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Platinum-Induced Filamentous Growth in *Escherichia coli*[1]

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ABSTRACT

Certain group VIIIB transition metal compounds were found to inhibit cell division in *Escherichia coli*, causing marked filamentous growth. Gram-negative bacilli were the most sensitive to this effect, whereas gram-positive bacilli responded only at near-toxic levels of the metal. None of the cocci tested showed any apparent effect. Cytokinesis (cross-septation) can be initiated by removal or decrease of platinum, but not by treatment with pantoyl lactone, divalent cations, or a temperature of 42 C.

Filamentous growth in bacteria may be induced by a variety of environmental conditions, all of which are more or less damaging to the cell. Three general classes of agents which may cause long forms to appear in culture are (i) poisons and metabolic inhibitors, (ii) nutritional deficiencies including those of metals, and (iii) physical conditions such as radiation, pressure, or temperature. For a review of this field, see Hughes (3).

In an investigation of the possible effects of an electric field on the growth processes of bacteria, it was noted that *Escherichia coli* formed long filaments, up to 300 times the length of a normal cell (6). Subsequent investigation indicated that a platinum salt produced from the electrode was the active agent responsible for this phenomenon. This platinum salt, identified as $[PtCl_6]^{-2}$, when present in media containing free ammonia and irradiated with ultraviolet (UV) radiation, was found to form three ligand-substituted ionic species, each with a different biological effect on *E. coli* (5). The doubly negative-charged species $[PtCl_6]^{-2}$ was bactericidal. The singly negative-charged species $[PtCl_5NH_3]^{-}$, which is an intermediate form, appeared to have no effect on *E. coli*. The neutral species $[PtCl_4(NH_3)_2]^{0}$, which is also the stable species, produced marked filamentous growth in bacteria by inhibiting cell division without any marked effect on cell growth.

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The present study was undertaken to explore further this phenomenon of filament formation and to study the biological effect of the active metal salts.

MATERIALS AND METHODS

Cultures. Stock cultures of the various bacteria were maintained on Nutrient Agar slants and were subcultured at monthly intervals. *E. coli* strain B, the organism used for most of these studies, was first adapted to the synthetic media by serial transfers before being used. Most experiments were carried out at 37 C in tubes containing the simple chemically defined mineral "C" medium of Roberts and co-workers (4), with 0.2% glucose as the energy source. Complex nutrient media were also used in some of the experiments. The tubes were fitted with glass frit bubblers through which compressed air was passed at a constant rate. The absorbancy of the culture was measured at 450 or 850 mμ in a Beckman model B spectrophotometer. Colony counts were done on Nutrient Agar plates.

Preparation of metal compounds. The various group VIIIB transition metal compounds were prepared as stock solutions in the buffered (*p*H 7) "C" medium (minus the glucose) at concentrations of 100 to 700 μg/ml of the metal. Ammonium hexachloroplatinate $[(NH_4)_2PtCl_6]$, the most active metal compound, was irradiated for 30 min at 3,650 A with a UV lamp to produce the "active" neutral ionic species (5). Working solutions were then prepared from these stock solutions by diluting with sterile culture media.

Microscopic observations. The total number of cells was determined with a Petroff-Hausser counting chamber at a magnification of 600× with a phase-contrast microscope. Cytokinesis was followed by placing a drop of the filamentous culture which had been suitably diluted in Nutrient Broth, on a microscope slide and sealing the cover slip with wax. Phase-contrast microphotographs were then taken at intervals at room temperature (24 C).

Estimation of filamentous growth. The filamentous growth is described subjectively and categorized by estimating the relative percentage of filamentous cells in a drop of culture fluid. The relative length of the filaments is also estimated by comparing them to a control of normal size cells.

RESULTS

Various group VIIIB transition metal compounds were tested for their ability to produce filamentous growth in *E. coli* (Table 1). The compounds were tested over a range of 10 to 100 μg/ml of the metal in synthetic medium. Only $RhCl_3$ and $(NH_4)_2RhCl_6$ produced significant elongation when compared with the most active platinum salt [$(NH_4)_2PtCl_6$]. In all cases, however, a much higher concentration of the metal was required to produce elongation than with the platinum salt.

If grown in nutrient media, almost all the gram-negative bacilli showed filamentous growth (Table 2). Some of the gram-positive bacilli also showed slight elongation, but only at near toxic levels of platinum. On the other hand, all the cocci tested showed no noticeable effect, even at relatively high concentrations of platinum.

Figure 1 illustrates the appearance of filamentous *E. coli* B when grown for 12 hr in Nutrient Broth containing 23 μg/ml of platinum in the form of the neutral species. This elongation effect also occurred readily on solid media. When normal or filamentous cells were plated on Nutrient Agar plates containing the neutral species of the platinum complex, they gave rise to macrocolonies of filamentous cells. These colonies were of normal size and shape, but were dull in appearance and very dry or granular in consistency.

TABLE 1. *Effects of certain group VIIIB transition metal compounds in producing elongation in Escherichia coli after 6 hr of incubation in synthetic medium*

Metal compound	Effective concn (μg/ml)	Elongation[a] (estimated)
$(NH_4)_2PtCl_6$	1–20	100%, 25–100×
$RhCl_3$	30–100	75%, 5–25×
$(NH_4)_2RhCl_6$	20–30	75%, 5–20×
K_2RuCl_6	40–50	50%, 5–20×
RuK_2NOCl_5	40–100	50%, 5–10×
RuI_2	20–30	25%, 10–20×
$RuNOCl_3$	20–40	25%, 4–6×
$RuBr_2$	10–20	10%, 10–20×
$RuNO(NO_3)_3$	10–100	10%, 4–6×
$K_3Rh(NO_2)_6$	20–60	10%, 3–5×
$(NH_4)_3RuCl_6$	15–30	10%, 3–5×
$UO_2(C_2H_2O_2)_2$	25–75	5%, 3–5×
$RuCl_3$	—	—

[a] Growth is the estimate of the relative percentage of filamentous cells in a drop of culture fluid, and the relative length of the filaments is expressed in comparison to a control of normal size cells.

When ammonium hexachloroplatinate was initially dissolved in the media, it was as the doubly negative species $[PtCl_6]^{-2}$, which is bright yellow in color. In this form, it was extremely toxic to bacteria and killed the cells. Viable cell counts showed that, upon continued incubation, growth was resumed and that the bacterial population then approached that of the control (Fig. 2). This resumption of growth was the result of the slow conversion of the double negative species to the stable neutral form under the experimental conditions and was not due to the emergence of resistant organisms by mutation or selection, since studies of these cells on Nutrient Agar plates with and without the platinum showed no increased resistance to the platinum.

Induced filamentous growth caused by other agents in many bacteria can be reversed and cytokinesis (cross-septation) may be initiated by exposure to various agents. In an attempt to determine whether divalent cations would induce cytokinesis in the elongated filaments, filamentous cells were inoculated into Nutrient Broth containing 20 μg/ml of platinum and 0.001 M amounts of various divalent cations (Mg^{++}, Ca^{++}, Mn^{++}, Sn^{++}, Zn^{++}, and Fe^{++}). In no instance was cytokinesis observed upon continued incubation. Filamentous cells grown for 3 hr in Nutrient Broth containing 20 μg/ml of platinum in the form of the neutral species were further incubated at 42 C for an additional 3 hr. In this instance also, induced cytokinesis or cell division was not observed. Filamentous cells were also inoculated into the mineral media containing 13 μg/ml of the platinum in the form of the neutral species and varying amounts of L-pantoyl lactone (Table 3). In no instance was cytokinesis observed in the filaments. Low concentrations of pantoyl lactone enhanced filamentous growth, whereas high concentrations (above 0.115 M) proved to be toxic to the microorganism.

Cytokinesis or cell division in platinum-induced filaments could only be initiated by removal or decrease in the concentration of the irradiated platinum complex. Figure 3 illustrates cytokinesis and cell division in a filament which had been grown for 3 hr in Nutrient Broth containing 13 μg/ml of platinum in the form of the neutral species. Figure 3a is the filament immediately after being washed free from platinum and transferred to a platinum-free Nutrient Broth on a microscope slide. Cross walls were then formed at intervals along the entire filament (Fig. 3b, c),

TABLE 2. *Elongation effect on various bacteria by three concentrations of the neutral ionic species of ammonium hexachloroplatinate in nutrient media after 12 to 24 hr of incubation.*

Organism	Elongation[a]		
	5 μg/ml[b]	25 μg/ml	75 μg/ml
Escherichia coli B	50%, 10–25×	95%, 10–50×	Toxic
E. coli C	40%, 10–15×	70%, 10–25×	Toxic
E. coli K-12	25%, 5–10×	20%, 10–20×	Toxic
Aerobacter aerogenes	50%, 2–15×	Toxic	Toxic
Alcaligenes faecalis	25%, 2–10×	10%, 10–15×	Toxic
Proteus mirabilis	10%, 2–5×	25%, 5–10×	Toxic
Pseudomonas aeruginosa	25%, 5–10×	Toxic	Toxic
Klebsiella pneumonia	50%, 2–10×	Toxic	Toxic
Serratia marcescens	20%, 2–5×	Toxic	Toxic
Bacillus cereus	Normal	Normal	25%, 2–10×
B. licheniformis	Normal	Normal	90%, 2–5×
B. megaterium	Normal	Normal	20%, 2–5×
B. subtilis	Normal	Normal	20%, 2–4×
Lactobacillus sp.	Normal	Toxic	Toxic
Clostridium butylicum	Normal	Normal	Toxic
Corynebacterium sp.	Normal	Normal	Toxic
Streptococcus lactis	Normal	Normal	Normal
S. faecalis	Normal	Normal	Normal
Staphylococcus aureus	Normal	Normal	Normal
Sarcina lutea	Normal	Normal	Normal
Neisseria catarrhalis	Normal	Normal	Normal

[a] Expressed in the same terms as those of Table 1.
[b] Concentration of platinum.

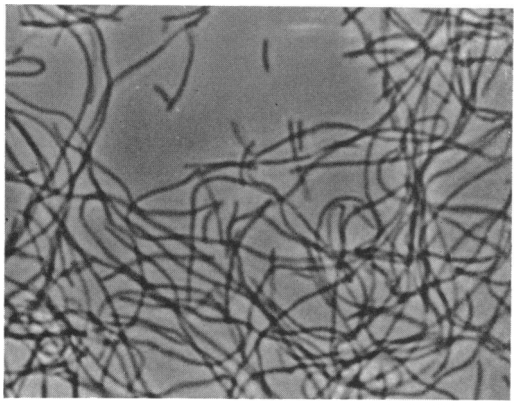

FIG. 1. *Example of filaments of Escherichia coli B when grown for 12 hr in Nutrient Broth containing 23 μg/ml of "active" neutral ionic species of ammonium hexachloroplatinate.*

but, in general, active cell division occurred near one or both ends of the filament (Fig. 3d, e). In some cases, however, active cell division was observed to occur anywhere along the filament. The normal length cells produced by this process appeared to be normal in all respects.

DISCUSSION

Various group VIIIB transition metal ions were found to inhibit cell division in *E. coli* although not apparently interfering with growth. Previous studies indicated that the most active metal ions were platinum, rhodium, and ruthenium compounds which were capable of undergoing photochemical replacement of an ion with ammonia in the culture medium (5, 6). Various available group VIIIB transition metal compounds were tested for their ability to inhibit cell division and produce filamentous growth in *E. coli* (Table 1). Those chosen were mostly rhodium and ruthenium compounds since we were looking for a metal ion which would approach platinum in activity and yet have a long half-life radioactive isotope which was easily obtainable for tracer studies. However, none of the rhodium or ruthenium compounds was as good as ammonium hexachloroplatinate, and they presented more difficulty in interpretation because their results were less clear-cut. These compounds formed many more ionic species than the platinum, and we were unable to find a stable ionic species which was responsible for the elongation effect. It was also noted that the same compound from different sources produced varying results; for example,

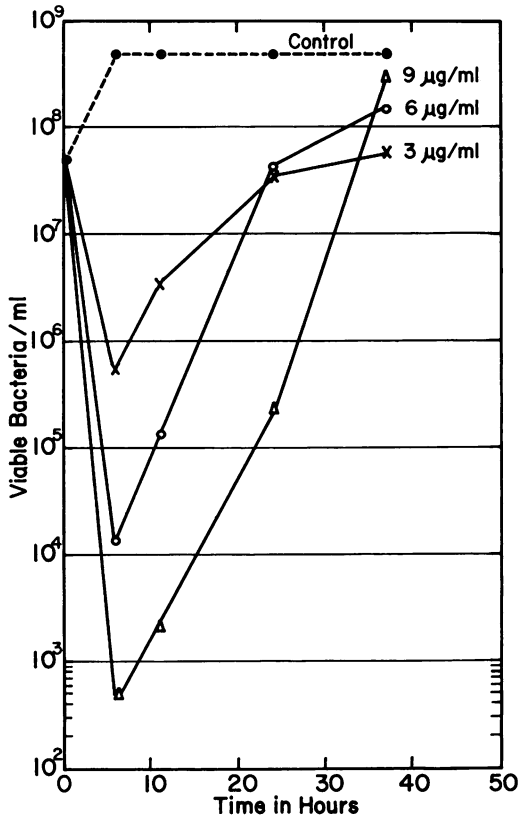

FIG. 2. *Effect of three concentrations of fresh ammonium hexachloroplatinate on Escherichia coli when grown in the dark in synthetic media.*

TABLE 3. *Effect of* L*-pantoyl lactone on filamentous growth of Escherichia coli after 6 hr of incubation in synthetic media containing 12 μg/ml of platinum*

Concn of L-pantoyl lactone	Elongation[a]
M	
0	80%, 25–50×
0.017	100%, 25–100×
0.042	90%, 25–75×
0.074	80%, 25–50×
0.115	70%, 20–30×
0.147	Toxic

[a] Expressed in the same terms as those of Table 1.

only one sample of rhodium trichloride (Engelhard Industries, Inc., Newark, N.J.) from five different sources was active in producing filamentous growth, although all samples appeared to be chemically identical.

This process of inhibition of cell division and filamentous growth induced by platinum appears to be confined to the gram-negative bacilli (Table 2). Some gram-positive bacilli also showed slight elongation, but only at near-toxic levels of platinum, and, therefore, this elongation may be due to a different mechanism. On the other hand, all cocci tested showed no apparent effect, even at high concentrations of the platinum. The various species of gram-negative bacilli vary greatly in the elongation effect and in their sensitivity to platinum, with *E. coli* B giving the greatest elongation.

When *E. coli* was incubated in the dark with fresh ammonium hexachloroplatinate $[PtCl_6]^{-2}$, the bacteria were immediately killed, with the bactericidal effect dependent upon the concentration of platinum (Fig. 2). It is of interest to note here that Sigler and Blow (7) found $[PtCl_6]^{-2}$ to be very similar to phenylmercuric acetate in action; both bound the same or similar sites of protein. When ammonium hexachloroplatinate was exposed to UV light or aged for a few days, the "active" stable neutral ionic species was formed (5). This ionic species produced the long, nonseptate multinucleated filaments in *E. coli* (Fig. 1), and these filaments appeared to be normal in most other respects. Filamentous growth was produced in both synthetic and nutrient media. In the synthetic medium, the optimal concentration of platinum for producing filaments was about 12 μg/ml with a range of 1 to 20 μg/ml. In nutrient media, approximately twice as much platinum was needed with an optimal concentration of about 25 μg/ml. At least a part of this increased platinum requirement may be due to a scavenging interaction of the platinum ions with the nutrient polypeptides.

Filamentous growth caused by other agents in many bacteria can be reversed by exposure to various agents. Cytokinesis can be initiated by pantoyl lactone or incubation at 42 C with the UV-irradiated induced filamentous form of *E. coli* (1), or it can be induced in filamentous forms of *Erwinia* by divalent cations and pantoyl lactone (2). These various methods of initiating cytokinesis, however, had no effect on the filaments induced by platinum. In fact, pantoyl lactone in low concentration enhanced filamentous growth in *E. coli* (Table 3). Cytokinesis and cell division could only be initiated by removal or decrease in the platinum from the culture medium (Fig. 3).

These observations indicate that the specific platinum-induced inhibition of cell division in *E. coli* differs from that of other reported methods and may provide a useful tool in the study of the cell division process.

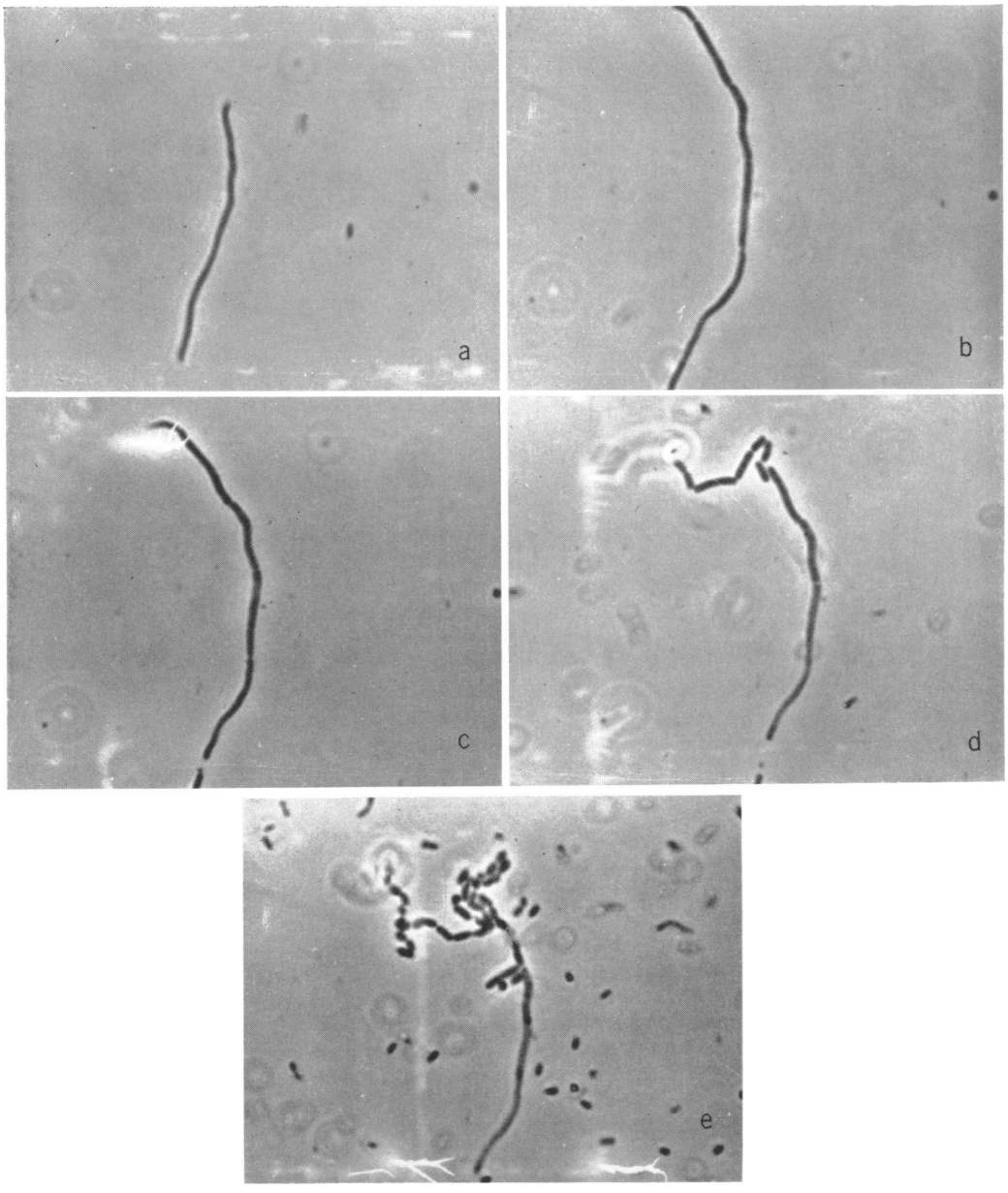

FIG. 3. *Cytokinesis and cell division in a filament of Escherichia coli after 3 hr of growth in Nutrient Broth containing 13 μg/ml of platinum and then transferred to a platinum-free Nutrient Broth on a microscope slide (24 C) at (a) zero time, (b) 40 min, (c) 75 min, (d) 150 min, and (e) 225 min.*

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