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Cytogenetic Effects of Psychoactive Drugs

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Introduction

The consumption of pharmacological agents for pleasure and therapeutic purposes dates far back in history. Initially, these agents were limited to naturally occurring compounds. Today, chemically synthesized psychoactive agents have added greatly to the abundance and availability of drugs for the modification of human behavior.

Within our generation, pharmacotherapy has revolutionized the treatment of major mental disorders (psychoses). Pharmacological agents are also used by nonpsychotic persons to relieve tension, anxiety, and depression, and merely to enhance 'normal' experiences.

Possible deleterious effects of these agents on the hereditary material of man are of serious concern. Reports suggesting damage to chromosomes (breaks and/or other abnormalities) as a result of drug ingestion have been sufficiently disturbing to warrant testing for possible mutagenic, teratogenic, and/or carcinogenic effects. According to *Moorhead et al.* (1971), an elevated frequency of chromosomal aberrations may be considered a reliable indicator of the presence of genetic changes.

It has become increasingly clear that gross chromosomal aberrations are associated with various forms of malignant neoplasia. For example, patients who underwent arteriography with thorium dioxide, a radioactive substance containing isotopes of the thorium-232 decay series, show a high frequency of chromo-

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some aberrations (*Fischer et al.*, 1967), and long-term follow-up studies have demonstrated a high frequency of carcinoma in these same individuals. An entirely different example is provided by persons afflicted with Down's syndrome (mongolism) who, since the advent of antibiotics, are able to survive intercurrent infections in early childhood and face a high risk of leukemia (*Miller*, 1970). It is believed that the extra chromosomal material in these patients (chromosome 21) may be responsible for the tendency to malignant neoplasia. Subjects with Bloom's syndrome and Fanconi's anemia constitute other groups where an elevated frequency of chromosome aberrations is associated with a high incidence of acute leukemia (*German*, 1969; *Swift and Hirschhorn*, 1966; *Hirschhorn and Bloch-Shtacher*, 1970; *Swift*, 1971). There is also the aberration known as the Philadelphia chromosome which is considered specific for chronic myelogenous leukemia, for it is observed only in this condition (*de Grouchy*, 1967; *Knudson et al.*, 1973). Whether chromosome abnormalities are the cause or the result of malignant tranformations, or both are caused by a common factor, is important, but not as important as the fact that an altered genome can provide cells with unlimited growth potential, and thus allow for the progression of tumors.

Presently, cytogenetic analysis constitutes the best available procedure for the *in vivo* testing of effects of drugs or other environmental agents on the genetic material of man. Human cytogenetics is a relatively new field. It was only in 1956 that the full chromosomal complement of man was determined (*Tjio and Levan*, 1956). It was even later that *Moorhead et al.* (1960) introduced human peripheral leukocyte cultures which, with modifications, constitute the technique most frequently used throughout the world for both *in vivo* and *in vitro* testing of drug effects on the human genome.

Two other cell types, bone marrow cells and fibroblasts, are also used in assessing the effects of drugs. Direct examination of bone marrow reflects *in vivo* effects, while fibroblast cultures permit long-term *in vitro* testing. However, in both cases, the procurement of cells for analysis requires special procedures (bone marrow aspiration or skin biopsy) in contrast to blood samples for leukocyte cultures which can be readily obtained from subjects, and repeat determinations easily made with little risk and discomfort to the individual. Therefore, leukocyte cultures have generally been preferred.

Although the human peripheral leukocyte culture technique is relatively simple, leukocytes are somatic cells and do not provide information on gonadal cells which are of primary genetic importance. Yet, *any* evidence of genetic damage may be indicative of similar damage in gonadal cells, and a drug causing such damage may, therefore, be considered potentially deleterious to future generations as well as a health risk to the individual himself. The inability of cytogenetic analysis to detect point mutations that may be induced by various compounds suggests an underestimate rather than an overestimate of the total mutagenic effect. In this regard, *Nichols* (1973) believes that chromosomal

damage is a good indicator of mutagenesis, stating that there is a 'high correlation between the ability of an agent to produce gene mutations and its ability to produce chromosome breakage'.

Inherent in cytogenetic testing is the ability to detect, under the microscope, visible morphological changes in the metaphase chromosomes. The recent discovery of techniques which make possible the identification of each chromosome by its characteristic banding pattern has brought cytogenetic analysis to new heights. (For a review of various techniques, see *Miller et al.*, 1973.) With these techniques, chromosome rearrangements, as a result of chromosome breaks caused by drug ingestion, are easily discernible, if present.

It is uncertain what importance should be attached to increased chromosome breaks detected in leukocyte cultures. Cells with breaks may be eliminated, or breaks may be repaired by simple rejoining. In either case, no detrimental effect is necessarily expected. It is only if cells with damaged chromosomes are sequestered and later come to expression as malignant neoplasia, or if damaged gonadal cells, upon fertilization, give rise to offspring with congenital anomalies that chromosome breaks become important. Therefore, cytogenetic monitoring is recommended so that potentially deleterious effects of drugs may be detected early.

With the above relationships in mind, a survey of the literature was undertaken and the following questions were considered: (1) What is available in the literature on the cytogenetic effects of psychoactive drugs? (2) Which drugs, if any, are capable of causing chromosome damage *in vivo* and/or *in vitro?* (3) For which drugs examined for mutagenicity is there evidence for or against teratogenicity?

It was noted, as in a previous extensive survey dating to 1970 (*Moorhead et al.*, 1971), that much of the information available was only assertion of fact without accompanying data, mere statement of opinion, editorial commenting, or interpretation of other investigators' findings, and expansion of earlier publications by the same investigators. Therefore, care was taken in determining the number of studies and subjects actually contributing to the data on cytogenetic effects of drugs lest they be overestimated by being counted more than once.

The following review of the cytogenetic literature on the more commonly used or abused psychoactive agents includes hallucinogens (LSD and marijuana); opiates (heroin, morphine, and methadone); antipsychotic drugs (phenothiazines: chlorpromazine, fluphenazine, perphenazine, and thioridazine; and lithium); antianxiety drugs (meprobamate, chlordiazepoxide, and diazepam); antidepressants (imipramine), and anticonvulsants.

It is hoped that by gathering the relevant literature, an accurate impression will be gained of the present knowledge, and the gaps in knowledge, about the effects of these drugs on the hereditary material of man, and the potential danger to present 'users' and to future generations.

Table I. Comparison of chromosome analyses in LSD 'users' and controls

Author	Year	LSD 'users'				
		subjects	age range years	cells analyzed	mean % breaks	range
Cohen et al.	1967a	18	19–52	4,282	13.2	5.3–25.1
Loughman et al.	1967	8	–	697	0.0	0
Abbo et al.	1968	2	19–20	60	10.0	6.7–13.3
Cohen et al.	1968	14	17–32	1,833	7.0	1.0–14
Egozcue et al.	1968	46[2]	17–43	9,140	18.8	8–45
Hulten et al.	1968	2	23–24	200	3.0	1.0–5.0
Jarvik et al.	1968	1	19	101	1.0	–
Sparkes et al.	1968	4	19–24	937	2.2	0.9–3.4
Judd et al.	1969	9	21–52	315	0.3	0–2.9
		8[4]	26–44	280	1.8	0–11.4
Nielsen et al.	1969	10	19.8 ± 3.0	635	2.5	–
Valenti	1969	10	15–25	894	2.0	0–5.8
Dorrance et al.	1970	14	17–27	1,284	0.8	0–2.0
Hsu et al.	1970	2	22–26	66	3.0	2.8–3.3
Stenchever and Jarvis	1970	12	18–31	2,525	1.3[5]	0–3.8
Dishotsky et al.	1971	14	–	1,412	0.4	–
Gilmour et al.	1971	8	–	400	1.2[5]	–

1 Two subjects with viral infections eliminated (14 and 31 % breaks).
2 Includes subjects reported by *Irwin and Egozcue* (1967).
3 Less two subjects with possible other drug use (18.5 and 24 % breaks).
4 Former users.
5 Percent cells with aberrations.

LSD

By far the most intensively studied agents are the hallucinogens, in particular LSD. The initial report on LSD was made by *Cohen et al.* (1967b) and showed that LSD was capable of causing extensive chromosome damage when added *in vitro*, and also *in vivo* as judged by the findings in one male schizophrenic following its use in treatment. This alarmed the public and medical community who had only recently weathered the thalidomide incident, and led to an intensive investigation of the effects of LSD on chromosomes. Yet, after 7 years of research and numerous publications there still remains the controversy as to the potential of LSD to cause chromosome damage. In an attempt to examine this controversy anew, the data in the various reports have been arranged into four groups: (1) LSD 'users' (i.e. users of illicit or street-obtained LSD with its attendant impurities and imprecise dosage); (2) medically treated

Table I (continued)

Author	Year	Controls				
		subjects	age range years	cells analyzed	mean % breaks	range
Cohen et al.	1967a	12[1]	22–55	2,674	3.8	2.0–5.5
Loughman et al.	1967	19	–	673	0.4	–
Abbo et al.	1968	32	24–59	1,022	3.5	0–?
Cohen et al.	1968	9	20–35	1,203	1.2	0–2.0
Egozcue et al.	1968	14	–	2,800	9.0	6.0–16.5
Hulten et al.	1968	5	0.5–38	250	2.4	0–6.0
Jarvik et al.	1968	11[3]	22–61	720	4.7	0–9.0
Sparkes et al.	1968	4	21–50	950	1.6	0.8–2.6
Judd et al.	1969	8	17–48	280	0.7	0–5.7
Nielsen et al.	1969	30	31.4 ± 9	1,030	0.2	–
Dorrance et al.	1970	10	18–44	1,018	0.8	0–2.0
Stenchever and Jarvis	1970	8	17–45	1,620	1.1	0–3.0
Dishotsky et al.	1971	8	–	805	0.6	–
Gilmour et al.	1971	16	–	771	0.5[5]	–

LSD subjects; (3) cytogenetic findings in infants exposed *in utero* to LSD (illicit and pure), and (4) *in vitro* studies. Finally, animal studies will be briefly reviewed.

Human Studies

LSD users. There are at least 16 studies of LSD users published in the literature. However, the lack of an adequate number of subjects in many of the studies (i.e. reports on only one or two subjects), makes it impossible to assign an equal weight to all studies for comparative purposes. Therefore, we have restricted our review to the ten studies reporting eight or more subjects, but for completeness have listed all studies in table I. Although *Valenti* (1969) reported on ten subjects, a control group was not available for comparison. Of the ten studies with a sample size of eight or more, five showed an increased frequency of breaks or aberrant cells (*Cohen et al.*, 1967a, 1968; *Egozcue et al.*, 1968;

Table II. Comparison of chromosome analyses in subjects medically treated with LSD and controls

Author	Year	Treated with LSD				
		subjects	age range years	cells analyzed	mean % breaks	range
Cohen et al.	1967b	1[1]	51	200	12.0	–
Abbo et al.	1968	5	24–57	266	8.3	3.3–13.3
Hungerford et al.[2]	1968	4[3,5]	–	1,375	3.9	1.0–7.0
		4[4,5]	–	350	1.1	0–6.0
Nielsen et al.	1968	5	29–48	358	1.7	0–6.9
Sparkes et al.	1968	4	28–45	914	1.5	0.4–2.5
Nielsen et al.	1969	9	36.1 ± 7.2	603	4.3	–
Siva Sankar et al.[7]	1969	15	7–15	1,500	0.8	0–2.0
		7[8]	7–15	700	1.0	0–3.0
Tjio et al.[9]	1969	11[10]	21–50	2,347	9.3	2.0–43.9
		21[11]	20–56	4,387	4.2	1.5–14.2
		5	23–35	–	3.2	1.0–5.7
		8	–	1,646	2.8	2.0–3.8
Corey et al.	1970	10[12]	–	1,000	4.9	2.0–8.0
		5	–	500	7.8	6.0–12.0
		6[13]	–	594	6.4	2.0–11.0
Warren et al.	1970	2	36–37	–	NS1[14]	–
Dishotsky et al.	1971	5	–	500	0.4	–
Fernandez et al.	1973	32	18–48	4,099	0.6	0–3.2
Jarvik et al.	1974	2[4]	24–45	800	1.4	0–3.0
		6[3]	24–45	1,650	2.2	0–4.0

1 Also used other drugs.
2 Intravenous administration.
3 During LSD bloods combined.
4 Combined follow-up bloods.
5 Chlorpromazine, 100 mg tablet prior to LSD.
6 Additional controls.
7 Includes *Bender and Siva Sankar* (1968).

Nielsen et al., 1969; *Gilmour et al.*, 1971), while five did not (*Loughman et al.*, 1967; *Judd et al.*, 1969; *Dorrance et al.*, 1970; *Stenchever and Jarvis*, 1970; *Dishotsky et al.*, 1971). In all of the above reports, no relationship was seen between chromosome damage and dosage, number of doses, duration of use, total amount used, and the interval from the last dose to chromosome analysis. The necessity to rely on the subjects' ability to recall their use patterns makes this information suspect, and is estimated rather than hard data. Furthermore, it is difficult to assess the true dosage in illicitly obtained LSD. Indeed, *Krippner*

Table II (continued)

Author	Year	Controls				
		subjects	age range years	cells analyzed	mean % breaks	range
Cohen et al.	1967b	–	–	–	3.7	–
Abbo et al.	1968	32	24–59	1,022	3.5	0–?
Hungerford et al.[2]	1968	3	–	600	1.0	1.0–7.0
		3[6]	–	900	1.2	0–3
Nielsen et al.	1968	40	17–59	1,312	0.4	–
Sparkes et al.	1968	4	21–50	950	1.6	0.8–2.6
Nielsen et al.	1969	30	31.4 ± 9	1,030	0.2	–
Siva Sankar et al.[7]	1969	25	7–15	2,500	0.7	–
		7	20–43	700	0.6	–
Tjio et al.[9]	1969	11	21–50	2,294	4.1	2.0–9.5
		21	20–56	4,404	4.4	1.5–19.4
		5	–	–	2.8	1.5–3.8
		2[8]	30–38	–	2.6	1.5–4.8
Corey et al.	1970	10	–	1,000	5.7	1.0–9.0
		13	–	1,300	7.0	2.0–17.0
		–	–	–	–	–
Warren et al.	1970	–	–	–	–	–
Dishotsky et al.	1971	8	–	805	0.6	–
Fernandez et al.	1973	32	age matched ± 5 years	4,271	0.4	0–1.6
Jarvik et al.	1974	2	24–45	200	2.5	2.0–3.0

8 UML (4.54 mg/day) also administered together with LSD. One patient with 9 % breaks not included.
9 Data are percent abnormal cells and not percent breaks.
10 Low dose (50 μg).
11 High dose (250–450 μg).
12 Pre- and post-bloods – subject serves as own control.
13 Also received mescaline.
14 No significant increase over controls.

(1970), on analyzing 12 tablets obtained from various sources and reportedly sold as containing 250 μg LSD, found one tablet without any LSD, and only two with more than 150 μg. Most contained less than 26 μg, and many had more STP (DOM) than LSD. Since it is most likely that many impurities are contained in illicitly obtained tablets, and since there have been frequent reports of the use of other drugs along with LSD, one concludes that drug users in general, and not specifically LSD users, show an elevated rate of chromosome breaks. In addition, personal hygiene, diet, general living conditions and exposure to infection differ

between controls and users, and may be directly responsible for increased chromosome damage found in LSD users. *Cohen et al.* (1967a) for example, eliminated two subjects with viral infections from their control population who showed 14 and 31 % breaks. The frequency of structural abnormalities roughly paralleled that of breaks, and structural abnormalities were seen in controls as well as drug users.

In the only study of human meiotic chromosomes, *Hulten et al.* (1968) could not demonstrate an elevated frequency of chromosomal abnormalities in the testicular material of one man.

Medically administered LSD. There are 13 reports of chromosome analyses on individuals administered LSD under medical supervision (table II). These studies are of two types. One type compares LSD-treated individuals to a separate control group. In the second type, subjects are studied before, during, and after treatment, thus serving as their own control. For purposes of comparison, we have focused our attention on those studies reporting five or more subjects. Six studies are of the first type, and three detected a statistically significant increase in chromosome breaks (*Abbo et al.*, 1968; *Nielsen et al.*, 1968, 1969). However, these are based on only 19 subjects, 14 of them from one laboratory. *Nielsen* also reported elevated frequencies of breaks in illicit users of LSD.

There are four reports of the second type which approach a scientifically sound experimental design to assess the chromosome damaging potential of LSD (*Hungerford et al.*, 1968; *Tjio et al.*, 1969; *Corey et al.*, 1970; *Jarvik et al.*, 1974). Pure LSD of known dosages was administered to subjects with chromosome examinations at specified intervals prior to, during, and following treatment. *Hungerford et al.* (1968) reported on three subjects for whom blood samples for chromosome analyses were taken 1 h before, during the course of treatment (three treatments at 1–2 week intervals), and 1–6 months following the last LSD administration. For a fourth subject, no predrug culture was available for analysis. In this study, LSD was administered intravenously at doses of 187–200 μg/treatment. Three control subjects matched for age and sex were also studied with three chromosome examinations for each at 1 week intervals. The mean percent breaks in these controls was 1.2 % (range of 0–3 %).

In LSD-treated individuals, the pretreatment mean was 1.0 %, during LSD treatment 3.9 %, and 1.1 % in follow-up examinations. Hence, the data suggest a transient increase in breaks with a return to predrug levels 1–6 months following termination of treatment. A number of structural abnormalities were recorded and these were seen only after LSD treatment had started, none having been observed in pretreatment cultures, or in control subjects.

In the second study, *Tjio et al.* (1969) reported on 32 subjects given a single dose of LSD orally (11 given 50 μg and 21 given 250–450 μg). 200 cells were analyzed for each culture. No dose response relationship was seen. Of the eleven

low dose subjects, pre- and postdrug values (mean % aberrant cells) were 4.1 and 9.3 % while in the high dose group the values were 4.4 and 4.2 %, respectively. The combined frequency of aberrant cells for both groups was 4.3 % pre-LSD and 5.9 % post-LSD. Although there was a slight increase in aberrant cells, this was not statistically significant. 31 of the 32 subjects were also exposed to other drugs during their hospitalization. Seven of them were on drugs at the time pre-LSD blood was drawn, but none showed an unusually high frequency of aberrations. Four had documented viral infections and only one had an extremely high aberration rate (43.9 %). An additional five LSD users, who were given pure LSD in known dosages as part of an NIH study, also failed to show a statistically significant increase in breaks (2.8 vs. 3.2 %, pre- and postdrug cultures). In addition, eight individuals studied after LSD use showed no significant increase in comparison to controls. Structural abnormalities were recorded in both pre- and post-LSD cultures. Data were also presented on two normal drug-free subjects which show the day-to-day variations in the frequency of chromosomal abnormalities in the same individual. In one subject with nine successive determinations the range was from 1.48 to 3.38 %, and in the second subject with eight cultures, from 1.96 to 4.76 %. In support of the findings by *Tjio et al.* (1969), *Corey et al.* (1970) reported no increase in breaks in ten subjects on whom blood samples were drawn immediately before and 24 h after a single LSD administration. The frequency of structural abnormalities was significantly reduced in post-LSD cultures and they were only found in those individuals who manifested them initially.

In the final report (*Jarvik et al.*, 1974), two subjects for whom pre- and post-LSD values were available showed, if anything, a reduction in mean break frequency after receiving LSD, dextroamphetamine or placebo. Six additional subjects with no pre-LSD values also failed to show an increase in breaks during LSD treatment.

Therefore, in all prospective studies carried out (4), no definitive evidence has been presented that LSD damages lymphocyte chromosomes *in vivo*. *Hungerford et al.* (1968) did report a transient increase but in follow-up cultures all returned to pre-LSD values.

In utero exposure. *Idanpaan-Heikkila and Schoolar* (1969), using radioactive LSD, presented evidence that LSD crosses the placenta in mice, thereby exposing the fetus. Nine reports are available on chromosome analyses on infants exposed to LSD *in utero* (table III). Doses *in utero* ranged from none (*Cohen et al.*, 1968, reports on four infants whose mothers took LSD only prior to conception) to 30 or more (*Aase et al.*, 1970). Of the 73 infants studied, one had a limb anomaly and one presented an aberrant karyotype (*Hsu et al.*, 1970). Again, only *Cohen et al.* (1968) and *Egozcue et al.* (1968) reported an elevated rate of chromosome damage. It is difficult to assess the action of LSD since most

Table III. Chromosome examinations in infants exposed to LSD *in utero*

Author	Year	Subjects	Age range	Cells analyzed	Mean % breaks	Range	*In utero* doses
Abbo et al.	1968	1[1]	3 days	60	3.3	–	twice during 1st trimester
Cohen et al.[2]	1968	9	0.17–5.5 years	1,625	8.6	0–19	1–30
		4	0.5–4.0 years	568	4.0	2.0–8.7	LSD prior to conception only
		6	0.3–2.0 years	800	1.2	0–2.4	controls
Egozcue et al.	1968	4	1–18 months	800	21.5	9.5–28	1–6
Hulten et al.	1968	1	5 weeks	100	13	–	
			8 months	100	4	–	1,600 mg total exposure *in utero*
Aase et al.	1970	10	within 48 h of birth	500	no evidence of damage	–	1–30?
Hsu et al.	1970	1	–	40[3]	0	–	LSD prior to conception only
Stenchever and Jarvis	1970	1	1.5 years	28	0	–	twice only during 2nd trimester
Warren et al.	1970	1	8 months	–	0	–	exposure during 1st 4 months
Dumars	1971	41	birth to 3 months	2,050	1.5	–	?
		20	–	1,000	1.0	–	controls

1 Born with limb anomaly.
2 Includes 4 subjects from *Cohen et al.* (1967a).
3 D-trisomy with congenital abnormalities.

mothers also took other drugs during their pregnancies, marijuana being one of the most prominent.

The potential teratogenic effect of LSD is far from clear. A survey of the literature led to reports on 244 children born to LSD users (*Aase et al.*, 1970; *Abbo et al.*, 1968; Anonymous, 1967; *Assemany et al.*, 1970; *Carakushansky et al.*, 1969; *Cohen et al.*, 1967a, 1968; *Dumars*, 1971; *Eller and Morton*, 1970; *Gelhrter*, 1970; *Hecht et al.*, 1968; *Hsu et al.*, 1970; *Hulten et al.*, 1968; *Jacobsen and Berlin*, 1972; *McGlothlin et al.*, 1970; *Sato and Pergament*, 1968; *Warren et al.*, 1970; *Zellweger et al.*, 1967). Of these, 213 were normal, seven were identified as cases of familial defects, and 24 (9.8 %) manifested congenital abnormalities. Six of the 24 were cases of limb deficiencies and further monitoring is warranted. However, most parents also used marijuana extensively making it difficult to indict LSD specifically. Extra care must be exercised in evaluating the literature since an inherent sample bias is present (i.e., malformed fetuses born to LSD users are reported more frequently than are normal infants born to LSD users, and more frequently than malformed infants born to normal drug-free individuals).

In vitro. There are eight reports of chromosome analyses following *in vitro* addition of LSD to lymphocyte cultures (table IV). Five reported an increase in breaks while three did not. All of the latter include only one or two subjects. Individual differences in susceptibility to chromosome breaks may constitute an important variable so far not sufficiently explored. In none of the positive studies was a dose response curve generated. The lowest dose tested was capable of producing the maximum damage possibly because low enough doses have not been tested. With one exception, the lowest dose tested, 0.001 μg/ml, was ten times higher than is calculated to be present in blood following ingestion of 100 μg LSD by a 70-kg man, and in that one exception, *Kato and Jarvik* (1969) did not find an increase in the frequency of breaks at a concentration of 0.0001 μg/ml. In general, it does appear that *in vitro* addition of LSD results in chromosome damage. However, care must be exercised in attempting to extrapolate *in vitro* results to *in vivo* findings. Drugs added to cells in culture are not subject to the body's metabolism and are readily available to the cells. Hence, they may act in a different manner than *in vivo*. Agents which exert a harmful effect *in vitro* may be detoxified and rendered harmless when consumed by the intact organism. And there is no reason to believe that the opposite situation may not also occur.

Animal Studies

The effects of LSD on the chromosomes of mice, rats, kangaroo rats, monkeys, and *Drosophila* have been reported.

Cohen and Mukherjee (1968), *Skakkebaek et al.* (1968) and *Skakkebaek*

Table IV. Chromosome analyses following *in vitro* addition of LSD. Survey of eight studies

Author	Year	No LSD added				
		subjects	age range years	cells analyzed	mean % breaks	range
Cohen et al.[1]	1967a	6	–	1,680	3.9	–
Jarvik et al.	1968	8	22–61	–	5.2	0–9.0
Mackenzie and Stone	1968	5	–	187	4.3[3]	–
Genest	1969	1	–	26	15	–
Kato and Jarvik	1969	2	–	100	5.0	–
Sturelid and Kihlman	1969	2	–	329	0.0	0
Corey et al.	1970	10	–	1,000	4.7	0–15.1
Jarvik et al.	1974	8	25–45	1,550	2.0	0.8–3.0

1 Includes *Cohen et al.* (1967b).
2 Two subjects showed no response.
3 Percent damaged cells.

and Beatty (1970) examined meiotic chromosomes in mice following LSD administration and detected a marked increase in abnormalities. *Cohen and Mukherjee* (1968) also studied bone marrow cells of LSD-treated mice and found a significant increase in structural aberrations, and a more than three-fold increase in breaks as compared to controls. However, *Egozcue and Irwin* (1969) and *Jagiello and Polani* (1969) could not replicate these observations, both reporting negative results.

All three studies available for rats had negative results. *Goetz et al.* (1972) studied meiotic chromosomes in male rats and found no increase in breaks with LSD. LSD administered orally (*Sato et al.*, 1971) and intraperitoneally (*Emerit et al.*, 1972) to pregnant rats produced no chromosome damage either in the mothers' bone marrow cells or in their embryos.

Bick (1970) found increased chromosome damage in leukocyte cultures of kangaroo rats following *in vitro* addition of LSD.

In monkeys *(Macaca mulatta)*, the chromosomal effects of LSD were assessed *in vitro* and *in vivo*. *Egozcue and Irwin* (1969) reported a statistically significant increase in chromosome damage following *in vitro* treatment of leukocyte cultures. However, examinations of meiotic chromosomes prior to and following acute or chronic treatment showed no significant increase. By contrast, blood cultures of monkeys treated *in vivo* showed a significant increase in breaks including structural abnormalities.

Table IV (continued)

LSD added					LSD information	
subjects	age range years	cells analyzed	mean % breaks	range	dosages μg/ml	duration of exposure, h
6	–	7,735	13.0	7.7–17.5	10, 1, 0.1, 0.01, 0.001	4, 24, 48
8[a]	22–61	498	10.2	0.0–15.0	0.01	4
5	–	453	7.7[b]	7.2–8.3	0.1, 0.01	48
1	–	64	12.5	7.1–20.8	10, 1.0, 0.1	24
2	–	600	5.0	2.0–12.0	1, 0.01, 0.0001	4, 24
2	–	372	0.3	0–0.5	10	24, 48
10	–	1,000	9.4	4.0–18.7	1	24
8	25–45	4,320	3.4	1.7–6.0	0.1, 0.01, 0.001	4

Kato et al. (1970) examined leukocyte chromosomes of four pregnant Rhesus macaques following LSD administration. Two additional monkeys served as controls. The two monkeys receiving the highest doses of LSD showed a transient increase in breaks paralleling the results of *Hungerford et al.* (1968) in humans. Monkeys receiving low doses did not exhibit chromosome damage. Further, no structural abnormalities were found in postdrug cultures.

The two control monkeys gave birth to normal offspring. However, of the four LSD-treated monkeys, two pregnancies ended in stillbirths and one offspring died of pneumonia 1 month after birth. The only survivor of an LSD-treated mother showed no increase in chromosome damage. The potential meaning of this high incidence of mortality in offspring of LSD-treated mothers is uncertain since these animals were en route from Asia during their pregnancy; further investigation is warranted.

Three studies have been reported on the potential mutagenic and chromosome damaging effects of LSD in *Drosophila. Grace et al.* (1968) reported no increase in mutations and/or translocations as a result of intra-abdominal injection of LSD into *Drosophila* males. *Tobin and Tobin* (1969) likewise found no increase in lethals in the progeny of males who were injected during the meiotic and postmeiotic stages of spermatogenesis, or from larval feeding experiments. Finally, *Browning* (1968) reported a marked induction of recessive lethals following intraperitoneal injection of extremely high dosages of LSD.

Table V. Reports of chromosome analyses in persons exposed to psychiatric drugs and controls

Author	Year	Users				
		subjects	age range years	cells analyzed	mean % breaks	range
Marijuana						
Dorrance et al.	1970	9	19–27	816	0.9	0–2.7
Gilmour et al.	1971	13[1]	–	650	0.8[3]	–
		11[2]	–	550	2.2[3]	–
Rubin et al.	1973	30[4]	–	–	2.4[5]	–
Stenchever et al.	1974	49[4]	17–34	4,900	3.4[3]	0–8.0
Opiates						
Gilmour et al.	1971	11	–	650	2.2[3]	–
Falek et al.	1972	16[6]	21–58	861	0.2	–
		16[7]	21–58	1,173	2.6	–
Falek and McFadden	1973	13	21–29	1,456	4.5[8]	0–10.7
Abrams and Liao	1974	16[10]	–	1,600	11.4	–
Gendel	1974	51	–	–	< 1	–
		89[10]	no increase over controls			
Chlorpromazine						
Cohen et al.	1967a	3	9–30	700	10.6	4.0–17.4
Cohen et al.	1969	6	26–54	533	2.8	1.3–4.0
Nielsen et al.	1969	13[6]	–	794	1.9	–
Jenkins	1970	3[11]	17–28	278	27.7	16.9–35.4
Gilmour et al.	1971	11	–	600	2.0[3]	–
Cohen et al.	1972	10	23–63	–	1.1[8]	1.0–1.2
Perphenazine						
Nielsen et al.	1969	15[12]	–	1,047	3.8	–
Cohen et al.	1972	9	23–63	–	1.3[8]	1.2–1.4
Lithium						
Friedrich and Nielsen	1969	3	45–70	215	5.1	1.6–8.6
Jarvik et al.	1971	16	30–64	1,433	3.3	0–12.0

1 Light users.
2 Heavy users ± LSD.
3 Percent cells with breaks.
4 Light and heavy users.
5 Percent cells with breaks and gaps.
6 48-hour cultures.
7 72-hour cultures.

Table V (continued)

Author	Year	Controls				
		subjects	age range years	cells analyzed	mean % breaks	range
Marijuana						
Dorrance et al.	1970	10	18–44	1,018	0.8	0–2.0
Gilmour et al.	1971	16	–	771	0.5[3]	–
Rubin et al.	1973	30	–	–	2.9[8]	–
Stenchever et al.	1974	20	13–52	2,000	1.2[3]	0–5.0
Opiates						
Gilmour et al.	1971	16	–	771	0.5[3]	
Falek et al.	1972	16	21–40	728	0.1	–
		16	21–40	771	0.4	–
Falek and McFadden	1973	13	21–29	728	3.2[9]	0–8.9
Abrams and Liao	1974	14	–	1,400	1.4	–
Gendel	1974	40	–	–	< 1	–
		48	–	–	–	–
Chlorpromazine						
Cohen et al.	1967a	12	22–59	2,674	3.8	2.0–5.5
Cohen et al.	1969	6	21–61	579	2.9	1.3–4.0
Nielsen et al.	1969	41	–	1,584	0.3	–
Jenkins	1970	4	20–30	308	10.1	8.2–14.1
Gilmour et al.	1971	16	–	771	0.5[3]	–
Cohen et al.	1972	10	23–63	–	1.5[9]	1.5–1.6
		6	–	–	1.3	0.5–2.0
Perphenazine						
Nielsen et al.	1969	41	–	1,584	0.3	–
Cohen et al.	1972	9	23–63	–	1.8[9]	1.5–2.1
		6	–	–	1.3	0.5–2.0
Lithium						
Friedrich and Nielsen	1969	11	67 ± 8.8	554	0.5	–
Jarvik et al.	1971	10[13]	33–73	850	1.5	0–6.0
		4[14]	34–73	320	0.9	0–2.0

8 Average of all postdrug values.
9 Average of washout and baseline values.
10 *In utero* exposure to heroin.
11 Also on stelazine.
12 Also on orphenadrine.
13 Drug-free controls.
14 Patients on placebo.

Table V (continued)

Author	Year	Users				
		subjects	age range years	cells analyzed	mean % breaks	range
Chlordiazepoxide						
Cohen et al.	1969	4	46–57	303	3.0	0–9.1
		6	–	1,720	3.0	1.0–7.0
Diazepam						
Cohen et al.	1969	6	12–24	555	2.9	1.0–5.1
Fluphenazine						
Cohen et al.	1969	3	31–44	296	4.4	3.0–7.0
Thioridazine						
Cohen et al.	1969	3	29–52	297	3.0	1.0–4.1

1 Light users.
2 Heavy users ± LSD.
3 Percent cells with breaks.
4 Light and heavy users.
5 Percent cells with breaks and gaps.
6 48-hour cultures.
7 72-hour cultures.

Marijuana

Human Studies

In vivo. There are four reports on chromosome examinations in marijuana users (table V). *Gilmour et al.* (1971) found a higher frequency of chromosome aberrations in eleven heavy users of marijuana, some of whom had also taken LSD, compared to 16 nonusers. However, no increase was seen in a group of 13 light users. *Stenchever et al.* (1974) confirmed an increase in chromosome breakage in 49 users in comparison to 20 controls. The remaining two studies, one by *Dorrance et al.* (1970) and a preliminary report on a clinical study conducted in Jamaica (*Rubin et al.*, 1973), gave negative results. In the Jamaican study, none of the subjects used any other drug.

On the basis of our own prospective study with chromosome examinations prior to, at weekly intervals during, and at 1 month after controlled marijuana use, we are unable to make a definitive statement concerning the chromosome-damaging potential of marijuana (*Matsuyama et al.*, in preparation). In this study, male subjects smoked marijuana under close supervision while confined to

Table V (continued)

Author	Year	Controls				
		subjects	age range years	cells analyzed	mean % breaks	range
Chlordiazepoxide						
Cohen et al.	1969	4	42–63	302	3.6	1.4–6.0
		6	–	1,798	3.4	0.0–7.0
Diazepam						
Cohen et al.	1969	6	14–19	473	2.5	1.4–4.7
Fluphenazine						
Cohen et al.	1969	6	21–61	579	2.9	1.3–4.0
Thioridazine						
Cohen et al.	1969	6	21–61	579	2.9	1.3–4.0

8 Average of all postdrug values.
9 Average of washout and baseline values.
10 *In utero* exposure to heroin.
11 Also on stelazine.
12 Also on orphenadrine.
13 Drug-free controls.
14 Patients on placebo.

a psychiatric ward for the duration of testing. Recently, *Nahas et al.* (1974) reported that 51 chronic marijuana smokers showed a significantly reduced responsiveness to phytohemagglutinin and leukocytes as compared to 80 'normal' individuals, and concluded that marijuana impairs cell-mediated immune reactions. Further studies with matched controls are required to confirm this finding. The relevance of immune changes to cytogenetic analysis rests in the fact that the cells showing reduced responsiveness in the above experiments are also the cells analyzed in the usual chromosome studies.

In vitro. Tetrahydrocannabinol, presumably the most relevant component of marijuana, has been studied *in vitro* (table VI). *Neu et al.* (1970) reported that Δ^8-THC, added to leukocyte cultures during the final 24 h of culture decreased the mitotic index with increasing concentrations but did not increase the number of breaks above that found in control cultures. No structural rearrangements were seen. Preliminary studies with Δ^9-THC yielded similar results. *Stenchever and Allen* (1972) also reported negative results with Δ^9-THC. By contrast, *Leuchtenberger et al.* (1973) reported that human lung explants ex-

Table VI. Reports of chromosome analyses on *in vitro* addition of drugs

Author	Year	No drug added			Drug added				
		cells analyzed	mean % breaks	range	cells analyzed	mean % breaks	range	dosage	duration, h
Marijuana									
Neu et al.[1]	1970	–	< 5	–	–	< 5	–	30, 35, 40, 45 μg/ml	24
Stenchever and Allen	1972	903	2.7	1.5–5.5	2,738	1.7	0–3.9	100, 10, 1, 0.1 μg/ml	72
Methadone									
Falek et al.	1972	no significant difference from treated values			450[2]	1.8	1.3–2.7	3 ×, 1 ×, 1/3 ×, 1/6 ×, 1/12 × 0.013 μg/ml	4, 24, 48
Morphine									
Falek et al.	1972	no breaks at all concentrations at all time intervals						3 ×, 1 ×, 1/3 ×, 1/6 ×, 1/12 × 0.002 μg/ml	4, 24, 48
Chlorpromazine									
Abdullah and Miller	1968	100	0	–	100	8.0	–	$8 \times 10^{-6} M$	48
Cohen et al.	1969	–	0.8	–	–	0–2.5	–	100, 10, 1 μg/ml[3]	6, 24, 48
Kamada et al.	1971	500	0.8[4]	–	1,436	1.1[4]	0.7–1.4	5×10^{-5}, 2×10^{-5}, 10^{-5}, $2 \times 10^{-6} M$	72
					2,500	1.0[4]	0.6–1.2	$2 \times 10^{-5} M$	24, 42, 54, 68, 72
Lithium									
Friedrich and Nielsen	1969	473	–	–	477	no significant increase over controls		1.2, 1.8, 2.4 mEq/l	48

Meprobamate									
Kamada et al.	1971	500	0.6[4]	–	1,500	0.8[4]	0.6–1.0	10^{-4}, 5×10^{-4}, $10^{-5}M$	72
					1,500	1.0[4]	1.0	$10^{-3}M$	24, 54, 72
Chlordiazepoxide									
Staiger	1969	602[5]	6.6	4.0–10.0	901[5]	7.3	1.0–17.0	50–200 μg/ml	8–72
Diazepam									
Staiger	1969	500[5]	9.2	6.0–14.0	800[5]	6.8	4.0–13.0	12.5–50 μg/ml	8–96
Stenchever and Frankel	1969	700	3.9	1.5–4.8	1,400	15.3[4]	3.0–22.2	20, 10, 1.0, 0.1 μg/ml	72
Staiger	1970	600[6]	4.0	1.0–10.0	793[6]	4.2	0–10.0	12.5–50 μg/ml	8–96
		800[7]	9.1	5.0–21.0	800[7]	7.1	4.0–12.0	10–40 μg/ml	72

1 Δ^8-THC added.
2 Cord blood used.
3 10 and 1 mg cytotoxic.
4 Percent abnormal cells.
5 Human diploid fibroblast cell lines.
6 Whole blood.
7 Separated leukocytes.

posed to marijuana smoke showed variability in DNA content (measured by Feulgen microfluorometry) and chromosome numbers greater than those seen in controls, suggesting an alteration of chromosomal complements.

Animal Studies

Martin (1969) reported on the effects of cannabis resin on chromosomes from cultured rat leukocytes, rat embryonic cells in tissue culture, and cells cultured from rat embryos of mothers treated with a teratogenic dose. She found no increase in breaks when compared to controls, but did find a decrease in mitotic activity with increasing concentrations.

Opiates

Human Studies

In vivo. Heroin and methadone have been studied *in vivo* (table V). *Gilmour et al.* (1971) showed an increase in the percent of cells with one or more aberrations in heroin users as compared to controls. In a study of 16 opiate addicts on methadone maintenance, *Falek et al.* (1972) found a significant increase in chromosome aberrations after 72 h of culture, but not after 48 h of culture. In a follow-up study (*Falek and McFadden,* 1973), serial determinations were carried out on 13 subjects on methadone. The frequency of breaks in predrug leukocyte cultures was 3.2 % compared to 5.0 % at 24 h postdrug (ten subjects). At 8, 20, and 30 weeks of maintenance, the values were 3.9, 4.8, and 4.2 %, respectively, and the mean percent breaks for all postdrug cultures was 4.5 %, a small but statistically significant increase over controls. However, *Gendel* (1974) found less than 1 % breaks in both 40 control mothers and 51 mothers addicted to heroin or methadone.

In utero. Abrams and Liao (1974) studied 16 newborn infants of mothers who used heroin during pregnancy and 14 newborn infants of non-drug-using mothers. They reported a 1.4 % frequency of chromosome breaks in controls, and a significantly elevated frequency of 11.4 % for heroin-exposed infants including a large number of structural abnormalities in contrast to controls where none was seen. *Gendel* (1974) again failed to find an increased frequency of breaks (89 newborn infants of addicted mothers in comparison to the 48 newborn controls). *Zelson et al.* (1971) reported that 4 of 384 infants born to heroin-addicted mothers were born with congenital anomalies, an incidence similar to that found in the general newborn population.

In vitro. In an attempt to determine if methadone was responsible for the elevated rate of breaks seen in their *in vivo* study, *Falek et al.* (1972) tested

methadone, morphine, and quinine *in vitro* (table VI). At different concentrations (based on established therapeutic doses) and various durations of exposure, none of these agents showed an increase in breaks.

Chlorpromazine (Thorazine)

Human Studies

In vivo. There are six reports in the literature on the effects of therapeutically administered chlorpromazine, three of which originated from the laboratory of Dr. *M. Cohen* (table V). In the first report (*Cohen et al.*, 1967a) an increased frequency of breaks was found when compared to controls. However, in a subsequent investigation of an additional six subjects, no increase was seen (*Cohen et al.*, 1969). *Nielsen et al.* (1969) and *Gilmour et al.* (1971) did find an increase in the frequency of breaks in patients treated with chlorpromazine when compared to control subjects as did *Jenkins* (1970) despite an unusually high control value (10.1 %). All of the subjects in *Jenkins'* study were also on stelazine. In a well-designed prospective study by *Cohen et al.* (1972) with chromosome examinations prior to, during, and following chlorpromazine treatment of ten patients, no increase in breaks was found. Values after 3 and 6 weeks of treatment, and at a 1-month follow-up were compared to baseline values. Six control subjects were also studied in parallel and no differences were found.

In vitro. In addition to the above *in vivo* studies, there are three studies of *in vitro* effects (table VI). *Abdullah and Miller* (1968) found an increased frequency of breaks when chlorpromazine was added to fibroblast cultures at a concentration of $8 \times 10^{-6} M$ (8 vs. 0 %). At a higher concentration of $8 \times 10^{-5} M$, no mitoses were seen. In a second experiment, fibroblast cultures from another individual were utilized, and again an increase was found (5 vs. 1 %). *Cohen et al.* (1969) added chlorpromazine (10 and 1 mg/ml, 100, 10 and 1 μg/ml) at 48, 24, and 6 h prior to harvest, and found that at concentrations of 1 and 10 mg/ml no metaphases were seen. For 1, 10, and 100 μg/ml, there was significant mitotic inhibition, and this effect was related to duration of exposure. However, at concentrations where cells for chromosome analyses were available, no increased damage was observed. *Kamada et al.* (1971) were also unable to demonstrate any chromosome-damaging effect following *in vitro* addition of chlorpromazine to lymphocyte cultures.

Animal Studies

Siva Sankar and Geisler (1971) administered chlorpromazine intravenously to mice, and blood for analysis was drawn 1 and 24 h later. At 1 h, a cytotoxic

effect was seen with very few metaphases available for examination. At 24 h there was no effect on growth, but a significant increase in total breaks was found. *Kelly-Garvert and Legator* (1973) designed an *in vitro* experiment on a Chinese hamster cell line based on the report that chlorpromazine forms stable ions when photoactivated with UV light. It is postulated that these radicals in turn intercalate into DNA, causing damage. Chlorpromazine, to give a final concentration of 3.5 mg/ml, was added to cultures and exposed to dark light for 0–10 min. They found an increasing frequency of cells with rearrangements with increasing duration of exposure to light.

Perphenazine (Trilafon)

Human Studies

In vivo. There are two reports on the cytogenetic effects of perphenazine (table V). In the first report, *Nielsen et al.* (1969) found a significantly higher frequency of breaks in those individuals taking perphenazine as compared to age-matched controls. However, these patients were also given orphenadrine (Disipal), and there is the possibility that this increase may be due to the synergistic effects of orphenadrine and perphenazine. Two additional cases with chromosome analyses prior to, and twice during treatment, also showed an increase in breaks. However, in a recent prospective study (*Cohen et al.*, 1972) where individuals served as their own control with two chromosome examinations prior to drug administration, one at 3- and one at 6-week intervals during treatment, and one at a 1-month follow-up, no increase in the frequency of breaks could be demonstrated.

Lithium

Human Studies

In vivo. There are two cytogenetic studies on lithium (table V). *Friedrich and Nielsen* (1969) found a significantly higher frequency of breaks and hypodiploid cells in three psychiatric patients on lithium therapy than in age-matched controls. In the study by *Jarvik et al.* (1971) the 16 patients on lithium also showed a higher frequency of breaks but the difference was not statistically significant when compared with drug-free controls. However, the placebo group had significantly fewer breaks than the lithium-treated patients. These findings must be interpreted with caution since five of the lithium patients were also receiving other drugs and their mean break frequency was 4.5 % compared to only 2.7 % for the remaining eleven patients on lithium alone.

In utero. While no chromosome data are available on infants exposed *in utero, Schou and Amdisen* (1970) reported that of 40 mothers on lithium ther-

apy during pregnancy, two gave birth to children with malformations. Unfortunately, there are no comparative malformation rates for the offspring of psychiatric patients not on lithium.

In vitro. Friedrich and Nielsen (1969) carried out *in vitro* studies on leukocyte cultures (table VI). Lithium at doses approximating serum concentrations added for the duration of culture (48 h) did not increase the frequency of breaks when compared to control cultures.

Animal Studies

Szabo (1970) reported palatal defects and increased fetal resorption in mice following oral administration of lithium, and believes lithium to be selective for palatal development since ear and eye defects were not seen. Two studies on rats (*Trautner et al.*, 1958; *Johansen and Ulrich*, 1969) failed to demonstrate a teratogenic effect following oral administration. In contrast, *Wright et al.* (1971) observed increased resorption rates and cranial malformations in the fetuses of rats given lithium intraperitoneally during pregnancy, and postulates that the route of administration may be responsible for the differing results.

Chlordiazepoxide (Librium)

Human Studies

In vivo. Cohen et al. (1969) studied four subjects on chlordiazepoxide and four controls, and found no increase in breaks (table V). In addition, serial determinations were done on six subjects given the drug and six controls at 2-week intervals during an 8-week period, and no increase in breaks or structural abnormalities was observed.

In vitro. An *in vitro* study by *Staiger* (1969) on human fibroblasts gave negative results (table VI). Concentrations used were 25–100 times higher than the blood level of 2 μg/ml obtained 2–4 h following a single oral dose of 30 mg.

Animal Studies

Schmid and Staiger (1969) studied the *in vivo* effect on Chinese hamsters following oral administration. Again the results were negative.

Diazepam (Valium)

Human Studies

In vivo. Cohen et al. (1969) studied six patients who had been on diazepam for 3–6 years and did not find an increased incidence of chromosome damage in comparison to controls (table V).

In utero. There are two case reports of congenital abnormalities in infants born to mothers who had received diazepam during their pregnancies (*Istvan,* 1970; *Ringrose,* 1972). Although the duration is not stated, it is known that in both cases diazepam was taken at least during the first trimester. However, other drugs were also taken during this period, in one case propoxyphene HCl (Darvon 65) as necessary for headaches, and in the other hydroxyprogesterone (Delalutin) over a prolonged period of time. The significance, if any, of isolated case reports must be carefully weighed due to the reporting bias.

In vitro. There are three reports on the *in vitro* effects of diazepam (table VI). For purposes of comparison with *in vivo* blood levels, it should be noted that a blood level of 1 μg/ml of diazepam is obtained following repeated daily doses of 30 mg (*de Silva et al.,* 1966). At concentrations 12–50 times higher than blood levels, *Staiger* (1969) found no increase in breaks or structural abnormalities in three different human diploid fibroblast cell lines. In 1970, *Staiger* investigated the effects on human lymphocytes, utilizing similar concentrations and two different culture techniques, but could not demonstrate any effect. In contrast to the above, using a wide range of concentrations including those reported to be within the therapeutic range, *Stenchever and Frankel* (1969) did show a dose-dependent increase in the number of cells with breaks. No structural abnormalities were seen; the increase was accounted for by chromatid and chromosome breaks exclusively.

Animal Studies

Schmid and Staiger (1969) examined bone marrow chromosomes of Chinese hamsters following oral administration (19 doses of 300 and 900 mg/kg), and found no increase in breaks or structural abnormalities. *Beall* (1972) tested the teratogenic potential of diazepam on rats. No effect on the number of pregnant rats, number of resorptions, and average litter size was seen; nor was the drug found to be teratogenic.

Other Psychotherapeutic Agents

Cohen et al. (1969) reported negative results for fluphenazine (Prolixin, Permitil) and thioridazine (Mellaril) (table V). However, these findings are based on a limited number of subjects (three in each group).

In vitro addition of various concentrations of meprobamate (Miltown) to leukocyte cultures and for different durations of exposure also gave negative results (*Kamada et al.,* 1971) (table VI). *Jagiello et al.* (1973) conducted *in vivo* and *in vitro* meiotic cytogenetic studies with meprobamate in mice. *In vitro* concentrations tested on mouse ova ranged from 1 to 10,000 μg/ml. At

750 μg/ml, meiotic processes were halted while at 500 μg/ml an increase in abnormal second metaphase figures was seen. However, acute or chronic *in vivo* treatment of mice had no effect on meiosis.

Bone marrow examinations of Chinese hamsters following oral administration of medazepam (Nobrium, ten doses of 450 mg/kg) showed no increase in breaks (*Schmid and Staiger*, 1969).

Imipramine (Tofranil) is considered teratogenic in man (*McBride*, 1972), yet *Levy* (1972) states that he has successfully treated 'hundreds of patients, many of whom were pregnant' without side effects. *Gilani* (1974) injected chick eggs with imipramine and found extensive toxic effects on developing chick embryos. *In vitro* addition of imipramine at different concentrations and for different durations decreased the number of mitoses, an index of cell division (*Fu et al.*, 1973). This effect was time and dose dependent.

Recently, cytogenetic and teratogenic effects of anticonvulsants have been studied. *Zellweger* (1974), in reviewing the literature, found a high incidence of tetraploid cells in four of six children exposed *in utero*. The significance of this finding, however, is unclear. Mention is also made of five additional children exposed *in utero*, and their mothers, all of whom manifested structural abnormalities (including chromosome breaks, triradial and quadriradial rearrangements). With regard to teratogenesis, *South* (1972), in a review of the literature, found a high incidence of congenital abnormalities in the offspring of 677 epileptic women on drugs during pregnancy as compared to no abnormalities in 201 epileptic women not on drugs.

Discussion and Conclusion

In recent years, there has been a tremendous upsurge of interest in the harmful effects of drugs on chromosomes. The research has elicited contradictory information which only adds to the seriousness of the situation, especially since greater numbers of people are continually becoming exposed to drugs.

In areas of medicine where drugs are life preserving, possible deleterious side effects, including genetic damage, are a necessary evil. In the treatment of mental disorders, however, the same risks need not be taken. Numerous psychoactive agents as well as alternate forms of therapy are available. Moreover, the accesibility of drugs to seemingly healthy people who use them for relatively minor complaints, further increases the number of persons exposed to possible genetic damage from psychoactive agents. It is of particular importance, therefore, that we have knowledge of the risk of genetic damage accompanying the use of psychoactive agents whether used therapeutically or abused.

Aside from the direct risk of genetic damage another far reaching consequence of modern pharmacotherapy is to reduce the length of hospitalization of

individuals undergoing treatment. In schizophrenia, for example, the result is an increased opportunity to procreate, leading to an increased reproductive rate in these patients (*Erlenmeyer-Kimling et al.*, 1969). This, in turn, causes a restructuring of the gene pool with the potential not only of increasing the number of individuals predisposed to schizophrenia but conceivably, as a result of pharmacotherapy, also those predisposed to malignant neoplasia and congenital anomalies.

In addition to the foregoing considerations there is, of course, the major problem presented by the increasing numbers of drug abusers in our present society who consist primarily of young persons still in their childbearing years.

Unfortunately, we lack precise information regarding the significance of chromosome breaks. If knowledge is lacking on the mutagenic and carcinogenic potential of these drugs, even less is known about their teratogenic potential. This survey has not attempted to include a thorough review of the latter, although an attempt has been made to include all available information on the former.

Cytogenetic studies have been carried out *in vivo* and *in vitro* on humans and animals. Human and animal studies have also been done *in utero*, but frequently two critical factors, the time and route of administration of the drugs, have not been mentioned, presenting difficulty in accurately evaluating the results.

There are numerous problems inherent in cytogenetic assessment of drug effects. Leukocyte cells from blood samples have been used almost exclusively because they are readily obtained, but these are not gonadal cells and inferences have to be made that changes in one cellular system reflect those in another when extrapolating from peripheral leukocytes to congenital malformations. In addition there is the problem that *in vivo* and *in vitro* findings do not always correspond.

Cytogenetic results may be influenced by a number of factors. Deficiencies in culture media (*Sparkes et al.*, 1968; *Freed and Schatz*, 1969), the source of leukocytes (whether cord, venous, or capillary blood), individual differences, and seasonal variation, have been proposed as explanations for the discrepant findings from study to study. Recent viral infections and radiation exposures of individuals whose cells are cultured represent another uncontrolled source of variability. Indeed, because of high aberration frequencies, some investigators have eliminated from their control groups individuals with a history of either of the latter. Observer scoring of aberrations may be yet another factor, particularly in light of the relative consistency with which positive and negative results emanate from the same laboratories. Finally, the tediousness of cytogenetic analysis allows for only a small number of individuals to be examined. All of the above interfere with gaining a clear picture of chromosome damage ascribable to any one drug.

A few investigators reporting negative results have been critical of the un-

usually elevated frequency of chromosome aberrations in control groups of positive studies. However, even in normal populations, there have been wide variations in the frequency with which aberrations have been reported, although basically the same techniques have been utilized in all laboratories. *Bloom and Tjio* (1964) reported no breaks in 20 subjects, while *Schmickel* (1967) found a frequency of 0.7 % in eleven subjects. In two large studies, *Court Brown et al.* (1966) and *Sandberg et al.* (1967) reported mean break frequencies of 0.4 and 1.7 %, respectively.

In contrast to the above studies, *Lubs and Samuelson* (1967) analyzed ten subjects and found a mean break frequency of 7.4 %. *Littlefield and Goh* (1973) reported their findings on serial determinations conducted on 31 subjects, ten men and 21 women. A total of 29,709 metaphases from 305 cultures were analyzed with a frequency of 7.1 %. Women showed considerably more variability from culture to culture and a higher frequency than men (7.6 and 6.4 %, respectively). For both men and women, a significantly increased overall frequency of breaks was found during the late spring and early summer months, as contrasted to other times of the year. The report of seasonal variation needs further investigation. For example, if drug users are more susceptible to viral infections, then studies conducted during a flu epidemic may show a significant increase in breaks unrelated to the drug under study. Despite such marked discrepancies, *Jarvik and Yen* (1974) reported that chromosome examinations done 2 years apart on seven individuals yielded nearly identical mean break frequencies (although there was considerable individual variability).

One new approach to the study of drug effects concentrates on cellular proliferation in response to mitogens (agents stimulating cell division). Response is measured by comparing the level of incorporation of DNA-specific radioactive thymidine into cells prior to, and following, acute administration of the drug in question, or by comparing responses of chronic drug users to responses of matched controls. However, it must be pointed out that there is no perfect correlation between response to mitogens and chromosome damage. Some drugs may have no effect on cell proliferation but are capable of producing extensive chromosome damage while other drugs may reduce cell proliferation without producing an increased frequency of chromosome damage (marijuana, according to *Neu et al.*, 1970; imipramine, according to *Fu et al.*, 1973; and chlorpromazine, according to *Cohen et al.*, 1969). Therefore, although the procedure returns a quick dividend, it does not appear to be sufficient to exonerate any drug from having potentially deleterious effects.

Certain limitations were generally encountered in attempting to evaluate the findings in the literature. They included lack of proper controls, inadequate sample sizes (some reports are based on one or two individuals), unknown composition of 'street' drugs (see section on LSD for example), scarcity of information on health care and socioeconomic status of the subjects, insufficient data on

exogenous agents (i.e. exposure to other drugs including caffeine, chemicals, radiation, and viral infections, all of which are known to be mutagenic agents), paucity of chromosome analyses before, during, and after administration of drugs, and lack of follow-up studies. Many, if not all of these, may contribute to the increased frequency of chromosome damage reported for drug 'users'. On the other hand, it is conceivable that these limitations may have prevented the surfacing of even more incriminating data.

The possibility of individual differences in susceptibility to chromosome damage must also be considered. Although the basic components of DNA are identical in all individuals, heterozygosity prevails and may characterize as much as 20% of genetic loci in man (*Harris and Hopkinson*, 1972). Hence, the well-known existence of interindividual variability at the biochemical level. Even if pathways of drug metabolism are similar, the rates of metabolism may differ markedly, and equivalent dosages may not necessarily mean equal serum levels. Genetic factors have been shown to play prominent roles in determining serum concentrations for nortryptiline (*Alexanderson et al.*, 1969) and alcohol (*Vesell et al.*, 1971). (For a more thorough discussion, see *Propping and Kopun*, 1973.) Thus, interindividual differences may operate at several levels to produce differences in susceptibility to chromosome damage. Nonetheless, most of the publications have ignored such differences.

In the light of the above, it is not surprising that the literature reviewed yielded few clear-cut conclusions. These may be summarized as follows:

LSD. In vitro addition of LSD to leukocyte cultures produces an increase in chromosome damage which is *not* dose dependent. *In vivo* studies of LSD users, persons given LSD under medical supervision, and infants exposed *in utero*, have been contradictory, some reporting increased damage, others not. No relationship has been found between dosage and/or duration of exposure and frequency of chromosome aberrations. In some studies, those individuals having used LSD the longest were among those showing the lowest frequency of breaks. The majority of the studies have been retrospective. The few well-controlled prospective studies with chromosome analyses prior to, during, and after drug administration failed to detect a significant effect of LSD on chromosomes.

Marijuana. Reports are contradictory, except that 'users' of 'street' drugs usually show increased frequency of breaks.

Other drugs. Data are insufficient for any summary statement at this time. There is further need for cytogenetic analyses to test the potentially harmful effects of drugs on the genetic material of man. Such studies should be carefully designed and include samples of adequate size with chromosome examinations prior to, during, and at intervals following treatment. However, the requirement

for highly skilled individuals to conduct the chromosome examinations, and the time-consuming nature of this type of study are distinct disadvantages of the method. It is hoped that these disadvantages will be overcome once computer-aided analysis has been developed into a routine procedure.

In the meantime, however, the data are such as to preclude complacency until more definitive information is acquired.

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