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Rheology of mucus and transepithelial potential difference: small airways *versus* trachea

E.M. App*†, J.G. Zayas*, M. King*

Rheology of mucus and transepithelial potential difference: small airways versus *trachea. E.M. App, J.G. Zayas, M. King.*

ABSTRACT: The transfer of water across the airway epithelium is closely related to the transepithelial potential difference (PD). Thus, PD should be directly involved in the regulation of airway intraluminal water content and, by extension, mucus rheology. Experiments by Boucher and co-workers (*J Appl Physiol*, 1980; 48: 169; and 1981; 51: 706) indicated that the values of PD in the small airways of dogs were considerably lower than in the trachea or mainstem bronchus. This fact suggests that water is increasingly removed from the airway lumen in the cephalad direction, and provides a possible mechanism whereby airway flooding is avoided as the total airway cross-section diminishes mouthward.

We investigated this possibility by collecting and analysing mucus from the small airways and trachea of anaesthetized dogs and comparing our findings with measurements of PD. Mucus was collected on a cytology brush placed against the wall of the airway. Tracheal samples were taken from the lower lateral or anterior trachea, while small airway samples were taken from a 6th or 7th generation bronchus, chosen at random from either side. Measurements of PD were made at comparable sites. The mucus was analysed for its viscoelastic properties using the magnetic microrheometer technique.

PD in the 6th–7th generation bronchus was significantly less than in the lower trachea (4.1±1.3 *vs* 17.2±7.1 mV). The rigidity of mucus collected from the small airways (log mechanical impedance (G*) at 100 rad·s^{-1}) was significantly less than in the trachea (2.81±0.22 *vs* 3.01±0.29). Small airway mucus also had a significantly lower solids content than tracheal mucus (12.1±3.5 *vs* 16.5±2.7%). The findings are consistent with the view that ion-modulated transepithelial water transfer contributes to the regulation of the water content of airway mucus.

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The protection afforded by airway mucus can be considered as the first line of defence of the lungs, and effective clearance of the mucus is vital to its basic function [1–3]. Alterations in the physicochemical properties of mucus can result in changes in its clearability by ciliary and cough mechanisms [4–6], and could affect its barrier function [7, 8]. The velocity of mucociliary clearance (MC) is known to increase in the cephalad direction along the tracheobronchial tree [9–11]. MC is affected by both ciliary and mucous factors, and part of the mouthward increase in MC can be attributed to changes in the beat frequency, distribution and length of the cilia [12]. We wondered, however, if there were also changes in properties of mucus which could contribute to the peripheral-central gradient in MC.

A critical factor affecting the physical properties of mucus and ultimately its rate of clearance is its water content. The movement of water across the airway epithelium is believed to follow the transfer of ions [13, 14], and is related to transepithelial electric potential difference (PD) [15–17]. The major contribution to PD generation in high resistance epithelia is made by sodium and chloride ion fluxes. Increased sodium absorption, or increased chloride secretion, would ultimately lead to an increased PD (negative potential across the epithelium). This is facilitated for sodium by passive sodium flow through Na^+ channels on the apical membrane and by further active extrusion from the cell by Na^+/K^+-adenosine triphosphatase (ATPase) located on the basolateral membrane. The basolateral membrane also contains a Na^+/H^+-antiporter, sensitive to sodium and pH changes, as well as a Na^+/Ca^{++}-exchanger, a Na^+-coupled solute cotransporter and Na^+/Cl^--cotransporter. For chloride secretion, Cl^- is actively transported into the cell by the Na^+/Cl^--cotransporter on the basolateral membrane and secreted through

Cl^--channels on the apical membrane into the airway lumen [18].

There are many hypotheses relating to the mechanism for the transfer of fluid across the epithelium; the two main theories on this subject relate to paracellular and transcellular pathways. The first concept suggests that isotonic water passes through the epithelium of absorptive tissue *via* the lateral intercellular space. This concept is supported by findings that amphotericin B dilated the lateral intercellular space, which completely collapsed after exposure to ouabain [19], as well as the finding that aminophylline caused a shift of isotonic water into the lumen of dog trachea [14]. Both mechanisms are effective with regard to ion transport; aminophylline stimulated net secretion of Cl^- and amphotericin B induced a significant increase in Na^+ and Cl^- absorption. The second concept is based on the model of CURRAN and MACINTOSH [13], who proposed a shift of isotonic fluid across the epithelium in a two membrane, three compartment model, suggesting that fluid transport passes directly through the epithelium. The driving forces in this model are concentration gradients, osmotic gradients and hydrostatic pressure. The common concept in these theories is the idea that water transport and ion fluxes are interdependent mechanisms.

Experiments by Boucher and co-workers [16, 20] demonstrated that PD is considerably lower in the small airways than in the trachea of mainstem bronchus. Assuming that this finding indicates increasing sodium absorption in the cephalad direction, this suggests that water is increasingly removed from the airway lumen as the trachea is approached, and thus we are provided with a possible mechanism whereby airway flooding is avoided as the total airway cross-section diminishes mouthward. As a consequence of this cephalad progression in airway potential difference, one would predict that mucus in the lower trachea should contain less water and be more rigid than mucus in the small airways.

The purposes of this study were, thus: a) to confirm previous findings regarding the difference in transepithelial potential difference between small airways and trachea; b) to determine the rheological properties of mucus from these two airway sites; and c) to explore the interrelations between PD, mucus water content, rheology, and clearance.

Materials and methods

Animals

We examined 17 healthy dogs aged 1–6 yrs. They included five beagles with chronic tracheostomies (3 M/2 F, wt *ca* 10 kg) and 12 conditioned, mixed-breed, non-beagle dogs (8 M/4 F, wt 20–30 kg). The beagles were studied on 3–4 occasions each, separated by at least three weeks, while the mongrels were studied only once, although duplicate mucus samples were obtained from several of them. The dogs were anaesthetized with intravenous sodium pentobarbital (25–30 $mg \cdot kg^{-1}$, supplemented as required) and placed in the supine position. The dogs were usually intubated shallowly with a shortened no. 9 endotracheal tube, but on some occasions the airways of the beagles were accessed *via* the tracheostomy. Some of the dogs received humidified air for part or all of the procedure (Ringer aerosol generated by a Pari jet nebulizer). They all breathed spontaneously throughout the studies.

Mucus sample collection

We collected mucus by means of the cytology brush technique [21, 22], which involves placing a soft-bristle cytology brush (Mill Rose no. 151) against the airway wall under bronchoscopic guidance, and removing the brush once it is covered with sufficient mucus for analysis. The mucus is then removed from the brush with a scalpel blade and stored, refrigerated under paraffin oil, until analysed. Care was taken to avoid injuring the airway wall during mucus collection.

Mucus was sampled from both the lower trachea, 2–3 cm above the carina along the anterior or side wall, and from a "small airway" chosen at random from either side. This was approximately the 6th or 7th generation bronchus, as determined by counting bifurcations. Tracheal samples of 1–10 mg were usually obtained in 5 min of sampling, while 10 min were generally required in small airways in order to obtain sufficient mucus for rheological analysis. Blood contaminated samples were rejected for analysis.

Mucus rheological analysis

The magnetic microrheometer technique [23] was used to measure the viscosity and elasticity of microlitre quantities of mucus. A microsphere (*ca* 100 μm diameter) was carefully positioned in a 1–10 μl sample of mucus and oscillated under the influence of an electromagnet. The image of the steel ball was projected onto a pair of photocells which produce a signal that corresponds to the displacement of the ball. The photocell signal was plotted against the signal corresponding to the oscillating magnetic field gradient driving the microsphere. The resulting elliptical figures on the oscilloscope were analysed for phase and amplitude, from which the viscoelastic properties of the mucus were obtained.

In this paper, we report two viscoelastic variables: mechanical impedance (G*) (vector sum of "viscosity + elasticity"); and loss tangent (tan δ) ("viscosity/elasticity"), at low (1 $rad \cdot s^{-1}$) and high (100 $rad \cdot s^{-1}$) oscillation frequency. All samples were analysed within 4 h of collection. Samples less than 1 mg were rejected for rheological analysis.

Two derivative parameters, mucociliary clearability (MC) and cough clearability (CC), were computed

from *in vitro* relationships derived from model studies of clearance [6, 24]. The mucociliary clearability index (MCI), indicating clearability by normalized ciliary function, was computed from G* and tan δ at 1 rad·s^{-1}, and the cough clearability index (CCI) was computed from G* and tan δ at 100 rad·s^{-1}. Both indices relate negatively with log G*; MCI also relates negatively with tan δ, but CCI relates positively with it. Their respective formulae are as follows:

$$MCI=1.62 - 0.22\times\log G^*_1 - 0.77\times\tan \delta_1 \quad (1)$$

$$CCI=3.44 - 1.07\times\log G^*_{100} + 0.89\times\tan \delta_{100} \quad (2)$$

Mucus solids content

After rheological analysis, the mucus samples were usually frozen at -80° for periods up to one month. They were then rewarmed to room temperature, rinsed in petroleum ether to remove adherent paraffin oil, weighed on tared glass slides (Mettler HK 60 microbalance) and evaporated to dryness in a microwave oven (30 min at 750 W). The ratio of dry to wet weight was computed and expressed as % solids. The mucus collection rate (wet weight divided by collection time) was also determined as an index of mucus flux or secretion rate. Samples less than 2 mg were rejected for % solids analysis.

Transepithelial potential difference (PD)

The electrical potential difference (PD in mV) across the epithelial membrane was measured using two microelectrodes connected with KCl-saturated agar bridges to calomel half-cells. The calomel half-cells were connected to the high input of an electrometer (Fisher Accumet 950), which was grounded. Because of the saturated agar bridges, a correction for diffusion potentials was not necessary; liquid junction problems associated with perfusion methods were also avoided.

The reference electrode was guided with a polyethylene catheter and placed intravenously, which was shown in a preliminary study to be isoelectric with the subcutaneous space relative to the adventitia of the airways. The measuring electrode was guided by bronchoscopy and carefully placed in contact with the epithelium at either location. Prior to each experiment, the electrodes and agar bridges were connected to a common Ringer solution bath. Whenever electrode pairs differed by more than 1 mV PD, the electrodes and bridges were discarded. The PD method [25] was modified after the technique described by KNAUF *et al.* [26].

PD was measured at a similar but non-identical location to the one where mucus was collected. The sequence was varied in order to control for the possibility of systematic variations due to interference between the component variables being investigated.

Tracheal mucus velocity (TMV)

We measured TMV (*i.e.* tracheal mucociliary clearance rate) by visual inspection and timing of the transport of pulverized charcoal placed in contact with the epithelium [27]. TMV was measured in a similar region of the lower trachea to that used for mucus collection but on the opposite side of the trachea to avoid contamination of the mucus samples with residual charcoal. Mucus linear velocity was not measured in the small airways.

Statistical analysis

Statistical analysis was performed using a Macintosh computer and the statistical package Statview II. Since multiple samples were obtained from some dogs, an analysis of variance for repeated measurements was first performed to check for variations with time, and when these were ruled out, the rheological and PD data were averaged for each dog. Paired t-tests were then used to compare small airway and lower tracheal parameters, using $p<0.05$ as the standard of statistical significance. Group comparisons were made using unpaired, Student's t-tests.

Results

A total of 29 sample pairs of lower trachea *versus* small airway mucus were obtained, 16 sample pairs from 10 mongrel dogs and 13 sample pairs from the 5 beagles. Twenty two sample pairs of lower trachea *versus* small airway PD measurements were also obtained (12 sample pairs from 9 mongrels and 10 sample pairs from the 5 beagles). The data set was first subjected to an analysis of variance for repeated measures. This revealed that there were no significant variations over time or order of measurement for PD or any of the rheological parameters tested. Multiple data points from individual dogs were then averaged before performing further statistical comparisons. The overall results (small airways *versus* lower trachea) for mucus rheology are presented graphically in figure 1, while the data for the other parameters studied (PD, solids content, collection rate, and predicted clearability) are presented in figure 2.

Regional PD

As indicated in figure 2, the mean±standard deviation PD value in the small airways (6th–7th generation bronchus) was -4.1±1.3 mV (lumen negative). These values of PD are similar to those reported by others [16, 18, 28]. At this level of the bronchial tree, the values of PD were usually quite constant. In the lower trachea, *ca* 2 cm above the main carina, the mean PD value was -17.2±7.1 mV.

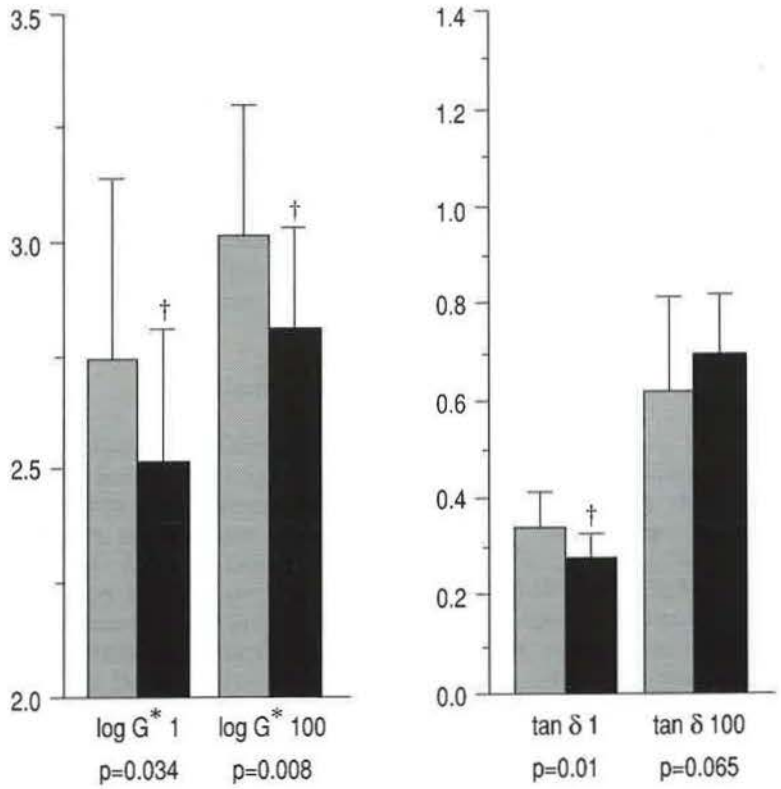

Fig. 1. – Comparison between lower trachea (▨) and small airways (■) for mucus rheological parameters (mechanical impedance (log G*), and loss tangent (tan δ), at 1 and 100 rad·s^{-1}). The different scales for the two parameters are indicated. The numbers beneath each pair represent the significant levels by paired t-tests; †: indicates significant difference between small airways and trachea.

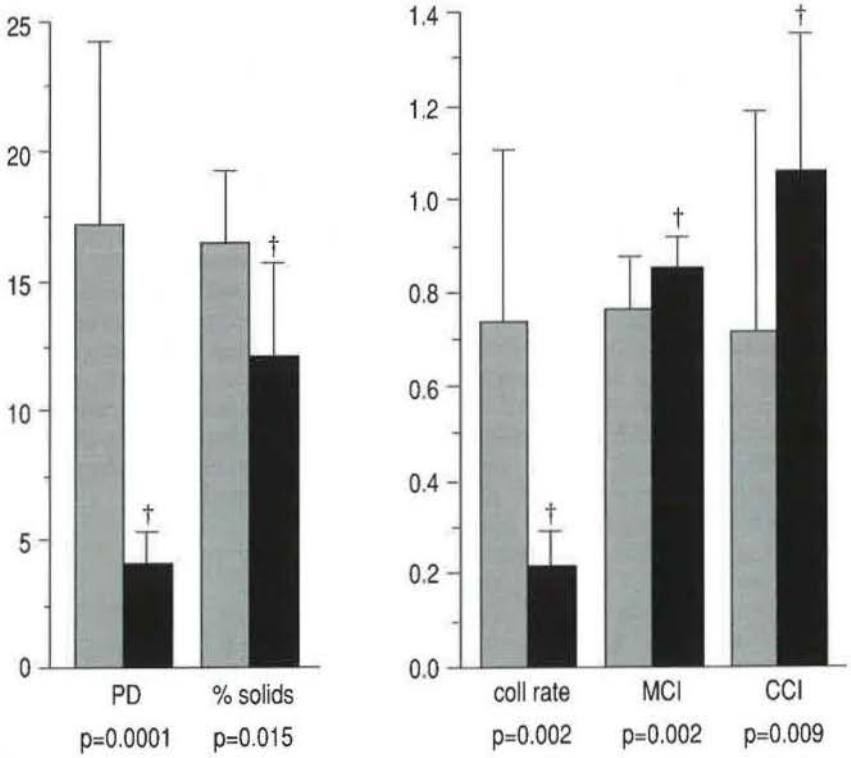

Fig. 2. – Comparison between lower trachea (▨) and small airways (■) for transepithelial potential difference (PD, -mV), mucus solids content (% solids), collection rate (coll rate, mg·min^{-1}), and predicted transportability by ciliary (MCI) and cough (CCI) mechanisms. Different scales for the various parameters are indicated. The numbers beneath each data pair represent the significance levels by paired t-tests; †: indicates significant difference between small airways and trachea.

The difference in the means of the PD values from the two locations was highly significant ($p<0.0001$), with complete separation of the two data sets. There was a significant degree of correlation ($p<0.01$) between the PD values at the two measuring sites in individual dogs, as indicated in figure 3.

Three of the dogs (all with chronic tracheostomies), while apparently healthy at the time of anaesthetization, had an obvious tracheitis, with an almost abolished PD in the lower trachea, while showing normal PD values in the smaller airways. Diagnostic procedures done by brushing the airways at this location and a bacteriological mucus analysis were negative, *i.e.* no detectable microorganisms or inclusion bodies. These dogs were re-studied about two months later, at which time we found a somewhat increased lower tracheal PD in the direction of normalization. On the other hand, some other dogs had PD values as high as -30 mV and confirm the PD values in the trachea of dogs reported by others.

Repeated bronchoscopy (including careful mucus collection) showed no effect on the PD, as long as the epithelium was not injured in a visible way. On the other hand, diagnostic procedures done by brushing the airways in order to obtain epithelial cytology specimens virtually abolished PD (PD=±0 mV), even when the PD value at a given location prior to brushing had been as high as -30 mV.

Regional mucus solids content

The small airway samples contained an average of 12.1±3.5% solids or dry weight compared to 16.5±2.7% for the tracheal samples ($p<0.02$). Thus, the water content of the small airway samples was greater than that of the tracheal samples (88 *vs* 84%).

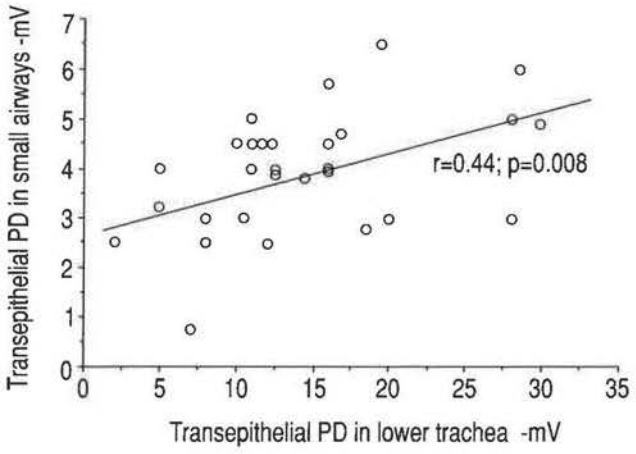

Fig. 3. – Transepithelial potential difference (PD) values (-mV) in small airways plotted against the corresponding values in the lower trachea. Age, previous environment and/or species differences may account for the broad overall range of PD values; however, within the same animal, there is a relatively constant relationship between small airways and lower tracheal PD.

The % solids content in these samples is higher than that seen in some other studies, presumably because of the effect of the pentobarbital anaesthesia [21].

Regional mucus rheology

The most important mucus rheological parameter, in terms of composition and function is G*, the mechanical impedance or "rigidity", measured at either 1 or 100 rad·s^{-1}. The variations in G* are usually expressed in logarithmic terms [6]. As indicated in figure 1, the mean value of log G* at both oscillation frequencies was less for small airway mucus than for mucus obtained from the lower trachea. The difference was statistically significant at both measurement frequencies ($p<0.01$ at 100 rad·s^{-1}, $p<0.05$ at 1 rad·s^{-1}); the variability at 100 rad·s^{-1} was less than at 1 rad·s^{-1}. In other words, the mucus in the 6th–7th generation bronchus is less rigid than in the lower trachea, and thus more appropriate for clearance by ciliary action. The values of G* in the lower trachea are consistent with our previous findings in pentobarbital anaesthetized dogs [21].

The other important mucus rheological parameter is the loss tangent tan δ. At low frequency (1 rad·s^{-1}), tan δ was significantly lower ($p<0.01$) for small airway mucus than for mucus in the lower trachea. At high frequency (100 rad·s^{-1}), tan δ was higher for small airway mucus, although the difference did not reach statistical significance. Both differences in tan δ project a more favourable clearability for small airway mucus, as do the differences in log G* (see Equations 1 and 2). Hence the derived parameters MCI and CCI, representing predicted mucociliary and cough clearability [6, 24], are significantly in favour of the mucus obtained from the 6th–7th generation bronchi ($p<0.01$ for both), suggesting that small airway mucus is much more suited for clearance than that found in the lower trachea.

Mucociliary clearance in the trachea

The tracheal mucociliary clearance rate, measured as transport of powdered charcoal as observed by bronchoscopy, showed a mean value of 11.6±6.1 mm·min^{-1}. These values represent typical data for anaesthetized dogs [27].

The TMV in the three dogs with evident tracheitis and low PD values (-PD <7 mV) was also very low (1–4 mm·min^{-1}) compared with data from the other dogs. TMV increased considerably in these dogs, into the normal range, when re-studied two months later.

Beagles vs *non-beagles*

The lower tracheal PD in the mixed breed, non-beagle dogs was considerably higher than that observed in beagles (-20.4±6.9 *vs* -11.4±0.6 mV;

p=0.014). This difference was maintained even when the low values obtained during apparent tracheitis were excluded. At the same time, the mucus samples obtained from the lower trachea of mongrel dogs had higher values of log G* and % solids than the corresponding specimens obtained from beagles, although these differences did not achieve statistical significance. There was no tendency for differences in small airways PD and mucus properties between the two groups of dogs. Within each group, the small airway/lower trachea differences in PD and mucus properties were still apparent, although the differences were more pronounced in the non-beagle dogs.

Humidification effect

We looked for differences in the bioelectrical and mucus rheological properties between dogs that were provided with a Ringer aerosol regularly throughout the experiment and those that were not. No significant differences were seen in PD, mucus rheology, or % solids in 18 matched pairs of lower tracheal measurements, although the small airway/lower trachea gradation in PD and mucus properties was still apparent in both treatment groups. There was a trend towards faster mucociliary clearance when the dogs were provided with humidified air (TMV 14.2±3.5 mm·min^{-1} after Ringer aerosol *vs* 8.9±6.0 mm·min^{-1} without).

PD vs *% solids and log G**

Figure 4A shows the relationship between % solids and PD. With increasing PD, the solids content in the lower trachea also increases. The wide range of solids content in small airways might be due to the physical presence of the mucus collection brush. The mean of the small airway samples had a low % solids content associated with a low PD, while with the tracheal samples, the higher % solids contents went along with the higher values of PD. The plotted correlation for the data from the lower trachea is significant (r=0.52; p<0.02). The relationship between log G* and PD was also positive (fig. 4B) r=0.35; p<0.05), but considerably weaker than the one obtained for solids content.

*log G** vs *% solids*

The relationship between log G* and % solids is shown in figure 5. In this plot, no overall relationship is visible, hence the regression line for each location is plotted separately. The expected correlation between log G* and % solids in the trachea is confirmed (r=0.46; p<0.01). The interrupted line shows a fictive regression for small airway samples, where there was no significant correlation (r=0.20) perhaps due to the presence of variable amounts of non-macromolecular components in the mucus.

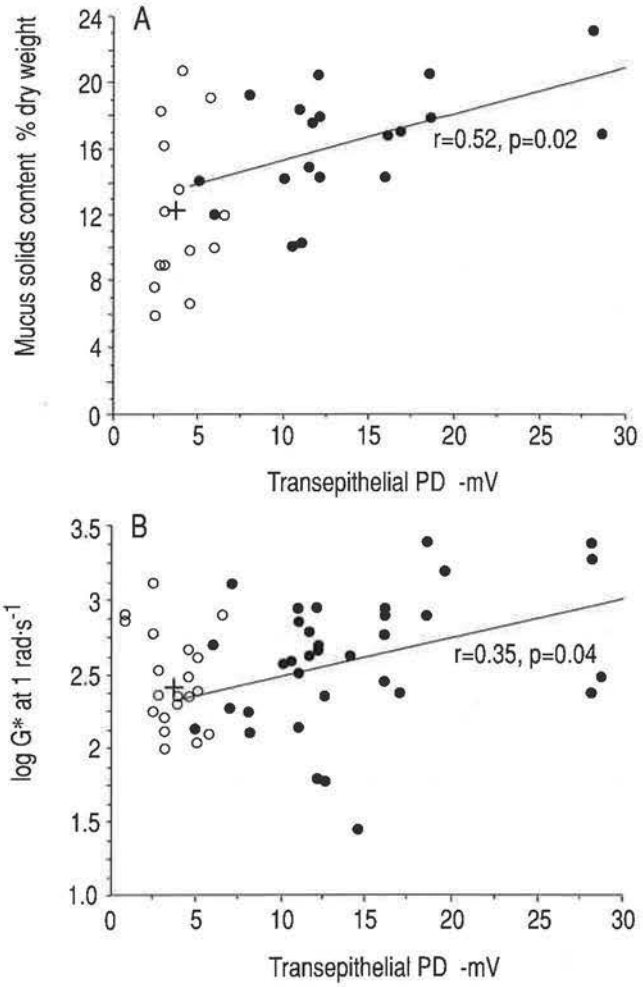

Fig. 4. – A) Mucus solids content (% dry weight) is plotted against transepithelial potential difference (PD) (-mV). B) Mucus viscoelasticity (G*) (at 1 rad·s^{-1}, plotted on a logarithmic scale) *versus* PD. The relationship in the lower trachea is also positive, but not as strong as that seen with % solids. The correlation indicated by the solid lines is for tracheal samples only. The mean data for small airways samples are indicated by the +. ○: small airway; ●: lower trachea.

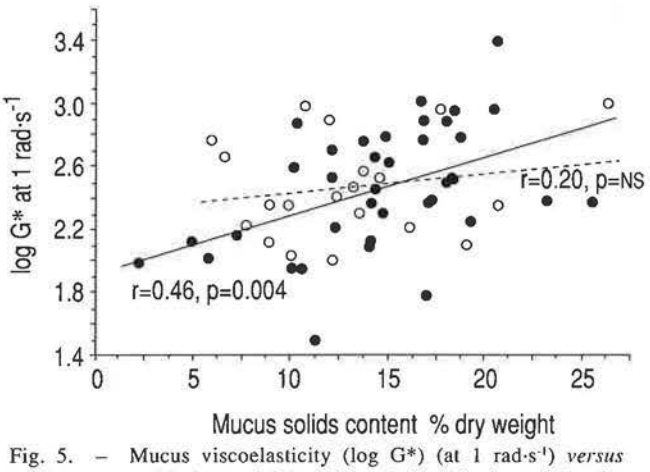

Fig. 5. – Mucus viscoelasticity (log G*) (at 1 rad·s^{-1}) *versus* solids content (% dry weight). The relationship is positive for lower tracheal mucus (solid line), as expected, but no significant relationship was seen for small airway mucus (dotted line). ○: small airway; ●: lower trachea; NS: nonsignificant.

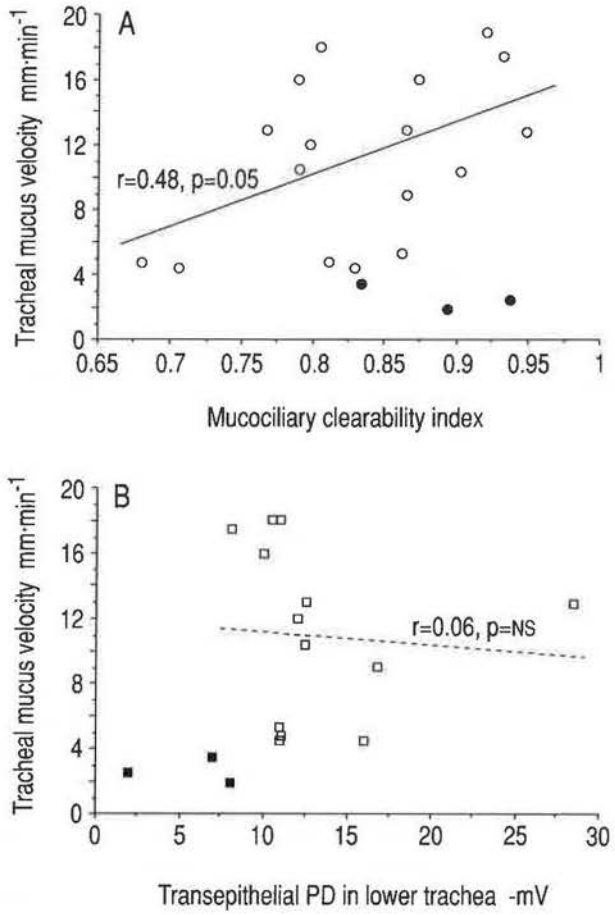

Fig. 6. – A) Tracheal mucus velocity (TMV) *versus* mucus clearability index (*i.e.* observed mucociliary clearance *versus* predicted clearability). The shaded symbols represent the dogs in which visible redness or apparent tracheitis was observed. There is no relationship until the three data points corresponding to the dogs with visible inflammation in the lower trachea are excluded. B) TMV *versus* PD. There is no significant correlation (r=0.06), even after exclusion of the inflammation points, indicated by the shaded symbols. PD: potential difference; NS: nonsignificant.

It may be an indication that other factors (such as low molecular weight proteins, cells, *etc.*) contribute to solids content, but not to cross-linking of the mucus in small airways.

TMV vs *PD and MCI*

Figure 6A shows the relationship between the observed tracheal clearance rate, TMV and the predicted MCI. The relationship is nonsignificant until the data points obtained for the three dogs with apparent tracheitis are excluded, in which case it becomes significantly positive ($r=0.48$; $p<0.05$), indicating a reasonable correspondence between observed clearance rate and that predicted from the mucus rheological properties. The relationship between TMV and log G* (not shown) became significantly negative with the same exclusions, because MCI depends principally on -log G* (Equation 1). The relation between TMV and PD (fig. 6B) was not significant, although there appears to be a minimum PD (*ca* -7 mV in beagle dogs), below which TMV becomes distinctly abnormal. In fact, the three observations of low PD and low TMV correspond to the occasions on which the trachea appeared to be inflamed. The low values of TMV cannot be attributed to rheologically inappropriate mucus, since the mucus on these occasions was relatively non-rigid, and gave relatively high values of the computed MCI. Thus, the poor mucociliary clearance rate on these occasions was probably due to ciliary dysfunction, associated with the inflammatory process or surface flooding affecting the interaction between mucus and cilia. Tracheal inflammation in these dogs appears to decrease TMV, PD and mucus rigidity, and increase mucus water content.

Discussion

The mean small airway PD value -3.7±1.5 mV measured between the 6th or 7th bronchus generation in the 17 dogs in the present study fits nicely with the data reported by others [16, 20]. Our tracheal PD data, (mean value -17.2±7.1 mV in the lower trachea) are somewhat smaller than those reported in the majority of published studies [16, 20, 28–31], although low values similar to ours have been reported by others [32]. The PD data reported above do not include the three dogs which had an obvious tracheitis associated with an almost abolished or highly decreased tracheal PD value; however, it is possible that other dogs had subacute inflammation that was not apparent to the eye or to routine analytical procedures. The other difference between our study and other investigations is the breed of dog used. We found that beagle dogs had considerably lower PD values than did non-beagle, mixed breed dogs, even when in apparent good health. The PD values that we found for the mongrel dogs (mean -20.4±6.9 mV) are not far from the ranges reported by others, and some of these mongrel dogs had lower tracheal values as high as 30 mV.

Other factors, which could have led to the unusually low values of PD in our study, include various technical factors, the effect of anaesthesia, an ageing effect, and the possibility of significant within trachea differences in PD. Indeed, we have now accumulated evidence [25, 33] that substantial intratracheal differences in PD exist, with a distinct caudal-cephalad gradient (more negative values subglottically). The ageing effect may be important to PD [33] as it is to mucociliary clearance [34, 35], but with the narrow age range of the dogs in the present study (1–6 yrs) this factor is an unlikely explanation for the observed discrepancies. The influence of technical factors, such as the possible influence of chance contact with the cytology brush on subsequent PD measurements, and the effect of anaesthesia cannot be ruled out.

Besides the deleterious effect on PD, deliberate brushing of the airways tended to reduce or abolish TMV, suggesting injury to the respiratory epithelial

membrane and damage to the ciliated cells. It appears that inflammation and mechanical injury decrease or abolish PD and TMV in the same way. Although the similarities suggest that similar pathways may be involved, this is not necessarily the case. It may be that after mechanical injury, the damage itself causes these changes directly, while during inflammation, a secondary response such as transudation through the epithelium could cause an increase in airway or periciliary fluid, thereby decreasing PD and TMV. On the other hand, repeated careful experimentation (mucus collection and PD measurements) did not result in important changes in either PD or in rheological parameters in a companion study [36], where tracheal mucus collection, TMV and PD measurements were repeated four times over the course of three hours during saline infusion (the control for a pharmacological manipulation). There was no significant change in any of these parameters over this time, although there was a trend to decreased PD over the experiment, about 5% per hour, which is more likely to be attributable to the effects of prolonged anaesthesia than repeated measurements.

Small airway mucus had, on average, a lower % solids content (12.1±3.5%) when compared with tracheal mucus (16.5±2.7%), thus the small airway mucus had a higher water content than the tracheal mucus. Correspondingly, the viscoelasticity (log G* at 100 rad·s^{-1}) in the small airway samples was also less (2.81±0.22) than in the tracheal samples (3.01±0.29). This means that the small airway mucus was more easily deformable, the effect of which should favour mucus clearance in the smaller airways. This might perhaps counterbalance other factors such as the lower ciliary beat frequency (CBF) or the decreased numbers and length of the cilia, which have been reported [12]. This is the first evidence that mucus rheology is different for mucus samples obtained at different lung levels. Since the ciliary function in the lung periphery is probably less efficient than in the central airways, an easily deformable, less rigid mucus, may thus have a teleological perspective in order to facilitate mucus clearance in the peripheral part of the lung. The main finding, that the water content of small airway mucus is greater than that of mucus in the trachea, is of course consistent with the original hypothesis that the caudal-cephalad PD gradient provides a mechanism to control the absorption of water as the airway cross-section diminishes in order to avoid flooding of the central airways.

We found a significant positive correlation between TMV (observed mucociliary clearance) and MCI (predicted clearance, based on mucus viscoelastic properties) (fig. 6A), which is equivalent to a negative correlation between TMV and log G* or % solids content. This finding is thus similar to the finding reported by GIORDANO *et al.* [4], who found an inverse relationship between tracheal mucociliary clearance in beagle dogs and the viscoelasticity of tracheal pouch mucus, as determined by magnetic rheometry.

Although there were no differences in tracheal PD or mucus rheology due to the two levels of humidification studied, there was, nevertheless, a tendency to an increased TMV when a Ringer aerosol was provided. This, perhaps, suggests that the periciliary fluid layer was better hydrated. The lack of any major effect of humidification is probably not too surprising, since experimental conditions in the present study were less extreme than those employed by MAN *et al.* [37], where significant differences in mucus electrolyte and water content were seen with two hours of mouth breathing of unhumidified air compared with nose breathing.

Some of the beagle dogs appeared to have a tracheitis on initial examination, which disappeared or diminished upon re-examination after several weeks. Inflammation in the form of a tracheitis in three of our dogs decreased or almost abolished the PD, as well as the TMV, despite the fact that the regional mucus had a higher water content and a lower log G* (viscoelasticity), which means an easily deformable and inelastic mucus, which should favour mucus clearance by ciliary action. The low TMV in these cases indicated either gross ciliary dysfunction or surface flooding affecting the interaction between mucus and cilia. When these dogs were re-studied two months later, the PD improved slightly, while TMV and MCI increased much more, suggesting that mucus properties and ciliary function improve or repair faster than defects on the epithelial membrane.

In summary, the findings are consistent with the view that ion-modulated transepithelial water transfer contributes to the regulation of the water content of airway mucus. Since it is believed that PD is governed by ion transport, the fact that PD was significantly different in the two airway locations examined probably indicates a different local ionic concentration between small airways and trachea. The fact that the water content of the mucus samples was significantly different between small airways and trachea confirms the same finding, because water is believed to follow ion fluxes. It has been suggested that the periciliary layer is governed by active ion transport across the epithelium, which may create an osmotic gradient as the driving force [31, 38]. A similar effect could occur in the mucus layer, since it may be influenced by ion transport, secondary water flow and changed PD.

References

1. Kilburn KH. – A hypothesis for pulmonary clearance and its implications. *Am Rev Respir Dis*, 1968; 98: 449–463.
2. Wanner A. – Clinical aspects of mucociliary transport. *Am Rev Respir Dis*, 1977; 116: 73–125.
3. King M. – Mucus, mucociliary clearance and coughing. *In*: Bates DV, ed. Respiratory Function in Disease. 3rd Edn. Philadelphia, Saunders 1989; Chapter 3.
4. Giordano AM, Holsclaw D, Litt M. – Mucus rheology and mucociliary clearance: normal physiologic state. Am *Rev Respir Dis*, 1978; 118: 245–250.

5. Puchelle E, Zahm JM, Duvivier C. – Spinability of bronchial mucus: relationship with viscoelasticity and mucus transport properties. *Biorheology*, 1983; 20: 239–249.
6. King M. – Interrelation between mechanical properties of mucus and mucociliary transport: effect of pharmacologic interventions. *Biorheology*, 1979; 16: 57–68.
7. De Sanctis GT, Kelly SM, Saetta MP, *et al.* – Hyporesponsiveness to aerosolized but not to infused methacholine in cigarette-smoking dogs. *Am Rev Respir Dis*, 1987; 135: 338–344.
8. Holma B. – Effects of inhaled acids on airway mucus and its consequences for health. *Environ Health Persp*, 1988; 79: 109–113.
9. Asmundsson T, Kilburn KH. – Mucociliary clearance rates at various levels in dog lungs. *Am Rev Respir Dis*, 1970; 102: 388–397.
10. Serafini SM, Wanner A, Michaelson ED. – Mucociliary transport in central and intermediate size airways: effect of aminophylline. *Bull Eur Physiopathol Respir*, 1976; 12: 415–422.
11. Foster WM, Langenback E, Bergofsky EH. – Measurement of tracheal and bronchial mucus velocities in man: relation to lung clearance. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1980; 48: 965–971.
12. Serafini SM, Michaelson ED. – Length and distribution of cilia in human and canine airways. *Bull Eur Physiopathol Respir*, 1977; 13: 551–559.
13. Curran PF, Macintosh JR. – A model system for biological water transport. *Nature*, 1962; 193: 347–348.
14. Welsh MJ, Widdicombe JH, Nadel JA. – Fluid transport across the canine tracheal epithelium. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1980; 49: 905–909.
15. Nathanson I, Nadel JA. – Movement of electrolytes and fluid across airways. *Lung*, 1984; 162: 125–137.
16. Boucher RC, Bromberg PA, Gatzy JT. – Airways transepithelial electric potential *in vivo*: species and regional differences. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1980; 48: 169–176.
17. Boucher RC, Stutts MJ, Bromberg PA, Gatzy JT. – Regional differences in airway surface liquid composition. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1981; 50: 613–620.
18. Kleyman TR, Cragoe EJ Jr. – Amiloride and its analogs as tools in the study of ion transport. *J Membr Biol*, 1988; 105: 1–21.
19. Nathanson IT, Widdicombe JH, Nadel JA. – Effects of amphotericin B on ion and fluid movement across dog tracheal epithelium. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1983; 55: 1257–1261.
20. Boucher RC, Stutts MJ, Gatzy JT. – Regional differences in canine airway epithelial ion transport. *J Appl Physiol: Respir Environ Exercise Physiol*, 1981; 51: 706–714.
21. King M, Engel LA, Macklem PT. – Effect of pentobarbital anesthesia on rheology and transport of canine tracheal mucus. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1979; 46: 504–510.
22. Jeanneret-Grosjean A, King M, Michoud MC, Lioté H, Amyot R. – Sampling technique and rheology of human tracheobronchial mucus. *Am Rev Respir Dis*, 1988; 137: 707–710.
23. King M, Macklem PT. – The rheological properties of microliter quantities of normal mucus. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1977; 42: 797–802.
24. King M, Brock G, Lundell C. – Clearance of mucus by simulated cough. *J Appl Physiol*, 1985; 58: 1776–1782.
25. App EM, King M. – Airway transepithelial electric potential difference (PD) measurements *in vivo* guided by bronchoscopy in animals and in man. *Eur Respir J*, 1989; 2: 278s.
26. Knauf H, Haag K, Lübcke R, Berger E, Gerok W. – Determination of short-circuit current in the *in vivo* perfused rat colon. *Am J Physiol (Gastrointest Liver Physiol 9)*, 1984; 246: G151–G158.
27. King M, Phillips DM, Gross D, *et al.* – Enhanced mucus clearance with high frequency chest wall compression. *Am Rev Respir Dis*, 1983; 128: 511–515.
28. Knowles MR, Buntin W, Bromberg PA, Gatzy JT, Boucher RC. – Measurements of transepithelial electric potential differences in the trachea and bronchi of human subjects *in vivo*. *Am Rev Respir Dis*, 1982; 126: 108–112.
29. Marin MG, Davis B, Nadel JA. – Effect of histamine on electrical and ion transport properties of tracheal epithelium. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1977; 42: 735–738.
30. Al-Bazzaz FJ, Al-Awqati Q. – Interaction between sodium and chloride transport in canine tracheal mucosa. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1979; 46: 111–119.
31. Nadel JA, Davis B, Phipps RJ. – Control of mucous secretion and ion transport in airways. *Ann Rev Physiol*, 1979; 41: 369–381.
32. Hulbert WC, Man SFP, Mehta J, *et al.* – The bioelectrical properties of tracheal tissues from the Basenji-Greyhound dog model of asthma are abnormal. *Am Rev Respir Dis*, 1986; 133: A90.
33. App EM, King M. – Tracheal mucus rheology and potential difference in two day old puppies. *Biorheology*, 1990; 27: 515–526.
34. Whaley SL, Muggenburg BA, Seiler FA, Wolff RK. – Effect of aging on tracheal mucociliary clearance in beagle dogs. *J Appl Physiol*, 1987; 62: 1331–1334.
35. App EM, Köhler D, Matthys H. – Mucociliary clearance and cough clearance in young patients with cystic fibrosis or chronic bronchitis: the effect of aging. *Eur Respir J*, 1988; 1: 250s.
36. King M, App EM, Ramirez O, Rubin BK, Zayas JG. – Effect of infused azelastine on canine tracheal mucus rheology, transepithelial potential difference and mucus clearability. *J Allergy Clin Immunol*, 1990; 85: 238, (Abstract).
37. Man SFP, Adams GK III, Proctor DF. – Effects of temperature, relative humidity and mode of breathing on canine airway secretions. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1979; 46: 205–210.
38. Knowles MR, Murray JF, Shallal JA, Gatzy JT, Boucher RC. – Ion transport in excised human bronchi and its neurohumoral control. *Chest*, 1982; 815 (Suppl.): 115–135.