Amniotic Fluid Phospholipid "Analysis"?

"How TLC Can Help 50,000 Infants This Year . . . and Every Year" was the eye-catching title to an article (advertisement) from the Lipid Reporter, April, 1979 (1). This was perhaps a fitting observation for the close of the decade that saw the introduction of analyses for phospholipids in amniotic fluid. Now, some 20 years since the first reports were published on the determination of lecithin/sphingomyelin ratios in amniotic fluid samples, perhaps it is time for a little sober reflection on what we have accomplished.

If I were to admit to remembering anything from those tortuous hours of undergraduate physical chemistry, it would have to be the Heisenberg uncertainty principle, especially the part about the uncertainty that is unavoidably introduced into the measured quantities by the measurement process itself. Probably the reason I retain even this small bit of wisdom is that I am so often reminded of it when I read articles on the analysis of amniotic fluid phospholipids. A decade ago Freer and Statland wrote a review on measuring amniotic fluid surfactant (2), in which they pointed out the numerous areas of confusion and contradiction in the dozens of publications in this field. At least two pages of this review were directed towards the preanalytical problems that needed to be addressed. Today these problems still have not been overcome. Should we continue to assume that anything we do to the sample does not introduce uncertainty into the measurement process? Dubin, in a recent editorial, has compared the assessment of fetal lung maturity to the search for the Holy Grail (3). In his brief review of the methods currently available to us, he points out that almost any of the procedures that we might choose to use, including visual inspection of the sample by the obstetrician, have a high probability of correctly identifying mature lungs. It is the correct prediction of immaturity that remains troublesome.

In a period when we, as a profession, have progressed from measuring total lipids by turbidity to meeting mandated CVs for cholesterol determinations of ±3% and, even more incredibly, from palpating tumors to homing in on the gene that causes colon cancer (4), have we really made any significant strides in predicting fetal lung maturity? Perhaps not. The Freer and Statland article was, in my opinion, a very interesting, enlightening, and refreshing approach; about the only thing they missed was mentioning the Heisenberg uncertainty principle by name. Unfortunately, 10 years later we still appear to be diligently ignoring all of the issues they raised. This attitude seems somewhat self-contradictory: the sheer number of modifications of the early procedures can easily be construed as an indication of the degree of dissatisfaction in the field. For would-be developers of methods of analysis for phospholipids in amniotic fluid samples, I strongly recommend a second reading of this review.

In a more recent review, Garite (5) refers to the original procedure for determining the lecithin/sphingomyelin (L/S) ratio as still being the "gold standard." Perhaps it is: after all, it is certainly the one that most reviewers will insist a new method be compared with; in all likelihood it is the one that obstetricians will insist be run; and it is laborious, time-consuming, expensive, and totally impractical for "stat" requests at 0300 h. By these criteria, it certainly fits the definition of a gold standard. However, is the standard accurate and reproducible? Garite also reports that the rate of false prediction of immaturity with this gold standard is 50%. In fact, he reports that this rate ranges from 50% to 75% for seven of the most common approaches to evaluating fetal lung maturity (the reported false-maturity rate was <2% for all of these same methods). Let us reflect: If we report the lungs to be mature, we have a better than 98% chance of being right, but if we predict them to be immature, we have only a 25–50% chance of being right. As a profession, how long would we last if we started reporting results of tests for human immunodeficiency virus antibody with this same degree of confidence? You have a 98% chance of being well or you have a 50% chance of becoming very ill. It does not seem to be reasonable that such a procedure be referred to as the gold standard. It might be more appropriate to accept nothing than it would be to accept anything with these characteristics.

So where do we go from here? We have progressed from thin-layer chromatography and observing bubbles on the surface of alcoholic solutions to fast atom bombardment mass spectrometry (6), which we are revisiting in this issue of Clinical Chemistry (7). If we are still not capable of doing any better than Garite or Dubin would have us believe, maybe we should reconsider our approaches. Perhaps we are looking at the wrong thing in the wrong way in the wrong sample at the wrong time—or some combination of the above. Maybe it is time to begin anew.

Lundell (8), of the National Bureau of Standards, 58 years ago wrote a somewhat critical commentary, "The Chemical Analysis of Things As They Are," because so many talks and articles on analytical subjects dealt with "The Chemical Analysis of Things As They Are Not." Although Lundell was not discussing phospholipids, the arguments presented are quite applicable to the subject at hand. So many of the reports I have read on phospholipid analysis deal with things as they are not. We tend to overlook sample preparation and get right to the final measurement part of the procedure, treating the sample as if it were a solution of pure material. Why? Freer and Statland warned us that it is not a
simple pure solution. As a profession, we take painstaking care in sample preparation for most other tests; why then do we allow such controversy for the evaluation of fetal lung maturity? How can we continue to ignore these factors, given the dearth of consensus in the field? The very first step in many procedures is centrifugation of the sample, yet this is also one of the most controversial steps. Why should it remain controversial when we have had nearly a quarter of a century to resolve this issue?

I do not consider it likely that within the next decade we will all be using fast atom bombardment mass spectrometry to analyze amniotic fluid phospholipids. In the face of the existing methodological controversy and fiscal restraints, some things are just not going to change that quickly. However, perhaps fast atom bombardment mass spectrometry is the analytical tool that some of the members of our profession could use to best resolve all of those controversial steps to which we have been alerted. If so, then for the first time since the inception of amniotic fluid analysis we can, "at least . . . begin to end the often heated discourse between laboratorian and clinician . . ." (3). And perhaps for the first time we will be able to view analysis for amniotic fluid phospholipids in terms of the chemical analysis of things as they are.

References
8. Lundell GEF. The chemical analysis of things as they are. Ind Eng Chem Anal Ed 1933;5:221–5.

Joseph D. Artiss

Department of Pathology
Wayne State University School of Medicine
Detroit, MI 48201