INHIBITORY EFFECT OF CHLORPROMAZINE ON RAT REPRODUCTION : A TWO WEEK ADMINISTRATION TEST BEFORE MATING

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Abstract.....The effect of chlorpromazine (CPZ) administration before mating on reproduction was studied using male and female Sprague Dawley strain rats, and was evaluated over three generations. CPZ in dose of 12.5, 25, 50 and 100 mg/kg was orally administered to both male and female rats (F0 generation) every day for 2 weeks before mating with the following results : 1) The incidence of insemination and pregnancy of the F0 generation rats decreased but did not decrease in the F1 generation. While no influence of CPZ on the number of myometrial glands, on the length of pregnancy or on delivery was noted in either the F0 or F1 females, the number of surviving fetuses and newborns decreased in a dose-dependent fashion. 2) The body weight of rats in the F0 and F1 generations decreased depending on the dosage of CPZ, but the body weight of the F2 generation increased. 3) The wet weight of the major organs of the male and female rats in the F0 generation was affected by CPZ when administered at 100 mg/kg. The weight of the adrenal, pituitary and prostate glands in males, the adrenal gland and ovary in females increased, whereas the weight of the pituitary and uterus in females decreased. These results suggest that administering CPZ to the F0 rats before mating exerts an influence on the reproduction in both the F0 and F1 generations.

Key words : Chlorpromazine, reproduction, rat, administration before mating.

INTRODUCTION

Chlorpromazine (CPZ) was introduced to psychiatric therapy about 40 years ago and has played a central role in psychotropic drug therapy in subsequent years (Gilman et al., 1980). Although this drug has been used for many years as a therapeutic agent, there are few studies on the influence of CPZ exposure on

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subsequent generations (Itoh, 1976a ; Fujii, 1987) except for a number of studies on the influence of CPZ on fetal toxicity (Beall, 1972 ; Dailey, 1978 ; Robertson et al., 1980).

In the present study, the effect of CPZ administration for 2 weeks before mating on the male and female rat's subsequent reproductive capacity was examined and compared to Saigo's results (Saigo, 1989) of CPZ administration for 9 weeks before mating.

MATERIALS AND METHODS

Animals and Drug : Male and female Sprague Dawley strain rats, purchased from Japan Clea Co. were used in the experiments. The rats were housed at a temperature of 22 to 26 °C, a humidity of 50 to 65 % and a 12-hour light-dark cycle with the light period from 7 : 00 to 19 : 00 hours. Solid feed for maintenance and for reproduction (Japan Clea Co.) and tap water were supplied to the rats ad libitum.

Chlorpromazine hydrochloride (CPZ ; Shionogi Pharmaceutical Co., Japan) was used as the test drug. The CPZ was dissolved in distilled water, preserved in a brown bottle in a cool dark place and discarded every 2 days when fresh solutions were prepared. The test doses of CPZ were 12.5, 25, 50 and 100 mg/kg/day, given at a dosage of 0.5 ml per 100 g of body weight through a metal needle used for the gavage. CPZ was administered once daily intragastrically. In the control group, on the same schedule and using the same technique, the same amount of distilled water was administered.

Procedures : Male and female rats of the F0 generation, 10 weeks of age, were maintained separately in groups of 10 males and 19 to 22 females. CPZ was administered for a 2-week period. During the experimental period, the general condition of the rats was observed daily, and the body weight as well as food and water intake was recorded once weekly. Mating was accomplished by housing one male with two or three females in a single cage over a period of 2 weeks from the day following the last administration of CPZ, allowing copulation between males and females who had been given the same dosage of CPZ. Pregnancy was confirmed by detection of spermatozoa in the vaginal smear test, and the day of detection was defined as "Day 0" of pregnancy. About half of the pregnant rats were sacrificed by chloroform anesthesia on the 14th day of pregnancy and underwent immediate autopsy. The number of live fetuses, dead fetuses and myometrial glands were recorded, and the gross morphology of the uterus was studied. At the same time, the heart, lungs, liver, kidneys, spleen, adrenal glands, ovaries, the whole brain, pituitary gland and submaxillary glands were removed and evaluated by their wet weight, appearance and gross morphology. Male rats at 14 weeks of age also underwent autopsy in a manner similar to female rats with an additional gross examination of the testis and prostate gland.

The remaining pregnant rats were allowed to deliver (F1 generation) in a natural

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course. Before, during and immediately after delivery, the mother rats were protected from outside stimuli as much as possible, being careful to avoid environmental changes or other stimulation. The mode of rearing the pups and the growth of the pups was carefully observed. In a similar study, Park (1968) adjusted the litter size to six. In the present study, the litter size of the F1 pups was limited to eight with four males and four females. The selection was made in a random fashion 48 hours after birth. A similar litter standardization procedure was repeated for the F2 pups.

After weaning the F1 rats, all F0 mothers, 20 weeks of age, and the F1 rats (10 males and 10 females) underwent autopsy using the same method as previously described. The remaining male and female rats were maintained separately. At the age of 10 weeks, 6 males and 12 to 14 females from different litters were mated. The F2 rats were thus obtained and studied in the same fashion as the F1 generation. In the present study, however, the teratogenicity of CPZ was not evaluated.

Statistical analysis : The significance of the difference between the treated and the control groups was evaluated using the multiple comparison analysis of Kruskal-Wallis and the direct probability analysis of Fisher.

RESULTS

1. F0 generation

Incidence of insemination and pregnancy : As shown in Table 1, the incidence of insemination and pregnancy decreased with CPZ in a dose-dependent fashion. In

Table 1. Insemination and pregnancy data for F0 rats exposed to chlorpromazine for 2 weeks before mating.

Observation		Chlorpromazine, mg/kg/day				
		0	12.5	25	50	100
No. of rats (F0)	M	10	10	10	10	10
	F	21	20	20	19	22
Insemination (%)		100.0	100.0	90.0	89.5	81.8
{ No. of female rats inseminated / No. of female rats paired with males }		(21/21)	(20/20)	(18/20)	(17/19)	(18/22)
Pregnancy (%)		95.2	75.0	72.2*	70.6*	61.1*
{ No. of pregnant rats / No. of female rats inseminated }		(20/21)	(15/20)	(13/18)	(12/17)	(11/18)

M: male, F: female.

* : Significantly different from the value of 0 mg/kg group, $P < 0.05$.

the case of the male and female rats administered 12.5 mg/kg of CPZ, the incidence of insemination was 100 %, but the incidence of pregnancy was only 75 %. Furthermore, the incidence was lower in the group of mating rats given 100 mg/kg of CPZ with the insemination rate at 81.1 % and the pregnancy rate at 61.1 %.

Autopsy findings on the 14th day of pregnancy : As shown in Table 2, the number of myometrial glands per litter in the groups of rats receiving CPZ was no different from the number observed in the control group. The number and weight of live fetuses, however, decreased significantly in a dose-dependent pattern. A macroscopic study of the uterus revealed no abnormalities.

General condition and body weight : With CPZ administration, the spontaneous locomotor activity of the F0 rats was inhibited and after about 5 or 10 minutes the rats entered a state of sedation. This state persisted from 30 minutes to several hours, with an intensity and duration depending on the dosage and occurred with every CPZ administration.

In both the male and female groups, the body weight gain was inhibited depending on the dosage of CPZ as well as on a decrease in food and water intake, showing a marked decrease in male rats receiving CPZ at 100 mg/kg. Furthermore, the estrus cycles in the F0 females prior to the CPZ administration at about 8 to 10 weeks of age were 4 to 10 days.

2. F1 generation

Growth of the F1 rats : The F1 rats from the F0 parents exposed to CPZ were evaluated. The length of pregnancy was 20.0 to 21.0 days, somewhat shorter than the 22.8 days noted in the control group, as shown in Table 3. Abortion or premature birth did not occur. The number of live pups per litter and the body weight of the pups at 48 hours after birth decreased significantly depending on the CPZ dosage to

Table 2. Autopsy findings on day 14 of pregnancy in F0 rats exposed to chlorpromazine for 2 weeks before mating.

Observation	Chlorpromazine, mg/kg/day				
	0	12.5	25	50	100
No. of dams (F0)	10	8	7	6	5
Myometrial glands / litter	12.8 ± 1.3	9.9 ± 1.8	10.8 ± 1.1	9.9 ± 1.6	9.4 ± 2.0
Live fetuses / litter	12.4 ± 0.7	9.1* ± 0.7	9.9* ± 0.7	8.8* ± 0.5	8.0* ± 0.7
Average live fetal weight (g)	2.72 ± 0.02	2.62* ± 0.01	2.30** ± 0.03	1.98** ± 0.08	2.04** ± 0.05

Mean ± S.E., * and ** : significantly different from the values of 0 mg/kg group, $P < 0.05$ and $P < 0.01$, respectively.

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Table 3. Data for pups (F1) from parental rats (F0) exposed to chlorpromazine for 2 weeks before mating.

Observation	Chlorpromazine, mg/kg/day					
	0	12.5	25	50	100	
No. of dams (F0)	10	7	6	6	6	
Length of pregnancy (days)	22.8	21.0	21.0	20.2	20.0	
	± 0.5	± 0.8	± 0.6	± 0.9	± 1.1	
Live pups / litter	12.4	9.7*	8.8*	8.3*	8.0*	
	± 0.8	± 0.7	± 1.1	± 1.0	± 1.0	
Average pup weight at hour 48 (g)	(M){	6.85	6.10*	6.17*	5.12*	5.07*
		± 0.05	± 0.14	± 0.36	± 0.54	± 0.84
	(F){	6.83	6.05*	5.95*	5.16*	5.05*
		± 0.06	± 0.13	± 0.32	± 0.45	± 0.65
Survival of pups at day 22 (%)	98.8	92.9	75.0*	58.3**	45.8**	

Mean ± S.E., * and **: significantly different from the values of 0 mg/kg group, $P < 0.05$ and $P < 0.01$, respectively.

M: males, F: female.

which the F0 parents had been exposed. Death of the F1 rats occurred mostly within a few days of birth, and no death occurred more than 2 weeks after birth. No great abnormalities were noted in their growth after 2 weeks. The mean body weight of the F1 rats up to the weaning period was lower depending on the CPZ dosage to which the F0 parents had been exposed, as shown in Table 4, when compared to the control group. However, no sex difference in the body weight gain was noted.

The survival of the F1 rats at the weaning period was also lower depending on the CPZ dosage to which the F0 parents had been exposed (Table 3). Especially in the mating groups of the F0 rats receiving 50 and 100 mg/kg of CPZ, the rate of survival of the F1 pups at the weaning period was 58.3 and 45.8 % respectively, a death of about half the number of newborns. However, after the weaning period, no great abnormalities were noted in the growth of the F1 rats or in their general condition. They followed almost the same course as the control group up to the 10th week of life. The estrus cycles of 12 females in the F1 control group at about 8 to 10 weeks of age were 4 to 5 days (8), 7 days (2), 9 days (1) and 10 days (1). In the F1 females from the parental rats (F0) exposed to CPZ the estrus cycles were : 1) with CPZ at 12.5 mg/kg, 4 to 5 days (10), 6 days (1) and 9 days (1) ; 2) with CPZ at 25 mg/kg, 4 to 5 days (8), 6 days (3), 7 days (1) and 10 days (1) ; 3) with CPZ at 50

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Table 4. Body weights of the F1 and F2 pups from their ancestors (F0) exposed to chlorpromazine for 2 weeks before mating.

Age (day)	Sex	Chlorpromazine, mg/kg/day									
		F1 pup					F2 pup				
		0	12.5	25	50	100	0	12.5	25	50	100
2	M	6.85 ±0.05	6.10* ±0.14	6.17* ±0.36	5.12* ±0.54	5.07* ±0.84	5.73 ±0.08	7.14* ±0.29	6.39* ±0.15	6.77* ±0.21	7.17* ±0.27
	F	6.83 ±0.06	6.05* ±0.13	5.95* ±0.32	5.16* ±0.45	5.05* ±0.65	5.89 ±0.06	6.93* ±0.18	6.41* ±0.34	6.59* ±0.68	7.03* ±0.34
7	M	14.83 ±0.58	13.46 ±0.65	11.34* ±0.71	10.64* ±0.84	10.34* ±0.71	14.87 ±0.23	16.01* ±0.38	16.14* ±0.86	16.52* ±0.75	18.36* ±0.53
	F	14.80 ±0.67	13.47 ±0.56	11.40* ±0.86	10.08* ±0.72	10.28* ±0.54	14.79 ±0.41	16.00* ±0.44	16.08* ±0.76	16.50* ±0.87	18.19* ±0.44
14	M	25.32 ±0.84	24.68 ±0.73	23.04 ±0.56	22.35* ±0.93	23.45 ±1.30	23.15 ±0.92	28.94* ±0.64	27.77* ±0.73	27.61* ±0.32	30.23* ±0.51
	F	24.06 ±0.75	23.34 ±0.64	22.67 ±0.36	22.36* ±0.83	23.06 ±0.93	23.26 ±0.93	28.89* ±0.56	27.75* ±0.74	27.56* ±0.81	30.33* ±0.56
21	M	42.34 ±1.04	37.65* ±0.81	32.46* ±1.32	30.30* ±2.34	30.18* ±2.65	34.14 ±0.10	42.63* ±0.84	42.34* ±1.34	41.07* ±1.46	45.89* ±1.63
	F	41.06 ±1.10	36.64* ±0.94	32.00* ±1.06	29.18* ±1.84	29.07* ±2.03	33.04 ±0.11	41.52* ±0.65	41.36* ±1.36	40.84* ±0.96	44.08* ±1.34

Mean ± S.E., *: significantly different from the value of 0 mg/kg group, $P < 0.05$.

M: male, F: female.

mg/kg, 4 to 5 days (8), 6 days (2), 9 days (1) and 10 days (1); 4) with CPZ at 100 mg/kg, 4 to 5 days (10), 6 days (1), 7 days (2) and 9 days (1), respectively. Thus, the estrus cycles at about 8 to 10 weeks of age in the F1 females from the F0 parents exposed to CPZ were found to be about 4 to 10 days, similar to the control group, regardless of the CPZ dosage to which the F0 parents had been exposed. No abnormalities were noted in the external appearance of the F1 rats with the macroscopic study.

Incidence of insemination and pregnancy: As shown in Table 5, the incidence of insemination and pregnancy in the F1 rats was not affected regardless of the CPZ dosage to which the F0 parents had been exposed.

Autopsy findings on the 14th day of pregnancy: An autopsy on the 14th day of pregnancy revealed no difference in the number of myometrial glands per litter as

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compared to the control (Table 6). The number and weight of live fetuses decreased depending on the CPZ dosage to which the F0 parents had been exposed. No abnormalities were observed in a macroscopic study of the uterus.

Table 5. Insemination and pregnancy data for F1 rats from parents (F0) exposed to chlorpromazine for 2 weeks before mating.

Observation		Chlorpromazine, mg/kg/day				
		0	12.5	25	50	100
No. of rats (F1)	M	6	6	6	6	6
	F	12	12	13	12	14
Insemination (%)		100.0	100.0	100.0	100.0	100.0
{ No. of female rats inseminated / No. of female rats paired with males }		(12/12)	(12/12)	(13/13)	(12/12)	(14/14)
Pregnancy (%)		100.0	100.0	100.0	100.0	92.9
{ No. of pregnant rats / No. of female rats inseminated }		(12/12)	(12/12)	(13/13)	(12/12)	(13/14)

M: male, F: female.

Table 6. Autopsy findings on day 14 of pregnancy in F1 rats from parents (F0) exposed to chlorpromazine for 2 weeks before mating.

Observation	Chlorpromazine, mg/kg/day				
	0	12.5	25	50	100
No. of dams (F1)	5	5	6	5	6
Myometrial glands / litter	12.4 ± 0.7	12.4 ± 0.5	11.2 ± 1.0	11.0 ± 0.8	11.2 ± 1.8
Live fetuses / litter	11.6 ± 0.5	11.6 ± 0.8	10.0 ± 1.1	10.0* ± 0.3	10.1* ± 0.3
Average live fetal weight (g)	2.61 ± 0.02	2.58 ± 0.04	2.52* ± 0.02	2.64 ± 0.08	2.43* ± 0.06

Mean ± S.E., *: significantly different from the values of 0 mg/kg group, $P < 0.05$.

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3. F2 generation

The length of pregnancy of the F1 mothers ranged from 22.1 to 22.7 days regardless of the CPZ exposure of the F0 rats (Table 7). The number of live pups per litter was no different from the control group. The body weight of the F2 pups 48 hours after birth was significantly heavier, when compared to the control, only in groups descended from the F0 rats exposed to CPZ. However, the F2 pups frequently died within a few days after birth but none died after the second week. Their subsequent growth was very good and the body weight was significantly heavier than that of the control group, with no notable male-female differences in the body weight gain (Table 4). The gain in body weight was unrelated to the CPZ dosage to which the F0 rats had been exposed. The rate of survival of the F2 pups at the time of weaning was no different in rats descended from the F0 rats exposed to less than 25 mg/kg of CPZ than in the control rats. In the F2 rats descended from the F0 rats with an exposure of 50 and 100 mg/kg of CPZ, a significant decrease in the survival rates (67.9 and 50.0 %) was noted at the time of weaning (Table 7). A macroscopic study of the F2 rats revealed no abnormalities.

Table 7. Data for F2 pups from F1 rats whose parents (F0) were exposed to chlorpromazine for 2 weeks before mating.

Observation	Chlorpromazine, mg/kg/day				
	0	12.5	25	50	100
No. of dams (F1)	6	7	7	7	7
Length of pregnancy (days)	22.2 ± 0.3	22.5 ± 0.5	22.7 ± 0.5	22.6 ± 0.4	22.1 ± 0.5
Live pups / litter	11.0 ± 0.3	10.8 ± 0.6	10.8 ± 0.4	11.0 ± 0.2	10.9 ± 0.5
Average pup weight at hour 48 (g)	(M) {	5.73	7.14*	6.39*	6.77*
		± 0.08	± 0.29	± 0.15	± 0.21
	(F) {	5.89	6.93*	6.41*	6.59*
		± 0.06	± 0.18	± 0.34	± 0.68
Survival of pups at day 22 (%)	100.0	89.3	100.0	67.9**	50.0**

Mean ± S.E., * and **: significantly different from the values of 0 mg/kg group, $P < 0.05$ and $P < 0.01$, respectively.

M: male, F: female.

4. Wet organ weights

The major organs in each generation of rats were weighed and the ratio of wet organ weight to body weight was calculated to evaluate the effect of CPZ on the wet organ weight. CPZ administration of less than 50 mg/kg produced no difference in the wet weight of major organs in either the male or female rats of each generation when compared to those of the control group. With the administration of 100 mg/kg of CPZ, as shown in Table 8, an autopsy of the F0 males at 14 weeks of age revealed an increase in the wet weight of the adrenal, pituitary and prostate, when compared to the control group. An autopsy of the F0 mothers, 20 weeks of age, at the time of weaning of the F1 pups, revealed an increased wet weight of the adrenal and ovary, with a marked decrease in the wet weight of the pituitary and uterus. However, such tendencies were not noted in the wet weight of the major organs of the F1 males and females at the time of weaning (Table 8).

Furthermore, in offspring from the F0 rats exposed to CPZ at 100 mg/kg, no difference from the control group was noted in the wet weight of the major organs of the F1 males at 12 weeks of age, of the F1 females at 18 weeks of age, and of the F2 males and females 3 weeks after birth.

Table 8. Comparison of organ weights in chlorpromazine (100 mg/kg)-exposed rats (F0) and their offspring (F1) with those in control rats.

Generation	Weeks of age	liver	kidney	adrenal	pituitary	testis [#] or ovary ^{##}	prostate [#] or uterus ^{##}
F0	14 (M, 10)	±	±	+	+	±	+
	20 (F, 4)	±	±	+	—	+	—
F1	3 (M, 10)	±	±	±	±	±	±
	3 (F, 10)	±	±	±	±	±	±

Marks of # and ##: for male and female. M or F and number in parentheses indicate male or female and number of rats, respectively. The effect of chlorpromazine on organ weights was evaluated by the following formula:

A (control) = organ weight/body weight

B (treated) = organ weight/body weight

C % = $100 \times (B - A) / A$

Marks of —, ±, + and # indicate the values when C value is less than —6 %, between —5 % and +5 %, between +6 % and 10 % and more than +10 %, respectively.

DISCUSSION

Since the tragic incident with thalidomide (Lenz, 1961), the teratogenic action of drugs has been recognized, and in 1966 WHO recommended the introduction of tests for teratogenicity, prenatal effect and reproductive effect as index tests for specific toxicity (Takagi et al., 1975).

CPZ has been used in the field of psychiatry for nearly 40 years. Studies on the reproductive effects of this drug have primarily relied on the tests for fetal toxicity (Beall, 1972 ; Itoh, 1976a ; Dailey, 1978 ; Robertson et al., 1980). The tests of CPZ influence on subsequent generations have been reported only by Itoh et al. (1976a ; 1978b), Robertson et al. (1980), Fujii (1987) and Saigo (1989). In the present study, CPZ was administered daily to male and female rats for 2 weeks prior to mating. The effect of CPZ on reproduction in three generations was evaluated.

The estrus cycle in mature female rats is usually reported to be 4 to 5 days (Takagi et al., 1975), but the estrus cycle prior to the CPZ administration, in the female Sprague Dawley strain rats (F0) at about 8 to 10 weeks of age used in the present study, was 4 to 10 days the same as in the report of Saigo (1989). Although in the present study the estrus cycle in the F0 females following CPZ administration could not be evaluated during housing and mating with the F0 males for 2 weeks, the estrus cycle in the F1 females from the F0 parents exposed to CPZ was from 4 to 10 days, the same as in the control group, with no correlation to the CPZ administration in the F0 generation. Saigo (1989) reported that in a test of CPZ administration for 9 weeks before breeding, the estrus cycle of the F0 females was prolonged due to the CPZ dosage to which the F0 females were exposed, leading to a characteristic prolongation of the proestrus which is in agreement with a report by Brraclough and Sawyer (1959). Thus, CPZ may induce the prolongation of the estrus cycle only in the parent generation receiving CPZ, as described by Saigo (1989), but have no influence on the estrus cycle in the following generation.

The incidence of insemination and pregnancy in the F0 rats decreased depending on the CPZ dosage, with a lower incidence in the latter than in the former. The results in the present study indicated a greater adverse influence of CPZ administration on pregnancy than in CPZ administration for 9 weeks by Saigo (1989). This is further supported by the reports of Hoekstra et al. (1984) and Kogo et al. (1986) on ovulation inhibition by CPZ, which showed a decrease in the degree of inhibition produced by a repeated administration of the drug, and also by the report of Sliwa (1984a, 1984b) on an inhibition in the number and motility of mouse spermatozoa resulting from CPZ administration. In addition, it is reported that in the study to evaluate the influence on reproduction of the benzodiazepine series of anti-anxiety drugs, administration of the drug to both males and females inhibited the mating performance of mice (Guerriero and Fox, 1976) and also the incidence of pregnancy of rats (McClain and Hoar, 1980), but the drug administration to male rats alone failed to have any influence on reproduction (Wilkinson et al., 1980). Thus, it

is thought that CPZ exerts an inhibitory influence on the insemination and pregnancy in a fashion different from that reported on the benzodiazepine series, because CPZ inhibits the reproductive capacity in both the males and females, and the degree of inhibition is modified by the dosage as well as by the duration of administration. Since the decrease in the incidence of insemination and pregnancy due to CPZ in the F0 generation was not found in the F1 generation, it is thought that CPZ has no influence on the incidence of insemination and pregnancy in successive generations.

The length of pregnancy of the F0 and F1 females was 20.0 to 22.7 days, the same as in the control group. These figures are also in agreement with the length of pregnancy in rats as previously reported by Saigo (1989). The natural delivery of litters was normal regardless of the CPZ dosage. Autopsy on the 14th day of pregnancy in the F0 and F1 rats revealed the same number of myometrial glands per litter as in the control group with no correlation to the CPZ dosage. However, the number and weight of the F1 and F2 fetuses decreased depending on the CPZ dosage. The degree of decrease was greater than that of the 9-week examination of Saigo (1989), indicating a greater adverse influence of short term administration of CPZ on the fetus than long term administration. This suggests the severity of the effect of CPZ on reproduction over a long term. The body weight of the F1 pups 48 hours after birth decreased, with no notable male-female differences, dependent on the CPZ dosage to which the F0 rats had been exposed, while the body weight of the F2 pups 48 hours after birth increased more than that of the control group. This degree of decrease and increase in the F1 and F2 pup weights was greater than that of the 9-week examination of Saigo (1989).

The growth of the F1 pups after the 14th day was favorable, however, rats born with a low birth weight had lower body weight than the control rats for about one or two weeks after weaning. At 48 hours after birth, the number of live pups (F1) per litter decreased depending on the CPZ dosage to which the F0 rats had been exposed. The degree of decrease was greater than that of the 9-week examination of Saigo (1989). The death of the newborns occurred mostly within the first several days after birth, and none died after the 14th day of life. Survival of the F1 rats at the weaning period decreased depending on the CPZ dosage to which the F0 rats had been exposed. Especially the survival of the F1 rats from the F0 rats, which had received 100 mg/kg of CPZ, showed a marked decrease of 45.8 %. Moreover, the survival of the F2 pups that descended from the F0 rats, which had received 50 and 100 mg/kg of CPZ, also showed a marked decrease of 67.9 % and 50.0 %, respectively, at the time of weaning. However, these results were not noted in the 9-week examination of Saigo (1989). Thus, it is suggested that CPZ exerts a greater adverse influence on the survival of F1 and F2 pups that descended from the F0 rats with a short-term administration before mating than on those with a long-term administration.

When the number of newborns per litter is small, it is well known that their growth is more rapid, with a marked increase in body weight (Takagi et al., 1975).

In the present study, the F2 pups had a growth pattern in agreement with this data. While the number of newborns was similar to the control group, their body weight 48 hours after birth was significantly heavier than that of the control. In addition, their subsequent growth was more favorable than that of the control group, with no notable male-female differences. This appears to be due to the influence of CPZ. It is well known that CPZ increases prolactin secretion from the anterior pituitary lobe, causing mammary hypertrophy and facilitating lactation (Hafs and Williams, 1964 ; Itoh, 1978c), but the relationship between possible CPZ-induced milk secretion and body-weight increase in newborns is still unclear.

McClain and Hoar (1980) have suggested that an increase of newborn death due to flunitrazepam results from a depression in the central nervous system of the mother animal due to drug administration during pregnancy, leading to indifference of the mother animal to the newborn. In the present study, CPZ was administered prior to pregnancy. Therefore, a similar conclusion in a relationship between newborn death and CPZ cannot be drawn. This is a problem for future research. However, the apparent influence on the mortality of both F1 and F2 generations that descended from the F0 rats administered CPZ prior to mating is of interest.

The influence of CPZ on wet organ weight was not significant following the administration of less than 50 mg/kg of CPZ in the F0, F1 or F2 generations. However, when administering CPZ at 100 mg/kg, the wet weight of the adrenal, pituitary and prostate glands in the F0 males increased. On the other hand, in the F0 females the weight of the adrenal gland and ovary increased, and the pituitary gland and uterus decreased. The results of the present study showed a trend similar to that in the 9-week examination of Saigo (1989). Hafs and Williams (1964) administered CPZ to goats for 8 weeks with no great influence on the reproductive organs, but their findings differ from the results we have obtained in rats.

In tests of CPZ on fetal toxicity in rats, it is reported that CPZ causes cleft palate (Beall, 1972 ; Tanimura, 1974 ; Dailey, 1978 ; McClain and Hoar, 1980 ; Robertson et al., 1980). However, cleft palate can also occur in fetuses when food and water are withheld from the mother (Szabo and Brent, 1974). Although CPZ decreases the maternal food and water intake, whether or not CPZ has a primary teratogenic effect has not been decided. In the present study, autopsy of the rats in the F0, F1 and F2 generations revealed no macroscopic abnormalities. Furthermore, in both male and female F0 rats receiving CPZ there was a decrease in the spontaneous motor activity and body weight, as well as food and water intake. This decrease was not noted in the F1 and F2 generations except for the influence of CPZ on body weight in the F1 generation. In fact, CPZ exerted an inhibitory influence on the growth of the F1 fetuses and newborns and a facilitatory influence on that of the F2 rats, when CPZ was administered to both male and female F0 rats before mating.

These results suggest that a short-term CPZ administration to both male and female F0 rats before mating exerts more inhibitory influence on the reproduction than a long-term CPZ administration (Saigo, 1989). Thus, the CPZ administration

may develop a tachyphylactic or adaptive response to the inhibition of reproduction in rats.

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