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NOTES

Bactericidal Activity of Cerumen†

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Freshly collected cerumen (dry form) suspended at a concentration of 3% in glycerol-sodium bicarbonate buffer showed bactericidal activity against some strains of bacteria tested. This suspension reduced the viability of *Haemophilus influenzae*, *Escherichia coli* K-12, and *Serratia marcescens* by more than 99%, whereas the viability of two *Pseudomonas aeruginosa* isolates, *E. coli* K-1, *Streptococcus*, and two *Staphylococcus aureus* isolates of human origin was reduced by 30 to 80%. The results support the hypothesis that cerumen functions to kill certain foreign organisms which enter the ear canal.

Cerumen, commonly known as earwax, is secreted by both ceruminous and sebaceous glands. Two distinct forms of human cerumen, dry and wet, are associated with race and controlled by two autosomal alleles (10). The dry allele is predominant in Mongoloid populations of Asia and in American Indians, whereas the wet allele is found predominantly in Caucasian and Negro populations (1, 10). Earwax has been found to contain amino acids, fatty acids, neurostearic acid, cerotic acid, cholesterol, triglyceride, hexone bases, lysozyme, immunoglobulin, glycopeptide, copper, and other components, although differences in composition between the cerumen types have been described (6, 7, 9, 15).

The function of cerumen in protecting the ear against invasion of microorganisms has long been a subject of controversy. It has been suggested that cerumen is unable to prevent infection and that the rich nutrients of earwax support luxuriant growth of bacteria and fungi (3, 8, 13, 14). On the other hand, it has been suggested that cerumen might have antimicrobial activity, although little evidence has been presented to support this contention (5, 9). Burtenshaw (2) extracted cerumen with either saline or an alcohol-ether solvent and showed that the saline extract promoted the growth of streptococci somewhat, whereas the alcohol-ether extract was inhibitory. However, the concentration of cerumen in the alcohol-ether extract used by this author was not specified.

In this communication we will describe a potent antibacterial activity of cerumen suspended in buffer against certain strains of common bacteria which are often encountered in humans.

Pooled cerumen was collected with a sterile earwax hook from 12 healthy individuals aged from 5 to 42, including males and females, and kept in a sterile bottle at 4°C. All cerumen belonged to the typical dry form, which appeared flaky or granular and yellowish white. The pooled cerumen was mixed well, weighed, and suspended in buffer (5% $NaHCO_3$, pH 8.2, containing 30% glycerol) at a concentration of 3.5% (wt/vol). The cerumen-buffer mixture was homogenized by repeated passage through a series of needles ranging from 19 to 23 gauge. This procedure broke the cerumen into fine particles distributed evenly in buffer and resulted in a milky suspension. Cerumen suspensions at concentrations over 3.5% were unsatisfactory because all the cerumen remained in big particles even after prolonged homogenization. The cerumen was sterilized by ethylene oxide or used without sterilization. The unsterilized preparation contained approximately 10^4 organisms per ml of cerumen-buffer suspension. The organisms were identified primarily as *Staphylococcus epidermidis*.

The strains used and their sources are listed in Table 1. Levinthal medium (12) and chocolate agar were used for growth of *Haemophilus influenzae*. For all other bacteria, proteose peptone beef extract medium (4) was used. One single colony was inoculated into 10 ml of broth in a 125-ml flask and incubated for 6 to 8 h at 37°C with vigorous agitation.

To determine the bactericidal activity of ce-

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TABLE 1. *Effect of cerumen (3%) on viability of bacteria*

Strain	Strain source	Viable counts (cells per ml)		% Killed by cerumen
		Buffer	Cerumen suspension[a]	
E. coli K-12 strain JF404	(4)	4.4×10^7	8.0×10^4	99.8
E. coli K-1	Stool[b]	1.2×10^7	2.5×10^6	79.2
S. marcescens JF246	(4)	1.6×10^7	3.8×10^4	99.7
H. influenzae (type b)	Cerebrospinal fluid[b]	1.5×10^7	1.0×10^5	99.3
H. influenzae (noncapsular)	Otitis media[b]	1.9×10^7	6.0×10^4	99.7
P. aeruginosa	Sputum[c]	5.5×10^8	2.6×10^8	52.7
P. aeruginosa	Urine[c]	1.7×10^8	1.2×10^8	29.4
Streptococcus	Skin lesion[c]	5.0×10^7	2.4×10^7	52.0
S. aureus	Wound[c]	1.6×10^8	6.7×10^7	58.1
S. aureus	Nose[c]	2.6×10^8	6.5×10^7	75.0

[a] Cerumen suspension contained 1.5×10^4 organisms per ml.
[b] Bureau of Biologics, Food and Drug Administration (Rachel Schneerson).
[c] Clinical Pathology Department, Clinical Center, National Institutes of Health (James D. MacLowry).

rumen, 0.15 ml of the cerumen suspension at 3.5% (wt/vol) was first mixed with 0.025 ml of each culture to be tested (final cerumen concentration, 3%), and the mixture was incubated at 25°C (room temperature) for 20 min. The mixture was examined for changes in cell morphology by phase-contrast microscopy. Next, the mixture was passed into a series of 10-fold dilutions with proteose peptone beef extract broth or Levinthal medium. Samples of 0.1 ml each of appropriate dilutions (10^{-4} to 10^{-6} for control, and 10^{-2} to 10^{-6} for cerumen suspensions) were plated in duplicate on proteose peptone beef extract agar or chocolate agar. Survivors were counted after 24 h of incubation at 37°C.

Cerumen at different concentrations, suspended in glycerol-sodium bicarbonate buffer, was measured for its bactericidal activity. Viability of *Escherichia coli* K-12 cells decreased as cerumen concentration increased (Fig. 1); at 0.1% cerumen, one-third of the cells were killed, whereas as the concentration of cerumen increased to 3%, cell survival was reduced to less than 1%. Since cerumen was only partially soluble in the buffer, a final concentration of 3% was selected for testing the bactericidal activity of cerumen suspensions. This suspension appeared to be homogeneous. Decrease in glycerol concentration below 30% in the buffer resulted in the unhomogenization of the cerumen preparation. Other tested solvents—ethanol-ether, 5% Triton, or saline—gave a poor suspension of cerumen.

Table 1 illustrates the bactericidal effect of cerumen on a variety of strains. *H. influenzae*, *E. coli* K-12, and *Serratia marcescens* strains were markedly susceptible to cerumen with more than 99% killed, whereas four other species

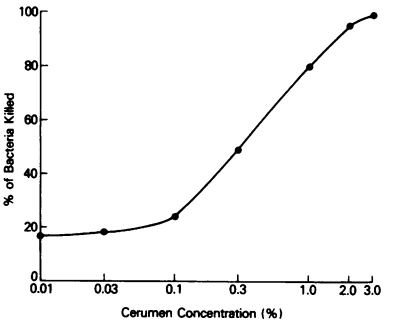

FIG. 1. *Killing of E. coli K-12 strain JF404 cells by cerumen. Cerumen at different concentrations suspended in glycerol-sodium bicarbonate buffer was mixed with freshly grown cells. After 20 min of incubation at 25°C, the surviving cells were determined as described previously. (Original count on cerumen buffer suspension was 1.3×10^4/ml.)*

tested (*E. coli* K-1, *Pseudomonas aeruginosa*, *Streptococcus*, and *Staphylococcus aureus*) were more resistant, with an overall lethality of 30% to 80%. The reduction in the number of viable colonies in treated samples was not due to cell aggregation, since in cerumen-treated cells no cell clumps were detected by microscopic examination.

The buffer alone showed no effect on the viability of the strains tested after incubation at 25°C for 20 min, with the exception of the two *H. influenzae* strains, where a 35% decrease in viability was observed.

Cerumen-treated *E. coli* and *S. marcescens* cells were converted to spheroplasts, many of which lysed as observed by microscopy during a 20- to 30-min incubation. To further determine whether cerumen lysozyme (9, 10) is the sole

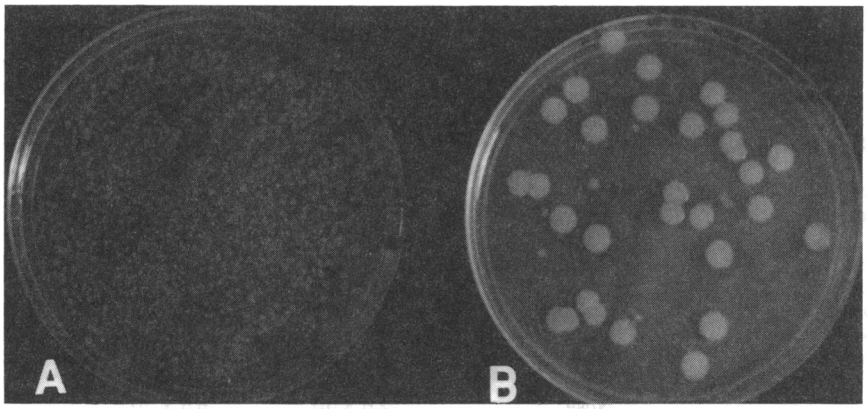

FIG. 2. *Colony appearance of E. coli K-12 strain JF404 cells after 3% cerumen treatment. (A) Control cells in buffer after 10^{-4} dilution; (B) cerumen-treated cells after 10^{-3} dilution.*

active agent leading to the death of cells, pure egg white lysozyme (Sigma) was dissolved in test cultures was dependent upon the concentrations of cerumen used. Perhaps a concentration buffer at various concentrations up to 0.5 mg/ml, which is much higher than the concentration of lysozyme present in cerumen, and was substituted for the cerumen suspension in the same procedure described above. Under these conditions, the lysozyme treatment resulted in a negligible number of spheroplasts and had no detectable effect on the viability of any of the eight strains tested. This indicated that some component(s) other than cerumen lysozyme was required for the bacteriolytic activity of this material. We cannot exclude that lysozyme by itself could become active in the presence of a specific immunoglobulin or that some other cerumen component(s) besides lysozyme is a potential bactericidal agent. The concentration of contaminating organisms present in the final cerumen suspension had no effect on the viability of test cultures under the test conditions. Results indicate that no difference in bactericidal activity was found between nonsterile and sterilized cerumen when *E. coli* and *H. influenzae* strains were tested.

After treatment of *E. coli* cells with cerumen, many survivors were larger and more mucoid than untreated cells (Fig. 2). Possibly, the mucoid material forms a barrier on the surface of the cell that prevents entry of the active component(s) of cerumen.

Our results showed that the susceptibility of of cerumen over 3% would result in a higher bactericidal effect on pathogenic strains. A more efficient buffer could be formulated, for the buffer we used had limited ability to suspend higher concentrations of cerumen. The correlation between cerumen concentration and decreased viability of tested organisms also indicated that some cerumen component(s) itself is responsible for the decreased viability. However, this correlation does not exclude the possibility of a synergistic effect of the buffer with the bactericidal component(s) in cerumen.

It is of interest that dry-form cerumen showed a high bactericidal activity against two strains of *H. influenzae* tested. *H. influenzae* type b is the major cause of meningitis in infants and young children (11); noncapsular *H. influenzae* strains are often involved in middle ear infections. Nevertheless, because cerumen is secreted into the external ear it normally does not come in contact with organisms involved in middle ear infection.

This work demonstrates that cerumen has antibacterial activity on each of 10 strains of bacteria tested. These results are in contrast to those reported by other investigators (3, 8). Differences in cerumen used are one possible explanation for this discrepancy. We used dry-form cerumen, whereas others may have used the wet form. Homogenization of cerumen may also be critical, e.g., a nonpolar buffer may be necessary to solubilize active hydrophobic antimicrobial components. Since the bacteriolytic principle in cerumen is likely to be hydrophobic, the cross-streaking test on agar plates is not suitable for testing the bactericidal activity of cerumen.

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LITERATURE CITED

1. **Bass, E. J., and J. F. Jackson.** 1977. Cerumen types in Eskimos. Am. J. Phys. Anthropol. **47**:209-210.
2. **Burtenshaw, J. M. L.** 1942. The mechanism of self-disinfection of the human skin and its appendages. J. Hyg. **42**:184-210.

3. **Creed, E., and V. E. Negus.** 1926. Investigations regarding the function of aural cerumen. J. Laryngol. Otol. **41:** 223-230.
4. **Foulds, J., and C. Barrett.** 1973. Characterization of *Escherichia coli* mutants tolerant to bacteriocin JF246: two new classes of tolerant mutants. J. Bacteriol. **116:** 885-892.
5. **Hyslop, N. E., Jr.** 1971. Earwax and host defense. N. Engl. J. Med. **284:**1099-1100.
6. **Kataura, A., and K. Kataura.** 1967. The comparison of lipids between dry and wet types of cerumen. Tohoku J. Exp. Med. **91:**227-237.
7. **Masuda, H., S. Shichijo, T. Goya, and M. Takeuchi.** 1978. Isolation and partial characterization of a glycopeptide from cerumen. Kureme Med. J. **25:**203-205.
8. **Perry, E. J., and A. C. Nichols.** 1956. Studies on the growth of bacteria in the human ear canal. J. Invest. Dermatol. **27:**165-170.
9. **Petrakis, N. L., M. Doherty, R. E. Lee, S. C. Smith, and N. L. Page.** 1971. Demonstration and implications of lysozyme and immunoglobulins in human earwax. Nature (London) **229:**119-120.
10. **Petrakis, N. L., U. Pingle, S. J. Petrakis, and S. L. Petrakis.** 1971. Evidence for a genetic cline in earwax types in the middle east and southeast Asia. Am. J. Phys. Anthropol. **35:**141-144.
11. **Robbins, J. B., R. Schneerson, M. Argaman, and Z. T. Handzel.** 1973. *Haemophilus influenzae* type b: disease and immunity in humans. Ann. Intern. Med. **78:**259-269.
12. **Rodrigues, L. P., R. Schneerson, and J. B. Robbins.** 1971. Immunity to *Haemophilus influenzae* type b. I. The isolation and some physicochemical, serologic and biologic properties of the capsular polysaccharide of *Haemophilus influenzae* type b. J. Immunol. **107:**1071-1080.
13. **Singer, D. E., E. Freeman, W. R. Hoffert, R. J. Keys, R. B. Mitchell, and A. V. Hardy.** 1952. Otitis externa: bacteriological and mycological studies. Ann. Otol. Rhinol. Laryngol. **61:**317-330.
14. **Syverton, J. L., W. R. Hess, and J. Kovafchuk.** 1946. Otitis externa: clinical observation and microbiological flora arch. Otolaryngology **43:**213-225.
15. **Yassin, A., M. A. Mostafa, and M. K. Moawad.** 1966. Cerumen and its micro-chemical analysis. J. Laryngol. Otol. **80:**933-938.