

Beat frequency of cilia from sites of purulent infection

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ABSTRACT Mucociliary clearance depends on the interaction between cilia and mucus; it is delayed in the presence of purulent secretions. Nasal mucociliary clearance was examined by the saccharin method and nasal ciliary beat frequency by a photometric technique. Four groups were studied: normal controls, patients with bronchiectasis without nasal symptoms, patients with chronic mucopurulent sinusitis alone, and patients with chronic mucopurulent sinusitis and bronchiectasis. Nasal mucociliary clearance was prolonged in infected patients. Cilia obtained from the site of purulent secretions were found to beat more slowly *in vitro* (mucopurulent sinusitis 12.1 Hz, mucopurulent sinusitis and bronchiectasis 11.6 Hz), than those obtained from normal controls (14.3 Hz) and from patients with bronchiectasis alone (13.6 Hz). The cause of the ciliary slowing seemed most likely to be the release of host factors during the inflammatory response, rather than the particular organism isolated. Ciliary slowing may contribute to the observed delay of mucociliary clearance in conditions in which purulent secretions are present.

Mucociliary clearance has been shown to be delayed in conditions in which purulent secretions are common: Lourenço *et al*¹ found slower clearance in bronchiectatic patients than in normal controls, Kollberg *et al*² and Wood *et al*³ found delayed clearance in patients with cystic fibrosis, and Stanley *et al* found delayed clearance in mucopurulent sinusitis.⁴

This delayed clearance is likely to be multifactorial. The mucociliary system depends on an interaction between cilia and mucus. The properties of mucus may change from low viscosity and high elastic recoil, which are ideal for mucociliary transport.⁵ Burgi⁶ showed that infected mucus has an increased viscosity that is mainly due to increased DNA fibre content. There may be loss of ciliated epithelium. Reimer *et al*⁷ studied biopsy specimens of maxillary sinus mucosa, with scanning electron microscopy, in patients with chronic mucopurulent sinusitis. Large areas of unciliated epithelium were seen.

The present study was designed to investigate the beating of cilia at sites of purulent infection.

Methods

PATIENTS

Four groups were studied. These comprised: 10 normal controls (five male and five female, aged 20-59

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years) with no past history of nasal or pulmonary disease; 10 patients with chronic mucopurulent sinusitis (three male and seven female, aged 12-75 years) but without any chest symptoms; 10 patients with bronchiectasis but no nasal symptoms (six male and four female, aged 16-60 years); and 10 patients with bronchiectasis and mucopurulent sinusitis (five male, five female; aged 16 to 44). Mucopurulent sinusitis was diagnosed by history, examination, and the appearance of sinus radiographs. Bronchiectasis was diagnosed by history, examination, the appearance of chest radiographs, and additional bronchographic information in nine patients. Only patients with purulent anterior nasal secretions on rhinoscopy were included. Serum immunoglobulin deficiency was excluded in all patients. No patient or normal control had received antibiotics or gave a history of viral infection in the four weeks before the study.

MEASUREMENT OF NASAL MUCOCILIARY CLEARANCE AND CILIARY BEAT FREQUENCY

Nasal mucociliary clearance was determined in an unobstructed nostril with the saccharin test.⁸ The time from placement of a 1 mm diameter particle of saccharin on the inferior nasal turbinate, 1 cm from its anterior end, to the subject's first experience of a sweet taste was recorded in minutes. The subject was positioned with head slightly forward and requested not to sniff, sneeze, cough, eat, or drink during the test. The test was terminated after 60 minutes and those subjects not tasting had their ability to taste

saccharin verified by placing a particle on the tongue. *In vitro* examination of ciliary beat frequency was performed by a photometric technique.⁹⁻¹¹ Cilia were obtained from the inferior turbinate of the same nostril without local anaesthesia by a brushing technique,⁹ the strips of ciliated epithelium being dislodged by brisk agitation of the cytology brush in 2 ml of medium 199 cell culture fluid (Flow laboratories). The suspension of ciliated epithelium was immediately transferred to a sealed microscope coverslip slide preparation with a pipette, and the preparation placed on an electronically controlled warmstage (Microtec) at 37°C, and allowed to equilibrate for 15 minutes. During this time strips of ciliated epithelium were identified on the slide and their positions marked. At least six strips of epithelium were identified and usually 10, single ciliated cells or small groups of ciliated cells being ignored as ciliary beat frequency measured at such sites is often inaccurate. If six strips of epithelium could not be identified a second nasal brushing was obtained from the same nostril.

For the measurement of ciliary beat frequency cilia were viewed directly on a Leitz Dialux 20 phase contrast microscope at magnification $\times 320$ by bright field illumination. This allowed an assessment of their beating pattern to be made. The cilia were positioned to interrupt the passage of light through a small diaphragm into a Leitz MPV compact microscope photometer, which converted light intensity into an electrical signal; this was subsequently converted into a reading of ciliary beat frequency (Hz) by a ciliary beat frequency processor unit.¹¹ Ten readings of ciliary beat frequency were made using each ciliated epithelial strip identified, and the mean was calculated.

BACTERIOLOGY

Cultures were set up from a swab of the anterior nares and from a sample of the medium 199 cell culture fluid containing ciliated epithelium. Standard and selective bacteriological plates¹⁰ were incubated aerobically and anaerobically overnight at 37°C.

OTHER STUDIES

The following studies were performed only on selected subjects.

Transmission electron microscopy of cilia obtained by brushing the inferior turbinate of three patients with a ciliary beat frequency less than 11 Hz (10.3, 9.8, 9.8 Hz) was performed. The brush was agitated in cacodylate buffered 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. After rinsing, the brushings of ciliated epithelium were embedded in a drop of 2% liquid agar and gently centrifuged. The agar was allowed to set, then processed through to embedding in Araldite as routine for tissues. Semithin

sections were cut and stained in 1% toluidine blue for light microscopy and suitable areas selected and trimmed for ultrathin sectioning. These were stained in uranyl acetate and lead nitrate for transmission electron microscopy.

Treatment In two patients (ciliary beat frequency 9.8 Hz for both) measurements of nasal mucociliary clearance and ciliary beat frequency were made after treatment appropriate to the isolated organism. Treatment was begun after the first brushing. Topical nasal treatment continued up to the time of the follow-up brushings. Betamethasone with neomycin nose drops have been shown to inhibit ciliary beating *in vitro* but not to affect adversely the ciliary beat frequency *in vivo*.¹²

Serial tests (1) A single normal volunteer underwent measurement of nasal mucociliary clearance and nasal ciliary beat frequency on 10 occasions on different days. This allowed assessment of the within subject variability of these tests. (2) Three patients with bronchiectasis and mucopurulent sinusitis each underwent measurement of nasal ciliary beat frequency on three successive days. On one of the days the ciliary beat frequency of the epithelium on the slide was measured after two and four hours at 37°C.

STATISTICS

The mean ciliary beat frequency of each group was compared with the unpaired Student's *t* test (18 degrees of freedom). The mean nasal mucociliary clearance of each group was compared with the same test, 60 being taken as the value for any subject who had not tasted saccharin after 60 minutes.

Results

Nasal mucociliary clearance (fig 1) was significantly slower in patients with mucopurulent sinusitis than in normal controls ($p < 0.001$), and in patients with bronchiectasis and mucopurulent sinusitis than in patients with bronchiectasis alone ($p < 0.001$). There was also a significant difference between the nasal mucociliary clearance of normal controls and patients with bronchiectasis alone ($p < 0.02$).

The mean ciliary beat frequency (fig 2) was significantly slower in patients with mucopurulent sinusitis (12.1) than in normal controls (14.3) ($p < 0.001$), and in those with bronchiectasis with mucopurulent sinusitis (11.6) than in those with bronchiectasis alone (13.6) ($p < 0.001$). There was no significant difference between the mean ciliary beat frequency of normal controls and of patients with bronchiectasis alone. As only 10 subjects were studied, however, only a gross difference would have been detected in this study.

The bacteriological results are shown in table 1.

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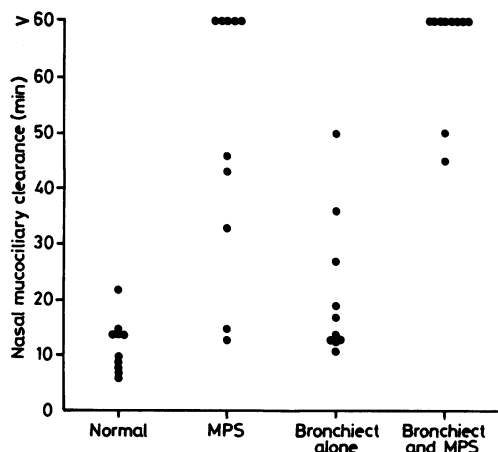


Fig 1 Nasal mucociliary clearance measured by the saccharin method in four groups of patients. MPS—mucopurulent sinusitis; Bronchiect—bronchiectasis.

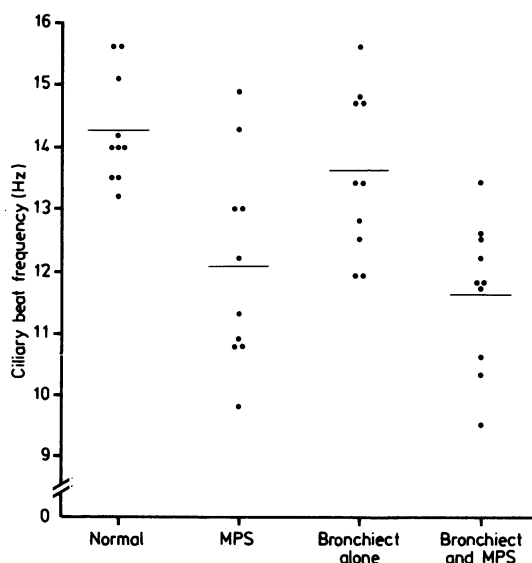


Fig 2 Ciliary beat frequency measured by a photometric technique in four groups of patients. MPS—mucopurulent sinusitis; Bronchiect—bronchiectasis.

Table 1 Bacteria isolated from culture of an anterior nasal swab and from a sample of the nasal brushing into medium 199

Group	Bacteria	No of isolates
Normal controls	No growth	6
	<i>Staphylococcus aureus</i>	2
	<i>Staphylococcus epidermidis</i>	2
Patients with bronchiectasis alone	No growth	8
	<i>Staphylococcus aureus</i>	2
Patients with mucopurulent sinusitis	No growth	2
	<i>Staphylococcus aureus</i>	4
	<i>Pseudomonas aeruginosa</i>	1
	<i>Streptococcus pneumoniae</i>	1
	<i>Staphylococcus epidermidis</i>	2
	Coliform	1
Patients with bronchiectasis and mucopurulent sinusitis	No growth	2
	<i>Staphylococcus aureus</i>	3
	<i>Pseudomonas aeruginosa</i>	2
	<i>Staphylococcus epidermidis</i>	2
	<i>Proteus vulgaris</i>	1

Staphylococcus aureus was the most frequent isolate, and as expected positive cultures were more frequent in the patients with purulent secretions.

The beating pattern of the cilia was normal in each case, with the exception of one patient whose nasal swab and brushing grew *Pseudomonas aeruginosa*. In this case the cilia appeared dyskinetic (loss of the usual coordinated beating pattern), but they returned to normal after clearance of the organism with antibiotic treatment. Static cilia were not observed in any specimen obtained.

No dynein arm defects were observed in the three specimens examined by transmission electron microscopy. An increased frequency of compound cilia was seen in one patient (eight of 179 cilia examined) and absence of some central microtubules observed in another.

With treatment (table 2) the infecting organism was

Table 2 Effect of treatment on ciliary beat frequency (CBF) and nasal mucociliary clearance (NMCC) in patients with mucopurulent sinusitis

		Duration of treatment		
		At diagnosis	6 weeks	12 weeks
Patient 1* (<i>Staphylococcus aureus</i>)	CBF (Hz)	9.8	10.8	12.1
	NMCC (min)	33	—	30
Patient 2† (<i>Pseudomonas aeruginosa</i>)	CBF (Hz)	9.8	11.8	—
	NMCC (min)	> 60	35	—

*Treatment: betamethasone with neomycin nose drops.

†Treatment: intravenous antibiotics followed by betamethasone with neomycin nose drops, gentamicin with hydrocortisone nose drops.

Table 3 Variability of ciliary beat frequency (CBF) measured on three successive days in three patients with mucopurulent sinusitis

Patient no	Bacteria isolated	NMCC (min)	Ciliary Beat Frequency (Hz)				
			Day 1	Day 2	Day 3	After 2 h*	After 4 h*
1	<i>Staphylococcus aureus</i>	55	11.8	11.5	12.0	11.1	11.3
2	<i>Neisseria</i> sp <i>Bacillus</i> sp	>60	10.7	11.2	10.9	12.1	13.5†
3	<i>Staphylococcus aureus</i>	>60	10.3	10.1	10.3	10.8	10.9

*Change in CBF with time in the specimen obtained on day 3

† $p < 0.001$ (unpaired Student's *t* test, 18 degrees of freedom) by comparison with CBF four hours earlier.

NMCC—nasal mucociliary clearance.

eradicated in both cases. Ciliary beat frequency improved ($p < 0.001$), and in one case there was an improvement in nasal mucociliary clearance.

In the volunteer subjects, the mean of 10 nasal mucociliary clearance values was 15 (range 11–25) minutes, coefficient of variation 27%. The mean ciliary beat frequency for 10 separate nasal brushings (each itself a mean of 10 readings) was 14.2 (range 13.0–16.2) Hz, coefficient of variation 8%.

In the three patients with mucopurulent sinusitis who each had ciliary beat frequency measured on three successive days (table 3) there was no significant within patient variation in ciliary beat frequency. Over four hours the frequency did not change significantly in two patients, but it increased ($p < 0.001$) in one patient.

Discussion

Several authors have suggested that slowed ciliary beating may contribute to the delay observed in mucociliary clearance in the presence of purulent secretions. Rutland and Cole¹³ examined the nasal ciliary beat frequency of 10 patients with bronchiectasis, and showed that the frequency was about 10% less than in normal controls. No attempt, however, was made in this study to correlate changes with the patients' symptoms, signs, or infecting organisms. Pedersen and Nielsen¹⁴ performed a similar study in patients with cystic fibrosis, and found a greater reduction in ciliary beat frequency (to about 25% of the control value), although these specimens were examined at room temperature, not at 37° as in our study and that of Rutland and Cole,¹³ making direct comparison between the studies difficult. Pedersen and Nielsen¹⁴ also found that the ciliary beat frequency of patients improved after treatment with antibiotics aimed at eradication of *Pseudomonas aeruginosa*.

Smallman *et al*¹⁵ showed that sputum sol phase, obtained from bronchiectatic sputum after rapid centrifugation, slowed the beating of normal human nasal cilia in vitro. This effect was neutralised by α_1 antitrypsin, suggesting that the effect was due to a serine protease, possibly elastase released from

neutrophils during the inflammatory response. Wilson *et al*¹⁰ showed in a similar in vitro system that products of *P aeruginosa* and *Haemophilus influenzae* may slow and disorganise normal human nasal ciliary beating, although products of *Staphylococcus aureus* did not do so.

In this study we have confirmed the finding of Stanley *et al*⁴ that nasal mucociliary clearance is delayed in the presence of mucopurulent sinusitis. The ciliary beat frequency results would suggest that this is partly due to slowed ciliary beating. The contribution of other factors, however, such as change in mucus volume or flow characteristics and loss of ciliated epithelium, is suggested by an analysis of the nasal mucociliary clearance compared with the ciliary beat frequency within the two purulent secretion groups. In fifteen patients the nasal mucociliary clearance time was over 60 minutes, with a mean ciliary beat frequency of 11.7 (SD 1.4) Hz; while in eight patients it was less than 60 minutes, with a mean ciliary beat frequency of 11.8 (1.4) Hz. The findings of Rutland *et al*,¹⁶ that nasal ciliary beat frequency is closely correlated with the beat frequency of intrathoracic cilia, would suggest that similar slowing may occur within the chest in the presence of purulent secretions.

The cause of the slowed ciliary beating cannot be determined from this study. *S aureus* was the most frequently isolated organism, and products of this organism did not affect human nasal ciliary beat frequency in vitro.¹⁰ The nasal mucociliary clearance and ciliary beat frequency of control subjects was not different when *S aureus* was isolated. Stockley *et al* have shown that the purulent appearance of sputum was closely related to its elastase content,¹⁷ and our infected patients had purulent anterior nasal secretions. Perhaps therefore factors released during the host response—for example, neutrophil elastase—are of greater importance than the bacterial products in the observed slowing of nasal ciliary beat frequency.

The coefficient of variation of ciliary beat frequency measured on 10 occasions in a normal volunteer was small (8%). We did not consider it reasonable to repeat the investigation on so many occasions in a patient. In three patients who each had

repeated brushings on three successive days, however, the ciliary beat frequency did not differ significantly (table 3). The technique used requires the "washing" of ciliated epithelium in medium 199 cell culture fluid when it is being dislodged from the cytology brush before transfer to the sealed microscope coverslip slide preparation. This may remove or dilute inhibitory factors present *in vivo*, perhaps leading to an increase in ciliary beat frequency. To minimise this effect only 2 ml of medium 199 were used and the ciliary beat frequency was measured immediately after 15 minutes' equilibration at 37°C. Smallman *et al*¹⁵ showed that in the presence of purulent sputum normal ciliary beat frequency continues to fall, and Wilson *et al*¹⁰ have shown that in the presence of bacteria that produce cilioinhibitory factors normal ciliary beat frequency similarly falls progressively. It would therefore be difficult to interpret changes in ciliary beat frequency over longer periods as the frequency would be predicted to increase or decrease according to the presence or absence of these factors (table 3).

The very low ciliary beat frequencies obtained in some patients raises the possibility of primary ciliary dyskinesia. Static cilia, however, were not observed; dyskinesia was observed only in the one patient with *P aeruginosa*, and the electron microscopic findings in three patients with a very slow ciliary beat frequency were not those associated with primary ciliary dyskinesia but were those found in the presence of infection.¹⁸ Treatment leading to improvement in symptoms and eradication of the infecting organism led to a significant improvement in ciliary beat frequency in two patients (table 2).

In the patient with the lowest ciliary beat frequency (the only patient to show ciliary dyskinesia) *P aeruginosa* was isolated from the nasal swab and brushing culture. Wilson *et al*¹⁰ have shown that products of this organism can slow and disorganise normal human ciliary beating *in vitro*. Eradication of the organism and clearing of symptoms and signs led to increase in ciliary beat frequency, return to a normal beating pattern and improvement of nasal mucociliary clearance. In two other patients from whom *P aeruginosa* was cultured, however, the frequency was not dramatically slowed (13.0 and 13.4 Hz); thus if products of this organism are important in causing ciliary dyskinesia *in vivo* they are not produced by all strains.

Nasal mucociliary clearance was also significantly longer in patients with bronchiectasis without nasal symptoms or abnormality on rhinoscopy (mean 20 min) than in normal controls (mean 12 min), although the ciliary beat frequency of these two groups did not differ. As only 10 patients were studied in each group this study would detect only gross

differences in beat frequency between groups. It would anyway seem likely that the previously reported slowing of ciliary beat frequency in bronchiectasis and cystic fibrosis is anyway likely to be a phenomenon secondary to the presence of purulent secretions, and not due to a primary ciliary defect.

We would conclude from this study that nasal cilia obtained from sites of purulent secretions beat more slowly *in vitro*. This may contribute to the observed delay of mucociliary clearance in conditions such as bronchiectasis and mucopurulent sinusitis in which purulent secretions are present.

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