The mucociliary activity of the upper respiratory tract

N. G. Toremalm, U. Mercke and A. Reimer, Lund, Sweden

ERASMUS UNIVERSITEIT MEDISCHE BIBLIOTHEEK ROTTERDAM

SUMMARY

A clinical in vivo - in vitro method for functional mucociliary studies via surface--light reflections is described as well as a method for intracellular recording of electrical activities in ciliated cells. Two types of surface-light reflections can be recognized – one from the secretion layer and one from the tops of lashing cilia. Internal and external cell activities als well as muco-rheological factors can be compared under the control of environmental influences such as air temperature, humidity and gas mixtures. The method can also be used for different clinical purposes.

IN the upper respiratory tract there are four essential defence mechanisms against inhaled particles such as bacteria, viruses, cancerogenic substances etc. They are:

1. The special airodynamic shape of the nose
2. The mucociliary mechanisms of transportation
3. The local immunity of the mucosa and secretions and
4. Phagocytosis.

The configuration of the nasal cavities with small inlets and outlets brings about a turbulent airflow with intimate contact between the inspired air and the mucous membranes as a result. This is essential for the

1. Warming
2. Moistening and
3. Cleansing of the inspired air.

The function of the cilia can be studied by two main approaches. One is qualitative observation of the transportation rate of small particles or quantitative observation of the removal of a known amount of deposited or inhaled particles, i.e. clearence estimations. The other is to record the beat frequency of the cilia or, more correctly, the frequency of mucus waves brought about by the beating cilia. The latter approach was used as early as 1884, when Martius estimated the ciliary beat frequency with a stroboscope. However, this technique does not permit recording of irregular changes in frequency, and more modern cinematographic

After a paper presented at the 6th Congress of the European Rhinologic Society, Erlangen, September 1975.

recording methods are time-consuming and expensive for routine experiments were immediate results are wanted.

In our opinion, there has been an urgent need for a relatively simple method for ciliary function studies, which can be easily reproduced in clinical laboratories. Our experimental arrangement consists of (1) a light source, (2) an experimental chamber for in vitro specimens, (3) a binocular microscope, (4) a photodiode (5) a frequency filter, and (6) an ink-writer as seen in the block diagram (Fig. 1). (Mercke et al., 1974a).

This method permits:

1. Instantaneous recording of wave movements in the secretion layer.
2. Sensitivity to rapid frequency changes.
3. Simple analysis of the recordings.
4. Simultaneous recording and regulation of temperature and humidity and
5. Use of routine laboratory equipments.

It is necessary to focus an area wide enough to present the wave movements initiated by a group of coordinated ciliated cells and small enough to exclude

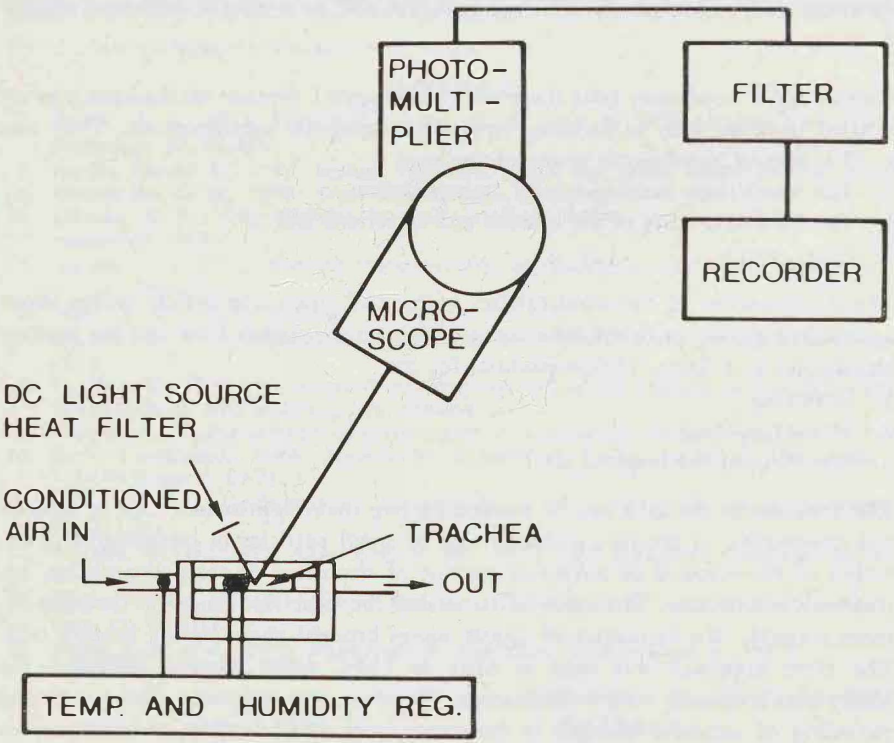

Figure 1. Block diagram of the experimental equipment including a light source, an experimental chamber, a microscope, a photomultiplier or photodiode, a frequency filter and an ink-writer.

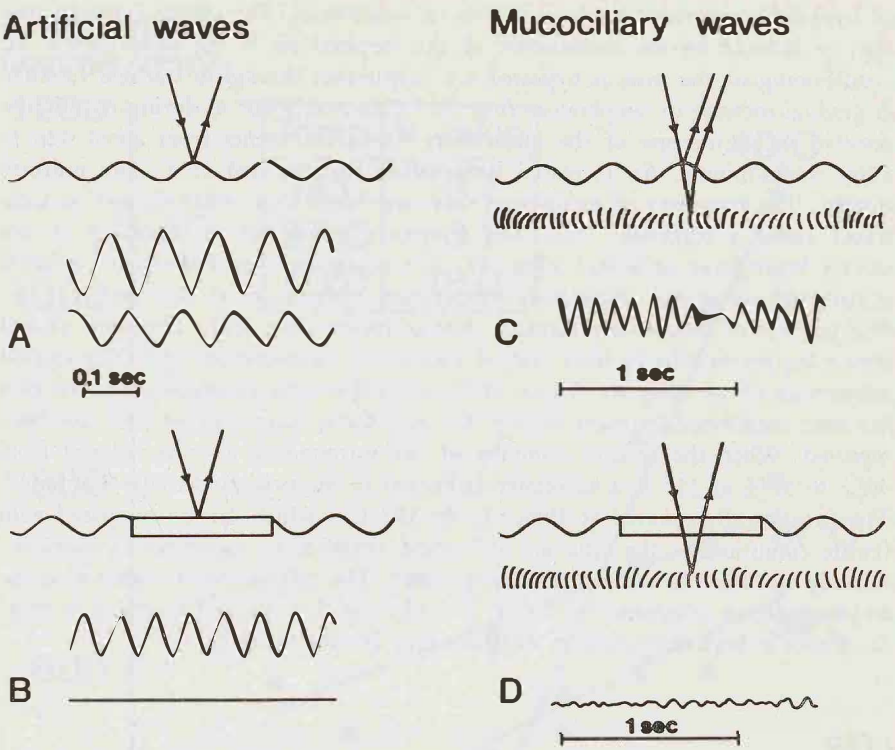

Figure 2. Recording of artificial waves on a water surface without (A) and with (B) a glass covering. Ordinary mucociliary waves from a rabbit trachea (C). In D the mucus waves are eliminated but the movements of lashing cilia are seen.

too many interchanging wave movements. In our method an area of about 380 μm^2 comprising about 10 ciliated cells is analyzed.

The light reflections may arise from the mucous layer or from the tops of the cilia beneath the surface. A simultaneous recording from a tone generator submerged in water and the reflections picked up by the described method from waves induced on a water surface is seen in fig. 2 (left). Down to the left the water surface is covered by a thin floating piece of glass. The reflections disappear. In the upper curve to the right there is an ordinary recording from the rabbit trachea with a frequency of twelve waves per second. The recording below is made through a piece of glass placed on the tracheal surface. The mucus waves are then eliminated, but nevertheless wave movements are still recorded. These reflections must emanate from the tops of the lashing cilia. That means that there are two more or less congruent reflection levels - (1) one on the mucus surface and (2) another on the underlying carpet of cilia. (Toremalm et al., 1974).

The mucociliary system in the nose, sinuses, trachea and bronchii may be influenced by temperature-changes during febrile conditions and also as a result

of hypothermia utilized in special types of anaesthesia. The ciliary function may also be reduced by the temperature of the inspired air if the physiological air conditioning of the nose is bypassed e.g. respiration through a tracheal cannula. A gradual increase in temperature from 21° C to nearly 40° C during 85 minutes resulted in an increase of the mucociliary wave movements from about 420 to 1000 waves/minute. An increased temperature also resulted in a more uniform rhythm. The frequency of mucociliary wave movements are relatively well concentrated along a regression line. The frequency/temperature relationship is not strictly logarithmic or linear. (Fig. 3). The regression line flattens out slightly at temperatures at and above body temperature. (Mercke et al., 1974b).

The pioneer of mucociliary research Arthur Proetz once said "The only natural enemy known to cilia in their line of function is excessive drying". The clinical importance of an adequate degree of humidity for retained mucociliary function has been mentioned by many authors but systematic investigations have not been reported. When the relative humidity of the surrounding air was reduced from 90% to 50% at 37° C a successive reduction in mucociliary activity was found. The function disappeared at 50% r.h. At 40° C – which can be compared with febrile conditions – the cilia are still more sensitive to reduction in humidity. Already at 70% r.h. the function disappeared. The relationship between function and humidity is illustrated in fig. 4. It is obvious that reduced humidity is more dangerous at high temperatures. (Mercke and Toremalm, 1975).

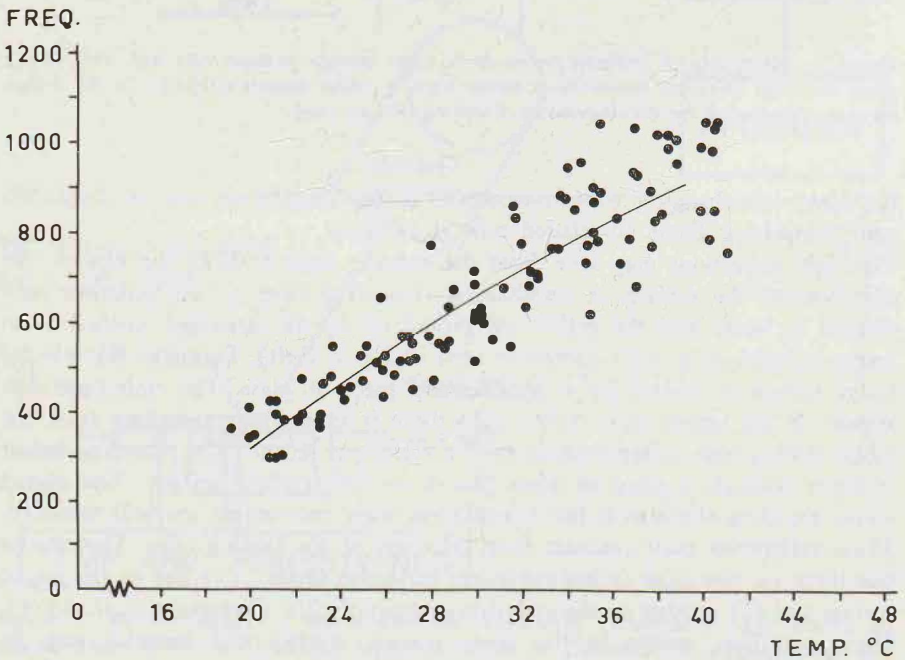

Figure 3. The frequency/temperature relationship of mucociliary activity.

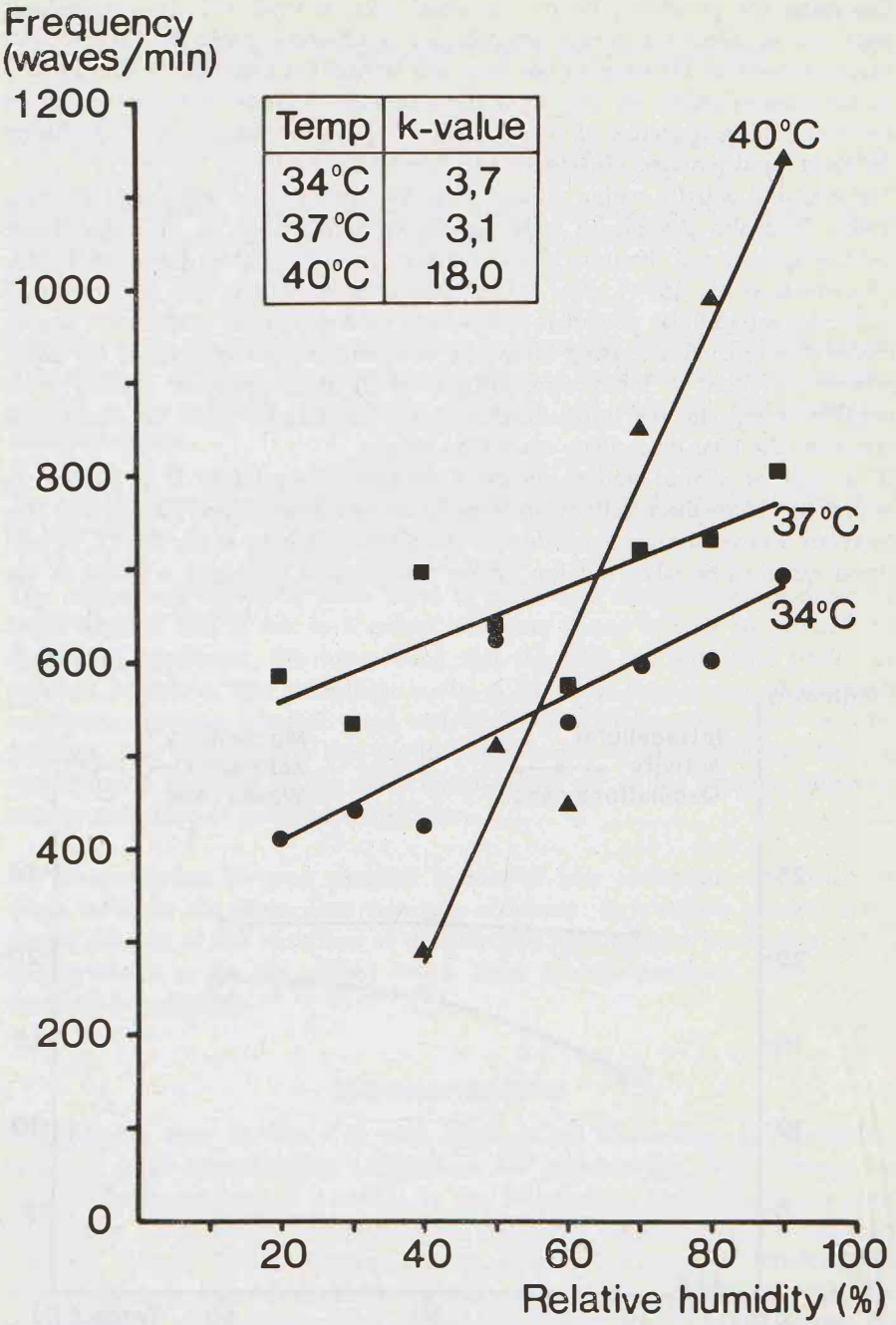

Figure 4. The frequency/humidity relationship of the mucociliary activity.

Regarding the possible influence on nasal cilia of cold and dry inspired air there are no problems during physiological ventilation thanks to the excellent heat-and moisture exchange of the nose and larynx. For example, we have found an air temperature of + 25° C in the nasopharynx in patients exposed to an environmental temperature of — 12° C which means an increase of 37° C during the short nasal passage. (Ingelstedt and Toremalm, 1960).

The electrical activity within ciliated cells has been studied with capillary electrodes. It is also possible to make simultaneous recordings of the extracellular light reflections and the intracellular potential recordings from the ciliated cells. (Toremalm et al., 1975). Fig. 5 illustrates a comparison of the frequencies of oscillating intracellular potentials and surface light reflections at different temperatures. There is an increasing difference between the intracellular and the extracellular activities at lower temperatures. With this combined method it is possible to separate the intracellular and the extracellular from the rheological factors of the total mucociliary evacuation system.

What type of clinical studies can these methods be used for? It is possible to record the mucociliary activity in vivo in human beings, for example, in the maxillary sinuses during Luc-Caldwell operations. (Reimer et al., 1975). A specimen can then be taken out for further experimental studies in a model of the

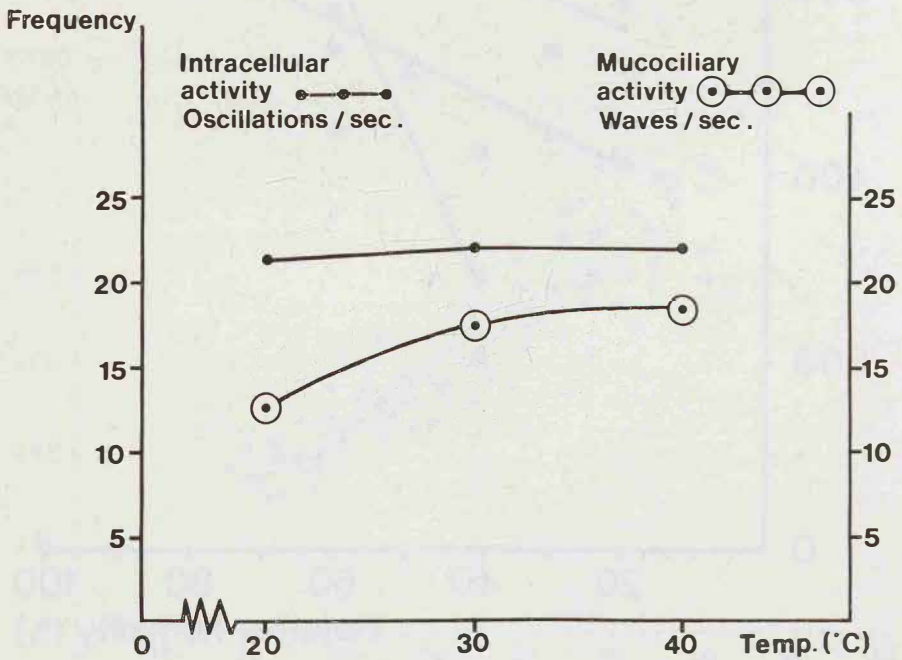

Figure 5. The relationship between intracellular electrical and extracellular mechanical activities at different temperatures.

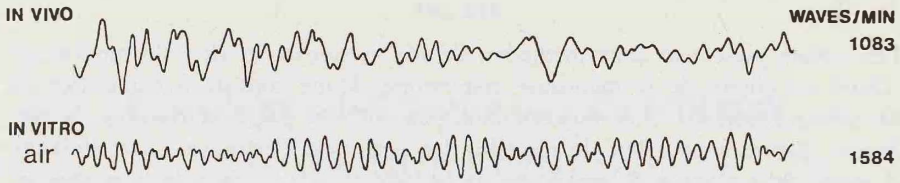

Figure 6. Mucociliary wave movements in a case of maxillary empyema in vivo during Luc Caldwell operation and in vitro from a specimen of the mucosa.

maxillary cavity. Not only the temperature and humidity factors can be regulated. The effect of different gas mixtures and pressure gradients can also be studied as well as the influence of allergens, chemicals and drugs on the mucociliary transport system.

The cilia are very little influenced by oxygen reduction and not intoxicated at all by pure oxygen.

The mucociliary activity is more rapid in vitro than in vivo but we don't yet know whether this is due to a neural inhibitory factor or only to extracellular rheological conditions. We have found that the cilia can withstand prolonged purulent infections. The recordings in fig. 6 are taken from a case of maxillary emphyema showing a rather good activity in vivo and a very good in vitro function. This may indicate that the infection is due to an insufficient drainage rather than a reduction of the mucociliary activity per se for example following oxygen reduction or bacterial intoxication.

An other question of great practical interest is how surfactants and mucolytic drugs influence the mucociliary transport efficiency. Is it only a physical effect due to dilution of the secretions or do they also have a direct biochemical effect on the mucus or on the ciliated cells? These are all questions which we are currently investigating.

ZUSAMMENFASSUNG

Beschreibung einer in vivo - in vitro Methode zur Darstellung de Mucoziliarfunktion durch oberflächliche Lichtreflexe mit gleichzeitiger Aufzeichnung der intrazellularen elektrischen Aktivität in den Zilienzellen. Zwei Typen von oberflächlichen Lichtreflexen werden erfasst – einer von der Sekretschicht und einer von den Spitzen der schlagenden Zilien. Interne und externe Zellaktivitäten sowohl als auch muco-rheologische Faktoren können bei Veränderung von Umwelteinflüssen, wie Lufttemperatur und -feuchtigkeit oder Zusammensetzung der Gasmischung, mit einander verglichen werden. Die Methode eignet sich auch für verschiedene klinische Zwecke.

RÉSUMÉ

Les auteurs présentent deux méthodes d'étude in vivo et in vitro du mouvement ciliaire au niveau de la muqueuse respiratoire. L'une consiste à enregistrer les variations d'intensité d'un faisceau lumineux réfléchi par la surface de la muqueuse; l'autre a pour but de recueillir les potentiels électriques intracellulaires. Il existe deux niveaux de réflextion de la lumière à la surface de la muqueuse: l'un correspondant à la surface de la couche de mucus, l'autre à la pointe des cils. Les activitiés interne et externe des cellules ciliées sont comparées sous l'action de diverses conditions ambiantes telles que température, humanité, mélanges gazeux. Les méthodes décrites sont également proposées dans les buts d'investigation clinique.

REFERENCES

1. Ingelstedt, S. and Toremalm, N. G., 1960: Aerodynamics within the larynx and trachea. Acta otolaryngol. 52, Suppl. 158.
2. Mercke, U., Håkansson, C. H. and Toremalm, N. G., 1974a: A method for standardized studies of mucociliary activity. Acta otolaryngol. 78, 118-123.
3. Mercke, U. Håkansson, C. H. and Toremalm, N. G., 1974b: The influence of temperature on mucociliary activity. Temp. range 20°C - 40°C. Acta otolaryngol. 78, 444-450.
4. Mercke, U. and Toremalm, N. G., 1975: Air humidity and mucociliary activity Ann. Otol. In press.
5. Reimer, A., Toremalm, N. G. and Håkansson, C. H., 1975: The mucociliary function of the upper respiratory tract. I. A method for experimental studies of human material. Acta otolaryngol. To be published.
6. Toremalm, N. G., Håkansson, C. H., Mercke, U. and Dahlerus, B., 1974: Mucociliary wave pattern. An analysis of surface light reflections Acta otolaryngol., 78, 247-252.
7. Toremalm, N. G., Håkansson, C. H., Mercke, U., Dahlerus, B. and Huberman, D., 1975: Mucociliary wave pattern. A comparative analysis of extracellular and intracellular activities. Acta otolaryngol., 79, 436-441.

Nils Gunnar Toremalm, M.D.
ENT-Department
Malmö General Hospital
S-214 01 Malmö, Sweden.