

SOME ACTIONS OF CHLORPROMAZINE

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Since the first account of the pharmacology of chlorpromazine by Courvoisier, Fournel, Ducrot, Kolsky, and Koetschet (1953), much work has been done in an attempt to elucidate the mode of action of this drug. Investigations have been made by Bradley and Hance (1955), Dasgupta and Werner (1954), Hiebel, Bonvallet, and Dell (1954), Holzbauer and Vogt (1954), Longo, Von Berger, and Bovet (1954), Cathala and Pocidalo (1952), Huidobro (1954), Kopera and Armitage (1954), Melville (1954), Cosnier and Drouin (1954), Salzman, Moran, and Brodie (1955), and others.

Chlorpromazine, like promethazine, is a phenothiazine derivative and possesses some of its actions to a greater degree, but it has negligible anti-histamine properties. Perhaps the most outstanding property of chlorpromazine is its central depressant action, which is different from that of barbiturates, and is more marked than with other related phenothiazines. Its main peripheral action is an antagonism to adrenaline, and Kopera and Armitage (1954) showed that chlorpromazine also caused a paralysis of striated muscle. These authors compared the properties of chlorpromazine, promethazine, and pethidine, and found that all three substances showed the same types of pharmacological action, but chlorpromazine was the most active except in its anti-acetylcholine and anti-histamine actions.

In the present investigation we have determined pA_2 values for the anti-acetylcholine and anti-histamine properties of chlorpromazine. Its actions on striated muscle, on the heart for a quinidine-like effect, on body temperature, on insulin hypoglycaemia, and on liver glycogen, were also studied.

METHODS

Anti-acetylcholine and antihistamine actions were determined quantitatively on the guinea-pig ileum by measurement of pA_2 values (Schild, 1947). The anti-histamine action was also estimated in guinea-pigs by the histamine aerosol method of Bovet and Walthert (1944). Atropine-like activity was determined on the pupil of the mouse by the method of Ing, Dawes, and Wajda (1945), the solutions of the compounds

being injected intraperitoneally, or instilled into the eye.

The hypothermic action of chlorpromazine was compared with that of promethazine in rabbits; the drugs were injected subcutaneously and the rectal temperatures were recorded with thermocouples.

The quinidine-like activity of chlorpromazine and promethazine was determined by the method of Dawes (1946), after the drugs had been in contact with the auricles for 10 and 20 min.

Actions of chlorpromazine and promethazine on striated muscle were studied on the phrenic nerve-diaphragm preparation (Bülbring, 1946) of the young rabbit, the sciatic-gastrocnemius preparation of the cat (Bülbring and Burn, 1942) and of the guinea-pig (Hall and Parkes, 1953). The muscle was stimulated, either indirectly or directly, with square wave pulses at a rate of 8/min. In the cat and guinea-pig, injections were made intravenously, or into the tied contralateral iliac artery, close to the bifurcation of the aorta. Guinea-pigs were anaesthetized with urethane intraperitoneally, and cats with chloralose given intravenously.

The effect of chlorpromazine on the volume of the skinned hind limb in cats was measured by means of a plethysmograph and a piston volume recorder.

The ability of chlorpromazine to alter the response to insulin in animals was studied by two methods. In one method, convulsions with insulin were produced in albino mice deprived of food for 24 hr.; the mice were placed in glass jars in an air chamber thermostatically controlled at 36° C. The number of animals convulsing within 3 hr. and the deaths within 18 hr. were recorded. Injections were made intraperitoneally. In the other method, the blood-sugar of rabbits (deprived of food for 18 hr. before the experiment) was determined by the method described by Hagedorn and Jensen (1923). The injections were made subcutaneously and the blood-sugar was determined either hourly or in a pooled sample of blood collected at hourly intervals over a 5 hr. period. The action of chlorpromazine on liver glycogen, which was estimated by the method of Kahan (1953), was investigated in albino rats.

The drugs used were: chlorpromazine hydrochloride, promethazine hydrochloride, tubocurarine chloride, acetylcholine chloride, and histamine acid phosphate, and the doses or concentrations given are all expressed in terms of the salt. Quinidine was weighed as the base, dissolved in sufficient dilute hydrochloric acid, and the amounts are expressed in terms of the base.

RESULTS

Anti-acetylcholine Action

On the guinea-pig ileum the mean pA_2 values for chlorpromazine were 6.07 (n =number of determinations=3) at 2 min. and 6.20 (n =2) at 14 min., with standard deviations of 0.22 and 0.04 respectively. Mean values for promethazine, obtained in these laboratories by Edge (1953), were 7.53 (n =2) and 7.81 (n =2), (S.D. 0.09 and 0.22 respectively). When a comparison was made with these results, chlorpromazine was 28 times less active than promethazine at 2 min. and 40 times less active at 14 min. Kopera and Armitage (1954) obtained a relative figure of approximately 8, but they left the antagonist in the bath for 1 min. before adding acetylcholine. Chlorpromazine was 1.3 times as potent at 14 min. as at 2 min. whereas promethazine was 1.9 times as potent at the longer period of contact.

The intraperitoneal injection of 5 mg./kg. of chlorpromazine had no effect on the pupil in mice observed for 1 hr. whereas this dose of promethazine caused a definite mydriasis in 15 min. However, the instillation of solutions of either chlorpromazine or promethazine into the eye produced a dilatation of the pupil. Ten mice at each concentration and three concentrations of each compound were used. Plotting log concentration against increase in pupil diameter gave dose-response curves which were similar in slope, and the relative potency of chlorpromazine to promethazine was 1/125. A concentration of 2.0% w/v chlorpromazine, for example, was found to be equivalent to a concentration of 0.016% w/v promethazine.

Antihistamine Action

On the guinea-pig ileum the mean pA_2 values for chlorpromazine were 7.67 (n =4) at 2 min. and 7.87 (n =3) at 14 min. (S.D. 0.34 and 0.10 respectively). Edge (1953) obtained corresponding values for promethazine of 8.01 (n =2) and 9.21 (n =2), (S.D. 0.06 and 0.05 respectively). A comparison of these two sets of figures showed that chlorpromazine was one half as potent as promethazine after 2 min. and 22 times less potent after 14 min. Kopera and Armitage found the relative potency to be one third after 1 min. contact. The activity of chlorpromazine at 14 min. was 1.6 times that at 2 min. whereas promethazine was 16.0 times as effective at 14 min. as at 2 min. (Edge, 1953). Thus the equilibrium condition, when the drug was exerting a maximum effect, was reached more quickly with chlorpromazine than it was with promethazine. At 2 min. contact the ratio of

antihistamine to anti-acetylcholine activity was 39.5:1 for chlorpromazine and 3.0:1 for promethazine. Kopera and Armitage obtained similar values (33:1 and 4:1 respectively). Under equilibrium conditions, however, this difference between the two compounds was less marked; the ratios were 47.0:1 for chlorpromazine and 25.4:1 for promethazine.

The results obtained by using histamine aerosols (2% w/v histamine acid phosphate) in guinea-pigs (10 or 12 at each dose) are shown in Fig. 1. Chlorpromazine was about 40 times less active than promethazine, i.e. 20 mg./kg. of chlorpromazine was approximately equivalent in its effect to 0.5 mg./kg. of promethazine.

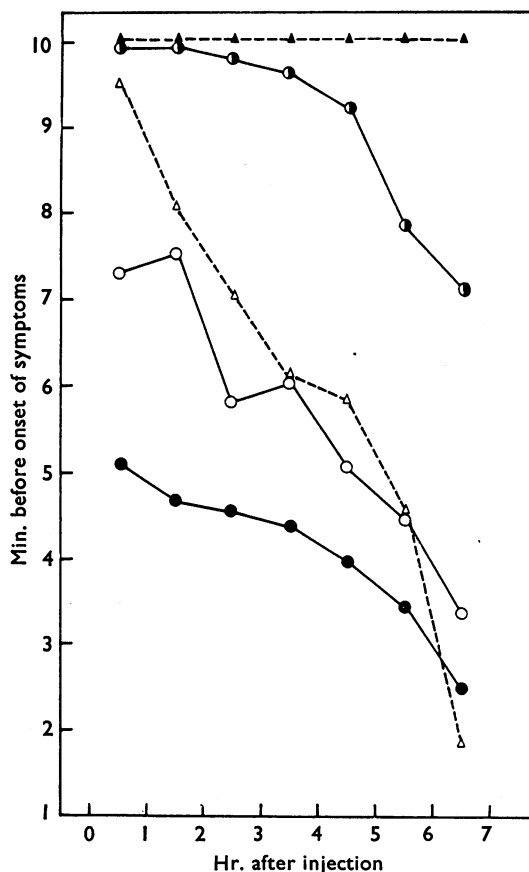


FIG. 1.—Antihistamine action by histamine aerosol method in guinea-pigs. Each point is the mean value of 10 or 12 animals. Ordinates: time (min.) before the animals collapsed due to respiratory distress. Abscissae: time (hr.) after subcutaneous injection of antihistamine compound. ●—●, chlorpromazine 10 mg./kg. ○—○, chlorpromazine 20 mg./kg. ○—○, chlorpromazine 40 mg./kg. △—△, promethazine 0.5 mg./kg. ▲—▲, promethazine 1.0 mg./kg.

Effect on Body Temperature

An assay was carried out in which three doses of both chlorpromazine and promethazine, and eight rabbits at each dose, were used.

The average maximum falls in temperature during the 4-hr. period following injection were plotted against the log dose (Fig. 2). Similar graphs were obtained when the mean sums of the eight deviations (at half-hourly intervals over four hours), from the temperature before injection, were plotted against the log dose. Relative potencies were determined from the antilog of the dose interval between the best fitting parallel straight lines, which were drawn by eye. Calculated from the maximum fall in temperature, the activity of chlorpromazine was 4.3 times that of promethazine, and the relative potency was 3.9 when calculated from the summed deviations in temperature.

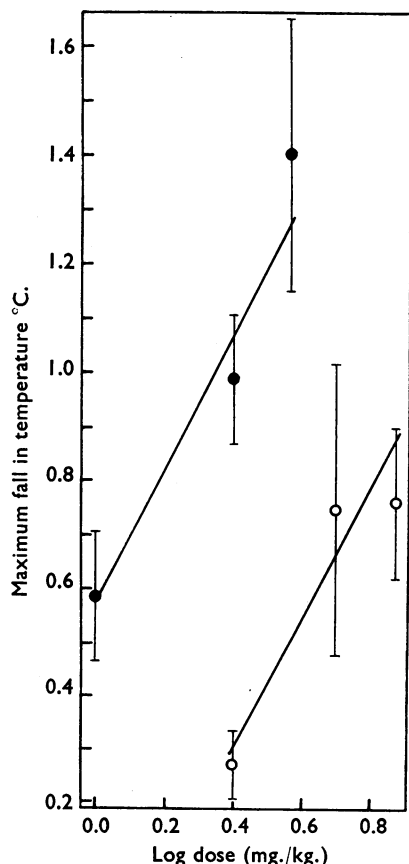


FIG. 2.—Hypothermic action in rabbits. Each point is the mean value of 8 animals. Subcutaneous injections. ●, chlorpromazine; ○, promethazine. Ordinates: maximum falls in temperature observed in 4 hr. Abscissae: log dose in mg./kg. Vertical bars are standard errors.

Quinidine-like Action

Both chlorpromazine and promethazine were as potent as quinidine in reducing the maximum rate of stimulation to which the auricles would respond without dropping beats. Each assay was carried out on a fresh preparation, in which two or three doses of quinidine, followed by only one dose of either chlorpromazine or promethazine, were used. The percentage reduction in the maximal rate of stimulation was plotted against the log dose. It was found that concentrations of about 5 $\mu\text{g./ml.}$ produced similar effects. The mean relative potencies (quinidine=1.0) were 1.06 (n =number of assays=5) at 10 min., and 1.02 (n =3) at 20 min., for chlorpromazine, and 0.93 (n =2) at 10 min., and 1.0 (n =1) at 20 min., for promethazine, with standard errors of 0.38, 0.056, 0.075 respectively for the first three of these values. After these results were obtained it was noted that Schallek (1956) had also found chlorpromazine to have a quinidine-like action.

Effects on Striated Muscle

In conscious rabbits doses of 2–12 mg./kg. intravenously of chlorpromazine produced drowsiness, but not a head drop characteristic of neuromuscular blocking substances. A dose of 16 mg./kg. was fatal.

On the phrenic nerve-diaphragm preparation both chlorpromazine and promethazine had a dual action. First, there was an inhibition of neuromuscular transmission, shown by a failure of the muscle to contract when stimulated through the phrenic nerve; but there was little effect on the response to direct stimulation (Fig. 3a). This neuromuscular blocking effect, which was usually obtained with a concentration of about 14 $\mu\text{g./ml.}$, was not reversed by neostigmine (0.4–1.2 $\mu\text{g./ml.}$). Secondly, there was an increase in resting tone and the heights of the contractions were reduced (Fig. 3b). This effect was observed after the concentration of drug in the bath was doubled, the response to indirect stimulation having been completely abolished at the lower concentration. A similar effect was obtained with the higher concentration, after abolition of the response to indirect stimulation with tubocurarine. It was noticed that after chlorpromazine had been washed out, following a period of contact of 5 min. or more, the responses to indirect and direct stimulation were reduced or even abolished, sometimes for only a few minutes and sometimes until the end of the experiment.

On the sciatic-gastrocnemius preparation of the cat, 3 mg./kg. of chlorpromazine or promethazine,

injected intra-arterially, first augmented and then decreased the contractions on indirect stimulation; these effects were similar to those obtained by Kopera and Armitage. However, intra-arterial injection of the same dose of chlorpromazine in cats previously curarized with tubocurarine produced inhibition of the contractions on direct stimulation, but there was no initial potentiation. In guinea-pigs, when chlorpromazine was given intra-arterially in the same dose, only inhibition of the contractions on indirect stimulation was obtained. On the other hand the effect of intravenous injection of chlorpromazine in cats was slight and often absent. In one cat about 30% inhibition of the contractions on indirect stimulation was obtained with a dose of 5 mg./kg.: in this cat there was a large fall in blood pressure. In four other cats no inhibition was observed after doses of 6 to 20 mg./kg. of chlorpromazine, when there was little effect on the blood pressure. The effect of chlorpromazine on the blood pressure was usually less after repeated doses. Intravenous injection in guinea-pigs of single or repeated doses of chlorpromazine up to 8 mg./kg. did not decrease the size of the muscle twitches on indirect stimulation.

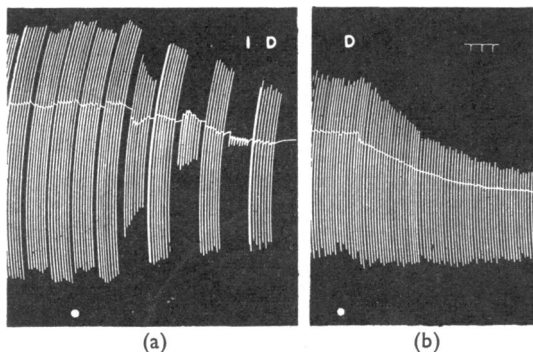


FIG. 3.—Rabbit phrenic nerve-diaphragm. Single supramaximal shocks (8 per min.). I, indirect stimulation (0.4 msec. duration). D, direct stimulation (15 msec. duration). Chlorpromazine, 2 mg. (bath capacity 140 ml.) at O. Between (a) and (b), 30 min. interval; preparation not washed out. Time scale, 30 sec. Contraction downwards.

In a cat under chloralose the intra-arterial injection of 1 mg./kg. of chlorpromazine produced a transient increase in limb volume indicating a dilator effect in the muscles, since the skin was removed. It is noteworthy that a peripheral vasodilatation has been demonstrated in man (Foster, O'Mullane, Gaskell, and Churchill-Davidson, 1954). It is therefore unlikely that any local vasoconstriction could have accounted for the observed inhibitory effects on the neuromuscular system.

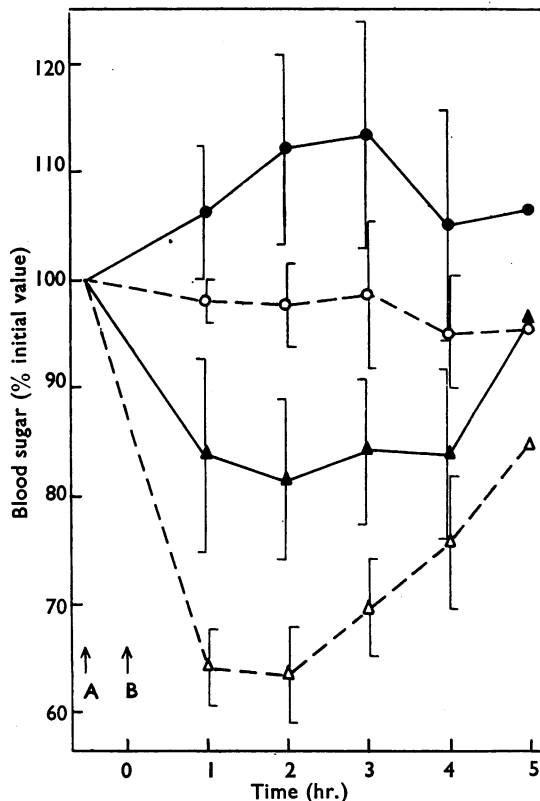


FIG. 4.—Blood-sugar values in rabbits, as % initial values. Each point is the mean of 8–11 animals. ●—●, chlorpromazine, 5 mg./kg. ○—○, controls. ▲—▲, chlorpromazine, 5 mg./kg.+insulin, 0.125 u./kg. △—△, insulin, 0.125 u./kg. Subcutaneous injections. Chlorpromazine injected at A and insulin at B. Ordinates: blood-sugar as % of initial value. Abscissae: time (hr.). Vertical bars are standard errors.

Effects on Blood-sugar and on Liver Glycogen

When chlorpromazine was given subcutaneously to rabbits in a dose of 5 mg./kg., there was a very small hyperglycaemia. More interest was attached to the effect chlorpromazine might have in modifying the effects of insulin on the blood-sugar in rabbits, and on the convulsive effect of insulin in mice.

Effect on Insulin Hypoglycaemia.—Chlorpromazine, given subcutaneously to rabbits in a dose of 5 mg./kg., half an hour before insulin, reduced the hypoglycaemia (Fig. 4). The mean blood-sugar values were not significantly different at any of the hourly points. However, the mean value over the 5 hr. period, for rabbits given chlorpromazine and insulin, was significantly higher than for animals given insulin alone ($P < 0.001$).

The results of an 8×8 Latin square experiment on eight rabbits are shown in Table I. The treatments were two doses of insulin, three doses of

TABLE I
EFFECT OF CHLORPROMAZINE ON MEAN BLOOD-SUGAR
VALUES IN RABBITS

Blood samples were pooled over 5 hr. and the values expressed as % of the initial value. (Subcutaneous injections)

Insulin (u./kg.)	Chlorpromazine (mg./kg.)	No. of Rabbits	Mean	S.E.
0.125	Nil	8	78.5	6.0
0.0625	„	7*	84.4	5.9
0.125	10.0	8	108.5	13.1
0.125	5.0	8	108.5	9.8
0.125	2.5	7*	85.3	11.8
Nil	10.0	7*	141.8	21.9
„	5.0	8	144.1	27.8
„	2.5	7*	112.1	2.1

* Because of the death of 1 rabbit before completion of the experiment, there were only 7 observations in each of these groups.

chlorpromazine, and three doses of chlorpromazine in conjunction with insulin. All injections were given subcutaneously. Doses of 5 and 10 mg./kg. of chlorpromazine abolished the hypoglycaemia due to insulin, and all three doses caused a significant hyperglycaemia, more marked than in the previous experiment. The blood-sugar values of the five animals which received insulin (i.e. three given insulin and chlorpromazine and two given insulin alone) were summed for each of the eight days of the experiment. The means of the figures so obtained, plotted against the day of the experiment, gave a straight line with a slope of 5.46 which differed significantly from zero ($P < 0.01$). This indicated either that chlorpromazine had a cumulative effect, or that there was a decreased sensitivity to insulin.

TABLE II
EFFECT OF CHLORPROMAZINE ON INSULIN CONVULSIONS AND DEATHS IN MICE
(Intraperitoneal injections)

Insulin (u./kg.)	Chlorpromazine (mg./kg.)	No. of Mice	Convulsed		Died	
			No.	%	No.	%
1.0	Nil	48	33	69	27	56
0.8	„	48	26	54	9	19
0.64	„	48	19	39	9	19
1.0	10	24	10	42	8	33
1.0	7.1	48	16	33	6	12
1.0	5.0	48	19	40	9	19
1.0	3.6	24	7	29	6	25

Effect on Insulin Convulsions.—Chlorpromazine reduced the number of mice convulsing and also the number of deaths after insulin (Table II). No relationship between the dose of chlorpromazine and the reduction of convulsions was obtained. However, there was a maximum protection against the lethal effects of insulin with a dose of 7.1 mg./kg. of chlorpromazine. The small protection given by

10.0 mg./kg. might have been due to a toxic action of chlorpromazine itself. χ^2 tests showed that the reduction in the number of mice convulsing after chlorpromazine was significant; the results on all groups which received chlorpromazine were summed and compared with a control group ($P < 0.001$). The number of animals which died was also significantly reduced after chlorpromazine ($P < 0.001$).

Effect on Liver Glycogen.—In order to determine whether the effects of chlorpromazine on blood-sugar were due to a depletion of the glycogen stores in the liver, the following experiments were carried out in albino rats. So that any inhibitory effect might be more readily detected, the stores of glycogen, in fasted rats, were previously raised either by the administration of glucose or by recent feeding. In some experiments the rats were deprived of food for 24 hr. and then 0.5 g. of glucose was given by mouth to each rat. This amount was found to be a convenient quantity to administer in order to produce a temporary rise in the liver glycogen (Barnes, 1953, used a similar amount in studying the effect of dinitro-orthocresol on glycogen deposition in the liver). The liver glycogens after glucose, where each figure was the mean result of determinations on 6 rats weighing 150–300 g., were:

Hours	0	0.35	1	2	4	6	8	24
Liver glycogen %	0.35	0.52	0.62	0.54	0.52	0.38	0.50	

0.5 g. glucose, orally

After 0.5 g. glucose there was a moderate rise in liver glycogen which was maintained between 1 and 6 hr. Consequently, to test the effect of chlorpromazine it was injected subcutaneously either 3 or 4½ hr. after glucose, and the first liver glycogen estimations were made between 1 and 2 hr. later. In other experiments, instead of giving glucose, each rat had 10 g. of a stock diet the evening before an experiment. These results are shown in Table III. The effects obtained with chlorpromazine in these experiments were not consistent. Although a temporary depletion of liver glycogen was obtained in experiment 1, where the difference at 1½ hr. was significant ($P < 0.05$), in the other experiments there was no marked depletion, and in fact in experiments 3, 4, and 5 there was a slight increase.

DISCUSSION

In these experiments some of the properties of chlorpromazine have been determined quantitatively. It was found, from pA_2 determinations against acetylcholine and histamine on the isolated guinea-pig ileum, that equilibrium conditions were not obtained with chlorpromazine at 2 min. period of contact. Edge (1953) obtained similar results for

TABLE III
EFFECT OF CHLORPROMAZINE ON LIVER GLYCOGEN

Liver glycogens (%), with standard errors: 6 or 8 rats were used for each determination; Expts. 1-3 after glucose, 4 and 5 after feeding a stock diet. In Expts. 1 and 2 chlorpromazine was given subcutaneously 4½ hr. after glucose, and 3 hr. after glucose in Expt. 3

Body Wt. (g.)	Expt. No.		mg./kg.	Hr. after Chlorpromazine						
				1	1½	2	3	3½	4	20
150-300	1	Chlorpromazine Controls	10	—	0.26±0.03 0.52±0.03	—	—	0.37±0.03 0.32±0.03	—	0.44±0.03 0.47±0.03
70-110	2	Chlorpromazine Controls	10	—	0.51±0.04 0.66±0.04	—	—	—	—	—
150-300	3	Chlorpromazine Controls	10	0.38±0.03 0.36±0.03	—	—	0.35±0.03 0.27±0.03	—	—	0.46±0.03 0.32±0.03
70-110	4	Chlorpromazine Controls	10	—	—	5.24±0.71 3.77±0.71	—	—	3.69±0.86 4.10±0.86	—
70-110	5	Chlorpromazine Controls	20	—	4.02±0.49 3.47±0.49	—	—	—	—	—

promethazine. It was found that pA_2 values determined under equilibrium conditions (i.e. at 14 min. period of contact) gave closer agreement with other results *in vivo*, obtained by the histamine aerosol and mouse pupil methods.

In rabbits chlorpromazine was only about four times as potent as promethazine in lowering body temperature, in contrast to a twenty-five times difference in mice (Courvoisier *et al.*, 1953). This difference between the relative potencies might be related to the part which surface cooling plays in the regulation of body temperature in the two species. That surface cooling is a factor in the hypothermic action is supported by the observations of Giaja and Markovic-Giaja (1954) that the degree of hypothermia after chlorpromazine in rats varied with the environmental temperature, and of Halpern and Liakopoulos (1954), who showed that the hypothermic action of chlorpromazine, but not of promethazine, was enhanced by a low environmental temperature. Chlorpromazine possibly affects surface cooling by means of its peripheral vasodilator action (Foster *et al.*, 1954).

It has been suggested that the fall in body temperature after chlorpromazine is partly due to a depressant effect on striated muscle (Kopera and Armitage, 1954). Though this might be true for some species, it could not be the explanation in guinea-pigs, at least where intravenous injection of large doses of the drug had no depressant effect on the gastrocnemius muscle, although the drug does produce a fall in body temperature (Halpern and Liakopoulos, 1954).

It was concluded that chlorpromazine depressed the activity of striated muscle only when the drug reached a very high concentration at the site of action, since inhibitory effects on the sciatic-gastrocnemius preparation were only consistently

obtained after intra-arterial injection; intravenous injection in cats gave inconsistent results. Both chlorpromazine and promethazine had a dual action on the phrenic nerve-diaphragm preparation of the rabbit, producing a neuromuscular block followed by a direct effect on the muscle. The neuromuscular block was not due to competitive inhibition, since the effect was not reversed by neostigmine. We noted the similarity between these effects and those described by Hajdu and McDowall (1949), who attributed the effects, on neuromuscular transmission, of phloridzin or lack of glucose to a failure of acetylcholine synthesis at the nerve endings.

Burn (1954) has pointed out that the quinidine-like action of chlorpromazine could be expected from a knowledge of its other properties. It was not surprising, therefore, that the drug resembled quinidine in its action on the electrically-driven isolated auricles of the rabbit, besides inhibiting the fibrillatory action of aconitine and other agents (Courvoisier *et al.*, 1953) and of pressor amines (Melville, 1954).

Chlorpromazine reduced the hypoglycaemia and convulsions due to insulin in experimental animals. Norman and Hiestand (1955) have also shown, in mice, that convulsions due to insulin were reduced after chlorpromazine. Since chlorpromazine and insulin therapy are sometimes used concurrently it is of interest that Lancaster and Jones (1954) were not able to demonstrate a significant effect upon hypoglycaemia due to insulin in man, but they used much smaller doses of insulin than would normally be employed for the treatment of psychiatric cases. However, Merivale and Hunter (1954) and Charatan and Bartlett (1955) showed that after chlorpromazine glucose-tolerance curves were abnormal, indicating

that chlorpromazine might have some action on carbohydrate metabolism in man.

The transient depletion, after chlorpromazine, of the liver glycogen in one experiment when glucose was given 4½ hr. previously was unlikely to be due to an effect on absorption, since in fed rats, and in rats which had been given glucose only 3 hr. previously, there was no depletion. It may have been that the alimentary hyperglycaemia, due to the absorption of carbohydrate from the intestine in the latter experiments, in some way prevented the slight depletion of glycogen stores. In any case, in view of its transient and inconsistent nature this effect cannot be considered very important. However, Cosnier and Drouin (1954) noted a depletion of the liver glycogen and an increase in the fat content of the liver cells, but these workers used a histological technique.

SUMMARY

1. Under equilibrium conditions (14 min. contact) on the isolated guinea-pig ileum, chlorpromazine was 40 times less potent than promethazine against acetylcholine and 22 times less potent against histamine. It was 125 times less active than promethazine on the mouse pupil and 40 times less active by the histamine aerosol method.

2. Chlorpromazine was four times as active as promethazine in lowering the body temperature in rabbits.

3. The quinidine-like action of chlorpromazine on rabbit auricles was similar to that of promethazine, and both of the phenothiazine compounds were as active as quinidine itself.

4. Chlorpromazine had an inhibitory action on skeletal muscle in cats and guinea-pigs only when injected intra-arterially; intravenous injection in cats gave variable results and was without effect in guinea-pigs. On the isolated phrenic nerve-diaphragm of the rabbit there was an inhibition of neuromuscular transmission and little effect on the maximum tension on direct stimulation, and, at a higher concentration, a direct effect on the muscle.

5. The incidence of convulsions due to insulin in mice was reduced by chlorpromazine. It produced a slight hyperglycaemia and reduced a hypoglycaemia due to insulin in rabbits. Chlorpromazine had little effect on the deposition of liver glycogen in rats.

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