Rhinology, 29, 17-26, 1991

Factors influencing ciliary beat measurements

K.J.A.O. Ingels, M.J.W. Kortmann, M.R. Nijziel, K. Graamans and E.H. Huizing

Dept. of O.R.L., University Hospital Utrecht, The Netherlands

SUMMARY

Investigation of the effect of environmental and pharmacological factors on human respiratory epithelium requires standardization of measuring conditions. Ciliary beat frequency (CBF) and its shift were determined photo-electrically in 43 biopsies of human nasal mucosa. Curette biopsies were compared to forceps biopsies. CBF variation between three different cells of one biopsy sample did not differ for the two biopsy techniques. The ciliated cells of forceps biopsy specimens showed a more constant beating pattern which resulted in a small CBF shift. It appeared that in studying ciliary activity a continuous layer of ciliated cells which is in contact with the basal membrane is required. Therefore forceps biopsies are preferable to curette biopsies. The environmental temperature has to remain constant since CBF is temperature dependent. The pH and osmolarity of the medium do not influence CBF when kept within a certain range. No effect of medium superfusion flow rate was seen.

INTRODUCTION

The photo-electrical method is a well accepted and direct means of studying ciliary beat frequency (CBF) (Dalhamn and Rylander, 1962; Rutland and Cole, 1980; Puchelle et al., 1981). It is known that a mucosal biopsy contains cells with cilia that beat at different frequencies. Consequently, the average CBF of several cells (3-30) in a sample is usually reported (Yager et al., 1978; Konietzko et al., 1981; Lopez-Vidriero, 1982; Rutland et al., 1982; Greenstone et al., 1983; Pedersen et al., 1983; Duchateau et al., 1985; Deitmer, 1986). Nevertheless, the results of many of these studies are difficult to compare since different parameters have been used. It would therefore be advantageous to standardize the conditions for measuring CBF in vitro (Deitmer, 1986). Lopez-Vidriero (1982) has already emphasized the use of objective criteria for measuring CBF.

For these reasons we analyzed the effect of various factors upon ciliary beat measurements i.e.: 1) biopsy technique, 2) temperature, 3) medium parameters (pH and osmolarity) and superfusion.

It is common practice to take curette biopsies for photo-electrical analysis of

CBF. However, the maximal difference in CBF between cells in these samples often exceeds the variance found between different subjects (Ingels et al., 1990). This might be the result of differences in the vitality of the cells in curette biopsies. Cells in samples obtained by means of a curette are often dispersed and sometimes clustered together. The question arises whether cilia of cells that are part of an intact lining show a more identical beat frequency. Therefore we decided to compare the ciliary beat in curette biopsies with that of forceps biopsies in which we may expect an intact ciliated lining.

CBF is known to increase with temperature but the exact relationship is not yet completely elucidated. Konietzko et al. (1981) demonstrated an exponential increase of CBF with temperature (20–40 °C) in human tracheobronchial samples. In the experiments by Kennedy and Duckett (1981), however, the CBF temperature curve appeared to have a sigmoidal shape. They found a linear increase up to 37 °C, where a maximum was reached that remained constant up to a temperature of 55 °C. On the other hand, Mercke et al. found a linear increase between mucociliary movements and temperature (20–40 °C) in the rabbit trachea (Mercke, 1974; Mercke et al., 1974; Toremalm et al., 1977). We were especially interested in CBF between 32 °C, being the temperature of the nasal mucosa (Duchateau et al., 1985), and 37 °C, the body temperature. Therefore we investigated CBF-temperature relationship in that range yet again.

The features of the medium in which the sample is suspended may also influence CBF studies. It is essential to use aqueous solutions as the viscosity of the medium may have an effect on CBF. Moreover pH is of importance as CBF was drastically reduced, or ceased, when the pH of the medium was decreased below 6.5 or increased above 9.5 (Luk and Dulfano, 1983). Van de Donk (1980) found, in chicken embryo tracheas, a reduction of CBF by about 20% when the pH was lowered to 6 and a more severe decrease when the pH was further diminished. Osmolarity can also influence CBF. Both hypotonic 0.45% NaCl as well as hypertonic 1.5% NaCl solution were found to decrease CBF by 50% in comparison to an isotonic solution. In studying the effects of pharmacological agents on ciliary beat the sample has to be superfused. Di Benedetto et al. (1986) investigated the effect of the superfusion flow rate on CBF in tracheal rings of rats and found it increased. CBF gradually decreased when the superfusion was stopped and it returned to baseline values within 15 minutes. Since studies on the effects of pH, osmolarity and superfusion flow rate on human material are still lacking, we have investigated the influence of these three factors on human nasal mucosal biopsies.

MATERIALS AND METHODS

Forty-three individuals participated in this study. They all signed a "volunteer declaration", as proposed in the 1964 "Declaration of Helsinki" of the World

Medical Association and reviewed in Tokyo in 1975. Exclusion criteria were: acute rhinitis during the two weeks preceding the study, chronic rhinitis, allergy, a history of nasal surgery, gross deformities of nasal anatomy, smoking, and all medications except contraceptives. Curette biopsies were scraped in 29 subjects by means of a 2 mm sharp ring curette (Entermed, Linschoten, The Netherlands), without local anaesthesia. Forceps biopsies were performed in 14 subjects by means of a Gerritsma forceps (Fokkens et al., 1988) under general anaesthesia immediately prior to surgery. Both the curette and the forceps specimens were taken from the posterior part of the inferior turbinate of the most patent side of the nose. They were brought into a culture medium (CMRL-1066 (R/); Gibco, Paisley, U.K.) to which had been added 5% inactivated fetal calf serum, hydrocortisone hemisuccinate (0.1 μg/ml), crystalline porcine insulin (1 μg/ml), beta-retinyl acetate (0.1 μg/ml), penicillin G (100 units/ml), and streptomycin (100 μg/ml), according to Yager et al. (1978).

The movements of the cilia of one cell were measured photo-electrically, perpendicular to the light beam of a phase contrast microscope. The signal, comprising various sinusoidal and cosinusoidal waves, was differentiated by fast Fourier transform (FFT) analysis (Kennedy and Duckett, 1981; Rutland et al., 1982). In the power spectrum obtained in this way the first harmonic was considered as the CBF. From the data the frequency shift, or CBF-variation with time, was calculated as well. For the details of the method we refer to a previous paper (Ingels et al., 1990).

Pilot studies showed that CBF does not change for at least five hours when preserved in the medium described above. All experiments in the present study were carried out within four hours, so time elapse did not influence our results.

1. Biopsy technique

Findings in seven forceps biopsies (seven subjects) were compared with those in 11 curette biopsies (11 subjects). Three different cells, chosen at random, were measured and analyzed photo-electrically as described above.

2. Temperature

In this part of the study 18 curette biopsies (18 subjects) were investigated. The CBF and shift of the same cell were measured at different temperatures ranging from 22.5–40 °C in steps of 2.5 °C.

3. Medium parameters (pH and osmolarity) and superfusion

The effect of both pH and osmolarity were investigated each in three forceps biopsies.

The influence of pH was studied by measuring CBF (and shift) of the same cell at pH 7.50, 7.25, 7.00, 6.75 and 6.5 by addition of NaOH to the medium. The effect of

osmolarity was studied by measuring CBF (and shift) of the same cell at 300, 350, 400, and 450 mosm/l. The osmolarity gradient was created by proportionally adding saccharose to the medium.

The mechanical effect of superfusion flow rate of the medium was studied in one forceps biopsy. Flow rates 1, 2, 3, 4 and 5 ml/6 minutes were investigated. The CBF and shift of the same cell were measured 1, 5, 10 and 15 minutes after each superfusion.

RESULTS

1. CBF in relation to biopsy technique

The CBF of the three different cells measured in the forceps and in the curette biopsies is shown in Figures 1a, b. The average CBF (SD) amounted to 9.1 Hz (1.8) for the forceps biopsies and 9.2 Hz (2.2) for the curette biopsies. No difference in CBF could be demonstrated between the two biopsy techniques (Student's t-test; $p > 0.2$). The forceps and curette biopsies were also similar with regard to the average CBF difference (SD) between the three cells measured in each specimen (forceps: 2.4 Hz (1.6); curette: 2.9 Hz (1.6); Student's t-test; $p > 0.5$). The CBF shift (SD), however, was 1.5 Hz (1.4) for the curette biopsies and 0.7 Hz (0.2) for the forceps biopsies. (Student's t-test; $p > 0.1$).

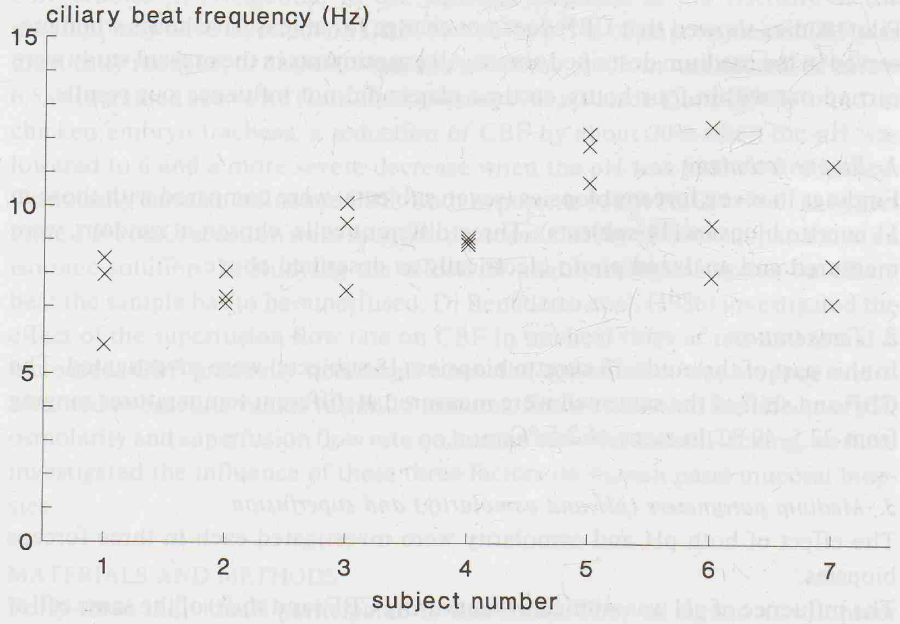

Figure 1a. Ciliary beat frequency (Hz) of three cells - forceps biopsy (seven subjects).

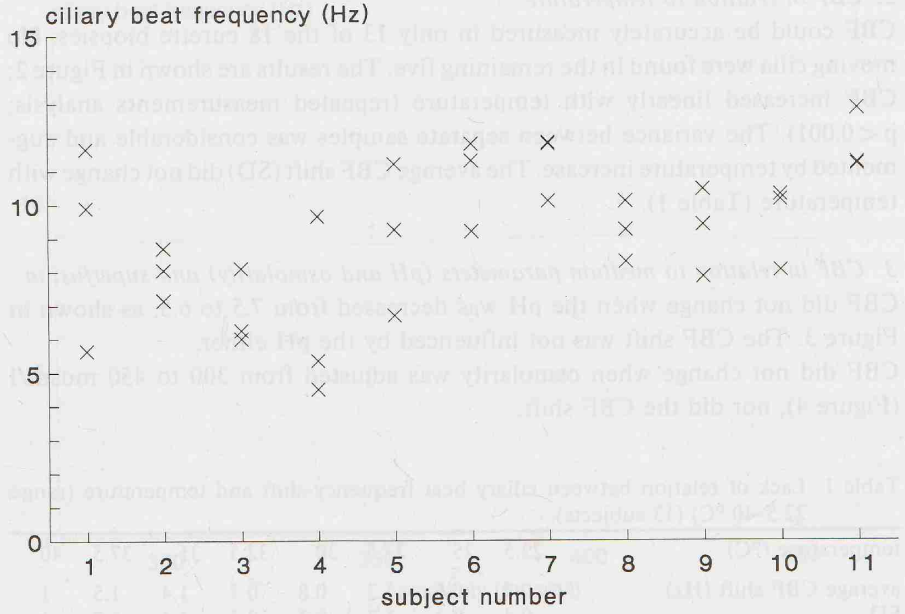

Figure 1b. Ciliary beat frequency (Hz) of three cells - curette biopsy (11 subjects).

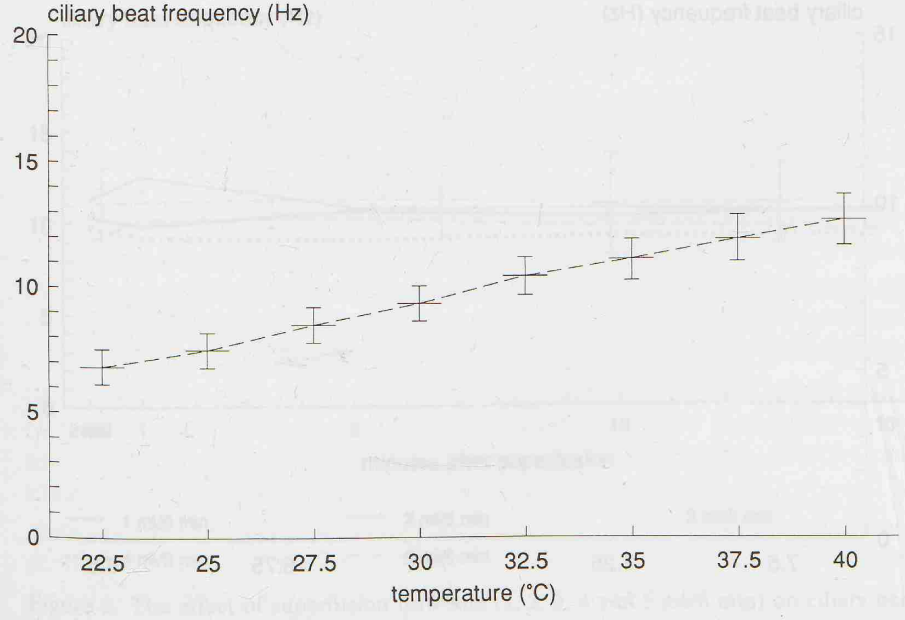

Figure 2. Average ciliary beat frequency (Hz) in relation to temperature (13 subjects).

2. CBF in relation to temperature

CBF could be accurately measured in only 13 of the 18 curette biopsies. No moving cilia were found in the remaining five. The results are shown in Figure 2; CBF increased linearly with temperature (repeated measurements analysis; $p < 0.001$). The variance between separate samples was considerable and augmented by temperature increase. The average CBF shift (SD) did not change with temperature (Table 1).

3. CBF in relation to medium parameters (pH and osmolarity) and superfusion

CBF did not change when the pH was decreased from 7.5 to 6.5, as shown in Figure 3. The CBF shift was not influenced by the pH either.

CBF did not change when osmolarity was adjusted from 300 to 450 mosm/l (Figure 4), nor did the CBF shift.

Table 1. Lack of relation between ciliary beat frequency-shift and temperature (range 22.5–40 °C) (13 subjects).

temperature (°C)	22.5	25	27.5	30	32.5	35	37.5	40
average CBF shift (Hz)	0.7	0.6	1.3	0.8	0.7	1.4	1.5	1
SD	0.4	0.3	1.7	0.3	0.3	1.4	1.7	1

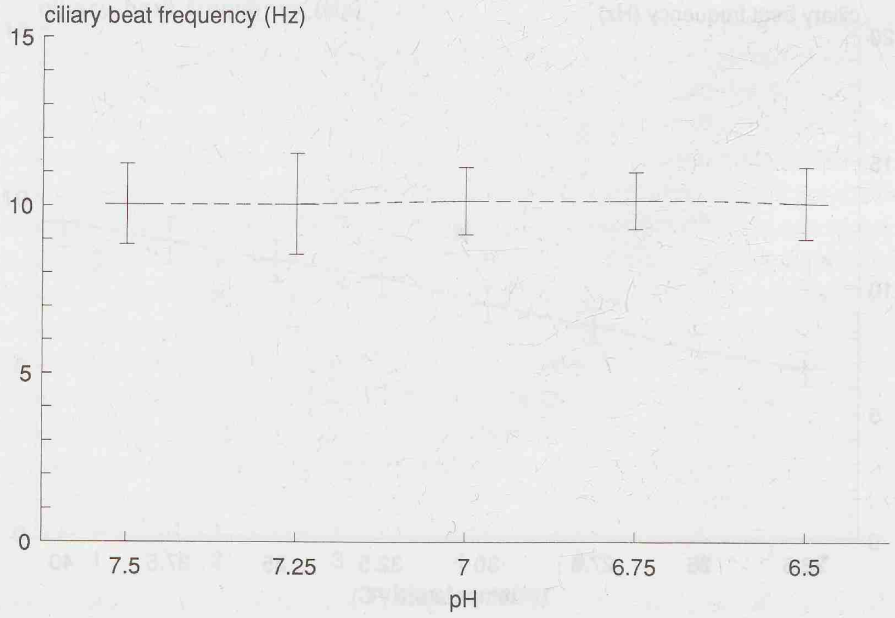

Figure 3. Lack of effect of pH on ciliary beat frequency (Hz) (three subjects; one cell).

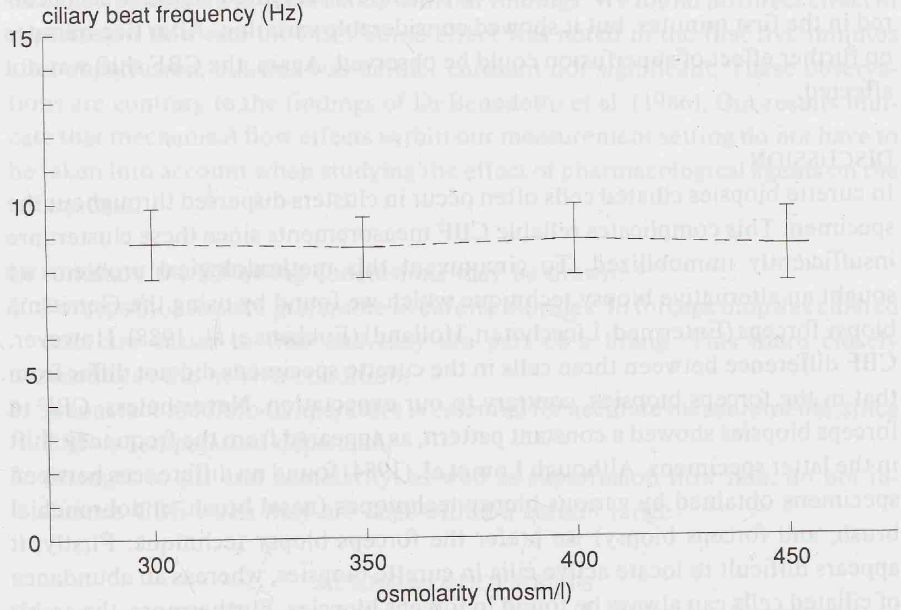

Figure 4. Lack of effect of osmolarity on ciliary beat frequency (Hz) (three subjects; one cell).

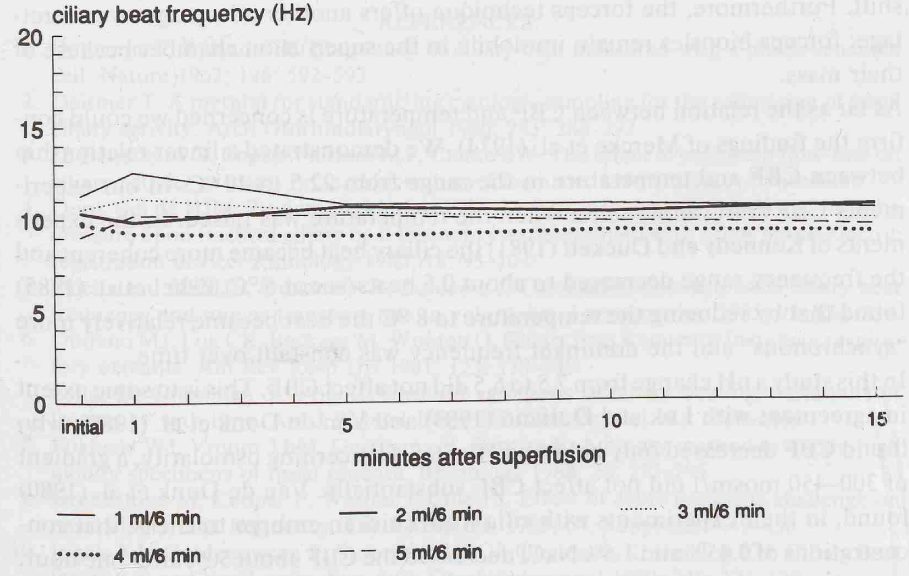

Figure 5. The effect of superfusion flow rate (1, 2, 3, 4 and 5 ml/6 min) on ciliary beat frequency (Hz).

In Figure 5 the influence of superfusion flow rate is presented. Some effect occurred in the first minutes, but it showed considerable variation. After five minutes no further effect of superfusion could be observed. Again, the CBF shift was not affected.

DISCUSSION

In curette biopsies ciliated cells often occur in clusters dispersed throughout the specimen. This complicates reliable CBF measurements since these clusters are insufficiently immobilized. To circumvent this methodological problem we sought an alternative biopsy technique which we found by using the Gerritsma biopsy forceps (Entermed, Linschoten, Holland) (Fokkens et al., 1988). However, CBF difference between three cells in the curette specimens did not differ from that in the forceps biopsies, contrary to our expectation. Nevertheless, CBF in forceps biopsies showed a constant pattern, as appeared from the frequency shift in the latter specimens. Although Low et al. (1984) found no differences between specimens obtained by various biopsy techniques (nasal brush, endobronchial brush, and forceps biopsy) we prefer the forceps biopsy technique. Firstly, it appears difficult to locate active cilia in curette biopsies, whereas an abundance of ciliated cells can always be found in forceps biopsies. Furthermore, the architecture of the pseudostratified epithelium remains intact in the forceps biopsies. This is more comparable to the in vivo condition, as the specimen is more homogeneous with intact intercellular links, probably resulting in a small frequency shift. Furthermore, the forceps technique offers another advantage over curettage: forceps biopsies remain immobile in the superfusion chamber because of their mass.

As far as the relation between CBF and temperature is concerned we could confirm the findings of Mercke et al. (1974). We demonstrated a linear relationship between CBF and temperature in the range from 22.5 to 40 °C. In our experiments CBF shift did not alter when the temperature was raised. In the experiments of Kennedy and Duckett (1981) the ciliary beat became more coherent and the frequency range decreased to about 0.5 beats/sec at 5 °C. Eshel et al. (1985) found that by reducing the temperature to 8 °C the beat became relatively more "synchronous" and the dominant frequency was constant over time.

In this study a pH change from 7.5 to 6.5 did not affect CBF. This is to some extent in agreement with Luk and Dulfano (1983) and Van de Donk et al. (1980), who found CBF decreased only below a pH of 6.5. Concerning osmolarity, a gradient of 300–450 mosm/l did not affect CBF substantially. Van de Donk et al. (1980) found, in their experiments with cilia from chicken embryo tracheas, that concentrations of 0.45% and 1.5% NaCl decreased the CBF about 50% after one hour, compared to the initial frequency. These concentrations can be expressed in solutions with an osmolarity of 154 mosm/l and 513 mosm/l, respectively. There-

fore their results are in accordance with our findings. We found no direct effect of superfusion flow rate on CBF. Some effect was noted in the first five minutes after superfusion, but this was neither constant nor significant. These observations are contrary to the findings of Di Benedetto et al. (1986). Our results indicate that mechanical flow effects within our measurement setting do not have to be taken into account when studying the effect of pharmacological agents on the ciliary beat.

In summary the following conclusions may be drawn:

1. Forceps biopsies are preferable to curette biopsies. In forceps biopsies ciliated cells are easier to find and they are part of a lining. This more closely resembles the in vivo condition.
2. A constant medium temperature is essential for accurate measurements, since CBF is temperature dependent.
3. Changes in pH and osmolarity, as well as superfusion flow rate, do not influence CBF when they are kept within a certain range.

ACKNOWLEDGEMENTS

We wish to thank Y. van der Graaf and J. Faber for their statistical work and advice. We also want to thank F. Meeuwsen and H.L.C.J. van Strien for sharing their technical knowledge and for their support.

REFERENCES

1. Dalhamn T, Rylander R. Frequency of ciliary beat measured with a photo-sensitive cell. Nature 1962; 196: 592–593.
2. Deitmer T. A method for standardizing cytologic sampling for the estimation of nasal ciliary activity. Arch Otorhinolaryngol 1986; 243: 288–292.
3. Di Benedetto G, Lopez-Vidriero MT, Clarke SW. The effect of perfusion flow-rate on ciliary activity in vitro. Prize "Mucociliary Clearance, 1986", LIRCA-Synthelabo
4. Donk van de HJM, Zuidema J, Merkus FWHM. The influence of the pH and osmotic pressure upon tracheal ciliary beat frequency as determined with a new photo-electric registration device. Rhinology 1980; 18: 93–104.
5. Duchateau GSMJE, Graamans K, Zuidema J. Correlation between nasal ciliary beat frequency and mucus transport rate in volunteers. Laryngoscope 1985; 95: 854–859.
6. Dulfano MJ, Luk CK, Beckage M, Wooten O. Ciliary beat frequency in human respiratory explants. Am Rev Resp Dis 1981; 123: 139–140.
7. Eshel D, Grossman Y, Priel Z. Spectral variations of ciliary beating: variations of frequency with time. Am J Physiol 1985; 249 (Cell Physiol 18): c160–c165.
8. Fokkens WJ, Vroom ThM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. Rhinology 1988; 26: 293–295.
9. Greenstone M, Cooper P, Warner J, Cole PJ. Effect of acute antigenic challenge on nasal ciliary beat frequency. Eur J Resp Dis 1983; 64, Suppl 129: 449–450.
10. Ingels KJAO, Meeuwsen F, Strien van HLCJ, Graamans K, Huizing EH. Ciliary beat frequency and nasal cycle. Eur Arch Otorhinolaryngol 1990; 248: 123–126.
11. Kennedy JR, Duckett KE. The study of ciliary frequencies with an optical spectrum analysis system. Exp Cell Res 1981; 135: 147–156.

12. Konietzko N, Nakhosteen JA, Mizera W, Kasparek R, Hesse H. Ciliary beat frequency of biopsy samples taken from normal persons and patients ith various lung diseases. Chest 1981; 80, Suppl 6: 855–857.
13. Lopez-Vidriero MT. Objective criteria for measuring ciliary beat frequency in vitro. Am Rev Resp Dis 1982; 125, 2 (Suppl): 244.
14. Low PM, Luk CK, Dulfano MJ, Finch PJP. Ciliary beat frequency of human respiratory tract by different sampling techniques. Am Rev Resp Dis 1984; 130: 497–498.
15. Luk CK, Dulfano MJ. Effect of pH, viscosity and ionic strength on ciliary beating frequency of human bronchial explants. Clin Sci 1983; 64: 449–451.
16. Mercke U, Hakansson CH, Toremalm NG. The influence of temperature on mucociliary activity. Temperature range 20–40 °C. Acta Otolaryngol (Stockh) 1974; 78: 444–450.
17. Mercke U. The influence of temperature on mucociliary activity. Temperature range 40–50 °C. Acta Otolaryngol (Stockh) 1974; 78: 253-258.
18. Ohashi Y, Nakai Y. Reduced ciliary action in chronic sinusitis. Acta Otolaryngol (Stockh) 1983; Suppl 397: 3–9.
19. Pedersen M, Sakakura Y, Winther B, Brofeldt S, Mygind N. Nasal mucociliary transport, number of ciliated cells and beating pattern in naturally acquired common cold. Eur J Respir Dis 1983; 64, Suppl 128: 355–364.
20. Puchelle E, Aug F, Pham QT, Bertrand A. Comparison of three methods for measuring nasal mucociliary clearance in man. Acta Otolaryngol (Stockh) 1981; 91: 297–303.
21. Rossman CM, Lee W, Forrest JB, Newhouse MT. Nasal ciliary ultrastructure and function in patients with primary ciliary dyskinesia compared with that in normal subjects and in subjects with various respiratory diseases. Am Rev Resp Dis 1984; 129: 161–167.
22. Rutland J, Griffin WM, Cole PJ. Human ciliary beat frequency in epithelium from intrathoracic and extrathoracic airways. Am Rev Resp Dis 1982; 125: 100–105.
23. Rutland J, Cole PJ. Non-invasive sampling of nasal cilia for measurement of beat frequency and study of ultrastructure. Lancet 1980; 2: 564–565.
24. Toremalm NG, Hakansson H, Mercke U, Reimer A. Intra-and extracellular activities of ciliated cells. Acta Otolaryngol (Stockh) 1977; 83: 34–38.
25. Yager J, Chen TM, Dulfano MJ. Measurement of frequency of ciliary beats of human respiratory epithelium. Chest 1978; 73, 5: 627–633.

K. Ingels, M.D.
Dept. of Otorhinolaryngology
University Hospital Utrecht
P.O. Box 85500
3508 GA Utrecht
The Netherlands