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# EFFECTS OF CHLORPROMAZINE ON CELL WALL BIOSYNTHESIS AND INCORPORATION OF OROTIC ACID INTO NUCLEIC ACIDS IN *BACILLUS MEGATERIUM**

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**Abstract**—The microorganism *Bacillus megaterium* was used as a model system for the study of the biochemical basis of action of chlorpromazine. At 33 μM, chlorpromazine (10 μg/ml) doubled the generation time of logarithmic phase cultures of *B. megaterium*. The effect of the drug on the incorporation of labeled precursors for macromolecules was compared at equivalent turbidities. Incorporation of $^{14}$C-orotic acid into RNA and DNA was immediately inhibited. In contrast, incorporation of $^{14}$C-adenine, $^{14}$C-formate and $^{14}$C-thymidine into nucleic acids was unaffected, indicating that nucleic acid synthesis was normal despite interference by the drug in the utilization of exogenous orotic acid.

Incorporation of $^{14}$C-L-lysine was unaffected, but chlorpromazine immediately inhibited the incorporation of $^{14}$-C-diaminopimelic acid into cell wall. Uridine nucleotide precursors (colorimetric assay) for glycopeptide polymer of cell wall accumulated promptly upon addition of the drug. Accumulation was dose dependent and reached eight times that of control cells. Other phenothiazines including trifluoperazine (3 μM), promazine (58 μM), promethazine (156 μM) and chlorpromazine sulfoxide (2 mM) also caused significant accumulation of cell wall precursors. Although the mechanism of action is unknown, it appears that chlorpromazine specifically interferes with cell wall synthesis in *B. megaterium*.

THE PSYCHOTROPIC drug chlorpromazine (CPZ) was introduced into widespread clinical use after the report of Delay *et al.*[1] on the effectiveness of the drug in the treatment of agitated psychotics. Numerous biochemical and pharmacological effects of the drug have been described[2,3] with special emphasis being given to effects on altered membrane permeability and ion transport.[4–6] However, the site and mechanisms by which CPZ exerts its psychotropic effect are unknown.[2,3]

Microorganisms including *Escherichia coli*,[7–10] *Lactobacillus plantarum*,[11] and *Tetrahymena pyriformis*[12–15] have been used as model systems to study the action of CPZ and related phenothiazine drugs. In a previous communication, we reported that CPZ, 0·10 mM, specifically inhibited the incorporation of $^{14}$C-diaminopimelic acid into wall of logarithmic phase cultures of *Bacillus cereus*. In contrast, when suitably labeled precursors were used, CPZ did not appear to affect the synthesis of RNA, DNA or protein. We concluded that CPZ might be specifically interfering with cell wall biosynthesis in *B. cereus*.[16] Examination of the effect of CPZ on accumulation of uridine nucleotide precursors of cell wall glycopeptide of *B. cereus* by a colorimetric method[17] revealed that the drug produced a small increase in the pool of these precursors in inhibited cells.† The extent of the accumulation of soluble cell wall pre-precursors produced by known inhibitors of cell wall synthesis varies among sensitive

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† P. Klubes, unpublished results.

bacterial species.[18,19] Therefore, we were prompted to examine the effect of CPZ on macromolecular synthesis in another bacterial species whose growth was inhibited by low concentrations of the drug.

This report describes the use of *Bacillus megaterium* as a model system for a study of the biochemical basis of action of CPZ and related phenothiazine drugs. In exponentially growing *B. megaterium* cells, CPZ specifically inhibited cell wall synthesis while RNA, DNA and protein synthesis were only depressed in accordance with decreased cell mass. In addition, the drug interfered with the incorporation of the pyrimidine precursor orotic acid-6-$^{14}$C into polynucleotides of *B. megaterium*. A preliminary report of this work has recently appeared.[20]

## MATERIALS AND METHODS

*Bacterial growth and media. Bacillus megaterium* JA, a laboratory strain derived from *Bacillus megaterium* ATCC 11478, was obtained from Dr. F. E. Hahn, Walter Reed Army Institute for Research, Washington, D.C. Bacteria were grown in a New Brunswick Gyrotory shaker at 37° in a medium of the following composition per liter: $K_2HPO_4$, 7 g; $KH_2PO_4$, 3 g; sodium citrate-2 $H_2O$, 0·47 g; $MgSO_4$-7 $H_2O$, 0·1 g; $(NH_4)_2SO_4$, 1 g; and D-glucose, 0·5 g; at pH 7·0. Growth was monitored turbidimetrically as extinction at 540 m$\mu$ ($E_{540}$) in a Beckman spectrophotometer model DU.

*Synthesis of cellular macromolecules.* Macromolecular biosynthesis was measured according to the membrane filtration method.[21,22] Radioactively labeled compounds were added to a suspension of log phase *B. megaterium* cells at $E_{540}$ 0·10. The suspension was then immediately divided into control and drug-treated cultures and chlorpromazine hydrochloride, freshly dissolved in water, was added to the latter culture. These supplementations had no effect on the pH of the bacterial medium. For most radioactive compounds, 0·01 to 0·1 $\mu$c was added per ml of medium. Cultures were sampled periodically by removing 2-ml aliquots, measuring turbidity, and mixing with 2 ml of 10% trichloroacetic acid or 1 N KOH prior to membrane (B-6 Bac-T-Flex, Schliecher and Schuell Co., Keene, N.H.) filtration. Radioactivity measurements were made in a Nuclear–Chicago windowless gas-flow counter.

*Accumulation of uridine nucleotides.* Measurements of accumulation of derivatives of uridine diphosphate acetyl muramic acids, which are cell wall precursors, was carried out as reported previously.[17] Samples of cultures, 100 or 250 ml, were harvested by centrifugation at 4°. The cells were washed by centrifugation in cold 0·9% NaCl and extracted with 1·5 ml water in a boiling water bath for 10 min. Acetylamino sugar-containing nucleotides in the hot water extract were measured colorimetrically using *N*-acetyl-D-glucosamine as standard.

*Phenothiazines.* The phenothiazine derivatives were kindly donated by the following manufactures: chlorpromazine hydrochloride, chlorpromazine sulfoxide hydrochloride and trifluoperazine dihydrochloride, Smith, Kline & French Laboratories, Philadelphia, Pa.; promethazine hydrochloride and promazine hydrochloride, Wyeth Laboratories, Philadelphia, Pa. Solutions of each drug, in water, were prepared immediately before use.

*Chemicals.* Adenine-8-$^{14}$C, guanine-8-$^{14}$C, orotic acid-6-$^{14}$C, uracil-2-$^{14}$C, thymidine-2-$^{14}$C, and sodium formate-$^{14}$C were obtained from New England Nuclear,

Boston, Mass. Diaminopimelic acid-1,7-$^{14}$C was obtained from International Chemical & Nuclear Corp., Irvine, Calif., and uniformly labeled L-lysine-$^{14}$C from Nuclear-Chicago, Chicago, Ill. D-Cycloserine was obtained from Sigma Chemical Co., St. Louis, Mo., and *N*-acetyl-D-glucosamine from Mann Research Laboratory, New York, N.Y. Other chemicals were from commercial sources.

## RESULTS

*Effect on growth.* The addition to exponentially growing cultures of *B. megaterium* at $E_{540}$ 0·10 of CPZ at 25 and 33 $\mu$M (10 $\mu$g/ml, final concentration) resulted in an instantaneous retardation of growth (Fig. 1). As can be seen, 33 $\mu$M CPZ, produced an approximate doubling in the generation time.

*Effect on macromolecular synthesis.* In an attempt to localize the site of action of CPZ, the effect of the drug on macromolecular synthesis was examined by the membrane filtration method.[21,22] Incorporation was plotted in relation to increased turbidity, as this method detected differences produced by the drug rather than those due to delayed growth.

*Protein synthesis.* At a concentration of 33 $\mu$M, CPZ did not specifically inhibit the incorporation of $^{14}$C-L-lysine into protein of *B. megaterium* (Fig. 2), indicating that the drug was not specifically affecting protein biosynthesis.

*RNA synthesis.* The incorporation of $^{14}$C-adenine into RNA was unaffected by 25 and 33 $\mu$M CPZ (Fig. 3). Although not shown, similar results were obtained with $^{14}$C-guanine and $^{14}$C-uracil. Their incorporation into RNA was considered to be unaffected by 25 and 33 $\mu$M CPZ, since when incorporation into RNA (cpm) was plotted against change in cell mass ($\Delta$ O.D.$_{540}$), the points obtained from control and drug-treated cells fell along the same line. In contrast, the incorporation of $^{14}$C-orotic

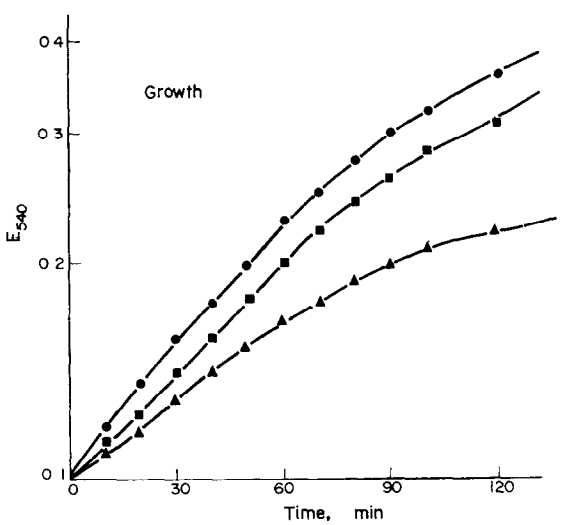


FIG. 1. Effect of chlorpromazine on growth of *B. megaterium*. Growth was measured turbidimetrically in a Beckman model DU spectrophotometer. The extinction at 540 m$\mu$ ($E_{540}$) was plotted against time. Chlorpromazine was added at time 0 at $E_{540}$ 0·10 at concentrations: ●—●, 0; ■—■, 25 $\mu$M; ▲—▲, 33 $\mu$M (10 $\mu$g/ml).

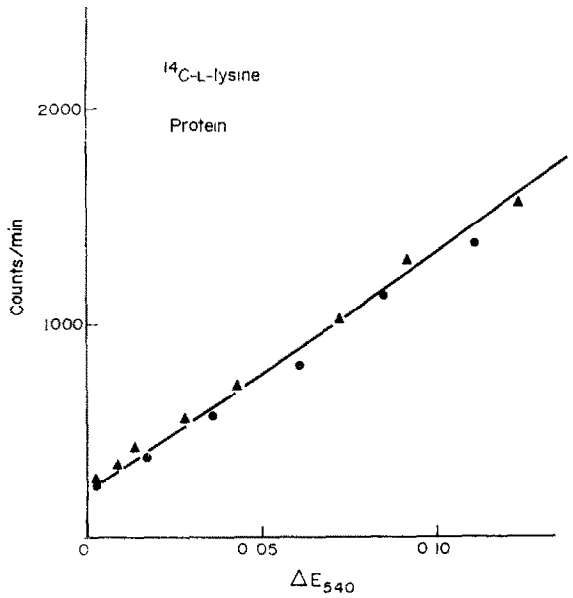


FIG. 2. Effect of chlorpromazine on incorporation of uniformly labelled L-lysine-$^{14}$C into protein. Cells were grown in the presence and absence of drug and comparisons were made for similar increases in bacterial turbidity ($E_{540}$). Incorporation is represented as total counts per minute in trichloroacetic acid-washed cells present in 2 ml bacterial suspension. Concentrations of drug: ●—●, 0; ▲—▲, 33 $\mu$M.

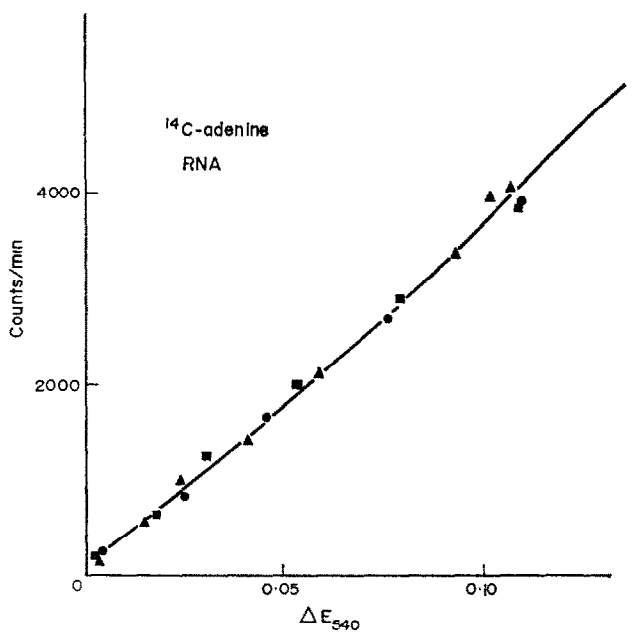


FIG. 3. Effect of chlorpromazine on incorporation of adenine-8-$^{14}$C into RNA. Concentrations of drug: ●—●, 0; ■—■, 25 $\mu$M; ▲—▲, 33 $\mu$M.

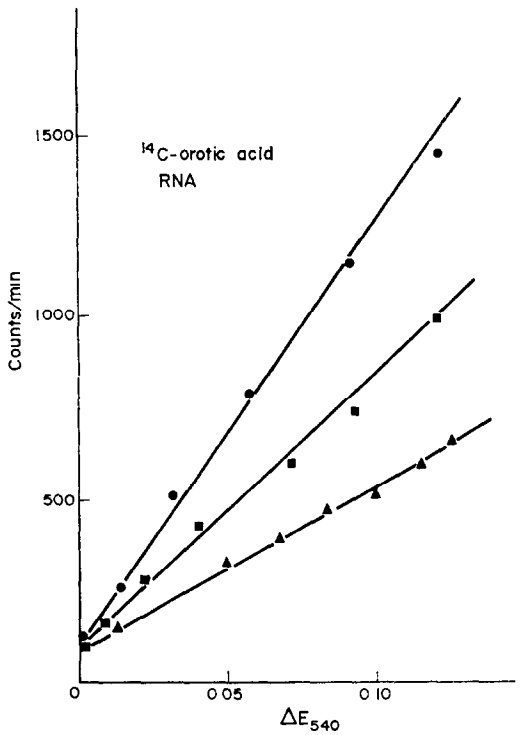


FIG. 4. Effect of chlorpromazine on incorporation of orotic acid-6-$^{14}$C into RNA. Concentrations of drug: ●—●, 0; ■—■, 25 $\mu$M; ▲—▲, 33 $\mu$M.

acid into RNA was immediately depressed in a dose-dependent manner by 25 and 33 $\mu$M CPZ (Fig. 4). Furthermore, the incorporation of $^{14}$C-formate into hot trichloroacetic acid-soluble material (RNA plus DNA) was not inhibited by 33 $\mu$M CPZ (Fig. 5). This indicated that CPZ was not specifically inhibiting *de novo* synthesis of RNA or DNA. Therefore RNA synthesis, as measured by the incorporation of $^{14}$C-adenine, $^{14}$C-formate, $^{14}$C-guanine and $^{14}$C-uracil, was not specifically depressed by CPZ, while at the same time the drug interfered in some manner, with the incorporation of exogenous $^{14}$C-orotic acid into RNA.

*DNA synthesis.* DNA synthesis was followed by measuring the incorporation of $^{14}$C-thymidine into cold trichloroacetic acid-washed cells. As can be seen, 33 $\mu$M CPZ had no effect on the incorporation of $^{14}$C-thymidine into DNA (Fig. 6). This indicated that the drug did not specifically inhibit DNA synthesis. Although not shown, similar results were obtained with $^{14}$C-adenine, $^{14}$C-guanine and $^{14}$C-uracil in that the incorporation of these isotopically labeled bases into KOH-insoluble material was not affected by 25 and 33 $\mu$M CPZ. This procedure normally measures incorporation of purines and pyrimidines into DNA exclusively.[21,22] When incorporation of each of these bases into KOH-insoluble material (cpm) was plotted against change in cell mass ($\Delta$ O.D.$_{540}$) the points obtained from control and drug-treated cells fell along the same line. In contrast, the incorporation of $^{14}$C-orotic acid into DNA, as measured by incorporation into KOH-insoluble material, was depressed by 25 and 33 $\mu$M CPZ (Fig. 7). Thus DNA synthesis was not specifically depressed by CPZ,

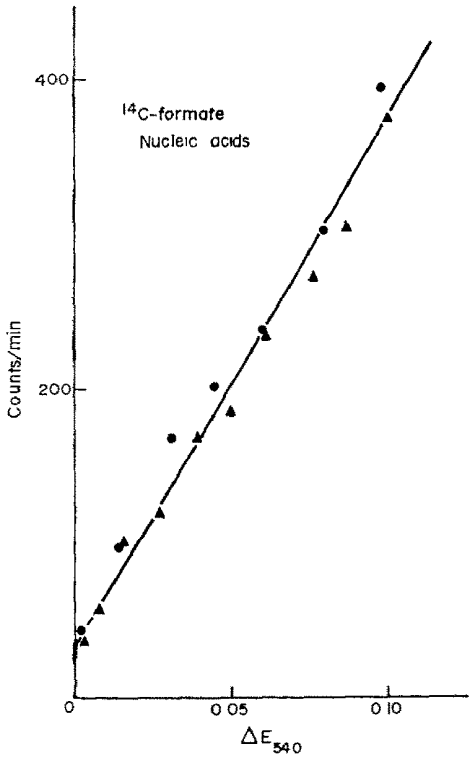


FIG. 5. Effect of chlorpromazine on incorporation of formate-$^{14}$C into hot trichloroacetic acid-soluble material (RNA plus DNA). Concentrations of drug: ●—●, 0; ▲—▲, 33 $\mu$M.

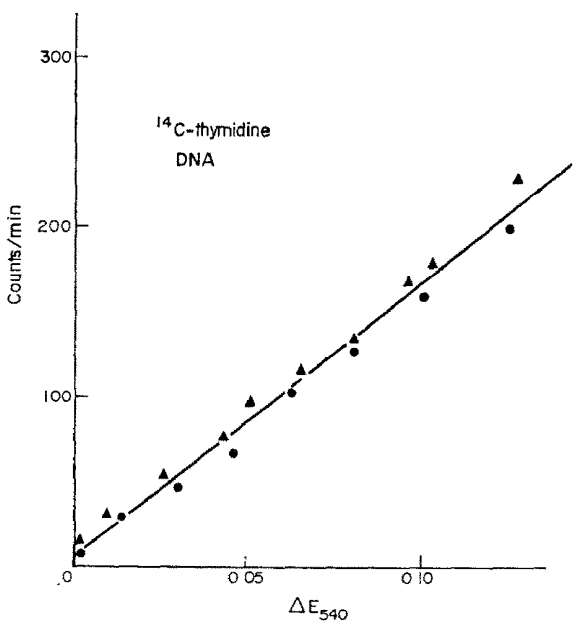


FIG. 6. Effect of chlorpromazine on incorporation of thymidine-2-$^{14}$C into DNA. Concentrations of drug: ●—●, 0; ▲—▲, 33 $\mu$M.

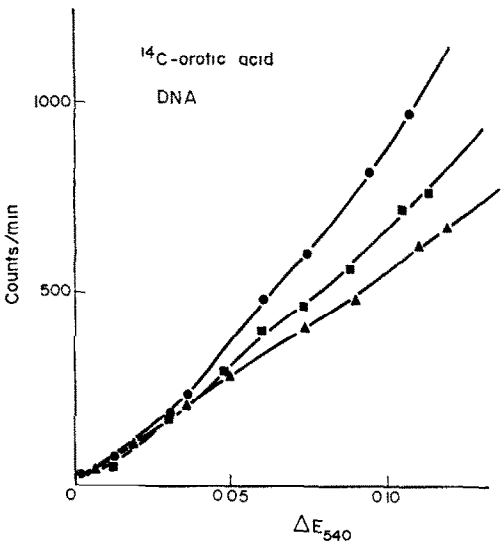


FIG. 7. Effect of chlorpromazine on incorporation of orotic acid-6-$^{14}$C into DNA. Concentrations of drug: ●—●, 0; ■—■, 25 $\mu$M; ▲—▲, 33 $\mu$M.

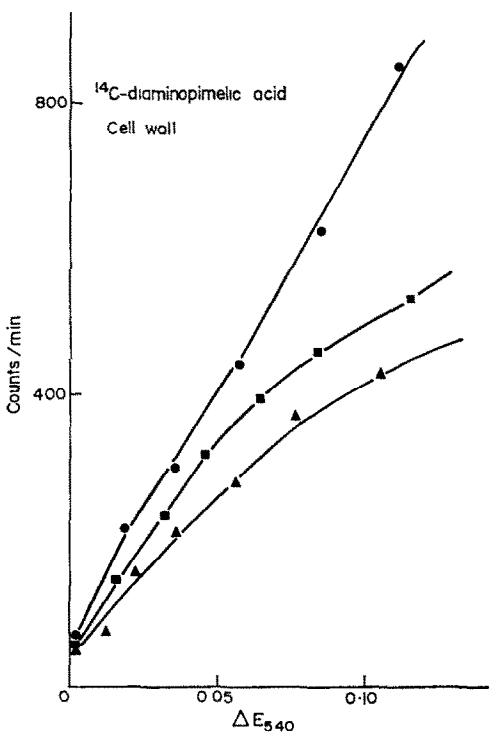


FIG. 8. Effect of chlorpromazine on incorporation of diaminopimelic acid-1,7-$^{14}$C into cell wall. Concentrations of drug: ●—●, 0; ■—■, 25 $\mu$M; ▲—▲, 33 $\mu$M.

although the drug selectively interfered with the utilization of exogenous $^{14}$C-orotic acid for DNA synthesis.

*Cell wall formation.* At concentrations of 25 and 33 $\mu$M, CPZ inhibited the incorporation of $^{14}$C-diaminopimelic acid, a bacterial cell wall precursor, into trichloroacetic acid-washed cells (Fig. 8). Therefore it was possible that the drug might be interfering with bacterial cell wall synthesis. Drugs which inhibit bacterial cell wall synthesis, including D-cycloserine and penicillin, often produce an increase in the intracellular accumulation of uridine-5′-pyrophosphate *N*-acetylamino sugar nucleotides.[23–26] Therefore, we examined colorimetrically[17] the effect of CPZ on the accumulation of uridine nucleotide cell wall precursors in *B. megaterium*.

TABLE 1. COMPARATIVE EFFECTS OF CHLORPROMAZINE AND D-CYCLOSERINE ON ACCUMULATION OF URIDINE DIPHOSPHATE ACETYLAMINO SUGAR COMPOUNDS IN *B. MEGATERIUM**

| | *N*-acetylamino sugar ($\mu$moles/l. of culture at $E_{540}$ 0·20) |
|---|---|
| Control | 1·14 |
| Chlorpromazine (33 $\mu$M) | 6·92 |
| D-Cycloserine (2·5 $\mu$M) | 11·5 |

* A log phase culture of *B. megaterium* at $E_{540}$ 0·10 was divided into control and two drug-treated portions. When a culture reached $E_{540}$ 0·20, a 250-ml aliquot was harvested by centrifugation at 4° and *N*-acetylamino sugar compounds were measured colorimetrically as described under Methods.

Table 1 is a representative experiment which shows the accumulation of uridine nucleotides in *B. megaterium* cells grown from $E_{540}$ 0·10 to 0·20 in the presence of 33 $\mu$M CPZ. For comparison, the accumulation produced by 2·5 $\mu$M D-cycloserine is also shown. This concentration of D-cycloserine produced growth inhibition comparable to that of 33 $\mu$M CPZ. When washed cells of *B. megaterium* were resuspended in medium which lacked D-glucose and citrate, and therefore could not support growth, CPZ at 33 $\mu$M failed to produce an accumulation of uridine nucleotide precursors.

*Relationship between chlorpromazine concentration and accumulation of uridine nucleotides.* As can be seen (Table 2), when *B. megaterium* was grown in the presence of varying concentrations of CPZ, the accumulation of uridine nucleotides was found to be dose dependent. At concentrations of CPZ which did not inhibit cell growth, there was no accumulation of uridine nucleotides.

*Accumulation of uridine nucleotides as a function of increasing cell mass.* Figure 9 shows the accumulation of uridine nucleotides produced by 33 $\mu$M CPZ as a function of increasing cell mass. As can be seen, the drug-treated cells showed an immediate and progressive increase in uridine nucleotide content which began to level off at $E_{540}$ 0·175. Although not apparent from this figure, the time for half-maximal and maximal accumulation of nucleotide was of the order of 15 and 90 min respectively.

TABLE 2. EFFECT OF CONCENTRATION OF CHLORPROMAZINE ON ACCUMULATION OF URIDINE DIPHOSPHATE ACETYLAMINO SUGAR COMPOUNDS IN *B. MEGATERIUM**

| | *N*-acetylamino sugar (μmoles/l. of culture at $E_{540}$ 0·15) |
|---|---|
| Control | 1·20 |
| Chlorpromazine (17 μM) | 2·16 |
| Chlorpromazine (25 μM) | 3·82 |
| Chlorpromazine (33 μM) | 4·80 |

* A log phase culture of *B. megaterium* at $E_{540}$ 0·10 was divided into control and three drug-treated portions. When a culture reached $E_{540}$ 0·15, a 250-ml aliquot was harvested by centrifugation at 4° and *N*-acetylamino sugar compounds were measured colorimetrically as described under Methods.

*Accumulation of uridine nucleotides produced by other phenothiazines.* Since CPZ had produced an accumulation of uridine nucleotide cell wall precursors in *B. megaterium*, it was of interest to see if other pharmacologically active phenothiazine derivatives could produce a similar accumulation. The concentration of each drug was chosen which caused an approximate doubling in the generation time compared to control cells. The accumulation of nucleotide cell wall precursors was determined by colorimetric assay.[17] As can be seen (Table 3), each of the phenothiazine derivatives tested produced an accumulation of uridine nucleotides in log phase cultures of *B. megaterium* comparable to that seen with 33 μM CPZ.

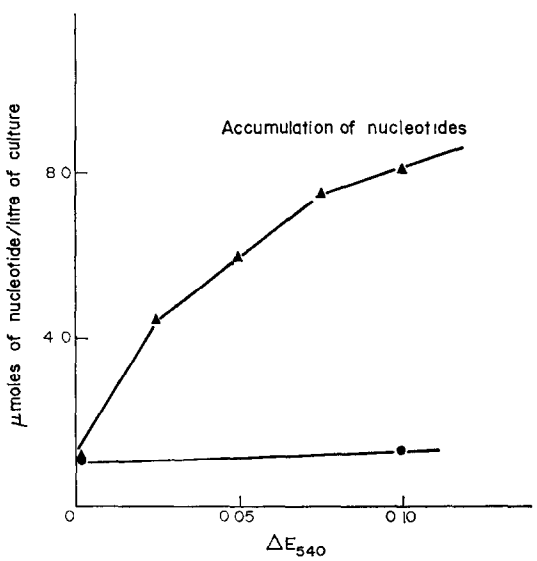


FIG. 9. Effect of chlorpromazine on accumulation of uridine diphosphate acetylamino sugar compounds. A log phase culture of *B. megaterium* at $E_{540}$ 0·10 was divided into control and drug-treated portions. At the indicated $E_{540}$ ,100-ml aliquots of control and drug-treated cultures were harvested by centrifugation at 4° and *N*-acetylamino sugar compounds measured colorimetrically as described under Methods. Concentrations of drug: ●—●, 0; ▲—▲, 33 μM.

TABLE 3. EFFECTS OF PHENOTHIAZINE DERIVATIVES ON ACCUMULATION OF URIDINE DIPHOSPHATE ACETYLAMINO SUGAR COMPOUNDS IN *B. MEGATERIUM**

| Experiment | *N*-acetylamino sugar ($\mu$moles/l. of culture at $E_{540}$ 0·15) |
|---|---|
| 1. Control | 0·96 |
| Chlorpromazine (33 $\mu$M) | 5·16 |
| 2. Control | 0·96 |
| Promazine (58 $\mu$M) | 3·30 |
| 3. Control | 1·02 |
| Chlorpromazine sulfoxide (2 mM) | 4·60 |
| 4. Control | 1·02 |
| Promethazine (156 $\mu$M) | 5·88 |
| 5. Control | 0·96 |
| Trifluoperazine (7 $\mu$M) | 3·12 |

* In each experiment, a log phase culture of *B. megaterium* at $E_{540}$ 0·100 was divided into control and drug-treated portions. When a culture reached $E_{540}$ 0·15, a 250-ml aliquot was harvested by centrifugation at 4° and *N*-acetylamino sugar compounds were measured colorimetrically as described under Methods.

## DISCUSSION

The inhibition of incorporation of $^{14}$C-diaminopimelic acid into cell wall as well as the accumulation of soluble cell wall precursors, measured by colorimetric assay, occurred shortly after addition of CPZ to log phase cultures of *B. megaterium* and at concentrations which are similar to the minimal growth inhibitory concentrations of the drug. Other isotope experiments demonstrated that the effect of CPZ was specifically restricted to interference with cell wall synthesis, since RNA, DNA and protein synthesis were only depressed in accordance with cell mass.

Trifluoperazine, CPZ and promazine at concentrations of 7, 33 and 58 $\mu$M, respectively, which caused an approximate doubling of the generation time of log phase *B. megaterium* cells, produced a significant accumulation of uridine nucleotide precursors of peptidoglycan. These concentrations correlate with the relative potencies of trifluoperazine, CPZ and promazine when these drugs are used as antipsychotic agents.[27]

The antihistaminic phenothiazine derivative, promethazine, which has a very low potency as an antipsychotic agent,[27] also produced an accumulation of uridine nucleotide cell wall precursors at a concentration of 156 $\mu$M, which was similar to the minimal growth inhibitory concentration of the drug.

Chlorpromazine sulfoxide, a relatively inactive metabolite of CPZ,[27] was the least potent phenothiazine derivative tested, since it required a concentration of 2 mM to cause a doubling of the generation time of *B. megaterium* and bring about an accumulation of uridine nucleotides. Apparently the ability of phenothiazine derivatives to produce an accumulation of uridine nucleotides in log phase cultures of *B. megaterium* is not restricted to those compounds which are clinically effective antipsychotic agents.

To our knowledge, there are no reports in the literature of effects of CPZ and related phenothiazines on bacterial cell wall biosynthesis. We found only one communication in which it was suggested, on the basis of changes in cell shape and staining properties, that CPZ may produce alterations in bacterial cell wall.[28] In addition, the lytic action of CPZ on *Escherichia coli* in the lag and early log phase of growth has been described; however, the effect was not believed to be the result of a direct action of the drug on bacterial cell wall biosynthesis.[8]

The mechanism by which CPZ interferes with cell wall biosynthesis in *B. megaterium* is unknown. CPZ has been shown to alter lipid and phospholipid biosynthesis in various systems.[3,15,29] A role for a phospholipid carrier in cell wall biosynthesis has been demonstrated.[30] Conceivably the action of CPZ on cell wall synthesis in *B. megaterium* may be due to interference by the drug with the biosynthesis of the phospholipid carrier which is essential for normal cell wall synthesis.

Alternatively, it has been shown that those steps in the synthesis of peptidoglycan which involve the phospholipid cycle can be inhibited by a number of antibiotics including novobiocin, ristocetin, vancomycin and bacitracin. Solvents and detergents including *n*-butyl alcohol, *n*-octyl alcohol, deoxycholate, sodium lauryl sulfate, and Tween 80 had similar effects.[30] CPZ has been shown to lower surface tension; chlorpromazine sulfoxide is much less active in this respect.[8,31,32] Promazine, promethazine and trifluoperazine are also surface active agents.[31] The ability of a number of psychoactive drugs including CPZ to penetrate lipid monolayers has led to the suggestion that these compounds may become bound to the lipids of natural membranes.[33] A study of the effect of CPZ on glucose metabolism in *E. coli* cells suggested that one of the actions of the drug was to exert a surface-active effect on the bacterial cell wall and membrane.[10] Therefore, it is possible that the action of CPZ on cell wall synthesis in *B. megaterium* is due to the surface-active properties of CPZ, which may result in interference with one or more steps in the phospholipid cycle of peptidoglycan synthesis.

Effects of CPZ on membrane permeability have been described.[4–6] Therefore, the possibility that the effect of CPZ on cell wall synthesis may be a consequence of the action of the drug on the bacterial membrane cannot be excluded.

By the use of radioisotope precursors including $^{14}$C-adenine, $^{14}$C-guanine, $^{14}$C-uracil, $^{14}$C-thymidine and $^{14}$C-formate, it was concluded that CPZ did not selectively inhibit the biosynthesis of RNA or DNA in log phase *B. megaterium* cells. However, the drug interfered, in a dose-dependent manner, with the incorporation of $^{14}$C-orotic acid into RNA and DNA. The effect of CPZ on utilization of orotic acid for RNA and DNA synthesis may occur at the membrane level, possibly by inhibition by the drug of a permease-like transport mechanism(s). This is suggested by the observation that in log phase *B. cereus* cells CPZ at 0·10 mM inhibited uptake of $^{14}$C-orotic acid into nucleic acids by about 50 per cent while its corresponding effect on $^{14}$C-adenine, $^{14}$C-guanine, $^{14}$C-uracil and $^{14}$C-formate was small.[16] Similar effects have been produced in log phase *B. cereus* cells by amobarbital at 1 mM, a concentration which is non-growth inhibitory. In this latter case, the effect has been clearly shown to be due to antagonism by amobarbital of the process which allows orotic acid to enter the cell; the structural relation between these two pyrimidines probably accounts for this antagonism.[34] Since CPZ is structurally unrelated to orotic acid, it appears likely that any action that CPZ may have on orotic acid transport would be by a different

mechanism than that of amobarbital. Finally, the possibility must also be considered that in the bacterial cells CPZ may be inhibiting the enzymes which convert exogenous orotic acid to orotidine-5'-phosphate[35] and uridine-5'-phosphate.[35]

Effects of CPZ and promazine on nucleic acid synthesis have been described in other systems. Promazine reduced the levels of RNA and DNA, as measured colorimetrically, in *T. pyriformis* during logarithmic growth.[14] In *E. coli*, CPZ depressed the incorporation of $^{14}$C-uracil and $^{14}$C-adenine into RNA, while incorporation of $^{14}$C-valine into protein was unaffected.[9] The incorporation of $^{3}$H-thymidine into DNA of bone marrow cells *in vitro* was depressed by CPZ.[36]

The utilization of $^{14}$C-orotic acid, $^{14}$C-adenine, $^{14}$C-guanine, $^{14}$C-uracil and $^{14}$C-formate for synthesis *in vivo* in cat and rat brain has been demonstrated.[37–39] As far as we can ascertain, there have been no studies on the effect of CPZ on the utilization of pyrimidine or purine bases for nucleic acid synthesis in brain. It would be of interest to see if the interference produced by CPZ on the utilization of orotic acid for nucleic acid synthesis in *B. megaterium* also occurred in mammalian brain.

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