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Role of the physicochemical properties of mucus in the protection of the respiratory epithelium

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Role of the physicochemical properties of mucus in the protection of the respiratory epithelium. S. Girod, J-M. Zahm, C. Plotkowski, G. Beck, E. Puchelle.
ABSTRACT: The respiratory mucus is a very complex biological material, which possesses both flow and deformation rheological properties, characterized by non-linear and time-dependent viscoelasticity and physical properties of adhesiveness and wettability. Viscosity and elasticity are directly involved in the transport capacity of mucus, whereas wettability and adhesiveness contribute to the optimal interface properties between the mucus and the epithelial surface. Optimal conditions for the protective and lubricant properties of respiratory mucus are represented by high wettability, and adhesiveness high enough not to induce flow of mucus in the respiratory bronchioles under gravity but low enough to mobilize mucus by airflow during coughing. An intermediate viscoelasticity is also required for an optimal mucociliary transport.

Different biochemical constituents such as glycoproteins, proteins, proteoglycans and lipids are involved in the gel properties of respiratory mucus. During bronchial infection and particularly in cystic fibrosis, the loss of water and the increase in macromolecules result in a marked increase in viscosity and adhesiveness responsible for the mucus transport impairment. The various lipids present in mucus contribute differently to the physicochemical properties. Surface-active phospholipids, such as phosphatidylcholine and phosphatidylglycerol improve the wettability of mucus, whereas neutral lipids and glycosphyngolipids contribute to the hyperviscosity of mucus during infection. Phospholipids and associated mucins are also implicated in the interaction between bacteria and epithelial cells. Therefore, the respiratory mucus needs appropriate physicochemical properties for the protection, hydration and lubrication of the underlying airway epithelium.
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The respiratory mucus is a gel or biopolymer composed of a network of high molecular weight glycoproteins, which fulfils a multitude of roles, directly or indirectly related to their biochemical and physical properties. One of the main missions ascribed to mucus is to form a continuous filter at the cell-air interface and, therefore, to constitute a barrier, protecting the epithelial cells from invasion and injury by micro-organisms and toxic agents present in the environment. Although the most widely studied function of the respiratory mucus is mucociliary clearance, a variety of other functions, such as airway hydration, regulation of the periciliary water, bacterial adhesion and clearance, filtration and diffusion barrier, are almost as important as mucus clearance in the protection of the underlying respiratory cells. All these functions are closely associated to the physical and biochemical characteristics of mucus.

In this paper we will, therefore, list the different rheological and physical properties of mucus, with the aim of relating these properties to their different functions.

In so far as it is difficult to dissociate rheology from biochemistry, we will briefly recall which biochemical components are involved in the rheological and physical pattern of mucus, in normal, as well as in pathological, conditions.

Collection of mucus

A major difficulty when studying the physical and biochemical properties of mucus, is that there are no simple, noninvasive methods of collecting normal mucus in sufficient quantity to make repeated or even single measurements.

Different methods have been proposed for collecting *in situ* normal mucus and studying the rheological properties in animals or in man. These methods involve either the bronchoscopy brush or a screen introduced into the airways, or endotracheal tube techniques [1–5]. However, the major difficulty with these methods is that only a small volume of mucus is collected and, moreover, it is difficult to assess how representative of "normal" the mucus is when collected in conditions which can mechanically stimulate the airway epithelial secretory cells. This stimulation would tend to induce water secretion, which may considerably alter the rheological properties of the local bronchial secretions. Furthermore, storing collected mucus frozen could alter the physical properties of mucus. The bronchofibroscopic technique offers the advantage of collecting mucus in a controlled area without salivary contamination, but this method cannot be easily repeated. Nevertheless, according to RUBIN *et al.* [6], the endotracheal tube technique enables us to study respiratory mucus from people with and without pulmonary disease at the time of incidental surgery. All these difficulties in collecting normal or pathological native mucus explain that most studies on the rheological, physical and biochemical properties of pathological respiratory mucus are carried out on sputum samples. As expectorated mucus is generally contaminated by saliva, a protected method of collecting saliva-free mucus has been developed [7]. Although the extrapolation of pathological to normal mucus remains questionable, the large available quantities of mucus can be used to analyse the relationship between physical and biochemical properties, as well as to correlate alterations in the latter properties with changes in functional properties.

Rheological and physical properties of mucus

The evaluation of the rheological and physical properties of mucus samples is essential for the understanding of their normal functional properties and for determining the factors which are responsible for their functional abnormalities during pulmonary diseases.

The respiratory mucus is a very complex biological material, in terms of physical properties, which possesses both flow and deformation (viscoelasticity) properties characterized by non-linear (non-Newtonian viscosity) and time-dependent flow (thixotropy) properties. Apart from these rheological characteristics, the respiratory mucus possesses surface properties, such as adhesivity and wettability. These physical characteristics, which are independent of the viscoelastic properties, determine the capacity of mucus to protect, hydrate and lubricate the underlying airway epithelium and, therefore, are probably almost as important or more important than the rheological properties.

Rheological properties of mucus

Viscoelasticity and time-dependence rheological properties. Mucus is a highly non-Newtonian viscoelastic material. Under a discontinuous stress, induced by ciliary motion during active stroke or by cough, the mucus starts to instantaneously deform and, once the stress is removed (as during the recovery period of beating or after cessation of coughing), the mucus relaxes. Dynamic or non-dynamic tests have been developed for measuring the viscoelastic properties of mucus. Whatever the apparatus used, two parameters are analysed: the stress which represents the force applied to the material and the result of this force which is the deformation or the strain. In dynamic conditions, the phase lag and the amplitude ratio between the stress and the strain allow calculation of the elastic G' and viscous G" dynamic components of mucus. The magnetic rheometer technique [5] is very elegant and particularly suitable for measuring the rheological properties of microsamples of normal mucus. However, several difficulties are related to this method, including, for example, the risk of rapid dehydration of the mucus due to the very small size of the sample. This method is in fact ideal for normal mucus, but in clinical practice remains difficult to apply to pathological heterogeneous samples, the viscoelastic properties of which are more often analysed using the creep test or the stress relaxation test. Creep experiments also give rapid information on the viscoelastic behaviour of mucus. In the creep test, a constant stress is applied to the sample and the resultant strain response is recorded against time (creep curve), allowing the determination of the instantaneous elastic and retarded viscoelastic response, as well as the steady-state viscosity.

Stress relaxation tests can also be easily applied to analyse the viscoelastic properties of pathological mucus. A special viscoelastometer has recently been developed for routine measurements of the viscoelasticity of mucus [8]. Using this apparatus, with a constant shear rate of 0.4 s, we reported that in patients with chronic bronchitis, the rheological viscoelastic properties of mucus may exhibit large variations, ranging between 1–80 Pa.s for viscosity, with relaxation time varying from 10 to >100 s. The higher the degree of infection, the higher the relaxation time and viscosity. Very low values of viscosity (<5 Pa.s) and relaxation time (<10 s) may be observed in bronchorrhea.

Whatever the techniques used for analysing the rheological properties of sputum under steady-state stress or strain, we should bear in mind that mucus is a highly pseudoplastic biological system, which means that the viscosity decreases as a function of increasing shear rates. To compare sputum sample viscosity, it is necessary to analyse the samples at the same shear rate, low enough not to irreversibly break down the internal structure of the sample.

Bronchial mucus possesses an internal structure with properties involving time-dependence, such as thixotropy. In response to an applied shear rate $>1\ s^{-1}$, the stress first increases markedly before showing a progressive shear thinning. This thixotropy is a reversible type of behaviour depending on the concentration of the coupling that maintains the structure of the mucus [9].

Using standardized preparations of biopolymers to simulate normal and pathological respiratory mucus, we were able to demonstrate that intermediate visco-elasticity with a viscosity between 10–15 Pa.s and a relaxation time of about 40 s, represents an optimal rheological profile to the mucus transport [10].

Spinnability. Spinnability (also called thread-forming capacity) is a property common to most types of mucus, either of respiratory, cervical or gastroduodenal origin. Spinnability characterizes the property of mucus that can be drawn into long threads under the effect of a traction. The spinnability of sputum [11] appears to be highly dependent on the degree of purulence. Purulent samples exhibit significantly lower values of spinnability, as compared to mucoid sputum samples. In patients with chronic obstructive pulmonary diseases, a wide variation of spinnability values (15–150 mm) can be observed from one mucus to another. An important feature of spinnability is that it gives information on the internal cohesion forces of mucus, as well as on its elasticity, although it does not directly depend on its degree of viscosity and elasticity. Sputum samples exhibiting quite different values of viscosity may be characterized by a similar value of spinnability. Furthermore, a non-elastic sputum sample may be spinnable [12].

Other physical properties

Apart from the rheological properties of sputum which imply flow and deformation properties, other properties, such as adhesivity (or tackiness) and wettability, represent two fundamental physical properties which play an important role in the transport of mucus, either by the ciliary or cough mechanism.

Adhesivity and tackiness of sputum. Adhesivity is a phenomenon which characterizes the attraction forces between an adherent surface and an adhesive system. At the respiratory level, mucociliary transport involves surface interactions between the cilia and the mucus. During coughing, it is likely that surface forces between the apical lining of the respiratory epithelial cells and the mucus, as well as interfacial forces between the sol and gel layer of mucus, may control the efficiency of mucus cough clearance. Little attention has so far been paid to the adhesivity of respiratory mucus. Recently, Rutte *et al.* [13] described a method for measuring the surface adhesive properties of sputum samples, using the plate detachment technique, which requires large sample volume. The adhesivity corresponds to the strength to be applied to achieve the separation between the adhesive fluid (the mucus) and the adherent surface (mucosa). We have developed a platinum ring method [14], which can also be applicable to the measurement of adhesivity of native mucus. In patients with chronic bronchitis, we observed large variations in mucus adhesivity ranging from 57–137 mN·m^{-1}. Purulent sputum samples, with a low water content, were generally characterized by high values of adhesivity and, in parallel, a low mucociliary transport rate. In patients, with cystic fibrosis (fig. 1), the adhesivity of mucus ranged between 50–165 mN·m^{-1}. A significant correlation was observed between the adhesivity of mucus, the dry weight (r=0.83, p<0.001) and the leucocyte numeration (r=0.60, p<0.05). The latter parameter was used to evaluate the purulence degree of mucus. Conversely, the decrease in hydration was associated with a lowering in mucociliary transport [15].

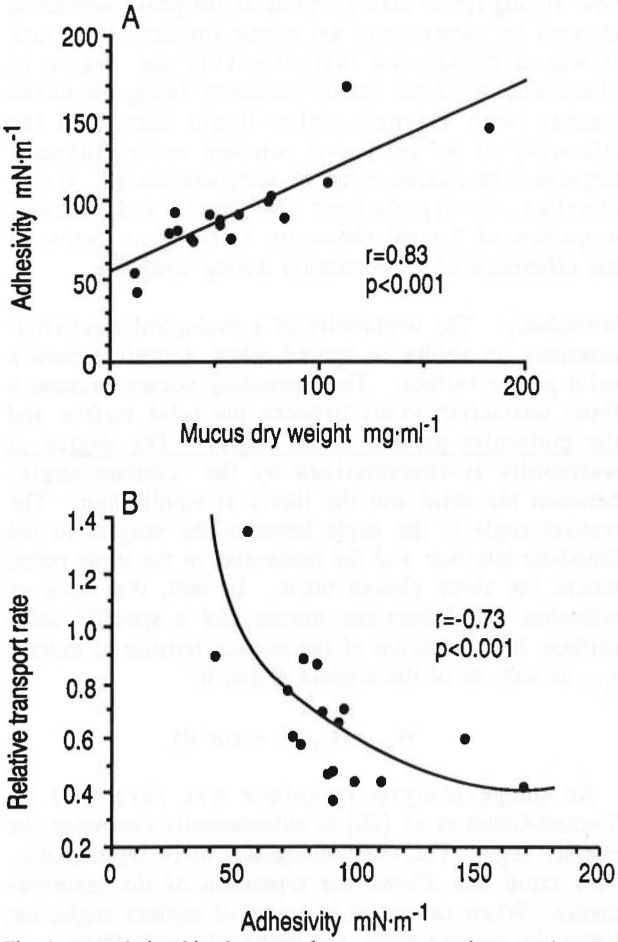


Fig. 1. – Relationships between the water content (expressed as dry weight), the adhesivity (A) and transportability (B) of the respiratory mucus collected in 17 cystic fibrosis (CF) patients. The lower the water content, the higher the adhesivity of mucus, and the lower its transport rate by ciliary activity.

These adhesive properties of mucus have also been studied by analysing their "tack" properties. According to Lopez-Vidriero [16], adhesive materials of different natures can exhibit "tack". "Tack" can be defined as the property of adhesive materials (of biological and industrial origin) that enables them to bind rapidly and tenaciously under conditions of light contact pressure and short-time contact. Therefore, these two latter parameters are of major importance for the standardization of the technique. "Tack" is, like adhesivity, a combination of viscosity, elasticity and surface tension. In chronic or acute mucus hypersecretion, all of these physical properties are of major importance in expectoration by cough. By using a "tack" tensile machine, LOPEZ-VIDRIERO [16] has reported, in preliminary experiments, that the gel phase of sputum exhibits a high "tack", whereas the sol phase does not. These preliminary results are to be related to recent results showing that the airway surface liquid sol phase interferes in the mucus cough transport by decreasing the adhesivity of mucus gel and is, therefore, essential in the efficiency of coughing. Using a simulated cough machine, developed by KING and co-workers [17, 18] and ZAHM *et al.* [19], we analysed how adding tensio-active liquids as sol phase simulants, affected the clearance of gel mucus simulants by cough. It was demonstrated that, whatever the degree of viscoelasticity of the mucus simulants, lining the model trachea with a tensio-active liquid decreased the adhesivity of the gel mucus simulant and significantly improved the clearance by the simulated cough. It can, therefore, be hypothesized that "tack" and adhesive properties of the sol phase are determinant factors in the efficiency of expectoration during coughing.

Wettability. The wettability of a biological fluid characterizes its ability to spread when deposited onto a solid planar surface. This spreading occurs because a finite interaction exists between the solid surface and the molecules present in the liquid. The degree of wettability is characterized by the "contact angle" between the solid and the liquid at equilibrium. The contact angle is the angle between the tangent to the liquid-air interface and the horizontal, at the triple point, where the three phases meet. In fact, the work of adhesion (W_{ad}) between mucus and a specific solid surface, is a reflection of the surface tension of mucus, γ_{LV}, as well as of the contact angle, θ:

$$W_{ad} = \gamma_{LV} (1 + \cos \theta)$$

An image analysis technique was developed by VAQUEZ-GIROD *et al.* [20] to automatically determine the contact angle. This method appears to be reproducible, very rapid and allows the repetition of the measurements. When expressed in terms of contact angle, the lower the contact angle, the higher the wettability.

Wettability, as well as adhesivity, are surface properties independent of rheological properties of mucus, such as viscoelasticity. A 100% decrease of viscosity may be induced simply by diluting the sputum sample without any significant change in its wettability. The wettability and adhesivity of respiratory mucus markedly contribute to the optimal interface properties between the respiratory epithelial surface and mucus and, therefore, interfere in ciliary clearance and cough clearance.

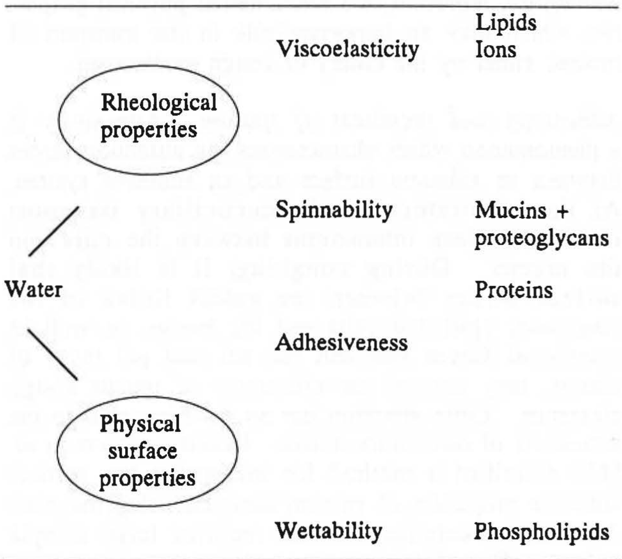
By extrapolating the results of HILLS [21] concerning the role of the wettability of gastric mucus in protecting the epithelial surface, we can hypothesize that an efficient protective mucus possesses a high wettability (*i.e.* it spreads over the epithelial surface) and an adhesivity, high enough not to flow into the peripheral bronchioles under gravity, but low enough not to adhere too markedly to the respiratory mucosa and to be easily mobilized during coughing.

From preliminary results obtained with sputum samples, we suggest that low adhesivity (about 70 $mN \cdot m^{-1}$) and wettability characterized by a low contact angle (<20°) should represent optimal conditions for the protective and lubricant properties of respiratory mucus.

Relationships between biochemical and physical properties of respiratory mucus

Different biochemical components and types of linkages are involved in the gel-like properties of respiratory mucus: ions, water and macromolecules directly interfere in the rheological and physical properties of respiratory mucus (table 1). In fact, mucus is composed of a mixture of glycoproteins, proteins, proteoglycans and lipids, to which are associated deoxy-ribonucleic acid (DNA) in infected samples. In healthy subjects, the water content of mucus (95–97%) is regulated by ionic movements of Cl^- at the epithelial cell surface. During infection, in chronic bronchitis and particularly in cystic fibrosis, the decrease in water and the increase in macromolecule secretion result in the total dry weight of mucus being from 5 to 10 times higher than normal [22]. As a consequence, a marked hyperviscosity, as well as an increase in adhesivity and a lowering of wettability, are observed. Glycoprotein-protein interactions play a major role in the rheological profile of sputum [23–27].

Table 1. – Relationships between physical and biochemical properties of respiratory mucus



Close and significant correlations have been demonstrated between the mucin content and the viscosity and elasticity of sputum samples collected in patients with chronic bronchitis. Among the polyanionic mucins, sialomucins were highly correlated to viscosity and elasticity [28]. The negatively charged carboxylic and sulphated glycoproteins may also interact, *via* electrostatic bonds, with positively charged proteins, such as lysozyme, or ions such as Ca^{++} or Mg^{++} [29–31]. In pathological situations, such as chronic bronchitis, these interactions may be considerably increased. We have demonstrated that the addition of pure proteins, such as immunoglobulins A, M (IgA, IgM) or lysozyme to lyophylized sputum samples considerably modifies their rheological properties. A positive and significant increase in viscosity and in elastic modulus has been observed after adding proteins, such as lysozyme or secretory IgA [31]. These results are consistent with the data of HARBITZ *et al.* [29], who suggest that lysozyme is an important restructuring molecule, as important as IgA in the mucus gel network formation. In chronic bronchitic patients with different stages of the disease, the secretory IgA content, and concurrently the viscoelasticity and the mucociliary transport rate, decrease with the severity and duration of the disease. The progressive degradation of the IgA is likely to be related to a degradative activity of bacterial proteases, rather than to a primary IgA bronchial deficiency. Therefore, a high IgA sputum content can be considered as an index of the functional mucosal activity. It has also been reported by NAKAMURA *et al.* [32] that a high IgA content correlates with a high spinnability and high mucociliary transport rate. Other secretory proteins, such as bronchotransferrin, IgG and IgM, may also restructure the human airway secretions. In purulent sputa collected from patients with cystic fibrosis, high molecular weight immunoglobulins, such as IgM, were frequently identified in high concentration [33]. We further observed that the IgM concentration in cystic fibrosis (CF) sputa was significantly correlated to the viscosity and that very high IgM levels ($>1.5\ g \cdot l^{-1}$) were associated with a low mucociliary transport rate.

The negative effect on the mucociliary transport rate, obtained after increasing the viscoelasticity beyond the optimal values previously defined, has been demonstrated by increasing the dry weight of reconstituted pathological mucus. These results agree with those of MARRIOTT *et al.* [34], who observed that an over-thickening of mucus gels, induced by addition of biopolymers to mucins, led to a progressive decrease in their transport rate, which was related to the concentration of the added biopolymer. When DNA, a major non-mucin component of purulent samples was added to cystic fibrosis sputa, an increase of as much as 30% in both elasticity and viscosity was observed [27]. This effect was not observed in the chronic bronchitic sputa.

Therefore, there is an optimal concentration of molecules in gels for maximal transport rate. Mucus transport rate is maximal in a given range of viscosity and elastic modulus [10, 35]. In fact, the concentration and cross-linking of glycoproteins need to be within an optimal range: too marked thickening or, conversely, thinning of mucus decrease the efficiency of mucus transport. The concentration of proteins and glycoproteins is not the only factor which controls the rheological and physical properties of sputum. Apart from the water content, ionic concentration and pH of sputum, which may alter the rheological properties of sputum by interfering in the degree of cross-linking of macromolecules, we must keep in mind that lipids represent a high percentage (1–2%) of macromolecules present in sputum. Although the role of lipids as potential determinants of the rheological properties of mucus has been suggested [36], only a small number of detailed analyses on the lipid composition of airway secretions has been carried out [37–39]. In addition, only a few studies have been devoted to the relationships between the lipid composition and the rheological properties of normal and pathological airway secretions [40–42]. Lipids present in pathological airway secretions may originate from alveolar surfactant, from epithelial secretory granules or epithelial membranes. GIROD *et al.* [43] have recently demonstrated that phospholipids are identified in the serous and mucous secretory granules of the respiratory submucosal glands and that they are also identified attached to the glycocalyx of the microvilli of the surface epithelial cells (Girod *et al.* personal communication). This demonstrates that phospholipids present in tracheobronchial mucus are locally synthesized and may play an important role in the protection of the epithelium, as well as in the transport of mucus. These data suggest that the airway mucosa, like the gastric mucosa [44], is coated by a layer of phospholipids, which, in association with mucins, may simultaneously lubricate and isolate the epithelium from aggressive agents [45]. Our concept of protection of the respiratory mucosa by phospholipids is schematized in figure 2.

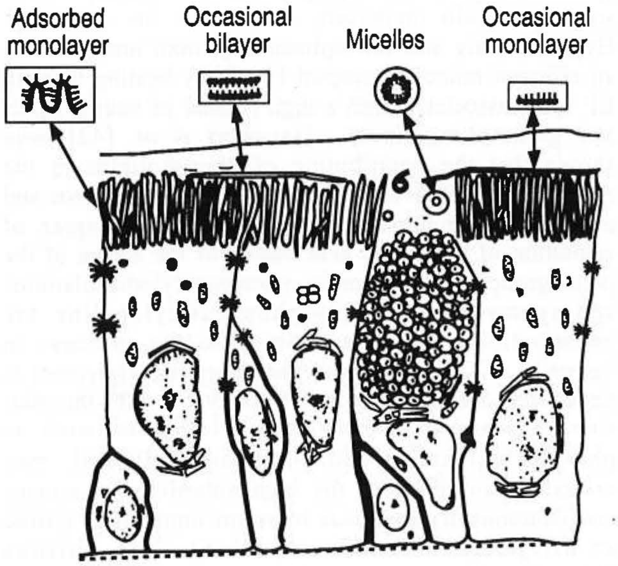


Fig. 2. – Potential roles of phospholipids in the protection of respiratory epithelium and in mucus transport: a first adsorbed layer of phospholipids, the presence of which was demonstrated by cytochemical labelling, constitutes a hydrophobic barrier against noxious particles and bacteria. At the sol-gel phase interface, phospholipids (in the form of micelles, mono-bilayer) play a lubricating role in mucus transport by cilia or by cough.

As previously proposed by Hills [21] for the gastric mucosa, the surface of respiratory epithelial cells is covered by an adsorbed layer of phospholipids, which constitutes a barrier against noxious particles and bacteria. Furthermore, phospholipids, which have been secreted by respiratory submucosal gland cells in the lumen, form either mono or multilayers, or micelles, located at the interface between sol and gel phases of respiratory mucus. At the tips of cilia, this second phospholipid layer lubricates the mucus and facilitates its transport by either cilia or cough. Lipids identified in airway secretions include a variety of neutral lipids, phospholipids and glycolipids. Several of these components, namely sphingomyelins, phosphatidylethanolamine, cholesterol and glycosphingolipids are considered to be factors which increase the viscosity of lipid-lipid/lipid-protein assemblies [42]. It has been shown [46] that in CF, infection is associated with markedly abnormal rheological and transport properties of airway secretions, which may be responsible for the severity of the disease. Houdret *et al.* [47] reported that the very frequent infections observed in CF produce increased amounts of lipids that stick to the respiratory mucins.

Galabert *et al.* [42] analysed the relationship between the total lipid content, as well as the proportion of the different fractions of lipids, and the rheological properties of CF airway secretions, taking into account the degree of infection, as judged by the leucocyte-count in the expectorated secretions. It was demonstrated that the total lipid content was higher in the superinfected CF secretions and, in particular, that the content in glycosphingolipid fractions and in cholesterol was markedly increased when the CF sputum samples were highly purulent. Glycolipids and sphyngomyelin increased, as well as the viscosity. Hyperviscosity and low spinnability, both unsuitable to an efficient mucous transport by ciliary beating were in CF sputa associated with a high content of neutral lipids and glycosphingolipids. Galabert *et al.* [42] have shown that the contribution of phospholipids to the rheological profile of CF sputum may be different and even quite the opposite, according to the degree of saturation of their fatty acid chains or the nature of the polar groups. For example, phosphatidylethanolamine, sphingomyelins and lysophosphatidylcholine are phospholipids which contribute to the increase in viscosity of CF sputa, whereas phosphatidylglycerol is negatively correlated to the viscosity. In CF, the relative decrease in surface-active fractions, such as phosphatidylcholine and phosphatidylglycerol, may markedly contribute to the high adhesivity of mucus, and consequently to mucus transport impairment (Girod *et al.* personal communication). The various phospholipids present in the airway secretions may contribute either positively or negatively to the transport properties of sputum. It is also important to consider, in particular in the cough mechanism, the role of phospholipids as macromolecules able to improve the wettability and the adhesive properties of mucus.

Physical and related functional properties of respiratory mucus

Mucus is secreted by epithelial tissues which, whatever their primary physiological functions, all transport ions and water across the epithelium. Whereas the function of mucus in gastric physiology has generally been considered as a protective function (against mechanical abuse, invading microorganisms, toxic substances, destructive enzymes or corrosive acidity), the functional properties of the respiratory mucus have, until now, been limited to microbial or particle clearance. In fact, the biochemical and physical properties of respiratory mucus are either directly or indirectly involved in controlling several main functions (table 2 and fig. 3) which include: 1) mucus hydration and humidification of airways; 2) mucus transport either by ciliary or cough mechanism; 3) antibacterial protection by means of bacterial adhesion; and 4) filtration and diffusion barrier. These different functions are fulfilled by several physical factors and parts of mucus (fig. 3).

Table 2. – Physical and related functional properties of respiratory mucus

Functions	Mucus layer concerned	Physical or rheological properties
Mucus hydration	Periciliary and mucus gel layer	Viscosity, spinnability Surface properties (wettability, adhesivity)
Mucus transport		
Cilia Cough	Periciliary layer and mucus gel	Viscoelasticity Spinnability Surface properties
Bacterial adhesion	Mucus gel	Adhesivity
Filtration barrier	Mucus gel	Viscosity

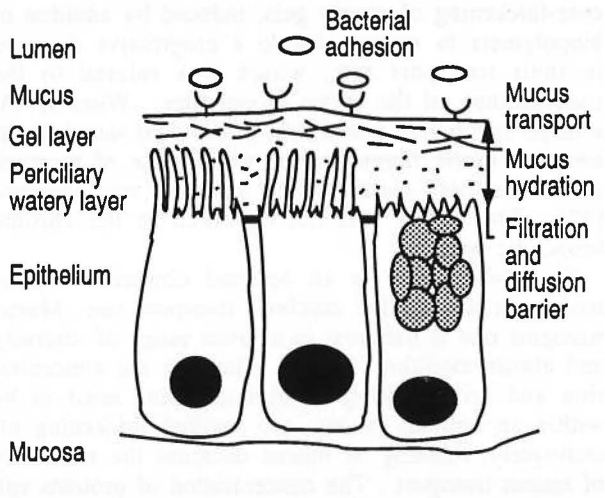


Fig. 3. – Diagram of the main functions of the respiratory mucus.

Airway humidification and mucus hydration

As a gel, respiratory mucus contains a high percentage of water which is able to supply a correct relative humidity to the inspired air. To continually replace the water loss, the surface epithelium of the conducting airways functions as a Cl^--secreting epithelium, and therefore can secrete water. Part of this water may be reabsorbed by Na^+ pump. The transfer of water, carried out by the apical microvilli and the ciliated cells, regulates the depth of the periciliary and gel layers of the mucus. It is thought that reabsorption of watery peripheral secretion is necessary to equilibrate the "excess" secretions, which would converge from thousands of bronchioles up to the trachea. Therefore, regulation of mucus hydration is not only required to humidify the inhaled gas at airway level, but also to optimize the efficiency of the mucociliary escalator.

Tam and Verdugo [48] have introduced the concept that changes in the rheological properties of mucus are directly related to changes in the degree of hydration, controlled by a Donnan equilibrium process. The volume expansion of such a Donnan system is dependent on the ionic composition of the bathing medium. Mucus hydration decreases with increasing pH and/or osmolality. Man *et al.* [49] have shown that mouth breathing increases the osmolality of respiratory mucus. The inhalation of dry air rapidly induces a decrease in the depth of the periciliary layer or an increase in the solid content of the gel mucus layer, with a resulting increased viscosity. The degree of mucus hydration also influences the thread-forming (spinnability) properties of mucus. Puchelle *et al.* [50] have shown in dogs that the inhalation of dry air decreases the spinnability of the gel mucus which, in parallel, induces a decreased mucociliary transport.

Therefore, by means of their gel properties, respiratory mucus, as well as mucus from other sources, possess very large polyanionic macromolecules which, due to their cations and water-binding properties, may control the local hydration of the epithelium, lubricate the mucosa and humidify inhaled gas.

By contrast, if there is a local dehydration, either due to an excessive epithelial Na^+ reabsorption or abnormal epithelial Cl^- secretion described in CF disease, these ionic abnormalities will give rise to absence or decrease in water transport to the lumen and, as a result, a dehydration and compaction of mucus. In terms of physical properties, this will result in increased viscosity and adhesivity, which will be followed by the impairment of mucus transport. In cystic fibrosis, the observation that we made, is that the mucus water content (expressed as dry weight) varies markedly from one patient to another, but is closely and significantly correlated to the adhesivity of mucus [15]. In the same way, the more adhesive the mucus, the lower the mucociliary transport (fig. 1).

Mucus transport

Mucociliary transport. The clearance of exogenous microorganisms and particles from the respiratory epithelium represents one of the fundamental functions of the mucociliary system [51]. In conducting airways, the mucociliary system consists of a watery periciliary layer, which lines the epithelium and in which the cilia beat and of an upper gel layer, which is propelled by cilia (fig. 4). The mucus gel layer acts as a coupler which transfers momentum from the tips of the cilia to the gel. In normal airways, the loads trapped by the gel mucus are transported by the ciliary beating, up to the oesophagus where they are swallowed and coughing has no effect. The efficiency of the mucociliary transport depends on several factors, such as the number and activity of the cilia, the thickness of the periciliary and upper gel layer of mucus, as well as their respective rheological properties. In healthy subjects, it has been shown that a large variation of nasal mucus transport may occur within different subjects and that it is related to the rheological properties of mucus and not to the ciliary activity [52].

Fig. 4. – The mucociliary system consists of a watery periciliary layer (electron-lucent phase) in which the cilia beat and of an upper gel layer (electron-dense phase) which is transported by the cilia. Bar = 2 μm.

Using the depleted frog palate as a model representative of human airway mucociliary epithelium, and polysaccharide xanthan as respiratory mucus simulant, a close dependence between the mucus rheological properties, ciliary beat frequency and mucociliary transport rate was reported [10]. When the palate is depleted of mucus, the transport of tracers is interrupted, but mucus from different sources (dog tracheal mucus, cow cervical mucus, mucus scrapped from fish scales), as well as non-mucus systems, such as egg-white or guar or xanthan gels, can restore the mucociliary transport. In fact, all of these systems possess one common property, that of being viscoelastic. Conversely, the mucociliary transport cannot be restored by adding purely viscous fluids. This clearly demonstrates that the elasticity of mucus plays a fundamental role in the mucociliary transport mechanism. In fact, a viscosity close to 12 Pa.s and an elastic modulus close to 1 Pa represent optimal ranges of values for the mucociliary transport to be efficient. The ciliary transport rate is also closely correlated to the spinnability: the higher the spinnability, the faster the mucus transport rates [12]. It is interesting to note that in patients with chronic bronchitis, the transport rate is normal, whatever the degree of viscoelasticity, provided the spinnability is similar or even higher than normal values (>30 mm).

Mucus transport by cough. Although, in healthy subjects, cough does not contribute to the airway clearance, in patients with hypersecretion, cough may account for more than 50% of clearance, thereby compensating for a possible defective mucociliary transport. Using a simple model in the form of a rigid tube for simulating trachea and gel simulants, several investigators described wave formation [53, 54]. Particles deposited in viscous mucus samples may be moved with high flow rate, whereas wave formation and particle clearance are inhibited in lining fluids of marked elasticity. According to KING *et al.* [18], the native mucus may exhibit intermediate levels of elasticity, because it must be capable of responding to both forms of clearance, *i.e.* mucociliary and cough. The cough clearability decreases with either increasing viscosity or elasticity of the mucus, but at constant mechanical impedance (ratio of elasticity and viscosity), cough clearance increases as the ratio viscosity/elasticity decreases. This contrasts with the opposite relationship observed for mucociliary clearance. The correlation with spinnability was found to be in the opposite direction to that obtained for mucociliary clearance on the frog palate. This indicates that a balance between elastic and viscous properties of the mucus might exist naturally to optimize both forms of clearance. Concerning the adhesivity, it was clearly shown that a lowering of mucus surface tension and, therefore, a lowering of adhesivity could be beneficial to both mucociliary and cough clearance. As mucus is a shear and time-dependent fluid, it seams reasonable to hypothesize that coughing, and particularly repetitive coughing, could induce changes in rheological properties with a parallel alteration in mucus transport. ZAHM *et al.* [55] have shown, with the model of the cough machine developed by KING *et al.* [17] that the shear-dependent property of mucus, associated with repetitive coughing, increases the efficiency of mucus clearance. Using a gel mucus simulant to which a sol phase simulant was added, ZAHM *et al.* [19] demonstrated that, in the presence of the sol phase, the clearance index was high and did not change with increased viscosity. In the absence of the sol phase, the cough clearance of the mucus simulant significantly decreased as the viscosity increased. The phospholipids present at the interface between the gel and sol layers, or associated to the cilia (in the form of vesicles) or luminal microvillous border, would most probably play a lubricant role in mucus transport either by cilia or cough (fig. 5).

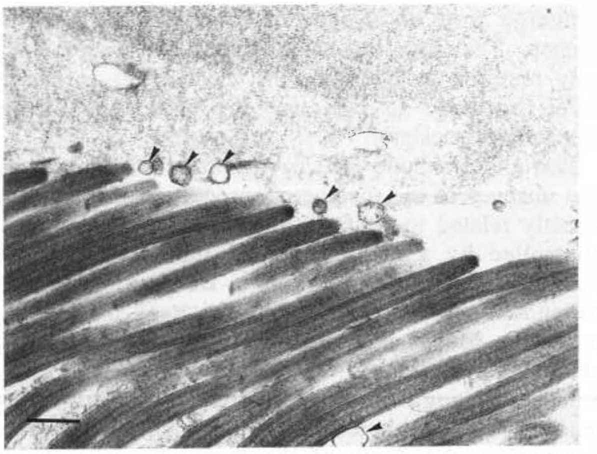


Fig. 5. – Small vesicles (arrows) identified at the interface between the sol and gel phase of mucus or between the cilia may represent phospholipid micelles with surface-active properties. Bar = 0.5 μm.

Antibacterial mucous protection and bacterial adhesion

Several proteins in respiratory mucus have been demonstrated to be active molecules possessing bacteriostatic or bactericidal properties. This is the case for IgA, lactoferrin and lysozyme, which are secreted by submucosal gland serous cells and leucocytes and, apart from their restructuring properties, are important factors in defending lungs against infection. Bronchial mucins, synthesized by the secretory cells of the surface epithelium and submucosal glands, are high molecular weight (10^6) glycoproteins which are composed of a polypeptidic chain to which are attached several hundred glycan chains [56]. These very heterogeneous chains represent a mosaic of receptors allowing the entrapment of aerocontaminants and bacteria, which are then transported to the pharynx by the ciliary activity (fig. 6). In fact, the entrapment of bacteria by respiratory mucus and their elimination by ciliary beating is the first stage in the defence of human respiratory epithelium. PLOTKOWSKI *et al.* [57] have shown that pneumococci adhere to mucus and not to the ciliated cells. After protease treatment and successive exfoliation of the epithelium, it was demonstrated that incubation of this injured epithelium with *Pseudomonas aeruginosa* was accompanied by a high bacterial adhesion to the mucus granules, as well as to the desquamating cells [58]. These results demonstrate once again the protective role of mucus for the epithelial cells, inasmuchas it avoids the adhesion of bacteria to the non-ciliated cells.

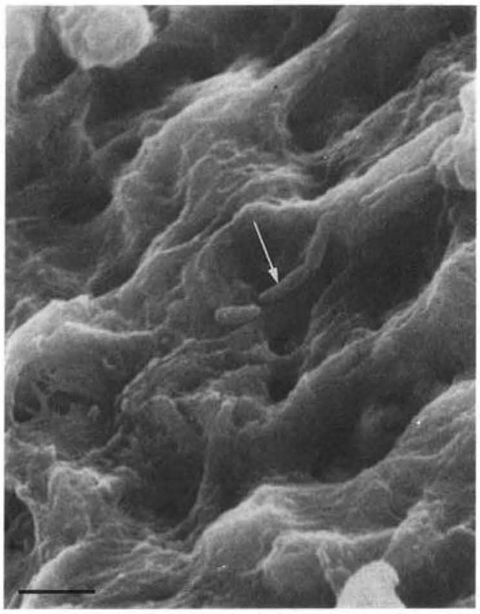


Fig. 6. – Bacteria (*Pseudomonas aeruginosa*) trapped by the mucus gel layer are transported by the cilia. Bar = 1 µm.

the bacterial cell wall hydrophobicity has been shown to directly correlate to the extent of bacterial adhesion [59]. Measuring the contact angle of a 0.1 M NaCl solution with the bacterial surface is representative of the bacterial hydrophobicity and has a predictive value for adhesion. Changes in the hydrophobicity of bacteria, following the addition of cationic proteins have been reported after lysozyme treatment; it can be hypothesized that mucins and phospholipids, which have been shown to be present at the surface of the respiratory epithelial cells, may modify the wettability (*i.e.* the opposite of hydrophobicity) of the epithelium, and that it is also quite likely that phospholipids and associated mucins locally synthetized and released in the mucus [43], may modify the interaction between bacteria and epithelial cells. Therefore, bacteria may adhere *in vivo* to epithelial cells by reacting with surface properties of the mucus gel coating.

Filtration barrier

In the gastrointestinal tract, it is well known that mucus serves as a mixing and ionic diffusion barrier, the diffusion coefficient of which is far lower in mucus than in saline solution [60]. In the respiratory

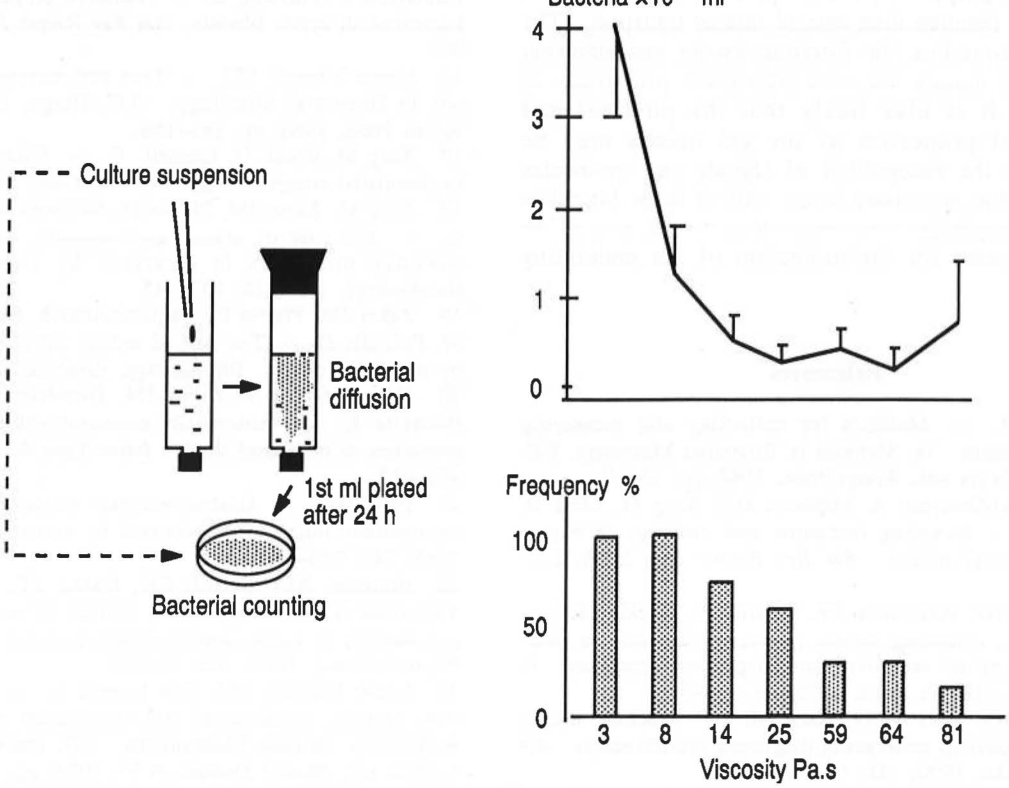


Fig. 7. – The diffusion of bacteria (*Pseudomonas aeruginosa*) through the mucus simulant gel decreases as the mucus viscosity increases.

Apart from specific interactions between microbial adhesins and host cell receptors, the surface properties of bacteria may also interfere in the bacterial adhesion to the mucus and mucosa. In particular,

tract, the coefficient of diffusion and the degree of permeability of mucus have not been studied. It is probably due to the fact that the barrier functions of gastrointestinal and respiratory mucus are fundamentally different. Whereas the gastrointestinal cells have to be protected from the acidic injury of gastric juice, the respiratory tract has to be protected from aerocontaminants and bacteria. Beck *et al.* (personal communication) carried out a simple and instructive experiment which demonstrates the efficiency of the filtration barrier properties of the mucus against bacteria. In this experiment (fig. 7), the mucus barrier is simulated by a 3 ml column of culture medium, introduced into a syringe, to which is added an increasing concentration (1 to 2.5% w/v) of a biopolymer in order to obtain a range of viscosity from 3–80 Pa.s. The filtration barrier property of mucus was analysed by collecting the lowest part (1 ml) of the mucus simulant and counting bacteria, 24 h after having added a 1.5 ml bacterial suspension (*Pseudomonas aeruginosa*) on the surface of the upper part of the culture medium. The results show that, whereas low viscosity allows a rapid progression and multiplication of the bacteria along the column of the mucus simulant, a viscosity higher than 8 Pa.s (which is the case for the gel mucus layer) acts as an efficient bacterial barrier or filter. This simple experiment demonstrates that an excessive fluidification of mucus not only hinders the mucociliary transport but can also favour bacterial diffusion and colonization of the respiratory epithelium.

In conclusion, by means of its physical and rheological properties, the respiratory mucus fulfils many more features than control mucus transport. The hydration properties, the filtration barrier and adhesion capacity of mucus are also important properties to consider. It is also likely that the physical and biochemical properties of the gel mucus may be involved in the recognition of signals and molecules secreted at the periciliary level. All of these functions of mucus emphasize that respiratory mucus acts as an efficient system for the protection of the underlying epithelium.

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