Ultrastructural Studies of the Effects Produced by Some Amino Acid Metal Systems on *Escherichia Coli* B

NORMA T. McARDLE, ALEXANDER J. CHARLSON

School of Chemistry, Macquarie University, North Ryde, N.S.W. 2113, Australia

CEDRIC D. SHOREY, ROBIN ARNOLD

Department of Histology and Embryology, University of Sydney, N.S.W. 2006, Australia

and NORMAN BARKER

School of Chemistry, University of New South Wales, Kensington, N.S.W. 2033, Australia

Received November 22, 1983

Escherichia coli *cells were grown in the presence of L-serine and gallium(III) nitrate at different molar ratios. Under these conditions ultrastructural changes were observed in the cells when examined under the electron microscope. Although some changes were seen inside the cell the major modifications were observed at the cell surface. These changes appeared to involve both the cell wall and the peptidoglycan layer. Autoradiography at the electron microscope level undertaken with similar mixtures and containing* L-*(3 – 3H) serine showed silver grains at or near the cell surface. In some cases, surface modifications were so pronounced that they resulted in the* E. coli *appearing as sheets of cells.*

No cell surface changes were detected when mixtures of L-*serine and potassium tetrachloropalladate-(II) were used as modifying agents. With the palladium(II) mixtures all changes observed were intracellular. These modifications included the appearance of membrane-bound vesicles, clumping of the cytoplasm and changes in the nucleoplasm. Autoradiography carried out in the presence of* L-*(3 – 3H) serine showed a significant proportion of silver grains over the nuclear region. A pure palladium(II) complex of* L-*serine was examined as a modifying agent in the concentration range 1–9 μg/cm³ resulting in very pronounced modification of the cells when exposed to higher concentrations.*

Introduction

Rosenberg and collaborators [1] showed that several platinum complexes can induce filamentous growth in *E. coli.* Filaments induced by one of these complexes, cis-dichlorodiammineplatinum(II) (cisplatin), have been reported to contain significant levels of *rec*-A product (protein X) [2]. Cisplatin has also been shown to cause induction of lysogenic cultures [3], and to be mutagenic in *E. coli* and in *Salmonella typhimurium* strains [4–8]. Cunningham and co-workers noted that many of the above phenomena are dependent on functional *rec*-A and *lex*-A genes [9]. It has also been proposed that cisplatin induces SOS functions [10]. Ultrastructural studies on cisplatin treated *E. coli* cells have been reported by Harder [11] and by Beck and co-workers [12].

In addition to platinum complexes, complexes of rhodium(III) [13], ruthenium(III) [13] and palladium(II) [14, 15] have been reported to induce filamentous growth in *E. coli.* It has also been shown that filamentation in *E. coli* can be produced by mixtures of either *L*-glutamine or *L*-serine with gallium-(III) nitrate [16], and that the copper(II) *L*-glutamine system can induce small filaments in *E. coli* [17]. In this communication we report ultrastructural studies on the effects of the gallium(III) *L*-serine system and the palladium(II) *L*-serine system on *E. coli* B.

Experimental

Materials

E. coli NCTCID 418 was obtained from the Commonwealth Serum Laboratory, Melbourne, Australia. Potassium tetrachloropalladate(II) was prepared from palladium metal according to Grube [18], and caesium *cis*-dichloro–*L*-serinatopalladium-(II) was prepared by the method which we described previously [15]. Gallium(III) nitrate octahydrate was obtained from E. Merck Darmstadt, and *L*-serine was obtained from the BDH company: these substances were used without further purification. *L*-(3 – 3H) serine obtained from the Radiochemical

0020-1693/84/$3.00

centre, Amersham, England, had an activity of 11 curies/mmol or 105 mCi/mg with a radioactive concentration of 1.0 mCi/cm^3.

Bacterial Cultures and Methods for Incorporating Test Substances

Cultures were grown under aerobic conditions in glucose enriched liquid medium C at pH 7 and 37 °C [17]. In preparing cultures (1–2 cm^3) for autoradiography and also when caesium *cis*-dichloro–*L*-serinatopalladium(II) was used as the test substance the aeration procedure previously described [17] was not used: cultures were incubated in a shaking water bath at 37 °C. The test solutions (3×10^{-3} *M*) were prepared using sterile distilled water, filtered through a millipore filter and mixed in the required ratios immediately prior to use. In a typical experiment a mixture of 5 cm^3 of *L*-serine and 5 cm^3 of gallium(III) nitrate (molar ratio 1:1) was added to 20 cm^3 of bacterial culture 6–8 h after subculturing and incubated a further 6 h at 37 °C. When caesium *cis*-dichloro–*L*-serinatopalladium(II) was used as the test substance, aqueous solutions of the complex were added to 2 cm^3 samples of the culture to give final concentrations in the range 1–9 μg/cm^3. In order to check for viability of a culture after incubation with a modifying agent, the cells were harvested by centrifugation and, after washing twice with 0.01 *M* phosphate buffer (pH 7), inoculated into fresh culture medium. The growth rate was shown to be similar to that of control cultures by monitoring increases of absorption at 650 nm.

Electron Microscopy

The cultures were cooled to 4 °C and centrifuged at 5000 g for 10 min. The cells were washed twice with 0.01 *M* phosphate buffer (pH 7) at 4 °C and then fixed in 1% gluteraldehyde in 0.01 *M* phosphate buffer (pH 7) for 30 min at 4 °C. After washing four times with 0.01 *M* phosphate buffer (pH 7) the cells were post-fixed in 1% OsO_4 in 0.01 *M* phosphate buffer (pH 7) for 3 h at 4 °C and dehydrated in a graded ethanol series. They were then stained in 4% uranyl acetate in 70% ethanol for 45 min at ambient temperature. Excess stain was removed by brief rinsing in 70% ethanol. The cells were then dehydrated in 90% ethanol and placed in three 15 minute changes of absolute acetone. After embedding in Spurr's resin [19], specimens were thin-sectioned on a Porter Blum MT2 200 microtome and stained with 2% uranyl acetate followed by Reynold's lead citrate [20]. The electron micrographs were taken at 60 KV using a JEM-100 CX electron microscope.

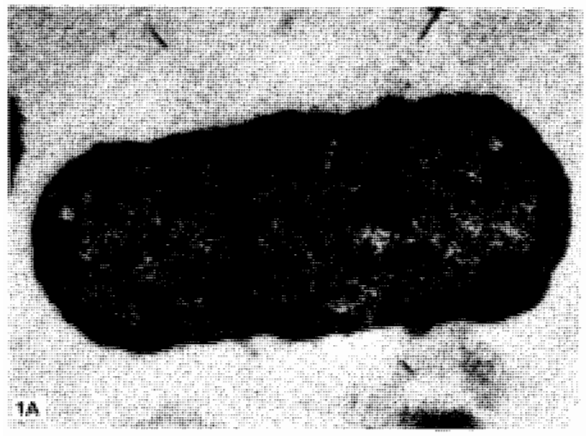
Autoradiography

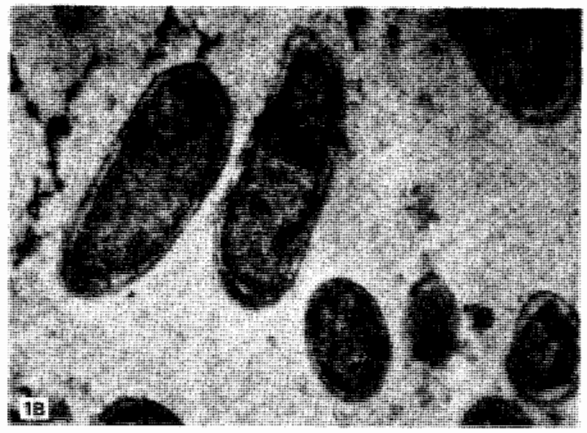
Mixtures consisting of 0.03 cm^3 *L*-(3 – 3H) serine, 10^{-4} *M* *L*-serine and 10^{-4} *M* metal salt were added to 1 cm^3 samples of bacterial culture in the log-phase (6 h growth) to give final volumes of 1.5 cm^3. The volumes of *L*-serine and metal salt were varied to obtain a set of different amino acid to metal ion ratios. The non-radioactive controls consisted of mixtures of bacterial culture and 0.5 cm^3 of distilled water, and 1 cm^3 of bacterial culture and 0.5 cm^3 of the metal salt solutions. After incubating a further 6–8 h at 37 °C in the presence of the test substances, the cells were harvested, washed, fixed, post-fixed, dehydrated and embedded in resin as described above for electron microscopy. (Preliminary experiments, using scintillation counting, had shown that the bacterial cells absorbed most of the radioactivity during incubation). The controls were processed in the same way as the radioactive samples. Thin sections approximately 100 nm thick of test samples and non-radioactive controls were placed on slides coated with nitrocellulose. The slides were covered with photographic emulsion whilst in total darkness by dipping them in a solution containing 13 cm^3 of Ilford L-4 emulsion sticks in 20 cm^3 of water. They were then stored in air-tight slide boxes over Drierite, and light was excluded by covering the boxes with thick black plastic. The exposure time was 10 weeks at 4 °C. Emulsions were developed and sections were prepared for electron microscopy according to the method of Kopriva [21]. The sections were finally stained with uranyl acetate followed by lead citrate prior to examination in the electron microscope.

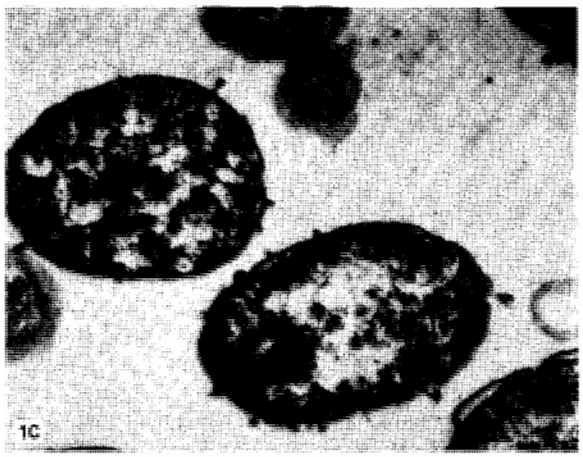
Results

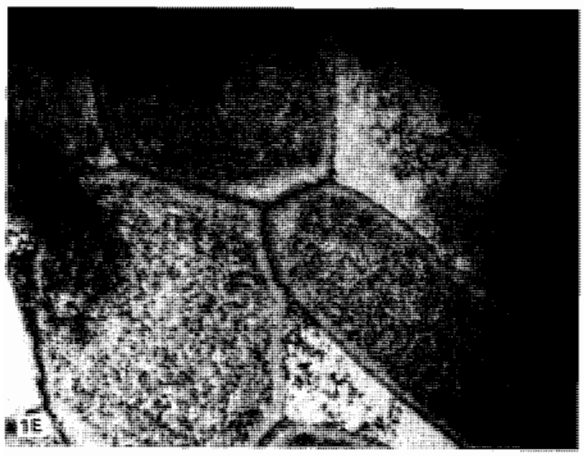
Effects produced on E. coli *by the* L*-Serine Gallium-(III) System*

Some metal ions are known to form several complex species with amino acids. In a previous investigation on induction of filamentous growth, we described a method of varying the ratios of amino acid to metal ions so as to cover the full range of the metal chelate system [17]. A similar approach was used in the present study. The preparation of thin sections for electron microscopy was a modification of the method of Zusman and collaborators [22]. The *E. coli* control is shown in Fig. 1A. Addition of *L*-serine alone as modifying agent appears to separate the plasma membrane from the cell wall: this is illustrated in Fig. 1B. Gallium(III) nitrate alone modified the outer envelope of the cells. Figure 1C shows the projections extending from the cell. These projections appear to involve the cell wall and the peptidoglycan layer. Figure 1D shows the effect of using a mixture of *L*-serine and gallium(III) nitrate (molar ratio 1:1) as test substance. The cell wall modifications were such that some of the cells appear to be linked together. The electron micro-









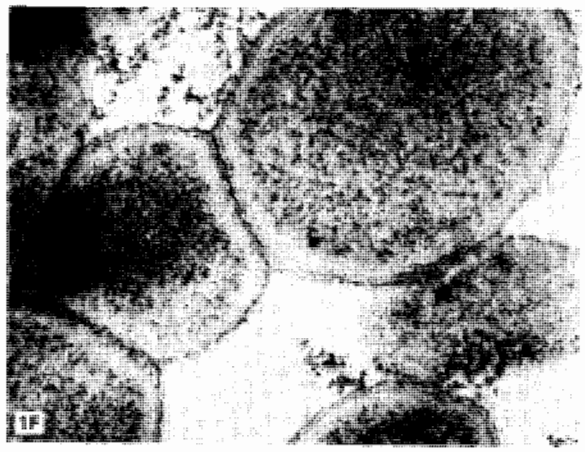


Fig. 1A. Electron micrograph of a normal *E. coli* cell cultured in mineral 'C' medium; 45 000× magnification.

Fig. 1B. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with *L*-serine to a final concentration of 10^{-3} *M*; 20 000× magnification.

Fig. 1C. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with gallium(III) nitrate to a final concentration of 10^{-3} *M*; 28 000× magnification.

Fig. 1D. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with a mixture of *L*-serine and gallium-(III) nitrate in a molar ratio of 1:1; 30 000× magnification. The arrow shows a small opening at the site of linkage providing a direct contact between the cytoplasm of the linked cells.

Fig. 1E. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with a mixture of *L*-serine and gallium-(III) nitrate in a molar ratio of 1:3; 30 000× magnification.

Fig. 1F. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with a mixture of *L*-serine and gallium-(III) nitrate in a molar ratio of 1:3; 45 000× magnification.

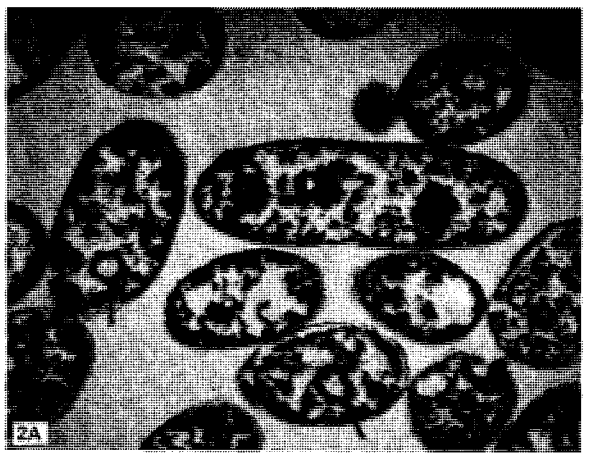


Fig. 2A. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with a mixture of *L*-serine and potassium tetrachloropalladate(II) in a molar ratio of 1:1; 30 000× magnification. The arrows show membrane-bound spherical bodies in several cells.

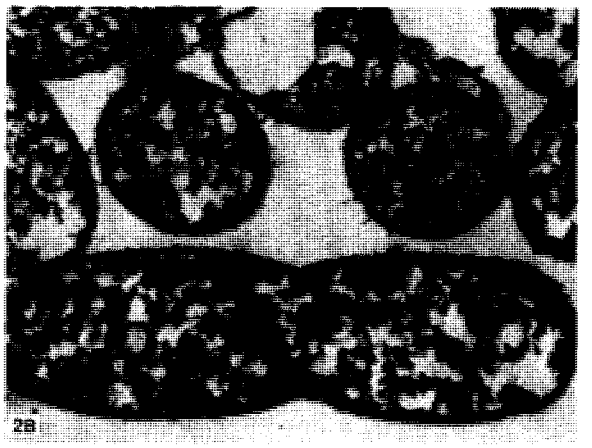


Fig. 2B. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with a mixture of *L*-serine and potassium tetrachloropalladate(II) in a molar ratio of 1:2; 30 000× magnification.

graph also shows a small opening (arrowed) at the site of linkage providing a direct contact between the cytoplasm of the linked cells. A pronounced cell wall modification was observed when the molar ratio of amino acid to metal ion was 1:3. Figure 1E shows that the bacteria have lost their rod-shaped, unicellular structure and appear as sheets of cells. This effect is illustrated at higher magnification in Fig. 1F.

Effects Produced on E. coli *by the L-Serine Palladium(II) System*

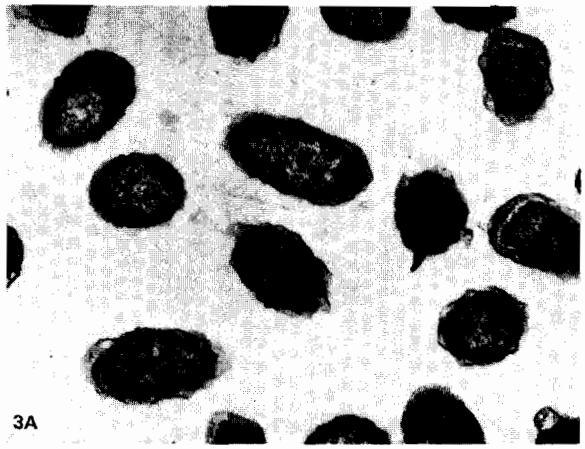
Mixtures of 3×10^{-3} *M* solutions of *L*-serine and potassium tetrachloropalladate(II) were used as modifying agents as described above for the *L*-serine gallium(III) system. When the amino acid and metal salt were used in a molar ratio of 1:1 (Fig. 2A) some of the cells are elongated and in the interior of the cells considerable clumping of the ribosomes is apparent. The large areas not stained, due to clumping, appeared to contain some fibrillar mateial. Figure 2A also shows some membrane-bound spherical bodies (arrowed). These membrane-bound structures were also observed in the cells when a mixture of the amino acid and metal salt in a molar ratio of 1:2 was used as a modifying agent (Fig. 2B).

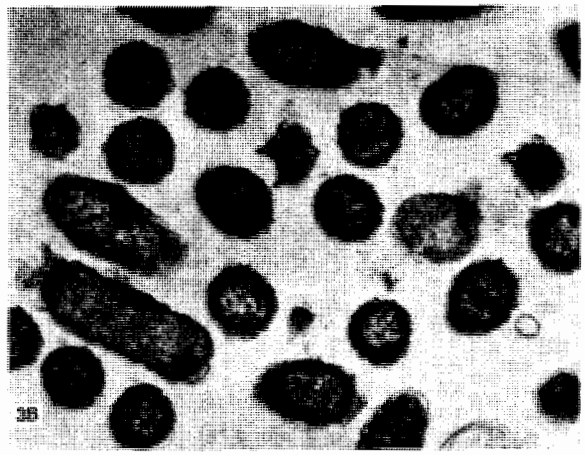
The Effects Produced on E. coli *by Caesium cis-dichloro-L-Serinatopalladium(II)*

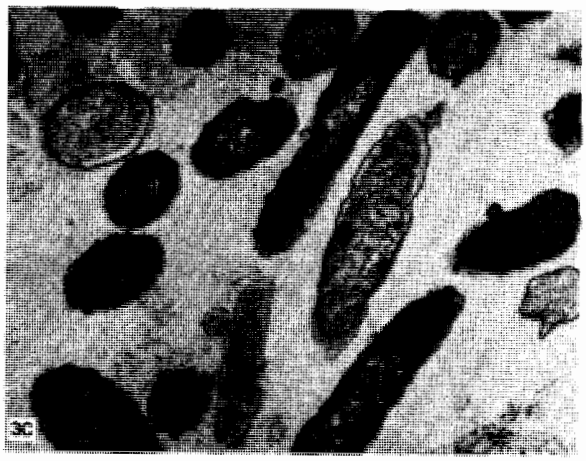
L-Serine can react with tetrachloropalladate(II) ions to yield two different complexes. One of these complexes, caesium *cis*-dichloro-*L*-serinatopalladium-(II), induced pronounced filamentous growth in *E. coli* [15]. Ultrastructural studies on this analytically pure palladium complex on *E. coli* have now been conducted in the concentration range 1–9 $\mu g/cm^3$. As previously indicated, the aeration procedure was not used when the *E. coli* were incubated with this test substance. The cultures were incubated in a shaking water bath. Comparison of electron micrographs of control cultures using aerators and a shaking water bath, where all other physical conditions were kept constant, showed no notable difference. At a concentration of 1.4 $\mu g/cm^3$ caesium *cis*-dichloro-*L*-serinatopalladium(II) produced no ultrastructural changes within the cell and the electron micrograph (Fig. 3B) appears similar to the control (Fig. 3A). On increasing the concentration of the complex to 2.8 $\mu g/cm^3$, Fig. 3C shows that many of the cells appear elongated. In the control sample (Fig. 3A) the ratios of length to width of the cells are in the range 1:1–2.3:1. Since these ratios in the test sample (Fig. 3C) are in the range 1.5:1–6.0:1, the observed elongation of the cells does not seem to be an effect produced by the plane of cutting. In many of the cells (Fig. 3C) the nucleoplasm appears electron transparent with small electron opaque inclusions. When caesium *cis*-dichloro-*L*-serinatopalladium(II) was used at a concentration of 5.5 $\mu g/cm^3$ (Fig. 3D) the cells again appear to be elongated and the nucleoplasm can be seen as relatively electron transparent areas containing fibrillar material. Figure 3E shows that these characteristics are more marked when the complex was used at a concentration of 8.3 $\mu g/cm^3$.

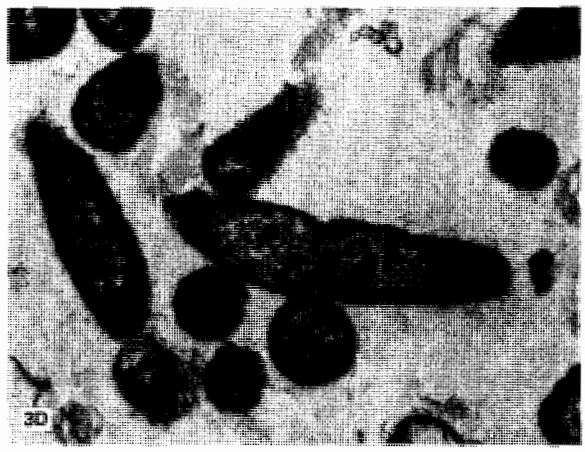
Investigation of the L-Serine Gallium III System on E. coli *using Autoradiography with L-(3 – 3H) Serine*

Figure 4A shows the normal control *E. coli* and Fig. 4B is an electron micrograph of the bacteria (control) grown in the presence of gallium(III) nitrate. When a mixture of *L*-(3 – 3H) serine was used









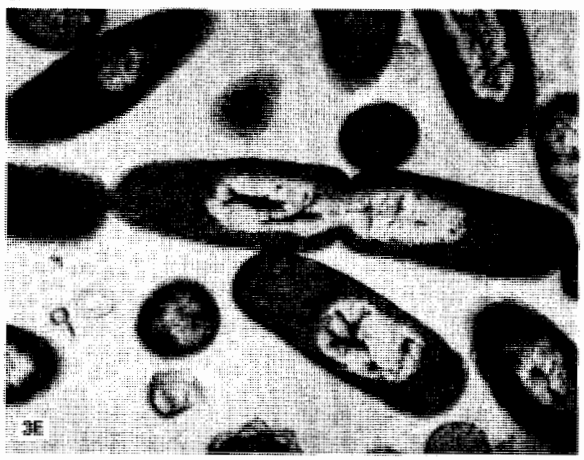


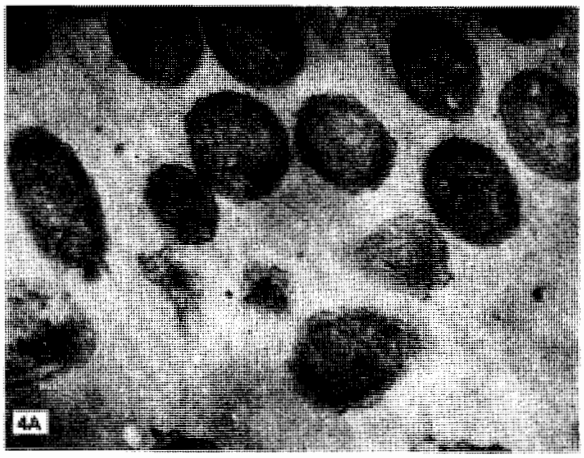
Fig. 3A. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with distilled water; 16 000× magnification.

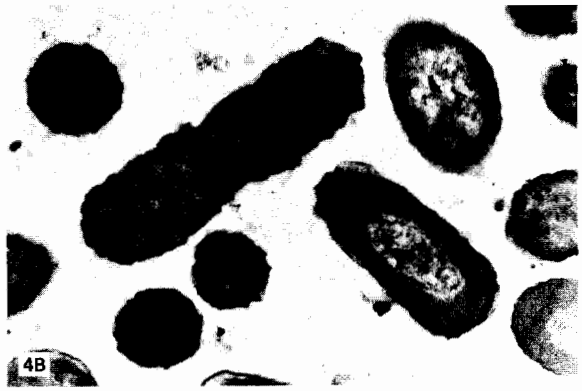
Fig. 3B. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with 1.4 $\mu g/cm^3$ of caesium *cis*-dichloro–*L*-serinatopalladium(II); 16 000× magnification.

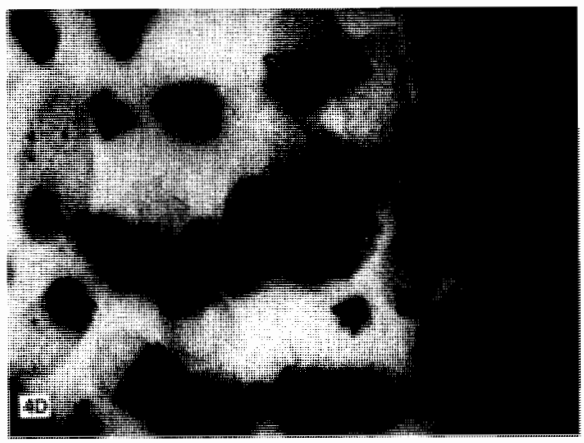
Fig. 3C. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with 2.8 $\mu g/cm^3$ of caesium *cis*-dichloro–*L*-serinatopalladium(II); 16 000× magnification.

Fig. 3D. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with 5.5 $\mu g/cm^3$ of caesium *cis*-dichloro–*L*-serinatopalladium(II); 16 000× magnification.

Fig. 3E. Electron micrograph of *E. coli* cells cultured in mineral 'C' medium supplemented with 8.3 $\mu g/cm^3$ of caesium *cis*-dichloro–*L*-serinatopalladium(II); 16 000× magnification.







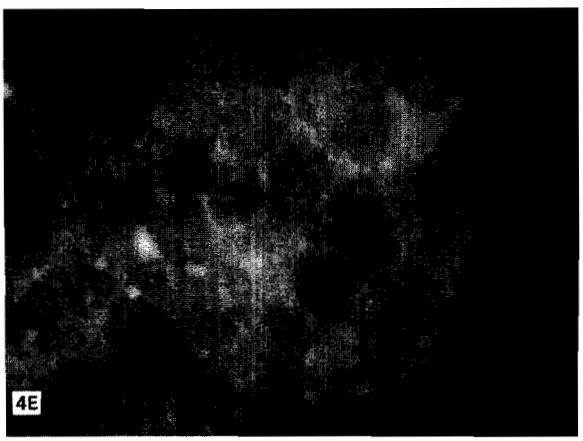


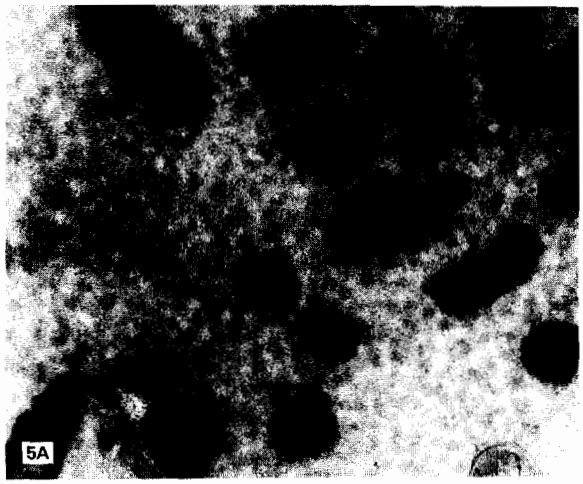
Fig. 4A. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with 0.5 cm^3 of distilled water; 17 000× magnification.

Fig. 4B. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with 0.5 cm^3 of 10^{-4} *M* gallium(III) nitrate; 20 000× magnification.

Fig. 4C. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with 0.47 cm^3 of 10^{-4} *M* *L*-serine and 0.03 cm^3 of *L*-(3 – 3H) serine; 17 000× magnification.

Fig. 4D. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with a mixture of 0.30 cm^3 of 10^{-4} *M* *L*-serine and 0.17 cm^3 of 10^{-4} *M* gallium(III) nitrate (molar ratio; 1.8:1) and 0.03 cm^3 of *L*-(3 – 3H)serine; 17 000× magnification.

Fig. 4E. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with a mixture of 0.22 cm^3 of 10^{-4} *M* *L*-serine, 0.25 cm^3 of 10^{-4} *M* gallium(III) nitrate and 0.03 cm^3 of *L*-(3 – 3H) serine; 17 000× magnification.



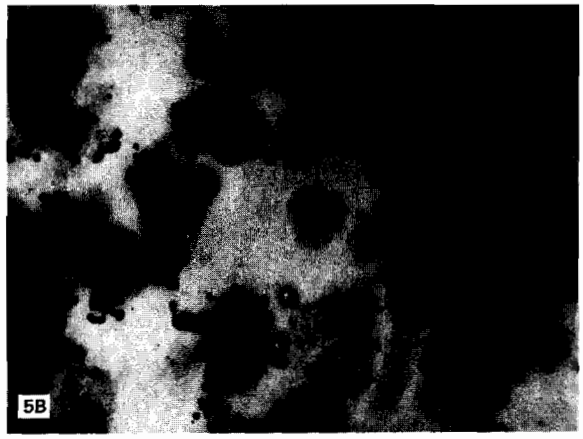


Fig. 5A. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with 0.5 cm^3 of distilled water; 13 000× magnification.

Fig. 5B. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with a mixture of 0.47 cm^3 of 10^{-4} *M L*-serine and 0.03 cm^3 of *L*-(3 – 3H) serine; 13 000× magnification.

Fig. 5C. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with a mixture of 0.37 cm^3 of 10^{-4} *M L*-serine, 0.10 cm^3 of 10^{-4} *M* potassium tetrachloropalladate(II) and 0.03 cm^3 of *L*-(3 – 3H) serine; 16 000× magnification.

Fig. 5D. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with a mixture of 0.34 cm^3 of 10^{-4} *M L*-serine, 0.13 cm^3 of 10^{-4} *M* potassium tetrachloropalladate(II) and 0.03 cm^3 of *L*-(3 – 3H) serine; 13 000× magnification.

Fig. 5E. Electron micrograph of *E. coli* cells cultured in 1 cm^3 of mineral 'C' medium supplemented with a mixture of 0.30 cm^3 of 10^{-4} *M L*-serine, 0.17 cm^3 of 10^{-4} *M* potassium tetrachloropalladate(II) and 0.03 cm^3 of *L*-(3 – 3H) serine; 13 000× magnification.

as the radioactive test substance, many of the silver grains can be seen in the cytoplasm (Fig. 4C). There are also a number of silver grains over the nuclear region. A similar result was obtained with a mixture of *L*-serine and gallium(III) nitrate (Molar ratio 1.8:1) containing some *L*-(3 – 3H) serine (Fig. 4D). However, much of the radioactivity appears to be located on the edge of the cytoplasm nearest to the cytoplasmic membrane. As shown in Fig. 4E sheets of bacterial cells were produced when *L*-serine and gallium(III) nitrate were mixed in a molar ratio of 1:1. Silver grains can be seen covering some of the cell junctions.

Investigation of the L-*Serine Palladium(II) System on* E. coli *using Autoradiography with* L-*(3 – 3H) Serine*

Figure 5A shows the normal control *E. coli* and Fig. 5B represents the result using *L*-serine in the presence of *L*-(3 – 3H) serine. The effect produced on *E. coli* by a mixture of *L*-serine and tetrachloropalladate(II) ions in a molar ratio of 4:1 and containing some *L*-(3 – 3H) serine is illustrated in Fig. 5C. In Fig. 5C there is evidence of condensation of the nuclear material and some cells show the presence of radioactivity in the nucleoplasm. These effects can also be seen in Fig. 5D which represents a mixture of amino acid and metal ion in a molar ratio of 2.0:1, and in Fig. 5E showing the result of using the amino acid and metal ion in a molar ratio of 2.6:1.

Discussion

Gallium(III) nitrate and mixtures of *L*-serine and gallium(III) ions produced pronounced ultrastructural changes in the cell wall and the plasma membrane. The gallium appears to cause surface convolution due to enhanced and uncoordinated growth of the cell wall. Some chemical evidence has been obtained that gallium(III) ions can react with *L*-serine to form complexes. The observation of silver grains at or near the bacterial surface in the autoradiography study using *L*-(3 – 3H) serine might thus be rationalized. However, the localization of radioactivity is not good enough to permit an unequivocal statement concerning the position of gallium-(III) complexes within the cell. Mertz *et al.* [23] reported that gallium(III) citrate has an affinity for plasma membranes of lymphocytes. On the basis of the results given in this paper, it would seem that gallium(III) compounds also have an affinity for the periphery of *E. coli* cells. In the present study, ultrastructural changes within the cells are also apparent, and Mertz *et al.* described similar observations with lymphocytes. Of particular interest is the production of aggregates of *E. coli* cells by the gallium(III) *L*-serine system. It is tempting to speculate that a gallium(III) complex can cross-link cell wall constituents of different *E. coli* cells. In the autoradiography study, silver grains were seen covering the junctions between some of the cells. Although this result may well be fortuitous, it would be in accordance with the above suggestion. Evidence has been obtained for the involvement of divalent magnesium and calcium ions in the agglomeration of cells of sea sponges. A model based on the cross-linking of surface glycoproteins by magnesium and calcium ions has been proposed [24].

By contrast with the *L*-serine gallium(III) system, cell surface changes of *E. coli* were not observed when either mixtures of *L*-serine or a pure *L*-serine palladium(II) complex were investigated as modifying agents. The palladium complexes produced pronounced changes in the interior of the bacterial cells. These modifications were seen as membrane-bound vesicles, clumping of the cytoplasm and a very marked effect on the nucleoplasm. In an ultrastructural study on the effects of ampicillin on *E. coli,* Burdett and Murray [25] observed similar membrane-bound vesicles and clumping of the cytoplasm. One of the most likely explanations for changes seen in the nuclear region is an interaction of palladium(II) complexes with bacterial chromatin. The observation of an apparent localization of silver grains over the nuclear region in the autoradiographs supports this conclusion.

Organic chemicals such as hydroxyurea or nalidixic acid can induce filamentous growth in *E. coli*. Filaments can also be induced by ultraviolet light. Evidence has been obtained that in these cases filamentation occurs as a result of modification of the nuclear material [26–28]. However, substances such as penicillin, which are known to cause changes at the cell surface, can induce filamentous growth in *E. coli* [29]. Burdett and Murray [25] suggested that enzymes responsible for septation can be inhibited by the antibiotic. Roberts [30] proposed that filamentous growth induced by cisplatin is a manifestation of the ability of this platinum compound to react with bacterial DNA. On the basis of the results obtained in the present study, we suggest that filamentous growth induced by some other metal ions or metal complexes may well involve mechanisms which do not depend on interaction with nuclear material.

Acknowledgements

The authors wish to thank Mr. A. Wipperman, Managing Director of Mathey Garrett and Co. for supplying palladium metal on the loan scheme, and Mr. Winston A. Shorland for preparing potassium tetrachloropalladate(II). We also thank Miss. J. Hubbard and Mr. P. James for advice and help with autoradiography.

References

1 B. Rosenberg, E. Renshaw, L. van Camp. J. Hartwick and J. Drobnik, *J. Bact., 93,* 716 (1967).
2 B. E. Markham and R. R. Brubaker, *J. Bact., 143,* 455 (1980).
3 S. Reslova, *Chem. Biol. Interact., 4,* 66 (1971).
4 K. S. Anderson, *Mutation Res., 67,* 209 (1979).
5 D. J. Beck and R. R. Brubaker, *Mutation Res., 27,* 181 (1975).
6 D. J. Beck and J. E. Fisch, *Mutation Res., 77,* 45 (1980).
7 P. Lecointe, J. P. Maquet, J. L. Butour and C. Paoletti, *Mutation Res., 48,* 139 (1977).
8 C. Monti-Bragadin, M. Tamaro and E. Banfi, *Chem. Biol. Interact., 11,* 469 (1975).
9 D. Cunningham, J. T. Pembroke and E. Stevens, *Mutation Res., 84,* 273 (1981).
10 M. Kelehan, E. Farrell and P. Smith, *Soc. Gen. Microbiol., 4,* 135 (1977).
11 H. C. Harder, 'Recent Results Cancer Res.', Vol. 48, Springer-Verlag, Belin, pp. 98–112, (1974).
12 D. J. Beck, D. Vasquez and R. Cook, *J. Clin. Hematol. Oncol., 7,* 726 (1977).
13 M. J. Cleare and P. C. Hydes, 'Metal Ions in Biological Systems', Vol. 11, Marcel Dekker Inc., New York and Basel, pp. 1–62 (1980).
14 M. Aresta, D. Giordanna, A. Ingrosso, S. Ricardi and E. Sinigaglia, *Chem. Biol. Interact., 28,* 61 (1979).
15 A. J. Charlson, N. T. McArdle and E. C. Watton, *Inorg. Chim. Acta, 56,* L35 (1981).
16 R. J. Banner, A. J. Charlson, R. P. Gale, N. T. McArdle, K. E. Trainor and E. C. Watton, *Cancer Treatment Rep., 61,* 469 (1977).
17 A. J. Charlson, K. E. Trainor and E. C. Watton, *J. and Proc. Royal Soc. New South Wales, 108,* 6 (1975).
18 H. L. Grube, 'Handbook of Preparative Inorganic Chemistry', 2nd Ed., G. Brauer, Ed. Vol. 2, Academic Press, New York, pp. 1560–1605 (1965).
19 A. R. Spurr, *J. Ultrastructure Res., 26,* 31 (1969).
20 E. S. Reynolds, *J. Cell Biol., 17,* 208 (1963).
21 B. M. Kopriva, *Histochemie, 37,* 1 (1973).
22 D. R. Zusman, A. Carbonell and J. Y. Haga, *J. Bact., 115,* 1167 (1973).
23 T. Mertz, L. Malmud, K. Mc Kusic and H. N. Wagner, Jr., *Cancer Res., 34,* 2495 (1974).
24 R. C. Hughes, *Essays Biochem., 11,* 1 (1975).
25 D. J. Burdett and R. G. E. Murray, *J. Bact., 119,* 1039 (1974).
26 J. Donch and J. Greenberg, *J. Bact., 95,* 1555 (1968).
27 G. J. Kantor and R. A. Deering, *J. Bact., 95,* 520 (1968).
28 D. R. Zusman and D. M. Krotoski, *J. Bact., 120,* 1427 (1974).
29 U. Schwartz, A. Asmus and H. Frank, *J. Mol. Biol., 41,* 419 (1969).
30 J. J. Roberts, 'Recent Results in Cancer Res.', Vol. 48, Springer-Verlag, Berlin, pp. 79–112 (1974).