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The following is the abstract of the article discussed in the subsequent letter:

**Cassin, S., and A. M. Perks.** Estimation of lung liquid production in fetal sheep with blue dye dextran and radioiodinated serum albumin. *J Appl Physiol* 92: 1531–1538, 2002; 10.1152/japplphysiol.00777.2001.—Lung liquid production and reabsorption rates and lung volumes were measured in 99 fetal sheep (119–148 days of gestation) by indicator-dilution methods with the simultaneous use of blue dye dextran (BDD) and radioiodinated serum albumin (RISA). There were no significant differences between rates of lung liquid production or reabsorption by the two methods (n = 71 pairs; paired t-test; Wilcoxon test; ANOVA); this was equally true for rates in milliliters per hour or milliliters per kilogram body weight per hour and was independent of age. Volumes measured by both methods showed a close linear relationship (r = 0.97; for slope P < 0.0001; n = 99), whether expressed as milliliters or milliliters per kilogram body weight. Either method could give the higher volume. Values differed by only ~4%, independent of age or parameter (ml or ml/kg body wt; volumes regressed to original volume, or as measured in untreated control hours). However, this small difference was significant by paired t-test or Wilcoxon test when all data were combined irrespective of age; it was not significant after allowance for gestational age (two-way ANOVA). Both indicators showed the same increase in lung volume toward birth and the same fall when related to body weight (slopes significant P = 0.0003–0.0004; r = 0.93). Two-way ANOVA showed that the declines were significant (P = 0.003). The data suggest that J) there was no significant difference in production or reabsorption rates measured by BDD or RISA, 2) differences in volumes measured by the two indicators were only significant if gestational age was ignored and were too small to have physiological importance, and 3) although BDD and RISA each may have methodological weaknesses, for purposes of measuring lung liquid volumes both are sufficiently accurate and reproducible to obtain meaningful physiological results.

**Measuring lung liquid volume and secretion using radioiodinated serum albumin and blue dextran**

To the Editor: The issue of appropriate tracer molecules for use in dye-dilution determination of lung liquid volume and secretion rate in the fetal lung is under debate. We recently reported an experimental study comparing blue dextran (BD) and radioiodinated serum albumin (RISA) (4). We found large errors associated with the use of BD, making estimates of lung liquid volume derived from BD unreliable, especially in near-term fetuses. By contrast, we found only small errors when RISA is used alone as a tracer. Cassin and Perks (1) have responded with the results of a retrospective analysis of their earlier work. They present evidence that, when BD and RISA are used simultaneously, lung liquid volumes and secretion rates derived from the two tracers do not differ significantly.

As an introductory comment, we wholeheartedly agree with Cassin and Perks (1) that both BD and RISA have aided our understanding of mechanisms and dynamics of liquid movement across the fetal pulmonary epithelium. We accept that BD can be used to detect relative changes in the rate and direction of lung liquid flow. If, however, it is important to determine absolute values for volume and secretion, we maintain that the choice of tracer is of crucial importance. This view is based on our observation that, when we used repeated saline washes to retrieve BD from the lung, the predictable exponential decline in BD concentration was observed in successive washes. When no further BD could be retrieved with saline washout, a 5% albumin wash retrieved a large amount of BD, and the concentration of BD in subsequent 5% albumin washes fell exponentially toward zero. No explanation, other than BD binding, can account for these observations. Consequently, lung liquid volume is overestimated when BD is used as the tracer. Because secretion rate is estimated from the rate of change in volume, it will also be overestimated.

Cassin and Perks (1) have not repeated the washout experiments that we performed to test for binding of BD. However, they take reassurance from their finding that, when BD and RISA are used together, estimates of volume and secretion derived from the two tracers are statistically indistinguishable (1). We contend that no such reassurance can be drawn from their observation. If, as we have shown, BD and RISA both bind to the epithelium when they are used together (4), volume and secretion must be overestimated with both tracers. Clearly, no inference can be drawn from their observation about the correctness of estimates derived from BD and RISA if they were to be used separately.

Our evaluation of BD and RISA as tracer molecules provides a plausible explanation for why several reports involving BD have claimed that lung liquid volume rises continuously over the last days of gestation (3), whereas studies that use RISA report a fall (2, 5). The important consequence of our evidence, which shows RISA as a reliable tracer in the late gestation fetal sheep lung and BD as not, is that we can confidently conclude that events occurring over the last days of gestation, as well as mechanisms initiated during labor, play a crucial role in reducing the amount of liquid contained in the lung, thereby facilitating pulmonary gas exchange immediately after delivery.
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REPLY

To the Editor: In their letter, Pfister et al. suggested that volumes of lung liquid estimated by blue dye dextran (BDD) are unreliable in near-term fetal sheep. This suggestion is based on the fact that these investigators could wash dye out of the lungs with albumin solutions after saline appeared to have cleared them. In an attempt to clarify this matter, we (1) reexamined large numbers of experiments to look for discrepancies when BDD and radioiodinated serum albumin (RISA) were used concurrently. We found that there were no significant differences between the two tracers in measuring rates of fluid production, reabsorption, lung volumes, and changes in volumes approaching term. These agreements were shown in large numbers of experiments (71 production rates, 99 original volumes), making it difficult to reject these data. However, in their letter, Pfister et al. do not accept our conclusions. They claim that both indicators bind to lung epithelium if they are used simultaneously, yet, in their experiments, lung washes appeared to liberate only BDD. They suggest that there can be no explanation for this other than the binding of BDD to the epithelium. We believe that there are other explanations for their data. First, there were marked differences in the methodology by which they and we added BDD to the lungs. In our experiments, BDD was added to the lung liquid as a solution. BDD was dissolved in saline at 39°C with vigorous stirring for at least 1 h. The solution was then filtered before it was added to the lung liquid. In contrast, Pfister et al. added powdered BDD directly to the lung liquid. If small undissolved particles persisted in the lung liquid, these could be engulfed by mucus or monocytes in the lungs or bronchi and become dislodged by final vigorous washing with albumin solution. It was implied that BDD could attach to proteins so albumin could extract it. However, BDD is covalently linked to dextran (1). Second, Pfister et al. did not add BDD and RISA to the lung liquid at the same time. BDD was present alone in the early hours of their experiments. It is known that groups of alveoli may open and close during experiments. This would have little effect on the apparent production rates and volumes because both fluid and tracer are trapped in proportional amounts. If pockets formed during the early hours of Pfister’s experiments, when only BDD was present, the heavy washing at the end of their experiments could have liberated free BDD. In younger fetuses, where even Pfister et al. found good agreement between BDD and RISA, the activity of the alveoli probably had not yet started.

We disagree with Pfister et al. on another point as well. An incorrect estimate of total volumes does not necessarily result in incorrect estimates of lung liquid production rates. The attachment of tracer to some surfaces might alter the volume, but the increment of volume as a function of time could still be useful in estimating production rates.

Finally, in nine early pilot experiments, we added known volumes of fluid to the fetal lungs by syringe, and the changes in volume were assessed by BDD. The volumes measured by syringe differed from those measured by BDD by only 3.2 ± 7.0% (SD).

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