Thorax 1985;**40**:125–131

Effect of bacterial products on human ciliary function *in vitro*

ROBERT WILSON, DAVID ROBERTS, PETER COLE

From the Host Defence Unit, Department of Medicine, Cardiothoracic Institute, Brompton Hospital, London

ABSTRACT Ciliary activity protects the respiratory tract against inhaled particles, including bacteria, by transporting them trapped in mucus towards the pharynx. We have studied the effect of bacteria (*Haemophilus influenzae, Staphylococcus aureus,* and *Pseudomonas aeruginosa*) on human nasal cilia, measuring their in vitro ciliary beat frequency by a photometric technique. Supernatant fluids were obtained from 18 hour broth cultures by centrifugation alone, by filtration, and by lysis. Supernatants obtained from *Ps aeruginosa* and *H influenzae* caused a significantly lower ciliary beat frequency than controls (broth alone). Slowed cilia were dyskinetic and at times of maximal slowing ciliostasis occurred in some areas of the epithelium. A dose related effect was demonstrated. Abrogation of cilioinhibitory properties was achieved by heating the lysate to 56°C for 30 minutes and by allowing the filtrate to stand at 37°C for 120 minutes. Staphylococcal products were not cilioinhibitory. It is concluded that *Ps aeruginosa* and *H influenzae* release a factor (or factors) which causes slowing of human nasal cilia *in vitro*. The role of this factor in the pathogenesis of infection is discussed.

Ciliary activity protects the respiratory tract against inhaled particles, including bacteria, by transporting them trapped in mucus towards the pharynx. Cilia beat in a coordinated manner in the periciliary fluid layer beneath overlying mucus. They have a stiff downstroke which propels the mucus forward, and are then withdrawn in a curved fashion within the periciliary fluid so as not to disturb the mucus layer.

There are few published reports of the effects of bacteria on cilia. In 1965 Hoorn and Lofkvist[1] reported that a staphylococcal strain producing α toxin was toxic in vitro to cilia obtained from tracheal and nasal explants of the adult rabbit and human fetus. Denny[2] in 1974 reported the in vitro ciliostatic effect of *H influenzae*, using tracheal cilia from the chick, rat, and human fetus.

There is, however, more evidence that purulent secretions affect ciliary motility. Patients with bronchiectasis have delayed mucociliary clearance in vivo.[3] Purulent sputum is reported to slow in vitro mucus transport on the frog palate,[4] and recently bronchiectatic sputum containing elastase activity has been reported to slow human nasal cilia in vitro.[5]

Address for reprint requests: Dr R Wilson, Cardiothoracic Institute, London SW3 6HP.

Accepted 29 October 1984

Leucocyte elastase has been shown to inhibit ciliary activity and damage respiratory epithelium in a rabbit model.[6]

Using a photometric technique,[7] we have studied the effects of bacteria cultured from the sputum of patients suffering from severe bronchiectasis on the in vitro beat frequency of human nasal cilia obtained by a brushing technique from normal volunteers. The organisms studied were those commonly isolated from the sputum of patients with chronic bronchial sepsis: *H influenzae, Staph aureus,* and *Ps aeruginosa*.

Methods

ISOLATION OF BACTERIAL STRAINS

Bacteria were isolated by standard and selective bacteriological techniques.[8] Briefly, sputum was homogenised by vigorous shaking in phosphate buffered saline and then streaked on to culture medium plates. *Ps aeruginosa* was isolated on plates containing cetrimide nalidixic acid agar (Oxoid) after aerobic incubation at 37°C overnight. *Staph aureus* was isolated after similar incubation on plates containing mannitol salt agar (Oxoid). *H influenzae* was isolated on medium containing blood agar base number 2 (Oxoid) 1 litre (supplemented by bacitracin 100 mg, haemin 2 mg (BDH Ltd), *N*-

acetyl-D-glucosamine 10 mg, glucose 120 mg) and incubated overnight anaerobically at 37°C with a single "V" disc containing 6 μg nicotinamide on the centre of the plate. The isolates were confirmed as being *H influenzae* using "V" and "X" (haemin) discs.

A pure growth of bacterial species was confirmed by subculture and further overnight incubation. Five isolates from sputum of five different patients were used in the case of each of the three species. The patients all suffered from bronchiectasis, in some cases due to cystic fibrosis.

Each isolate was incubated for 18 hours at 37°C in 20 ml of broth: staphylococci and pseudomonads were grown in Oxoid nutrient broth No 2, while *Haemophilus* was grown in the same broth supplemented with liver digest, yeast extract, and magnesium chloride. Growth factors for *Haemophilus* were also added: haemin hydrochloride (British Drug Houses) and nicotinamide adenine dinucleotide (Sigma Chemicals). Incorporation of 0·12% glucose into the broth was found on occasions to increase the viable count of *Haemophilus*.

PREPARATION OF BACTERIAL SUPERNATANTS

Supernatants were prepared from the 18 hour broth cultures by three methods:

1 *Crude* The broth culture was centrifuged for 30 minutes (at 2880 *g* for *Ps aeruginosa,* 2270 *g* for *Staph aureus*, 890 *g* for *H influenzae*) and the supernatant carefully aspirated so as not to disturb the bacterial pellet.
2 *Filtration* The supernatant obtained by method (1) was passed through an 0·2 μm filter (Acrodisc, Gelman) to obtain a bacteria free preparation. The sterility of this preparation was tested by overnight culture at 37°C.
3 *Lysis* Ciprofloxacin 100 mg/l (Bayer Pharmaceuticals), to which all organisms had been shown to be sensitive, was added to the 18 hour broth culture (without centrifugation) and the mixture was gently rolled at 37°C for 60 minutes. Lysis of bacteria was achieved by ultrasonication for two minutes in the case of *Pseudomonas* and *Haemophilus* or, in the case of staphylococci, by addition of 50 mg/l lysostaphin (Sigma Chemicals) and incubation for 15 minutes at 37°C.[9]

VIABLE COUNTS

Viable counts were performed on all broth cultures at the end of the 18 hour incubation, and also on the crude supernatants after centrifugation and aspiration. Aliquots of 100 μl were diluted serially in 100 fold dilutions of phosphate buffered saline, and 100 μl of each dilution was plated to enable a viable count to be performed after overnight culture.

TEST FOR CILIOTOXIC PROPERTIES OF SUPERNATANTS

Human nasal cilia were obtained from normal volunteers without local anaesthesia by brushing the lateral aspect of the inferior turbinate of the nostril with a cytology brush and dispersing the strips of ciliated epithelium so obtained by agitation in tissue culture medium[7] (Medium 199 with Earle's salts and HEPES, Flow Laboratories). This provided a suspension of ciliated epithelium sufficient for one or two experiments.

The bacterial supernatant was mixed 50:50 with Medium 199 containing nasal ciliated epithelium and placed in a sealed microscope coverslip slide preparation at 37°C for measurement of ciliary beat frequency. Three supernatants were prepared as described above for each of the 15 separate isolates and then tested for their effect on ciliary beat frequency. Where statistically significant slowing of cilia occurred after additon of lysed bacteria, a further experiment was carried out after the lysate had been heated to 56°C for 30 minutes.

PHOTOMETRIC TECHNIQUE FOR MEASUREMENT OF CILIARY BEAT FREQUENCY

For the measurement of ciliary beat frequency by the photometric technique[7] the slide coverslip preparation of epithelium is placed on an electrically controlled warm stage (Microtec) at 37°C, mounted on a Leitz Dialux 20 phase contrast microscope. A Leitz MPV compact microscope photometer transduces light intensity into an electrical signal. Strips of epithelium with beating cilia are viewed directly at magnification ×320 by bright field illumination. The cilia are positioned to interrupt simultaneously the passage of light through a small diaphragm into the photometer, and the electrical signal generated is converted into a reading of ciliary beat frequency. Viewing the cilia during the experiment allows an assessment of their beating pattern to be made directly after measurement of ciliary beat frequency.

During experiments using the crude supernatant the ciliary beat frequency was measured at 0, 15, 30, 60, 90, and 120 minutes. Additional readings were taken at 180 and 240 minutes in all other experiments. Initially, the slide coverslip preparation was allowed to equilibrate to 37°C over 10 minutes. Under low power (×320) six or more strips of ciliated epithelium were identified and the slide marked for reidentification at subsequent timepoints (if less than six strips were present a fresh slide was prepared). Single ciliated cells were ignored. These six strips were all used at sites of 10 subsequent ciliary beat frequency readings at each timepoint. Every

time a reading was taken on such a strip of epithelium the actual point on the strip at which ciliary beat frequency was determined was randomly chosen. The mean of these 10 ciliary beat frequency readings at each timepoint was calculated. Because counts were made only on moving cilia, ciliary dyskinesia and ciliostasis were noted if present. Each experiment was controlled by exposing a similar sample of nasal cilia from the same brushing to broth which had been treated identically, but to which no bacteria had been added. Ten readings of ciliary beat frequency were again taken at identical timepoints during the experiment.

DOSE RESPONSE EFFECT

Three isolates of both *Pseudomonas* and *Haemophilus* were each grown for 18 hours in Medium 199 cell culture fluid. The culture was centrifuged, and the supernatant was then carefully aspirated and filtered (0·2 μm) to yield a bacteria free preparation (sterility being tested by overnight culture at 37°C). Cilia were added without prior dilution in fresh Medium 199. In the control experiment cilia were taken from the same volunteer, added to Medium 199 which had been kept at 37°C for 18 hours; ciliary beat frequency was again measured at regular intervals for four hours.

H influenzae was found not to grow well in Medium 199 alone, but did so after addition of 1 ml Lab-lemco (Oxoid) 10% to 100 ml of Medium 199. Controls were similarly treated.

STABILITY

The stability of the above six filtered supernatants was assessed by incubating them at 37°C for 60 and 120 minutes before addition of the cilia. Ciliary beat frequency was again measured at regular intervals for four hours.

In all experiments the pH of each test preparation was checked at the beginning of the experiment with a pH meter and at the end with a pH indicator strip (universal indicator, Merck Pharmaceuticals). If the pH did not lie between 7·2 and 7·4 at the beginning of the experiment (unusual) it was adjusted to this range by adding small volumes of 1% hydrochloric acid or 1/5 normal sodium hydroxide. No pH changes were detected during the course of the experiments.

Every effort was made to prevent degradation of factors in the test material. Broth was cooled before ultrasonication, centrifugation was carried out at 4°C, and filtration and ultrasonication were carried out in universal containers surrounded by crushed ice. Test material was added to the cilia immediately after it had been prepared.

STATISTICAL ANALYSIS

The ciliary slowing reached a maximum at various

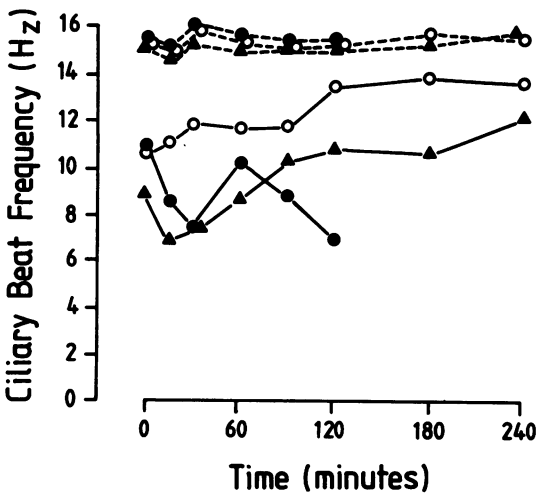

Fig 1 *Ciliary beat frequency of human nasal epithelium in vitro after exposure to supernatants (50:50 dilution) from 18 hour broth cultures of Pseudomonas aeruginosa. Test values are shown with continuous line, control values with interrupted line.* ● *Crude supernatant;* ○ *filtrate;* ▲ *lysate.*

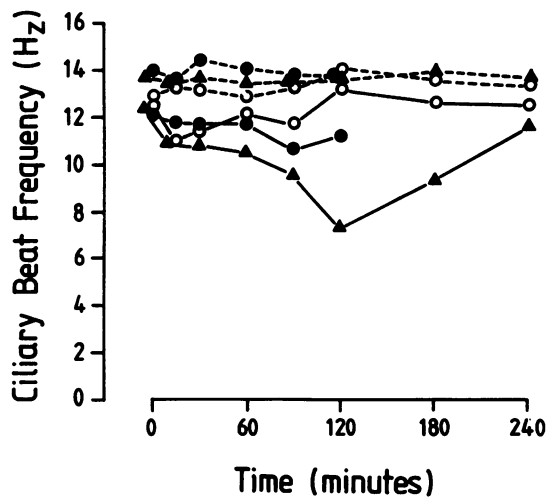

Fig 2 *Ciliary beat frequency of human nasal epithelium in vitro after exposure to supernatants (50:50 dilution) from 18 hour broth cultures of Haemophilus influenzae. Test values are shown with continuous line, control values with interrupted line.* ● *Crude supernatant;* ○ *filtrate;* ▲ *lysate.*

Table 1 *In vitro human nasal ciliary beat frequency after exposure to products of Pseudomonas aeruginosa**

Isolate No	*Unfiltered supernatant*	*Filtrate*	*Lysed bacteria*	*Heated lysate*
1 Control	16·0 (1·1)	15·5 (1·2)	14·4 (0·9)	13·9 (1·1)
Test	5·9 (2·5)	10·5 (1·3)	6·8 (2·0)	12·9 (1·2)
2 Control	16·1 (0·8)	14·7 (0·9)	14·0 (1·5)	14·3 (1·6)
Test	9·3 (1·0)	11·0 (1·3)	7·5 (1·8)	13·2 (1·4)
3 Control	13·8 (0·9)	13·1 (1·1)	15·6 (1·3)	14·4 (1·0)
Test	7·4 (2·3)	10·4 (1·5)	8·6 (1·5)	13·6 (0·7)
4 Control	13·8 (0·9)	14·7 (1·1)	15·2 (0·8)	13·8 (1·1)
Test	7·6 (1·6)	12·9 (1·3)	12·2 (0·6)	13·5 (0·6)
5 Control	16·1 (0·8)	14·9 (0·9)	14·9 (0·7)	14·5 (1·3)
Test	10·1 (1·9)	13·2 (1·4)	10·8 (1·9)	14·2 (1·2)

*All values represent the mean maximum slowing (n = 10) of ciliary beat frequency (SD in parentheses) compared with control mean (SD) at the same experimental timepoint.

timepoints in different experiments. Each experiment yielded a series of 10 ciliary beat frequency readings at each timepoint and the mean frequency at each timepoint was calculated for the test and control preparations. The maximum mean slowing of the test preparation (compared with the control) was identified and an unpaired *t* test was performed on the 20 readings (10 test, 10 control) at the time of maximum slowing (18 degress of freedom).

As noted above, only moving cilia were counted. In some experiments ciliostasis was seen to occur after addition of bacterial supernatants and in these experiments the means represent an underestimate of the effect of the bacterial supernatant. Static cilia were not counted as having a zero ciliary beat frequency because after stasis the epithelium became blurred and impossible to differentiate from unciliated epithelium.

Results

Figures 1 and 2 show the mean (n = 10) ciliary beat frequency values obtained during experiments with one isolate of *Pseudomonas* and one of *Haemophilus*. A number of features are illustrated. First, the mean ciliary beat frequency in control experiments does not vary significantly during the four hours. The onset of cilioinhibition is extremely rapid, so that the test value at time 0, when the slide has reached 37°C, is already less than the control value. Usually a further decrease in ciliary beat frequency then occurred to reach a point of maximum cilioinhibition. After this, in the experiments using lysate and filtered supernatants, apparent recovery of ciliary beat frequency occurred, although over the time course of the experiment control values were not reached. In experiments using crude supernatant, however, when live organisms were present the trend was toward continued slowing of the ciliary beat frequency.

A pronounced slowing of ciliary beat frequency was seen with lysate preparations of both species. The slowing of ciliary beat frequency when *Haemophilus* was used occurred more gradually, however, and maximum cilioinhibition occurred later.

For clarity, further results are shown as means and standard deviations (SD) of the 10 readings taken at the time of maximum cilioinhibition, and the means and SD of the 10 control readings taken at the same experimental timepoint.

Tables 1, 2 and 3 show the results for each species of bacterium. Ciliary slowing occurred after addition of the crude supernatant, filtrate, and lysate of each isolate of *Pseudomonas* and *Haemophilus*. Heating the lysate to 56°C for 30 minutes completely abro-

Table 2 *In vitro human nasal ciliary beat frequency after exposure to products of Haemophilus influenzae**

Isolate No	*Unfiltered supernatant*	*Filtrate*	*Lysed bacteria*	*Heated lysate*
1 Control	15·3 (1·3)	14·2 (0·4)	13·6 (1·3)	14·8 (1·0)
Test	12·1 (1·6)	11·1 (1·5)	7·3 (2·0)	14·3 (1·7)
2 Control	13·7 (1·9)	15·1 (1·4)	15·0 (1·2)	13·8 (0·9)
Test	10·7 (2·5)	13·3 (2·0)	8·3 (1·5)	13·5 (1·0)
3 Control	15·3 (1·4)	14·2 (1·1)	15·0 (0·9)	14·8 (1·0)
Test	10·9 (1·2)	11·9 (1·3)	11·9 (2·0)	14·5 (1·6)
4 Control	14·3 (1·1)	13·7 (1·0)	13·6 (1·2)	13·5 (1·2)
Test	12·3 (1·0)	11·0 (1·5)	11·2 (1·3)	12·8 (1·0)
5 Control	14·9 (0·9)	14·0 (1·1)	14·9 (0·9)	13·8 (1·3)
Test	12·3 (0·9)	12·9 (1·7)	12·1 (1·6)	13·3 (1·2)

*All values represent the mean maximum slowing (n = 10) of ciliary beat frequency (SD in parentheses) compared with control mean (SD) at the same experimental timepoint.

Table 3 *In vitro human nasal ciliary beat frequency after exposure to products of Staphylococcus aureus**

Isolate No	*Unfiltered supernatant*	*Filtrate*	*Lysed bacteria*
1 Control	13·4 (0·8)	13·4 (0·9)	14·4 (1·1)
Test	13·1 (1·1)	13·2 (1·0)	13·9 (1·2)
2 Control	14·0 (1·7)	13·4 (1·0)	14·0 (0·8)
Test	13·4 (1·1)	13·1 (1·1)	13·7 (0·8)
3 Control	13·8 (1·4)	14·9 (0·8)	13·9 (1·4)
Test	13·8 (1·4)	13·9 (1·0)	13·3 (0·9)
4 Control	14·0 (1·4)	14·9 (0·8)	14·0 (0·6)
Test	13·6 (0·6)	14·2 (1·2)	13·2 (0·9)
5 Control	14·0 (1·8)	15·1 (1·3)	13·9 (1·0)
Test	13·6 (1·4)	14·7 (0·8)	13·7 (1·3)

*All values represent the mean maximum slowing (n = 10) of ciliary beat frequency (SD in parentheses) compared with control mean (SD) at the same experimental timepoint.

gated its cilioinhibitory properties. The staphylococcal preparations did not cause ciliary slowing.

Significant differences ($p < 0·005$) were seen between the control and the test readings at maximal slowing with all the *Pseudomonas* and *Haemophilus* crude supernatants, filtrates, and lysates. Significant differences did not occur between test and control values in any staphylococcal experiment or with the lysates after heating.

In some experiments, at times of maximum slowing (when ciliary beat frequency values were very slow) ciliary dyskinesia was noted. This appeared to be loss of the normal coordinated beating pattern, so that cilia at different ends of the epithelium appeared to be beating in opposite directions; in more severe cases the cilia remained almost vertical, oscillating from side to side. On these occasions complete ciliostasis was also seen to occur in some areas of the epithelium.

Viable counts performed on the 18 hour broth cultures yielded similar results with pseudomonads (3–9·5 × 10^8/ml) and staphylococci (2·5–8·6 × 10^8/ml) but lower values with *Haemophilus* (0·7–2·6 × 10^8/ml). After centifugation viable counts on the crude supernatants were similar in all the species (2–8 × 10^6/ml).

The results in table 4 show the effect of using the bacterial filtrates without dilution, and the stability of these filtrates at 37°C. The effect of increasing the filtrate concentration was to increase the cilioinhibitory properties in each case. Allowing the filtrate to stand at 37°C for 60 minutes reduced the cilioinhibitory properties, in some cases considerably. Allowing the filtrate to stand for 120 minutes left little residual cilioinhibitory activity.

Discussion

Cash *et al*[10] showed in a rat model of *Pseudomonas* respiratory infection that substantial breakdown of organisms occurs, with release of bacterial products into surrounding airways and alveoli. In such circumstances in vivo cilia are likely to be bathed in mucus containing live and dead organisms and their breakdown products.

We have shown in vitro that *Ps aeruginosa* and *H influenzae* release a factor (or factors) which slows cilia, disorganises their beating, and in some cases completely stops their activity. It is therefore tempting to speculate that cilia in vivo might be similarly affected by bacterial products.

This property of *Pseudomonas* and *Haemophilus* products may be important in establishing the infectious process by rendering mucociliary clearance less efficient, thus allowing time for the organism to multiply in the lung. It might also assist in the perpetuation and spread of existing infection within the lung. In patients with chronic pulmonary sepsis the release of such factors would tend to weaken local host defences and predispose to secondary infection

Table 4 *In vitro human nasal ciliary beat frequency after exposure to filtrates of Pseudomonas aeruginosa and Haemophilus influenzae**

Isolate No	*Dilution 50:50*	*Neat*	*Neat 60 min*	*Neat 120 min*
Pseudomonas				
1 Control	15·5 (1·2)	14·5 (1·1)	15·0 (1·1)	13·6 (1·4)
Test	10·5 (1·3)	7·0 (2·0)	9·9 (1·9)	12·6 (0·6)
2 Control	14·7 (0·9)	14·0 (0·9)	15·0 (1·1)	13·0 (0·8)
Test	11·0 (1·3)	8·8 (1·4)	10·6 (2·1)	12·9 (0·9)
3 Control	13·1 (1·1)	15·0 (0·7)	13·9 (1·5)	13·4 (1·4)
Test	10·4 (1·5)	9·9 (3·0)	10·1 (1·1)	12·1 (2·3)
Haemophilus				
1 Control	14·2 (0·4)	12·0 (0·8)	12·0 (0·8)	14·3 (0·6)
Test	11·1 (0·5)	7·7 (2·9)	9·1 (1·7)	12·0 (0·8)
2 Control	13·7 (1·4)	13·7 (1·3)	13·3 (0·8)	14·3 (0·7)
Test	11·0 (2·0)	10·4 (1·0)	11·4 (1·2)	12·9 (1·2)
3 Control	14·2 (1·1)	12·6 (1·2)	13·2 (0·7)	14·3 (0·7)
Test	11·9 (1·3)	9·8 (1·1)	11·5 (1·1)	12·8 (0·6)

*All values represent the mean maximum slowing (n = 10) of ciliary beat frequency (SD in parentheses) compared with control mean (SD) at the same experimental timepoint.

by other organisms, leading to acute exacerbations of chronic disease.

Human leucocyte elastase is capable of inhibiting ciliary activity and damaging respiratory epithelium.[6] During the infectious process the release of this enzyme from leucocytes trafficking to the lung may overwhelm the lung's antiproteases, such as α_1 protease inhibitor, leaving free elastase present in the respiratory tract.[11]

Leucocyte elastase may complement bacterial products in inhibiting ciliary activity. The investigation of these interactions could form the basis of future work. Bacterial products may, however, be a more important factor early in the infectious process, before the inflammatory reaction (and thus cell traffic) has become established.

Denny[2] demonstrated a heat stable factor produced by *H influenzae*, which he postulated to be an endotoxin. The factor caused ciliary slowing followed by ciliostasis and epithelial damage. It took 12 to 24 hours, however, to cause ciliary slowing in rat tracheal rings, and eight to 10 days to cause ciliostasis in human fetal trachea. We have demonstrated much more rapidly acting factor or factors produced by *Haemophilus* and *Pseudomonas*. For the factor or factors to be important in the early stages of the infectious process in vivo such rapidity would be required.

Apparent recovery of ciliary beat frequency occurs, however, during the four hour experiment. The early stages of this recovery may be an artefact of our decision to count only moving cilia. At the lower ciliary beat frequencies some ciliostasis is seen. Thus the slowest beating cilia may stop completely and then no longer be counted in our assay, giving an apparent increase in the mean ciliary beat frequency. The decision to count only moving cilia was taken because after ciliostasis occurs the epithelial surface becomes blurred, and it is impossible to differentiate such areas from previously unciliated epithelium.

Later in the experiments, however, obvious true recovery in ciliary beat frequency does occur in experiments using filtrates and lysates. This may be due to instability of the supernatant (which we have demonstrated—table 4) and is supported by the observation that recovery does not occur in experiments using the crude supernatant in which live organisms can continue producing the factor or factors.

In general, *Pseudomonas* preparations seem more cilioinhibitory than those of *Haemophilus*. This may be partly explained by the larger viable counts obtained from the 18 hour cultures of *Pseudomonas*.

The dyskinesia seen at times of maximal slowing is of interest. The mechanism of mucus transport by cilia requires both a specific pattern of movement by single cilia and coordinated movement of many cilia together. Both of these appear to be disrupted by the cilioinhibitory factor.

The loss of activity of the supernatant at 37°C may indicate that the cilioinhibitory factor (or factors) is labile, but perhaps more probably that the organism produces other substances which neutralise the factor (for example, a polypeptide cilioinhibitory factor destroyed by the bacterial proteolytic enzymes). Unfortunately, this makes interpretation of efforts to neutralise the factor within the supernatant (including the demonstration of the heat lability of the lysate preparation) difficult. Considerable activity does remain in some supernatants after 60 minutes, however, and we consider, therefore, that these results based on heated lysate are worth presenting. Further investigation of the factor requires either its separation or a method of stabilising the supernatant.

Potent cilioinhibitory activity remains in the supernatant after removal of bacteria by filtration. This has also been shown to have a dose-response effect.

Pseudomonas produces several proteases and toxins which have been shown to have appreciable virulence properties in vitro and in vivo.[12] This is a less well studied property of *Haemophilus*. Unlike Hoorn and Lofkvist,[1] we have been unable to demonstrate any ciliotoxic properties of staphylococci.

We conclude that *Ps aeruginosa* and *H influenzae* produce a factor or factors inhibiting human nasal cilia in vitro.

RW is supported by Janssen Pharmaceuticals Ltd, and this work was supported by the Wellcome Trust; the Chest, Heart and Stroke Association; and the National Fund for Research into Crippling Diseases.

References

1 Hoorn B, Lofkvist T. The effect of staphylococcal alpha toxin and preparations of staphylococcal antigens on ciliated respiratory epithelium. *Acta Otolaryngol* 1965;**60**:452–60.
2 Denny F. Effect of a toxin produced by *Haemophilus influenzae* on ciliated respiratory epithelium. *J Infect Dis* 1974;**129**:93–100.
3 Laurenco RV, Loddinkemper R, Canton RW. Patterns of distribution and clearance of aerosols in patients with bronchiectasis. *Am Rev Respir Dis* 1972;**106**:857–66.
4 Puchelle E, Tournier JM, Pettit A, *et al*. The frog

palate for studying mucus transport velocity and mucociliary frequency. *Eur J Respir Dis* 1983;**64** suppl 128:293–303.
5 Smallman LA, Hill SL, Stockley RA. Reduction of ciliary beat frequencies in vitro by sputum from patients with bronchiectasis: a serine proteinase effect. *Thorax* 1984;**39**:663–7.
6 Tegner H, Ohlsson K, Toremalm NG, von Mecklenburg C. Effect of human leucocyte enzymes on tracheal mucosa and its mucociliary activity. *Rhinology* 1979;**17**:199–206.
7 Rutland J, Cole PJ. Non-invasive sampling of nasal cilia for measurement of beat frequency and study of ultrastructure. *Lancet* 1980;ii:564.
8 Roberts DE, Cole PJ. Use of a selective media in bacteriological investigation of patients with chronic suppurative respiratory infection. *Lancet* 1980;i:796–7.
9 Easmon CSF, Lanyon H, Cole PJ. Use of lysostaphin to remove cell-adherent staphylococci during in-vitro assays of phagocyte function. *Br J Exp Pathol* 1978;**59**:381–5.
10 Cash HA, Strauss DC, Bass JA. *Pseudomonas aeruginosa* exoproducts as pulmonary virulence factors. *Can J Microbiol* 1983;**29**:448–56.
11 Stockley RA, Burnett D. Serum derived protease inhibitors and leucocyte elastase in sputum and the effect of infection. *Bull Eur Physiopathol Respir* 1980;**16,** suppl:261–71.
12 Liu PV. Extracellular toxins of *Pseudomonas aeruginosa. J Infect Dis* 1974;**130,** suppl:594–9.