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# Electron Microscopy of Colicin I-Producing Cells

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Electron microscope examination of mitomycin C-induced strains of *Escherichia coli* colicinogenic for colicins Ia or Ib reveals particles of approximately 15 to 20 nm in diameter associated with the cell surface. Such structures are not present on uninduced colicinogenic strains nor on a mitomycin C-treated non-colicinogenic strain. In contrast to wild-type colicinogenic strains, induction of a colicinogenic strain known to be lacking the specific colicin I receptor was shown to result in the release of colicin activity into the cell medium. Examination of such induced mutants by electron microscopy showed their cell surfaces to be free of the particles observed on the wild-type strain. The cell medium of such induced mutant strains contained large numbers of particles of similar size and shape to those found on the surface of induced colicinogenic wild-type strains.

Colicinogenic factors (Col factors) are nonessential extrachromosomal genetic elements found in a variety of strains of the *Enterobacteriaceae*. The presence of a particular Col factor confers on the host strain the ability to produce bactericidal proteins called colicins. Under normal growth conditions, the colicin structural gene is repressed such that the majority of cells in the population are not producing significant amounts of colicin. However, treatment of such cells with various agents such as ultraviolet light (9) or mitomycin C (3) leads to induction of the production of certain colicins.

Monk and Clowes (7) observed that when colonies of an *Escherichia coli* (Col I) strain were overlaid with an appropriate sensitive indicator strain, the resultant zone of inhibition was smaller than the zones observed with a *Salmonella* (Col I) strain. Since *Salmonella* is naturally colicin resistant (10), they suggested that I colicin was adsorbed by the nonproducing cells of the colonies of *E. coli*. That the I colicin was adsorbed to colicin I receptors was supported by the finding that an *E. coli* Col I strain which had been made colicin I resistant exhibited zones of inhibition of similar size to those found in *Salmonella* (Col I).

In this paper we investigated the state of colicin I-producing cells by direct observation in the electron microscope. We concluded that upon induction, some of the colicin I molecules become associated with their cognate receptors on the surface of most of the cells in the mitomycin C-treated bacterial population. These results support the conclusions of Monk and Clowes (7).

## MATERIALS AND METHODS

**Organisms.** The bacterial strains used are described in Table 1. Conditions of cell growth and induction are described in the legends to figures and footnotes to tables.

**Electron microscopy.** The 200-mesh copper grids (Pellco) were coated with a Formvar membrane (Ladd Chemical) and then coated with a layer of carbon. Cells were stained by mixing equal volumes of cells (2 $\times$ $10^9$ per ml) and 2% potassium phosphotungstate (adjusted to *p*H 7.0 with 5 N KOH). A 2-$\mu$liter amount of this mixture was immediately applied to coated grids, and, after 15 to 30 sec, excess liquid was removed with filter paper and the grids were allowed to air-dry. The grids were observed in an RCA EMU-3C electron microscope.

## RESULTS

**Examination of colicin-producing cells.** Treatment of cells colicinogenic for colicin Ia or Ib with mitomycin C leads to a 4- to 64-fold increase in colicin production (Table 2). Previous studies showed that after 4 hr of induction, the colicin produced by such cells is not found free in the growth medium, but is associated with cells (5). Induction of colicinogenic strain JK4, which lacks the colicin I receptor (6), leads to release of colicin into the growth medium (Table 3). This suggests that after synthesis, colicin I molecules become associated with the colicin I receptor. Under certain conditions, the killing action of colicins Ia and Ib can be

TABLE 1. *Bacterial strains used*

| Strain | Synonym | Comments |
|---|---|---|
| JK1 | *Escherichia coli* W3110 str-r | |
| JK16 | *E. coli* W3110 str-r (Col Ia-CA53) | Produces colicin Ia-CA53 |
| JK20 | *E. coli* W3110 str-r (Col Ib-P9) | Produces colicin Ib-P9 |
| JK4 | *E. coli* W3110 str-(Col Ib-P9) I-r | Produces colicin Ib-P9; resistant to colicins Ia-CA53 and Ib-P9 and does not adsorb these colicins (6) |

TABLE 2. *Induction of colicin Ia and Ib*[a]

| Strain | Colicin produced (units) | |
|---|---|---|
| | -MTC[b] | +MTC |
| JK1 | <1 | <1 |
| JK4 (Col Ib) I-r | 8 | 64 |
| JK16 (Col Ia) | 4 | 16 |
| JK20 (Col Ib) | 1 | 64 |

[a] Strains were induced as previously described (6). After 16 hr of induction, the cells were lysed by lysozyme-ethylenediaminetetraacetic acid treatment (2), and the resulting lysates were centrifuged at 12,000 × *g* for 15 min. The supernatant fractions were assayed for colicin activity by the end point dilution method (6). The colicin activity measured in this experiment was taken as a measure of the total intracellular colicin, free colicin found in the medium, and any bound colicin activity that was freed by ethylenediaminetetraacetic acid-lysozyme treatment.
[b] MTC, mitomycin C.

reversed by incubation of colicin-treated cells with trypsin (6). This suggests that the receptor is on the cell surface and that it might be possible to observe cell-bound colicin directly in the electron microscope.

Figure 1 is a representative electron micrograph of negatively stained colicinogenic strain JK16, which produces colicin Ia-CA53. Examination of the cell periphery shows round particles with a mean diameter of approximately 20.6 ± 2.4 nm. In some instances, these particles seem to be aggregated into oligomeric structures. Figure 2 shows this same strain after 16 hr of mitomycin C induction. Such preparations show irregularly shaped structures distributed on the cell surface. These structures have a mean diameter of approximately 20.3 ± 3.5 nm and appear heterogeneous with respect to size and shape. Figures 3 and 4 show similar electron micrographs of noninduced (Fig. 3) and mitomycin C-induced (Fig. 4) colicinogenic strain JK20, which produces colicin Ib-P9. Figure 4 shows surface particles on the induced culture which have mean diameters of approximately 14.5 ± 4.1 nm. Although not shown, induced cultures of both colicinogenic strains exhibit peripheral particles. If the particles observed on the surface of these strains are colicin I, one would not expect to find similar structures associated with the non-colicinogenic parent strain JK1. Figure 5 shows a representative cell from a non-colicinogenic culture of strain JK1 which had been treated with mitomycin C. In marked contrast to the mitomycin C-treated colicinogenic derivatives, the surface of such cells lack the structures seen in Fig. 2 and 4. The surfaces of uninduced cells of this strain have the same appearance. Examination of the mitomycin C-induced population of either colicinogenic strain shows that approximately 65% of the total cells have an appearance similar to the cell shown in Fig. 2 or 4, whereas 35% of the cells appear similar to the cell shown in Fig. 1 or 3.

If the structures seen on the surface of induced colicinogenic cells are receptor-associated colicin, strain JK4, which produces colicin Ib-P9 but lacks the colicin I receptor, should not exhibit bound structures. As seen in Table 3, after 16 hr of induction, the medium of induced cells of this strain exhibits a 7.5-fold higher lever of colicin activity than does that of the wild-type strain. Figure 6 shows that mitomycin C-induced strain JK4 exhibits none of the structures seen on the surface of induced wild-type cells (Fig. 2, 4). Furthermore, unlike the case of induced wild-type colicinogenic cells, the medium of the receptor-defective strain contains a large amount of round structures with a diameter of 19.6 ± 2.5 nm. These can be clearly seen around the cell shown in Fig. 6.

TABLE 3. *Release of colicin Ib from JK4 (Col Ib) I-r and JK20 (Col Ib)*[a]

| Time after mitomycin C addition (hr) | Colicin activity | |
|---|---|---|
| | JK20 (Col Ib) | JK4 (Col Ib) I-r |
| 0 | <1 | <1 |
| 4 | <1 | 18 |
| 16 | 48 | 360 |

[a] Strains were induced as previously described (6). At the indicated times, the cultures were centrifuged at 12,000 × *g* for 15 min. The supernatant fractions were centrifuged a second time, and the resultant supernatant fraction was assayed for colicin activity as in Table 2.

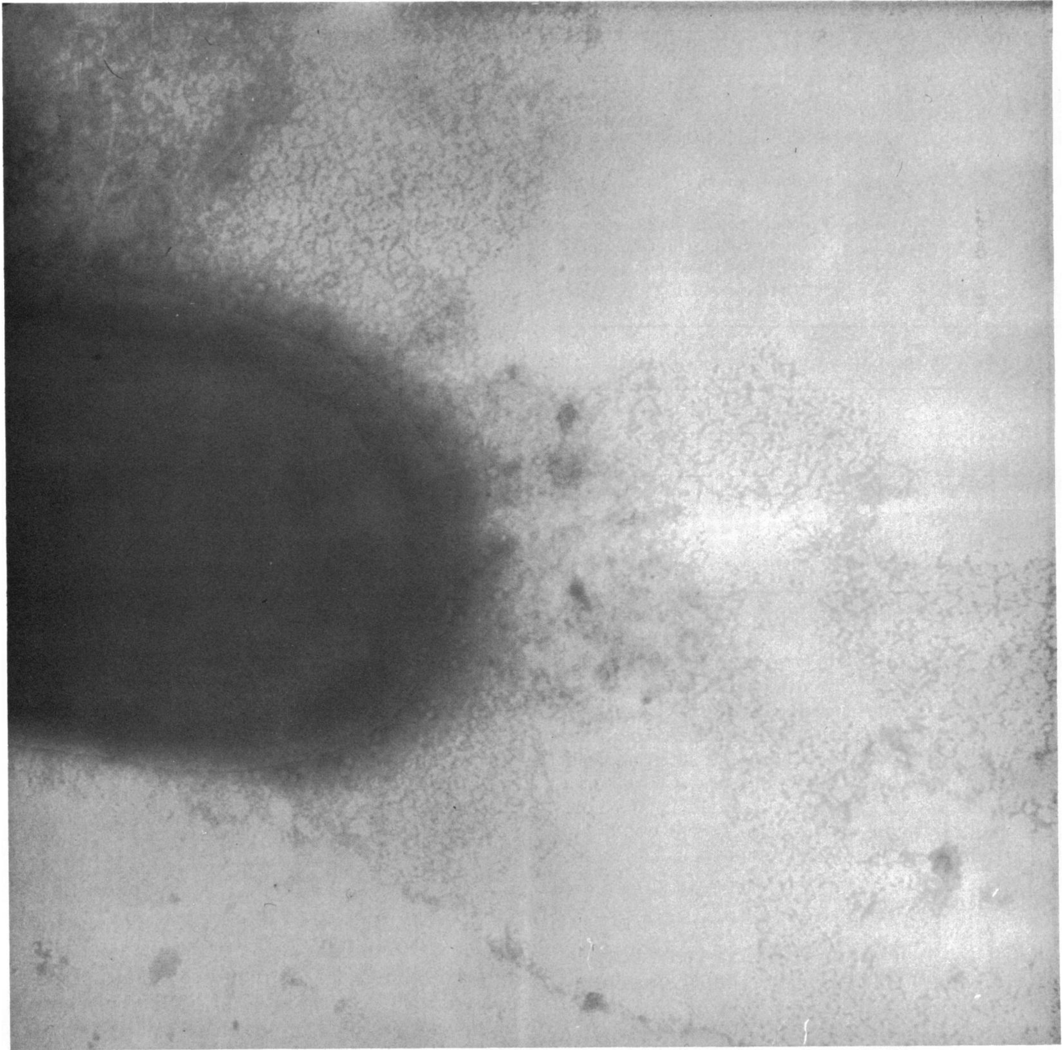

FIG. 1. *Electron micrograph of uninduced JK16 (Col Ia). Grids were prepared as in Materials and Methods.*

## DISCUSSION

As described here, electron microscope examination of mitomycin C-induced cells of strains colicinogenic for colicins Ia and Ib exhibit particles on the cell surface which are lacking in either non-colicinogenic or uninduced colicinogenic strains. Examination of mitomycin C-induced colicinogenic strain JK4 which lacks the specific I colicin receptor does not exhibit such cell-bound particles. Furthermore, unlike the colicinogenic parent, the cell-free medium of this receptorless mutant contains particles of approximately the same size as those found on the surface of wild-type colicinogenic cells. The simplest interpretation of these results is that upon induction, some synthesized colicin becomes associated with the I-specific receptor which is on the cell surface. The fact that after induction the receptorless mutant contains 7.5-fold more colicin activity in the cell medium than does the wild-type parent strain supports this interpretation. These results do not argue that a colicin molecule associated with a particular receptor was synthesized in that same cell. It is possible that the 65% of the total cell population showing bound structures have not produced any colicin, but have merely scavenged colicin molecules secreted into the medium by a minority of the bacterial population.

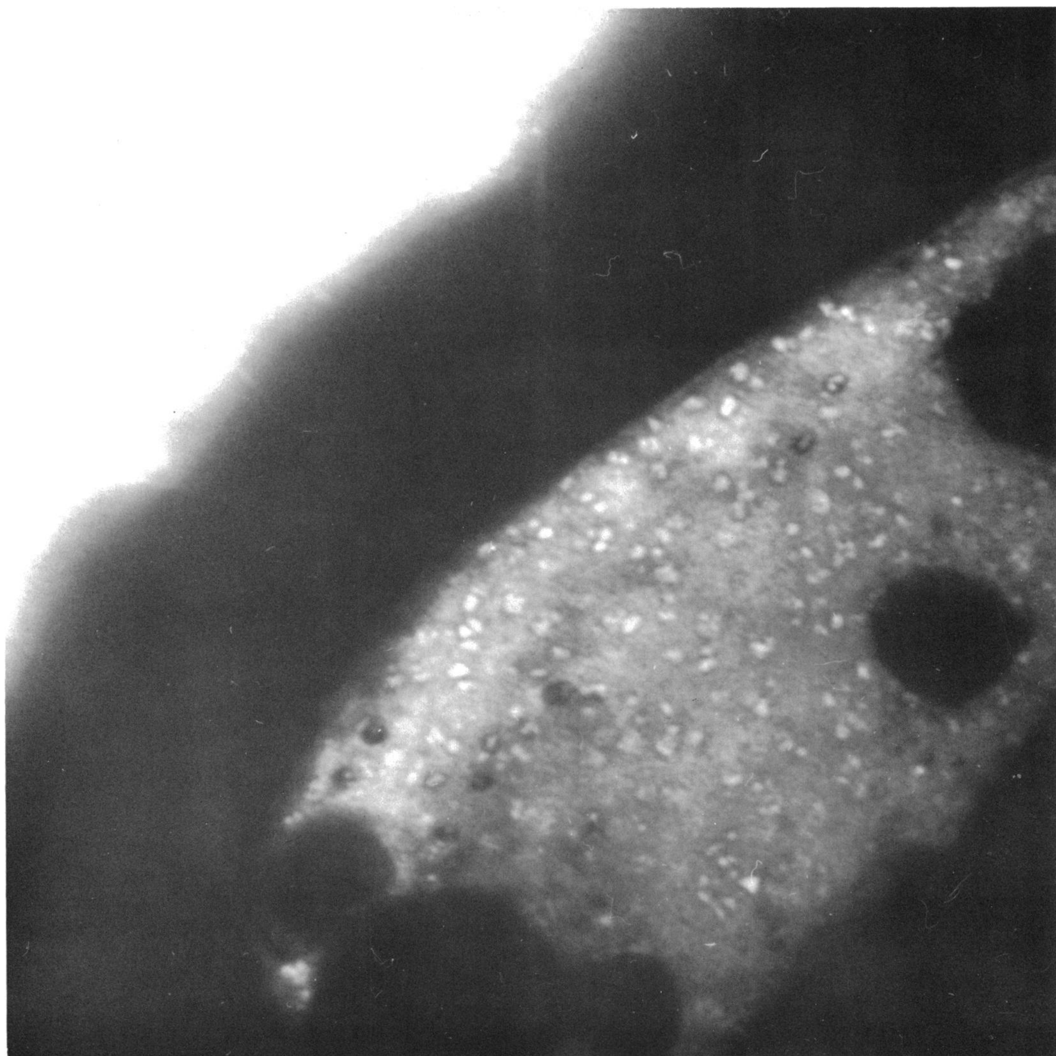

FIG. 2. *Electron micrograph of induced strain JK16 (Col Ia). Cells were induced for 16 hr with mitomycin C (0.2 μg per ml) before preparation of grids.*

Purification of E colicins was greatly simplified by the finding that some of the cell-bound colicin can be extracted by treatment of mitomycin C-induced cells with a 1 M NaCl solution (1, 11). This is in contrast to the situation with colicin I-producing cells, which are refractory to such extraction (5). This suggests that the state of induced molecules is different for I colicins and E colicins. However, it does not rule out the possibility that the E colicins become receptor associated after synthesis. There is no a priori reason for expecting that the interactions between the E or I colicins and their respective cognate receptors would be sensitive to the same dissociative agents. Furthermore, it is possible that although salt treatment does lead to colicin I extraction, colicin I may not be released in an active form. For example, colicin I may be inactivated upon adsorption, whereas colicin E2 is not. Since the I colicins are stable in 1 M NaCl, the possibility that NaCl extracts, yet inactivates, the I colicins would seem to be ruled out. From the purification data for colicins E2 and E3 (1), one can calculate that, on the average, each cell makes 2 to 3 $\times 10^5$ colicin molecules. Of the total colicin produced, 10% is found free in the medium, 36% can be extracted with salt, and 54% ($1.5 \times 10^5$ molecules) remains associated with cells in a state refractory to salt extraction.

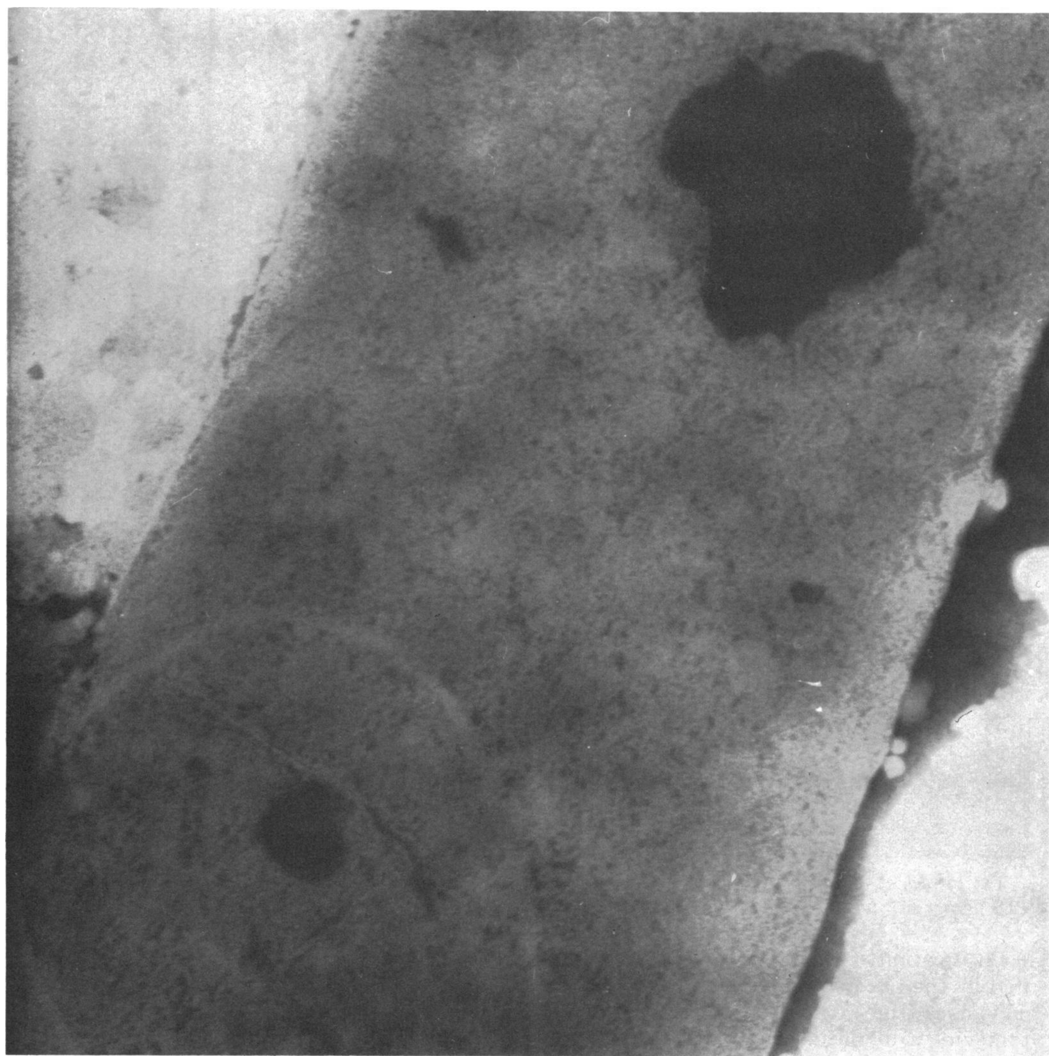

FIG. 3. *Electron micrograph of uninduced strain JK20 (Col Ib). Procedure was the same as in Fig. 1.*

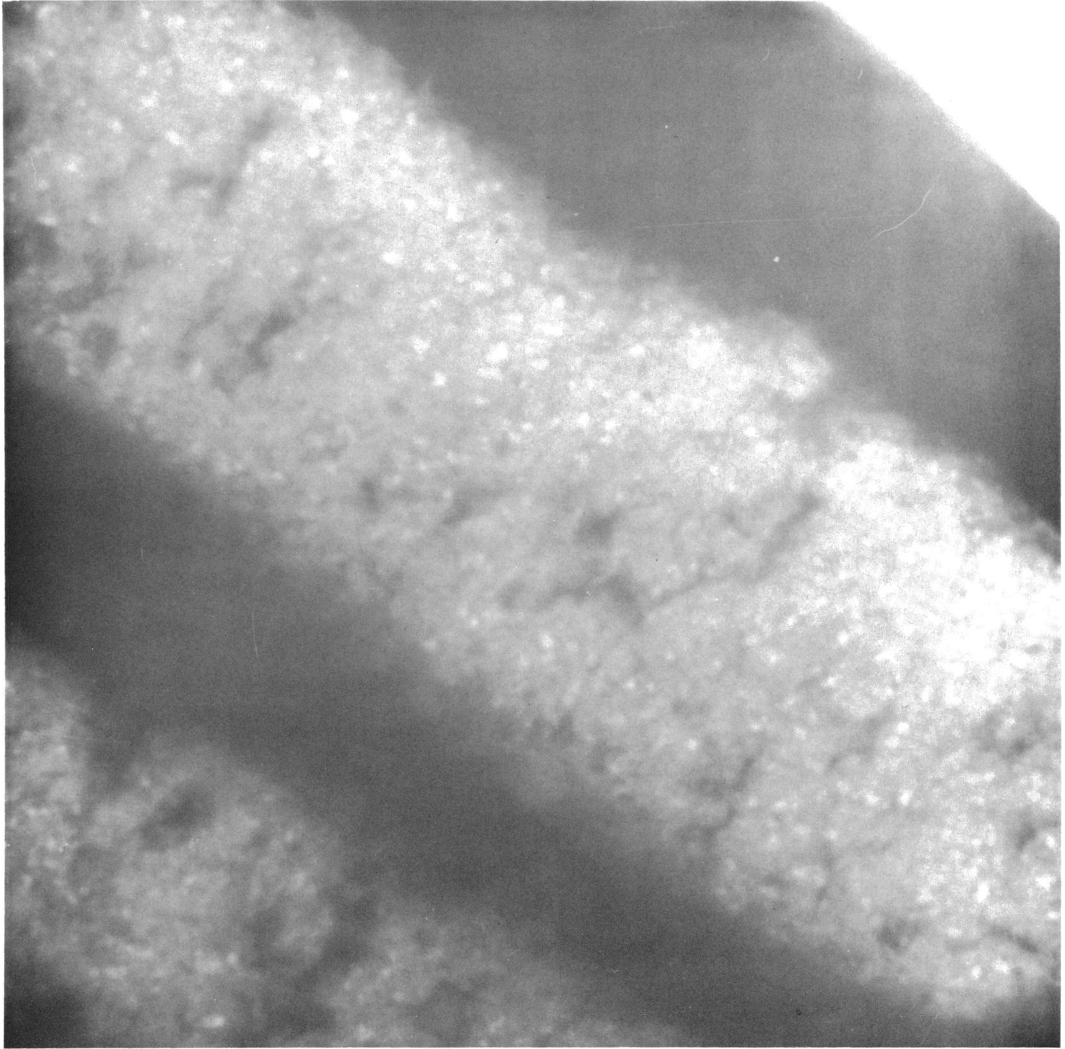

Fig. 4. *Electron micrograph of induced strain JK20 (Col Ib). Procedure was the same as in Fig. 2.*

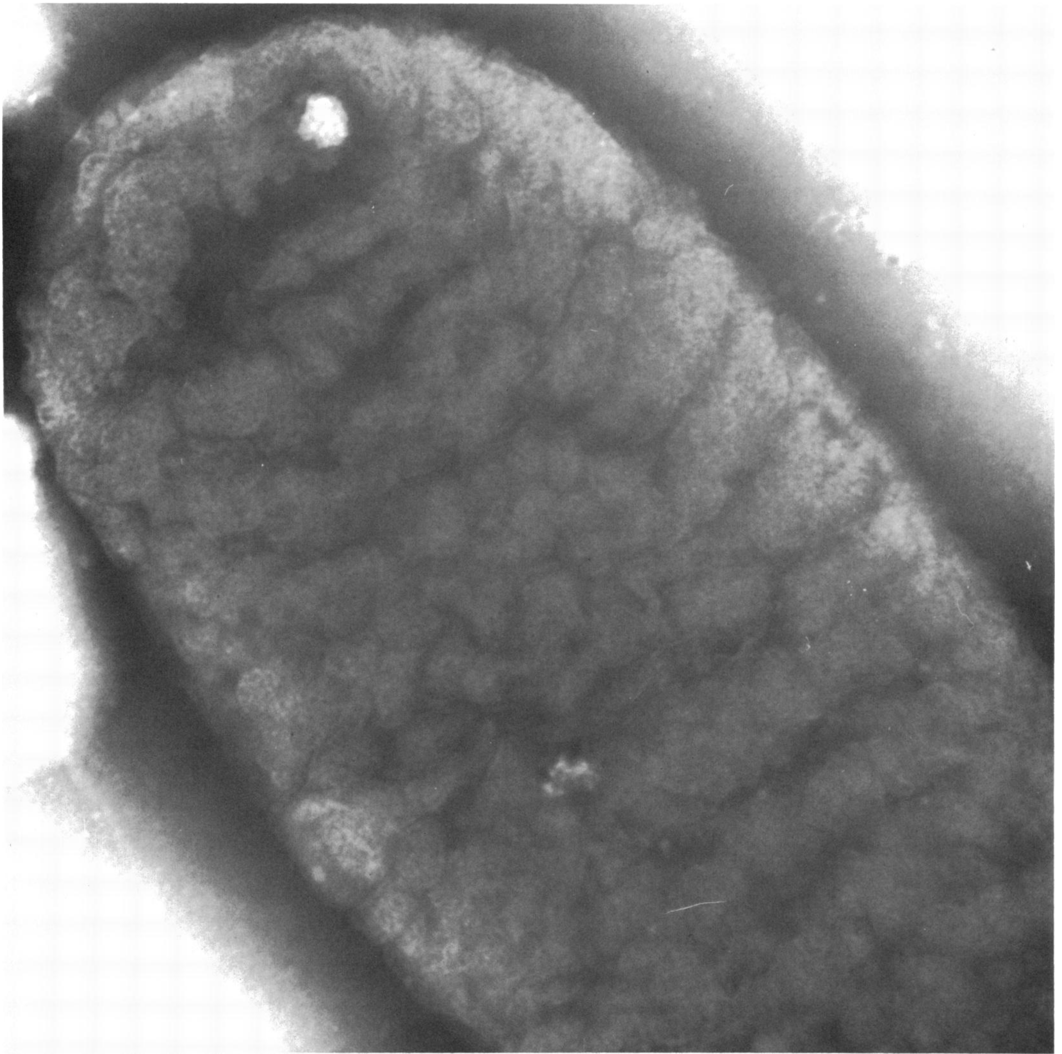

FIG. 5. *Electron micrograph of mitomycin C-treated strain JK1. Procedure was the same as in Fig. 2.*

Colicin I production yields approximately $10^4$ molecules per cell, of which 95% is cell associated and less than 3% is extracted by salt (5). Since the number of E receptors is probably in the order of 3,000 (7), only 1% of the total colicin synthesized may become receptor associated. The remaining colicin could then exist free in the medium, adsorbed to the cell surface in a salt-extractable state or cell-bound in a nonextractable state, but not bound to receptors. Colicin I, on the other hand, might be made in such quantities that binding is primarily to receptors and not to sites from which it can be removed by salt treatment. Strain JK16 (Col Ia) and JK20 (Col Ib) each have approximately 5,000 to 30,000 colicin I receptor sites (4; Konisky, *unpublished results*). In the case of strain JK4 (Col Ib) I-r, approximately 25% of the total colicin activity (cell medium plus lysed cells) is released into the medium. As described above, the surface of such cells does not exhibit colicin-like structures.

The studies presented here lead to the suggestion that it might be possible to observe colicin adsorbed to sensitive cells (strain JK1) by direct electron microscope examination. However, such attempts have been unsuccessful. This suggests that the cell receptor interaction between sensitive cells and added colicin may not be strictly analogous to the situation of

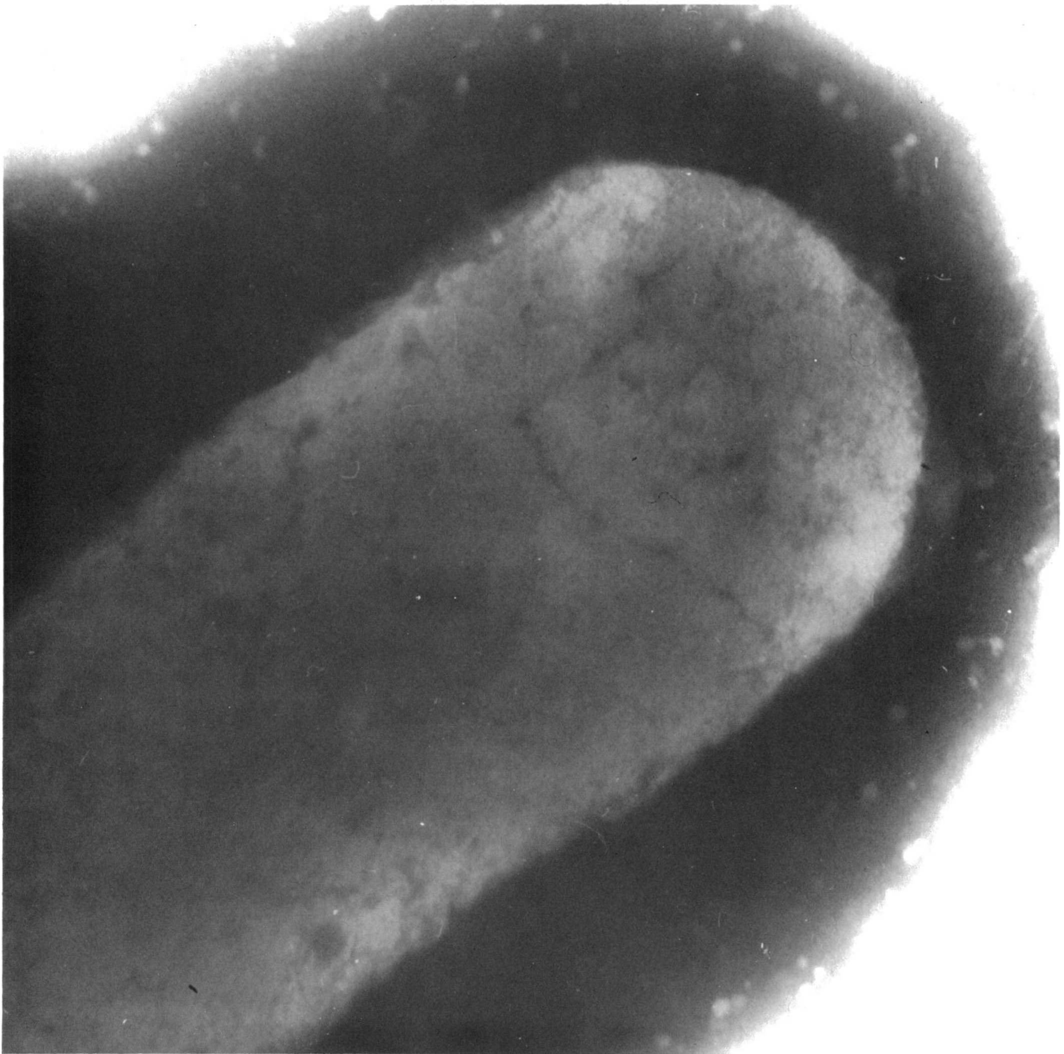

FIG. 6. *Electron micrograph of induced strain JK4 (Col Ib) I-r. induction was as described in Fig. 2.*

colicin molecules synthesized after induction. The possibility that added colicin has penetrated into the cell seems unlikely, since treatment of cells with radioactive colicin I followed by trypsin treatment releases approximately 75% of the bound radioactivity in the form of trichloroacetic acid-soluble counts (data not shown). An understanding of this interaction must await further study.

#### ACKNOWLEDGMENTS

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