

Estimation of nasal epithelial lining fluid using urea as a marker

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Background: The luminal surface of respiratory mucous membranes is lined with an epithelial lining fluid (ELF) layer. Previous attempts to determine ELF volumes in airways have used dyes or freely diffusible molecules such as urea, yet have not led to a universally accepted method. The nasal mucous membrane provides an accessible area to examine whether urea is an appropriate marker of respiratory ELF volume.

Methods and Results: Collection of undiluted nasal secretions after either glandular stimulation or induction of vascular permeability confirmed that plasma urea and nasal urea concentrations are equivalent. Baseline ELF volume was calculated as 800 μ l/nostril. The calculated molar concentrations of urea in ELF did not vary with either methacholine or histamine challenge.

Conclusions: These data indicate the plasma, interstitial, glandular, and ELF urea concentrations are equivalent and, therefore, that urea is a useful marker of ELF volume in the nasal mucosa. (*J ALLERGY CLIN IMMUNOL* 1993;92:457-65.)

Key words: Epithelial lining fluid, glandular secretion, vascular permeability, urea

Nasal secretions are a complex mixture of water, electrolytes, proteins, lipids, and sugars derived from many sources. Serous glands, which secrete the host-defense proteins, lysozyme, lactoferrin, and secretory IgA (SIgA), and mucous glands, which secrete mucoglycoproteins, are plentiful in the nasal mucosa.^{1, 2} Plasma proteins, such as albumin, IgG, and nonsecretory IgA (NSIgA), may be secreted during periods of vascular permeability or may diffuse from the interstitial tissues into glands, becoming incorporated into the glandular secretions.³⁻⁵ Cellular products from epithelial cells, goblet cells, plasma cells, mast cells, and inflammatory cells in the nasal mucosa may also contribute to nasal secretions.

The luminal surface of respiratory mucous membranes are lined with an epithelial lining fluid (ELF) layer. ELF lubricates, humidifies, and

Abbreviations used

BAL: Bronchoalveolar lavage
ELF: Epithelial lining fluid
HSA: Human serum albumin
NSIgA: Nonsecretory IgA
SIgA: Secretory IgA

protects the mucosal epithelium. ELF also plays an important role in host defense, being the site where SIgA, lysozyme, lactoferrin, and IgG reside. In 1934, Lucas and Douglas initiated the concept of the bilaminar mucous layer.^{6, 7} The outer viscous gel (or mucous) layer, 5 to 10 μ m deep, is composed of products of the mucous glands and goblet cells and rides on the tips of the cilia.^{8, 9} The stems of the cilia beat in the watery inner sol (or serous) layer, 5 to 8 μ m deep, which originates from serous glands or transudates from the cells lining the airways.

The proteins and cells recovered during bronchial lavage procedures vary from patient to patient. In repeated lavage procedures, variation occurs secondarily to inconstant recovery of washings. In an effort to estimate recovery and thereby standardize protein concentrations, early investigators reported protein concentrations in bronchoalveolar lavage (BAL) fluid relative to the concentration of albumin.¹⁰⁻¹² However, in disease

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states, the concentration of albumin in ELF can be quite elevated because of vascular leakage.¹³ In 1983, Baughman et al.,¹⁴ using methylene blue as an external marker, calculated the volume of ELF to be 11 ml in the lavaged lung segment. Critics considered this volume to be too high because of binding of methylene blue to tissue.¹⁴ In 1986, Rennard et al.¹⁵ used endogenous urea as a dilution marker in BAL aspirates. The dilution of urea by the BAL fluid was used to calculate the ELF volume (1.7 ml in the lavaged lung segment, for a total lung ELF volume of 20 to 40 ml). Jones et al.¹³ noted urea to be a more consistent marker of ELF volume than albumin in both normal subjects and patients with interstitial lung disease. However, Marcy et al.¹⁶ argued that urea had limited use as a marker of ELF volume. They suggested that the high concentration gradient generated by the urea-free lavages caused a rapid diffusion of urea into the residual lavage fluid, which resulted in an inappropriate increase in the level of urea in BAL fluid during sequential lavage procedures, thus leading to an overestimate of the ELF volume.

The upper airway is lined by respiratory epithelium overlaying a mucous membrane that resembles that of the conducting airways. The nasal mucous membrane is easily accessible for frequent, atraumatic lavage and affords the opportunity to test the hypothesis that urea is a reasonable marker of ELF volume. More important, by measuring the protein constituents of nasal ELF relative to urea, it may be possible to further understand the factors that control protein and solute movement into the ELF and to determine the effects of certain physiologic responses on ELF.

METHODS

Subjects

Thirty-six volunteers were studied, 22 men and 14 women aged 19 to 42 years, in a total of 48 observations. Patients were considered to be atopic on the basis of a history of seasonal symptoms and confirmatory skin tests. Though 22 patients were atopic, 14 patients were nonatopic, inasmuch as they had negative histories of allergic symptoms and negative skin tests. In each protocol, no subject was studied within 3 weeks of recovery from an upper respiratory tract infection. No patients were receiving immunotherapy. Subjects with systemic diseases (renal, hepatic, cardiovascular, or respiratory), with active asthma, or with a history of systemic anaphylaxis were excluded. All medications were discontinued before the subjects entered the studies, and no medications were allowed during the protocols. Informed consent was obtained from all patients.

Serum collections

Blood (5 ml) was collected by antecubital venipuncture for serum urea measurement.

Nasal lavage methods

Aspiration/nasal lavage technique. The aspiration/nasal lavage technique, which has been described in detail,^{1, 3, 4, 17} was used for both the baseline ELF protocol and the protocol involving calculation of the molar concentrations of proteins. A nasal catheter was inserted 4 cm into the nasal cavity below the inferior turbinate. All challenges were performed by instillation of 0.13 ml challenge substance (dose delivered will be reported in each protocol) by aerosol spray. Ten minutes later, each nostril was subjected to lavage with 4 ml normal saline with simultaneous collection into separate specimen traps via wall suction. Patients sat comfortably with their heads level throughout the procedures. No changes in head position were required. With this technique the secretions from each nasal cavity could be collected and analyzed separately. In 140 samples that were analyzed, an average of 3.58 ml was recovered (89.5% recovery).

Expulsion method. An expulsion method described elsewhere was used to determine the ELF volume of the entire nasopharyngeal cavity in 24 volunteers. Saline (5 ml) was instilled into each naris while subjects tilted their heads back at a 60 degree angle with the glottis closed. After 5 to 10 seconds, lavage fluid was forcibly expelled into a collection vessel. Recovery volume was 4 to 8 ml.

Collection of undiluted nasal secretions

To determine the urea concentration in undiluted nasal secretions, six volunteers (four men, two women, four with atopy, aged 27 to 40) underwent provocation studies with methacholine and histamine (see Fig. 1 for graphic depiction). The right nostril was challenged with 32.5 mg methacholine. The resulting secretions were collected during a 10-minute period. The nostril was not subjected to lavage; the volume of secretions was sufficient to permit aspiration of undiluted mucus. One hundred micrograms of atropine sulfate, 0.025% (courtesy of Muro Pharmaceuticals, Inc., Tewksbury, Mass.), was applied to the left nostril to reduce reflex glandular secretions.¹ After 10 minutes, the nostril was subjected to lavage to remove residual atropine. Histamine 1.3 mg was then applied to the same nostril that received atropine. The resulting secretions were collected during a 10-minute period. The nostril was not subjected to lavage; again, the volume of secretions was sufficient to permit collection of undiluted mucus. Nasal lavage samples were kept on ice until the end of each protocol, then centrifuged (45,000 g for 5 minutes), aliquoted, and stored at -70°C for subsequent analysis.

Assays

Total protein (TP). Protein in each sample was measured by either the standard or enhanced protocols

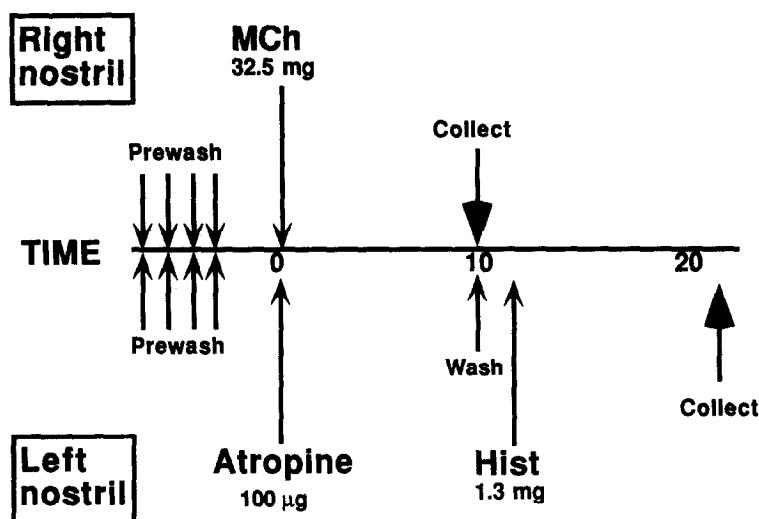


FIG. 1. Collection of undiluted nasal secretions. After four saline prewashes, right nostril was challenged with 32.5 mg methacholine (MCh). Resulting secretions were collected by aspiration during 10-minute period. Left nostril was pretreated with 100 µg atropine sulfate to reduce reflux glandular secretions. Ten minutes later, after lavage with saline, left nostril was challenged with 1.3 mg histamine (Hist). Resulting secretions were collected during 10-minute period.

of the microtiter bicinchoninic acid assay (Pierce Chemical Co., Rockford, Ill.) with bovine serum albumin used as the standard. The assay range for the standard protocol was between 15.6 and 2000 µg/ml and for the enhanced reaction between 3.9 and 250 µg/ml.

Albumin. Human albumin was measured by a competitive ELISA. A microtiter plate was incubated with 100 µl per well anti-human serum albumin (HSA; Organon Teknika-Cappel, Malvern, Pa.) diluted 1:500 in 0.1 mol/L carbonate buffer, pH 9.6, for 1.5 hours at 25° C. The wells were then blocked with 0.05% gelatin in a buffer consisting of phosphate-buffered saline, pH 7.4, and 0.05% Tween 80 (Fisher Scientific Co., Pittsburgh, Pa.). In separate tubes, 200 µl of a mixture of peroxidase-conjugated anti-HSA (Organon Teknika-Cappel) diluted 1:500 in the phosphate-buffered saline/Tween 80 buffer and 2 µg/ml HSA (Sigma Chemical Co., St. Louis, Mo.) was added to 200 µl of each sample or HSA standard. Triplicate aliquots of these combinations were transferred to a fresh microtiter plate and incubated at 25° C for 30 minutes, then transferred into the blocked plate (100 µl per well) and incubated at 25° C for 1 hour. The reaction was developed with an *o*-phenylenediamine dihydrochloride substrate (Sigma Chemical Co.) and read at 490 nm. The assay range was between 0.75 and 100 µg/ml.

Lactoferrin. Human lactoferrin was measured by a noncompetitive ELISA as previously reported.^{2, 19} The assay range was between 1.5 and 200 ng/ml.

Lysozyme. Human lysozyme was measured by a turbidimetric assay based on the enzymatic hydrolysis of bacterial cell walls as previously described.² The assay range was between 3.0 and 100 µg/ml.

SIgA and NSIgA. Human total IgA and SIgA were assayed by direct, noncompetitive ELISAs as previously described.¹ The assay ranges were between 3.1 and 400

ng/ml. NSIgA was calculated as the difference between total IgA and SIgA.

IgG. Human IgG was measured by a noncompetitive ELISA as previously described.⁵ The assay range was between 3.1 and 100 ng/ml.

Urea. Urea was measured with Sigma Diagnostics Kit No. 66-UV (Sigma Chemical Co.), a spectrophotometric assay based on the enzymatic hydrolysis of urea. For serum samples, 5-µl samples were incubated with 1000 µl reagent. For nasal lavages, 20-µl samples were incubated with 300 µl reagent. The average absorbance from 336 to 344 nm was measured. Sigma glucose/urea nitrogen combined standard (Sigma Chemical Co.) was used as the standard.

Statistics

All data are presented as means and standard errors.

Calculations

With the use of serum and nasal urea, one can calculate the dilution of ELF by the lavage fluid according to the following equation:

$$Df = \frac{[\text{Urea}]_{\text{serum}}(\text{mg/dl})}{[\text{Urea}]_{\text{nasal}}(\text{mg/dl})}$$

With this dilution factor (Df), one can then calculate the concentration of various proteins in the nasal ELF as follows:

$$\begin{aligned} \text{Molar concentration of protein in ELF} &= \frac{[\text{Protein}](\text{mole/L}) \times (Df)}{\text{or}} \\ &= \frac{[[\text{Protein}]_{\text{nasal}}(\mu\text{g/ml}) \times [Df] \times [10^3 \text{ ml/l}]]}{[\text{Molecular weight}](\text{gm/mole}) \times [10^6 \mu\text{g/gm}]} = \\ &[\text{Protein}](\text{mol/L ELF}) \end{aligned}$$

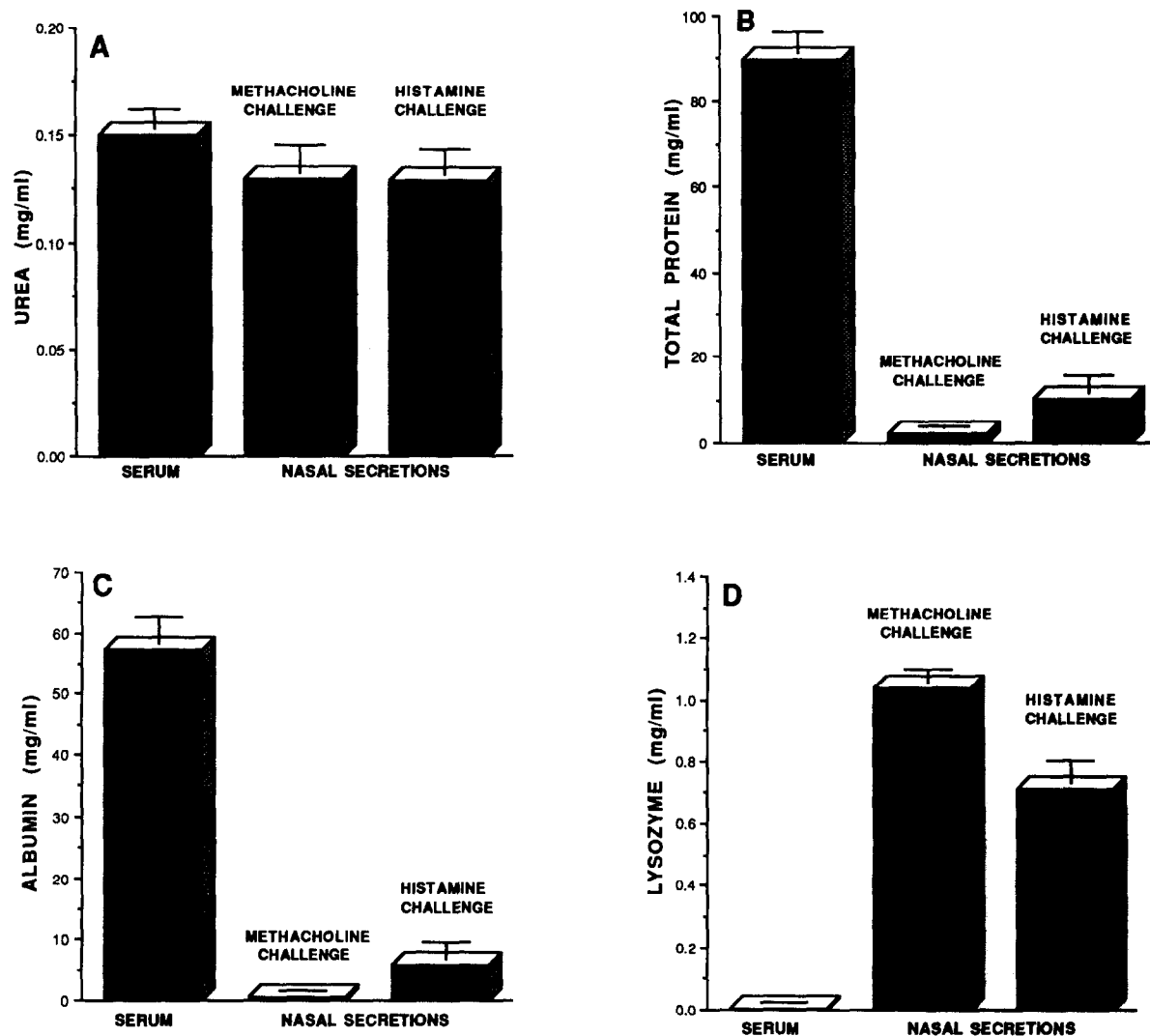


FIG. 2. Comparison of components in undiluted nasal secretions and serum. Nasal secretions were collected after stimulation with 32.5 mg methacholine or 1.3 mg histamine. Serum was obtained at time of challenge. Levels of serum urea, nasal urea after methacholine, and nasal urea after histamine were equivalent (**A**). In contrast, levels of serum total protein and albumin were 40-fold and 100-fold higher, respectively, than levels of nasal total protein and albumin after methacholine challenge (**B** and **C**). After histamine challenge, levels of nasal total protein and albumin increased to 9-fold and 10-fold less than levels of serum total protein and albumin. Serum lysozyme levels were 200-fold and 150-fold lower than those of nasal lysozyme after methacholine and histamine challenges (**D**).

With the use of this dilution factor, one can also calculate the volume of ELF recovered in lavage fluids thus: Volume of ELF in lavage fluid ($\text{Vol}_{\text{ELF Lavage}}$) = Collection volume/Df. Assuming that all wash fluids equilibrate with the ELF being sampled, one can estimate the volume of ELF sampled by accounting for both lost and recovered wash fluids as follows:

Volume of ELF sampled =

$$\frac{\text{Vol}_{\text{ELF Lavage}}}{\frac{\text{Collection volume} - \text{Vol}_{\text{ELF Lavage}}}{\text{Lavage volume}}}$$

RESULTS

Analysis of undiluted secretions

To examine the premise that urea is in equilibrium in body fluids (and thus can be used to calculate ELF volume), the concentrations of urea and proteins in undiluted nasal secretions were compared with those in serum (Fig. 2). Levels of serum urea (15.02 ± 0.93 mg/dl), nasal urea after methacholine challenge (13.02 ± 1.29 mg/dl), and nasal urea after histamine challenges (12.32 ± 1.00 mg/dl) were equivalent (Fig. 2, A).

TABLE I. Molar concentrations of nasal secretory proteins

	Resting ELF concentration*	After methacholine†	After histamine‡
Albumin ($\mu\text{mol/L}$)	0.76 ± 0.20	0.59 ± 0.34	96.59 ± 40.26
IgG ($\mu\text{mol/L}$)	0.22 ± 0.07	0.38 ± 0.19	27.65 ± 9.01
NSIgA ($\mu\text{mol/L}$)	0.45 ± 0.15	0.61 ± 0.15	3.55 ± 0.82
Lysozyme ($\mu\text{mol/L}$)	20.13 ± 4.32	33.54 ± 4.42	34.23 ± 4.64
Lactoferrin ($\mu\text{mol/L}$)	0.43 ± 0.12	2.07 ± 0.49	3.73 ± 1.52
SIgA ($\mu\text{mol/L}$)	0.32 ± 0.04	0.53 ± 0.10	0.54 ± 0.10
UREA (mmol/L)	3.33 ± 0.21	2.96 ± 0.03	3.00 ± 0.00
ELF volume (μl)	136 ± 17	827 ± 166	882 ± 162

*Initial lavage.

†Lavage after stimulation with 32.5 mg methacholine.

‡Lavage after stimulation with 1.3 mg histamine.

In contrast, serum total protein (89.71 mg/ml) was 40-fold higher than the total protein in nasal secretions after methacholine challenge (2.21 mg/ml) and 9-fold higher than the total protein in nasal secretions after histamine challenge (10.10 mg/ml; Fig. 2, B). The level of albumin in serum (57.37 mg/ml) was more than 100-fold higher than that found in nasal secretions after methacholine challenge (0.51 mg/ml; Fig. 2, C). After induction of vascular permeability by histamine challenge, the level of albumin in nasal secretions increased to 5.79 mg/ml, only 10-fold less than that in serum. The level of serum lysozyme (0.005 mg/ml) was significantly lower than that of nasal lysozyme after methacholine (1.04 mg/ml) or histamine (0.71 mg/ml) challenges (Fig. 2, D). These data indicate that, in contrast to a marked disequilibrium between the proteins in nasal secretions and plasma, urea is in equilibrium throughout these compartments.

Molar concentrations of proteins in ELF

The molarity of each protein, on the basis of its concentration, molecular weight, serum urea concentration, and nasal urea concentration, was calculated at baseline, after instillation of 32.5 mg methacholine, and after instillation of 1.3 mg histamine, by the aspiration/nasal lavage method (described in the Methods section) (Table I). Whereas the molar concentration of each protein in nasal lavage fluid was found in micromolar per liter concentrations, urea was measured at 100 to 10,000 times these levels (3 mmol/L). In baseline ELF, the vascular proteins, albumin, IgG, and NSIgA, were found at concentrations of 0.76, 0.22, and 0.45 $\mu\text{mol/L}$, respectively. After stimulation with methacholine, these values did not significantly change. In contrast, after stimulation

with histamine, both albumin and IgG increased 126-fold to 97 and 28 $\mu\text{mol/L}$, respectively. NSIgA increased 8-fold to 3.6 $\mu\text{mol/L}$. The glandular proteins, lysozyme, lactoferrin, and SIgA, were found in baseline ELF at concentrations of 20, 0.43, and 0.32 $\mu\text{mol/L}$, respectively. After both methacholine and histamine stimulation, there was a similar increase in glandular proteins. Lactoferrin increased 5-fold to 2.1 $\mu\text{mol/L}$ after methacholine and 8-fold to 3.7 $\mu\text{mol/L}$ after histamine challenge, although the increase was not significantly different between the stimuli. Lysozyme and SIgA increased 1.7-fold after both stimuli to 34 and 0.54 $\mu\text{mol/L}$, respectively. Urea remained steady at 3 mmol/L in resting ELF, after methacholine challenge, and after histamine challenge.

Baseline ELF volume

Baseline samples of nasal lavage fluids were collected from 18 volunteers by the aspiration/nasal lavage technique. The volume of ELF in the lavage fluid was calculated by dividing the recovery volume by a dilution factor composed of the concentration of urea in serum divided by the concentration of urea in nasal secretions, a modification of the equation described by Rennard et al.¹⁵ The estimated volume of ELF sampled by the 4-ml lavage was then calculated, taking into account the lost lavage fluid and the recovered ELF (see Methods section). The volume of ELF sampled in these patients was $136 \pm 17 \mu\text{l}$ (Table II). The lavage method used in this experiment sampled the anterior portion of the nasal cavity. Therefore, to determine the total volume of ELF in the nasopharyngeal cavity, 24 volunteers underwent lavage by an expulsion method, which washes most of the nasal cavity, as well as the

TABLE II. Determination of ELF urea and calculated ELF volumes

Aspiration method*			Expulsion method†		
Patient	Baseline urea (mg/dl)	Baseline ELF volume (μl)	Patient	Baseline urea (mg/dl)	Baseline ELF volume (μl)
1	0.57	111	1	1.68	821
2	0.75	159	2	1.97	918
3	0.86	199	3	2.00	1019
4	1.43	286	4	2.32	1006
5	1.12	143	5	0.95	536
6	1.43	123	6	1.49	475
7	0.22	62	7	0.73	265
8	0.47	103	8	1.28	657
9	0.04	11	9	0.62	324
10	0.52	116	10	1.94	932
11	0.51	113	11	1.03	358
12	0.89	200	12	1.88	732
13	1.03	132	13	2.66	2494
14	1.42	286	14	0.81	260
15	0.39	89	15	0.71	377
16	0.61	141	16	0.95	593
17	0.26	70	17	0.95	365
18	0.43	99	18	0.75	464
Mean	0.67	136	19	1.52	746
SEM	0.09	17	20	2.09	899
			21	1.39	1229
			22	2.75	2127
			23	0.85	513
			24	1.49	1087
			Mean	1.45	800
			SEM	0.13	111

*Four milliliters lavage fluid aspirated with inserted catheter.

†Five milliliters lavage fluid instilled in nostril, then expelled.

upper pharynx. The calculated ELF volume of each nasal cavity by this method was 800 ± 111 μl. Although the aspiration method samples only 17% of the nasopharyngeal mucosal surface, because of patient acceptability and ease of performance this method was used for the study of the molar concentrations of proteins in ELF. From a baseline of 136 μl, ELF volume was calculated to increase similarly to 827 and 882 μl after methacholine and histamine challenge, respectively.

DISCUSSION

The usefulness of urea as a marker of the ELF volume on the nasal mucosa was examined. Although much work has been done examining ELF in the lung, there have been no recent studies of nasal ELF. Urea, a 60-dalton plasma molecule that equilibrates throughout tissues, moves across the renal tubular epithelium via a specialized urea transporter, also found in the mammalian red

blood cell.²⁰ [¹⁴C]-urea enters erythrocytes within 0.1 seconds.²¹ A polar molecule, urea is moderately soluble in lipid. Galluci et al.²² calculated the urea permeability coefficient of phospholipid (lecithin) bilayers as 4.0×10^{-6} cm/sec. The permeability coefficient across dog alveolar epithelium was determined to be 2.7×10^{-6} cm/sec²³ and 2.3×10^{-6} cm/sec (compared with 0.7×10^{-6} cm/sec for ²⁴Na).²⁴ In comparison, the maximal permeability of albumin across endothelium and epithelium in sheep lungs is 4.9×10^{-9} and 0.58×10^{-9} cm/sec, respectively.²⁵

Whereas the transport mechanism of urea in the nasal mucosa is unknown, the transport of materials across the lung has been extensively studied. According to Enna and Shanker,²⁶ the adsorption of urea from the air space of the lung into the blood occurs within 4 minutes. In an attempt to compare markers of ELF volume, Effros et al.²⁷ filled rat lungs with lavage fluid for

5 seconds, 2 minutes, and 6 minutes. The BAL/plasma urea ratio increased rapidly after the 2- and 6-minute dwell times, whereas the ratio for the electrolytes, Na⁺, Cl⁻, and Ca⁺⁺, remained unchanged, which indicated that the urea had diffused into the air spaces. In addition, after only 1 minute, radiolabeled [¹⁴C]-urea had leaked into the air spaces at twice the rate of ²²Na⁺. The authors believed that urea could only be used for calculating ELF dilution if the lavage could be completed within 1 minute. Juettner et al.²⁸ used a closed, nonregenerating fluid reservoir to examine pulmonary exchange of solutes in pigs. They noted that urea most closely resembled Na⁺, in that both had high permeability constants that increased exponentially in the lavage fluid.

Rennard et al.¹⁵ noted that increased dwell times (1 and 5 minutes) resulted in increased concentrations of urea, glucose, and albumin in BAL fluid. The increase, which was inversely related to the molecular weight, was greatest for urea. Seitsema et al.²⁹ noted that urea more than doubled its level in BAL fluid within 5 minutes. In isolated dog lungs, Cross et al.³⁰ noted that urea levels in alveolar fluid reached 55% of those for blood urea after 15 minutes of dwell time. It has also been shown that water enters residual BAL solution from the interstitium during lavage.³¹ Kelly et al.,³² with the use of tritiated water, demonstrated a bidirectional flux of water across the alveolar membrane during lavages with minimal dwell time. However, according to Taylor et al.,²⁴ the permeability coefficient of urea over the alveolar membrane is one twentieth that of tritiated water. In a recent study, Van de Graaf et al.³³ found urea influx to be equal in a healthy subject during BAL procedures with durations of 185 and 380 seconds.

In our experiments, because nasal lavages take only 30 to 60 seconds to perform and there is minimal dwell time inasmuch as secretions are collected continuously, the urea content of the collected nasal lavage fluids should reflect the concentration of urea in ELF and not the rapid flux of urea. Moreover, the exchange of urea in the experimental models cited herein occurs in the alveoli, which are specialized tissues designed for the free exchange of diffusible molecules. The nasal mucous membranes, by contrast, more closely resembles conducting airways with a tight control over fluid exchange. Indeed, after an initial washout of resident nasal secretions, we found the urea concentration and estimated ELF volume to be constant in multiple lavage procedures

separated by various time intervals ranging from 2 minutes to 2 hours (data not shown).

On stimulation with methacholine or histamine, urea in undiluted nasal secretions was discovered to be equal to urea levels in serum. This observation confirms the hypothesis that urea can be used to reflect levels of nasal ELF. In contrast, the level of the plasma protein albumin was 100-fold lower in ELF than in serum. After the induction of vascular permeability with topical histamine, the level of albumin increased 10-fold in ELF, achieving the ratio of albumin/total protein concentrations that was found in serum. As noted by Persson,³⁴ the existence of this ratio in secretions suggests that there is a bulk flow of proteinaceous plasma into the ELF during periods of vascular permeability. As expected, the glandular protein lysozyme was consistently 150- to 200-fold higher in ELF than in serum.

These data confirm that there exists an equilibrium between vascular urea and nasal urea (as well as interstitial urea). The calculation of ELF volume on the basis of dilution of nasal urea by the lavage fluid is therefore valid. Moreover, urea can be used to measure relative changes in ELF volume and the concentration of various proteins. On the basis of these studies, nasal ELF may be collected and the relative dynamics of secretion assessed by measuring urea and representative plasma and glandular proteins.

The baseline anterior mucosa ELF volume was calculated in 18 volunteers as $136 \pm 17 \mu\text{l}$. The variation among individual subjects may have been secondary to individual anatomic differences. The lavage fluids, collected by an aspiration technique, washed the anterior portion of the nasal cavity but probably failed to wash the posterior aspect. To determine the baseline ELF volume in the entire nasal cavity (including some undetermined portion of the upper pharynx), 24 volunteers underwent baseline nasal lavage procedures by an expulsion method, which washes most of the inner surface of the nasal cavity. The ELF volume was calculated as $800 \pm 111 \mu\text{l}/\text{nostril}$, which indicated that the aspiration method samples about 15% of the nasopharyngeal mucosal surface washed by the expulsion procedure.

The dynamic flux of proteins into nasal secretions is illustrated by Table I, which displays the calculated molarity of each protein and urea at baseline and after methacholine and histamine provocations. Methacholine-induced glandular secretion caused the ELF to become enriched with

glandular proteins, whereas vascular proteins remained relatively unchanged. Histamine-induced microvascular permeability caused elevations in the concentrations of albumin and other plasma proteins more than 100-fold. However, after both methacholine and histamine challenges, the concentration of urea remained constant. Thus the increase in urea reflects the increase of ELF volume, whereas the increase in proteins reflects the specific secretion of vascular proteins during periods of vascular permeability or of glandular proteins during glandular secretion. Earlier studies have also demonstrated that glandular proteins become relatively enriched in secretions after cholinergic stimulation and that plasma proteins are selectively enriched in secretions after histamine or allergen challenges on a microgram per milliliter basis.¹⁻⁵ Therefore the current analysis, which used actual molar concentrations, may provide an improved basis of comparing protein concentrations after various stimuli.

Notwithstanding the problems associated with the use of urea to measure ELF by BAL, the data reported herein indicate that this method can be used in the nasal mucosa and probably in the conducting airways. On the basis of these observations, lavage procedures of the lower airway that use techniques that restrict access to alveoli might be enhanced by measurement of urea and estimation of volume.

It seems likely that the capacity to generate respiratory ELF with such greatly varied constituents must reflect an important host-defense mechanism. Glandular secretion and plasma exudation may serve as primary mucosal defense reactions.³⁵ Therefore the ability to pharmacologically manipulate the proteins in ELF may be a novel means to treat or prevent infections.

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REFERENCES

- Raphael GD, Druce HM, Baraniuk JN, Kaliner MA. Pathophysiology of rhinitis: I. Assessment of the sources of protein in methacholine-induced nasal secretions. *Am Rev Respir Dis* 1988;138:413-20.
- Raphael GD, Jeney EV, Baraniuk JN, Kim I, Meredith SD, Kaliner MA. Pathophysiology of rhinitis: lactoferrin and lysozyme in nasal secretions. *J Clin Invest* 1989;84:1528-35.
- Raphael GD, Meredith SD, Baraniuk JN, Druce HM, Banks SM, Kaliner MA. The pathophysiology of rhinitis: II. Assessment of the sources of protein in histamine-induced nasal secretions. *Am Rev Respir Dis* 1989;139:791-800.
- Raphael GD, Igarashi Y, White MV, Kaliner MA. The pathophysiology of rhinitis: V. Sources of protein in allergen-induced nasal secretions. *J ALLERGY CLIN IMMUNOL* 1991;88:33-42.
- Meredith SD, Raphael GD, Baraniuk JN, Banks SM, Kaliner MA. The pathophysiology of rhinitis: III. The control of IgG secretion. *J ALLERGY CLIN IMMUNOL* 1989;84:920-30.
- Lucas AM, Douglas LC. Principles underlying ciliary activity in the respiratory tract: II. A comparison of nasal clearance in man, monkey and other mammals. *Arch Otolaryngol* 1934;19:518-41.
- Dulfano MJ. Sputum: fundamentals and clinical pathology. Springfield, Ill.: Thomas Books, 1973:113-5.
- Welsh MJ. Electrolyte transport by airway epithelia. *Physiol Rev* 1987;67:1143-84.
- Berger HA, Welsh MJ. Electrolyte transport in the lungs. *Hosp Pract* 1991;26:53-9.
- Keimowitz RI. Immunoglobulins in normal human tracheobronchial washings: a qualitative and quantitative study. *J Lab Clin Med* 1964;63:54-9.
- Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med* 1974;84:559-73.
- Hunninghake GW, Gadek JE, Kawanami O, Ferrans VJ, Crystal RG. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am J Pathol* 1979;97:149-206.
- Jones KP, Edwards JH, Reynolds SP, Peters TJ, Davies BH. A comparison of albumin and urea as reference markers in bronchoalveolar lavage fluid from patients with interstitial lung disease. *Eur Respir J* 1990;3:152-6.
- Baughman RP, Bosken CH, Loudon RG, Hurtubise P, Wesseler T. Quantitation of bronchoalveolar lavage with methylene blue. *Am Rev Respir Dis* 1983;128:266-70.
- Rennard SI, Basset G, Lecossier D, et al. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J Appl Physiol* 1986;60:532-8.
- Marcy TW, Merrill WW, Rankin JA, Reynolds HY. Limitations of using urea to quantify epithelial lining fluid recovered by bronchoalveolar lavage. *Am Rev Respir Dis* 1987;135:1276-80.
- Kaulbach HC, Igarashi Y, Mullol J, White MV, Kaliner MA. Effects of nedocromil sodium on allergen-induced rhinitis in humans. *J ALLERGY CLIN IMMUNOL* 1992;89:599-610.
- Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
- White MV, Kaliner MA. Neutrophils and mast cells. I. Human neutrophil-derived histamine-releasing activity. *J Immunol* 1987;139:1624-30.
- Knepper MA, Star RA. The vasopressin-regulated urea transporter in renal inner medullary collecting duct. *Am J Physiol* 1990;259:F393-F401.
- Garrick RA, Chinard FP. Permeability of the dog erythrocytes to ¹⁴C-urea. *Microvasc Res* 1980;20:88-91.
- Galluci E, Micelli S, Lippe C. Non-electrolyte permeability across thin lipid membranes. *Arch Int Physiol Biochim* 1971;79:881-7.
- Theodore J, Robin ED, Gaudio R, Acevedo J. Transalveolar transport of large polar solutes (sucrose, inulin, and dextran). *Am J Physiol* 1975;229:989-96.
- Taylor AE, Guyton AC, Bishop VS. Permeability of the alveolar membrane to solutes. *Circ Res* 1965;16:353-62.

25. Gorin AB, Stewart PA. Differential permeability of endothelial and epithelial barriers to albumin flux. *J Appl Physiol* 1979;47:1315-24.
26. Enna SJ, Schanker LS. Absorption of saccharides and urea from the rat lung. *Am J Physiol* 1972;222:409-14.
27. Effros RM, Feng N, Mason G, Sietsema K, Silverman P, Hukkanen J. Solute concentrations of the pulmonary epithelial lining fluid of anesthetized rats. *J Appl Physiol* 1990;68:275-81.
28. Juettner FM, Smolle J, Popper H, Rehak P, Petek W, Friehs G. Pulmonary exchange of solutes during experimental lung lavage in pigs. *Respiration* 1988;54:226-34.
29. Sietsema K, Effros RM, Siu ST, Mason GR. Solute concentrations of bronchoalveolar fluid [Abstract]. *Am Rev Respir Dis* 1986;133:A20.
30. Cross CE, Rieben PA, Salisbury PF. Urea permeability of alveolar membrane: hemodynamic effects of liquid in the alveolar spaces. *Am J Physiol* 1960;198:1029-31.
31. Kelly CA, Kotre CJ, Ward C, Hendrick DL, Walter EH. Anatomical distribution of bronchoalveolar lavage fluid as assessed by digital subtraction radiography. *Thorax* 1987;42:624-8.
32. Kelly CA, Fenwick JD, Corris PA, Fleetwood A, Hendrick DJ, Walters EH. Fluid dynamics during bronchoalveolar lavage. *Am Rev Respir Dis* 1988;138:81-4.
33. Van de Graaf EA, Jansen HM, Weber JA, Koolen MGJ, Out TA. Influx of urea during bronchoalveolar lavage depends on the permeability of the respiratory membrane. *Clin Chim Acta* 1991;196:27-40.
34. Persson CGA. Leakage of macromolecules from the tracheobronchial microcirculation. *Am Rev Respir Dis* 1987;135:571-5.
35. Persson CGA. Plasma exudation in tracheobronchial and nasal airways: a mucosal defense mechanism becomes pathogenic in asthma and rhinitis. *Eur Respir J* 1990;3:652s-7s.