer et al. (1) recently reported the evaluation of four commercially available 125I-labeled digoxin radioimmunoassay kits with regard to their cross reaction with the relatively cardioinactive digoxin metabolite, dihydropdigoxin. The kit we currently use for digoxin, Nuclear Medical Laboratories’ Digi-Tab, was not one of those evaluated. We have checked its cross reactivity with dihydropdigoxin.

Dihydropdigoxin (Bio-Dynamics/bmc, Indianapolis, Ind. 46250), was diluted to 1 liter with de-ionized water, and this dilution was further diluted 100-fold with de-ionized water. Digoxin-free serum was used to dilute the 50 µg/liter dilution to concentrations of 0.5, 1.0, 2.5, 4.0, and 5 µg/liter. These samples were then assayed in duplicate exactly as instructed by NML’s procedure booklet. We obtained eight values for each sample and standard curve by performing four assays in duplicate. The radioactivity was measured in a calibrated Automatic Gamma Counter (Model 1185; Searle Analytic, Inc., Chicago, Ill. 60666), and the data were analyzed with a Searle PDS/3 Computer (SRA/2 System).

The percentage of 125I-labeled digoxin bound (B/T) for each standard was plotted vs. dose, yielding a hyperbolic curve, and the controls and dihydropdigoxin standard values were read off this curve by the computer.

The Ortho I and Ortho II controls assayed with each run were well within ±2 SD of their mean values in this laboratory, and the curve SD never exceeded 0.2; the CV for the curve never exceeded 10%.

The digoxin-free serum used to prepare the dihydropdigoxin working standards was also assayed, and the apparent digoxin (222 ng/liter) due to nonspecific binding was subtracted from each dihydropdigoxin standard result. The results, as digoxin, for the dihydropdigoxin standards were as follows:

<table>
<thead>
<tr>
<th>Added dihydropdigoxin concentration (µg/liter)</th>
<th>Mean cross reactivity as % of digoxin concentration</th>
<th>Required digoxin equiv.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>1.0</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>2.5</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td>4.0</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td>5.0</td>
<td>16.7</td>
<td>16.7</td>
</tr>
</tbody>
</table>

* Dihydropdigoxin may comprise up to 30% of total glycosides (1); therefore, these are the minimum digoxin concentrations in the serum necessary to produce the amounts of dihydropdigoxin we tested. For instance, if a patient’s digoxin concentration is 3.3 µg/liter, the maximum dihydropdigoxin metabolite expected would be 1.0 µg/liter, which would result in a maximum of 0.344 µg/liter increase in the di- 
goxin result.

We conclude that the amount of dihydropdigoxin that might bind to the digoxin antibody in NML’s Digi-Tab is not clinically significant at those digoxin concentrations we encounter. At 1.7 and 3.3 µg of digoxin per liter, variations of 0.2 and 0.3 µg/liter, respectively, might be expected merely from duplicate assays of serum samples for digoxin.

Reference

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Application of Technicon’s SMAC to the Analysis of Amniotic Fluid

To the Editor:
We analyzed some amniotic fluids by use of the SMAC continuous-flow analyzer (1). Determinations were made on 27 amniotic fluids having lecithin/sphingomyelin (L/S) ratios greater than 2.0 (2). Although not obtained in our study, the gestational ages may safely be assumed to range upwards from 34 weeks, because L/S ratios rarely exceed 2.0 before 34 weeks of gestation.

Table 1 compares our results to those of Benzie et al. (3). The differences are small when compared to the physiological variation. New normal values for pregnancies near term are provided for measurement of calcium, phosphate, and aspartate aminotransferase (EC 2.6.1.1).

We looked for correlations between the concentrations of some chemical constituents and the L/S ratio in 37 amniotic fluids having an L/S ratio between 0.5 and 8.5. Creatinine and alkaline phosphatase were found to increase with increasing L/S ratio, but the correlations were poor (r = 0.5). No L/S ratios corresponding to immaturity were encountered when either the creatinine concentration was greater than 22 µg/liter or the alkaline phosphatase activity exceeded 40 U/liter. Thirteen of our 37 specimens exceeded these limits and had an L/S ratio greater than 2.0. These observations are consistent with the previous reports (4).

The tabulated SMAC reference values at maturity have been compared to results obtained by Weisberg for 12–20 weeks of gestation (5). The differences...
Table 1. Amniotic Fluid Reference Values

<table>
<thead>
<tr>
<th></th>
<th>Benzie et al. (3) (36–38 weeks gestation)</th>
<th>This work (L/S &gt;2; estimated 34–44 weeks gestation)</th>
<th>Benzie et al. (3) (39–41 weeks gestation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Glucose, mg/liter</td>
<td>405</td>
<td>116</td>
<td>335</td>
</tr>
<tr>
<td>Urea, mg/liter</td>
<td>180</td>
<td>54</td>
<td>150.0</td>
</tr>
<tr>
<td>Creatinine, mg/liter</td>
<td>21</td>
<td>5</td>
<td>18.42</td>
</tr>
<tr>
<td>Uric acid, mg/liter</td>
<td>102</td>
<td>33</td>
<td>88.2*</td>
</tr>
<tr>
<td>Calcium, mg/liter</td>
<td>—</td>
<td>—</td>
<td>60.9</td>
</tr>
<tr>
<td>Phosphate, mg/liter</td>
<td>—</td>
<td>—</td>
<td>12.56</td>
</tr>
<tr>
<td>Sodium, mmol/liter</td>
<td>126.3</td>
<td>7.4</td>
<td>129.7b</td>
</tr>
<tr>
<td>Potassium, mmol/liter</td>
<td>—</td>
<td>—</td>
<td>4.19*</td>
</tr>
<tr>
<td>Chloride, mmol/liter</td>
<td>104.4</td>
<td>3.7</td>
<td>109.4</td>
</tr>
<tr>
<td>Alkaline phosphatase, d U/liter</td>
<td>—</td>
<td>—</td>
<td>35.8</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/liter)</td>
<td>—</td>
<td>—</td>
<td>9.81</td>
</tr>
</tbody>
</table>

* Omitting one outlier >150 mg/liter.

b Omitting one outlier at 103 mmol/liter.

c Omitting two outliers > 5.6 mmol/liter.

d SMAC method not comparable to that in ref. 3.

are in agreement with the changes known to occur with increasing gestational age. Constituents for which concentrations decrease during gestation are glucose, phosphate, sodium, and chloride; in contrast, creatinine, uric acid, potassium, and alkaline phosphatase increase.

References
1. Technicon Instruments Corp., Tarrytown, N.Y. 10591.

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M. M. Cook
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