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Effects of Chlorpromazine on the Cell Envelope Proteins of *Escherichia coli*

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Chlorpromazine (CPZ), at a concentration of 60 μg/ml of medium completely inhibited the replication of *Escherichia coli*. At concentrations below this MIC, CPZ caused transient induction of filamentation, such that by the end of 5 h, all of the cells were filaments, and by the end of 24 h, only rod-shaped *E. coli* were present. The reversion to normal morphology in the presence of CPZ was not due to either the degradation of CPZ or the selection of CPZ-resistant mutants. The electrophoretic pattern of proteins extracted from isolated cell envelopes of CPZ-induced filaments as well as from *E. coli* that reverted to normal morphology was distinctly different from that of the controls.

The antibacterial activity of the phenothiazine chlorpromazine (CPZ), a compound employed for the treatment of psychosis, was first described in 1959 (6) and confirmed in 1961 (3). Recent studies have demonstrated that CPZ has activity against a wide gamut of bacteria (4, 5). The mechanism by which CPZ exerts its antibacterial activity is not yet understood, and therefore, the studies to be described were undertaken.

Escherichia coli ATCC 25922 was grown in Trypticase soy broth overnight to an optical density of 1.500 U at a wavelength of 578 nM. From this culture, approximately 100,000 CFU were seeded into tubes containing Trypticase soy broth and various concentrations of CPZ that ranged from 0 to 100 μg/ml of medium. The cultures were incubated at 37°C and optically scanned at the end of various intervals. At the end of these intervals, aliquots (0.1 ml) were removed for microscopic examination. By the end of 3 h of incubation, the cultures containing concentrations of CPZ below 60 and greater than 40 μg/ml caused the filamentation of *E. coli*. By the end of 5 h, essentially all of the organisms were filaments exceeding 20 μm in length. By the end of 8 h, the culture showed a mixture of rods and filaments; by the end of 24 h, all organisms were rods.

The determination of the effects of CPZ on the growth of *E. coli* during those intervals during which filaments exist was determined by the use of the thymidine incorporation method (2).

Aliquots (1 ml) from duplicate cultures taken at the intervals described were transferred to tubes containing 0.1 μCi of tritiated thymidine with a specific activity of 23 Ci/μm (New England Corporation) and incubated for an additional 15 min at 37°C. The cultures were terminated with the addition of 10% trichloroacetic acid, the precipitates containing incorporated thymidine were collected with the aid of filters (Millipore; pore size, 0.47 μm), and the filters were washed three times with 10 ml volumes of trichloroacetic acid. The filters were air-dried and placed into scintillation vials containing scintillant. The radioactivity present on the filters was determined with the aid of a Beckman 100 C scintillation counter. The effects of CPZ on the incorporation of tritiated thymidine are depicted in Fig. 1. Briefly, concentrations of CPZ higher than 40 μg/ml of medium yielded inhibitions during the first 3 h of culture. The inhibitions noted increased in magnitude with time. However, by the end of 24 h, only concentrations of 60 μg/ml or greater completely inhibited the incorporation of thymidine. Optical examination of parallel cultures at the end of 24 h (valid because all of the organisms present were rod shaped) indicated that at a concentration of 60 μg/ml of medium, the optical density noted was comparable to that at the beginning of the incubation. Therefore, this concentration was taken as the MIC.

The question of whether the reversion to normal morphology, noted at the end of 24 h, was due to the alteration of the CPZ in the medium, by metabolic or other means, was examined by the use of thin-layer chromatography of CPZ extracted from the medium after 24 h of incubation. The chromatograph indicated no change in the recovered CPZ, thus indicating that reversion to normal morphology was not the result of degradation of CPZ to an ineffective compound.

The reversion of *E. coli* to normal morphology was not due to the selection for CPZ-resistant mutants, because subculturing of the reverted cells in drug-free medium and subsequent culturing in CPZ at 40 μg/ml induced *E. coli* to become filaments within 5 h. The effects of CPZ on the

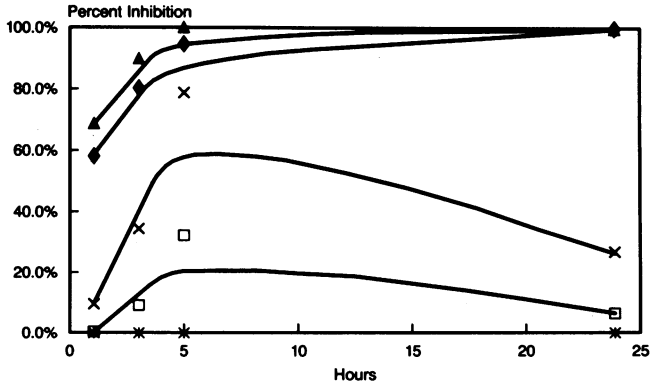

FIG. 1. Effects of CPZ concentration on incorporation of tritiated thymidine by *E. coli*. Symbols (CPZ, in micrograms per milliliter): ●, 10; +, 20; ∗, 30; □, 40; ×, 50; ◆, 60; ▲, 70.

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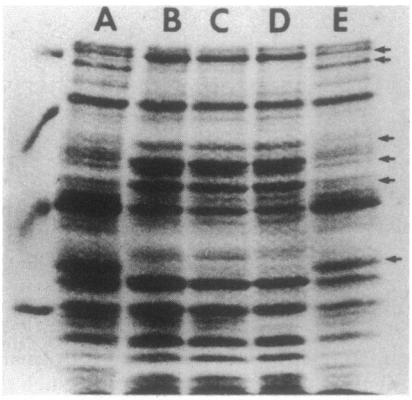

FIG. 2. Electrophoretic patterns of proteins extracted from *E. coli*. Lanes: A, control, 100 μg of protein; B, 5 h in CPZ (only filaments), 100 μg of protein; C, 16 h in CPZ (rods and filaments), 100 μg of protein; D, 24 h in CPZ (rods), 100 μg of protein; E, control, 60 μg of protein. Arrows indicate bands that are different from those of controls.

proteins of the cell envelope of *E. coli* exposed to a subinhibitory concentration of the drug for various periods was studied via the use of acrylamide slab electrophoresis (1). *E. coli* was cultured in the presence and absence of CPZ at a concentration of 50 μg/ml of medium. Five 200-ml cultures were thus prepared, and at the end of 5, 16, and 24 h, the contents of each flask was centrifuged at 8,000 × *g* for 15 min and the pellets were washed with saline and recentrifuged. The pellets were resuspended in ice-cold phosphate-buffered saline, pH 7.4, and sonicated at 100 W for three 30-s bursts while being maintained in an ice bath.

The disrupted cells were centrifuged at 100,000 × *g* for 40 min, and the pellets were resuspended in ice-cold phosphate-buffered saline. The protein concentration was determined by the method of Lowry and adjusted to 10 mg/ml of buffer. Sonicated product (100 μl) from each of the cultures was extracted with 10 μl of 20% Sarkosyl. The Sarkosyl-extracted proteins were electrophoresed and stained as previously described (1). The electrophoretic patterns of Sarkosyl-extracted proteins from the cell envelopes of *E. coli* incubated with and without 50 μg of CPZ per ml of medium for various periods is depicted by Fig. 2. A number of electrophoretic bands that are significantly different from those of the controls are detected in the cell envelopes of the filaments of *E. coli* within 5 h of incubation with CPZ (see arrows).

These electrophoretic changes noted with cultures that exhibit only filaments persist for the remainder of the 24 h of incubation, even with cultures containing only rod-shaped cells. These results suggest that perhaps the alteration of these proteins is a response to the presence of a subinhibitory concentration of CPZ. The alteration of these proteins may in turn result in a decreased permeability to CPZ which allows the reversion of the organism to normal, rod-shaped morphology.

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