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A Teratogenic Study of Chlorpromazine, Orphenadrine, Perphenazine, and LSD-25 in Rats

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A Teratogenic Study of Chlorpromazine, Orphenadrine, Perphenazine, and LSD-25 in Rats. BEALL, JAMES R. (1972). *Toxicol. Appl. Pharmacol.* 21, 230–236. Pregnant rats were treated po with either perphenazine, chlorpromazine, orphenadrine or LSD-25 on days 6 through 15 after mating. On day 21, the pups were removed and examined for gross, visceral, and skeletal defects. The dams were examined for nidations, resorptions and reproductive performance. In all, 265 dams and 3454 offspring were evaluated. The results indicate that pregnant rats treated with near-lethal doses of perphenazine or chlorpromazine have more resorptions than controls. At one or more doses of all drugs, average offspring weights were less than control values. Visceral examination of the pups revealed that 7 dams from the 2 highest orphenadrine groups had abnormal offspring. In these groups, 8 of 157 pups had enlarged, darkened bladders. One abnormally developed pup was produced in the low-dose chlorpromazine group. All other rats treated with perphenazine, chlorpromazine or LSD-25 had normally developed offspring.

Teratogenic studies of LSD administered to pregnant rats (Alexander *et al.*, 1967; Warkany and Takacs, 1968), mice (Auerback and Rogowski, 1967; Hanaway, 1969), and hamsters (Geber, 1967) yielded inconclusive data. Chromosomal studies of leukocytes from persons taking LSD have likewise produced conflicting results, some studies indicating increased damage (Irwin and Egozcue, 1967; Egozcue *et al.*, 1968; Cohen *et al.*, 1967; Zellweger *et al.*, 1967; Nielsen *et al.*, 1969) and others indicating no damage (Bender and Sankar, 1968; Sparkes *et al.*, 1968; Tjio *et al.*, 1969; Corey *et al.*, 1970).

In the study by Nielsen *et al.* (1969), other psychotropic drugs were implicated in chromosomal damage. These workers reported that LSD and perphenazine (with orphenadrine) caused higher total frequencies of chromosome fragments, dicentric chromosomes, and ring chromosomes. They stated that such damage would "Probably predispose to malignant disorders and an increased risk of abortions, chromosome abnormalities, and congenital abnormalities in the offspring." Yet, definitive teratogenic studies of chlorpromazine, perhenazine, and orphenadrine have not been reported (Julou *et al.*, 1968). Since implications of chromosomal damage include developmental abnormalities (Shaw, 1970), a teratogenic study of these 4 compounds was performed. This paper reports the data obtained.

METHODS

The following compounds were used: (1) chlorpromazine hydrochloride; Smith, Kline, and French Labs, Lot No. 5238796, received as tablets; (2) orphenadrine hydrochloride; Riker Laboratories, Lot No. 9912, received as tablets; (3) perphenazine

hydrochloride; Schering Corporation, Lot No. PTP-9K-3, received as powder; and (4) LSD-25 (LSD); Sandoz S.A., Batch No. 88601, received as powder.[1]

Mature rats [CAW; CFE (SD) spf strain, Carworth, Inc.], were kept in rooms with controlled lighting (12 hr on, 12 hr off) and provided continuously with Purina Rat Chow and water. Females were bred, and mating was confirmed the next morning by the presence of sperm in the vaginal smear. The day that sperm was found was designated as day 0. Based on this time designation, the females were dosed from day 6 through day 15 after mating.

The tablets were pulverized. All drugs were administered by esophageal intubation in 2.5% aqueous Tween 80. Drug concentrations were adjusted so that no rat received a dose volume in excess of 2.5 ml. The doses (Table 1) were selected on the weights of the salts or ethers, not the active bases.

The study was separated into two parts (Table 1): Chlorpromazine and perphenazine were studied in Part A; orphenadrine and LSD-25 were studied in Part B. Control groups were included in each part.

TABLE 1
NUMBER OF ANIMALS EVALUATED[a]

Dose group	Number of pregnant rats which: Started on study	Died during study	Had complete resorptions	Produced live offspring
Part A				
Controls I	24	0	0	24
Chlorpromazine				
5.0 mg/kg (1)[b]	19	0	0	19
25.0 mg/kg (5)	20	0	0	20
35.0 mg/kg (7)	22	3	1	18
Perphenazine				
0.7 mg/kg (1)	21	0	1	20
3.5 mg/kg (5)	21	0	0	21
7.0 mg/kg (10)	25	10	0	15
Part B				
Controls II	20	0	0	20
Orphenadrine				
3.0 mg/kg (1)	19	0	0	19
15.0 mg/kg (5)	19	0	0	19
30.0 mg/kg (10)	19	0	0	19
LSD-25				
3.0 μg/kg (1)	16	0	0	16
30.0 μg/kg (10)	18	0	0	18
90.0 μg/kg (30)	18	1	0	17
	281	14	2	265

[a] The study utilized 2 control groups because 5 months elapsed between Parts A and B.

[b] These values indicate the relation to an average human dose given to *hospitalized* patients (Nielsen *et al.*, 1969). On a mg/kg/day basis, (1) means equal to human dose and (5) means 5 times human dose. For perphenazine, the doses are about 3, 15, and 30 times the amount recommended by the package insert.

[1] The LSD-25 was supplied by the National Institute of Mental Health, Bethesda, Maryland.

On day 21, the dams were sacrificed (by overdose of chloroform) and the offspring removed. The following data were collected: (1) the numbers of live fetuses, resorptions, and nidations, and (2) the sex and weight of each live fetus. Differences among the groups were evaluated by Dunnett's multiple comparison procedure (Dunnett, 1955).

The embryos were inspected for gross abnormalities and segregated into 2 lots for internal examination; every third fetus in each litter was prepared for serial slicing and examined for soft tissue defects by the method of Wilson (1965). Remaining fetuses were prepared by the KOH-Alizarin Red S method (Staples and Schnell, 1964) and examined for skeletal defects.

RESULTS

The data indicate that treatment of pregnant rats with chlorpromazine, perphenazine, and LSD did not cause abnormal offspring. Some pregnant rats treated with orphenadrine produced abnormal offspring. These 4 drugs influenced, to varying degrees, resorptions and offspring body weights.

Tables 1 and 2 present the number of rats which were used and the number of

TABLE 2

NIDATIONS, RESORPTIONS AND LITTER SIZES

Group	Nidation sites		Dams with resorptions	Resorptions	Litter size
	Total	Mean	No. (%)	No. (%)[a]	
Part A					
Control I	333	13.9 ± 0.4[b]	4 (16.7)	6 (1.8)	13.6 ± 0.4[b]
Chlorpromazine					
5.0 mg/kg	232	12.2 ± 0.8	7 (36.8)	13 (5.6) C[c]	11.5 ± 0.8 C
25.0 mg/kg	283	14.2 ± 0.4	9 (45.0) B	17 (6.7) A	13.3 ± 0.5
35.0 mg/kg	251	13.2 ± 0.5	12 (63.2) A	51 (20.3) A	10.5 ± 1.0 B
Perphenazine					
0.7 mg/kg	268	12.8 ± 0.8	6 (28.6)	17 (6.3) B	12.0 ± 1.0 C
3.5 mg/kg	278	13.2 ± 0.8	8 (39.1)	9 (3.2)	12.8 ± 0.8
7.0 mg/kg	215	14.3 ± 0.4	8 (53.3) B	14 (6.5) A	13.4 ± 0.5
Part B					
Controls II	272	13.6 ± 0.5	3 (13.0)	4 (1.5)	13.4 ± 0.5
Orphenadrine					
3.0 mg/kg	270	14.2 ± 0.3	6 (31.6)	7 (2.6)	13.8 ± 0.4
15.0 mg/kg	259	13.6 ± 0.4	7 (36.8)	8 (3.1)	13.2 ± 0.4
30.0 mg/kg	268	14.1 ± 0.2	8 (42.1) C	9 (3.4)	13.6 ± 0.3
LSD-25					
3.0 μg/kg	228	13.4 ± 0.4	6 (29.4)	5 (2.2)	13.1 ± 0.4
30.0 μg/kg	252	14.0 ± 0.4	9 (50.0) B	10 (4.0)	13.4 ± 0.4
90.0 μg/kg	225	14.1 ± 0.5	5 (37.5)	10 (4.)	13.4 ± 0.5

[a] Percentage of nidation sites which resorbed.
[b] Mean ± SE.
[c] Statistical differences between control and treatment groups are indicated as follows: A = $P < 0.01$, B = $P < 0.05$, C = $P < 0.10$.

offspring which were evaluated. The number of dams which died during the study (Table 1) indicates that near toxic quantities were used in the high-dose perphenazine and chlorpromazine groups. A minimum of 200 pups per group were produced by the surviving dams.

Reproductive Performance and Embryotoxicity

The data which reflect the reproductive performance of the dams are presented in Table 2. None of the compounds influenced the number of nidations. Compared to controls, the percentage (as analyzed by the methods of Dixon and Massey, 1969) of pregnant rats which had 1 or more resorptions were greater in the high-dose perphenazine ($p < 0.05$) and chlorpromazine groups ($p < 0.01$). The percentage of dams with resorptions may have been greater ($p < 0.10$) in the high-dose orphenadrine group than in the control group. While the percentage in the high-dose LSD group was similar to the control value, the percentage of dams with resorptions in the intermediate-dose group was greater than the control value ($p < 0.05$).

Similar results were obtained in all groups when the number (not percentage) of dams with resorptions were expressed as functions of the total in each group.

The average litter size in the high-dose chlorpromazine group was less than that in the controls ($p < 0.05$). Litter sizes in the low-dose perphenazine and chlorpromazine groups may have been smaller than the control values ($p < 0.10$). Litter sizes were not significantly different between the control and other treatment groups ($p > 0.10$). In all cases, dams which resorbed entire litters were included in the analyses.

Rats from the low- and high-dose perphenazine groups had significantly more resorptions, as percentages of nidation sites, than the control group ($p < 0.05$ and $p < 0.01$, respectively). While the percent resorptions in the low-dose chlorpromazine group may have been greater than control values ($p < 0.10$), the intermediate- and high-dose groups had more resorptions ($p < 0.01$) than controls. The percent resorptions were not different among controls, orphenadrine, and LSD-25-treated rats.

The averages of individual pup weights revealed differences between pups from control and treated rats (Table 3). In Part A, pups from the high-dose perphenazine group and from the low- and intermediate-dose chlorpromazine groups weighed less than controls ($p < 0.01$). In Part B, pups from all treated groups weighed less than controls ($p < 0.05$). Body weights from the Part A control pups were less than the Part B control pups. This difference could not be ascribed to differences in sizes of the dams; analyses of the weights on day 6 showed no differences between the 2 groups ($p > 0.10$). Analysis of the average litter weights showed only the high-dose perphenazine pups to weigh significantly less ($p < 0.05$) than control pups.

Developmental Abnormalities

In Part A, none of the control or perphenazine treated dams had abnormally developed offspring. Of the pups produced by dams treated with chlorpromazine, 1 was deformed. This pup was produced in the low-dose group and had the following defects: (1) the tail was absent; (2) the 3 posterior lumbar vertebrae were absent; (3) the first lumbar vertebra had split ossification centers; (4) the second lumbar arch was prematurely fused.

In Part B, visceral examination revealed that 1 control pup had an enlarged atrium.

TABLE 3

DATA OBTAINED FROM OFFSPRING

Group	No. examined	Body weight[a]	Examinations: Skeletal	Examinations: Visceral	No. abnormal[b]
Part A					
Controls I	327	5.36 ± 0.03	228	99	0
Chlorpromazine					
5.0 mg/kg	219	5.16 ± 0.06 A[c]	149	70	1 S, V
25.0 mg/kg	266	5.05 ± 0.03	188	78	0
35.0 mg/kg	200	5.32 ± 0.03	149	51	0
	685		486	199	1
Perphenazine					
0.7 mg/kg	251	5.40 ± 0.04	176	75	0
3.5 mg/kg	269	5.31 ± 0.02	189	80	0
7.0 mg/kg	201	5.00 ± 0.04 A	139	62	0
	721		504	217	0
Part B					
Control II	268	5.59 ± 0.02	185	83	1 V
Orphenadrine					
3.0 mg/kg	263	5.50 ± 0.02 A	183	80	0
15.0 mg/kg	251	5.43 ± 0.02 A	173	78	6 V[d]
30.0 mg/kg	259	5.51 ± 0.02 B	180	79	2 V
	773		536	239	8
LSD-25					
3.0 μg/kg	223	5.45 ± 0.03 A	158	65	0
30.0 μg/kg	242	5.48 ± 0.02 A	173	69	0
90.0 μg/kg	215	5.35 ± 0.03 A	150	65	0
	680		481	199	0

[a] Mean ± SE, on a per group basis.
[b] S represents skeletal abnormalities; V represents visceral abnormalities.
[c] Significant differences between control and treatment groups are indicated as follows: A = $P < 0.01$, B = $P < 0.05$.
[d] While control data were taken from only 44 dams, examination of over 6000 control pups from other studies performed in these laboratories has failed to reveal similar bladder abnormalities (see text).

Eight pups from 7 orphenadrine-treated dams had abnormal urinary bladders: The bladders were dark, occupied about 1/3 of the abdominal cavity, and contained urine with traces of blood. Of 78 pups examined for visceral defects in the intermediate-dose group, 6 had enlarged bladders; of the 79 pups examined in the high-dose group, 2 had enlarged bladders.

Aside from a normal and infrequent incidence of extra ribs and dermal hematomas in pups from all groups, abnormalities were not seen in any other pups from Parts A and B.

DISCUSSION

While chromosome analyses were not performed, the data obtained do not support the statement of Nielsen *et al.* (1969) which implied that the types of chromosome damage caused by perphenazine and LSD would probably predispose offspring to an

increased risk of congenital abnormalities. Congenital abnormalities did not occur in pups from dams treated with perphenazine, LSD, or chlorpromazine.

In the study of Nielsen *et al.* (1969) patients who received perphenazine also received orphenadrine, and those taking LSD were concurrently taking other psychoactive drugs (Ayd, 1969). The possible synergistic actions of perphenazine and orphenadrine were not evaluated in the present teratologic study. Abnormalities did occur in the urinary system of 8 pups from 7 orphenadrine-treated dams; aside from the abnormal bladders, the pups appeared morphologically normal.

In her report on "clastogens," Shaw (1970) stated, "In spite of the conflicting chromosome results, there is general agreement that LSD, bromolysergic acid, and mescaline are teratogenic to mice, rats, and hamsters." As mentioned above, the literature indicates that the teratogenic properties of LSD are dubious. Alexander *et al.* (1967) reported that LSD treatment resulted in stunted stillborn rats, but Warkany and Takacs (1968) reported that LSD treatment did not affect fetal development in rats. The study of Alexander *et al.* (1967) evaluated only 136 pups of which 51 were controls while Warkany and Takacs evaluated 887 pups from treated dams alone.

The teratogenic action of LSD in other species is also questionable. For example, LSD has been reported both to cause (Geber, 1967) and not to cause (DiPaolo *et al.*, 1968) deformities in hamsters. While in mice LSD freely crosses the placenta (Idänpään-Heikklia and Schoolar, 1969) and causes deformities (Auerback and Rugowski, 1967; Hanaway, 1969), the response may be strain dependent (DiPaolo *et al.*, 1968).

Of the 680 pups from LSD-treated dams examined in the present study, none was grossly deformed. Hence, the data support the work of Warkany and Takacs (1968). LSD-treated dams produced pups with mean body weights which were less than control values. This effect of LSD has been previously reported for rats (Alexander *et al.*, 1967), hamsters (Geber, 1967), and rabbits (Fabro and Sieber, 1968). Although the body weights of pups from several treatment groups were statistically less than control values, only those from the perphenazine groups had a dose-related decrease in weight. However, many drugs when given to pregnant dams in toxic doses will reduce growth rates in the offspring.

It is noteworthy that the doses of perphenazine and chlorpromazine were lethal to some treated dams; yet, teratologic changes did not occur in these groups. In contrast, rats received less toxic doses in the orphenadrine group where visceral changes occurred.

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