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LYTIC ACTION OF CHLORPROMAZINE HYDROCHLORIDE ON *ESCHERICHIA COLI* G. CELLS

BY

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The pharmacological properties of chlorpromazine are now well known. Besides pharmacological studies, some interesting results of its action on growth and metabolism in different organisms have been reported (Eyal & Eyal-Giladi, 1963). The inhibition by chlorpromazine of growth of organisms precedes the inhibition of activities of certain enzymes (Dawkins, Judah & Rees, 1960; Courvoiser, Fournel, Ducrot, Kolsky & Koetschet, 1953). Nathan (1961) and Nathan & Friedman (1962) have shown an increase in cellular permeability in some micro-organisms with this compound. We have noted that chlorpromazine hydrochloride lyses *Escherichia coli* G. strain only during the lag and early log phases. In the middle of the log phase of the growth, it has practically no lytic effect.

METHODS

E. coli G. strain was grown in synthetic medium (in g: NH_4Cl 1.0, $MgSO_4$ 0.1, KH_2PO_4 1.5, Na_2HPO_4 3.5 and glucose 5.0; dissolved in 1 l. of distilled water). Chlorpromazine hydrochloride (kindly supplied by May & Baker) was dissolved in the synthetic medium. The *p*H of the solution was 6.9. In all the experiments with chlorpromazine, the titre of bacteria in the suspensions, as measured by the turbidity, was the same.

The optical density of the bacterial suspensions was measured with a photcmeter using a 640-mμ filter. The growth of the bacteria was also checked by counting the viable cells on agar plates, following conventional technique. The nature of the growth curve, obtained by plotting log of number of colonies against time in hours (Fig. 1,*a*), was the same as that obtained with the optical density measurements (Fig. 1,*b*). The slope in the log phase in the former case is 10.8 colonies/hr, and that in the latter is 0.05 $\Delta OD_{640m\mu}$/hr. For the sake of brevity, only the optical density/time curves are shown in this paper.

RESULTS

The effect of chlorpromazine on E. coli

The lag period of growth in synthetic medium as determined by optical density is 1 hr (Fig. 1,*b*), similar to that shown by viable counts (Fig. 1,*a*). When the compound at a concentration of 3.3 mg/ml. was added to a culture of bacteria grown for 1 hr, the $OD_{640m\mu}$ decreased to such a degree that the sample became almost clear after 2 hr of incubation at 37° C (Fig. 2).

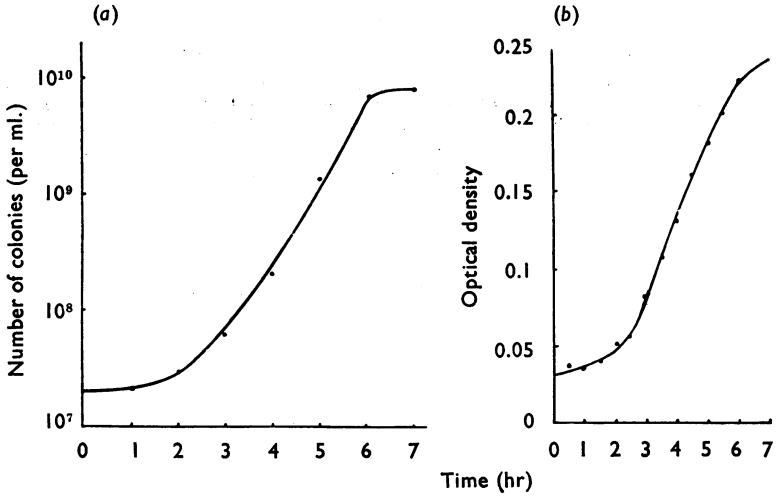

Fig. 1. (*a*) Growth curve of bacteria measured in terms of number of colonies per ml. (ordinate) against time of incubation in hours (abscissa). (*b*) Growth curve of bacteria measured in terms of rise in optical density of the bacterial suspension (ordinate) against time of incubation in hours (abscissa).

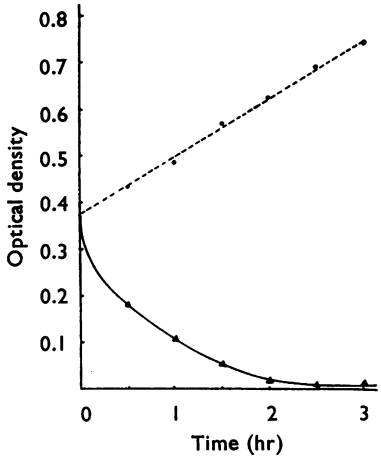

Fig. 2. The effect of chlorpromazine on 2-hr-old cells incubated at 37° C for different times. The culture became almost clear within 3 hr of incubation. Ordinate: optical density of the bacterial suspension; abscissa: time of incubation in hours. Interrupted line: untreated culture; continuous line: culture treated with chlorpromazine.

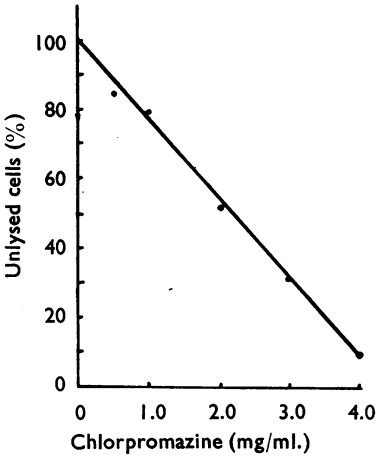

Fig. 3. Effect of concentrations of chlorpromazine on 2-hr-old cells incubated for 2 hr at 37° C. Ordinate: percentage of unlysed (intact) cells calculated from (100× $OD_{640m\mu}$ at 2 hr)/($OD_{640m\mu}$ at 0 hr); abscissa: concentration of chlorpromazine hydrochloride in mg/ml.

The age-dependent action of chlorpromazine

When chlorpromazine was added at a concentration of 3.3 mg/ml. to bacterial suspensions of different ages and incubated for 2 hr at 37° C, the lytic phenomenon was observed only with the cells up to 3.5 hr of age (Table 1).

TABLE 1

EFFECT OF AGE OF BACTERIA ON THE LYTIC ACTION OF CHLORPROMAZINE

Values (means and standard errors) give the percentage of lysis of bacteria of different ages with 3·3 mg of chlorpromazine hydrochloride per ml. of the culture when incubated for 2 hr at 37° C, keeping the turbidity of the suspension in each of the samples the same in all the experiments

$$\text{Percentage of lysis} = 100 - \left[\frac{OD_{640m\mu} \text{ at 2 hr}}{OD_{640m\mu} \text{ at 0 hr}} \times 100\right]$$

Age of bacteria (hr)	Percentage of lysis
0	70±6·2
0·5	75±8·7
1	71±7·8
1·5	71±6·5
2	72±7·4
2·5	69±5·9
3	40±8·2
3·5	22±6·2
4	0
4·5	0
5	0

Kinetic studies of lysis by chlorpromazine

Fig. 3 shows the percentage of intact cells (unlysed) at different concentrations of chlorpromazine, when 1-hr-old cells were incubated at 37° C for 2 hr after the addition of drug. The curve indicates that the concentration of drug is linearly related to the lysis of bacteria. The rate constants of the lytic effect of various concentrations of drug (Fig. 4) indicate that it requires at least 1 hr for starting the lytic action. This lag period of chlorpromazine action (namely 1 hr) is the same for all concentrations of the compound. The corresponding changes in the cell wall, as observed after staining, show that during the first hour the cells become very swollen and that bursting of the cells is observed only after 1 hr of contact.

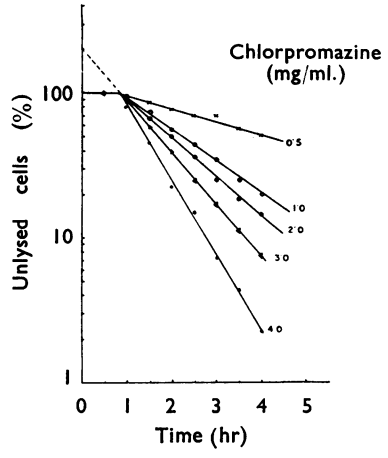

Fig. 4. Kinetics of action of different concentrations of chlorpromazine on 2-hr-old cells. Ordinate: percentage of unlysed (intact) cells at different concentrations of chlorpromazine, calculated from $(100 \times OD_{640m\mu} \text{ at } t \text{ hr})/(OD_{640m\mu} \text{ at 0 hr})$; abscissa: time of incubation (in hours) of bacteria with drug. Concentrations of chlorpromazine hydrochloride are given on right.

DISCUSSION

The action of chlorpromazine at a particular stage of development of the hen's egg yolk has been observed by Miller & Pasciuto (1963). Our observations also indicate an age-dependent action of the compound on a micro-organism. The lag period in the bacterial culture is mainly the period of profuse synthesis of deoxyribonucleic acid (Belozersky, 1959) and only during this period has the cell wall been found to be highly vulnerable to chlorpromazine. Moreover, we have observed that the compound produces a considerable decrease in the surface tension of the medium. The addition of 3.3 mg/ml. reduced the surface tension of the medium from 74.39 to 45.0 dynes/cm at 20° C. The kinetics (Fig. 4) demonstrate that chlorpromazine requires about 1 hr before causing the cells to burst. Therefore, the bursting of the cells is not due simply to action on cell walls but may involve formation of some lysogenic complex within the material of the cell (extrapolation of kinetic data of various concentrations shows two different functions before starting lysis). We have found that, after 1.5 hr of incubation with chlorpromazine, the cells stained deeply with Feulgen in a diffused pattern, while, up to 1 hr of incubation, organized Feulgen-stained threads are observable similar to those in normal cells.

SUMMARY

1. Chlorpromazine hydrochloride showed a lytic effect during the lag and early log phases of *E. coli* G. cells grown in synthetic medium. In older cells, no lysis was shown.

2. Rate kinetic studies showed that at least 1 hr was required to start the lytic action at all concentrations of drug (from 0.5 to 4 mg/ml.).

3. From the rate kinetic studies it is concluded that the bursting of the cells is not due simply to action on the cell wall, but that it probably involves the formation of some lysogenic complex in the material of the cell.

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