Roles of hydration, sodium, and chloride in regulation of canine mucociliary transport system

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Winters, Scot L., and Donovan B. Yeates. Roles of hydration, sodium, and chloride in regulation of canine mucociliary transport system. J. Appl. Physiol. 83(4): 1360–1369, 1997.—To gain insight into the homeostatic mechanisms regulating airway ion/water fluxes and mucociliary transport, the canine tracheobronchial airway fluid was perturbed by deposition of hypo- and hyperosmotic aerosols for >1 h. Tracheal ciliary beat frequency (CBF) was measured by using heterodyne laser light scattering. Tracheal mucus velocity (TMV) and bronchial mucociliary clearance (BMC) were measured by using radioaerosols and nuclear imaging. Respiratory tract fluid output (RTFO) was collected by using a secretion-collecting endotracheal tube. In six dogs, CBF increased during water deposition in the airways to 180 ± 30 mg/min and RTFO increased from 2.2 ± 0.5 to 18.3 ± 1.6 mg/min, accounting for <10% of the fluid deposition. TMV and BMC were unchanged. CBF, TMV, and BMC were markedly increased by inhalation of aerosolized 3.4 M NaCl. Aerosolized 0.85 M NaCl, in contrast, decreased BMC. In this case, RTFO represented 24% of aerosol deposition. Aerosolized 0.85 M choline chloride and 0.85 M sodium gluconate enhanced BMC and TMV concurrent with a decrease in CBF. RTFO of sodium gluconate studies exceeded 50% of aerosol deposition. Thus the airways appear to have transepithelial compensatory mechanisms that reduce the impact of a moderate increase in NaCl and hydration load, but when these responses cannot adequately respond because of the delivery of impermeable ions or very high tonicity, removal of the challenges are affected by a stimulation of mucociliary transport.

The effective transport of airway mucus atop cilia extending 5–7 µm from the subjacent epithelium is critically dependent on the depth of the periciliary fluid. Acute changes in the hydration of the fluid lining the airways must, therefore, induce compensatory mechanisms within the epithelium to regulate its depth. The mechanisms sensing the depth and composition of this fluid, as well as the regulatory processes responsible for preserving and maintaining effective mucociliary transport, are still to be elucidated. It has been proposed that the epithelia respond to osmotic challenges in such a way as to protect the Na+/Cl− ratio within the airway (13). Because Na+ and Cl− are the predominant osmolytes in unperturbed airway lining fluid (24), an investigation into their role in the maintenance of the mucociliary transport system is warranted. In a companion paper (31), we have shown that the inspiration of dry air decreases bronchial mucociliary clearance (BMC) while transiently decreasing tracheal ciliary beat frequency (CBF) and possibly tracheal mucus velocity (TMV). Both CBF and TMV seem to recover after prolonged exposure, indicating the induction of compensatory mechanisms. Airway dehydration increases the tonicity of airway secretions (13). However, the response of the airway to an increase in tonicity caused by dehydration may differ from that due to a change in tonicity without dehydration (9). In this paper, we focus on the responses of the mucociliary transport system, and thus the ciliated airway epithelium, to near steady-state anisotonic fluid perturbations in which both the total concentrations of Na+ or Cl− ([Na+] and [Cl−], respectively), as well as the Na+/Cl− ratio, are manipulated.

We hypothesized that the unperturbed tracheobronchial airways favor absorption of water and NaCl into the mucosa, consistent with in vitro data (12), leading to a low basal level of mucociliary transport. We developed four postulates as to how the mucociliary transport system, and thus the ciliated airway epithelium, may respond when perturbed: 1) with hypotonic deposition, the airway epithelium has the capacity to increase water absorption sufficiently to maintain effective mucociliary transport; 2) with hypertonic deposition containing high [Na+] and [Cl−], the limits of NaCl and water absorption by the airway epithelium may be approached, leading to increased mucociliary transport to remove the excess airway fluid (mediator release or nerve activation induced by hypertonicity might also stimulate transport); 3) with hypertonic deposition containing moderate [Na+] and [Cl−], the airway epithelium may still respond with NaCl and water absorption (21), although at a greater relative magnitude, to maintain stable mucociliary transport; and 4) with an increase or decrease in the Na+/Cl− ratio (such as by respective ion replacement with less permeable ions), the absorption of fluid by the airway epithelium may be impaired (or secretion induced), leading again to increased mucociliary transport to remove the excess airway fluid.

By investigating responses of the canine mucociliary transport system in vivo to continuous inspiration of aerosolized water, 0.85 M NaCl, 0.85 M sodium gluconate, 0.85 M choline gluconate, and 3.4 M NaCl, we attempted to corroborate the above hypotheses regarding the joint regulation of airway ion/water fluxes and mucociliary transport. The parameters monitored, individually or in combination, included CBF, TMV, BMC, and volumetric respiratory tract fluid output (RTFO) at the larynx in complementary experiments.
METHODS

The experimental procedures and protocols for these studies on dogs were approved by the Animal Care Committee of the Biological Resources Laboratory at the University of Illinois at Chicago. The Biological Resources Laboratory is sanctioned by the American Association for Accreditation for Laboratory Animal Care.

Dog preparation. Six healthy adult mongrel dogs (5 male, 1 female; 1–3 yr old; and 20–30 kg in weight) were studied, as described in the companion paper (31). Each dog was fasted for 12 h before each study but was allowed free access to water. The dogs were anesthetized with intravenous (iv) thiamylal sodium and placed in the supine position. The head and neck of the dog were extended, and the maxilla was stabilized with gauze across the premolar teeth. Airway patency was maintained in the control study (common to this study and the companion study) by a size 10 endotracheal tube through which the dog was allowed to breathe spontaneously. For perturbation studies, airway patency was maintained by using a 37-Fr double-lumen endobronchial tube with the endobronchial portion distal to the tracheal cuff trimmed. Thus spontaneous inspiration was achieved through an aerosol nebulizer directly attached to one lumen while expiration proceeded through the other lumen without aerosol dilution. The endotracheal tube cuff in every experiment was positioned within or just distal to the larynx. The dog was allowed to inhale room air spontaneously or through a nebulizer. The resulting eucapnic ventilation was confirmed by monitoring end-expired carbon dioxide concentration, which remained stable (±4.5%) throughout all experiments.

Arterial blood samples were drawn from an indwelling femoral artery catheter at 30-min intervals throughout the experiment and were analyzed for pH, oxygen, and carbon dioxide content to rule out the occurrence of inadequate blood oxygenation or acid-base homeostasis. Mean values (±SE) for pH were 7.34 ± 0.00, and the partial pressures of carbon dioxide and oxygen were 42.4 ± 0.3 and 79.8 ± 0.8 Torr, respectively. Electrocardiogram and femoral arterial blood pressure were monitored to verify normal circulation hemodynamics. Rectal temperature was monitored, and warm water blankets and electrical heating pads were used to maintain body temperature as necessary (36–38°C). Supporting fluid (0.9% NaCl injection, US Pharmacopoeia) at a nominal rate of 0.5 ml/min and maintenance doses of thiamylal sodium were delivered through a cephalic iv catheter. The dose of anesthetic was titrated to approach extinction of the palpebral reflex and jaw muscle tone and yet maintain minimal effect on the other monitored physiological parameters. Each dog was allowed to stabilize for at least 30 min after intubation before data collection.

Estimation of aerosol deposition in the lung during perturbation. The radioaerosol techniques used for quantifying deposition of aerosolized solutions within the lung conformed to those used in other investigations (6, 31). The animal model was calibrated for radioactivity count sensitivity to lung geometry and tissue absorption by iv injection of radiolabeled macroaggregated albumin. The macroaggregated albumin was labeled with ~3.7 MBq of 99mTc and was assumed to be totally trapped within the pulmonary alveolar capillary bed within a few minutes of the injection. The known injection activity was correlated with the activity of a point source (typically 50 MBq) placed 50 cm above the gamma camera. The gamma-camera sensitivity was recalibrated to a similar reference point source each day of experimentation and in general was ~4,000 counts·min⁻¹·MBq⁻¹ in the lung. Aerosols used to perturb the airway lining fluid were characterized for volumetric rate of deposition within the lung by aerosolization of solutions of human serum albumin labeled with 99mTc. The radioactively labeled albumin was dispersed in the particular solution to be aerosolized at ~70 µg/ml albumin and 7.4 MBq/ml radioactivity. Aerosol delivery conditions to the dog mimicked the specific perturbation. The duration of aerosol delivery during these characterization studies varied between 1 and 4 min.

Measurement of CBF. CBF within the trachea was measured by nonstationary time-frequency analysis of laser light scattering described elsewhere in detail (3, 31). In this system, a helium-neon laser beam was transmitted down the axis of a hollow stainless steel probe (30 cm long, 8 mm OD). The beam exited perpendicular to the probe such that the ~7-µm focal spot, 5 mm from the probe surface, was coincident with the surface of the ciliated epithelium in the midtrachea of the anesthetized animal. The endotracheal tube was positioned cephalad and dorsal to the probe. Time-frequency analysis of the signals from the backscattered photons determined CBF values approximately every 3 s. Data collection continued for at least 60 min after the perturbation was begun.

Aerosol deposition for mucociliary transport studies. An iron oxide colloid was prepared (15) and labeled with 99mTc (29) as in the companion paper (31). The system to generate the concentrated aerosol has been described elsewhere (19) and is summarized in the companion paper. Briefly, the radioaerosol delivery system was maintained at 2 kPa positive pressure, and aerosol of mass median diameter of 10 µm (31) was delivered to the animal through a solenoid-controlled valve that opened 1 s every 10 s; a second valve allowed exhalation for 3 s immediately after inhalation. The radioactivity deposited was monitored throughout the 2-min inhalation period with a gamma camera positioned beneath the table supporting the animal. The inhalation period was considered complete when 3 MBq were deposited in the lungs or when 2 min had elapsed, whichever occurred first.

Measurement of BMC. Retention of radioactivity-labeled iron oxide particles within the lungs was measured each minute beginning immediately after radioaerosol delivery and continuing for 90 min. Scintigrams were collected and processed by using a computer interfaced with the camera. Perturbations of airway lining fluid were begun ~20 min after radioaerosol delivery to allow stabilization of mucociliary transport indexes before perturbation. The endotracheal tube was not in the camera field and was left in place throughout the study. The entire camera field was used in the determination of iron oxide retention.

At ~24 h after each delivery of iron oxide radioaerosol, the dog was returned to the gamma camera table for a measurement of the radioactivity retained within the lungs. The dogs had been previously trained to lie motionless supine on the gamma-camera table for up to 10 min without administration of anesthesia (chemical restraint). The animals were fed before this measurement, and ingestion of food assisted clearance of any radioactivity from the esophagus and stomach. The iron oxide retained in the lungs, expressed as a percentage of that present at the end of radioaerosol deposition (R24%), was considered an index of deposition pattern correlated to alveolar deposition.

Measurements of retained radioactivity within the lung were corrected for background and radioactive decay from the end of radioaerosol deposition. The R24% was subtracted from the measurements of retention in the lung to estimate retention in the bronchi. With respect to the iron oxide present within the bronchi, clearance of iron oxide particles

from the bronchi by BMC was normalized to 0% at the start of each perturbation. Measurement of TMV. Impaction of radioaerosol particles at bifurcations of the airways created local concentrations of radioactivity. The average rate at which these radioactive boluses were transported along the trachea by mucociliary activity was measured by using six aligned 8-mm-thick scintillation detectors in conjunction with a six-channel count/time recorder, as previously described (31, 38). From time-response curves, the time at which a bolus was in direct apposition to a given detector was estimated (31) and TMV for each bolus was determined by linear regression. Only peaks that were evident in at least three channels were considered. Data collection continued up to 90 min after challenge was begun (up to 120 min after radioaerosol deposition).

Measurement of RTFO. RTFO was collected by using a modified endotracheal tube (Fig. 1). The concept takes advantage of the streamlining of tracheobronchial secretions on approach to the larynx to a small path width through the interarytenoid area or posterior commissure of the dog (2). The device was fabricated onto the double-lumen and standard endotracheal tubes described earlier (see Dog preparation). First, the cranial end of the deflated cuff was restricted circumferentially with fast-setting, flexible epoxy (DP-190, 3M Industrial Specialties Division, St. Paul, MN) so that only the caudal 2 cm of the cuff could be inflated. A stainless steel tube, 2 mm ID, was also affixed axially across one side of the deflated cuff, and secured circumferentially at both ends. A mucus collection catheter (polytetrafluoroethylene; 1.29 mm ID, 1.90 mm OD) was passed inside this tube so that its tip just protruded from the distal tube end and the retention foot. The catheter was free to move within this pathway and was adjusted independently of the endotracheal tube so that the catheter was close enough to the mucosa to allow fluid collection without obstruction of the catheter tip. A V-shaped retention foot, conforming to the interarytenoid groove and in line with the mucus collection catheter, was molded in epoxy near the caudal end of the endotracheal tube cuff, both to assist placement and to stabilize the device by preventing both caudal movement and rotation. The cuff was inflated in the larynx immediately distal to the vocal cords.

The other end of the catheter was connected to a test tube maintained in an incubator at 37°C. Gentle intermittent suction applied to the test tube caused any airway lining fluid at the posterior commissure to be transported within the catheter and collected in the test tube. Vacuum of up to 15 kPa was applied to the collection test tubes (and the RTFO collection catheter) during three sampling periods: 0–15, 30–45, and 60–75 min.

Collected samples sealed within the tubes were allowed to stand in the incubator for up to 1 h after the end of the experiment to allow for foam coalescence. The samples were then withdrawn into a graduated syringe to estimate RTFO volume. RTFO rates were estimated only from the volumetric sums of the last two samples (30–45 and 60–75 min) to avoid the influence of animal preparation before collection. These sums were divided by the time span from the end of the first collection to the end of the third collection, usually 1 h.

The representative nature of RTFO-collection sampling was examined by assaying samples for radioactivity (CRC-5; Capintec Instruments, Ramsey, NJ) and comparing the results to BMC of radioactivity calculated from gamma-camera measurements taken during the sampling. The samples assayed were from four experiments in which the airway lining fluid was perturbed with delivery of aerosolized 0.85 M NaCl. The cumulative recovery of radioactively labeled iron oxide in consecutive sampling periods was evaluated as a percentage of the estimated bronchial radioactivity deposition, determined from the gamma-camera calibration of count sensitivity (counts·min⁻¹·MBq⁻¹).

The canine tracheal circumference was used in calculation of an RTFO transport depth (see Data analysis and statistical significance). It was estimated by magnification scaling of radiographic imaging and was confirmed in postmortem study in one of the dogs considered representative of the cohort.

Perturbations of airway lining fluid. Aerosolized water, 3.4 M NaCl (20% solution by weight), 0.85 M NaCl (5% solution by weight), 0.85 M sodium gluconate, and 0.85 M choline chloride were deposited by spontaneous breathing of room air through an aerosol nebulizer by using a one-way valve on the nebulizer inlet and another on the outlet side of the double-lumen endotracheal tube. The 0.85 M concentration level was chosen to approach the solubility limit of NaCl for maximal perturbation. The 0.85 M concentration level was chosen to approach the solubility limit of sodium gluconate. Aerosols were generated by using a Microstat ultrasonic nebulizer (Mountain Medical Equipment, Littleton, CO) directly attached to the endotracheal tube inlet, with minor nebulizer modifications designed to increase delivered aerosol dose. Spiral grooves on the baffle were removed, and two mouthpiece tubes were joined vertically to increase the aerosol chamber volume. Under similar conditions, this nebulizer is reported to produce mass median diameters of 2.6–3.4 µm (14), although particle-size distribution for these aerosols was not measured during these studies. The unit was constructed of transparent plastic so that reservoir volume and aerosol output were easily visualized. Deposition continued throughout the 90-min study; the nebulizer reservoir was refilled between breaths when it appeared to be low.

Dog protocol. Each dog underwent six studies: one control study and five studies of differing airway lining fluid perturbations. In study 1, to evaluate the response of the airways to additional water only, water aerosol was delivered. In studies 2 and 3, to evaluate the response of the airways to added water and NaCl, aerosolized solutions of 0.85 and 3.4 M NaCl were delivered. Finally, in studies 4 and 5, to evaluate the response of the airways to perturbation of the Na⁺/Cl⁻ ratio

![Fig. 1. Respiratory tract fluid output (RTFO) collection system at posterior commissure of larynx. Catheter was attached to a test tube maintained at 37°C. Application of gentle suction to test tube caused fluid at posterior commissure to move along catheter for collection within tube.](image-url)
in mucosal fluid, aerosolized solutions of 0.85 M choline chloride and sodium gluconate were delivered. These aerosols were delivered continuously during spontaneous ventilation over the period of 1 h. The control study for each dog included 2 min of mechanical ventilation with conditioned air, with an inspiratory flow of 60 l/min, tidal volume of 600 ml, and 16 inspirations/min, as in the companion paper (31). Perturbations began immediately after this ventilation. The perturbation was repeated up to three times in complementary experiments while one or more of the following assays were recorded: measurement of CBF, measurement of TMV and BMC, and measurement of RTFO. CBF was always recorded alone, as a separate set of experiments, to eliminate any interference caused by the presence of the stainless steel CBF probe within the trachea on the other assays.

RTFO was recorded during TMV-BMC experiments in some studies and was recorded during separate experiments in other studies because these assays were fully developed only after some studies were already complete. The control study and the water aerosol study consisted of three sets of experiments (CBF, TMV-BMC, and RTFO). Four studies consisted of two sets of experiments (CBF and either TMV-BMC or TMV-BMC-RTFO). In three of these studies (0.85 M NaCl, sodium gluconate, and choline chloride), RTFO was recorded during the experiment coincident with recording of TMV-BMC. In the other study, with 3.4 M NaCl, only CBF and TMV-BMC were measured. This protocol resulted in a total of 14 experiments for each dog, not including gamma-camera calibration and aerosol characterization studies. These experiments were separated by a quasi-random period of weeks to months. Animals were not studied more than once per week.

Data analysis and statistical significance. The same cohort of dogs was used in the control and perturbation studies. Statistical analysis was performed by using commercially available software (SigmaStat 1.0; Jandel Scientific Software, San Rafael, CA). R24% was examined for differences among the treatment groups by using the Friedman repeated-measure analysis of variance on ranks. Blood-gas results, monitored indexes (CBF, TMV, BMC, and RTFO), and the derived indexes described below were all analyzed by using the Kruskal-Wallis analysis of variance by ranks to evaluate significant differences among the treatment groups. When significant differences in the median values among the treatment groups were detected, Dunn’s method was used to isolate the group or groups that differed from the control. A P value of <0.05 was considered significant. The indexes were evaluated in time periods of 15 min, with time 0 coinciding with the start of the perturbation. The time span of 15 min was chosen arbitrarily. For ease of notation, subscripts separated by commas refer to the period in minutes from which the values were derived (e.g., BMC0,15). The results, which did not satisfy testing for distribution normality, are nevertheless related in text and figures as means ± SE.

To evaluate the effects of the perturbations on the depth of airway lining fluid being transported along the trachea, an estimated depth in micrometers was derived. If it is assumed that uniform transport occurred along the essentially circular, cylindrical surface of the trachea, individual RTFO (cm²/time) can be factored by the TMV (cm/time) and the estimated circumference of the trachea (cm) to obtain an estimate of the tracheal fluid depth (cm) for each RTFO collection period, i.e., 15–45 or 45–75 min. Comparisons were based on the cohort over a span of 15–75 min.

To compare the effects of airway lining fluid perturbations on the interrelationship between airway regions, an index of the coordination was derived in percent bronchial clearance per centimeter of tracheal mucus transport from individual BMC and TMV results during the 30 min after the start of the perturbation. TMV values were assumed to be indicative of the TMV from either time 0 or any previous TMV value to the next TMV value or, if no following values existed, to the end of the experiment. Specifically, the BMC over each minute was divided by the TMV at that time. Comparisons were based on the set of all animals over the 30 min after the beginning of challenge.

To compare the effects of airway lining fluid perturbations on the conversion of tracheal ciliary beat into mucus transport, an index of cilia-mucus interaction was derived in micrometers transport per ciliary beat from individual TMV and CBF results. By using the same assumptions and time intervals as above, TMV-CBF could be estimated for each CBF data point. Comparisons were based on the set of all animals over the entire experiment.

RESULTS

The rate of water aerosol deposition within the lungs, evaluated by using water as the diluent of a radioactive albumin solution, was estimated at 180 ± 30 mg/min. When 3.4 M NaCl aerosol was administered, 70 ± 20 mg/min were deposited in the lungs. This equates to deposition rates of 14 ± 4 mmol/h for Na+ and Cl−. When 0.85 M NaCl, 0.85 M choline chloride, and 0.85 M sodium gluconate solutions were administered by inhalation of aerosol, measured radioactivity indicated deposition rates of 140 ± 50, 130 ± 50, and 70 ± 30 mg/min, respectively. This equates to respective deposition rates (in mmol/h) of 7.1 ± 2.6 NaCl, 6.6 ± 2.6 choline chloride, and 3.6 ± 1.5 sodium gluconate.

The temporal responses of CBF, TMV, and BMC for the control, water, and 3.4 M NaCl experiments in six dogs can be compared in Fig. 2. For the study in which 3.4 M NaCl was aerosolized, CBF was slightly but significantly higher than the control study in the 15 min before the perturbation. The CBF from the first 15 min after the sham control perturbation was started was also slightly but significantly elevated with respect to subsequent time periods (Fig. 2A). This is possibly caused by mechanical disturbance of the system inherent in moving equipment and the coupling of ventilatory circuits.

CBF throughout 75-min inhalation of aerosolized 3.4 M NaCl (Fig. 2A) was markedly increased from the control of 9.0 ± 0.1 to 11.1 ± 0.2 Hz. CBF was most elevated between 15 and 45 min of exposure. CBF also increased slightly during 75-min inhalation of water aerosol (CBF 0.75, 9.6 ± 0.1 Hz) in comparison with the control study (CBF 0.75, 9.0 ± 0.1 Hz), being most elevated after 30 min of exposure. There was no consistent trend in TMV over the time course of the control study (Fig. 2B). TMV increased in the study when aerosolized 3.4 M NaCl was inhaled (TMV 0.07, 18.0 ± 0.7 mm/min) far above the control TMV values (TMV 0.07, 10.9 ± 0.7 mm/min). During an hour of exposure, water aerosol had little if any effect on TMV, with a possible trend toward an increase in the subsequent 30 min.

In the radioactivity retention studies, the R24% for the control study was 36.9 ± 3.0% while R24% were 35.4 ± 3.8 and 28.9 ± 10.1% for the studies in which
aerosolized water or 3.4 M NaCl were administered, respectively. This indicator of radioaerosol deposition pattern did not significantly vary either between test perturbations or between individual dogs. In Fig. 2, it can be seen that BMC increased during continuous inhalation of aerosolized 3.4 M NaCl (18.3 ± 1.2 vs. 14.0 ± 1.4%, BMC30,45 vs. control, respectively). Mucociliary clearance in the 15 min before the airway perturbation was started was slightly lower than in the control study for both aerosolized water and 3.4 M NaCl. Water aerosol may have slightly reduced BMC during the first 15 min of delivery, but BMC was not significantly different from the control study thereafter.

The temporal response of the mucociliary transport system, as indicated by CBF, TMV, and BMC, to depositions of aerosolized 0.85 M NaCl, sodium gluconate, and choline chloride, is shown in Fig. 3. CBF during continuous inhalation of aerosolized 0.85 M NaCl is initially slightly higher than CBF during the control study (Fig. 3A). During inhalation of aerosolized 0.85 M sodium gluconate (CBF30,75, 8.1 ± 0.1 Hz) and choline chloride (CBF30,75, 7.4 ± 0.1 Hz), CBF was lower than the control study (CBF30,75, 8.8 ± 0.2 Hz), particularly between 30 and 75 min of exposure. However, before the perturbations were started, CBF was lower than the control CBF, with the study in which aerosolized choline chloride was inhaled attaining statistical significance. TMV increased during the first 30 min of exposures to aerosolized 0.85 M NaCl, sodium gluconate, and choline chloride (Fig. 3B). In the following 30–75 min, TMV in the study using aerosolized 0.85 M NaCl (TMV30,75, 12.1 ± 1.1 mm/min) appears to return towards the values of the control study (TMV30,75, 10.9 ± 0.7 mm/min). During this same time, however, TMV remained elevated during aerosolized sodium gluconate (TMV30,75, 16.4 ± 1.0 mm/min) and choline chloride exposures (TMV30,75, 15.6 ± 0.8 mm/min). In the radioactivity retention studies, the R24% values were 37.7 ± 8.1, 47.5 ± 6.9, and 51.9 ± 3.4% for the studies in which 0.85 M NaCl, sodium gluconate, or choline chloride, respectively, were administered. Again, R24% was not significantly different between control and test perturbations or between individual dogs. BMC was slightly lower in the 15 min before inhalation of the NaCl, choline chloride, or sodium gluconate aerosol than was BMC in the control study during the comparable 15-min period. The BMC during delivery of aerosolized 0.85 M NaCl (Fig. 3C) was also slightly lower than the comparable BMC in the control study (14.0 ± 1.4 vs. 10.9 ± 0.7%, control vs. BMC30,45, respectively). Compared with the control study, BMC increased during inhalation of both aerosolized sodium gluconate (BMC30,45, 18.4 ± 1.0%) and aerosolized choline chloride (BMC30,45, 18.1 ± 1.1%).

Coordination of regional tracheobronchial transport, i.e., between BMC and TMV, exhibited increased tracheal transport relative to bronchial clearance for all perturbations compared with the control (Fig. 4), including water aerosol and aerosolized choline chloride, sodium gluconate, and both NaCl solutions. As can be seen in Fig. 5, inhalation of aerosolized water was the only perturbation to significantly decrease the conversion of ciliary beat into mucus transport. This decreased from 57.8 ± 0.7 µm/beat in the control study to 50.5 ± 0.6 µm/beat with aerosolized water.
water. The cilia-mucus interaction was slightly increased by inhalation of aerosolized 0.85 M NaCl (73.0 ± 0.7 µm/beat). The coupling was most markedly improved during exposure to aerosolized 0.85 M sodium gluconate or choline chloride (98.1 ± 0.9 and 103.7 ± 0.9 µm/beat, respectively).

The accumulated collection of radioactivity for the three successive sampling periods in four experiments (by using aerosolized 0.85 M NaCl challenge) was similar to the temporal trend in BMC evaluated during these same experiments (Fig. 6). RTFO for the control and perturbation studies for each of the sampling periods is compared in Fig. 7.

RTFO collection rate, based on the latter two sampling periods, was significantly elevated above the
control study (2.2 ± 0.5 mg/min) by water aerosol (18 ± 2 mg/min) and aerosolized 0.85 M NaCl, choline chloride, and sodium gluconate (33 ± 7, 38 ± 3, and 38 ± 7 mg/min, respectively). RTFO was not measured during delivery of aerosolized 3.4 M NaCl. RTFO during inhalation of aerosolized 0.85 M NaCl was not significantly increased compared with RTFO during water aerosol. RTFO was increased by inhalation of aerosolized hypertonic solutions of choline chloride and sodium gluconate vs. aerosolized water alone. The mean diameter of the trachea for the dogs used in these studies was estimated as 19 mm, based on postmortem measurement of one dog, and radiographic image scaling from another. By elimination of the time dimension from RTFO and TMV and by assumption of a uniform circular cylinder of transport, the estimated airway lining fluid transport depth in the control study was 4.5 ± 0.9 µm. Estimated transport depths for perturbation by using water aerosol and aerosolized 0.85 M NaCl, choline chloride, and sodium gluconate were 41 ± 7, 48 ± 8, 42 ± 7, and 53 ± 12 µm, respectively.

**DISCUSSION**

Consistent with our first postulate, the airways exhibit an absorptive capacity when presented with a purely aqueous perturbation. The increase in RTFO from 2.2 ± 0.5 to 18.3 ± 1.6 mg/min during water aerosol delivery, while quite remarkable, was still an order of magnitude less than the rate of aerosol deposition, 180 ± 30 mg/min, indicating a considerable portion of the water deposited either was absorbed or not accessible to mucociliary transport. Other indexes of mucociliary transport (BMC, TMV) remained similar to the control study, in agreement with the observations of Parks et al. (17), although TMV trended upwards at extended times (Fig. 2B). The fact that CBF in the control study was elevated during the first 15-min period compared with the following periods is likely caused by disturbance of the dog inherent in moving equipment and the coupling of ventilatory circuits. The small, but significant, stimulation of CBF during continuous inhalation of water aerosol is consistent with CBF increase caused by hypotonic swelling-induced release of intracellular calcium (25). This is also consistent with CBF increase associated with an increase in the intracellular [Na\(^+\)]/[Cl\(^-\)] ratio (34). This is predicted to result from K\(^+\) and Cl\(^-\) efflux in response to cell swelling.

Consistent with our second postulate, hypertonic perturbation with high [Na\(^+\)] and [Cl\(^-\)] (continuous inhalation of aerosolized 3.4 M NaCl, 70 ± 20 mg/min) increased BMC, TMV, and CBF (Fig. 2). These increases are also in agreement with the enhanced mucociliary clearance in patients with chronic obstructive lung disease (18), or in patients with cystic fibrosis after inhalation of aerosolized 1.2 M NaCl (23), or in asthmatic and healthy subjects after inhalation of 2.4 M NaCl (4), although these inhalations were administered for a much shorter duration. These responses contrast markedly with those observed during dry-air inspiration, which acutely decreased CBF, TMV, and BMC (31). In addition to direct effects on epithelial ion and water transport, high NaCl concentration in the airway lining fluid may activate sensory nerves (20) and induce neurogenic inflammation (28) as well as release mediators from mast cells and basophils. Mediators such as histamine may augment transepithelial water transport induced by a hyperosmotic perturbation (5). Such mediator release might also activate adrenergic, cholinergic, peptidergic, or other cell receptors to stimulate CBF (32, 33).

In comparison, continuous deposition of a larger fluid load (140 ± 50 mg/min) at a lesser NaCl concentration (aerosolized 0.85 M NaCl), induced increases in TMV and CBF that were smaller and not sustained, whereas BMC marginally decreased (Fig. 3) instead of increasing. It can be concluded that the lesser NaCl deposition (7.1 ± 2.6 mmol/h) activated mechanisms within the...
airway which were similar to those activated by the greater NaCl deposition (14 ± 4 mmol/h), although not to the same extent. Furthermore, the lesser NaCl deposition may not have surmounted the ability of the airways to actively absorb Na⁺ and Cl⁻ ions (without substantial water flux, Ref. 21), as indicated by the minimal perturbation of CBF, TMV, and BMC, consistent with our third postulate. The recovery of fluid from the airways, RTFO, expressed as a percentage of total fluid deposition, was greater during inhalation of aerosolized 0.85 M NaCl (24%) than during inhalation of water aerosol (10%), suggesting that the NaCl in solution reduced the ability of the airways to absorb fluid.

During continuous inhalation of aerosols containing ions of lower epithelial permeability (0.85 M sodium gluconate or choline chloride; see Fig. 3), cellular volume was likely reduced by osmotic water movement, similar to the volume reduction observed with mucosal fluid hypertonic in mannitol (30). Consistent with our fourth postulate, mucociliary transport was stimulated by ions of lower epithelial permeability, even though there may have been a concurrent decrease in CBF. Although equimolar solutions were aerosolized, less sodium gluconate was delivered to the lung (70 ± 30 mg/min) compared with the other salts (130 ± 50 and 140 ± 50 mg/min for sodium gluconate and choline chloride, respectively), based on deposition studies. The cause for this is unknown, but it might result from the lower water solubility of sodium gluconate, which may affect aerosol particle hygroscopic growth and equilibrium particle size in the airways. Considering the dose of agent administered, the mucociliary transport system response was largest during continuous inhalation of aerosolized 0.85 M sodium gluconate, indicated by both an increase in BMC (Fig. 3C) and by a sustained increase in TMV (Fig. 3B). During the aerosolized 0.85 M choline chloride and sodium gluconate depositions, the RTFO recoveries (Fig. 7) with respect to deposition were higher (29 and 54%, respectively) vs. aerosolized 0.85 M NaCl (24%) or water (10%), consistent with ions of lower epithelial permeability further impairing the ability of the airways to absorb excess fluid.

TMV increase relative to BMC during all perturbations with increased fluid load (Fig. 4) is predictable, considering the necessity for the transport rate in the trachea to be larger than its tributaries, to avoid the accumulation of secretions at the tracheobronchial junction (36). Interaction of the airway lining fluid with the cilia is required for effective transport (37). As all perturbations, except one (0.85 M NaCl), increased the rate of clearance of radioactivity from the lungs (Fig. 3), it would appear that the mucociliary transport system can cope effectively with moderate increases of airway fluid to maintain airway function. However, these experiments were conducted in the horizontal supine dog, not in an upright lung where gravity may be expected to play a larger role. The fact that the translation of ciliary beat into mucus movement was increased by all perturbations (Fig. 5) except one (water aerosol) suggests that decreasing the net water flux into the epithelium will improve ciliary mucus coupling.

The samples of RTFO collected appear to be representative of the airway lining fluid transported by mucociliary interaction, based on comparison of the cumulative airway output of radioactivity and BMC (Fig. 6). The collection of RTFO [2.2 ± 0.5 mg/min (0.13 ± 0.03 ml·kg⁻¹·day⁻¹)] during the control study in dogs with a mean weight of ~25 kg (Fig. 7) is consistent with daily tracheobronchial secretory output in humans of 0.1-0.3 ml·kg⁻¹·day⁻¹ (27), supporting the relevance of our dog model to the understanding of the regulation of the mucociliary transport system in humans.

It is notable that all perturbations resulted in similar estimated fluid transport depths (ranging from 41 ± 7 to 53 ± 12 µm), much greater than the control value (4.5 ± 0.9 µm). This indicates a relatively constant association between RTFO and TMV during the aerosol perturbations, possibly representing an upper limit of cilia-mucus interaction. Alternatively, this might simply illustrate an overpowering effect on RTFO of aerosol deposition local to the tip of the collection catheter.

The responses of the mucociliary transport system to these aerosol perturbations are consistent with the following model. The transport of water across the epithelium is bidirectional, with the direction of net water transport across the epithelium being regulated by intracellular Cl⁻ and Na⁺ contents and by cell volume, acting as second messengers. That is, an increase of cell volume promotes an increase in net water flux from the airway lumen to the submucosa, and a decrease in cell volume (due to relatively membrane-impermeable ions in the luminal fluid). This promotes an increase of water transport from the submucosa to the mucosa. Induced cell volume changes cause regulatory adjustment of ion and water transport in and out the cell, as well as across the epithelium, in an effort to return the intracellular concentrations of macromolecules (16) to the basal conditions and in so doing regulate the depth and toxicity of the airway lining fluid. When ions that are relatively permeable to transepithelial transport, such as Na⁺ and Cl⁻, are added with excess fluid, the airways respond in such a manner as to reduce the excess fluid load, consistent with the observations of Price et al. (21). High concentrations of hyperosmolar solutions might also activate additional (mediator-induced) mechanisms as discussed in the companion paper (31). Thus we propose that the airway epithelium possesses mechanisms to stabilize the osmolarity, the ion content, and the volume of the surface airway lining fluid. However, the signal transduction mechanisms whereby the airways sense airway fluid depth and ion content remain to be elucidated. The integration of these signal transduction mechanisms with the respective roles of the potential transepithelial water transport mechanisms, i.e., diffusion, electroosmosis, active water transport, and aquaporin expression (7, 10), into the performance of the mucociliary transport system, will provide information on the pathophysiology of impaired mucociliary function and its alleviation.
In concert, these data indicate that there is a low basal level of airway hydration, and the mucociliary transport system can cope with markedly increased fluid loads. Deposition of high NaCl concentration generates mucociliary responses (increased BMC, TMV, and CBF) that contrast with results from dry-air inspiration (decreased BMC, TMV, and CBF). Whereas in the companion paper we suggest that there are other responsive alterations (decreased BMC, TMV, and CBF) that are involved in the response to fluid challenges of decreased and increased osmolarity. The airways appear to have transepithelial compensatory mechanisms that reduce the impact of moderate increases in NaCl and hydration load. When these responses cannot adequately respond, due to the delivery of impermeable ions or very high tonicity, removal of the perturbation in these cases is effected by a stimulation of mucociliary transport. Both improved coupling and enhanced clearance can be achieved by the hydration of the airway lumen by using an aqueous hypertonic aerosol with judicious choice of the osmolyte. We thank A. Daza, who assisted in data collection, and particularly Dr. L. B. Wong for direction and assistance in the use of the CBF-measurement system. The project was supported by National Institute of Environmental Health Sciences Grant 5R01-ES-04317–07 and by the Veterans Affairs Medical Research Service. This work is based on a thesis of S. Winters, submitted in partial fulfillment of the requirements for the PhD degree in chemical engineering at the University of Illinois at Chicago, 1995. This work was previously presented in abstract form: S. Winters, A. V. Daza, and D. B. Yeates. Am. Rev. Respir. Dis. 147: A1010, 1993; S. Winters and D. B. Yeates. Clin. Res. 41: 675A, 1993; and S. Winters and D. B. Yeates. Am. J. Respir. Crit. Care Med. 149: A28, 1994. Address for reprint requests: D. B. Yeates, Dept. of Medicine (MC 788), Univ. of Illinois at Chicago, 1940 West Taylor St., Rm. 212, Chicago, IL 60612 (E-mail: YEATES-D@UIC.EDU). Received 7 March 1996; accepted in final form 11 June 1997.

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