Chromosomal Effect in vivo of Exposure to Lysergic Acid Diethylamide

Abstract. *Chromosome studies of persons exposed to lysergic acid diethylamide, either self-administered or received during medical therapy, failed to demonstrate significant chromosomal damage.*

Effects of various environmental factors on human genes and chromosomes are receiving increased attention. Recent studies indicate that certain drugs, including lysergic acid diethylamide (LSD), may lead to chromosomal damage; although some workers have demonstrated damage by LSD (*1–3*), others have not (*4*). Two studies agree on the effects of LSD on chromosomes in vitro (*3, 5*), but reported effects in vivo are conflicting. Because relatively little is known of the biochemical effects of LSD in relation to human chromosomes, the answer to the question of whether it does damage chromosomes depends on further observations. We have found no significant damage in vivo.

Our control group was chosen from hospital volunteers who had not been exposed to known causes of chromosomal damage; it comprised two women aged 21 and 50 and two men aged 21 and 25. The subjects who had taken LSD privately were selected from patients seeking psychiatric help, usually after use of LSD; they had been exposed to other drugs also. The third group comprised patients who had been treated with LSD for medical reasons. Information on both LSD groups is summarized in Table 1.

Unknown factors may cause the results of chromosome studies to vary in different laboratories, so we performed duplicate experiments in two laboratories on each patient. Furthermore persons who use LSD privately may not know the amount of LSD to which they are exposed, or indeed whether it is only LSD or really LSD. For this reason we studied two groups of exposed persons: those who used the drug privately and those who had been treated medically.

A sample of fresh, sterile, heparinized blood from each person was divided into two portions which were processed separately in the two laboratories. At one laboratory six to eight drops of whole blood were added to 5 ml of medium contained in plastic culture flasks (Falcon). The tissue-culture medium was TC-199 (Microbiological Associates) containing L-glutamine and 20 percent fetal calf serum (Microbiological Associates). To this was added 0.1 ml of phytohemagglutinin (Burroughs, Wellcome) reconstituted according to directions. The cells were incubated for 72 hours at 37°C in an atmosphere containing 5 percent CO_2. Three hours before the end of the culture period, colchicine was added (0.05 ml at $10^{-5}M$). The cells were harvested in standard fashion with 20-minute hypotonic treatment in 0.95 percent sodium citrate; they were fixed in a 3:1 mixture of ethanol and acetic acid, flame-dried on microscope slides, and stained with Giemsa. The slides were scanned under a microscope (× 250), and unbroken cells having minimum chromosome overlaps were selected for analysis. After selection of the metaphase, a photomicrograph (× 1000) was made on 35-mm film with bright-field illumination and oil immersion.

At the second laboratory the method differed in these respects: 0.3 ml of whole blood was added to 8 ml of F-10 culture medium containing L-glutamine (Hyland Laboratories) with 20 percent fetal calf serum in plastic test tubes (Falcon); and incubation was in closed tubes rather than an atmosphere containing 5 percent CO_2. The film negatives were viewed on a reader-printer by two of us. Cells showing definite damage or rearrangement were printed on stabilization paper and later characterized according to the type of chromosomal abnormality.

Reported criteria (*1*) were used for the scoring of chromosomal breakage. Chromosomal damage is scored on the basis of breaks and their equivalents: chromatid breaks, isochromatid breaks, and chromosomal fragments are scored as single breaks. Rearrangement figures, such as ring chromosomes or dicentric chromosomes, are scored on the basis of minimal numbers of changes needed for their formation. Quadriradials are graded as two-break aberrations. Moreover, chromatid and isochromatid gaps also were noted and are added to the breaks as an alternate means of evaluating chromosomal damage. Percentages of aberrations and aberrant cells were recorded separately in each laboratory; the results are combined in Tables 2 and 3 since there was no significant difference between results from the two laboratories.

Our percentages of aberrations based on only the chromosomal breaks are lower in our control group than percentages from the laboratories that have reported positive results (*2, 3*). Statistical evaluation of the data with the

Table 1. Summary of exposures to LSD of our subjects; none had had unusual exposure to radiation or recent viral infection. Control patients (not included) were chosen on the basis of no exposure to drugs or radiation and of no recent viral infections. Abbreviations: "Pot," probable marijuana; DMT, dimethyltryptamine; STP, 4-methyl-2,5-dimethoxy-α-methylphenethylamine.

Subject	Age (yr)	Sex	Diagnosis	Doses				Last dose		Other drugs
				Each (μg)	No.	Total (μg)	Duration (mo)	Amount (μg)	Months before bleeding	
				"Users"						
A	21	F	Psychotic reaction to LSD	250–1000	50	25,000	24	500	1	"Pot," DMT, STP
B	19	M	Schizoid personality	?	75	?	12	?	3	"Pot"
C	20	F	Dysocial personality	?	?	?	18	300–1000	0.25	"Pot"
D	24	M	Schizophrenic reaction	350	10	3,500	5	350	6	"Pot"
				Medically treated (all had psychoneurotic reactions)						
E	33	M		300	50	15,000	12	300	60	None
F	45	F		250	63	15,750	48	250	2	"Pot"
G	31	M		250	100	25,000	24	250	3	Ritalin
H	28	M		250–500	12	3,500	48	250	1	None

Table 2. Combined observations of chromosomal damage from both laboratories. Numbers of cells analyzed appear in parentheses.

Subject	Gaps		Breaks			
	Chromatid	Isochromatid	Chromatid	Isochromatid	Quadriradial	Chromosome interchange
			Controls			
I (241)	15	1	2	3	0	0
II (236)	27	5	0	2	0	0
III (230)	7	0	4	2	0	0
IV (243)	6	0	0	0	0	1*
			"Users"			
A (225)	0	0	1	1	0	0
B (235)	4	0	2	0	1	0
C (244)	23	2	6	1	0	0
D (233)	4	1	0	0	0	3†
			Medically treated			
E (244)	17	3	1	0	1	0
F (198)	11	1	1	4	0	0
G (245)	6	1	2	1	1	0
H (227)	6	0	1	0	0	0

* Ring chromosome. † One tricentric and two dicentric chromosomes.

Mann-Whitney U-test (nonparametric *T*) (*6*) indicates no significant difference between the control group and the two groups of LSD users for the following comparisons: results from each laboratory; laboratory versus laboratory; and group versus group on the basis of combined data from the two laboratories. Moreover the results show no correlation between the total degree of exposure to LSD, or the proximity of the last dose, and chromosomal damage. However, one should note that quadriradials were observed only in persons exposed to LSD, while chromosome interchanges were found in one control and one "user" (Table 2). There was no difference in distribution of the chromosome counts between the controls and the subjects exposed to LSD.

Our results resemble those of some workers (*4*) but differ from those of two laboratories (*1–3*) that have demonstrated chromosomal damage in vivo. The reason for this inconsistency is not obvious, but techniques and culture media varied, and examination of the constituents of the culture media suggests that the laboratories producing positive results used less complete media than did the negative laboratories. The two teams that demonstrated that LSD does cause chromosomal damage also found higher rates of chromosomal breakage in their control groups (*1–3*).

The various aspects of this problem must be critically examined. If one assumes that LSD damages chromosomes, some persons may be more susceptible to this effect than are others because of inherent or unrecognized environmental factors. Thus data obtained before and after exposure to LSD of the same person could be more informative than comparison of results between unrelated individuals. The positive effect of LSD on isolated cells in vitro may not be representative of exposure of these or other cells in the intact person. Moreover, so far all have studied only one special type of cell—the lymphocyte. The usual 72-hour culture period for these cells permits some cells to undergo two or three successive cell divisions, during which time chromosomal damage may be modified. Although exposure to LSD is a common factor among the subjects studied, most had been exposed to other drugs, so that correlation of any observed chromosomal changes with LSD is less clear. Finally it is troublesome that one finds in studies that have demonstrated a positive effect of LSD no apparent dose-effect relation.

Apart from meiotic studies, which have not been reported for persons exposed to LSD, one can evaluate children of parents who have taken LSD, before or at the time of conception, to determine whether or not the gametic cells are affected by the drug. On the basis of our experience and the literature there appears to be no definite effect of LSD in this regard on humans. Reasoning by analogy suggests that chromosomal damage may increase one's potential for malignant change, but this suggestion should not be misinterpreted as evidence of an oncogenic effect of LSD. The final answer will probably result from additional studies of persons exposed to LSD and from efforts to explain the differences in the results so far reported.

Table 3. Summary of results from Table 2, showing percentages of chromosomal damage (both laboratories).

Subject	Breaks plus gaps (%)		Breaks (%)	
	Aberrant cells	Aberrations	Aberrant cells	Aberrations
		Controls		
I	7.1 (17/241)	8.7 (21/241)	2.1 (5/241)	2.1 (5/241)
II	6.4 (15/236)	14.4 (34/236)	0.8 (2/236)	0.8 (2/236)
III	4.8 (11/230)	5.6 (13/230)	2.2 (5/230)	2.6 (6/230)
IV	3.3 (8/243)	3.3 (8/243)	0.8 (2/243)	0.8 (2/243)
		Averages		
	5.4 (51/950)	8.0 (76/950)	1.5 (14/950)	1.6 (15/950)
		"Users"		
A	0.9 (2/225)	0.9 (2/225)	0.9 (2/225)	0.9 (2/225)
B	2.6 (6/235)	3.4 (8/235)	1.3 (3/235)	1.7 (4/235)
C	11.5 (28/244)	13.1 (32/244)	2.4 (6/244)	2.9 (7/244)
D	3.4 (8/233)	5.6 (13/233)	0.9 (2/233)	3.4 (8/233)
		Averages		
	4.7 (44/937)	5.9 (55/937)	1.4 (13/937)	2.2 (21/937)
		Medically treated		
E	5.7 (14/244)	9.4 (23/244)	1.2 (3/244)	1.2 (3/244)
F	7.1 (14/198)	8.6 (17/198)	2.5 (5/198)	2.5 (5/198)
G	3.7 (9/245)	4.9 (12/245)	1.6 (4/245)	2.0 (5/245)
H	3.1 (7/227)	3.1 (7/227)	0.4 (1/227)	0.4 (1/227)
		Averages		
	4.8 (44/914)	6.5 (59/914)	1.4 (13/914)	1.5 (14/914)

ROBERT S. SPARKES
Department of Medicine, University of California School of Medicine, Los Angeles 90024
JOHN MELNYK
Children's Hospital of Los Angeles, University of Southern California Medical School, Los Angeles 90027
LOUIS P. BOZZETTI
Department of Psychiatry, University of California School of Medicine

References and Notes

1. M. M. Cohen, M. J. Marinello, N. Back, *Science* **155**, 1417 (1967).
2. S. Irwin and J. Egozcue, *ibid.* **157**, 313 (1967).
3. M. M. Cohen, K. Hirschhorn, W. A. Frosch, *New Engl. J. Med.* **277**, 1043 (1967).
4. W. D. Loughman, T. W. Sargent, D. M. Israelstam, *Science* **158**, 508 (1967).
5. L. Bender and D. V. S. Sankar, *ibid.* **159**, 749 (1968).
6. L. F. Jarvik and T. Kato, *Lancet* **1968-I**, 250 (1968).
7. H. B. Mann and D. R. Whitney, *Ann. Math. Statist.* **18**, 50 (1947).

8. Aided by PHS grant MR 0504B67 and California state grant DMH 67-14-9; HEW Children's Bureau project 422. We thank J. T. Ungerleider for making patients available for study, and David Thomas for statistical analysis.

22 April 1968

Mechanism for Multiplication of Atmospheric Ice Crystals: Apparent Charge Distribution on Laboratory Crystals

Abstract. *Replication of ice crystals with vapor of methyl 2-cyanoacrylate has produced evidence of whiskers on them; an additional mechanism for the necessary multiplication of ice crystals in the atmosphere is suggested. This replication technique also confirms crystal clustering in the atmosphere and appears to confirm the distribution of electrical charge on ice crystals.*

Photography of three-dimensional replicas of ice crystals produced by use of methyl 2-cyanoacrylate monomer (*1*), with a scanning electron microscope, revealed whiskers on the surfaces of growing crystals (*2*) and confirmed charge-induced clustering of ice crystals in the atmosphere (*3*). These results suggest a mechanism for multiplication of ice crystals and the existence of a charge distribution on the surface of a growing crystal.

The manner of multiplication of ice crystals in the atmosphere is potentially important in the ice-nucleus economy of clouds. Langham and Mason (*4*) suggested the importance of splintering and explosive rupture of droplets in the formation of ice nuclei. It was proposed (*5*) that ice whiskers serve as sources of new ice nuclei. We suggest that ice whiskers present on growing ice crystals, smaller by an order of magnitude than those reported (*5*), may serve this purpose.

The small diameter of these whiskers (of the order of 0.5 μ) and the large length-to-diameter ratio suggest that they may be easily broken. A collision between two whiskered ice crystals, or between a whiskered crystal and a water drop, could fracture many of these fragile whiskers, with the consequent production of new ice nuclei. The nature of the whiskers depends on the conditions of growth. Replicas of whiskered ice crystals, grown under different conditions, are shown in Fig. 1.

The electrical charge of ice crystals grown in a laboratory cloud chamber has been reported (*6*); its polarity appeared to depend on the temperature, and the sign-transition temperatures appeared to coincide with those of the reported (*7*) habit changes. Furthermore, modification of habit by impurities produced a corresponding modification of charge (*8*).

These results suggest that the crystal growth produces an excess of negative electrical charges on the basal faces and an excess of positive charges on the prism faces. Because ice is a poor conductor, while the crystal is growing, the negative charges appear to be concentrated near the center of the basal plane faces; the positive charges, on the edges between the prism faces.

The ice crystals agglomerate, fuse together (*9*), fall onto a metal pedestal, and are replicated. The metal substrate effectively prevents charge-induced rafting. An example is shown (see cover) of air clustering of two platelets grown at $-10°C$, each approximately 30 μ in diameter. Photographs of replicated clusters of ice crystals produced in a

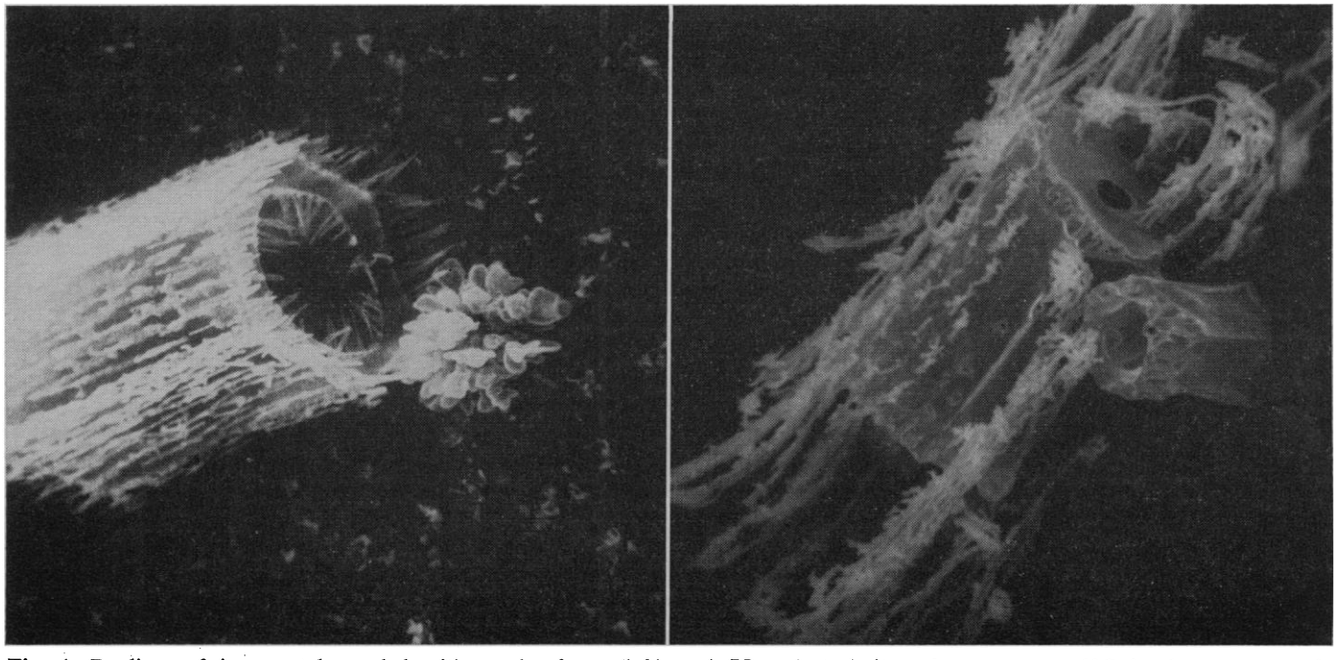

Fig. 1. Replicas of ice crystals seeded with smoke from (left) a $AgIO_3$ pyrotechnic and grown at $-6°C$ in the presence of NH_4OH contamination, and (right) from a $AgIO_3$–KIO_3 pyrotechnic and grown at $-7°C$ in air not deliberately contaminated; $\times$ 1000.