METALLIC CATIONS AND THE ANTIBACTERIAL ACTION OF OXINE

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THE binding of cobaltous and manganous ions by *Staphylococcus aureus* suspensions, alone and in combination with oxine and with iron, has been examined as part of a study of the mechanism of antibacterial action of oxine[1]. The antibacterial properties of oxine solutions containing iron, cobalt and manganese ions, alone or in combination, have been evaluated. These metal ions were chosen because Albert and others have reported the unique action of cobalt in reversing the bacteriostatic and bactericidal actions of iron-oxine solutions against Gram-positive bacteria and that oxine solutions containing cobalt or manganese were much less toxic to *Staph. aureus* than those containing iron (ferrous or ferric), copper or cadmium[2].

The concentrations of metal ions remaining in solution after contact with the bacterial suspensions were determined colorimetrically: total iron was determined as the ferrous-*o*-phenanthroline complex, manganese as the permanganate ion and cobalt as a complex with α-nitroso-β-naphthol 3,6-disulphonic acid (Nitroso R salt). Quantitative recoveries of each of these metal ions were achieved under the conditions obtaining in the biological investigations.

The extent of iron, cobalt and manganese ion binding by *Staph. aureus* suspensions was independent of the contact time between 20 and 60 minutes; the initial metal ion concentrations were sufficient to achieve maximum uptake by bacteria (Fig. 1). Binding was at least 90 per cent complete within 2 minutes.

The curves obtained by plotting the molar concentration of ions bound by *Staph. aureus* suspensions (standardised nephelometrically) against the equilibrium concentration were similar in shape for iron, cobalt and manganese although the maximum uptake of iron and manganese exceeded that for cobalt. Mass Law plots (Rothstein and Hayes[3]) of these results indicate that iron and manganese ions are each bound at two different receptor sites whereas cobalt is bound at only one, thus supporting the previous postulate of binding at an anionic receptor site and a chelating site[1].

An estimation of the number of iron and manganese atoms bound per bacterium is $6{\cdot}5 \times 10^7$, whereas the corresponding figure for cobalt is 4×10^7. The divalent metal ions have similar ionic atomic radii (cobalt 0·72, iron 0·75 and manganese 0·8 Å, neglecting water of hydration) and a common co-ordination number of 6. Although the relative affinities of bivalent cations of the first transition series is $Mn^{++} < Fe^{++} < Co^{++}$

$< Ni^{++} < Cu^{++} > Zn^{++}$[4], the oxidation state of the metal ions on binding at the bacterial surface is uncertain and this order will not necessarily be observed. Experiments with solutions initially containing equimolar proportions of iron and manganese showed that manganese was preferentially bound by *Staph. aureus* suspensions. Further, the total concentration of iron and manganese bound reached a constant value equivalent to the maximum uptake of either iron or manganese alone, thus implying interchangeability at common binding sites. Using solutions containing iron and cobalt, however, the total concentrations of ions bound varies with the relative proportions of the two ions; it may exceed the level of maximum uptake for cobalt alone but does not attain that for iron alone. Cobalt, therefore, appears to compete with iron for one binding site and to prevent normal iron binding at the second; the latter effect might be caused by bound cobalt atoms partially masking adjacent binding sites.

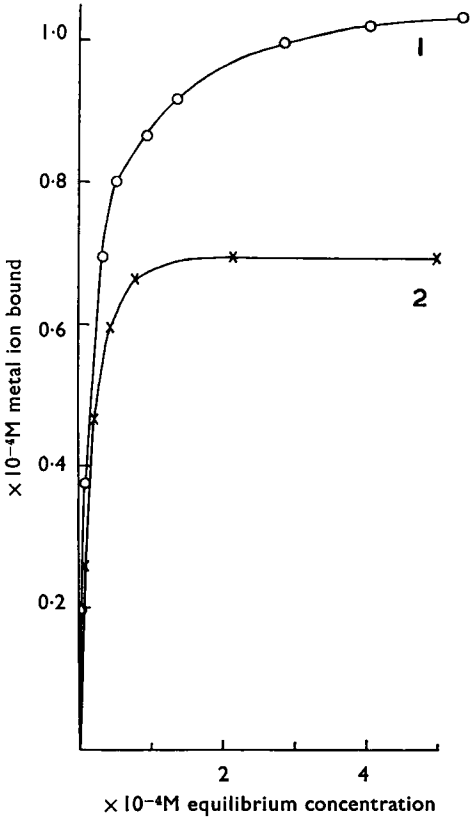

FIG. 1. The uptake of iron (1) and cobalt (2) from their separate solutions by *Staph. aureus* suspensions containing approximately 10^9 organisms/ml. The results for manganese are similar to those for iron.

Slight potentiation of metal binding by *Staph. aureus* was effected by the addition of a small proportion of oxine (1×10^{-5} M) to the contact solutions (molar ratio of oxine : metal ion 1 : 10 or less). The analytical methods were insufficiently sensitive to allow a reduction of the molar ratio of oxine : iron to 1 : 1.

Bactericidal evaluation was carried out under similar conditions to those for the uptake work; the metal ions, *per se*, at maximum concentrations of 1×10^{-4} M for iron, cobalt and manganese were inactive, as was oxine at 1×10^{-5} M. The maximum contact time in all these experiments was 150 minutes. Using solutions containing a constant concentration of oxine (1×10^{-5} M) and varying proportions of iron, optimum bactericidal activity against *Staph. aureus* was attained when the molar ratio of iron : oxine was about 3 : 1; the activity decreased markedly

if the ratio was reduced to 0·5 : 1. Solutions containing equimolar proportions of oxine (1×10^{-5} M) and either cobalt or manganese were devoid of activity. Addition of a 5 mole excess of manganese to an iron-oxine solution (1 : 1 molar ratio, 1×10^{-5} M) did not reduce the bactericidal activity, whereas some loss occurred on substitution of a similar molar proportion of cobalt for the manganese.

Thus, traces of cobalt were less effective in reversing the bactericidal effects of iron-oxine solutions against *Staph. aureus* in this system than in the one used by Albert, who found that $0{\cdot}2 \times 10^{-4}$ M cobalt would abolish the bactericidal action of a solution containing iron ($0{\cdot}2 \times 10^{-4}$ M and oxine ($0{\cdot}4 \times 10^{-4}$ M) against *Staph. aureus*[5]. Variations in the degree of efficiency of cobalt in this rôle may probably be attributed to the differences in the experimental procedures adopted.

References

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After Dr. Robinson presented the communication there was a DISCUSSION.