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Influence of hyperpnea on airway surface fluid volume and osmolarity in normal humans

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Kotaru, C., Rana B. Hejal, J. H. Finigan, A. J. Coreno, M. E. Skowronski, L. J. Brianas, and E. R. McFadden, Jr. Influence of hyperpnea on airway surface fluid volume and osmolarity in normal humans. *J Appl Physiol* 93: 154–160, 2002. First published March 8, 2002; 10.1152/japplphysiol.00830.2001.—To determine the effect of hyperpnea on the characteristics of periciliary liquid, we collected airway surface fluid (ASF) and measured its osmolarity in 11 normal people while they breathed dry, frigid air (−17 ± 1.2°C) at minute ventilations ($\dot{V}_E$) of 10, 40, and 80 l/min through a heat exchanger. The ASF was collected at the fifth tracheal ring by absorption onto filter paper pledgets inserted via fiber-optic bronchoscopy. Hyperpnea had no influence on the amount of ASF recovered (ASF volume at a $\dot{V}_E$ of 10 l/min = 12.0 ± 2.0 μl; at 80 l/min = 8.8 ± 1.5 μl; $P = 0.28$) or its osmolarity (at a $\dot{V}_E$ of 10, 40, and 80 l/min = 326 ± 15, 323 ± 11, and 337 ± 12 mosM, respectively; $P = 0.65$). These findings demonstrate that the tracheal mucosa of normal subjects does not desiccate during hyperpnea and that hypertonicity of the periciliary fluid does not develop even at high levels of ventilation.

airway drying; hypertonicity

IT IS WELL ESTABLISHED THAT the respiratory tract of humans actively participates in thermal homeostasis. Measurements of the temperature fluxes that occur within the tracheobronchial tree demonstrate that, whenever ventilation rises, the intrathoracic airways are called upon to heat and humidify the incoming air to full saturation at body temperature before it reaches the alveoli (11, 26, 30, 37). Throughout inspiration, the air is actively warmed by conduction and convection as it moves down the tracheobronchial tree, and water evaporates passively from the mucosa along the vapor pressure gradients that exist in any given region (11, 26, 30, 37). During this process, the bronchi cool, and on expiration the flow of energy reverses. As air temperature falls, its ability to hold moisture decreases, and water condenses back onto the epithelium (11, 26, 30, 37). Although reasonably efficient, this countercurrent mechanism is imperfect, and some heat and water are routinely lost to the environment (11, 26, 30, 37). The greater the ventilation and the drier the inspirate, the larger the losses, and the more the need for replenishment (11, 26, 30, 37).

Even though the overall physics of respiratory heat exchange have been reasonably well worked out, little is known about the kinetics of the conditioning process at the mucosal level, particularly that of water. One school of thought holds that the process is unbalanced so that water losses always exceed replenishment. In this construct, hyperpnea is invariably associated with airway drying and the formation of a hypertonic periciliary fluid (1, 2). In contrast, a second postulate maintains that such phenomena are unlikely because of the branching pattern of the tracheobronchial tree and the distributed nature of respiratory thermal transfers (13, 14). In the present study, we reasoned that it should be possible to determine which hypothesis is correct by collecting the surface fluid from the intrathoracic airways during hyperpnea and measuring its osmolarity. If water losses exceed replenishment, the surface should dry and osmolarity rise. If, however, evaporation and replacement are homeostatically regulated, osmolarity should remain unchanged. Our observations form the basis of this report.

METHODS

Eleven normal volunteers (4 men and 7 women), aged 32 ± 3 yr (mean ± SE), served as our subjects. None used tobacco products or experienced an upper respiratory tract infection in the 6 wk preceding the investigation. Each participant inhaled frigid air through a heat exchanger at an imposed ventilation of 10 l/min to standardize resting conditions. Moderate and high levels of physical activity were then simulated by having the participants perform isocapnic hyperventilation at minute ventilations ($\dot{V}_E$) of 40 and 80 l/min by using standard techniques (7, 15, 27). Each period of study (rest, moderate and high levels of $\dot{V}_E$) lasted 4 min (7, 15, 27) and was separated by a 5-min wait. The water content of the inspirate was <1 mg H_2O/l, which, for the purposes of this study, was considered zero. The expired air was directed away from the heat exchanger into a reservoir balloon that

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was being constantly evacuated at a known rate through a calibrated rotameter. The subjects were coached to keep the balloon filled, and, in so doing, their $\dot{V}_E$ could be controlled at any desired value. End-tidal CO_2 concentrations were monitored with a Nellcor N-1000 analyzer (Mallinckrodt, Kansas City, KS), and sufficient CO_2 was added to the inspiratory port of the exchanger during hyperpnea to maintain end-tidal CO_2 at eucapnic levels.

Airway surface fluid (ASF) was collected from the trachea with previously described techniques that employed fiberoptic bronchoscopy and absorption onto filter paper strips (19, 20). The nasal passage with the largest opening was anesthetized with 2% lidocaine gel, and 1–2 ml of lidocaine liquid were applied to the vocal cords under direct visualization. No premedication was given (13, 14, 15, 29, 30). After a wait of 15 min, the bronchoscope was inserted and secured at the nares with the tip in the upper trachea. The subjects then inhaled through the heat exchanger at the above levels of ventilation in sequential order. Before each experiment, we removed the sample brushes from three protected double-lumen microbiology catheters (Microbiology Brush, Microinvasive Division, Boston Scientific, Watertown, MA) and replaced them with pediatric alligator forceps (Mill Rose Laboratory, Mentor, OH) holding pledgets of five preweighed 15 mm × 5 mm strips of hardened ashless filter paper (Whartman 42, W&R Balston). Care was taken not to disturb the wax plugs at the distal end of the outer catheters, and the paper was washed three times in double-distilled water and dried before use. The sealed system was then inserted through the channel of the bronchoscope 1 min before the sampling period. The catheter containing the forceps was kept recessed until it was needed. Periciliary fluid was collected during the 4th min of each experimental period by moving the catheter into the trachea and knocking out the plug. The forceps were then extended until the pledget touched the mucosal surface at the level of the fifth ring. They were held there for 30 s. A new plugged collection system was used for each sample period in each subject. Time was carefully monitored, and surface droplets of all types were avoided. After collection was complete, the forceps and filter paper were drawn back into the catheter sheath so that the tip was 3–5 mm from the opening. The latter was resealed with capillary action by touching it against the airway wall for 5 s. The catheter assembly was quickly removed, and the filter paper strips were immediately placed into dried, preweighed glass vials and sealed for analysis. The vials were reweighed, and the volume of liquid collected was determined by subtracting the weights of the dry tubes from those with the pledgets. All weights were obtained with the same calibrated precision balance (Mettler H30 balance, Mettler Instrument). The time from resealing the catheter opening to closing the vial was also carefully recorded for each sample for all experiments. The "handling time" was then used to correct for the evaporation of water from the filter paper (see below).

After the tared weights were recorded, 100 μl of double-distilled deionized water were added to the tubes, after which they were reweighed and centrifuged for 60 s to ensure thorough mixing. The samples were left to stand overnight to allow the fluid on the paper to elute into the water. The osmolarity of the mixture was measured by freezing-point depression (Advanced Micro-osmometer, Advanced Instruments, Norwood, MA).

The accuracy and reproducibility of the osmolarity measurements were assessed by comparing the results from the experimental filter paper technique against direct measurements of known standards. The influence of sample handling on evaporative water losses from the filter paper was also determined. Because we did not know the magnitude of the increases in osmolarity, if any, that would accompany hyperpnea, we performed our validation experiments with solutions of 284 mosM (isosmolar), 463 mosM (moderate hypertonicity), and 742 mosM (high ionic content). Each test fluid was prepared by adding measured quantities of sodium chloride to double-distilled deionized water and measuring them with the osmometer. These ions were used because they most closely match the expected composition of the periciliary fluid (4, 5). In 10 separate experiments, 20 μl of each osmolar standard were pipetted into petri dishes, absorbed by the filter paper strips, and analyzed as above. Mean values and the coefficients of variation (CV) were calculated.

The rate of evaporation of water from the filter paper was assessed by placing dry weighed pledgets into a 37°C water bath and reweighing after 15, 30, 60, and 120 s of drying at room temperature and humidity. An equation regressing water loss against time was constructed from 16 measures at each sampling point. By knowing the handling time in each experiment and the slope of the regression line, the initial weight of each pledget in the experimental protocols could be back extrapolated.

The amount of water absorbed by the filter paper from the airstream was measured in vitro by holding dried pledgets in the outflow tract of the heat exchanger while fully saturated air at 25°C was blown by them for 30 s at rates of 40 and 80 l/min. Ten experiments were performed at each level. The pledgets were analyzed as described above, and the mean values were subtracted from the measured ASF volumes before correction to zero time. The temperature and humidity of the airstream matched the thermal profiles known to exist in the upper trachea when frigid air is inhaled at the levels of ventilation used here (13, 14) and were generated with the heat exchanger and water bath as in previous studies (7, 30, 39).

To determine the integrity of the collection technique in preventing contact with fluid from sources other than the airway surface, three subjects underwent a second bronchoscopy while breathing frigid air, as previously described. In this trial, the pledgets were extended into the airstream for 30 s during the last minute of hyperpnea but not touched to the tracheal wall. They were then withdrawn into the catheter sheath and analyzed as in the main study. The catheter tip was not touched to the mucosa in this set of experiments, because the extrusion of the pledget through the resulting film of fluid would have negated the purpose of the experiment.

The total quantity of water that the pledgets could hold was determined in 10 trials by immersing them into double-distilled water for 30 s and immediately reweighing them.

Maximum forced exhalations were performed in triplicate with a waterless spirometer before the start of the study, 10 min after the administration of lidocaine before bronchoscopy and 5 min after the completion of the last bout of hyperpnea. The curve with the largest 1-s forced expiratory volume (FEV_1) was chosen for analysis.

The institutional review board for human investigation approved the protocol, and all participants gave informed consent.

The data were analyzed statistically by paired *t*-tests and one-factor analysis of variance. A two-tailed P value ≤ 0.05 was considered significant. The study was powered to detect a minimum increase in osmolarity of ~100 mosM. This level corresponds to the smallest osmolar thresholds previously reported to produce a ≥10% release of histamine from isolated mast cells and basophils in vitro and is similar to that

found in the nose when respiratory water recovery is totally prevented (8, 38, 40).

RESULTS

Validation experiments. The initial proof of concept studies is contained in Fig. 1. The individual CV of the osmolarities measured by the filter paper technique were <6% (normal CV: 5.1%, moderate: 2.1%, and high osmolar standard: 2.3%). Each experimental assessment was statistically identical to its directly measured counterpart in the reference solutions (filter paper vs. direct measure of standards: normal, $P = 0.24$; moderate, $P = 0.24$; high, $P = 0.28$).

The mean amount of water picked up from the airstream by the pledgets in 30 s in the in vitro studies was <0.6 μl, and there were no significant differences between ventilatory trials ($F = 0.32$; $P = 0.73$, Fig. 2). On average, the total quantity of water that the filter papers could hold when completely saturated was 43.1 ± 4.4 μl.

The mean handling times for the samples were 13.0 ± 1.1, 11.9 ± 0.7, and 12.3 ± 1.3 s for the resting, moderate-, and high-ventilation experiments, respectively ($F = 0.30$; $P = 0.75$). As expected, the weight of the filter paper pledgets decreased with time, but the effect was not significant over 2 min ($P = 0.25$) (Fig. 3). A calculated evaporation factor of 16.4 μg/s was used to back correct the experimental samples to zero time.

Human studies. The demographic and physiological data of our subjects are contained in Table 1 and Fig. 4. The FEV_1 averaged 99 ± 4% (3.38 ± 0.24 liters), and the temperature of the inspired air during hyperpnea was −17 ± 1°C. Neither lidocaine nor hyperventilation had any significant effect on the FEV_1 ($P = 0.98$) (Fig. 4).

The amount of fluid collected over 30 s at rest averaged 12.0 ± 2.0 μl (Fig. 5). Correcting for evaporation and absorption from the airstream had negligible effects. Fluid volume fell slightly as $\dot{V}_E$ rose; however, the changes were not statistically significant (corrected ASF volume at a $\dot{V}_E$ of 40 and 80 l/min = 10.5 ± 1.1 and 8.8 ± 1.5 μl, respectively; $F = 1.34$; $P = 0.28$).

The effect of hyperpnea on ASF osmolarity is shown in Fig. 6. The values for the uncorrected samples were 333 ± 15 mosM at rest and 329 ± 11 and 346 ± 12 mosM, respectively, in the moderate- and high-ventilation studies. Correcting the data for evaporative losses and absorption from the airstream resulted in a small (6–9 mosM), but significant, reduction in the absolute values. Note that increasing $\dot{V}_E$ had no discernable impact on ASF osmolarity, irrespective of how the data were expressed. The corrected values at rest averaged 326 ± 15 mosM and remained constant as ventilation rose ($F = 0.4$, $P = 0.65$). Even at a $\dot{V}_E$ of 80 l/min, the mean osmolarity was within 3.5% of control.

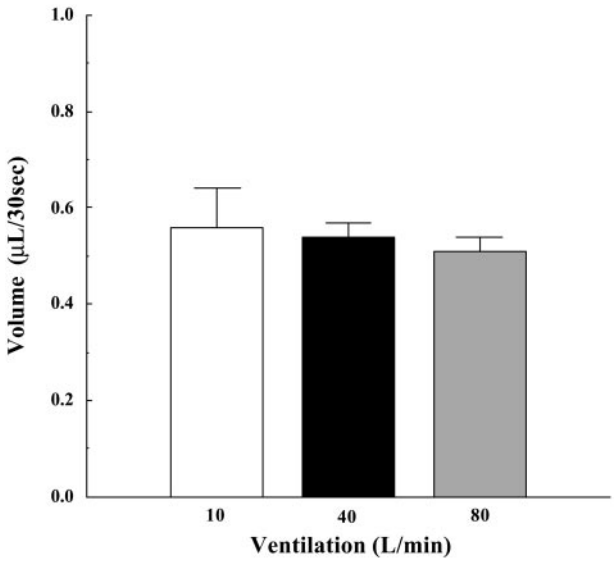

Fig. 2. Amount of water absorbed from the airstream in 30 s at ventilations of 10, 40, and 80 l/min. Bars indicate mean values, and error bars indicate SE. The abscissa is ventilation, and the ordinate is sample volume.

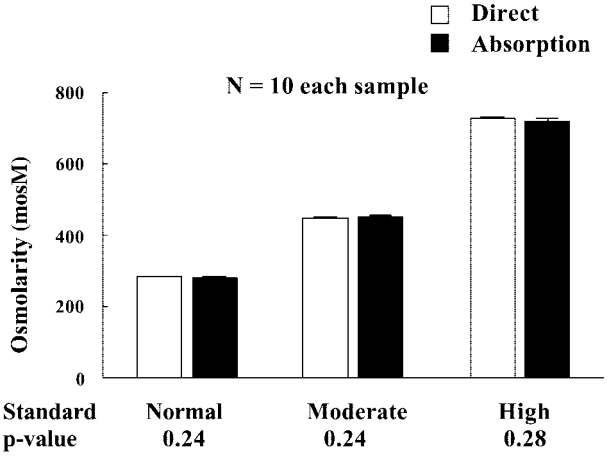

Fig. 1. Comparison of osmolarity measurements of standard solutions made directly and by absorption onto filter paper. Osmolarity is displayed on the ordinate, and the standards are on the abscissa. Normal standard = 284 mosM, moderate = 468 mosM, and high = 742 mosM. Bars indicate mean values, and error bars indicate SE. Open bars, direct measurements; solid bars, measurements made through filter paper absorption. The *P* values beneath each data pair are derived from paired comparisons.

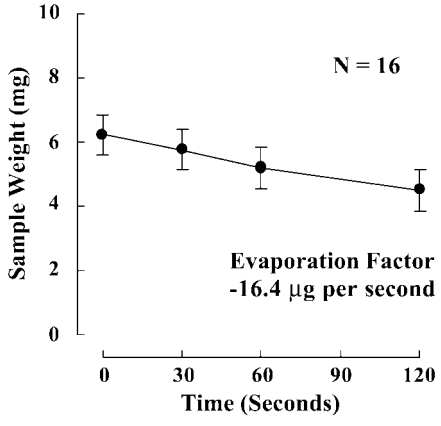

Fig. 3. Rate of evaporation of water from filter paper pledgets. Sample weight is displayed on the ordinate, and the time the sample spent in air is on the abscissa. Values are means ± SE. The slope of the regression equation = 16.4 μg/s loss of water. Each data point represents 16 sets of measurements.

Table 1. *Demographic and physiological data*

Subject No.	Gender	Age, yr	FEV_1, %	T_I, °C
1	F	30	92	−14.6
2	F	31	110	−16.0
3	M	40	94	−9.5
4	M	41	100	−19.3
5	F	25	100	−19.3
6	F	19	75	−24.4
7	F	45	106	−20.4
8	M	39	89	−14.3
9	F	26	93	−16.9
10	F	27	110	−16.9
11	M	29	115	−16.0
Mean	7 F/4 M	32	99	−17.0
SE		3	4	1.2

F, female; M, male; FEV_1, 1-s forced expiratory volume as a percentage of predicted normal; T_I, temperature of the inspired air during hyperpnea.

In the in vivo studies assessing possible contamination, the temperature of the inspired air was −16 ± 2°C. The pledgets absorbed an average of 0.4 ± 0.2 μl of water from the tracheal air in 30 s. This value is statistically similar to that found in the in vitro trials (P = 0.49). The liquid was free of any detectable osmotic concentration (0 ± 0 mosM).

DISCUSSION

The results of the present study demonstrate that, during periods of severe thermal stress, the human respiratory tract can condition large volumes of air without altering the physiology of its lining fluid in any detectable fashion. Inhaling a dry inspirate at a $\dot{V}_E$ four and then eight times resting levels markedly enhances the movement of water from the mucosa to the air and greatly aggravates replacement mechanisms (1, 11, 26, 37); nonetheless, there is no significant impact on either the volume of periciliary liquid available or its tonicity. The amount of ASF that was collected in 30 s varied between 9 and 12 μl as ventilation rose, and the osmolarity remained constant at <4% of its resting value. Because the temperature and water profiles in the lungs during exercise and voluntary hyperventilation are identical when the appropriate variables are matched (14, 29), and because the imposed ventilations in this study and their associated thermal burdens approached the upper tolerable limits seen in individuals working heavily out of doors in winter, our data indicate that airway drying and hypertonicity are not features of respiratory heat exchange in normal humans.

To our knowledge, there have not been any previous studies in humans that have measured ASF availability and osmolarity in the intrathoracic airways during

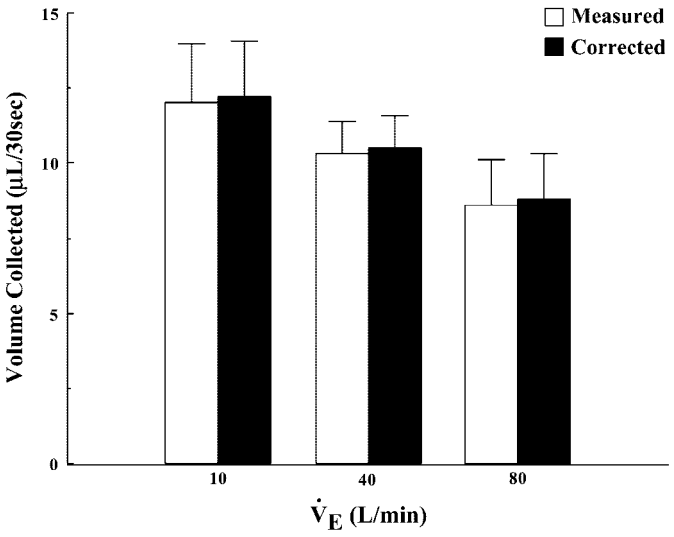

Fig. 5. Effect of hyperpnea of airway surface fluid volume collection in normal subjects. The ordinate presents the volume of fluid collected in 30 s. Minute ventilation ($\dot{V}_E$) is shown on the abscissa. The measured and corrected volumes are shown by the open and solid bars, respectively. Bars indicate mean values, and error bars indicate SE.

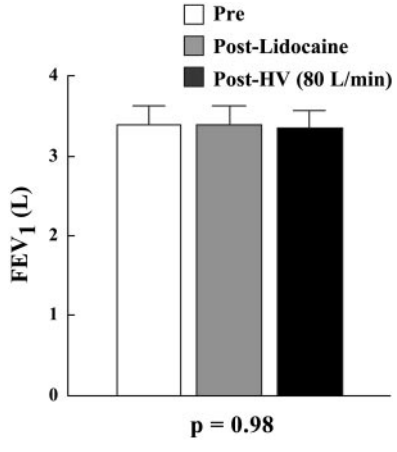

Fig. 4. Lung function measurements during the collection of airway surface fluid. The ordinate presents the 1-s forced expiratory volume (FEV_1). Bars indicate mean values, and error bars indicate SE. Open bar, data before the start of the study (Pre); gray bar, effect of lidocaine; solid bar, consequences of hyperventilation (HV) of 80 l/min. The P value derives from a 1-factor analysis of variance.

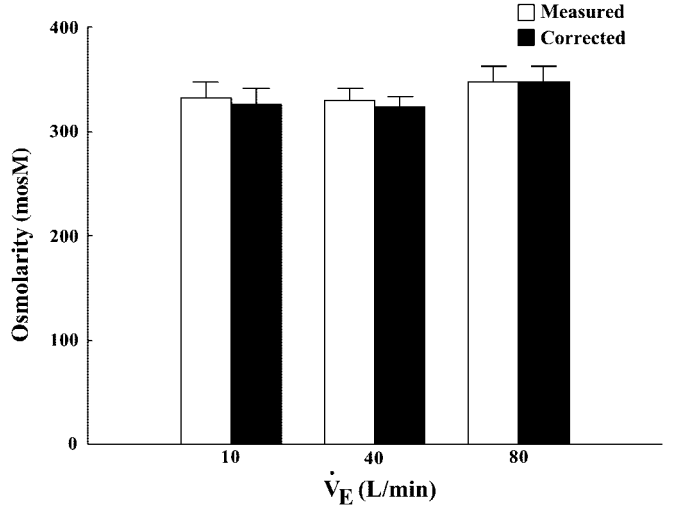

Fig. 6. Effect of airway surface fluid osmolarity in normal subjects. The ordinate shows osmolarity, and the abscissa shows $\dot{V}_E$. The format is identical to Fig. 5.

the conditioning process; hence, direct comparisons are not possible. Investigations in dogs have suggested that bypassing the upper airways can increase the osmolarity of the fluid lining the trachea (6), but, given the present findings, it does not seem that a similar effect is operational in humans. Because our subjects breathed through their mouths without any change in ASF dynamics or tonicity, our protocol already presents the worst case scenario. Nasal breathing would have limited the need for intrathoracic thermal exchanges, thereby minimizing any tendency toward drying. It would not have worsened it (26, 37). Several papers have indicated that mucosal desiccation and hyperosmolarity can occur in the nares of cold-air-sensitive patients (40) and in the peripheral airways of an animal model (12). Togias et al. (40) noted surface osmolarity to rise when their subjects inhaled frigid air through their noses and exhaled out of their mouths, and Freed and Davis (12) found similar effects in dogs when they blew dry air through segmental bronchi subtended to a wedged bronchoscope. In both instances, airflow limitation developed, and a cause-and-effect relationship was postulated. It was also suggested that exertional asthma shared a similar mechanism.

In light of the present work, it is probable that the unidirectional flow of air in each class of experiments produced the changes in surface fluid physiology reported, and recent data suggest that the mechanism for obstruction in this form of asthma derives from a different source (22). The ventilatory paradigms employed in the studies cited totally subvert the normal countercurrent replacement mechanisms for water and so force the airways to dry. When both the inspiratory and expiratory phases of ventilation are allowed to proceed naturally, such adverse mucosal events do not develop. Even if they did, it is now recognized that the bronchial narrowing that follows hyperpnea in asthma does not depend on either the amount of the surface liquid available or an increase in osmolarity (22). Even though evaporation is the major cause of airway cooling (3, 7, 13, 14, 17) and, as such, correlates with the severity of obstruction (3, 7, 17), it only serves as a means of initiating the reaction. Desiccation and hypertonicity do not appear to be critical factors (22, 28).

Although it is true that nasal congestion can be made to occur with normal respiratory patterns of hyperpnea (33), it takes 45 min of hyperventilation before it is evident, and it may be that thermally induced nitric oxide production with resulting hyperemia plays a role (16, 21, 23). This mediator is produced when the surface temperatures in the upper and/or lower respiratory tract fall (16, 21), and recent work indicates that it is pathogenically important in thermally induced asthma (21, 23).

Only fragmentary information exists on the thickness and composition of the ASF layer in humankind, and nothing at all is known about its kinetics during hyperpnea. We understand that our collection technique could not determine breath-by-breath water movement, and this was not our intent. Rather, we hoped that, by making measurements over a fixed time, we could assess whether there were quantitative changes in the amounts of fluid available in the region of interest and determine what happened when ventilation rose. Based on published estimates, we feel that we were able to achieve this goal in a reasonable fashion. Knowles et al. (20) found that 10–20 μl of fluid could be absorbed onto filter paper strips in 20 s from the airways of normal subjects and that there were no differences between them and patients with various forms of airway disease. Our values clearly fall well within these ranges.

The proof of concept studies allowed us to be confident that our collection techniques were neither limited by the ability of the filter paper strips to hold liquid or compromised by extraneous contamination. The pledgets could have contained more than twice the amount of fluid gathered; therefore, saturation was not an issue. Similarly, the in vitro and in vivo data show that the maximum amount of water that entered the pledgets from the surrounding air was too small to have influenced the results (Figs. 2, 5, and 6). Equally important, the lack of any osmolar activity in the filter paper strips that were held in the tracheal airstream demonstrates that contamination by residual or nonsurface liquids anywhere in the system was not a source of error. Given that our sampling technique is quite similar to that used to obtain and culture infectious organisms from the lungs, such isolation is precisely what was anticipated. The wax plug in the opening of the catheter sheath makes it physically impossible to contaminate the lumen during insertion into the tracheobronchial tree.

We recognize that pulling the pledgets into the catheter could have squeezed fluid out of them and thus have underestimated ASF volume. However, closing the catheter tip by capillary action would have prevented liquid from going back onto the airway surface. This action also allowed us to reverse the process when the pledgets were extruded so that we could recapture any such losses. Because the ASF is a continuous layer (4, 5) and because the fluid on the filter paper and in the catheter tip both came from the same mucosal sources, no bias was introduced. Resealing the catheter with ASF before removal merely adds a bit more liquid for analysis and is not terribly dissimilar to collecting with a pipette (17). As described above, the combination of presealing the collection system with a wax plug and the use of a new assembly for each trial in each subject prevented any possible admixtures with fluids remaining in the bronchoscope lumen between sampling periods or inadvertently entering the catheter when the collection system was introduced.

We sampled at the fifth tracheal ring because it had been previously determined that this region is where the maximum temperature and vapor gradients develop during hyperpnea, and thus it is here where the greatest impacts on fluid volume and tonicity are expected (15, 16, 29, 30). It is important to appreciate that the previously defined linear distribution of the conditioning process makes it physically impossible for

larger alterations in volume or tonicity to have existed anywhere downstream from this level (13, 14, 29, 30).

The observation that ASF volumes and tonicity remained stable in our experiment is not entirely unexpected and, in fact, actually fulfills both known physiological events and earlier predictions (10, 13, 24, 28). Data demonstrate that the airway epithelial surface cannot allow an osmotic gradient to exist; therefore, ventilatory-induced hypertonicity via evaporation is unlikely to ever occur in normal situations (10, 24). Moreover, thermal maps of the respiratory tract show that, when $\dot{V}_E$ rises, only 50% of the water transferred derives from the intrathoracic airways (13, 14). Of this, no more than 10–15% is evaporated from the regions encompassing the trachea to the subsegmental bronchi, and the remainder comes from the myriad of peripheral airways (13, 14). The overall consequence is that water fluxes per unit area are always small and easily replaced. Finally, calculations indicate that the surface of the conducting airways contains far more water than is needed to completely saturate incoming air at maximum ventilations, even if it is at zero relative humidity (13). The physiological net effect is that a functional reservoir for surface water exists in the tracheobronchial tree that can be called on as needed to meet increasing demands.

What has not been fully recognized until now is the interplay of the homeostatic elements that maintain the reserves. For all of the measured features of the ASF to remain unchanged in our study, losses must have been actively replaced. Otherwise, fluid availability should have fallen and the ionic content risen as hyperpnea continued, because of the inefficiencies in the passive countercurrent mechanism (26, 37). The elements governing dynamic replenishment are not yet fully established, but, based on what is known, increased secretion by glandular elements is the dominant source (4, 5). In support of this, we could easily visualize increased mucous and serous fluids on the surface shortly after starting hyperventilation. Pools of either were deliberately avoided. Because, as mentioned above, the airway epithelium is unable to sustain an osmotic gradient, this phenomenon is also apt to be an important mechanism to support water availability (10, 24). The movement of water to the surface through trans- and paracellular pathways could provide additional means of quickly neutralizing transient increases in surface tonicity that might develop with evaporation (4, 5, 42).

Irrespective of mechanisms, there are data to suggest that the quantity of fluid actively produced by the respiratory tract can be quite high, at least for short periods. The experiments noted above that bypassed the normal recovery processes for water recorded surprisingly diminutive increases in ASF tonicity. In the dog model, blowing 2 l/min of dry air through a sublobar segment of the lung for 5 min resulted in the vaporization and loss of 0.44 g H_2O from the mucosa but only a 43% increase in osmolarity (12). The involvement of a larger surface area in humans produced even much smaller consequences. In this case, inhaling dry air through the nose for 15 min at a ventilation of 12.5 l/min only raised osmolarity 25% for a water loss of 8.2 g (i.e., 8 ml H_2O) (40). The latter is the equivalent of what would have been lost from the lower respiratory tract during several minutes of moderately severe exertion (28). As yet, there is no information on what happens with longer periods of hyperventilation (33).

The osmolarity of the periciliary fluid has been reported to lie between 225 and 367 mosM (18, 25, 31, 32, 35, 36, 41). Our data fall within this range and in the extreme are within 8.5% of the values normally reported for serum. Because there were no differences in the results found in the standard solutions when they were assayed directly or by absorption onto and elution from filter paper, it is unlikely that simple measurement issues account for the size of our estimates. It is possible, however, that the collection technique we used may have itself influenced the experimental sample. Some (9, 12), but not all, studies (20) have shown that filter paper results in higher osmolarities than those found with electrodes or pipettes. Presumably, this is because the paper exerts a capillary pressure that may pull macromolecules through the epithelium (9). We readily acknowledge the imperfections in our technique, but because our only purpose was to determine how hyperpnea impacted osmolarity and not to ascertain the composition of the ASF per se, we do not feel that our main findings are compromised in any fashion. The important point is that osmolarity remained constant at all levels of ventilation studied. The addition of large molecules would merely have driven the overall values upward and would not have affected the pattern seen. The contact time of the filter paper with the mucosa was precisely timed for all collections so that any transepithelial effect would have been constant from sample to sample.

In summary, our data demonstrate that the conditioning process of inspired air is a far more highly regulated process in normal subjects than previously thought. The movement of heat and water from the mucosa is homeostatically balanced so that regional epithelial losses never impair physiological integrity. Desiccation of the respiratory epithelium and hypertonicity of the periciliary fluid are not features of normal respiration, even during prolonged periods of hyperpnea of dry frigid air.

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