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In Vivo Effects of Illicit Hallucinogens on Human Lymphocyte Chromosomes

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Fourteen subjects exposed to illicit lysergic acid diethylamide (LSD) and nine exposed to marihuana were compared to age-matched controls for effects upon lymphocyte chromosomes. Eight out of 1,284 in the group given LSD, or 0.76%, exhibited chromosomal abnormalities; seven out of 816, or 0.86%, in the group given marihuana; and eight out of 1,018, or 0.79%, in the control group. Cells were grown in medium unenriched with nucleotide precursors. Thus, our negative results mitigate against medium deficiency as contributing toward positive effects. Similarly, our results on individuals whose last dosage was within an hour or so of specimen procurement indicates that this factor may not explain conflicting results.

Our interest in the possible effects of hallucinogens on chromosomes derived from the obvious public health problem and the conflicting nature of published reports. In addition to a study of illicit lysergic acid diethylamide (LSD), a similar review of the effects of marihuana was undertaken. As specimens were available under a wide variety of conditions, and times and culture medium varied, it was thought that some areas of conflict might be resolved.

The difficulties are obvious. In vivo, in vitro, meiotic, and teratogenic effects have been reported and all with ambiguous reports.[1-14]

In our study, the population exposed to LSD was composed of hospital inpatients and volunteers, all of whom had taken LSD alone or with other drugs (Table 1). The population exposed to marihuana was composed of volunteers who used marihuana in varying amounts and who had not used other psychedelic drugs (Table 2). Both groups were questioned about possible causes of chromosome damage; history was obtained of exposure to x-rays, recent viral illness, drug (nonhallucinogens) ingestion, and of genetically determined diseases, especially those affecting chromosome structure (Bloom's syndrome, Fanconi's anemia, and ataxia telangiectasia).

Materials and Methods

For the group exposed to LSD, 10 ml of peripheral venous blood was drawn from each subject into

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Table 1.—Effect of LSD on Lymphocyte Chromosomes

Case	Age (yr)	Sex	No. of Doses	Last Dose	Breaks (%)*	Other Drugs†	X-ray Films
1	20	M	24	2 mo	0 (0/100)	Me, P, STP, DMT, H, Cpr, B	Chest (2)
2	21	M	75	2 mo	1 (1/100)	Me, P, STP, DMT, H, Thio	Dental, chest
3	25	M	1	16 hr	1.27 (1/79)	P	...
4	27	M	1	16 hr	1.96 (2/102)	P	...
5	25	M	2	1½ hr	0 (0/102)	P	...
6	25	M	1	16 hr	0 (0/107)	P	...
7	17	M	100+	2 weeks	0.85 (7/117)	Me, H, DMT, Cpr, STP MGS, Co, B	Dental, chest
8	18	M	175	15 days	0 (0/94)	Me, H, P, STP, Mesc, MGS	Dental, hand, chest, skull
9	26	M	150	1 week	1.54 (1/65)	Me, P	Dental, chest
10	25	M	2	16 hr	0 (0/54)	P, Mesc	...
11	26	M	1	16 hr	0 (0/63)	P	Chest
12	18	F	6	3 mo	0 (0/100)	P, Cpr	...
13	18	F	25	2 mo	1 (1/100)	P, Me, H	Chest
14	20	F	50	2 mo	1 (1/101)	P, Me, H, Co, MGS	Chest

*Mean, 0.76% (8/1,284).
†Me signifies methamphetamine hydrochloride; P, marihuana; DMT, dimethyltryptamine; H, heroin; B, barbituates; MGS, morning-glory seeds, Co, cocaine; Mesc, mescaline; Cpr, chlorpromazine hydrochloride; Thio, thioridazine hydrochloride; STP, an amphetamine derivative.

Table 2.—Effect of Marihuana on Human Lymphocyte Chromosomes

Case	Age (yr)	Sex	No. of Doses*	Last Dose	Breaks (%)†	Gaps	X-ray Films	Viral Illness	Other Drugs
15	27	M	50c in 2 yr 8c in 7 days	12 hr	0 (0/87)	0	Chest, to head	...	...
16	26	M	100c in 1 yr 8c in 7 days	12 hr	0 (0/100)	0	...	...	...
17	27	M	2-3c/week for 1.5 yr	1 week	2.67 (2/75)	2	Chest	...	Dextroamphetamine sulfate, aspirin with codeine, and aspirin, butalbital, phenacetin, and caffeine (Fiorinal)
18	25	M	2c/mo in 9 mo	1 mo	2 (2/100)	2	Chest	"Cold before specimen" 5-days	Penicillin for "cold"
19	24	F	2c/mo in 9 mo	1 mo	1 (1/100)	0	...	...	Ethynodiol diacetate and mestronol (ovulen)
20	20	F	1c/day for 3 mo	1 day	0 (0/86)	1	Chest	...	...
21	19	F	1c/week for 2 yr	1 day	1 (1/100)	1	...	...	...
22	25	M	1c/week for 9 mo	1 day	0 (0/101)	3	...	...	...
23	22	F	4c in 1 yr	1 week	1.49 (1/67)	0	...	...	...

*The abbreviation C signifies marihuana cigarette.
†Mean, 0.86% (7/816).

tubes containing heparin sodium. Macrocultures were made, using a modification[15] of the method of Moorhead,[16] and microcultures were made according to a standard technique used in this laboratory and described below. Macrocultures contained approximately 40% inactivated fetal bovine serum, 60% Eagle's minimum essential medium or Eagle's basal medium, 1.2 ml phytohemagglutinin M, 0.2 ml of 0.1% glutamine, and 0.0025 ml of neomycin. Microcultures were prepared from 0.25 ml whole blood, 40% inactivated fetal bovine serum, 5 ml Eagle's minimum essential medium, 0.1 ml phytohemagglutinin M, 0.1 ml of 0.1% glutamine, and 0.0015 ml neomycin. All cultures were incubated at 37 C for 72 hours. Colchicine was added four hours before harvest to accumulate cells in metaphase. Cells were washed in saline and expanded in 0.6% potassium chloride hypotonic solution for 10 to 30 minutes. Fixation was accomplished in anhydrous 100% alcohol and acetic acid solution (3:1). Cells then were flame-dried on microscope slides and Giemsa stained.

For the group exposed to marihuana, 1 ml of peripheral venous blood was drawn from each volunteer, and triplicate microcultures were made. The whole blood was placed in containers of 12 ml Eagle's basal medium, 40% inactivated fetal bovine serum, 100 units of heparin, and 0.0025 ml of neomycin. This mixture was stored by refrigeration for up to 50 hours (previous studies

had shown no ill effects on mitotic index or chromosome structure). When cultures were initiated, the mixture was divided into three aliquots. We added 0.1 ml phytohemagglutinin M and 0.1 ml of 0.1% glutamine to each culture and incubated them at 37 C for 72 hours. The remainder of the procedure was as for the LSD experiment.

Results

For both groups, where possible, 100 metaphase spreads were analyzed for chromosome number and abnormalities. Abnormalities were scored according to the method of German and Grippa.[17] Six karyotypes were made for each case. There were ten controls, using both LSD and marihuana culturing techniques. Since control results were similar for both techniques, the results were pooled.

The results of a wide range of dosages of LSD appear in Table 1. The experimental group shows from 1 to 175 doses. The time of last exposure to LSD before sampling ranged from 1½ hours to three months. Among LSD users a total of 1,284 metaphase spreads were analyzed, and eight breaks were found, for a 0.76% mean break rate with a range of 0.0% to 1.96%. This compares with a 0.79% mean break rate for 1,018 metaphase spreads of normal controls (range, 0.0% to 1.4%) (Table 3). The only abnormalities noted in any group were chromatid breaks and fragments. No ring chromosomes, acentric fragments, dicentric chromosomes, or exchange figures were present. In no single case was the LSD break rate significantly different from the range of breaks in the control group.

The results of the marihuana experiment appear in Table 2. Nine marihuana users were studied. Both light and relatively heavy users were represented in the sample. The time of the last dose of marihuana ranged from 12 hours to one month prior to sampling. Exact dosage of marihuana was difficult to quantify due to the different strengths and amounts smoked, but the number of marihuana cigarettes is given. A total of 816 metaphase spreads was analyzed yielding a total of seven breaks, for a 0.86% mean break rate. Abnormalities were chromatid breaks and fragments. Again, no rings, dicentric chromosomes, acentric fragments, or exchange chromosomes were present.

Table 3.—Normal Controls

Case	Age (yr)	Sex	Breaks	X-ray Films
1	25	F	1/106	Chest
2	26	F	1/105	...
3	25	M	0/122	Chest
4	18	M	1/70	...
5	24	F	1/101	...
6	24	M	0/114	Chest
7	27	F	1/100	Chest
8	23	M	2/100	Chest
9	25	F	0/100	...
10	44	M	1/100	Chest, dental

†Mean, 0.79% (8/1,018).

T-test analysis of the data on the mean percentages of the groups in both comparisons (LSD vs control, marihuana vs control) was employed. The normal approximation was assumed arbitrarily. In a second determination of statistical significance, the proportions of aberrations in a group were transformed by the formula

$$Y = \text{SIN}^{-1}\sqrt{\frac{X}{N+1}} + \text{SIN}^{-1}\frac{X+1}{N+1}$$

to stabilize the variance (where X = number of chromosomal aberrations and N = number of metaphase spreads enumerated. The *t*-test was then applied to the transformed data. Both procedures indicated no statistical significance between the control and experimental group.

Comment

Some of the problems of interpretation are exemplified by the following: LSD was implicated as a teratogen in a case of unilateral fibular aplasia,[14] although unilaterality of malformations generally vitiates such an interpretation. Similarly, the finding of a "Philadelphia-like" chromosome[18] in a 19-year-old boy with leukemia who had taken LSD, suggested a causal relationship. Chromosomal abnormalities are often demonstrable in leukemic cells,[15] therefore such a finding in this patient is merely confirmation of the fact. Further, lymphocytes cultured from the same patient were free of chromosomal anomalies. Thus, LSD exposure in a leukemic patient who has chromosomal abnormalities seems mere coincidence.

The explanation for contradictory findings in different laboratories studying the effect of LSD on chromosome structure has yet to be clarified. Variations in composition of illicit LSD might be significant, and is not resolved in the literature.[1-4] The problem of time of last dose before sampling could be critical in view of the findings of Hungerford et al.[4] But, in our series, a wide temporal spectrum is included, from 1½ hours to three months after last ingestion, without significant abnormalities. This report includes subjects hospitalized during acute toxic manifestations of drug (illicit LSD) exposure who were without significant evidence of undesirable chromosomal effect. Thus, timing of specimen procurement was not decisive, although the precise composition of drugs taken could not be determined.

Sparkes et al[2] suggested that laboratories reporting chromosome abnormalities in LSD users employed less fortified culture media, which presumably might detrimentally affect chromosome repair. Our negative results using either Eagle's minimal essential medium or Eagle's basal medium, speaks against this hypothesis; neither contains nucleotide precursors. The possibility still exists that differing results may be attributed to technical variation in cell culturing (source of media, se-

rum, etc) and chromosome evaluation. The higher control chromosome break rate reported by those laboratories finding significant LSD chromosome damage suggests this possibility.[5,11] However, in one laboratory,[13] a subsequent study showing LSD-related chromosome damage was reported with control break rates within our usual limits of variation (2% or less).

The results of our LSD and marihuana studies leads to the following possible interpretations: (1) Illicit LSD or marihuana or both does not induce damage to human lymphocyte chromosomes in vivo. (2) LSD or marihuana or both does cause chromosome damage, but damaged chromosomes went undetected by us because of (*a*) selective elimination of chromosome-damaged cells, or (*b*) chromosome repair before analysis. The selection hypothesis, while possible, is unlikely, since cells damaged by other means, such as radiation, viruses, and various chemical agents persist for long periods of time. The alternative hypothesis is currently under study in our laboratory.

Studies of the effects of pure LSD and illicit LSD clearly may not be comparable. While both are important, illicit materials are those commonly taken and therefore immediately relevant to the public health problem. Drugs mixed with the hallucinogen may either potentiate or minimize chromosomal damage. Local practices among various sources may account for differing results in such studies. Likewise, the mode of administration with the attendant dangers of contaminated injections may contribute to positive effects. These possibilities remain to be resolved.

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Nonproprietary and Trade Names of Drug

Thioridazine hydrochloride—*Mellaril.*

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