Because aminopterin and dichloromethotrexate inhibit dihydrofolate reductase activity with, respectively, 86.9% and 93.4% cross reactivity, the described method can be used to measure these folate analogs separately. The sensitivity for 2,4-diamino-N\textsuperscript{10}methylpteroyl acid was 17%; it was less than 1% for 7-hydroxy-methotrexate. There was no interference by folic acid or folinic acid (which is often used for "rescue").

We compared this method with the protein-binding assay previously described by Myers et al. (2), because its sensitivity (10\textsuperscript{-8} mol/L) exceeds that of liquid chromatography (10\textsuperscript{-7} mol/L).

Results obtained by the two methods correlated closely (n = 120; r = 0.966; y = 0.901x + 1.21 \times 10^{-8} \text{ mol/L}, where y = Cobas-Bio assay, x = protein-binding assay).

The proposed method provides a precise methotrexate analysis (day-to-day variation ranges from 1.9% to 6.7%), and it is especially useful when many samples are to be tested. Twenty assays can be done in less than 10 min with sufficient sensitivity at less than the toxicity threshold (10\textsuperscript{-7} mol/L), and the method can be used as an emergency technique to monitor methotrexate in plasma.

References

A. M. Imbert
T. Pignon
N. Lena

Institut J. Paoli-I. Calmettes
Lab. Of Biochem.
232, boulevard Sainte Marguerite
B.P. 156
Marseille Cedex 9, France

Aluminum, Barium, Silicon, and Strontium In Amniotic Fluid by Emission Spectrometry

To the Editor:

Several reports (1, 2) deal with trace-element analysis of human amniotic fluid. Most of the analyses involve atomic absorption, a single-element technique, although neutron activation analysis, a multi-element technique, has been used in a few cases for analysis of amniotic fluid collected at parturition (3).

However, rather than limit ourselves to a single-element analysis procedure that could be biased by preconceptions of what is present, we adopted a more systematic approach to trace-element analysis of amniotic fluid. We use a combination of proton-induced x-ray emission (PIXE) (4) and direct current plasma-atomic emission spectrometry (DCP-AES), which allows simultaneous multi-element analysis for the elements lithium to uranium. The detection limits of these techniques approach <10\textsuperscript{-6} g/L (ng/mL). Because these are multi-element techniques we can determine concentrations of unsuspected elements, which can lead to serendipitous discoveries.

Here we report the first determination of aluminum, barium, silicon, and strontium in samples of human amniotic fluid, obtained at 16–19 weeks of gestation mainly from cases of advanced maternal age. None of the samples assayed were bloody. A questionnaire answered by the subjects revealed that 50% drank alcohol, 40% smoked, 30% were vegetarians, 65% lived in cities, and 30% were nonwhite. Their ages ranged from 17 to 42 years and their occupations from construction worker to physician.

The analyses were performed by DCP-AES (Beckman SpectraSpan IIIIB; SpectraMetrics, Inc., Andover, MA 01810) and the detection limits for Al, Ba, Si, and Sr were 2, 2, 15, and 2 \mu g/L, respectively. A certified standard [SRM-1643a (water); National Bureau of Standards, Washington, DC 20234], was analyzed as every tenth sample, and the CVs for Ba and Sr were always <5%; Al and Si are not certified in the standard. The results for these four elements in 97 amniotic-fluid samples were as follows:

<table>
<thead>
<tr>
<th>Element</th>
<th>Conc., \mu g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>37–149</td>
</tr>
<tr>
<td>Sr</td>
<td>13–35</td>
</tr>
<tr>
<td>Ba</td>
<td>34–800</td>
</tr>
<tr>
<td>Si</td>
<td>34–800</td>
</tr>
</tbody>
</table>

*Less than the detection limit for Ba: 2 \mu g/L.

The concentrations of Zn, Fe, K, Cu, and Ca were similar to those reported by others (1, 2) and data on the concentrations of other elements from Li to U are available upon request.

In the 97 samples studied, the concentration range for Sr is small, even though the subjects were from a large and diverse group. This suggests that measuring the concentration of Sr in amniotic fluid might be useful in assessing fetal well-being.

Silicon is an essential element in mucopolysaccharide synthesis in cartilage and connective tissue.

Very little is known about the toxicity of barium, and there seems to be no conclusive evidence that it performs any essential function in man.

Several reports link excessive aluminum exposure with increased concentrations of the metal in the brain, which may result in various encephalopathies (5). Another report (6) has shown a statistically significant relationship between dyslexia and abnormal Al in children's hair. In our study, the concentration of aluminum (y) in amniotic fluid was highly correlated (r = 0.901, p < .001) with the concentration of Ca (55.3 (SD 7.2) mg/L, range 28–76) in amniotic fluid: y = 0.721 + 0.150x (n = 97). Because the fetus inhales and swallows amniotic fluid (7) at a turnover rate of one-third of its volume every hour, maternal exposure to aluminum should perhaps be of concern.

The objective of our study was to establish a data base that could be used as a diagnostic tool for identifying a fetus at risk. The significance of Al, Ba, Si, and Sr, as well as some 20 other elements in amniotic fluid, will become clearer as our data base increases.

References

Gene S. Hall
Dept. of Chem.
Michael J. Carr
Dept. of Geol. Sci.
Rutgers, The State Univ. of New Jersey
New Brunswick, NJ 08903

Emilie Cummings
Ming-liang Lee
Dept. of Pediatrics
Univ. of Med. and Dentistry
of New Jersey–Rutgers Med. Sch.
New Brunswick, NJ 08903