NASAL MEDICATION and CILIARY MOVEMENT

huib van de donk

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ACADEMISCH PROEFSCHRIFT

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HUBERTUS JOHANNES MARIA VAN DE DONK

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Promotor : Prof. dr. F.W.H.M. Merkus
Coreferenten : Prof. dr. E.H. Huizing
Prof. dr. P.A. van Zwieten

- Wijzen zijn geen bruikbare lieden -

Maar het zou nog enigszins te dragen zijn als ze zich slechts ten opzichte van openbare ambten als ezels gedroegen; op geen enkel terrein van het leven zijn ze echter een greintje handiger.
Nodig maar eens een wijze uit voor een diner: hij zal het door somber zwijgen of vervelende vraagjes in de war schoppen. Vraag hem ten dans: u zult denken dat er een kameel rondhuppelt! Neem hem mee naar publieke vermakelijkheden: alleen al door zijn blik zal hij het plezier van het volk bederven en hij zal als de wijze Cato gedwongen worden uit het theater te verdwijnen daar hij zijn ernstige frons niet kan afleggen. Valt hij in een gesprek, dan passeert er onmiddellijk een dominee. Als er iets gekocht moet worden of een overeenkomst getekend, kortom, als er een van die dingen gedaan moet worden zonder welke ons dagelijks leven niet kan bestaan, dan lijkt die wijze wel een blok hout in plaats van een mens.....

D. Erasmus Laus Stultitiae.

In Memoriam Gallinae

CONTENTS

Nasal drops are extensively used as they are often "over-the-counter" drugs and indicated for frequently occurring diseases like the common cold. The self-cleaning capacity of the nose effectuated by the ciliary epithelium, necessary to remove dust, allergens and bacteria, should not be decreased by medication. However, this capacity called "nasal clearance" can be influenced in a negative way not only by the drugs in the medication but also by the additives that are present in nasal preparations.
It is the purpose of the investigations of this thesis to develop a method to study the influence of drugs, additives, pH and osmotic pressure on the ciliary movement.

In the human nose each ciliary cell has about 250-300 cilia on its surface and each cilium is about 8 µm long and 0.3 µm in diameter. Each beats with a frequency of about 20 beats/sec and the movement of the cilia is probably regulated by an intracellular pacemaker system, which also maintains the movement in vitro. The mucus layer is transported towards the throat at about 0.5 cm/min, where the mucus, which includes foreign particles is swallowed. Every 24 h about 1 l of mucus is excreted and transported by the ciliary epithelium.
The ciliary activity is influenced by many factors.
Dry air decreases the activity to a large extent: a relative humidity of less than 30% results in ciliary arrest within 5 min.
Low temperatures decrease ciliary activity as well: temperatures below 10° C result in stagnation of ciliary movement.
Smoking also has a negative influence: three cigarettes smoked successively arrest ciliary activity.

The eventual effect of arresting ciliary movement is difficult to estimate, but from patients with "Immotile Cilia Syndrome" it is known that chronic ciliary arrest leads to recurrent infections of the airways. Of course, this permanent lack of ciliary movement is at the extreme end of a range of effects on cilia, but if we have the opportunity to choose among several drugs or additives, which are, in their desired therapeutic effects, equipotent, it is obvious that we should use the most "cilio-friendly" substances.
Therefore, a simple in vitro method is needed to measure the effects of substances on ciliary movement.

To prevent microbial contamination, nasal drops have to be preserved when they are used as multidose preparations. As there are many preservatives that can protect nasal drops effectively against contamination and as preservatives appear in almost all nasal drops (some drops containing antibiotics form an exception), the study of the effects of preservatives on ciliary movement is most relevant.

Knowing the effects of the preservatives, it is obvious to study the effects of nasal drops that are available on the market and to focus the attention on three groups of active components:
Decongestants: xylometazoline HCl, oxymetazoline HCl, tramazoline HCl, naphazoline nitrate, ephedrine HCl, phenylephrine HCl or phenylpropanolamine HCl. Some of these nasal drops contain menthol, camphor or eucalyptol as well.
Antibiotics and chemotherapeutics: e.g. neomycine sulphate, chloramphenicol, sulphanilamide, sulphacetamide or mild silver protein.
Anti-allergic agents: e.g. antazoline sulphate, sodium cromoglycate, prednisolone phosphate or dexamethasone.

It will however, be necessary to check the in vitro results with in vivo results. Therefore, it is also one of the purposes of this thesis to investi-

gate the correlation between in vitro results and the effects on the transport time of mucus in vivo.

Recently, it has been suggested that drugs with a large first-pass effect like propranolol should be administrated through the nose. Therefore propranolol's ciliotoxicity is also studied.

The results of these investigations will contribute to the improvement of the pharmaceutical formulation of nasal medication.

CHAPTER I

Rhinology, 18, 93-104, 1980

The influence of the pH and osmotic pressure upon tracheal ciliary beat frequency as determined with a new photo-electric registration device

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SUMMARY

A method for measurement of tracheal ciliary beat frequency in vitro is described. Light transmitted through the cilia is detected by a phototransistor mounted in a microscope, while the frequency is measured instantaneously and the waveform is displayed by an oscilloscope, connected to a transient recorder. Due to the magnification and the method of illumination, the movement of approximately 30 cilia is projected on the phototransistor. In Locke-Ringer solution the waveform shows a very constant amplitude. Interference arises after a noxious influence and is dependent on the frequency of the ciliary movement. The effect of pH and osmotic pressure on chicken embryo and rat tracheal ciliary beat frequency is assessed. The frequency is not influenced between pH=7 and pH=10, but higher and lower pH values decrease the frequency. Hypertonic NaCl solutions decrease the frequency of chicken embryo cilia as much as hypotonic NaCl solutions. Rat cilia turned out to be less sensitive for hypotonic NaCl solutions.

INTRODUCTION

Nasal drops have been used during the last decennia, however only little information is available about their influence upon the ciliary function of the mucosa. Cilia were described by De Heide and Leeuwenhoek in the seventeenth century, but it took untill the thirties before Proetz emphasized the importance of studying the ciliary function and physiology (Proetz, 1932). The ability of the mucosal epithelium to remove foreign particles is essential to human health. The nasal cilia are covered by a blanket of mucus. The aerodynamic characteristics of the nose force a great deal of the particles in the inspired air to precipitate on the mucus. Mucus and particles are transported together by the cilia to the throat. Nasal medications can change and even destroy the epithelial cells and dependent on the agent the recovery takes a few hours to a few months. In order to improve the pharmaceutical formula of drugs administered in nose and respiratory tract, a model and method is needed to investigate the influence of these drugs and additives on ciliary motion.

Dalhamn (1955) estimated the frequency of ciliary waves with high speed cinematography. A motion picture is recorded at high speed and afterwards projected at low speed. The method is accurate, but expensive and laborious. Gallay (1960) assessed the activity of cilia of guinea pigs by determining the time, necessary to provoke a cessation of movement. Mirimanoff and Paley (1966) modified the method so that a rough indication could be obtained about the time, needed for recovery. Andersen (1971) illuminated cilia with stroboscopic light, which consisted of a variable number of flashes per second. When this number equals the frequency of the ciliary movement, the cilia are perceived stationary. This method is simple, but is not applicable for frequencies lower than 10 Hz and prolonged observations are extremely strenuous. Dalhamn and Rylander (1962) used a CdS cell and Mercke et al. (1974) used a photomultiplier to transform light variations, resulting from mucociliary waves into voltage variations. After suitable amplification the frequency can be assessed. This method is sensitive to vibrations, but accurate and quickly performable. Lee and Verdugo (1976, 1977) illuminated cilia from the rabbit oviduct with a laser beam. The spectrum of the scattered light can be analysed and gives information about the frequency of the ciliary movement. This method is sophisticated, and highly accurate, but very expensive.

All these methods involve more or less complicated electronic equipment. Some authors described more simple methods. An important disadvantage is the diminished precision of these methods.

Cherry (1970) estimated the function of the cilia as a percentage of its initial extension and vigor. This method is simple but very subjective. Schleppy (1975) measured the velocity of an erythrocyte placed on a piece of epithelium of a mouse. This model appproximates reality more than the methods mentioned above, but appeared to be little reproducible in our experiments. Ballenger and Orr (1963) took ciliary tissue from young human subjects under general anaesthesia by gently scraping over the tracheal rings. The shreds of tissue were observed under the microscope. Aggregates of epithelial cells are in a rotating movement caused by the outward directed cilia. The rotation speed can be measured and is dependent on the co-ordination and the force of the beating. The need of sufficient volunteers makes this elegant method not generally applicable. Some characteristics of the different methods are given in table 1. The development of a quick and precise method for direct monitoring the frequency of mucociliary waves would provide a useful tool to investigate medications for the respiratory tract.

We developed a simple and objective technique as a modification of the photoelectric method, that can be employed to study ciliary activity. The method was applied firstly for the determination of the influence of pH and osmotic pressure on ciliary movement.

Table 1. Comparison of several methods to investigate ciliary activity.
Valuation: + positive
0 indifferent
− negative

method	expenses	velocity	simpleness	precision	amount of information	author	species	vitro/vivo
high speed cinem.	0	−	−	+	+	Dalhamn (1955)	rat, guinea pig	vivo
stroboscopy	+	+	+	0	0	Andersen (1971)	rabbit	vitro and vivo
photo-electric	0	+	0	+	+	Mercke (1974)	rabbit	vitro
light scattering	−	+	−	+	+	Lee (1976, 1977)	rabbit (oviduct)	vitro
direct observation	+	+	+	0	−	Galley (1960)	guinea pig	vitro
particle transport	+	+	0	0	+	Schleppy (1975)	mouse	vitro
aggregate rotation	+	−	−	0	+	Ballanger (1963)	human	vitro

MATERIALS AND METHODS

Solutions

Experiments to investigate the effect of difference pH's were performed in solutions, containing:

NaCl	7.72 g
KCl	0.42 g
$CaCl_2 \cdot 2\ H_2O$	0.16 g
Dextrose	1.00 g/1000 ml

The pH was adjusted to 5, 6, 7, 8, 9, 10, 11, with an isotonic HCl solution, or an isotonic NaOH solution. pH was controlled before and after the experiments with a micro-electrode.

Experiments to investigate the effect of osmotic pressure were performed with solutions, containing resp. 0.45%, 0.9% and 1.5% NaCl. A Locke-Ringer (LR) solution was used for a comparison.

LR:	NaCl	7.72 g
	KCl	0.42 g
	$CaCl_2 \cdot 2\ H_2O$	0.16 g
	Dextrose	1.00 g
	$NaHCO_3$	0.15 g/1000 ml (pH adjusted at 7.4).

Tissue preparation

Inseminated chicken eggs (White Leghorns) were incubated for 19 days at 37°C and at an appropriate humidity in a breeding machine. After decapitation of the embryo, the trachea was removed, rinced with Locke-Ringer and sliced in rings, approximately 0.5 mm thick. A second set of experiments was performed with rings of tracheas of Wistar Albino Glaxo rats, sliced as described above.

Procedure

The rings were incubated in Locke-Ringer solution at 25°C for 45 minutes. Between the measurements the rings were stored in petri dishes at 25°C. Before a measurement the ring with a drop of the solution under investigation was transported from the petri dish to a slide with a 0.5 mm deep well. The correct position of the slide under the microscope could be found with the aid of an oscilloscope. Measurements were performed at four rings of one trachea of which one served as a reference. The other three rings were incubated in different test solutions. This procedure was repeated six times with different tracheas.

Microscope

The rings were studied with a Zeiss binocular microscope. The condensor was adjusted to the "Köhler" illimination. The microscope table was connected with a

cryo-thermostate (Colora WK6) to maintain a temperature of 25°C. The microscope was placed on a 350 kg marble slab, which was mounted on shock absorbers. A thermocouple at the place of the tracheal ring demonstrated no variation in temperature during 45 minutes, independent of the power of the illumination. The lamp of the microscope was fed by a stabilized direct current power supply (Eurocart 1035-0680) and was used at full power (6V, 2.5A).

Photo-electric Registration Device.

The device consisted of a phototransistor (BPX25), an amplifier and counter with adjustable trigger level (home made), a dual trace oscilloscope (Jiwatsu SS-5212), a transient recorder (Pauly-DMS-4000) and a T-Y recorder (Goerz RE 511); see fig. 1. On top of the microscope a photocamera was placed. In stead of the film the phototransistor, with a photosensitive surface of 0.64 mm^2, was mounted in the filmcassette. A preamplifier was constructed in the filmcassette to avoid pick up.

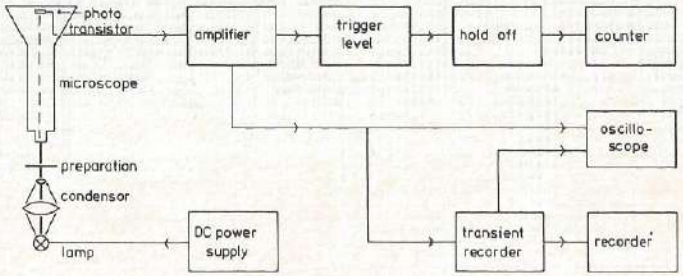

Figure 1. Block diagram of the photo-electric device, including a light source, microscope, phototransistor, amplifier, counter with adjustable trigger level and hold off, oscilloscope, transient recorder and an ink writer.

After amplification the signal was passed to the counter, which counted the times that the trigger level was exceeded. The counter was provided with a hold-off, which blocks the counter, during a time adjustable between 16 and 250 msec. after the trigger level was exceeded. This blocking effect could be visualised on the oscilloscope. The gate of the counter was open during 5 or 10 seconds at choise, the frequency was displayed afterwards for a second before counting starts again. The trigger level was adjusted in a way that peaks, which were three times shorter than the highest peaks, were just counted. When the microscope was focused on an object micrometer, the device was most sensitive to vibrations. In this situation it was checked that no counts were accomplished. The transient recorder made it possible to store a signal. This signal could be transported at a slower rate to the recorder (plotter). The waveform could be displayed flicker free on the oscilloscope, using the roll mode.

RESULTS

Figure 2a, b and c show representative examples of ciliary waves, obtained from the trachea of a chicken embryo. The variation of amplitude appeared to be dependent on the frequency of the ciliary beat. Lower frequencies caused more rapid variation, while very high frequencies caused no variation at all (fig. 2c). The frequencies found for the chicken embryo at pH = 7.4 were 17 to 20 Hz and for the rat 14 to 16 Hz, both at 25°C and during at least 6 hours.

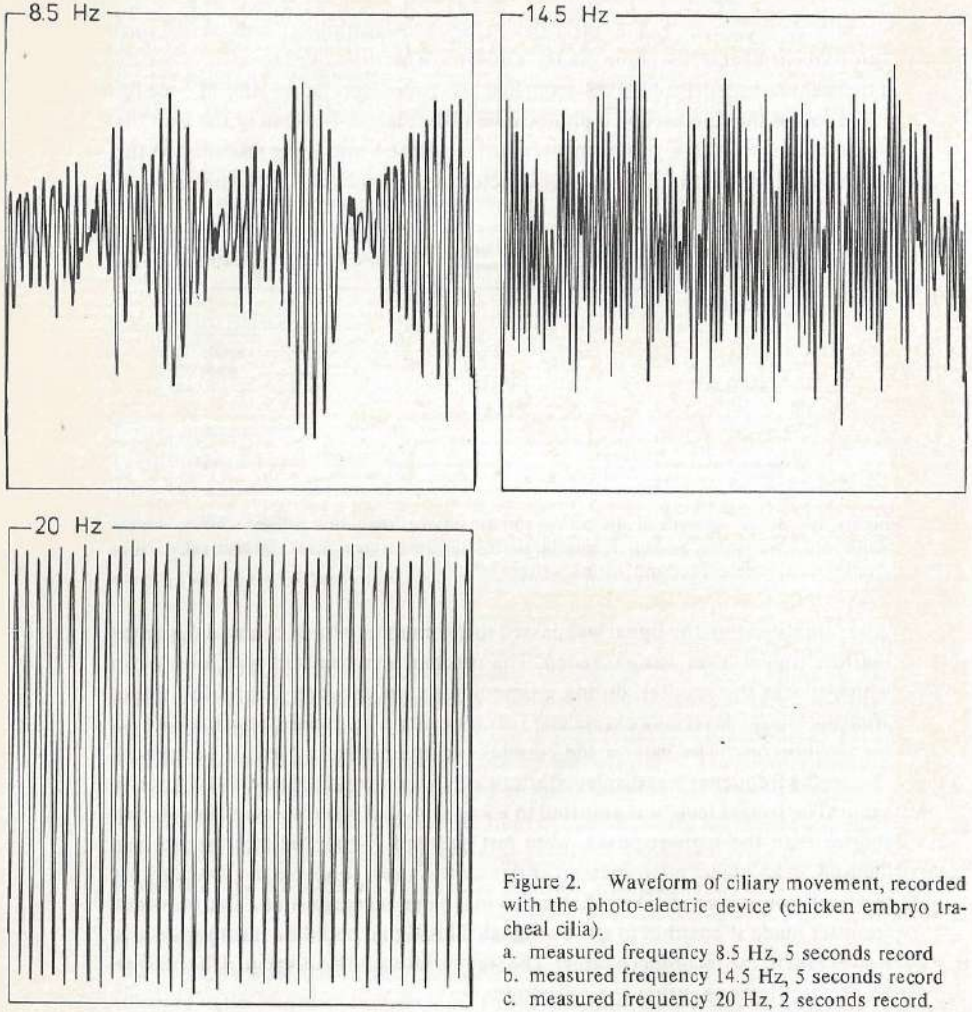

Figure 2. Waveform of ciliary movement, recorded with the photo-electric device (chicken embryo tracheal cilia).
a. measured frequency 8.5 Hz, 5 seconds record
b. measured frequency 14.5 Hz, 5 seconds record
c. measured frequency 20 Hz, 2 seconds record.

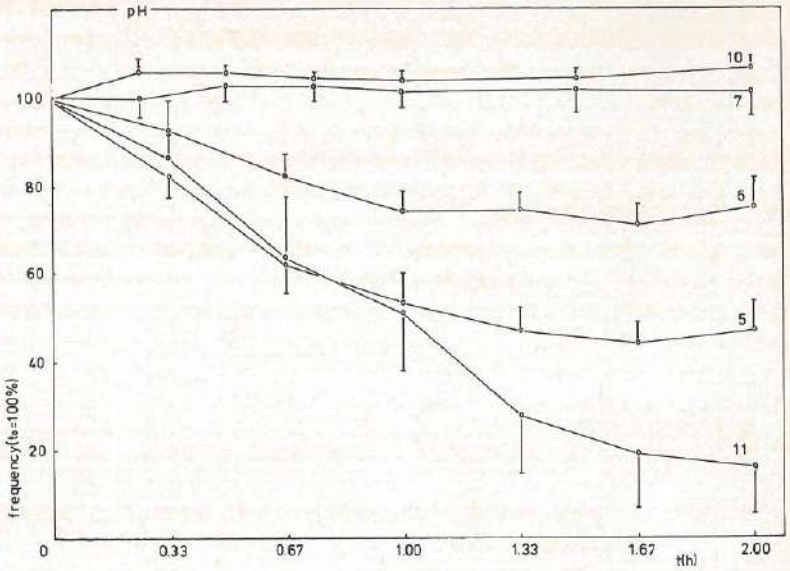

Figure 3. Time versus frequency plot pH = 5, 6, 7, 10 and 11 (chicken embryo tracheal cilia). S.E.M. is indicated.

The influence of the pH is shown in fig. 3. No different effect was found on rat or chicken cilia. The highest ciliary frequency was found from pH = 7 till pH = 10. At pH = 6 the ciliary frequency was decreased about 20%, compared with the frequency at pH = 7, each measured after 0.75 h incubation. Values lower than pH = 6 and higher than or equal to pH = 11 resulted in severe decrease of the ciliary frequency.

Figures 4 and 5 show the results of differences in the NaCl content or osmotic pressure. The isotonic (0.9%) solution shows the best results of the NaCl solutions. Increased and diminished concentrations result in a steeper decay. In the experiments with chicken cilia a concentration of both 0.45% and 1.5% NaCl decreased the frequency about 50% after 1 hour in comparison with the initial frequency. A concentration of 0.9% NaCl decreased the frequency 27% after 1 hour in comparison with the initial frequency. In the experiments with rat cilia a concentration of 0.45% NaCl decreased the frequency 14% and 0.9% NaCl decreased the frequency 5%, both after 1 hour in comparison with the initial frequency. With 1.5% NaCl solution the frequency decreased 38% after 0.75 hour in comparison with the initial frequency.

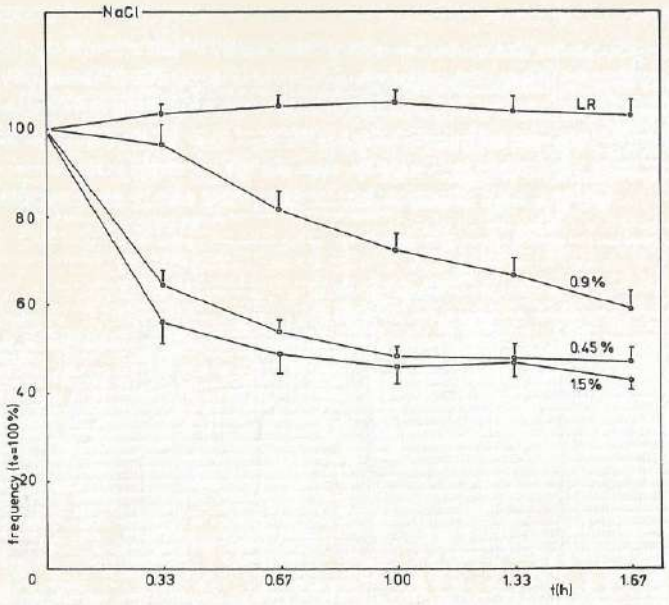

Figure 4. Time versus frequency plot for Locke Ringer, 0.45% NaCl, 0.9% NaCl and 1.5% NaCl solution (chicken embryo tracheal cilia). S.E.M. is indicated.

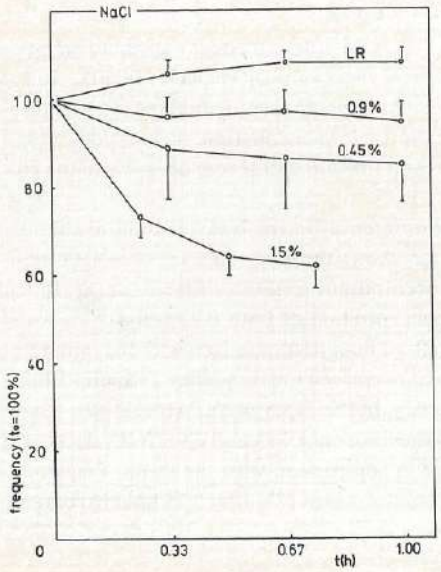

Figure 5. Time versus frequency plot for Locke Ringer, 0.45% NaCl, 0,9% NaCl and 1.5% NaCl solution (rat tracheal cilia). S.E.M. is indicated.

DISCUSSION

One of the questions at the start of this investigation was, which animals could be used. Wanner writes: "The cilia of human (. . .) respiratory epithelia or cilia from lower animals are remarkably similar when compared by transmission and scanning electron microscopy".

So, for practical reasons, we choose the chicken embryo and performed some experiments with rats for comparement.

The number of cilia under investigations differs much from other authors. Mercke et al. (1974) and Yager et al. (1978) observed a larger area and used a different illumination (table 2). Mercke et al. worked with a light beam reflected by the cilia and Yager et al. used light, transmitted through a piece of epithelium. We employed light perpendicular to the cilia. An area of 36 μm^2 was projected on the photosensitive part of the phototransistor. This area contained about 220 cilia (Andersen, 1971) and one row about 15 to 22 cilia.

Table 2. Comparison of several methods to employ a photo-electric registration device.

authors	area	illumination	number of cilia under investigation
Mercke	380 μm^2	reflection	2,400
Yager	314–490 μm^2	transmission through preparation	2,000–3,100
Van de Donk	36 μm^2	transmission through cilia	15–44

The microscope could be focused on one or two rows which corresponded with 15–44 cilia. Our results indicated, that enough cilia were observed to receive a good impression of the co-ordination between several cilia. In Locke Ringer the waveform was regular (fig. 2c), after storage or a contact with drugs which resulted in frequency slowing, the waveform always showed an interference pattern (fig. 2a and b). This interference pattern might be explained by a small difference in the frequencies of neighbouring cilia, due to a lack of co-ordination. The number of ciliary beats, found for the rings in Locke Ringer, was in agreement with the average "normal" frequency for mammals of about 16 Hz (Wanner 1977). The average frequency of chicken cilia was somewhat higher than the average frequency of rat cilia. This might be due to the fact that embryonic tissue is more intact. The pH influence on the frequency is in agreement with the results of Hée and Guillerm (1973) but different from the results of Gallay (1960). Gallay found a very marked decrease of the time, necessary to provoke a cessation of movement for pH smaller than 6.5 or greater than 7.5 (vitro experiments on tracheas of guinea pigs). This might be explained by the fact that Gallay used borate buffers, which are toxic to ciliary epithelium (Grumbach et al., 1965).

The effect of a hypertonic solution on rat cilia is nearly the same as the effect on chicken embryo cilia.

The decrease of the frequency of rat cilia in 0.9% and 0.45% NaCl solutions was less than the decrease of the frequency of chicken cilia in those solutions. An explanation (though not satisfying) of this phenomenon can be, that the tracheal rings of a rat are much bigger than the tracheal rings of a chicken embryo and might give more protection. The frequency of cilia in Locke Ringer had the tendency to increase a little, which was probably due to recovering after the slicing of the trachea.

The effect of the osmotic pressure on chicken cilia is in agreement with the results of Stepper et al. (1965, human mucosa in vitro), but differs from the results of Gallay (1960). Gallay found only a negative effect on the activity with hypotonic NaCl solutions and hardly with hypertonic solutions.

Sumarizing it can be concluded, that our method is adequate for studying the ciliary movement, is easy to handle and very suitable for routine measurements.

RÉSUMÉ

Une méthode de mesure de la fréquence du battement ciliaire in vitro est décrivée. Les variations d'intensité lumineuse, produites par le déplacement des cils, sont détectées à l'aide d'un phototransistor, monté sur un microscope, et la fréquence du battement ciliaire est mesurée immédiatement et le patron d'ondes est montré à l'aide d'un oscilloscope et d'un enregistreur des phénomènes transistoires.

Le degré d'amplification et le mode d'illumination sont choisis de façon qu'environ 30 cils sont projectés sur le phototransistor.

Dans la solution de Locke-Ringer, l'amplitude des signaux enregistrés est très constante. Des interférences de la fréquence ciliaire surviennent sous l'effet de facteurs nocifs et sont dépendantes de la fréquence du battement ciliaire.

L'influence du pH et de la pression osmotique est recherchée au niveau de l'epithélium trachéal du rat et de l'embryon de poulet. La fréquence ciliaire n'est pas influencée par des variations de pH entre 7 et 10; mais elle diminue par des pH inférieurs à 7 ou supérieurs à 10. Des solutions hypertoniques de NaCl aussi bien que des solutions hypotoniques diminuent la fréquence du battement ciliaire de l'embryon de poulet.

ZUSAMMENFASSUNG

Es wird eine Methode zur Messung der trachealen Flimmerfrequenz in vitro beschrieben. Durch die Zilien geführtes Licht wird mit Hilfe eines in einem Mikroskop montierten Fototransistor detektiert. Die Frequenz wird unmittelbar gemessen und zugleich wird die Wellenform in einem Oszilloskop, das an einen Transient Speicher angeschlossen ist, sichtbar. Vergrößerung und Beleuchtungsweise werden so gewählt, dasz etwa 30 Zilien auf dem Fototransistor abgebildet werden. In Locke-Ringer Lösung zeigt die Wellenform eine sehr konstante Amplitude. Durch schädliche Einflüsse auf das Zilienepithel treten von der Frequenz der Zilienbewegung bedingte Interferenzen auf.

In dieser Arbeit wurde der Effekt des pH sowie der Tonizität auf die tracheale Flimmerfrequenz des Hühnerembryos und der Ratte untersucht. Die Frequenz wird zwischen pH = 7 und pH = 10 nicht beeinfluszt, während höhere und niedrigere pH-Werte die Frequenz vermindern. Hypertonische und hypotonische Kochsalzlösungen vermindern in gleichem Masze die Frequenz der Hühnerembryonenzilien, Rattenzilien waren dagegen weniger empfindlich für hypotonische Kochsalzlösungen.

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CHAPTER II

Rhinology, 18, 119–133, 1980

The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas

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SUMMARY

The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas are determined. Polar compounds like benzalkonium chloride in commonly used concentrations, decrease the frequency less than 30% after a 20 minutes' exposure. The effect is not reversible after rinsing with Locke-Ringer solution.
Lipophilic compounds however, like chlorbutol, cause an arrest of the ciliary movement within 10 minutes. The effect, different from the polar compounds, is reversible; but only after a limited exposure-time. Mercuric compounds, like thiomersal, decrease the frequency non-reversibly 30 to 90% after a 20 minutes' exposure. EDTA decreases the frequency 40 to 50%, independent of the exposure-time and in a reversible way.
The combination of benzalkonium chloride 0.01% and EDTA 0.05% is recommended to preserve nasal drops.

INTRODUCTION

Nasal drops should neither be a medium for bacterial growth, nor decrease the mucociliary clearance. Bacterial growth may lead to (1) instability of the dosage form, (2) decrease of the drug concentration and (3) infection in the patient. Therefore, it is important that nasal drops contain preservatives.
The immotile cilia syndrome causes often rhinitis and sinusitis (Afzelius, 1979). Although the effects of a temporary impediment of the ciliary movement are unknown, nasal drops should not disturb substantially the nasal clearance.
The influence of some preservatives on ciliary movement has been investigated by Gallay (1960) and Perrault et al. (1978). Generally it is stated that preservatives can diminish the ciliary movement in the nasal cavity and trachea. Our investigations were performed to study the effects of preservatives on the ciliary beat frequency, with respect to the influence of concentration of the preservative, exposure time and the reversibility of the effects.
From the results of this study a proposition will be made for a preservative which

can be used in nasal drops and which has to be preferred, regarding its effects on mucociliary clearance.

MATERIALS AND METHODS

Pharmacopoeial or analytical grade chemicals were used without further purification.

Most experiments were performed in Locke-Ringer solution (LR) (pH = 7.4). Some experiments were performed in Locke-Ringer solution without $CaCl_2$ (LR-Ca). The ciliary frequency was assessed by a photo-electric registration device (Van de Donk et al., 1980). The effects of each concentration was assessed on six different chicken embryo tracheas.

Tabel 1. Effects of preservatives, in commonly used concentrations, on ciliary movement.

Compound	(1) Freq. t=0.33 h	(2) Freq. t=1 h	(3) $t_{50\%}$ (h)	(4) $t_{0\%}$ (h)	(5) R	Fig.
Lipophilic:						
chlorbutol 0.5%	0%	0%	0.04	0.08	+	1a, 1b
chlorocresol 0.05%	0%	0%	0.01	0.02	+	1c
methyl-p-hydroxybenzoate 0.15%	0%	0%	0.06	0.33	+	1d
propyl-p-hydroxybenzoate 0.02%	0%	0%	0.10	0.33	+	1d
methyl-p-hydroxybenzoate 0.15%+ propyl-p-hydroxybenzoate 0.02%	0%	0%	0.06	0.33	+	1d
Polar:						
benzalkonium chloride 0.01%	100%	65%	1.13	>2	−	2a, 2f
benzalkonium chloride 0.01%+ EDTA 0.05%	75%	58%	1.12	>2	−	2b
benzalkonium chloride 0.01%+ EDTA 0.1%	50%	26%	0.33	1.33	−	2c
domiphen bromide 0.01%	87%	62%	1.12	>2	−	2d, 2f
chlorhexidine gluconate 0.01%	72%	4%	0.48	1.33	−−	2e, 2f
Mercuric:						
thiomersal 0.01%	9%	0%	0.19	0.50	−−	3a, 3c
thiomersal 0.005%	30%	0%	0.24	0.67	−−	3a, 3c
phenylmercuric borate 0.002%	62%	0%	0.47	1.0	−−	3b, 3c
Others:						
EDTA 0.1%	61%	49%	0.86	>2	++	4a, 4c
EDTA 0.025 to 0.1% (in LR-Ca)	62%	58%	>2	>2	++	4b, 4c

(1) Percentage of frequency of the reference after a contact of 20 min.
(2) Percentage of the frequency of the reference after a contact of 1 h.
(3) Exposure time in hours after which the frequency was 50% of the reference frequency.
(4) Exposure time in hours after which motility lacked completely.
(5) Reversibility: ++ complete; + dependent on exposure time; − nihil; −− decreasing effect continues after rinsing with LR.

RESULTS

The effects of preservatives on the frequency of the ciliary beat after a contact of 20 min, respectively 1 h are demonstrated by Table 1. The initial frequency was defined as 100%. The time necessary to diminish the frequency 50%, compared to the reference and the duration of motion are shown as well. In the fifth column an indication about the reversibility of their effects is given. Detailed data are shown in the Figures 1-4. S.E.M. values are indicated by vertical bars.

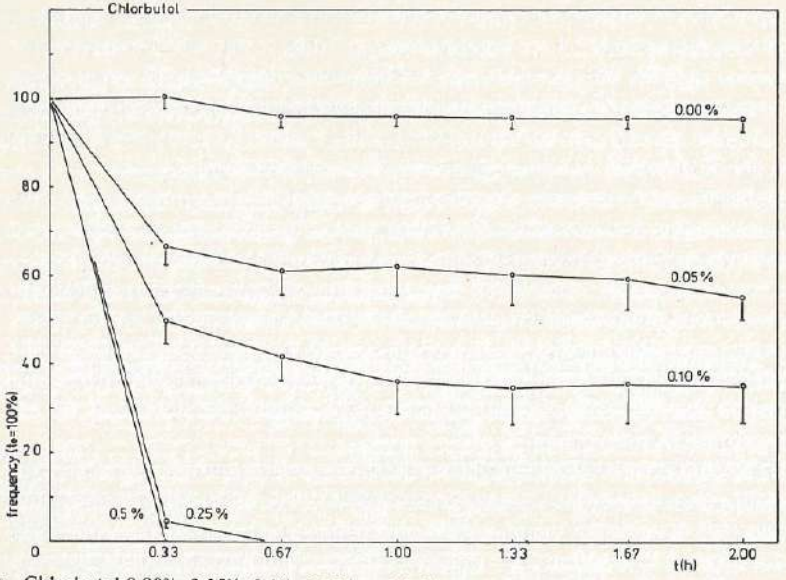
Lipophilic compounds like chlorbutol, chlorocresol and the p-hydroxybenzoates cause a strong decrease of the ciliary frequency. The effects are reversible when the exposure time is limited (Figures 1a–1d). More polar substances like the quaternary ammonium compounds (benzalkonium chloride, domiphen bromide) and chlorhexidine gluconate decrease the frequency more slowly, but the effects are not reversible: the decline in frequency even may continue after rinsing with LR solution (Figures 2a–2f). The mercuric compounds like thiomersal and phenylmercuric borate decrease the frequency severely and the effects are not reversible (Figures 3a–3c).

The last compound disodium edetate (EDTA) is not a preservative but merely an additive to augment the potency of some preservatives (especially benzalkonium chloride). It is a polar compound with a moderate effect on ciliary movement and this effect is highly reversible (Figures 4a–4c). Combined with benzalkonium chloride it accelerates the onset of action on the ciliary movement (Figures 2a–2c) as can be seen after a contact of 20 min.

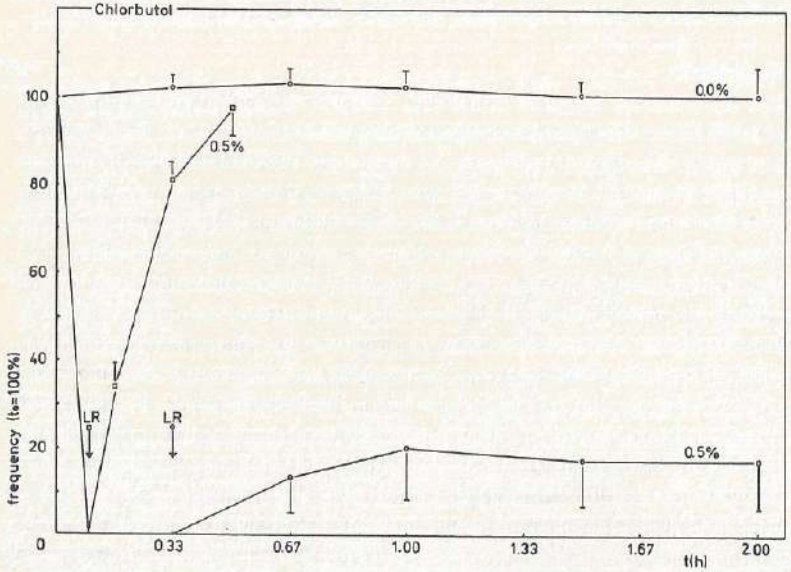
DISCUSSION

From these results it can be concluded, that different effects in relation to the exposure time, of lipophilic and hydrophilic preservatives occur. The lipophilic compounds like chlorbutol (Figure 1b), turned out to provoke a rapid decrease of the ciliary frequency, in normal preservative doses, but the effect was reversible. Quaternary ammonium compounds like benzalkonium chloride and domiphen bromide (Figures 2a and 2d) have a slow onset of their decreasing effect. The effect continues after rinsing with LR (Figure 2f). The lipophilic preservatives act probably by diffusion through the lipophilic membrane of the micro-organism. The reversibility of their effects can be explained by return-diffusion from the cell; a long exposition will damage in a non reversible way (Figure 1b). The quaternary ammonium compounds develop their action by damaging the cell wall of the micro-organism (Richards and Cavill, 1976). The decreasing effect on the ciliary frequency by these compounds will not be reversed since the cell membranes are damaged and the process of cell degeneration will continue (Figure 2f). The effects of benzalkonium chloride and domiphen bromide are quite comparable. Chlorhexidine gluconate decreases the frequency more than the quaternary ammonium compounds (Figure 2e). Both concentrations of phenylmercuric bo-

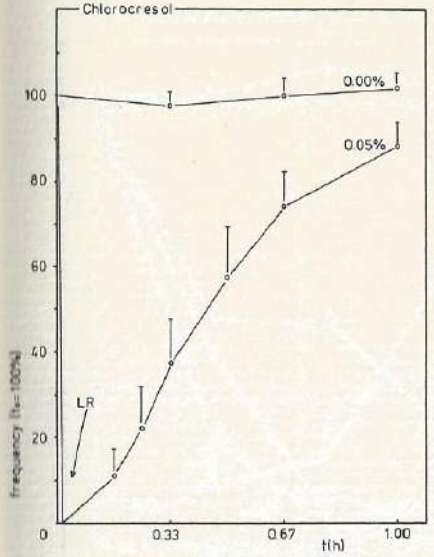
Figure 1. Time versus frequency plot.



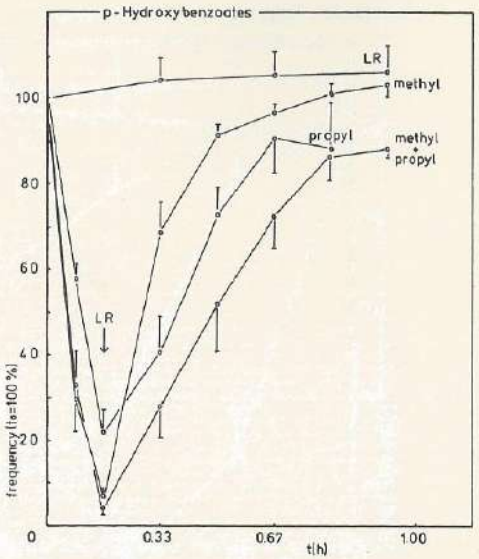
a. Chlorbutol 0.00%, 0.05%, 0.1%, 0.25% and 0.5%.



b. Chlorbutol 0.00%, 0.5% washed after 5 min. and 0.5% washed after 20 min. with LR.



c. Chlorocresol 0.00% and 0.05% washed after 1 min. with LR.

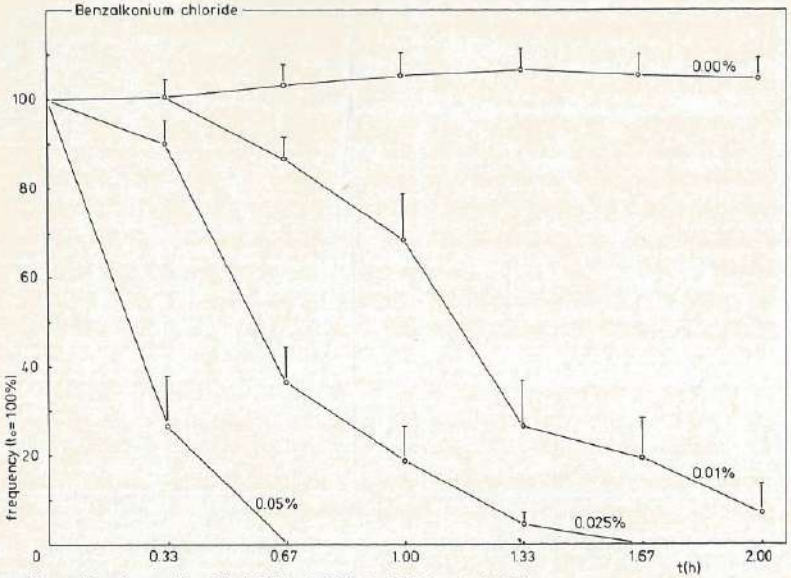


d. Methyl-p-hydroxybenzoate 0.15%, propyl-p-hydroxybenzoate 0.02%, methyl-p-hydroxybenzoate 0.15%+propyl-p-hydroxybenzoate 0.02%, all washed after 10 min. with LR, and as a reference LR.

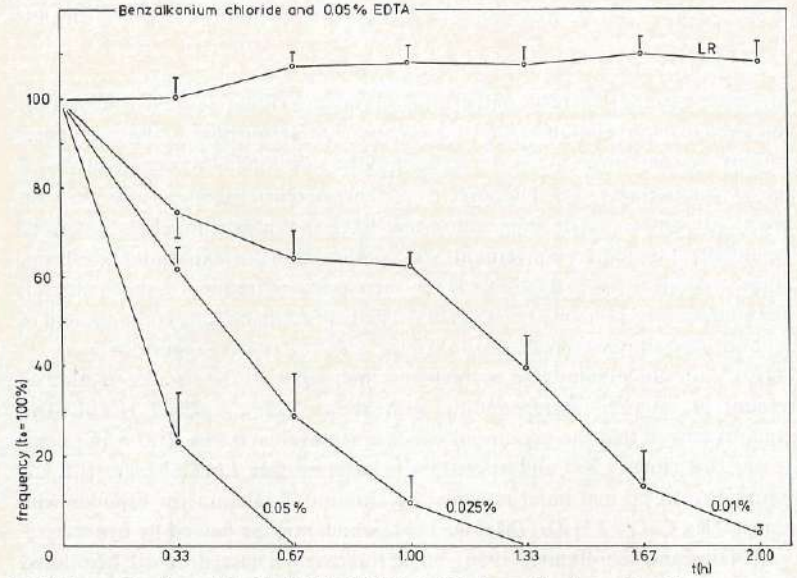
rate cause a similar decrease of the ciliary frequency (Figure 3b). So there is little objection to use 0.004% instead of 0.002% phenylmercuric borate. Higher concentrations are sometimes preferred because of the sorption of this preservative into many packing materials. The effect of phenylmercuric borate is less than the effect of thiomersal. The expectation that EDTA, as a calcium chelating agent, should affect the ciliary movement, was confirmed in our experiments. Normal concentrations (0.1%, 0.05%) of EDTA decreased the frequency approximately 50% after a contact of 1 h. The effect of 0.05% EDTA can be fully compensated by doubling the amount of calcium to 0.32 g $CaCl_2 \cdot 2\ H_2O$/l (Figure 4c).

This is quite understandable by realising that 0.05% EDTA is able to bind an amount of calcium, corresponding with about 0.22 g $CaCl_2 \cdot 2\ H_2O$/l. This explains as well that the maximum effect is achieved at 0.05% EDTA in Locke-Ringer (LR, Figure 4a) and at 0.025% in calcium free Locke-Ringer (LR-Ca, Figure 4b). In normal nasal mucous the amount of calcium corresponds with almost 0.8 g $CaCl_2 \cdot 2\ H_2O$/l (Melon, 1967) which may be halved by hypersecretion (Melon and Schoffeniels, 1966), but in this case the nasal drop will be diluted to a higher degree. The different effects of rinsing with LR-Ca (no effect) and LR

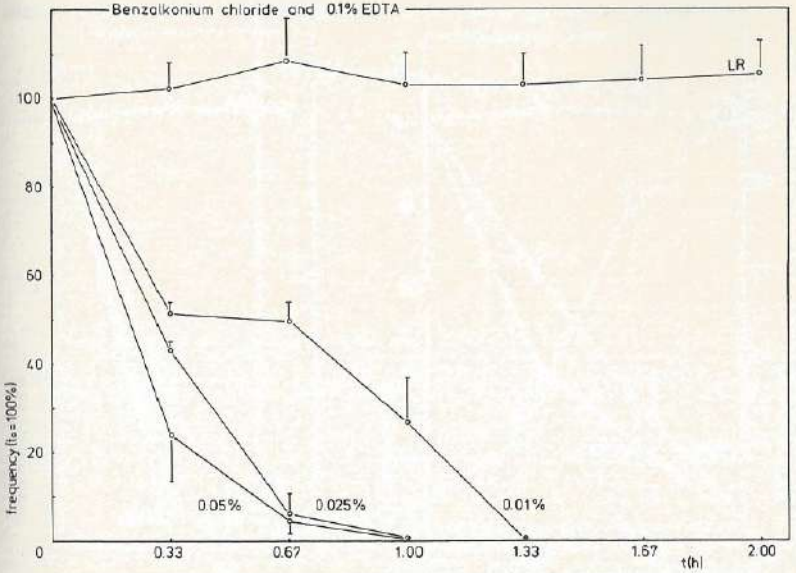
Figure 2. Time versus frequency plot.



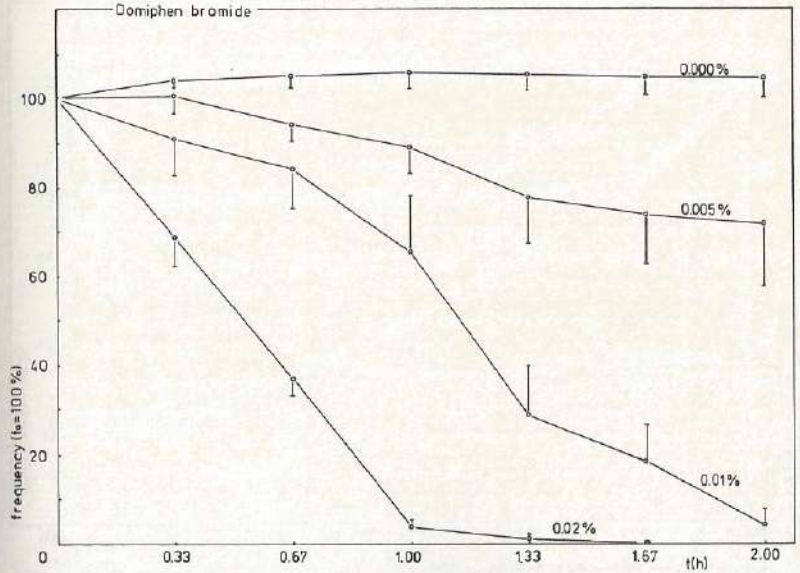
a. Benzalkonium chloride 0.00%, 0.01%, 0.025% and 0.05%.



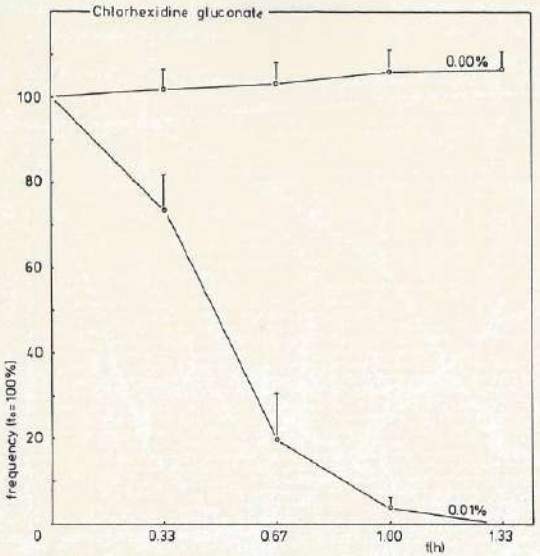
b. LR, benzalkonium chloride 0.01%+EDTA 0.05%, benzalkonium chloride 0.025%+EDTA 0.05% and benzalkonium chloride 0.05%+EDTA 0.05%.



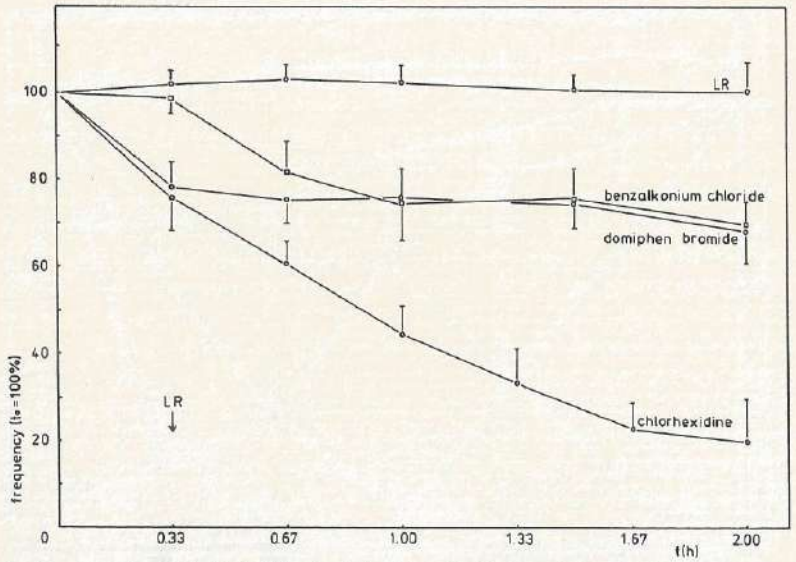
c. LR, benzalkonium chloride 0.01%+EDTA 0.1%, benzalkonium chloride 0.025%+EDTA 0.1% and benzalkonium chloride 0.05%+EDTA 0.1%.



d. Domiphen bromide 0.000%, 0.005%, 0.01% and 0.02%.

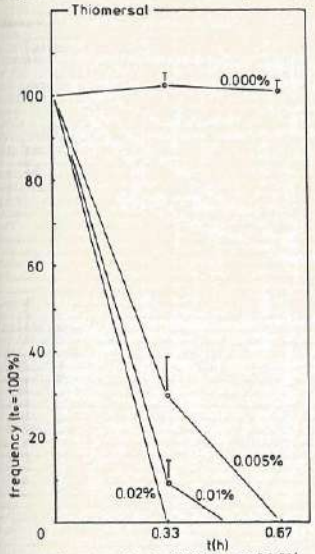


e. Chlorhexidine gluconate 0.00%, and 0.01%.

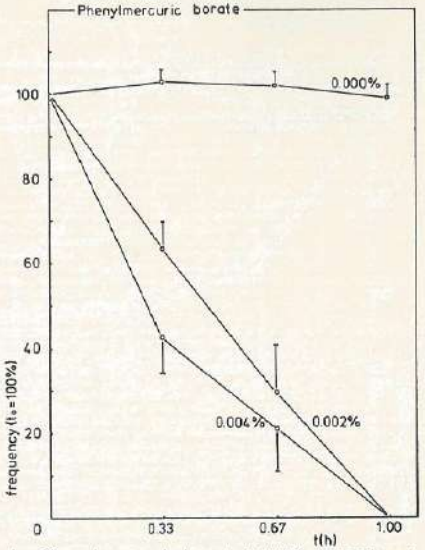


f. Benzalkonium chloride 0.01%, domiphen bromide 0.01% and chlorhedixine gluconate 0.01%, all washed after 20 min. with LR, and as a reference LR.

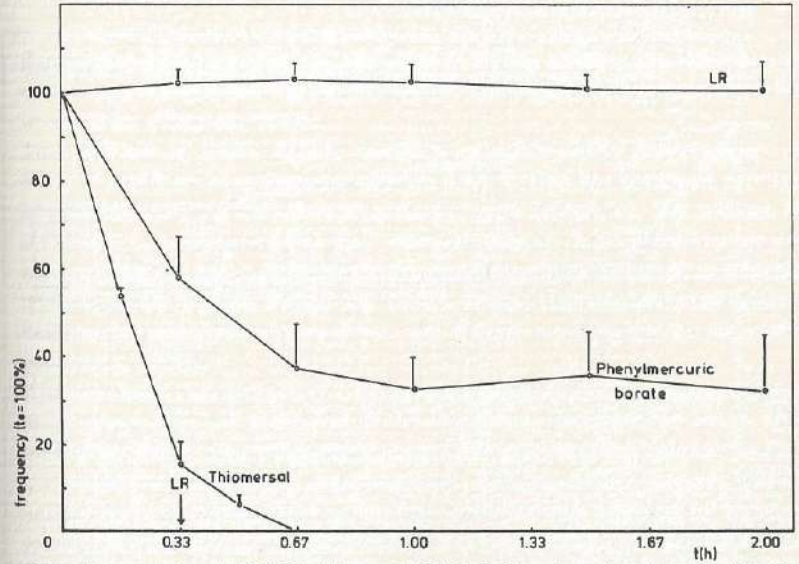
Figure 3. Time versus frequency plot.



a. Thiomersal 0.000%, 0.005%, 0.01% and 0.02%.

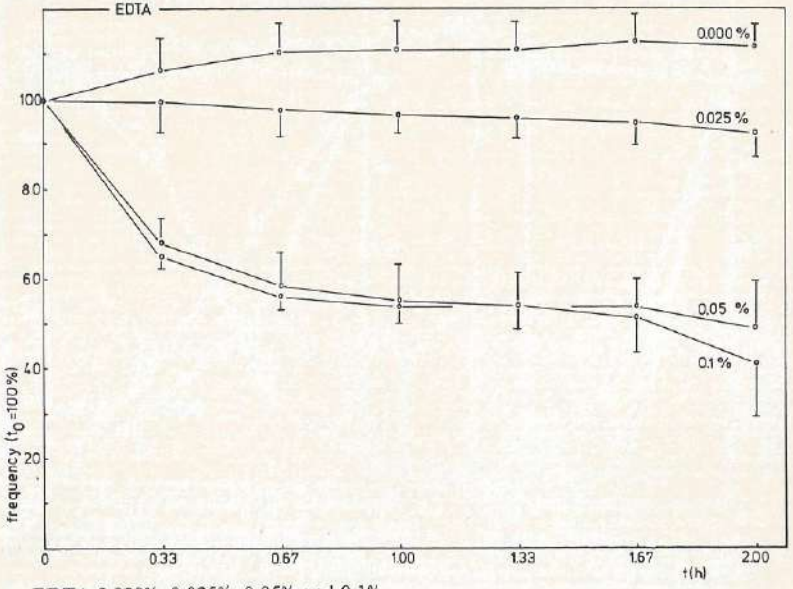


b. Phenylmercuric borate 0.000%, 0.002% and 0.004%.

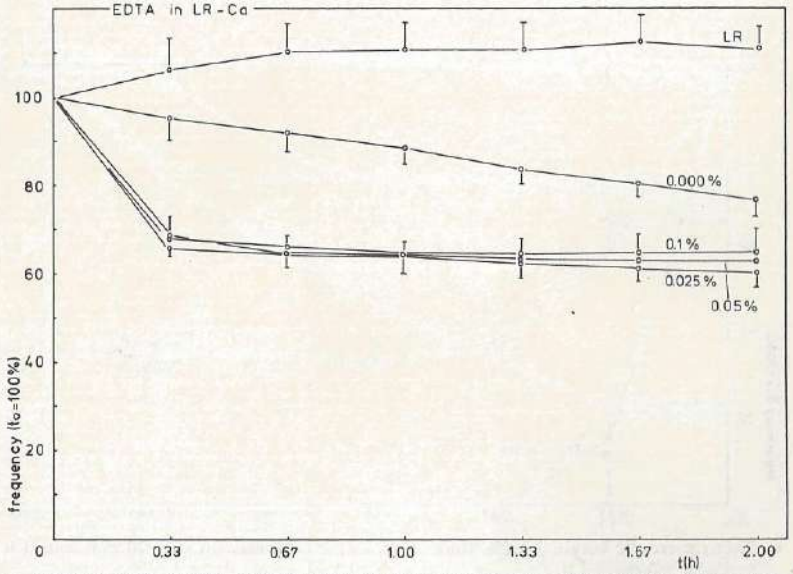


c. Phenylmercuric borate 0.002%, thiomersal 0.01%, both washed after 20 min. with LR and as a reference LR.

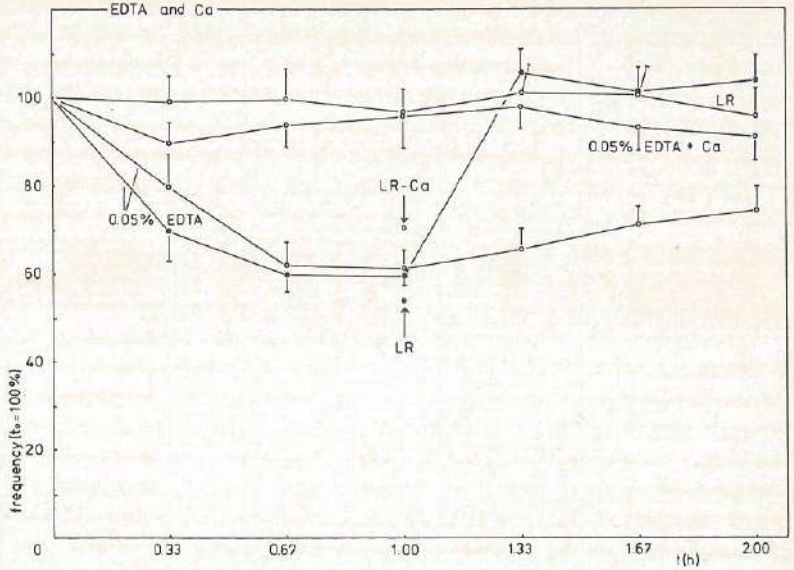
Figure 4. Time versus frequency plot.



a. EDTA 0.000%, 0.025%, 0.05% and 0.1%.



b. EDTA 0.000%, 0.025%, 0.05% and 0.1%, all in calcium free LR, and as a reference LR.



c. EDTA 0.05%+0.16 g $CaCl_2 \cdot 2\,H_2O$/l, EDTA 0.05% washed after 1 h with calcium free LR (o), EDTA 0.05% washed after 1 h with LR (•), and as a reference LR.

(restoration of the activity) and the effect of adding calcium (no inhibition of movement) indicate that the calcium binding capacity of EDTA is responsible for its effect on ciliary movement.

The addition of 0.05% EDTA, resulting in a free Ca^{2+} concentration of 54.10^{-12} mol/l, to benzalkonium chloride (0.01–0.05%) has hardly an additive effect in diminishing the ciliary frequency (Figure 2a and 2b). The addition of 0.1% EDTA, resulting in a free Ca^{2+} concentration of 12.10^{-12} mol/l to benzalkonium chloride, has an additive effect especially on low benzalkonium chloride concentrations (Figures 2a and 2c). EDTA favours the penetration of benzalkonium chloride into the layers of the cells of Gram-negative bacteria (Richards and Cavill, 1976).

It seems likely, that the ciliated cells are fairly accessible for quaternary ammonium compounds, but in the case of low concentrations the effects will be augmented by EDTA. This will result in a small difference in effect between the several benzalkonium chloride concentrations. Richards (1971) proposes to add 0.05% EDTA to antibacterial agents like benzalkonium chloride, in order to increase the germ-killing capacity. Table 2 shows the results of other authors. The results of Greenwood et al. (1946) are in contradiction with the results of Gallay (1960) but in agreement with the results reported in this paper. Gallay found that

Table 2. Comparison of the results from other "in vitro" studies.

Author	Compound	Specimen	Activity	Reversibility (1)
Greenwood, G. et al. (1946)	benzalkonium chloride 0.1%, NaCl 0.9%	rabbit nose	+ after one hour	
	benzalkonium chloride 0.05%, NaCl 0.9%	rabbit nose	+ after one hour	
Gallay, C. (1960)	methyl-p-hydroxybenzoate 0.1%, pH = 7, glucose 5%	guinea pig trachea	0 after 4 minutes	
	benzalkonium chloride 0.025%, pH = 6.7., glucose 5%	guinea pig trachea	0 after 5 minutes	
	benzalkonium chloride 0.0125%, pH = 6.7, glucose 5%	guinea pig trachea	0 after 13 minutes	
	benzalkonium chloride 0.0125%, pH = 7, glucose 5%	guinea pig trachea	0 after 45 minutes	
	phenylmercuric borate 0.025%, pH = 7, glucose 5%	guinea pig trachea	0 after 45 minutes	
Perrault, G. et al. (1978)	chlorbutol 0.5%, pH = 7.7	guinea pig trachea	0 after 20 minutes	+
	methyl-p-hydroxybenzoate 0.05%, pH = 7.7	guinea pig trachea	0 after 20 minutes	+
	methyl-p-hydroxybenzoate 0.025% + propyl-hydroxybenzoate 0.015%, pH = 7.7	guinea pig trachea	0 after 20 minutes	+
	thiomersal 0.004%, pH = 7.7	guinea pig trachea	0 after 20 minutes	0
Mostow, S. R. et al. (1979)	methyl-p-hydroxybenzoate 0.2%, pH = 6.5–7	ferret trachea	0 after one hour	0
	methyl-p-hydroxybenzoate 0.1%, pH = 6.5–7	ferret trachea	0 after one hour	+

(1) + reversible, 0 not reversible.

a modest lowering of the pH of the benzalkonium chloride solution reduces the duration of ciliary movement of a guinea pig trachea quite severely. Greenwood et al. do not give details about the pH of the benzalkonium chloride solution they used, but the pH must have been smaller than 6 as benzalkonium chloride is a salt with a slight acidic reaction. We have found indications for the concept that a limited lowering of the pH of the benzalkonium chloride solution (from pH = 7.4 to pH = 6) reduces the duration of movement moderately (from 1.33 to 1 h). The results of Perrault et al. (1978) and Mostow et al. (1979) are in agreement with our results.

CONCLUSIONS

Within the group of the lipophilic compounds only small differences occur with respect to the onset, speed and reversibility of their effects on ciliary movement. This can be stated with regard to the polar compounds as well. The choice between these groups is dependent on many other factors e.g.: compatibility with other substances, pH and interference with the packing materials. The present knowledge of nasal physiology does not give a clear indication whether a slow decrease of the ciliary frequency or a rapid reversibility is of major importance. The effect of EDTA can be neglected in both physiological and pathological conditions in the human nose as a result of the high calcium concentration "in situ". The effects of preservatives in the human nose will be less dramatic than in an "in vitro" situation, because of the dilution by the nasal mucus and elimination by the nasal clearance. The latter however, will be of a smaller extension for the lipophilic compounds as they diminish the ciliary movement very rapidly.
Until more information is available regarding the influence of nasal medications in different pathological situations, we would prefer 0.01% benzalkonium chloride with 0.05% EDTA. It is a potent preservative and has only moderate influence on the cilia.

RÉSUMÉ

Les effets de conservateurs sur la fréquence du battement ciliaire de l'embryon de poulet sont déterminés.
Des substances polaires, comme le chlorure de benzalkonium, aux concentrations usuelles, diminuent la fréquence avec moins que 30% après une exposition de 20 minutes.
L'effet n'est pas réversible après rinçage avec une solution de Locke-Ringer. Des substances lipophiles par contre, comme le chlorobutanol, produisent un arrêt du mouvement ciliaire en moins que 10 minutes. L'effet est réversible, autrement qu'avec les substances polaires, mais seulement après un temps d'exposition limiteé.
Des composés de mercure, comme mercurothiolate, diminuent la fréquence avec

30 à 90% après une exposition de 20 minutes, de façon irréversible.
L'édétate de sodium diminue la fréquence avec 40 à 50%, dans l'indépendence du temps d'exposition et de façon réversible. La combinaison de chlorure de benzalkonium 0,01% et d'édétate de sodium 0,05% est recommandée pour la conservation des gouttes nasales.

ZUSAMMENFASSUNG

Es wird die Wirkung von Konservierungsmitteln auf die tracheale Flimmerfrequenz des Hühnerembryos ermitteld.
Poläre Stoffe, wie Benzalkoniumchlorid, in den gebräuchlichen Konzentrationen, vermindern nach einer Einwirkungsdauer von 20 Minuten, die Frequenz um weniger als 30%. Diese Wirkung ist nicht durch Waschen mit Locke-Ringer-Lösung aufzuheben. Fettlösliche Stoffe jedoch, wie Chlorbutanol, verursachen innerhalb von 10 Minuten einen Stillstand der Flimmerbewegung. Diese Reaktion kann, anders als bei den polären Verbindungen, nach einer kurzen Einwirkungszeit, rückgänging gemacht werden.
Quecksilberverbindungen, wie Thiomersal, vermindern, nach einer Einwirkungsdauer von 20 Minuten, die Frequenz um 30 bis 90%. In diesem Fall kann die Wirkung nicht rückgängig gemacht werden.
EDTA verursacht eine Verminderung der Frequenz von 40 bis 50%, die unabhängig von der Einwirkungsdauer und leicht rückgängig zu machen ist.
Für die Konservierung von Nasentropfen ist die Kombination Benzalkoniumchlorid 0,01% und EDTA 0,05% zu empfehlen.

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The effects of nasal drops on the ciliary beat frequency of chicken embryo tracheas

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SUMMARY

The effects of proprietary preparations in the Netherlands and nasal preparations according to the F.N.A. (Formulary of the Netherlands' Pharmacists Association) on the ciliary beat frequency of chicken embryo tracheas have been determined. In general the preservatives, used in the nasal drops, turned out to have a more decisive influence on the ciliary motion than the pharmacologically active constituents.

The average time, necessary to decrease the frequency 50% with 1 = 5 diluted nasal drops (average $t_{50\%(1=5)}$) containing a mercury compound or chlorbutol, is 0.4 h. Quaternary ammonium compounds containing preparations, however, have less influence, average $t_{50\%(1=5)} > 1.22$ h. They are to be used preferably.

Nasal drops, used as decongestants, provided that they are preserved with quaternary ammonium compounds, have little influence on the ciliary motion (average $t_{50\%(1=5)} > 1.38$ h).

Nasal drops containing antihistamines, in contrast, inhibit the ciliary motion largely and almost independently of the preservatives (average $t_{50\%(1=5)} = 0.22$ h); the cromoglycate containing preparation, on the contrary, has only little effect in this respect ($t_{50\%(1=5)} > 2$ h).

Antimicrobial preparations are not preserved and have an intermediate ciliotoxic effect (average $t_{50\%(1=5)} = 0.7$ h).

INTRODUCTION

The extensive use of nasal drops and sprays is explained by the frequent occurence of nasal diseases, like the common cold. These preparations are easily available as "over the counter" drugs in most countries.

In the nasal cavity a mucociliary structure exists to remove particles, microorganisms, viruses and allergens.

Wanner (1977) called this capability "the nasal clearance". Most nasal medications influence the nasal clearance in a negative way. Dudley and Cherry (1978)

investigated the effects of dilutions of 10 proprietary preparations, registered in the U.S.A., on the ciliary movement after a contact of 5 minutes, three times a day. All the nasal drops showed, to some degree, ciliotoxicity, but the damaging component was not mentioned. Perrault et al. (1978) investigated some preparations containing preservatives like chlorbutol, thiomersal and p.hydroxybenzoates. They did not study quaternary ammonium compounds. So, the available literature does not offer a comprehensive survey of the ciliotoxic effects of nasal preparations and their common components.

In a former publication (Van de Donk et al., 1980b) we described the effects of preservatives on ciliary movement. In this study we want to elucidate the effects of proprietary preparations in the Netherlands and nasal preparations according to the F.N.A. (F.N.A. preparations) on ciliary movement.

The F.N.A. is the formulary of the Netherlands' Pharmacists Association.

Table I. Nasal preparations

names	lot-numbers	manufacturers
Argyrophedrine®	1490	Aron
Rhinamide®	776 81	Bailly
Rhinogutt®	78J25	Boehringer Ingelheim
Fenox®	3X	Boots
Chloramphenicol®	78K16	Bournonville-Pharma
Chloransulfa®	78C22	Bournonville-Pharma
Antistine-Privine®	78F02AF	Ciba
Otricorten®	78B27HH	Ciba
Otrivin® 0.1%	80A11AB	Ciba
Otrivin® 0.05%	80A10AB	Ciba
Privine® 0.1%	77H30AK	Ciba
Privine® 0.05%	747L	Ciba
Lomusol®	78K08	Fisons
Nasivin® 0.05%	78A10M205 1430	Merck
Nasivin® 0.025%	80B15U074 4362	Merck
Codelsol®	79A08NC0656	Merck, Sharp & Dohme
Rhinofral®	31489	Philips-Duphar
Sinex®	9E	Richardson-Merrell
Endrine®	78J06	Wyeth
Rhinoguttae antazolini 0.5%		FNA
Rh. antazolini et naphazolini		FNA
Rh. argenti nucleinici comp.		FNA
Rh. ephedrini 1%		FNA
Rh. ephedrini comp.		FNA
Rh. naphazolini 0.05%		FNA
Rh. phenylephrini 0.025%		FNA
Rh. xylometazolini 0.1%		FNA
Rh. xylometazolini 0.05%		FNA
Rh. xylometazolini 0.025%		FNA

MATERIALS AND METHODS

Names, lot-numbers and manufacturers' names of the investigated proprietary preparations are given in Table I. Some experiments were performed in the pure preparations, other experiments in dilutions: 20% of the preparation in Locke-Ringer solution. The ciliary beat frequency was assessed by a photo-electric registration device (Van de Donk et al., 1980a). The effects of diluted preparations and the effects of the reference (Locke-Ringer) were determined on six different chicken embryo tracheas. The effects of the pure preparations were determined on four different tracheas.

The concentrations of the quarternary ammonium compounds in the nasal preparations were determined with high performance liquid chromatography (HPLC), according to the method of Meyer (1980). Although with this method each homolog can be separated, concentrations of the benzalkonium homologs were listed as a total only.

The mercury concentrations were determined with atomic absorption spectrophotometry (AAS) after destruction with nitric acid.

RESULTS

Assays

Table II lists the concentrations of benzalkonium chloride, determined with the United States Pharmacopeia reference as a standard, and the concentrations of benzalkonium chloride as claimed by the manufacturer.

The table includes the contents of two commercially available brands of benzalkonium chloride. All assays resulted in roughly three quarters of the concentrations as claimed by the manufacturer. The assays of mercury and domiphen bromide (compared to domiphen bromide Lot 7002121, Ciba-Geigy) and all benzalkonium chloride concentrations are summarized in table III.

Table II. Concentrations of benzalkonium chloride in some nasal drops

	Benzalkonium chloride assayed	Benzalkonium chloride as claimed by the manufacturer
Rhinogutt®	0.0154%	0.02%
Lomusol®	0.0087%	0.01%
Nasivin® 0.05%	0.0074%	0.01%
Nasivin® 0.025%	0.0073%	0.01%
Sinex®	0.015%	-
Benzalkonium chloride[1]	0.006%	0.01%
Benzalkonium chloride[2]	0.009%	0.01%

[1] Used by Van de Donk et al., 1980b and in all the FNA preparations.
[2] Merck 9159612.

Tabel III. Composition of nasal drops

preparation	composition: preservative[1]	drug
Nasivin® 0.05%	benzalkonium chloride 0.0074%[2]	oxymetazoline HCl 0.05%
Nasivin® 0.025%	benzalkonium chloride 0.0073%[2]	oxymetazoline HCl 0.025%
Rhinogutt®	benzalkonium chloride 0.0154%[2]	tramazoline HCl 0.117%
Sinex®	benzalkonium chloride 0.015%[2]	oxymetazoline HCl 0.05%; menthol 0.025%; camphor 0.015%; eucalyptol 0.0075%
Lomusol®	benzalkonium chloride 0.0087%[2]	sodium cromoglycate 2%
Rhinoguttae antazolini 0.5%	benzalkonium chloride 0.006%; EDTA 0.1%	antazoline HCl 0.5%
Rh. antazolini et naphazolini	benzalkonium chloride 0.006%; EDTA 0.1%	antazoline HCl 0.5%; naphazoline nitrate 0.025%
Rh. ephedrini 1%	benzalkonium chloride 0.006%; EDTA 0.1%	ephedrine HCl 1%
Rh. ephedrini comp.	benzalkonium chloride 0.006%; EDTA 0.1%	ephedrine HCl 1%; menthol 0.015%; camphor 0.015%; eucalyptol 0.1%
Rh. naphazolini 0.05%	benzalkonium chloride 0.006%; EDTA 0.1%	naphazoline nitrate 0.05%
Rh. phenylephrini 0.25%	benzalkonium chloride 0.006%; EDTA 0.1%	phenylephrine HCl 0.25%
Rh. xylometazolini 0.1%	benzalkonium chloride 0.006%; EDTA 0.1%	xylometazoline HCl 0.1%
Rh. xylometazolini 0.05%	benzalkonium chloride 0.006%; EDTA 0.1%	xylometazoline HCl 0.05%
Rh. xylometazolini 0.025%	benzalkonium chloride 0.006%; EDTA 0.1%	xylometazoline HCl 0.025%
Endrine®	chlorbutol 0.25%	ephedrine HCl 0.5%; eucalyptol; menthol and camphor
Fenox®	chlorbutol 0.5%; cetrimide 0.02%	phenylephrine HCl 0.5%; menthol 0.025%; eucalyptol 0.025%
Otrivin® 0.1%	domiphen bromide 0.008%[2] a mercuric compound: 9.6 µg Hg/ml[3]	xylometazoline HCl 0.1%
Otrivin® 0.05%	domiphen bromide 0.008%[2] a mercuric compound: 9.6 µg Hg/ml[3]	xylometazoline HCl 0.05%
Privine® 0.1%	a mercuric compound: 7.0 µg Hg/ml[3]	naphazoline nitrate 0.1%
Privine®0.05%	a mercuric compound: 9.2 µg Hg/ml[3]	naphazoline nitrate 0.05%
Otricorten®	a mercuric compound: 10.0 µg Hg/ml[3]	xylometazoline HCl 0.05%; dexamethazone 0.01%
Antistine-Privine®	a mercuric compound: 8.0 µg Hg/ml[3]	naphazoline nitrate 0.02%; antazoline sulphate 0.5%
Codelsol®	phenylmercuric acetate 0.004%	prednisolone phosphate 0.1%; neomycin 0.35%; phenylephrine HCl 0.25%; phenylpropanolamine HCl 0.75%
Rhinofral®	aminacrine HCl 0.1%	naphazoline HCl 0.05%
Rhinamide®	benzalkonium chloride 0.0015%; benzoic acid 0.1%	sulphanilamide 0.4%; ephedrine HCl 1%; butacaine sulphate 0.03%
Argyrophedrine®	none	mild silver protein 0.5%; ephedrine laevulate 1%
Chloramphenicol®	none	chloramphenicol 0.4%
Chloransulfa®	none	chloramphenicol 0.5%; sulphacetamide 10%
Rhinoguttae argenti nucleinici comp.	none	mild silver protein 0.5%; ephedrine sulphate 1%

[1] Preservative content as claimed by the manufacturer with the exception of [2] and [3]
[2] Assayed with HPLC.
[3] Assayed with AAS.

Table IV. Effects of nasal drops on ciliary activity

preservative	preparation	activity: undiluted $t_{0\%}$(h)[1]	undiluted pH	diluted (1=5) freq.[2] t=0.33 h	freq.[3] t=1 h	freq.[4] t=2 h	$t_{50\%}$(h)[5]	$t_{0\%}$(h)[1]	pH	figure
benzalkonium chloride	Nasivin® 0.05%	0.58	6.8	95	92	59	>2	>2	6.9	3
	Nasivin® 0.025%	0.72	7.0	98	96	57	>2	>2	7.2	3
	Rhinogutt®	0.65	6.2	98	76	28	1.59	>2	6.6	3
	Sinex®	0.30	6.8	47	11	0	0.31	1.67	6.9	3
	Lomusol®	0.68	5.4	100	92	83	>2	>2	7.7	6
	Rhinoguttae antazolini 0.5%	0.43	4.7	20	0	0	0.21	0.67	7.2	6
	Rh. antazolini et naphazolini	0.37	4.8	12	0	0	0.19	0.67	7.2	6
	Rh. ephedrini 1%	0.99	4.7	82	45	10	0.90	>2	7.7	1
	Rh. ephedrini comp.	0.27	4.7	39	4	0	0.27	1.33	7.3	1
	Rh. naphazolini 0.05%	0.82	4.7	88	62	29	1.18	>2	7.2	4
	Rh. phenylephrini 0.25%	1.51	4.7	103	75	45	1.17	>2	7.2	1
	Rh. xylometazolini 0.1%	0.83	6.0	97	73	22	1.45	>2	7.1	2
	Rh. xylometazolini 0.05%	0.98	6.0	90	86	38	1.74	>2	7.1	2
	Rh. xylometazolini 0.025%	0.96	6.0	94	94	55	2	>2	7.1	2
chlorbutol	Endrine®	0.13	5.0	45	7	0	0.30	2	7.7	1
	Fenox®	0.07	5.2	31	5	0	0.24	1.33	7.6	1
mercury containing preservatives	Otrivin® 0.1%	0.25	6.4	60	29	0	0.54	2	6.9	2
	Otrivin® 0.05%	0.28	6.4	75	41	6	0.78	>2	6.9	2
	Privine® 0.1%	0.12	5.0	53	15	0	0.38	2	7.1	4
	Privine® 0.05%	0.13	4.9	50	24	0	0.33	1.67	7.4	4
	Otricorten®	0.07	5.0	39	0	0	0.27	1	5.3	6
	Antistine-Privine®	0.05	5.0	35	0	0	0.25	1	7.4	6
	Codelsol®	0.52	6.1	59	33	18	0.52	>2	7.3	6
others	Rhinofral®	0.13	6.5	33	0	0	0.25	1	7.3	4
	Rhinamide®	0.80	6.8	43	14	5	0.30	>2	7.7	5
none	Argyrophedrine®	0.22	8.1	85	50	0	1	2	8.0	5
	Chloramphenicol®	0.18	7.2	50	21	12	0.33	>2	7.6	5
	Chloransulfa®	0.01	7.7	67	20	0	0.54	2	7.7	5
	Rhinoguttae argenti nucleinici comp.	0.30	7.7	64	47	24	0.91	>2	7.8	5

[1] $t_{0\%}$(h) = time necessary to arrest ciliary movement in hours.
[2] freq. t=0.33 h = frequency of ciliary movement after a contact of 20 min.
[3] freq. t=1 h = frequency of ciliary movement after a contact of 1 h.
[4] freq. t=2 h = frequency of ciliary movement after a contact of 2 h.
[5] $t_{50\%}$(h) = time necessary to decrease the frequency of ciliary movement with 50% in hours.

Ciliary movement

The effects of some proprietary preparations and F.N.A. preparations on the ciliary beat frequency are demonstrated in Table IV.

The preparations are ranged according to their preservatives. Those containing more than one preservative are ranged according to the preservative with the strongest effect on ciliary movement, as published by Van de Donk et al. (1980b). Thiomersal and other mercury compounds appeared to have the strongest effect.

The first column shows the duration of movement in the case of undiluted preparations (mean of 4 experiments).

The second column gives the pH of these preparations.

The next columns reveal the effects of the preparations diluted with Locke-Ringer (1 = 5): the activity after a contact of 20 min, 1 h and 2 h.

The sixth column shows the time necessary to decrease the frequency 50% compared to the initial frequency, and the last two columns the duration of movement and the pH after the measurements.

The Figures 1–6 show the effects in detail. The S.E.M. values are indicated by vertical bars. The preparations are grouped, according to their indications.

Figure 1 shows the effect of preparations with short acting decongestants: ephe-

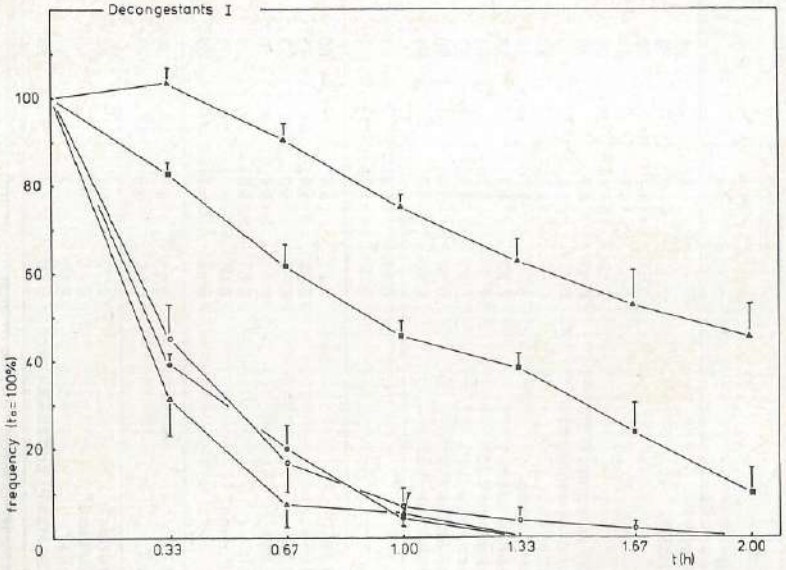

Figure 1. Time versus frequency plot for Rhinoguttae phenylephrini (FNA) 0.25% (▲), Rhinoguttae ephedrini (FNA) 1% (■), Endrine® (○), Rhinoguttae ephedrini compositae (FNA) (●) and Fenox® (△).

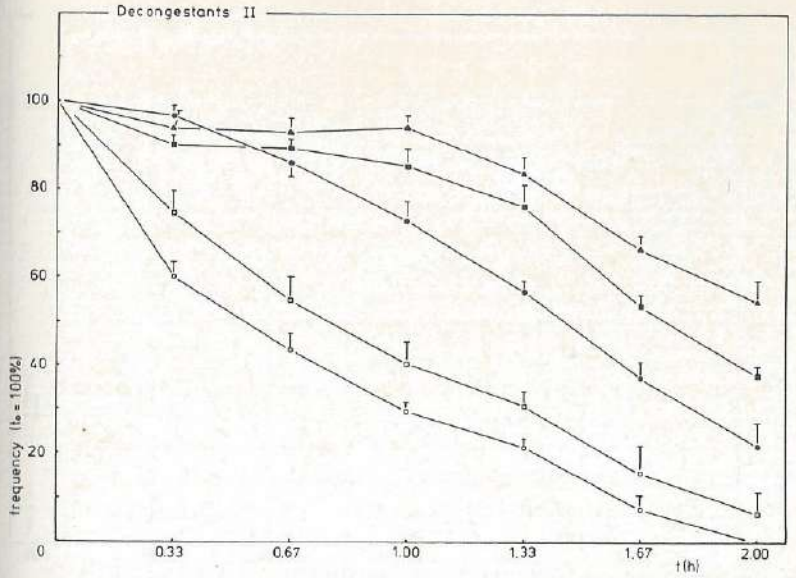

Figure 2. Time versus frequency plot for Rhinoguttae xylometazolini (FNA) 0.025% (▲), 0.05% (■) and 0.1% (●) and Otrivin® 0.05% (□) and 0.1% (○).

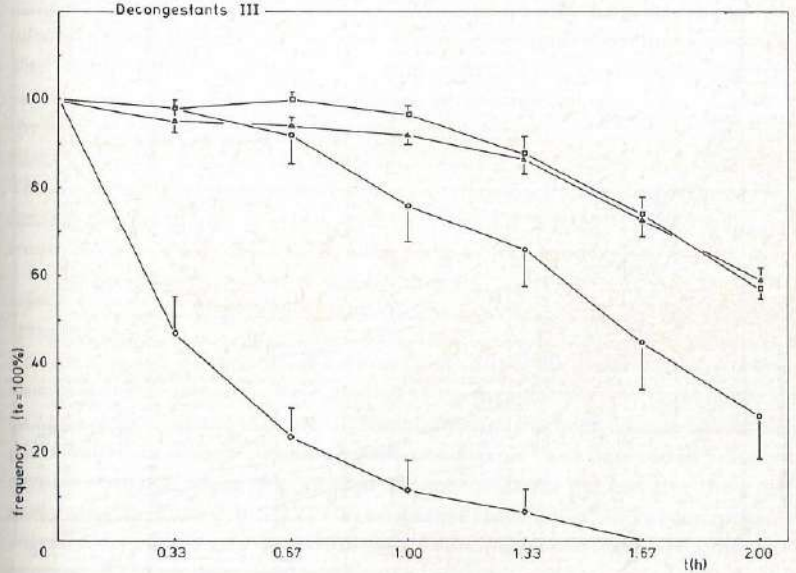

Figure 3. Time versus frequency plot for Nasivin® 0.025% (□) and 0.05% (△), Rhinogutt® (○) and Sinex® (◇).

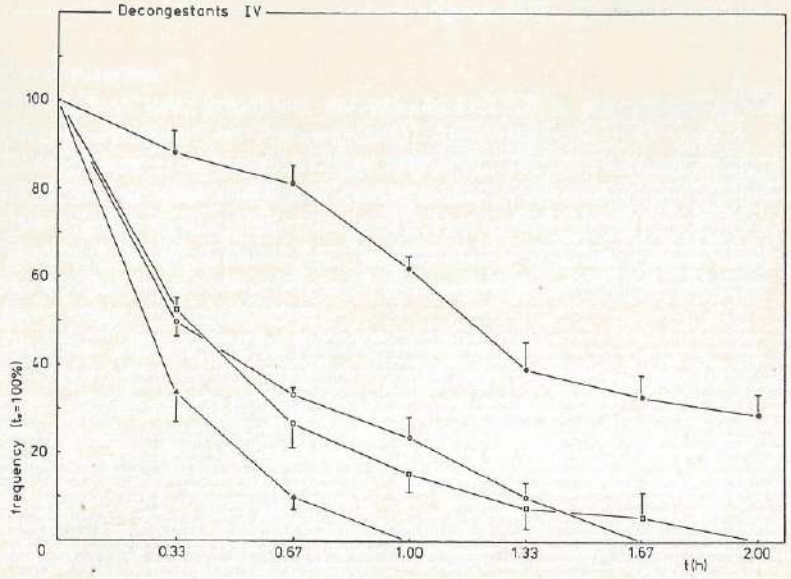

Figure 4. Time versus frequency plot for Rhinoguttae naphazolini (FNA) 0.05% (●), Privine® 0.1% (□) and 0.05% (○) and Rhinofral® (△).

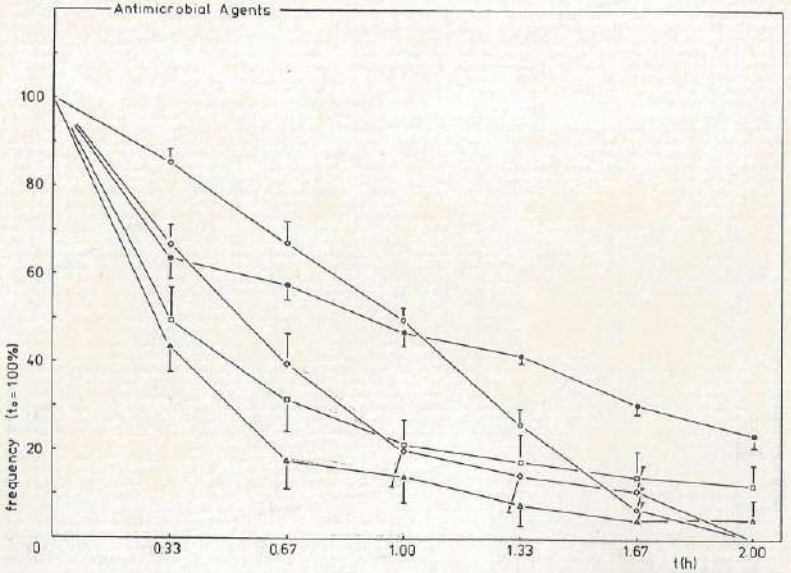

Figure 5. Time versus frequency plot for Argyrophedrine® (○), Chloransulfa® (◇), Rhinoguttae nucleinici compositae (FNA) (●), Chloramphenicol® (□) and Rhinamide® (△).

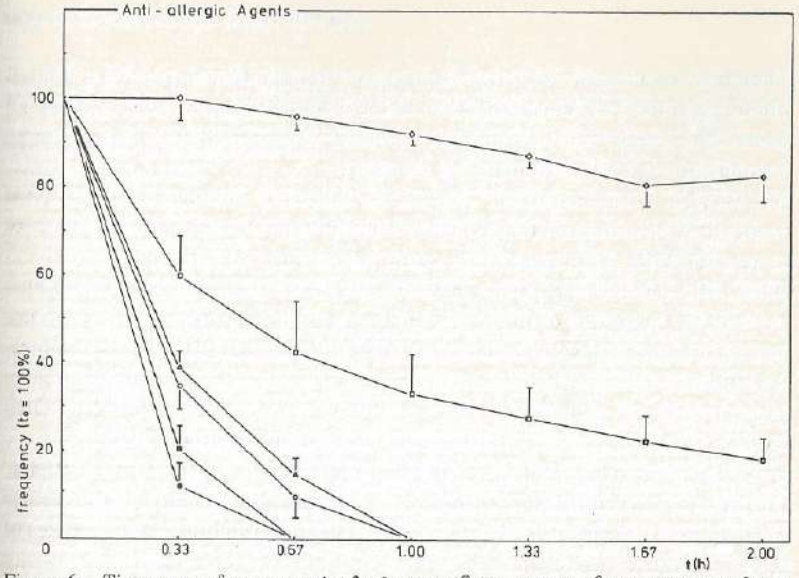

Figure 6. Time versus frequency plot for Lomusol® (◇), Codelsol® (□), Otricorten® (△), Antistine-Privine® (○), Rhinoguttae antazolini (FNA) 0.5% (■) and Rhinoguttae antazolini et naphazolini (FNA) (●).

drine and phenylephrine. The Figures 2, 3 and 4 deal with the preparations containing decongestants with a prolonged action: the imidazoline derivatives.

The effects of the preparations with antimicrobial agents are shown in Figure 5 and refer to antibiotics, sulphonamides and silver protein. Figure 6 demonstrates the effects of preparations containing drugs which are used against allergic diseases and sometimes against vasomotor rhinitis.

The activity of preparations serving as a reference (Locke-Ringer), assessed during all measurements, was always between 97% to 107% of the initial frequency.

DISCUSSSION

Assays

The identity and amounts of all the drugs and most of the preservatives in the investigated nasal drops have been granted by the manufacturer, partly analyzed by us and are summarized in Table III. No information has been obtained about the preservatives of Otrivin®, Privine®, Antistine-Privine® and Octricorten®. Sinex® contains benzalkonium chloride, but the manufacturer did not give the exact concentration. From F.A.S.S. (1978) we learned that Otrivin® in Sweden contains a quaternary ammonium compound (0.01% domiphen bromide)* and a mercury compound (20 µg thiomersal/ml).*

* This has recently been confirmed by Ciba with respect to the Netherlands' market.

Therefore we assayed the concentration and identity of the quaternary ammonium compounds in all nasal drops mentioned above. Only Otrivin® and Sinex® contained a quaternary ammonium compound: respectivily 0.008% domiphen bromide and 0.015% benzalkonium chloride.
The label of Antistine-Privine® reports thiomersal as the preservative without mentioning the concentration. A normal concentration would be 50–200 µg thiomersal/ml. With HPLC we could not find more than 1 µg thiomersal/ml.
When we added 20 µg thiomersal/ml before the analysis, we detected this amount. The amount of mercury, found by atomic absorption spectrophotometry, corresponded with an amount of 16 µg thiomersal/ml. Either the thiomersal of this nasal drop has been decomposed or a part of the mercury belongs to another mercuric compound. Otrivin®, Privine®, Antistine-Privine® and Otricorten® have been analysed with atomic absorption spectrophotometry.
We did not exclude the presence of other preservatives in these preparations.
Finally, we essayed the concentrations of benzalkonium chloride in the other nasal drops. All preparations containing benzalkonium chloride contained less of this preservative than claimed by the manufacturers (Table II).
The Extra Pharmacopoeia (Martindale, 1977) demands that benzalkonium chloride contains not more than 15% of water. Benzalkonium chloride is highly hygroscopic. After storage the water content may exceed 15%, which explains the rather low benzalkonium chloride concentrations.

Ciliary movement

It turned out that all preparations decreased the ciliary beat frequency though to a very different extent.
Some undiluted preparations arrested ciliary movement very fast ($t_{0\%} < 0.2$ h). These results were less reproducible than the effects of the diluted preparations. Moreover, the nasal drops will be diluted by the nasal mucus after application. Therefore we focussed our investigations on the diluted preparations and assessed the effects of undiluted preparations only in fourfold.
A good parameter to evaluate the effects of the nasal drops on ciliary movement is the time necessary to decrease the frequency 50% ($t_{50\%}$, table IV) in the case of fivefold dilutions.
Relating the effects of nasal drops to the preservatives which they contain, it appears that the chlorbutol or mercury containing products have a strong effect on ciliary movement in contrast to the preparations which contain only benzalkonium chloride as a preservative (Table V).
These results are in agreement with our former publication (Van de Donk et al., 1980b). Table V compares average $t_{50\%}$ of nasal drops with a specific preservative or a specific drug regardless other components of the nasal drops. The preparations with chlorbutol as a preservative contain more than one drug, which makes

Table V. Effects of some groups of nasal drops

preparation	number	$\bar{t}_{50\%(1=5)}$ [1]
benzalkonium chloride containing[2]	14	1.22
chlorbutol containing[3]	2	0.27
mercury containing	7	0.44
antazoline containing	3	0.22
combination preparations[4]	10	0.38

[1] Time necessary to decrease frequency of ciliary movement 50% of 1=5 diluted preparations in hours.
[2] These preparations contain only benzalkonium chloride as a preservative.
[3] These preparations form a subgroup of the combination preparations as mentioned in foot-note 4.
[4] Preparations containing more than one drug.

it difficult to differentiate between the effects of the preservative and those of the drugs. In a next study we will focus our attention on the effects of single drugs.
The effects of the mercury containing preservatives is more obvious. The preparations Rhinoguttae xylometazolini 0.1%, 0.05%; Rh. naphazolini 0.05% (average $t_{50\%} = 1.46$ h) and the preparations Otrivin® 0.1%, 0.05%; Privine® 0.05% (average $t_{50\%} = 0.55$ h) are quite comparable as to their composition, but the latter three contain a mercury compound.
The preservatives aminacrine and benzoic acid probably have a strong effect on ciliary movement, regarding the effects of Rhinofral® ($t_{50\%} = 0.25$ h) and Rhinamide® ($t_{50\%} = 0.3$ h). The effects of these preservatives have not been investigated separately in our former study as they are seldomly used in nasal drops.
In Table V the average $t_{50\%}$ of antazoline containing preparations is given as well. This drug is mentioned separately since the two nasal drops containing both antazoline and benzalkonium chloride (Rhinoguttae antazolini 0.5% and Rhinoguttae antazolini et naphazolini) appeared to be an exception in the group of nasal drops, preserved with benzalkonium chloride in showing a marked decrease of ciliary motion.
The mercury containing Antistine-Privine® has a smaller effect than Rhinoguttae antazolini and Rhinoguttae antazolini et naphazolini (after dilution with Locke-Ringer).
This can be explained by the fact that the two last mentioned products contain 20% more antazoline than Antistine-Privine®. The dilution moderates the effects of mercury containing preparations fairly, but the effects of antazoline hardly. This is in agreement with our recent findings that antazoline has a weaker effect at pH = 5 than at pH = 7.4 (to be published) and that the pH rises after dilution.

Generally, the combination preparations (nasal drops, containing more than one drug) have a worse effect on ciliary movement than the single drug containing

preparations. The effects of Fenox®, Endrine®, Sinex® and Rhinoguttae ephedrini compositae may, at least partially, be explained by the effects of drugs like menthol, camphor and eucalyptol, which are added to the main drugs in these preparations. The differences in effect between Rhinoguttae ephedrini 1% ($t_{50\%} = 0.9$ h) and Rhinoguttae ephedrini compositae ($t_{50\%} = 0.27$ h) indicate that the combination menthol 0.015%, camphor 0.015% and eucalyptol 0.1% has a strong effect on ciliary movement. Proetz (1953) found no effect for camphor 1% or menthol 1% on ciliary activity of human and rabbit tissues. But this author performed these experiments in liquid paraffin from which medium the penetration into the tissues is very limited. Secondly, mineral oils should never be used in nasal preparations as they have often been reported to cause oil-inspiration pneumonia.

The strong effect of Chloransulfa® is a.o. explained by the large drug concentration, which is about three times the isotonic concentration. This effect is very sensitive to dilution.

Many preparations had a more physiological pH after dilution than before, but their effect on ciliary movement diminished little. Sometimes the pH exceeds 7.4 after dilution of an acid preparation, which is due to the loss of carbon dioxide during the experiments.

Table VI reports the results of other authors. Only Schleppy and Blaser (1978) revealed the exact composition of their nasal drops. The preservatives of 4 nasal drops investigated by Dudley and Cherry (1978) are listed in the P.D.R. (1979). Hutcheon and Cullen (1955) assayed the concentrations of phenylephrine, naphazoline and ephedrine, that resulted in the same ciliotoxicity. These drugs are present in the nasal drops Rhinoguttae phenylephrini 0.25%, Rhinoguttae naphazolini 0.05% and Rhinoguttae ephedrini 1%. These concentrations are respectively 17.2, 8 and 2.4 times lower than the concentrations in the publication of Hutcheon and Cullen. This means that phenylephrine has the least and ephedrine the largest ciliotoxic effect, which is in agreement with the effects of the related nasal drops.

The effects of Privine® as reported by Bos and Jongkees (1966) are in agreement with our results.

In contrast with our results Bos and Jongkees (1966) did not find frequency decrease for Otrivin®. The composition of Otrivin® in 1965 in the Netherlands however is not known. Possibly this preparation did not contain a mercury compound at the time of their experiments, as it is still the case in the United States.

Like Hutcheon and Cullen, Mirimanoff (1969) studied drugs instead of nasal drops and found a strong effect for antazoline HCl 0.5% and a very slight effect for ephedrine HCl 1%. These effects are in agreement with the effects of Antistine-

Table VI. Comparison of the results from other "in vitro" studies

author	preparation	drug	preservative	activity of the cilia	dilution	tissue
Hutcheon and Cullen (1955)		ephedrine 2.4% naphazoline 0.4% phenylephrine 4.3%	none none none	50% of the ring dead after 15 min	1=1 1=1 1=1	trachea, rat trachea, rat trachea, rat
Bos and Jongkees (1966)	Privine® Otrivin® Ephedrine HCl 1% Rhinospray®[1]	naphazoline 0.1% xylometazoline 0.1% ephedrine HCl 1% tramazoline HCl 0.1%	unknown unknown unknown unknown	<5 min >1 h >1 h >1 h	1=1 1=1 1=1 1=1	adenoid, human adenoid, human adenoid, human adenoid, human
Mirimanoff (1969)		antazoline HCl 0.5% ephedrine HCl 1%	none none	immediate frequency decrease modest decrease after one hour	1=1 1=1	trachea, guinea pig. trachea, guinea pig.
Schleppy and Blaser (1978)	Rhinapan®	phenylephrine HCl 0.25%	chlorhexidine gluconate 0.01% dequalinium acetate 0.01%	>20 min	1=1	trachea, mouse
Dudley and Cherry (1978)	Privine® Otrivin® NTZ® Afrin®	naphazoline HCl 0.05% xylometazoline HCl 0.1% phenylephrine HCl 0.1% thenyldiamine HCl 0.1% oxymetazoline HCl 0.05%	benzalk. chl. 0.02% benzalk. chl. 0.02% benzalk. chl. 0.02% phenylmercuric ac. 0.002% benzalk. chl. 0.02%	50% of the act. after 12 h[2] 50% of the act. after 12 h[2] 50% of the act. after 3 h[2] 50% of the act. within 1 h[2]	1=6 1=6 1=6 1=6 1=6	trachea, chicken embryo

[1] New name is Rhinoguti®.
[2] 5 min contact every 8 hours.

Privine®, Rhinoguttae antazolini 0.5% and Rhinoguttae antazolini et naphazolini, resp. Rhinoguttae ephedrini 1% in our experiments.
The effect of Rhinipan® is moderate and comparable with Rhinoguttae phenylephrini 0.25% (Schleppy and Blaser, 1978). This is in agreement with the weak effects found for chlorhexidine gluconate and quaternary ammonium compounds (Van de Donk et al., 1980b). Dudley and Cherry (1978) assessed the effects of nasal drops after 5 min. contact every 8 hours. After each contact the rings were rinced and stored in a rich medium (HEPES-BME).
It appears that the nasal drops containing quaternary ammonium compounds have a moderate effect, e.g. Privine® and Otrivin®.
The addition of the antihistamine thenyldiamine HCl makes the effect worse, e.g. NTZ®. the mercury compound in Afrin® is likely the cause of the very strong effect of this preparation.

To reduce the adverse reactions of locally applicated nasal drops three possibilities exist: (1) preservatives are omitted and to avoid microbial contamination of the nasal drop sterilized single dose units are used; (2) drugs and additives are selected according to their effects on ciliary acticity; (3) the drug is administered otherwise, e.g.: orally.
The first will be rather expensive, whereas the third might give systemic adverse reactions. Empey et al. (1980) reports a good decrease of the nasal airway resistance for 60 mg pseudo-ephedrine orally without increasing pulse and systolic blood pressure. After pseudo-ephedrine 120 mg orally significant increases in pulse and systolic blood pressure occurred. Benson (1971) found approximately the same effect for pseudo-ephedrine (60 mg, oral) and one drop ephedrine 1% on the maximal nasal inspiratory flow rate. The onset of action of the nasal drop was faster.
It is however, unlikely that systemic administration will ever be preferable to local administration. It is more reasonable to advise the use of the most harmless preparation within a therapeutical group.

CONCLUSION

Realizing the limitations of our "in vitro" model with a single application, it is possible to draw some preliminary conclusions. Some of our results will be checked in an "in vivo" model and described in a next study.

- For the preservation of nasal drops quaternary ammonium compounds should be preferred to mercury compounds. As appeared from our previous study (Van de Donk et al., 1980b), these compounds can be combined with EDTA. Nasal drops containing a mercury compound should not be used.
- The ciliotoxic effects of decongestants are moderate, provided that they are preserved with a quaternary ammonium compound and contain one drug only.

- The anti-allergic agents have different effects. Sodium cromoglycate hardly influences the ciliary motion, whereas antihistamines are likely to be very ciliotoxic (Figure 6). They need further study, especially while this group of drugs is often used in chronic diseases.

RÉSUMÉ

Les effets des gouttes nasales, sous forme de spécialités pharmaceutiques ainsi que de préparations magistrales, conformes à l'F.N.A. (Formulaire de l'Association des Pharmaciens Néerlandais), sur la fréquence du battement ciliaire de la trachea de l'embryon de poulet sont déterminés.
En général l'influence sur le battement ciliaire des conservateurs utilisés dans les gouttes nasales se réléva beaucoup plus forte que celle des éléments pharmacologiquement actifs. Le temps moyen, nécessaire à diminuer la frequence avec 50% en cas de gouttes nasales délayées 1 = 5 (le moyen $t_{50\%(1=5)}$) et contenantes des composés mercuriels ou du chlorobutanol est 0,4 h.
Les préparations contenants des composés d'ammonium quaternaire montrent cet effet à un moindre degré, le moyen $t_{50\%(1=5)} > 1{,}22$ h. Elles sont par conséquent à préférer.
Les gouttes nasales décongestives, à moins que conservées à l'ammonium quaternaire, ont peu d'influence sur le battement ciliaire, le moyen $t_{50\%(1=5)} > 1{,}38$ h.
Les gouttes nasales antihistaminiques ont, par contre, un effet inhibiteur prononcé sur le mouvement ciliaire, indépendent des conservateurs utilisés, le moyen $t_{50\%(1=5)} = 0{,}22$ h.
D'autre part l'effet faible à cet égard de la préparation de cromoglycate est remarquable, le $t_{50\%(1=5)} > 2$ h.
Les préparations antimicrobiennes ne sont pas préservées et retardent le mouvement ciliaire intermédiairement, le moyen $t_{50\%(1=5)} = 0{,}7$ h.

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CHAPTER IV

CORRELATION BETWEEN THE SENSITIVITY OF THE CILIARY BEAT FREQUENCY OF HUMAN ADENOID TISSUE AND CHICKEN EMBRYO TRACHEAS FOR SOME DRUGS

H.J.M. van de Donk, J. Zuidema, F.W.H.M. Merkus

Summary

The effects of benzalkonium chloride, chlorbutol, xylometazoline and naphazoline on the ciliary beat frequency of human adenoids and chicken embryo tracheas have been determined and compared.
Chlorbutol 0.5% appeared to arrest ciliary motion in both tissues within 5 min. Rinsing with Locke Ringer solution (LR) restored the ciliary motion almost completely in both cases.
Benzalkonium chloride 0.006% + EDTA 0.1% decreased the ciliary beat frequency 35% for the human tissues and 50% for the chicken tissues after a contact of 20 min. In both cases the frequency hardly changed after rinsing with LR.
Naphazoline nitrate 0.1% and xylometazoline HCl 0.05% have reversible effects on the ciliary beat frequency of both human adenoids and chicken embryo tracheas. Cilia of human adenoids appeared to be more sensitive for xylometazoline than for naphazoline; whereas cilia of chicken embryo tracheas were more affected by naphazoline than by xylometazoline.
The results with human adenoids and chicken embryo tracheas show a strong correlation (correlation coeff.= 0.82, $P<0.005$). In the initial response the differences in sensitivity to preservatives and drugs were in many cases statistically significant, but the final effects were similar.

Introduction

In a former study Van de Donk et al. (1980a) described a photo-electric registration device with which the ciliary beat frequency can be determined in vitro. With this instrument the influence of preservatives (Van de Donk et al., 1980b) and nasal drops (Van de Donk et al., 1981) on the ciliary beat frequency of chicken embryo tracheas have been determined.
The question arises whether a correlation exists between the sensitivity of the ciliary activity of chicken embryo tracheas and that of the human nasal epithelium. An indication has been given by the results of investigations with transmission electron microscopy: the anatomy of cilia of humans and all animals are quite similar (Satir, 1980). However, anatomical identity does not guarantee physiological identity. Therefore we investigated the influence of two preservatives (chlorbutol and benzalkonium chloride + EDTA) and two drugs (xylometazoline HCl and naphazoline nitrate) on the ciliary beat frequency of human adenoids and compared these results with the effects on the ciliary beat frequency of chicken embryo tracheas.

Materials and methods

The experiments were performed in Locke Ringer solution (LR), pH=7.4 and a temperature of 25°C. The ciliary beat frequency was assessed by a photo-electric registration device (Van de Donk et al., 1980a). The effect of each compound was assessed on six different chicken embryo tracheas and on six different human adenoids. The preparation of the chicken embryo tracheas is described by Van de Donk et al. (1980a).
The human adenoids were obtained from children (younger than 13 years) with infected adenoids. Anaesthesia was performed with a nitrous oxide/oxygen mixture;

local anesthetics were not used. The adenoids were removed by adenoidectomy. The adenoids were collected in LR and kept at room temperature. The time between the adenoidectomy and the start of the experiments was about two hours. Slices were cut off of approximately 1 mm thick. The slices were turned on their sides and inspected for the presence of motile cilia. When the frequency of the ciliary beat exceeded 8 Hz, which was sufficient for our experiments, the piece of tissue was judged suitable for the experiments.
The effects of chlorbutol 0.5%, benzalkonium chloride 0.006% + EDTA 0.1%, naphazoline nitrate 0.1% and xylometazoline HCl 0.05% in LR on both human adenoids and chicken embryo tracheas were determined.
The differences in sensitivity were submitted to a student test.
The correlation was assessed with a spearman-rank correlation test.

Results

On about 50% of the adenoids we were able to find motile cilia in the not inflamed areas of the adenoids. The ciliary beat frequency of the adenoids, used for the experiments, was 11.1 ± 1.7 Hz and of the chicken tracheas 16.9 ± 1.8 Hz (mean ± s.d.). The effects of chlorbutol 0.5% and of benzalkonium chloride 0.006% + EDTA 0.1%, xylometazoline HCl 0.05% and naphazoline nitrate 0.1% are demonstrated by table I.
The third column shows the ciliary beat frequency just before the experiment started. The next column gives the frequencies at the first measurement, as a percentage of the initial frequency.
The effect of chlorbutol was first measured after a 5 min contact, the others after a 20 min contact. After the first measurements the tissues were rinsed with LR solution. The last columns show the frequency as a percentage of the initial frequency after rinsing with LR. The probabilities of the differences between the ciliary beat frequencies of human adenoids and chicken embryo tracheas at the same drug concentrations and contact time are indicated next to the frequencies.
The figures 1-4 demonstrate the effects of the preservatives and the two decongestants in more details. The SEM values are indicated by vertical bars.

Table I The effects of chlorbutol 0.5%, benzalkonium chloride 0.006% + EDTA 0.1% xylometazoline HCl 0.05% and naphazoline nitrate 0.1% on ciliary movement.

compound	species	initial a) frequency	b) t=5 min	frequency c) t=0.33 h	P d)				
chlorbutol	human	10.6 ± 1.6	0% (LR)	65%	0.95				
	chicken	17.1 ± 2.0	0% (LR)	81%					
				frequency					
			t=0.33 h	P d)	t=0.67 h	P d)	t=1 h	P d)	
benzalkonium chl. + EDTA	human	11.4 ± 1.7	65% (LR)	0.995	65%	0.9	66%	0.995	
	chicken	17.4 ± 1.9	50% (LR)		57%		49%		
xylometazol.	human	11.4 ± 1.2	57% (LR)	0.95	88%	0.75	102%	0.75	
	chicken	16.6 ± 2.0	75% (LR)		101%		100%		
naphazoline	human	11.0 ± 1.8	67% (LR)	0.9	95%	0.9	100%	0.75	
	chicken	16.3 ± 1.4	52% (LR)		79%		92%		

a) Initial frequency and standard deviation in beats/sec.
b) Tissues were rinsed with LR after this measurement.
c) Percentage of the initial frequency after the indicated time.
d) Probability of the differences between the beat frequency of human and chicken cilia.

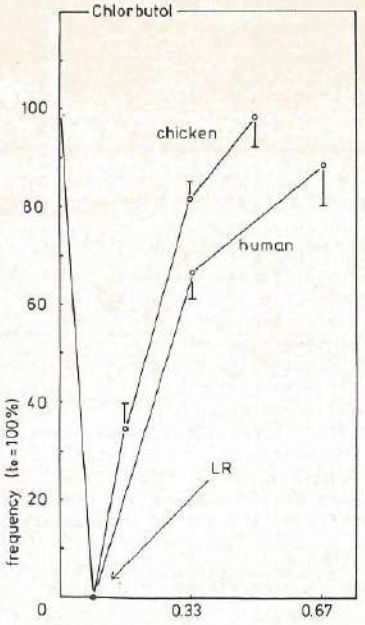

Fig. 1 Time versus frequency plot: chlorbutol 0.5% washed after 5 min with LR.

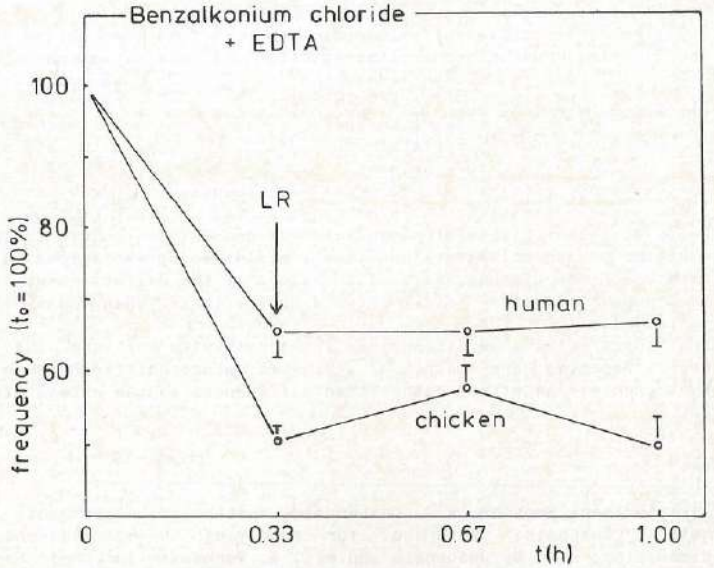

Fig. 2 Time versus frequency plot: benzalkonium chloride 0.006% + EDTA 0.1% washed after 20 min with LR.

Discussion

The purpose of this study was to compare ciliary epithelium of humans and chicken embryos.
The pieces of tissues must have a surface of at least a few square millimeters to perform the experiments. As we had human adenoids at our disposal we did not ask volunteers to provide us with pieces of ciliary epithelium.
We investigated chlorbutol as a representative of the lipophilic preservatives with fast but reversible effects on ciliary motion. Benzalkonium chloride, mostly combined with EDTA, belongs to the polair preservatives with slow but irreversible effects on ciliary motion. Naphazoline nitrate and oxymetazoline HCl were tested because they are extensively used as drugs in nasal medications. With the human adenoids, we performed experiments without pieces of tissue that could serve as a reference, because the intact area was mostly too small. Therefore we focused our experiments on the reversibility of the effects. In case of complete reversibility, the tissues served as their own references.
This procedure was effective in the cases of chlorbutol, xylometazoline and naphazoline; the ciliary beat frequency returned respectively to 87%, 102% and 100% of the initial value.
The effects of chlorbutol 0.5% on the ciliary beat frequency of human adenoids and chicken embryo tracheas (table I, fig 1) are quite comparable. In both cases the cilia are immotile after a 5 min contact. This effect is a bit more reversible for the chicken tracheas, which can be explained by the condition of these tissues. The difference after 20 min is significant ($P<0.05$).
The chicken trachea tissues turned out to be significantly more sensitive than the adenoid tissues for the effects of benzalkonium chloride 0.006% + EDTA 0.1% (table I, fig 2). The decrease of the ciliary beat frequency during contact with this preserving combination and subsequently no more change in frequency after rinsing with LR, is however the same for both tissues.
Human adenoids are more sensitive for xylometazoline HCl 0.05% than chicken tracheas are (table I, fig 3). For naphazoline nitrate 0.1% this situation is just the reverse (table I, fig 4). In both cases the differences are significant after 0.33 h and 0.67 h, but not after 1 h. Again the tendency: decrease of ciliary beat frequency during contact with the drugs and increase of the frequency up to about 100% of the initial frequency after rinsing with LR, is for both drugs and both kinds of tissue the same.
The overall correlation coefficient for all the experiments with regard to the sensitivity of human adenoids and chicken embryo tracheas is 0.82 ($p<0.005$).
The differences in the sensitivity of human adenoids and chicken embryo tracheas to the drugs and the preservatives are not likely explained by variations in the pathological status of the tissues, as the SEM values of the ciliary beat frequency of the human adenoids (fig 1-4) are hardly larger than those of the chicken embryo tracheas.
We conclude that there exist some differences in sensitivity between the two species. The differences are mainly of a kinetic nature: differences in the time necessary to achieve an effect rather than differences in the extent of the eventual effect.

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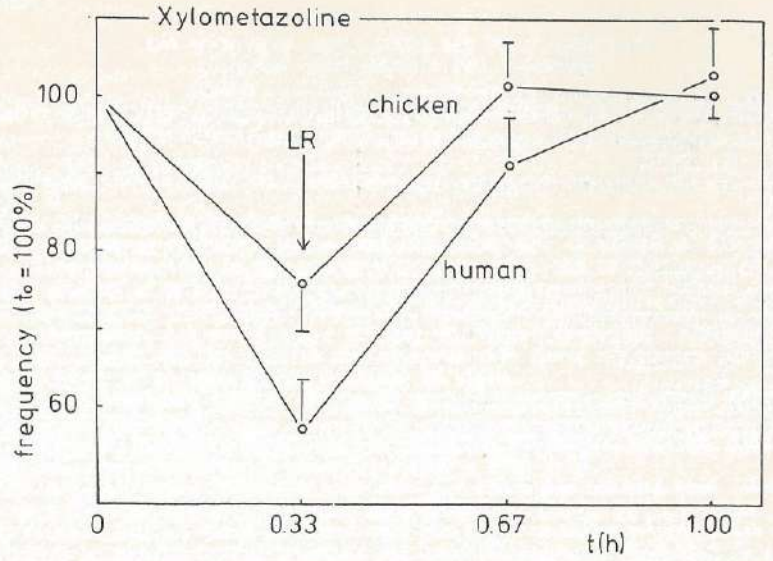

Fig. 3 Time versus frequency plot: xylometazoline HCl 0.05% washed after 20 min with LR.

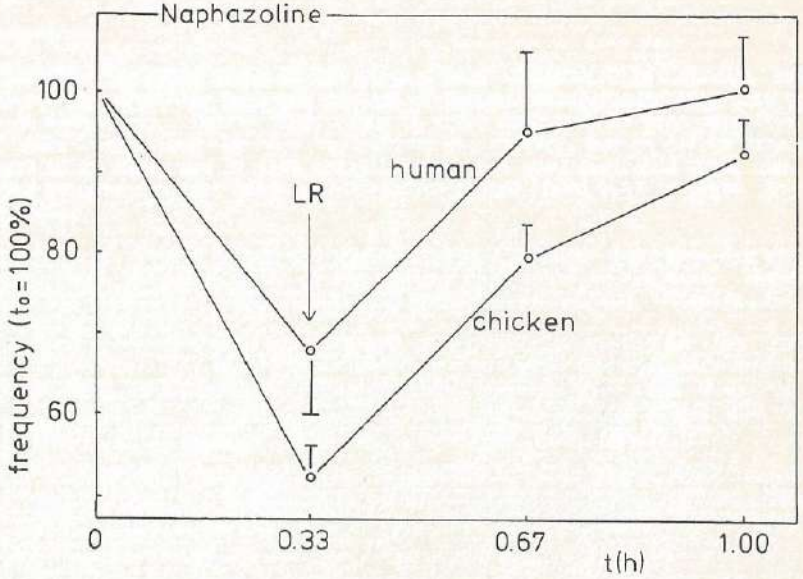

Fig. 4 Time versus frequency plot: naphazoline nitrate 0.1% washed after 20 min with LR.

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CHAPTER V

THE EFFECTS OF NASAL DROPS AND THEIR ADDITIVES ON HUMAN NASAL MUCOCILIARY CLEARANCE

H.J.M. van de Donk, A.G.M. van den Heuvel, J. Zuidema and F.W.H.M. Merkus

Summary

A method for measurement of nasal mucociliary clearance in vivo is described. A drop, containing saccharin sodium and indigo carmine is placed on the edge of the ciliary epithelium in the entrance to the nose. The time between placement and the sensing of the sweet taste as well as the appearance of a blue line in the nasopharyngeal cavity is measured and called the transport time.
Two preservatives, two nasal drops and one viscosity-increasing substance have been investigated and the results are compared with their effects on the ciliary beat frequency of chicken embryo tracheas in vitro.
The more the transport time is increased by a compound the more the ciliary beat frequency is decreased.
Chlorbutol 0.5% increases transport time more and decreases ciliary beat frequency more than benzalkonium chloride 0.006% + EDTA 0.1%.
Otrivin 0.1% increases transport time more and decreases ciliary beat frequency more than Rhinoguttae xylometazolini 0.1% (FNA`).
These results support those obtained with the photo-electric registration device applicated on chicken embryo tracheas and human adenoids as described in earlier publications.

`) FNA is the Formulary of the Netherlands' Pharmacists Association.

Introduction

The influence of nasal drops on the ciliary beat frequency in chicken embryo tracheas in vitro has been the subject of earlier publications from this group (Van de Donk et al., 1981). Many other in vitro methods have been described and they were summarized by Van de Donk et al. (1980a), but no paired data with respect to in vitro and in vivo investigations has been available until now.
Several in vivo methods, divisible into four groups, have been applied to the human nose:

Sakakura et al. (1973) placed one particle with a diameter less than 0.5 mm and labeled with 50 µCi of 99Tc on the ciliary epithelium in the nose. The movement of the particle was registered by a gamma-camera and the velocity of the particles calculated.
Andersen et al. (1974) used one particle with a diameter of 0.5 mm and labeled with 3 µCi 99Tc per experiment. Simon et al. (1977) modified this method and used particles with a smaller size (diameter between 0.01 and 0.05 mm) to prevent impairment of ciliary movement. The particles were tagged with 30 µCi 51Cr which resulted in lower irradiation than 3 µCi 99Tc (Jung, 1977).

Sackner (1978) blew radiopaque Teflon discs (1.0 by 0.8 mm with BiO_3) into the nose. The velocity was computed from a roentgenographic image. A poor correlation was found between the "saccharin time" (a method described below) and the results of this method.

Van Ree and Dishoeck (1962) blew some edicol orange onto a position just behind the head of the concha inferior. The time taken until the powder was seen arriving around the tuba wall, with rhinoscopia posterior was measured. Ewert

(1965) used edicol supra Orange AG and watched for the transport of this powder with a special microscope focused on the epithelium.

Andersen et al. (1974) placed a particle of saccharin (with a diameter of 0.5 mm) on the superior surface of the interior turbinate. The subject was instructed to swallow every 30 sec and to report when a sweet taste was noticed. The time was measured from the moment of placement until the sensation of sweetness. A significant correlation between the flow rate measured by this saccharin test and the flow rate measured by the tagged-particle technique was demonstrated.

The main disadvantages of the first two methods are the use of radiation and the expensive equipment. The authors of the reports on the last two methods did not describe the effects of the test substance on ciliary clearance. Their main advantage, however, is the simplicity of the procedures.
We modified and combined the last two methods, studied their reliability and checked the validity of our in vitro studies. Therefore saccharin and indigo carmine were tested in vitro for their usefulness as tracer substances. Otrivin 0.1%, Rhinoguttae xylometazolini 0.1% (FNA), chlorbutol 0.5%, benzalkonium chloride 0.006% + EDTA 0.1% and hypromellose 1% (=hydroxypropylmethylcellulose) were investigated in vivo, and the in vitro and in vivo results were compared.

Materials and methods

The experiments were performed with healthy volunteers, who had given their informed consent. The subjects were 20-57 years old and consisted of smokers and non-smokers, women and men. The subjects had no history of respiratory diseases, except for the common cold. Only one volunteer had to be excluded because she had recently broken her nose. Experiments with subjects that had a cold were postponed till the subjects had recovered.
The substances used were of pharmacopoeial quality or analytical grade; 0.006% benzalkonium chloride (anhydrous) = 0.01% benzalkonium chloride, commercial quality.
The mucociliary clearance was measured with a test solution:

indigo carmine 8 mg
saccharin sodium 2 mg
dextrose 50 mg/ml (pH adjusted to 7.4)

Substances under investigation were: chlorbutol 0.5%, benzalkonium chloride 0.006% + EDTA 0.1%, hypromellose (2%=4000cps) 1%, hypromellose 1% + chlorbutol 0.5% and hypromellose 1% + benzalkonium chloride 0.006% + EDTA 0.1%.
All solutions were made isotonic with NaCl and the pH was adjusted to 7.4.
Two nasal drops were also investigated: Otrivin 0.1%, containing a.o. xylometazoline HCl 0.1%, domiphen bromide 0.008% and a mercury compound with 9.6 µg Hg/ml, and Rhinoguttae xylometazolini (FNA) 0.1%, containing xylometazoline HCl 0.1%, benzalkonium chloride 0.006%, EDTA 0.1%, hypromellose 0.5%, $NaH_2PO_4.12H_2O$ 0.15%, $Na_2HPO_4.2H_2O$ 0.1% and NaCl 0.8%.
The effects of indigo carmine 0.8% and saccharin sodium 0.2% (both solutions were isotonic with dextrose and the pH was adjusted to 7.4) on the ciliary beat frequency of chicken embryo tracheas in vitro were assayed according to the method of Van de Donk et al. (1980a).

The indigo carmine-saccharin test.

On arrival the volunteers rinsed their noses with saline. After 15 min one 25 µl drop of the solution, containing saccharin sodium and indigo carmine, was placed 20 mm from the tip of the nose at the bottom of the meatus nasi inferior of one nostril. After 2 min the subject was requested to swallow every 20 sec and the throat was inspected every 60 sec with the aid of an ophthalmoscope.

The subject was asked to indicate and to stop swallowing when a sweet taste appeared. Both the time necessary for the transport (transport time) of saccharin and that for indigo carmine were recorded. The nose was rinsed with saline and after 15 min one nasal drop or one drop of saline, containing the additive under investigation was given in the same nostril. The subject bent his or her head backwards while receiving the drop under investigation and the relevant side of the nose was softly massaged. After 15 min the test procedure was repeated, beginning with the placement of the 25 µl drop of the solution, containing saccharin sodium and indigo carmine. The subjects were requested to remain silent during the experiments, not to shake or bend their heads, nor to eat, smoke or drink. The results were tested with the rank-sign-test with a level of significance $P<0.05$. The test design was a non-randomized blind cross-over study.
We performed the next experiments as cross-over studies: chlorbutol versus benzalkonium chloride + EDTA; chlorbutol + hypromellose versus benzalkonium chloride + EDTA + hypromellose; and Otrivin versus Rhinoguttae xylometazolini.

Results

The effects of the test substances on the ciliary beat frequency of chicken embryo trachea were determined with the photo-electric registration device and the results are shown in table I and figure 1.

Table I Effects of the test substances on the ciliary beat frequency of chicken embryo tracheas.

substance		Activity a) freq. t=0.33 h	freq. t=0.67 h	freq. t=1 h
indigo carmine + dextrose	0.8% 5%	61	63	61
saccharin sodium + dextrose	0.2% 5%	66	54	53
dextrose	5%	55	49	37

a) Percentage of the initial frequency after 0.33, 0.67 and 1 h contact.

Subsequently, the transport time was assayed twice successively, each time 15 min after rinsing with saline and without giving a nasal drop or one of its constituents. The second transport time was significantly decreased, compared to the first transport time as measured with saccharin sodium and indigo carmine (table II, fig 2).
The effects of two preservatives, one viscosity-increasing substance and two nasal drops on the transport time are also shown in table II. The preservative chlorbutol 0.5% (fig 3) increased the transport time significantly as measured with saccharin and indigo carmine. The other preservative, benzalkonium chloride 0.006% + EDTA 0.1% (fig 4), provoked a non-significant increase for both saccharin sodium and indigo carmine. One outlier was recorded and when this was omitted, benzalkonium + EDTA even showed a small decrease in the transport time (not significant for saccharin sodium nor for indigo carmine). The influence of hypromellose 1% was assayed before the assay of hypromellose + chlorbutol and that of hypromellose + benzalkonium chloride + EDTA. The combined results for hypromellose 1% (12 subjects) showed a mean increase of the transport time of 19% with saccharin sodium and 21% with indigo carmine (both $P<0.05$). Both the combination of hypromellose 1% and chlorbutol 0.5% and the combination of hypromellose 1% and benzalkonium chloride 0.006% + EDTA 0.1% increased the transport time non-significantly (for saccharin sodium and indigo carmine).

Table II Effects of nasal drops and their additives on the nasal mucociliary transport time.

compound	parameter a)	ta b)	tp c)	(ta-tp)/ta d)	number of volunteers	signi-ficance
none	S	11.35	8.8	-21%	10	P<0.05
	I	12.85	9.6	-23%	10	P<0.05
chlorbutol 0.5%	S	8.1	11.8	+52%	12	P<0.01
	I	10.4	12.7	+54%	11	P<0.05
benzalkonium chl 0.006% + EDTA 0.1%	S	8.7	9.9	+23%	11	N.S.
	I	12.0	13.3	+19%	12	N.S.
hypromellose 1%	S	5.7	6.6	+16%	6	N.S.
	I	8.9	9.6	+11%	6	N.S.
+ chlorbutol 0.5%	S	-	8.8	+58%	6	N.S. e)
	I	-	11.5	+43%	6	N.S. e)
hypromellose 1%	S	5.7	6.8	+23%	6	N.S.
	I	6.3	8.3	+31%	6	P<0.05
+ benz chl 0.006% + EDTA 0.1%	S	-	7.7	+40%	6	N.S. e)
	I	-	8.5	+35%	6	N.S. e)
Rhinoguttae xylometazolini 0.1%	S	7.7	7.0	-3%	8	N.S.
	I	9.5	8.3	-8%	8	N.S.
Otrivin 0.1%	S	7.3	8.9	+27%	8	P<0.05
	I	8.9	9.7	+15%	8	N.S.

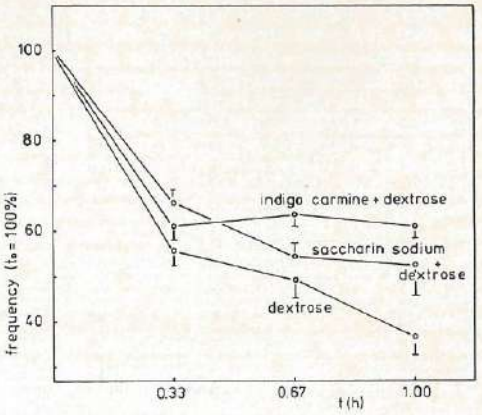
a) S is saccharin taste, I is indigo carmine colour.
b) ta is mean transport time before application of the compound under investigation in min.
c) tp is mean transport time after application of the compound under investigation in min.
d) (tp-ta)/ta is mean of relative increase of transport time after application of the compound under investigation.
e) with respect to transport time before application of hypromellose.

The nasal drop Rhinoguttae xylometazolini 0.1% (FNA,fig 5) produced a small decrease in the transport time (not significant for both saccharin sodium and indigo carmine). Again an outlier was recorded and when this was omitted, the transport time decreased 22% (significantly for both saccharin sodium and indigo carmine). Otrivin 0.1% (fig 6) increased the transport time, significantly for saccharin sodium only. No correlation was found between the effects of the investigated substances with respect to sex, age or smoking-behaviour.

Discussion

The object of this study was to investigate whether a correlation exists between results of in vitro experiments with nasal drops and their constituents on the ciliary beat frequency and the effects of these compounds on the nasal clearance in humans.
We used a method which is easy to apply, harmless to the volunteers and does not need a large financial investment.
The test was non-randomized as we always compared the transport time after application of a drug or additive with that of the reference substance (transport time before application). An increased transport time was interpreted as a decrease in nasal clearance.
The test was performed blind: the volunteers did not know which substance was applied. However, the investigator was informed about the composition of the drop under investigation as the smell or viscosity could not be concealed.
We preferred the use of a solution containing indigo carmine and saccharin sodium rather than a solid grain of these substances. A drop of solution is dif-

Fig. 1 Time versus ciliary beat frequency plot: indigo carmine 0.8% + dextrose 5%, saccharin sodium 0.2% + dextrose 5% and dextrose 5%.



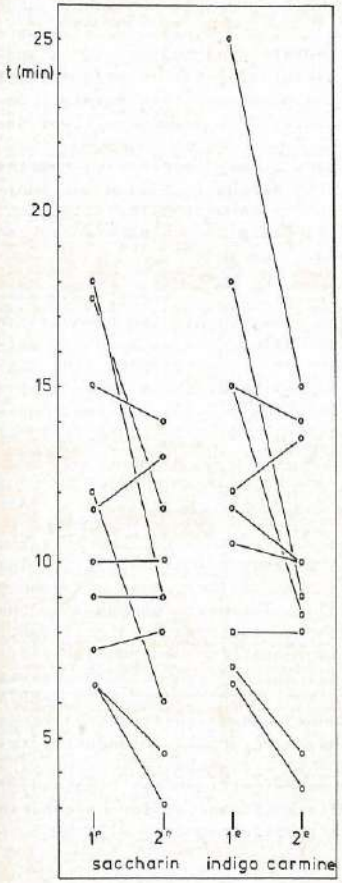
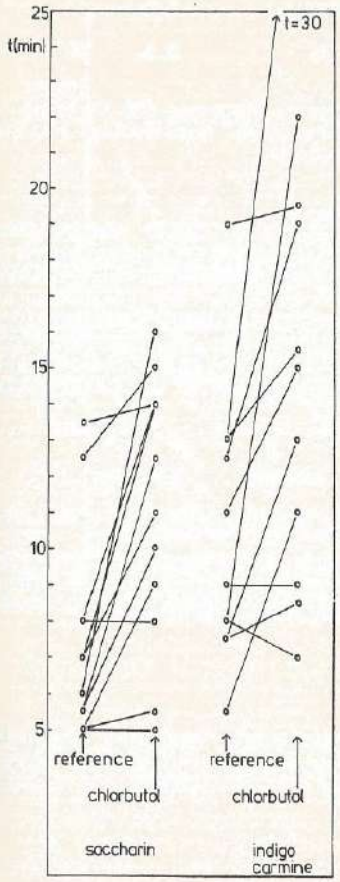


Fig. 2 Nasal mucociliary transport time: assayed twice with saccharin (10 volunteers) and indigo carmine (10 volunteers).

fused faster than a grain and transported over a larger area. The placement of a drop of solution is therefore less critical because there is less chance that the test solution will find an obstacle on its way. Moreover the solution can easily be made to physiologic pH and physiologic osmotic pressure. One 25 µl drop of the test solution contained approximately the same amount of saccharin sodium as the particles used by Andersen et al. (1974). Indigo carmine is chemically incompatible with many substances like NaCl and $CaCl_2$. Therefore the solution was made iso-osmotic using dextrose and no other substances were added. Initially the effects of indigo carmine 0.8% and saccharin sodium 0.2% on the ciliary beat frequency of chicken embryo tracheas were determined. Both dextrose 5% + indigo carmine 0.8% and dextrose 5% + saccharin sodium 0.2% decreased the ciliary beat frequency less than dextrose 5% alone (fig 1, table I). This can probably be explained by the fact that both saccharin sodium and indigo carmine contain sodium in contrast with the reference substance. So, a decreasing effect of saccharin sodium and indigo carmine on nasal clearance was not to be expected.
When we assayed the nasal transport time twice, without giving any medications, the second assay usually showed a shorter transport time. The volunteers were instructed to rinse the nostril under investigation before each assay to standardize the experiments. However, after the first assay the intensive sweet taste probably motivated the volunteers to rinse the nose more intensively. Saline dilutes the nasal mucus and so the viscosity of the mucus will be decreased, which in turn increases nasal clearance.
From this experiment it appears that if the transport time decreases, remains constant or even increases less than 21% and 23% with saccharin sodium and indigo carmine respectively after application of the substance under investigation, the nasal clearance will be de facto decreased. In this manner substances can be compared with respect to their influence on nasal clearance.

From table II it appears that chlorbutol 0.5% increased the transport time more than benzalkonium chloride 0.006% + EDTA 0.1% and that the latter combination increased the transport time as well, as amply compensating the stimulating effect of rinsing with saline. Hypromellose 1% increased the transport time significantly. Addition of hypromellose to chlorbutol had hardly no effect on the transport time, whereas addition to benzalkonium chloride + EDTA resulted in almost additive increases in the transport time. In the first case the effect of hypromellose is probably masked by the large effect of chlorbutol.
With respect to the nasal drops it appears that Otrivin 0.1% increased the transport time more than Rhinoguttae xylometazolini 0.1%, which in turn decreased the transport time less than rinsing with saline only.
The results of this study are summarized and compared with those of the in vitro investigations of the effects on ciliary beat frequency in table III. The second column shows the time necessary to decrease the ciliary beat frequency 50% as reported by Van de Donk et al. (1980b,1981). The next columns show the increase of the nasal transport time and whether these results are significant. A clear correlation between the effects on ciliary beat frequency and the effects on nasal transport time could be demonstrated: chlorbutol 0.5% decreases the ciliary beat frequency much faster than benzalkonium chloride 0.006% + EDTA 0.1% and also increases the nasal transport time more (and significantly) than benzalkonium chloride + EDTA. With respect to the nasal drops: Rhinoguttae xylometazolini 0.1% decreases the ciliary beat frequency less than Otrivin 0.1% and decreases the nasal transport time (though not significantly), instead of Otrivin, that increases the nasal transport time (significantly for saccharin sodium). The effects of hypromellose on the ciliary beat frequency will be published later.

Fig. 3 Nasal mucociliary transport time: assayed before and after the application of chlorbutol 0.5%, with saccharin (12 volunteers) and indigo carmine (11 volunteers).



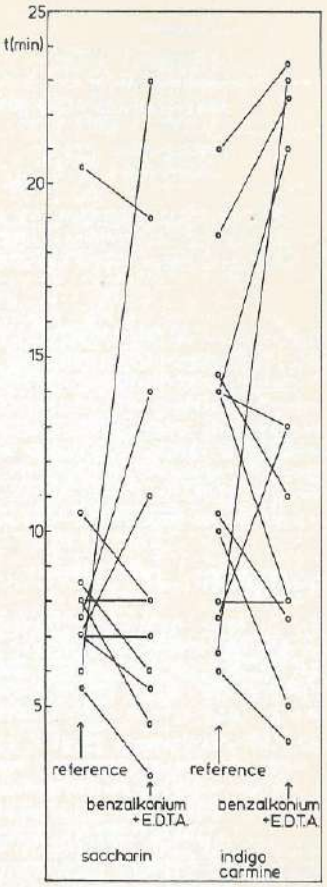


Fig. 4 Nasal mucociliary transport time: assayed before and after the application of benzalkonium chloride 0.006% + EDTA 0.1% with saccharin (11 volunteers) and indigo carmine (12 volunteers).

Table III Comparison of the effects on the ciliary beat frequency of chicken embryo tracheas and the effects on the nasal mucociliary transport time.

compound	t50% a)	(tp-ta)/ta b)	significance
chlorbutol 0.5%	0.04	+52%	P<0.01
Otrivin 0.1%	0.13	+27%	P<0.05
benzalkonium chloride 0.006% + EDTA 0.1%	0.33	+23%	N.S.
Rhinoguttae xylometazolini 0.1%	0.42	-3%	N.S.

a) t50% is time necessary to decrease the ciliary beat frequency 50% in hours.
b) (tp-ta)/ta is relative increase of transport time after application of the compound under investigation, assayed with saccharin.

The results of other authors are paradoxical. Simon et al. (1977) investigated the influence of Otrivin and found that the nasal transport time increased from 7 to 19 min (P<0.05), whereas Van Ree and Van Dishoeck (1962) found that Otrivin had no effect on the nasal transport time. However, the composition of proprietary preparations may very well vary with respect to their additives from country to country and even from time to time. Moreover Bos and Jongkees (1966) found no effect of Otrivin on the ciliary beat frequency of human adenoids whereas Van de Donk et al. (1981) found a strong decrease of the ciliary beat frequency of chicken embryo tracheas.

Conclusions

The in vivo results obtained with saccharin sodium and those obtained with indigo carmine are very similar.
The effects on the ciliary beat frequency of chicken embryo tracheas show a good correlation with the effects on the nasal mucociliary transport time in humans and so will have a good predictive value in studying the effects of nasal medications.

Acknowledgement

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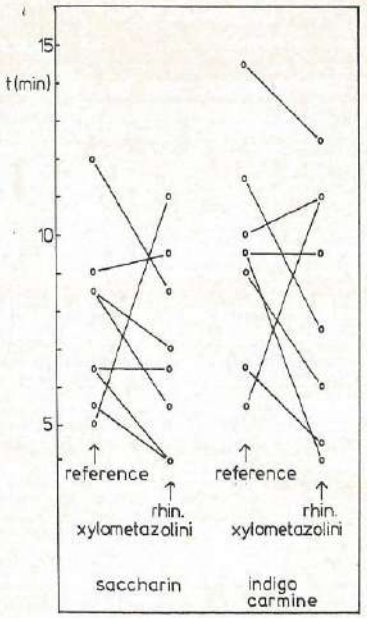
Fig. 5 Nasal mucociliary transport time: assayed before and after the application of Rhinoguttae xylometazolini HCl 0.1% with saccharin (8 volunteers) and indigo carmine (8 volunteers).



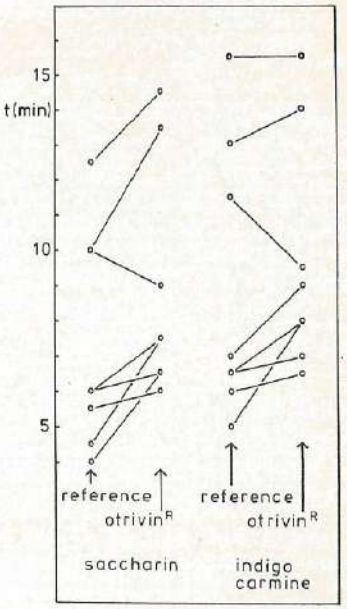


Fig. 6 Nasal mucociliary transport time: assayed before and after the application of Otrivin 0.1%, with saccharin (8 volunteers) and indigo carmine (8 volunteers).

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CHAPTER VI

THE EFFECTS OF DRUGS ON CILIARY MOTILITY

Part I: DECONGESTANTS

H.J.M. van de Donk, B. Jadoenath, J. Zuidema and F.W.H.M. Merkus

Summary

The effects of some decongestants at different pH's on the ciliary beat frequency of chicken embryo tracheas have been investigated. Ephedrine, phenylephrine, xylometazoline and oxymetazoline exerted the smallest effect on the ciliary beat frequency at pH 7.4, whereas phenylpropanolamine, tramazoline and naphazoline had the smallest effect at pH 6. The decongestants investigated did not cause a 50% decrease in the ciliary beat frequency within 20 min at the pH at which the ciliotoxicity was minimal. The effects of all decongestants on the ciliary beat frequency at pH 7.4, after exposure for 20 min, were reversible. Phenylephrine and oxymetazoline appeared to be the least ciliotoxic.

Introduction

A large part of the respiratory tract is covered with ciliated epithelia which removes dust, allergens and microorganisms in the direction of the pharynx. If ciliary motility is impeded for a long time, recurrent infections often appear, as is known from the Immotile Cilia Syndrome (Afzelius,1979). The effect of temporary ciliary arrest is unknown, but ciliodepression should be avoided especially in chronic therapy.
However it is well-known that drugs can influence ciliary motility in a negative way. In former studies we described the effects of preservatives and nasal preparations on the ciliary beat frequency of chicken embryo tracheas (Van de Donk et al.,1980b and 1981). We found a good correlation between the effects of drugs on the ciliary beat frequency of chicken embryo tracheas and human adenoids in vitro and also between these in vitro results and the in vivo effects of drugs on nasal clearance in volunteers (Van de Donk et al.,1 and 2).
The influence of alpha-sympathomimetic drugs on ciliary activity has received little attention until now. Hutcheon and Cullen (1955) investigated the effects of phenylephrine, ephedrine, naphazoline and tetrahydrozoline on rat tracheas. Verdugo et al. (1980) found a stimulating effect for isoproterenol. This compound has mainly beta-sympathomimetic properties. The stimulation could be compensated according to Verdugo et al., with the beta-blocking agent propranolol, indicating that the stimulating effect of isoproterenol was merely caused by its beta-sympathomimetic effect. In this study the effects of decongestants, acting like alpha-sympathomimetics, at different pH's on the ciliary beat frequency of chicken embryo tracheas are described.

Methods and Materials

The ciliary beat frequency was determined with a photo-electric registration device at 25° C in Locke Ringer (LR) solution (Van de Donk et al.,1980a). The effects of each drug at a fixed pH were assayed six times. Reversibility was studied at pH 7.4 by washing the tissues with LR after 20 min contact with the decongestant. For each experiment a piece of tissue from the same trachea, and placed in pure LR, served as a reference. Table I lists the substances investigated and their lot-numbers.

Table I List of investigated decongestants

substance	lot-number	manufacturer
ephedrine HCl	108 635/79F26	Brocacef
phenylephrine HCl	110 107/79F12	Brocacef
phenylpropanolamine HCl	19C-0559	Sigma
xylometazoline HCl	790717	Multipharma
oxymetazoline HCl	0080766	Merck
tramazoline HCl	11441	Karl Thomae gmbh
naphazoline nitrate	111 082/80D10	Brocacef

Results

The effects of the drugs at different pH's on the ciliary beat frequency of chicken embryo tracheas are shown in table II. The effects of rinsing with LR at pH 7.4 after 20 min contact have been assayed as well. The second column shows the pH, the next columns the ciliary beat frequency as a percentage of the initial beat frequency after 20, 40 and 60 min. The contact time necessary to reduce the beat frequency to 95% and 90% of the initial beat frequency are indicated by the last two columns.
The effects of the decongestants appeared to be reversible because the ciliary beat frequency was restored by washing with LR after 20 min contact.
Figures 1-7 show the effects in more detail. The SEM is indicated by vertical bars.
The ciliary beat frequency of the references remained between 98% and 109% during all experiments.

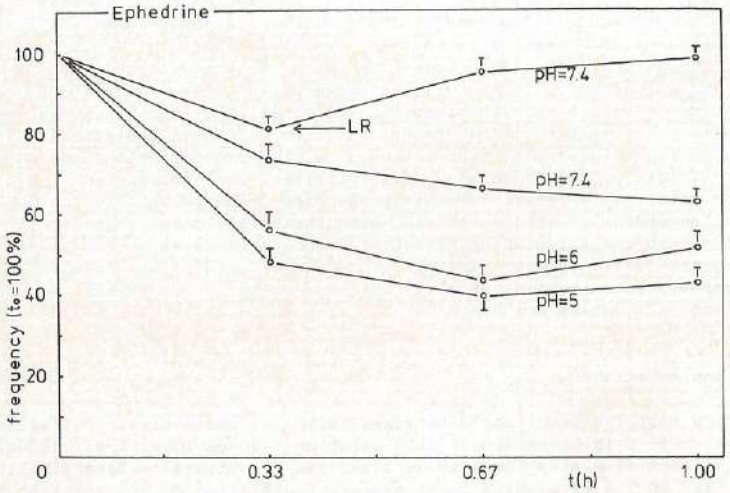

Fig. 1 Time versus frequency plot of ephedrine HCl 0.5% at pH 5, 6 and 7 and at pH 7.4 washed after 20 min with LR.

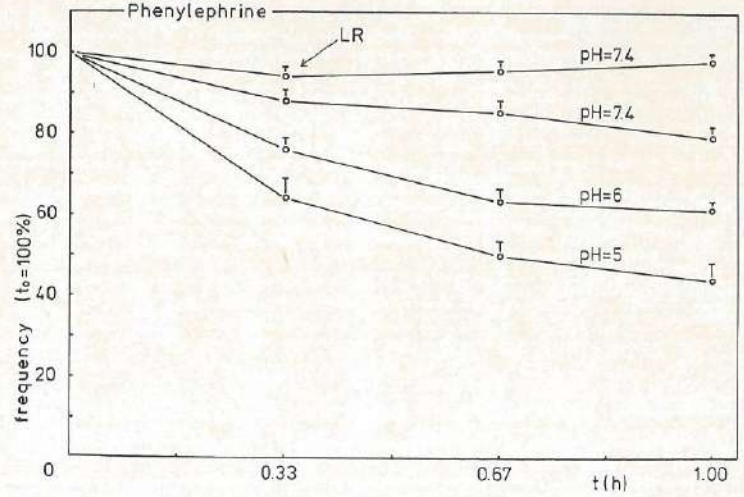

Fig. 2 Time versus frequency plot for phenylephrine HCl 0.5% at pH 5, 6 and 7.4 continuously and at pH 7.4 washed after 20 min with LR.

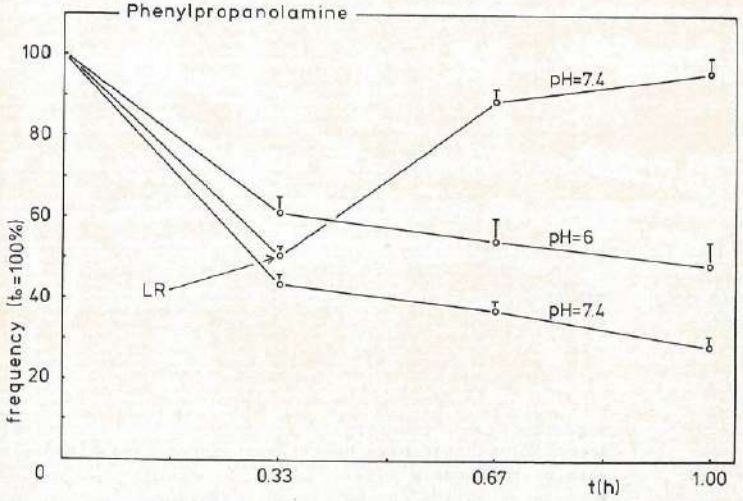

Fig. 3 Time versus frequency plot for phenylpropanolamine HCl 0.75% at pH 7.4 continuously, pH 7.4 washed after 20 min with LR and at pH 6.

Table II Effects of decongestants on the ciliary beat frequency

Compound	pH	Frequency a) t=0.33h	t=0.67h	t=1.00h	t95% b)	t90% c)
	7.4	82 d)	95	98	0.09	0.18
ephedrine HCl	7.4	74	66	63	0.06	0.13
0.5%	6	56	44	51	0.04	0.08
	5	48	40	43	0.03	0.06
	7.4	95 d)	96	99	0.30	
phenylephrine HCl	7.4	88	86	80	0.14	0.29
0.5%	6	77	64	62	0.07	0.14
	5	68	50	44	0.05	0.10
phenylpropanolamine	7.4	50 d)	89	95	0.03	0.07
HCl 0.75%	7.4	43	37	28	0.03	0.06
	6	61	54	48	0.04	0.09
	7.4	78 d)	101	100	0.08	0.15
xylometazoline HCl	7.4	76	63	50	0.07	0.14
0.05%	6	65	55	42	0.05	0.10
	5	54	48	37	0.04	0.07
	7.4	84 d)	96	99	0.11	0.21
oxymetazoline HCl	7.4	90	75	65	0.17	0.33
0.05%	6	64	59	52	0.05	0.09
	5	56	49	37	0.04	0.07
	7.4	18 d)	34	75	0.02	0.04
tramazoline HCl	7.4	28	11	0	0.02	0.05
0.117%	6	62	59	53	0.04	0.09
	5	50	48	45	0.03	0.07
	7.4	48 d)	79	92	0.03	0.06
naphazoline nitrate	7.4	52	33	23	0.03	0.07
0.1%	6	50	58	51	0.03	0.07
	5	42	35	32	0.03	0.06

a) Ciliary beat frequency as a percentage of the initial frequency, after 0.33, 0.67 and 1 h.
b) Time necessary to decrease the ciliary beat frequency to 95% of the initial value.
c) Time necessary to decrease the ciliary beat frequency to 90% of the initial value.
d) After this measurement the tissue was rinsed with LR and the experiment was continued in LR.

Discussion

The effects of the decongestants were investigated at only one concentration. Table III shows the concentrations that have been investigated and those that are recommended for adults by Martindale (1977).
Tramazoline HCl was investigated at its concentration in Rhinogutt as Martindale gives no data.
The concentrations chosen were close to those normally used for adults, and in such a way that the effects of chemically similar substances could be compared. Therefore, the same concentrations were used for ephedrine HCl and phenylephrine

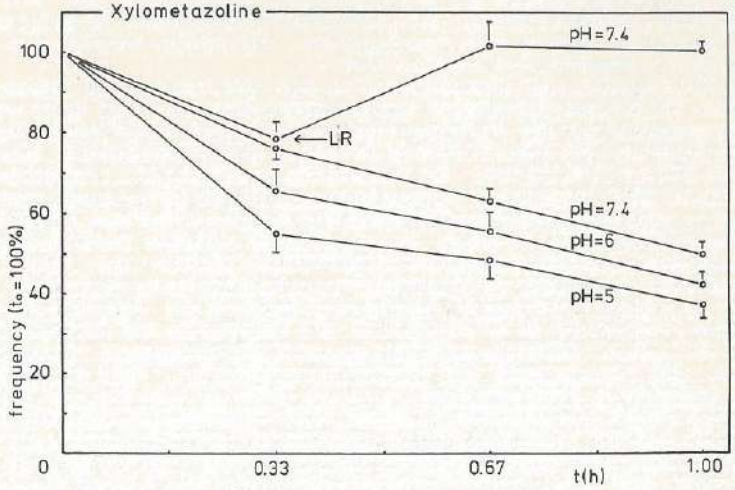

Fig. 4 Time versus frequency plot for xylometazoline HCl 0.05% at pH 5, 6 and 7.4 continuously and at pH 7.4 washed after 20 min with LR.

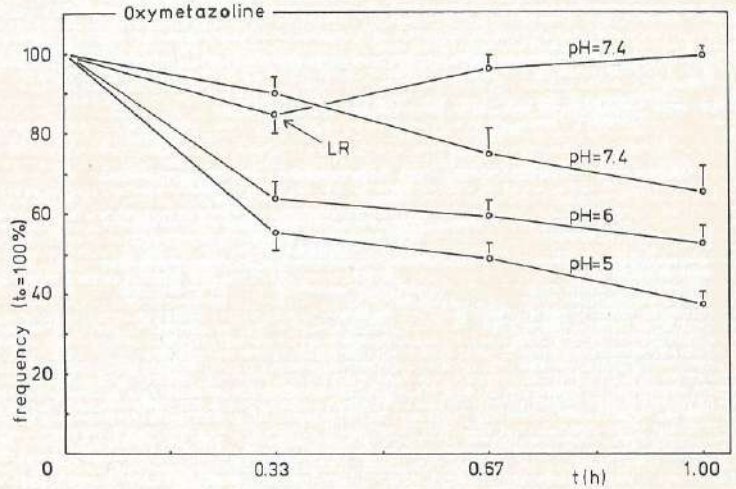

Fig. 5 Time versus frequency plot for oxymetazoline HCl 0.05% at pH 5, 6 and 7.4 continuously and at pH 7.4 washed after 20 min with LR.

HCl (0.5%) and for xylometazoline HCl and oxymetazoline HCl (0.05%). In the Netherlands phenylpropanolamine HCl is not used as a single agent but only in combination with a corticosteroid. Its concentration in Codelsol is 0.75% which was the concentration used in this investigation. Naphazoline nitrate was investigated at the concentration normally used.
From table II it appears that phenylephrine is less ciliotoxic than ephedrine. Phenylephrine HCl is more hydrophilic than ephedrine HCl; the Merck Index (1976) states that phenylephrine HCl is freely soluble and ephedrine HCl 1:4 in water. Also, oxymetazoline HCl (freely soluble in water) is more hydrophilic than xylometazoline HCl (1:33 in water). The hydrophilic substances are less ciliotoxic than their more lipophilic counter-parts, which is in agreement with our former results that lipophilic preservatives damage ciliary motility faster than polar preservatives (Van de Donk et al.,1980b). We also found that polar preservatives decrease ciliary movement irreversibly. However the effects of the decongestants, regardless of lipophilicity, were found to be reversible.
The effects on ciliary motility at pH lower than 7.4 can be expected to increase, since lowering the pH of LR resulted in a decrease in the frequency (Van de Donk et al.,1980a). This phenomenon is reversed for the naphtyl derivatives (naphazoline and tramazoline) and for phenylpropanolamine. This reversed pH-dependency could not be explained .
Hutcheon and Cullen (1955) found that at therapeutic concentrations phenylephrine was far less ciliotoxic than ephedrine, and ephedrine was to a limited extent less ciliotoxic than naphazoline at pH 7.2. The results of our study indicate that at equal concentrations phenylephrine is less ciliotoxic than ephedrine, and, since phenylephrine is used at lower concentrations than ephedrine, this difference in effect on ciliary movement will be more pronounced at therapeutic concentrations. This study shows that 0.1% naphazoline nitrate is slightly more ciliotoxic than 0.5% ephedrine HCl but as ephedrine HCl is often used at a concentration of 1% the difference in ciliotoxicity at therapeutic concentrations will be very small.

Conclusion

The effects of decongestants on the ciliary beat frequency appear to be very small.
Oxymetazoline and phenylephrine are the least ciliotoxic.

Table III Investigated and therapeutic concentrations

compound	concentration investigated	concentration for adults
ephedrine HCl	0.5%	0.5-2%
phenylephrine HCl	0.5%	0.25-0.5%
phenylpropanolamine HCl	0.75%	1-3%
xylometazoline HCl	0.05%	0.1%
oxymetazoline HCl	0.05%	0.05%
tramazoline HCl	0.117%	0.117%
naphazoline nitrate	0.1%	0.05-0.1%

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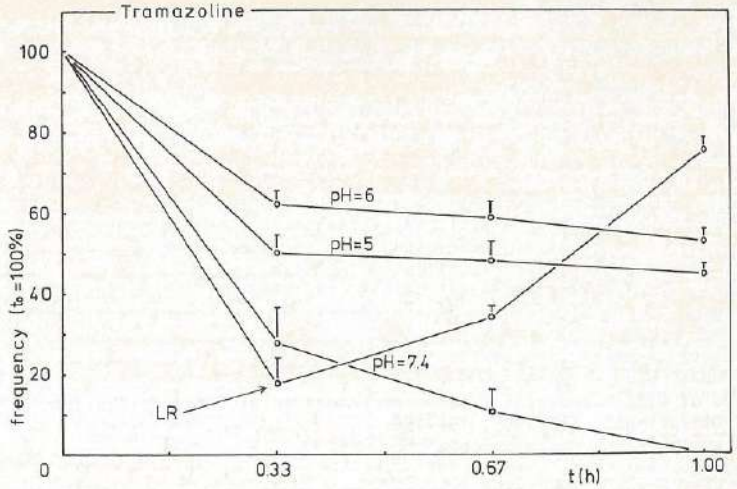

Fig. 6 Time versus frequency plot for tramazoline HCl 0.117% at pH 7.4 washed after 20 min with LR; and continuously at pH 7.4, 5 and 6.

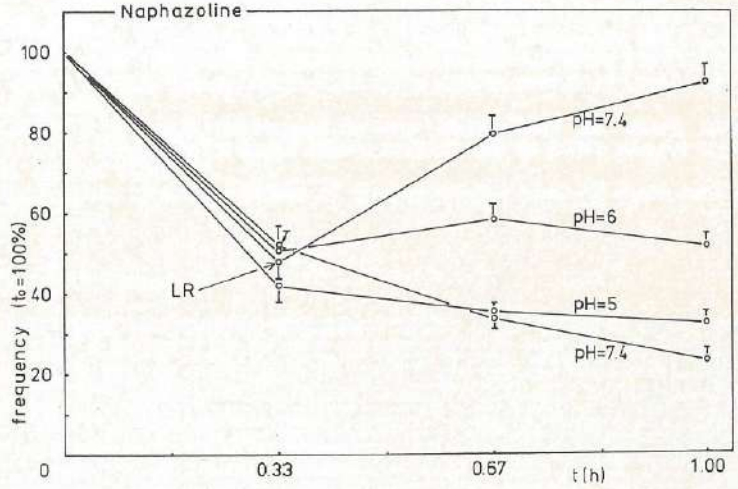

Fig. 7 Time versus frequency plot for naphazoline nitrate 0.1% at pH 5 continuously, at pH 7.4 washed after 20 min with LR; and continuously at pH 6 and 7.4.

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CHAPTER VII

THE EFFECTS OF DRUGS ON CILIARY MOTILITY

II ANTIMICROBIAL AGENTS

H.J.M. van de Donk, A.L.M. van Egmond, J. Zuidema and F.W.H.M. Merkus

Summary

The effects of antimicrobial agents at different pH's on the ciliary beat frequency of chicken embryo tracheas have been investigated.
Benzylpenicillin sodium 10,000 U/ml, ampicillin sodium 1%, neomycin sulphate 0.35%, polymyxin 0.1%, sulphanilamide sodium 0.4% and mild silver protein 0.5% do not decrease the ciliary beat frequency by more than 50% in 1 h.
Chloramphenicol 0.4%, bacitracin 10,000 U/ml and sulphacetamide sodium 10% arrest ciliary movement within 1 h, and only the effects of bacitracin were not reversible.
Lowering the pH of the antibiotic containing solutions aggravated their ciliotoxicity in proportion to the increase in effect of lowering the pH in agent-free Locke Ringer solution.

Introduction

Further to part I: "decongestants" (Van de Donk et al., 1), this study presents the effects of antimicrobial agents on the ciliary beat frequency of chicken embryo tracheas.
In general, local application of antimicrobial agents leads more frequently to sensibilisation and resistance than systemic administration. Moreover, natural defense by the ciliary epithelium might be decreased. Only a few authors have reported the effects of antimicrobial agents on ciliary motility: Greenwood et al. (1946) found no apparent deleterious effect for penicillin 200 and 500 U/ml on rabbit upper respiratory tract both in vivo and in vitro and Pacilio (1961) found ciliary arrest for neomycin 0.1% and a modest effect of bacitracin on frog palates in vitro.
The advantages of local application are the possibility of using antibiotics that are too toxic for systemic administration and to limit adverse reaction to a small area of the body.
This study deals with the effects of antimicrobial agents of which most are used in nasal drops.

Methods and materials

The ciliary beat frequency is determined with a photo-electric registration device at 25° C in Locke Ringer (LR) solution (Van de Donk et al., 1980). The effects of each drug at a fixed pH was assayed six times. During all the experiments a piece of tissue from the same trachea, and placed in pure LR served as a reference. Reversibility was studied at pH 7.4 by washing with LR after 20 min contact with the drug . Reversibility was only studied for those drugs that showed a substantial frequency decrease. Table I lists the substances investigated and their lot-numbers. Macrogol 400 is used as a solvent for chloramphenicol and, therefore, was also assayed.

Table I List of antimicrobial agents investigated and macrogol

substance	lot-number	manufacturer
benzylpenicillin sodium	78H04	Gist-Brocades
ampicillin sodium	79J01	Gist-Brocades
neomycin sulphate	108835/79F05	Brocacef
chloramphenicol	104104/75F24	Brocacef
sulphanilamide sodium	106906/79B07	Brocacef
sulphacetamide sodium	110159/79L31	Brocacef
polymyxin	901-61104	Pfizer
bacitracin	80C05D	Pharmachemie
mild silver protein	79L18-84389	OPG
macrogol 400	106852-77C14	Brocacef

Results

The effects of the drugs at different pH's on the ciliary beat frequency of chicken embryo tracheas are shown in table II. The effects at pH 7.4 of rinsing with LR after 20 min contact are also presented. The second column shows the pH, the next columns show the ciliary beat frequency as a percentage of the initial beat frequency after 20, 40 and 60 minutes. The contact time necessary to reduce the beat frequency to 95% and 90% of the initial beat frequency are listed in the last two columns. Figures 1-7 show the effects in more detail. The SEM values are indicated by vertical bars. The ciliary beat frequency of the references remained between 97% and 107% during all the experiments.

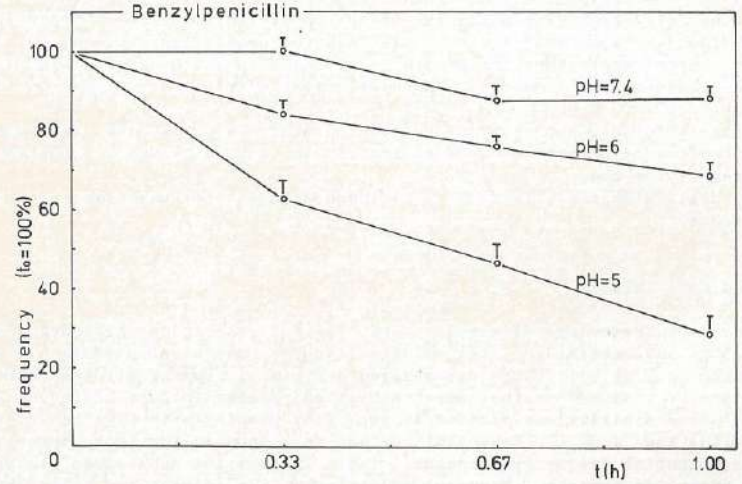

Fig. 1 Time versus frequency plot for benzylpenicillin sodium 10,000 U/ml; at pH 5, 6 and 7.4.

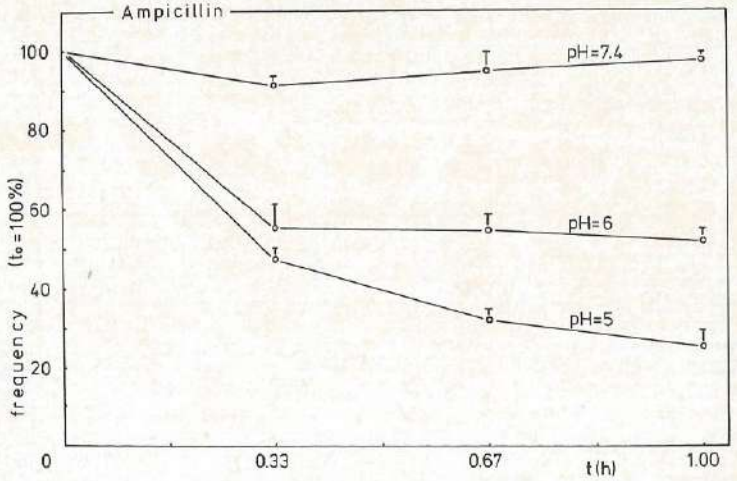

Fig. 2 Time versus frequency plot for ampicillin sodium 1%; at pH 5, 6 and 7.4

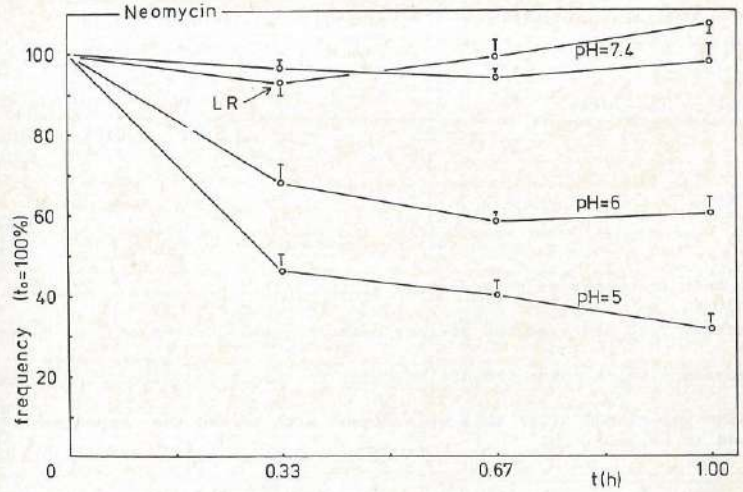

Fig. 3 Time versus frequency plot for neomycin sulphate 0.35%; at pH 5 and 6, at 7.4 washed after 20 min with LR and at pH 7.4 continuously.

Table II Effects of antimicrobial agents on the ciliary beat frequency

Compound	pH	Frequency a) t=0.33h	t=0.67h	t=1.00h	b) t95%	c) t90%
benzylpenicillin sodium	7.4	100	87	88	0.46	0.59
10,000 U/ml	6	84	76	69	0.10	0.21
	5	63	47	29	0.04	0.09
	7.4	91	95	97	0.19	
ampicillin sodium 1%	6	55	55	52	0.04	0.07
	5	47	52	25	0.03	0.06
	7.4	93 d)	99	107	0.23	
neomycin sulphate	7.4	96	94	97	0.48	
0.35%	6	68	58	60	0.05	0.10
	5	46	40	31	0.03	0.06
chloramphenicol 0.4%	7.4	1 d)	79	104	0.02	0.03
+ macrogol 5%	7.4	17	4	2	0.02	0.04
	6	2	0	0	0.02	0.03
macrogol 5%	7.4	75 d)	96	99	0.07	0.13
	7.4	77	75	71	0.07	0.14
	7.4	66 d)	95	105	0.05	0.10
sulphanilamide sodium	7.4	72	65	63	0.06	0.12
0.4%	6	51	43	38	0.03	0.07
	5	42	27	19	0.03	0.06
sulphacetamide sodium 10%	7.4	0 d)	43	79	0.017	0.033
polymyxin 0.1%	7.4	97	95	97	0.58	
bacitracin 10,000 U/ml	7.4	22	1 d)	0	0.02	0.04
	7.4	93 d)	105	105	0.24	
mild silver protein 0.5%	7.4	91	75	53	0.20	0.36
	8	98	91	80	0.49	0.70
	6	66	48	35	0.05	0.10
Locke Ringer	7–10	103	104	103		
solution	6	93	82	74	0.24	0.42
	5	82	62	54	0.09	0.19

a) Ciliary beat frequency as a percentage of the initial frequency, after 0.33, 0.67 and 1 h.
b) Time necessary to decrease the ciliary beat frequency to 95% of the initial value.
c) Time necessary to decrease the ciliary beat frequency to 90% of the initial value.
d) The tissue was rinsed after this measurement with LR and the experiment was continued in LR.

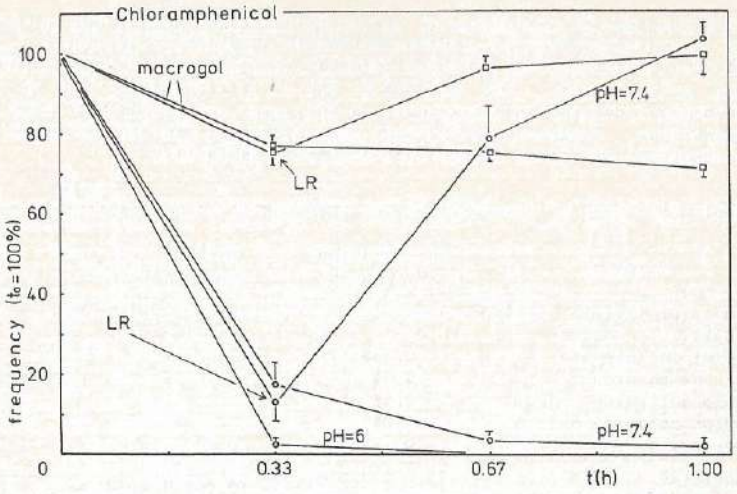

Fig. 4 Time versus frequency plot for chloramphenicol 0.4% + macrogol 5% at pH 6 and at 7.4 washed after 20 min with LR; at pH 7.4 continuously and for macrogol 5% only at pH 7.4 washed after 20 min with LR and at pH 7.4 continuously.

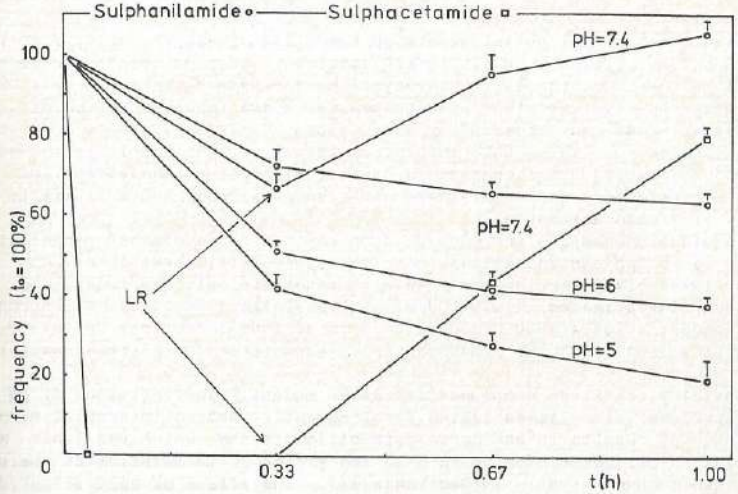

Fig. 5 Time versus frequency plot for sulphanilamide sodium 0.4% (○) at pH 5 and 6; at 7.4 washed after 20 min with LR and at pH 7.4 continuously and for sulphacetamide sodium 10% (□) at pH 7.4 washed after 20 min with LR.

Discussion

The concentrations of the antimicrobial agents investigated are listed in table III. As far as the antimicrobial agents present in nasal drops available in the Netherlands are concerned, they were investigated at the concentrations found in these nasal preparations. The effects on the ciliary beat frequency of some other antibiotics were also investigated. As they are not commonly used in nasal preparations, their concentrations in eye drops were investigated and these concentrations are given in the last column of table III.

Table III List of investigated and therapeutic concentrations

Compound	concentration investigated	therapeutic concentration
benzylpenicillin sodium	10,000 U/ml	2,500-10,000 U/ml a)
ampicillin sodium	1%	1% a)
neomycin sulphate	0.35%	0.35% b)
chloramphenicol	0.4%	0.4% b)
sulphanilamide sodium	0.4%	0.4% b)
sulphacetamide sodium	10%	10% b)
polymyxin	0.1%	0.1-0.25% b)
bacitracin	10,000 U/ml	500-1,000 U/ml a)c) 10,000 U/ml d)
mild silver protein	0.5%	0.5% b)

a) Concentration in eye drops, according to Martindale (1977).
b) Concentration in nasal drops in the Netherlands.
c) In combination with other antibiotics.
d) Concentration in eye drops, according to the "Informatorium Medicamentorum" (1980).

The effects of most antimicrobial agents on the ciliary beat frequency are very modest. Both penicillins show little ciliotoxicity. Neomycin and chloramphenicol inhibit protein synthesis, but neomycin hardly passes through cell membranes. Chloramphenicol, however, penetrates cells and is known to inhibit protein synthesis in eukaryotic cells to some extent and is indeed much more ciliotoxic than neomycin. Chloramphenicol is only slightly soluble in water, therefore 5% macrogol 400 has been added to the LR solution. The effect of macrogol is quite modest and highly reversible and will contribute little to the ciliotoxicity of chloramphenicol.
The effects of sulphonamides are more pronounced than those of the penicillins, but even at very high concentrations the effects are still reversible.
Bacitracin, like polymyxin, is hardly able to penetrate cell membranes, but the concentration investigated (10,000 U/ml) is very high. Bacitracin is normally used in a concentration of 500 U/ml and only in serious infections up to 10,000 U/ml. Bacitracin 10,000 U/ml appeared to depress ciliary activity dramatically and irreversibly.
The ciliotoxicity of silver compounds is rather modest. The influence of pH on ciliotoxicity was also investigated for frequently-used antimicrobial agents. Decreasing the pH results in an increase in ciliotoxicity, which was a bit more pronounced for all these substances than the effect of LR solution at the same pH values (Van de Donk et al., 1980a; table II). The effect of mild silver protein was investigated at pH 8 as it is mostly used at this pH.
The effects of benzylpenicillin are in agreement with the results of Greenwood et al. (1946).
The highest concentration of bacitracin (0.1%) investigated by Pacilio (1961)

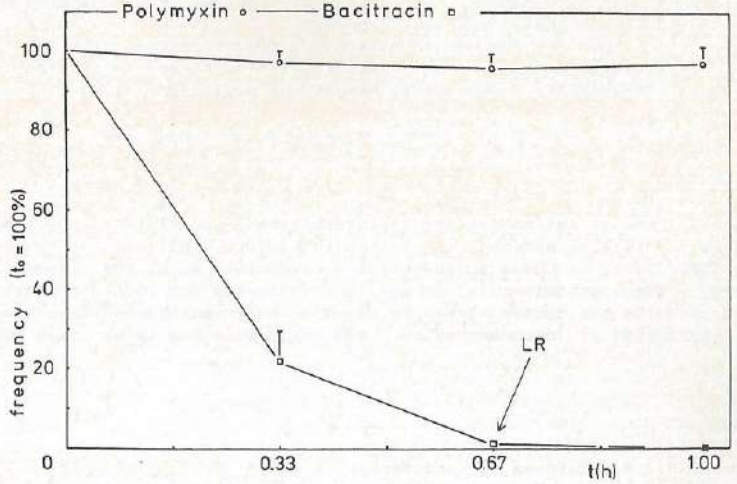

Fig. 6 Time versus frequency plot for polymyxin 0.1% (○) at pH 7.4 and for bacitracin 10,000 U/ml (□) at pH 7.4 washed after 40 min with LR.

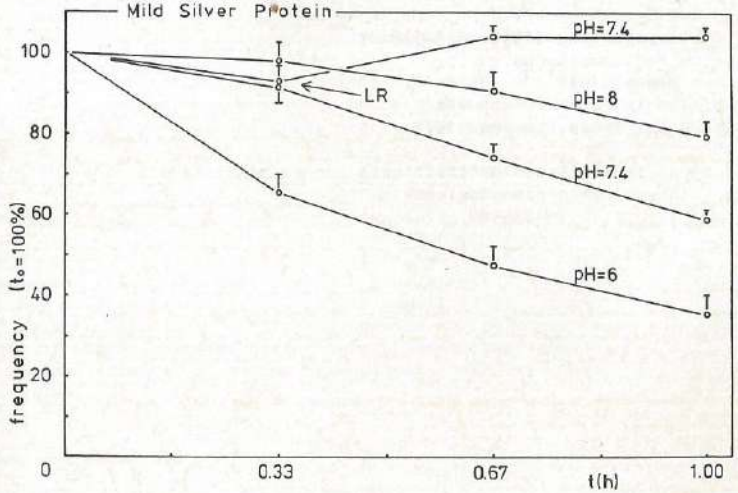

Fig. 7 Time versus frequency plot for mild silver protein 0.5%; at pH 6 and 7.4; at pH 7.4 washed after 20 min with LR and continuously at pH 8.

increased the time of transport over a piece of frog palate by 53% after 25 min contact. So there must have been some depression of ciliary activity but not ciliary arrest. However, our concentration was approximately 135 times higher. Neomycin 0.1% arrested ciliary motility within 25 min in Pacilio's experiments. This is in contrast with our results: hardly any ciliodepression with neomycin 0.35%.

Conclusions

The effects of the antimicrobial agents investigated on ciliary motility are moderate with the exception of bacitracin (10,000 U/ml) and chloramphenicol (0.4%). The effects of these substances are reversible with the exception of bacitracin. When antimicrobial drugs are indicated for local application on ciliated epithelia the choice should be based on their antibacterial action, but the ciliotoxicity of chloramphenicol and bacitracin has to be taken into account.

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CHAPTER VIII

THE EFFECTS OF DRUGS ON CILIARY MOTILITY

Part III: LOCAL ANAESTHETICS AND ANTI-ALLERGIC DRUGS

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Summary

The effects of local anaesthetics and anti-allergic drugs at different pH's on the ciliary beat frequency of chicken embryo tracheas have been investigated. The local anaesthetics arrested ciliary movement reversibly within 2.5 min at pH 7, whereas the antihistamines arrested ciliary movement irreversibly within 20 min at pH 7.4. Lowering the pH made both groups of drugs less ciliotoxic. The effects of a solution of prednisolone sodium phosphate are very limited, whereas solubilisation of dexamethasone in polysorbate decreases the ciliary beat frequency more than 60% in 20 min. The latter effect was due largely to the polysorbate. Cromoglycate sodium was found to be only slightly ciliotoxic.

Introduction

This final part, which continues from part I "decongestants" and part II "antimicrobial agents" (Van de Donk et al., 1 and 2), describes the effects of local anaesthetics and anti-allergic drugs on the ciliary beat frequency of chicken embryo tracheas. Local anaesthetics can come into contact with ciliated epithelia when used before or during nasal surgery and bronchoscopy, when added in low concentrations to nasal drops or when injected into the spinal cord from where it may reach the ciliated ependymal lining of the brain and the spinal cord.
The drugs used in the therapy of allergic diseases are antihistamines, corticosteroids and sodium cromoglycate. Antihistamines are used in nasal drops often in combination with a decongestant; corticosteroids and sodium cromoglycate are used in preparations intended for the lower as well for the upper respiratory tract. These drugs have received little attention until now as far as their effects on ciliated epithelia are concerned. The effects of local anaesthetics (lignocaine and others) have been investigated by Manawadu et al. (1978) and Mastow et al. (1979). The study presented here describes the effects of lignocaine, cocaine and butacaine. The latter two were not included in the studies of Manawadu et al. and Mastow et al. The effect of only one antihistamine (antazoline) has been investigated by Mirimanoff (1969); the study presented here also reveals the effects of diphenhydramine, tripelennamine and antazoline. The effects of corticosteroids have not been investigated until now as far as we know. The effects of sodium cromoglycate have been investigated by Blair and Woods (1969).

Methods and materials

The ciliary beat frequency is determined with a photo-electric registration device at 25° C in Locke Ringer (LR) solution (Van de Donk et al., 1980). The effects of each drug at a fixed pH were assayed six times. The local anaesthetics were dissolved in enough distilled water to obtain an iso-osmotic solution, which was diluted with a solution containing 2.5% dextrose and 0.45% NaCl, which also served as a reference. The local anaesthetics were investigated in a medium containing only NaCl and dextrose because the $CaCl_2$ of LR precipitates buta-

caine sulphate. Cocaine HCl appeared to precipitate above pH 7. Reversibility was investigated by washing with LR or with a solution containing 0.45% NaCl and 2.5% dextrose (reference). Table I lists the substances investigated and their lot-numbers.

Table I List of substances investigated

substance	lot-number	manufacturer
lignocaine HCl	112015/80H30	Brocacef
cocaine HCl	111089/79J08	Brocacef
butacaine sulphate	118C-0479	Sigma
diphenhydramine HCl	108814/78K24	Brocacef
tripelennamine HCl	103834/74H30	Brocacef
antazoline HCl	109380/79I10	Brocacef
prednisolone sodium phosphate	5644 1111	Organon
dexamethasone	109567/79F13	Brocacef
sodium cromoglycate	025980P760	Fisons
polysorbate	104215/74L30	Brocacef

Results

The effects of the drugs at different pH's on the ciliary beat frequency of chicken embryo tracheas are shown in table II. The effects at pH 7.4 of rinsing with LR after 20 min contact (for the local anaesthetics, at pH 7 and rinsing with dextrose 2.5% + NaCl 0.45% after 5 min contact) have been assayed as well. The second column shows the pH, the next columns show the ciliary beat frequency as a percentage of the initial frequency after 2.5, 5, 10, 20, 40 and 60 min. The contact time necessary to reduce the beat frequency to 95% and to 90% of the initial beat frequency are indicated in the last two columns. Figures 1-8 show the effects in more detail. The SEM values are indicated by vertical bars. The effects of the pH of pure LR have been added to table II (Van de Donk et al., 1980). The ciliary beat frequency of the trachea rings in LR remained between 96% and 106% during all experiments.

Discussion

The effects on the ciliary beat frequency of the local anaesthetics were quite severe but they were reversible; the reversibility diminished from lignocaine, cocaine to butacaine. The effects at pH 6 were small compared to the effects at pH 7. However, the effects of butacaine were still much more severe than those of lignocaine and cocaine.
The antihistamines were all very ciliotoxic and irreversible . Diphenhydramine appeared to have the strongest effects, whereas antazoline was the least ciliotoxic of the three antihistamines investigated. Lowering the pH diminished the effects on the ciliary beat frequency, as was found for the local anaesthetics. This can be explained by the fact that in both groups of drugs pKa values range from 8 to 10. Under such conditions it applies that the lower the pH, the larger the protonated fraction and the smaller the fraction of the drug that diffuses through the cell membranes.
Both Manawadu et al. (1978) and Mastow et al. (1979) found irreversible ciliary arrest within 30 min for 2% lignocaine on ferret tracheal rings. However, Mastow et al. performed their experiments at pH 6.5 to 7. Manawadu et al. do not give information on the pH. In both studies the rings were not washed earlier than after 1 h contact, whereas we washed the tissues after 5 min contact. Mirimanoff (1969) found an immediate decrease in frequency for a gel containing

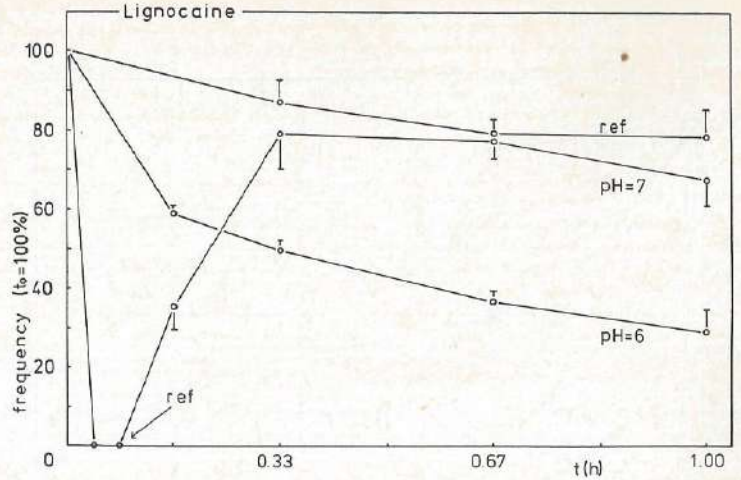


Fig. 1 Time versus frequency plot for lignocaine HCl 2%; at pH 7 washed after 5 min with the reference and continuously at pH 6 and for the reference.

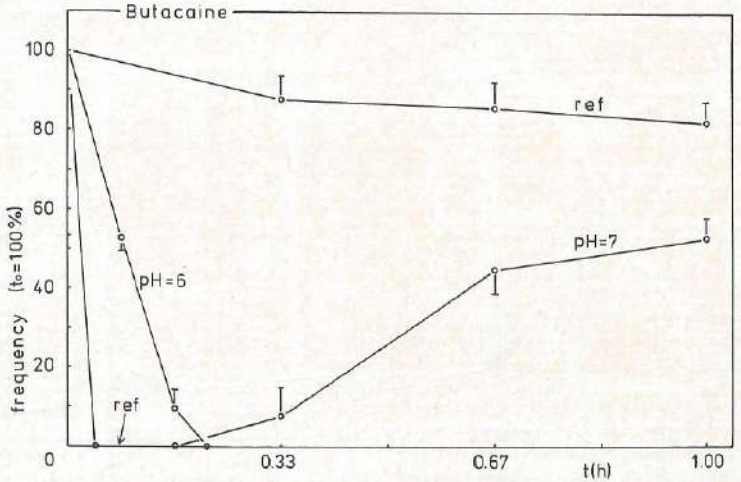


Fig. 2 Time versus frequency plot for butacaine sulphate 2%; at pH 7 washed after 5 min with the reference and continuously at pH 6 and for the reference.

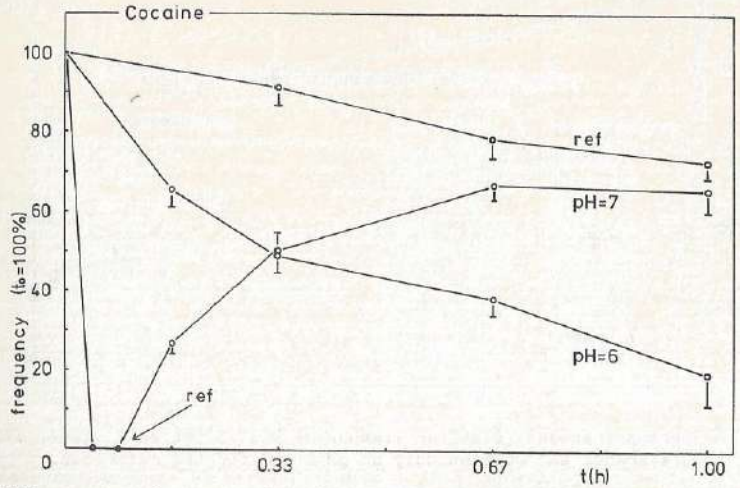


Fig. 3 Time versus frequency plot for cocaine HCl 5%; at pH 7 washed after 5 min with the reference and continuously at pH 6 and for the reference.

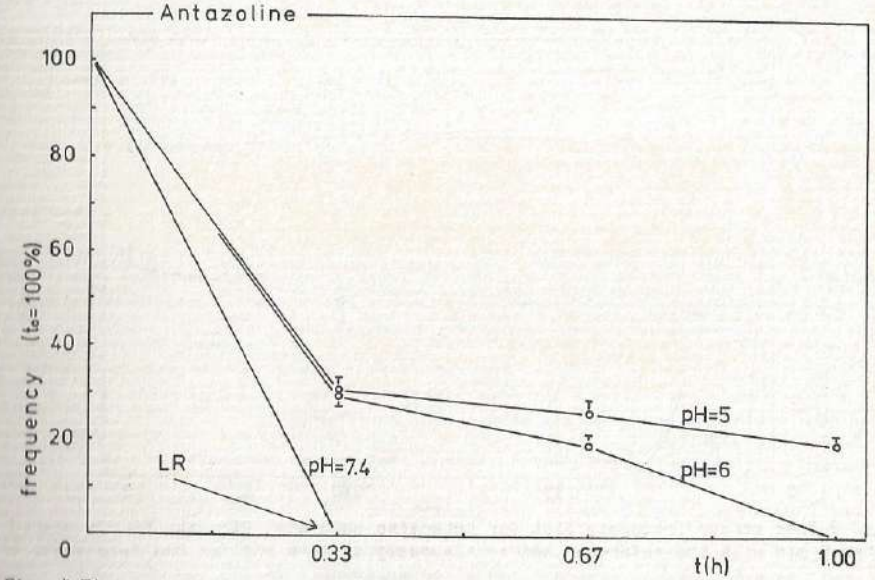
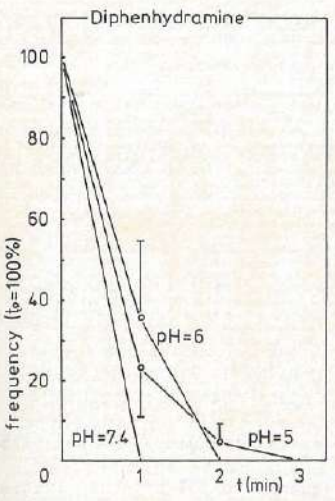


Fig. 4 Time versus frequency plot for antazoline HCl 0.5%; at pH 7.4 washed after 20 min with LR and continuously at pH 6 and 5.

0.5% antazoline (pH 6.9) on guinea pig tracheas, but even after 1 h contact ciliary arrest appeared in only 3 of 10 experiments. Probably the high viscosity prevented intensive contact between the drug and the tissue. The benefit of administrating antihistamines intranasally is doubtful.
Cirillo and Tempero (1976) reported the occurrence of severe irritative rhinitis and allergic reactions. Moreover, no information on the therapeutic effects of such preparations can be found. This, added to their ciliotoxicity, indicates that the intranasal application of antihistamines should be discouraged.
The corticosteroids have a very modest and reversible effect on the ciliary beat frequency. In comparison with the soluble salts (prednisolone sodium phosphate, dexamethasone sodium phosphate) , the effects on ciliary motility of the pure corticosteroids solubilisated in polysorbate 80 are much worse. This difference, however, is caused largely by the polysorbate (fig 7). The polysorbate effect is reversible. Solubilisation of corticosteroids is often used because the salts tend to precipitate at pH<7.5. This makes the combination of a corticosteroid salt and a decongestant impossible. The effects on ciliary motility at different pH's are almost equal.
Sodium cromoglycate exerts at different pH's very little influence on the ciliary beat frequency and the effects are reversible. Also, Blair and Woods (1969) found hardly any effect for sodium cromoglycate on the ciliary beat frequency of cat trachea.

Fig. 5 Time versus frequency plot for diphenhydramine HCl 2%; at pH 7.4, 5 and 6.



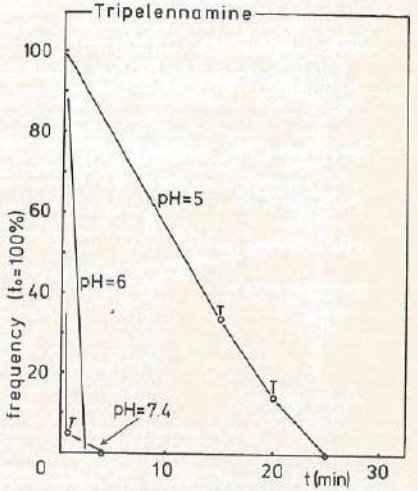


Fig. 6 Time versus frequency plot for tripelennamine HCl 2%; at pH 7.4 washed after 5 min with LR and continuously at pH 6 and 5.

Table II Effects on the ciliary beat frequency

compound	pH	Frequency a) 2.5 0.04	5 0.08	10 0.16	20 0.33	40 0.67	60 min 1.00 h	b) t95%	c) t90%
lignocaine HCl 2%	7	0	d)	36	79	77	67	0.00	0.00
	6			59	50	37	29	0.02	0.05
butacaine sulphate	7	0	d)	0	8	45	53	0.00	0.00
2%	6		51	10	0			0.01	0.02
cocaine HCl 5%	7	0	d)	27	50	67	65	0.00	0.00
	6			66	49	39	20	0.02	0.05
reference	7				87	81	78	0.13	0.26
antazoline HCl	7.4				0 e)	0	0	0.02	0.03
0.5%	6				29	19	0	0.02	0.03
	5				31	26	20	0.02	0.05
diphenhydramine	7.4	0	e)		0	0	0	0.00	0.00
HCl 2%	6	0						0.00	0.00
	5	3	0					0.00	0.00
tripelennamine	7.4	2	e)		0	0	0	0.00	0.00
HCl 2%	6	0						0.00	0.00
	5			58	14			0.02	0.04
prednisolone	7.4				93 e)	102	105	0.24	
sodium	7.4				91	87	86	0.19	0.43
phosphate	8				97	99	98		
0.1%	9				95	93	94	0.35	
dexamethasone	7.4				39 e)	75	89	0.03	0.05
0.01% +	7.4				36	33	26	0.03	0.05
polysorbate	6				34	33	16	0.03	0.05
0.4%	5				32	23	14	0.02	0.05
polysorbate 0.4%	7.4				48	39	37	0.03	0.06
cromoglycate	7.4				98 e)	107	109		
sodium	7.4				96	93	84	0.47	0.77
2%	8				85	88	81	0.11	0.22
	6				65	61	67	0.05	0.10
Locke Ringer	7-10				103	104	103		
solution	6				93	82	74	0.24	0.42
	5				82	62	54	0.09	0.19

a) Ciliary beat frequency as a percentage of the initial frequency, after 2.5, 5, 10, 20, 40 and 60 min.
b) Time necessary to decrease the ciliary beat frequency to 95% of the initial value.
c) Time necessary to decrease the ciliary beat frequency to 90% of the initial value.
d) The tissue was rinsed with NaCl 0.45% + dextrose 2.5% after 5 min and the experiment was continued in this medium.
e) The tissue was rinsed with LR at the indicated time and the experiment was

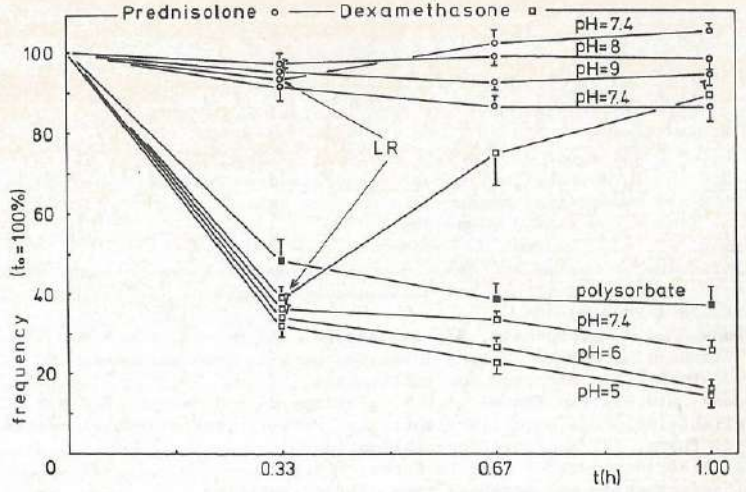


Fig. 7 Time versus frequency plot for prednisolone sodium phosphate (o) 0.1% at pH 7.4; at pH 7.4 washed after 20 min with LR and at pH 9 and 8; and for dexamethasone 0.01% + polysorbate 0.4% (□) at pH 5, 6 and 7.4 and at pH 7.4 washed after 20 min with LR; and for polysorbate 0.4% (■) at pH 7.4.

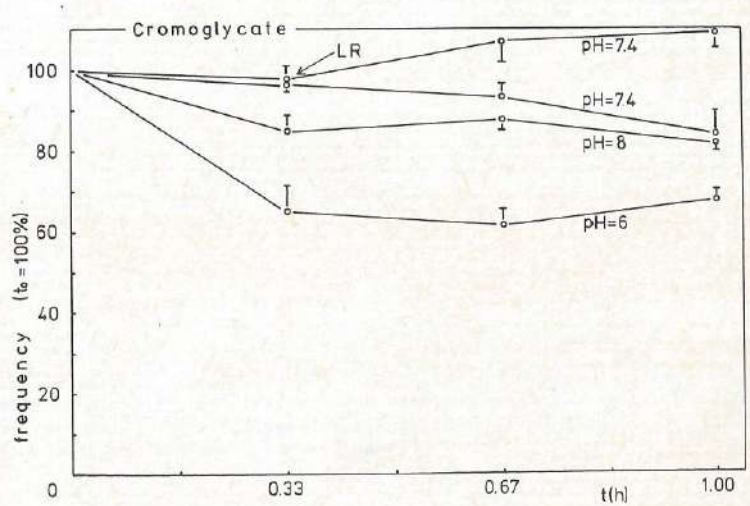


Fig. 8 Time versus frequency plot for cromoglycate sodium 2% at pH 6, 8 and 7.4 and at pH 7.4 washed after 20 min with LR.

continued in LR.

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CHAPTER IX

INTRANASAL ADMINISTRATION OF PROPRANOLOL DECREASES CILIARY BEAT FREQUENCY

H.J.M. van de Donk and F.W.H.M. Merkus

Abstract

Recently the intranasal application of 5% propranolol was proposed in order to prevent the extensive first pass metabolism of this drug.
The ciliary epithelium in the nose effects the removal of dust, allergens and microorganisms. With a photo-electric registration device the decreasing effect of propranolol on the ciliary beat frequency of human adenoid tissue and chicken embryo tracheas was measured. After nasal application of 5% propranolol, the drop is diluted by nasal mucus. It was found that 0.1% propranolol had a deleterious effect on the cilia of chicken and human epithelial tissue. Ciliary movement was arrested irreversibly within 20 minutes.

Introduction

The extensive use of nasal drops urged us to investigate the effects of nasal medication on the ciliary beat frequency. The nasal ciliary epithelium effects the removing of dust, allergens and microorganisms, which are precipitated after inhalation. This "nasal clearance" is a physiological defense mechanism that should not be disturbed. A method has been developed to investigate the influence of drugs on the ciliary beat frequency (1). With this method the ciliary beat frequency of chicken embryo tracheas is measured with a photo-electric registration device. The effects of preservatives (2) and nasal drops (3) have already been investigated. A good correlation has been found between the ciliary beat frequency of ciliary epithelium of human adenoids and of chicken embryo tracheas (4).
In order to prevent the first pass effect of propranolol after oral administration Hussain et al. (5) suggested recently the intranasal application of this drug. As propranolol is used in chronic therapies the effects of the intranasal application on the ciliary beat frequency may be important. We therefore investigated the effect of propranolol on the ciliary beat frequency of chicken embryo tracheas and human adenoid tissue.

Experimental

The effects of a solution containing 1% propranolol hydrochloride (B.P. 0504001/4 PO 82 1A ICI), made isotonic with sodium chloride and of a 10-fold dilution of this solution in Locke Ringer, were investigated. The pH of both solutions was adjusted at 7.4. The effects on the ciliary beat frequency was assessed on six different tracheas for each concentration and for the reference (Locke Ringer), and on pieces of six different human adenoids for the 10-fold dilution and the reference (Locke Ringer).

Results

The effects of 1% and 0.1% propranolol are demonstrated in table I. The frequencies are listed as a percentage of the frequency just before the start of the experiments. The solution containing 1% propranolol arrested ciliary movement of chicken embryo tracheas within 2 minutes.

Table I The decreasing effect of propranolol on the ciliary beat frequency of chicken embryo tracheal epithelium and human adenoid epithelium.

compound	frequency a) t=2 min	t=10 min	t=20 min	species
propranolol HCl 1%	0%	0%	0%	chicken
propranolol HCl 0.1%	70%	8%	0%	chicken
propranolol HCl 0.1%	77%	25%	0%	human

a) Frequency as a percentage of the initial frequency

The 10-fold dilution arrested the ciliary movement of both human adenoids and chicken embryo tracheas within 20 minutes. This effect was irreversible: rinsing with Locke Ringer solution after a 20 minutes' contact with 0.1% propranolol did not restore ciliary movement within 2 hours.
The effects of 0.1% propranolol are shown in fig. 1 in more detail. The SEM values are indicated by vertical bars.

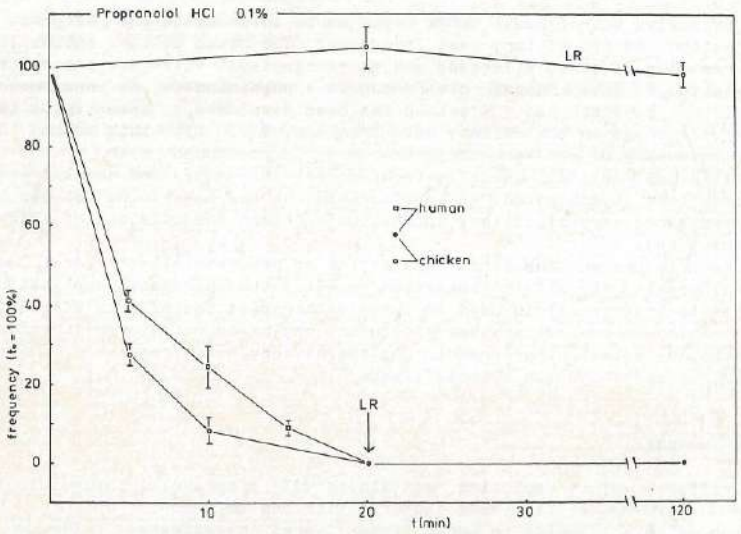

Fig. 1 Time versus ciliary beat frequency plot: effect of 0.1% propranolol HCl on the cilia of human adenoids and chicken embryo tracheas. LR was used as a reference.

Discussion

Hussain et al. suggested the use of a nasal drop containing 5% propranolol. This nasal drop is diluted by the nasal mucus after application. Therefore we started with 1% propranolol which had a deleterious effect on ciliary movement of chicken embryo tracheas. But even 0.1% propranolol arrested the ciliary movement of chicken and human cilia irreversibly within 20 minutes. It is not likely that the nasal drop will be diluted more than 50 times within 20 minutes,

especially since it interferes with the nasal clearance.
For repeated intranasal administration of propranolol, its ciliotoxicity should be taken into account.

Acknowledgement

The authors wish to thank mrs. N. Verhoeven for her assistance in performing the experiments, dr. N. van Proosdij (R.C. Hospital, Sittard) for supplying the human adenoids and mrs. B. Eckmann for secretarial work.

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CHAPTER X

BENZALKONIUM CHLORIDE IN MORE DETAIL

Summary

In previous chapters benzalkonium chloride with EDTA has been advised for the preservation of nasal drops. This chapter describes its ciliotoxicity in more detail. The effects of benzalkonium chloride 0.006% + EDTA 0.1% on the ciliary beat frequency of chicken embryo tracheas have therefore been investigated at different pH's. The t50% (time necessary to decrease the ciliary beat frequency by 50%) is 0.57, 0.25 and 0.22·h for pH 7, 6 and 5 respectively.
The effects of hypromellose 1% are modest: the maximal frequency decrease is 20% which is achieved within 0.33 h. The combination of hypromellose 1% and benzalkonium chloride 0.006% + EDTA 0.1% is as ciliotoxic as the preservative alone. The effects of this combination appeared to be reversible in contrast with the effects of benzalkonium chloride + EDTA alone. The combination of hypromellose 1% and chlorbutol 0.5% is as ciliotoxic as chlorbutol alone.
The effects on the ciliary beat frequency of exposure, twice a day during two days to chlorbutol and to benzalkonium chloride + EDTA appeared to be almost equal. But since chlorbutol decreases the nasal clearance more and hence increases its contact time with the ciliated epithelia, benzalkonium chloride + EDTA is still to be preferred for the preservation of nasal drops.

Introduction

In a former chapter benzalkonium chloride + EDTA has been recommended for the preservation of nasal drops. This preference was based upon "single dose" in vitro experiments, without further additives, in which a slow but irreversible effect on ciliary movement was found. This was in contrast with the effects of chlorbutol which gave a very rapid, but reversible effect after a short contact (Van de Donk et al.,1980b). Nasal drops are mostly used in a two or three times a day dosing schedule. In nasal drops the preservatives are often used in the presence of viscosity-enhancing substances such as hypromellose at pH values fixed by the stability requirements of the drug. Ionized substances often have a pH-dependent effect on ciliary activity: the more the substances are ionized, the less the ciliotoxic effect. This study has been performed to gather additional data on the influence of pH and hypromellose on the effects of benzalkonium chloride + EDTA. Also a "multiple-contact" experiment was done to investigate the longer term effects.

Methods and Materials

The ciliary beat frequency was determined with a photo-electric registration device at 25° C in Locke Ringer (LR) solution on chicken embryo tracheas. The effects of benzalkonium chloride (USP XVII,OPG Utrecht) 0.006% + EDTA 0.1% were investigated at pH 5, 6 and 7. Benzalkonium chloride 0.006% anhydrous = benzalkonium chloride 0.01% commercial quality. The influence of increased viscosity was studied with hypromellose (44354 72D12,OPG,Utrecht). Hypromellose 1%, hypromellose 1% + benzalkonium chloride 0.006% + EDTA 0.1% and hypromellose 1% + chlorbutol (74723 58177,OPG,Utrecht) 0.5% were studied. Reversibility was investigated by rinsing with LR after 20 min with the exception of chlorbutol where the tissues were rinsed after 5 min (because of the rapid ciliary arrest by chlorbutol). The effects of repeated exposures were investigated by bringing the tissues in contact with 1=5 dilutions of benzalkonium chloride 0.006% + EDTA 0.1% or of chlorbutol 0.5% at 0, 5, 24 and 29 h and rinsing with LR 20 min later

each time. All experiments were performed six times and during all experiments a piece of tissue in pure LR served as a reference.

Results

The effects of benzalkonium chloride 0.006% + EDTA 0.1% at pH 5, 6 and 7 are shown in figure 1. The ciliary beat frequencies, as a percentage of the initial beat frequency are listed after 0.33, 0.67 and 1 h contact in table I. Finally, the time necessary to decrease the ciliary beat frequency by 50% is given. The effects of hypromellose 1% alone and combined with benzalkonium chloride 0.006% + EDTA 0.1% or chlorbutol 0.5% are shown in fig 2. The reversibility was investigated as well: hypromellose alone and combined with benzalkonium chloride + EDTA were rinsed with LR after 20 min; hypromellose + chlorbutol was rinsed with LR after 5 min. Table I shows the ciliary beat frequencies at different times for hypromellose alone and also combined with one of the two preservatives. The ciliary beat frequency of the references in the experiments mentioned so far remained between 96% and 105%. The effects of immersion in benzalkonium chloride 0.006% + EDTA 0.1% (1=5 diluted) and in chlorbutol 0.5% (1=5) for 20 min followed by rinsing with LR twice a day during 48 h are shown in figure 3. Table II summarizes the ciliary beat frequencies for both preservatives at different times. The SEM values are indicated by vertical bars in figures 1-3.

Table I Effects on the ciliary beat frequency

Compound	pH	Frequency a) t=0.33h	t=0.67h	t=1h	t50%(h) b)
benzalkonium chloride	7	62	45	20	0.57
0.006% + EDTA 0.1%	6	32	15	3	0.25
	5	24	0	0	0.22
hypromellose 1%	7.4	80	81	81	>2
	7.4 c)	80	97	100	>2
hypromellose 1% + benz.	7.4	63	48	35	0.62
0.006% + EDTA 0.1%	7.4 c)	63	92	96	>2
		t=5min	t=10min	t=20min	t=25min	t50% (h)
hypromellose 1% + chlorbutol 0.5%	7.4	0 c)	25	68	85	0.04

a) Ciliary beat frequency as a percentage of the initial frequency.
b) Time necessary to decrease the ciliary beat frequency to 50% of the initial value.
c) The tissue was rinsed after this measurement with LR and the experiment was continued in LR.

Discussion

The ciliotoxicity of benzalkonium chloride 0.006% + EDTA 0.1% increased steadily with decreasing pH from 7 to 5, parallel with the effects in pure LR solution. Gallay (1960) found a strong increase in ciliotoxicity from pH 7 to 6.7: benzalkonium chloride 0.0125% arrested ciliary movement in 45 min and 13 min, respectively. In a benzalkonium chloride-free reference this author found ciliary arrest at pH 7 and 6.5 occurring within 60 and 25 min respectively. Unfortunately, Gallay did not give any details about the additives used such as buffers.
The maximum decrease in the ciliary beat frequency for hypromellose 1% was 20% and was reached within 0.33 h. The effects were reversible as appeared from

Table II Effects on the ciliary beat frequency during multi-exposure

Time min	Time hours	Frequency a) benzalkonium chloride 0.006% + EDTA 0.1% (1=5)	chlorbutol 0.5% (1=5)	b) pres.-LR
0	0.00	100	100	pres.
20	0.33	91	7	LR
40	0.67	95	102	
300	5	75	98	pres.
320	5.33	70	17	LR
340	5.67	63	99	
1440	24	43	67	pres.
1460	24.33	44	13	LR
1480	24.67	41	65	
1740	29	35	29	pres.
1760	29.33	26	5	LR
1780	29.67	23	30	
2880	48	0	0	

a) Ciliary beat frequency as a percentage of the initial frequency.
b) pres. and LR indicate whether the tissues were immersed in a solution containing the preservative or were rinsed with LR, respectively.

washing with LR after 0.33 h. These effects in vitro are modest in contrast with those in vivo (Van de Donk et al., 1). However, increasing of the viscosity of the mucus will not only decrease the ciliary beat frequency but will also increase the tendency of the mucus layer to lag behind the ciliary movement. The combination of hypromellose 1% and benzalkonium chloride 0.006% + EDTA 0.1% was approximately as ciliotoxic as benzalkonium chloride + EDTA alone. The effects of benzalkonium chloride + EDTA when combined with hypromellose were reversible as appeared from washing after 0.33 h with LR however, after more than one h the beat frequency tends to decrease again (fig 2). The increase in viscosity is likely to impede the ciliary movement on the one hand but seems, on the other, to diminish the uptake of benzalkonium chloride from the gel by the cell membranes. Another explanation might be that if benzalkonium chloride did penetrate the cell membrane, leakage of the cell contents is impeded by the increased viscosity of the medium around the cell. This protective effect of hypromellose has not been found in our in vivo experiments. The effects of benzalkonium chloride 0.006% + EDTA 0.1% and hypromellose 1% on the nasal transport time were almost additive when combined (Van de Donk et al., 1). Hypromellose 1% + chlorbutol 0.5% arrested ciliary movement within 5 min and the effects were reversible when washed after 5 min with LR. Both effects are similar to those of chlorbutol 0.5% alone (Van de Donk et al., 1980b). The ciliary arrest by chlorbutol is achieved in such a short time because the diffusion of this lipophilic compound occurs so easily that the viscosity of the medium appears to be of no relevance.
The repeated exposure to 1=5 dilutions of chlorbutol 0.5% and benzalkonium chloride 0.006% + EDTA 0.1% showed a steady decrease of the ciliary beat frequency with an arrest after 48 h for the preservatives as well as for the LR solution (fig 3). A single exposure to chlorbutol had a far more dramatic effect than one exposure to benzalkonium chloride + EDTA, both in vitro and in vivo (Van de Donk et al., 1980b, 1). Since the effect in vitro of benzalkonium chloride + EDTA is irreversible and the effect of chlorbutol is reversible (provided the exposure time is short), it would be interesting to know what the effects are after multiple exposures.

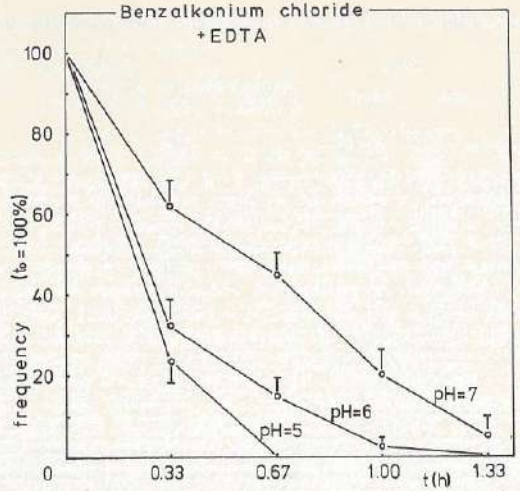

Fig. 1 Time versus frequency plot for benzalkonium chloride 0.006% + EDTA 0.1% at pH 7, 6 and 5.

As is shown in fig 3 the second and third exposure were in favour of chlorbutol, but at the fourth exposure the effects of both preservatives were well-balanced. Moreover at 29 h the ciliary beat frequencies of the tissues exposed three times to chlorbutol were lower than those exposed three times to benzalkonium chloride + EDTA.

Still, two important differences in vivo remain: nasal clearance will, at least temporarily, be more decreased by chlorbutol, as a result of the strong frequency decrease, than by benzalkonium chloride which will lead to a longer exposure to the former than to the latter preservative. Too long a contact may cause the effects of chlorbutol to become irreversible as well.

The second point to keep in mind is that our model cannot simulate recovery mechanisms by cell-replacement nor the possible protective effects of the mucus layer. Such effects will have more chance with mild substances. Until more is known about these phenomena the less damaging effects of benzalkonium chloride + EDTA on ciliary epithelia justify our preference for this preservative.

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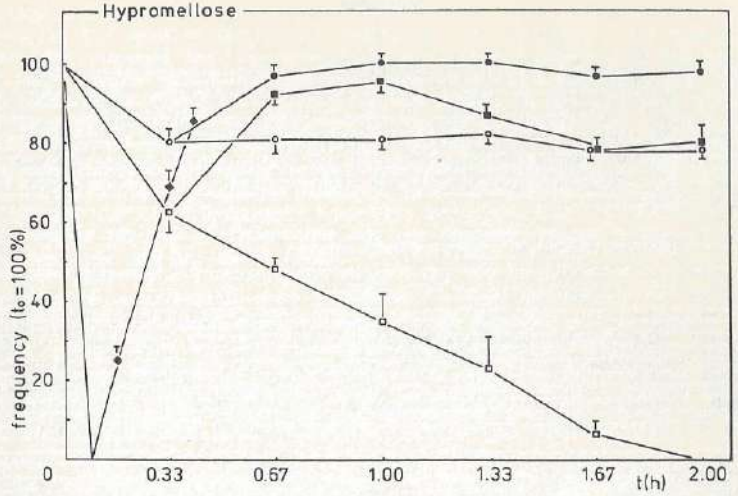

Fig. 2 Time versus frequency plot for hypromellose 1% (○) and rinsed with LR after 0.33 h (●); hypromellose 1% + benzalkonium chloride 0.006% + EDTA 0.1% (□) and rinsed with LR after 0.33 h (■) and hypromellose 1% + chlorbutol 0.5% rinsed with LR after 5 min (◆).

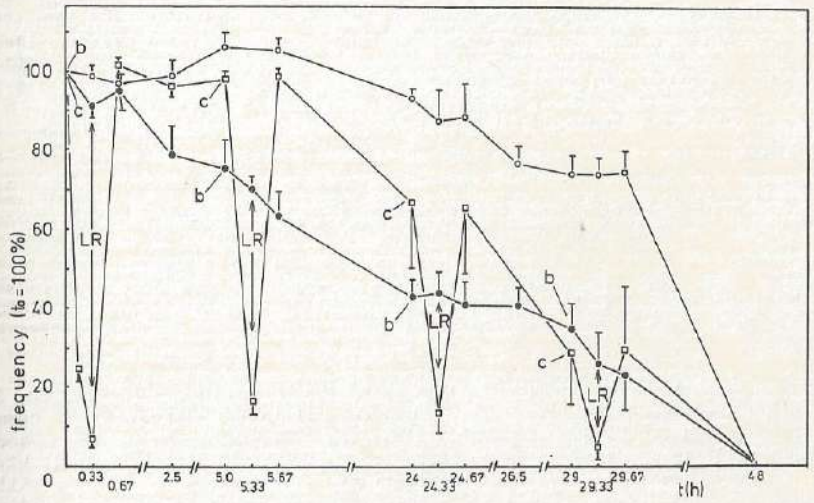

Fig. 3 Time versus frequency plot for LR (○); benzalkonium chloride 0.006% + EDTA 0.1% (1=5,●) and chlorbutol 0.5% (1=5,□) applied at 0, 5, 24 and 29 h, both rinsed with LR at 0.33, 5.33, 24.33 and 29,33 h.

SUMMARY

The surface in the nasal cavity is covered with ciliary epithelium which cleans the nose by a continuous stream of mucus towards the throat. This nasal clearance ought to be affected as little as possible by nasal medications. Therefore the effects of nasal drops and their constituents on ciliary activity and nasal clearance have been investigated. With the presented results the pharmaceutical formulation of these medications can be improved with respect to their effects on ciliary activity.

Method: the photo-electrical registration device chap. I
A light beam is transmitted through the moving cilia and after magnification by a microscope the flickering light is projected on a photo-cell. The electrical signal, generated by this photo-cell is visualized by an oscilloscope and the frequency of this signal is estimated electronically and displayed. In this way, the ciliary beat frequency of ciliated epithelia in vitro can be measured. The chicken embryo trachea has been chosen as the test-tissue. It is more intact than tissues of older specimens and it is easy to obtain. The device appeared to be easy to handle and allows reliable studies of ciliated epithelia.

pH and osmotic pressure chap. I
The values of pH and NaCl concentrations at which the ciliary beat frequency reached a maximum have been established: pH 7-10 and 0.9% NaCl.

Preservatives chap. II
The effects on the ciliary beat frequency of preservatives, often used in nasal drops, have been investigated. The mercury containing preservatives are most ciliotoxic. They decrease the frequency rapidly and irreversibly, for instance: the mean time necessary for thiomersal 0.01% to decrease the ciliary beat frequency 50% (t50%) is 0.19 h. Lipophilic compounds arrest ciliary activity very fast (e.g. chlorbutol 0.5%, t50% is 0.04 h), but the effects are to some extent reversible. However, the polar compounds provoked an irreversible but slowly achieved ciliotoxicity (e.g. benzalkonium chloride 0.006%, t50% is 1.13 h). Quaternary ammonium compounds + EDTA are recommended for the preservation of nasal drops.

Nasal drops chap. III
Accordingly, the effects of nasal drops on the ciliary beat frequency have been investigated. In many nasal drops ciliotoxicity can be traced to the effects of the preservatives. For 1=5 diluted nasal drops containing a mercury compound or chlorbutol the t50% is 0.04 h, containing a quaternary ammonium compound the t50% is greater than 1.22 h. Some nasal preparations, such as those containing antihistamines, are more ciliotoxic than would be expected from the preservatives alone.

Correlation between human adenoids and chicken embryo tracheas chap. IV
A correlation between the sensitivity of the ciliated epithelia of human adenoids and chicken embryo tracheas to ciliotoxic effects has been established. The human adenoids appeared to be a little less sensitive, in a quantitative point of view to most of the investigated drugs. Chlorbutol 0.5% arrests ciliary motion in both tissues reversibly within 5 min Benzalkonium chloride 0.006% + EDTA 0.1% decreases the ciliary beat frequency 35% for human tissues and 50% for chicken tissues after a 20 min contact, both irreversibly. The effects of xylometazoline and naphazoline were only to a small extent tissue-dependent. The overall correlation coefficient for the 4 substances investigated is 0.82 ($p<0.005$, spearman-rank).

Human nasal transport chap. V
Nevertheless, the need to check the results in vivo remained. A method has been developed to measure the time necessary to transport mucus from the tip of the nose to the throat. A drop containing a colourant and a sweet-tasting substance has been used as an indicator. This drop was placed in the entrance of the nose and the time necessary for tranport to the throat was measured. When the nasal transport time is measured twice successively it decreases 22%, probably by decreasing the viscosity of nasal mucus. Benzalkonium chloride 0.006% + EDTA 0.1% increases the nasal transport time 21%, non-significantly, whereas chlorbutol 0.5% increases the nasal transport time 53%, significantly. A correlation can be demonstrated between ciliotoxicity and the increase in the transport time for two preservatives and two nasal drops.

Decongestants chap. VI
The influence of pure drugs at different pH's on the ciliary motility has been investigated as well. Firstly, 7 decongestants have been explored. Ephedrine, phenylephrine, xylometazoline and oxymetazoline have the least effect at pH 7.4 and phenylpropanolamine, tramazoline and naphazoline at pH 6. At these optimal pH's the t50% was greater than 0.33 h. The effects at pH 7.4 were reversible.

Antimicrobial agents chap. VII
Most antimicrobial agents also show little ciliotoxicity: benzylpenicillin sodium 10,000 U/ml, ampicillin sodium 1%, neomycin sulphate 0.35%, polymyxin 0.1%, sulphanilamide sodium 0.4% and mild silver protein 0.5% do not decrease the ciliary beat frequency more than 50% in 1 h. Chloramphenicol 0.4%, bacitracin 10,000 U/ml and sulphacetamide sodium 10% arrest ciliary movement within 1 h and only the effects of bacitracin were not reversible.

Local anaesthetics and antihistamines chap. VIII
The local anaesthetics arrest ciliary movement reversibly within 2.5 min at pH 7, whereas the antihistamines arrest ciliary movement irreversibly within 20 min at pH 7.4. Corticosteroids and cromoglycate sodium hardly interfere with the ciliary beat frequency.

Propranolol chap. IX
Recently it has been suggested that drugs with a large first pass effect like propranolol should be administered through the nose for systemic absorption. However, even a 50-fold dilution of the proposed nasal drop arrests ciliary motion irreversibly within 20 min and chronic use should therefore be discouraged

Benzalkonium chloride chap. X
Finally the effects of benzalkonium chloride 0.006% + EDTA 0.1% are described with respect to different pH's, increased viscosity and repeated administration The t50% is 0.57, 0.25 and 0.22 h for pH 7, 6 and 5 respectively. The ciliotoxicity of benzalkonium chloride decreases with increasing viscosity. The effects on the ciliary beat frequency of exposures twice a day for two days to chlorbutol and benzalkonium chloride + EDTA are almost equal. But since chlorbutol decreases the nasal clearance more and hence increases its contact time with the ciliated epithelia, benzalkonium chloride + EDTA is still to be preferred for the preservation of nasal drops.

SAMENVATTING

Het oppervlak van de neusholte is bekleed met epitheel dat bezet is met trilhaartjes (cilia), die voortdurend slijm in de richting van de keelholte sturen en zo de neus reinigen. Deze reiniging van de neus moet zo weinig mogelijk door geneesmiddelen worden verstoord.
Om meer inzicht te verkrijgen in de effekten van neusdruppels op de aktiviteit van de trilhaartjes en op de reiniging van de neus is dit onderzoek gestart. Met de in dit proefschrift beschreven resultaten kunnen voorschriften volgens welke neusdruppels worden bereid verbeterd worden.

Methode: de foto-elektrische registratie apparatuur hfst I
Een lichtstraal wordt tussen de bewegende trilharen door gezonden en na vergroting door een mikroskoop wordt de pulserende lichtstraal op een foto-cel gericht. Het elektrische signaal, dat door deze foto-cel wordt opgewekt, wordt door een oscilloskoop zichtbaar gemaakt en de frequentie van dit signaal wordt elektronisch geschat en digitaal weergegeven.
Zo kan de frequentie van de trilhaarbeweging van ciliair epitheel in vitro worden bepaald.
De luchtpijp van kippe embryo's is voor het onderzoek gebruikt. Dit weefsel is intakter dan weefsel van oudere dieren en het is gemakkelijk te verkrijgen.
De apparatuur bleek eenvoudig te bedienen en maakt betrouwbare meting aan trilhaarepitheel mogelijk.

pH en osmotische druk hfst I
De waarden voor pH en NaCl koncentratie, waarbij de trilhaarfrequentie een maximum bereikt zijn bepaald: pH 7-10 en 0,9% NaCl.

Konserveermiddelen hfst II
De invloed op de trilhaarfrequentie van konserveermiddelen, die vaak in neusdruppels gebruikt worden, zijn onderzocht.
De kwik bevattende konserveermiddelen zijn het meest schadelijk voor de trilharen (ciliotoxisch). Zij doen de frequentie snel en onherstelbaar afnemen, b.v.: de gemiddelde tijd nodig om de trilhaarfrequentie te halveren (t50%) is voor 0,01% thiomersal 0,19 h.
Lipofiele stoffen beëindigen de ciliaire aktiviteit erg snel (b.v. 0,5% chloorbutanol t50% 0,04 h), echter de effekten zijn tot op zekere hoogte omkeerbaar.
De polaire stoffen daarentegen gaven een onherstelbare doch langzaam bereikte ciliotoxiciteit (b.v. 0,006% benzalkonium chloride t50% is 1.13 h).
Kwaternaire ammonium verbindingen in kombinatie met EDTA worden voor de konservering van neusdruppels aanbevolen.

Neusdruppels hfst III
Vervolgens zijn de effekten van neusdruppels op de trilhaarfrequentie onderzocht. Bij veel neusdruppels blijkt de ciliotoxiciteit van die van hun konserveermiddelen te kunnen worden afgeleid.
Voor de 1=5 verdunde neusdruppels, die een kwik verbinding of chloorbutanol bevatten is de t50% 0,04 h, voor de neusdruppels met kwaternaire ammonium verbindingen is de t50% groter dan 1,12 h.
Sommige neusdruppels, zoals degene die antihistaminica bevatten, zijn meer ciliotoxisch dan alleen van hun konserveermiddelen te verwachten is.

Korrelatie tussen menselijke neusamandelen en kippe embryo luchtpijpen hfst IV
Een korrelatie tussen de gevoeligheid van trilhaarepitheel van menselijke neusamandelen en luchtpijpen van kippe embryo's met betrekking tot ciliotoxische effekten kon worden vastgesteld.
De neusamandelen bleken iets minder gevoelig, in kwantitatief opzicht, voor de meeste onderzochte geneesmiddelen.
Chloorbutanol 0,5% beëindigt de ciliaire beweging herstelbaar binnen 5 min, voor beide weefsels. Benzalkonium chloride 0,006% + EDTA 0,1% deed de trilhaarfrequentie met 35% afnemen wat betreft de menselijke weefsels en 50% wat betreft de kippe weefsels na een kontakt van 20 min, beiden echter onherstelbaar. Het effekt van xylometazoline en nafazoline waren slechts in geringe mate weefsel afhankelijk.
De gezamenlijke korrelatie koefficient voor de 4 onderzochte stoffen is 0,82 ($p<0,005$, spearman-rang-test).

Transport in de menselijke neus hfst V
Niettemin bleef er behoefte om de resultaten in vivo te kontroleren. De ontwikkeling van een methode wordt beschreven, om de tijd, nodig om slijm van het begin van de neus tot de keelholte te transporteren, te meten. Een druppel met een kleurstof en een zoet-smakende stof is als indikator gebruikt. Deze druppel werd in de ingang van de neus geplaatst en de tijd nodig voor transport naar de keelholte werd gemeten.
Wanneer de neus transporttijd twee keer achtereen gemeten wordt neemt deze met 22% af, waarschijnlijk door verlaging van de viskositeit van het neusslijm.
Benzalkonium chloride 0,006% + EDTA 0,1% doet de neus transporttijd met 21% (niet signifikant) toenemen en chloorbutanol 0,5% met 53% (signifikant). Er kan een korrelatie worden aangetoond tussen de ciliotoxiciteit en de toename in transporttijd voor deze twee konserveermiddelen en twee neusdruppels.

Decongestiva hfst VI
De invloed van geneesmiddelen sec op de beweeglijkheid van trilharen bij verschillende pH's is eveneens onderzocht. Allereerst zijn de effekten van 7 decongestiva ondergezocht.
Efedrine, fenylefrine, xylometazoline en oxymetazoline hebben bij pH 7,4 het geringste effekt en fenylpropanolamine, tramazoline en nafazoline bij pH 6. Bij deze optimale pH's was de t50% groter dan 0,33 h. De effekten waren bij pH 7,4 herstelbaar.

Antimikrobiële stoffen hfst VII
De meeste antimikrobiële stoffen vertonen een geringe ciliotoxiciteit: natrium penicilline 10.000 E/ml, natrium ampicilline 1%, neomycine sulfaat 0,35%, polymycine 1%, natrium sulfanilamide 0,4% en nucleinezilver 0,5% doen de trilhaarfrequentie minder dan 50% afnemen in een uur.
Chlooramfenicol 0,4%, bacitracine 10.000 E/ml en natrium sulfacetamide 10% doen de trilharen binnen een uur stilstaan en slechts de effekten van bacitracine zijn niet herstelbaar.

Lokale anaesthetica en antihistaminica hfst VIII
De lokale anaesthetica beëindigen de trilhaarbeweging herstelbaar binnen 2,5 min bij pH 7, de antihistaminica daarentegen stoppen de trilhaarbeweging onherstelbaar binnen 20 min bij pH 7,4.
Corticosteroiden en natrium cromoglycaat hebben nauwelijks invloed op de trilhaarfrequentie.

Propranolol hfst IX
Onlangs is de suggestie geopperd dat geneesmiddelen, met een groot "first pass effect" (snelle omzetting van het geneesmiddel bij de eerste lever passage) zoals propranolol via de neus voor systemisch gebruik zouden kunnen worden toegediend.
Echter, zelfs een 50-voudige verdunning van de voorgestelde neusdruppel beëindigt de trilhaarbeweging onherstelbaar binnen 20 min en chronisch gebruik moet derhalve worden ontraden.

Tenslotte worden de effekten van benzalkonium chloride 0,006% + EDTA 0,1% beschreven, met betrekking tot verschillende pH's, verhoogde viskositeit en herhaalde toediening.
De t50% bij pH 7, 6 en 5 is resp. 0,57, 0,25 en 0,22 h.
De ciliotoxiciteit van benzalkonium chloride neemt af bij verhoogde viskositeit.
De effekten op de trilhaarfrequentie van toedieningen twee maal daags gedurende twee dagen van chloorbutanol en benzalkonium chloride + EDTA zijn nagenoeg gelijk.
Maar aangezien chloorbutanol de reinigende werking van de neus meer doet afnemen dan benzalkonium chloride + EDTA en daardoor langer in kontakt met het trilhaar epitheel zal zijn, moet aan de kombinatie benzalkonium chloride + EDTA wat betreft de konservering van neusdruppels, de voorkeur worden gegeven.

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Prof.dr.F.W.H.M. Merkus heeft mij als promotor en hoofd van de vakgroep Artsenijbereidkunde de mogelijkheid tot het uitvoeren van dit onderzoek gegeven, zowel in materiele zin als door het geven van waardevolle adviezen, korrigeren van artikelen en aanzetten tot een snelle start. Verder ben ik je dankbaar voor de grote vrijheid waarin ik heb mogen werken.

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STELLINGEN

1. De foto-elektrische registratie apparatuur zoals beschreven in dit proefschrift, leent zich goed voor routine-matige kontrole op ciliotoxiciteit van geneesmiddelen, hulpstoffen en farmaceutische preparaten.
   — Dit proefschrift, hoofdstuk I.

2. Als konserveermiddel van neusdruppels voor meervoudige toepassing dient bij voorkeur de kombinatie van benzalkonium chloride + EDTA gebruikt te worden.
   — Dit proefschrift, hoofdstuk II.

3. Voor het bepalen van de transporttijd van neusslijm, van neus tot keelholte, is de toepassing van radio-aktieve labeling onnodig, en gezien het ontbreken van een veilige ondergrens bij stralingsdoses, ongewenst.
   — Dit proefschrift, hoofdstuk V.

4. De effekten van decongestiva op het zelfreinigend mechanisme van de neus zijn gering; dit geldt in het bijzonder voor fenylefrine en oxymetazoline.
   — Dit proefschrift, hoofdstuk VI.

5. Het gebruik van antibiotica in neusdruppels dient in de eerste plaats bepaald te worden door de indikatie, daarna door de ciliotoxiciteit.
   — Dit proefschrift, hoofdstuk VII.

6. Het nut van de lokale toepassing in de neus van antihistaminica dient, gezien hun ciliotoxische eigenschappen, nog meer dan voorheen in twijfel te worden getrokken.
   — Cirillo, V.J. en Tempero, K.F., Am. J. Hosp. Pharm. 33(1976)1200-1207.
   — Dit proefschrift, hoofdstuk VIII.

7. Bij het toedienen van geneesmiddelen via de neus voor niet lokaal gebruik dient rekening gehouden te worden met de effekten op het trilhaarepitheel.
   — Dit proefschrift, hoofdstuk IX.

8. Bij het voorschrijven en afleveren van doxycycline voor orale toediening verdient de tablet de voorkeur boven de kapsule, teneinde de kans op een slokdarmbeschadiging zo gering mogelijk te maken.
   — Merkus, F.W.H.M., Ned. T.v.G. 126(1982)203

9. Geneesmiddelen die N-7 gesubstitueerde theofyllinederivaten bevatten worden ten onrechte, dikwijls gelijkgesteld met theofyllinebevattende preparaten.
   — Zuidema, J. en Merkus, F.W.H.M., Pharm. Int. 1(1980)80-83.

10. De ipratropium bevattende neusspray, voorgesteld door Borum et al. met als indikatie verkoudheid, is een voorbeeld van symptoombestrijding die de uiteindelijke geneesduur kan verlengen.
   — Borum, P. et al., Am. Rev. Resp. Dis. 123(1981)418-420.

11. Etiketten op geneesmiddelverpakkingen dienen naast het werkzame bestanddeel alle hulpstoffen te vermelden, enerzijds ten behoeve van patiënten met allergieën en, anderzijds, om artsen en apothekers ervan te doordringen dat een farmaceutisch preparaat meer is dan alleen een farmakologisch aktieve stof.

12. Bij wetenschappelijk onderzoek van farmaceutische preparaten en bij het voorschrijven van deze preparaten op basis van buitenlandse of niet recente gegevens, dient rekening te worden gehouden met een afwijkende samenstelling van merkpreparaten in verschillende landen, respektievelijk met een verandering van samenstelling van het preparaat door de fabrikant in de loop der tijd.

13. Het veelal kategorisch denken doet een digitale grondslag van het menselijk geheugen vermoeden.

14. Alvorens te beslissen tot in gebruikneming van de snelle kweekreaktor te Kalkar (Duitsland) dient de eventuele onjuistheid van het door Webb berekende, zeer hoge nucleaire explosiepotentiëel te worden aangetoond.
   — Het Nucleaire Explosie Potentiëel van de SNR-300 in Kalkar. (Uitgave Landelijk Energie Komitee).

Stellingen behorend bij het proefschrift van H.J.M. van de Donk
12 mei 1982