Eur Respir J 1999; 13: 1177–1188
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European Respiratory Journal
ISSN 0903-1936

SERIES 'CHEST PHYSIOTHERAPY'
Edited by S.L. Hill
Number 1 in this Series

Regulation of mucociliary clearance in health and disease

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Regulation of mucociliary clearance in health and disease. E. Houtmeyers, R. Gosselink, G. Gayan-Ramirez, M. Decramer. ©ERS Journals Ltd 1999.

ABSTRACT: Airway secretions are cleared by mucociliary clearance (MCC), in addition to other mechanisms such as cough, peristalsis, two-phase gas–liquid flow and alveolar clearance. MCC comprises the cephalad movement of mucus caused by the cilia lining the conducting airways until it can be swallowed or expectorated. MCC is a very complex process in which many variables are involved, all of which may modify the final outcome. The structure, number, movement and co-ordination of the cilia present in the airways as well as the amount, composition and rheological properties of the periciliary and mucus layers are determinants of MCC.

Physiological factors such as age, sex, posture, sleep and exercise are reported to influence MCC due to a change in the cilia, the mucus or the periciliary layer, or a combination of these. Environmental pollution is suspected to have a depressant effect on MCC dependent on different factors such as pollutant concentration and the duration of exposure. Most studies focus on sulphur dioxide, sulphuric acid, nitrogen dioxide and ozone. Tobacco smoke and hairspray have been noted to have a negative influence on MCC. Some diseases are known to affect MCC, mostly negatively. The underlying mechanism differs from one illness to another. Immotile cilia syndrome, asthma, bronchiectasis, chronic bronchitis, cystic fibrosis and some acute respiratory tract infections are among the most frequently reported.

The present paper reviews normal mucociliary clearance and the effects of diseases on this process.

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Keywords: Cilia
environmental pollutants
lung diseases
mucus
physiological determinants

Received: December 2 1998
Accepted after revision December 15 1998

This work and G. Gayan-Ramirez were supported by the Fonds voor Wetenschappelijk Onderzoek–Vlaanderen.

A variety of different particles, such as bacterial or viral agents and environmental pollutants, are deposited in the lungs while breathing. Three main clearance mechanisms operate in the lungs to remove the inhaled and deposited material as well as cellular debris in order to keep the airways relatively clean and sterile. Mucociliary clearance (MCC) clears the conducting airways of its own secreted mucus, together with substances trapped in it, by means of the mucociliary escalator. Coughing serves as a back-up system when MCC fails [1]. Consensus has, however, still not been reached as to which airway generations this cough clearance mechanism is effective in. Finally, alveolar clearance removes insoluble particles deposited on the respiratory surface of the lungs [2].

This review focuses on one of these clearance mechanisms, namely MCC. A great difficulty concerning MCC is the diversity of investigational methods designed to study this process. This diversity indicates that, at present, no method of studying MCC is ideal. These methodological problems lead to difficulties in the comparison and interpretation of the published literature. BRAGA and ALLEGRA [3] have written a book of >400 pages describing different methods of studying MCC and its component functions, such as ciliary motion and the rheological properties of mucus.

Despite the methodological problems it is important to study MCC and its component functions. Gaining insight into MCC may lead to improved or new treatment modalities for diseases associated with impaired MCC. Prevention of derangement of MCC may also be important. For example some bronchodilators and anaesthetics are known to have a negative influence on the functioning of the mucociliary escalator.

First, the normal functioning of MCC must be understood. Then, how disease may affect MCC is reviewed.

Cilia

Structure of the cilia

The two human lungs contain ~0.5 m^2 of ciliated epithelia, with a total number of cilia in the order of 3×10^{12}. Apart from the lungs with their bronchi and bronchioles, ciliated epithelia also occur in the nasal cavity, paranasal sinuses, eustachian tubes, middle ear, pharynx, trachea, oviducts and cervix in females, ductuli efferentes in males, and ependymal lining of the brain [4, 5].

For editorial comments see page 949.

Immediately below the larynx, the tracheobronchial tree is formed by a pseudostratified columnar epithelium, on which surface cilia are found down to about the sixteenth bronchial division of WEIBEL [6]. The ciliated cells, characterized by their long cytoplasmic projections and numerous microvilli, have about 200 cilia·cell^{-1}. Each cilium has a length of 5–7 μm in the trachea and 2–3 μm in the seventh airway generation, and a diameter of 0.25–0.33 μm [7, 8].

Cilia have a 9+2 ciliary axoneme (fig. 1), which comprises nine interconnected doublet microtubules surrounding and joined by cross-bridges to two centrally positioned microtubules. Each doublet is formed by an A- and a B-subfibre or microtubule. Subfibre A is a complete microtubule whereas subfibre B has a smaller number of protofilaments, but shares part of the A subfibre as well. Paired adenosine triphosphatase (ATPase) or dynein arms are located on subfibre A. Adjacent to these dynein arms, subfibre A also has radial links or spokes. These structures join the outer doublets to the so-called sheath which surrounds the two central microtubules. Each radial link terminates in a head near the surface of the central sheath. Interdoublet links or nexin links appear to connect the terminal portion of the inner arm to the adjacent B-subfibre. Cilia have a surrounding membrane which is an extension of the cell membrane [9].

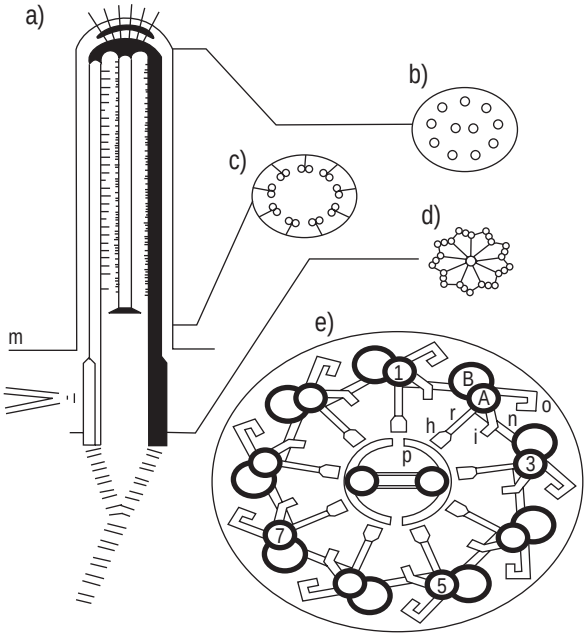

Fig. 1. – a) Longitudinal; and b–e) cross-sectional views of the structure of a cilium from the respiratory tract. The ciliary shaft, which is surrounded by a membrane continuous with that around the cell (m), terminates in a crown of "claws" attached to a dense cap at the tip of the longitudinal microtubules of the axoneme (a). The microtubules continue into the basal body, which lies in the cell cortex and bears rootlet fibres. A detailed representation of the cross-sectional arrangement of a cilium is depicted (e), including the arrangement and conventional numbering of 9 doublets (as seen looking from the base towards the tip). The doublets are connected by nexin links (n). The A-subfibre (A) of each doublet bears outer (o) and inner (i) dynein arms, projecting towards the next doublet. Radial spokes (r) with dilated heads (h) can attach to projections (p) associated with the central microtubules. Changes in microtubules pattern at different levels are shown (b–d). B: B-subfibre (From [10], with permission.)

Movement and co-ordination of the cilia in the respiratory tract

Mucus is propelled by ciliary movement. Cilia start moving from the rest position by bending sideways and backwards. This is called the recovery stroke. During this recovery stroke, ciliary movement takes place near the cell surface (fig. 2) [10, 11]. The recovery phase amounts to three-quarters of the cycle time [7]. This is followed by an effective stroke during which the cilia move in a plane perpendicular to the cell surface. This phase ends with the cilium bent over in its rest position and with its tip pointing in the direction of propulsion, thereby minimizing resistance to mucus flow [10, 11]. During this active phase, claws on the tip of the cilia engage in the overlying mucus and sweep it in a cephalic direction. The active phase occupies one-quarter of the total cycle time [7].

On normal airway epithelium, all of the ciliary bases have the same alignment of their basal feet and therefore all of the effective strokes take place in approximately the same direction, thereby cooperating to propel the overlying mucus in a cephalic direction [10, 12].

The cilium moves in a layer of periciliary fluid whose depth is a little less than the ciliary length. This means that the overlying mucus is only penetrated by the ciliary tips in the effective stroke and not in the recovery stroke, thereby optimizing the propulsive force of the ciliary beating on mucociliary transport. The thickness of the periciliary layer is critical for effective propulsion of mucus [10, 11].

The normal beating of cilia results from active sliding movements between adjacent doublets of the axoneme, as has been described by AFZELIUS [12]. This sliding is powered by an adenosine triphosphate (ATP)-driven mechanochemical cycle in which the dynein arms of one doublet interact with successive binding sites along the B-microtubule of an adjacent doublet. Other structures in the cilium, notably the cross-links between all peripheral microtubules of the axoneme in the basal body and the radial spokes that link together all doublets through

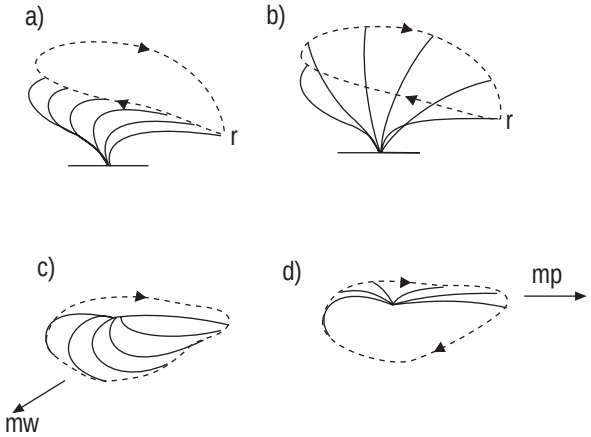

Fig. 2. – Schematic representation of the beat cycle of a rabbit tracheal cilium as seen: a, b) from the side; and c, d) from above. In the recovery stroke (a, c), the cilium starts moving from the resting position (r) and unrolls clockwise (a) to the left. In the effective stroke (b, d), it remains extended and bends over to reach the resting position, to the right. Mucus is propelled (mp) towards the right, and the metachronal wave (mw) is propagated backwards and towards the left. (From [13], with permission.)

the central complex of the axoneme, tend to restrict the sliding between peripheral doublets and cause the axoneme to bend rather than slide apart. The nexin links probably limit the extent of relative sliding and help to maintain the integrity of the cylinder of doublets [10, 13, 14].

Ciliary movement is co-ordinated by metachronal waves, thereby coupling the beat of each cilium to that of neighbouring cilia [10, 11]. Waves of ciliary activity are propagated *via* antilaeoplectic co-ordination, *i.e.* if an observer faces in the direction in which the waves are propagated, the effective stroke takes place backwards and towards the left. Each metachronal wave passes across a few ciliated cells before dying away. The direction of propagation of the waves depends upon the clockwise movement of the bent cilia in the recovery stroke, during which movement of neighbouring cilia is initiated, and upon the slight delay between the mechanical activation of adjacent cilia. Many small areas of co-ordinated activity, operating independently of each other, collaborate to propel the overlying mucus.

A wide range of ciliary beating frequencies (CBFs) in the central airways of mammals, including humans, have been reported. Various factors are probably responsible for these differences in CBF found in the literature. It would be expected that differences in CBF would be found depending on whether it is determined *in vivo* or *in vitro*. *In vitro*, the influences of airway blood flow and innervation are absent. Moreover, *in vitro*, whether the measurement is made in isolated ciliated cells or in airway epithelia may make a difference. Differences in CBF may be attributed to experimental conditions, such as temperature [15–18], humidity [19, 20] or duration [20]. The presence or absence of mucus may also play a role in determining the CBF. Not only the presence of mucus but also the load of the mucus have been suggested to change the CBF locally [10, 21]. The invasiveness of the experimental procedure probably has an influence on CBF. Indeed, mechanical stimulation, by means of mechanical deformation of the cell surface of cultured rabbit respiratory tract ciliated cells, induced a rapid-but-transient increase in CBF [21].

All these possible influencing factors account for the variation in normal CBF values in the central airways of mammals, including humans. HASTIE *et al.* [22], for example, reported the CBF in rabbit tracheal explants to be approximately 11 Hz. KARTTUNEN *et al.* [23] showed that the baseline CBF in rat tracheal explants varied between 18.3 and 22.4 Hz. ROTH and KRONENBERG [24] reported that the mean CBF in hamster tracheal rings was 16.1 Hz. The CBF, measured *in vivo* in healthy subjects by HELLEDAY *et al.* [25], was found to be highly homogenous between subjects under baseline conditions, with a range of 12–15 Hz.

Respiratory mucus

Composition of respiratory mucus

Mucus is composed of ~1% by weight of salts and other dialysable components, 0.5–1% free pro-tein, a similar proportion of carbohydrate-rich glycoproteins (also called "mucins" or "mucoproteins"), and ≥95% water. In bronchial mucus, there is additionally a significant amount of lipid [26], and, in pathological conditions, deoxyribonucleic acid (DNA) may also be present [26, 27]. LETHEM *et al.* [28] demonstrated that the DNA in the purulent sputum of cystic fibrosis (CF) patients was almost entirely derived from the host and, thus, not from the bacteria.

JORIS *et al.* [29] studied the electrolyte composition of the airway surface fluid (ASF) in healthy persons as well as in patients with sustained airway irritation, infection, CF and asthma. ASF collected from healthy airways contained approximately 45% less Na^+ and Cl^- and 600% more K^+ than plasma. These data suggest that ASF is hypotonic in normal subjects compared to plasma. In patients with sustained airway irritation, infection or CF, the ASF composition becomes more isotonic compared to plasma. In asthmatics, however, ASF was hypertonic compared to plasma.

Composition of respiratory mucus glycoproteins

Mucus behaves as a viscoelastic gel, consisting of water and high relative molecular mass cross-linked glycoproteins mixed with serum and cellular proteins, including albumin, enzymes and immunoglobulins, and lipids [30]. Mucus glycoproteins are composed of protein and carbohydrate components [31]. A high density of oligosaccharide units attached to a long polypeptide chain is a predominant feature of most mucus glycoproteins and might be expected to contribute greatly to the biological properties of these molecules [32].

SACHDEV *et al.* [33] studied respiratory mucus collected from healthy young male volunteers by means of fibreoptic bronchoscopy. Chemical analysis showed that tracheobronchial mucins consisted of 73% carbohydrate, 4% sulphate monoester and 23% protein.

All the monosaccharides of mucus are pyranoses. Monosaccharides, when linked together, are capable of forming a large number of different oligosaccharide units, which are attached to the polypeptide chain [31]. The sugars found in glycoproteins are fucose, galactose, glucosamine, galactosamine and sialic acid [32, 33]. Variations in the sugar content of the bronchial glycoproteins are found, depending on the blood group [32].

Threonine, serine and proline make up 40–50% of all amino acids in bronchial mucus glycoproteins [32]. The amino acid composition showed a high content (29%) of the combined hydroxyamino acids, serine and threonine, threonine being the predominant amino acid. The presence of carbohydrate chains linked to these hydroxyamino acids has been demonstrated [33]. KING and RUBIN [34] reported these linkages to be *O*-glycosidic bonds between *N*-acetylgalactosamine and serine or threonine.

The high degree of glycosylation and the presence of acidic groups, both sialic acid and sulphate monoester, are typical features of respiratory mucins. Other typical features of mucus-type glycoproteins, namely a high content of proline, glycine and alanine, a significant number of cysteine residues not involved in disulphide bonds and a low content of aromatic amino acids are also quite evident [33]. Mannose residues [32, 33] and uronic acid [32] are absent from the prosthetic group. The chemical composition of the respiratory mucus glycoproteins is suspected to influence the formation of the molecular network of mucus, thereby altering its rheological properties.

Secretion of respiratory mucus

Airway mucus is a mixture of products from several sources: 1) alveolar liquid, 2) secretory products from a variety of cells along the surface of the conducting airways, 3) submucosal gland secretory cell products, and 4) serum transudate. Epithelial cell turnover may also add membrane and cytoplasmic components to these secretions [35].

In the nose, trachea and larger bronchi, there are goblet cells and submucosal glands, consisting of both serous and mucous cells. The volume of the submucosal glands is approximately 40 times that of the goblet cells in the human tracheobronchial tract. The ciliated and goblet cells and submucosal glands decrease in number peripherally in the tracheobronchial tree, where simple cuboidal cells become increasingly common in the epithelium. In the smallest ciliated bronchioles, there is another type of secretory cell, the Clara cell, which may be transformed into a goblet cell in disease states [36].

Mucins produced by the goblet cells of the respiratory epithelium are condensed while stored in secretory granules. The condensation of mucins as well as their decondensation upon exocytosis may be explained by the theory of TANAKA [37] regarding polymer–gel phase transition. After receptor activation, which leads to the docking of the secretory granule to the plasma membrane, the formation of a secretory pore and the switching of the pore to high ionic conductance, Ca^{2+} inside the granule is exchanged for extracellular Na^{+}; Na^{+}/Ca^{2+} exchange triggers a polymer–gel phase transition, whereby the mucin polymer matrix undergoes extensive swelling and, thereby, changes from the condensed to the hydrated phase. Swelling of the granular content is driven by a Donnan potential and results in the release of secretory product and the formation of small mucin gels, which ultimately form the airway mucus layer [38, 39]. This secretion process is illustrated in figure 3.

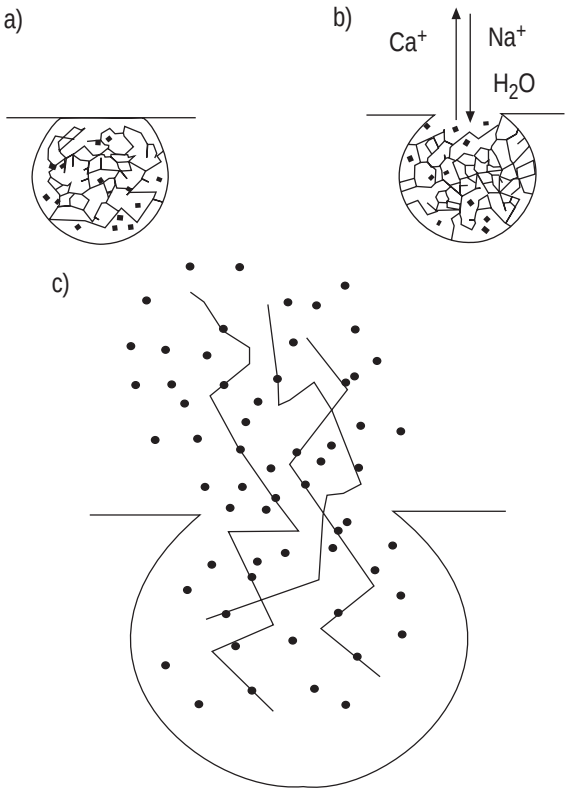

Fig. 3. – Mechanism of mucin exocytosis. By analogy to the sequence of activation in muscle contraction, in exocytosis, it is convenient to separate the stimulus-coupling steps from the actual mechanical phenomena that drive product release. a) The stimulus-coupling steps encompass the events that start with receptor activation and lead to the docking of the secretory granule to the plasma membrane, formation of a secretory pore and switching of the pore to high conductance. b) Once a high conductance channel is established between intragranular and extracellular compartments, Na^{+}/Ca^{2+} exchange follows, triggering a phase transition that changes the mucin polymer from the condensed to the hydrated phase. c) At this stage, electrochemical energy stored in the mucin polymer network is transformed into mechanical energy, *via* electrostatic interactions, driving the final release of secretory products. (From [39], with permission.)

The role of respiratory mucus

MCC is the most widely studied function of the respiratory mucus. A traditional and an alternative model have been proposed for the MCC mechanism.

Figures 4 and 5a show a schematic representation of the traditional concept of the anatomy of the mucociliary apparatus in the airways. The mucus blanket, once established, is usually thought to consist of a sol layer, which bathes the cilia, and a gel layer, which lies on top of the sol (or periciliary) fluid, in the proximal airways. Clearly there is an equilibrium between these two layers and it may be argued that the sol layer, which is thin and watery, allows the cilia to beat and sweep the overlying gel in a cephalic direction on the lubricant-like sol [7].

More recently, an alternative model has been proposed in which mucins form a tangled network that is concentrated at the air–liquid interface but extends to the epithelial surface, as illustrated in figure 5b [39, 40].

YEATES *et al.* [41] reported mucus flow in the trachea of 42 healthy nonsmoking adults to average 3.6 $mm \cdot min^{-1}$ with a coefficient of variation of 75%. FOSTER *et al.* [42] showed that the mucus velocity in the main bronchi averaged 2.4 $mm \cdot min^{-1}$ and tracheal mucus velocity (TMV) 5.5 $mm \cdot min^{-1}$ in a group of seven healthy subjects. FRIEDMAN *et al.* [43] measured TMV in 12 normal nonsmoking subjects and found values ranging 6.7–11.4 $mm \cdot min^{-1}$. Mucus flow in the human trachea has been shown to be variable, diminishing progressively towards the lung periphery, where the secretions are thought to be more watery, and reabsorption of the secretions takes place during movement of mucus from the peripheral to the central airways [44]. Consequently, differences in mucus transport velocity might occur, depending on the airway generation in which it is measured. However, the method chosen to measure mucus transport velocity may also affect the results, as is the case for the determination of CBF. Moreover, there are studies showing a wide range of MCC values within a group of healthy persons [45, 46].

Although the most widely studied function of the respiratory mucus is MCC, a variety of other functions, such as airway hydration, regulation of the thickness of the periciliary layer, bacterial adhesion and clearance, and acting as a filtration and diffusion barrier, are almost as important as the clearance of mucus in the protection of the underlying respiratory cells. All these functions are closely associated with the physical and biochemical character-

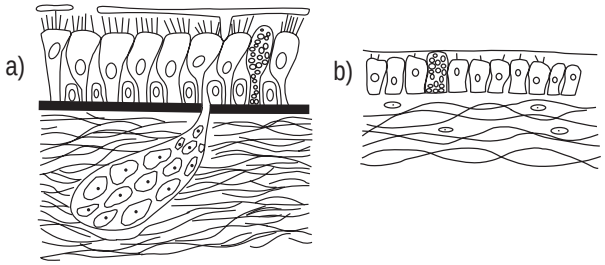

Fig. 4. – Schematic representation of the normal adult mucociliary apparatus in: a) central (*e.g.* trachea), and b) peripheral (*e.g.* terminal bronchioles) airways. The components of the apparatus in the central airway are, from top to bottom, the mucus layer (gel), periciliary fluid layer (sol), surface epithelial layer consisting of ciliated and nonciliated (basal and secretory granule-containing goblet) cells and submucosal gland. By contrast, in the peripheral airways, there is no mucus layer, the epithelium is flat and less densely ciliated and mucus-producing cells are replaced with other secretory (Clara) cells. (From [85], with permission.)

istics of mucus [47]. Other functions of mucus rely on its cellular components. Neutrophils and macrophages are the scavengers of the lung. Substances produced locally, *e.g.* lysozyme, bronchotransferrin and antiproteases, which have antimicrobial and antiproteolytic actions, are also present in mucus [48].

The rheological and physical properties of mucus and their effect on mucociliary clearance

The respiratory mucus is a viscoelastic material characterized by nonlinear (non-Newtonian viscosity) and time-dependent flow (thixotropic) properties. Spinability (see below) is also a property common to respiratory mucus. Besides these rheological properties, the respiratory mucus possesses surface properties, such as adhesivity and wettability. These physical characteristics determine the capacity of mucus to protect, hydrate and lubricate the underlying airway epithelium and, therefore, are probably almost as important or even more important than the rheological properties [47].

Owing to the cross-linking of glycoproteins [34], mucus can be considered a viscoelastic fluid which exhibits both liquid-like (viscous) and solid-like (elastic) properties [30]. Viscosity is the resistance to flow and represents the

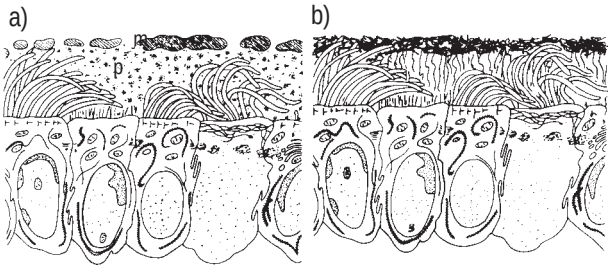

Fig. 5. – Schematic representation of the mucociliary transport mechanism. Superficial epithelial cells are depicted predominantly as ciliated cells. a) The conventional view of the organization of the surface liquid layers. A discrete mucus layer is present (m), resting on a periciliary liquid layer (p). b) An organization in which the mucus layer is not discrete but consists of a "tangled network", concentrated at the air–liquid interface but extending to the epithelial surface. (From [40], with permission.)

capacity of a material to absorb energy when moving. Elasticity is the capacity of a material to store the energy used to move or deform it. As a viscoelastic gel, mucus exhibits a response to stress that is neither solid-like nor liquid-like, but some combination of the two. A true solid (and this includes gels with stable cross-links) responds to stress with a finite deformation from which it totally recovers after the stress is removed. A true liquid responds to the same stress by deforming or flowing continuously for the time that the stress is applied. After the removal of the stress, the flow ceases and there is no recovery of the strain [30].

Owing to the recently proposed alternative model of the mucociliary apparatus in which the mucin network has a tangled rather than a cross-linked topology, the rheological properties of mucus are believed to be physiologically regulated by hydration *via* control of the transepithelial movement of water, ions and soluble proteins [38, 39], rather than by variations in the degree of covalent cross-linking between glycoprotein chains.

Mucus exhibits shear thinning, *i.e.* following exposure to high shear forces, it shows a decreased viscosity at lower shear forces. Some shear thinning may be permanent, with a permanently reduced viscosity (altered molecular structure), whereas some shear thinning may be reversible (thixotropy) [30, 34]. This is particularly important during coughing.

Mucus is non-Newtonian and behaves as a pseudoplastic liquid in which viscosity decreases as the applied force is increased [13]. The ratio of stress to rate of strain is thus nonlinear [30]. This implies that the more forcefully the cilia beat, the more easily the mucus moves [13].

Spinability (filance, Spinnbarkeit) is the thread-forming capacity of mucus under the influence of large amplitude elastic deformation [30, 34]. An important feature of spinability is that it gives information on the internal cohesive forces of mucus as well as on its elasticity, although it does not directly depend on its degree of viscosity and elasticity [47]. It has been reported that the spinability of normal respiratory mucus ranges between 40 and 100 mm and decreases, often markedly, in pathological conditions as its purulence increases. Normal mucus has been obtained from the human nose, the dog trachea and the frog palate, and abnormal mucus from chronic bronchitis (CB) patients. Spinability has been correlated positively with MCC on the frog palate [49]. A major disadvantage of the measurement of spinability is that it does not correlate well with other more basic measures of viscoelasticity [30].

Adhesiveness is the ability of mucus to bind to a solid surface, and this can be measured as the force needed to separate one or more solid surfaces and the adhesive material. Adhesiveness is dependent on mucus surface tension, hydration, wettability and contact (dwell) time [30, 34]. At the respiratory level, mucociliary transport involves surface interactions between the cilia and the mucus. Adhesivity has been found to correlate inversely with MCC [50]. PUCHELLE *et al.* [51] measured values ranging 62.2–118.4 $mN \cdot m^{-1}$ in human bronchial secretions obtained from subjects with pathological conditions.

The wettability of a biological fluid characterizes its ability to spread when deposited onto a solid planar surface. The degree of wettability is characterized by the "contact

angle" between the solid and the liquid at equilibrium; the lower the contact angle, the greater the wettability [47].

Physiological factors affecting mucociliary clearance

Age

YAGER *et al.* [52] measured the CBF of human respiratory epithelium *in vitro*. Their results indicate that CBF decreases with increasing age. However, some groups were represented by only one or a few subjects in their study, and, hence, no significant data could be obtained. In addition, there appears to be an inverse relationship between MCC and age, although this is still debated [53]. GOODMAN *et al.* [54] showed that the mean TMV in a group of 10 young nonsmoking subjects (mean age 23 yrs) was 10.1 $mm \cdot min^{-1}$ and in a group of seven elderly nonsmokers (mean age 63 yrs) 5.8 $mm \cdot min^{-1}$.

Sex

HASANI *et al.* [55] showed that there was no statistically significant difference between young healthy males and females, of comparable age, in the rate of clearance of inhaled radioaerosol over a 6-h observation period. In the literature, there is however still some doubt about the absence of an influence of sex on MCC [53]. The results of SVARTENGREN *et al.* [56], for instance, indicated that MCC was faster in females than in males. Two hours after inhalation of 6 μm Teflon particles labelled with technetium-99m, the percentage retention (an indication of the rate of MCC, with a higher percentage retention indicating slower MCC and *vice versa*) in healthy nonsmoking females and males was significantly different (26±17% mean±SD for the female and 41±23% for the male subjects respectively).

Posture

Posture may influence MCC by making use of gravitational forces. Gravity does not aid mucociliary transport in healthy subjects [57–58]. In patients with impaired MCC, the influence of gravity on the transport of mucus becomes more important [57]. Postural drainage makes use of gravity to enhance MCC [59, 60]. By inclining patients downwards on an adjustable bed, the bronchi are brought into a vertical position. Postural drainage is reported only to be effective when there are relatively large quantities of mucus of a low adhesiveness present in the airways [60, 61]. In the literature, however there is controversy about the effects of this technique [62].

Sleep

Sleep has a depressant effect on lung MCC, both in healthy subjects [63] and in asthmatic patients [63, 64].

Exercise

WOLFF *et al.* [65] showed a significant enhancement of MCC during exercise in healthy, nonsmoking subjects compared to controlled eucapnic hyperventilation. OLDENBURG *et al.* [62] showed that exercise increased total lung clearance in patients with CB. PAVIA [63] found, however, that simple exercise, such as performing routine duties within a hospital environment, compared to relaxing (throughout the 6 h observation period) did not have any effect on the rate of removal of lung secretions in healthy subjects.

Environmental pollutants affecting mucociliary clearance

Common environmental pollutants such as sulphur dioxide, sulphuric acid, nitrogen dioxide and ozone are known to affect the functioning of the mucociliary apparatus.

Sulphur dioxide and sulphuric acid

SO_2 is one of the major airborne pollutants in industrialized countries [66]. The primary outdoor source of SO_2 is the domestic, commercial and industrial combustion of sulphur-containing fossil fuels (coal and oil). Coal-fired power plants, in some areas, are also large point sources, with tall stacks that can disperse pollutants at higher altitudes and promote long-distance transport [67]. The concentrations of SO_2 which may not be exceeded are fixed at 0.14 parts per million (ppm)$\cdot day^{-1}$ or 365 $\mu g \cdot m^{-3} \cdot day^{-1}$ and 0.03 $ppm \cdot yr^{-1}$ or 80 $\mu g \cdot m^{-3} \cdot yr^{-1}$, as prescribed by the national ambient air quality standards. The 24-h limit may not be exceeded more than once a year. The industrial threshold limit value, which is the concentration allowed for 8-h exposure, is 2 ppm [67, 68].

RIECHELMANN *et al.* [66] reported on the functional and ultrastructural alterations to guinea-pig respiratory epithelium exposed *in vitro* to SO_2 for 30 min. Despite a 56% decrease in CBF, only minor morphological alterations were observed following exposure to 7.5 $mg \cdot m^{-3}$ SO_2. However, following exposure to ≥ 15 $mg \cdot m^{-3}$ SO_2, structural alterations to the respiratory mucosa such as epithelial sloughing, intracellular oedema and mitochondrial swelling, widened intercellular spaces and ciliary cytoplasmic extrusions were found. CBF thus seems to be a more sensitive indicator of airway toxicity than structural alterations alone. SO_2-induced impairment of mucociliary function increases the contact time of particles, bacteria and allergens with the airway mucosa. In addition, widening of intercellular spaces suggests that the barrier function of the airway epithelium is impaired, predisposing to invasion by inhaled pathogens. Although the concentrations used in these short-term toxicity experiments are approximately 25 times higher than ambient SO_2 concentrations in polluted areas, it is likely that these alterations are important pathophysiological factors and may lead to an increased incidence of upper airway disease in polluted areas.

Conversely, NEWHOUSE *et al.* [69] noted that MCC was significantly faster in 10 healthy exercising nonsmoking adults after exposure to 5 ppm SO_2 for 2.5 h. The same observation was made in another group of 10 healthy exercising nonsmoking males after exposure to 1 $mg \cdot m^{-3}$ H_2SO_4 for the same period of time. The faster clearance following exposure was suggested to be an irritant response caused by the exposure.

MAN *et al.* [70] examined the influence of SO_2 exposure for 75 min on mucociliary transport in dogs *in vivo*. Following 100 ppm SO_2 exposure, abnormal mucociliary transport was present, and lasted for a period of days before recovery occurred. For 500 ppm SO_2, as expected, the derangement of mucociliary transport was more profound and took several weeks to return to normal in some animals. It is, however, important to note that mucociliary transport returned to normal in these dogs, even after exposure to a high concentration of SO_2 such as 100 ppm.

SCHLESINGER *et al.* [71] studied the effect of exposure to 125 $\mu g \cdot m^{-3}$ H_2SO_4 for 2 $h \cdot day^{-1}$ and 5 $days \cdot week^{-1}$ for up to 1 yr in rabbits. Tracheobronchial MCC became progressively slower towards the end of the exposure regimen. An increase in the number of secretory cells in the small airways was found by 12 months of exposure.

Nitrogen dioxide

NO_2 is one of the most common air pollutants. NO_2 is mainly produced by various combustion processes, especially in industrial locations and urban areas, and also in homes with gas stoves. In European countries, the exposure limits for an 8-h work shift range 2–5 ppm (5–9 $mg \cdot m^{-3}$) [25]. The national ambient air quality primary standards prescribe that the NO_2 concentration may not exceed 0.05 $ppm \cdot yr^{-1}$. The industrial threshold limit value is fixed at 3 ppm [67, 68].

HELLEDAY *et al.* [25] examined the influence of NO_2 exposure on the CBF in healthy subjects. They demonstrated a significant reduction in CBF 45 min after exposure to either 1.5 ppm or 3.5 ppm NO_2, concentrations often found in industrial workrooms. This effect ceases within 24 h but could potentially be of importance in the MCC of, for example, allergens, viruses or other microorganisms.

RASMUSSEN *et al.* [72] studied the influence of long-term (4 $h \cdot day^{-1}$ and 5 $day \cdot week^{-1}$ for 8 or 15 weeks) exposure to 0.5 or 10 ppm NO_2 on respiratory tract clearance in 6-week-old ferrets. They found no effect of NO_2 exposure on MCC in the head airways region, which extends from the tip of the nose to the midpoint of the trachea. NO_2 reduced MCC in the thoracic region, which includes the lower half of the trachea and the lungs, but this reduction in particle clearance reached significance only in the 10 ppm group. Their results indicated that the impairment in clearance function may only recover slowly after chronic exposure.

Ozone

As prescribed by the National Ambient Air Quality Standards, the concentration of O_3 which may not be exceeded for 1 h, is fixed at 0.12 ppm or 235 $\mu g \cdot m^{-3}$. This limit may not be exceeded more than once a year. The industrial threshold limit value for O_3 is 0.1 ppm [67, 68].

GERRITY *et al.* [73] exposed 15 healthy male and female nonsmoking subjects, on different occasions, to clean air and 0.4 ppm O_3 for 1 h while they exercised continuously. Their results indicated that despite significant changes in pulmonary function, mucociliary transport was unaffected by ozone exposure if measured 2 h after exposure.

FOSTER *et al.* [74] studied the effect of 0.2 and 0.4 ppm O_3 compared with filtered air in seven healthy males. They were exposed to these concentrations for 2 h, during which time periods of rest were alternated with periods of light exercise. Contrary to the findings of GERRITY *et al.* [73], mucus transport was significantly increased during exposure to 0.4 ppm O_3 and was associated with impaired lung function (*e.g.* reductions in forced vital capacity, midmaximal flow rate and forced expiratory volume in one second). Regional analysis indicated that mucus flow from the distal airways into the central bronchi was significantly increased by exposure to 0.2 ppm O_3. This peripheral effect was buffered by there being only a marginal influence of 0.2 ppm O_3 on the larger bronchi. Consequently, the resultant mucus transport, in all airways of the lung, differed only slightly from that found on exposure to filtered air. Differences in the regional diffusion of O_3 may, however have affected these results.

SCHLESINGER *et al.* [71] investigated the effect of long-term exposure to O_3 in rabbits. They exposed the animals to 0.1 ppm O_3 for 2 $h \cdot day^{-1}$ and 5 $days \cdot week^{-1}$ for up to 1 yr. O_3 was shown not to have a statistically significant effect on tracheobronchial MCC. An increase in secretory cell number was noticed by 4 months, but the response became attenuated with continued exposure.

Hairspray

The effect of hairspray on TMV was investigated in healthy subjects [43]. The aerosol spray, directed towards the hair, was delivered for 20 s. At 1 h postexposure, TMV was found to be depressed by as much as 57% of baseline values despite unaltered pulmonary function tests. This reduction in TMV was transient since it could no longer be demonstrated after 3 h. No significant changes in TMV or pulmonary function tests were observed in the five control subjects exposed to the Freon propellant alone.

Tobacco smoke

The influence of smoking on MCC appears to be subject to interindividual variation. Although five of 13 young smokers in the study of GOODMAN *et al.* [54] had TMV values within the range of age-matched nonsmokers, the remainder had markedly depressed values. They noticed the same trend in exsmokers. Five of nine young exsmokers had values within the range of age-matched nonsmokers, whereas in the other four TMV was markedly depressed. MORTENSEN *et al.* [53] reported MCC to be faster in lifelong nonsmokers than in exsmokers.

GOODMAN *et al.* [54] measured TMV before and within 10 min after smoking a cigarette in a group of smokers. They noticed no consistent change in this parameter.

Chronic smoking has been shown to induce an increased number of abnormal (bronchial) cilia, which could participate in the impairment of tracheobronchial clearance. These ciliary abnormalities appear to be independent of the aetiology of chronic sputum production. Even after a long period of smoking cessation, the ultrastructural ciliary damage seems nonreversible and respiratory symptoms persist [75].

The effects of environmental pollutants on MCC have been covered in two excellent reviews by WOLFF [68] in 1986 and, more recently, by GONG [67] in 1992.

Diseases affecting mucociliary clearance

Immotile cilia syndrome – primary ciliary dyskinesia – Kartagener's syndrome

Immotile cilia syndrome (ICS) is a congenital disease. ICS patients have impaired ciliary activity and, as a consequence, the incidence of respiratory infection is high. The reason for impaired ciliary activity is the absence of the dynein arms normally found in the nine peripheral, microtubular doublets. These arms contain the ATPase-containing protein dynein [76]. Although the absence of the outer dynein arms is the most common defect, missing radial spokes and translocation of the outer doublet microtubules have also been observed [77].

ICS patients exhibit a spectrum of motility abnormalities of their cilia, ranging from complete immotility to considerable but abnormal motility, resulting in inefficient transport of the mucus blanket. Even when motility occurs, ciliary motion is grossly abnormal and not co-ordinated into the normal metachronal waves owing to the ultrastructural defect being incomplete and perhaps of varying penetrance in the individual cells or cilia. Because some motility may occur, the syndrome is alternatively referred to as primary ciliary dyskinesia [36].

Approximately half of ICS cases have situs inversus totalis, *i.e.* a complete transposition of the organs in the abdomen and chest. This subgroup of ICS, if combined with bronchiectasis, is a previously recognized entity named Kartagener's syndrome [4]. Kartagener's syndrome is the association of situs inversus with chronic sinusitis and bronchiectasis. Visceral asymmetry is probably determined through the movement of the cilia of some embryonic epithelial tissue [5].

Because the cilia (or the sperm flagellum) of persons with ICS are immotile (or inefficient or absent), a number of common clinical findings occur such as nasal polyposis, bronchitis, rhinitis, sinusitis, otitis media and often obstructive lung disease associated with bronchiectasis, sometimes acquired at an early age. Males are usually sterile due to sperm immotility, whereas females have lowered fertility. The respiratory tract, sinuses and auditory ducts are, thus, sites of repeated infection in these patients, as might be expected in a person with no mucociliary transport [4, 5].

CAMNER *et al.* [78] measured tracheobronchial clearance in 20 patients who fulfilled the criteria for ICS, based on combinations of a typical history of bronchitis, rhinitis and sinusitis since childhood, hereditary data, situs inversus, sperm immotility and characteristic defects of respiratory tract cilia and sperm tails. These data were compared with those of eight subjects suspected of suffering from the syndrome, but who did not fulfil the criteria. All patients who fulfilled the criteria had an extremely slow, and probably no, tracheobronchial MCC, whereas all those who did not fulfil the criteria had some clearance.

It has for a long time been thought that failure of the ciliary mechanism in the respiratory tract might lead to death. Surprisingly, ICS patients have respiratory disease and decreased fertility, but in most cases live a normal and active life. Indeed, they are able to compensate for their mucociliary transport deficiency caused by ciliary failure by other mechanisms such as coughing and hawking [4].

Asthma

Mucociliary dysfunction has been shown to be a feature of asthma [79]. Even during remission periods, tracheobronchial MCC seems to be slower in asthmatic patients than in healthy subjects. SVARTENGREN *et al.* [80] studied tracheobronchial clearance in asthma-discordant monozygotic twins. According to this study, mucociliary transport may be either increased or decreased in asthma. Indeed, the authors assumed that there may be constant irritation in asthmatics, caused by inflammation, and therefore, at least in the earlier stages, increased mucociliary transport. On a long-term basis, hyperstimulation of mucociliary transport itself, or other factors associated with asthma (*e.g.* epithelial damage and hypersecretion), might lead to impaired mucociliary transport. The clearance impairment, when observed, is thus likely to be secondary to the disease.

Because the mucociliary dysfunction in asthma has been closely linked to airway inflammation, a search for inflammatory mediators causing mucociliary dysfunction was initiated. The effects of platelet activating factor [81], endothelin-1 [82], major basic protein [22], slow reacting substance of anaphylaxis [83], bradykinin [84] and the prostaglandins E_2 and $F_{2\alpha}$ [24] on MCC or its component functions were, for example, studied. In addition to inflammatory cell products, neuropeptides may also participate in mucociliary dysfunction. Extensive research has been performed on the effect of substance P on MCC. The role of inflammatory mediators and neuropeptides in mucociliary impairment was summarized by WANNER *et al.* [85].

Bronchiectasis

Bronchiectasis develops when mucus plugging and infection occur together in the absence of functioning cilia [86].

In patients with bronchiectasis, the clearance of mucus varied from normal to extremely slow [56]. Indeed, the more impaired the clearance was, the more generalized the airway symptoms were (affecting upper as well as lower airways), the more continuous they were and the earlier in life they had started. An impairment of clearance is usually generalized in patients with bronchiectasis, regardless of the localization of the bronchiectasis.

CAMNER and MOSSBERG [36] suggest that, in a subgroup of bronchiectatic patients, generalized impairment of mucociliary transport is a major factor in the later development of bronchiectasis. In this subgroup, patients with congenital defects as well as patients acquiring widespread damage to their mucociliary system early in life, possibly due to infections, are included. In fact, for most bronchiectatic patients, local damage to the respiratory tract epithelium or bronchial wall along with a local clearance defect (*e.g.* due to infection) might be the cause of the mucociliary transport dysfunction.

In the study of WILLS *et al.* [17], sputum from patients with bronchiectasis was transported slowly, at a mean rate of 15% of that of control mucus on the mucus-depleted bovine trachea. Their results suggest that there was a serious defect in the ciliary transportability of sputum, unrelated to the presence of infection, as neither the purulence of the sputum nor the presence of *Pseudomonas aeruginosa* in the mucus influenced its transportability. This study also indicated that mucus retention is not simply due to a larger quantity of normal mucus being produced, as sputum was transported more slowly than an equal quantity of control mucus.

Chronic bronchitis

Common CB, usually caused by many years of tobacco smoking, is characterized by symptoms of cough and expectoration quite similar to those found in ICS. Many patients with CB develop varying degrees of more or less fixed airway obstruction, which also tends to occur in ICS patients. There are thus some striking similarities between these diseases, in spite of the obvious dissimilarities present, such as their occurrence in very different age groups and the fact that obstructive CB in the elderly is often associated with emphysema [36].

Various studies agree that MCC is impaired in patients with simple as well as obstructive CB. Simple CB is defined as chronic expectoration of mucoid secretions without major airway obstruction, whereas in obstructive CB airway obstruction is present. ERICSSON *et al.* [87] found a slight impairment of MCC in the tracheobronchial tract of patients with mainly simple CB. GOODMAN *et al.* [54] reported a markedly decreased TMV in patients with simple as well as obstructive CB. In contrast, MORETTI *et al.* [88] noted that patients with obstructive CB had faster tracheobronchial clearance when their bronchial reversibility was >15% compared to patients with a bronchial reversibility of <15%. VAN DER SCHANS *et al.* [89] compared the clearance of mucus in an emphysema group and a CB patient group. All patients had chronic airway obstruction and productive cough, but lung elastic recoil pressure was low in the emphysema patients, and normal in the CB patients. Spontaneous clearance from the peripheral lung region was higher in the emphysema group than in the CB group. There was no difference between the two groups in central clearance. The clearance of mucus from a peripheral lung region, however, increased significantly during forced expirations and cough in the patients with CB, but not in those with emphysema. In agreement with these findings, ERICSON *et al.* [90] suggested that, in patients with CB, coughing may compensate for decreased MCC. This would lead to a fairly effective overall tracheobronchial clearance. It is noteworthy that all patients selected for these studies were smokers or exsmokers.

PUCHELLE and ZAHM [91] showed that the CBF, measured photometrically on the depleted frog palate, was significantly lower using sputum samples collected from patients with chronic obstructive pulmonary disease (COPD) (11.3±3.3 Hz) than with the frog mucus (16.9±3.3 Hz) used as control. The relative (*i.e.* the ratio of sputum to control frog mucus) transport rate of the different sputum samples closely correlated ($r = -0.68$, $p<0.001$) with the relative CBF.

PRESCOTT *et al.* [92] found that chronic hypersecretion of mucus was a significant predictor of pulmonary infectious death but not of noninfectious death in COPD.

Cystic fibrosis

CF is a common lethal genetic disease that affects epithelia. Although different organs are affected, including the pulmonary airways, pancreas, sweat glands, intestine and male genital tract, lung disease is the major cause of morbidity and mortality [93].

CF patients have defective epithelial Cl^- permeability and, hence, a reduced capacity for Cl^- secretion, as well as an increased rate of Na^+ absorption, which may generate dehydrated respiratory tract fluid [93]. Abnormal amounts of viscous mucus are thus produced [36]. As a result, the efficiency of the normal MCC defence mechanism may be impaired, and the lungs may become more susceptible to bacterial infection [93]. S*taphylococcus aureus* and *P. aeruginosa* are the most common sources of bacterial colonization [77], resulting in bronchiectasis and chronic airway obstruction [36].

APP *et al.* [94] noted that baseline MCC values were significantly lower in younger CF patients (≤14 yrs) than in older ones (>14 yrs). The authors suggested that this might represent a maturation effect of MCC [95].

Acute respiratory tract infections

Respiratory tract infections may influence MCC or its component functions. CEESAY *et al.* [96] showed *in vitro* that the mucus transport velocity was lower in rats with acute bronchitis than in normal rats, possibly due to an early phase of viral or bacterial infection. SALATHÉ *et al.* [77] agreed that respiratory tract infection may impair MCC by means of two principal mechanisms, namely through cytotoxic effects on the airway epithelium (*e.g.* viruses) or through production of substances by microorganisms (*e.g.* pyocyanin) that impair MCC.

Viral agents such as myxovirus as well as different types of the rhinovirus or influenza virus, bacterial agents such as *Haemophilus influenzae* or *Streptococcus pneumoniae* and various types of mycoplasma including *Mycoplasma pneumoniae* are described as influencing MCC, mostly negatively. Reviews on this subject have been published by LINDBERG [97] and PAVIA [98].

Conclusions

MCC is an important mechanism for the clearance of airway secretions from the lungs. Studies on MCC have yielded considerable knowledge about its function, both in health and disease. Many questions, however, remain. There is still uncertainty about, for example, the composition of the periciliary fluid layer since it is extremely difficult to sample. Continued research is important to finding answers to some of the remaining questions.

Numerous publications are available about MCC itself and on its component functions. However, different studies have not always yielded similar results; in some cases opposite results have been obtained. This may, in part, be

explained by the methods chosen to evaluate MCC. There are many methods of studying the MCC mechanism and its component functions, but none has been shown to be superior. They all have their own shortcomings.

Although the methodology for studying the mucociliary apparatus is not optimal, research should continue. It may be important to know the cause of MCC impairment in patients. Specific knowledge about MCC deficiencies may lead to specific therapy. Indeed, defective Na^+/Cl^- transport across the airway epithelium is a major cause of the impaired functioning of the mucociliary apparatus in CF patients. Based on this knowledge, drugs, such as amiloride, were identified that influence the defective ion transport and, hence, also the impaired MCC mechanism. Moreover, an abnormally high concentration of DNA has been noted in CF mucus. This causes the mucus to become more viscous. Consequently, the prescription of a drug, such as DNase, that makes the mucus less viscous may be desirable to improve MCC in these patients.

Different studies have investigated the effect of environmental pollutants on MCC. Mostly, their data show that environmental pollution has a negative influence on this process. Many variables are expected to modify the final result of environmental pollution on the functioning of the mucociliary apparatus. Indeed, the concentration and duration of exposure to the environmental pollutant are probably important in this matter. However, the state of mucociliary functioning before exposure to the environmental pollutant may also be important. It might be suspected that, in a person with an impaired MCC mechanism, the effect of environmental pollution on this process would be more pronounced than in persons with a normally functioning mucociliary apparatus.

Finally, it is important to keep in mind that cough serves as a back-up system for the clearance of mucus if the mucociliary transport mechanism fails. This is certainly true for patients who suffer from immotile cilia syndrome. There are, however, numerous diseases such as chronic bronchitis where patients do not only have impaired mucociliary clearance, but also encounter problems with cough. Indeed, the amount and rheological properties of mucus influence not only mucociliary clearance, but also cough. Other patients have normally functioning mucociliary clearance but are unable to cough efficiently, for instance because of muscle weakness. These patients will have difficulty in clearing their airways if mucociliary clearance also becomes impaired, for example due to an acute respiratory tract infection.

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