RESEARCH PAPERS

THE INTERACTION OF CHELATING AGENTS WITH BACTERIA

PART II. CATION BINDING AND THE ANTIBACTERIAL EFFECTS OF 8-HYDROXYQUINOLINE (OXINE)*

BY ANN E. ROBINSON, A. H. BECKETT AND R. N. DAR

From Chelsea School of Pharmacy, Chelsea College of Science and Technology, Manresa Road, London, S.W.3

Received March 7, 1960

The binding of iron, cobalt and manganese ions from their aqueous solutions by viable and heat-killed suspensions of *Staph. aureus* is investigated. Competitive uptake of any two of these metal ions by viable and heat-killed organisms was also studied. The cation binding properties of viable and nonviable suspensions of *Staph. aureus* were compared and discussed. Data for the uptake of metal ions are also discussed in relation to the bactericidal effects of oxine solutions containing an equimolar concentration of iron and similar solutions containing additional iron, or cobalt or manganese.

WE have postulated previously[2] that the bactericidal action of solutions containing oxine and iron might be the consequence of the metal ion forming a bridge to link the oxine molecule to an important site in the cytoplasmic membrane of the bacteria. Since Rubbo, Albert and Gibson[3] have demonstrated the unique action of cobalt in reversing the bactericidal action of iron-oxine solutions against *Staphylococcus aureus* suspensions, the uptake of cobalt was studied under conditions similar to those used previously with iron. Manganese was also investigated as an example of a metal ion devoid of antibacterial activity in the presence of oxine and apparently incapable of reversing the toxic effects of iron-oxine solutions against *Staph. aureus* suspensions. Binding studies of the separate cations by *Staph. aureus* suspensions using solutions containing various combinations of cobalt and manganese, together with evaluation of the bactericidal action of oxine solutions containing one or more of these metal ions, were undertaken and similar uptake studies have been carried out using heat-killed bacterial suspensions.

Thus it was hoped that some pattern would emerge to contribute towards a greater understanding of the mechanism of antibacterial action of oxine.

METHODS

Reagents. Analar grade chemicals were used unless otherwise stated. 8-Hydroxyquinoline (oxine) was as described previously[2]. Solutions of ferrous ammonium sulphate ($FeSO_4(NH_4)_2SO_4.6H_2O$), cobalt sulphate ($CoSO_4.7H_2O$) and manganese sulphate ($MnSO_4.6H_2O$) in water were

* A summary of part of the work described in this paper was presented at the British Pharmaceutical Conference, September 1959[1].

prepared immediately before use. If oxine was added to any solution of a metallic salt, the mixture was stored at least 24 hours before use.

Precautions taken to minimise contamination with metal ions. Distilled water (subsequently referred to as water) was obtained from a Baracop still (Baird and Tatlock Ltd.). Glassware was treated with acid and alkali before use following the method of Waring and Werkman[4].

Spectrophotometer. A Hilger H 700 spectrophotometer was used in conjunction with matched fused silica cuvettes.

Preparation of bacterial suspensions. The test bacteria (*Staph. aureus*) were harvested from surface cultures with water; the resulting suspension was centrifuged at 8,500 *g* for 10 minutes and the cells resuspended and again washed with water. The final bacterial suspension was standardised nephelometrically to contain 10^{10} organisms/ml.

Preparation of suspensions of heat-killed bacteria. Washed suspensions of *Staph. aureus* in water were heated in flowing steam at 98–100° for 15 minutes. After centrifuging and washing once, the bacteria were resuspended in water and diluted to the original volume.

General method for the uptake experiments. Solutions containing either iron, cobalt or manganese, or oxine or a mixture of two or more of these substances (total volume 45 ml.), were introduced into glass centrifuge tubes which were immersed in a water bath at 25 $\pm$ 1°. After allowing sufficient time for temperature equilibration, 5 ml. portions of the standardised bacterial suspensions were added to each of the solutions to give a final concentration of 10^9 organisms/ml. One hour later, unless otherwise stated, the bacteria were removed by centrifuging and the supernatant solutions analysed quantitatively for the components of the initial solution.

Determination of unchanged oxine in solutions after contact with bacteria. Oxine was determined by measuring the optical density of the solution at 252 mμ after adding an equal volume of 0·2 N hydrochloric acid. This figure was corrected for the presence of cell exudate by measuring the optical density at 252 mμ of a portion of the solution after chloroform extraction to remove oxine and oxine-metal chelates (cf. reference 2 for procedure and reference 5 for precision).

Colorimetric determination of iron. The method was as described previously[2] except that 5 ml. instead of 2 ml. portions of the *o*-phenanthroline reagent were used. The optical densities of the solutions containing less than $0{\cdot}17 \times 10^{-4}$ M iron were measured in 4 cm. cuvettes; 1 cm. cuvettes were used for more concentrated solutions up to a maximum of $0{\cdot}8 \times 10^{-4}$ M iron. Neither cobalt nor manganese, at molar ratios to iron of 10:1 and 50:1 respectively, interfered with colour formation.

Colorimetric determination of cobalt. Cobalt was determined by the formation of a coloured complex with α-nitroso-β-naphthol-3,6-disulphonic acid (Nitroso R. salt): to a suitable volume of solution was added 5 ml. of 2 per cent v/v phosphoric acid (H_3PO_4), 5 ml. of 0·5 per cent. w/v solution of Nitroso R salt in water and 2·5 ml. of 50 per cent w/v solution of sodium acetate ($CH_3COONa.3H_2O$). The mixture was heated on a boiling water bath and 1 ml. of concentrated hydrochloric

acid added after 6 minutes; heating was continued for a further 4 minutes. The solution was cooled and diluted to 100 ml. with water. The optical density of the solution was measued at 530 mμ using 1 cm. cuvettes for solutions containing between $0{\cdot}13 \times 10^{-4}$ M and $0{\cdot}5 \times 10^{-4}$ M cobalt and 4 cm. cuvettes for more dilute solutions. A straight line relation was obtained by plotting the optical density at 530 mμ of the coloured

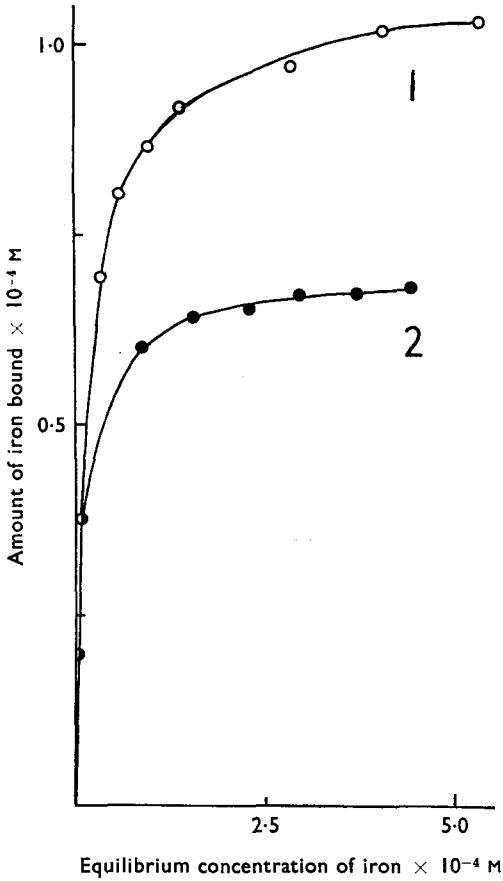

FIG. 1. The uptake of iron by viable (curve 1) and heat-killed (curve 2) suspensions of *Staph. aureus*.

solutions against the cobalt concentration. The colour was stable for at least 3 hours. Neither iron, up to molar ratio of iron to cobalt of 50:1, nor oxine nor an arbitrary concentration of cell exudate (cf. reference 5) interfered with the above method. The effect of widely varying proportions of manganese on the formation of the cobalt-nitroso R complex, using solutions containing between $6{\cdot}2 \times 10^{-6}$ M and $3{\cdot}0 \times 10^{-5}$ M cobalt, were investigated. The deviation from the cobalt calibration curve ($0{\cdot}062$–$0{\cdot}45 \times 10^{-4}$ M) was almost independent of the amount of

manganese ($0{\cdot}20$–$2{\cdot}5 \times 10^{-4}$ M) added to the solutions and of the relative molar proportions of the two metal ions.

Colorimetric determination of manganese. To a suitable volume of solution was added 10 ml. of concentrated nitric acid and 10 ml. of water. The solution was boiled gently for 10 minutes, 0·5 g. potassium periodate (KIO_4) was added and heating was continued by steaming for 60 minutes. The cooled solution was diluted to 100 ml. The optical density of the permanganate solution thus obtained was measured at λ max 525 mμ; 4 cm. cuvettes were used for solutions containing less than $0{\cdot}5 \times 10^{-4}$ M manganese and 1 cm. for those containing between $0{\cdot}5 \times 10^{-4}$ and $3{\cdot}0 \times 10^{-4}$ M manganese. A straight line relation was observed

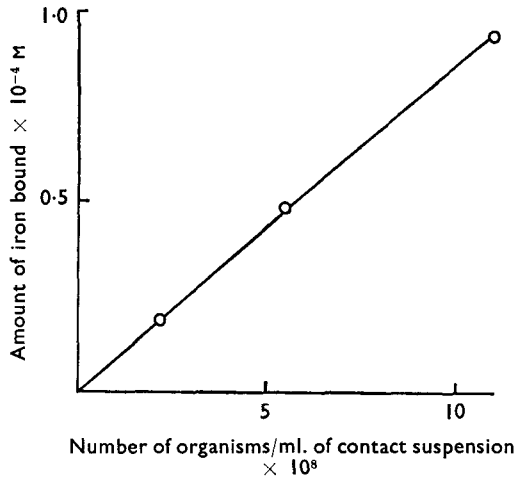

FIG. 2. The relation between the amount of iron bound and the number of organisms in the contact suspension.

on plotting the optical density at 525 mμ against the manganese concentration. Neither iron nor cobalt up to a molar ratio of iron or cobalt to manganese of 50:1, nor oxine nor an arbitrary concentration of cell exudate affected this method.

Bactericidal evaluation. The method was similar to that for the uptake measurements except that sterile materials were used and aseptic conditions were employed. Solutions containing oxine and metal ions, etc., were filtered through sintered glass filters (5/3) and the concentrations checked before use. Suspensions of *Staph. aureus* were prepared in sterile water and standardised to contain $11{\cdot}2 \times 10^8$ organisms/ml. 5 drops (0·09 ml.) of this suspension were added to 10 ml. volumes of the solutions under test (final bacterial concentration of 10^7 organisms/ml.) and 5 drop portions of this mixture were immediately transferred to dry sterile test tubes. The reactions were quenched at timed intervals by the addition of 5 ml. of sterile lemco-peptone broth and then incubated for 24 hours at 37° before examination for the presence of visible growth.

The experiments were replicated (usually 10-fold). Solution temperatures were maintained at 25 $\pm$ 1° before incubation. An oxine solution (1×10^{-5} M) containing an equivalent concentration of iron was included in each set of experiments to act as a daily standard. The percentage deviation of the extinction time observed for the bacteria in the standard oxine iron solution (44 $\pm$ 10 minutes) from the average was calculated and the results for the other solutions adjusted to take into account the day to day variations in the results.

RESULTS

The Uptake of Iron by Viable Staph. aureus *Suspensions*

The results obtained for solutions initially containing up to $6{\cdot}5 \times 10^{-4}$ M iron are represented by curve 1 of Figure 1. Curve 2 of the same diagram shows the results for a heat-killed suspension of the bacteria.

TABLE I

A COMPARISON OF THE UPTAKE OF COBALT AND MANGANESE BY VIABLE AND HEAT-KILLED *Staph. aureus* CELLS

	Initial concentration $\times 10^{-4}$ M	Uptake by	
		Viable organisms $\times 10^{-4}$ M	Heat-killed organisms $\times 10^{-4}$ M
Co^{++}	1·16	0·51	0·44
	1·86	0·54	0·52
	2·79	0·59	0·56
	4·66	0·65	0·62
Mn^{++}	2·55	0·85	0·77
	6·39	1·07	1·01
	7·66	1·11	0·99

Thus the maximum amount of iron bound is much reduced by heat-killing the organisms.

With an initial concentration of $5{\cdot}1 \times 10^{-4}$ M iron, an amount sufficient to allow the maximum uptake by the bacteria, the extent of iron binding was independent of the contact time between 20 and 60 minutes. Sorption was at least 90 per cent complete within 2 minutes.

Iron binding is proportional to the number of bacteria present, at least over a limited range, if the initial concentration is sufficient to attain maximum uptake (Fig. 2).

The Uptake of Cobalt by Staph. aureus *Suspensions*

The results for solutions containing up to $7{\cdot}0 \times 10^{-4}$ M cobalt initially are represented by curve 2 of the Mass Law plot, Figure 7. The graph relating the amount of cobalt bound by the bacteria to the equilibrium concentration of cobalt (see reference 1) showed that the maximum uptake of cobalt was much less than the maximum uptake of either iron or manganese.

The speed of cobalt binding by *Staph. aureus* suspensions was similar to that noted for iron with solutions initially containing $1{\cdot}77 \times 10^{-4}$ M cobalt.

The amount of cobalt bound was reduced slightly by heat-killing the bacterial suspension, as shown by the data in Table I. These results were obtained from a single suspension of the bacteria, a portion of which was heated; precautions were taken to minimise the loss of bacteria during the latter treatment.

The Uptake of Manganese by Staph. aureus *Suspensions*

A plot relating the amount of manganese bound to the equilibrium concentration of manganese is virtually superimposable on the curve

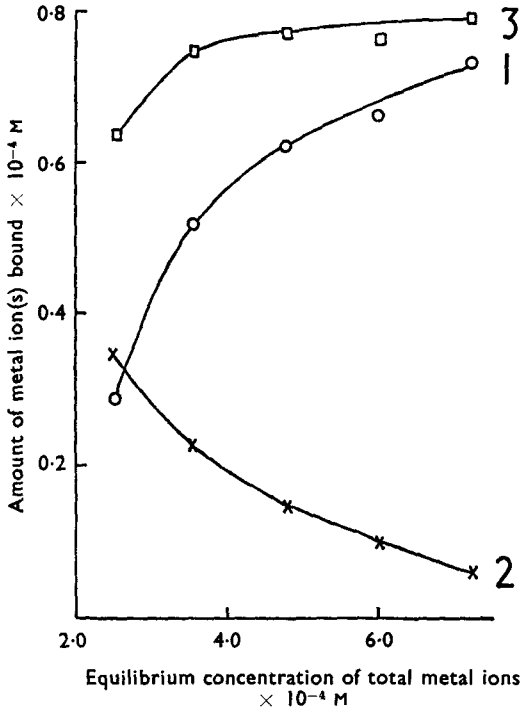

FIG. 3. Competitive binding of iron and cobalt by viable suspensions of *Staph. aureus*. The initial cobalt concentration was constant at $1{\cdot}8 \times 10^{-4}$ M.

Curve 1, the amount of iron bound.
2, the amount of cobalt bound.
3, the total amount of iron plus cobalt bound.

for iron (i.e. curve 1 of Fig. 1). A Mass Law plot of the data for manganese is included in Figure 7 (curve 1).

The speed of uptake of manganese from solution by *Staph. aureus* was similar to that noted for iron. The initial solutions contained $6{\cdot}7 \times 10^{-4}$ M manganese.

The relative amounts of manganese bound by viable and heat-killed suspensions of *Staph. aureus* are shown in Table I. These results, obtained from a single original bacterial suspension, indicate a slight reduction in manganese binding capacity in the heated suspensions.

The relative maximum amounts of iron, cobalt and manganese bound by a *Staph. aureus* suspension containing 10^9 organisms/ml. are $1{\cdot}07 \times 10^{-4}$ M, $0{\cdot}67 \times 10^{-4}$ M and $1{\cdot}06 \times 10^{-4}$ M respectively. These results were obtained using the same initial bacterial suspension; the total number of bacteria present in the contact suspensions was determined microscopically. These precautions were necessary as there were small day to day numerical variations in the bacterial suspensions standardised by the nephelometric method.

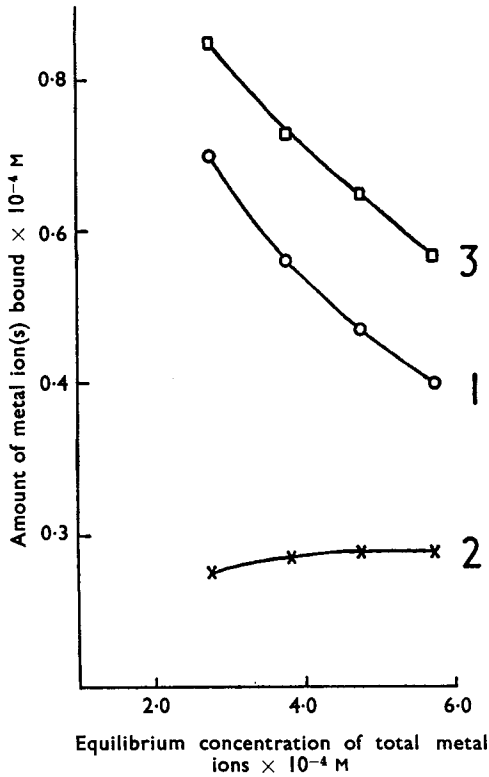

FIG. 4. Competitive binding of iron and cobalt by viable suspensions of *Staph. aureus*. The initial iron concentration was constant at $2{\cdot}7 \times 10^{-4}$ M.

Curve 1, the amount of iron bound.
2, the amount of cobalt bound.
3, the total amount of iron plus cobalt bound.

Competitive Binding of Metal Ions by Living Staph. aureus *Cells*

Iron and cobalt. Figure 3 shows the results obtained for the uptake of iron (curve 1) and cobalt (curve 2) from solutions initially containing a constant concentration of cobalt and varying proportions of iron. The maximum total amount of metal ions bound (curve 3) exceeded the maximum uptake of cobalt alone but was less than the corresponding value for iron alone.

The results for the uptake of iron (curve 1) and cobalt (curve 2) from solutions initially containing a constant concentration of iron and varying amounts of cobalt are presented in Figure 4. Curve 3 of the same diagram shows the total amount of the metal ions bound.

Iron and manganese. Figure 8 shows the plot of the amount of iron (curve 1), and of manganese (curve 2) and of total metal ions (curve 3) bound by a *Staph. aureus* suspension plotted against the equilibrium concentration of the total metal ions when the initial solution contained a constant concentration of manganese and varying proportions of iron. A similar graph was obtained when the iron content of the initial solutions

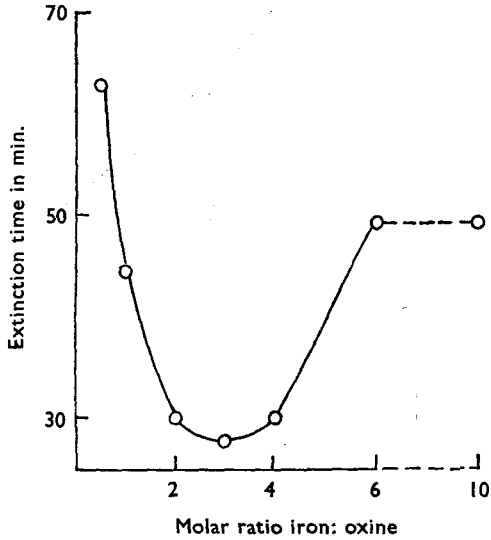

FIG. 5. Extinction times of *Staph. aureus* in solutions containing oxine (1×10^{-5}M) and varying proportions of iron.

was constant and the proportion of manganese was varied. The maximum total amount of bound iron and manganese corresponded to the maximum uptake of either of the individual metal ions in both cases.

Cobalt and manganese. The results for this system were exactly analogous with those for the iron and cobalt competition studies described above.

Competitive Binding of Metal Ions by Heat-killed Staph. aureus *Cells*

Iron and cobalt. The results for iron and cobalt binding by heat-killed organisms from solutions containing both ions are similar to those described above for iron and manganese binding by viable *Staph. aureus* suspensions; the maximum concentration of metal ions bound, however, is lower. Figure 9 shows the results for iron and cobalt binding by *Staph. aureus* suspensions using solutions initially containing varying concentrations of cobalt and a constant concentration level of iron.

Iron and manganese. Figure 10 shows the results obtained when the manganese content of the initial solution was kept constant and the iron

concentration was varied. The result was similar when this position was reversed. The maximum combined total uptake of iron and manganese is about 1 per cent less than the corresponding value for manganese on heat-killed organisms.

Cobalt and manganese. The results were similar to those obtained for the competition studies with iron and manganese with heat-killed suspensions of *Staph. aureus*.

Bactericidal evaluation. The results for the bactericidal evaluation of oxine solutions containing varying proportions of iron are summarised in Figure 5.

The effect of adding cobalt to the oxine-iron solution is shown in Figure 6; addition of a one or two molar equivalents of cobalt (cobalt: oxine: iron of 1 : 1 : 1 or 2 : 1 : 1) caused a slight potentiation of bactericidal

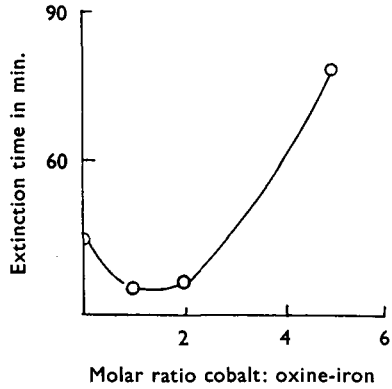

FIG. 6. Effect of cobalt on the extinction time of *Staph. aureus* in a solution containing oxine and iron (both at 1×10^{-5} M).

activity against *Staph. aureus*, whereas, a five molar equivalent of cobalt caused a reduction in activity.

No reduction in activity was observed in solutions containing oxine and iron and manganese at ratios of 1 : 1 : 1, 1 : 1 : 2 and 1 : 1 : 5.

Solutions containing oxine and either cobalt or manganese (up to a five molar equivalent) were non-toxic to *Staph. aureus* under similar conditions.

DISCUSSION

Cation Binding by Viable Staph. aureus *Cells*

Iron. When the initial iron concentration was sufficient to achieve maximum uptake by the bacteria, binding was almost complete within 2 minutes of contact. This is in contrast to the results reported previously[2] using lower concentrations of iron in the contact suspensions in which the metal ion uptake remained incomplete after 60 minutes contact with bacteria.

Presentation of the results for iron binding by a *Staph. aureus* suspension as a Mass Law plot (Rothstein and Hayes[6]) is made in curve 1 of Figure 7. Since two intersecting straight lines are obtained, it may be concluded

that two types of binding site are involved. This supports the previous postulation of anionic and chelating sites for iron binding by this organism[2]. The results of the binding studies using solutions containing iron and cobalt also support this postulate.

Cobalt. The maximum amount of cobalt bound by *Staph. aureus* suspensions is about 70 per cent less than the corresponding amount of iron (see Table I) although the speed of uptake is similar. The Mass

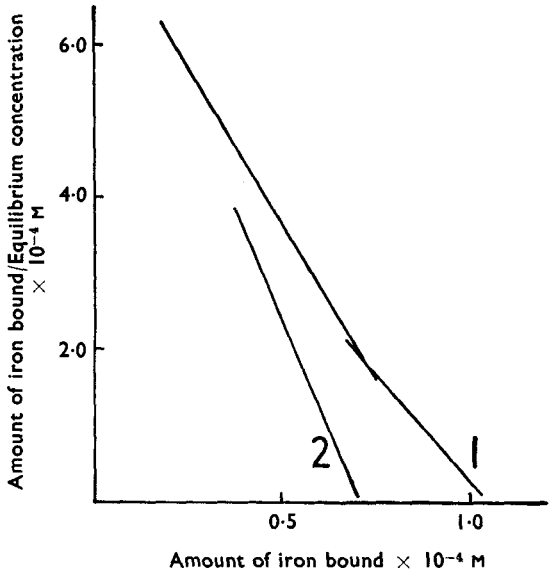

FIG. 7. Mass Law plots for the uptake of manganese or iron (curve 1) and cobalt (curve 2) by viable suspensions of *Staph. aureus*.

Law plot of the uptake data, a straight line (curve 2 of Fig. 7), indicates that there is only one type of binding site for cobalt on the bacterial surface.

Manganese. The marked similarity between the results for iron binding by viable *Staph. aureus* suspensions and those for manganese suggested the existence of a common binding site for these ions on the bacterial surface.

TABLE II

SUMMARY OF THE NUMBER OF TYPES OF CATION BINDING SITES ON *Staph. aureus*

Metal ion	Viable organisms	Heat-killed organisms
Fe^{++}	2	1
Co^{++}	1	1
Mn^{++}	2	2

The number of metal ions bound per bacterium at maximum uptake was estimated; the values obtained were $6{\cdot}5 \times 10^7$ atoms per bacterium for iron and manganese and 4×10^7 atoms per bacterium for cobalt.

Cation Binding by Heat-killed Staph. aureus *Cells*

The maximum amount of iron bound by *Staph. aureus* suspensions was much reduced by heat-killing the organisms (see Fig. 1); the seeming *slight* reductions in cobalt and manganese binding by heat-killed suspensions compared with viable suspensions may be disregarded since some losses of bacteria must inevitably occur during the preparation of the former. Since the light scattering properties of bacterial suspensions

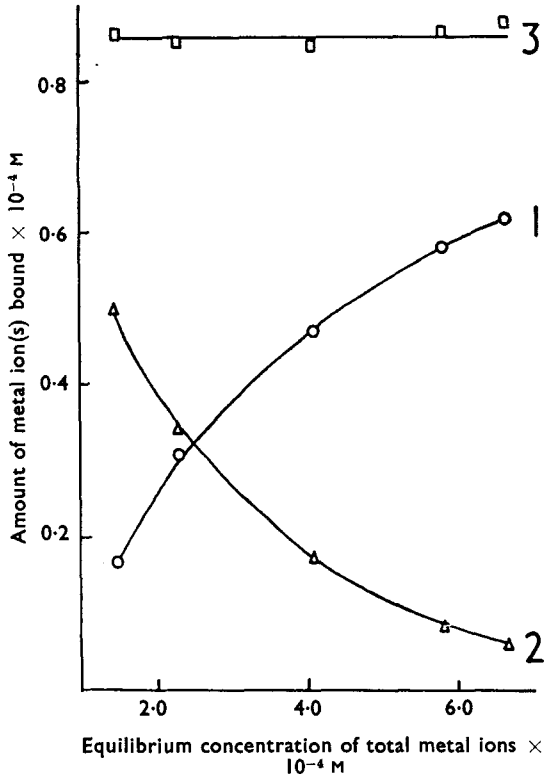

FIG. 8. Competitive binding of iron and manganese by viable suspensions of *Staph. aureus*. The initial manganese concentration was constant at $1{\cdot}67 \times 10^{-4}$ M.

Curve 1, the amount of iron bound.
2, the amount of manganese bound.
3, the total amount of iron plus manganese bound.

change during heat treatment, corrections would be applicable only if viable and total counts were made on the suspensions before and after heating.

Thus with *Staph. aureus* suspensions the maximum amount of iron bound by viable organisms is reduced by heat-killing to a level similar to that observed for cobalt-binding by the same bacteria (viable or heat-killed). The extent of manganese binding was hardly affected by similar treatment.

Mass Law plots of the uptake data for the three metal ions by heat-killed *Staph. aureus* suspensions indicated the number of binding sites summarised in Table II. The results of the competition studies using heat-killed bacteria support these conclusions.

Competitive Binding of Metal Ions by Living Staph. aureus *Cells*

Iron and cobalt with constant initial cobalt concentration. The data presented in Figure 3 show that the amount of cobalt bound decreases as the amount of iron bound increases. The greatest amount of both is about 0·75–0·80 $\times$ 10^{-4} M, whereas, the corresponding figure for

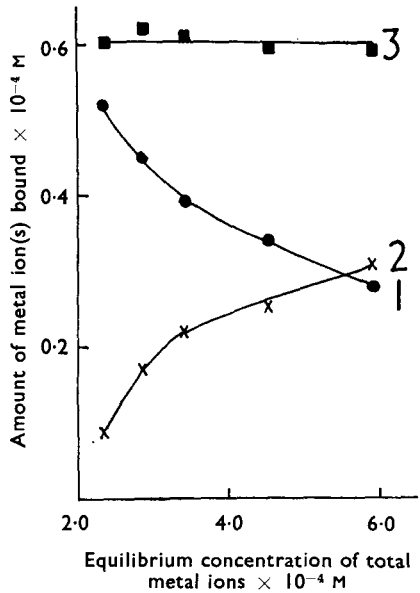

FIG. 9. Competitive binding of iron and cobalt by heat-killed suspensions of *Staph. aureus*. The initial iron concentration was constant at 2·40 $\times$ 10^{-4} M.

Curve 1, the amount of iron bound.
2, the amount of cobalt bound.
3, the total amount of iron plus cobalt bound.

iron is 0·95–1·00 $\times$ 10^{-4} M and for cobalt 0·65–0·70 $\times$ 10^{-4} M. Assuming that both ions are bound at a common site and that a second site is available for iron binding, then the maximum metal ion uptake of the two should correspond to the maximum uptake of iron. But this was not found. Therefore, cobalt ions, either bound or free, apparently interfere with normal binding at the second iron site.

Iron and cobalt with constant initial iron concentration. The above suggestion is further supported by the results (Figure 4) which show that the amount of iron bound by *Staph. aureus* suspensions is reduced as the initial cobalt concentration is increased, whereas, the amount of cobalt bound does not alter significantly. This indicates that the *free*

cobalt ions appear to exert a great effect on the extent of iron binding by this organism.

Iron and manganese. Since similar plots are obtained for iron and manganese binding by *Staph. aureus* suspensions from solutions containing both metal ions, irrespective of which metal is kept at a constant initial concentration (see Fig. 8), it may be deduced that these metal ions are bound at similar sites. Further, the combined amount of iron and manganese bound is equivalent to the maximum uptake of either of these ions alone. By interpolation, the ratio of iron to manganese in the initial solution at the point of intersection of curves 1 and 2 in Figure 8 is 1 : 1; a similar value was also obtained from the data for iron and manganese binding by *Staph. aureus* when the iron content of the initial solution was kept constant.

Cobalt and manganese. The remarks above concerning iron and cobalt also apply to the results obtained for the cobalt and manganese studies with viable organisms.

Competitive Binding of Metal Ions by Heat-killed Staph. aureus *Cells*

Iron and cobalt. Iron and cobalt ions appear to be interchangeably bound at a single site on the bacterial surface (Fig. 9); the maximum total uptake of the two ions corresponds approximately to the maximum uptake of either of the individual ions by heat-killed suspensions of this organism. Iron is bound to a greater extent than cobalt in the proportion of 1 : 0·67. Although changes must occur in the bacterial surface during the heat treatment it may be assumed that these metal ions share a common binding site on the heated bacteria as well as on the viable organisms. Thus the major effect of heating appears to be a modification of the second iron binding site, i.e. the chelating site.

Iron and manganese. Interchangeability of binding of these two ions by heat-killed suspensions of *Staph. aureus* is evident from the results presented in Figure 10. The relative proportions of iron and manganese present in the initial solution from which equivalent concentrations of the two ions are bound by the bacteria indicate that iron is bound preferentially to manganese at a ratio of 1 : 0·84. Since iron is bound preferentially and at only one type of site by heat-killed suspensions of *Staph. aureus*, whereas, manganese is bound at two types of sites (Table II), the slight reduction in the maximum uptake level for the combined metal ions compared with the corresponding figure for manganese binding was not unexpected.

Comparison of Cation Binding by Viable and Heat-killed Staph. aureus *Cells*

Table II shows the number of binding sites for iron, cobalt and manganese available on the surface of the bacteria. The loss of the second iron binding site after heat-killing the bacteria was not unexpected as some molecular rearrangement of the bacterial surface might be anticipated (cf. reference 7). However, the lack of change in the manganese binding sites contradicts this suggestion especially since iron

and manganese ions were interchangeably bound by the viable bacteria. These results might be explained by steric effects or the oxidation state of the metal ions. A change in the avidities of the binding groups on the bacterial surface could be the result of a rearrangement of intermolecular bonding brought about by heat. Changes in the physical orientation of groups at the surface could obviously alter the nature

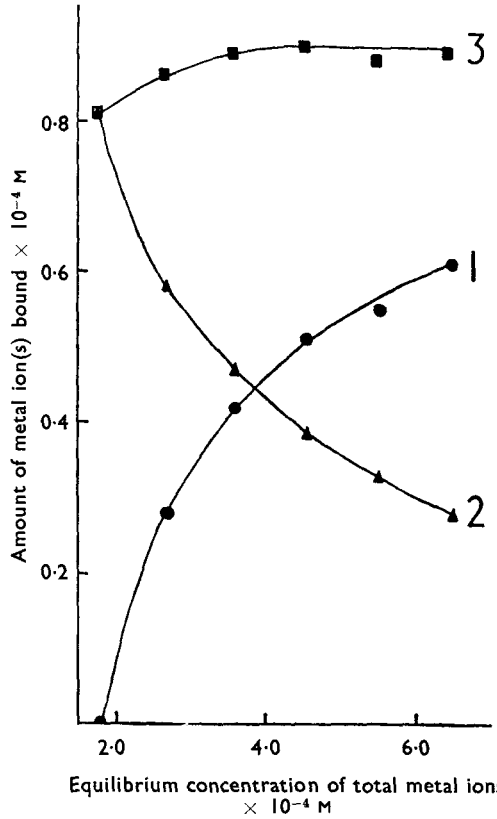

FIG. 10. Competitive binding of iron and manganese by heat-killed suspensions of *Staph. aureus*. The initial manganese concentration was constant at 2·57 × 10^{-4} M.

Curve 1, the amount of iron bound.
2, the amount of manganese bound.
3, the total amount of iron plus manganese bound.

and extent of ions or molecules subsequently bound thereto. Alternatively, if a change in the oxidation state of some of the metal ions occurs at the bacterial surface and is a pre-requisite for their binding, heat-inactivation of the enzyme system responsible for the change could prevent binding of the metal ions presented in an oxidation state unacceptable to the organism.

The Effect on Bactericidal Properties

Some trends may be noted from the results: (i) that the optimum bactericidal activity against *Staph. aureus* was exhibited by solutions containing iron and oxine at a molar ratio of 1:3,

(ii) that addition of an equivalent molar ratio of cobalt to the iron-oxine solution causes a slight potentiation in bactericidal activity, whereas, a five mole excess of cobalt reduced the toxicity of the 1:1 iron-oxine solution to *Staph. aureus* and gave a solution which was also less toxic than a solution containing oxine and a five mole equivalent of iron,

(iii) that addition of manganese to the iron-oxine solution only slightly affected the bactericidal activity against *Staph. aureus.*

Although the object of this work has been incompletely realised, there may be some correlation between the bactericidal results for oxine-metal ion solutions and the competition studies using solutions containing mixtures of two metal ions. For example, if the toxic effect of iron in the presence of oxine is a consequence of iron binding at one particular site, then a five molar equivalent of cobalt may displace sufficient iron atoms to reduce the toxicity. Also, with the interchangeably bound iron and manganese ions, the latter may be unable to displace the former from the sites where it exerts its toxic effect so that the bactericidal activity is hardly affected by relatively small proportions of manganese.

References

1. Beckett, Dar and Robinson, *J. Pharm. Pharmacol.*, 1959, **11**, 195*T*.
2. Beckett, Vahora and Robinson, *ibid.*, 1958, **10**, 160*T*.
3. Rubbo, Albert and Gibson, *Brit. J. exp. Path.*, 1950, **31**, 425.
4. Waring and Werkman, *Arch. Biochem.*, 1942, **1**, 303.
5. Beckett, Patki and Robinson, *J. Pharm. Pharmacol.*, 1959, **11**, 352.
6. Rothstein and Hayes, *Arch. Biochem. Biophys.*, 1956, **63**, 87.
7. Beckett, Patki and Robinson, *J. Pharm. Pharmacol.*, 1959, **11**, 367.