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SHORT COMMUNICATION

The bactericidal effect of silver ions on *Pseudomonas aeruginosa*

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THERE are frequent reports of *Pseudomonas aeruginosa* infections in hospitals, with contamination of hospital equipment and pharmaceutical preparations (Editorial, 1967). Cason, Jackson & others (1966) report a high incidence of this organism in burn patients and found silver nitrate compresses to be an effective prophylactic measure.

Little information is available on the action of silver ions on *Ps. aeruginosa*, and the present communication reports the initial stages of such a study. Using cultures whose growth was eventually limited by the magnesium concentration in the culture medium, Brown & Melling (1968) found that *Ps. aeruginosa* resistance to EDTA was related to the degree of magnesium-limitation. We have examined the effect of magnesium-limitation on sensitivity to silver.

EXPERIMENTAL

Ps. aeruginosa NCTC 6750 was grown in the following medium: D-(+)-Glucose 0·001M, $(NH_4)_2HPO_4$ 0·01M, $(NH_4)_2SO_4$ 0·01M, NaCl 0·0005M, KCl 0·0005M with either 1×10^{-6} or 2×10^{-6} or 1×10^{-4}M $MgSO_4$ as described by Brown & Melling (1968). After 24 hr at 37·5°, cultures were allowed to cool slowly to 25°, and were then centrifuged and washed three times with water at 25°; this procedure resulted in no loss of viability. The cell concentration was adjusted to give an absorbance of 0·20 measured in a 10 mm cell at 470 mμ using a Unicam SP 600 spectrophotometer. This corresponded to a viable count of about 4×10^8 cells/ml. 1 ml of a washed suspension was added to 99 ml of a test solution containing silver nitrate in acetate buffer at 25° to give a final concentration of 1×10^{-5}M silver, ionic strength 2×10^{-4}, pH 5·85 or 6·25, and containing initially about 4×10^6 cells/ml. Viable counts were made at 2·5 min intervals by adding 0·5 ml test suspension to 19·5 ml of $1{\cdot}5 \times 10^{-2}$M thioglycollate in Oxoid nutrient broth No. 1 at 25°. After standing for 35 min at room temperature (20–25°), further dilutions were made in nutrient broth and appropriate volumes spread on over-dried nutrient agar plates. Colonies were counted after 20 to 40 hr at

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37·5°. Quenching in $1{\cdot}5 \times 10^{-2}$M thioglycollate in broth for 35 min before dilution was shown to give optimal recovery of organisms which had been exposed to 1×10^{-5}M silver at pH 6·25 at 25° for 10 min. Preliminary experiments showed that nutrient broth with and without additional sodium chloride or polysorbate 80, or both, effectively neutralized much higher silver concentrations (e.g., 0·5 ml 10^{-3}M of silver to 19·5 ml of neutralizer) when undamaged cells were tested, but these were much less effective than thioglycollate in broth for the recovery of silver-damaged cells. This is consistent with the suggestion of Richards & El Khouly (1967) that penetration of thioglycollate into cells damaged by phenylmercuric nitrate is required for optimal recovery.

RESULTS AND DISCUSSION

Cells obtained from the medium containing 1×10^{-4}M (i.e., excess) magnesium abruptly stopped dividing when the medium became depleted of glucose and will be referred to as "glucose-limited" cells. In the media containing the lower magnesium concentrations, cell division did not cease abruptly but became slower as growth proceeded and as the magnesium levels in the media fell. Cells grown in the medium containing 2×10^{-6}M magnesium had ceased dividing after 24 hr at 37·5° because of glucose depletion (glucose-magnesium-limited cells). Cells grown in 1×10^{-6}M magnesium were still dividing slowly after 24 hr because the glucose had not been completely used, they were thus magnesium-limited but not glucose-limited.

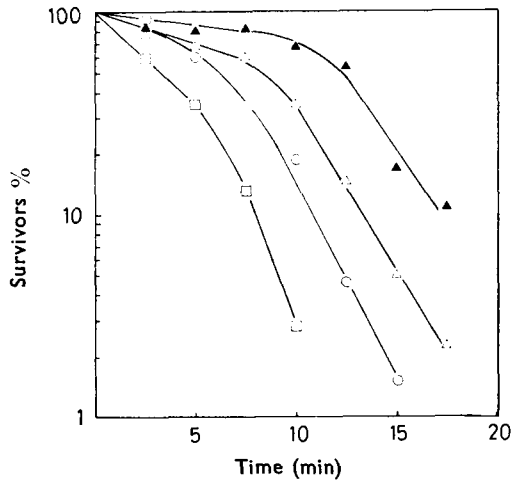

FIG. 1. Rate of kill of *Ps. aeruginosa* in 1×10^{-5}M silver nitrate at 25°. ▲ Glucose-limited cells at pH 5·85. △ Glucose-limited cells at pH 6·25. ○ Glucose-magnesium limited cells at pH 6·25. □ Magnesium-limited cells at pH 6·25.

Representative results given in Fig. 1 show that the glucose-limited cells were more resistant to silver ions than the glucose-magnesium-limited cells. This suggests that cell walls of magnesium-limited cells provide a less effective barrier first to the adsorption by the cell and later

perhaps to entry into the cell of the silver ions. The even lower resistance of cells grown in the medium containing 1×10^{-6}M magnesium may be due to more extensive changes in the cell wall but may be due to differences in glucose-limitation. The difference in sensitivity between magnesium-limited and non magnesium-limited cells may reflect a difference in cell wall composition. Differences in cell wall composition of magnesium limited and non-magnesium limited cells have been shown with *Aerobacter aerogenes* (Ellwood & Tempest, 1967).

Fig. 1 also shows that silver ions are less effective at the lower pH suggesting competition between metal cations and hydrogen ions for anionic sites on the bacteria. Variation of effectiveness with pH may explain the differences in activity of different silver salts reported by Foord, McOmie & Salle (1938).

None of the survivor curves are linear and a shoulder seems to be characteristic of silver-damaged organisms recovered in thioglycollate. These shoulders were not found when test systems (containing glucose-limited cells) were neutralized in nutrient broth; in this case linear survivor curves suggesting much more rapid kill were obtained. It seems likely that although silver is taken up by the cell very quickly, reaction with a vital centre occurs more slowly so that if thioglycollate becomes available during this time it combines with the silver and permits the cell to recover and multiply.

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