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LSD: No Chromosomal Breakage in Mother and Embryos During Rat Pregnancy

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ABSTRACT LSD (500 μg/kg/day ip) given to pregnant rats from the 7th–13th days of pregnancy produced no *in vivo* chromosome damage, either in maternal bone marrow or in the embryos. These negative cytogenetic results parallel the lack of teratogenicity observed under these conditions.

The lack of teratological effects of LSD-25 in Wistar rats was recently described (Roux et al., '70). We now report the results of studies of chromosomes of pregnant Wistar rats and their embryos treated with LSD-25.

Lysergic acid diethylamide was first reported to induce breakage in human chromosomes *in vitro* by Cohen et al. ('67). Although these results were confirmed by others (Abbo and Norris, '68; Jarvik and Kato, '68) the effect of LSD on chromosomes *in vivo* is still in question. An elevated frequency of chromosome breaks in LSD-25 users (Irwin and Egozcue, '67; Hungerford et al., '68) and in children exposed to LSD *in utero* was reported by Cohen et al. ('68) and Hsu et al. ('70). Negative results, however, were obtained by several others (Bender and Sankar, '68; Sato and Pergament, '68; Hecht et al., '68; Hultén et al., '68; Tjio et al., '69; Aase et al., '70; Loughman et al., '67) found no structural chromosome abnormalities even after intake of high doses of LSD.

Reports of chromosome studies in animal experiments were also contradictory. Skakkebaek et al. ('68) and Cohen and Mukherjee ('68) noted a high frequency of breaks as well as chromosomal rearrangements in bone marrow and testicular tissue of mice. Jagiello and Polani ('69), however, failed to confirm these findings. Lack of chromosome damage was also reported for meiotic chromosomes in monkeys (Egozcue and Irwin, '69) and men (Hultén et al., '68).

At present there is no explanation for these inconsistent cytogenetic results. Whether LSD is a teratogen or not also remains an open question.

MATERIALS AND METHODS

In the present study LSD (lot 18017, kindly provided by Sandoz Laboratories, Paris) was tested in preliminary experiments for its effects on chromosomes *in vitro*. Increasing amounts of the drug were added to the culture medium and human lymphocytes were exposed during the entire duration of incubation (3 days). The frequency of chromatid and chromosome breaks was increased, but chromosome-type aberrations were not observed (table 1).

The highest dosage used for teratological investigations (Roux et al., '70) was chosen for the *in vivo* cytogenetic studies. Nine pregnant Wistar rats were injected ip with 500 μg/kg/day of LSD dissolved in 0.5 ml of distilled water on the 7th to 13th days of pregnancy (time of finding sperm in vaginal smear called 1st day). The animals were killed 2 h after the last treatment. Bone marrow of both maternal femurs was used for direct examination of chromosomes. A satisfactory number of mitoses was obtained by ip injection of 0.01 ml/g colchicine (0.25 mg%) 1 h prior to death. The cell suspension was exposed to hypotonic citrate solution for 30 min and fixed in 3:1 methanol-glacial acetic acid for 1 h. Slides were prepared by flame drying and staining by Giemsa after hydrolysis in 1 N HCl for 6 min at 60°C.

For direct examination of mitoses in the 13th-day embryos a cell suspension was obtained from each whole litter by a crusher and handled as described above for bone marrow. The preparations were examined under low-power microscopy. All apparently complete mitosis were pho-

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TABLE 1

Results of in-vitro *tests on human lymphocytes with increasing amounts of LSD*

LSD in culture medium	Mitoses examined	Cells with gaps	Cells with breaks	Cells with other abnormalities
μg/ml	*no.*	*%*	*%*	*%*
0.01	34	11.8	2.9	0
0.1	33	12.1	6.1	0
1.0	31	25.8	9.7	0
5.0	67	13.4	20.9	0
Control culture	50	6.0	2.0	0
Control culture	50	4.0	2.0	0
Control culture	50	6.0	4.0	0
Control culture	50	8.0	0.0	0

TABLE 2

Results of chromosome studies in bone marrow of LSD-treated pregnant rats and their embryos

	Bone marrow of pregnant rats		Embryonic tissue	
	Treated	Controls [1]	Treated	Controls
Mitoses examined	606	328	670	331
Abnormal mitoses (%)	7.0	7.0	1.45	20.3
Cells with gaps (%)	3.7	4.9	8.0	12.4
Cells with breaks (%)	2.3	2.1	5.2	5.5
Cells with fragments (%)	1.0	0	1.1	1.8
Cells with other aberrations (%)	0	0	0.2 [2]	0.6 [3]

[1] Controls from previous series (Roux et al., '71).
[2] 1 centric fusion and 1 chromosome with satellites on both ends.
[3] 1 centric fusion and 1 chromatid exchange.

tographed at × 100 magnification. Chromosomes were counted and scored for aberrations using 9 × 12-cm photographic prints. Cells in which the presence of aberrations was unclear were scored as normal. Controls consisted of offspring from pregnant rats injected with physiological saline, prepared in the same manner and examined by the same observer, who did not know whether he examined chromosomes from treated animals or controls. For each experiment 50–80 mitoses were examined.

RESULTS AND DISCUSSION

The results are shown in table 2. In bone marrow of the pregnant rats individual results ranged from 3.8–16% of mitoses with structural chromosome abnormalities, consisting of gaps, breaks, and acentric fragments. For the total of mitoses studied the data did not differ from those obtained with previous control material (Roux et al., '71). For this reason the control preparations were not examined.

In embryonic tissue the frequency of mitoses with structural chromosome aberrations was higher in controls than in embryos exposed to LSD-25 *in utero* ($P < 0.05$), but when gaps were excluded from the total of abnormalities the difference disappeared. Individual results varied considerably in treated as well as in control embryos, depending on the technical quality of the preparation. Chromosome counts did not reveal differences between the experimental group and the controls, in either bone marrow or embryonic tissue. It may be concluded, therefore, that there was no evidence of an increased frequency of structural chromosome aberrations in rats after exposure to LSD-25.

Negative results were also reported by Sato et al. ('71) who also studied bone marrow from pregnant rats by a direct method and embryonic tissues after 12–14 days' culture. The drug used in our experiments had chromosome-breaking properties in human peripheral blood cultures, as noted for equal doses by others (Cohen et al., '67). Our negative cytogenetic results in rats may either reflect

differences in species sensitivity or simply represent an example of the fact that compounds that are toxic *in vitro* do not have necessarily the same effect *in vivo*.

These negative cytogenetic results were paralleled by negative teratological results. No teratogenic action was noted in rat fetuses exposed to 5–500 μg/kg/day (Roux et al., '70). To our knowledge this is the only study with LSD in which teratological and cytogenetic experiments were coordinated.

These findings after exposure to LSD are in contrast to previous results with three other agents examined in this laboratory, thalidomide, rubidomycine, and trypan blue (Roux et al., '71), which produced chromosomal breakage *in vitro* (human chromosomes) and *in vivo*, in pregnant animals (rabbits, rats) and their embryos, together with severe malformations.

LITERATURE CITED

Aase, J. M., N. Laestadius and D. W. Smith 1970 Children of mothers who took LSD in pregnancy. Lancet, *2:* 100–101.

Abbo, G., A. Norris and H. Zellweger 1968 Lysergic acid diethylamide (LSD-25) and chromosome breaks. Humangenetik, *6:* 253–258.

Bender, L., and D. V. S. Sankar 1968 Chromosome damage not found in leukocytes of children treated with LSD-25. Science, *159:* 749.

Cohen, M. M., K. Hirschorn, S. Verbo, W. A. Frosch and M. M. Groeschel 1968 The effect of LSD-25 on the chromosomes of children exposed *in utero*. Pediat. Res., *2:* 486–492.

Cohen, M. M., M. J. Marinello and N. Back 1967 Chromosomal damage in human leukocytes induced by lysergic acid diethylamide. Science, *155:* 1417–1419.

Cohen, M. M., and A. B. Mukherjee 1968 Meiotic chromosome damage induced by LSD-25. Nature, *219:* 1072–1074.

Dishotsky, N. I., W. D. Loughman, R. E. Mogar and W. R. Lipscomb 1971 LSD and genetic damage. Is LSD chromosome damaging, carcinogenic, mutagenic or teratogenic? Science, *172:* 431–440.

Egozcue, J., and S. Irwin 1969 Effect of LSD-25 on mitotic and meiotic chromosomes of mice and monkeys. Humangenetik, *8:* 86–93.

Hecht, F., R. K. Beals, M. H. Lees, H. Jolly and P. Roberts 1968 Lysergic-acid-diethylamide and cannabis as possible teratogens in man. Lancet, *2:* 1087.

Hsu, L. Y., L. Strauss and K. Hirschhorn 1970 Chromosome abnormality in child of LSD user. D trisomy with D/D translocation. J. Am. Med. Ass., *211:* 987–990.

Hultén, M., J. Lindsten, L. Lidberg and H. Ekelund 1968 Studies on mitotic and meiotic chromosomes in subjects exposed to LSD. Ann. Génét., *11:* 201–210.

Hungerford, D. A., K. M. Taylor, C. Shagass, G. U. LaBadie, G. B. Balabon and G. R. Paton 1968 Cytogenetic effects of LSD-25 therapy in man. J. Am. Med. Ass., *206:* 2287–2291.

Irwin, S., and J. Egozcue 1967 Chromosomal abnormalities in leukocytes from LSD-25 users. Science, *157:* 313–314.

Jagiello, G., and P. E. Polani 1969 Mouse germ cells and LSD-25. Cytogenetics, *8:* 136–147.

Jarvik, L. F., and T. Kato 1968 Is lysergide a teratogen? Lancet, *1:* 250.

Loughman, W. D., T. W. Sargent and D. M. Israelstam 1967 Leucocytes of humans exposed to lysergic acid diethylamide: lack of chromosomal damage. Science, *158:* 508–510.

Roux, C., I. Emerit and J. L. Taillemite 1971 Chromosomal breakage and teratogenesis. Teratology, *4:* 303–315.

Roux, C., R. Dupuis and M. Aubry 1970 LSD: no teratogenic action in rats, mice, and hamsters. Science, *169:* 588–589.

Sato, H., and E. Pergament 1968 Is lysergide a teratogen? Lancet, *1:* 639–640.

Sato, H., E. Pergament and V. Nair 1971 LSD in pregnancy: chromosomal effects. Life Sci., *10:* 773–779.

Skakkebaek, N. E., J. Philip and O. J. Rafaelsen 1968 LSD in mice: abnormalities in meiotic chromosomes. Science, *160:* 1246–1248.

Tjio, J., W. N. Pahnke and A. A. Kurland 1969 LSD and chromosomes. A controlled study. J. Am. Med. Ass., *210:* 849–856.