Biochemical Pharmacology, Vol. 26, pp. 1205–1211. Pergamon Press, 1977. Printed in Great Britain.

NEW ASPECTS CONCERNING THE MECHANISM OF ACTION OF TRANQUILIZERS. THE INFLUENCE OF SOME TRANQUILIZERS ON PROTEIN AND NUCLEIC ACID SYNTHESES

EKRAM Z. KHAFAGY*, A. F. EL-LAITHY, H. K. EL-MAKKAWI and Z. I. EL-DARAWY

National Centre for Social and Criminological Research and Laboratory of Biochemistry, National Research Centre, El-Dokki, Cairo, Egypt

(*Received* 20 *March* 1976; *accepted* 31 *December* 1976)

Abstract—In *Escherichia coli B*, nucleic acid and protein syntheses were inhibited in the presence of chlorpromazine, promethazine, 2-chlorophenothiazine and librium. The presence of the dimethylaminoalkyl side chain of chorpromazine is essential for potent inhibition of these macromolecular syntheses. In parallel with the inhibition of protein synthesis, the processes of de-repression of alkaline phosphatase and induction of β-galactosidase were also inhibited in the presence of chlorpromazine and librium. We have found that all of the effects of these drugs on protein and nucleic acid syntheses in *E. coli B* are not due to an increased destruction of ATP in the drug-treated cultures. The interference with protein and nucleic acid syntheses may be due to the interaction of these drugs and deoxyribonucleic acid (DNA). Chlorpromazine is found to be effective in displacing methyl green (MG) from its complex with DNA and form insoluble complex with DNA.

In bacterial systems and in cell cultures, protein synthesis was inhibited in the presence of narcotic drugs. *E. coli* grown in the presence of levorphanol had less protein synthesis [1], and an inhibition of synthesis of RNA in *E. coli* in the presence of levorphanol was first reported by Simon [2]. The incorporation of ^{32}P into RNA was inhibited when the cells were grown in the presence of 1.35×10^{-3} M levorphanol with the inhibition limited to ribosomal RNA. Noteboom and Mueller [3], have ascribed the effect of the narcotic drug on RNA synthesis to a primary inhibition of protein synthesis. Complete cessation of all incorporation of [^{14}C]uracil into RNA and of [^{14}C]thymidine into deoxyribonucleic acid (DNA) was found when *E. coli* were grown in the presence of 3×10^{-3} M levallorphan [4].

It has also been reported [5] that bone marrow of chlorpromazine-sensitive patients was devoid of DNA-synthesizing cells during agranulocytosis. Incubation of marrow cells from chlorpromazine-sensitive patients with [^{3}H]thymidine for 3 hr under standard conditions resulted in a significant lower percent of labeled granulocytes as compared with nonsensitive normals. Marrow cells from chlorpromazine persons incubated with chlorpromazine *in vitro* failed to divide. They indicated that the index of cell division in the presence of chlorpromazine lagged behind that of control preparation. Kraus and Vinarova [6] indicated that *in vitro* chlorpromazine inhibited protein

On the other hand, it has been reported [7] that chlorpromazine did not accelerate bacterial multiplication *in vitro*. Later, Bourdon [8] has indicated that *in vitro* chlorpromazine shows antibiotic activity against gram-positive bacteria only.

In addition, librium (a member of the benzodiazepine class of tranquilizer) has been reported [9] to increase survival times in mice with staphylococcal infection. Histological changes in the nucleic acid constituents of nerve cells of rat has occurred [10] following administration of librium (5 mg/100 gm, given intramuscularly).

These previous findings have promoted us to undertake a comparative study not only between three derivatives from the phenothiazines group, namely chlorpromazine (potent tranquilizer), promethazine (antihistaminic) and 2-chlorophenothiazine, but also between these three derivatives and librium and diazepam which are agents from the benzodiazepine class of tranquilizer and central muscle relaxant for structural activity relationship with respect to their influence on macromolecular synthesis, since protein and RNA synthesis are required in the nervous system for the development of tolerance to narcotic drug [11–14].

MATERIALS AND METHODS

Bacteria. The bacteria employed was *Escherichia coli* NRRL B-210.

Culture media. Media contained 2.0 g beef extract, 2.0 g yeast extract, 5.0 g peptone, 5.0 g NaCl per 1 liter distilled water. This medium was used for the cultivation of bacteria. All incubations were carried out on a rotary shaker at 30°.

Biosynthesis of macromolecules. Overnight cultures of *E. coli B* were diluted to a concentration of 10^8 cells per ml† and were grown with aeration to a density of about 2×10^8 cells per ml, at which time the cultures were divided into equal portions and the drug under investigation was added in ascending concentrations‡ and incubation was carried out for 4 hr

* To whom inquiries should be addressed; Biochemistry Lab., National Research Centre, El-Dokki, Cairo, Egypt.

† Viable cell counts were carried out by the plate out procedure.

‡ Tween 80 has been used to solubilize drugs which are not readily soluble in the media. Up to 5% Tween 80 has no effect on bacterial growth.

with hourly samples taken. Aliquots 10 ml of cultures were chilled, adjusted to 0.25 M perchloric acid, allowed to remain in the cold for 30 min, and the precipitated cells collected by centrifugation at 4000 rev/min at 0°.

The packed cells were washed twice with 2 ml of 0.25 N perchloric acid at 0° and resuspended in 3 ml of 0.5 M perchloric acid. They were then extracted for 30 min at 75°, the supernatant fluid collected and used for nucleic acid determinations. The pellets were carefully drained, and taken up in 2 ml of 1 N NaOH and extracted at 90° for 30 min. The supernatant fluids of this extraction were used for protein analyses.

Protein determinations were carried out by the procedure of Lowry *et al.* [15], RNA by the orcinol reaction [16], and DNA by the procedure of Burton [17]. The following were used as standards: bovine-serum albumin, purified yeast RNA and calf thymus DNA (all were obtained from Sigma Chem. Co.).

Induction of β-galactosidase in E. coli B. Overnight cultures (100 ml) of *E. coli B* in nutrient broth were washed with sterile saline and resuspended in 100 ml of medium (Davis and Mingioli) [18] supplemented with 5 mg of thiamine HCl and 10 g of casein hydrolysate per liter, free of glucose but adjusted to 1% lactose. The culture was divided into equal portions, one remained as the control, to the other different concentrations of each of chlorpromazine HCl and librium HCl were added, and all the cultures were aerated for 1.5 hr at 30°. At the end of the incubation period 5 ml of the control culture and the other cultures receiving the drugs investigated were kept cold to estimate the induced enzyme activity in cell suspension. The cells from the remaining cultures were each collected by centrifugation at 0° for 30 min at 4000 rev/min. After washing with 0.03 M Tris pH 7.4 in 0.01 M $MgCl_2$, the cells were ground with sand and extracted with three portions of the Tris-Mg^2 buffer. The sand and cell debris were removed by centrifugation at 0°.

Assay of β-galactosidase activity. For assay of the enzyme activity in cell suspension, 1 ml aliquots of control culture and cultures treated with different concentration of chlorpromazine and librium were pipetted into tubes containing 1 drop of toluene. The tubes were shaken vigorously and were incubated for 30 min at 37°. They were then brought to 28°, 0.2 ml of a solution of M/75 *o*-nitrophenyl-β-D-galactoside in M/4 sodium phosphate (pH 7.0) was added, and the tubes were incubated for 30 min at 28°. At the end of incubation time, the reaction was halted by addition of 0.5 ml of 1 M Na_2CO_3 and the optical density of the yellow color developed was measured at 420 nm.*

In case of estimating the enzyme activity in cell extract, the same procedure outlined above was applied, but incubation with toluene is omitted. The amount of *o*-nitrophenol liberated in the sample was calculated from a standard curve of *o*-nitrophenol. One unit of enzyme activity is defined as producing 1 nmole *o*-nitrophenol/min at 28°, pH 7.0.

De-repression of alkaline phosphatase. Overnight cultures (100 ml) of *E. coli B* in nutrient broth were harvested, washed and resuspended in 100 ml of the Tris (phosphorus-free) medium of Torriani [19] supplemented with amino acids and thiamine. The cultures were divided into equal portions, one portion served as control, to the others different concentrations of chlorpromazine and librium were added, then all the cultures were aerated for 1.5 hr. At the end of the incubation period, a sample of each culture was taken for assay of enzyme activity in cell suspension. The remaining cultures were each centrifuged at 4000 rpm at 0° for 30 min. The cells were ground with sand and extracted with 1 M Tris pH 8.8.

Assay of alkaline phosphatase activity [20]. For assay of alkaline phosphatase activity in cell suspension, 1 ml of culture from each of control and those treated with the drugs under investigation were placed in a centrifuge tube (containing 0.2 ml of 1:1000 merthiolate in order to block instantly further enzyme formation).

The killed cells were resuspended in exactly 1 ml of buffer. This suspension was either used directly or was first treated by shaking with 0.02 ml toluene/ml at 37° for 30 min. Then to 1 ml 1 M Tris buffer pH 8.8 and 0.5 ml of either cell suspension treated as above or cell extract were added and then brought to 37° for 10 min, 0.5 ml of 0.04 M NPP (*p*-nitrophenyl phosphate at 37°) was added with rapid mixing and incubation was carried out at 37° for 60 min. The appearance of free NP is measured at 420 nm. The unit of enzyme activity is defined as that amount of enzyme which liberates 1 nmole of NP per hr under the prescribed conditions.

Effect of ATP addition on the reversal of the inhibitory action of chlorpromazine and librium on E. coli B *multiplication.* An inoculum from an overnight culture was added to nutrient broth-NaCl media, and the content incubated until viable-cell count had reached almost 1×10^8 cells/ml. A series of culture flasks, were prepared by the addition of 0.16 mg/ml chlorpromazine and 1.2 mg/ml librium and these were incubated for a period of 2 hr. Samples for turbidity measurement at 600 nm were removed from the control flask at zero time, and from all flasks at the end of incubation period. At that time graded increments of ATP were added to the cultures inhibited by chlorpromazine and librium. Incubation was resumed; turbidity measurements were determined for each culture for the next 3 hr.

Release of methyl-green from a DNA-MG complex. This method is based on the observation that at pH 7.5 the DNA-methyl green complex is intensely green, where as the unbound methyl green is colorless [21]. Therefore a compound that displaces methyl green from its complex with DNA decreases the intensity of the green-colored solution. A solution containing the DNA-methyl green complex was prepared by placing 15 mg of the DNA-methyl green compound into 100 ml of 0.05 M Tris–HCl buffer (pH 7.5) containing 7.5×10^{-3} M $MgSO_4$. The mixture was gently stirred in the dark at 25 ± 1° until dissolved. The assay was carried out by incubating 2 ml of the DNA-methyl green solution and 0.5 ml of the drug solution for 18 hr in the dark at 25 ± 1°. A control tube was prepared containing no drug. The absorbancies of the solutions at 640 nm were measured.

* The yellow color developed was measured after centrifugation to remove turbidity due to cell suspension.

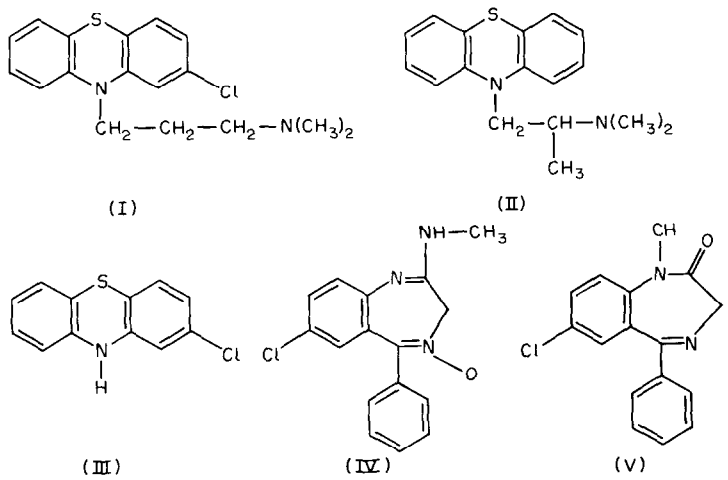

Fig. 1. Molecular formulae of chlorpromazine (I), promethazine (II), 2-chlorophenothiazine (III), librium (IV) and diazepam (V).

Formation of insoluble complex between chlorpromazine and DNA. The ability of chlorpromazine and librium to form complex with DNA was examined in the following manner: increasing amounts of chlorpromazine and librium were added to test tubes containing 30 μ/ml calf thymus DNA in saline-citrate (0.15 M NaCl containing 0.015 M sodium citrate). When insoluble complexes were formed, an immediate turbidity was observed which was read at 600 nm. The increase in O.D. follow an increase in the concentration of chlorpromazine used. Librium did not form complex with DNA. The effect of heat on the solubility of such complex was studied. Results are indicated in Table 6.

RESULTS

The influence of chlorpromazine, promethazine, 2-chlorophenothiazine, librium and diazepam (Fig. 1) on the synthesis of DNA, RNA and protein by *E. coli B* were studied. In the case of chlorpromazine, it is evident from Figs 2A, B & C that with increase in the concentration of the drug up to 0.16 mg/ml, there is a progressive inhibition of synthesis of these

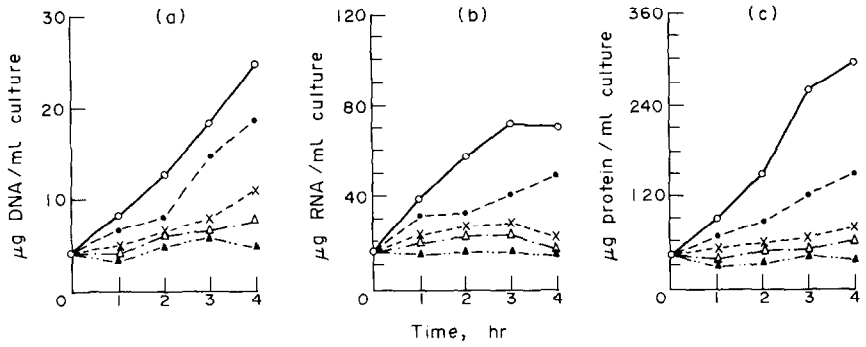

Fig. 2. Effect of chlorpromazine HCl on DNA, RNA and protein syntheses in *E. coli B*. O——O Control, ●– –● 0.048 mg/ml, ×-----× 0.08 mg/ml, △–·–△ 0.12 mg/ml and ▲–···–▲ 0.16 mg/ml.

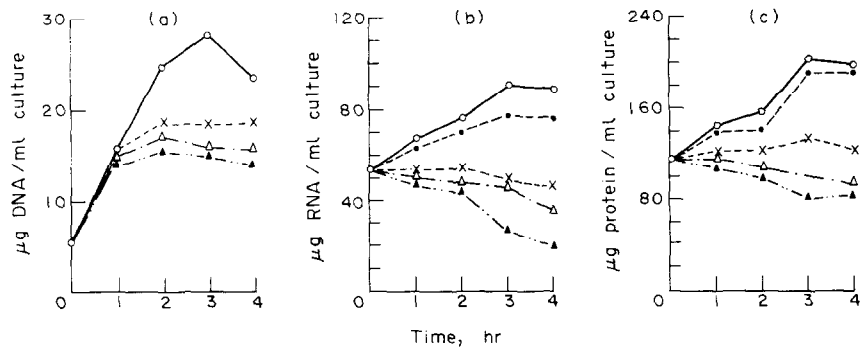

Fig. 3. Effect of promethazine on DNA, RNA and protein syntheses in *E. coli B*. O——O Control. ●– –● 0.04 mg/ml, ×-----× 0.08 mg/ml, △–·–△ 0.12 mg/ml and ▲–···–▲ 0.16 mg/ml.

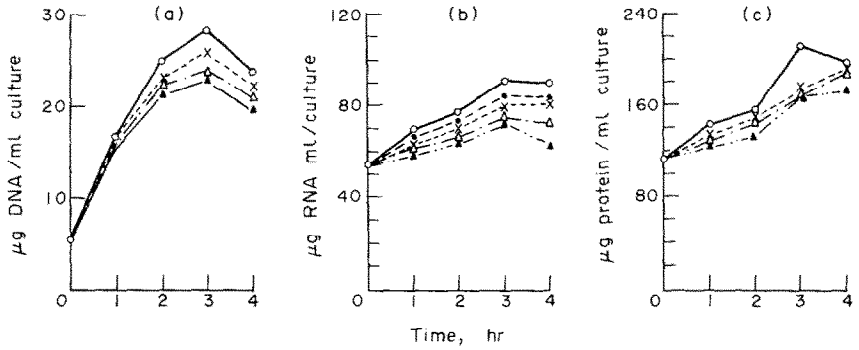

Fig. 4. Effect of 2-chlorophenothiazine on DNA, RNA and protein syntheses in *E. coli B*. ○—○ Control, ●—●—● 0.04 mg/ml, ×-----× 0.08 mg/ml △ ··· △ 0.12 mg/ml and ▲- ··· ▲ 0.16 mg/ml.

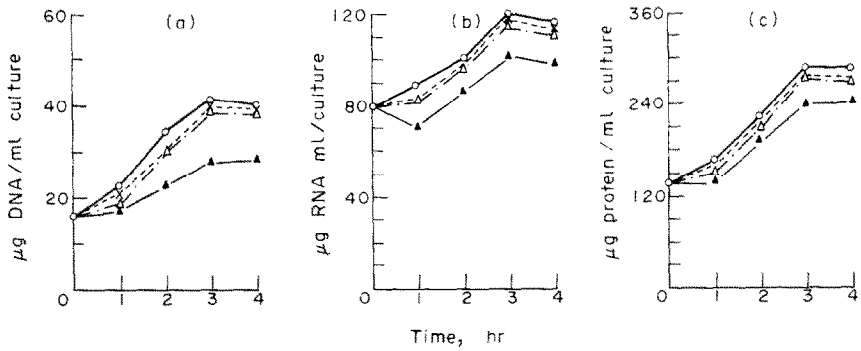

Fig. 5. Effect of librium on DNA, RNA and protein syntheses in *E. coli B*. ○—○ Control, ×-----× 0.8 mg/ml, △—·—△ 1.0 mg/ml and ▲— ··· —▲ 1.2 mg/ml.

three macromolecules. The inhibition of DNA, RNA and protein occur almost at the same rate, since at a concentration 0.16 mg/ml chlorpromazine complete cessation of DNA, RNA and protein have occurred.

Promethazine at a concentration ranging from 0.08 up to 0.16 mg/ml cause a gradual inhibition of DNA, RNA and protein synthesis. RNA and protein synthesis were more inhibited than DNA synthesis under the influence of the same concentration of the drug tested. No complete cessation of DNA has occurred at 0.16 mg/ml of promethazin (Figs 3A, B & C respectively).

2-Chlorophenothiazine at a concentration ranging from 0.08 up to 0.16 mg/ml cause a gradual inhibition of DNA, RNA and protein synthesis. This inhibition is progressing with increase in the concentration of the drug tested, while no cessation in the synthesis of these three macromolecules have occurred at a concentration of 0.16 mg/ml (Figs. 4A, B & C).

Librium at concentrations as those used for the above three drugs have no effect on the synthesis of these three macromolecules. With increase in the concentration of the drug used up to 1.2 mg/ml, DNA, RNA and protein synthesis were progressively inhibited as the concentration of the drug increased. The rate of inhibition of DNA, RNA and protein synthesis was almost the same (Figs. 5A, B & C). Diazepam has no effect on the synthesis of these three macromolecules (Table 1).

The interference of narcotic drugs in nucleic acid metabolism of micro-organisms may be dependent on: a decrease in the rate of synthesis of a particular protein, or may be related to energy (ATP) levels, or dependent on an initial drug nucleic acid chemical interaction.

So the influence of chlorpromazine and librium on enzyme induction and de-repression were studied. Upon enzyme induction, the synthesis of the structural protein is proceeded by the formation of a specific messenger RNA (m RNA) (Nakada and Magasanik) [22]. The levels of induced β-galactosidase of bacteria (*E. coli B*) exposed to several levels of chlorpromazine and librium are summarized in Table 2.

In parallel with the inhibition of total protein syn-

Table 1. Effect of diazepam on the synthesis of macromolecules in *E. coli B*

	Time (min)	μg of macromolecules/ml cultures DNA synthesis	RNA synthesis	Protein synthesis
Control	0	18.8	50.0	150
	60	22.2	54.6	238
	120	50.0	82.6	654
	180	43.2	98.1	794
Inhibited (1)*	0	18.8	50.0	150
	60	25.5	53.1	260
	120	51.7	88.4	580
	180	44.3	92.3	678
Inhibited (2)*	0	18.8	50.0	150
	60	26.2	53.8	266
	120	53.4	90.3	654
	180	44.3	94.2	714

* The concentration of diazepam was 0.8 mg (1) and 1.2 mg/ml (2) culture respectively.

Table 2. Effect of chlorpromazine and librium on the β-galactosidase content of induced *Escherichia coli B* cells

Concn. of chlorpromazine	β-galactosidase activity in cell suspension	Cell extract β-galactosidase activity	Cell extract Specific enzyme activity*	Concn. of librium	β-galactosidase activity in cell suspension	Cell extract β-galactosidase activity	Cell extract Specific enzyme activity*
μg/ml	units/ml	units/ml		μg/ml	units/ml	units/ml	
0†	104	878	1097	0†	36.5	537	981
20	65	719	922	600	36.5	452	861
40	52	661	801	800	29.8	357	713
80	24	55	76	1000	24.5	339	679
120	23	48	66	1200	23.9	311	625
160	17	24	33				

* Expressed as units per milligram protein.
† Control cultures of *E. coli B* received no inhibitor.

Table 3. Effect of chlorpromazine and librium on the enzyme content of the alkaline phosphatase constitutive bacterium *Escherichia coli B*

Conc. of chlorpromazine	Alkaline phosphatase activity in cell suspension	Cell extract Alkaline phosphatase activity	Cell extract Specific enzyme activity*	Concn. of librium	Alkaline phosphatase activity in cell suspension	Cell extract Alkaline phosphatase activity	Cell extract Specific enzyme activity*
μg/ml	units/ml	units/ml		μg/ml	units/ml	units/ml	
0†	216	81	350	0†	216	90	340
20	144	72	342	600	199	86	328
40	59	66	221	800	196	72	300
80	58	65	221	1000	196	65	289
120	49	65	196	1200	187	50	262
160	48	55	182				

* Expressed as units per milligram protein.
† Control cultures of *E. coli B* received no inhibitor.

thesis brought about by chlorpromazine and librium, the synthesis of alkaline phosphatase (constitutive enzyme) was also stopped (Table 3).

Attempt to antagonize the inhibitory action of chlorpromazine and librium on growth of *E. coli B* by the addition of ATP to a culture of *E. coli B* after multiplication had been inhibited by chlorpromazine and librium resulted in no return of growth (Table 4).

Table 4. Effect of ATP on the reversal of the inhibitory action of chlorpromazine and librium on *E. coli B* multiplication

	O.D. at 600 nm of culture during growth Time (min) 60	120	180*
Control	0.24	0.42	0.56
Control plus ATP (200 μg/ml)	0.25	0.46	0.58
Inhibited (1)†	0.10	0.09	0.08
plus ATP (50 μg/ml) Inhibited (1)†	0.10	0.09	0.08
plus ATP (100 μg/ml) Inhibited (1)†	0.09	0.07	0.07
plus ATP (150 μg/ml) Inhibited (1)†	0.09	0.07	0.07
plus ATP (200 μg/ml) Inhibited (2)‡	0.2	0.32	0.34
plus ATP (100 μg/ml) Inhibited (2)‡	0.21	0.29	0.31
plus ATP (200 μg/ml)			

* These time intervals (60, 120, 180 mins) indicate time of incubation after ATP addition.
† Cultures inhibited by 0.16 mg/ml chlorpromazine.
‡ Cultures inhibited by 1.2 mg/ml librium.

Drug nucleic acid interaction. Methyl green forms a complex with DNA and compounds which bind DNA, e.g., quinacrine [23] have been reported to displace methyl green from its complex with DNA. Table 5 shows that chlorpromazine was effective in displacing methyl green from the DNA-MG complex, while librium was less effective.

From Table 6, it is evident that when increasing amounts of chlorpromazine were added to a highly polymerized calf thymus DNA an immediate turbidity was observed which was read at 600 nm, the increase in O.D. follow an increase in the concentration of chlorpromazine used. Librium did not form insoluble complex with DNA, and an attempt to see

Table 5. Release of methyl green from DNA-MG complex by chlorpromazine and librium

Concn. of chlorpromazine	Percent release of MG	Concn. of librium	Percent release of MG
μg/ml		μg/ml	
100	5.9	600	5.9
200	17.1	800	9.4
300	19.7	1000	11.1
400	19.7	1200	12.8

Table 6. Formation of insoluble complex between chlorpromazine and DNA and the effect of heat on the solubility of such a complex

Concn. of chlorpromazine	O.D. at 600 nm at different temperature			
	30	50	70	90
100 μg/3 ml	0.22	0.19	0.17	0.15
200 μg/3 ml	0.45	0.42	0.37	0.35
300 μg/3 ml	0.65	0.58	0.51	0.49
400 μg/3 ml	0.85	0.73	0.66	0.61

its effect on the thermal melting of DNA was not possible since very small concentration of librium shows a high absorption at 260 nm. Table 6 shows that, the increase in temperature resulted in a slight solubility of the formed complex as evident from a decrease in O.D. at 600 nm with increase in temperature.

DISCUSSION

From our results, it is evident that there is a relation between structure and function, chlorpromazine a well known tranquilizer showed at a lower concentration, a complete cessation of DNA, RNA and protein synthesis. The percent inhibition of DNA, RNA and protein synthesis at the end of incubation period at a concentration of 0.16 mg/ml chlorpromazine are: 81.4, 78.6 and 88.8 respectively. It is known that by separating the N atoms of the dimethyl-aminoalkyl side chain of chlorpromazine by two carbon yield promethazine (Fig. 1) an antihistaminic drug. Upon investigating its effect on DNA, RNA and protein synthesis, it is evident (Figs. 3A, B & C) that RNA is the primary target with a percent of inhibition in the synthesis of DNA, RNA, and protein at the end of incubation period at a concentration of 0.16 mg/ml are as follows: 40.3, 77.6 and 57.8 respectively.

2-Chlorophenothiazine at the same concentration used as that for chlorpromazine and promethazine cause a slight inhibition of DNA, RNA and protein synthesis. The percent inhibition at 0.16 mg/ml at the end of incubation period are 18.7, 28.9 and 13.2 respectively.

Comparing the structure of these three phenothiazine derivatives (Fig. 1) with the percent inhibition of DNA, RNA and protein synthesis, it is evident that chlorpromazine is the potent inhibitor for these synthetic processes. Protein synthesis is the most sensitive to inhibition by chlorpromazine (Fig. 2C), the inhibition in the synthesis of these three macromolecules starts during 1 hr of incubation. Following in potency with respect to inhibition of DNA, RNA and protein synthesis is promethazine and then 2-chlorophenothiazine. In case of these two drugs, DNA inhibition starts after hr 1 of incubation, while inhibition of RNA and protein synthesis took place during hr 1 of incubation (Figs. 3A, B & C, and 4A, B & C) and continue to hr 4 of incubation. From these results, we could conclude that the dimethyl-aminoalkyl side chain of chlorpromazine is essential for potent inhibition of synthesis of these three macromolecules.

It is evident from our results that librium when compared to diazepam upon its effect on DNA, RNA and protein synthesis; diazepam has insignificant effect on the synthesis of these three macromolecules, while librium at a concentration of 1.2 mg/ml at the end of incubation period, gave the following percent inhibition of DNA, RNA and protein synthesis as follows: 26.7, 16.3 and 13.2 respectively. It is evident that DNA is inhibited the most, and inhibition of the three macromolecules start during hr 1 of incubation and continue until the end of incubation period. It is also evident that librium is less effective than chlorpromazine and promethazine.

Upon enzyme induction and derepression, it is evident that in parallel with the inhibition of the total protein synthesis, the synthesis of induced enzyme (β-galactosidase) in cell free extract and cell suspension is inhibited to 97 and 83.7 per cent by 0.16 mg/ml chlorpromazine respectively. While librium at a concentration of 1.2 mg/ml cause 36.3 and 34.6 per cent inhibition in β-galactosidase induction, in cell free extract and in cell suspension, respectively.

Also the synthesis of constitutive enzyme (alkaline phosphatase) is also inhibited by chlorpromazine and less by librium. Chlorpromazine at a concentration 0.16 mg/ml cause 48 and 77.8 per cent of inhibition in both cell free extract and cell suspension respectively. Librium at a concentration of 1.2 mg/ml bring about 15.2 and 13.5 per cent inhibition in both cell free extract and cell suspension respectively. The inhibition of new enzyme synthesis was the direct result of inhibition in the formation or expression of m RNA brought about by chlorpromazine and to a less extent by librium. The process of enzyme induction can be used as an index of m RNA function and synthesis [22].

We have suggested that all of the effect of the drugs investigated on protein and nucleic acids synthesis in *E. coli B* are due to an increased destruction of ATP in the drug treated culture, since an initial decrease in ATP levels leads to subsequent inhibition of all synthetic processes as already have been observed by Greene and Magasanki (1967) [4]. They have indicated that when the bacterial cell nucleotides were prelabeled by exposing the cells to labeled adenine before adding levallorphan, there was a shift from ATP to AMP within the cells and leakage of labeled nucleotides from the cells in the presence of narcotic drug.

From our results, it is evident that addition of different concentrations of ATP to cultures of *E. coli B* which have been inhibited by 0.16 mg/ml chlorpromazine or 1.2 mg/ml librium has no antagonistic effect. This eliminates the possibility that the interference of chlorpromazine and librium in nucleic acids and protein synthesis may be related to energy (ATP) levels.

So, the interference of these drugs in nucleic acid and protein synthesis may be dependent on an initial drug nucleic acid chemical interaction. That narcotic drugs do react with nucleic acids have been shown by changes in the temperature of thermal denaturation of the nucleic acids in the presence of morphine, and in the quantitative precipitation of SRNA by levallorphan in the test tube (Clouet, 1967) [24]. With this assumption, we looked for evidences to sup-

port it. From our results in Table 5, it is evident that chlorpromazine and to a lesser extent librium were effective in displacing MG from MG-DNA complex. This result suggest that chlorpromazine and to a lesser extent librium bind to DNA, since compound which bind to DNA, e.g., quinacrine have been reported [23] to displace MG from its complex with DNA. This result suggest that chlorpromazine and librium bind to DNA. Also a parallelism was observed between the antibacterial effect of chlorpromazine and its ability to form insoluble complex with DNA. Librium did not form an insoluble complex with DNA, and an attempt to study its effect on the Tm of DNA was not possible, owing to the absorbancy of librium in the ultraviolet.

In conclusion, the dimethyl-aminoalkyl side chain of chlorpromazine is essential for potent inhibition of DNA, RNA and protein synthesis. We suggest that these drugs exert their action on macromolecular synthesis by forming a complex with DNA which in turn prevents the DNA from participating as a template in the biosynthesis of nucleic acids. It has also been found that the metabolic function sensitive to the inhibitory action of chlorpromazine appeared to be the process of enzyme induction and, possibly, the synthesis of messenger ribonucleic acid.

According to the findings of Cohen and his collaborators (1965) [11], Yamamoto *et al.* (1967) [12], Spoerlein and Scrafani (1967) [13] and Smith *et al.* (1967) [14], that mice and rats which had received actinomycin D, 8-azoguanine or, puromycine (inhibitors of RNA, and consequently protein synthesis) developed tolerance to the chronic administration of morphine at a rate significantly ($P < 0.001$) slower than those receiving morphine alone. They have also indicated that puromycin and actinomycin D blocked long term tolerance to levorphanol administration. We suggest that a tranquilizing agent which has an inhibitory action on the synthesis of RNA and consequently protein will develop less tolerance to their administration.

REFERENCES

1. E. J. Simon and D. Vanpraag, *Proc. natn. Acad. Sci. U.S.A.* **51**, 877 (1964).
2. E. J. Simon, *Nature, Lond.* **198**, 794 (1963).
3. W. D. Noteboom and G. C. Mueller, *Molec. Pharmac.* **2**, 534 (1966).
4. R. Greene and B. Magasanik, *Molec. Pharmac.* **3**, 453 (1967).
5. A. V. Pisciotta and A. S. Santos, *J. Lab. clin. Med.* **65**, 228 (1965), *ibid.* **63**, 445 (1964).
6. P. Kraus and M. Vinarova, *Eur. J. Pharmac.* **3**, 355 (1968); C. A. **69**, 85197s (1968).
7. M. Faguet *C. r. hebd. Séanc. Acad. Sci., Paris* **255**, 1155 (1962).
8. J. L. Boudon, *Annls Inst. Pasteur, Paris* **101**, 878 (1961); C. A. **56**, 13345b (1962).
9. I. M. Smith, S. S. Lendell, E. C. Hazard and S. Rabinovich, *Nature, Lond.* **211**, 720 (1966).
10. S. Ghosh and J. J. Ghosh, *Sci. Cult.* **23**, 246 (1966); C. A. **65**, 20721a (1966).
11. M. Cohen, A. S. Keats, W. Krivory and G. Ungar, *Proc. Soc. exp. Biol. Med.* **119**, 381 (1965).
12. I. Yamamoto, R. Inoki, Y. Tamari and K. Iwatsubo, *Jap. J. Pharmac.* **17**, 140 (1967).
13. M. T. Spoerlein and J. Scrafani, *Life Sci.* **6**, 1549 (1967).
14. A. A. Smith, M. Karmin and J. Gavitt, *J. Pharmac. exp. Ther.* **156**, 85 (1967).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. W. Mejbaum, *J. phys. Chem.* **258**, 117 (1939).
17. K. Burton, *Biochem. J.* **62**, 315 (1956).
18. B. D. Davis and E. S. Mingioli, *J. Bact.* **60**, 17 (1950).
19. A. Torriani, *Biochem. biophys. Acta* **38**, 460 (1960).
20. H. A. Echols, S. Garen and Torriani, *J. molec. Biol.* **3**, 425 (1961).
21. H. M. Bates, W. Kuenzig and W. B. Watson, *Cancer Res.* **29**, 2195 (1969).
22. D. Nakada and B. Magasanik, *J. molec. Biol.* **8**, 105 (1964).
23. N. B. Kurnick and I. E. Radcliffe, *J. Lab. clin. Med.* **60**, 669 (1962).
24. D. H. Clouet, *Psychopharmac. Serv. Cent. Bull.* **4**, 34 (1967).