Tolerance of the depressant effect with a moderate dose of chlordiazepoxide.

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Tolerance of the Depressant Effect with a Moderate Dose of Chlordiazepoxide.

A Thesis Presented
by
Timothy L. Ralph

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TOLERANCE OF THE DEPRESSANT EFFECT WITH A MODERATE DOSE OF CHLORDIAZEPoxide.

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September, 1970
ABSTRACT

Two opposing effects on behavior, a depressant effect and a disinhibitory effect, have been attributed to the benzodiazepine tranquilizers including chlordiazepoxide. The depressant effect has been reported to undergo tolerance with repeated doses over several days. The first experiment here was designed to determine the number of days required for tolerance at the 15 mg/kg dose. When no tolerance was found in a test of spontaneous activity, further studies were conducted to determine why no tolerance had occurred and to study conditions under which tolerance might be found.

The second study found that, using the 15 mg/kg per day dose, the spontaneous activity rates of chronically treated subjects were lower than those of control subjects, and were not higher than those of acutely treated subjects. With a dose of 100 mg/kg per day, chronically treated animals still had lower activity rates than controls, but higher rates than acutely treated subjects. This would indicate some tolerance of the depressant effect at the larger dose.

The third study, investigating latency in one-way avoidance behavior under chronic and acute administration of CDP, found no differences between drug-naive and drug-sophisticated groups.

The fourth study incorporated a two-way avoidance task which presented a conflict situation. Two measures were used, latency to respond, and number of conditioned avoidance responses (CAR's) per session. Control subjects had the shortest latencies, but made the fewest CAR's. The latencies of the drug groups did not differ significantly from each other except for the second day when the drug-sophisticated
group was faster. The drug-naive group, however, eventually made the highest number of CAR's.

Possibly the CDP enabled some of the drug-naive animals to overcome the tendency to freeze allowing them to learn the appropriate avoidance response.

The results of these four studies indicate that with the 15 mg/kg dose there is no tolerance of the depressant effect of CDP in a spontaneous activity situation or in a one-way avoidance task. There was some tolerance at the very large dose used by those investigators which have reported tolerance. In the two-way avoidance task the results indicated some tolerance of the effect where the action of CDP was apparently to reduce the value of the negative consequences of the conflict situation.
ACKNOWLEDGEMENTS

The author wishes to express gratitude to the members of his thesis committee, Dr. Neil R. Carlson, Dr. Robert S. Feldman, and Dr. Jay A. Trowill, for their advice and guidance in the completion of this research. I am particularly obliged to my wife Hannelore, who provided the often necessary impetus in writing this thesis, and who patiently typed the several drafts.
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INTRODUCTION

Benzodiazepine tranquilizers, including chlordiazepoxide (CDP), diazepam, oxazepam, etc., have been said to have two opposing effects on behavior - a depressant effect and a disinhibitory effect. Margules and Stein (1968) have defined the disinhibitory effect as a facilitation of the tendency to respond, especially if the behavior is under some inhibitory suppression such as satiety, punishment, or non-reinforcement, while the depressant effect has been described as a reduction in the tendency to respond.

The depressant effect reportedly undergoes tolerance with repeated doses over several days (Goldberg, Manian, and Efron, 1967; Margules and Stein, 1968), i.e., the subject develops the ability to resist the effects of continued doses of the drug (Goodman and Gilman, 1965). Other investigators, citing these studies, have accepted as fact that, after three or four days of treatment, the depressant effect of CDP is tolerated and may thereafter be ignored (e.g., Stein and Berger, 1969).

A possible metabolic basis for this tolerance was reported by Hoogland, Miya, and Bousquet (1966). They found that rats, which had been given 50 mg/kg injections of CDP twice daily for five days, i.e., 100 mg/kg per day, showed increased rates of tissue disappearance and excretion of \(^{14}C\)-labeled CDP.

In a detailed study of tolerance of the depressant effect Goldberg et al., (1967) used several behavioral tests, one of which was the measurement of the rate of spontaneous activity, to compare the performance of chronically dosed, drug-sophisticated animals with that of acutely dosed, drug-naive subjects. They gave their rats a fourteen day
pretreatment period and then tested them on the fifteenth day. The "Acute Single" rats were injected with physiological saline twice each day for the pretreatment period. Then, 60 minutes before being tested, they received a 50 mg/kg injection of CDP. "Acute Double" rats received the same treatment except that they received 100 mg/kg of CDP before being tested. "Chronic + Acute Single" rats received 50 mg/kg injections of CDP twice daily during the pretreatment period, and then received a 50 mg/kg CDP injection on the test day. The "Chronic + Acute Double" rats were given the twice-daily pretreatment injections of 50 mg/kg CDP, but on test day they received a 100 mg/kg dose before being tested. They found "several instances of tolerance to the depressant actions of CDP," and reported that this tolerance was evident in all the tests used, (Goldberg et al., 1967).

The first study reported here was designed to determine the course of tolerance of the depressant effect of CDP using a spontaneous activity test (one of the tests used by Goldberg et al., 1967), but with the commonly reported 15 mg/kg dose (Feldman, 1962; Feldman, 1968; Sachs, Weingarten, and Klein, 1966; Gandelman and Trowill, 1968). When the expected tolerance did not appear, further studies were conducted to ascertain why the results contradicted previous reports, and to establish the circumstances under which tolerance might occur.
EXPERIMENT I

Daily measurement of spontaneous activity should show the time required for rats which were initially drug-naive to tolerate the depressant effect produced by moderate daily injections of CDP. At the start of the study the activity rate for animals treated with CDP shortly before each test session was expected to be relatively low, and relatively high for subjects which were not receiving the drug. Then, after a few days of testing, the higher scores of the undrugged groups were expected to drop due to habituation to the task, while the depressed rate of the drug group was expected to increase, showing tolerance to the depressant effect. If the tolerance were complete, the activity rates of the drugged and non-drugged groups should thereafter be indistinguishable.

Method

Subjects. Subjects were twenty naive male Charles River albino rats, weighing about 450 grams. The animals were housed in individual cages and had food and water available ad lib.

Apparatus. The test cage for this study of spontaneous activity was a galvanized steel cylinder, 12" in diameter, 8" deep, with a wire-mesh floor. A photoelectric cell was mounted on the outside of the cage in a position such that the light beam bisected the cage 2" above the floor. Each time that the beam was broken a count was registered.

Procedure. The subjects were separated into four treatment groups of five rats each. In the first stage which lasted for twelve days,
animals of these groups were tested under one drug schedule, then, after one day of no testing, the treatment schedules were switched, and the subjects were tested for another six days.

In Stage I the subjects in the Drug-Before/Saline-Before group (DB/SB), were given a 15 mg/kg i.p. injection of CDP in sterile water solvent 30 minutes prior to the start of a one-hour activity test session. In the second stage these subjects each received an injection of saline before the test sessions. During the first stage members of the Drug-After/Drug-Before group (DA/DB), were given the same dose of CDP, but at the end of their activity test session, i.e., after being removed from the activity cage. After the switch these subjects received the 15 mg/kg injection of CDP one-half hour before the test session. Subjects in the Saline-Before/Drug-Before group (SB/DB) received injections of physiological saline 30 minutes prior to the test sessions in the first stage, but in the second stage they were given injections of CDP before testing. Subjects in the Saline-After/Saline-Before group (SA/SB) received injections of saline at the end of their test sessions in Stage I, and before the test sessions in Stage II.

All injections were administered at the animals' home cages. Subjects were tested singly, and at the same time each day. During the test sessions the room lights were left on, and the experimenter was out of the room.
Table 1. CDP and Saline treatment groups. Stage I lasted for twelve days. Then, after one day of no testing, Stage II extended for another six days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Stage I</th>
<th>Stage II</th>
</tr>
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<tbody>
<tr>
<td>DB/SB</td>
<td>15mg/kg CDP, before the session</td>
<td>Saline, before the session</td>
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<tr>
<td>DA/DB</td>
<td>15mg/kg CDP, after the session</td>
<td>15mg/kg CDP, before the session</td>
</tr>
<tr>
<td>SB/DB</td>
<td>Saline, before the session</td>
<td>15mg/kg CDP, before the session</td>
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<tr>
<td>SA/S3</td>
<td>Saline, after the session</td>
<td>Saline, before the session</td>
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</table>
Results

In the first stage the activity rates of the "Drug-After", "Saline-Before", and "Saline-After" groups were expected to start out relatively high, while the rate of the "Drug-Before" group was expected to be relatively low. Then, after four or five days the higher scores of the first three groups were expected to decrease due to habituation to the test apparatus, while the depressed rate of the Drug-Before group was expected to increase, showing tolerance to the depressant effect of CDP. In the second stage, after the switch, with CDP administered prior to the test sessions, the DA/DB group, with twelve days of drug experience, should show no activity decrement, while the scores of the drug-naive SB/DB subjects were expected to drop significantly.

The results of these tests are shown in Fig. 1. The curves on the left of the figure show that groups which were not drugged during the test sessions showed high activity levels which decreased markedly through the fourth day and then returned to a relatively high level. The DB/SB subjects, which in Stage I received CDP prior to each activity session, started out with lower rates which were maintained approximately throughout the first stage.

The median activity counts of the groups, summed across the twelve days of Stage I were 1524 for group DA/DB, 1790 for group SB/DB, and 1554 for group SA/SB. The Drug-Before (DB/SB) group count was 660. The difference between the activity rate of the DB/SB group and each of the other groups, using the two-tailed Mann-Whitney U test, was significant at p < .004.
Fig. 1. Median spontaneous activity rates of the four treatment groups in Experiment I.
The expected decline due to habituation to the test situation was seen in the activity rates of the three groups which were undrugged during the test sessions. The observation that the Drug-Before group activity rate never deviated from the low level of the first few days was, however, unexpected. The findings of Goldberg et al. and Margules and Stein would have predicted that this depressed rate should show a consistent rise, demonstrating tolerance.

At the start of Stage II the activity rate of the DB/SB group which had been depressed throughout the first stage rose to the same high level of the other three groups at the onset of Stage I. This would indicate that either there had been no habituation during the first stage on the part of this group, or that any first stage habituation was dependent upon drug-produced cues. The activity rate of the SA/SB group remained at approximately the level maintained during the first stage.

Comparison of the Stage II performance of the drug-sophisticated DA/DB group with that of the drug-naive SB/DB group provides further indication of a lack of tolerance. During the second stage subjects of both groups received injections of CDP 30 minutes prior to their activity test sessions. Had the depressant effect been tolerated the DA/DB group would have had a significantly higher activity level than the SB/DB group. The activity levels, as seen on the right side of Fig. 1 were uniformly low, and nearly identical. Thus, with a 15 mg/kg dose, rats in a spontaneous activity situation do not show tolerance to the depressant effect of CDP.
EXPERIMENT II

There were several differences in the method used by Goldberg et al. and that used in Experiment I. They housed two rats per cage while the rats in this study were housed individually. Their subjects were food-deprived for 16-18 hours prior to the administration of the test dose while rats used here were given food ad lib. Also, they used saline as solvent for the drug whereas sterile water was used in this study.

A more significant difference between the two studies was that Goldberg et al. used a pretreatment period to establish differences in drug-sophistication, i.e., the animals were tested in the activity situation only once, while rats in this study had daily activity sessions throughout the study. Also, there were large differences in the dose sizes. Goldberg et al. used the heavy dose of 100 mg/kg per day compared with the more moderate 15 mg/kg used in this experiment - a dose commonly reported in studies using CDP.

This second study was designed to investigate the possibility that the difference in dose size was responsible for the apparent contradiction between Goldberg's findings and the results of Experiment I just reported.

Two sets of groups were used. Each set included a chronic group, an acute group, and a saline control group. In one set the subjects received a single injection per day of either 15 mg/kg CDP or physiological saline. In the other set all subjects were given two injections per day of either physiological saline or 50 mg/kg CDP. The chronic group of each set corresponded with Goldberg's "Chronic + Acute Single" group, the acute group with their "Acute Single" group.
Method

**Apparatus.** The apparatus used in this study was the same as that used in Experiment I.

**Subjects and Procedure.** Thirty naive rats from the same population used in Experiment I were separated into six groups of five rats each. As seen in Table 2, subjects in the Control-15 group received an i.p. injection of physiological saline once a day for a fourteen-day pretreatment period. On the 15th or test day, they received another saline injection 30 minutes before their individual test sessions. The Acute-15 group subjects received the same pretreatment, but on test day, half an hour before the hour-long activity test session each subject received a 15 mg/kg i.p. injection of CDP dissolved in physiological saline. Subjects in the Chronic-15 group received the 15 mg/kg dose of CDP each day of the pretreatment period, and then were given the same injection thirty minutes before their activity test session.

Rats of the Control-100 group were administered two injections of saline, separated by at least six hours, each day of the pretreatment period. Then on test day they received a single injection of saline prior to being tested. The Acute-100 group subjects were given the same saline pretreatment as the Control-100 group, but before the activity test they received a 50 mg/kg dose of CDP. Subjects of the Chronic-100 group received 50 mg/kg injections of CDP, twice a day, i.e., 100 mg/kg per day, for the fourteen days, and then received a single dose of 50 mg/kg prior to the test sessions.

During this study three animals died, and the data of a fourth was unusable. The dead animals included one from the Control-15 group.
Table 2. Treatment groups in Study II.

<table>
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<tr>
<th>Group</th>
<th>Pretreatment</th>
<th>Test Treatment</th>
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<tr>
<td>Control 15</td>
<td>Saline, 1/day for 14 days</td>
<td>Saline, 1/2 Hr. before test</td>
</tr>
<tr>
<td>Acute 15</td>
<td>Saline, 1/day</td>
<td>15mg/kg CDP, 1/2 Hr. before test</td>
</tr>
<tr>
<td>Chronic 15</td>
<td>15mg/kg CDP, 1/day (&quot;&quot;&quot;)</td>
<td>15mg/kg CDP, 1/2 Hr. before test</td>
</tr>
<tr>
<td>Control 100</td>
<td>Saline, 2/day</td>
<td>Saline, 1/2 Hr. before test</td>
</tr>
<tr>
<td>Acute 100</td>
<td>Saline, 2/day</td>
<td>50mg/kg CDP, 1/2 Hr. before test</td>
</tr>
<tr>
<td>Chronic 100</td>
<td>50mg/kg CDP, 2/day (&quot;&quot;&quot;)</td>
<td>50mg/kg CDP, 1/2 Hr. before test</td>
</tr>
</tbody>
</table>
which had a greatly swollen head, and two rats of the Chronic-100 group, one of which appeared to have some intestinal blockage, while the other had no immediately apparent symptoms. This last subject expired after receiving the first 50 mg/kg injection on the first day of the study so a replacement was possible. In the Chronic-15 group, one subject developed the symptoms of an inner ear infection so its data, although near the median, were not included in the results.

Results

The median spontaneous activity rates of these groups are shown in Fig. 2. The rate of the Control-15 group (171) was significantly higher (p < .02 U test) than that of either the Chronic-15 group (53.5) or the Acute-15 group (26). The difference between the Chronic-15 and Acute-15 groups was not significant (p = .143 U test).

The median activity rate of the Control-100 group (195) was also significantly greater (p < .01 U test) than that of either the Chronic-100 group (102.5) or the Acute-100 group (52). However, the rate of the Chronic-100 group was significantly higher (p < .04 U test) than that of the Acute-100 group. Therefore, it appears that there was some degree of tolerance of the depressant effect of CDP at the 100 mg/kg per day dose.

These results show that rats treated chronically for fourteen days with very heavy doses of CDP have slightly higher rates of spontaneous activity than do acutely dosed, drug-naive rats. These drug-sophisticated animals were, however, significantly less active than saline controls. With the commonly reported 15 mg/kg dose of CDP the chronically
Fig. 2. Comparisons of the median spontaneous activity rates of Control, Chronic, and Acute groups at the moderate and high levels of CDF. Vertical bars indicate range.
drugged animals are not only significantly less active than controls, but are not significantly more active than the acutely dosed, drug-naive animals.
EXPERIMENT III

The first two studies reported here have shown that tolerance of the depressant effect of CDP depends considerably upon the size of the dose. It was also found that the depressant effect of a moderate dose of CDP does not undergo tolerance in a spontaneous activity situation, i.e., one with no extrinsic motivation. To determine whether such motivation would affect the development of tolerance this third experiment was conducted studying the depressant effect in a one-way avoidance situation.

Method

Apparatus. The apparatus consisted of a masonite box $2^{1/4}$" square and $2^{1/4}$" deep with a grid floor. The box was partitioned into two compartments of equal size. In the center of the partition, at floor level, was a $4$" square guillotine door. In order to maximize discriminability the start compartment was painted black while the goal side was painted white.

Subjects and Procedure. As in the other experiments, subjects were naive male Charles River albino rats of about 450 grams each. For this study fifteen rats were separated into three groups of five rats each. Subjects in the SS (Saline-Saline) group, and the SD (Saline-Drug) group received injections of physiological saline i.p. after each daily training session. The DD (Drug-Drug) group subjects were administered 15 mg/kg injections of CDP in physiological saline i.p. after each daily session.

To learn the avoidance task the subjects were given five training
trials per day for ten days. Each trial began with a one-minute wait in the start compartment. This allowed the animal to quiet down, and to orient itself in whatever way it wanted - usually toward the escape door. After the wait, the beginning of the timed trial was marked by the onset of a 1000 cps tone and the opening of the guillotine door. For the first five days of training the CS was a loud buzzer, but since the buzzer seemed to be aversive, and it was feared that escape from the buzzer might obscure shock avoidance, the tone was substituted beginning with the sixth day.

After a 5-second interstimulus interval (ISI), a 2.5 mA current from a shock scrambler was delivered to the grid floor. The shock and tone were terminated at the moment the rat left the start compartment. The guillotine door was closed gently a few seconds after the animal entered the goal compartment. After each crossing the animal was allowed to remain in the safe compartment for about two minutes before being removed to the start side, beginning a new trial.

On the eleventh day of the study each rat was given an injection thirty minutes prior to the start of that animal's test session. Subjects of group SS again received physiological saline. DD group subjects, now ten days drug-sophisticated, again received a 15 mg/kg injection of CDP, but this time the drug-naive SD group animals which had received injections of saline during the training phase now were given their first 15 mg/kg dose of CDP.

It was predicted that if the drug-sophisticated group DD subjects showed any tolerance of the depressant effect of CDP their latency to cross would be significantly lower than that of group SD subjects.
Table 3. Treatment and Control groups in Studies III and IV.

<table>
<thead>
<tr>
<th>Group</th>
<th>Training Treatment or Pretreatment</th>
<th>Test Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>Saline, after session</td>
<td>Saline, $\frac{1}{2}$ Hr. before test</td>
</tr>
<tr>
<td>SD</td>
<td>Saline, after session</td>
<td>15mg/kg CDP, $\frac{1}{2}$ Hr. before test</td>
</tr>
<tr>
<td>DD</td>
<td>15mg/kg CDP, after session</td>
<td>15mg/kg CDP, $\frac{1}{2}$ Hr. before test</td>
</tr>
</tbody>
</table>
If the tolerance were complete the latency of group DD should be as low as the latency of the control group.

To prevent a ceiling effect from obscuring group differences, the ISI was extended from the 5 seconds used during training to 30 seconds in the test. If there were a tolerance-induced difference in latency, it would be more apparent over thirty seconds than if restricted to five seconds. If a given rat's performance were not depressed by the drug, i.e., if the depressant effect had been tolerated, the latency to cross should still be less than the original five seconds. Those subjects which were still under any depressant effect should be more likely to wait for the shock onset before crossing to the safe compartment.

Actually, the SS group was run several weeks after the other groups with the apparatus in a different room. These changes, plus the fact that due to differences between the two rooms, the experimenter was in the test room for the entire session for the SD and DD groups, but present only during part of the ITI for the SS group, probably account for the differences apparent throughout the training portion of this study, and must be suspected in any test differences between the SS group and the two drug groups.

Results

The scores of the three groups shown in Fig. 3 were found by taking each subject's total latency over the five trials per day. The median of the five totals within each group was then plotted as that group's score for that day. As mentioned earlier, the CS was changed from a
Fig. 3. Median avoidance latencies of the drug-sophisticated, drug-naive, and control groups during training and test sessions in the one-way avoidance task. The break between the 5th and 6th training session was due to a change of CS.
buzzer to a tone on the sixth day of training. This change accounts for the break and the increase in latency seen in both drug groups from the fifth to the sixth days.

During training the median latencies of the two experimental groups (DD and SD) were nearly indistinguishable, and in no place did they differ significantly from each other. This was expected since all injections were administered after the completion of each animal's training session, i.e., none of the subjects of either group were under tranquilization during the actual training session. Unfortunately, the median score of the SS control group was significantly different (p < .05 U test) from both experimental groups on the first three days of training. All differences, however, had disappeared by the end of training.

The median test latencies of the two groups (37.8 for the drug-sophisticated DD group, and 37.9 for the drug-naive SD group), were again nearly identical. The latency for the SS group was 10.8, but due to the considerable within group variability, the difference between the control group and either drug group was not significant.

Since there was no difference between the latencies of the drug-sophisticated and drug-naive groups, any depressant effect of CDP does not seem to be tolerated in an avoidance task which does not involve conflict.
EXPERIMENT IV

The preceding studies have shown that, with a moderate daily dose of CDP, the depressant action of the drug is not tolerated in a spontaneous activity situation, nor is there any difference between drug-sophisticated and drug-naive groups in an avoidance task which doesn't involve conflict.

Since the disinhibitory effect was defined by Margules and Stein (1968) as the facilitation of the tendency to respond, especially (perhaps only), if the behavior is under some inhibitory suppression, e.g., in some conflict situation, it was decided to test for tolerance of the depressant effect of CDP in a situation where the disinhibitory effect would be at work. For this study a two-way avoidance task was designed which would put the subjects into a high conflict situation.

METHOD

Apparatus. The apparatus was the same used in the one-way task of the previous experiment. In this study, however, both compartments were painted black, and the guillotine door in the partition separating the compartments was completely removed.

Subjects and Pretreatment. The subjects were from the same population used in the previous studies. Eighteen rats were separated into three groups of six each. For ten days of pretest treatment the subjects in group SS (control group) and those of group SD (drug-naive group) received daily injections of physiological saline. The rats in group DD (drug-sophisticated group) were given daily i.p. injections of 15 mg/kg of CDP in physiological saline solvent.
Test Procedure. After ten days of pretreatment, the animals were tested in the two-way avoidance situation in daily sessions for eighteen days. Each day, thirty minutes before being tested, each rat of the two drug groups received a 15 mg/kg injection of CDP, while control group subjects were given injections of physiological saline.

Test sessions began with the placement of the subject on one side of the shuttle box. The side from which the animal started was alternated every day. After a wait of about one minute a 1000 cps tone was presented. After a five-second I.S.I., a scrambled 2.5 mA current was delivered to the grid floor of the start compartment. The tone ceased as soon as the animal crossed to the other side, but the grid current was left on to punish spontaneous returns. If the animal crossed in less than five seconds it received no shock, and scored an avoidance response. If a subject failed to escape, the shock and tone continued together for twenty-five seconds. When this occurred the rat was left where it was and the subsequent trial was run from the same start compartment.

Each test session consisted of ten trials, and each trial began approximately forty-five seconds after the beginning of the previous trial. The tests were conducted with the room lights turned on, and with the experimenter out of the room.

Results

Two responses were measured in this study, the number of conditioned avoidance responses (CAR's), and the median latency for crossing. The former measure is the total number of CAR's made by the six subjects in each group for each daily test session. Since there were ten trials per
subject, the possible number of CAR's each day for each group was 60. None of the groups ever approached this figure. The median latency was calculated by taking each subject's median response latency for the ten trials each day. Then the median of the six scores in each group was plotted as that group's daily score.

The results of this experiment provide, once again, no evidence for tolerance of the depressant effect of CDP as seen in Fig. 4. Examination of the first five days of the test, where tolerance would be expected to occur, shows that summed over the five days, the latency of the control group (SS) was significantly shorter than the drug-sophisticated DD group (p < .03 U test) as well as the drug-naive SD group (p < .01 U test). Further, the difference between the two drug groups was not significant (p < .12 U test). Day by day comparisons show that the latency of the drug-sophisticated DD group was significantly shorter (p < .03 U test) than that of the drug-naive SD group only on the second test day, and somewhat although not significantly shorter on the third and fourth days. After the fourth day there were no differences between the two drug groups.

This could be considered as evidence that the latency of the acutely dosed group was depressed, while the chronically dosed group had already tolerated the depressant effect. Then, after a few days of drug exposure, the SD animals would have also undergone tolerance to do as well as the DD subjects.

This explanation, however, is unable to account for the results of the first test day when there was no difference between the latencies of the drug-sophisticated and drug-naive groups. If there were tolerance of the depressant effect, the DD group's superiority should have been most
Fig. 4. Median avoidance latencies in the two-way avoidance task.
apparent on that first day. That is, unless some exposure to the situation were necessary for the display of tolerance. It may be possible that the tolerance appears only in a familiar conflict situation, and until such familiarity is gained the depression remains unabated. If the first day is eliminated the difference between the DD and SD groups for days 2 through 5 is significant (p < .03 U test).

The number of CAR's was extremely low throughout the study, especially during the early days. Fig. 5 shows that there were no CAR's at all on the first day by any group, and that the highest number produced by any group (24 by group SD on day 18) was only 40% of the number possible. This low number of avoidance responses is probably attributable to the degree of conflict produced in the subject by having to enter the opposite chamber, where the animal had previously received a considerable shock.

Through the fifth day the number of avoidance responses of the three groups was indistinguishable. Beginning with the sixth day, members of the SD group, which had been drug-naive at the start of the test phase, began to make more CAR's than either of the other groups. This trend was maintained throughout the study. The drug-sophisticated DD group and the SS saline control group produced similar numbers of CAR's through the tenth test day, but from the eleventh day on, the DD group made slightly but insignificantly more CAR's than did the control group.

An observation made during the eighteen days of testing was that the behavior of the animals of both drug groups varied considerably among subjects and even as individuals from trial to trial, while that of the control group subjects was quite constant. All group SS animals made very quick escapes, but few avoidances. At the onset of the tone
Fig. 5. Total number of Conditioned Avoidance Responses per session for each group in the two-way avoidance task.
these animals would orient towards the door and assume a crouching position, but they would rarely avoid the shock onset. Certain subjects of the drug groups, on the other hand, would make a rapid avoidance response on a given trial and then, perhaps on the subsequent trial, take several seconds of shock before making an escape. A few subjects made relatively high numbers of CAR's, while others (SD-5, DD-1, & DD-2) made only one avoidance response each in the entire 180 test trials.
DISCUSSION

The results of the first three experiments indicate that there is no tolerance of any depressant effect of CDP at the 15 mg/kg dose. The results of the fourth experiment were not so easy to characterize.

The daily measurement of spontaneous activity in the first stage of Experiment I had been expected to show the development of tolerance to the depressant effect. The second stage was then expected to show that animals with several days of drug sophistication would have developed tolerance. Neither of these expectations were fulfilled.

The depressed activity rate of the tranquilized subjects in the first stage remained low for as long as they continued to receive the drug. In the second stage there was no difference in spontaneous activity between the drug-sophisticated and drug-naive groups. Both groups had nearly identical activity rates, both of which were similar to the depressed rate of the Drug-Before group of the first stage.

These results disagree with the findings of Goldberg et al. (1967), who reported that the depressant effect of CDP underwent tolerance after several days of treatment; and with Margules and Stein (1963) who reported that the depressant effect of the benzodiazepine tranquilizers is completely tolerated after repeated doses over several days.

Hoogland et al. (1966) reported a possible mechanism for tolerance of the depressant effect. They found that, after five days, rats which had received two 50 mg/kg injections of CDP daily showed increased rates of tissue disappearance and excretion of C\(^{14}\)-labeled CDP. They hypothesized that this increased excretion involved a drug-induced stimulation of hepatic microsomal enzymes which are responsible for metabolism of the
drug. Such a mechanism could account for the findings of Hoogland et al., and for Goldberg et al. who used the 100 mg/kg per day for their chronically drugged groups, leaving the possibility that the 15 mg/kg dose does not stimulate the numbers of hepatic microsomal enzymes necessary for the development of tolerance.

The second experiment was designed to determine if in fact the dose size caused the discrepancy between the results of the first experiment here and those of Goldberg et al. It was found that at the 15 mg/kg dose the median spontaneous activity rate of the chronically dosed, drug-sophisticated group was significantly lower than that of the saline control group, and was not significantly different from that of the acutely dosed, drug-naive group. At the 100 mg/kg per day level the drug-sophisticated group again had a significantly lower activity rate than the control group, but at this dose the chronically drugged group did have a significantly higher rate than the drug-naive group. These results confirm the findings of Experiment I here for the lower dose, and agree essentially with Goldberg et al. (1967) for the larger dose indicating that dose size has considerable influence on the tolerance of the depressant effect of CDP.

In the third study, investigating one-way avoidance latency, the depressant effect was not adequately displayed since there was no significant difference between the control group and the drug groups. It did show, however, that in a familiar situation, with extrinsic motivation (active shock avoidance), but with no conflict, there was no difference in performance between drug-sophisticated and drug-naive groups, i.e., in such a motivated task the depressant effect of CDP does not
undergo tolerance at the dose level used.

The results of the fourth study using a two-way avoidance task are not so clear-cut. If the depressant effect of a moderate dose were to undergo tolerance after several days of pretreatment, the latency measure from the beginning of testing should have shown a depressed rate for the drug-naive group, but a normal, or at least significantly shorter latency for the drug-sophisticated group. Then, as the drug-naive group developed its own tolerance to the depressant effect its latency should have come to match that of the drug-sophisticated group.

The latter prediction was borne out as expected, but not the first. On the first day the latencies of the two drug groups were virtually identical, and both were significantly longer than that of the undrugged control group. The first-day latency of the drug-sophisticated group might be accounted for if the tolerance showed up only in an already learned response or a familiar situation, and the fact that the latency of the drug-sophisticated group was significantly shorter than the drug-naive group's on the second day would apparently tend to support such an explanation. That is, except that not one of the DD group animals made a single CAR on the first, or for that matter, the second day. So it would be difficult to maintain that the CAR had been learned from the first to the second day. Further evidence against such an argument is provided by Experiment III, were it was found that no tolerance occurred even with a previously learned (but not conflictful) response.

As mentioned above, the control group subjects made from the beginning very quick escapes and had the shortest latencies, but they made almost no avoidance responses. These subjects despite (or perhaps due
to) being acutely alert, resorted to a freezing response while certain of the drugged subjects were much less apt to freeze and were able to make more of the appropriate avoidance responses.

In general, these findings agree with Feldman's (1964) report that CDP prevents conflict-induced behavior fixations. They also apparently agree with Sachs, Weingarten, and Klein (1966) who found that rats injected with CDP acquire a two-way avoidance response faster than saline injected controls. The agreement, however, of these findings with Sachs, Weingarten, and Klein is one only of direction. They reported that their subjects acquired a high level of conditioned avoidance responding after only a few sessions, and that the rate of CAR acquisition was enhanced under CDP with the superiority of the drug group most evident on the very first training session. In this study there was not one single CAR on the first day, and there was no significant difference between the numbers of CAR's made by the SD drug group and the saline control group until the last (eighteenth) day of testing. And of the sixty daily test trials for each group, the number of CAR's only finally reached 40%, in one group, with the substantial number of these (21 of 24 CAR's) being made by three of the subjects of the group.

Cicala and Hartley (1967) reported that CDP attenuates the expression and possibly the acquisition of fear. Assuming that the freezing response is an expression of fear, the undrugged control group should have and did make fewer CAR's than either of the drug groups.

These reports and the present findings indicate that CDP provides some effect which reduces the tendency to make the freezing response and increases the likelihood that the subject will learn to make the appro-
priate response. This effect is apparently to reduce the aversiveness of a situation which in turn permits the subject to make a "sober assessment" of the situation and eventually learn the optimal response. Without the drug in the two-way avoidance situation, i.e., with the unmitigated negative consequences, the normal rats, afraid of returning to the chamber in which earlier shocks had been received, resort to freezing - the behavior exhibited by rats in situations where action is perceived as being unlikely to lead to safety (Weiss, Kriechhaus, & Conte, 1968).

CDP might reduce the offensiveness of the shock, or perhaps reduce the reluctance of the rat to enter the opposite chamber enabling it to replace the prepotent but inappropriate freezing response with the least painful avoidance response.

The effect which CDP was observed to have on conflict behavior was somewhat transient since the drug-naive animals were more apt to show the effect than were the drug-sophisticated subjects. But the effect which underwent tolerance is not easily characterized. It would at first appear incorrect to consider it a depressant effect since rather than reducing the tendency to respond, the avoidance response was enhanced. Margules and Stein's (1968) opposing effects on behavior would then lead one to conclude that the small enhancement of CAR acquisition seen in the drug-naive group was a case of disinhibition where the avoidance response (or the "return to the shock chamber response"), was released from the inhibition of the punishment permitting these subjects to make the CAR at a higher rate than either of the other groups.

Richelle, Xhenseval, Fontaine, and Thone (1962) found that rats
working on a FI 2 schedule for food had an increased response rate under CDP. These results could be interpreted as a case where the response during the interval, in undrugged subjects, is under the inhibition of nonreinforcement. With the disinhibitory action of CDP these nonreinforced responses would be released increasing the response rate.

These same results, however, are explained even more adequately by the reduction of the negative consequences of the nonreinforcement. That is, the nonreinforcement is a negative experience for the subject, the effects of which may be reduced by CDP. In other words, the concept of disinhibition depends upon a reduction of the effect of nonreinforcement, punishment, or in general the negative consequences of a given situation.

Other studies such as Heise and Boff, (1962), and Bernstein and Cancro, (1962) found that CDP reduced avoidance responding in continuous avoidance procedures. It is difficult to discern any response that is released from suppression in these studies. Rather the response seems to have become less important to the subject, or the effect of the punishment was reduced.

Further difficulty for the disinhibitory interpretation is seen in the results of McConnell, (1962), who found that rats under CDP would tolerate higher intensity of shock as measured in a fractional escape procedure than animals without the drug. Again, there is no response being facilitated, but apparently the noxiousness of the shock at a given intensity is not as great to drugged subjects as it is to undrugged ones.

The reduction of punishment or of the negative consequences provides
a basis for the concept of disinhibition in that there must be some negative component of a situation which causes inhibition in the first place. For disinhibition the value of the negative influence must be reduced. CDP in some way appears to reduce that value as perceived by the subject, and if some response had been suppressed it is apt to be disinhibited.

In Experiment IV certain of the drug-naive animals acquired the CAR before any of the drug-sophisticated subjects. This would indicate that the drug’s effect was partially tolerated. Margules and Stein (1968), however, reported that the disinhibitory effect of the benzodiazepine tranquilizers not only failed to show tolerance, but even appeared to increase substantially during chronic dosing. If this effect of CDP were a disinhibitory action, and if such an effect were to increase during chronic dosing, the drug-sophisticated group should have made the greater number of CARs.

It is interesting that those animals under the undiminished effect of the drug tended to acquire the CAR faster than controls or drug-sophisticated subjects, but as has been argued, these same animals would have had the least fear of the shock due to the reduced punishing effect. This apparent paradox can perhaps be explained as a situation in which CDP depressed the tendency of the rat to make the freezing response.

The prepotent response of the rat in the two-way avoidance situation is to freeze. The CDP could reduce the negative value of the shock or punishment, making the freezing response less likely to occur and as a result allowing the subject to make a less potent response— in this situation the avoidance response.
In conclusion, from the results of the experiments reported here and the others cited it appears that rather than two effects, CDP has but one — depression. This is not necessarily inconsistent with Margules and Stein (1968) since they wrote of two effects on behavior. The single depressant effect of CDP would of course affect components of more than one behavioral system, e.g., the motor system and probably some negative or aversive system. The effect on the motor system in the spontaneous activity situation was not tolerated with the 15 mg/kg dose. The effect upon the quickness of a motivated response in the avoidance situation was not tolerated. But some tolerance of the effect did appear where the action was to reduce the value of the negative consequences of a conflict situation.
References

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