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IN VIVO AND IN VITRO CHROMOSOMAL DAMAGE INDUCED BY LSD-25*

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THE induction of chromosomal aberrations by exogenous agents, such as viruses, radiation and chemicals, is an area of active cytogenetic investigation.[1] Recently, the psychotomimetic hallucinogen, lysergic acid diethylamide (LSD-25), has been added to the list of chemicals capable of causing abnormalities in the chromosomes of human leukocytes.[2,3] Such preliminary studies report the in vitro and in vivo chromosomal effects of LSD, and the present communication extends these observations to include additional in vitro findings and a significant sample of patients.

MATERIALS AND METHODS

In Vitro Studies

Peripheral leukocyte cultures were initiated from 6 normal, healthy persons (3 males and 3 females) with no history of prior drug ingestion, radiation exposure or recent viral infection. Chromosomes were obtained from cells of whole-blood inoculum (3 to 4 drops) grown in commercially available microculture kits (Chromosome Medium 1A, Grand Island Biological Company, Grand Island, New York) and incubated at 37°C for seventy-two hours. Two hours before harvest colcemide was added at a final concentration of 0.05 μg per milliliter to accumulate metaphases. The cells were harvested, and cytologic preparations were made after a slight modification of the procedure of Moorhead et al.[4] Slides were stained with aceto-orcein and examined with phase-contrast microscopy.

During the period of incubation, LSD dissolved in sterile distilled water was added to selected cultures in final concentrations of 10, 1, 0.1, 0.01, 0.001 μg per milliliter at forty-eight, twenty-four and four hours prior to harvest of the cells. Duplicate cultures were initiated for each treatment. Two slides were made from each culture, and it was hoped to obtain 25 metaphases from each, or a total of 100 cells per treatment per subject. Cultures that did not receive any LSD were used as controls.

All slides were coded and evaluated "blindly." A mitotic index was ascertained by scoring of the number of mitoses observed in 250 cells on each slide. Chromosome damage was classified as either chromatid or isochromatid breaks, and other structural abnormalities were also noted. A chromatid break was defined as a visual discontinuity of the chromatin material in which the distal fragment of a single chromatid was displaced from the axis of the proximal portion (Fig. 1*a*). An isochromatid break was defined identically except that both chromatids were "broken" at the same level and the distal portion displaced (Fig. 1*b*). Both these abnormalities were scored as single breaks. "Gaps" or visual discontinuities of the chromatin material without displacement of the distal portion were noted but not considered as breaks. Exchange figures and dicentric chromosomes (Fig. 1*c* and *d*) were counted as two breaks. Each break, where possible, was assigned to a given chromosome or chromosome group. In addition, the break was visually placed in a given segment of a chromosome arm – proximal, central or distal. Another area included the breaks observed at the centromere region. A number of unidentifiable fragments (Fig. 1*c*) were not assigned to any chromosome.

In Vivo Studies

Peripheral leukocyte cultures were initiated from 22 persons who had ingested LSD (Table 1) or been exposed to the drug *in utero* (Table 2); from 6 patients receiving various other drugs but no LSD (Table 3) and from 14 drug-free controls (Table 4). Two replicate blood samples per subject were collected and coded by one of us (W.A.F.). Two drops

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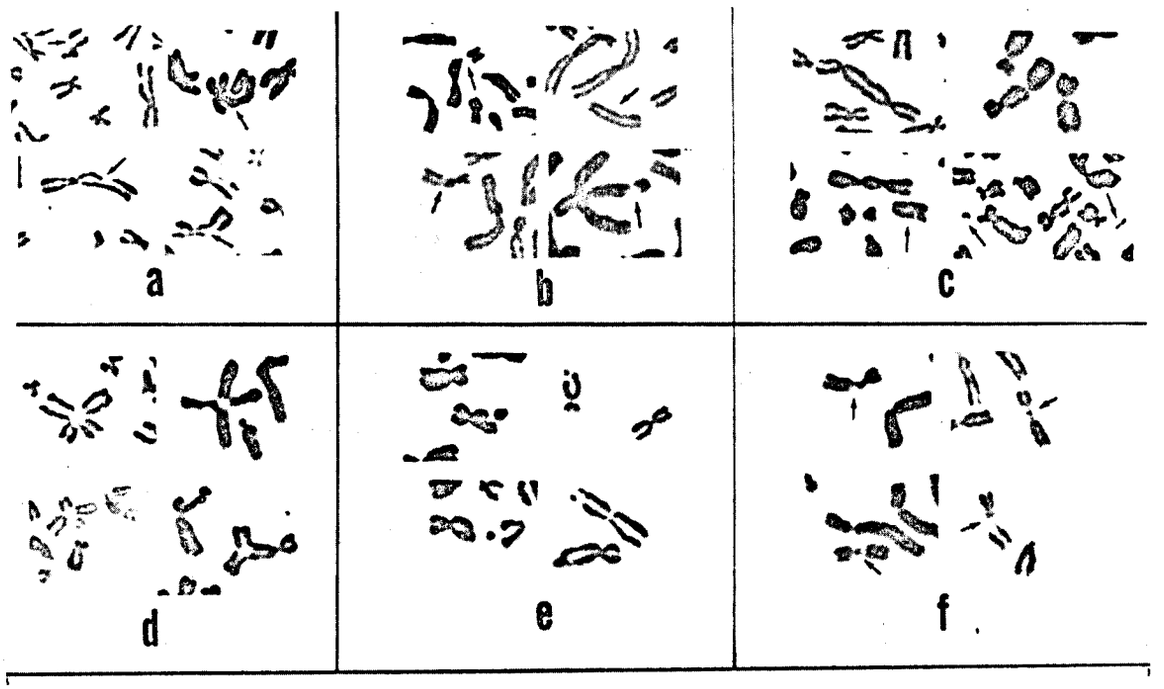

FIGURE 1. *Types of Chromosomal Abnormalities Observed in Both the in Vitro Experiments and from the Leukocyte Cultures Derived from Drug "Users," Showing Chromatid Breaks* (a), *Isochromatid Breaks* (b), *Dicentric Chromosomes* (c) *(Arrows Indicate Acentric Fragments), Exchange Figures* (d), *Chromatid Breaks Giving Rise to Terminal Deletions and Acentric Fragments* (e) *and Attenuation and Breakage at the Secondary Constriction in Chromosome No. 9* (f).

of capillary blood were placed into the microculture tubes and cultured at 37°C for seventy-two hours, the last two hours after the addition of 0.5 μg of vinblastine* per tube. Incubation of cultures and slide preparations, as described above, were carried out at another laboratory (K.H.). Four to 6 cover slips (22 mm²) were prepared from each subject and mounted randomly, 2 to a microscope slide. Blood specimens from several subjects were cultured on two separate occasions. The coded slides were sent to Buffalo for analysis (M.M.C.). Fifty well spread metaphases per cover slip were selected under low power (X250) by workers not involved in the scoring of chromosomal damage. Detailed analysis of the cells for chromosome abnormalities (oil immersion, X2500) was done by one person and was identical to that followed in the in vitro studies.

RESULTS

In Vitro Studies

Figure 2 demonstrates the effect of LSD on the mitotic rate in the leukocyte cultures. Sixteen thousand cells were scored on the control slides, and approximately 6000 cells were counted for each treatment. The rate of mitosis in the controls was 6.2 per cent. A significant mitotic inhibition was apparent on addition of the drug in any concentration. However, the longer the exposure time, the greater the suppression of mitosis.

Table 5 shows the induction of chromosome breakage by LSD. Since no statistically significant differences were demonstrable among the 6 subjects or between the sexes, the data from all subjects were pooled for each treatment. Among the controls 3.9 per cent of cells exhibited chromosomal abnormalities. Among the treated cultures, the lowest frequency observed was almost twice the control (7.7 per cent) and ranged to over four times control values (17.5 per cent). The vast majority of the abnormalities scored were chromatid and isochromatid breaks although a few chromatid exchanges and dicentric chromosomes were observed (Fig. 1*c* and *d*). No ring chromosomes were seen.

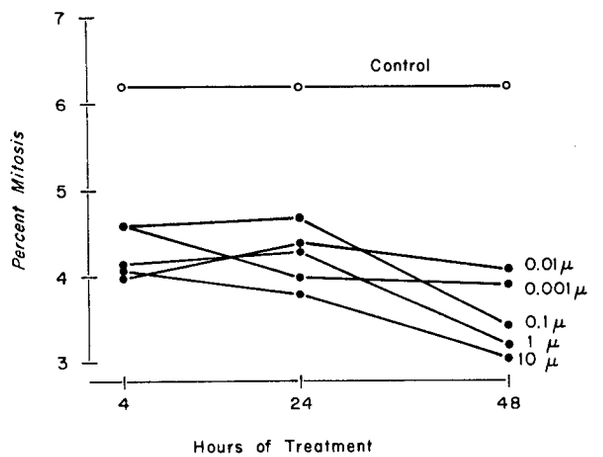

FIGURE 2. *Effect of LSD on Mitotic Rates of Leukocytes Derived from 6 "Drug-Free" Controls.*

*In the form of Velban, Eli Lilly and Company, Indianapolis, Indiana.

TABLE 1. *Chromosomal Damage in Patients Ingesting LSD.*

CASE NO.	AGE	SEX	NO. OF DOSES	INTERVAL BETWEEN LAST DOSE & BLEEDING	PER CENT BREAKS	OTHER DRUGS ACCORDING TO HISTORY*
	yr.					
10	19	Male	300	2 wk.	9.0 (18/200)	A,M,O,P
3	24	Male	200	1 mo.	9.8 (28/285)	A,H,M,O
34	22	Male	100	1 mo.	25.1 (44/175)	A,H
9	28	Female	100	2 mo.	11.0 (22/200)†	A,B,C,H,M,P
22	29	Female	30-60	1 mo.	15.3 (46/300)	A,M,P
29	22	Male	50	1 mo.	5.5 (10/183)	H,M,O,P
7	24	Male	50	7 mo.	11.6 (29/250)	A,B,C,H,P
32	20	Male	35	1 mo.	12.0 (30/250)	A,B
6	19	Male	20-25	4 mo.	10.3 (19/184)	A,H,M,P‡
20	22	Male	15-20	4 mo.	12.6 (35/278)	A,H,M
37	52	Male	15	8 mo.	13.0 (39/300)	P
14	25	Female	15	1 mo.	20.3 (61/300)	A,H,M
36	26	Male	15	1 mo.	22.0 (58/264)	A,H,M,P
39	23	Male	6	7 mo.	24.0 (36/150)	A,H,M,O,P
2	21	Female	4	2 mo.	6.4 (5/ 78)	A,B,C,H,M,O
26	20	Female	4	1 mo.	14.0 (42/300)	M
38	22	Male	3	6 mo.	9.8 (28/285)	A,H,M
28	21	Female	2	1 mo.	5.3 (16/300)†	A,C,M,P‡
					13.2 (566/4282)	

*A indicates amphetamines, B barbiturates, C cocaine, H hallucinogens, M marijuana, O opiates, & P phenothiazines.
†Low dose (50-100 μg).
‡Phenothiazine at time of bleeding.

The distribution of chromosome breaks among the various chromosomes or chromosome groups is not random according to length (Table 6). With the use of length values described in the Denver classification[5] and multiplication by the total number of breaks observed, it is possible to derive the expected number of breaks per unit length of chromatin. It is obvious that a significant deviation from randomness is present in this distribution (p less than 0.001). This is ascribed to a substantial excess of breaks in chromosomes No. 1 and 2 and Group C and a paucity of breaks in Groups D, E, F and G.

In addition, the distribution of breaks within the chromosomes (or groups) does not appear uniform (Fig. 3). Since it is sometimes difficult to ascertain long and short arm in chromosomes No. 1 and 3, breaks in these elements were classed as centromere, proximal, central or distal. The number of breaks in Groups F and G were too small to be plotted. The centromeric regions of all elements (except Group D) appeared to be very susceptible to breakage by LSD. In addition, several other areas of localization were noted that appeared to coincide with regions already described as being heterochromatic secondary constrictions[6] — for example, the paracentric region of No. 1, the central portion of the long arms of the Group B chromosomes, the

TABLE 2. *Chromosomal Damage in Offspring of Mothers Who Ingested LSD during Pregnancy.*

CASE NO.	NO. OF MOTHERS	AGE	SEX	NO. OF DOSES IN UTERO	PER CENT BREAKS	OTHER DRUGS USED IN UTERO*
1	2	11 mo.	Female	4	19.0 (57/ 300)	A,C,M
15	14	2½ yr.	Male	1	13.0 (39/ 300)	—
12	9†	5½ yr.	Female	2	7.5 (15/ 200)	M
11	9†	2 mo.	Male	4	4.0 (8/ 200)	C,M
					11.9 (119/1000)	

*See key Table 1 for abbreviations.
†Low dose (50-100 μg).

TABLE 3. *Chromosomal Damage in Patients Ingesting Drugs Other than LSD.*

CASE NO.	AGE	SEX	RACE	DRUGS	PER CENT BREAKS
	yr.				
8	20	Male	W	Chlorpromazine, 200 mg/day, for 1 mo. until day of bleeding	17.4 (34/195)
16	34	Female	N	M* — 10-yr. duration; chlorpromazine, 50 mg/day for 2 days until day of bleeding.	21.5 (43/200)
31	30	Male	N	Chlorpromazine, 200 mg/day, for 3 wk. until day of bleeding.	13.7 (28/205)
18	31	Male	W	A* — 4-yr. period up to 1 gm/day (last dose 2 wk. before bleeding); O* — 6 yr.; M* — 15 yr.; B* — intermittent.	9.0 (18/200)
21	9	Male	N	Diphenhydramine, 100 mg/day for 1 mo. up to time of bleeding.	10.0 (26/261)
23	9	Male	N	Chlorpromazine & thioridazine for 1½ yr. up to 1 mo. before bleeding; diphenhydramine for 1 wk. before bleeding; patient addicted to opiates at birth — exposed throughout pregnancy.	4.0 (12/300)

*See key to Table 1 for abbreviations.

TABLE 4. *Chromosomal Damage in Subjects Used as Normal "Drug-Free" Controls.*

CASE NO.	AGE yr.	SEX	PER CENT BREAKS*
5	35	Male	5.0 (5/ 100)
17	39	Female	4.9 (9/ 182)
19	29	Male	5.5 (11/ 200)
24	23	Male	4.3 (13/ 300)
25	28	Female	4.1 (13/ 314)
27	26	Female	4.5 (9/ 200)
30	23	Male	2.8 (7/ 250)
33	24	Male	2.3 (6/ 257)
35	23	Male	5.0 (11/ 221)
40	55	Male	2.0 (4/ 200)
41	23	Male	3.0 (6/ 200)
42	22	Male	3.2 (8/ 250)
			3.8 (102/2674)
4	37	Female	31.0† (31/ 100)
13	23	Female	14.0† (28/ 200)

*Figures in parentheses denote number of breaks per cells investigated.
†Viral infection, with high fever & diarrhea, developed within 24 hr. of bleeding.

middle short arm of a C chromosome (No. 6), the paracentric region of another Group C element (No. 9) (Fig. 1*f*), the middle of the arm of a D chromosome (No. 13) and the paracentric region of an E chromosome (No. 16).

In Vivo Studies

Fourteen subjects, without drug ingestion, of an age and sex distribution similar to that of the LSD patients, served as the control group (Table 4). Of these, 2 (Cases 4 and 13) contracted a virus infection with high fever and diarrhea less than twenty-four hours after initiation of their cultures, and these subjects demonstrated a high frequency of chromosomal breakage. The remaining 12 controls, all whites except Case 24, who was Negro, showed a range of chromosomal break frequencies of 2.0 to 5.5 per cent (mean, 3.8 per cent) on the basis of 2674 metaphases scored. This mean frequency is almost identical to that found in the controls for the in vitro study (Table 5) and with previously published findings.[2]

The 18 patients with a history of LSD ingestion are listed in Table 1. All were white persons with a history of some additional drug use. In only 2 (Cases 6 and 28), however, was a drug (chlorpromazine) being taken at the time of investigation. The usual dose of LSD taken by these subjects was between 300 and 600 μg. In every case, there was an increased frequency of chromosomal damage above the mean control value. The range in the "treated" group was much wider, from 5.3 to 25.1 per cent, with a mean of 13.2 per cent, on the basis of the scoring of 4282 metaphases. This increase is more than three times the control mean and is highly significant. There was no apparent correlation between number of doses, amount per dose or the interval between last dose and time of bleeding for leukocyte culture and the frequency of chromosome breaks. There was also no apparent relation between the type or number of other drugs and the break frequency. Elevated frequencies of chromosome damage were seen regardless of which drugs were ingested in combination with LSD. As in the in vitro studies, the majority of abnormalities were chromatid and isochromatid breaks. However, 12 dicentrics and 5 exchange figures were observed in the LSD group whereas no structural rearrangements were noted in the control group.

TABLE 5. *Distribution of Chromosomal Breaks Induced in Cultured Human Leukocytes by Various Dosages and Exposure Times to LSD-25.**

HR. BEFORE HARVEST	DISTRIBUTION OF BREAKS WITH DOSAGE INDICATED				
	10 μg/ml	*1 μg/ml*	*0.1 μg/ml*	*0.01 μg/ml*	*0.001 μg/ml*
48	50/398 (12.6)	30/390 (7.7)	77/439 (17.5)	55/515 (10.7)	53/425 (12.5)
24	61/530 (11.5)	93/587 (15.8)	73/560 (13.0)	81/527 (15.4)	57/529 (10.8)
4	95/554 (17.1)	62/587 (10.6)	76/582 (13.1)	81/534 (15.2)	65/578 (11.2)
Control	65/1680 = (3.9)				

*Figures in parentheses denote % of cells with breaks.

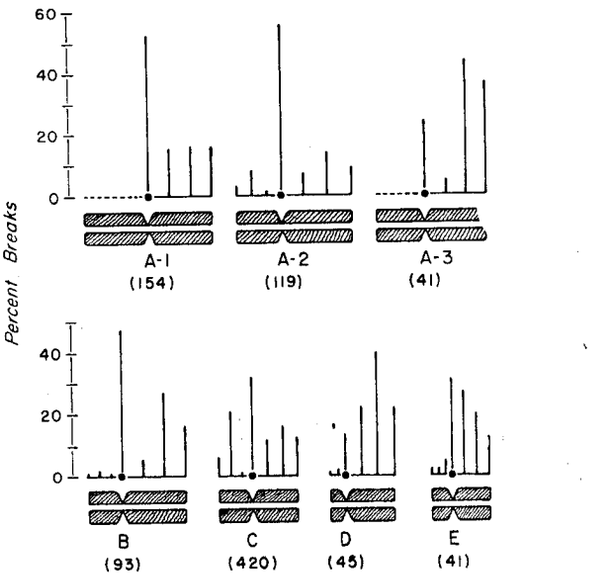

FIGURE 3. *Distribution of Chromosomal Breaks within Given Chromosomes or Chromosomal Groups (Figures in Parentheses Indicate the Number of Breaks).*

We also examined the chromosomes of 4 children born to 3 mothers who had ingested LSD during pregnancy (Table 2). In the 2 children (Cases 1 and 15) of the 2 mothers taking the usual doses of LSD (300 to 600 μg per dose), during the third and fourth months of pregnancy, highly elevated frequencies (13.0 per cent and 19.0 per cent) of breaks were observed, while the 2 children exposed to low maternal doses (50 to 100 μg/dose) late in pregnancy showed only 4.0 per cent and 7.5 per cent breaks. In one of these pregnancies (Case 15), no other drugs were taken, but 13.0 per cent breaks were found two and a half years after birth.

TABLE 6. *Distribution of Chromosome Breaks According to Individually Identifiable Chromosomes or Chromosome Groups.*

	Chromosome Group										Unidentifiable Fragments & Breaks
	A1	A2	A3	B	C	D	E	F	G	TOTALS	
Observed	154	119	41	93	420	45	41	11	6	930	79
Expected	80.82	75.70	63.43	113.0	346.52	93.65	80.26	42.22	34.32	929.9	
χ^2	66.26	24.77	7.93	3.54	15.58	25.27	19.20	23.09	23.37	209.01	

$df = 8$; $p < 0.001$

Discussion

The occurrence of spontaneous chromosomal aberrations, as ascertained through abnormal clinical phenotypes, coupled with the induction of similar cytogenetic anomalies by exogenous agents in vitro, has led to speculation concerning the in vivo cytogenetic effects of such agents commonly available in the environment. That LSD should come under scrutiny at this time is particularly pertinent considering the supposed widespread use of this drug and almost total lack of knowledge concerning its basic biochemical or pharmacologic properties.

It is now apparent that LSD is capable of causing chromosomal damage in human leukocytes in vitro and in the circulating white blood cells of the "user."[2,3] One obvious drawback of in vitro studies is the difficulty of extrapolation to a meaningful in vivo comparison. Our studies (Tables 1 and 5) indicate comparable results in the yields of aberrations whether the cells were exposed to the drug in vivo or in vitro. The fact that the frequency of chromosome breaks produced by in vitro dosages of 0.001 and 0.01 μg per milliliter corresponds closely to the mean frequency observed in the patients lends support to the supposition that these doses correspond to those to which the cells are exposed in vivo. Yields of chromosomal aberrations in vivo and in vitro have been used similarly as a quantitative dosimeter to assess the degree of radiation exposure.[7-10] Therefore, such cytogenetic methods may also have merit in quantitating certain drug effects.

Although LSD produced a clear depression of mitosis in vitro, this effect was not as obvious in vivo. This may be explained by the variability of the interval between exposure to the drug and study of the cells, a factor that is constant and very short in the in vitro experiment. Differences between subjects in lymphocyte growth rate and division in response to phytohemagglutinin is well known. In our study, the results for a given subject showed remarkably good agreement, whether comparison was made among cover slips, culture tubes or repeat cultures. For example, 1 subject (Case 30), a normal male control, showed the following results from 5 cover slips derived from 2 replicate culture tubes: 1, 2, 2, 1, 1 breaks per 50 metaphases each. Successive cultures on another normal male control (Case 19) showed 5/100 and 6/100 breaks, respectively. Similarly in a male subject (Case 36) we found 9/50, 11/45, 9/36, 12/50, 8/50 and 9/33 breaks in 6 cover slips from 2 replicate tubes. Successive cultures on a baby exposed to LSD *in utero* (Case 1) showed 24/100 and 33/200 breaks, respectively. It is apparent that there was close agreement between replicate cover slips, culture tubes and cultures from the same subject grown at different times.

Although iso-chromatid and chromatid aberrations generally originate from damage caused during pre-DNA and post-DNA synthetic periods respectively, we have chosen to combine the 2 classes in our analysis. The reasons for this are as follows: lymphocyte cultures are not synchronous populations of cells; furthermore, cells observed after seventy-two hours of culture are not infrequently in the second or third division, and breaks that would have been isochromatid (chromosome) at the first metaphase will appear in only 1 chromatid in subsequent divisions; and isochromatid breaks may appear as chromatid breaks owing to partial restitution. In fact, it has been shown that 95 per cent of chromosome damage induced by radiation is indeed restituted.[11] Most of the observed increase in cytogenetic abnormalities, both in vivo and in vitro, appeared as chromatid breaks, but chromosomal aberrations also increased in both groups. Dicentrics and exchange figures, however, were seen only in the cells exposed to LSD from both experiments. A high number of small acentric fragments (Fig. 1*c* and *e*), presumably because of near terminal breaks, were seen. Such breaks, occurring in 2 chromosomes of the same cell, may, by healing with each other, be responsible for the origin of the large yield of dicentric chromosomes observed by us and Irwin and Egozcue.[3] In fact, such end-to-end associations are commonly seen early in SV-40-virus-infected cultures, giving rise a high number of dicentrics later in the culture period.[12]

The vast majority of LSD "users" are inveterate experimenters with other drugs (Table 1).[13] For this reason, we have included a group of subjects exposed to such drugs, but not to LSD (Table 3). The drugs include phenothiazines, amphetamines, opiates and antihistamines. The patients receiving chlorpromazine showed a marked increase in chro-

mosome breaks. It is well known that this drug is often used to stop a bad LSD "trip." However, 8 of the 18 patients on LSD were not exposed to chlorpromazine or other phenothiazines and still demonstrated increased abnormalities. It should be noted that the patients not taking LSD, but exhibiting increased chromosome damage associated with chlorpromazine, were exposed to this drug at the time of testing. It cannot be stated, however, whether a synergistic effect can be produced by a combination of the 2 agents. This problem and controlled in vitro and in vivo studies with chlorpromazine are now in progress.

Although the mechanism of chromosome breakage is unknown, experimental data suggest several phenomena capable of damaging chromosomes: free radical formation due to ionizing radiation[11]; incorporation of nucleotide analogues into DNA[14]; cross-linking of the DNA molecule by agents such as mitomycin C[15]; faulty ATP and protein synthesis needed for repair[16,17]; and release of lysosomal DNAase and cathepsins by agents incorporated into lysosomes,[18-20] possibly including certain viruses, carcinogens and other chemicals. Some of these are amenable to investigation with relation to the drug in question.

Chromosome aberrations, similar to those induced by LSD, have been noted to occur spontaneously in 3 autosomal recessive diseases, Bloom's syndrome, Fanconi's anemia and ataxia telangiectasia.[21-28] Patients with these syndromes also show a high propensity to leukemia and other neoplasms. In this context, cells of neoplastic origin almost invariably illustrate a variety of chromosomal aberrations, many of which are not unlike those seen after LSD exposure. Moreover, many agents known to produce similar chromosome aberrations (such as radiation in man and other species and viruses in laboratory animals) are known carcinogens. One of the obvious potential dangers, therefore, of exposure to agents such as LSD is the possible future increase in the incidence of leukemia and other neoplasms in the persons exposed.

In addition, less evident dangers exist. In view of the demonstration of transplacental transport of LSD, as indicated by chromosome damage in the exposed children, the production of congenital defects is a distinct possibility. In fact the teratogenic properties of the drug through the production of congenital anomalies and increased abortions has been observed in pregnant rats and mice injected with LSD.[29,30] Two of the 4 offspring exposed to LSD *in utero* in this study showed high frequency of chromosomal damage (Cases 1 and 15, Table 2). The other 2 (Cases 11 and 12), both from the same mother, were exposed to low doses (50 to 100 μg) *in utero*, late in pregnancy, and exhibited little or no increase in breaks. It is not known, however, what effect exposure to the drug may have at various times during pregnancy. For example, early exposure, may have a more detrimental effect, in terms of congenital anomalies. Case 15, a child two and a half years of age, whose last exposure to the drug had been *in utero*, still demonstrated 13 per cent of cells with chromosome damage, including the observation of a dicentric chromosome. Similar long lived damage is evident in a number of the other patients as well. By analogy, chromosomal damage of long duration has been documented after therapeutic radiation for ankylosing spondylitis,[31] and these studies not only demonstrate the long intermitotic life span (over two years) of the circulating lymphocyte but again emphasize the association of such chromosome damage with the later appearance of an increased incidence of leukemia.

One of the well documented dangers of LSD exposure is prolonged or acute behavioral derangements. Recurrence of psychiatric disorders has been observed at least a year after the last exposure to the drug.[32] There seems to be no direct correlation between behavioral disorders, dosage, frequency of ingestion or time since last dose and the incidence of chromosomal damage in the group studied. Also, no obvious physical or behavioral abnormalities have been noted as yet in the 4 children exposed *in utero*. A larger sample of mother-child pairs has already been assembled for further assessment of *in utero* chromosome damage.

Perhaps the greatest danger of such agents lies in potential chromosome damage to the gametes. Since LSD appears in the circulation, it presumably has free access to germ cells. Another agent, streptonigrin, known to cause chromosomal damage in human leukocytes[33,34] and to have teratogenic effects in rats,[35] has recently demonstrated the ability to inflict damage on the meiotic chromosomes of the ova of the mouse.[36] The existence of chromosome damage in these cells can only be ascertained through direct observation of germinal cells from gonadal biopsies. Chromosome rearrangements produced by breaks and subsequent incorrect healing result in structural anomalies, including balanced reciprocal translocations. Ample evidence already exists that segregation of such translocations results in chromosomal imbalance, leading to fetal wastage and a high percentage of offspring with congenital anomalies and mental retardation. Since the carrier of such balanced translocations is clinically normal, as are many of his offspring, the consequences of the chromosomal imbalance may not appear for several generations. The total damage caused by LSD to the human population, both genetically and psychologically, therefore, may not be assessable for some time to come.

SUMMARY

The effects of LSD on the chromosomes of leukocytes were investigated both in vitro and in vivo. A marked depression of mitosis was demonstrated upon addition of the drug to leukocyte cultures of

normal persons. Elevated frequencies of chromosomal aberrations (particularly dicentrics) were evident in cultures treated in vitro, derived from "users" of the drug and from children exposed *in utero*.

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