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LYSERGIC ACID DIETHYLAMIDE INTAKE IN PREGNANCY: FETAL DAMAGE IN RATS[1, 2]

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ABSTRACT

ALEXANDER, GEORGE J., GEORGE M. GOLD, BRUNO E. MILES and RITA B. ALEXANDER: Lysergic acid diethylamide intake in pregnancy: Fetal damage in rats. J. Pharmacol. Exp. Ther. **173:** 48–59, 1970. Oral or subcutaneous administration of lysergic acid diethylamide (LSD) to hundreds of pregnant Wistar-O'Grady rats resulted in damage to litters which was 3 to 4 times higher than in corresponding controls given distilled water or saline. The proportion of deaths during gestation, abortions, resorptions, runting, offspring stillbirths and the rate of offspring mortality was increased. However, no unusual number of specific deformities was encountered. Teratogenicity during any particular organogenetic period was not demonstrated. Treatment during the first seven days was found harmful; treatment later in pregnancy was ineffective. The LSD effects appear to be dose-related and persist into the second generation.

Harmful side effects of lysergic acid diethylamide (LSD) during pregnancy have been reported in experimental animals (Alexander *et al.*, 1967; Auerbach and Rugowski, 1967; Geber, 1967; Roizin *et al.*, 1968) although not by all investigators (Abramson, 1960; Warkany and Takacs, 1968). Until recently no case of human fetal deformities directly and solely ascribable to LSD intake in pregnancy has been known. Now, however, one such possible case exists although the evidence, as usual in such cases, is far from incontrovertible (Zellweger *et al.*, 1967).

The exact nature of the damage due to LSD treatment in animals during pregnancy has not been detailed heretofore; only preliminary observations have been published. In this paper, we attempt to describe in detail the gross effects of treatment with LSD early in pregnancy on one strain of rats.

METHODS. Lysergic acid diethylamide tartrate (LSD-25) was obtained from Sandoz Pharmaceuticals (Hanover, N.J.) as a dark yellow amorphous powder. It was kept dry in the dark and dissolved as needed. The compound was analyzed in the ultraviolet and spectrophotofluorimeter for decomposition products and was found to contain no noticeable admixture of oxidized material, such as was visible, for example, after treatment with hydrogen peroxide. A solution of LSD (5 μg/ml) standing in the refrigerator for three months showed 50% decomposition. However, frozen stock solution (1 mg/ml) showed no significant change in six months. Fresh dilute solutions were routinely made before use: in dis-

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tilled water for oral treatment and in physiologic saline for injection.

Wistar rats were purchased originally from Flora O'Grady (Bronx, N.Y.) and were bred in our colony at the Bronx State Hospital. They were fed *ad libitum* a standard Teklad laboratory diet containing 6% fat. One or two females were placed with each male in a cage for four days to ensure conception. When the knowledge of exact date of conception was deemed essential, the animals were left together overnight only. This method, although wasteful, was considered superior to all others that we have tried, since it avoided all the pitfalls inherent in observing vaginal plugs, smear tests for presence of sperm, etc. In routine cases, once it was established that LSD does not affect the very regular 22-day gestation period of these rats, it was possible to calculate backwards from date of delivery to date of conception. The animals were permitted to come to term, and the gross appearance of the offspring at birth, their growth rate and health and finally their fertility and progeny were observed.

Animals which failed to deliver on the due date were autopsied a few days later, as were animals which died during gestation. In a few cases the animals were obviously very sick and unable to deliver healthy offspring. To alleviate their suffering, they had to be sacrificed during gestation. After a gross examination, they were etherized, and their brain, heart, lungs, liver, spleen, adrenals, intestines, bladder, kidneys, ovaries and uterus were inspected. Excised organs were preserved in formalin for future pathologic examination. A similar procedure was followed with the offspring. Stillborn young, or those dying within one month, were examined as soon as obtained and then preserved in formalin. Surviving offspring (no more than 8/litter) were kept for one month: those needed for mating were spared, and the rest were sacrificed and examined. When mature (usually at three months of age), the offspring were mated with each other or with controls to determine second-generation fertility and health. Care was exercised, however, to avoid inbreeding.

LSD was administered by s.c. injection in the dorsal region at the beginning of the study. Later it was also given p.o. with an eyedropper introduced into the back of the mouth. The dose was varied from 5.0 μg/kg to 100 μg/kg. It was given only once to any animal, and the time of administration varied from 10 A.M. to 7 P.M. Single doses were given between the 1st and 16th day of gestation during either first, second or third pregnancy. Results of the pregnancies were compared with each other as well as with results obtained with control animals carefully matched according to weight, age and apparent health. The procedure of selecting animals for treatment with LSD or placebo, respectively, was deliberately varied from experiment to experiment. The animals were always numbered first and labeled with picrate, placed in cages and mated, usually two females to one male. The specifics of the selection for treatment were not decided upon until later. In some experiments either even- or odd-numbered rats received LSD; in others, the first five received the placebo, the next five LSD, the next five placebo again and so on. These variations also insured that housing and mating were deliberately varied. Sometimes one of two females housed together and impregnated by one male received LSD, the other placebo; at other times both females from one cage received LSD, whereas both from another received the placebo. In a separate experiment LSD was also given in drinking water after delivery to test for an effect, if any, on lactation.

RESULTS. *Overall statistics.* The total number of female rats mated in the experiments described in this report exceeded 260. They were mated with over 200 males and produced almost 1800 offspring. In the first series of experiments which involved a study of the effects of LSD intake early in pregnancy on fetal damage, 108 pregnant females were divided into matched groups: 55 were given the drug, and 53 were given placebo (table 1). Although 15% of litters of the control rats showed some evidence of damage, 47% of the rats treated with LSD failed to deliver undamaged litters. Probability of ob-

TABLE 1

LSD early in pregnancy in Wistar-O'Grady rats (s.c. and p.o. treatment)[a]

	Placebo Total	LSD Treatment		
		s.c. inj.	p.o.	Total
Number of pregnancies	53	26	29	55
Resorptions	2	2	3	5
Deaths during gestation	3	1	1	2
Litters of 6 or less	0	1	2	3
Litters containing stillborn	3	3	5	8
Litters containing sickly young	0	5	3	8
Total of abnormal pregnancies	8	12	14	26
Offspring, total	612	212	309	521
Sick or stillborn	14	29	29	58

[a] LSD was administered p.o. or by s.c. injection as indicated, between the first and fourth day of pregnancy. p.o. dose, 20 μg/kg in distilled water; s.c., 5 μg kg in saline. Concentration, 20 μg/ml and 5 μg/ml, respectively. Placebo, 0.3 ml of distilled water or saline, as required.

taining these results by chance, according to the Student's t test, was less than 0.1%. Control litters varied from 8 to 17 offspring, and LSD litters varied from 0 to 16. Average litter size was 12.2 offspring/litter for controls and 12.7 for treated animals. However, although only 2% of 612 offspring of control rats were stillborn and none died during the first 10 days, 11% of the 521 offspring of LSD-treated mothers were either stillborn or died within 10 days after birth. Statistical analysis of the data (14:612 *vs.* 58:521), also according to the Student's t test, indicated that the likelihood of obtaining these results by chance was minimal ($Z = 6.28$; $P < .001$). All surviving offspring, as well as those which were found *in utero* during autopsies or were stillborn, were carefully checked for gross abnormalities. Few obvious defects were found, and these will be discussed later. Almost 70% of the surviving offspring of LSD-treated rats developed normally until maturity and did not show any effects of the drug. However, 3% did not grow as well as controls, 18% died during the first month of life and another 9% died when they matured and were mated. By comparison, 4% of offspring of control rats died during the first month. In addition, a sizeable proportion of apparently normal offspring from LSD-treated animals, showing no visible damage while growing to maturity, revealed the presence of damage through production, in turn, of damaged offspring. As described below, when both parents were LSD offspring, 70% of their litters showed some evidence of damage. Thus, the overall value of 11% of damaged offspring after treatment with LSD is low because it reflects only that pronounced damage which prevented survival for more than a few days. If damage as revealed by second-generation animals during their pregnancies, were to be included, the figure would rise considerably.

Comparison of s.c. and p.o. treatment of rats with LSD. Of the 55 pregnant rats treated with LSD early in pregnancy, 26 received the drug by s.c. injection, and the remainder received the drug p.o. Possibly the group given p.o. treatment showed somewhat less damage, fewer of their offspring were affected and the litter size was larger. However, none of these differences appear statistically significant (table 1). The route of administration apparently makes little difference.

Effects of single s.c. injection of LSD. A single dose of 5 μg/kg was given to five pregnant rats on the fourth day of their first gestation, while matched controls were given physiologic saline (table 2). The experiment was repeated with

TABLE 2

Subcutaneous injection of LSD early in pregnancy[a]

Expt.	Control			LSD Treatment		
	Size of litter	Sick or stillborn	Comments	Size of litter	Sick or stillborn	Comments
1a	11	0	All offspring normal	0	0	Interrupted pregnancy
1b	11	0	All offspring normal	9	9	All offspring stillborn, two stunted
1c	13	0	All offspring normal	13	6	Six offspring stillborn
1d	13	0	All offspring normal	8	1	One offspring stunted
1e	16	0	All offspring normal	16	0	All offspring normal
2a	8	0	All offspring normal	0	0	Interrupted pregnancy
2b	12	0	All offspring normal	14	4	Three offspring stillborn, one died 2nd day
2c	13	0	All offspring normal	11	1	One offspring stillborn
2d	15	0	All offspring normal	4	0	All offspring normal but size of litter small
2e	17	0	All offspring normal	10	0	All offspring normal

[a] Only pregnant females, 250 ± 20 g, were used, matched in five pairs per experiment. On fourth day of pregnancy, control animals received 0.25 ml of saline, others 5 μg of LSD per kg in saline (5 μg/ml). LSD animals 1a and 2a were autopsied 25 days after conception. No embryos were found in the uteri, which were, however, enlarged. From time and weight curves it appears that pregnancy was interrupted within three days after LSD administration.

10 more rats. All 10 control animals (5 in each experiment) delivered normal litters of 8 to 17 young; whereas, only 2 of 10 treated animals had apparently normal litters, 2 resorbed their fetuses and 6 delivered litters containing either stillborn or stunted offspring. Control rats delivered 128 healthy offspring, and LSD-treated rats delivered 85 offspring, of which 21 were obviously abnormal. Statistical analysis of the data with the Student's *t* test established that the differences in litter sizes (8–17 for controls *vs.* 0–16 for LSD-treated rats) were significant at the 5% level, which is generally acceptable in the biologic work. Other *t* test comparisons involved the proportions of abnormal pregnancies, defined as interrupted pregnancies, identifiable resorptions and pregnancies resulting in litters containing one or more stillborn, runts or sick offspring that died within 10 days. These data were highly significant ($P < .001$). Similarly, a comparison of the numbers of damaged offspring out of the total born (0:129 *vs.* 21:85) proved the data significant, also at the level of 0.1%.

Effect of treatment of rats with LSD after the eighth day of gestation. Five pregnant animals were injected with 5 μg/kg of LSD once, late in pregnancy (*i.e.*, between the 8th–16th day). In no case were the offspring of these matings visibly affected by the drug. Litter size was possibly somewhat smaller than in the five matched controls given saline (controls, 10–16 offspring; LSD, 8–13 offspring). The LSD animals delivered 51, and controls delivered 65 offspring. In addition, seven animals received LSD p.o. on or after eighth day of pregnancy, and they, too, delivered only normal offspring (see below).

Comparison between first and second litters of the same females, with and without LSD. To ensure that the observed LSD effect was not due

TABLE 3

Comparison of first and second litters of the same mothers, with and without LSD injection

Expt.	First Mating			Second Mating		
	Litter size	Sick or stillborn	Comments	Litter size	Sick or stillborn	Comments
			No treatment			Placebo
4a	8	0	All offspring normal	9	3	Two died on 12th day, one on 17th
b	8	0	All offspring normal	11	0	All offspring normal
c	10	0	All offspring normal	14	0	All offspring normal
d	16	0	All offspring normal	17	4	Two died on 13th day, one on 18th, one on 25th
e	13	0	All offspring normal	11	2	Two died on 18th day
f	11	0	All offspring normal	12	0	All offspring normal
g	10	1	One died on 17th day	14	0	All offspring normal
h	16	0	All offspring normal	16	0	All offspring normal
			No treatment			LSD
i	13	0	All offspring normal	4	3	Two died on 5th day, one on 13th day, litter small
j	11	0	All offspring normal	0	0	Pregnancy interrupted
k	15	0	All offspring normal	13	0	All offspring normal
l	12	0	All offspring normal	10	10	All ten died, three on 10th day, three on 11th day, two on 16th day, two on 18th day
m	11	0	All offspring normal	11	4	Two died on 11th day, one on 12th day, one on 22nd day
n	13	0	All offspring normal	10	2	Two died on 14th day
o	13	0	All offspring normal	11	3	One died on 1st day, two on 13th day
p	11	0	All offspring normal	14	6	Three stillborn, three died on 3rd day

merely to difference between first and second litter of the same female, records were kept of all pregnancies, and 20 animals which had a healthy, normal first litter were mated again three months later. Sixteen which became pregnant were divided into matched groups: one group was given 5 μg/kg of LSD s.c. on the fourth day of gestation, and the other group was given 0.2 ml of physiologic saline. The control group delivered eight normal litters. None of the offspring were stillborn, and none died during the first 10 days of life. Of the 8 LSD-treated rats, 1 failed to deliver any offspring, 1 delivered a normal litter of 13 and the remaining 6, all of which had previously had normal healthy litters, now produced litters which showed some damage due to the drug (table 3). Twelve of 73 offspring of LSD-treated rats were damaged, either stillborn or died during 10 days. In addition, 16 more offspring from this batch died between the 10th and 30th day of life. In a previous (untreated) mating of this batch of rats, no offspring died within the month. In a matched control batch, first pregnancy resulted in 1 of 92 offspring dying on the 17th day. Second pregnancy in this control batch resulted in 104 offspring, 9 of which died within a month. In all three "control" pregnancies, as seen in table 3, no offspring died within 10 days after birth. Statistical analysis (Student's t test) showed that the data were significant at the 0.1% level when offspring dying in 10 days in first (untreated) and second (LSD) pregnancies were compared.

Damage to offspring after p.o. LSD intake. In a batch of 16 pregnant rats (from 20 pairs mated), one-half were given 20 μg/kg of LSD in the back of the mouth on the fourth day of pregnancy, and the other half were given a placebo. One of the control rats delivered a litter containing seven stillborn offspring, but the other litters were normal. Treated rats produced a much higher percentage of damaged offspring (table 4). Student's t test indicated that this result could not have been obtained by chance more than 0.1% of the time. The proportion of abnormal pregnancies after LSD intake was, however, not significant at the 5% level. Changing the dose from 5 to 100 μg/kg produced an increase in the number of damaged litters at higher concentrations. Each dose group consisted of five rats but one animal in five never became pregnant. None of the four control litters showed any damage; p.o. treatment with 5 or 10 μg/kg of LSD produced slight damage; treatment with 20 μg/kg produced severe damage (table 5). The number of animals was not sufficient to give more than a mere indication of possible results. Even at the highest dose tested (100 μg/kg), one rat delivered a normal litter.

TABLE 4

Effect of LSD (20 μg/kg p.o.)[a]

Placebo			LSD Treatment		
Litter size	Sick or stillborn	Comments	Litter size	Sick or stillborn	Comments
12	0	All offspring normal	5	0	Litter small, but normal
10	7	Seven offspring stillborn	13	3	One offspring died on 21st day, two died on 26th day
13	0	All offspring normal	16	7	Five offspring died on 1st day, two died on 2nd day
11	0	All offspring normal	13	4	Four offspring were stillborn
13	0	All offspring normal	13	0?	Mother died 1 week after delivery[b]
12	0	All offspring normal	6	2	Litter small, two offspring died on 1st day
11	0	All offspring normal	13	0	All offspring normal
12	0	All offspring normal	11	0	Resorption (11 sites)

[a] Twenty animals, in 10 matched pairs, were used; 16 became pregnant, one-half received LSD on fourth day of pregnancy.

[b] Offspring of this animal appeared normal at the time of mother's death, but they were too small to survive on their own. Attempts to find substitute mother failed. They had to be sacrificed.

Treatment on any of the first seven days of gestation produced roughly comparable damage to offspring. As with s.c. injections, p.o. treatment after the eighth day of gestation produced no visible harm.

Effect of LSD on lactation. Immediately after delivery by normal, untreated rats, water containing 20 μg of LSD per kg per day was provided to some nursing mothers. Fresh solution was given daily for three weeks. Offspring (19 in the control experiment and 15 in the LSD experiment) were weighed after 5 days and then were weighed daily thereafter. Animals with mothers on LSD grew as well as controls. Presence of LSD in the drinking water during lactation apparently did not affect the offspring.

Results of autopsies of treated rats. Few gross changes were observed and none that could reliably be ascribed to a direct effect of LSD administration. Pregnant animals, of course, had an increased vascular bed around the uterus and associated sex structures. Hearts of some LSD-treated rats were often engorged and were larger than those of comparable controls. Lungs were often congested and infected. In animals pregnant for the first time, which were treated with LSD and which resorbed their fetuses, the uteri were moderately enlarged and showed three to nine pinhead-size, well delimited areas of dark red-to-black discoloration in each horn. These changes suggested individual foci of vascularization. The ovaries were slightly enlarged. Animals which died towards the end of gestation had, of course, *in utero,* embryos or fetuses of varying size depending on stage of development. Non-

TABLE 5

Oral administration of LSD (dose effect)

Dose of LSD	Number of Pregnancies	Offspring Total	Stillborn Offspring and Offspring Dying in 10 Days	
			No.	%
μg/kg				
0	4	48	0	0
5	4	40	2	5
10	4	49	4	8
20	4	47	13	28
50	4	37	14	38
100	4	39	9	23

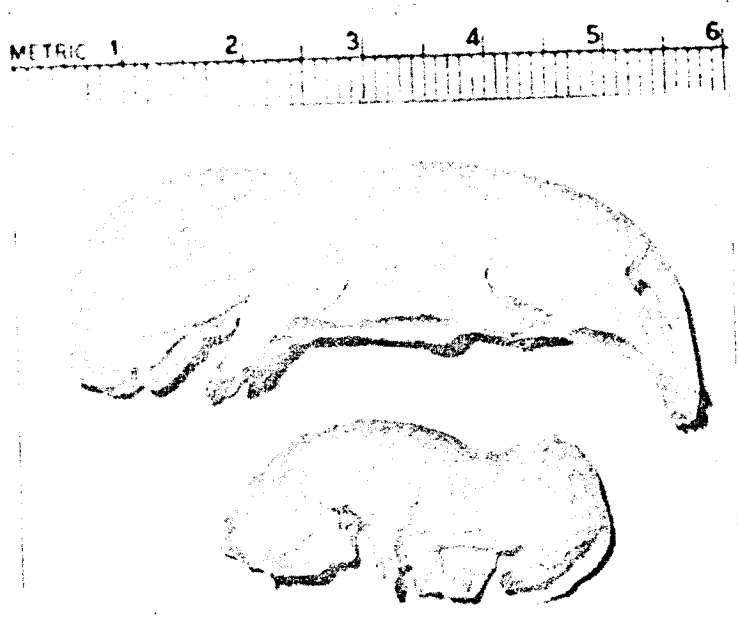

FIG. 1. Two stillborn fetuses of a rat injected with LSD (5 μg/kg) on the fourth day of pregnancy. One is fully developed; the other is immature but shows no obvious deformities.

pregnant LSD-treated animals displayed normal uteri, ovaries and Fallopian tubes.

Description of offspring. Obviously, seriously damaged offspring did not survive until birth. Embryos whose development ceased early were completely resorbed, their previous presence indicated only by dark spots on the inside wall of the uterus. Stillborn young usually appeared paler than normal. In general, the gross appearance of their heads and location of eyes was normal. Extremities and tail were fully developed and normal. In a number of cases, however, some of the stillborn animals were severely stunted although well formed. They looked younger, as if their growth had been arrested several days prior to their delivery (figs. 1 and 2). Judging from gross appearance, damage to bone structure was a distinct possibility in a few cases but was not examined further. In a few isolated cases bladders were abnormally distended; livers, kidneys, intestines, thymus or spleen were irregular in shape, enlarged or unusually dark; but in general these and other organs were grossly normal.

Gross examination of hundreds of surviving

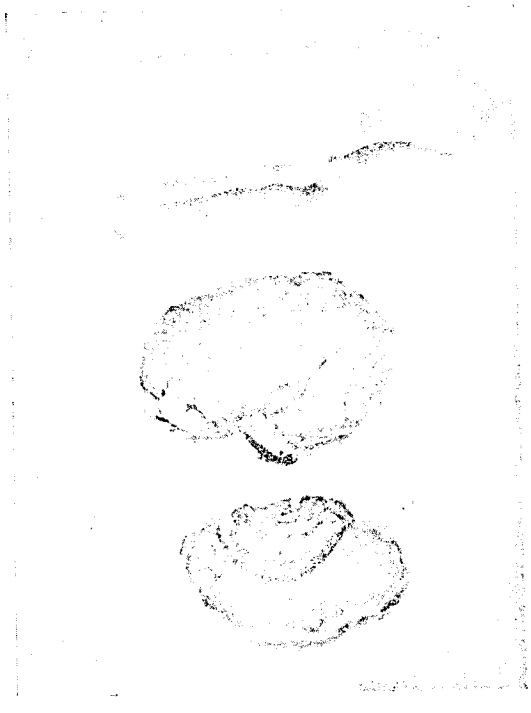

FIG. 2. Three embryos from an LSD rat injected with 20 μg/kg on the fourth day of pregnancy.

TABLE 6

Second-generation mortality after s.c. LSD[a]

	Litters	Offspring	Stillborn	Mortality (days)		
				10	20	30
Control	16	199	0	0	5	3
LSD	16	178	18	10	13	6

[a] LSD was given to mothers on fourth day of gestation. Offspring themselves were not treated in any way, but they were already conceived, though probably not yet implanted in uterine wall.

second-generation animals revealed few obvious deformities. Shape of head, skin and extremities appeared normal at birth. No exact data can be given at this time, but a few impressions of the observers are given below. They may prove useful as hints for further work. Large differences in size were found among offspring of the same litter of LSD-treated mothers. These size differences were larger than those found among offspring of controls. Stunted animals appeared less well-developed and weaker, and they generally died within a few days. Among survivors, some differences appeared with time: the eyes of some did not open, or they opened 3 to 10 days later than in presumably healthy litter mates or controls. Some LSD-treated animals were more lethargic than others. Their fur did not look healthy, their gait was often peculiar with their rear limbs spread apart. The shape of their heads was more rounded. Two to 3% bled occasionally from their noses or mouths. No such traits were observed in offspring of control rats. Eventually most of the visibly affected offspring died: some immediately after birth, some as much as a month later (table 6). One survived for six weeks in spite of a probable skeletal deformity (fig. 3). The mortality data is statistically significant at the 0.1% level. The brains and viscera of offspring of LSD-treated rats were generally grossly normal. Approximately 20%, however, showed lung infection and heart engorgement, as compared to less than 1% of such symptoms among matched controls.

Litters were arbitrarily reduced to 8 offspring, both in case of controls and LSD-treated mothers. The offspring were kept for three months, during which time most grew at a normal rate. On the 8th day the average weight of 64 control

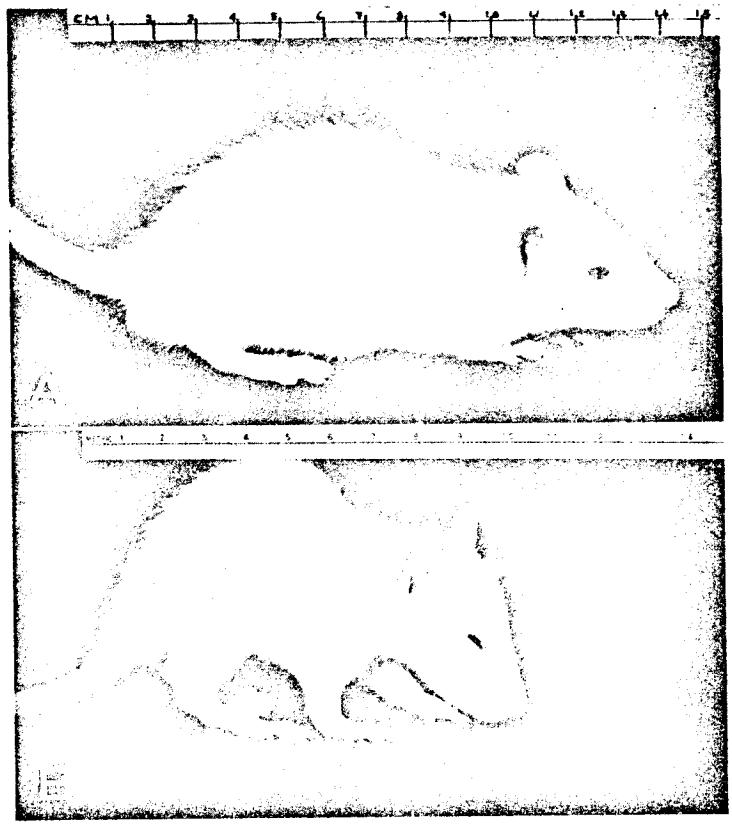

FIG. 3. Two one-month-old offspring of LSD-treated mother. One, small, showing a possible skeletal deformity; the other apparently normal. The small rat died at six weeks of age.

young was 18.4 g; the average weight of those derived from LSD-treated mothers was 19.4 g; the weights at 22 days (weaning) were 53 g and 51 g, respectively. After 6 weeks, controls weighed, on the average, 163 g, and LSD rats weighed 153 g. Upon autopsy, the surviving group of apparently healthy second-generation LSD animals usually had normally appearing brain and viscera, but occasionally, 3 to 4 times as often as in the control group, various gross pathologic findings were manifested. Hearts were engorged, vascularization was greater than normal and the spleen was often enlarged. In addition, thick-walled, round or slightly elliptical, grayish-white opaque cysts, 3 to 4 mm in diameter were found in the livers or small intestines of 1% of the animals. Again, a significant proportion (approximately 20%) showed lung congestion suggesting that the offspring of LSD-treated rats had a lower resistance to infection than controls which only rarely manifested such symptoms.

Offspring of rats treated with LSD early in pregnancy were mated in turn at three months of age and proved fertile, but a significant proportion bore damaged young. These animals, used now as parents, were already conceived when LSD was injected into their mothers on the fourth day of gestation but were not themselves treated in any way after birth. The damage to third-generation rats was negligible when only one parent (either male or female) was derived from an LSD-treated mother but was severe when both parents were so derived. Five of six control rats delivered "normal" litters. When one parent was derived from an LSD-treated mother, 13 of 19 litters were normal, but when both parents were derived from LSD-treated mothers, only 3 of 10 litters were normal (table 7). The type of damage is shown in table 8.

Statistical analysis was difficult. Comparison of numbers of offspring actually born (including stillborn) indicated no significant difference at

TABLE 7

Overall results of pregnancies of second-generation LSD rats[a]

	Parents			
	Control (♂) and Control (♀)	Control (♂) and LSD (♀)	LSD (♂) and Control (♀)	LSD (♂) and LSD (♀)
Pregnancies	6	10	9	10
Resorptions	0	0	1	2
Deaths during gestation	0	1 (13)[b]	0	1 (11)[b]
Offspring (3rd generation)				
Total	64	83	92	79
Stillborn	7	0	1	13
Sick and dying in 10 days	0	3	1	3
Surviving	57	80	90	63

[a] Offspring of LSD-treated rats were mated with control rats or with each other.

[b] Figures in parentheses represent the number of embryos found *in utero* during autopsies. They are not included in the offspring totals (stillborn animals are included).

TABLE 8

Issue of second-generation rats[a]

Rat	Litter Size	Stillborn Offspring	Offspring Mortality (days)			Remarks
			10	20	30	
6 a	0					Mother died (11 embryos)
6 b	2					Litter small
6 c	0					Resorption
6 d	14					All normal
6 e	0					Resorption
8 a	14	3	3	3	1	Two stillborn were runts
8 c	13			3		Mother died after delivery
8 e	10	10				All ten stillborn
8 d	12					All normal
8 f	14					One died on 35th day; one died on 40th day; one was blind

[a] Individual results of LSD rats described in the last column of table 7; both parents were offspring of LSD-treated females.

the 5% level between the control/control group (7:64), control/LSD group (5:175) and LSD/LSD group (16:79). However, inclusion of offspring observed *in utero* in rats which died during gestation, as well as of potential offspring (11/litter) resorbed after conception, changed the data considerably. The difference between the number of surviving control/control offspring out of an estimated total conceived (57:64) compared to a similar number of LSD/LSD offspring (63:112) was significant at the 0.1% level (Student's *t* test). Offspring of LSD/control parents were, however, not significantly different from offspring of control/control parents.

Discussion. A batch of five four-day pregnant rats was initially injected with LSD and permitted to come to term. Eighteen days later these rats delivered some stillborn offspring. It was decided to repeat the first experiment; hence, the second batch of rats again was treated on the fourth day of gestation. Again, the animals were permitted to come to term, and again, offspring abnormalities were encountered. These data, although preliminary, were striking. The number of resorptions and of stillborn offspring was significantly higher in LSD-treated animals than in matched controls or in the untreated population in our colony.

The conditions of the preliminary experiments largely determined further developments. Since the initial sample was very small, we decided to continue with larger numbers of animals from the same strain, treated in exactly the same manner as the initial batch. Our present data, compiled from a number of experiments, largely confirm our preliminary report. A significant increase in fetal abnormalities occurred among litters of Wistar-O'Grady rats injected s.c. with LSD early in pregnancy but not among those injected after the eighth day. In addition, it appears that when apparently normal second-generation animals grew to maturity and were mated, some died during gestation or soon after delivery; whereas, others produced a higher-than-normal percentage of abortions and abnormal litters. They must have carried some hidden damage not visible until the demands of pregnancy brought it to the surface.

Our present first-generation data (table 1) indicate that 9.1% of pregnant females treated with LSD early in pregnancy aborted or resorbed their fetuses, 3.6% died during gestation, 14.5% delivered litters containing one or more stillborn young, another 14.5% delivered litters containing sick or stunted young and 5.4% delivered unusually small litters. Thus, 47% of all LSD-treated females had abnormal pregnancies as compared to 15% of control females. Obvious damage among second-generation animals reached 11.1% as contrasted to 2.3% among offspring of control females (table 1). It made essentially no difference whether the LSD was

administered s.c. or p.o. Statistical analysis of the data showed that the probability of obtaining these results by accident is minimal ($P < .001$), whether a mother and litter or the offspring individually were considered as the unit tested.

As stated above, we did not interrupt the pregnancies after treatment but permitted our rats to come to term. Interruption four days after LSD treatment of seven-day pregnant mice was carried out by Auerbach and Rugowski (1967), who thereupon found 6-fold increase in the number of observed embryo deformities. These deformities included brain defects such as shift and enlargement of midbrain, improper closure of mid- and hindbrain regions, modification of fourth ventricle, abnormalities of the lower jaw, shifts in position of eyes and modification of facial contours. Four strains of mice were examined and large variations in susceptibility to LSD were found from strain to strain. Malformation of brain, but also of spinal cord, liver as well as fetus resorptions, dead fetuses and runts were observed in fetal hamsters from mothers injected s.c. with a single dose of LSD on the eighth day of pregnancy and sacrificed four days later (Geber, 1967). Similar results were obtained with larger concentrations of the psychotomimetic alkaloid mescaline and with a derivative of LSD, 2-bromo-D-lysergic acid diethylamide. In our initial experiments, injection of LSD prior to the fourth day after copulation, thus, prior to the implantation of the blastocysts in the uterus, produced the described undesirable effects on fetuses, but injection after the seventh day did not. Auerbach and Rugowski (1967) also found that injection in mice after the seventh day failed to produce any obvious teratogenic effects. Our present data indicate that there was only small difference when LSD was given during any of the first seven days of gestation. Dose effect was present. Percentage of abnormal offspring increased with doses from 5μg/kg to 50 μg/kg. In addition, higher doses caused more resorptions than lower ones and fewer sick offspring.

The effect of LSD treatment was transferred to surviving offspring. When they matured and were mated, they displayed damage very similar to that shown by their LSD-treated mothers. A total of 70% of all pregnancies with both parents derived from LSD-treated mothers showed abnormalities, as opposed to 32% for pregnancies where only one parent was derived from an LSD-treated mother and to 17% for controls. The sample was small, however, and the figures may have to be modified as more information is obtained.

We found no particular focus or foci for the toxic activity of LSD. No pattern has emerged from the gross pathologic examinations of treated animals or their offspring, except possibly an increase in vascularity. The numbers of abortions, resorptions, runting, stillbirths and offspring mortality were increased; but no unusual number of obvious deformities was encountered. Of course, detailed pathologic and hematologic work may yet reveal changes which we were unable to detect.

No assay of chromosome damage was attempted in our animals. The occurrence of chromosome damage in human leukocytes *in vitro* in presence of LSD or *in vivo* after LSD intake has been reported (Cohen *et al.*, 1967a,b; Irwin and Egozcue, 1967; Zellweger *et al.*, 1967; Egozcue *et al.*, 1968) but could not be reproduced (Loughman *et al.*, 1967; Bender and Siva Sankar, 1968; Slatis, 1968; Sparkes *et al.*, 1968), and the entire problem awaits resolution. The significance of chromosome breakage in circulating leukocytes, if any, is uncertain (Jarvik and Kato, 1968; Kato and Jarvik, 1969). Recently chromosome damage in gametes of mice treated with high doses of LSD has been observed (Skakkebaek *et al.*, 1968).

It is not possible yet to conclude whether or not there exists a direct relationship between the two striking effects of LSD: the psychotomimetic and the teratogenic. Brain deformations found in mice treated with LSD might be due entirely to the fact that the mice were injected on the seventh day, and this is the stage where neural structures are first being formed, a stage uniquely sensitive to induced abnormalities of the brain (Auerbach and Rugowski, 1967). Mescaline, like LSD, produced congenital malformations (Geber, 1967). The potency of mescaline as a teratogen appeared to parallel its potency as a hallucinogen. On the other hand, 2-bromolysergic acid diethylamide, which has practically no psychotomimetic attributes, caused damage. Both drugs produced damage which was observed not only in the brain but in viscera as well. Investigations begun in 1957 at the New

York State Psychiatric Institute by Roizin and co-workers (Taylor *et al.*, 1960; Denber *et al.*, 1964; Roizin *et al.*, 1968), indicate that LSD may affect the structure as well as function of the central nervous system. Further work with other hallucinogens and their nonhallucinogenic derivatives, already under way, will hopefully provide an answer to this question.

No conclusions concerning teratogenicity of LSD in man can be drawn for our data. Toxic activity of a drug in one species need not imply toxicity in another. The mechanism of the effect in rats described above is not yet known. Adequate statistics on human LSD consumption and effect of the drug, if any, on pregnancy remain to be assembled. Should they indicate that LSD is indeed teratogenic in man (current data are not convincing), then the rat results will be of help in establishing the mechanism of LSD action. In the meantime, caution is indicated in the human use of LSD for therapeutic or hedonistic purposes, and further work on side-effects of LSD and its derivatives and other psychotomimetic agents is mandatory.

CONCLUSIONS. 1) Intake of LSD early in pregnancy was harmful in the Wistar-O'Grady strain of rats. The number of resorptions was higher among LSD-treated groups than among matched control groups. The proportion of stillborn offspring was 5 times greater in litters of LSD-treated rats than in litters of matched controls. The proportion of sick offspring which died within 10 days of age was similarly higher in LSD-treated group than in the control group. Offspring mortality was significantly higher in the LSD-treated group than in the control group. On the other hand, there was no observable difference between the two groups in the proportion of limb deformities or other abnormalities, although runting in the offspring population was more prevalent after LSD treatment.

2) The rats were found susceptible to LSD only during the first one-third of their period of gestation. The LSD effect was dose-related. The LSD-induced damage was transmitted to the offspring. Mating of matured offspring of LSD-treated mothers resulted in a higher-than-average proportion of deaths during gestation and production of litters containing stillborn and sick offspring.

3) Rat data are not directly applicable to man. Caution is, however, recommended in human use of LSD, whether for scientific, therapeutic or hedonistic purposes.

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