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# The effects of nasal drops and their additives on human nasal mucociliary clearance

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SUMMARY

*A method for measurement of nasal mucociliary clearance in vivo is described. A drop, containing saccharine 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% (F.N.A.)*.*

*These results support those obtained with the photo-electric registration device applicated on chicken embryo tracheas and human adenoids as described in earlier publications.*

## 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 $^{99}Tc^{m}$ on the ciliary epithelium in the nose. The movement of the particle was registrated by a gamma-camera and the velocity of the particles calculated.

* F.N.A. = Formulary of the Netherlands' Pharmacists Association.

Andersen et al. (1974) used one particle with a diameter of 0.5 mm and labeled with 3$\mu$Ci $^{99}Tc^{m}$ 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 $^{51}Cr$ which resulted in lower irradiation than 3 µCi $^{99}Tc^{m}$. (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 Van 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 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 seconds 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 expensive equipment. The authors of the reports on the last two methods did not describe the effects of the test substances 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% (F.N.A.), 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 are 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 lung 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* 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 9.6µg Hg/ml, and Rhinoguttae xylometazoline (F.N.A.) 0.1%, containing xylometazoline HCl 0.1%, benzalkonium chloride 0.006%, EDTA 0.1%, hypromellose* 0.5%, $NaH_2PO_4 \cdot 12\ H_2O$ 0.15%, $Na_2HPO_4 \cdot 2\ H_2O$ 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 minutes 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 minutes the subject was requested to swallow every 20 seconds and the throat was inspected every 60 seconds 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 minutes 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 minutes 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.

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* hypromellose (2% = 4000 cps.).

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 1 and Figure 1.

Subsequently, the transport time was assayed twice successively, each time 15 minutes 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 2, Figure 2). The effects of two preservatives, one viscosity-increasing substance and two nasal drops on the transport time are also shown in Table 2.

The preservative chlorbutol 0.5% (Figure 3) increased the transport time significantly as measured with saccharin and indigo carmine. The other preservative, benzalkonium chloride 0.006% + EDTA 0.1% (Figure 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

Table 1. Effects of the test substances on the ciliary beat frequency of chicken embryo tracheas.

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

[1] Percentage of the intitial frequency after respectively 0.33 h, 0.67 h and 1 h's contact.

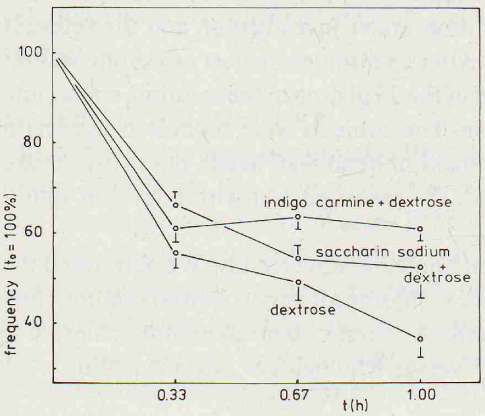


Figure 1. Time versus ciliary beat frequency plot: indigo carmine 0.8% + dextrose 5%, saccharin sodium 0.2% + dextrose 5% and dextrose 5%.

Table 2. Effects of nasal drops and their additives on the nasal mucociliary transport time.

| compound | parameter[1] | $\bar{t}_a$[2] | $\bar{t}_p$[3] | $\overline{(t_p - t_a)/t_a}$[4] | number of volunteers | significance |
|---|---|---|---|---|---|---|
| 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 chloride 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.[5] |
| | I | | 11.5 | +43% | 6 | N.S.[5] |
| hypromellose 1% | S | 5.7 | 6.8 | +23% | 6 | N.S. |
| | I | 6.3 | 8.3 | +31% | 6 | $P < 0.05$ |
| +benzalkonium chloride 0.006% +EDTA 0.1% | S | | 7.7 | +40% | 6 | N.S.[5] |
| | I | | 8.5 | +35% | 6 | N.S.[5] |
| 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. |

[1] S = saccharin (taste).
I = indigo carmine (colour).
[2] $\bar{t}_a$ = mean transport time before application of the compound under investigation in min.
[3] $\bar{t}_p$ = mean transport time after application of the compound under investigation in min.
[4] $\overline{(t_p - t_a)/t_a}$ = mean of relative increase of transport time after application of the compound under investigation.
[5] with respect to transport time before application of hypromellose.

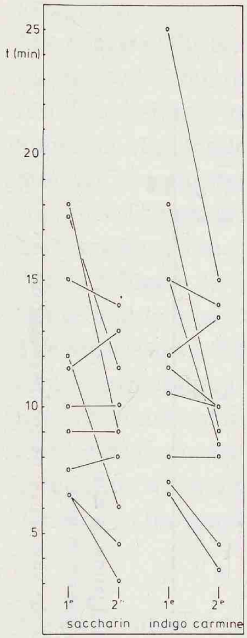


Figure 2. Nasal mucociliary transport time: assayed twice with saccharin (10 volunteers) and indigo carmine (10 volunteers).

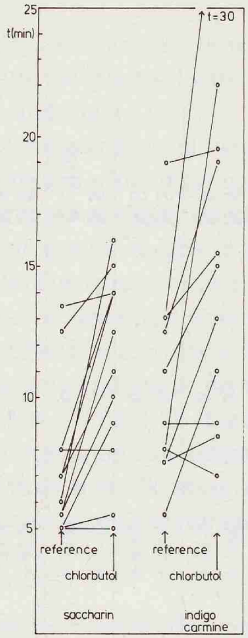


Figure 3. Nasal mucociliary transport time: assayed before and after the application of chlorbutol 0.5%, with saccharin (12 volunteers) and indigo carmine (11 volunteers).

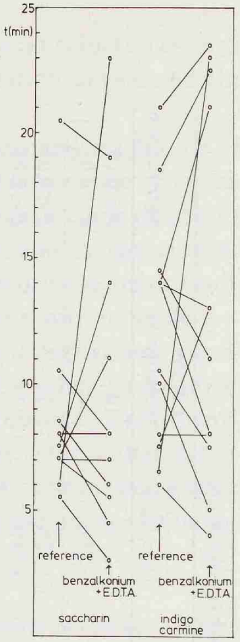


Figure 4. Nasal mucociliary transport time: assayed before and after the application of benzalkonium chloride 0.006% + EDTA 0.1%, with saccharin (11 volunteers) and indigo carmine (12 volunteers).

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 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 0.0006% + EDTA 0.1% increased the transport time non-significantly (for saccharin sodium and indigo carmine).

The nasal drop Rhinoguttae xylometazolini 0.1% (F.N.A.) (Figure 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% (Figure 6) increased the transport time, significantly for saccharin

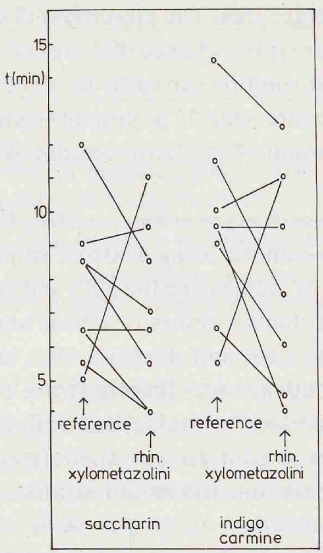


Figure 5. Nasal mucociliary transport time: assayed before and after the application of Rhinoguttae xylometazolini 0.1%, with saccharin (8 volunteers) and indigo carmine (8 volunteers).

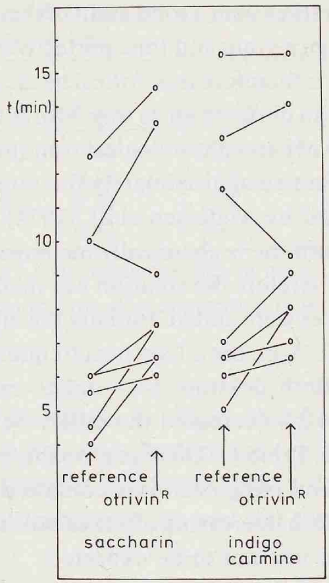


Figure 6. Nasal mucociliary transport time: assayed before and after the application of Otrivin® 0.1%, with saccharin (8 volunteers) and indigo carmine (8 volunteers).

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 prefered the use of a solution containing indigo carmine and saccharin

sodium rather than a solid grain of these substances. A drop of solution is diffused 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 physiological pH and physiological 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 (Figure 1, Table 1). 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 saccharine 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 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 2 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

Table 3. Comparison of the effects on the ciliary beat frequency of chicken embryo tracheas and the effects on the nasal mucociliary transport time.

| compound | $t_{50\%}$ [1] | $\overline{(t_p - t_a)/t_a}$ [2] | 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. |

[1] $t_{50\%}$ = Time necessary to decrease the ciliary beat frequency 50% in hours.

[2] $\overline{(t_p - t_a)/t_a}$ = mean relative increase of transport time after application of the compound under investigation, assayed with saccharin.

those of the "in vitro" investigations of the effects on ciliary beat frequency in Table 3. The first column shows the time necessary to decrease the ciliary beat frequency 50% as reported by Van de Donk et al. (1980b, 1981). The second column shows the increase of the nasal transport time and the third column 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.

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 minutes ($P < 0.05$), 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 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.

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RÉSUMÉ

Une méthode de mesure de la mucociliaire "clearance" nasale in vivo est décrivée.

Une goutte, contenant du saccharinate de sodium et du carmin d'indigo, a été placée au bord de l'épithélium cilié des orifices extérieurs des cavités nasales. On a mesuré le temps qui écoulait entre le placement de la goutte et le moment où le sujet d'expérience percevait une saveur douce et qu'une ligne bleue apparaissait à l'arrière-bouche; ce temps a été appelé temps de transport.

Ensuite, en appliquant la méthode décrivée ci-dessus, les effets de deux conservateurs, deux gouttes nasales et un gélifiant ont été examinés et comparés aux effets qu'avaient ces mêmes substances sur la fréquence du battement ciliaire des trachéas de poulet in vitro. On a constaté que plus une substance élève le temps de transport, plus elle diminue la fréquence du battement ciliaire. Le chlorobutanol 0,5% cause une plus grande élévation du temps de transport et une plus grande diminution de la fréquence du battement ciliaire que le chlorure de benzalkonium 0,006% + l'édétate de sodium 0,1%. De même, l'Otrivin® 0,1% cause une plus grande élévation du temps de transport et une plus grande diminution de la fréquence du battement ciliaire que les Rhinoguttae xylometazolini 0,1% (F.N.A.*).

Ces résultats confirment ceux obtenus par les expériences faites à l'aide de l'appareil de régistration photo-électrique, sur les trachéas des embryons de poulet et des adenoides humaines, lesquelles expériences ont été décrivées dans des publications précédentes.

* F.N.A. = Formulaire de l'Association des Pharmaciens Néerlandais.

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