OBSERVATIONS ON THE BEHAVIOR AND RECONSTITUTION OF CHLORPROMAZINE-TREATED PLANARIA (*DUGESIA TIGRINA*)*

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Dundee (1954) reviewed the effects of chlorpromazine (10-(3-dimethylamino-propyl)-2-chlorphenothiazine) on the behavior and physiology of man as well as a number of other higher mammals. In what manner the effects of this tranquilizer are mediated, together with its site of action are subjects requiring more extensive and thorough investigation. According to Dundee's review, the locus of action is the hypothalamus (particularly the reticular formation) of the brain. In this review, it was noted that chlorpromazine has marked anti-adrenaline and feebler anti-acetylcholine reaction. However, it produces neither competition nor depolarizing block of myoneural junctions.

The fourth edition of the *Thorazine Reference Manual* (1961) adds that the drowsiness caused by the drug is believed to result from its inhibition of the reticular activating system, but that its tranquilizing effect was due to its action on other areas of the diencephalon, including the basal ganglia. In this same work the antiemetic effect of chlorpromazine is attributed to medullary activity—a selective inhibition of the chemoreceptor trigger zone. It is possible that it affects in some way or another all living cells without any particular affinity for those of the nervous system.

The study of a simpler organism with its less complex structure has often lead to an explanation of events occurring in the higher, more complex animals. The simple triclad flatworm, *Dugesia tigrina*, commonly known as planaria, seemed a nearly ideal subject. This hardy little creature lends itself well to laboratory conditions and possesses one of the simplest nervous systems among bilaterally symmetrical, free-living, animals.

The specimens used in the observations and experiments described in this report were selected from a stock of approximately 700 individuals. This supply was obtained from nearby Jefferson Lake (Jefferson Co., Ohio) in the summer of 1961 and maintained successfully in the laboratory ever since. They were allowed to eat to satiety once a week on beef liver. After each feeding the 8-inch diameter glass finger bowls in which the worms were kept had to be thoroughly cleaned and replenished with fresh pond water. The bowls, each containing approximately 150 worms, were kept on the open shelves of a small metal cabinet in the laboratory. An opaque plastic sheet was hung over the front to cut down the light.

During two months preliminary observations were made on the effects of chlorpromazine on the behavior of planaria. Groups of five worms were placed in plastic containers (3-inch diam) of approximately 50 ml of test solution. These test solutions comprised a spectrum of concentrations ranging from 0.1 to 0.01 mg of chlorpromazine per liter of pond water. After 15 min in any of the solutions with concentrations from 0.1 to 0.06 mg/liter of chlorpromazine, the worms were dead. The minimum lethal concentration appeared to be 0.06 mg of chlorpromazine per liter of pond water. The stock solution of chlorpromazine was that supplied in the one-ml ampules by Smith, Kline, and French in a concentration of 25 mg/ml under the trade name "Thorazine."

Within 15 min after exposure to concentrations of 0.05 or 0.04 mg/liter of

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chlorpromazine, the behavior of the worms was markedly affected. The most conspicuous change was the complete absence of the usual gliding (often described as creeping or crawling) locomotion. However, after 24 hr in the same solution the observed effects gradually disappear. According to the *Thorazine Reference Manual* (1961) the tranquilizer is photosensitive and a 5 per cent aqueous solution at a pH between 4.9 and 4.6 is stable for more than 24 hr. Since the worms began to resume their gliding locomotion after 24 hr, we took this to be an indication of the deterioration of the chlorpromazine. When observations were made for a period longer than a day, the worms were placed in a freshly prepared solution of the compound every 24 hr.

Hyman (1951) contends that ciliary action can account for the gliding locomotion only in those forms less than 2 or 3 mm long. In the larger individuals, this type of locomotion results from an almost invisible wave of muscular contraction that moves from the anterior end backward. Hyman states further that in the presence of lithium chloride, a ciliary poison, the planarians can still glide.

Instead of the usual gliding, the chlorpromazine-treated worms substituted a variation of what Hyman (1951) described as "hurried" locomotion. Ordinarily this is resorted to briefly upon being disturbed after which the worms resume gliding. In this "hurried" type of movement, the animal loops along not unlike a leech. It quickly extends the body, flattens the head against the substrate, and then contracts the rest of its length pulling forward the posterior end. The chief difference between this type of locomotion and that manifest by the worms under the influence of chlorpromazine, is that the latter is anything but hurried. It is rather a slow-motion version of the former. The animal begins by stretching to an almost unbelievable degree the part of its body anterior to the pharynx. During this time it slowly turns its head from side-to-side. The post-pharyngeal area of the body stretches some but not to such an extreme degree as does the anterior part. The stretching so distorts the head that the auricles cannot be distinguished. After lowering its head to the substrate, the worm slowly contracts the rest of its length thus pulling the body forward.

Hyman (1961) described the nervous system of the triclad flatworms as consisting of a subepidermal plexus and a submuscular plexus. In the latter were the nerve cords which supplied nerves to the muscles. The unusual degree of stretching as part of the looping locomotion may indicate that the chlorpromazine lowered the response threshold of the circular muscles. Or possibly the effect resulted from an increase of the drug within the cytoplasm because the chlorpromazine had raised the cell permeability. Nathan and Friedman (1962) reported an increase in cell permeability to occur in *Tetrahymena pyriformis* following chlorpromazine treatment.

According to Schmidt-Nielsen (1960), many invertebrate muscles, particularly in crustaceans, are supplied with two or more nerves. A single muscle fiber may be connected with two types of nerve fibers, one inhibitory the other excitatory. The same muscle fiber responds differently to the impulses in the two nerves. In this way different stimuli elicit different responses on the part of such a doubly supplied muscle fiber. If planarians had such an anatomical arrangement, it might explain why chlorpromazine inhibits gliding but facilitates looping locomotion on the part of planaria. As far as we know, however, such a nerve-muscle arrangement has not been discovered in these flatworms.

The desirability of more precise information concerning the behavioral effects of chlorpromazine on these animals prompted us to turn our attention to the righting reaction. When a planarian is turned over on its dorsal surface, it immediately rights itself. This is a rather simple reaction, but it does require a degree of coordinated activity. The worm first turns its head over and fixes it to the substrate. Then beginning with the region just posterior to the head, turning proceeds backward in a smooth, flowing motion. Pieces severed posterior to the head of a planarian worm, likewise are capable of righting themselves although more slowly than when the head is attached (Hyman, 1951). Manifestly the cerebral ganglia are not required for this bit of coordinated behavior.

We decided to compare the time required for treated animals to right themselves with that required by a control group. The first experiments were performed on a group of 40 individuals—20 treated worms and 20 serving as controls. During the course of the experiments the worms were kept in plastic dishes of pond water, ten to a dish. The four dishes were set in a metal trough filled with a continuous flow of tap water. A rubber hose attached to one end of the trough drained the overflow into the sink. By this means the temperature of the cultures was kept between 22 and 25.5 C during the entire procedure. For an experimental trial, a worm was removed from its container, placed on a clean glass slide and flooded with pond water. Then, with a toothpick, it was turned onto its dorsal surface. The time required for it to right itself completely was measured with a stopwatch and recorded. Each of the 20 worms was flipped over five times before it was returned to its container. This gave a total of 100 righting reactions. The average of the length of time required for these 100 righting reactions is given in table 1 for each of the three trials comprising a series. In between runs, the containers in the trough were kept covered with a black cloth. Both the pond water and the chlorpromazine solution were changed daily.

TABLE 1

*The minimum-maximum, median, mean, and mode of the lengths of time required for 20 intact, chlorpromazine-treated planaria to right themselves 5 times in succession**

Day	A Min-Max	A Md	A Mn	A Mo	B Min-Max	B Md	B Mn	B Mo
1	1.9–13.8	4.7	4.9	3.5	3.0–33.4	8.7	9.9	5.7
2	3.1–21.2	6.1	6.7	4.8	4.0–43.0	10.3	13.1	8.3
3	2.5–14.5	5.4	6.3	5.5	5.0–37.0	9.4	11.9	8.8
		A'				B'		
1	2.8–20.1	6.7	7.1	5.5	5.5–70.5	17.5	19.0	17.0
2	2.8–22.3	5.4	6.5	4.4	5.3–66.5	18.1	21.7	13.8
3	2.4–12.7	4.9	5.5	4.0	5.1–65.0	13.8	16.1	12.2

*Data under B and B' were obtained while using chlorpromazine concentrations of 0.05 and 0.04 mg/liter respectively. A and A' represent data from controls. Time is in seconds.

The three trials during which each of 20 worms was flipped five times occurred on successive days. In the first series the experimental worms were in a solution of 0.05 mg/liter of chlorpromazine (table 1: B1, B2, and B3). In the second series (table 1: B'1, B'2, and B'3), the concentration of chlorpromazine was 0.04 mg/liter. In both series the first trial was made 24 hr after the worms had been placed in the chlorpromazine solution; the second trial, 48 hr after; and the third (B3 and B'3), 72 hr after. Table 1 also shows the minimum and maximum lengths of time (seconds) required for a righting reaction in a trial of 100. In the first series where the mean time in seconds for the first trial (B1) was 9.99, 60 per cent of the values ranged between 4.1 and 11.7; the mean for the second trial (B2) was 13.09, with 60 per cent of the values falling between 7.3 and 14.9; the mean for the third trial (B3) was 11.89, with 65 per cent of the values falling between 6.1 and 12.6. In the control groups the mean for the first trial (A1) was 4.99, with 60 per cent of the values falling between 3.0 and 6.2; for the second trial (A2) the mean was 6.73, 72 per cent of the values ranging from 3.2 to 7.5; for the third trial (A3) the mean was 6.29, with 60 per cent of the values between 3.8 and 7.1. In terms of the averages of the time in seconds required for 20 planaria to right themselves five times in succession during three trial runs, the worms in the 0.05 mg/liter concentration of chlorpromazine required approximately twice the time to right themselves as did the individuals used as controls. However, on the same basis of comparison, it required approximately three times as long for the worms in the 0.04 mg/liter concentration of chlorpromazine as it did the control groups.

In this second series, in which the experimental worms were treated with a 0.04 mg/liter concentration of chlorpromazine, the mean time in seconds for the first trial (B′1) was 19.03 and 60 per cent of the values ranged from 12.1 to 24.1. For the second trial (B′2) the mean was 21.7, with 60 per cent of the values between 10.0 and 25.0; for the third trial (B′3) the mean was 16.14, with 60 per cent of the values between 8.4 and 17.1. In the control groups, the mean for the first trial (A′1) was 7.12, with 60 per cent of the values between 5.0 and 8.2; for the second trail (A′2), the mean was 6.48 with 60 per cent of the values 3.9 and 7.1; for the third trial (A′3), the mean was 5.47 with 61 per cent of the values between 3.5 and 6.7. In neither of the two series of experiments were the lengths of time required for the worms to right themselves as closely clustered about the mean as were the values among the control individuals. The spread between minimum and maximum values expressing the time required for the righting reactions was also much greater for the treated worms than for the untreated ones.

Following the foregoing series of experiments in which the whole worms were used, all of the worms were transected just anterior to the proboscis. The anterior and posterior segments were segregated in separate plastic dishes. In the two dishes containing each control group, there were 20 anterior segments in the one and 20 posterior in the other. The experimental groups were similarly divided. As in the first two series of experiments in which the whole worms were used, the experimental sections were placed in a freshly prepared solution of chlorpromazine each day and, at the same time, the control group was given a change of pond water. The containers were again kept in the trough of running tap water.

Ten segments were chosen, one at a time, from each container; placed on a clean glass slide and flooded with pond water; turned over with a toothpick; and allowed to right themselves. The length of time required for each segment to do so was measured with a stopwatch. For each series of trials, only 10 segments were taken from a given container although 20 were present in each In this way even if half the segments in a container died, we would have a constant number with which to work.

Tables 2 and 3 show the results of the trials in which the pieces of transected worms were used and in which both of the previously used concentrations of chlorpromazine were repeated. In both tables, in the series involving the use of 0.05 mg/liter of chlorpromazine, the control trials were run 1.5, 27, 49, 72, 95, and 123 hr respectively after sectioning. The trials with the treated sections were made 2½; 25½, 48, 72, 96, and 123 hours after sectioning. In the series involving the use of 0.04 mg/liter concentration of chlorpromazine, the trials of the control groups were made 24, 48, 77, 92, 120, and 144 hr respectively after sectioning. The trials for the treated sections took place at the same intervals after sectioning. If any of the treated sections required more than 90 sec to right themselves, the time was recorded as 90 sec and no additional time was allowed the individual. Not one of the sections used in the control groups died during the course of the experiments using the 0.05 mg/liter concentration of the tranquilizer but, 10 anterior and 9 posterior treated sections disintegrated. One anterior and one posterior section failed to survive among those subjected to the 0.04 mg/liter concentration. Among the controls, one anterior and two posterior sections died.

In table 2 the tabulations are all in reference to the performance of anterior sections. For both of the control groups, the length of time required for the righting reaction varies scarcely at all throughout the trials. In the group treated with a chlorpromazine concentration of 0.05 mg/liter, the table indicates that they took longer to right themselves than did the controls when the means are compared. However, when the minimum-maximum times are compared a con-

TABLE 2

The minimum-maximum, median, mean, and mode of the lengths of time (seconds) required for 10 anterior sections of chlorpromazine-treated planaria to right themselves 5 times in succession

Day	Control				Experimental*			
	Min-Max	Md	Mn	Mo	Min-Max	Md	Mn	Mo
0	1.6– 9.2	3.4	3.7	2.1	3.9–19.2	7.9	8.3	5.5
1	1.4– 9.0	2.9	3.5	3.0	3.5–18.1	5.9	7.2	5.1
2	1.6–16.4	2.9	4.2	2.1	2.2–14.2	6.1	6.1	5.4
3	1.2– 9.9	3.8	4.2	2.8	3.2–90.0	11.5	19.2	—
4	1.2–15.8	4.1	4.9	2.3	4.4–54.2	13.5	18.2	—
5	1.0– 9.0	2.7	2.9	2.0	5.4–90.0	25.2	52.0	—

*These data were obtained using sections of planaria treated with a chlorpromazine concentration of 0.05 mg/liter; the data in the right hand columns below, after treatment with a concentration of 0.04 mg/liter.

Day	Min-Max	Md	Mn	Mo	Min-Max	Md	Mn	Mo
0	—	—	—	—	—	—	—	—
1	1.5–14.4	5.2	6.1	4.2	5.7–90.1	16.3	20.6	—
2	1.4–19.5	4.2	5.6	3.0	4.7–90.0	18.5	21.5	—
3	1.8–20.0	5.2	6.6	4.5	4.2–90.0	20.7	27.8	—
4	1.6–19.1	5.1	6.2	5.4	8.4–54.6	18.0	19.4	—
5	1.6–16.9	4.9	6.0	4.3	4.4–84.6	18.9	24.3	—
6	2.5–11.2	4.8	5.2	3, 5.2	7.9–66.5	16.8	18.9	—

TABLE 3

The minimum-maximum, median, mean, and mode of the lengths of time (seconds) required for 10 posterior sections of chlorpromazine-treated planaria to right themselves 5 times in succession

Day	Control				Experimental*			
	Min-Max	Md	Mn	Mo	Min-Max	Md	Mn	Mo
0	4.0–70.7	22.6	24.0	—	6.5–90.0	38.4	48.5	—
1	5.2–47.2	12.9	15.4	—	12.7–90.0	30.3	47.0	—
2	6.0–51.4	21.4	22.4	—	6.9–90.0	13.6	18.9	—
3	7.4–45.2	15.1	15.5	—	6.3–90.0	18.5	23.9	—
4	2.2–28.5	8.0	9.4	—	4.5–39.2	12.9	15.2	—
5	1.6–14.7	4.1	4.7	2.6	4.2–35.8	10.9	12.3	—

*These data were obtained using sections of planaria treated with a chlorpromazine concentration of 0.05 mg/liter; the data in the right hand columns below, after treatment with a concentration of 0.04 mg/liter.

Day	Min-Max	Md	Mn	Mo	Min-Max	Md	Mn	Mo
0	—	—	—	—	—	—	—	—
1	9.0–77.9	22.4	29.9	—	11.3–90.0	26.6	31.8	—
2	7.7–54.3	23.6	26.6	—	10.2–67.6	34.4	42.3	—
3	10.6–77.6	33.5	37.7	—	10.6–64.7	28.2	33.5	—
4	6.2–90.0	22.7	35.5	—	12.4–90.0	60.5	55.0	—
5	4.2–42.7	15.3	16.3	—	10.3–90.0	25.6	34.3	—
6	2.7–51.9	11.1	16.1	7.6	8.1–46.5	18.2	21.2	16.3

siderable overlap is revealed. Up until, and including, the second day, there is little difference in the average lengths of time required for the righting reaction in this group. But, beginning with the third day, there is a considerable increase in the spread between minimum and maximum. There is likewise such an even distribution between these two extremes that no mode is established.

The anterior sections in the chlorpromazine concentration of 0.04 mg/liter require on the average (table 2) more time to right themselves than do the controls. Only slight differences are noticed between them and the group in the 0.05 mg/liter concentration. For one thing, a considerable spread between the minimum and maximum time requirements is evidenced from the day following that on which the worms were sectioned. Here too, the distribution of the average times was such that no modal intervals were established.

Table 3 contains only data in reference to posterior sections. A comparison of these data with those in table 2 suggests that in terms of average and median time, the posterior sections require a longer time to right themselves after being turned over. The generally greater spread between minimum and maximum time as well the absence of modes, indicate the poor coordination seen in this group. This is true for both the controls and the experimentals but the condition is more marked in the treated worms. The tabulations in table 3 suggest that the prolonged effects of the 0.04 mg/liter concentration on the coordination are greater than those produced by the 0.05 mg/liter concentration of chlorpromazine.

An inspection of table 3 shows that on the fourth day in most instances and on the fifth day in all, the medians and the means show a shortening of the time required for the righting reaction. On the sixth day a mode is established for the righting time in both of the control groups and for the treated worms in the 0.04 mg/liter concentration of the tranquilizer. This suggests that on about the fifth day a stable point is reached. Could this point to the fourth day as being a critical one for regenerating posterior segments of planaria?

TABLE 4*

Pattern and sequence of regeneration in planaria

Trial	Beginning pigmentation	Definite eyes	Definite auricles	Pigmentation completed	Av. temp.	No. of sections
I	2nd day	4th day	6–7th day	14th day	——	32
II	4th day	5th day	6th day	14–15th day	19.2 C	48
III	4-5th day	5th day	5th day	14–15th day	19.5	48
IV	3rd day	6th day	6th day	————	20.3	48

*After Regoli (1962).

McWhinnie (1955) in her study of the effects of colchicine, at similar temperatures, on regeneration of planaria (*Dugesia dorotocephala*), indicated that the fourth day following transection was a critical period. Regoli (1962) working in this laboratory with a total of 88 worms found that 48 posterior sections reconstituted eyes on the fifth day following sectioning; 16 on the fourth day and 24 on the sixth day (table 4). More than half had completed eyes by the fifth day while the remaining segments completed their eyes either the day before or the the day following. Wolff (1962) states that the appearance of the eyes generally certifies in planaria that the brain has been reconstituted. In his work with *Polycelis nigra* he found that if he excised the eyes and the brain at the same time and that if he prevented the regeneration of the brain by extirpation of its blastema every two days, the eyes would not regenerate. As long as the brain is not reconstituted, the eyes will not reform. Seven days after the last extirpation of the brain, the eyes appear. This would suggest that in the work of Regoli referred to above, appearance of eyes on the fifth day means that the cerebral ganglia were completed on the fourth.

It may be that the critical period which seems to hover around the fourth day after sectioning is connected primarily with the completion of the reconstituted brain. If this is the situation, then the shortening of the average lengths of time required for the posterior sections to right themselves (mean and medians values in table 3) noted to occur on the fifth day, may mean that the presence of these cephalic ganglia appreciably affects the coordination in these worms. This improvement in coordination which shows up as a decrease in the median and mean lengths of time required for the righting reaction on the fifth day, is found among both the control and experimental groups (table 3). However, the drop in the median and mean values for the experimentals is considerably greater on any given day than for their counterparts in the control group. The drug appears to interfere with coordination requiring a generally longer time for the posterior sections to right themselves. If the improved coordination appearing on the fifth day indicates a brain has been reconstituted by the fourth, then the drug has not appreciably interfered with the brain regeneration process which, judging from this line of evidence, appears at the same time in both the experimental and the control groups.

We next experimented to find the time required for worms to recover from the chlorpromazine treatment. Three groups of 5 specimens each were placed in a concentration of 0.04 mg/liter of chlorpromazine. One group was removed and returned to pond water after 24 hr; a second group after 48 hr; a third group after 72 hr. The return to the usual gliding method of locomotion was used as a criterion for recovery. The group treated for 24 hr showed complete recovery 9 days after the return to pond water; individuals treated 48 hr recovered in 11 days; those treated 72 hr took 14 days to recover. In each case gliding was gradually resumed and at first used only occasionally by one or two worms. Fo some time, given specimens would alternate between gliding and the looping manner of travel. The figures cited for recovery, however, refer to complete resumption of the normally used gliding. That is, as far as we were able to determine within the limits of our observation times.

We repeated the entire procedure once just as described above but using different worms. Here again the worms subjected to the chlorpromazine for the longer periods, required a longer time for recovery. There were some interesting differences from the first groups. Worms treated for 24 hr recovered in 17 days; those treated 48 hr took 15 days; and those treated 72 hr required 22 days. All three groups required considerably longer time for recovery than did their counterparts in the first series. The group exposed to the drug for 48 hr recovered two days sooner than the group treated for 24 hr. We cannot account for these differences. Work in progress may enable us to do so later.

SUMMARY

1. The tranquilizing drug, chlorpromazine, in concentrations of 0.06 mg/liter or over is lethal to the planarian worm, *Dugesia tigrina.*

2. Worms exposed to both 0.04 and 0.05 mg/liter concentrations of chloropromazine substitute a type of looping locomotion for the usual gliding manner.

3. When turned over on their dorsal surface, treated worms required a considerably longer time than did the controls to right themselves. Generally, however, the worms in the 0.04 mg/liter concentration of chlorpromazine required a longer time to right themselves than did those individuals in the more concentrated solution.

4. Pieces of worms transected anterior to the proboscis were allowed to regenerate in either 0.04 mg/liter or 0.05 mg/liter concentration of chlorpromazine at a temperature ranging from 22 to 25.3 C. An equal number of segments was placed in pond water in similar conditions. After 24 hr and on each succeeding day for five days, 10 anterior and 10 posterior segments from each group were required to right themselves. Mean and median values indicate that the chlorpromazine-treated worms took a greater length of time to right themselves

than did the controls. These same figures suggest that the worms in the stronger solution are able to right themselves more quickly than those segments in the less concentrated solution.

5. On the average, the anterior segments in both the experimental and control groups required less time to right themselves than did the posterior sections.

6. We observed that the posterior sections were better coordinated in their righting reaction around the fourth or fifth day after sectioning. The mean and median values of the lengths of time required for this reaction are appreciably less on the fifth day. Wolff (1962) showed that the eyes of planaria would not regenerate unless the brain was present. Since the eyes most frequently appeared on the fifth day, the cerebral ganglia must have been reformed by that time. This suggests that while the righting reaction can occur in the absence of a brain, the brain does, nevertheless, influence this reaction as indicated by the improvement in speed and coordination.

7. After exposure for 24, 48, and 72 hours respectively to a concentration of 0.04 mg/liter of chlorpromazine, worms completely recovered on being returned to pond water. The longer the time during which the worms were exposed to the drug, the longer was the time necessary for their recovery.

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