HPLC method we used was 1.7, 2.0, and 1.8%, respectively, (n = 18) in the concentrations studied. Interassay variability (n = 19) of the Seralyzer Aris method for a theophylline concentration of 15 mg/L was 5.9%.

According to the manufacturer, the results of the Sera-
lyzer Aris method vary linearly with concentrations only between 3.0 and 30 mg/L. Theophylline concentrations greater than 30 mg/L can be measured by diluting the sample with distilled water.

We tested metabolites of theophylline and other xan-
thines for possible interference with the Seralyzer Aris method. Samples containing 1- and 3-methylxanthines (17 mg/L), β-hydroxethyltheophylline (20 mg/L), or caffeine (30 mg/L) showed no apparent theophylline when measured with the Seralyzer Aris method. Theobromine at 20 mg/L in drug-free serum gave an apparent theophylline value of 6.4 mg/L; however, theophylline isn’t metabolized to theobro-
mine in humans (9), so possible interference is practically negligible. We noticed no interference in patients’ samples containing drugs other than theophylline.

Studies with asthmatic patients indicate that serum concentrations of theophylline between 10 and 20 mg/L are associated with therapeutic effectiveness (5). In our investigation, we found that the Seralyzer Aris method gave accurate and reproducible results at the clinically important concentration range. The method is rapid and simple, and only 30 µL of serum is needed. It is suitable for monitoring theophylline concentrations in emergencies such as acute severe asthmatic attacks and suspected cases of theophyl-
line intoxication.

We thank Mrs. Ritva Pohjola and Mrs. Liisa Jarvinen for valuable technical assistance.

References
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Amniotic Fluid Acetylcholinesterase Activity and Alpha-Fetoprotein in Chromosomal Anomalies and Neural Tube Defects

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Measurement of alpha-fetoprotein concentration and acetyl-
cholinesterase activity in amniotic fluid can be used to iden-
tify chromosomal defects as well as neural tube defects. In seven cases of trisomy 21 and one case of partial trisomy 3, alpha-fetoprotein concentrations were below the reference range but values for acetylcholinesterase activity were nor-
mal for the appropriate gestational age. One case of trisomy 13 had an increase in acetylcholinesterase activity and normal alpha-fetoprotein concentration.

Additional Keyphrases: chromosomal anomalies · neural tube defects · heritable disorders · antenatal screening

We have previously demonstrated that in trisomy 21 the acetylcholinesterase (AChE, EC 3.1.1.7) isoenzyme pattern in amniotic fluid may be identical to that found in cases of neural tube defects, and thus it may be necessary to determine the fetal karyotype of amniotic-fluid cells to differentiate the two (1). The longer incubation period required to demonstrate the AChE isoenzyme pattern in chromosomal anomalies (Buamah, unpublished observa-
tion) suggests that the total AChE activity in these cases is within normal limits.

Concentrations of alpha-fetoprotein (AFP) in maternal serum have been shown to be lower when the fetus has a chromosomal abnormality (2, 3). We have attempted to determine a relationship between the total AChE activity and the concentrations of AFP in amniotic fluid in cases of chromosomal anomalies and neural tube defects. The results for these analytes may be useful in antenatal screening for chromosomal anomalies, especially for trisomy 21, which occurs about once per 1000 live births.

Patients and Methods

Samples of amniotic fluid were obtained by percutaneous amniocentesis from 29 normal pregnancies, 12 pregnancies associated with neural tube defects, and nine pregnancies resulting in chromosomal abnormalities. The samples were obtained at known gestational ages during the second trimester of pregnancy and they were stored at −20°C until analyzed. The procedure was performed on patients in hospitals in the geographical areas served by the Depart-
ment of Human Genetics, University of Newcastle upon Tyne. The trisomies were determined from fetal karyotypes.
Amniotic fluid AFP was measured by a single-antibody radioimmunoassay with polyethylene glycol precipitation (4). AChE activity was measured at 30 °C by a reaction rate method (5) in a Cobas centrifugal analyzer.

Results and Discussion

Table 1 shows the individual AFP concentration and AChE activity for each pregnancy with a chromosomal anomaly; Table 2 shows the results for 30 pregnancies with normal outcome. The total AChE activity for the seven cases of trisomy 21 and a case of partial trisomy 3 was within the reference range for normal pregnancies. However, a single case of trisomy 13 had an above-normal AChE value, 23.9 U/L—similar to that found in pregnancies associated with neural tube defects. We have already shown (1) that electrophoresis of amniotic fluid on polyacrylamide gel allows differentiation between trisomy 13 and neural tube defects by the presence of three and two AChE isoenzymes, respectively.

The AFP values for the seven cases of trisomy 21 and the case of partial trisomy 3 were much lower than the cutoff level of 3.5 multiples of the normal median (MoM) used to identify neural tube defects (6); however, the single case of trisomy 13 had an AFP value of 195 mg/L, greater than 12 MoM (Table 1). The 12 cases of neural tube defects gave AFP values ranging from 3.8 to 27.9 MoM (Table 3). The advantage of using MoM's to express AFP values is that laboratories need not establish numerical agreement in their values, and each laboratory can express AFP as a multiple of its own median value for each gestational week. A median value also can be used with relatively small numbers of samples and is relatively unaffected by occasional outliers and by random errors of measurement.

Concentrations of AFP in amniotic fluid are significantly lower in pregnancies associated with trisomy 21 than pregnancies with normal outcome, but the total AChE activity in the two groups is similar. Thus, neural tube defects are associated with increased AFP and increased AChE activity, whereas trisomy 21 and partial trisomy 3 are associated with AFP concentrations below the reference range and normal AChE activity. The rare case of trisomy 13, with increased AFP and AChE, has a characteristic isoenzyme pattern (7) that clearly differentiates it from the pattern found in neural tube defects. The measurement of AFP in amniotic fluid and the AChE isoenzyme test may form a basis of antenatal screening for trisomy 21 (Down’s syndrome) and complement the time-consuming determination of fetal karyotype.

References

Table 1. Acetylcholinesterase and α-Fetoprotein in Amniotic Fluid from Pregnancies with Chromosomal Anomalies

<table>
<thead>
<tr>
<th>Case number</th>
<th>Gestational age, weeks</th>
<th>Chromosome anomaly</th>
<th>AChE activity, U/L</th>
<th>Total AFP, mg/L</th>
<th>MoM (UKCS + 0.5)</th>
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<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>47XY + 21</td>
<td>3.0</td>
<td>34</td>
<td>1.78</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>47XY + 21</td>
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<td>10</td>
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</tr>
<tr>
<td>3</td>
<td>16</td>
<td>47XY + 21</td>
<td>3.8</td>
<td>26</td>
<td>1.36</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
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<td>2.2</td>
<td>22</td>
<td>1.15</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>47XY + 21</td>
<td>3.6</td>
<td>26</td>
<td>1.36</td>
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<td>5.3</td>
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<td>1.10</td>
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<tr>
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<tr>
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<td>12.18</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>47XY + 3*</td>
<td>5.4</td>
<td>28</td>
<td>2.0</td>
</tr>
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</table>

*Partial trisomy. *UKCS = United Kingdom Collaborative Study; MoM = multiples of the normal median.

Table 2. Acetylcholinesterase and α-Fetoprotein in Amniotic Fluid from Normal Pregnancies

<table>
<thead>
<tr>
<th>Gestational age, weeks completed</th>
<th>AChE activity, U/L</th>
<th>Cutoff limit</th>
<th>AFP</th>
<th>AFP (UKCS + 0.5)</th>
<th>MoM</th>
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<tbody>
<tr>
<td>Controls</td>
<td>16</td>
<td>Median 3.8</td>
<td>19</td>
<td>68.5</td>
<td>3.5</td>
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<td>16</td>
<td>Range 2.6–5.6</td>
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<td>56</td>
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<tr>
<td>10</td>
<td>17</td>
<td>Median 4.7</td>
<td>14</td>
<td>49</td>
<td>3.5</td>
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<tr>
<td>10</td>
<td>18</td>
<td>Range 2.7–12.7</td>
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<td></td>
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</tbody>
</table>