Estimation of lung liquid production in fetal sheep with blue dye dextran and radioiodinated serum albumin

S. CASSIN AND A. M. PERKS
Department of Physiology, University of Florida College of Medicine, Gainesville, Florida 32610; and Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, British Columbia, Canada V6H 3V5

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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.—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 per hour 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 1) 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.

IN THE FETUS, ACTIVE PRODUCTION of lung liquid by a Na\(^+\)-K\(^-\)-2Cl\(^-\) cotransport system is important for lung development (16, 33), but, at time of birth, this liquid must be reabsorbed by an amiloride-sensitive, Na\(^+\)-based reabsorptive system, probably aided by other mechanisms (8, 26). It is this reabsorption that has generated considerable interest among investigators of the perinatal period because it is vital to survival of the newborn and can fail in the potentially fatal respiratory distress syndrome.

Our most important advance in understanding lung liquid reabsorption came in 1971, with the introduction of the indicator-dilution technique by Strang and coworkers (33). In 1973, this same group introduced radioiodinated serum albumin (RISA) as the impermeant tracer to be used in fetal lung liquid studies. For the first time, fluid production and reabsorption could be detected, followed in its time course, and quantified (24, 27). Since then, we have come to understand many complex factors such as epinephrine (35), arginine vasopressin (6), 3,5,3\(^-\)-triiodothyronine and hydrocortisone (1, 7), glucagon (10), expansion (14, 28), fall in temperature (13), and products of the pulmonary neuroendocrine system (8, 9), which may help to drain the lungs at birth.

Later studies were greatly helped by the introduction of the massive molecule of blue dye dextran 2000 (BDD) as an alternative indicator to RISA. It had little chance of passing through the pulmonary epithelium, far less than RISA, and avoided problems intrinsic to the use of radioactive isotopes. BDD formed a second check on results obtained by RISA, and, in our careful studies of fetal sheep, the two were used together (5). However, recent reports by Pfister et al. (31) have suggested that although BDD worked well in younger fetuses, it was in some way sequestered in those of 141 days of gestation, relatively close to term. Therefore, BDD was considered less reliable than RISA, even though it was already known that both albumin and RISA could escape the lungs and therefore did not satisfy the strictest requirements for indicator-dilution studies. It was suggested that this anomalous behavior of BDD could explain the differences in results from different investigators, whereby those who used BDD reported a steady increase in lung volume as birth approached (15, 20) whereas those who utilized RISA found a “massive” decline at some point between 140 days of gestation and end labor (3, 11).

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Although these overall changes in lung volume were interesting, they were not the most reliable criteria for analyzing changes near to term, because they could be affected by loss of fluid into the amniotic cavity, perhaps related to fetal movements. Rates of fluid production or reabsorption give the clearest information on the mechanisms operating close to birth, and, even if absolute volumes were in error for some reason, their increment with time could still give correct rates of fluid movement. Therefore, it was important to reexamine our earlier work to determine whether problems existed, especially in regard to rates, because these had been the basis of our earlier investigations.

In the work presented here, extensive data from previous studies were reexamined to determine three things: 1) whether there were discrepancies between rates of fluid production as determined by simultaneous use of BDD and RISA; 2) whether there were discrepancies in volumes, and, if so, whether they were sufficient to have physiological significance; and 3) whether the two indicators used together gave different results for the apparent changes in total lung volume as birth approached.

METHODS

Animals and General Methods

Studies are based on chronic preparations of fetuses from sheep with dated pregnancies [n = 99; 119–148 days of gestation; average body weight 3.28 (1.8–4.2) kg]. Indicator-dilution methods are based on Normand et al. (24), Martins et al. (23), and Liu and Chiou (21). They utilized and compared BDD [Pharmacia; molecular mass, 2,000,000 Da, 

\[
\text{radius} = 270 \, \text{Å} \quad (1 \, \text{Å} = 1 \, \text{nm}), \quad \text{radius of gyration,} \quad 380 \, \text{Å}
\]

\text{Å} = 1 \, \text{nm})], RISA (125I-labeled albumin; molecular mass 111001100 Å, radius 0.1 nm), radius of gyration, 380 Å] and RISA ([125I]-labeled albumin; molecular mass ~68,000 Da; 400,000–800,000 dpm; dialyzed for 12 h before use to remove traces of free iodide).

Surgery

Pregnant ewes were fasted for 24 h (water was provided ad libitum) and then anesthetized with ketamine followed by halothane (2.0%) in oxygen. Suitable aseptic procedures were used to place polyvinyl catheters into the maternal femoral artery and vein. A part of the pregnant uterus was delivered through the maternal cervical os and Perks, Ref. 5). Rates were estimated from plots of the slopes of their regressions, and thoroughly mixed. A 0.5-ml sample was then withdrawn from the warmed reservoir to estimate the quantity of BDD and RISA present at the onset of the experiment. The contents of the reservoir were then returned to the lungs. A side arm of the reservoir syringe allowed tracheal pressures to be monitored by a pressure transducer, so that excessive pressures could be avoided during movements of the lung liquid (pressures never exceeded 15 cmH2O). During the experimental period, samples of lung liquid (0.5 ml) were taken at regular 10-min intervals; withdrawal and return of the fluid also took place at the 5- and 8-min time points between sampling to ensure proper mixing of fluid within the lungs. On withdrawal, samples were automatically filtered through a sterilizing filter and then diluted 1:10 and centrifuged for 30 min at 250 g on a clinical centrifuge. Finally, the fluid was allowed to stand for 24 h in polypropylene tubes at 2°C before estimations were made. The concentration of BDD was measured with a Beckman DU-2 spectrophotometer (λ = 620 nm); measurements were made five to nine times, with consecutive runs carried out in opposite directions through the series of tubes. RISA was measured with an LKB Wallach 1282 gamma counter; all samples were measured three times. The concentrations of BDD or RISA gave the volume of lung liquid, whereas the change in volume over time provided the rate of secretion or reabsorption of lung liquid. Checks were made for the presence of BDD or RISA in fetal blood. There was never any evidence for the existence of either indicator in plasma samples in any study, at any time.

Quantitation of Results

Volumes of lung liquid and rates of production or reabsorption were calculated from the concentrations of BDD or RISA and their change with time, as described previously (Cassin and Perks, Ref. 5). Rates were estimated from plots of the total volume of fluid against time, with readings recorded every 10 min; the total volume of fluid was the sum of that within the lungs and that removed for study. Appropriate sequential adjustments were made every 10 min for the removal of both fluid and indicator(s) throughout the experimental period. The rates of production of fluid over 1-h intervals were calculated from the volume plots by using the slopes of their regressions, fitted by the method of least squares (34) (Sigma Stat and Sigma Plot). The first hour
after addition of indicators was not regressed but left for equilibration of indicators throughout the lung. Equilibration was aided by the withdrawal and return of fluid at regular intervals for the duration of the experiment. The volumes obtained in the subsequent control hour were regressed to obtain the volume of fluid before the experiment began (Vo). Differences between rates or volumes obtained jointly by BDD and RISA were analyzed for significance both parametrically (paired t-test; one-way and two-way ANOVA) and nonparametrically (Wilcoxon paired-sample test), as appropriate (36). Significance of slopes of regressions was estimated as above. Statistical significance was accepted at or below $P = 0.05$.

**RESULTS**

**Analysis of Rates of Lung Liquid Production Or Reabsorption by BDD or RISA**

Table 1 shows results of 71 pairs of measurements of rates of lung liquid production as indicated by both BDD and RISA (total 142 measurements). No statistical test, whether parametric (Student’s paired t-test; ANOVA) or nonparametric (Wilcoxon paired-sample test), showed any significant difference between rates obtained by the two methods. This was true for rates as milliliters per kilogram body weight per hour or as milliliters per hour and for rates over all periods of study ($n = 71$) or for rates limited to untreated control hours ($n = 16$). It applied equally to the group close to 141 days of gestation ($n = 28$) and to the younger fetuses (131–138 days of gestation; $n = 43$). $P$ values were most frequently $> 0.5$. When fetuses were divided according to their exact gestational ages (8 groups: 131, 134, 135, 136, 137, 138, 139, and 141 days of gestation), there were still no significant differences in rates by the two indicators ($P > 0.5$ for six ages; 0.5–0.2 for two ages; ANOVA). Analysis of periods of lung liquid reabsorption ($n = 12$) also showed no significant differences between the two indicators by paired t-test, Wilcoxon test, or ANOVA ($P = 0.2–0.1$).

**Analysis of Lung Volumes and Their Changes With Age, As Estimated by BDD and RISA**

It has been suggested that changes in lung volume toward birth appear different when assessed by BDD or RISA. We therefore compared lung volumes and their change with time in 99 studies of chronic fetal sheep in which both indicators were used simultaneously. Fetuses were studied over the range of 119–148 days of gestation (all data) and also divided by age, with each group covering 3 days, except for the youngest group (119–124, 125–127, 128–130, 131–133, 134–136, 137–139, 140–142, 143–145, and 146–148 days of gestation). Volumes were analyzed in two ways: (1) as volumes expressed as Vo either in total milliliters or as milliliters per kilogram body weight, where Vo was the original volume before the onset of the experiment (estimated by regression of volumes for the first control hour, after equilibration and before treatment), $n = 99$; and (2) volumes from control (untreated) hours, $n = 102$.

**Volumes from all data.** Figure 1 shows the clear linear relationship between Vo (ml) as estimated by both methods, independent of age (BDD = 0.172 + 1.05·RISA; $r = 0.97$, $P$ for slope $< 0.0001$; $n = 99$). Figure 2 shows the same data expressed in terms of body weight (ml/kg body wt); the results were closely similar (BDD/kg = 1.62 + 0.996·RISA/kg; $r = 0.97$; $P$ for slope $< 0.0001$; $n = 99$). Clearly, there was no gross difference between the two methods.

Differences between the volumes by the two methods were notably small, −4%, independent of parameter [3.8 ± 11.5 (SD)% for Vo for all fetuses, 4.9 ± 6.2% for Vo for fetuses close to birth (140–148 days of gestation), 4.9 ± 9.3% for all estimates during untreated hours, and 3.1 ± 5.9% for estimates in untreated hours in fetuses close to 141 days of gestation]. Either indicator could give the higher volume, as shown by the standard deviations. Nevertheless, there was a significant difference between the two indicators by all pa-

<table>
<thead>
<tr>
<th>Rates Estimated</th>
<th>Units of Rates</th>
<th>Fetal Ages, days of gestation</th>
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<th>Rates by RISA means ± SD</th>
<th>Rates by BDD means ± SD</th>
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<tr>
<td>Rates from all periods of study</td>
<td>ml·kg⁻¹ body weight·h⁻¹</td>
<td>131–141</td>
<td>71</td>
<td>3.53 ± 2.80</td>
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<td>139–141</td>
<td>28</td>
<td>3.85 ± 2.72</td>
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<td>131–138</td>
<td>43</td>
<td>3.33 ± 2.86</td>
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<td>131–141</td>
<td>71</td>
<td>10.61 ± 8.20</td>
<td>10.11 ± 6.59</td>
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<td>139–141</td>
<td>28</td>
<td>12.01 ± 8.83</td>
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<td>131–138</td>
<td>43</td>
<td>9.70 ± 7.72</td>
<td>9.40 ± 6.29</td>
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<td>Rates for periods limited to those before treatments</td>
<td>ml·kg⁻¹ body weight·h⁻¹</td>
<td>131–141</td>
<td>16</td>
<td>5.28 ± 2.42</td>
<td>4.89 ± 1.53</td>
<td>0.5–0.2</td>
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<td>139–141</td>
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<td>4.84 ± 3.51</td>
<td>4.73 ± 1.80</td>
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<td>5.54 ± 1.65</td>
<td>4.99 ± 1.45</td>
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<td>16</td>
<td>15.44 ± 7.04</td>
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<td>15.25 ± 10.59</td>
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<td>15.55 ± 4.51</td>
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RISA, radioiodinated serum albumin; BDD, blue dye dextran; n, no. of fetuses; ns, not significant.

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parameters as judged by paired t- and Wilcoxon tests (P < 0.001). This significance depended on the large numbers of observations and the exclusion of age as a variable.

Despite the statistical significance, the small ~4% difference in volume was considered too small to have physiological significance; this was supported by the good agreement in rates, as shown above.

**Volumes related to age.** Figure 3 shows the change in volumes (Vo in ml) toward birth, as estimated by BDD;

there was a steady increase with age (29% increase over the whole period; r = 0.97; P for slope < 0.0001; n = 99).

**Fig. 3.** Change in Vo (ml) determined by BDD as a function of gestational age. Values are means ± SD. Means are not statistically different from one another (ANOVA), but there is an increase in the slope of the regression for the means.

**Fig. 4.** Change in Vo (ml) determined by RISA as function of gestational age. Values are means ± SD. Means are not different (ANOVA), but there is an increase in the slope of the regression toward birth.
DISCUSSION

Most workers in this field have based their conclusions on changes in rates of lung liquid production or reabsorption, because absolute volumes can be influenced by loss of fluid into the amniotic sac, perhaps associated with fetal movements (4, 5, 8). In addition, even if absolute volumes are incorrect for some reason, such as incorrect addition or concentration of indicator or rapid attachment of a proportion of the tracer to lung surfaces on first entry to the lung, the increment in volumes can still be correct, with their changes still indicating the net amount of fluid added or withdrawn from the system. It is rarely crucial to determine absolute volumes, but rates of fluid movement can tell us much concerning the mechanisms of lung liquid production and the vital reabsorption at time of birth. Only indicator-dilution methods can detect and quantify this reabsorption.

In the indicator-dilution work presented here, statistical tests did not show any significant difference between rates of fluid production or reabsorption as measured by BDD or RISA. The work was based on unusually large numbers (n = 71), and P values were almost always >0.5. The lack of difference between the two indicators was true for rates expressed in milliliters per hour and milliliters per kilogram body weight per hour, and for fetuses divided into major groups according to age (all data, 139–141 days; “younger,” 131–138 days; “near term,” 139–141 days of gestation). It was also true for fetuses divided according to their exact gestational ages (P > 0.5 or 0.5–0.2) This division included fetuses of 141 days of gestation, in which discrepancies have been suggested (P > 0.5; n = 8). Again, there were no significant differences between the indicators by any of the statistical tests.

In general, the differences between average rates by BDD or RISA were small, only 2.6% for all 71 comparisons. This similarity showed that rates by BDD were acceptable, and this was supported by our studies of guinea pigs, although by a different comparison. In the guinea pig studies, in which large numbers of observations could be obtained, rates of fluid production by BDD could be compared with those from a simple drip method, which, although considered less accurate and incapable of measuring reabsorption, was free of the potential errors of indicator-dilution studies: average values for BDD were 2.14 ± 0.08 (n = 450) and by direct collection 1.96 ± 0.71 ml·kg body wt⁻¹·h⁻¹ (n = 10), a remarkably close agreement for independent experiments, again with no significant difference (29). It is concluded that, in terms of rates, BDD is as acceptable an indicator as RISA.

In regard to volumes, the differences between the two methods were also remarkably small. This confirms many early checks for the validity of BDD and RISA, given most clearly by Beierle et al. (2). In that paper, it was shown that the agreement between volumes from BDD and RISA was excellent (r = 0.99; P < 0.001). This was found even though the ages included
the now disputed age of 141 days of gestation. In the
results given here, far greater numbers of observations
gave a similar result ($r = 0.97; P < 0.0001; n = 99$; Fig.
1); this was true independent of age and for volumes
expressed in various parameters. The difference in
volumes by the two methods was always close to 4%,
whether volumes were expressed as Vo or as volumes
in untreated hours; this was independent of age and
with or without allowance for body weight.

It has been suggested that volumes might be affected
by an unexplained jump in optical density of lung
liquid between the first, “blank” sample and later sam-
bles, a jump that slowly subsided over the next hours
(31). The significance of this effect is difficult to assess.
However, we believe it to be unlikely for the following
reason. Control experiments in many studies showed
no such phenomenon. This failure to show distortion in
control experiments was even clearer in fetal guinea
pigs, in which large numbers of controls were carried
out in successive projects [e.g., $n = 30$ (29); $n = 6, 12,$
and 18 (30); $n = 10$ and 18 (14)]. Fluid production
through 3 h was remarkably constant. Even if changes
in optical density did occur, they were too small to
produce significant effects on our results.

Examination of control experiments can also give us
information concerning the chief possible artifact that
might be associated with use of BDD (or any other
indicator): attachment to pulmonary surfaces. As
pointed out earlier, if this were to occur on first entry to
the lung, the later increments in volume might be
completely unaffected, even though the absolute vol-
umes were in error. If the process continued into the
experiment, but halted at some point because of satu-
ration, this would be shown by significant changes in
slope in control experiments; this was not seen. If the
process continued steadily throughout the study, ap-
parent rates would all be affected equally, and re-
sponses to external agents would still be clear. In
sheep, no direct studies have demonstrated attach-
ment of BDD to pulmonary epithelia, although our
simple opening of pulmonary tissues gave no evidence
of this. In contrast, in many examinations of lungs of
near-term experimental fetal guinea pigs by scanning
electron microscope, sometimes in conjunction with
lung expansions, no sign of BDD was ever found
on pulmonary surfaces (unpublished observations; Ref.
14).

In general, our data suggest that BDD is an accept-
able indicator for studying fluid movements in fetal
lungs, in at least partial disagreement with Pfister et
al. (31). However, it is difficult to compare methods
from different laboratories. Methods for adding BDD
were different and might account for the differing
results. Sometimes small, apparently unimportant dif-
ferences can be crucial. Much detail is omitted here in
the need for brevity. Clearly, we cannot know the true
situation, but results presented here show good agree-
ment between BDD and RISA in the conditions of our
experiments.

There was also good agreement in the changes of
lung volume toward birth, as tested by the two indica-
tors. It has been suggested that differences in the
properties of BDD and RISA might explain discrepant
results concerning the time at which lung liquid vol-
ume begins to decline around delivery. Also, it was
suggested that volumes appeared to increase steadily
up until birth if BDD was used (15, 20) but showed a
massive decline somewhere between 140 days of ges-
tation and “end-labor” if RISA was the indicator (3, 11).
In the work reported here, in which both tracers were
used simultaneously, there was close similarity in the
changes shown by both methods. Vo (ml) showed a
continuous increase in lung volume from 119 days of
gestation until term (significant by slope), although the
slope fell off close to delivery. However, both indicators
showed the same decline in Vo in terms of body weight
(ml/kg), and this was clearly significant by analyses of
slope and ANOVA. This suggests that increase in vol-
ume due to growth was counteracted by a slow turning
down of secretory mechanisms. This agreed well with
changes shown by extensive studies of guinea pigs in
which only BDD was used, and there was no chance of
interactions between indicators ($n = 874$; unpublished
observations). In the guinea pig it was particularly
clear that there was an increased variability in lung
volume on the day before and the day of delivery, with
values showing the highest and lowest volumes seen
(coefficients of variation: 60 days, 15% ($n = 106$); 66
and 67 days (term), both 36% ($n = 23$ and 17, respec-
tively). The time at which lung liquid volume begins to
decline around delivery must not be due to the indica-
tor used, because both indicators showed the same
results in the present studies. Therefore, it is probable
that preparation for birth starts at different times in
the different fetuses and the system is less tightly
organized than we would like to think.

It is sometimes stated, or implied, that BDD has a
high affinity for proteins and that this could complicate
its use in dye-dilution studies (31). This is not strictly
true. The cibacron blue F3-GA moiety of BDD will
attach to proteins, but in BDD it is tightly covalently
coupled to the giant dextran molecule (personal com-
munication, E. Brassard, Pharmacia). BDD was devel-
oped and marketed for marking the front during gel-
filtration chromatography of proteins, and it has been
widely used for this in our own and other laboratories.
If it were to attach to proteins, or dissociate so that the
dye element could do so, even in the more stringent
environment of elution fluids, it would have been use-
less for the purpose for which it was made. It is there-
fore most unlikely that it would attach to RISA, and
even if it did, it does not affect our measurements of
rates of fluid production. Its use has definite assets. It
avoids the problems of handling radioactive materials.
The great size of the molecule (molecular mass,
2,000,000 Da; Stokes’ radius; 270 Å) makes it poten-
tially excellent for dye-dilution studies, and it has been
used this way in situations as widely different as the
lungs, the eye, and cerebrospinal fluid, in which it was
originally validated against radioiodinated albumin (5,
21, 23). This great molecular size represents one of its
potential assets over radioiodinated albumin, which,
with its far lower molecular weight (~68,000), is more likely to exert osmotic effects, and which, despite the ability of the fetal lung to severely restrict its passage, does come close to the limit of alveolar permeability, especially during spontaneous breathing (12, 25). Therefore, for studies of lung expansion, BDD would be the safer agent to use (14). In fact, no one has been able to detect BDD in the blood of fetal sheep and goats after placing it in lung liquid (unpublished observations; 28, 31). Similarly, during many experiments on in vitro lungs of fetal guinea pigs, there was never any escape of BDD into the outer saline, except when ligu- tures were poor or there was damage to the lung, when its escape was readily detected (14). In contrast, RISA is known to escape the lungs (12, 17, 22, 31).

Early studies of lung liquid production using drop or bag collection methods had the merit of simplicity and avoided potential problems of indicator-dilution studies, but they were unable to show the time course of rapid responses, and, more importantly, incapable of demonstrating reabsorption. Strang and co-workers (33) were able to overcome these problems by using RISA. However, they recognized early on that RISA did not entirely meet the fundamental requirements of dilution methods, because it could escape the lungs. In fact, albumin is unusually efficient at passing the epithelium compared with other agents of similar size, and there is a specific albumin-binding protein in monolayers of alveolar cells, one probably concerned in transport (22, 18). Clearly, albumin was not a perfect indicator and was capable of both leaving the lung and attaching to alveolar cells. However, it was felt that these imperfections were too small to invalidate its use; we tend to agree (12). We may well have to make parallel allowances for BDD.

We thank Hilkin Kuck for able technical assistance, Dr. Charles Wood for useful consultations, Dr. Jonathan Shuster of the Division of Medical Statistics for statistical advice, and all our former colleagues for enthusiastic help with the experiments.

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REFERENCES


