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# Chlorhexidine kinetics of hydrophilic contact lenses

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An average chlorhexidine concentration of 4·5 $\mu$g mg$^{-1}$ lens (wet weight) was measured in HEMA-based contact lenses after 16 days of the following treatment. The lenses were maintained 16 h daily in 1·5 ml of a solution containing chlorhexidine digluconate 0·005%, and then worn by adult albino rabbits 8 h daily. Similarly maintained, but unworn lenses contained approximately 23 $\mu$g chlorhexidine mg$^{-1}$ wet lens weight after 16 days. A plot of the results indicates that continued exposure to chlorhexidine following initial immersion increased its concentration in unworn lenses while that in the worn lenses reached a peak at 8 days, then decreased slightly in the subsequent days of wearing. Continuous exposure of lenses to the disinfecting solution (1 lens/167 ml) in which the chlorhexidine concentration was maintained at 0·005% for 16 days gave a maximal concentration of 44 $\mu$g mg$^{-1}$ wet lens weight. The comparatively small concentration in a worn lens results from: (1) daily loss from the lens during wear, a large percentage of which is apparently absorbed to tear proteins which subsequently flow from the eyes via the canaliculi; (2) limited uptake because of the small immersion volume employed in routine storage; and (3) the possible competition between chlorhexidine and tear components for lens binding sites or establishment of an equilibrium condition between uptake and release.

Soft hydrophilic (polyHEMA-based) contact lenses have a strong affinity for a variety of disinfecting agents commonly used in ophthalmic solutions (MacKeen, 1974). Their accumulation in and subsequent release from the lens could result in corneal damage. For example, the corneal toxicity caused by benzalkonium released from HEMA-based lenses (Green & Tonjum, 1971, 1975) precludes its use as a preservative. Experimental and clinical findings with soft lenses routinely maintained as directed in solutions containing 0·005% chlorhexidine digluconate have been found to be safe and effective (Charles, Callender & Grosvenor, 1973; Feldman & Bailey; 1974, Roth, 1978) even though the preservative binds strongly to HEMA lens material (Hubbard, 1975; Refojo, 1976).

Chlorhexidine uptake and release from HEMA lenses *in vitro* have been reported (Hubbard, 1975; Refojo, 1976), but the findings have only been semi-quantative because of difficulties in measuring either lens-bound chlorhexidine or the small quantities released from these lenses into immersion fluids.

The use of $^{14}$C-labelled chlorhexidine digluconate permits quantitation of the preservative taken up, by measurement of the radioactivity after the lens has been dissolved in acid, and also the measurement of small quantities desorbed from lenses into immersion fluids. We used this approach to measure the uptake and release of chlorhexidine from lenses during various times of immersion and with access to various quantities of chlorhexidine at 0·005% concentration.

One group of lenses was maintained by the complete recommended procedure for cold disinfection and was worn daily by rabbits. This was done to mimic the clinical situation as closely as possible.

## MATERIAL AND METHODS

$^{14}$C-Labelled (hexane-1,6-$^{14}$C) chlorhexidine digluconate was obtained from the New England Nuclear Corporation, Boston, MA as an aqueous solution containing 185·6 mg ml$^{-1}$ (0·442 mCi ml$^{-1}$). Proprietary lens solutions [Flexsol disinfecting solution: chlorhexidine digluconate 0·005%, thimerosal sodium 0·001% and edetate disodium 0·1%, Adsorbobase (povidone with other water soluble polymers) borate buffer and sodium chloride and Normol rinsing solution (formulation identical to the disinfection solution without Adsorbobase)] were prepared without the chlorhexidine digluconate. This was subsequently added as the radioactive material to give a concentration as the digluconate of 0·005% in both solutions. The chlorhexidine concentration was corroborated by both the standard spectrophotometric method (Holbrook, 1958) and counting in a scintillation counter. All subsequent measurements were made by the latter method.

Hydrated HEMA-based lenses (Deltafilcon A, supplied by G&S lenses, Kensington, MD) were

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used (43% water at equilibrium hydration) and were stored in normal saline before the experiment. Lenses ranged from 7·40 to 8·00 base curves and were fitted to the eyes of albino rabbits of 2–4 kg. No lens had been exposed to chlorhexidine before use in these experiments.

To determine the radioactivity of lenses, they were heated (approximately 50°) in concentrated sulphuric–nitric acid (2:1) contained in capped scintillation vials; 100 $\mu$l samples of the resultant solution were taken and diluted to 1 ml with distilled water and then scintillation fluid (Aquasol, New England Nuclear, Boston, MA) was added. The final volume of all samples was identical. After cooling for at least 24 h the samples were counted in a Packard Tricarb Scintillation Counter. All counts were corrected by means of a quench curve.

Several types of experiments were made to determine the rates of uptake and release of chlorhexidine from the lenses.

*Daily wearing of lenses by rabbits*

This experiment was over 16 days. Lenses were worn in both eyes by 15 rabbits; another group of 30 lenses were used as controls. The lenses were immersed in 1·5 ml amounts of the disinfecting solution for 16 h before insertion. This was done for the remainder of the experiment: that is, overnight storage for 16 h in 1·5 ml of fresh disinfecting solution (1·5 ml is approximately the volume of solution per contact lens in a lens case). In the morning each experimental lens was rinsed with the rinsing solution then replaced on the appropriate eye. At this time each control lens was rinsed briefly with saline, shaken to remove excess saline and placed in a humidity chamber. Each wearing lens was placed in an eye which was then closed and taped lightly to prevent loss of the lens. This did not prevent the animal from making movements of certain extraocular muscles associated with blinking.

At the end of 8 h the lenses were removed, experimental lenses were cleaned by means of a rubber gloved hand and the lens cleaner (Preflex: tyloxapol, hydroxyethylcellulose, polyvinyl alcohol, preserved with thimerosal sodium and edetate disodium) and rinsed with the rinsing solution. Both experimental and control lenses were returned to fresh 1·5 ml amounts of the disinfection solution.

After the end of 2, 4, 8, 12* and 16 days of wearing an equal number of lenses were taken for radioactive counting from control and wearing groups (* the values for the 12 day group were done some time after the original experiment).

*Comparison of chlorhexidine loss from lenses exposed to 'maximal' and 'minimal' uptake conditions*

'Maximal' uptake lenses were immersed in the disinfecting solution 24 h a day for 16 days (384 h) at a solution–lens ratio of 167 ml per lens. During the immersion the solution was stirred with a Teflon-coated stirring bar to reduce the thickness of unstirred boundary layer. The chlorhexidine concentration was adjusted to 0·005% daily as needed with the addition of more $^{14}$C-labelled material. Each 'minimal' uptake lens was immersed for 1·6 h in 1·5 ml of the disinfecting solution.

*Comparison of the release of chlorhexidine into saline or artificial tears from 'maximal' and 'minimal' uptake lenses*

The release of $^{14}$C into either 0·9% sodium chloride solution (saline) or an artificial tear solution from lenses containing either 'maximal' or 'minimal' chlorhexidine concentration was assessed. The tear solution contained (% w/v) NaCl, 0·8; bovine submaxillary mucin, 0·22; bovine serum albumin, 0·20; gamma globulin, 0·10; and egg lysozyme 0·08 (Holly-personal communication). Each lens was immersed, concave side up, in 8 ml of either saline or 'tears' in a capped scintillation vial which was kept at room temperature (25°) without stirring.

After 8 h, 100 $\mu$l of the supernatant was sampled for $^{14}$C. Also, each lens was weighed, solubilized and a 100 $\mu$l sample taken. Then 2 ml of 20% trichloroacetic acid was added to each vial of artificial tear solution. Half an hour later the resultant suspensions were centrifuged (5000 rev min$^{-1}$) for 10 min, the supernatant decanted and the precipitate weighed; approximately 100 mg was weighed, solubilized with heat (37°) in a solubilizing solution designed for use in scintillation experiments (Protosol, New England Nuclear).

## RESULTS

*Daily wearing of lenses by rabbits*

During the first few days of wear there was a deposition of mucus-like material on many lenses but this subsequently disappeared. The deposits were readily removed by gently rubbing with cleaner and subsequent rinsing with the rinsing solution.

The average values of chlorhexidine was significantly different between control and worn lenses ($P < 0{\cdot}005$ for all time intervals). The average values with the standard error of the mean are shown in Fig. 1. The lines in the figure were fitted by least square analysis. The rate of uptake of chlorhexidine ($\mu$g mg$^{-1}$ lens wet weight day$^{-1}$) by control lenses

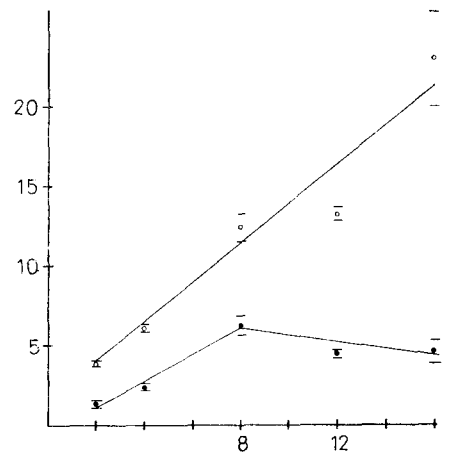


FIG. 1. Plot of chlorhexidine concentration in soft contact lens, *vs* time of immersion. Each point is the mean, vertical bars indicate s.e.m. Lenses immersed in 1·5 ml disinfecting solution (chlorhexidine digluconate 0·005%) per lens for 16 h daily and either worn for 8 h, (closed circles, worn lenses) or stored in an humidity chamber 8 h (open circles, control lenses). Ordinate: $\mu$g chlorhexidine mg$^{-1}$ lens wet weight. Abscissa: Time (days).

was greater than that of the initial uptake rate of the worn lenses (Phase I—days 2–8: 1·26 *vs* 0·84 $\mu$g mg$^{-1}$ wet lens weight day$^{-1}$). During Phase II (from day 8 to 16) the rate of uptake in the worn lenses decreased to 0·22 $\mu$g mg$^{-1}$ wet lens weight day$^{-1}$. The differences between the slopes of worn lenses in Phase I and II was significant. However, the average value at 8 days was not significantly different ($P = 0·12$) from that at 16 days.

*Comparison of the release of chlorhexidine into saline or artificial tears*

Table 1 lists the results of the desorption experiment into saline. A similar proportion of the calculated pre-immersion content of chlorhexidine in each lens was desorbed into the saline for both the minimal (7·2%) and the maximal (7·6%) group. The pre-immersion quantity of chlorhexidine was the sum of the material remaining in the lens following the desorption, plus the total amount desorbed. The amount desorbed was proportionate to the lens concentration.

Table 2 shows the desorption values of chlorhexidine into artificial tears. A slightly greater proportion ($P = 0·03$) of the total lens chlorhexidine was extracted from the lenses in the minimal group.

No additional radiolabelled chlorhexidine digluconate was required to maintain the disinfecting solution at a concentration of 0·005% w/v during the last week of lens immersion. Despite the high solution-lens ratio, significant depletion of chlorhexidine had occurred in the solution over each of the initial seven days. It was found that loss resulted from uptake by the Teflon-coated stirring bar. Separate experiments showed that a Teflon-coated stirring bar immersed in 25 ml chlorhexidine solution removed

Table 1. *Chlorhexidine* in soft hydrophilic contact lenses and in saline lens immersion fluid†.*

| | Lens | | Amount desorbed into 8 ml saline | |
|---|---|---|---|---|
| | I | II | III | IV |
| Lens no. | Orig. concn $\mu$g mg$^{-1}$ | Total amount in lens after desorption ($\mu$g) | Total $\mu$g | % Original lens total |
| | | Minimum uptake group | | |
| 1 | 0·55 | 34·25 | 3·32 | 8·8 |
| 2 | 1·33 | 78·72 | 4·21 | 5·1 |
| 3 | 0·57 | 35·26 | 2·89 | 7·6 |
| Mean | 0·57 | | | 7·2 |
| s.e.m. | 0·19 | | | 1·1 |
| | | Maximum uptake group | | |
| 6 | 32·35 | 946·8 | 92·19 | 8·9 |
| 7 | 36·61 | 993·4 | 82·26 | 7·7 |
| 8 | 31·24 | 886·4 | 89·74 | 9·2 |
| 9 | 38·67 | 1058·9 | 88·82 | 7·7 |
| 10 | 54·97 | 1806·1 | 88·16 | 4·7 |
| Mean | 38·77 | | | 7·6 |
| s.e.m. | 4·1 | | | 0·82 |

* Calculated as the digluconate salt.

† Each lens was immersed in 8 ml of normal saline without stirring at 25° for 8 h. Each value in columns II and III is the product of measured concentration in each lens and immersion fluid, times the lens weight or 8 ml respectively. Minimum Group: each lens immersed in 1·5 ml of disinfecting solution for 1·6 h. Maximum Group: each lens immersed in disinfecting solution for 384 h at ratio of 167 ml per lens.

Table 2. *Chlorhexidine* in soft hydrophilic contact lenses and in artificial tear † lens immersion fluid‡.*

| | I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| | Lens | | Artificial tears Solution | | Precipitate | |
| | Original | remaining | | | | % Eluted |
| Lens no. | $\mu$g mg$^{-1}$ | $\mu$g | Total $\mu$g | % Lens total | Total $\mu$g | material in ppt |
| | | | Minimum uptake group | | | |
| 1 | 2·49 | 81·9 | 20·48 | 19·99 | 6·5 | 31·7 |
| 2 | 1·00 | 57·1 | 10·55 | 15·61 | 4·1 | 38·7 |
| 3 | 1·46 | 39·1 | 6·54 | 14·35 | 3·1 | 46·8 |
| Mean | 1·65 | | | 16·65 | | 39·1 |
| s.e.m. | ±0·44 | | | 1·71 | | 4·4 |
| | | | Maximum uptake group | | | |
| 4 | 33·95 | 912·3 | 133·7 | 12·78 | 56·00 | 41·9 |
| 5 | 34·67 | 880·2 | 101·2 | 10·31 | 46·1 | 45·5 |
| 6 | 65·16 | 1537·7 | 161·0 | 9·48 | 71·4 | 44·4 |
| 7 | 39·83 | 931·3 | 160·6 | 14·71 | 59·4 | 37·0 |
| 8 | 70·37 | 1438·7 | 189·7 | 11·65 | 86·8 | 45·7 |
| Mean | 48·79 | | | 11·78 | | 42·9 |
| s.e.m. | ±7·85 | | | 0·92 | | 1·6 |

* Calculated as digluconate salt.

† NaCl 0·8%, bovine submaxillary mucin, 0·22%; bovine serum albumin, 0·2%; gamma globulin 0·1% and egg lysozyme 0·08%.

‡ Each lens was immersed in 8 ml of normal saline without stirring at 25° for 8 h. Each value in columns II and III is the product of measured concentration in each lens and immersion fluid, times the lens weight or 8 ml respectively. Minimum group: Each lens immersed in 1·5 ml of disinfecting solution for 16 h; Maximum group: Each lens immersed in disinfecting solution for 384 h at a ratio of 167 ml per lens.

Columns II, III and V represent measured values times lens weight, 8 ml or total precipitate weight respectively.

approximately 25% of the compound from solution in 24 h.

The desorbing solution of these lenses contained 16·6% of the calculated amount of chlorhexidine taken up by these lenses while that of the maximal uptake group contained 11·8%. The percentages of desorbed chlorhexidine associated with the trichloroacetic acid-precipitated proteins were proportionally similar for both minimal and maximal groups, i.e., 39·1 and 42·2% respectively.

Some of the results from these experiments were combined with control data in Fig. 1 to make Fig. 2, the concentration of chlorhexidine in HEMA lenses *vs* time of immersion in 0·005% chlorhexidine digluconate solution. The concentration after 384 h

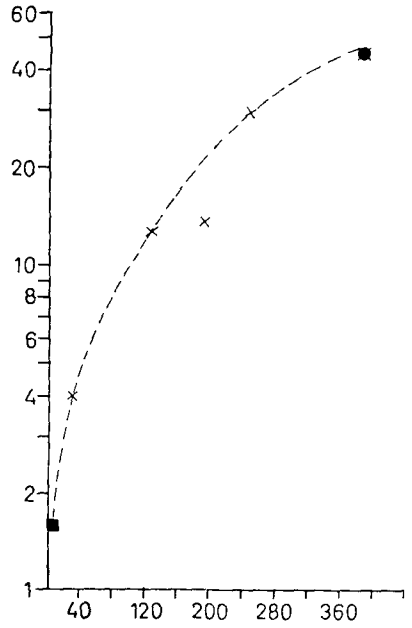


FIG. 2. Chlorhexidine concentration in soft contact lenses *vs* total immersion time in disinfecting solution (chlorhexidine digluconate 0·005%). All points average values. ● constant immersion in 166·6 ml per lens; ×, 1·5 ml per lens for 16 h a day; ■, 1·5 ml per lens for 1·6 h. Ordinate: μg chlorhexidine $mg^{-1}$ less wet weight. Abscissa: time (h).

(16 days continuous immersion) was 43·8 μg $mg^{-1}$ wet lens weight. The mean value for 1·6 h was 1·20 μg $mg^{-1}$ wet lens weight. These two values are the mean concentration values (column I from Tables 1 and 2), minimal and maximal uptake respectively. The chlorhexidine uptake data can be extrapolated to an instantaneous uptake value of approximately 1 μg $mg^{-1}$ lens wet weight at time zero (surface uptake). The maximum value is equivalent to 76·8 μg $mg^{-1}$ dry polymer gel (hydrated material contains 43% water).

## DISCUSSION

After identical recommended maintenance in the disinfecting solution for 16 days, the worn lenses contained about 1/5th of the amount of the chlorhexidine as did the control lenses. The difference arose from the chlorhexidine lost during wear—possibly a small amount was removed during the use of the cleaning agent; however, after cleaning, the lens was rinsed with a solution containing 0·005% chlorhexidine. The experiment was devised to determine the uptake by lenses during exposure to chlorhexidine without any opportunity for loss with that remaining in lenses after recommended daily cleaning and wearing.

The data points of the wearing lenses (Fig. 1) would probably be better fitted in a curvilinear fashion, but the straight lines fit the data reasonably and simplify the rate determinations.

Phase II of the wearing curve in Fig. 1 should provide information about the chlorhexidine concentration in lenses during clinical wear. Although the slope is slightly negative the difference between the values at 8 and 16 days is not significant ($P = 0·14$).

This permits two interpretations: first, if the slope is actually negative it suggests competition between chlorhexidine and small components in the tears for binding sites on the HEMA. Second, if the curve in this phase is actually a plateau, it would reflect an equilibrium state in which the uptake during immersion equalled the loss during wearing.

The 12 day values in Fig. 1 were obtained in a separate experiment. These relatively smaller values may have resulted from a multitude of minor variations related to lenses made from a different lot of HEMA, a new batch of [$^{14}$C]chlorhexidine and a different batch of solutions.

The percentage of chlorhexidine taken up by the lens and that desorbed into the artificial tear solution was greater than into saline. The strong affinity of chlorhexidine for proteins has been previously reported (Hjeljord, Rølla & Bonesvoll, 1973). A large percentage of the desorbed material was present in the trichloroacetic acid-precipitate of the solutions (Table 2). Possibly a greater percentage of chlorhexidine would have been desorbed if the solutions had been stirred to reduce boundary layers. This is another example of the problems that arise in attempts to make an *in vitro* model of lens wear. The solutions were not stirred in an attempt to simu-

late a situation in which at any one time the lens would be in contact with less than 10 $\mu$l of tears, although the total daily turn-over volume probably was similar to the volume of desorbing liquid employed.

The findings indicate that the tears act as a sink for chlorhexidine taken up by the lens during storage. The rate of desorption during wear is probably a function of blink rate and tear flow. Once chlorhexidine is desorbed from the lens, the probability of its secondary adsorption to tear proteins is great because of its strong affinity for soluble proteins, the relatively large concentration of these proteins in the tears, and the close proximity of the two. Chlorhexidine molecules complexed with tear proteins would be expelled from the eye via the canaliculus. The replacement tears contain unbound proteins which are available for binding by subsequently desorbed chlorhexidine.

The experiments show that concentration of chlorhexidine in worn lenses remains low when the lenses are maintained as prescribed: cleaning, rinsing and storage in approximately 1·5 ml disinfecting solution. However, repeated exposure to fresh amounts of chlorhexidine solutions without a subsequent washout, or exposure to a very large volume of chlorhexidine will result in increasing concentrations of chlorhexidine in the lens.

These findings offer another example of the need to conduct *in vivo* experiments in addition to *in vitro* measurements. An *in vitro* model that simulates eye-lens interactions cannot be created because of the task of duplicating the complex and often unknown pertinent physiological interactions between ocular tissue and a lens.

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