J. Pharm. Pharmacol. 1990, 42: 145–151
Received October 25, 1989

The Effect of Some Preservatives Used in Nasal Preparations on the Mucus and Ciliary Components of Mucociliary Clearance

A. H. BATTS†, C. MARRIOTT, G. P. MARTIN, C. F. WOOD AND S. W. BOND*

*Pharmaceutical Sciences Research Group, Department of Pharmacy, Brighton Polytechnic, Brighton BN2 4GJ, UK and *The Wellcome Foundation Ltd, Dartford, Kent, UK*

Abstract—Efficient mucociliary clearance is a function of the physical properties of the mucus coupled to appropriately functioning cilia and may be altered by substances affecting ciliary beat frequency (CBF). Therefore the effect of preservatives on CBF was investigated using a photoelectronic technique. Methyl-*p*-hydroxybenzoate, propyl-*p*-hydroxybenzoate, chlorbutol and chlorocresol inhibited beat frequency, an effect which was reversible upon rinsing out the first three compounds but not chlorocresol. The effect of chlorhexidine and phenylmercuric borate on CBF was complicated by an interaction with chloride ions in the media used. EDTA did not appear to be ciliotoxic, while the effect of benzalkonium chloride was variable. Thiomersal halted ciliary beating after 40–100 min. Mucociliary clearance may also be affected by an alteration of the physical properties of the mucus layer, therefore the effect of each compound on the rheological properties of purified pig gastric mucus glycoprotein was investigated. None of the preservatives significantly altered the viscoelastic properties of the gel, measured using dynamic techniques.

Most nasal drops and sprays contain a preservative in their formulation to prevent the growth of micro-organisms which might contaminate the preparation during its repeated use. Any substance administered intranasally has the potential to alter mucociliary clearance, which is the mechanism by which inhaled and deposited particles are cleared from the upper respiratory tract. Efficient mucociliary clearance depends upon a successful relationship between cilia, mucus and periciliary fluid. Changes in any of these may alter the characteristics of transport and produce an accumulation of secretions in airways or give toxic materials a longer residence time in the airways (Wolff 1986). Abnormal elimination of airways mucus is associated with a variety of conditions such as chronic rhinitis, sinusitis and bronchitis (Afzelius 1979). Thus it is important to determine the response of the mucociliary system to substances administered by the respiratory route, such as drugs and other pharmaceutical excipients, including preservatives. There are a number of efficient preservatives, but at present the choice of preservative is largely determined by its compatibility with other formulation ingredients and the packaging of the preparation. No concern is paid to the effect of preservatives on nasal mucociliary clearance.

A number of in-vitro studies have been carried out to investigate the effects of preservatives on the mucociliary apparatus. Most have monitored the ciliary component of mucociliary clearance (Greenwood et al 1946; Gallay 1960; Perrault et al 1978; Mostow et al 1979; Van de Donk et al 1980; Stanley et al 1985). In this paper we report the effect of preservatives on the ciliary beat frequency of explants of chicken embryo trachea, where the effect of mucus is negligible. This model was selected because it had been used previously to investigate the ciliotoxicity of preservatives. The results are compared with the effect of the same preservatives on the transport rate of graphite particles over the frog palate (Batts et al 1989), which possesses a ciliated epithelium protected by a layer of viscoelastic mucus (Morgan et al 1984). As such it might have been expected to offer a good comparison with the human nasal cavity. In addition, mucociliary transport rate over the frog palate has been shown to correlate with in-vivo tracheal clearance in dogs (Giordano et al 1977) and bronchial clearance in man (Puchelle et al 1980).

Viscoelasticity is considered to be prerequisite to transport of mucus by cilia (King et al 1974) and studies have shown that an optimum range of elastic modulus exists for efficient transport, implying that an alteration of elasticity outside this range would be detrimental to transport (Litt 1977). Therefore the effect of the same range of preservatives on the viscoelastic properties of a mucus glycoprotein gel was investigated to assess whether an alteration of the mucus component of the mucociliary clearance apparatus was responsible for, or contributed to, the action of the preservative on mucociliary transport over the frog palate. Each preservative was tested at the concentration at which it is generally employed in pharmaceutical dosage forms to prevent microbial growth.

Materials and Methods

Preparation of solutions

The preservative solutions investigated in ciliary beat frequency (CBF) and rheological studies were made by dissolving the required amount of preservative in the appropriate control solution. The compounds and concentrations inves-

Correspondence to: C. Marriott, Pharmaceutical Sciences Research Group, Department of Pharmacy, Brighton Polytechnic, Brighton BN2 4GJ, UK.

† Current address: Department of Anatomy and Cell Biology, University of California, Los Angeles, School of Medicine, California 90024, USA.

tigated were: methyl-*p*-hydroxybenzoate 0·15%; propyl-*p*-hydroxybenzoate 0·02%; benzalkonium chloride 0·01% (Sigma Chemical Company, UK); 4-chloro-*m*-cresol 0·1%; chlorbutol 0·5%; thiomersal 0·01% (BDH Chemicals Ltd, UK); ethylenediaminetetraacetic acid, disodium salt, dihydride (EDTA) 0·1% (Aldrich Chemical Company Ltd, UK); phenylmercuric borate 0·002% (Zyma SA, Switzerland); chlorhexidine gluconate 0·01% (ICI plc, UK). Phenylmercuric nitrate 0·002% (BDH Chemicals Ltd, UK) was investigated in rheological studies only.

The control solution used for CBF studies was Locke-Ringer (LR) containing (mM): NaCl 132 (May and Baker Ltd, UK), KCl 5·6, dextrose 5·5, $NaHCO_3$ 1·8 (BDH Chemicals Ltd, UK) and $CaCl_2$, dihydrate 1·1 (Hopkin and Williams Ltd, UK). The pH was adjusted to 7·4.

The control solution used for rheological studies was 10 mM Tris/HCl (BDH Chemicals Ltd, UK) buffer, with the exceptions of phenylmercuric borate and nitrate, and chlorhexidine when distilled water, at pH 7·4, was used for investigations using the Controlled Stress Rheometer (Carri-Med Ltd, UK).

CBF studies

Tissue preparation. Fertilized chicken eggs were incubated at 37°C and a relative humidity of 100%. At 16–19 days post-insemination the trachea was dissected from the embryo, sliced into rings and stored in LR until required (30–40 min). Rings were transferred to welled-slides containing LR and observed under the microscope (Vickers M41 Photoplan) to establish that ciliary motion was present. Measurements were performed on four rings from one trachea; one ring remained in LR to serve as control, and the three other rings were each placed in a different preservative solution. The procedure was repeated with five different tracheae and all measurements were made at room temperature (20–22°C).

Optical equipment and CBF analysis. The slide coverslip preparation of the tracheal explant was placed on the stage of the microscope with an anti-vibratory base and examined using a ×40 dry objective and ×10 eyepiece. A video camera attached to the side port of the microscope enabled an image of the ciliated epithelium to be displayed on a television monitor and simultaneously recorded on a videocassette. The system provided a magnification of ×1700. Each of the four tracheal rings was recorded every 20 min for 2 h. The movements of the cilia were detected using a hand held light probe, containing a photodiode, placed against the monitor screen over regions of beating cilia (0·5 cm^2). CBF was analysed by sampling the amplified and filtered output using a microcomputer and performing real-time spectral analysis using the fast Fourier transform (FFT) on the wave form obtained. This provided a power spectrum of the frequency components present in the photoelectronic signal, from which a dominant frequency was determined which represented the mean beat frequency of the cilia in the selected area over the sample period (Figs 1, 2). Measurements were taken from different areas of the tracheal ring where contrast was sufficient to allow a reasonable signal to be obtained. Six measurements of CBF were made from each recording.

Preparation of mucus glycoprotein for rheology

Mucus was obtained from the stomachs of freshly slaughtered pigs (*Suis scrofa domestica*). Samples were homogenized in five volumes of a protease inhibiting solution (NaCl 200, EDTA 5, phenylmethylsulphonyl fluoride (Sigma Chemical Company, UK) 1 mM and 0·02% sodium azide (BDH Chemicals Ltd, UK)), centrifuged, to remove any insoluble food matter, and the supernatant filtered through glass wool. Samples were applied to a Sepharose CL4B gel exclusion column at 4°C and the excluded fraction, containing the glycoprotein, was collected. Following concentration by ultrafiltration, using a Millipore Minitan Ultrafiltration System (Millipore Corporation, USA), the product was exhaustively dialysed, at 4°C, against 10 mM Tris/HCl buffer or distilled water, at pH 7·4. The glycoprotein was then concentrated further to a gel by ultrafiltration through an Amicon PM30 membrane (Amicon Corporation, UK). Dry weight determinations were carried out on samples of the gel. The remaining gel was divided into six portions, each was adjusted either with control solution alone or with that containing the appropriate concentrations of preservative to obtain six, 8% w/w gel samples. The gels were left at 4°C overnight to ensure that a homogeneous preparation was achieved.

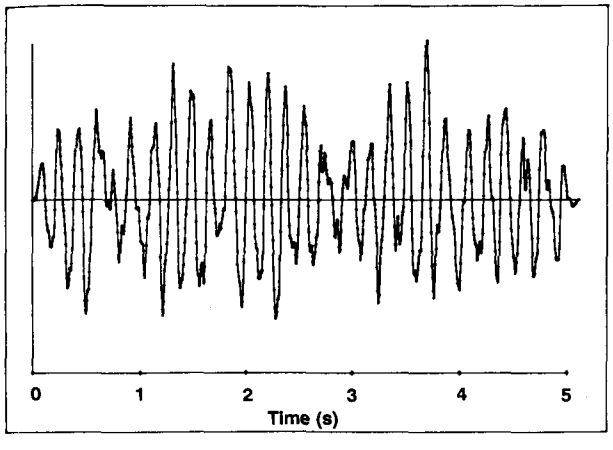

FIG. 1. Wave form of ciliary movement.

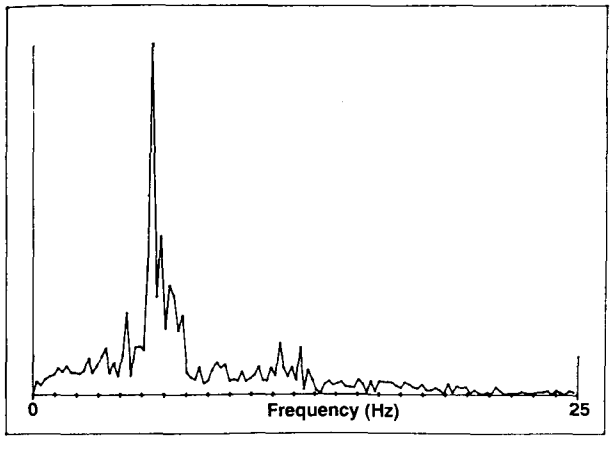

FIG. 2. Power spectrum of selected area of beating cilia.

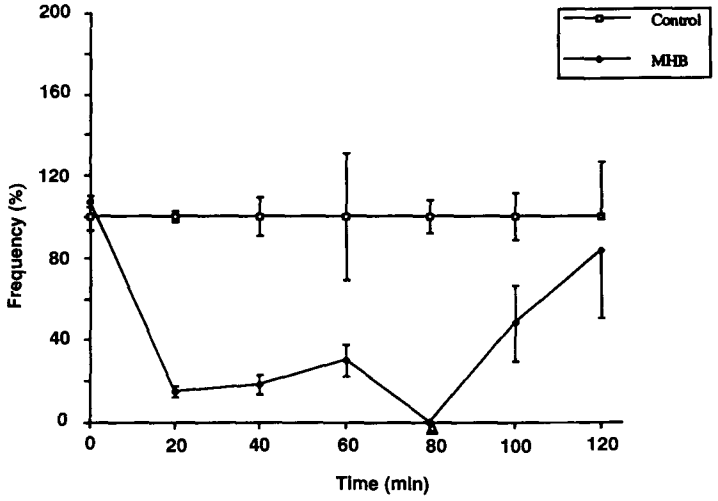

FIG. 3. Effect of methyl-*p*-hydroxybenzoate (●) on ciliary beat frequency. Means ± s.d. are indicated. △ Rinsing of tissue with LR. Each point represents six measurements of CBF from one piece of tissue. □ Control.

Rheological testing

Rheological investigations were conducted using an oscillating sphere magnetic microrheometer (James & Marriott 1982) over the frequency range 0·2–20 Hz and a controlled stress rheometer over the range 0·1–10 Hz.

Each preservative was tested on up to five different batches of mucus, with three being the minimum number used. In order to compare the results from different batches, the data were normalized. A grand mean was calculated from all the control values of all the batches of mucus. In addition, another mean was calculated from the control values of individual batches of mucus. The test value was divided by the mean of the control values which came from the same batch of mucus as did the test, and then multiplied by the grand mean. Data obtained using the oscillating sphere magnetic microrheometer were not combined with data obtained using the controlled stress rheometer.

Results

Sections of chick embryo trachea were observed daily from 12 days post-insemination. It was not until the sixteenth day that cilia were sufficiently well developed to permit measurements of CBF. The effect of methyl-*p*-hydroxybenzoate, chlorocresol and phenylmercuric borate are shown in Figs 3, 4 and 5. These Figures have been selected as examples of reversible toxicity, irreversible toxicity and no apparent toxicity, respectively. The frequency of the control at each time interval was defined as 100% and CBF of the test portion of trachea was plotted relative to this. In reality, the beat frequency of control tissue diminished during the course of the experiments such that after 2 h it was 50–75% of the initial frequency.

The compounds methyl- and propyl-*p*-hydroxybenzoates, chlorbutol, and chlorocresol were ciliotoxic. Rinsing the

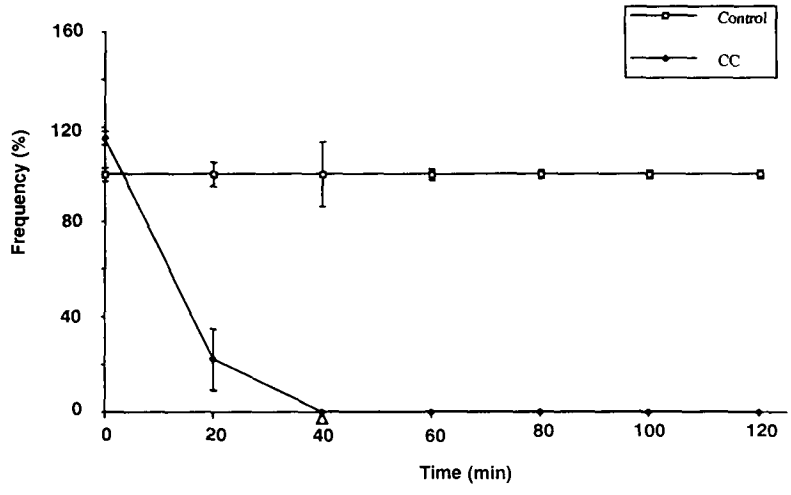

FIG. 4. Effect of chlorocresol (●) on ciliary beat frequency. Means ± s.d. are indicated. △ Rinsing of tissue with LR. Each point represents six measurements of CBF from one piece of tissue. □ Control.

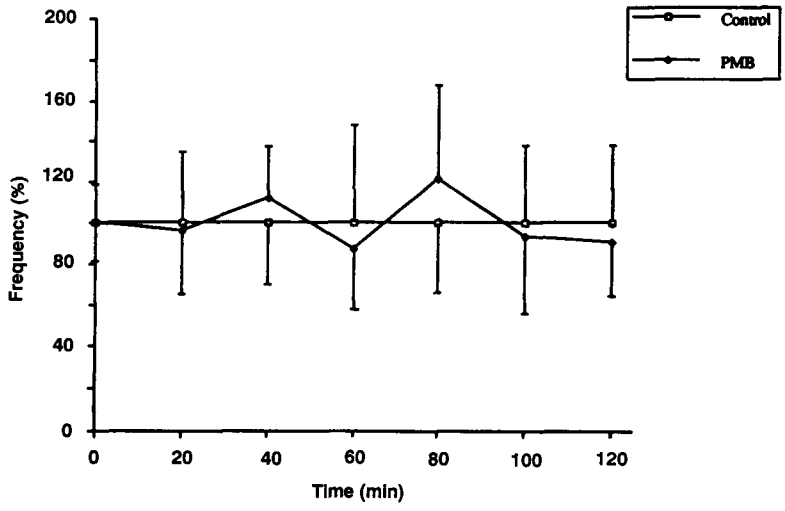

FIG. 5. Effect of phenylmercuric borate (●) on ciliary beat frequency. Means ± s.d. are indicated. Each point represents the combined means from five pieces of tissue. □ Control.

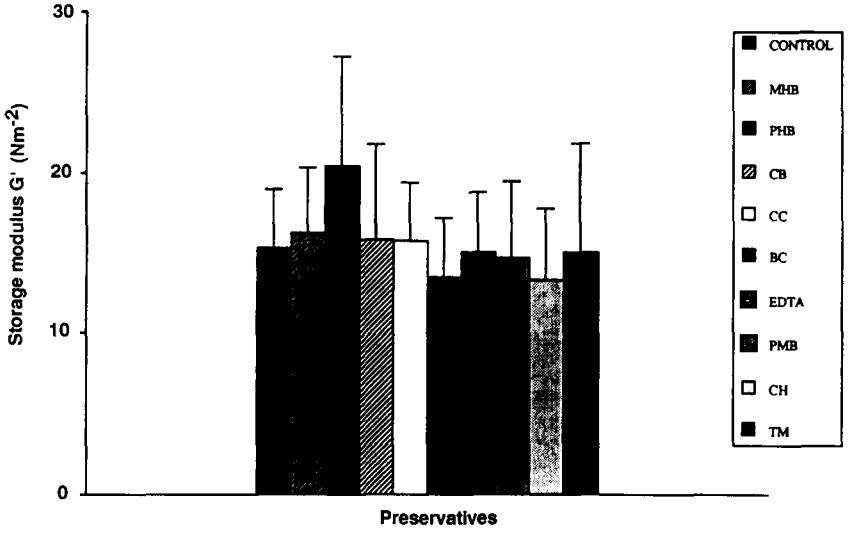

FIG. 6. Effect of preservatives on the storage modulus G′ at 10 Hz measured using the oscillating sphere magnetic microrheometer. Means ± s.d. are indicated. MBH methyl-*p*-hydroxybenzoate. PHB propyl-*p*-hydroxybenzoate. CB chlorbutol. CC chloro-*m*-cresol. BC benzalkonium chloride. PMB phenylmercuric borate. CH chlorhexidine gluconate. TM thiomersal.

tissue with LR abrogated the effect of the first three, but not chlorocresol. Rinsing was carried out in response to the decrease or absence of ciliary activity which was observed at different times after immersing the tracheal rings in preservative. It was not possible therefore to combine data from different experiments with the same compound. Consequently, Figs 3 and 4 represent examples from only one of the five pieces of trachea studied.

Fig. 5 shows the mean data (with standard deviation bars) from all five portions of trachea exposed to PMB. The Mann–Whitney U-test (Siegel 1956) was used to test for any difference between test and control values at each 20 min interval. Phenylmercuric borate did not appear to affect CBF and a similar response (not shown) was obtained with chlorhexidine gluconate and EDTA. After 2 h the CBF of tissue exposed to chlorohexidine was seen to decrease ($P < 0{\cdot}01$), whereas the CBF of the tissue exposed to EDTA was not significantly different from the control. Thiomersal appeared ciliotoxic after 40–100 min whereas benzalkonium chloride appeared toxic in three of the five tracheal portions and non-toxic in the other two.

Results of rheological testing were expressed as the storage modulus (G′) and the loss modulus (G″) at each frequency. Using the Mann–Whitney U-test, at the frequencies 0·6 and 10 Hz, it appeared that there was no significant difference between the treated and untreated gels ($P < 0{\cdot}05$) for all the preservatives tested. Fig. 6 compares normalized data for G′ at 10 Hz, which approximates to the rate of ciliary beating,

providing a comparison between the treated and untreated gels. Similar results were obtained for G′ and G″ using both rheometers.

Discussion

The experimental system described is similar to that of Teichtahl et al (1986) and is capable of giving real-time measurements of CBF while recordings may be stored and analysed retrospectively. The software was designed to perform a FFT analysis on the waveform of ciliary beating. This is a commonly employed technique of analysing in-vitro CBF and, despite the likelihood of the analysis containing frequency components attributable to the biphasic nature of ciliary beating and metachronal waves (Sanderson & Dirksen 1985), was considered adequate for assessing the ciliotoxicity of preservatives.

The observed decrease of CBF with time may reflect a lack of nutrition to the cilia due to the small volume of LR in the slide coverslip preparation. In a previous study, tracheal rings were kept in a petri dish of solution under test and only transferred to the slide when measuring CBF (Van de Donk et al 1980). This was avoided in the current study because it has been shown that the mechanical stimulation to the cilia involved in such manoeuvres is likely to result in the measurement of an abnormally high CBF (Sanderson & Dirksen 1986).

Mucociliary transport depends upon the coordinated beating of cilia coupled to mucus with appropriate rheological properties. Preservatives which halted transport over the frog palate (Batts et al 1989) but appeared innocuous to cilia when the effect of mucus was negligible were believed to be exerting their effect via an interaction with the mucus component of the system. Therefore the effect of each preservative on the rheological properties of a purified mucus glycoprotein gel was investigated to determine whether the viscoelastic properties were altered beyond the range required for optimum transport. A glycoprotein gel was selected because it is the glycoprotein molecule which is considered to be responsible for the rheological properties of mucus (Carlstedt et al 1985). Of the rheometers used, the microrheometer has been shown to be sufficiently sensitive to detect cation-induced changes in the properties of mucus (Crowther et al 1984) and would therefore be likely to detect any significant changes in mucus viscoelasticity caused by the preservatives. Results obtained using the microrheometer were supported by those obtained using the controlled stress rheometer.

Methyl- and propyl-*p*-hydroxybenzoates and chlorbutol are lipophilic preservatives which all exhibited reversible toxicity to cilia in the absence of mucus. This was in agreement with the results of previous studies (Gallay 1960; Perrault et al 1978; Mostow et al 1979; Van de Donk et al 1980). In addition, these preservatives halted transport over the frog palate in a reversible manner (Batts et al 1989). Since the effect of the compounds was the same in the presence and absence of mucus, the role of mucus was unlikely to be important in their mode of action. This was supported by the fact that none of the compounds affected the rheological properties of a purified mucus glycoprotein gel.

Chlorocresol halted the ciliary beat of the chicken embryo trachea in an irreversible manner. It also halted transport irreversibly over the frog palate (Batts et al 1989). Since it did not appear to alter the rheological properties of a purified mucus glycoprotein gel, it is likely that it exerts its effect on the ciliary component of mucociliary clearance.

Phenylmercuric borate and chlorhexidine have been found to halt beating of chicken embryo tracheal cilia within 60 and 80 min, respectively (Van de Donk et al 1980). In our study the former did not appear toxic and chlorhexidine only decreased CBF after 2 h. These differences between studies were believed to be due to the formation of the less soluble chloride salts of these two preservatives in the presence of chloride ions of LR solution, since a fine precipitate was observed in both solutions. This was further substantiated by experiments using the frog palate, when the application of either compound in normal saline halted transport much less rapidly than the application of the same compound in an isotonic solution of mannitol (Batts et al 1989). Nasal drops and sprays are usually formulated to be isotonic with natural secretions. Therefore the possibility exists of an interaction between phenylmercuric preservatives or chlorhexidine and other formulation components which would reduce the efficiency of the preservative. Assuming care is taken to avoid precipitation of the preservative within the formulation, subsequent precipitation may occur upon intranasal delivery of the formulation due to an interaction with the chloride ions present in nasal secretion. This would reduce the ciliotoxicity of the compound, whose preservative function would now be redundant, and the precipitate would be removed by mucociliary clearance.

Tris/HCl buffer and distilled water were both used as control solutions in rheological studies because precipitation of the chloride salts of phenylmercuric borate and nitrate and chlorhexidine may have rendered them less likely to affect the physical properties of the gel. However, no alteration of the rheological properties of the gel was observed with either solution.

The effect of benzalkonium chloride (0·01%) on CBF of chicken embryo trachea was variable. In some experiments it was well tolerated while in others it appeared toxic. A similar variability in its effect on CBF has been found previously; at 0·01% it was observed to cause ciliostasis within 5 min (Stanley et al 1985) and 45 min (Gallay 1960). However, ciliary activity was still observed after exposure to 0·1% benzalkonium for 1 h (Greenwood et al 1946), and 0·01% benzalkonium for 2 h (Van de Donk et al 1980). Benzalkonium (0·01%) halted transport over the frog palate, irreversibly, after one or two 10 min applications (Batts et al 1989). Such differences in reported observations have been explained by differences in species sensitivity to the preservative (Stanley et al 1985), since it halted ciliary beating in human, guinea-pig and frog tissue but not in rabbit and chicken embryo tissue.

Benzalkonium chloride is surface-active, and micellar aggregation in solutions of different ionic strength could affect its activity and also explain its different effects in the above studies when different media were used. But this does not explain the variety of results obtained in the current study. Surfactants have been shown to exhibit mucolytic properties (Martin et al 1978) which might affect mucociliary transport. In fact benzalkonium chloride did not alter the

rheological properties of a purified mucus glycoprotein gel and therefore its toxicity is likely to be to the ciliary component of mucociliary clearance since surfactants can damage the ciliary membrane (Satir & Dirksen 1985).

EDTA has been observed to halt transport over the frog palate after one or two 10 min applications, although in the presence of calcium ions in excess of those required to saturate the chelation sites of EDTA, the latter was well tolerated (Batts et al 1989). In the present study EDTA did not appear to affect CBF despite insufficient calcium ions being present to saturate all chelation sites.

Although EDTA has been reported as having mucolytic activity at concentrations in excess of 0·25 M (Lieberman 1968), it did not appear to affect mucus rheology in this study, when it was used at $2{\cdot}7 \times 10^{-3}$ M. Marriott et al (1979) reported that at concentrations in the range $1\text{–}2{\cdot}5 \times 10^{-3}$, it did not affect the rheological properties of mucus gels, although transport over a toad palate was observed to halt. Those observations support results in the current study and suggest that the difference of EDTA on CBF and mucociliary transport may be explained by the response of cilia to Ca^{2+} rather than to an alteration of the physical properties of the mucus.

Calcium ions are believed to play a role in the control of ciliary motion. Intracellular ions are believed to be actively involved in modulating the frequency of ciliary beat (Girard & Kennedy 1986), and it has been hypothesized that rabbit cultured tracheal cilia beat faster in response to mechanical stimulation owing to an increase in intracellular calcium ions (Sanderson & Dirksen 1986). The cilia of the frog palate beat, and therefore transport, in response to mechanical stimulation and are otherwise quiescent (Spungin & Silberberg 1984), whereas cilia of higher vertebrates beat in the absence of stimulation (Sleigh et al 1988). The removal of extracellular calcium by EDTA is likely to be of greater significance to the cilia of the frog palate, which will need to increase intracellular calcium (ultimately from extracellular sources) in order to respond to stimulation, than to cilia of higher vertebrates which seem to maintain a baseline activity that might not depend so critically on extracellular calcium ions. Such variations in species sensitivity suggests a requirement for confirmatory in-vitro studies using human airway tissue. The effect of benzalkonium chloride, EDTA and thiomersal on in-vivo mucociliary clearance of volunteers has been examined with this in mind (Batts 1989).

Thiomersal appeared toxic to the cilia of the chicken embryo trachea. This was in agreement with previous results (Perrault et al 1978; Van de Donk et al 1980; Stanley et al 1985). However, it was well tolerated by the frog palate with transport still present after nine 10 min applications (Batts et al 1989). Thiomersal did not alter the rheological properties of a purified mucus glycoprotein gel and it seemed likely that the mucus of the frog palate was preventing it reaching the cilia. Diffusion experiments showed that it was capable of diffusing through a layer of purified mucus glycoprotein 1 mm thick (Batts 1989) and was therefore likely to reach the cilia of the frog palate. However, there was a considerable lag time before the mean preservative front penetrated the purified mucus glycoprotein in-vitro. Although this lag time is likely to be considerably reduced in-vivo because of the reduced thickness of the mucus layer, in combination with mucociliary clearance the compound might not be retained on the palate long enough to exert its toxic effect.

While offering a degree of protection to the ciliated mucosa by provision of a barrier, mucus is also able to dilute the applied substance perhaps rendering it less toxic. Therefore although preservatives are generally used in nasal preparations at the lowest concentration able to inhibit bacterial growth, a place exists for examining dilutions of this concentration on CBF. However, little information is available concerning the degree of dilution that might be expected to occur in the nose.

From the results of this investigation it would appear that those preservatives modifying mucociliary transport in-vitro do not act via an effect on the physical properties of the glycoprotein component of the mucus gel. This does not preclude the possibility of them altering the physical properties of the native secretion. Although the glycoprotein molecule is considered to be of prime importance for the physical properties of mucus, other constituents of the native secretion, such as the water content, ions, both native and denatured proteins and nucleic acids, may modify the rheological properties of the gel (Pain 1980). An interaction between the preservatives and one or more of these constituents could alter the viscoelastic properties of the secretion and thus its transportability. It is also possible that the preservatives could be interacting with the glycoprotein molecules in a manner which does not alter the rheological properties of the gel.

This investigation has reported the effect of a range of compounds on the ciliary and mucus components of the mucociliary apparatus, and compared these results with the effects exerted by the same compounds on a system where the two components interact. This strategy provided a better understanding of the action of preservatives on mucociliary clearance, aiding the selection of the least toxic for inclusion in nasal drops and sprays. With the use of nasal preparations being extended to include chronic delivery of drugs for systemic therapy, the effect on mucociliary clearance of drugs, preservatives and other excipients, such as absorption enhancers, should be investigated so that the most appropriate compounds may be selected for inclusion in preparations intended for intranasal delivery.

References

Afzelius, B. A. (1979) The immotile-cilia syndrome and other ciliary diseases. Int. Rev. Exp. Pathol. 19: 1–43

Batts, A. H. (1989) A study of the ciliotoxicity of some pharmaceutical preservatives. Ph.D. Thesis, Brighton Polytechnic

Batts, A. H., Marriott, C., Martin, G. P., Bond, S. W. (1989) The effect of some preservatives used in nasal preparations on mucociliary clearance. J. Pharm. Pharmacol. 41: 156–159

Carlstedt, I., Sheehan, J. K., Corfield, A. P., Gallagher, J. T. (1985) Mucous glycoproteins: A gel of a problem. Essays in Biochemistry 20: 40–76

Crowther, R. S., Marriott, C., James, S. L. (1984) Cation-induced changes in the rheological properties of purified mucus glycoprotein gels. Biorheology 21: 253–263

Gallay, C. (1960) Essays physiologuiques des gouttes nasales. Pharm. Acta Helv. 35: 358–374

Giordano, A. M., Shih, C. K., Holsclaw, D. S., Khan, M. A., Litt, M. (1977) Mucus clearance: in vivo canine tracheal clearance vs. in vitro bullfrog palate studies. J. Appl. Physiol. 42: 761–766

Girard, P. R., Kennedy, J. R. (1986) Calcium regulation of ciliary

activity in rabbit tracheal epithelial explants and outgrowth. Eur. J. Cell Biol. 40: 203–209

Greenwood, G., Pittenger, R. E., Constant, G. A., Ivy, A. C. (1946) Effect of zephiran chloride, tyrothricin, penicillin and streptomycin on ciliary action. Arch. Otolaryngol. 43: 623–628

James, S. L., Marriott, C. (1982) A modified oscillating sphere magnetic microrheometer for use with biological secretions. J. Phys. E: Sci. Instrum. 15: 179–180

King, M., Gilboa, A., Meyer, F. A., Silberberg, A. (1974) On the transport of mucus and its rheological stimulants in ciliated systems. Am. Rev. Respir. Dis. 110: 740–745

Lieberman, J. (1968) Measurement of sputum viscosity in a cone and plate viscometer II: an evaluation of mucolytic agents in vitro. Am. Rev. Respir. Dis. 97: 662–672

Litt, M. (1977) Mucus rheology and mucociliary clearance. Mod. Probl. Paediatr. 19: 175–181

Marriott, C., Shih, C. K., Litt, M. (1979) Changes in gel properties of tracheal mucus induced by divalent cations. Biorheology 16: 331–337

Martin, G. P., Marriott, C., Kellaway, I. W. (1978) Direct effect of bile salts and phospholipids on the physical properties of mucus. Gut 19: 103–107

Morgan, K. T., Patterson, D. L., Gross, E. A. (1984) Frog palate mucociliary apparatus: Structure, function and response to formaldehyde gas. Fundam. Appl. Toxicol. 4: 58–68

Mostow, S. R., Dreisen, R. B., Manawadu, B. R., La Force, F. M. (1979) Adverse effects of lidocaine and methyl paraben on tracheal ciliary activity. Laryngoscope 89: 1697–1701

Pain, R. H. (1980) The viscoelasticity of mucus: A molecular model. Symp. Soc. Exp. Biol. 34: 359–376

Perrault, G., Dreyfuss, M. F., Saunie, R. (1978) Etude de la function ciliaire. Application aux essais de tolerance des gouttes nasales. Sci. Tech. Pharm. 7: 383–388

Puchelle, E., Zahm, J. M., Girard, F., Berrand, A., Polu, J., Aug, F., Sadoul, P. (1980) Mucociliary transport in vivo and in vitro. Eur. J. Respir. Dis. 61: 254–264

Sanderson, M. J., Dirksen, E. R. (1985) A versatile and quantitative computer assisted photoelectronic technique used for the analysis of ciliary beat cycles. Cell Motility 5: 267–292

Sanderson, M. J., Dirksen, E. R. (1986) Mechanosensitivity of cultured ciliated cells from the mammalian respiratory tract: Implications for the regulation of mucociliary transport. Proc. Natl. Acad. Sci. USA 83: 7302–7306

Satir, P., Dirksen, E. R. (1985) Function–structure correlations in cilia from mammalian respiratory tract. In: Fishman, A. P., Fisher, A. B. (eds) Handbook of Physiology; The respiratory system I., Circulation and non-respiratory functions. Am. Physiol. Soc. pp 473–494

Siegel, S. (1956) In: Nonparametric statistics for the behavioural sciences, McGraw Hill, London

Sleigh, M. A., Blake, J. R., Liron, N. (1988) The propulsion of mucus by cilia. Am. Rev. Respir. Dis. 137: 726–741

Spungin, B., Silberberg, A. (1984) Stimulation of mucus secretion, ciliary activity and transport in frog palate epithelium. Am. J. Physiol. 24: C299–C308

Stanley, P. J., Griffin, W. M., Wilson, R., Greenstone, M. A., Mackay, I. S., Cole, P. J. (1985) Effect of betamethasone and betamethasone with neomycin nasal drops on human nasal mucociliary clearance and ciliary beat frequency. Thorax 40: 607–612

Teichtahl, H., Wright, P. L., Kirsner, R. L. G. (1986) Measurement of in vitro ciliary beat frequency: a television–video modification of the transmitted light technique. Med. Biol. Eng. Comput. 24: 193–196

Van de Donk, H. J. M., Muller-Plantema, I. P., Zuidema, J., Merkus, F. W. H. M. (1980) The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas. Rhinology 18: 119–133

Wolff, R. K. (1986) Effects of airbourne pollutants on mucociliary clearance. Environ. Health Perspect. 66: 223–237