New Engl.J.Med. 282, 939-943 (1970). LSD 1984

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REFERENCES

1. Graham GG: Effect of infantile malnutrition on growth. Fed Proc 26:139-143, 1967
2. Cabak V, Najdanvic R: Effect of undernutrition in early life on physical and mental development. Arch Dis Child 40:532-534, 1965
3. Stoch MB, Smythe PM: Does undernutrition during infancy inhibit brain growth and subsequent intellectual development? Arch Dis Child 38:546-552, 1963
4. Cravioto J, Robles B: Evolution of adaptive and motor behavior during rehabilitation from kwashiorkor. Amer J Orthopsychiat 35: 449-464, 1965
5. Cravioto J, DeLicardie ER, Birch HG: Nutrition, growth and neurointegrative development: an experimental and ecologic study. Pediatrics 38: Suppl 2:321-333, 1966
6. Snedecor GW: Statistical Methods Applied to Experiments in Agriculture and Biology. Fifth edition. Ames, Iowa, Iowa State College Press, 1956, p 45
7. Nellhaus G: Head circumference from birth to eighteen years: practical composite international and interracial graphs. Pediatrics 41:106-114, 1968
8. Frankenburg WK, Dodds JB: The Denver developmental screening test. J Pediat 71:181-191, 1967
9. Wechsler D: Manual for the Wechsler Adult Intelligence Scale. New York, The Psychological Corporation, 1955, pp 42-43 and 63-75
10. Caldwell BM: Descriptive evaluations of child development and of developmental settings. Pediatrics 40:46-54, 1967
11. Chope HD, Blackford L: The chronic problem family: San Mateo County's experience. Amer J Orthopsychiat 33:462-469, 1963
12. Cochran WG: Some methods for strengthening the common X^2 tests. Biometrics 10:417-451, 1954
13. Cramér H: Mathematical Methods of Statistics. Seventh edition. Princeton, New Jersey, Princeton University Press, 1957, pp 214-215
14. Bancroft H: Introduction to Biostatistics. New York, Hoeber Medical Division, Harper and Row, 1957
15. Chase HP, Dorsey J, McKhann GM: The effect of malnutrition on the synthesis of a myelin lipid. Pediatrics 40:551-559, 1967
16. Benton JW, Moser HW, Dodge PR, et al: Modification of the schedule of myelination in the rat by early nutritional deprivation. Pediatrics 38:801-807, 1966
17. Howard E: Effects of corticosterone and food restriction on growth and on DNA, RNA and cholesterol contents of the brain and liver in infant mice. J Neurochem 12:181-191, 1965
18. Winick M, Noble A: Cellular response with increased feeding in neonatal rats. J Nutr 91:179-182, 1967
19. Chase HP, Lindsley WFB Jr, O'Brien D: Undernutrition and cerebellar development. Nature (London) 221:554-555, 1969
20. Winick M, Rosso P: The effect of severe early malnutrition on cellular growth of human brain. Pediat Res 3:181-184, 1969
21. Chase HP, Canosa CA, Rosal J, et al: Unpublished data
22. Cazden CB: Three sociolinguistic views of the language and speech of lower-class children — with special attention to the work of Basil Bernstein. Develop Med Child Neurol 10:600-612, 1968
23. Hess RD, Shipman VC: Early experience and the socialization of cognitive modes in children. Child Develop 36:869-886, 1965

CHROMOSOME STUDIES ON PATIENTS (IN VIVO) AND CELLS (IN VITRO) TREATED WITH LYSERGIC ACID DIETHYLAMIDE*

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Abstract In a prospective study of 10 patients given d-lysergic acid diethylamide 25, there was no difference in frequency of chromosome breakage between samples obtained immediately before and 24 hours after treatment. In 11 patients, treated over periods ranging from 24 hours to eight years before sampling, the frequency of chromosomal breaks did not differ from that found in untreated controls. In an in vitro study the frequency of chromosomal breaks was increased in replicate cultures from each of 10 subjects when 1 μg per milliliter of d-lysergic acid diethylamide 25 was added during the last 24 hours of culture. There is no cytogenetic evidence that d-lysergic acid diethylamide 25 given therapeutically produces chromosomal damage. The chromosomal aberrations found after illicit use of the drug remain unexplained.

THE possibility that d-lysergic acid diethylamide 25 (d-LSD 25) ingestion induces chromosomal abnormalities was raised in 1967 by Cohen and his associates[1] after in vitro studies. Since that time numerous reports of the effects of LSD on chromosomes of circulating lymphocytes have appeared. Some have indicated an increased frequency of chromosome breakage,[2-5] others have found no increase,[6-8] and one report has suggested a slight transient increase.[9]

In addition to the lack of agreement of reports from different laboratories, there are some unusual features within the results of studies that have reported an increase in chromosomal damage. Within each study, increased aberrations have not been found in all subjects exposed, and the frequency of aberrations appears to have no relation to the dose or to the time elapsed between exposure and testing.[2,5] Although this suggests a variation in response to the drug the relation of the variation to the nature of the drug is difficult to assess because these studies have used experimental groups in which information concerning the source, composition, frequency of use and dosage of the drug could not be accurately determined.

In an attempt to clarify some of these unexplained effects, a series of studies were carried out using as experimental subjects patients who were treated under clinical conditions with known amounts of d-LSD 25. This report deals with the results from three separate studies of the frequency and types of chromosomal aberrations, including in

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vivo prospective and retrospective studies and an in vitro study.

Materials and Methods

Experimental Subjects

All subjects included in the study were interviewed to obtain information concerning exposure to known chromosome-breaking agents. None had received extensive therapy with or exposure to radiation or antitumor agents. Exposure to routine x-rays was noted, but those who had received routine chest or dental x-rays were not excluded from the study. All subjects had normal karyotypes.

All treated patients were drawn from a private psychiatric-hospital population. Details of selection for each experiment are presented with the results.

Drug Administration

The LSD used in all experiments was d-lysergic acid diethylamide 25 (Sandoz). The "mescaline" was mescaline sulfate (Light).

The d-LSD 25 was dissolved in distilled water and administered orally. The mescaline sulfate was administered in capsule form. No other drugs were administered to modify or terminate the effects of the psychoactive drug.

Cytogenetic Technics

All chromosome analyses were carried out on short-term leukocyte cultures in a commercially prepared medium (GIBCO chromosome medium 1A). In all experiments cultures designated as controls were incubated in the same medium and at the same time.

Ten slides from each treated and each control culture were coded to ensure that the examiner could not identify the source of a slide, and, where possible, 10 cells from each slide were analyzed. Cells for analysis were selected with a low-power objective, and once a cell had been selected, it was included in the final analysis. Cells were analyzed under the microscope and photographed, and a second analysis carried out on a photographic print. Analysis of each cell included a chromosome count as well as examination for structural aberrations. For the purpose of this study, a break was defined as a lesion in which the proximal end of the acentric fragment had been displaced from a position of alignment with the distal end of the centric fragment. Achromatic lesions that did not meet this criterion were scored as gaps and were not included as breakage events in the analysis. Chromatid and isochromatid breaks, acentric fragments and deletions were scored as single-break events, whereas exchange configurations, dicentric chromosomes, ring chromosomes and translocations were scored as two-break events. Slides were not decoded until scoring had been completed.

Although specific types of aberrations were scored individually, the values were too low for statistical analysis. However, an analysis was carried out on total single-break aberrations and total two-break aberrations as well as total breaks.

Results

In Vivo Prospective Study — Frequency and Type of Chromosomal Aberrations before and after Treatment with LSD

Ten subjects with no prior use of d-LSD 25, other "psychedelic" agents or narcotics were drawn in consecutive order of treatment with d-LSD 25.

Blood samples for chromosome analysis were obtained immediately before LSD therapy and a second sample was obtained 24 hours later. In this experiment each sample obtained before the initiation of treatment was used as a control for the sample obtained from the same subject 24 hours after treatment, and the difference between the two samples was used as a measure of the effect of treatment. Drug doses ranged from 200 to 600 μg.

Before treatment the frequencies of total chromosome breakage events ranged from 1 to 9 per 100 cells, with a mean of 5.7. After treatment the frequencies ranged from 2 to 8 breaks per 100 cells, with a mean of 4.9.

The differences between total breaks per 100 cells before and after LSD therapy ranged from −4.0 to +4.0, with a mean of −0.80. Single-break aberration differences ranged from −4.0 to 5.0, with a mean value of 0.20 breaks, and two-break aberration differences from 0 to −4.0, with a mean of −1.0 per 100 cells scored.

The results of paired t-test analysis are presented in Table 1. There was no significant change in the frequency of single-break aberrations, total breaks or gaps.

The analysis did indicate a significant reduction in the frequency of aberrations attributed to two-break events. The frequency of these events in both samples was low, with 1.8 per 100 cells before and 0.8 per 100 cells after treatment. Two-break events were never observed after treatment in persons who had not displayed similar events before treatment, and in four out of five, the frequency was de-

TABLE 1. *Prospective in Vivo Study — Paired t-Test Analysis of Chromosomal Aberrations before and after Treatment with d-LSD 25.*

	Difference between before & after Samples*			
	Gaps	1-break aberrations	2-break aberrations	Total breaks
Mean	−0.30	0.20	−1.00	−0.80
t-value	−0.131	0.200	−2.236	−0.873
Probability	0.867	0.825	0.050†	0.409

*100 cells analyzed from each before & each after samples from 10 patients.

†Significant.

creased in the sample obtained 24 hours after treatment.

In Vivo Retrospective Study — Frequency and Type of Chromosomal Aberrations in Patients Treated with LSD, Mescaline or LSD and Mescaline and a Control Group

The treatment center from which the patient population was being drawn had available persons who had been treated with varying LSD dosages over a number of years, and a pilot project was carried out to determine whether or not some indication of dose-time response might be elicited. Since those who had received the largest dosage of LSD had also received mescaline, a group who had been treated solely with mescaline was also included.

This study included four groups: five patients treated with d-LSD 25 only; five treated with mescaline sulfate only; six treated with both d-LSD 25 and mescaline sulfate; and 13 control subjects exposed to neither drug.

Illness and treatment with other drugs were uncontrolled and largely undocumented although four patients in the treated group are known to have used prescribed or illicit drugs.

Patients included in this study had LSD dosages varying from 200 to 4350 μg over periods ranging from 24 hours to eight years. The total number of breaks per 100 cells ranged from six to 12 for LSD, two to 13 for mescaline, two to 11 for LSD plus mescaline and two to 17 in the control group.

With the exception that the maximum number of single breaks in an LSD-treated patient was 12, and the maximum in the control population was 11 breaks per 100 cells, the range of aberration frequencies in each of the categories for all treated groups was within the range observed in the control group. The results of an analysis of variance (Table 2) indicated no significant differences between treated and control groups for single-break, two-break or total-break events. Within the LSD-treated population there was no relation between the frequency of aberrations and either LSD dosage or the time elapsed between treatment and sampling.

In Vitro Study — Chromosomal Aberration Frequencies in Replicate Samples Cultured with and without the Addition of d-LSD 25

The experimental group for this study consisted of 10 university student and faculty members who had never ingested LSD. Blood samples obtained from each were used to initiate replicate cultures. d-LSD 25 in aqueous solution was added to one replicate of each culture in a final concentration of 1 μg per milliliter 24 hours before the culture was terminated. An equal volume of sterile distilled water was added to the control replicates.

The number of breaks per 100 cells in the control cultures ranged from 0 to 15.12, with a mean of 4.72. In the treated replicates the number of breaks per 100 cells ranged from 4.0 to 18.70, with a mean of 9.37.

An increase in both single-break aberrations and total breaks was observed in each of the 10 treated replicates. The differences between treated and control replicates for single breaks ranged from 2.0 to 6.0, with a mean of 3.96, and the differences in total number of breaks per 100 cells scored ranged from 3.0 to 7.9, with a mean of 4.65. Two-break aberrations were increased in four treated replicates and decreased in one, and no difference was observed in five samples. Differences ranged from −2.0 to 3.3, with amean of 0.69 per 100 cells scored.

A paired t-test analysis (Table 3) indicated a significant increase in single breaks and total breaks but not in two-break aberrations.

Discussion

The in vitro study reported here partially duplicates the experiments reported by Cohen, Marinello and Back[1] and Cohen, Hirschhorn and Frosch.[2] Using culture, treatment and scoring technics similar to those described by the above authors, we obtained very similar results. Cohen, Hirschhorn and Frosch[2] reported a range of 3.7 to 17.5 breaks per 100 cells in the treated cultures as compared to an average of 3.8 breaks per 100 cells in control cultures. In the study reported here the frequency of breaks in treated cultures ranged from 4.0 to 18.7, with a mean control value of 4.7 breaks per 100 cells.

An analysis of differences between untreated and treated cultures indicated a consistent and highly significant increase of chromosomal breakage in the treated replicate cultures. Although the in vitro concentration was much greater than any known ingested

Table 2. *Retrospective in Vivo Study — Analysis of Variance.*

Group	No. of Patients	Total Cells	1-Break Aberrations	2-Break Aberrations	Total Breaks	Gaps
				mean/100 cells		
LSD	5	500	6.200	1.600	7.800	20.000
Mescaline	5	500	3.600	2.000	5.600	7.400
LSD + mescaline	6	594	4.708	1.600	6.400	15.418
Control	13	1300	5.923	1.077	7.000	16.923
F value			1.06	0.38	0.31	2.35
p value			0.386	0.773	0.819	0.095

TABLE 3. *In Vitro Study — Paired t-Test Analysis of Chromosomal Aberrations in Replicate Samples Cultured with and without d-LSD 25.**

	DIFFERENCE BETWEEN TREATED & UNTREATED REPLICATES			
	GAPS	1-BREAK ABERRATIONS	2-BREAK ABERRATIONS	TOTAL BREAKS
	per 100 cells			
Mean	7.04	3.96	0.69	4.65
t-value	3.481	9.764	1.428	9.598
Probability	0.007†	0.000†	0.185	0.000†

*Approximately 100 cells analyzed from each treated & each untreated replicate from 10 subjects (1010 treated cells, 1094 untreated cells).

†Highly significant.

dosage the mean increase of 4.65 breaks per 100 cells is small as compared to the range of breakage frequencies (0.0 to 15.2) observed in the untreated cultures. The deviation range of less than 5 breaks per 100 cells indicated no variation in response from subject to subject.

Using the same culture and scoring technics, we found no indication of an increased frequency of chromosomal breakage in subjects who had ingested LSD. Neither the direct comparison of samples obtained immediately before and again 24 hours after treatment nor the study of persons exposed to varying doses over a given time indicated any increased frequency in chromosome breaks. The latter group is similar to those reported by Cohen, Hirschhorn and Frosch[2] and Egozcue, Irwin and Maruffo[5] with one exception: these previous reports involved subjects who had ingested self-administered illicit "LSD" whereas the present study was carried out on patients treated with pharmaceutical d-LSD 25 under clinical conditions.

Since the results of the in vitro studies indicate that the scoring systems are sufficiently alike to produce similar results, some difference between the experimental groups appears to be the most likely source of lack of concurrence in the results of in vivo studies.

There is further evidence that differences in the experimental groups influence the results. To date all indications that LSD ingestion is associated with significant increase in the frequency of chromosomal breakage are based on the study of subjects who have ingested "LSD" under nonclinical conditions.[2-5] On the other hand, studies on patients treated with LSD under clinical conditions have not indicated similar increases.[7,8]

One serial study on four clinically treated subjects[9] indicated a slight transient increased breakage frequency that returned to normal on follow-up observation. In that report LSD was injected, and treatment was in some cases combined with chlorpromazine. In the present prospective study LSD was administered by mouth, with no modifying drugs. In the larger population involved in this study the pretreatment sample shows a greater variation in aberration frequencies than is indicated in the study reported by Hungerford and his associates.[9] However, the deviation between samples before and after treatment had a mean value of −0.80 breaks per 100 cells and was not significant.

Although both Hungerford et al.,[9] in a prospective in vivo study, and Cohen, Hirschhorn and Frosch,[2] in an in vitro study, have reported the occurrence of two-break aberrations (that is, dicentric, exchange figures and so forth) only in samples from treated patients or cultures, a low frequency of similar aberrations was observed in samples from untreated subjects in both the prospective and the in vitro studies reported here. This discrepancy may be due to the selection of subjects. In the present study the effect of LSD was measured by the difference between treated and untreated cultures from the same person, and study of possible variation in response was one of the objectives. Thus, the use of rigid selection criteria was both unnecessary and undesirable. However, the control values are not necessarily representative of a "normal" or a "drug-free" population.

In the prospective study the frequency of two-break events was significantly reduced in samples obtained after treatment, and two-break aberrations were observed after treatment only in patients who had similar aberrations before treatment. In the in vitro study the frequency of two-break aberrations was not significantly increased.

The reduction in two-break aberrations in the prospective study is difficult to explain. However, the 24-hour interval between samplings is remarkable in that the patients were exposed to virtually nothing but LSD. Assuming that the LSD had no influence on the chromosomes, the reduction might be due to the absence of other common environmental agents that contribute to the breakage frequencies observed in control groups.

In conclusion, there appears to be no cytogenetic evidence that d-LSD 25 given therapeutically produces chromosomal damage. On the other hand, there is increasing evidence that populations ingesting "illicit" drugs should be further investigated to identify the source of the increased frequency of chromosome aberrations.

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REFERENCES

1. Cohen MM, Marinello MJ, Back N: Chromosomal damage in human leukocytes induced by lysergic acid diethylamide. Science 155:1417-1419, 1967
2. Cohen MM, Hirschhorn K, Frosch WA: In vivo and in vitro chromosomal damage induced by LSD-25. New Eng J Med 277: 1043-1049, 1967
3. Cohen MM, Hirschhorn K, Verbo S, et al: The effect of LSD-25 on the chromosomes of children exposed in utero. Pediat Res 2: 486-492, 1968

4. Irwin S, Egozcue J: Chromosomal abnormalities in leukocytes from LSD-25 users. Science 157:313-314, 1967
5. Egozcue J, Irwin S, Maruffo CA: Chromosomal damage in LSD users. JAMA 204:214-218, 1968
6. Laughman WD, Sargent TW, Israelstam DM: Leukocytes of humans exposed to lysergic acid diethylamide: lack of chromosomal damage. Science 158:508-510, 1967
7. Bender L, Sankar DVS: Chromosome damage not found in leukocytes of children treated with LSD-25. Science 159:749, 1968
8. Sparkes RS, Melnyk J, Bozzetti LP: Chromosomal effect in vivo of exposure to lysergic acid diethylamide. Science 160: 1343-1345, 1968
9. Hungerford DA, Taylor KM, Shagass C, et al: Cytogenetic effects of LSD 25 therapy in man. JAMA 206:2287-2291, 1968

COMPARISON OF OXYGEN POISONING OF THE LUNG IN CYANOTIC AND ACYANOTIC DOGS*

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Abstract Cyanosis was produced in dogs by the surgical creation of large intracardiac venoarterial shunts. Eight cyanotic and 10 acyanotic dogs were exposed, awake and unrestricted, to 1 atmosphere of 98 per cent to 100 per cent oxygen for two days. In each of the two groups of animals, a similar frequency and extent of pulmonary oxygen damage was found, as judged by the production of respiratory distress, atelectasis, alveolar edema and hemorrhage and abnormal surface tension of whole-lung extracts. Cyanosis did not protect against pulmonary injury from 100 per cent oxygen at 1 atmosphere for two days.

IN patients requiring intensive treatment of hypoxemia, oxygen intoxication of the lung is being recognized with increasing frequency. Among those at risk are a large number of infants and children with cyanotic congenital cardiac defects. Recent experiments with hyperbaric oxygen exposures[1] have shown that pulmonary atelectasis and hemorrhage are significantly greater in normal dogs than in those with cyanosis from intracardiac shunts. These findings suggest that the lower systemic arterial oxygen tensions resulting from large venoarterial shunts provide a certain measure of tolerance against hyperbaric oxygen damage of the lung.

The purpose of the studies reported here is to evaluate the possibility of a similar "protective" effect of cyanosis in animals exposed to 1 atmosphere of 100 per cent oxygen – a dose more applicable to that used in the treatment of patients with cyanosis from congenital cardiac defects or pulmonary diseases.

MATERIAL AND METHODS

Eighteen healthy adult mongrel dogs were studied. Ten were normal dogs, and eight had marked cyanosis from intracardiac shunts that were produced surgically. Two months before study each of the 18 animals was dewormed, immunized against distemper and quarantined to prevent pulmonary infection. In the eight experimental dogs, large venoarterial shunts were created one to two months before study. This was accomplished during three minutes of inflow occlusion of the venae cavae. The right atrium was opened, and the atrial septum was incised. The superior border of the atrial opening was repositioned so that most of the blood from the inferior vena cava passed to the left atrium, whereas blood from the superior vena cava continued in its normal course through the right side of the heart.

On the day before oxygen exposure, each dog was anesthetized with sodium pentobarbital (30 mg per kilogram of body weight). Catheters were placed in the right carotid artery and the right jugular vein, and their tips were advanced to the descending aorta and right atrium, respectively. The proximal ends were tunneled subcutaneously to the back of the neck and exteriorized. The animals were allowed to recover for 24 hours.

The dogs were placed in individual cages within a large oxygen chamber.† The chamber was designed so that observers could enter and leave through double airlock doors without changing the gas environment. The constancy of gas concentration within the chamber was verified by measurement of oxygen and carbon dioxide concentrations every 15 minutes. A high inflow rate of 100 per cent oxygen (150 liters per minute) resulted in a chamber concentration of 98 to 100 per cent oxygen at 1 atmosphere. Excess oxygen and expired carbon dioxide was eliminated from the chamber through one-way outflow valves. Chamber carbon dioxide concentration was maintained below 1 per cent at all times; temperature was kept at 20°C, and relative humidity was 50 to 60 per cent. The animals were observed throughout the exposure on television monitors located outside the chamber.

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