Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution

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RENNARD, STEPHEN I., GUY BASSET, D. LECOSSIER, KATHLEEN M. O’DONNELL, PAULA PINKSTON, PATRICIA G. MARTIN, AND RONALD G. CRYSTAL. Estimation of volume of epithelial lining fluid recovered by lavage using urea as a marker of dilution. J. Appl. Physiol. 60(2): 532-538, 1986.—Bronchoalveolar lavage is a powerful technique for sampling the epithelial lining fluid (ELF) of the lower respiratory tract but also results in a significant dilution of that fluid. To quantify the apparent volume of ELF obtained by bronchoalveolar lavage, urea was used as an endogenous marker of ELF dilution. Since urea diffuses readily through the body, plasma and in situ ELF urea concentrations are identical; thus ELF volume can be calculated using simple dilution principles. Using this approach, we determined that with a standard lavage procedure, the volume of ELF recovered from a normal human is 1.0 ± 0.1 ml/100 ml of recovered lavage fluid. Time course experiments in which the saline used for lavage was permitted to remain in the lower respiratory tract for various “dwell times” suggested that differences of urea from sources other than recovered ELF can contribute to the total urea recovered resulting in an overestimate of the volume of ELF recovered. Thus, while reasonably accurate, the volume of ELF determined by urea must be considered an overestimate, or “apparent” volume. The ELF albumin concentration based on the apparent ELF volume was 3.7 ± 0.3 mg/ml, a value that is in good agreement with direct measurements made by other techniques in experimental animals. The density of all inflammatory and immune effector cells on the epithelial surface of the lower respiratory tract, based on the apparent ELF volume, was 21,000 ± 3,000 cells/µl, a value that is twofold greater than that in blood. The ability to estimate the concentration of molecular and cellular components of ELF should be of great utility in understanding the role of these components in the lower respiratory tract in health and disease.

bronchoalveolar lavage, diffusion of urea, lower respiratory tract, inflammatory and immune effector cells

The technique is based on the concept that aliquots of sterile, physiological saline infused through the bronchoscope mix with the ELF, and when the saline is recovered by aspiration ELF and its components are recovered along with it. With this approach, remarkable advances have been made in the understanding of inflammatory and immune processes in the lower respiratory tract in health and disease (3, 6, 10, 11, 13, 14, 17, 20, 21).

Despite the enormous utility of bronchoalveolar lavage in providing access to the lower respiratory tract, one of its limitations is the fact that the ELF can only be recovered by the instillation of fluid into the lower respiratory tract; i.e., although the instillation of saline permits sampling of ELF, the recovered fluids are a variable mixture of saline, ELF, and ELF components. Thus, it has been difficult to estimate the actual concentration of recovered molecules and cells in the ELF in situ.

To circumvent this problem, the present study was designed to directly quantify the volume of ELF recovered by bronchoalveolar lavage. To accomplish this, we have taken advantage of the fact that urea, a plasma component that is easily measured, diffuses freely throughout the body, including the alveolar wall (26, 27). In this context, since the concentration of urea can be quantified in plasma and the total amount of urea recovered by lavage can be measured, the total volume of recovered ELF can be calculated by simple dilution principles. The results are promising and suggest that the volume of recovered ELF can be easily quantified with reasonable accuracy.

MATERIALS AND METHODS

Study population. All individuals were normal nonsmoking volunteers. All had normal lungs as judged by history, physical examination, pulmonary function testing, and chest roentgenograms. All studies were performed on the in-patient service of the Pulmonary Branch, Clinical Center, National Institutes of Health, after informed consent had been obtained. These studies were approved under the auspices of the National Heart, Lung, and Blood Institute Research Review Board under approved protocol 77-H-61. Thirteen subjects were studied in a standard fashion, and nine subjects were studied with slight modifications as indicated below.

Bronchoalveolar lavage protocols. Flexible fiberoptic
bronchoscopy and lavage were performed with an Olympus bronchoscope as previously described (20, 21). Briefly, following local anesthesia, the bronchoscope was inserted and gently wedged in a subsegmental bronchus. The standard lavage protocol was performed by infusing a 20-ml aliquot of sterile 0.9% saline through the aspiration port and collecting it via the same port into a plastic trap using wall suction. A total of five 20-ml aliquots (100 ml) were used to lavage each site for a total of three sites (300 ml).

To evaluate the diffusion of molecules into the recovered fluid, the standard lavage protocol was modified slightly to allow the saline to remain in the lower respiratory tract for variable lengths of time (referred to as "dwell time"). The lavage was performed by infusing a 50-ml aliquot of sterile 0.9% saline through the aspiration port and immediately aspirating back (referred to as "zero dwell time"). The bronchoscope was moved to the second location, where another 50-ml aliquot was instilled, allowed to remain for 1 min dwell time, and then aspirated back. Finally, the bronchoscope was moved to a third site where a 50-ml aliquot was instilled, allowed to remain for 5 min dwell time, and then aspirated. These subjects received a total of 150 ml saline infused and each sample recovered was processed separately.

Prior to any centrifugation and cell washing procedures, an aliquot of the lavage (pooled samples in the standard lavage protocol) was analyzed for cell number by hemocytometer and cell differential using millipore filter preparations (23). Lavage fluid samples were then centrifuged (5 min, 800 g) to separate cells from the fluid components. The supernatant fluid was divided into aliquots and stored in liquid N₂ vapor until analysis.

Use of urea to determine the volume of epithelial lining fluid recovered by bronchoalveolar lavage. The use of urea to quantify the amount of ELF recovered by bronchoalveolar lavage is based on the knowledge that urea is freely diffusible through most body compartments including the lung (26, 27). In this context, if the concentration of urea in plasma is known, and the quantity of urea in a lavage sample is known, the volume of ELF obtained can be calculated as: volume ELF (ml) = [total amount of urea in lavage fluid recovered (mg)]/[concentration of urea in plasma (mg/ml)] (see APPENDIX). Once the volume of ELF recovered is known, then any acellular component (i.e., albumin or cellular component (i.e., alveolar macrophages) can be referenced to the volume of ELF from which it was obtained.

To determine the urea content of lavage fluid samples, a commercially available kit (Sigma 65 UV) was used with slight modifications. This kit determines the urea concentration by cleaving urea with urease to CO₂ and NH₃, by combining the NH₃ generated with α-ketoglutarate, and by measuring the change in optical density of the nicotine-adenine dinucleotide (NADH) consumed by this latter reaction (NH₃ + α-ketoglutarate + NADH → glutamic acid + NAD⁺). Preliminary tests demonstrated that using a reaction mixture of 2.5 ml (in contrast to the 3.0 ml recommended by the supplier) and analyzing 0.5 ml of lavage fluid sample (rather than the 0.005 ml recommended in the instructions) yielded a test that could reliably measure the urea concentration in bronchoalveolar lavage fluid. However, with these modifications, it was necessary to include a saline blank to correct for dilution of the reactants by the sample and to include a blank with lavage fluid alone to correct for optical absorbance of the sample. As modified, the test was reproducible to within ±10%.

Contribution of urea from sources other than the recovered ELF to the apparent volume of ELF as sampled by bronchoalveolar lavage. One problem relating to the use of urea as an endogenous marker to quantify ELF volume relates to the theoretical possibility that urea present in the alveolar wall or in ELF that is not recovered might diffuse into the saline used for lavage, i.e., that some of the urea recovered by lavage may be from sources other than the ELF that is recovered. To determine to what extent diffusion from sources other than the recovered ELF contributes to the apparent volume of ELF as sampled by bronchoalveolar lavage, lavages were performed using a modified bronchoalveolar lavage protocol.

These studies were performed with 50-ml aliquots of saline (rather than the standard 20-ml aliquots) because preliminary studies showed that for dwell times of 1 min or more the study subjects experienced some coughing and the recovery of the instilled saline was reduced significantly. For each individual studied, three separate lavages were performed, each in a different subsegment, and each for a different dwell time (see Bronchoalveolar Lavage Protocols). The lavage fluid recovered from each lobe was processed and analyzed separately for cellular and acellular components.

For a comparison with the rate of diffusion of urea [60 daltons (Da)] into the lavage fluid during these periods, the concentrations of glucose (180 Da) and albumin (69,000 Da) were also determined. Glucose was measured using a commercially available kit (Sigma 15-UV) with a slight modification. Each assay was performed by reconstituting the reagents to 2.5 ml (rather than the recommended 3.1 ml) and by adding 1.0 ml of lavage fluid sample (rather than 0.02 ml of sample indicated in the manufacturer's instructions). It was necessary to include a correction for dilution and for absorbance due to the lavage fluid itself. With these modifications, the test could reliably measure the glucose content of lavage fluid samples with a reproducibility of ±10%. Albumin concentration in bronchoalveolar lavage fluid was determined by enzyme-linked immunoassay with a reproducibility of 10% (19).

Statistical evaluation. All data are presented as means ± SE.

RESULTS

Volume of ELF recovered by bronchoalveolar lavage. By use of the standard lavage procedure, 58 ± 2% of the 300 ml of infused saline was recovered. In all cases it was possible to measure the urea concentration in the recovered fluid (Table 1, Fig. 1). The lavage fluid urea concentration averaged 1.0 ± 0.1% of the simultaneously
TABLE 1. Urea and albumin concentrations in bronchoalveolar lavage fluid and plasma from 13 normal subjects

<table>
<thead>
<tr>
<th>Subj</th>
<th>Percent Fluid Recovered, mg/100 ml</th>
<th>UreaBAL, mg/100 ml</th>
<th>UreaPlasma, mg/100 ml</th>
<th>AlbuminBAL, µg/ml</th>
<th>AlbuminPlasma, g/100 ml</th>
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<tr>
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Mean ± SE  58±2  0.12±0±0.01  13±1  34±2  4.5±0.1

* Each subject received a total infusion of 300 ml of 0.9% saline.

measured plasma urea. Based on the measurements of the total amount of urea in the lavage fluid recovered with a standard 300-ml lavage, and the concentration of urea in plasma, the calculated volume of epithelial fluid recovered averaged 1.7 ± 0.2 ml. In terms of the recovered lavage fluid, 1.0 ± 0.1 ml of epithelial lining fluid was recovered for each 100 ml of lavage fluid that was recovered.

Using the enzyme-linked immunoassay, albumin was detectable in all lavage samples (Table 1). Two approaches were used to calculate the apparent ELF volume based on albumin (see APPENDIX). First, if a concentration of albumin in ELF is assumed to be equal to the concentration of albumin in plasma, the recovered volume of ELF was very small, as expected. Since the measured albumin concentration in ELF was much lower than that of plasma, the calculated apparent ELF volume recovered was markedly low. Second, assuming a concentration of albumin in ELF that is 10% of the plasma concentration, based on previously determined values (1, 18, 25), the volume of ELF recovered was of the same order of magnitude as the volume of ELF recovered based on urea. For the 13 individuals studied, the volume of ELF recovered, using the measured lavage albumin and assuming the albumin in ELF is 10% of the albumin in plasma, was 1.9 ± 0.1 ml/100 ml recovered lavage fluid.

Thus, when the correct estimation of the concentration of albumin in ELF was used, the volume of ELF based on urea was not significantly different from the volume of ELF based on albumin (P > 0.2, paired t-test).

Apparent diffusion of molecules into bronchoalveolar lavage fluid during the lavage procedure. Evaluation of the concentration of urea, glucose, and albumin in the fluid recovered after the saline used for lavage was allowed to remain in the lower respiratory tract for varying dwell times, suggested that a time-dependent diffusion of molecules into the lavage fluid from sources other than the recovered ELF occurred during the performance of bronchoalveolar lavage. Increased dwell times resulted in increased concentration of urea, glucose, and albumin in the recovered bronchoalveolar lavage fluid (Fig. 2). This time dependent increase was inversely dependent on the molecular mass. Urea (60 Da) demonstrated the greatest increase, glucose (180 Da) an intermediate increase, and albumin (69,000 Da) the smallest increase. These findings were consistent with the concept that some molecules diffuse into the bronchoalveolar lavage fluid during the lavage procedure. Although it was likely that increasing dwell time also increased the amount of ELF recovered (as a fraction of total bronchoalveolar lavage fluid recovered), if this were the only reason for the increased amount of urea, glucose and albumin recovered when the dwell time was increased, there would have been a parallel increase in urea, glucose, and albumin concentration rather than the divergent increases which were actually observed (Fig. 2). Importantly, a dramatic absorption of the saline used for the lavage also would have resulted in a parallel increase in all solutions. The divergent increase observed, therefore, suggests that absorption of saline during the lavage procedure is not likely to be a significant source of error.

While the standard technique of immediate aspiration of instilled saline (i.e., zero dwell time) allowed the least possible amount of diffusion of molecules from source other than the recovered ELF into the recovered lavage fluid, a definite time period was required for the instillation and aspiration. As a result, the calculated volume of ELF based on urea must be considered an "apparent volume" and the calculation of the concentrations of molecules and the density of cells in this volume are probably underestimates of the actual concentrations; i.e., they are "apparent concentrations." However, the length of time required to perform the instillation and aspiration of saline is brief, 20 s at most, and places an
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FIG. 2. Recovery of urea, glucose, and albumin in bronchoalveolar lavage fluid as a function of amount of time lavage fluid remains in lower respiratory tract (referred to as dwell time). Shown are relative amounts of urea (filled circles), glucose (filled triangles), and albumin (filled squares) recovered when fluid was immediately aspirated after instillation, when fluid was aspirated after a dwell time of 1 min, and when fluid was aspirated after a dwell time of 5 min. Each lavage was performed in a different site with a single 50-ml aliquot of saline. Data are presented as percent increase in value of each parameter compared to a zero for 9 normal volunteers studied.

upper limit on the time during which diffusion of urea might occur. With this upper limit and assuming free concentration dependent diffusion, by extrapolating to zero time an estimate can be made of the relative percentage of urea recovered in bronchoalveolar lavage fluid which derives from the urea that was in the ELF prior to the instillation of the saline and that which derives from diffusions from other sources. Using a time of 20 s, this calculation suggests that a minimum of 80% of the urea recovered in bronchoalveolar lavage fluid is derived from the present in situ in ELF and a maximum of 20% derives from diffusion.

Estimates of the albumin concentration and cell density in ELF of normal humans. The standard lavage procedure involves the sequential instillation and aspiration of five 20-ml aliquots of sterile saline into the same subsegmental bronchus. The returns from all 5 infusions and aspirations are then pooled. Utilizing this procedure, the measured lavage fluid albumin concentration was 33 \( \pm 2 \) mg/ml (Fig. 3). When urea was used as a marker of epithelial lining fluid dilution to calculate the apparent ELF volume, ELF albumin concentration was calculated to be 3.7 \( \pm 0.3 \) mg/ml. This value represented 8.8 \( \pm 0.4 \) of the plasma albumin concentration in the same individuals.

Since the duration of the lavage procedure, i.e., the dwell time, can influence the results, it was of interest to compare the five 20-ml sequential lavage with the dwell-time experiment. This was done by calculating the apparent albumin concentration for the 0, 1, and 5 min dwell times. The five 20-ml sequential lavage corresponded to an “apparent” dwell time of 30–45 s. The apparent dwell time reflects the partial recovery of some of the infused saline with a subsequent lavage.

When determination of the absolute volume of recovered ELF was combined with determination of the number of the recovered cells, it was possible to estimate the density of cells on the epithelial surface of the lower respiratory tract. In the normal population evaluated, the total cells recovered were 210 \( \pm 30 \times 10^3 \) cells/ml lavage. When expressed in terms of the apparent ELF volume recovered, the total cell density in the ELF of the lower respiratory tract was 21 \( \pm 3 \times 10^3 \) cells/\( \mu l \), a concentration twofold greater than the density of cells in peripheral blood. In terms of the specific cell types recovered (macrophages 83 \( \pm 8 \%), lymphocytes 17 \( \pm 2 \), and neutrophils 1.0 \( \pm 0.3 \%), the density of macrophages in ELF was 17 \( \pm 3 \times 10^3 \) cells/\( \mu l \) (25-fold higher than blood), lymphocytes 4 \( \pm 0.4 \times 10^3 \) cells/\( \mu l \) (twofold higher than blood) and neutrophils 150 \( \pm 50 \) cells/\( \mu l \) (2.5% that of blood).

DISCUSSION

This study evaluates the use of urea as an endogenous marker to determine the volume of the epithelial lining fluid of the lower respiratory tract recovered by bron-
choanalveolar lavage. With this approach, the apparent volume of ELF recovered by bronchoalveolar lavage can be measured with reasonable accuracy, thus permitting measurement of the absolute concentration of molecular and cellular components in epithelial lining fluid in situ.

Use of urea to quantify ELF volumes. The major advantage in using urea to quantify the amount of ELF recovered by bronchoalveolar lavage is that it is an endogenous marker of dilution; i.e., it is not necessary to administer a tracer molecule to estimate the dilution of the recovered ELF by the saline used for lavage. Furthermore, since urea has a low molecular mass it diffuses across the alveolar wall and lung urea will be in equilibrium with plasma urea (26, 27), thus the urea concentration in ELF is assumed to be equal to the urea concentration in plasma. In support of this premise, direct measurements of lung epithelial fluid urea in fetal sheep have demonstrated the equivalence of plasma and lung urea concentrations (1, 25). Moreover, urea does not have a net charge at physiologic pH, is not consumed or produced by lung cells. In addition, since the concentration of urea in plasma (and therefore ELF) is relatively high, even after dilution by bronchoalveolar lavage fluid, it is technically easy to measure the concentration of urea in the recovered lavage fluid. Thus the dilution of a small volume of ELF by a large volume of saline results in a large decrease in the concentration of urea, a change that can be determined accurately. Since the accuracy of the estimate of dilution of ELF by the saline used for lavage depends on the ability to measure the difference in the concentration of the marker of dilution being used, urea provides an excellent marker of this dilution.

The major disadvantage of using urea as a marker is that some urea from sources other than the recovered ELF is likely to diffuse into the recovered lavage fluid during the lavage procedure. Thus, the volume of ELF determined by the urea method probably represents an overestimate of the true ELF volume, and should correctly be termed the apparent ELF volume. In this regard, the demonstration of a time-dependent diffusion of urea into the bronchoalveolar lavage fluid emphasizes the point that variations in technique of lavage can lead to differences in the estimates of ELF recovered. For this reason, until lavage methodology becomes standardized, it is important for investigators to be explicit in their descriptions of the method of lavage used.

However, even though the volume of ELF estimated by urea is an apparent volume, several lines of evidence suggest that the value is a reasonably accurate measure of the actual volume of ELF recovered.

First, measurements of the apparent dwell time of the bronchoalveolar lavage procedure suggest that only a limited time is available for diffusion. With a single instillation and immediate aspiration, it is likely that a minimum of 80% of the recovered urea derives from the urea present in situ in ELF prior to the lavage procedure. Thus, while the resulting estimates must be considered apparent volumes, the brief duration of the procedure suggest that the estimates are likely to be very reasonable.

Second, the value of epithelial lining fluid albumin concentration of 8.8% that of plasma is in good agreement with studies in experimental animals that have directly measured lung epithelial fluid albumin concentration. In this regard, the albumin concentration in the epithelial lining fluid obtained from fetal lamb alveoli has been estimated to be 3% that of plasma (25), from fetal lamb tracheal fluid, 5% that of plasma (1), and from rat alveolar epithelial fluid obtained by micropuncture, 13% that of plasma (18). Similarly, Mentz et al. reported the albumin concentration of tracheal and bronchial epithelial lining fluid obtained by adsorption to a filter to be 48 and 73 mg/100 ml (15), in close agreement with the above studies and with our results for alveolar fluid. By way of contrast, these estimates compare with normal lung lymphatic fluid which has an albumin concentration of 60-80% that of plasma (24), consistent with the concept that the alveolar epithelium is a greater barrier to diffusion than is the alveolar vascular endothelium (9, 24, 26, 27).

Third, comparison of the calculated total lung ELF volume obtained by combining the estimate of the ELF volume recovered by lavage together with an estimate of the fraction of lung lavaged by a standard method is consistent with the estimates of total lung ELF volume from morphometric techniques. For a standard lavage, using five 20-ml aliquots in three sites, 1.7 ml of ELF is recovered. Since the bronchoscope wedges in fourth to fifth order bronchi, ~1.5-3% of the lung is lavaged at each site. If the recovery of ELF were complete, total ELF volume would be 20-40 ml. Since ELF recovery by lavage is likely to be incomplete, this value probably represents a lower limit of total ELF volume. Although this estimate for total ELF volume is based on assumptions that are difficult to validate, it is, nevertheless, in good agreement with estimates of ELF volume based on morphometry, i.e., 15-70 ml (9, 28, 29).

Other methods to quantify molecular and cellular components recovered by bronchoalveolar lavage. The most common approach to quantifying the molecular components of bronchoalveolar lavage has been to compare the amount of the various components recovered to an endogenous reference substance, usually albumin (3, 10, 11, 13, 14, 20, 21). The utility of albumin for this purpose stems from the fact that it is not produced in the lung, it is present in blood plasma in high concentration, its molecular mass of 69,000 Da permits sufficient diffusion from plasma to ELF that it can be considered to be of average molecular mass, and it can be easily measured in the recovered lavage fluid. In this context, it is assumed that the amount of albumin in the recovered lavage fluid reflects the average normal transfer of plasma proteins into the ELF and thus the relative proportions of molecules of albumin in the lavage fluid can be contrasted to those in plasma.

Unfortunately, while the basic assumptions are probably correct, the use of albumin as a reference standard has limitations. First, it permits only relative comparisons to plasma and does not lead to estimates of absolute concentrations of molecules in ELF. Second, it is used as a molecule of average molecular mass; the validity of albumin as a reference standard diminishes for molecules...
of mass that are much higher and lower. Third, although it permits comparison of the concentrations of molecules in lavage fluid to the concentrations of molecules in plasma, it is not meaningful to compare lavage cell numbers to the lavage albumin concentration. Fourth, lung diseases are associated with inflammation of the lower respiratory tract and are likely to be associated with altered lung permeability; i.e., the concentration of albumin in lavage fluid may be altered differently than that of other molecules being evaluated, particularly those of different molecular weight. Thus, even though the use of albumin as a lavage reference substance has been useful in a number of applications, it is not an ideal approach to the problem of quantifying ELF components.

Other markers of dilution such as potassium or calcium have been suggested (8, 12). However, perhaps because of the effects of the large intracellular content of potassium on the ionic gradients established by epithelia, these have generally not been found to be useful (12).

The urea method provides an estimate of the absolute volume of ELF recovered by bronchoalveolar lavage and therefore permits calculation of the absolute concentration of the various molecules and cells in ELF in situ. Using the urea technique, it is estimated that ELF represents 1.0% of the recovered lavage fluid volume. This estimate of ELF volume represents a much smaller proportion of recovered lavage fluid than that reported by Baughman and co-workers (2) using methylene blue as an exogenous marker of dilution. The methylene blue technique results in an estimate of 11 ml of epithelial lining fluid recovered in 30 ml of lavage fluid recovered, i.e., 37%. In this regard, since the urea technique is likely to result in an overestimate of the recovered epithelial lining fluid volume, the methylene blue technique probably represents a much larger overestimate, most likely because methylene blue is lost through diffusion or binding to cells (2).

Uses of measurements of the apparent ELF volume:
The ability to quantify an apparent ELF volume should prove useful in a variety of applications in health and disease.

First, with knowledge of the apparent ELF volume recovered, it is possible to estimate the actual concentration of molecules in ELF in situ and thus determine whether the concentration of the various molecules is relevant to biological function. For example, the normal lung has been suggested to be functionally deficient in the C5 component of the complement pathway (22). Calculating the absolute concentration of C5 in the normal lung, based on the published data relative to albumin and the calculated ELF albumin concentration, confirms that C5 levels are much lower than those thought to be functionally potent. Furthermore, a number of molecules known to function as regulatory mediators, i.e., prostaglandins, have been detected in the lower respiratory tract (16). The ability to determine the absolute concentration of these mediators in ELF is critical to establishing their role in both normal physiological processes and in disease states. In this context, the urea technique has been utilized to estimate the absolute concentration of prostaglandin E2 in the lower respiratory tract, and reveals that absolute concentrations are well within the range expected to mediate physiological effects (4, 16).

Second, since the concentration of albumin in bronchoalveolar lavage fluid depends on transudation of this molecule from plasma, increases in albumin concentration have been suggested to reflect increased capillary leak (24). The ability to determine absolute albumin concentration of the epithelial lining fluid of the lower respiratory tract will help to determine whether this capillary leak results from an increased leak of fluid with an abnormally increased protein concentration and/or fluid with a normal composition. In support of this, Buchalter (5) has reported that the alveolitis and alveolar derangement of interstitial lung disease can be associated with both increased volume of alveolar fluid and with increased protein content of that fluid. Distinguishing between these two possibilities in specific clinical settings in these lungs diseases will help to understand the pathogenesis of these diseases.

Third, the use of the apparent ELF volume to estimate the density of inflammatory and immune cells on the epithelial surface of the lower respiratory tract may help characterize the alveolitis of acute and chronic lung disorders. The alveolitis of these disorders is properly characterized by three parameters: 1) the types of cells present, 2) total numbers of cells present, and 3) the state of activation of these cells (7). Therefore, only the first and third characteristics could be defined using conventional lavage methods. With the additional knowledge of the apparent ELF volume recovered, the density of these cells can now also be estimated. This should prove useful in both understanding the pathogenesis of inflammatory disorders as well as staging such patients and following their responses to therapy.

APPENDIX
Sample Calculation of Epithelial Lining Fluid (ELF) Volume Based on Urea Concentration

ASSUMPTION

\[
\text{[urea}_{ELF} = \text{[urea}_{plasma} \quad (A1)
\]

For subject I (see Table 1), volume recovered = 151 ml, \([\text{urea}_{BAL}] = 0.154 \text{mg/100 ml}, \text{total urea}_{B(AL)} = 0.154 \text{mg/100 ml} \times 151 \text{ ml} = 0.233 \text{ mg}, \text{ and ELF volume} = (\text{total urea}_{BAL})/([\text{urea}_{plasma}] - 1.94 \text{ ml}.

Sample Calculation of ELF Volume Based on Albumin Concentration

ASSUMPTION

\[
\text{[albumin}_{ELF} = \text{[albumin}_{plasma} \quad (A2)
\]

For subject 1, volume recovered = 151 ml, \text{albumin}_{BAL} = 40 \mu g/mL, \text{albumin}_{plasma} = 46 \mu g/mL, \text{total albumin}_{B(AL)} = 40 \mu g/mL \times 151 \text{ ml} = 6.04 \text{ mg}, \text{ and ELF volume} = \text{total albumin}_{BAL}/[\text{albumin}_{plasma}] = 0.13 \text{ ml}.

ASSUMPTION

\[
\text{[albumin}_{ELF} = 10\% \text{[albumin}_{plasma} \quad (A3)
\]

For subject 1, volume recovered = 151 ml, \text{albumin}_{BAL} = 40 \mu g/mL, 10\% \text{[albumin}_{plasma}] = 0.46 \text{ g/100 ml} - 4.6 \text{ mg/mL, total albumin}_{B(AL)} = 40 \mu g/\text{ml} \times 151 \text{ ml} = 6.04 \text{ mg, ELF volume} = \text{total albumin}_{BAL}/[\text{albumin}_{plasma}] = 1.31 \text{ ml}.

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REFERENCES


