Lecithin/Sphingomyelin Ratio in Respiratory Distress Syndrome

To the Editor:

Gluck et al. (1) have published observations on the "Diagnosis of the Respiratory Distress Syndrome by Amniocentesis." This test has, understandably, been of much interest to physicians. The report speaks of a lecithin/sphingomyelin ratio. In setting up this test, I obtained consistently higher ratios than reported there. Attempting to resolve this problem, I had a split sample analyzed by their laboratory and ours. I obtained a ratio of 1.51, whereas they found it to be 0.82. A conversation with personnel in Dr. Gluck's laboratory revealed the apparent cause of the discrepancy. The reported lecithin/sphingomyelin ratio is in reality a lecithin/(phosphoglyceryl + phosphatidyl serine + phosphatidyl inositol) ratio. Because sphingomyelin, phosphatidyl serine, and phosphatidyl inositol migrate closely in the solvent system used by these authors, the three compounds together were termed "sphingomyelin." (They can easily be separated by substituting ammonium hydroxide for water in the solvent system -- i.e., by using chloroform: methanol:conc. NH₄OH, 65:25:4 by vol.)

All who use the procedure should be aware of this clarifying observation. Such "difficult separations" can be achieved, and the results reported correctly.

Reference


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This letter was referred to Dr. Gluck for comment. His reply (in part) follows:

To the Editor:

Thank you for calling Dr. Simon's letter to my attention. This lecithin/sphingomyelin ratio is exactly as represented; it's a lecithin/phospholipid ratio. The system we use incorporating 5% ammonium sulfate with silica Gel H separates phosphatidyl inositol from sphingomyelin. Dr. Simon thus is mistaken. As a matter of fact, even if there were the two compounds, from experience we know they act as an internal standard and the procedure retains its validity and reproducibility.

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Editor's note: The November 1971 issue of Hospital Practice includes an article by Dr. Gluck, reviewing research on "Pulmonary Surfactant and Neonatal Respiratory Distress." This will provide the reader with further background for these letters.

A biochemist consultant, expert in lipid analysis, who was asked to try and ascertain the basis for these disparate results summarizes his findings as follows:

"At your request we separated phosphatidylserine (ps), phosphatidylinositol (pi), phosphatidyleholine (pc), sphingomyelin (s), and a mixture (mix) of these compounds by thin-layer chromatography, with the system described by Gluck et al. [Amer. J. Obstet. Gynecol. 109, 440 (1971)] (Figure 1).

"Thirty grams of silica Gel H (Merck, Lot No. F3084) was slurried in 65 ml of aqueous 5% ammonium sulfate and spread on glass plates to a thickness of 250 μ. The plate was air-dried, then activated at 110°C for 30 min before use. The phospholipids were separated with a solvent system of methanol–distilled water–chloroform (25:4:65, by vol), and the spots on the thin-layer plate made visible with iodine.

"Obviously ps, pi, and pc all clearly separate from sphingomyelin with this system. Consequently, assuming Dr. Simon is separating phospholipid in this same manner, his failure to reproduce Dr. Gluck's procedure would not appear to be due to contamination of sphingomyelin with either ps or pc.

"Perhaps another factor that should be considered is a possible explanation for Dr. Simon's failure to reproduce Dr. Gluck's results is the method of quantification of individual phospholipids by directly scanning the thin-layer plate. I am under the impression that these procedures are quite variable unless conditions of both separation and quantification are stringently controlled. In addition, the relative responses of individual phospholipids are often differ-

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