Adaptation and Growth of *Serratia marcescens* in Contact Lens Disinfectant Solutions Containing Chlorhexidine Gluconate

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Serratia marcescens (11 of 12 strains) demonstrated an ability to grow in certain chlorhexidine-based disinfecting solutions recommended for rigid gas-permeable contact lenses. For a representative strain, cells that were grown in nutrient-rich medium, washed, and inoculated into disinfecting solution went into a nonrecoverable phase within 24 h. However, after 4 days, cells that had the ability to grow in the disinfectant (doubling time, $g = 5.7$ h) emerged. Solutions supporting growth of *S. marcescens* were filter sterilized. These solutions, even after removal of the cells, showed bactericidal activity against *Pseudomonas aeruginosa* and a biphasic survival curve when rechallenged with *S. marcescens*. Adaptation to chlorhexidine by *S. marcescens* was not observed in solutions formulated with borate ions. For chlorhexidine-adapted cells, the MIC of chlorhexidine in saline was eightfold higher than that for unadapted cells. Cells adapted to chlorhexidine showed alterations in the proteins of the outer membrane and increased adherence to polyethylene. Cells adapted to chlorhexidine persisted or grew in several other contact lens solutions with different antimicrobial agents, including benzalkonium chloride.

A number of investigators have reported that various contact lens solutions, particularly certain formulations with chlorhexidine digluconate, a bisbiguanide (1,6-dichlorophenyldiguanido hexane), are susceptible to contamination by *Serratia marcescens* (1, 8, 19, 22). The inability of these contact lens solutions to kill *S. marcescens* is of particular concern because of the association of contaminated solutions with ocular infections (19, 22).

Chlorhexidine digluconate has been reported to have an MIC of 0.003% for *S. marcescens*, and at a concentration of 0.05%, chlorhexidine digluconate has been reported to reduce the population of *S. marcescens* by greater than 99.999% within 10 min (7). Formulations containing 0.05% chlorhexidine are commonly used as skin disinfectants. However, because of ocular toxicity near these concentrations and binding of chlorhexidine to certain contact lens polymers, contact lens solutions are formulated with 0.005 to 0.006% concentrations of the chlorhexidine gluconate salt. The U.S. Food and Drug Administration guidelines for determining the efficacy of chemical disinfection suggest the use of a U.S. Pharmacopia-type multi-item microbial challenge test (26). Briefly, the test recommends inoculation of 20 lenses each with 2×10^6 cells in proteinaceous materials (to mimic eye secretions), and after 3 to 10 min of contact between the lens and the microorganism, application of the disinfection solution as directed for patient use. The disinfection solution passes the test if it renders all lenses negative for microorganisms. The guidance document recommends the use of *S. marcescens* as one of the challenge organisms. The use of decimal reduction times or D values is also recommended in the document for measurement of disinfection efficacies of contact lens solutions. The D value is defined as the time required to reduce the population of viable microorganisms by 90% i.e., 10-fold. Lowe et al. (17)

have recommended that D values be used in a formula ($P = $ minimum recommended disinfection time/D value) to compare efficacies of contact lens solutions.

At sublethal concentrations, chlorhexidine induces changes in cell permeability that cause leakage of K^+, 260-nm light-absorbing material, and pentoses (15). At concentrations of chlorhexidine higher than that which causes maximum leakage, no leakage is detected but the cytoplasm is precipitated (6, 16). At physiologic pH, chlorhexidine exists as a cation which probably exerts its bactericidal effect by combining with the negatively charged protein molecules in the cell wall and cell membrane, altering osmotic equilibrium and perhaps the protein configuration itself (4, 11). Russell and Furr (24) examined the activity of chlorhexidine and benzalkonium chloride against wild-type, outer membrane protein (OMP)-deficient, and lipopolysaccharide-deficient strains of *Escherichia coli*. Chlorhexidine was equally effective against all types of strains, indicating a common mode of action in both wild-type and mutant strains. A chlorhexidine-sensitive strain of *Providencia stuartii* showed greater inner membrane damage and a more rapid decrease in cell electrophoretic mobility than a resistant strain (9). The disparity in sensitivity between the two strains was attributed to changes in the outer membrane which could prevent drug penetration to its presumed target, i.e., the inner membrane. This study investigated the physiological changes that permit *S. marcescens* to survive in chlorhexidine-containing contact lens solutions.

MATERIALS AND METHODS

Cultures. Eleven isolates of *S. marcescens*, mostly from in-use contact lens solutions, were obtained from the lyophilized culture collection at Georgia State University. Working cultures were kept on Trypticase soy agar (Difco Laboratories, Detroit, Mich.) at 4°C with transfers every 2 or

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TABLE 1. Preservative contents and types of contact lens solutions tested

Solution	Constituent(s)[a]	Solution recommendation(s)	Lens type(s)[b]
A	0.006% CHX, 0.5% EDTA	Wetting, soaking, disinfecting	RGP
B	0.005% CHX, 0.02% EDTA, phosphate[c]	Wetting, soaking, disinfecting	RGP, hard
C	0.004% BZK	Wetting, soaking	RGP, hard
D	0.005% CHX, 0.1% EDTA, borate[c]	Rinsing, storing, disinfecting	SCL, RGP
E	0.005% CHX, borate[c]	Disinfecting	SCL
F	0.001% PQ-1, citrate[c]	Rinsing, disinfecting	SCL
G	0.001% PQ-1, borate[c]	Rinsing, storing, disinfecting	SCL

[a] CHX, chlorhexidine digluconate; PQ-1, polyquaternium-1; BZK, benzalkonium chloride.

[b] RGP, rigid gas permeable; SCL, soft hydrophilic contact lenses.

[c] Buffer.

4 weeks. An isolate of *S. marcescens* ATCC 8100 and an isolate of *P. aeruginosa* GSU 3 were employed as controls.

Microbial challenge of solutions. Seven different types of commercially available contact lens solutions were purchased from retail stores. These solutions (Table 1) are recommended for use with rigid gas-permeable and soft (hydrophilic) contact lenses. The challenge organism *S. marcescens* or *P. aeruginosa* (control organism) was grown overnight in Trypticase soy broth (TSB, Difco), harvested by centrifugation, and suspended in saline. The solutions were challenged in their original containers with saline-suspended cells to give a final concentration of 10^6 CFU/ml of solution. To minimize dilution of the contact lens solution, we used a maximal inoculum volume of 100 μl. One milliliter of each challenged solution was inoculated into DE neutralizing broth (Difco), serially diluted, and also plated on DE agar. Solutions that did not eradicate the challenge organisms were filtered through a 0.22-μm-pore-size membrane, and the filtrate was collected in sterile test tubes. The filter-sterilized solutions were rechallenged. In certain experiments, cells that survived in the contact lens solutions (adapted cells) were concentrated with centrifugation and used as challenge inocula.

D-value determinations. D values (24°C) were determined in sterile glass test tubes by the standard procedure recommended by the Food and Drug Administration for contact lens solutions (26). Solutions (9.9 ml) were challenged with 0.1-ml inocula (10^7 cells) for 0.5 to 180 min. In certain experiments, survivals were monitored for over 30 days. The challenged solutions were serially diluted into DE neutralizing broth. Because of the imminence of sampling times, the dilutions from the shorter challenge intervals were kept on ice for not more than 20 min prior to inoculation of 0.1 ml of the cell suspensions into pour plates (triplicate) of 12 ml of Trypticase soy agar. Viable cell numbers were obtained and plotted on a logarithmic scale against time. The slopes representing decreases in cell populations to a minimal count were used to calculate D values. When biphasic survival curves were observed or when cells were observed to increase in number after an initial decline, D values for the decline phases are noted in parentheses (see Table 2). Chlorhexidine digluconate (Sigma Chemical Co., St. Louis, Mo.), at 0.005% in normal saline and in boric acid (0.2 M)-borax (0.05 M) buffer (pH 7.2) was used as a control solution with adapted and nonadapted cells of strain GSU 86-828. The MIC of chlorhexidine digluconate for GSU 86-828 in TSB (one-half strength) was determined with a standard microtiter system. Both adapted and nonadapted cells were inoculated in TSB as positive controls, and TSB alone was used as a negative control. The microtiter plates

were incubated at 37°C for 24 h and then incubated at 25°C for 7 days. The MIC was the maximum dilution of chlorhexidine that showed inhibition of growth at 7 days.

OMPs. The OMPs of cells of *S. marcescens* GSU 86-828 (unadapted and adapted) were examined with procedures modified from those of Manning et al. (18) and Hancock and Nikaido (12). A loopful of cells from a stock culture grown on Trypticase soy agar was inoculated into 15 ml of TSB, which was incubated with periodic agitation for 5 h at 37°C. This culture was transferred to 1 liter of TSB contained in a 2-liter flask. The cells were grown at 37°C with shaking for 16 h. For adapted cells, chlorhexidine digluconate at a subinhibitory concentration of 0.0025% was included in the medium. The culture was cooled in an ice bath for 30 min and harvested in a centrifuge (IEC B-20A rotor) at 6,000 × *g* for 6 min. The cells were then suspended in 80 ml of ice-cold 10 mM sodium phosphate buffer (pH 7.0). 2-Mercaptoethanol (final concentration, 0.14 M) was added to the cells in the ice bath, and the cells were ruptured by three 30-s pulses of sonication (60 MHz; W140 Sonifier Cell Disrupter, Heat Systems-Ultrasonics, Inc., Plainview, N.Y.). This preparation was centrifuged at 8,000 × *g* and 4°C for 20 min. The supernatant containing the cell membranes was centrifuged at 100,000 × *g* (Beckman 35 Ti rotor) and 4°C for 40 min. The pelleted membranes were suspended in 20 ml of cold phosphate buffer, washed twice by centrifugation at 100,000 × *g*, and stored in phosphate buffer (4 to 5 ml) at −80°C. The protein concentration (10 to 12 mg of protein per ml) was determined from the absorbancies at two different wavelengths by the following formula: $[1.56(O.D._{280})]$ − $[0.76(O.D._{260})]$, where O.D. is optical density. The cell membrane preparation consisted of an inner (cytoplasmic) membrane and an outer membrane with peptidoglycan. Two milliliters of the cell membrane was extracted twice with 10-ml volumes of a solution composed of 2% Triton X-100 and 5 mM magnesium chloride in 10 mM Tris-HCl buffer (pH 8). Each extraction was done for 10 min at 23°C and was followed by centrifugation in the cold at 200,000 × *g* for 45 min (Beckman 75 Ti rotor and UltraClear tubes). The pellet (outer membrane with peptidoglycan) was then extracted twice with 2% Triton X-100 and 5 mM EDTA in Tris-HCl buffer. This procedure solubilized approximately 60% of the OMPs. Two volumes of ice-cold 95% ethanol were added, with stirring, to the pooled supernatant (Triton-MgCl$_2$-solubilized OMPs). The solubilized OMPs were stored overnight at −20°C, and the precipitated protein was sedimented at 20,000 × *g* (IEC B-20A rotor). The protein concentrations of the samples were determined with the BCA Protein Assay Reagent kit (Pierce, Rockford, Ill.). The protein was then suspended in 800 μl of polyacrylamide gel electrophoresis

TABLE 2. Recovery of bacteria[a] from chlorhexidine solutions

Conditions[b]	D value, min	Coefficient of determination (r^2)	Time to no recovery	Density after 10 days (CFU/ml)
Solution A				
GSU 86-828	(3.9)[c]	(0.91)	60–120 min	10^{10}
GSU 86-828 adapted to solution A	NA[d]	NA	NA	8.1×10^6
GSU 86-828 adapted to solution F	34	0.92	120–180 min	<1
ATCC 8100	(35.6)	(0.96)	80–120 min	10^5
P. aeruginosa GSU 3	0.4	0.97	1–3 min	<1
Solution A, filter sterilized				
GSU 86-828	(21.3)	(0.91)	80–120 min	10^5
P. aeruginosa GSU 3	2.4	0.95	5–10 min	<1
Solution D				
GSU 86-828	3.1	0.99	10–20 min	<1
GSU 86-828 adapted to solution A	(97.3, 4,023.5)	(0.92, 0.90)	12–13 days	$<10^1$
Solution E				
GSU 86-828	3.9	0.99	15–27 min	<1
GSU 86-828 adapted to solution A	(1.8, 146.5)	(0.90, 0.91)	3–24 h	<1
Controls				
NS, GSU 86-828[e]	<1	NA	0.5–1 min	<1
BI, GSU 86-828[e]	<1	NA	0.5–1 min	<1

[a] Inoculum concentration, 10^6 cells per ml.

[b] Code numbers indicate strains of *S. marcescens* unless otherwise specified.

[c] Parentheses indicate a decline in cells followed by increased recovery; if two values are present, the second value represents the second phase of a biphasic decline curve.

[d] NA, not applicable.

[e] Includes values for both adapted and unadapted cells. Adapted cells typically had D values lower by 0.1 to 0.2 min, but the data are not significantly different. Control solutions contained 0.005% chlorhexidine in normal saline (NS) or with borate ions (BI).

loading buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Proteins (adjusted to 20 ng/ml of sample) were separated on sodium dodecyl sulfate-polyacrylamide minigels by the PhastSystem (Pharmacia LKB Biotechnology, Piscataway, N.J.).

Adherence studies. Adherence of chlorhexidine-adapted and wild-type *S. marcescens* GSU 86-828 to polyethylene was determined with cells radiolabelled with tritiated leucine (20, 27). Bacteria were grown in TSB at 37°C on a rotary shaker for 12 to 18 h. Cells were harvested, washed twice with 0.9% saline, and suspended in minimal broth (1.0 g of D-glucose, 7.0 g of K_2HPO_4, 2.0 g of KH_2PO_4, 0.5 g of sodium citrate, 1.9 g of ammonium sulfate, 0.1 g of $MgSO_4$ in 1 liter of distilled water) with shaking at 25°C for 1 h. L-[3,4,5-^{3}H]leucine (0.037 to 0.111 MBq/ml) (NEN Research Products, Du Pont Co., Wilmington, Del.) was added, and the suspension was incubated for an additional 20 min. These cells were washed four times and suspended in 0.9% saline to a concentration of about 10^8/ml. Individual polyethylene disks (8-mm diameter) were incubated with 2 ml of a radiolabelled cell suspension for 2 h at 25°C. Disks were removed with a sterile forceps and immersed five times in each of three successive changes (250 ml) of 0.9% saline. The disks were shaken free of excess saline and transferred to 20-ml glass scintillation vials. Ten milliliters of scintillation cocktail (Opti-Fluor; Packard Instrument Co., Downers Grove, Ill.) was added to each vial; the vials were vortexed and counted in a liquid scintillation counter. Samples (100 µl) of serially diluted radiolabelled cell suspensions of known concentrations were distributed into scintillation vials and counted as described above. Counts per minute were converted to cell numbers from a calibration curve relating counts per minute to viable cell numbers. Calibration curves were constructed from counts on pour plates of serial dilutions of labeled cells. Disks without labeled cells were included as controls. All tests were performed in triplicate on three separate days.

RESULTS

Persistence of *S. marcescens*. In preliminary studies, 11 of 12 strains of *S. marcescens* (2 pigmented, 10 nonpigmented) persisted in chlorhexidine solution A at densities equivalent to or greater than the inoculum for over 30 days. All surviving isolates showed an initial decline in recoverable numbers of 30- to 60-fold prior to attaining the initial inoculum levels within 5 days. By 10 days, cell densities usually exceeded inoculum levels by 20- to 30-fold (data not shown). Strain GSU 86-828 was used as a representative organism in further experiments.

Seven different solutions were challenged in their original containers, as well as in test tubes (D-value experiments), with *S. marcescens* GSU 86-828. Recovery of *S. marcescens* from challenged solutions at 24 h was negligible; however, after 7 days, three solutions (A, B, and F) yielded numbers of *S. marcescens* organisms equal to or greater than the original inocula in both test tubes (Tables 2 and 3) and the original containers. A representative survival curve for solution A is presented in Fig. 1. The cell population at day 10 was greater than the inoculum, which indicated logarithmic growth ($g = 5.7$ h). These cells, adapted to solution A, showed no decrease in density when reintroduced to solution A and showed continued growth. In all solutions in which cell densities increased (solutions A, B, and F), viable *S. marcescens* organisms persisted in the original containers over the entire time course (1 to 3 years, depending on the solution) of this study.

TABLE 3. Recovery of chlorhexidine-adapted *S. marcescens* GSU 86-828 from selected contact lens solutions[a]

Conditions[b]	D value, min	Coefficient of determination (r^2)	Time to no recovery	Density after 10 days (CFU/ml)
Solution C				
Control[c]	2.4	0.90	10–15 min	<1
Solution A-adapted cells	NA[d]	NA	NA	7.8×10^4
Solution F				
Control	(414.6)[e]	(0.98)	1–2 days	3×10^3
Solution A-adapted cells	(2,605)	(0.95)	4–5 days	3×10^3
Solution F-adapted cells	(1,030.9)	(0.90)	NA	10^6
Solution G				
Control	316.8	0.91	1–2 days	<1
Solution A-adapted cells	33.0	0.97	2–33 h	<1
Solution F-adapted cells	578.4	0.91	1–2 days	<1

[a] Inoculum concentration, 10^6 cells per ml.
[b] See Table 1 for solution compositions.
[c] Control, cells grown in TSB.
[d] NA, not applicable. Cells persisted at inoculum levels or at least 10^3/ml.
[e] Values in parentheses apply to initial decline phases.

D values. The D values of unadapted and adapted cells of *S. marcescens* GSU 86-828 in chlorhexidine digluconate solutions are given in Table 2. D values for unadapted cells in two (D and E) of the four chlorhexidine solutions tested (A, filter-sterilized A, D, and E) were in agreement with time to no recovery. These two solutions also showed excellent coefficients of correlation for linear regression (r^2 = 0.99). Although the coefficients of correlation for unadapted cells in the other two solutions were acceptable as linear killing (r^2 > 0.90), the calculated D value was not in agreement with the time to no recovery. The control solutions (0.005% chlorhexidine in normal saline with and without borate) gave D values of <1 min for unadapted cells (Table 2). The MICs of chlorhexidine digluconate in TSB for solution A-adapted and unadapted cells of *S. marcescens* GSU 86-828 were 50 and 6.25 μg/ml, respectively. For unadapted cells, the control chlorhexidine solution containing borate ions gave an MIC in TSB of about 10 μg/ml, whereas the MIC for solution A-adapted cells was less than 0.001 μg/ml.

Adapted cells survived for significantly longer periods in solutions D and E, and linear regression analyses indicated biphasic killing curves. Cells adapted to polyquaternium-1
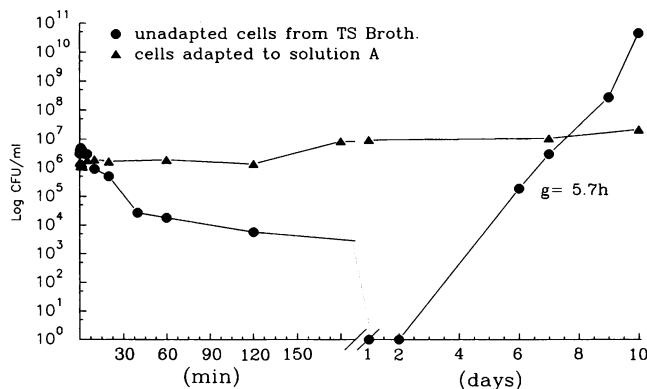

FIG. 1. Killing kinetics of *S. marcescens* GSU 86-828 in solution A (0.006% chlorhexidine digluconate, 0.5% EDTA). These data represent five replications with GSU 86-828 and survival curves of 11 of 12 isolates of *S. marcescens*.

(solution F) did not persist in solution A. The D values obtained in filtered solution A that had previously supported the growth of *S. marcescens* were higher, indicating loss of preservative either due to bacterial growth or during filter sterilization. The control organism *P. aeruginosa* did not persist in solution A or the other solutions, including filtered solutions A and B that had previously supported the growth of *S. marcescens* (Table 2). Solution A-adapted cells, after 24 h of growth in TSB, showed initial susceptibility to chlorhexidine when inoculated into solution A, again followed by adaptation to chlorhexidine (data not shown).

The D values of chlorhexidine-adapted cells in benzalkonium chloride and polyquaternium-1 solutions are shown in Table 3. Unadapted cells of *S. marcescens* GSU 86-828 did not survive in solution C (0.004% benzalkonium chloride); however, solution A-adapted cells, after an initial rapid decline of 30-fold, increased in numbers. In solution F (polyquaternium-1 with citrate buffer [PQ-C]), both solution A-adapted and unadapted cells were not recovered at 2 to 5 days postinoculation but after 10 days both types of cells were recovered at >10^3 CFU/ml. These cells, now adapted to the PQ-C solution when inoculated into the same sterile solution, showed no decline in cell numbers. Solution G, also containing polyquaternium-1 but in a borate buffer system (PQ-B), eliminated both solution A-adapted and PQ-C-adapted and unadapted cells. Solution A-adapted cells had a D value 18-fold lower than that of PQ-C-adapted cells and 10-fold lower than that of unadapted cells (Table 3).

Changes in OMPs. Densitometric scanning of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels of OMPs showed that the 21-kDa protein was about four times more concentrated in adapted cells than in unadapted cells of GSU 86-828 (Fig. 2). The bands corresponding to 134.8 and 92.3 kDa in unadapted cells were not observed in solution A-adapted cells. Modification of the OMPs of adapted cells was further indicated by [^{3}H]leucine uptake, which was greater by a factor of about 1.3 than in unadapted cells.

Adherence studies. Solution A-adapted cells showed a greater level of adherence (1.6 times) to polyethylene (solution bottle material) than did unadapted cells in all tests (Fig. 3). Comparison of data by the unpaired t test gave a P value of 3.338×10^{-3}, and the means of the two sets of data differed at the 0.05 level.

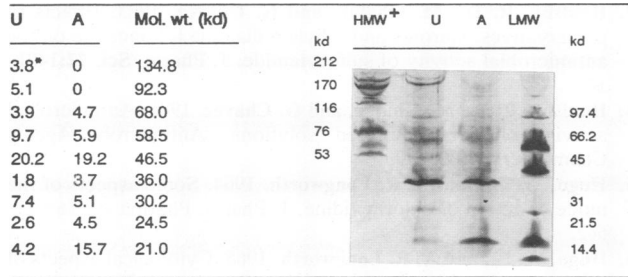| U	A	Mol. wt. (kd)
3.8*	0	134.8
5.1	0	92.3
3.9	4.7	68.0
9.7	5.9	58.5
20.2	19.2	46.5
1.8	3.7	36.0
7.4	5.1	30.2
2.6	4.5	24.5
4.2	15.7	21.0

FIG. 2. OMPs of *S. marcescens* GSU 86-828 unadapted (U) and adapted (A) to solution A (0.006% chlorhexidine digluconate). Relative concentrations of resolved proteins measured as area with a densitometric scanner and electrophoretic patterns compared with high-molecular-weight (HMW⁺) and low-molecular-weight (LMW) standards are shown. Mol. wt. (kd), molecular mass in kilodaltons.

DISCUSSION

The persistence and growth of isolates of *S. marcescens* in solutions containing chlorhexidine varied with the formulation and adapted or unadapted state of the inocula. In contrast to inhibitory borate solutions, solutions with a phosphate or citrate buffer were associated with bacterial survival or growth. Solutions containing borate ions in combination with chlorhexidine effectively inhibited unadapted *S. marcescens* with linear killing kinetics, whereas cells adapted to phosphate-buffered chlorhexidine showed biphasic killing curves in borate-buffered chlorhexidine. Antimicrobial activity of borate solutions for ophthalmic products has been noted by Houlsby et al. (14). Ogunbiyi et al. (21) reported that borate ions enhanced the activity of polyhexamethylene biguanides, and Wilson et al. (27) noted relatively greater inhibitory activity for several contact lens solutions containing borate versus phosphate buffers. Definitive evidence that borate ions were the sole synergistic agent in the various solutions was not established, because most contained additives such as EDTA, wetting agents, and viscosity agents. Addition of EDTA to variously preserved solutions has been known to improve the killing rate but not the MIC for *Pseudomonas*, *Serratia*, and *Candida* species
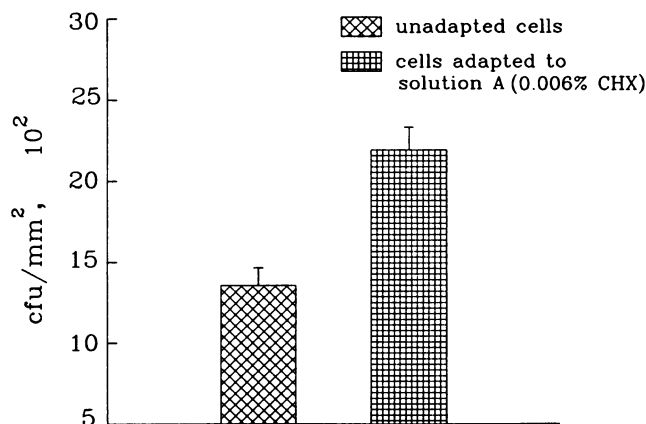

FIG. 3. Adherence of *S. marcescens* GSU 86-828 to polyethylene lens case material. The bars indicate mean results from three different days. Results for each day were derived from triplicate samples. Error bars are standard errors of the means. CHX, chlorhexidine digluconate.

(13). Various substances in contact lens solutions also may reduce overall preservative activity (note herein the activity of control solutions), but their inclusion is necessary because of toxicity or stability needs (13, 24). The 10,000-fold lower MIC obtained for chlorhexidine-adapted versus unadapted cells in the control chlorhexidine solution with borate ions further suggests that adapted cells carry bound chlorhexidine and other ingredients from solution A that are lethal in the presence of borate ions.

D values for the various contact lens solutions were calculated with a statistical approach that applied regression analysis. First-order killing kinetics are assumed when describing a D value. When biphasic killing is observed or when growth occurs, D-value determinations may not apply or may be difficult to interpret. Doubts have been raised as to the validity of the D-value test as a measure of the disinfecting activity of contact lens solutions because of the common occurrence of biphasic killing curves (25). Our data suggest that these biphasic curves are a result of emerging populations of adapted cells. Therefore, biphasic curves for a contact lens solution may be an indication of the potential of the organism to persist in the solution. Because *S. marcescens* appeared to follow a linear killing curve in most solutions during early sampling and sometimes was periodically nonrecoverable, the failure to pursue sampling for periods substantially beyond those that yielded <1 CFU/ml could give misleading information. Therefore, D-value determinations for contact lens disinfecting solutions should be supplemented with samples taken at least 7 days beyond the first negative recovery.

Several theories may be proposed to describe the killing kinetics and survival observed for *S. marcescens* in contact lens solutions containing chlorhexidine. One possibility is that introduced cells initially adhere to the sides of the container. Goldberg et al. (10) have shown that chlorhexidine exposure increased the cell surface hydrophobicity of *E. coli*. Hence, cell surface changes in the presence of chlorhexidine may enhance biofilm formation, resulting in rapid disappearance of the cells from the disinfectant solution. After an adhered population increases in the biofilm, the cells may be shed into the solution in large numbers. In this study, chlorhexidine-adapted cells showed increased adherence to polyethylene. Bacteria in biofilms behave differently (e.g., show more resistance to antimicrobial agents) from planktonic bacteria (5, 21). A second possibility is adaptation. Initial exposure to chlorhexidine only damages the cells. The cells become nonculturable with standard neutralization and recovery procedures until protein-synthesizing repair systems function. Subsequently, the cells proliferate with a sudden increase in population, suggesting logarithmic growth rates. Adaptation of *S. marcescens* to chlorhexidine was indicated by alterations on the OMPs (Fig. 2). A third possibility is that the initial cell population is selected for resistant cells that eventually repopulate the solution. However, our data do not support this possibility because adapted cells became susceptible when passaged in nutrient-rich media. The consistency of growth of *S. marcescens* in phosphate-buffered, chlorhexidine-based contact lens solutions argues against random mutations. Various studies have been unable to demonstrate that chlorhexidine resistance in gram-negative bacteria is plasmid related or transferable (2, 3, 23). Therefore, adaptation and enhanced adherence probably are involved in the persistence of different strains of *S. marcescens* in chlorhexidine-preserved contact lens solutions.

Adaptation of isolates of *S. marcescens* for persistence in

multipurpose disinfecting solutions and, at least in the case of chlorhexidine, enhancement of adherence by the preservative are pertinent in the epidemiology of contact lens-associated infections. Cross-adaptation to polyquaternium-1 and (especially) benzalkonium chloride is of extreme concern because almost 80% of all ophthalmic pharmaceutical eye drops and over-the-counter eye care products are preserved with benzalkonium chloride (at lower concentrations than in disinfecting solutions) and most contact lens users use other ophthalmic products.

REFERENCES

1. Ahearn, D. G., C. A. Penley, and L. A. Wilson. 1984. Growth and survival of *Serratia marcescens* in hard contact lens wetting solutions. CLAO J. 10:172–174.
2. Ahonkhai, I., W. J. Pugh, and A. D. Russell. 1984. Sensitivity to antimicrobial agents of some mercury-sensitive and mercury-resistant strains of gram-negative bacteria. Curr. Microbiol. 11:183–185.
3. Ahonkhai, I., and A. D. Russell. 1979. Response of RPI$^+$ and RPI$^-$ strains of *Escherichia coli* to antibacterial agents and transfer of resistance to *Pseudomonas aeruginosa*. Curr. Microbiol. 3:89–94.
4. Al-Najjar, A. R., and L. B. Quesnel. 1979. Synergism between chlorhexidine and polymyxins against *Pseudomonas aeruginosa*. J. Appl. Bacteriol. 47:469–476.
5. Costerton, J. W., and H. M. Lappin-Scott. 1989. Behavior of bacteria in biofilms. ASM News 55:650–654.
6. Davies, A., and B. S. Field. 1968. The bactericidal activity of biguanides. Biochem. J. 106:14.
7. Denton, G. W. 1991. Chlorhexidine, p. 322–333. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation. Lea & Febiger, Philadelphia.
8. Donzis, P. B., B. J. Mondino, B. A. Weissman, and D. A. Bruckner. 1987. Microbial contamination of contact lens care systems. Am. J. Ophthalmol. 104:325–333.
9. El-Moug, T., D. T. Rogers, J. R. Furr, B. M. A. El-Falaha, and A. D. Russell. 1985. Antiseptic induced changes in the cell surface of a chlorhexidine-sensitive and a chlorhexidine-resistant strain of *Providencia stuartii*. J. Antimicrob. Chemother. 16:685–689.
10. Goldberg, S., Y. Konis, and M. Rosenberg. 1990. Effect of cetylpyridinium chloride on microbial adhesion to hexadecane and polystyrene. Appl. Environ. Microbiol. 56:1678–1682.
11. Greenstein, G., C. Berman, and R. Jaffin. 1986. Chlorhexidine. An adjunct periodontal therapy. J. Periodontol. 57:370–375.
12. Hancock, R. E. W., and H. Nikaido. 1978. Outer membranes of gram-negative bacteria. XIX. Isolation from *Pseudomonas aeruginosa* PAO1 and use in reconstitution and definition of the permeability barrier. J. Bacteriol. 136:381–390.
13. Houlsby, R. D., M. Ghajar, and G. Chavez. 1983. Effects of preservatives, steroids and ethylenediamine tetraacetate on the antimicrobial activity of sulfacetamide. J. Pharm. Sci. 72:1401–1403.
14. Houlsby, R. D., M. Ghajar, and G. Chavez. 1986. Antimicrobial activity of borate-buffered solutions. Antimicrob. Agents Chemother. 29:803–806.
15. Hugo, W. B., and A. R. Longworth. 1964. Some aspects of the mode of action of chlorhexidine. J. Pharm. Pharmacol. 16:655–662.
16. Hugo, W. B., and A. R. Longworth. 1965. Cytological aspects of the mode of action of chlorhexidine diacetate. J. Pharm. Pharmacol. 17:28–32.
17. Lowe, R., V. Vallas, and N. A. Brennan. 1991. Comparative efficacy of contact lens disinfection solutions. CLAO J. 18:34–40.
18. Manning, P. A., L. Beutin, and M. Achtman. 1980. Outer membrane of *Escherichia coli*: properties of the F sex factor *traT* protein which is involved in surface exclusion. J. Bacteriol. 142:285–294.
19. Mayo, M. S., R. L. Schlitzer, M. A. Ward, L. A. Wilson, and D. G. Ahearn. 1987. Association of *Pseudomonas* and *Serratia* corneal ulcers with use of contaminated solutions. J. Clin. Microbiol. 25:1398–1400.
20. Miller, M. J., and D. G. Ahearn. 1987. Adherence of *Pseudomonas aeruginosa* to hydrophilic contact lenses and other substrata. J. Clin. Microbiol. 25:1392–1397.
21. Ogunbiyi, L., F. X. Smith, and T. M. Reidhammer. July 1988. U.S. patent 4,758,595.
22. Parment, P. A., R. Ronnerstam, and M. Walder. 1986. Persistence of *Serratia marcescens*, *Serratia liquefaciens* and *Escherichia coli* in solutions for contact lenses. Acta Ophthalmol. 64:456–462.
23. Russell, A. D. 1985. The role of plasmids in the bacterial resistance to antiseptics, disinfectants and preservatives. J. Hosp. Infect. 6:9–19.
24. Russell, A. D., and J. R. Furr. 1986. Susceptibility of porin- and lipopolysaccharide-deficient strains of *Escherichia coli* to some antiseptics and disinfectants. J. Hosp. Infect. 8:47–56.
25. Sutton, S. V. W., R. J. Franco, D. A. Porter, M. F. Mowrey-McKee, S. C. Busschaert, J. F. Hamberger, and D. W. Proud. 1991. D-value determinations are an inappropriate measure of disinfecting activity of common contact lens disinfecting solutions. Appl. Environ. Microbiol. 57:2021–2026.
26. U.S. Food and Drug Administration. 1989. Guidelines for soft (hydrophilic) contact lens solutions. Department of Health and Human Services, Washington, D.C.
27. Wilson, L. A., A. D. Sawant, and D. G. Ahearn. 1991. Comparative efficacies of soft contact lens disinfectant solutions against microbial films in lens cases. Arch. Ophthalmol. 109:1155–1157.