ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1980, p. 63-68
0066-4804/80/07-0063/06$02.00/0
Vol. 18, No. 1

Antibacterial Effect of Scandium and Indium Complexes of Enterochelin on *Klebsiella pneumoniae*

HENRY J. ROGERS,* CHARLOTTE SYNGE, AND VIVIENNE E. WOODS
National Institute for Medical Research, Mill Hill, London NW7 1AA, England

A number of studies point to the conclusion that enterochelin, the iron chelator produced by a number of pathogenic enterobacteria, may be an essential metabolite for bacterial multiplication within the host. The compound removes iron from complexes with the host iron-binding proteins transferrin and lactoferrin, and the resulting ferric enterochelin is assimilated by the bacterial cell. It was reasoned that complexes of enterochelin with ions other than Fe^{3+} might act as antimetabolites and inhibit bacterial multiplication by interfering with the assimilation of ferric enterochelin. Enterochelin forms complexes with a number of group III and transition metal ions. The complex containing scandium exerts a bacteriostatic effect on *Klebsiella pneumoniae* in serum, whereas the indium complex induces a large increase in the generation time. The Fe^{3+} complexes of other microbial iron-transporting compounds are capable of reversing the bacteriostatic effect of the Sc^{3+} complex of enterochelin, suggesting that the compound acts solely by interfering with the enterochelin system of iron transport. Preliminary experiments show that the Sc^{3+} complex probably acts as a competitive inhibitor of ferric enterochelin. The Sc^{3+} complex of enterochelin exerts a therapeutic effect on intraperitoneal *K. pneumoniae* infections in mice similar to that obtained with kanamycin sulfate.

Iron is essential for the growth of pathogenic bacteria (8, 30). Invading organisms which reach either mucosal surfaces or the circulating plasma become exposed to the iron-binding proteins lactoferrin and transferrin, respectively. The ferric ion is associated very strongly with these proteins (1, 3), and it was postulated that to multiply in these situations the bacteria must possess an active means of assimilating this protein-bound iron (10). In species such as *Escherichia coli*, *Klebsiella pneumoniae* (*Aerobacter aerogenes*) (26), and *Salmonella typhimurium* (27), this could be achieved by the production of enterochelin, the cyclic trimer of 2,3-dihydroxy-*N*-benzoyl serine (Fig. 1) in response to the iron restriction imposed by the iron-binding proteins (1). In fact, it was found that *E. coli* O141 secreted enterochelin and its degradation products when growing in serum. One of these products was shown to be capable of removing Fe^{3+} from its complex with transferrin and abolished the inoculum-dependent growth of the organism in serum. It also enhanced the growth of the less virulent *E. coli* O111 in mice (28). Although *E. coli* O111 failed to multiply in either normal serum (28) or human milk (9), it was capable of synthesizing enterochelin in a minimal medium (29). Further analysis showed that antibody to lipopolysaccharide probably interfered with the release of enterochelin from the bacterial cell (15). A number of enterobacterial pathogens are now known to produce iron chelators (22). On the basis of this evidence, it was suggested that enterochelin was an essential factor for the multiplication of *E. coli* in vivo (28). The finding that *ent* mutants of *S. typhimurium* are less virulent than the wild type (31), together with the observation that enterochelin can be detected in animals suffering from fatal *E. coli* infections (7), only strengthens this view.

Since enterochelin appears to be an endogenous growth factor for certain enterobacterial pathogens, it was reasoned that it might be possible to use complexes of enterochelin with ions other than Fe^{3+} as antimetabolites to ferric enterochelin. The experiments described in this paper show that this is the case for complexes of enterochelin with scandium and indium.

(A preliminary account of this work is available [United Kindom Patent 2022076, 1979]).

MATERIALS AND METHODS

Bacteria. The strain of *K. pneumoniae* used in this work was a human isolate. The 50% lethal dose for 23- to 25-g female TO mice was approximately 5×10^3 organisms by the intraperitoneal route. The main culture was stored in liquid N_2, and subcultures were stored at −70°C in broth containing 30% (wt/vol) glycerol. Cells from 8 ml of a 2-h subculture in Trypticase soy broth (BBL Microbiology Systems) were collected by centrifugation and suspended in 3.0 ml of

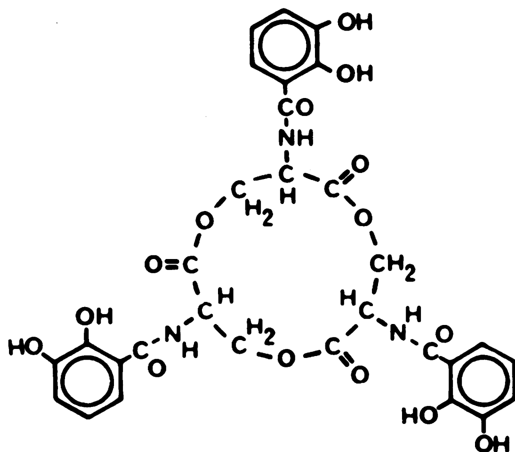

FIG. 1. *Structure of the enterochelin molecule.*

10% broth in saline. The population was estimated by nephelometry with a standard curve. Viable counts were made by spreading 0.10-ml volumes of suitable dilutions on blood agar plates, which were then incubated for 18 h at 37°C.

Sera. Normal horse serum (no. 3) was obtained from Wellcome Reagents Ltd. and stored at −20°C. Normal rabbit serum and heparinized rabbit plasma were inactivated by heating for 30 min at 56°C and then stored at −20°C.

Bacterial growth in serum. Serum or plasma in 3.0-ml volumes was stirred by means of magnetic followers in jacketed culture vessels at 37°C supplied with a gas mixture containing 10% O_2–5% CO_2–85% N_2 at 50 ml/min (28). Bacteria and test samples were each added in 0.03-ml volumes. Samples were diluted 1:10 in broth-saline and homogenized for 1 min in an ice bath (MSE microhomogenizer) before viable counting. To compare the activity of five or more substances simultaneously, the samples, in volumes not exceeding 0.1 ml, were placed in 25-ml bottles followed by 1.0 ml of heat-inactivated rabbit plasma previously inoculated with bacteria. The bottles were incubated at 37°C on a rotary shaker operating at 150 rpm. The contents were gassed with a stream of moist 5% CO_2 in air.

Microbial iron chelators. The method of culturing *E. coli* K-12 Hfr H *thi* for the production of enterochelin has been described previously (29). The compound was isolated by a modified procedure which increased the recovery from the culture fluid. 2,3-Dihydroxy-*N*-benzoyl serine was isolated after alkaline hydrolysis of crude enterochelin for 2 h at pH 11 under nitrogen at room temperature. The product was freed from sodium ion by extraction into ethyl acetate at pH 2.5 and purified by column chromatography on G25 Sephadex (superfine) in 6% acetic acid. The product was finally dissolved in water to give a 14.6 mM solution which was stored at −20°C.

Terregens factor was isolated by the method of Linke et al. (19) from the culture fluid of *Arthrobacter globiformis* NCIB 8910 grown for 48 h at pH 5.8 and 25°C in the medium of Burton et al. (11), to which 1 μM ferric ammonium citrate had been added after sterilization. Ferrichrome was isolated by the method of Neilands (24, 25) from culture fluids of *Ustilago maydis*. This organism was kindly supplied by R. Holliday of this institute. Rhodotorulic acid was isolated as described by Atkin et al. (4) from a 5-day culture of *Rhodotorula rubra* NCYC 65 grown at 35°C. Desferal (desferrioxamine mesylate) was obtained from CIBA.

Chemicals. $Al_2(SO_4)_3 \cdot 16\ H_2O$, $CuSO_4 \cdot 5\ H_2O$, $CoCl_2 \cdot 6\ H_2O$, $InCl_3 \cdot 3\ H_2O$, $MoCl_5$, $MnCl_2 \cdot 4\ H_2O$, $NbCl_5$, $RuCl_3 \cdot H_2O$, $TaCl_5$, $TiCl_4$, VCl_3, $VOCl_2 \cdot 2\ H_2O$ (50% solution), WCl_6, $Y(NO_3)_3 \cdot 6\ H_2O$ and $ZrCl_4$ were obtained from British Drug Houses Ltd. $Ga(NO_3)_3 \cdot 9\ H_2O$, $HfCl_4$, and $RhCl_3$ were obtained from Kock-Light Labs. $CrCl_2$ and $VOCl_3$ were obtained from Alfa Products, and kanamycin sulfate was from Sigma. Analytical grade reagents were used if available.

Preparation of enterochelin complexes. $MoCl_5$, $NbCl_5$, $TaCl_5$, $TiCl_4$, $VOCl_3$, and WCl_6 were used as 0.01 M solutions in ethanol. $HfCl_4$, $RhCl_3$, $RuCl_3$, VCl_3, and $ZrCl_4$ were dissolved in 1 N HCl to give 0.01 M solutions, and the remaining compounds were prepared as 5 mM solutions in 0.1 N HCl. Equivalent amounts of these solutions and enterochelin, dissolved in *n*-butanone, were mixed together, and sufficient water was then added to give a final concentration of 0.2 mM complex. Solid $NaHCO_3$ was added to raise the pH to approximately 7. To test the stability of the complexes, equal volumes of the complex and 0.2 mM ferric nitrilotriacetate (FeNTA), pH 7.5 (5), were mixed together, and the rate of appearance of the red color of ferric enterochelin was noted. The chromic complex of enterochelin was prepared by the method of Isied et al. (17).

Preparation of compounds for mouse protection tests. The details of the preparation of compounds for mouse protection tests are given elsewhere (United Kingdom Patent 2022076, 1979). Kanamycin sulfate, 1.0 mM in saline containing 1% $NaHCO_3$, was sterilized by membrane filtration.

Mice. Female TO mice weighing 23 to 25 g were used.

RESULTS

Reaction of metal ions with enterochelin. The ions were allowed to react with enterochelin dissolved in *n*-butanone under acidic conditions to minimize the formation of insoluble metal hydroxides. After neutralization, equivalent quantities of the complex and FeNTA were mixed together, and the rate of appearance of the red ferric enterochelin complex was noted to obtain some indication of the stability of the complex in the presence of readily exchangeable ferric ion (5). Al^{3+} was displaced only slowly over 2 days, and the order of stability among the group IIIb ions appeared to be $Al^{3+} > Ga^{3+} > In^{3+}$. The stability of the complexes with group IIIa ions appeared to be $Sc^{3+} \gg Y^{3+} > La^{3+}$. Sc^{3+} appeared to form an equilibrium mixture, whereas La^{3+} probably failed to form a complex. Ti^{4+}, Zr^{4+}, and Hf^{4+} all formed complexes, that

of Ti^{4+} being the least stable. Nb^{5+}, Ta^{5+}, Mo^{5+}, and W^{6+} all formed complexes which were decomposed in a few minutes by FeNTA. The colorless Ru^{3+} complex was decomposed in a few hours, whereas the Rh^{3+} complex was decomposed in a few minutes. Surprisingly, Cu^{2+} formed a pale blue complex which was decomposed immediately upon the addition of FeNTA. Mn^{2+} formed a blue complex, whereas Co^{2+} formed a yellow complex; both solutions turned brown over a period of 24 h, indicating oxidation of the ligand. Enterochelin formed a pale blue complex with V^{3+} at acid pH; on neutralization, a dark blue solution was formed. Both V^{4+} as $VOCl_2$ and V^{5+} as $VOCl_3$ formed a similar dark blue complex at acid pH. This complex appeared to form an equilibrium mixture with the Fe^{3+} complex in the presence of FeNTA.

Antibacterial activity. The enterochelin complexes of Sc^{3+}, Y^{3+}, La^{3+}, Ti^{4+}, Zr^{4+}, Hf^{4+}, VO^{2+}, Nb^{5+}, Ta^{5+}, Mo^{5+}, W^{6+}, Mn^{2+}, Ru^{3+}, Co^{2+}, Rh^{3+}, Cu^{2+}, Al^{3+}, Ga^{3+}, and In^{3+} were each added at a concentration of 2 μM to samples of normal horse serum inoculated 2 h previously with *K. pneumoniae*. Only the complexes of Sc^{3+} and In^{3+} showed any antibacterial activity which, in the case of Sc^{3+}, took the form of a bacteriostatic effect (Fig. 2). The growth curve obtained in the presence of the Ga^{3+} complex is shown for comparison. The VO^{2+}, Ru^{3+}, and Cr^{3+} complexes appeared to stimulate growth, whereas the Zr^{4+} complex delayed growth for an hour or so (data not shown). Clearly, however, only the Sc^{3+} and In^{3+} complexes displayed significant antibacterial effects, and subsequent work has been concerned mainly with these compounds.

Most of the subsequent experiments have been carried out in rabbit serum or plasma since the organism multiplies after only a very short lag phase. A comparison of the antibacterial effects of the complexes is shown in Fig. 3. From these results it can be seen that the Sc^{3+} complex is active at 0.2 μM (0.14 μg/ml). The In^{3+} complex does not produce complete bacteriostasis, but rather a marked increase in the generation time. A mixture of the two compounds produced an intermediate growth rate between those of the individual components (Fig. 4). No synergistic action could be detected between the more stable complexes such as those of Al^{3+} or Zr^{4+} when mixed at a concentration of 2 μM with the Sc^{3+} complex at either 0.02 or 2 μM (data not shown).

Role of scandium. To examine the role of the scandium ion, bacteria, 10^4/ml in horse serum, were exposed at 2 h to either 20 μM scandium citrate (16) or the scandium complex of 2,3-dihydroxy-*N*-benzoyl serine. At 7 h the count in the control was 3.6 × 10^7/ml, whereas the

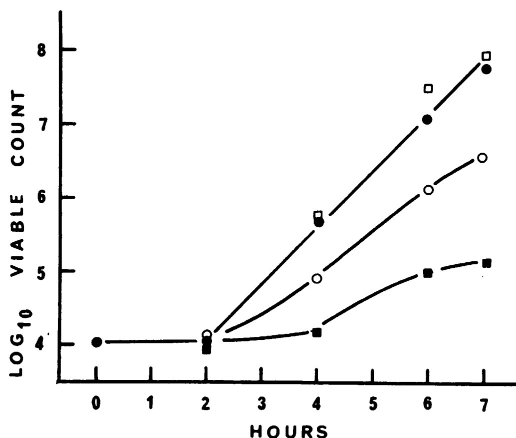

FIG. 2. *Antibacterial effect of the addition at 2 h of complexes of enterochelin (2 μM) to K. pneumoniae in normal horse serum. Symbols: ●, control, no addition; ○, indium complex; ■, scandium complex; □, gallium complex.*

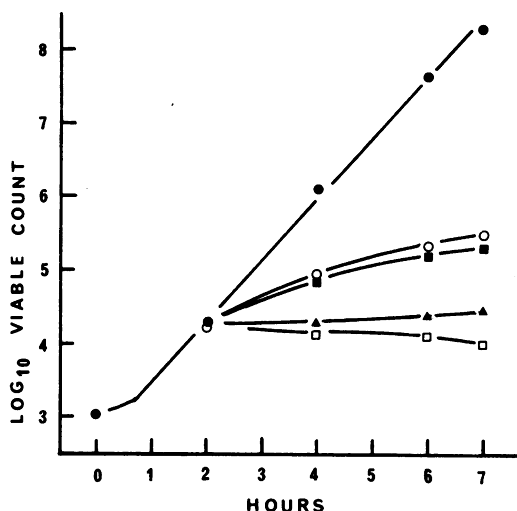

FIG. 3. *Antibacterial effect of complexes of enterochelin added at 2 h to K. pneumoniae growing in heat-inactivated rabbit plasma. Symbols: ●, control, no addition; ○, 2 μM indium complex; ■, 0.2 μM indium complex; □, 2 μM scandium complex; ▲, 0.2 μM scandium complex.*

counts from the serum containing the complexes were 3.5 × 10^7 and 2.0 × 10^7/ml, respectively. This lack of activity suggested that Sc^{3+} itself was probably not the toxic entity.

Stoichiometry of the reaction between Sc^{3+} and enterochelin. A 0.0917 M solution of $ScCl_3 \cdot 6H_2O$ in ethanol was standardized by acid-base titration of the complex formed with sodium ethylenediaminetetraacetate. A 1.0 mM solution of scandium nitrilotriacetate (ScNTA) was then prepared by the published method for

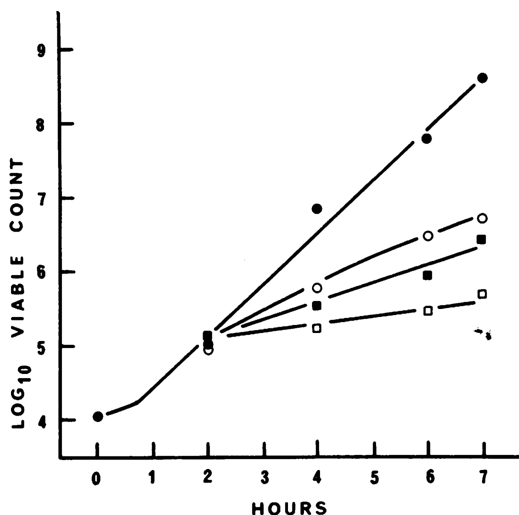

FIG. 4. *Effect of adding a mixture of the indium and scandium complexes of enterochelin at 2 h on the growth of K. pneumoniae in heat-inactivated rabbit plasma. Symbols:* ●, *control, no addition;* ○, *2 μM indium complex;* □, *2 μM scandium complex;* ■, *mixture.*

the Fe^{3+} complex (5). Volumes (3 ml) of 89.4 μM enterochelin in 0.01 M tris(hydroxymethyl)aminomethane were allowed to react for 30 min at 25°C with 0.10-, 0.15-, and 0.15-ml portions of ScNTA. To determine the free enterochelin remaining at this time, 0.2 ml of 1.0 mM FeNTA was added, and the optical densities at 495 nm were recorded at 1-min intervals for 10 min. From these three measurements, the ratio of Sc^{3+} to enterochelin in the complex was calculated to be 0.98, 0.94, and 1.1 to 1.0, respectively.

Effect of exogenous sideramines. It has been established that mutants of both *S. typhimurium* (21) and *E. coli* (18) which are unable to synthesize enterochelin can, in fact, utilize sideramines produced by a number of unrelated microorganisms. Sideramines have been used as a means of examining the mechanism of action of Sc enterochelin. In these experiments, Sc enterochelin (2 μM) was present from the beginning; addition of either 1 μM ferrichrome or 10 μM ferric rhodotorulate at 2 h suppressed the bacteriostatic effect (Fig. 5). Table 1 shows the effect of a number of iron compounds, including sideramines, on the bacteriostatic effect of Sc enterochelin in heated rabbit plasma. Ferric ammonium citrate and heme, both of which can abolish the antibacterial effects of serum (2), had little or no effect in this instance. Ferric enterochelin abolished the inhibitory effect at a concentration of 10 μM, but in separate, repeated experiments it produced little stimulation of growth at a concentration of 2 μM.

Mouse protection tests. In view of the antibacterial effects exerted by both the Sc^{3+} and the In^{3+} complexes of enterochelin in vitro, it was decided to carry out mouse protection tests. Kanamycin sulfate, an antibiotic which has been used against *K. pneumoniae* infections (6), was also tested for comparison. Mice were infected intraperitoneally with approximately 1.0×10^5 *K. pneumoniae* cells (50 50% lethal doses), and the test compounds were administered intraperitoneally as 0.2-ml volumes of 1.0 mM solutions at 6, 24, 31, 48, 55, 72, and 79 h postinfection.

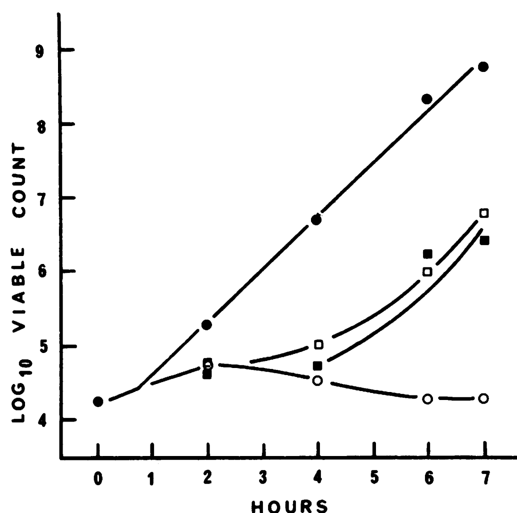

FIG. 5. *Effect of sideramines on the bacteriostatic action of the scandium complex of enterochelin (2 μM) on K. pneumoniae in heat-inactivated rabbit plasma (scandium complex present in all samples except the control from zero time.) Symbols:* ●, *control, no addition;* ○, *scandium complex only present;* ■, *1 μM ferrichrome added at 2 h;* □, *10 μM ferric rhodotorulate added at 2 h.*

TABLE 1. *Effect of iron compounds on the antibacterial action of Sc enterochelin on K. pneumoniae[a] in heat-inactivated rabbit plasma*

Addition[b]	Viable count[c]
None	5.0×10^8
Sc enterochelin + ferric ammonium citrate	5.5×10^5 / 1.1×10^6
Sc enterochelin + heme	5.6×10^5
Sc enterochelin + Fe enterochelin	3.5×10^8
Sc enterochelin + ferrioxamine B	1.9×10^7
Sc enterochelin + ferric rhodotorulate	6.0×10^7
Sc enterochelin + terregens factor	4.0×10^8

[a] Inoculum, 1.0×10^4/ml

[b] Sc complex concentration, 2 μM; iron compounds, 10 μM.

[c] Viable counts made at 6 h.

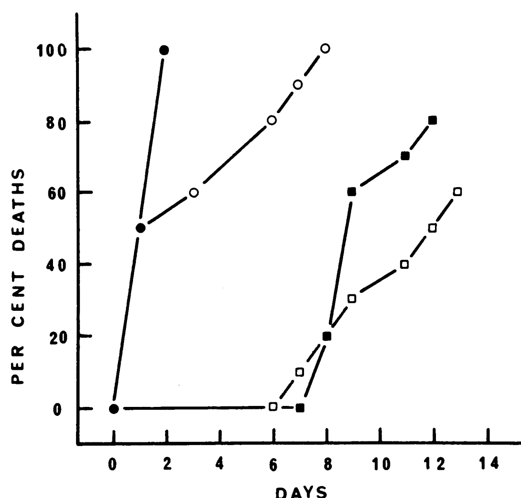

FIG. 6. Mouse protection tests. Mice were infected intraperitoneally with 10^5 K. pneumoniae cells. Treatment consisted of intraperitoneal injections of 0.2-ml 1.0 mM solutions of the compounds at 6, 24, 31, 48, 72, and 79 h postinfection. Symbols: ●, control, no treatment; ○, indium enterochelin; ■, scandium enterochelin; □, kanamycin sulfate.

Figure 6 shows the death rate during the course of the experiment. The Sc^{3+} complex protected the animals for 6 days and gave a survival rate of 20%. In an experiment carried out later in which only the Sc^{3+} complex was tested, the survival rate was 70%. All the survivors remained healthy for 2 months, at which time they were killed. When treatment was delayed for 17 h, by which time some of the animals were obviously ill, the survival rates obtained with Sc enterochelin and kanamycin sulfate were 0 and 10%, respectively. After the start of treatment, however, the condition of some of the animals improved for 2 to 3 days.

DISCUSSION

In the iron-restricted environment imposed by the transferrin and lactoferrin present in the plasma and on mucosal surfaces, respectively, certain pathogenic bacteria respond by secreting enterochelin (Fig. 1), which transports Fe^{3+} from its complex with the iron-binding protein to the bacterial cell. The work described in this paper is based on the hypothesis that complexes of enterochelin with ions other than Fe^{3+} may act as antimetabolites to ferric enterochelin, thus interrupting the bacterial iron supply and, hence, bacterial multiplication.

Enterochelin appears to form complexes with a number of group III and transition metal ions. Of these, only the scandium and indium complexes possessed antibacterial activity, the scandium complex being the most active (Fig. 2 and 3). It was clearly established that Sc^{3+} forms a 1:1 complex with enterochelin, as does Fe^{3+} (28). The complexes containing Ru^{3+}, VO^{2+}, or Cr^{3+} appeared to stimulate bacterial growth. In the case of VO^{2+} and Cr^{3+}, this could arise by exchange with transferrin, which is known to react with these ions (2, 12,); the resulting free enterochelin would then be available to transport Fe^{3+} to the bacterial cells. The Al^{3+}, Zr^{4+}, and Hf^{4+} complexes appeared to be stable in the presence of transferrin but exerted little or no antibacterial effect. Attempts to enhance the activity of the Sc^{3+} complex by addition of the inactive complexes were uniformly unsuccessful. The fact that mixtures of the Sc^{3+} and In^{3+} complexes exert an intermediate effect between those of the individual components (Fig. 4) suggests that they may have similar affinities for the same site within the bacterial cell.

It is interesting to consider the relationship between the ionic radii and biological activities of the complexes in view of the proposed model which suggests that Fe^{3+} is completely enclosed by the three catecholate rings of the enterochelin molecule (26). Representative values of the ionic radii (in picometers) are Fe^{3+}, 53; Cr^{3+}, 53; Al^{3+}, 45; Ga^{3+}, 60; In^{3+}, 81; Sc^{3+}, 68; Zr^{4+}, 77; and Y^{3+}, 90 (13). From these figures, it appears that only the complexes containing tripositive ions somewhat larger than Fe^{3+} possess antibacterial activity. Many years ago, Muroma (23) studied the bactericidal effect of aqueous solutions of a number of inner transition metal ions and scandium. Sc^{3+} appeared to be the most active, particularly against gram-negative bacteria, including *K. pneumoniae*. Unfortunately, the relevance of these findings to the present work is not clear at this time.

The fact that complexes of Sc^{3+} with ligands other than enterochelin are devoid of antibacterial activity suggests that Sc^{3+} is probably not the toxic entity. There appears to be competition between the Sc^{3+} and Fe^{3+} complexes of enterochelin, presumably, for a specific site (Table 1). Previous work showed that the concentration of iron-binding catechols in a serum culture of *E. coli* O141 was 0.48 μM (28). Since 2 μM Fe enterochelin fails to reverse the inhibitory effect, it seems most unlikely that bacterial production of enterochelin will interfere with the antibacterial effect of the scandium complex. Since other sideramines can reverse the inhibitory effect (Fig. 5, Table 1), it appears that there is no derangement of bacterial metabolism other than an interference with the assimilation of iron via the enterochelin pathway.

The relative protective effects, in mice, of the Sc^{3+} and In^{3+} complexes appear to parallel their antibacterial effects in vitro (Fig. 2 and 6). The

Sc^{3+} complex prolongs the survival of infected animals, although, as would be expected, the time at which treatment begins is important. Survival after treatment with the scandium complex is similar to that obtained with kanamycin sulfate.

Further work, details of which will be published later, has shown that the scandium complex of enterochelin also exerts an antibacterial effect against *E. coli*, *S. typhimurium*, and *P. aeruginosa* (United Kingdom patent 2022076, 1979), although the latter does not produce enterochelin (14, 20).

1. **Aasa, R., B. G. Malmstrom, P. Saltman, and T. Vänngård.** 1963. The specific binding of iron (III) and copper (II) to transferrin and conalbumin. Biochim. Biophys. Acta **75**:203–222.
2. **Aisen, P., R. Aasa, and A. G. Redfield.** 1969. The chromium, manganese and cobalt complexes of transferrin. J. Biol. Chem. **244**:4628–4633.
3. **Aisen, P., and A. Leibman.** 1972. Lactoferrin and transferrin: a comparative study. Biochim. Biophys. Acta **257**:314–323.
4. **Atkin, C. L., J. B. Neilands, and H. Phaff.** 1970. Rhodotorulic acid from species of *Leucosporidium, Rhodosporidium, Rhodotorula, Sporidiobolus* and *Sporobolomyces*, and a new alanine-containing ferrichrome from *Cryptococcus melbibiosum*. J. Bacterial. **103**:722–733.
5. **Bates, G. W., C. Billups, and P. Saltman.** 1967. The kinetics and mechanism of iron (III) exchange between chelates and transferrin. I. The complexes of citrate and nitrilotriacetic acid. J. Biol. Chem. **242**:2810–2815.
6. **Berendt, R. F., G. G. Long, and J. S. Walker.** 1975. Treatment of respiratory *Klebsiella pneumoniae* infection in mice with aerosols of kanamycin. Antimicrob. Agents Chemother. **8**:585–590.
7. **Buck, M., J. Humphreys, and E. Griffiths.** 1979. Iron-binding catechols, fever, and growth of *Escherichia coli* in lethally infected animals. Proc. Soc. Gen. Microbiol. **7**:9–10.
8. **Bullen, J. J., H. J. Rogers, and E. Griffiths.** 1978. Role of iron in bacterial infection. Curr. Top. Microbiol. Immunol. **80**:1–35.
9. **Bullen, J. J., H. J. Rogers, and L. Leigh.** 1972. Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. Br. Med. J. **1**:69–75.
10. **Bullen, J. J., H. J. Rogers, and J. E. Lewin.** 1971. The bacteriostatic effect of serum on *Pasteurella septica* and its abolition by iron compounds. Immunology **20**: 391–406.
11. **Burton, M. O., F. J. Sowden, and A. G. Lockhead.** 1954. Studies on the isolation and nature of "terregens factor." Can. J. Biochem. Physiol. **32**:400–406.
12. **Cannon, J. C., and N. D. Chasteen.** 1975. Nonequivalence of the metal binding sites in vanadyl-labeled human serum transferrin. Biochemistry **14**:4573–4577.
13. **Cotton, F. A., and G. Wilkinson.** 1972. Advanced inorganic chemistry, 3rd ed., p. 52. Interscience Publishers, Inc., New York.
14. **Cox, C. D., and R. Graham.** 1979. Isolation of an iron-binding compound from *Pseudomonas aeruginosa*. J. Bacteriol. **137**:357–364.
15. **Fitzgerald, S.P., and H. J. Rogers.** 1980. Bacteriostatic effect of serum: role of antibody to lipopolysaccharide. Infect. Immun. **27**:302–308.
16. **Ford-Hutchinson, A. W., and D. J. Perkins.** 1971. The binding of scandium ions to transferrin *in vivo* and *in vitro*. Eur. J. Biochem. **21**:55–59.
17. **Isied, S. S., G. Kuo, and K. N. Raymond.** 1976. Coordination isomers of biological iron transport compounds. V. The preparation and chirality of the chromium (III) enterobactin complex and model tris (catechol) chromium (III) analogues. J. Am. Chem. Soc. **98**: 1763–1767.
18. **Leong, J., and J. B. Neilands.** 1976. Mechanisms of siderophore iron transport in enteric bacteria. J. Bacteriol. **126**:823–830.
19. **Linke, W. D., A. Crueger, and H. Diekmann.** 1972. Stoffwechselprodukte von Mikroorganismen 106. Mitteilung. Zur Konstitution des Terregens-Faktors. Arch. Mikrobiol. **85**:44–50.
20. **Liu, P. V., and F. Shokrani.** 1978. Biological activities of pyochelins: iron-chelating agents of *Pseudomonas aeruginosa*. Infect. Immun. **22**:878–890.
21. **Luckey, M., J. R. Pollack, R. Wayne, B. N. Ames, and J. B. Neilands.** 1972. Iron uptake in *Salmonella typhimurium*: utilization of exogenous siderochromes as iron carriers. J. Bacteriol. **111**:731–738.
22. **Miles, A. A., and P. L. Khimji.** 1975. Enterobacterial chelators of iron: their occurrence, detection and relation to pathogenicity. J. Med. Microbiol. **8**:477–490.
23. **Muroma, A.** 1958. Studies in the bactericidal action of salts of certain rare earth metals. Ann. Med. Exp. Biol. Fenn. **36**(Suppl. 6):1–54.
24. **Neilands, J. B.** 1952. A crystalline organo-iron pigment from a rust fungus (*Ustilago sphaerogena*). J. Am. Chem. Soc. **74**:4846–4847.
25. **Neilands, J. B.** 1953. Factors affecting the microbial production of ferrichrome. J. Biol. Chem. **205**:647–650.
26. **O'Brien, J. G., and F. Gibson.** 1970. The structure of enterochelin and related 2,3-dihydroxy-N-benzoylserine conjugates from *Eschericha coli*. Biochim. Biophys. Acta **215**:392–402.
27. **Pollack, J. R., and J. B. Neilands.** 1970. Enterobactin, an iron transport compound from *Salmonella typhimurium*. Biochem. Biophys. Res. Commun. **38**:989–992.
28. **Rogers, H. J.** 1973. Iron-binding catechols and virulence in *Escherichia coli*. Infect. Immun. **7**:445–456.
29. **Rogers, H. J., C. Synge, B. Kimber, and P. M. Bayley.** 1977. Production of enterochelin by *Escherichia coli* O111. Biochim. Biophys. Acta **497**:548–557.
30. **Weinberg, E. D.** 1978. Iron and infection. Microbiol. Rev. **42**:45–66.
31. **Yancey, R. J., S. A. L. Breeding, and C. E. Lankford.** 1979. Enterochelin (enterobactin) virulence factor for *Salmonella typhimurium*. Infect. Immun. **24**:174–180.