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Interaction of aluminium with bacteria isolated from wounds

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The effect of aluminium as metal foil and as a weak solution of potassium aluminium sulphate on the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris*, has been examined over a range of pH values. Aluminium foil, and an aluminium metal film deposited by evaporation on a bonded cellulose fabric, failed to inhibit the growth of the organisms on agar plates. In contact with saline metal foil yielded trace amounts of aluminium in solution but no detectable amounts of aluminium were found in human serum which had been in prolonged contact with aluminium metal. *Staph. aureus* and *Ps. aeruginosa* were adsorbed from aqueous suspension by aluminium surfaces. Below pH 5 the growth of these organisms was inhibited by low concentrations of aluminium in aqueous solution. Preliminary measurements of the uptake of aluminium by these sensitive organisms have been made.

ALUMINIUM foil has been used surgically as a wound covering since the time of Lister. Brown, Farmer & Franks (1948) described the use of aluminium foil in the management of burns and, more recently, other applications of aluminium foil have been reported such as the treatment of venous ulcer of the leg (Haeger, 1963). An aluminised cellulose fabric dressing has also been described (Meyer, 1960).

Aluminium is reputed to have antibacterial properties at very low concentrations (Berger & Einstmann, 1959) and the present work was undertaken to provide more information on the interaction of aluminium and its ions with bacterial cells.

Methods and results

MATERIALS

The aluminium foil and chemicals were analytical grade unless otherwise stated. The aluminium-coated cellulose bonded-fibre fabric was supplied by Messrs. Wallace, Cameron & Co. Ltd., Glasgow, S.5. 2,3,5-*Triphenyltetrazolium chloride* (B.P.) in aqueous solution (10%) was sterilised by filtration. *De-ionised water* had a resistance of 4 mega ohms. *Nutrient agar.* Oxoid granules (C.M.3). *Peptone-free nutrient agar.* Oxoid beef extract (1%), Oxoid yeast extract (2%), dextrose (1%), sodium chloride (0·5%), Oxoid Ion agar No. 2 (1%) in de-ionised water. *Agarose.* Prepared by the method of Hjertèn (1962).

Organisms. Staphylococcus aureus (893), *Pseudomonas aeruginosa* (899), *Escherichia coli* (891), and *Proteus vulgaris* (894) were isolated from infected wounds. Cultures were freeze-dried and catalogued (bracketted numbers) in the Department of Applied Microbiology, University of Strathclyde. Bacterial suspensions in de-ionised water were prepared by washing 24 hr cultures from nutrient agar slopes which had been

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incubated at 31°. Suspensions were centrifuged and resuspended alternately to wash cells free from nutrient material. The final suspensions contained about 120×10^6 viable cells per ml.

EFFECT OF ALUMINIUM ON THE GROWTH OF ORGANISMS

By gel diffusion. Pieces (4 cm^2) of aluminium foil and aluminium-coated cellulose fabric were sterilised by heating in an autoclave (120°/20 min). They were placed in petri dishes and covered with a thin layer of peptone-free nutrient agar containing triphenyltetrazolium chloride solution (0·1 ml %) and seeded with the organism under test. After storage at 4° for 24 hr the plates were incubated (31°/7 days), then examined.

Similar experiments were made replacing the 1% Oxoid Ion agar in the peptone-free nutrient agar with 0·3% agarose, to enhance ion mobility.

No zones of inhibition were observed around the pieces of aluminium foil or aluminised fabric in any of the tests performed.

In aqueous suspension. Sterilised pieces (4 cm^2) of aluminium foil and aluminium-coated cellulose fabric were placed in normal saline (100 ml) containing triphenyltetrazolium chloride solution (0·1 ml). Each flask was separately inoculated with 0·1 ml cell suspension of the organism under examination and maintained at 31° for 72 hr. Subcultures were taken at intervals to assess viability.

Suspensions of the organisms were exposed, under the same conditions, to the cellulose fabric without the aluminium coating.

In contact with aluminium, *E. coli* and *P. vulgaris* gave positive subcultures throughout the experimental period of 72 hr. *Staph. aureus* and *Ps. aeruginosa* were negative on subculture after contact periods of 18 and 24 hr, respectively, and in both cases a red film was observed on the aluminium. This feature, *viz*, the red film of formazan, the reduced form of triphenyltetrazolium (Barnes, 1956), indicated that the organisms had been adsorbed on the metal surface from aqueous suspension. The adsorption was confirmed by microscopic examination.

DETERMINATION OF ALUMINIUM LEACHED OUT OF METALLIC ALUMINIUM BY SERUM AND SALINE

Using plastic containers, pieces (4 cm^2) of aluminium foil and aluminium-coated cellulose fabric were placed in separate 10 ml volumes of fresh human serum or in 0·9% sodium chloride solution in de-ionised water. Solutions were maintained at 31° for 40 days. The aluminium squares were removed and the fluid assayed for aluminium content by the method of Jones & Thurman (1958). A wet ash extraction procedure was used for the human serum (Sandel, 1950). Some samples of ash from the experiments using human serum were extracted with hydrochloric acid/water (1 : 1), the extracts mixed and evaporated to dryness on a rotary film evaporator and the residue of chlorides examined for aluminium by mass spectrometry.

No aluminium was detected in any of the experiments using human serum or when the aluminium-coated fabric had been immersed in saline.

After 40 days at 31° the sodium chloride solution in which the foil had been immersed contained 0·08–0·9 μg/ml aluminium, mean value 0·33 μg/ml (s.d. = 0·29).

EFFECT OF A WEAK SOLUTION OF POTASSIUM ALUMINIUM SULPHATE ON THE GROWTH OF ORGANISMS OVER A pH GRADIENT

Plates (16 cm × 24 cm) of peptone-free nutrient agar containing the following systems in wedge-shaped portions, one superimposed on the other, were prepared as described by Sacks (1956), 150 ml volumes composing each wedge to eliminate any irregularities in the surfaces and to give sufficient material for pH measurement after dissecting out. Sodium hydroxide solution (20 g/litre; 15 ml) was included in the lower wedge which was poured with the plate suitably tilted; the plate was levelled after 1 hr and the upper wedge containing potassium aluminium sulphate solution (176 g/litre, pH 3·2; 15 ml) poured and allowed to set. To provide other pH gradient plates without the aluminium salt, for comparison, similar plates were prepared incorporating sodium hydrogen sulphate solution (103 g/litre, pH 1·1; 15 ml) in the upper wedge. The prepared plates were dried at 31° for 1 hr and the standard aqueous suspensions of wound organisms containing 0·1% of the triphenyltetrazolium chloride solution streaked on each plate in the direction of the pH gradient. The plates were incubated at 31° for 72 hr and the pH at the limits of growth measured, first by laying narrow-range indicator papers on the agar surface adjacent to the upper and lower limits of growth and subsequently, by dissecting out a narrow strip of agar at the boundaries of growth, melting, cooling to 45° and inserting a glass electrode assembly. The pH meter was standardised to buffer solutions at 31°.

The upper limit of growth was in the region of pH 10 in all cases. On the sodium hydrogen sulphate pH gradient plates, the lower limits of growth occurred at pH values 4·0 for *E. coli* and 4·5 for *P. vulgaris*, *Ps. aeruginosa* and *Staph. aureus*. In the presence of the aluminium salt the lower limits of growth had the following pH values: *E. coli* 5·2, *P. vulgaris* 5·7, *Ps. aeruginosa* 5·5 and *Staph. aureus* 6·5. The calculated concentration of aluminium in the areas of the lower growth boundaries was about 8 μg/ml.

EVALUATION OF THE BACTERICIDAL EFFECT OF AN ALUMINIUM SOLUTION ON *Staph. aureus* AND *Ps. aeruginosa* AND ESTIMATION OF THE ALUMINIUM BOUND

Volumes (50 ml) of potassium aluminium sulphate solution (0·3518 g/litre in de-ionised water, adjusted to pH 4·5 (using 0·1N ammonium hydroxide solution), were mixed with 50 ml volumes of suspensions of the organisms containing about 12×10^6 viable cells per ml and maintained at 18°. At intervals, samples (1·0 ml) were suitably diluted and 0·5 ml used for surface counting on dried agar plates. Simultaneously, 10 ml samples were withdrawn and centrifuged (10 min/2° at 3,200 revs/min); 5 ml of the supernatant liquid was assayed for aluminium content by the method

of Jones & Thurman (1958). A similar suspension of the organisms in a solution containing sodium hydrogen sulphate (0·1024 g/litre) was sampled to compare the viable count at the same pH level in the absence of aluminium.

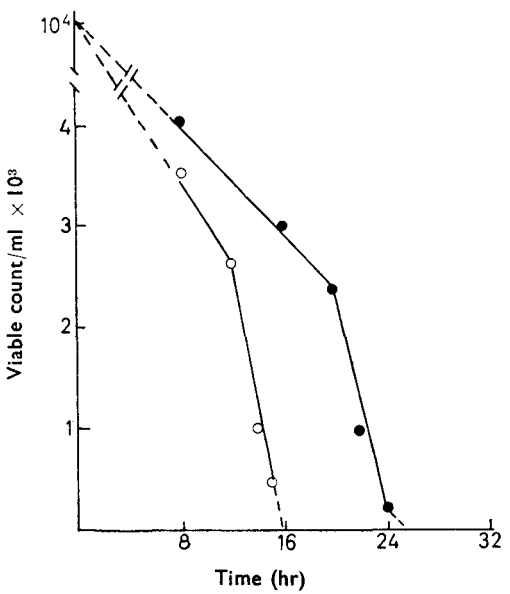

FIG. 1. The fall in viable count of suspensions of *Staph. aureus* and *Ps. aeruginosa* in a solution of potassium aluminium sulphate containing 10·1 μg aluminium/ml; at 18° and pH 4·5. ○ = *Staph. aureus*. ● = *Ps. aeruginosa*.

There was no significant fall in the viable count of the aluminium-free suspensions. The viable count of the suspensions which did contain aluminium salt, fell as indicated in Fig. 1 in which each point represents the mean of 10 counts. The count of *Staph. aureus* fell to zero in 16 hr and that of *Ps. aeruginosa* within 30 hr.

Staph. aureus bound about 60% of the aluminium ions available at the level of 10·1 μg/ml and about 75% of this total binding occurred within 2 min of contact: uptake by *Ps. aeruginosa* reached about 50% of available aluminium and about 90% of this was bound in the first 2 min (Fig. 2).

Discussion

The absence of zones of inhibition in the gel diffusion studies and the absence of detectable amounts of aluminium in human serum which had been in contact with the metal for 40 days, indicate that ions do not pass into solution under these conditions. Whereas aluminium ions have been detected in solution when aluminium foil remained in contact with saline for 40 days, no ions were detected when the foil was replaced by aluminised fabric. The layer of oxide present on aluminium exposed to

the air was probably much thicker on the metal film deposited by evaporation and this could account for the failure to remove ions from the metallised fabric.

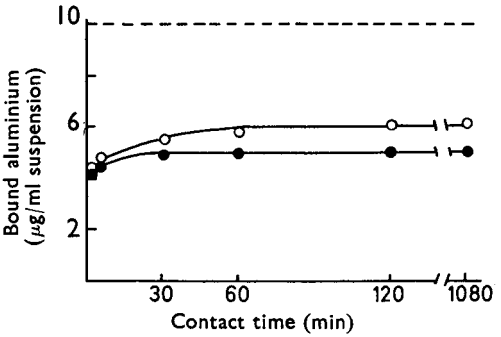

FIG. 2. The rate of binding of aluminium by *Staph. aureus* and *Ps. aeruginosa* from aqueous solution, pH 4·5 at 18°. ○ = *Staph. aureus.* ● = *Ps. aeruginosa.* – – – – Initial concentration of aluminium 10·1 μg/ml.

The oxide layer is responsible for adsorbing certain organisms from aqueous suspension as indicated by the appearance of a red film on the metal when the sensitive growth indicator triphenyltetrazolium chloride was used. It is significant that in the case of organisms which were adsorbed, *Staph. aureus* and *Ps. aeruginosa*, no growth was obtained on subcultures after 18 and 24 hr, respectively. With organisms which were not adsorbed, *E. coli* and *P. vulgaris*, growth was obtained from subcultures after 72 hr.

To investigate the inhibitory effect a soluble aluminium salt was used. Blank & Dawes (1960) have shown that aluminium acetate and basic aluminium chloride, in solution at pH 4·5 and 4·0 respectively, inhibit the growth of micrococci on thin sheets of human callus. Their work indicates that the inhibition is due not to low pH values alone, but to aluminium ions both free and complexed with cutaneous proteins. In the present work the inhibitory effect of aluminium ions in solution was clearly demonstrated on pH gradient plates where all the organisms studied were inhibited below pH 5·2; in this region the concentration of aluminium ions was about 8 μg/ml.

When suspensions of *Staph. aureus* and *Ps. aeruginosa* were exposed to a concentration of 10 μg/ml of aluminium at pH 4·5, the organisms were killed within 16 hr and 30 hr respectively. The cells of both organisms bound aluminium rapidly from solution, the uptake curves being similar to those obtained with *Staph. aureus* for the binding of iron (Beckett, Vahora & Robinson, 1958) and iodine (Hugo & Newton, 1964). The lethal effect demonstrated is probably due to an interaction between aluminium ions and bacterial proteins facilitated by the surface characteristics of these sensitive organisms.

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References

Barnes, E. M. (1956). *J. gen. Microbiol.*, **14**, 57–66.
Beckett, A. H., Vahora, A. A. & Robinson, A. E. (1958). *J. Pharm. Pharmacol.*, **10**, *Suppl.* 160*T*–170*T*.
Berger, U. & Einstmann, E. (1959). *Arch. Hyg. u. Bakteriol.*, **143**, 634–640.
Blank, I. H. & Dawes, R. K. (1960). *A.M.A. Arch. Dermatol.*, **81**, 565–569.
Brown, W. A., Farmer, A. W. & Franks, W. R. (1948). *Amer. J. Surg.*, **76**, 594–604.
Haeger, K. (1963). *Acta. chir. scand.*, **125**, 32–41.
Hjertèn, S. (1962). *Biochem. Biophys. Acta.*, **62**, 445–450.
Hugo, W. B. & Newton, J. M. (1964). *J. Pharm. Pharmacol.*, **16**, 49–55.
Jones, L. H. & Thurman, D. A. (1958). *Plant and Soil*, **9**, 131–142.
Meyer, A. (1960). *Der. Chirurg.*, **31**, 541–545.
Sacks, L. E. (1956). *Nature, Lond.*, **178**, 269.
Sandel, E. B. (1950). *Colorimetric Determination of Traces of Metals*, 2nd ed., p. 153, London: Interscience Publications.