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The effects of drugs on ciliary motility II. Antimicrobial agents

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Summary

The effects of antimicrobial agents at different pHs 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., 1982), 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 sensitization 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

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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 6 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 1 lists the substances investigated and their lot numbers. Macrogol 400 is used as a solvent for chloramphenicol and, therefore, was also assayed.

Results

The effects of the drugs at different pHs on the ciliary beat frequency of chicken embryo tracheas are shown in Table 2. The effects at pH 7.4 of rinsing with LR after 20 min contact are also presented. The first 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 min. 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. Figs. 1–7 show the effects in more detail. The S.E.M. values are indicated by vertical bars. The ciliary beat frequency of the references remained between 97% and 107% during all the experiments.

Discussion

The concentrations of the antimicrobial agents investigated are listed in Table 3. 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 3.

The effects of most antimicrobial agents on the ciliary beat frequency are very modest. Both penicillins show little ciliotoxicity. Neomycin and chloramphenicol

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

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 Locke Ringer 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

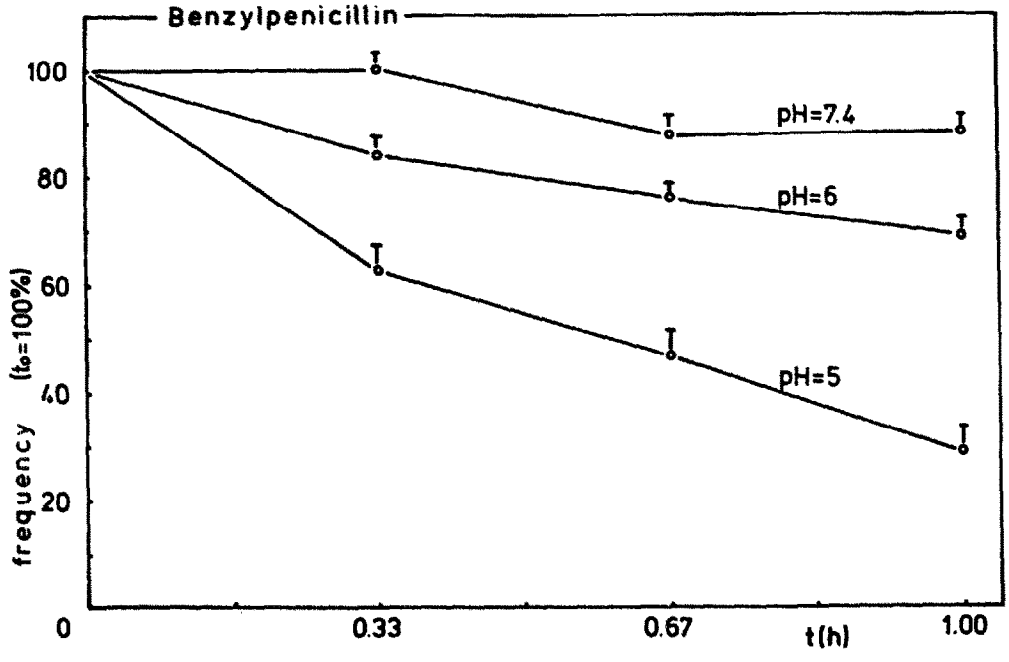

Fig. 1. Time versus frequency plot for benzylpenicillin sodium 10,000 U/ml; at pH=5, 6 and pH 7.4.

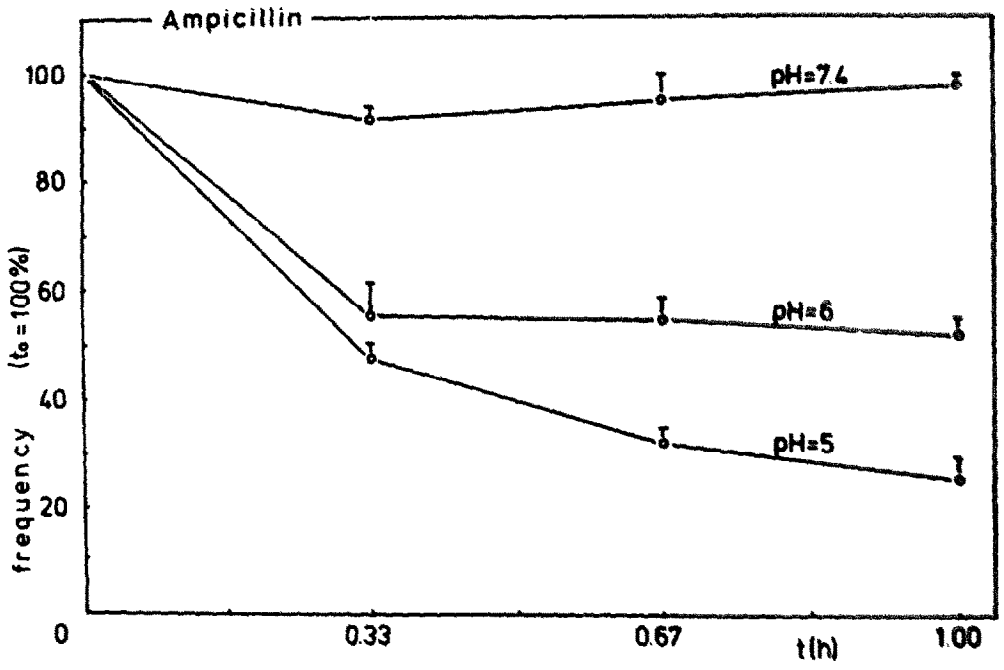

Fig. 2. Time versus frequency plot for ampicillin sodium 1%; at pH 5, 6 and at pH 7.4.

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

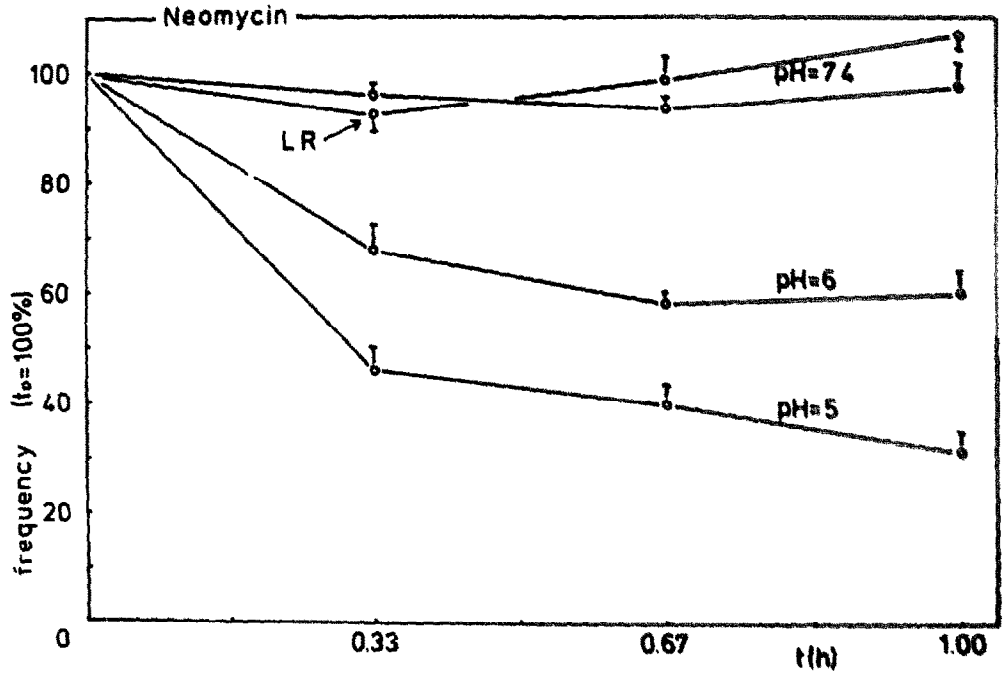

Fig. 3. Time versus frequency plot for neomycin sulphate 0.35%; at pH 5, 6, pH 7.4 washed after 20 min with LR and at pH 7.4 continuously.

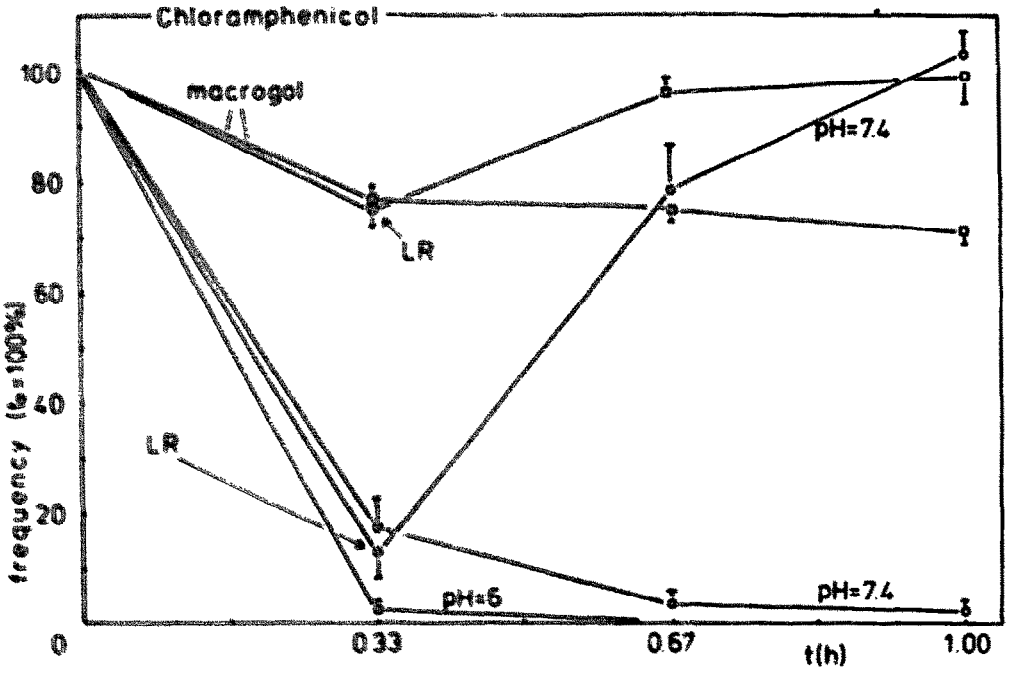

Fig. 4. Time versus frequency plot for chloramphenicol (0.4%) + macrogol (5%); at pH 6; pH 7.4 washed after 20 min with LR and at pH 7.4 continuously and for macrogol 5% only at pH 7.4 washed after 20 min with LR and at pH 7.4 continuously.

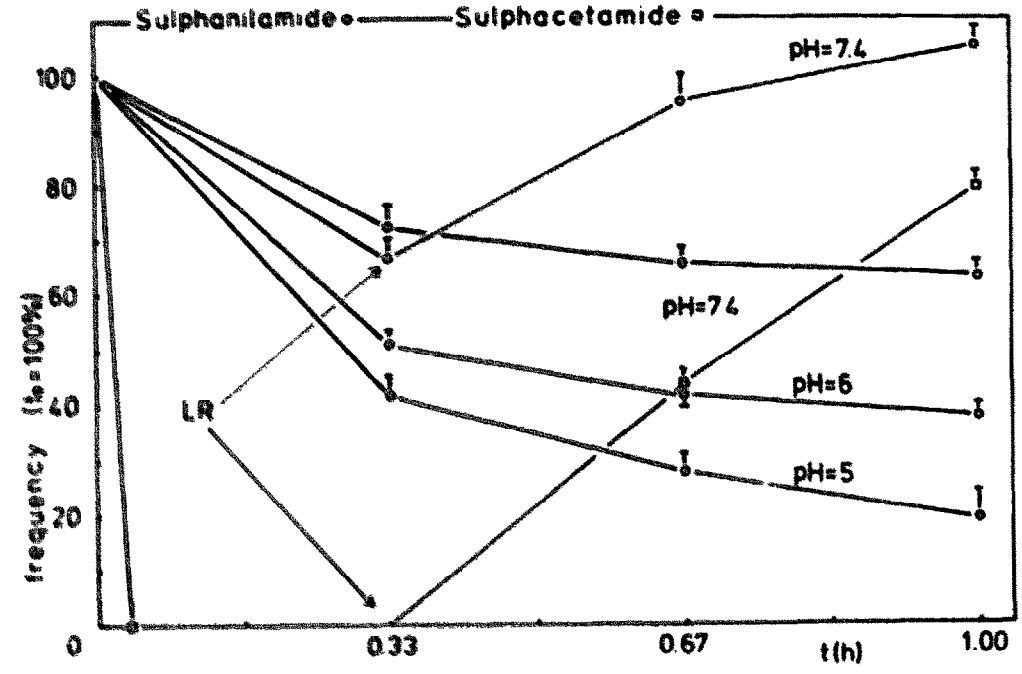

Fig. 5. Time versus frequency plot for sulphanilamide sodium 0.4% (○) at pH 5, 6 and at pH 7.4 washed after 20 min with LR and at pH 7.4 continuously and for sulphacetamide 10% (□) at pH 7.4 after 20 min with LR.

TABLE 2

EFFECTS OF ANTIMICROBIAL AGENTS ON THE CILIARY BEAT FREQUENCY

Compound	pH	Frequency [a]			$t_{95\%}$ [b]	$t_{90\%}$ [c]
		t=0.33 h	t=0.67 h	t=1.00 h		
Benzylpenicillin sodium (10,000 U/ml)	7.4	100	87	88	0.46	0.59
	6	84	76	69	0.10	0.21
	5	63	47	29	0.04	0.09
Ampicillin sodium (1%)	7.4	91	95	97	0.19	
	6	55	55	52	0.04	0.07
	5	47	52	25	0.03	0.06
Neomycin sulphate (0.35%)	7.4	93 [d]	99	107	0.23	
	7.4	96	94	97	0.48	
	6	68	58	60	0.05	0.10
	5	46	40	31	0.03	0.06
Chloroamphenicol (0.4%)+macrogol (5%)	7.4	1 [d]	79	104	0.02	0.03
	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

Sulphanilamide sodium (0.4%)	7.4	66 [d]	95	105	0.05	0.10
	7.4	72	65	63	0.06	0.12
	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
Mild silver protein (0.5%)	7.4	93 [d]	105	105	0.24	
	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 solution	7-10	103	104	103		
	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.

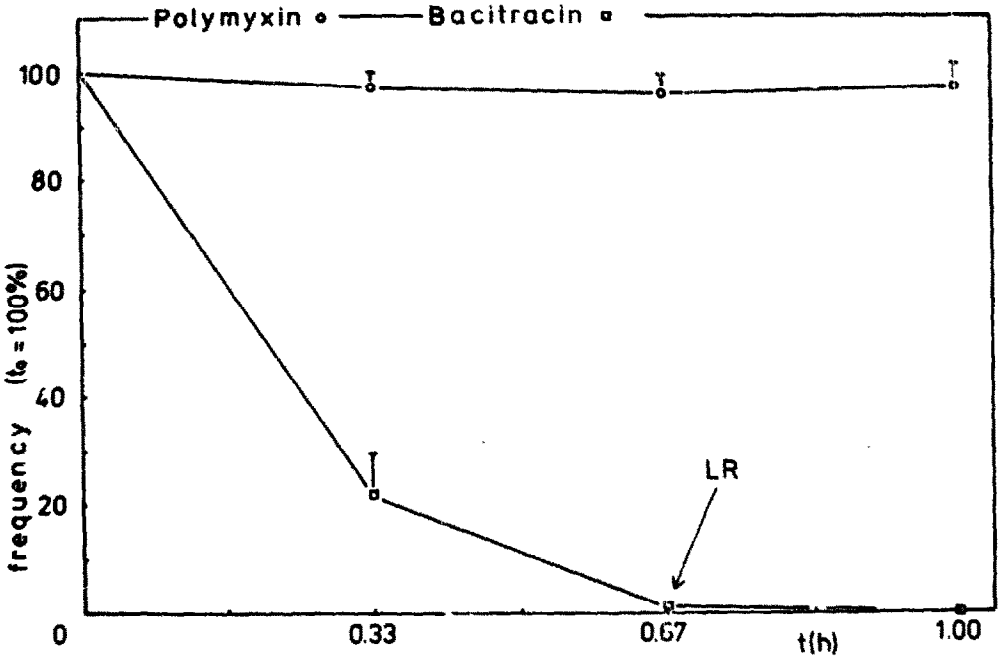

Fig. 6. Time versus frequently plot for polymyxin 0.1% (○) at pH 7.4 and for bacitracin 10,000 U/ml (□) at pH 7.4 and washed after 40 min with LR.

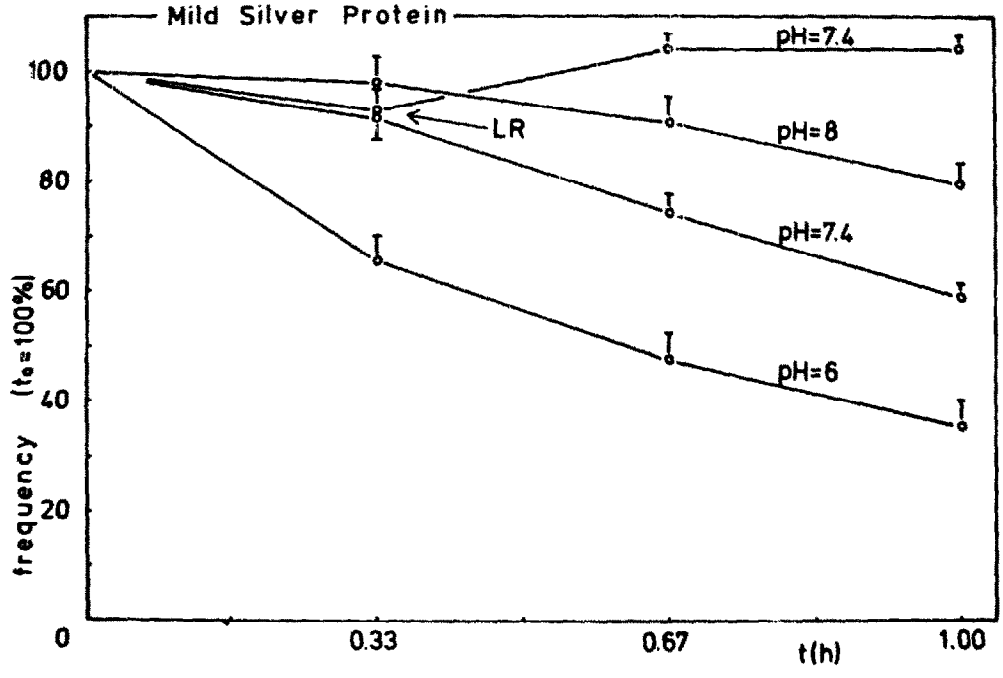

Fig. 7. Time versus frequency plot for mild silver protein (0.5%); at pH 6, 7.4 continuously and at pH 7.4 washed after 20 min with LR and at pH 8.

TABLE 3

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).

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., 1980; Table 2). 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) 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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