ABSTRACT

Joseph Franklin Holson, Jr. TERATOGENIC POTENTIAL OF D-LYSERGIC ACID DIETHYLAMIDE (DELYSID) IN THE ALBINO RAT. (Under the direction of Everett C. Simpson) Department of Biology, 1969.

D-lysergic acid diethylamide (LSD-tartrate) in doses of 5-20 ug per kilogram of body weight was given to pregnant rats (Holtzman strain) at preimplantation (day 4) and organogenic (day 9) periods. Twenty rats were given 5 ug LSD/kg b.w. on day 4 (10 rats) or day 9 (10 rats) of pregnancy. Another 20 rats were given similar injections of 20 ug LSD/kg b.w. Thirty pregnant rats served as control animals for intrauterine treatments with LSD. Ten of these animals were not treated. The remaining 20 animals were given control intrauterine injections. A total of 40 pregnant rats were given injections as follows:

Control unilateral intrauterine on day 4 of pregnancy

Control unilateral intrauterine on day 9 of pregnancy

LSD unilateral intrauterine on day 4 of pregnancy

LSD unilateral intrauterine on day 9 of pregnancy

The control intrauterine injections provided simulative operational treatment. Control groups (3 groups of 10 animals each) were utilized for each experimental phase to negate any seasonal variation of reproductive competence. Examination of the resultant 1,233 fetuses revealed no significant frequency of congenital defects in the control or experimental animals. Intrauterine LSD injections on day 4 of pregnancy evoked significantly decreased mean fetal weights. There was a significant increase of resorption sites in those animals given

intraperitoneal injections of LSD (5.0 ug/kg b.w.) on day 9 of pregnancy ($p < 0.05$). Results of the intrauterine treatments with LSD did not substantiate a direct target-tissue mechanism involving reported LSD teratogenicity. The systemic injections of LSD failed to cause a significant frequency of detectable malformations in offspring of treated adults.

TERATOGENIC POTENTIAL OF D-LYSERGIC ACID DIETHYLAMIDE (DELYSID) IN THE ALBINO RAT

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LOVINGLY DEDICATED TO MY MOTHER AND FATHER, MR. AND MRS. JOSEPH F. HOLSON, AND TO MY WIFE, JUDITH, AND DAUGHTERS, PHAEDRA AND SABRINA.

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INTRODUCTION

The period between 1958 and 1962 represents a very important time in the development of mammalian experimental teratology. When Widerkind Lenz discovered the relationship between thalidomide and birth of numerous malformed infants, scientists realized the need for more thorough drug studies.

During the last decade there has been widespread increase in abuse of drug use. One aspect of this increased consumption of drugs can be relegated to the "younger generation." The model psychotomimetic, lysergic acid diethylamide (LSD), has attracted much scientific and social consideration.

LSD was first prepared by Stoll and Hofmann in 1938 (Stoll and Hofmann, 1965). It was not until a few years later that the psychotropic properties of LSD were detected by Hofman while re-investigating the drug (Stoll and Hofmann, 1965). More recently, LSD has had limited use in the therapeutic treatment of alcoholics and as an analgesic (Pfeiffer and Murphree, 1965). Of primary concern in this investigation is the possible teratogenicity of LSD in the albino rat. It is generally accepted that inferences can be made to the human from research involving the rat.

However, consideration is maintained with reference to existence of inter-specific variables which might pertain to interpretation of this research. It must be emphasized that the probability of a pregnant woman consuming LSD at an early stage of pregnancy is high enough to warrant its investigation. This early stage of pregnancy,

from onset of tissue differentiation until eight weeks of age, is an especially critical insult period for the human (Wilson, 1964). This period of pregnancy is often the "uncertain period" (Auerbach and Rugowski, 1967). Therefore, an unknowing mother might endanger fetal development by ingesting LSD. Likewise, it is necessary to realize the potential of this drug with respect to its continued use in psychotherapy.

Specifically, this research was concerned with any effect of LSD upon fetal development in the albino rat. Major consideration was given to the mode of treatment, dosage, and the stage of pregnancy at which the LSD was administered.

THE REVIEW OF LITERATURE

The initial report of LSD teratogenicity was by George J. Alexander, et al., 1967. In these experiments, female rats of the Wistar strain (250 g.) were mated with healthy males (450 g.). All experimental animals were given subcutaneous injections of 5 ug LSD/kg of body weight. This dosage was selected because it corresponds in the rat to the human hallucinogenic dose (100-400 ug per person--1.7 to 6.7 ug/kg body weight). Control animals received subcutaneous injections of physiological saline.

The first two experiments consisted of a total of 10 rats which were treated with the LSD injections on day four of pregnancy. Of the first five animals, only one was reported to have delivered a normal litter. The other four rats either contained resorbed fetuses, had stillborn fetuses, or gave birth to runted offspring. In experiment two, three of the five rats either aborted or gave birth to varying numbers of stillborn fetuses. Two litters were normal, except that one litter was unusually small (4 offspring). The third group of animals was injected on days 8, 9, 10, 11, or 12 of pregnancy. There were no observed effects upon the offspring of animals in this last group. The treated animals delivered 51 apparently healthy offspring, and the control animals gave birth to 65 ratlets. Gross observation of the young from all these experiments revealed no malformations. There was some stunting of development in the case of the three stillborn offspring. Offspring of animals treated with LSD had comparable weights

to the control offspring, but some failed to grow as rapidly as the control animals.

The injection of LSD-tartrate into mice during early stages of pregnancy caused a 57 percent frequency of abnormal embryos (Auerbach and Rugowski, 1967). Mice of the strains BALB/Cau, C57BL6/Au, C3H/HeAu, and F_1 (BALB/Cx57BL) were utilized for this investigation. The appearance of a copulatory plug was designated as day 0 of pregnancy. Before injection, the LSD-tartrate was diluted in Tyrode's solution to obtain suitable concentrations. All animals were given I.P. control or I.P. LSD injections on day 7 of pregnancy. The embryos were removed and examined by gross inspection 4 days following the injection. The doses of LSD injected ranged from 1×10^{-6}g to 5×10^{-8}g. Treated animals exhibited a 57 percent incidence of deformities, while the control animals had fewer than 10 percent malformations. In all cases, malformations were characteristic brain defects and additional abnormalities of the lower jaw, shifts in eye position, and modified facial contours. To determine stage specificity of the produced malformations, mice were given injections of LSD on days 6, 8, or 9 of pregnancy. Brain abnormalities were observed in the day 6 treated animals, but no gross observable effects were discerned in offspring of animals treated later than day 7 of pregnancy.

Congenital malformations induced by Bromolysergic acid, mescaline, and lysergic acid diethylamide in the hamster were reported by W. F. Geber (1967). Pregnant hamsters were housed in quiet quarters and illuminated by natural light (roof window). The alkaloids used as insult agents were dissolved in sterile physiological saline solution prior to injection. Injections were given subcutaneously on the 8th

day of pregnancy. All control animals were injected with sterile saline in the same manner. After single injections they were returned to quarters and Caesarian sectioned on day 12 of pregnancy. The dose range of LSD given was from 84 ug/kg body weight to 240 ug/kg body weight. No major abnormalities were detected in the 300 control fetuses, but there was a limited number of stillborn fetuses, resorbed fetuses, and runts. In the experimental animals, abnormalities of this nature were much greater on a percentage basis. An average of 6.2% of experimental (LSD-treated fetuses) exhibited one or more types of congenital malformation. Approximately 10% of the malformed fetuses possessed more than one type congenital defect. The types of gross defects observed were exencephaly, spina bifida, interparietal meningocele, omphalocele, hydrocephalus, myelocele, edema along the spinal axis, generalized edema and hemorrhages of brain areas (parietal and frontal) and the neck region. This study indicated no correlation between dose (ug/kg of body weight) of the drug given to the parent and percentage of malformations contained in litters of treated animals. A dose-response relationship did occur in reference to two other parameters. As litter size decreased, resorption rate and fetal mortality rate increased. Runting was augmented as drug dosage was increased.

Lysergic acid diethylamide (Delysid) administered to pregnant Wistar rats during periods of organogenesis did not cause a significant frequency of congenital defects (Warkany and Takacs, 1968). LSD was administered orally or intraperitoneally in single doses to pregnant rats on day 7, 8, or 9 of gestation or in multiple doses from day 7 through day 12 of pregnancy. The dosage of individual treatments ranged from 1.5 to 300 ug. Of the 55 pregnant rats treated, 4 had

litters completely resorbed; 47 rats were sacrificed on day 21 and the young were removed; four were allowed to go to term and these offspring were raised (44 pups were born, some were raised and bred). The mean weight of the 21-day fetuses was 3.53 ± 0.44 g (controls, 4.14 ± 0.71 g). The mean litter size of the 21-day fetuses was 10.2 ± 1.8 (controls, 10.0 ± 2.8). There was no significant statistical difference between the control and experimental fetuses. The remaining 504 fetuses were cleared or dissected (409 dissected and 95 cleared) and considered normal. There was no discernible pattern common to the four abnormal fetuses; one was hydrocephalic; one had short extremities and syndactylism; and the remaining two were small (1.7 and 2.1 g).

Warkany and Takacs (1968) also gave LSD injections to pregnant Wistar rats on day 4 or 5 of pregnancy. Thirty-four rats were given doses ranging from 1 to 100 ug LSD (Delysid). Two of the treated rats had no litters. Of 355 offspring from the 32 treated rats, 296 were removed on day 21 of pregnancy by Caesarean section. Except for one small fetus, all appeared normal following gross inspection, dissection, or clearing. Thirty-five young were delivered and raised, all appeared normal and healthy. There was a resorption rate of 5.9% which was not different from those animals treated with saline solution injections.

Fabro and Sieber (1968) did not find lysergide tartrate teratogenic in the New Zealand white rabbit. Lysergide tartrate was administered orally (20 or 100 ug/kg b.w.) to pregnant does for various intervals both early and late in pregnancy. Animals given lysergide tartrate did not significantly differ from the control animals in respect to average number of implantations, incidence of resorption,

and number of malformations. There was a significant decrease in the average fetal weight of offspring from lysergide tartrate treated mothers (100 ug/kg b.w. on days 7 to 9 of pregnancy).

High doses (5 ug) of LSD-25 administered to Swiss-Webster mice on days 6, 7, 8, or 9 of pregnancy induced a significantly high incidence of abnormalities of the anterior subcapsular lens (Hanaway, 1969). Single interperitoneal doses (5 ug) of LSD-25 were given to a total of 18 mice on gestation days 6, 7, 8, or 9. The embryos were removed on day 18 and the whole heads were fixed for subsequent histological analysis. Earlier experiments had shown that treatment with LSD-25 on days 4 or 5 had no observable effect on the developing lens. However, fetuses treated on days 6, 7, 8, or 9 (prior to closure of the neural tube and differentiation of the eye) revealed drastic effects. Animals injected on day 6 had 81% incidence, day 8 had 55%, and day 9 revealed 79% incidence of abnormal subcapsular lens development. The observed lens defect was an accumulation of globular, eosinophilic material outside the anterior lens epithelium and under the capsule. Accumulation of this substance over the total anterior surface area of the lens occurred in 50% of the abnormal eyes. Hyperplasia was common in the germinal zones and often the lens bow was posteriorly broadened.

Roux, et al. (1970), treated pregnant rats, mice, and hamsters with doses of LSD ranging from 5-500 ug/kg b.w. over varied intervals of pregnancy. No deleterious effects were noted in offspring treated with the low or high doses of LSD. Fetal mortality, mean weight, and incidence of malformations were not significantly altered in either of these species when treated throughout their respective pre-implantation or organogenic periods of development. LSD was given to 22 pregnant

hamsters, 98 pregnant rats, and 67 pregnant mice. Some 189 hamster fetuses, 521 mouse fetuses, and 1,003 rat fetuses were examined prior to partuition, with no significant variation of the considered parameters.

There have been several clinical reports (case studies) on human pregnancies following LSD ingestion. These reports are conflicting and in general not well controlled; thus they are not reported in detail here. Cohen, et al. (1967), found increased frequencies of chromosomal aberrations, but no congenital malformations in two offspring whose mothers consumed LSD during the third and fourth months of pregnancy. Zellweger, et al. (1967), encountered a malformed girl infant whose mother and father had taken LSD. The mother had ingested LSD on the 25th day after her last menstrual period and three times between the 45th and 98th days. The infant had unilateral fibular aplastic syndrome. Chromatid breaks were found in peripheral white blood cells of the father (2 breaks), mother (5 breaks), and child (3 breaks). Since the mother ingested the second dose of LSD during the period critical for the production of leg deformities, one might suspect a causal relationship to exist. Sato and Pergament (1968) studied the birth of an apparently normal girl child to a mother who had taken LSD on the 43rd and 57th days of pregnancy. Karyotype studies did not discern significant frequencies of chromosomal abnormalities.

MATERIALS AND METHODS

Animals

Albino nulliparous female rats (200-305 g) of the Holtzman strain were used for this research. Males of proven fertility were employed for mating activity. All females were housed separately from males until they had two or more successive 4-5 day estrus cycles. These females were selected as they entered proestrus and housed with one male in designated cages. Males and females were numbered with ear markings and charts were kept pertaining to their cage position in the battery. Vaginal lavages for all females housed with males were obtained daily between 8:00 and 12:00 A.M.

Animals were housed in a room with 14 hours of light (5:00 A.M.-7:00 P.M.) and 10 hours of darkness (7:00 P.M.-5:00 A.M.). Care was exercised to keep extraneous activity and noise to a minimum. Unnecessary handling or moving of the animals was avoided to maintain a minimum of environmental stress. All animals had free access to water and received Purina Laboratory Chow ad lib.

Design of Experiment

Part I of this investigation involved three groups of 10 animals each. One group of pregnant subjects was given control injections of sterile physiological saline on days 4 and 9 of pregnancy. These injections were based on body weight-LSD volume basis as rendered to the experimental animals. Injections were given intraperitoneally (I.P.). Another group of 10 pregnant rats (experimental group 1) was

given one I.P. injection of 5 ug LSD-tartrate/kg body weight on day 4 of pregnancy. The remaining group (experimental group 2) of animals was administered 5.0 ug LSD/kg body weight on day 9 of pregnancy.

Day 4 of pregnancy represents the critical preimplantation and fetal membrane development period, and day 9 is the vital period of neurogenesis and osteogenesis (Nicholas, 1962). Typically, days 4 and 9 are insult-susceptible periods of development in the rat (Wilson, 1964). The injection doses of LSD-tartrate were extrapolated, on a body weight basis from the hallucinogenic dose for the human (Pfeiffer and Murphree, 1965).

Part II also involved three groups of 10 animals each. The control group rats (control group 2) were given I.P. injections of sterile physiological saline on day 4 and day 9 of pregnancy. Experimental groups 3 and 4 were treated as were experimental groups 1 and 2 except that the dosage of LSD was increased 4X that used in Part I (20 ug/kg b.w.). This dosage of LSD is comparable to very potent hallucinogenic human doses (Cohen, 1967). The dose given experimental groups 3 and 4 animals was 20.0 ug LSD/kg b.w. The LD_{50} (LSD) for the rat is 16 mg/kg b.w. (Cohen, 1967).

Part III of this investigation consisted of one control group and four experimental groups. Control group number 3 involved no treatment. These 10 animals were bred, weighed at particular stages of pregnancy and finally autopsied. Experimental groups 5 and 7 were administered unilateral intrauterine injections of sterile physiological saline. Group number 5 received treatment on day 4 of pregnancy, and group number 7 received the injection on day 9 of pregnancy. Experimental groups 6 and 8 received unilateral intrauterine injections of LSD on

days 4 and 9 of pregnancy, respectively. Injection doses for groups 6 and 8 were based on 2.52 ug LSD/kg of body weight. Control intrauterine injections for groups 5 and 7 were of corresponding volumes to injections in groups 6 and 8. The intrauterine injections of LSD and of physiological saline were volume-matched to minimize any adverse effects due to mechanical stress of the intrauterine injection. One-half (5 animals) of each group was injected in the right uterine horn, and the remaining 5 animals received the injection in the left uterine horn.

Control unilateral intrauterine injections provided simulative operational treatment for groups 5 and 7. Contention was that stress afforded by the 4th or 9th day operation might affect development of fetuses in the LSD treated animals. Therefore, groups 5 and 7 were utilized as secondary controls and served to effectively demonstrate any resultant operational effects.

The unilateral treatment of pregnant rats on either day 4 or 9 provided another control facet. With LSD presented to only one uterine horn, the contralateral horn provided a more discriminating control situation. Caesarian sectioning quickly revealed the difference in treated versus untreated uterine positions.

Experimental Procedures

Vaginal Lavages

Vaginal lavages were taken daily from those females housed with males. Vaginal lavages were always taken between the hours of 8:00 A.M. and 12:00 P.M. Vaginal cell stages were determined according to Turner (1966). Approximately $\frac{1}{4}$cc of warm water was introduced into the

vagina and asperated by means of a small pipette 3 or 4 times to obtain an optimal amount of sloughed cells. One drop of the lavage was placed on a microscope slide. Since the rats were numbered, several lavages were placed on the same slide. Each lavage was examined microscopically under 10X and the stage of estrus cycle recorded. Presence of spermatozoa in the vaginal lavages or of a copulatory plug established day 0 of pregnancy.

Parenteral Injections

All injections were administered intraperitoneally with a sterile 1 cc capacity tuberculin syringe and 26 gauge needle.

Laparotomy Technique and Intrauterine Injections

The animals were anesthesized with intraperitoneal injections of aqueous chloral hydrate (120 mg/cc; dosage equals 30 mg/100 g body weight). The pelvic region of the abdomen was shaved and swabbed with 70% alcohol,and a mid-ventral longitudinal incision approximately 3 cm in length was made about 1 cm anterior to the vaginal orifice. The uterine horns were carefully exteriorized with broad tipped forceps. In all 9-day pregnant animals both the number and approximate position of the embryos were recorded. The appropriate uterine horn was then injected in its anterior one-third. The intrauterine injection was performed with a sterile tuberculin syringe (27 gauge needle). The uterine cornua were then replaced into the abdominal cavity, the muscle wall sutured, and the skin incision closed with sterile surgical clamps (11 mm). Post-operative care entailed isolation of the animal for about one hour or until the animal was active enough not to be injured by cage companions.

Autopsy

All animals were autopsied one day prior to term (20th day of pregnancy). Each animal was weighed and then killed with an overdose of ether, but promptly removed from the ether with the cessation of cardiac activity to prevent fetal death. A mid-ventral longitudinal incision through the abdominal wall was made from the vaginal orifice to the xiphoid process of the sternum. The uterine cornua were exteriorized and the number and position of fetuses was recorded. Fetuses were then checked for a heart beat to determine whether they were living. Each ovary was examined and the number of corpora lutea noted. The uterus, containing fetuses, was removed and weighed and all fetuses were removed from the uterus and the condition of the endometrium observed. The presence and location of resorption sites were recorded. Metrial gland positions and numbers were compared to fetal numbers and positions. The uterus was then weighed. Fetuses were separated from the fetal membranes and the umbilical cord severed near the abdomen of the fetus. Fetuses were weighed and body lengths measured with a vernier caliper, after which they were individually and punctiliously examined for gross malformations. Abnormal appearance of the placenta, umbilical cord, fetal membranes, and of fetal skin were noted. Fetuses having abnormalities as well as every fourth fetus removed were placed in 95% alcohol and kept for subsequent clearing procedures (Wilson, 1964). The alizarin Red S staining and clearing procedure was used (Humason, 1967).

RESULTS

Part I

Effects of Intraperitoneal Injections of 5.0 ug LSD/kg Body Weight on Day Four or Nine of Pregnancy

A summary of the mean values for all parameters considered in Part I is presented in Table 1. Significant difference was found between the weight gains of the parents in the control group and the day 4 injected parents ($p < 0.01$). That the maternal weight gains of experimental group 1, during pregnancy, were less than control group 1 is perhaps due to the smaller litter size of the experimental group. This smaller litter size is the result of fetal resorption within the experimental group. However, fetal resorption was not significantly greater in the day 4 injected group than in the control group. The fertilization factor (percent corpora lutea represented at autopsy by fetuses) is 11.6% lower in the treated group of animals. This reduced aspect of fecundity is partially due to the greater fetal mortality of the day 4 treated animals. Since the resorption rate is slight, there is a possibility that treatment with LSD at the critical pre-implantation period may have interfered with necessary or integral processes of successful implantation. Average fetal weights and number of resorptions and runts did not significantly differ in the control group and the day 4 treated group.

Experimental group 2 (9th day injections) revealed a significantly lowered average weight gain as compared to the control group ($p < 0.01$). Again it would appear that the significant resorption rate of this group was responsible for the weight gain discrepancy. The number of

Table 1. Mean values for 5.0 ug LSD/kg body weight injections on day four or nine of pregnancy

	Control I.P. inj. on days 4 & 9	LSD inj. on day 4 (Exp. Grp. 1)	LSD inj. on day 9 (Exp. Grp. 2)
Weight Gain (g)	142.6	114.7**	95.9**
No. Fetuses	12.6	11.3	11.4
No. Corpora Lutea	14.0	14.4	15.4
Mean Fetal Weight (g)	4.4	4.4	4.1
Mean Fetal Length (cm)	3.8	3.8	3.9
No. Resorptions	0.0	0.3	0.6*
No. Runts	0.0	0.1	0.5
Fertilization Factor (%)	90.0	78.4	74.1

* ($p < 0.05$)
** ($p < 0.01$)

(10 animals per group)

runts in the day 9 treated group would lower the mean maternal weight gain, thereby decreasing the summed mean value for the group. The mean fetal weight was less in this group, thereby affecting the maternal weight gain mean. There was no significant change in the mean fetal length for this series of experiments.

The number of resorption sites was significantly increased in the day 9 injected group as compared to the control group ($p < 0.05$). The reduction of the fertilization factor was obviously due to the increase in fetal mortality. Augmented fetal mortality was represented by the numerous resorption sites.

Part II

Results of Intraperitoneal Injections of 20.0 ug LSD/kg Body Weight on Day Four or Nine of Pregnancy

Table 2 consists of the mean values for measurements taken from the control and two experimental groups testing the effect of an increased dose (20 ug LSD/kg b.w.) of LSD on fetal development. Application of Student t-test analysis for these categories yielded no significant differences. These results imply that this dose-level of LSD did not act teratogenically. Although the day 4 and 9 injected groups had relatively large resorption rates, there was a corresponding increase of resorption in the control animals for this experiment over those in control group 1. Perhaps this alteration of fecundity can be related to seasonal influences. The fertilization factors for these groups show that there was very little variation of reproductive competence within the groups tested. The day 4 treated group had the highest resorption rate and thus the lowest fertilization factor.

Table 2. Mean values for 20 ug LSD/kg body weight injections on day four or nine of pregnancy

	Control I.P. inj. on days 4 & 9	LSD inj. on day 4 (Exp. Grp. 3)	LSD inj. on day 9 (Exp. Grp. 4)
Weight Gain	121.55	115.6	103.1
No. Fetuses	11.6	11.0	12.1
No. Corpora Lutea	13.4	15.0	14.6
Mean Fetal Weight (g)	4.1	3.9	4.1
Mean Fetal Length (cm)	3.9	3.9	3.9
No. Resorptions	0.4	0.9	0.5
No. Runts	0.0	0.6	1.0
Fertilization Factor (%)	86.3	73.8	82.9

(10 animals per group)

Part III

Results of Unilateral Intrauterine Control and LSD Injections on Day Four or Nine of Pregnancy

Control and LSD Unilateral Intrauterine Treatment on Day Four of Pregnancy

Unilateral intrauterine treatment with LSD was employed to elucidate the potential direct or indirect modality of LSD activity. Subjecting the developing embryo to an environment rich with LSD could also present data which would provide negative or positive evidence for its proposed chromosomal effects as reported by Shakkebaek, et al. (1968).

The intrauterine application of LSD was accompanied by two types of control groups. Control group 3 was the basic control group. Group 3 animals were bred but were not treated experimentally. This untampered group of animals served to exhibit any seasonal or unrealized exogenous influences upon reproductive capacity. Control unilateral intrauterine injections on day 4 of pregnancy simulated experimental surgical stress. The simulative treatment was necessary to negate any effect due to the laparotomy performed during intrauterine treatments with LSD. Without the intrauterine controls, results of LSD intrauterine experiments would have been less defined as to causation and any physical effect induced by uterine distension. Although intrauterine injection volumes were minimized, some degree of uterine expansion was observed.

Summed data (mean values) from unilateral intrauterine injections on day 4 of pregnancy is presented in Table 3. The maternal weight gains are significantly lowered ($p < 0.01$) in the group given an intrauterine injection of LSD on day 4 of pregnancy (experimental group

Table 3. Mean values for control intrauterine (IU) and 2.52 ug LSD/kg body weight intrauterine injected animals (day four of pregnancy)

	Controls (No inj.)	IU control inj. on day 4 (Exp. Grp. 5)	IU LSD inj. on day 4 (Exp. Grp. 6)
Weight Gain (g)	113.3	90.0	65.3**
No. Fetuses	13.4	8.0	7.1
No. Corpora Lutea	15.2	12.9	12.9
Mean Fetal Weight (g)	4.0	4.2*	3.9
Mean Fetal Length (cm)	3.9	3.8	3.8
No. Resorptions	0.4	2.5**	3.6**
No. Runts	0.0	0.0	0.0
Fertilization Factor (%)	88.4	61.8	55.8

Comments: one malformed placenta in IU LSD group

** ($p < 0.01$)
* ($p < 0.05$)

(10 animals per group)

6). The decreased maternal weight gain is largely due to the high resorption rate of this group, which represents a significant increase over the control animals (control group 3). Neither the number of fetuses nor the number of corpora lutea of the control or LSD injected groups differed significantly from those values of the control group. The mean fetal weight of the control intrauterine treated group (experimental group 5) does significantly differ from that of the control group ($p < 0.05$). This increased mean fetal weight for the control intrauterine treated animals may be the result of decreased litter size, since reduced litter size often results in larger offspring. No runts were observed in control group 1 or experimental groups 5 and 6. One fetus, in group number 6, which appeared normal in all respects, had an elongated and discolored placenta. Experimental groups 5 and 6 did exhibit large decreases in their fertilization factors. Obviously, these decreases were due to the greater fetal mortality represented by the increased resorption rates.

Control and LSD Unilateral Intrauterine Injections on Day Nine of Pregnancy

Results of control and LSD intrauterine injections on day 9 of pregnancy are given in Table 4. None of the categories in either case gave significant statistical differences when compared with control group 3. From the data in Table 4, it is apparent that laparotomy did not affect fetal development at day 9 as drastically as at day 4 of pregnancy. Litter size, average fetal weight, and average fetal length were closely correlated throughout the three groups. The resorption rate increase in LSD injected animals, though not statistically significant, is a noticeable variation from the control values. The

Table 4. Mean values for control intrauterine (IU) and 2.52 ug LSD/kg body weight intrauterine injected animals (day nine of pregnancy)

	Controls (No inj.)	IU control inj. on day 9 (Exp. Grp. 7)	IU LSD inj. on day 9 (Exp. Grp. 8)
Weight Gain (g)	113.3	93.9	107.0
No. Fetuses	13.4	12.4	11.3
No. Corpora Lutea	15.2	13.7	13.8
Mean Fetal Weight (g)	4.0	4.1	4.1
Mean Fetal Length (cm)	3.9	3.8	3.9
No. Resorptions	0.4	0.3	0.6
No. Runts	0.0	0.0	0.5
Fertilization Factor (%)	88.4	90.4	82.0

Comments: one spina bifida in IU LSD group

(10 animals per group)

LSD treated group also displayed a sizeable increment in runting. The fertilization factor was relatively uniform in all three groups. One LSD treated rat contained a malformed fetus which was dead at autopsy (experimental group 8). Spina bifida was the primary congenital defect, as well as dorso-ventral compression and underdeveloped limbs. There was a total of 643 fetuses from LSD treated animals (only one anomalous condition was observed). A total of 580 offspring were taken from parents comprising the various control groups; no spontaneous congenital malformations were detected.

Table 5 represents a statistical comparison (Student t-test) between the day 4 control and LSD intrauterine injected groups of animals (experimental groups 5 and 6). There were no significant differences between data of day 9 control and LSD intrauterine treated animals. Therefore, these two groups (experimental groups 7 and 8) were not statistically compared. Comparison of experimental groups 5 and 6 showed a significant difference between the mean fetal weight categories only. The LSD treated animals produced offspring which were significantly lighter (0.3 g mean). Though not statistically different, there is a noticeable increase of the resorption rate for LSD treated animals over those given control injections.

Table 5. Comparison of mean values for control intrauterine (IU) and 2.52 ug LSD/kg of body weight intrauterine injected animals (day four of pregnancy)

	IU control inj. on day 4 (Exp. Grp. 5)	IU LSD inj. on day 4 (Exp. Grp. 6)
Weight Gain (g)	90.0	65.3
No. Fetuses	8.0	7.1
No. Corpora Lutea	12.9	12.9
Mean Fetal Weight (g)	4.2	3.9**
Mean Fetal Length (cm)	3.8	3.8
No. Resorptions	2.5	3.6
No. Runts	0.0	0.0
Fertilization Factor (%)	61.8	55.8

Comments: one malformed placenta in IU LSD group

** ($p < 0.01$)

(10 animals per group)

DISCUSSION

Part I of this study was conducted to reproduce the previously reported teratogenic action of LSD (Alexander, et al., 1967, and Auerbach and Rugowski, 1967). The original intention was to reproduce this teratogenic effect and to subsequently research its causal mechanisms, though initial findings indicated a need for modification of this approach. Part II was conducted (4x dose of LSD) to investigate the possibility of a dose-response relationship in our laboratory animals which might have explained the apparent lack of LSD teratogenicity in the described experiments. Experiments involving the increased dosages of LSD (Part II) did not yield data which supported the proposed teratogenicity of LSD. Part III (unilateral intrauterine injections) was designed and performed to further investigate the potential teratogenicity of LSD. Unilateral injections of LSD were used in order to obtain greater restriction of control, the contralateral uterine horn serving as an added control feature. As suggested by Saxen and Rapola (1969), the best possible control might be to use embryos located in the same uterus (opposite uterine horn). The technique of Brent (1965, uterine clamping) was not employed for these intrauterine tests to avoid additional variables. Intrauterine application of LSD was utilized because this allowed the presentation of LSD directly to the developing conceptus without prior biotransformation of large portions of the injected LSD. According to Pfeiffer and Murphree (1965), LSD is rapidly transformed to 2-hydroxy-LSD (no significant psychotomimetic activity) by the liver microsomes. According to the

autoradiographic studies of Idanpaan-Heikkila and Schoolar (1969), ^{14}C-LSD readily penetrates cell membranes and therefore should gain ready access through the extra-embryonic membranes. Idanpaan-Heikkila and Schoolar (1969) also demonstrated that 2.5% (early in pregnancy) of I.V. injected ^{14}C-LSD passed the placenta into the fetus and 5.0% entered the fetus later in pregnancy. These amounts passed the placental-fetal interface within five minutes following injection.

Another important aspect of the intrauterine LSD experiments is that the technique allowed delimitation of the direct versus the indirect modality of proposed LSD effects. Obviously, a teratogenic agent may exert its influence in a "target tissue" fashion or it might affect development indirectly via "generalized physiological stress." Any deleterious influence LSD might have upon embryogenesis should be dramatically evident in the late fetus following intrauterine application of the drug.

The parenteral (IP) injections of 5.0 ug LSD/kg b.w. on day 4 or 9 of pregnancy (Part I) were not teratogenic under these experimental conditions. Strictly speaking, teratogenic implies the causation of anomalous (monster) development. Significant numbers of developmental abnormalities were not detected in the offspring of the treated rats. However, there were significant statistical differences between control and experimental animals with reference to two other parameters listed in Table 1. The maternal weight gains of both the day 4 and 9 groups were significantly lower than the control animals. This appears to be a reflection of the increased resorption frequencies demonstrated by these two experimental groups. The experimental groups (1 and 2) displayed reduced fertilization factors. This appeared to be

correlated to the augmented resorption frequencies. Thus, injections of 5.0 ug LSD/kg b.w. on day 9 of pregnancy increased fetal mortality in these experiments. It should be realized that this rate of resorption (as in experimental group 2) is not actually very high (6.0 resorptions for ten animals) when compared to the resorption frequencies of subsequent control groups (control 2 = 4 resorptions, and control 3 = 3 resorptions). Control group 1 appears atypical in that there were no resorptions in the 10 litters, whereas rats in this laboratory minimally approximate 2-3% spontaneous resorption rates.

The fertilization factor for experimental group 1 (day 4 injection of LSD) revealed an 11.6% drop from the control value. This group of animals did not have a significant rate of resorption; therefore, it would appear that LSD treatment at this period of development (pre-implantation) reduced fecundity--possibly by upsetting mechanisms critical to successful implantation. This is speculation, since these experiments do not provide unquestionable evidence for this hypothesis.

Because this experiment (experimental group 2) involved administration of LSD during the osteogenic and neurogenic phases of rat development, one would expect teratogenic insults to appear in the form of gross malformations (spina bifida, exencephaly, etc.). No anomalies were observed, thus any insult afforded by LSD treatment was radical enough to terminate development and not induce non-lethal developmental alterations.

LSD did not act teratogenically when given rats on days 4 and 9 of pregnancy at a dose level of 20 ug LSD/kg b.w. Examination of the data from these experiments (Table 2) indicates that LSD was apparently ineffectual with respect to reproduction in this group of animals. Not

one of the measured parameters revealed any apparent alteration which might have resulted from LSD activity. These results are quite similar to those published by Warkany and Takacs (1968) and Roux, et al. (1970). It is difficult to explain a decrease in effect of LSD at higher doses as compared with results of experiments in Part I. There are dose-effect thresholds but no known instances where too great a dose of the substance renders it non-teratogenic. Part II results when compared to Part I results are somewhat conflicting. However, the results in Part II are quite clear--LSD did not act teratogenically. The data obtained from experiments in Part I are somewhat more ambiguous.

Part III of this investigation consisted of a series of experiments involving the intrauterine application of LSD on days 4 and 9 of pregnancy. Part III was comprised of one control group of animals (control group 3), which was not experimentally manipulated and four experimental groups (groups 5, 6, 7, and 8). Experimental groups 5 and 7 were mock or control treated groups. These two groups functioned to negate any effects due to surgical trauma brought about by the laparotomies performed. Groups 5 and 7 received volume-matched intrauterine injections of sterile physiological saline on days 4 and 9 of pregnancy, respectively. Groups 5 and 7 were then matched to the intrauterine LSD injected groups (6 and 8). To date, these are the first published experiments utilizing unilateral intrauterine injections to investigate the teratogenic potential of LSD. Analysis of raw data collected revealed no detectable significant differences between treated and untreated uterine horns in any of the experimental groups.

Table 3 presents data comparing control group 3 to day 4 control and LSD intrauterine injected groups (5 and 6). The mean maternal weight was significantly lowered ($p < 0.01$) in the LSD injected animals as compared to the untampered control animals. The lowered maternal mean weight gain is obviously a result of the increased fetal mortality as illustrated by resorption frequency. Control and LSD injected animals displayed significantly augmented levels of fetal mortality when compared with controls. Thus, it appears that the laparotomy or at least some facet of the technique disturbed normal reproductive processes. Reduced fecundity is dramatically illustrated by the radical drop in the fertilization factor for experimental groups 5 and 6. These decrements were 26.6% and 32.6%, respectively, the greater reduction being in the LSD treated group. The fertilized ova were not implanted at the time of operation (day 4), so that the mechanical process of aggitation at the time of injection may have contributed to reduced fecundity in groups 5 and 6.

There were significant differences between resorption rates for control and LSD intrauterine treated groups when compared to the untampered control group ($p < 0.01$). Some proportion of this increased resorption may have been evoked by surgical manipulations. Table 5, which represents the comparison of data from control and LSD intrauterine (day 4) experiments (5 and 6), illustrates significant difference ($p < 0.01$) when comparing the mean fetal weight of the LSD and control treated groups. Intrauterine LSD injection on day 4 of pregnancy appeared to effect a decrease in the mean fetal weight. The differential mean fetal weight value was the only parameter displaying

any significant statistical variation between the control and LSD intrauterine injected groups.

Intrauterine injections of LSD on day 9 of pregnancy did not exhibit deleterious effects, as shown in Table 4. The laparotomies performed for experiments on day 9 of pregnancy did not interfere with development as was the case in the day 4 intrauterine experiments. Results of intrauterine LSD injections on day 9 of pregnancy did not indicate that LSD acted teratogenically. The single incidence of malformation (spina bifida) which occurred did not constitute sufficient evidence to declare LSD a teratogen. Conversely, this single incidence of malformation would tend to dispute the alleged teratogenicity of LSD in the rat. Injections of LSD were given during the organogenic period of development in the rat, a period which is typically sensitive to drug insults (Saxen and Rapola, 1969). In these experiments LSD did not elicit deranged development, as shown by the lack of malformations. Furthermore, LSD did not appear to interfere with development to the extent of halting its progression, as evidenced by the absence of significantly increased fetal mortality in experimental animals.

None of the previously described experiments indicated that injection of LSD into pregnant rats would induce malformations in their offspring. However, LSD injections did produce statistically significant differences between control and experimental animals with respect to two other parameters. LSD injection (5.0 ug/kg b.w.) on day 4 of pregnancy did increase fetal mortality significantly ($p < 0.05$). Whether this is to be accepted as valid proof of an abortifacient effect by LSD depends on one's statistical viewpoint. However, 20 ug LSD/kg b.w. injection did not evoke significantly increased fetal

mortality in experiments 3 and 4. Prenatal mortality was increased over the control conditions but not at statistically significant levels. In all groups which were treated with LSD, fetal mortality was usually increased slightly.

The second parameter which significantly varied from control values in animals injected with LSD was mean fetal weight. The mean fetal weight of offspring from day 4 intrauterine LSD injected adults was significantly decreased. This parameter was significantly different at the $p<0.01$ level, agreeing with results published by Fabro and Sieber (1968) for LSD experiments with the New Zealand white rabbit. Fabro and Sieber's results indicated reduced fetal weights for offspring of LSD treated does, which might be considered as involving a general growth inhibiting factor. Experiments by Yielding, et al. (1969), have indicated that LSD binds to deoxyribonucleic acid (DNA) in vitro. The LSD absorption spectrum was not depressed by the addition of RNA to an LSD solution. These experiments, with imaginative interpretation, might lend some consideration for an LSD role in growth inhibition.

One important reservation, the extent of fetal examination, might be mentioned, since this project did not entail histological analysis of offspring. Serial sectioning is the ideal technique for searching out subtle malformations, but it is not a practical procedure (Wilson, 1965). The techniques of examination used in this study are those currently accepted and utilized by the majority of teratologists. Therefore, it is with some reservation that these results have been interpreted as not supporting the proposed teratogenicity of LSD in the rat. These experiments have indicated the inability of LSD to induce

gross malformations in offspring of treated rats under the described conditions.

SUMMARY

The effect of lysergic acid diethylamide (LSD) upon the developing rat embryo (Holtzman strain) was studied. All injections were given on days 4 or 9 of pregnancy. Both systemic (5.0 ug and 20.0 ug LSD/kg b.w.) and intrauterine modes (2.52 ug/kg b.w.) of application were tested.

Developmental malformations or congenital anomalies represent one of the most immediate manifestations of reproductive failure. This was the basis upon which adverse effects of LSD upon development were monitored.

Based on data accumulated from autopsies and fetal examinations during the described experiments, the following inferences were made:

1. In the described experiments LSD did not elicit significant incidence of detectable anomalies.
2. Injection of 5.0 ug LSD/kg b.w. on day 9 of pregnancy significantly increased prenatal mortality ($p < 0.05$) under the described experimental conditions. This statement is made with reservations because of the level of significance obtained and because the control animals (control group 1) of this experiment were not typical of subsequent control animals with respect to their resorption rates.
3. Unilateral IU injections of 2.52 ug LSD/kg b.w. on day 4 of pregnancy reduced mean fetal weights.

4. These results are not complete proof that LSD is non-teratogenic. Care must be exercised when extrapolation from these data to human concern is desired. This is especially important since this strain of rat appears relatively resistant to exogenous influences as exhibited by their slight rates of spontaneous malformation and fetal mortality.

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