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RESEARCH ARTICLES

Pharmacology of Mono- and Disubstituted Chlorpromazine Metabolites

JOSEPH P. BUCKLEY▲, MARIE L. STEENBERG, HERBERT BARRY, III, and ALBERT A. MANIAN

Abstract □ Chlorpromazine and 10 of its mono- and disubstituted metabolites were investigated for their effects on the CNS of male rats and mice. All of the compounds decreased motor activity and respiration, and all but two of the compounds decreased heart rate in rats. The most potent metabolite in depressing spontaneous motor activity of mice was 3,7-dihydroxychlorpromazine, with an ED_{50} of 5.1 mg./kg. i.p. in comparison to chlorpromazine which had an ED_{50} of 2.0 mg./kg. i.p. However, the 3,7-dihydroxy derivative was approximately twice as toxic as chlorpromazine in mice. 7,8-Dihydroxychlorpromazine did not alter forced motor activity, but it did induce a dose-related depression of spontaneous motor activity; 7-hydroxy-8-methoxy derivative also produced a marked decrease in spontaneous motor activity with minimal effects on forced motor activity. None of the compounds demonstrated anticonvulsant activity, and only 7-hydroxy-chlorpromazine elicited effects suggesting possible antidepressant activity. Barbiturate sleeping time was potentiated by all of the compounds, mainly due to their CNS depressant properties.

Keyphrases □ Chlorpromazine and 10 mono- and disubstituted metabolites—effects on CNS of rats and mice □ CNS depression—effects of chlorpromazine and 10 mono- and disubstituted metabolites, rats, mice □ Motor activity, spontaneous and forced—effects of chlorpromazine and disubstituted metabolites compared, rats, mice

Fishman and Goldenberg (1) identified several metabolites of promazine and chlorpromazine in human urine including 3-hydroxyphenothiazine and 7-hydroxychlorpromazine. These and other monohydroxylated and methoxylated derivatives have been investigated and found to be pharmacologically active (2–7); it also

Table I—Experimental Compounds

Compound Number	Compound Name
I	8-Hydroxy-7-methoxychlorpromazine
II	7-Hydroxy-8-methoxychlorpromazine
III	7-Hydroxy-8-methoxy-nor$_1$-chlorpromazine
IV	7-Hydroxy-8-methoxy-nor$_2$-chlorpromazine hydrochloride
V	3,7-Dihydroxychlorpromazine
VI	7,8-Dihydroxychlorpromazine hydrochloride
VII	7-Hydroxychlorpromazine
VIII	7-Hydroxy-nor$_1$-chlorpromazine
IX	7-Hydroxy-nor$_2$-chlorpromazine
X	3-(2-Chloro-7-hydroxy-10-phenothiazinyl)-propionic acid

has been shown that some metabolites contribute to the total effect of the parent drug. The purpose of this present investigation was to evaluate several mono- and disubstituted metabolites of chlorpromazine for their possible effects on the CNS.

EXPERIMENTAL

Nine mono- and disubstituted metabolites of chlorpromazine and 3-(2-chloro-7-hydroxy-10-phenothiazinyl)propionic acid (Table I) were used.

Male Swiss-Webster mice[1], weighing 20–30 g., and Wistar descendent rats[2], 200–300 g., were used after being acclimated to laboratory conditions for 4–5 days. Each compound or solvent was usually tested in groups of 10 mice or six rats, which were permitted food and water *ad libitum*. The free bases were dissolved in sufficient 0.1 *N* HCl and then adjusted to volume with normal saline, except for Compounds IV and IX for which propylene glycol USP was used and Compound X for which 0.5 *N* NaOH was used. Compound VI, synthesized as the hydrochloride salt, was dissolved in normal saline; however, since this solution changed color almost immediately, 0.1% ascorbic acid was added to prevent oxidation. The solutions were freshly prepared and the injection volume was kept constant at 10 ml./kg. for mice or 1.0 ml./kg. for rats. The injection time was over a period of 15 sec. The results that differed from control values at $p < 0.05$ level (Student's t test) were considered to be statistically significant.

METHODS

The acute 72-hr. intraperitoneal lethal effects were determined in mice using three dose levels, and the LD_{50} was estimated using probit analysis as described by Goldstein (8). The intravenous LD_{50} of Compound VI was also determined with three dose levels.

Gross Behavior in Rats—All 10 compounds were evaluated by a gross-observation rating scale as described by Watzman *et al.* (7). The time course of the drug effect was ascertained by checking items on the scale at 15 min. prior to and 30, 60, 120, 180, and 240 min. following drug administration with special emphasis on behavioral and autonomic effects.

Spontaneous Motor Activity—The effects of the compounds on the spontaneous motor activity of mice were measured in three photocell cages[3]. Two animals treated with identical doses of the same compound were placed in each of two photocell cages 0.5–1 hr. after drug administration, and counts were recorded every 15 min. over a 1-hr. period. Each dose was tested in a factorial design in each of the three activity cages to negate the differences in sensitivity among units. Control animals were tested simultaneously in one activity cage at the same time intervals after administration of an equal volume of saline or the particular solvent used, and the ED_{50} (defined as the dose that decreased the level of performance to 50% of the control scores) was calculated for each compound.

Forced and Spontaneous Motor Activity—The effects of the compounds on forced motor activity of mice were studied using the rotarod as described by Watzman and Barry (9). The wooden rod rotated at 4 r.p.m. during the first 30 sec., at 6 r.p.m. during the next 30 sec., and at progressively increasing speeds thereafter at 30-sec. intervals until the animals fell off. Six animals were tested simultaneously and were given two trials spaced 4–6 hr. apart on each of 2 consecutive days for a total of four trials. The last trial was preceded by a 0.5–1-hr. interval before the administration of saline, solvent, or one of the selected doses of chlorpromazine or the metabolites. The drug or placebo effect for each animal was computed as the ratio of performance time on the fourth trial divided by performance time on the third trial. Ten minutes following the initiation of the fourth rotarod test, the six animals were put into three photocell activity cages as described for spontaneous motor activity and the counts were recorded for 15 min. The percentage of control activity (spontaneous and forced motor activity) at each dose level of the compounds used was plotted in an attempt to make an early evaluation of the possible type of activity of each compound.

Table II—Acute LD_{50}[a], ED_{50}, and Safety Index of the Compounds in Mice

Compounds	LD_{50}, mg./kg. i.p.	ED_{50}, mg./kg. i.p.	Safety Index, $\frac{LD_{50}}{ED_{50}}$
Chlorpromazine	119.7	2.0	59.9
I	114.6	44.5	2.6
II	130.3	27.2	4.8
III	77.8	35.4	2.2
IV	67.5	31.7	2.1
V	52.8	5.1	10.4
VI	24.8	6.6	3.8
VII	119.4	7.5	15.9
VIII	58.1	16.1	3.6
IX	48.2	13.7	3.5
X	133.7	75.3	1.8

[a] The 72-hr. LD_{50}.

Anticonvulsant Activity—The effects of the compounds on convulsions and death produced by pentylenetetrazol (100 mg./kg. s.c.) and strychnine sulfate (2 mg./kg. i.p.) were studied in mice, as described by Watzman *et al.* (7), and on maximal electroshock seizure, as described by Manian *et al.* (6). The doses of chlorpromazine and of the metabolites were identical to those used in the spontaneous activity studies.

Antireserpine Activity—Male mice, weighing 20–25 g., were used. Each experiment was carried out on five groups (five animals in each group) (10). The doses of the metabolites were identical to those used in the spontaneous motor activity studies. The animals were permitted food and water *ad libitum*, and the experiment was conducted at a temperature of 18–20° (64.5–68°F.). One hour was allowed for the animals to stabilize in the room, and then the rectal temperature was determined by inserting the thermometer[4] 2 cm. into the rectum. Five groups were used simultaneously: Groups I, II, and III received different doses of chlorpromazine or a metabolite; Group IV received desipramine (desmethylimipramine) (20 mg./kg. i.p.); and Group V received saline (10 ml./kg. i.p.). One hour following treatment, the rectal temperature was again determined and the animals were examined for development of ptosis, diarrhea, and change in motor activity. Reserpine (1 mg./kg. i.p.) was then administered, and 4 hr. later the rectal temperature was obtained and alterations in ptosis, diarrhea, and motor activity were noted using the saline group as the control. Hypothermia was calculated in terms of the decrease in rectal temperature from the individual control value of each mouse. The control value was the temperature recorded after chlorpromazine or a metabolite was administered, since these compounds were found to decrease body temperature.

Barbiturate Sleeping Time—One hundred and twelve male mice, 20–24 g., were used. Half of these (56 animals) were divided into 14 groups of four animals each. Each group received normal saline (10 ml./kg.), a dose of chlorpromazine, or one of five test compounds intraperitoneally. Thirty to sixty minutes after administration of the drug, either sodium hexobarbital (100 mg./kg. i.p.) or sodium bar-

[1] Carworth Laboratories.
[2] Hilltop Laboratories.
[3] Woodard Research Corp.
[4] Tele-thermometer, Yellow Spring Instrument Co.

bital (300 mg./kg. i.p.) was injected. The onset of sleeping time (loss of righting reflex) and the length of sleeping time (recovery from immobility) were determined. Sleeping time is defined as the interval between the loss and return of the righting reflex. The criterion for the loss and return of the righting reflex is defined as the inability and ability, respectively, of the animal to right itself within 5 sec. in three successive trials when placed on its back. The test was terminated if the mouse continued to sleep 4 hr. after the loss of the righting reflex.

On the following day the study was repeated with the remaining five test compounds using the remaining 56 mice. The 2-day experiment was replicated so that a total of eight mice received one dose of each compound. Each metabolite was tested for effects on sodium hexobarbital sleeping time at three dose levels and on sodium barbital sleeping time at two dose levels in mice. Since sodium barbital is not metabolized by the liver microsomal enzymes, these data can distinguish between effects on the CNS *per se* and inhibition or enhancement of liver microsomal enzyme activity.

Since mice receiving the highest doses of chlorpromazine and of some of its derivatives did not recover their righting reflex in less than 4 hr., the problem was approached by using the HD_{10} of barbital (11). Male mice, in groups of 10, received various doses of sodium barbital intraperitoneally in order to estimate the HD_{10} of barbital. HD_{10} is defined as the dose of sodium barbital estimated to cause 20% of the mice to lose their righting reflex for at least 5 min. during the 1st hr. following the injection of sodium barbital. The intraperitoneal HD_{10} of sodium barbital was 147.9 mg./kg., determined according to the method of Litchfield and Wilcoxon (12). Male mice, 20–24 g., in groups of eight received the highest dose of either chlorpromazine or one of the 10 metabolites 30–60 min. prior to the administration of the HD_{10} of sodium barbital.

RESULTS

Gross Behavior—Chlorpromazine and its metabolites decreased motor activity and rate of respiration within 30 min. Heart rate was decreased by all compounds except III (7-hydroxy-8-methoxy-nor$_1$-chlorpromazine) and VIII (7-hydroxy-nor$_1$-chlorpromazine). The pupil was slightly dilated by all compounds except IX. Body temperature was decreased by all compounds except V, and ptosis occurred with all of the phenothiazines except III and V.

The intraperitoneal 72-hr. LD_{50}, ED_{50} (effects on spontaneous motor activity), and the safety index (LD_{50}/ED_{50}) in mice are summarized in Table II. Compound VI was the most toxic compound tested on a milligram per kilogram basis; however, Compound X had the lowest safety index and, therefore, the lowest safety range. As can be seen in Table II, chlorpromazine was more potent on a milligram per kilogram basis in depressing spontaneous motor activity than the metabolites and had a greater safety index than any of the compounds investigated. The 7-hydroxy (Compound VII) and 3,7-dihydroxy (Compound V) derivatives had the highest safety indexes of the metabolites investigated, having indexes of 15.9 and 10.4, respectively.

Table III—Effects of the Experimental Compounds on Spontaneous Motor Activity in Mice[a]

Compound	Dose, mg./kg. i.p.	Percent of Control	Dose, mg./kg. i.p.	Percent of Control	Dose, mg./kg. i.p.	Percent of Control
Chlorpromazine	1	87.0	2	53.8	4	5.2[b]
I	20	122.5	40	68.2	60	11.5[b]
II	13	101.6	26	50.0[b]	39	15.9[b]
III	16	97.2	32	59.4	48	23.4[b]
IV	12.5	85.1	25	61.7	50	30.3[b]
V	5	49.6	10	32.5[b]	20	11.8[b]
VI	3	90.2	5	66.7	10	28.5[b]
VII	6.25	63.8	9	34.1	12.5	9.0[b]
VIII	12	78.9	18	32.0[b]	24	22.8[b]
IX	5	110.4	10	47.1	30	17.3[b]
X	40	94.6	60	87.3	80	37.8[b]

[a] The 0.5-hr. counting time. [b] Significantly different from saline-treated control groups ($p < 0.05$).

Table IV—ED_{50} for Spontaneous Motor Activity Data for 0.25-hr. Test Shown in Figs. 1 and 2 and 10 min. following the Test of Forced Motor Activity

Compound	ED_{50}, mg./kg. i.p.	95% Confidence Limit
Chlorpromazine	1.9	1.3–2.4
I	47.2	28.3–78.9
II	22.2	15.4–31.9
III	28.8	19.7–42.3
IV	53.2	30.0–94.4
V	8.5	4.8–15.1
VI	11.6	5.2–25.7
VII	8.2	4.9–13.9
VIII	27.6	19.7–38.6
IX	24.6	12.0–50.4
X	197.2	95.3–408.3

Spontaneous Motor Activity—As seen in Table III, almost all doses of the 10 compounds exhibited a dose-dependent depressant effect on spontaneous motor activity as measured in the photocell activity cage.

Forced and Spontaneous Motor Activity—Figures 1 and 2 show the comparative effects of chlorpromazine and the 10 experimental compounds using data in which effects on spontaneous activity were investigated 10 min. following initiation of the rotarod test. Chlorpromazine clearly decreased motor coordination in doses of 1, 2, and 4 mg./kg. (Fig. 1), whereas much higher doses of the experimental compounds were needed to produce detrimental effects on forced and spontaneous motor activities. Most of the compounds had a greater detrimental effect on spontaneous than on forced motor activity. In particular, Compounds II, III, and VI decreased spontaneous motor activity with minimal effects on forced motor activity. However, Compounds V, VIII, and IX produced similar magnitudes of effect on both forced and spontaneous motor activities, suggesting maximal effects on cerebral cortical activity. Compound X did not significantly alter either forced or spontaneous motor activity under these experimental conditions, even though it did produce some decrease in spontaneous motor activity in the initial studies (Table III). This difference in activity could possibly be due to stimulating effects induced during the rotarod portion of this phase of the study, although the ED_{50}'s for depression of spontaneous motor activity in this phase of the study (Table IV) were similar for most of the compounds to those obtained earlier (Table II).

Anticonvulsant Activity—None of the compounds investigated protected the animals from convulsions produced by pentylenetetrazol (100 mg./kg. s.c.), strychnine sulfate (2 mg./kg. i.p.), or maximal electroshock.

Antireserpine Activity—Since chlorpromazine and its derivatives decreased body temperature, the rectal temperature was measured after the administration of the test compounds and again 4 hr. following the administration of reserpine. Compound VII was the only experimental compound that significantly counteracted the decrease in body temperature due to reserpine (Table V), and then the difference was significant only at the lowest dose used, 6.25 mg./kg. i.p. Two compounds, IV and V, significantly potentiated the hypothermic effect of reserpine (Table VI). None of the 11 compounds tested showed any effect on the reserpine-induced ptosis, and only des-

Table V—Antagonism of Hypothermic Effects of Reserpine (1 mg./kg. i.p.) by Compound VII

Drugs	Dose, mg./kg. i.p.[a]	N[b]	Δ Temperature[c], $\bar{X} \pm SE$	p
Saline	10 ml./kg.	5	3.62 ± 0.34°	—
Desmethylimipramine	20	5	1.32 ± 0.26°	<0.001
VII	6.25	5	2.10 ± 0.54°	<0.025
VII	9.00	5	2.68 ± 0.53°	<0.10
VII	12.50	5	2.74 ± 0.49°	<0.10

[a] Administered 1 hr. prior to reserpine. [b] Groups of five mice each. [c] Decrease in rectal temperature 4 hr. after the administration of reserpine.

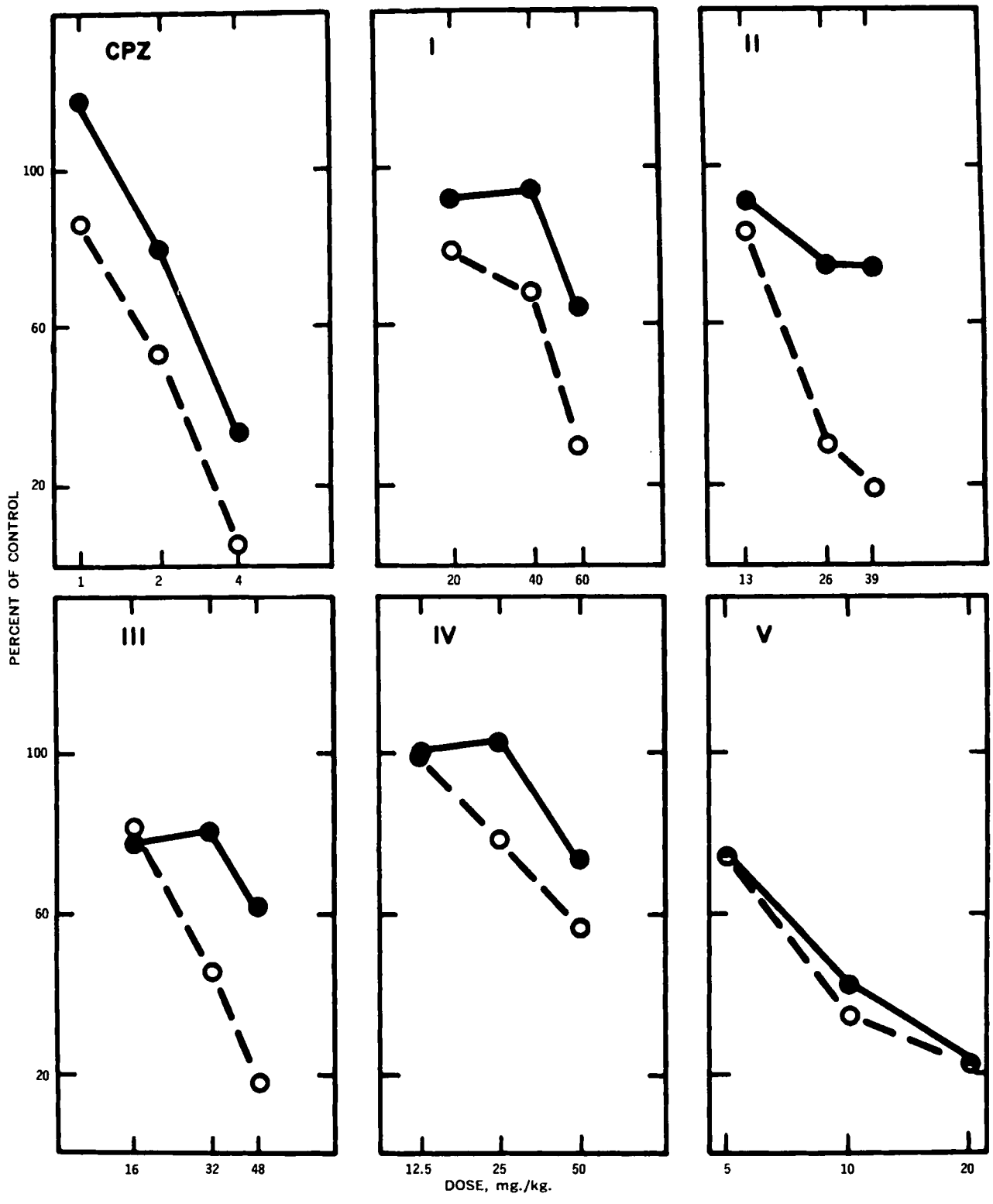

Figure 1—*Effects of chlorpromazine and Compounds I–V on spontaneous* (○) *and forced* (●) *motor activities in mice.*

ipramine was capable of blocking this effect. All animals showed a decrease in motor activity, and diarrhea developed in all mice except those receiving desipramine.

Barbiturate Sleeping Time—Only chlorpromazine and Compounds VII and VIII significantly increased hexobarbital sleeping time in the lowest dose used (Fig. 4). The low doses of Compounds VII and VIII increased hexobarbital sleeping time from 38.6 ± 1.5 to 52.0 ± 7.3 min. (34.7%) and from 38.6 ± 1.5 to 57.9 ± 5.1 min. (50.0%), respectively. In the medium dose range, all compounds except III, V, VI, and X increased sleeping time whereas the high doses of all compounds produced a significant increase in the sleeping time; however, chlorpromazine was by far the most potent of the compounds tested (Figs. 3 and 4).

In medium doses, chlorpromazine and Compounds III, VI, VII, VIII, and IX produced a significant increase in the duration of barbital sleeping time. The mean sleeping time of sodium barbital in the saline-treated animals ranged from 65.0 ± 4.8 to 88.3 ± 13.2 min. Table VII shows that when the HD_{20} dose of sodium barbital was given to mice that were pretreated 30–60 min. prior to the administration of sodium barbital with the high dose of either chlor-

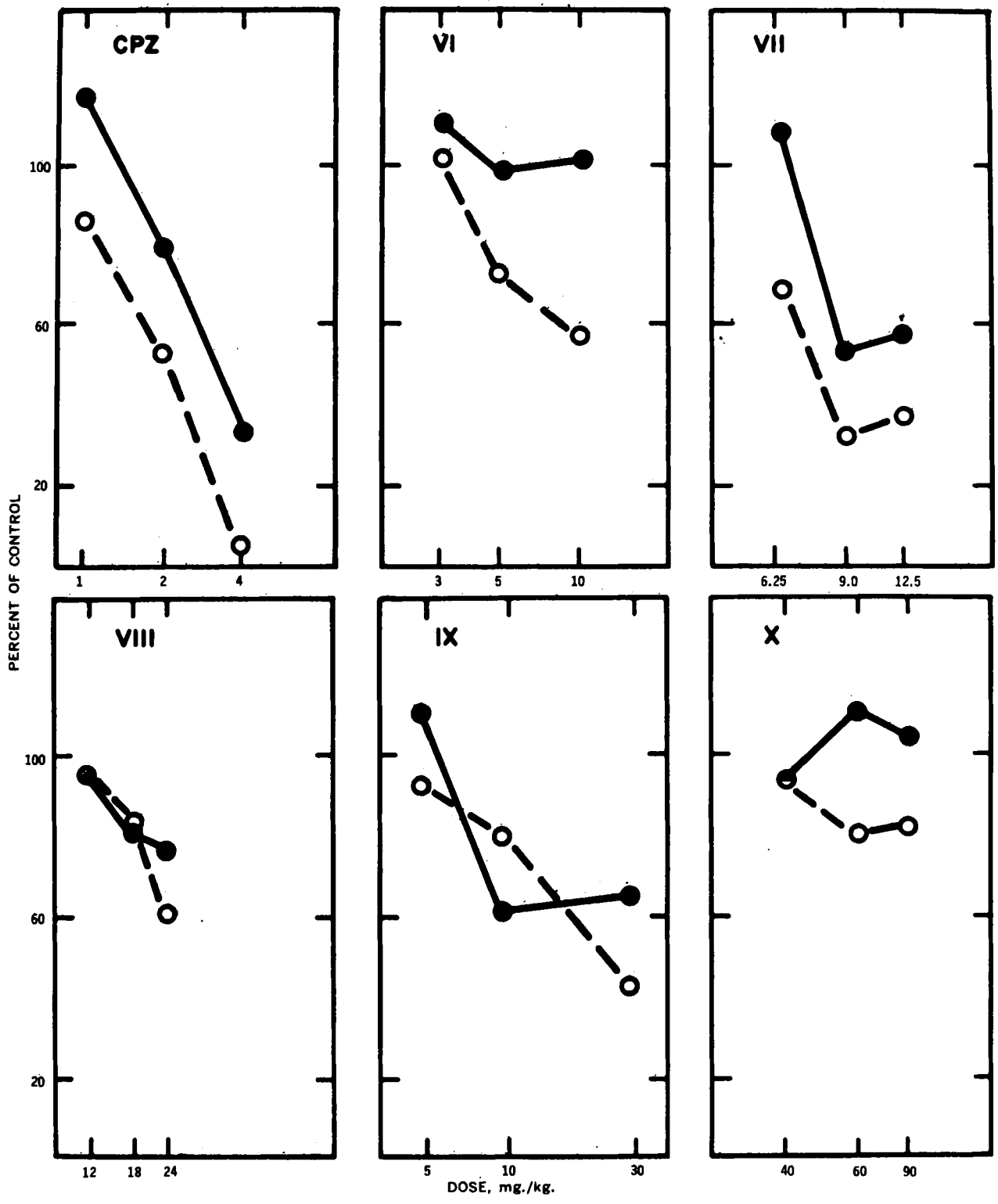

Figure 2—*Effects of chlorpromazine and Compounds VI–X on spontaneous* (○) *and forced* (●) *motor activities in mice.*

promazine or one of its 10 metabolites, all but Compound X potentiated the sedative action of sodium barbital; however, Compounds VII and VIII produced only a slight increase in the number of animals losing their righting reflex.

DISCUSSION

Chlorpromazine and 10 mono- and disubstituted metabolites were studied in a battery of tests designed to compare their effects on the CNS. The tests used were: gross behavior, spontaneous motor activity, forced motor activity, chemoshock antagonism, maximal electroshock seizures, antireserpine activity, and barbiturate sleeping time.

Chlorpromazine was by far the most potent compound in inhibiting spontaneous motor activity (ED_{50} 2.0 mg./kg. i.p.). Since the LD_{50} of chlorpromazine was higher than most of the metabolites and the safety index was 3.8–33.2 times greater than the metabolites investigated, it would appear that the metabolites not only are less

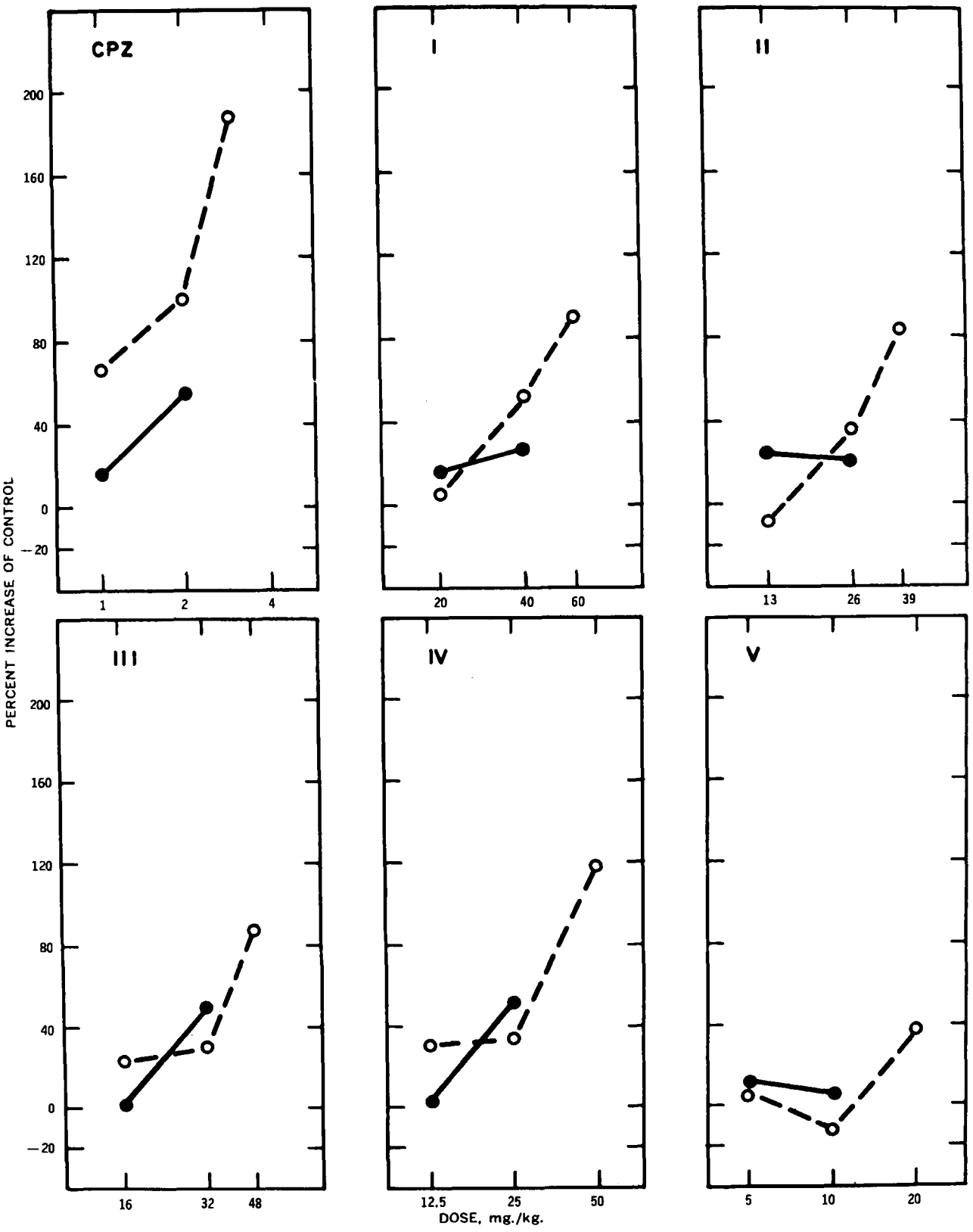

Figure 3—*Effects of chlorpromazine and Compounds I–V on hexobarbital* (○) *and barbital* (●) *sleeping time in mice.*

effective in depressing CNS activity but also are more toxic. Although Compound VI was extremely toxic, Compound II had an LD_{50} 5 times greater than that of Compound VI. Therefore, the replacement of the 8-hydroxy by an 8-methoxy group markedly decreased the toxicity of the compound; however, it still took a dose of Compound II approximately 5 times greater to equal the depressant effects produced by Compound VI. Compound V produced approximately equivalent dose–response effects on spontaneous and forced motor activities, suggesting maximal effects on cerebral cortical activity, whereas Compounds II, IV, and VI produced marked effects on spontaneous motor activity with minimal or no effects on forced motor activity. Compound VII had the highest

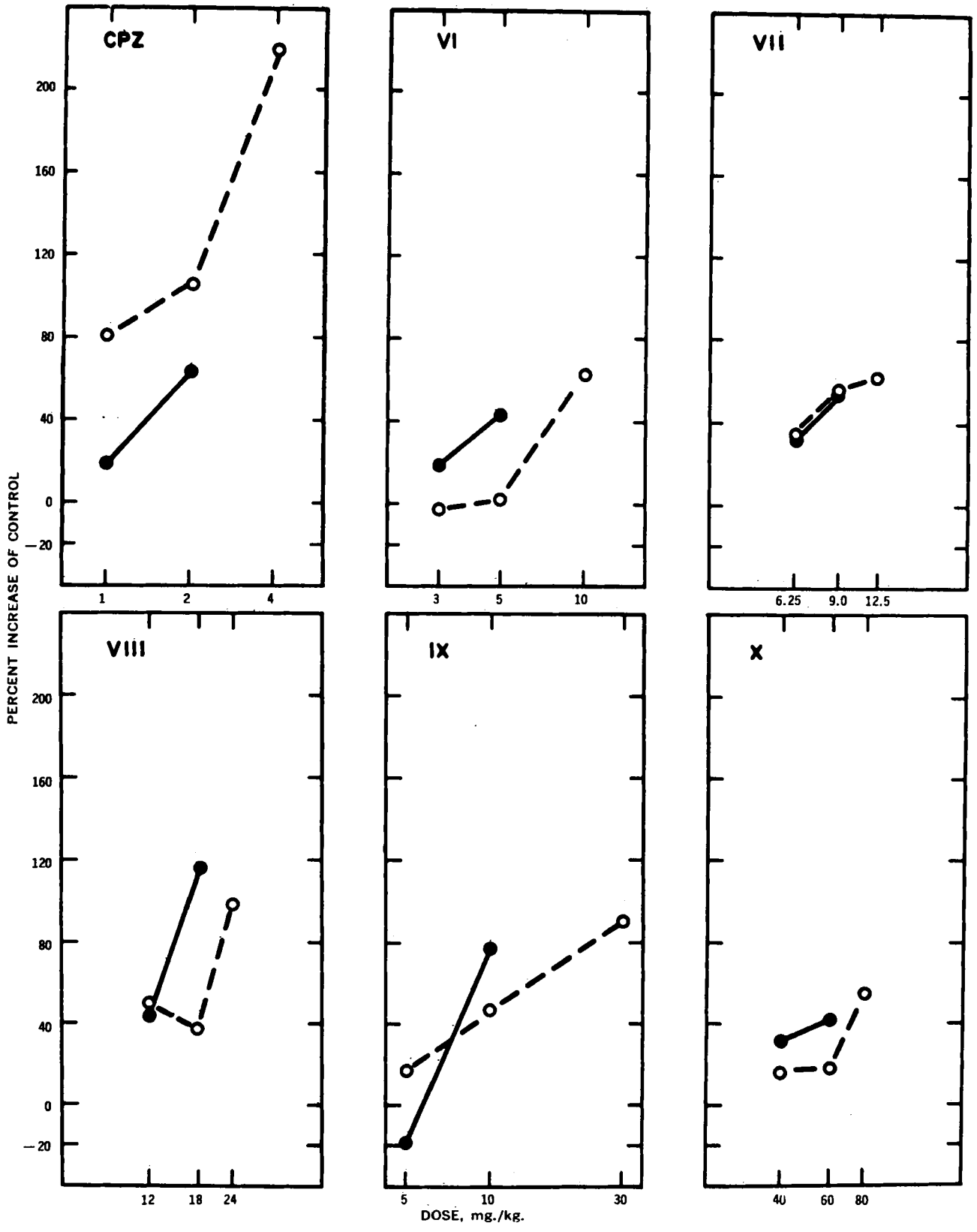

Figure 4—*Effects of chlorpromazine and Compounds VI–X on hexobarbital* (○) *and barbital* (●) *sleeping time in mice.*

safety index of the metabolites studied; however, it was slightly less than one-third as effective as chlorpromazine in inhibiting spontaneous motor activity, whereas the LD_{50}'s of the two compounds were approximately equal. The data obtained in this portion of the study suggest that the metabolites are less efficient in depressing CNS activity and more toxic than chlorpromazine.

None of the phenothiazine compounds investigated protected the animals from convulsions produced by pentylenetetrazol, strychnine sulfate, or maximal electroshock seizures.

Only Compound VII showed significant antireserpine activity in the low dose tested, and Compounds IV and V showed a significant potentiation of the effect of reserpine in decreasing rectal tempera-

Table VI—Potentiation of Hypothermic Effects of Reserpine (1 mg./kg. i.p.) by Compounds IV and V

Drugs	Dose, mg./kg. i.p.[a]	N[b]	Δ Temperature[c], $\bar{X} \pm SE$	p
Saline	10 ml./kg.	5	4.02 ± 0.26°	—
Desmethylimipramine	20	5	1.62 ± 0.17°	<0.001
V	20	5	5.48 ± 0.39°	<0.01
IV	50	5	5.58 ± 0.25	<0.01

[a] Administered 1 hr. prior to reserpine. [b] Groups of five mice each. [c] Decrease in rectal temperature 4 hr. after the administration of reserpine.

ture. No conclusion can be drawn from these data as to the effects of the dihydroxy and methoxy substitution to the chlorpromazine molecule as related to potential antidepressant activity. Halliwell *et al.* (13) tested imipramine, desipramine, chlorpromazine, and compounds structurally related to chlorpromazine. They found that while imipramine and its derivative markedly antagonized the reserpine-induced mydriasis and ptosis, chlorpromazine did not demonstrate these effects; thus their results were similar to the present findings.

The compounds affected sleeping time induced by both sodium hexobarbital, which is oxidatively metabolized by the hepatic microsomal enzymes (14), and sodium barbital, which is excreted unmetabolized (15). There were quantitative differences in the effects of each compound on the degree of potentiation of hexobarbital and barbital sleeping time. For example, if the effects of the medium dose of the compounds were compared, chlorpromazine and Compounds I and II had a greater effect on hexobarbital sleeping time; Compounds III, IV, VI, VIII, IX, and X had a greater effect on barbital sleeping time; Compound VII produced equivalent effects on both barbiturates; and Compound V produced a slight decrease in hexobarbital sleeping time without significantly affecting barbital activity. The enhancement of sleeping time induced by barbital, which is not metabolized, suggests that in those instances where the compounds potentiated hexobarbital, the activity was due to a direct depressant effect on the CNS and not merely to inhibition of hexobarbital metabolism. Compound V, the compound that had the lowest ED_{50} of the metabolites investigated (using effects on spontaneous motor activity as the assay procedure), produced minimal effects on barbital sleeping time in both doses used, increasing hexobarbital sleeping time only 6.5% with the low dose (5 mg./kg. i.p.) and 38.6% with the high dose (20 mg./kg. i.p.).

Compound X, in which the 10-[3-(dimethylamino)propyl] group of chlorpromazine is replaced by propionic acid, was the least active compound tested.

The inhibitory effects of Compound VII on spontaneous and forced motor activity, its relatively high safety index, and its antireserpine activity suggest that the metabolite may have potential utility as a therapeutic agent.

Table VII—Percentage of Mice Losing the Righting Reflex after Intraperitoneal Administration of Sodium Barbital (147.9 mg./kg.) at 30–60 min. after Intraperitoneal Injection of Chlorpromazine or the Experimental Compound

Compound	Dose, mg./kg.	Percent Losing Righting Reflex
Chlorpromazine	4	88
I	60	88
II	39	100
III	48	75
IV	50	75
V	20	75
VI	10	75
VII	12.5	38
VIII	24	38
IX	30	88
X	80	13
Saline	10 ml./kg.	25

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