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GENETIC TOXICOLOGY OF LYSERGIC ACID DIETHYLAMIDE (LSD-25)

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Summary

The acute and the chronic psychotomimetic potentials of the hallucinogen lysergic acid diethylamide (LSD-25) have been recognized for almost 40 years. That additional types of the biological effects should have come under scrutiny was directly attributable to widespread use and abuse of this drug on a worldwide basis. Although "genetic toxicology" encompasses a broad spectrum of disciplines, including many areas of highly specialized research, perhaps the most germane, and those on which this review has concentrated, are Clastogenicity, Mutagenicity, Teratogenicity and Oncogenicity. Based on our current understanding and interpretation of the available data, the genetic toxicology of LSD provides an excellent example of Newton's "third law of motion", e.g., to every force there is an equal and opposite reaction force.

From the published material it is impossible to draw clear cut conclusions regarding any of the above "problem areas" in spite of the considerable scientific effort invested. Most of the in vitro studies performed on the clastogenicity of LSD indicate either suppression of mitosis or enhanced chromosome damage. However, extrapolation of such results to the in vivo situation is very difficult. With regard to in vivo human use of the drug, no concensus is attainable as to chromosome breakage and the inconsistencies within and between studies remain inexplicable. However, several of the "controlled" investigations assessing the in vivo effect of chemically pure LSD suggest a transient increase in lymphocyte chromosome breakage. On the other hand, the results of cytogenetic studies on experimental animals are contradictory. Although human studies are nonexistent, in those experimental organisms tested, using accepted techniques, LSD proved to be, at best, a weak mutagen, if mutagenic at all. Teratogenicity studies in animals are confusing due to the multitude of organisms and plethora of discriminant parameters studied. However, with regard to man there has been ample opportunity and one can conclude that LSD is not teratogenic. As to the drug's oncogenic potential, the 3 reported cases of leukemia in LSD users are most likely the result of coincidence.

Introduction

The intensive abuse of lysergic acid diethylamide (LSD-25) some years ago created an international sense of alarm due to the uncertainty and possible permanency of its effects. The most serious complications included prolonged psychiatric reactions, spontaneous recurrence of the LSD experience without subsequent exposure to the drug ("flashback"), disturbed reactions such as acute panic, depression and confusion; and, less frequently, suicide, homocide and convulsions. It was also suggested that LSD could achieve toxic levels as well as lead to addiction; however, these latter effects have never been demonstrated. Although for a time the "mind expanding" properties of this hallucinogen held forth some promise in the treatment of alcoholism and certain psychotic states, this no longer appears valid.

The use of hallucinogenic drugs in many primitive groups has long been recognized as an adjunct to religious ritual and experience. However, in more advanced societies, drugs have served more limited goals; those of the treatment and prevention of disease. Nearly all of the psychotropic drugs are naturally occurring and derive from plant materials possessing somewhat similar chemical structures. LSD, on the other hand, was synthesized in 1938 by Albert Hoffman and Arthur Stall who were interested in obtaining a vasoconstrictive agent (similar to ergot) to be used as an anticonvulsant. The 25th experiment in the series produced the compound *d*-lysergic acid diethylamide (hence the name LSD-25). The drug failed to possess the desired properties but evoked unexpected results in animal tests. Some of the animals became strikingly excited after receiving the chemical while others entered a catatonic state. Initially by accident, but then purposefully, Hoffman conducted a series of experiments on himself which indicated that, in small doses, LSD was an extremely active substance exerting an unusual effect on both mental activity and physical experience.

Chemically, LSD is an amine alkaloid resembling ergonovine — one constituent of ergot — and promotes uterine contractions in a comparable way. Unlike the amino acid alkaloids, such as ergotamine which is also found in ergot, LSD produces only slight vasoconstriction. The significant effects of LSD are almost entirely upon the central nervous system. Responses of the autonomic and parasympathetic nervous systems also occur but they are all centrally mediated. For example, pupillary dilation is common on a "trip" but is not elicited by local application of the drug to the eye. In doses that affect the central nervous system in man, little effect can be detected in other organ systems that can be directly attributable to the drug. Apart from man, the effects of LSD have been investigated in a great variety of animals including mice, rats, hamsters, monkeys, clams, snails, spiders, flies, Siamese fighting fish, guppies and even an elephant.

A decade has elapsed since the initial report of a possible "genetic" effect of LSD was described. During the intervening 10 years, a great deal of scientific energy has been expended in investigating the possible relationship between this hallucinogen and an entire gamut of experimental systems. Extremely diverse disciplines are represented in these studies but many are beyond the scope of this review. Therefore, emphasis will be placed primarily on that

research which addressed questions relating to the potential of LSD with respect to four basic areas of interest: Clastogenicity; Mutagenicity; Teratogenicity; and Oncogenicity. A multiplicity of approaches was exploited including test organisms ranging from bacteria to humans with utilization of both in vivo and in vitro systems and the assessment of both meiotic and somatic tissues. With such an impressive effort, it might have been expected that definitive answers to the basic questions posed would be forthcoming. However, conflicting and contradictory results have been obtained for almost every observation in the specific area studied. It is our purpose to review these data, to point out the inconsistencies which might exist and to offer possible explanations for these obvious incongruities.

The LSD literature, at first glance, seems voluminous. However, a critical examination indicates that many of these publications add little to the controversy as they represent "editorial" comments and literature reviews with no experimental results. Also adding significantly to this large number of articles is the repeated appearance of identical data, or repeated publication of partial series in several different journals. For this reason, we have chosen to include in this review only those publications contributing original experimental data and those papers which seem to summarize the total efforts of various groups of investigators.

Clastogenicity

Human chromosomes

(1) In vitro studies. The first observation concerning LSD and the human genetic apparatus suggested a potential to induce chromosome breakage in vitro. Cohen et al. [31,34] added LSD in a wide range of concentrations and over many exposure times to PHA-stimulated lymphocyte cultures of 6 normal individuals and observed a marked decrease in mitotic rate as well as a significant increase in chromosome damage including both simple breaks and structural rearrangements. Studying cultures of an additional five subjects exposed in vitro for 48 h, MacKenzie and Stone [96] found twice the frequency of chromosome damage in treated cultures when compared to normals. In vitro studies by Corey et al. [38] on 10 subjects demonstrated a significant increase in single and total chromosome breaks in treated cultures, but not in double-break aberrations or chromosomal rearrangements. Emerit, Roux and Feingold [49] demonstrated a direct dose—response relationship with chromosome breakage over a range of LSD concentrations between 0.01—5.0 μg/ml in 3-day peripheral-blood cultures. In trying to repeat the dose—response work of Bick [18] on animal cells (see below), Dorrance, Janiger and Teplitz [43] found chromosome damage only at LSD concentrations greater than 1.0 μg/ml in both human lymphocytes and fibroblasts. A series of studies by Jarvik et al. [79] on a total of 8 subjects indicated that although LSD caused significant chromosome damage in vitro, similar breakage frequencies could be achieved with other drugs all of which were commonly used and considered innocuous [78]. However, streptonigrin, a known potent clastogen [29,37] induced 3 times as much damage as LSD. An experiment on a single subject exposing his lymphocytes to 0.1, 1.0 and 10.0 μg LDS/ml for 24 h led to no appar-

ent increase in chromosome damage. However the mitotic rate was suppressed by increasing drug concentrations [59]. Sturelid and Kihlman [140], although also noting a suppression of the mitotic index in two LSD-treated human lymphocyte cultures, found no demonstrable evidence that the drug could be considered a clastogen.

Although a wide range of drug concentrations (0.01—20.0 μg/ml) and exposure times were used (4—48 h), all the above studies used human peripheral-blood lymphocytes derived from normal individuals. These investigations suggest an in vitro effect of LSD, either as a mitotic inhibitor and/or a clastogen.

(2) In vivo studies. These reports constitute, by far, the largest group of studies performed and concentrated principally on two types of subjects: (a) the LSD "users" or individuals exposed to illicit LSD; and, (b) patients and/or volunteers treated with pure LSD because of medical indications. Results obtained from LSD users represent the first major point of controversy. Those studies indicating "positive results" follow. Cohen, Hirschhorn and Frosch observed increased rates of chromosome breakage among 18 adults who had ingested various numbers of doses of "street LSD". Structural aberrations were observed as well as a nonrandom distribution of damage based on chromosomal length [31]. 46 users were studied by Egozcue, Irwin and Maruffo [46], with an apparent 2-fold increase of chromosome breakage when compared with controls. A nonrandom distribution of damage among simple breaks as well as dicentric chromosomes and exchange figures were observed. However, no mitotic depression was noted. An earlier report on 6 of these patients [74] indicated a Ph^1 like chromosome in some subjects. Jacobson et al. [76] reported increased chromosome damage in peripheral blood cultures of more than 60 LSD users.

In addition, other users, examined because of LSD ingestion during pregnancy were examined cytologically. Among 11 adults in this category Cohen et al. found increased chromosome damage [33]. Abbo, Norris and Zellweger [2] studied both peripheral leukocytes and skin fibroblasts in a couple using LSD for a period prior to and during pregnancy. Increased chromosome damage was observed in both tissues examined in both subjects.

Other reports of studies of similar groups of patients contain no evidence for LSD clastogenicity. Loughman, Sargent and Israelstam [92] examined leukocytes of 8 users shortly after recent exposure to large doses of LSD and found no differences from their control data. However, these authors noted the occasional presence of "large cells with multiple micronuclei" in drug users' cells. Sparkes, Melnyk and Bozzetti [134] studied 4 "users" by dividing the samples with the cytogenetic analysis being performed in two different laboratories. No significant differences were observed in breakage rates between users and controls although the authors mention that "quadriradial" (QR) formation was observed only in a person exposed to LSD. An additional 13 users were studied by Stenchever and Jarvis [139] with no evidence of increased breakage in the exposed individuals. In this report, a QR was observed in one of the 8 controls but in none of the study group. 5 "users", serving as a control group for medically administered LSD treatment (see below), showed no increased chromosome damage [143]. Hultén et al. [69] studied an additional two subjects

whereas Kato and Jarvik [85] studied a single individual and reported no increased breakage. Two series of 14 LSD users and one with 20 subjects, each with negative results, have been published [41,43,94]. A comparison of current "heavy users", users who had used the drug for some time before examination and controls revealed no differences in chromosome breakage among the three groups [82].

Among those individuals examined because of LSD ingestion prior to or during pregnancy with no indication of increased chromosome damage are the mother of a child with amputation deformities [9] and the mothers of normal children [67,120]. 47 couples and their offspring were studied by Dumars [44] and single couples by Hultén et al. [69] and Warren, Rimoin and Sly [155]. In none of the couples was there evidence for increased chromosome damage, but Warren, Rimoin and Sly [155] cite the existence of micronuclei in the cells of the father, thus supporting a similar observation made previously by Loughman, Sargent and Israelstam [92].

(3) Children exposed in utero. Another, perhaps small and specialized, group of "users" are those children who were exposed via parental use of illicit LSD prior to and/or during pregnancy. As with the above group of individuals, controversy over the results exist. Increased chromosomal damage was reported in a series of publications in a total of 16 children and 5 abortuses exposed during pregnancy [2,31,33,46,75,76,159]. However, other authors examined 63 similarly exposed children and found no evidence of elevated chromosome damage [1,9,27,44,52,68,69,80,120,123,139,155]. Although increased breakage was not observed in the case of Hsu, Strauss and Hirschhorn [68], the child had trisomy D with a D/D de novo translocation. In individual cells of the parents, structural abnormalities were observed. Additionally, Sato and Pergament [120] note that hypodiploidy was significantly higher in their patients than the rate normally encountered.

The latter two groups of subjects who were exposed to illicit LSD pose great problems in interpretation of the data obtained vis-a-vis the clastogenic potential of the drug. The possible reasons for these difficulties will be discussed below. However, one possible approach to clarification of this problem and one which may remove some of the difficulties is better control and design of the experiments. This was attempted by investigating patients who were being treated with pure LSD under "controlled conditions".

(4) In vivo exposure to pure LSD. This group of individuals perhaps provides the best estimate of the in vivo effects of LSD on human chromosomes. A large number of patients has been studied in a variety of experimental settings. Because of the different experimental aims of these studies, it may be worthwhile to examine them in greater detail than the previous data, which are not as amenable to control. These studies will be presented in chronological order of appearance.

In 1967, Cohen, Marinello and Back [34] reported cytogenetic studies on a single paranoid schizophrenic whose psychotherapeutic regimen included 15 LSD treatments over a 6-year period. The total ingested dose of the drug was 2500 mg. Leukocyte cultures were prepared for chromosomal analysis 8 months following the last treatment. During this interval, no other hallucinogenic drugs were ingested. An analysis of 200 cells revealed an approximate

3-fold increase in chromosome breakage when compared to control cultures (12% vs. 3.7%), and included both single breaks and structural rearrangements. This patient was treated briefly with the tranquilizers chlorpromazine and chlordiazepoxide. Subsequent studies [32,70,109] did not find these drugs clastogenic.

The first negative report was published soon after. Bender and Siva-Sankar [15] reported 7 schizophrenic children receiving daily doses of LSD up to 500 μg for periods of 5.5 to 35 months. This series was expanded [126] to total 15 patients receiving LSD, 9 treated with UML-491 and 8 patients treated with both drugs. Controls included 25 psychiatric patients receiving no drugs, 7 normal adult employees and a normal 14-year old. Cytogenetic evaluation was performed 2—4 years after treatment had been terminated. There were no statistically significant differences in chromosome damage among any of these treatment groups.

Nielsen et al. [107,108] studied lymphocytes from 5 patients treated for between one half to three years prior to chromosomal evaluation. As controls, 40 individuals, equally divided between patients, some of whom had received psychotropic drugs other than LSD and staff personnel were used. Replicate control cultures were studied after both 48 and 72 h and no differences found in the frequency of cytogenetic abnormalities between incubation times. Significant increases ($P < 0.001$) however were observed in the frequency of gaps, breaks and hyperdiploid cells (trisomies) among the 5 LSD-treated patients when compared to the 40 controls. However, it must be pointed out that most of this damage was contributed by only two of the patients. In the preliminary study [107], an increase in chromosome breakage was also noted among those control patients treated with other unspecified psychotropic drugs. Sparkes, Melnyk and Bozzetti [134] found no increased chromosome damage in 4 patients who had had undergone psychoneurotic reactions and had been medically treated with LSD, with accumulated doses reaching high levels. The interval between last treatment and cytogenetic study ranged from 1 month to 5 years.

An additional 9 male patients who had received between 200—800 μg LSD for psychotherapeutic reasons were studied between 9 months and 3.5 years following termination of the treatments [2]. A control group of 32 age-matched males was also studied. Statistically significant differences (by two different methods of analysis) were demonstrated between the LSD treated group (8.3% breaks) and the controls (3.5% breaks). The majority of abnormalities observed were chromatid breaks. No structural rearrangements were observed in these lymphocyte cultures.

The first prospective multiple investigation study of therapeutically administered LSD was performed by Hungerford et al. [70]. In this investigation, LSD (approximately 200 μg) was administered intravenously to 4 patients; a regimen of three treatments was followed with intervals of 1—2 weeks between doses. Each LSD treatment was preceded by either 100 mg chlorpromazine or placebo. Lymphocyte cultures were examined from blood samples obtained 1 h prior to drug administration as well as 1 and 24 h following treatment. Cultures were incubated both for 46—52 h as well as for the usual three-day period. The incidence of abnormalities was identical in cultures incubated for

either period of time. Follow-up samples were taken between 1 to 6 months after the course of LSD therapy was completed. Three controls were included who were subjected to the same protocol. The results indicate an increase in aberration frequency following drug administration. Dicentric and multiradial chromosomes were observed in patients following LSD therapy. This increase in chromosome damage was of a transitory nature since the follow-up studies revealed normal frequencies of aberrations. The continual elevation of damage in the 4th patient was attributed to his possible occupational exposure to radiation. These data can also be interpreted, as suggested by the authors, as indicating no clear clastogenic or synergistic effect of chlorpromazine [32,70, 109]. This study made an important contribution to the field since it was based on careful design and did not rely on retrospective or anamnestic data. It also helps to explain those studies indicating no evidence of chromosome damage years after exposure.

Following the experiment of Hungerford et al. [70] several other groups published similar investigations in rapid succession. Tjio, Pahnke and Kurland [143,144] studied the most extensive group of subjects and introduced a great deal of "finesse" to the methodology and critical analysis of the problem. 32 patients were studied in "double-blind" fashion both before and after being treated with LSD. In this way, each individual served as his own control. "Before" treatment samples ("controls") were obtained between 1 and 66 days prior to LSD therapy. With each of the two dosage levels used ("Low" 50 μg and "High" 250—450 μg), blood samples were obtained between 1 and 10 days following treatment. The overall analysis showed no statistical differences between the pre- and post-LSD treatment levels of chromosome breakage for either dosage group or for all the patients combined.

Several additional observations were made in this study which are worthy of mention. 4 of the 32 patients had documented upper respiratory infections (URI) within the 40 days prior to the "pre-LSD" sample. One of these, whose URI ended 20 days before the sample was obtained, showed an elevated incidence of chromosome damage (9.50%). Interestingly, this same patient had a recurrence of the URI, after the above sample was obtained and 10 days before his LSD session. His post-treatment level of breakage was 43.92% aberrant cells, the highest in the entire series. The possible role of viral infections in chromosome breakage has also been demonstrated previously [31]. Another possible confounding factor was the concurrent or previous use of other psychoactive drugs. Almost all the subjects, some 7, were on or had received additional medication. However in 7, no history of drug use or infection was noted. Results obtained both pre- and post-LSD treatment showed no significant differences in chromosome breakage in this group. Two patients in the series showed increases in aberration rates following LSD treatment and were re-examined after 71 and 229 days. On follow-up both had returned to pre-treatment levels thus suggesting, at best, a transitory rise as was observed by Hungerford et al. [70]. However, follow-up studies on 6 other patients showed no differences, but immediate post-LSD values were already low and no further changes were observed.

Several other study groups were included in this investigation in the hope of controlling other possible variables. 5 chronic users of illicit LSD were studied

before, during and after administration of pure LSD. Blood samples were taken daily for 7—10 days prior to multiple oral administration of the drug. 2 normal subjects, who were not LSD users, were also studied serially for 8—10 days. An additional 8 subjects were studied only after LSD ingestion (intervals since last dose ranging from 2—15 months). No significant differences were observed in any comparison among these 3 separate experimental groups neither prior to nor following LSD administration.

A similar "before and after" study was also performed by Corey et al. [38] as well as a retrospective in vivo investigation and an in vitro experiment. 10 subjects, with no prior exposure to LSD or other "psychedelics" or narcotics were investigated immediately prior to and 24 h after LSD therapy. Drug dosage ranged between 200—600 μg. No significant differences were observed in the before (control) and after LSD evaluation of chromosome damage with regard to gaps, single or double break aberrations. The in vivo retrospective study with pure LSD included 4 groups of patients: 5 individuals on LSD alone; 5 treated solely with mescaline sulfate; 6 treated with both drugs and 13 control subjects treated with neither drug. 4 patients in the treated group were also known to have used illicit drugs. Total LSD dosages ranged from 200—435 μg administered over periods of 24 h to 8 years prior to the study. No statistically significant differences with regard to chromosome breakage were observed between any of these 4 groups.

A recent "single-blind" retrospective study of pure LSD administration addresses the problem of adequate controls and the possible parameters contributing the sources of variability [53]. This investigation compared 32 LSD-treated patients with a like number of similar patients who were not treated with the drug. Great care was devoted to the clinico-physical considerations of matching patients and controls. Similarly, the laboratory and microscopic procedures were presented in great detail. Analysis of the raw data indicated no significant differences between the two groups. However, the authors pointed out several important points to be considered before drawing conclusions. These included the problem of matching controls, variability in breakage rates in the normal population, and the numbers of cells studied. A statistical analysis partitioning the variance among some of these possible contributory components indicated that increased chromosome damage could not be attributed to the ingestion of pure LSD.

A more recent study by Jarvik et al. [79] described "before and after" examination of two patients and only "after treatment" with LSD and dextroamphetamine in 6 others. No increase in chromosome breakage was observed in the paired cultures of those patients receiving both drugs and the remainder of the cultures fell with normal limits. A study of 4 schizrophenic children treated weekly with LSD for 6—10 weeks totalling doses between 270 to 450 μg [124] paid attention to other drugs and upper respiratory and viral infections. Blood samples were obtained prior to therapy, following the last course of LSD, and follow-up studies at intervals of three months and approximately one year after treatment. In comparison to the patients' "pre-LSD" levels as well as to normal laboratory personnel, no significant chromosome damage was observed associated with the treatment. The largest study using pure-LSD administration is that of Robinson et al. [116] which reported a

retrospective investigation of 50 psychiatric patients treated with various LSD doses for different periods of time and 50 controls matched for age, sex and marital status. Except for difficulties in matching the controls by sex, no additional description is given. The mean interval between termination of treatment and cytogenetic analysis was 37 months. No significant differences were found between patients and controls. However, the authors noted a decrease in all chromosome anomalies as the study proceeded.

The foregoing section of this review represents a compilation of all the published studies on the clastogenic effects of pure LSD, administered for medical reasons. Although a tendency to negative results is obvious, these observations are not unanimous.

(5) Meiotic studies. Only one study of LSD effects on human meiotic chromosomes has appeared. Hultén et al. [69] investigated testicular biopsies from two healthy males, one of whom was a heavy LSD user, and the other a normal control. Although the authors admit to technical difficulties with the material, they concluded that the LSD "user" did not demonstrate an obvious increase in numerical or structural aberrations in germ-line cells 6 months after the last drug ingestion.

Other organisms

(1) Primates

Two studies used Rhesus monkeys (*Maccaca mulatta*) to investigate the chromosomal effects of LSD. Egozcue and Irwin [45] performed in vitro and in vivo experiments using both meiotic and mitotic material. Although occasional breaks were present, no differences in breakage rates in meiotic chromosomes were observed between control and LSD-treated (both acute and chronic) male macaques. However, studies of blood cultures revealed significantly higher rates of breakage in LSD treated animals both in vitro and in vivo. Chromosomal rearrangements were observed only in treated cultures. Variability was observed among animals treated chronically in vivo since only 2 of the 4 macaques showed increased chromosomal breaks. Kato et al. [86] studied leukocyte cultures from 4 pregnant treated macaques (plus 2 controls) following multiple LSD doses. Control cultures were obtained on all animals before treatment began. Chromosome analysis was also performed twice on infants born to one treated mother and the two control mothers. The other treated females delivered two stillborn infants and one who died at one month of age. In 3 of 4 treated mothers, a transient increase in chromosome damage was observed following LSD ingestion of 0.125—1.0 mg/kg. The abnormalities observed consisted of chromatid and isochromatid breaks, but chromosomal rearrangements were not observed. At 7 months of age, increased chromosome breaks were still noted in the offspring of the "treated" mother. However, in the blood culture of the offspring of a control mother, chromosomal aberrations (including a quadriradial) were observed at 14 months of age.

(2) Marsupials

Bick [18] studied in vitro effects of several mutagens, including LSD, on chromosomes of leukocyte cultures of the rat kangaroo, *Potorus tridactylis.*

Both chromatid and isochromatid breaks increased linearly with doses ranging from 0.4—6.65 μg/ml. The damage observed at 4.2 μg/ml LSD was comparable to that induced by 100 R radiation delivered at 25 R per minute. LSD also suppressed mitosis, an observation made previously [31,34,59,140].

(3) Rodents

(a) Mice

Meiotic investigations. Perhaps due to technical ease, most of the meiotic clastogenic studies were performed in mice. Cohen and Mukherjee [35] showed increased chromosome damage (breaks and rearrangements) in 10 mice receiving a single intraperitoneal injection of 25 μg/kg LSD when compared to 3 controls. The animals were sacrificed at various intervals between 1—21 days after treatment. The peak frequency of meiotic abnormalities was observed 2—7 days following injection, but returned to normal after 3 weeks. Skakkebaek and Philip [128] studied meiotic preparations from 6 pairs of treated and control mice — total doses between 68—138 μg/kg with multiple injections of 1 mg/kg. Chromosome damage was found only among the treated animals and was expressed at diakinesis or metaphase I and concentrated mainly in the X chromosome. This may be due to the ease with which the X—Y bivalent is recognizable. These findings were corroborated in a later study [127] in which increased meiotic chromosome damage and disturbances of spermatozoan morphology were observed in LSD-treated male mice. By contrast, Egozcue and Irwin [45] failed to find increased meiotic aberrations in 10 mice receiving dialy injections of 5 μg/kg LSD and 10 mice receiving single injections of 10, 15, 30, 45 and 60 μg/kg LSD. In this series, one testis was removed prior to injection and used as control. Jagiello and Polani [77] studied the effect of both acute and chronic administration of LSD on male and female meiosis in the mouse. Over a wide range of doses, which produced chromosome damage in male meiosis and leukocyte cultures in other studies, these authors could find no convincing evidence for breakage in either the first or second meiotic division in either sex. In 10 male mice who received a single LSD dose of 1000 μg/kg, Goetz, Srám and Zudova [60] observed no significant differences between treated and control animals. However, there was a significant increase in both autosomal and sex chromosome univalents in treated mice.

Bone marrow. In connection with their meiotic study, Cohen and Mukherjee [35] also examined chromosomal preparations of bone marrow made at the time of sacrifice. These results indicated that bone marrow chromosomes were much more susceptible to LSD-induced damage than meiotic chromosomes, but the results were not confirmed by other investigations [45,65,77].

(b) Rats

None of the studies assessing clastogenicity of LSD in rats has yielded positive results. The drug was administered to pregnant animals and bone marrow preparations of adults as well as embryonic tissues were examined cytogenetically. No differences in chromosomal damage between LSD-treated animals and controls were observed [49,65,121,122]. A meiotic chromosome study was undertaken by Goetz, Srám and Zudova [60] on male Wistar rats

receiving either 10 or 1000 μg/kg LSD in a single intraperitoneal injection. Spermatocytes at diakinesis/metaphase I were examined. Although no increased chromosome damage was observed, a significant increase in spermatocytes with univalents was noted in the treated animals.

(c) Hamsters

In vitro chromosome studies performed on Syrian hamster embryo cultures were carried out by DiPaolo, Givelber and Erwin [40]. LSD in concentrations between 0.9—45 μg/ml was added to the medium for 24—72 h. There was no decrease in mitotic index nor increase in chromosome aberrations in the treated cultures. Nicholson, Pace and Davis [106] studied the in vivo effects of chronic LSD exposure on bone marrow chromosomes of the golden hamster. Varying doses were injected daily for a period of 10 days and the animals sacrificed at different intervals. No differences in chromosomal anomalies were observed between control and treated groups.

(d) Rabbits

Amarose, Schuster and Muller [5] performed chromosomal studies on 72-h lymphocyte cultures of New Zealand white rabbits. After a series of treatment regimens, simulating acute and chronic exposures, administered intravenously, no increase in chromosome damage was seen in pre- and post-treatment blood cultures.

(4) Insects

(a) Drosophila

Although several investigations of LSD mutagenicity in Drosophila have been performed (see below) conclusions concerning chromosome breakage were mainly inferred based on other types of tests. For example, the appearance of a certain type of morphologic mutant attested to non-disjunction, deletion or translocation. Grace, Carlson and Goodman [61] found no evidence of chromosome breaks nor mutations of any kind after injection of massive doses of LSD into *Drosophila melanogaster* males and concluded that if the drug is indeed a clastogen or mutagen at all, it is not a very powerful one. Although chromosomal damage could not be shown, Browning [26] presented evidence for increased nondisjunction of the X and Y chromosomes after treating Drosophila males. Srám [135] strengthened Browning's observation by noting a 6-fold increase in non-disjunction and a 20-fold increase in the loss of X and Y chromosomes following the injection of 500 μg/ml LSD into males. Markowitz, Brosseau and Markowitz [98] found no evidence for chromosomal rearrangements. A direct cytological study of meiotic preparations by Srám and Zudova [137] revealed neither translocations nor breaks in the X chromosome, nor chromosomal rearrangements at the spermatogonial stage. Barnett and Muñoz [13] analyzed mature sperm from LSD treated males and found no increase in II—III translocations, which would indicate chromosome breakage.

(b) Antheraea eucalypti

Quinn [114] studied cultures from ovarian cells of diapausing pupae which

were treated with an aqueous solution of LSD for 5 h followed by a 5-h recovery period. Chromosomal preparations revealed a significant elevation in the frequency of aberrations in treated cultures. Most breaks occurred near the centromeric region.

(5) Plants

Chromosomal studies were performed on several different plant species exposed to LSD. Sturelid and Kihlman [140] treated the lateral roots of *Vicia faba* with various concentrations (1, 10 and 50 mg/l) for periods of time ranging between 2—24 h followed by similar recovery periods in tap water. 100 cells were examined for each experimental condition and not a single abnormal metaphase was observed at even 50 mg/l nor was any effect on mitosis discerned. Similar results were obtained by Riley and Neuroth [115] using root tips of *Vicia faba* and *Allium cepa* treated with LSD concentrations of between 0.01—50 mg/l. Exposure time ranged between 1—8 h with recovery periods of 1 h for Vicia and 3 days for Allium. No significant differences in chromosomal aberrations were found in any of the treatment groups when compared to controls. Truhaut and Deysson [147] exposed root meristems of *Allium sativum* for periods of up to 7 days using LSD concentrations of 0.1—80 mg/100 g. 500 metaphases were counted in each of 10 meristems for each experimental condition. No significant effect on either mitotic activity or chromosomal aberrations was found.

Positive clastogenic results of LSD treatment of plant chromosomes have been published by Singh, Kalia and Jain [125], Kalia et al. [83] and Sadasivaiash, Collins and Davis [118]. The first two of these studies reported extensive chromosome damage in barley root tips treated with 25 μg LSD/ml with both treatment and recovery periods of 4 or 8 h. Most of the aberrations observed appeared as chromosome breaks, nearly half of which were located at the centromeres, resulting in a large number of acentric fragments. Both treatment periods appeared to increase the mitotic index, an effect described as the "stimulatory effect of LSD on cell division". However, this influence was not reflected in seedling height when measured after 10 days since the treated plants showed a considerable reduction in height. Sadasivaiash, Collins and Davis [118] demonstrated less extensive, but yet significantly increased, chromosomal damage in *Allium cepa*, *Hordeum vulgare* and *Serale cereale* root tips treated with a 30 μg/ml solution of LSD for 4—12 h, followed by recovery periods of equal length. Most of the damage consisted of chromatid and isochromatid breaks which failed to repair. The absence of dicentric chromosomes also indicated a lack of rejoining of the broken ends. Again, the distribution of breaks was not uniform over the length of the chromosome with localization at the centromeric regions. Allium chromosomes appeared to be more susceptible to breakage than those of the other two species. However, no significant differences were found when comparing diploid and tetraploid rye. Meiotic investigations of diploid rye also revealed chromosome damage induced by LSD. At metaphase, a significant increase in chromosome breakage leading to acentric fragments was observed. The chiasma frequency was reduced due to the presence of univalent and rod bivalents. At anaphase I, a considerable number of cells contained laggards, fragments and demonstrated delayed separa-

tion of the bivalents and chromosome stickiness. Similar observations were also made in meiosis II with production of micronuclei at the tetrad stage. 25—35% of the pollen of treated plants was non-viable and these plants showed poor seed production.

Possible chemical interaction of LSD with the genetic apparatus

The possible effects of LSD on chromosomes led to the reasonable assumption that this drug may interact directly or indirectly with nucleic acids or other "genetically" active biochemical macromolecules. Various types of experiments were performed representing different approaches to this problem. In these studies, as with those concerning chromosome-breakage experiments, conflicting results have been obtained. This may seem somewhat surprising since many of the variables, obvious in the clastogenic studies, do not exist in these experiments. Yielding and Sterglanz [157] followed the binding of LSD to calf-thymus DNA by use of both fluorescent techniques and UV absorption spectra. They showed a maximum binding ratio of 1 molecule LSD per base moiety in the DNA chain and concluded that the binding was specific for LSD and represented a general property of the drug. Smythies and Antun [132] also claimed that LSD bound to DNA. Extension of this concept was supplied by Wagner [152] who, through circular dichroism studies, supported th idea that LSD intercalated into the double helix of calf-thymus DNA, leading to conformational changes similar to those obtained with ethidium bromide complexes. This type of intercalation could cause histone dissociation rendering chromosomal DNA susceptible to breakage.

Attempts to repeat these studies yielded opposite results. Waring [153] states that DNA uncoiling is a prerequisite to intercalation and sought evidence for local extension or uncoiling of the double helix of the replicative form of ΦX174 bacteriophage without success. Brady, Brady and Boucek [22] using calf-thymus DNA essentially repeated the circular dichroism measurements [152] and fluorescence studies [157] with negative results indicating that LSD neither binds to nor intercalates into DNA. Smit and Borst [131], using viscosity measurements on DNA of bacteriophage PM2, failed to show any effect of LSD on DNA conformation and that LSD-DNA complexes have no behavioral similarity to the intercalating ethidium—DNA complexes. These authors conclude that LSD does not intercalate into the DNA.

It was also suggested that perhaps LSD clastogenicity may be due to effects on DNA-repair mechanisms. Papirmeister and Wolpert [111] showed decreased efficiency of the repair of UV-induced damage following LSD treatment of *E. coli* and T1 bacteriophage. These authors conclude that DNA was the target of the photodynamic inactivation of the LSD-sensitized organisms. However, investigations of the DNA-repair mechanisms in human cells revealed this process to be normal following LSD treatment. Trosko [146] subjected human amnion cells to LSD, labeled them with [^{3}H]thymidine and irradiated them with UV light. He studied the excision-repair mechanism of UV-induced pyrimidine dimers and found it to be unaffected by LSD treatment. In addition, the rates of DNA synthesis, the linking of small DNA fragments during scheduled DNA synthesis and the breakage of pre-existing DNA were not

influenced by LSD. Dorrance et al. [42] also studied the repair of UV-induced DNA damage in human lymphocytes and fibroblasts and found no evidence for a defect in this process.

Gayer and Pribys [56] observed the variable disappearance of nucleoli in LSD-treated human cells (HEp) and suggested this may be due to the drug's influence on RNA–protein metabolism leading to the observed decrease in mitosis and chromosome damage. However, although it was suggested by Andersen, Gibbs and Kubinski [6] that, as a rule, neuropharmacological agents inhibit reactions between DNA, proteins, ribosomal RNA and calf-thymus histone, LSD seemed to be an exception in that studies showed decreased binding of DNA and no inhibition of interaction with histones. On the other hand, Brown [25] showed a stimulatory effect of LSD on both nucleoplasmic and nucleolar RNA synthesis in isolated rabbit brain nuclei. The main activity was due to nucleoplasmic RNA polymerase.

Mutagenicity

(1) Drosphila

Most of the research in this area was carried out in *Drosophila melanogaster*. Browning [26] and Grace, Carlson and Goodman [61] injected varying doses of LSD intraperitoneally into Drosophila males and examined the induction of mutations in sperm. The results obtained were contradictory. Browning [26] noted a marked increase of X-linked recessive lethal mutations following massive doses (up to 4000 μg/g body weight, i.e., 2000 times higher than that consumed by humans). The results obtained were dose-related. On the other hand, injection of similar doses, induced no X-linked lethals nor X-linked visible mutations in either premeiotic, meiotic or postmeiotic sperm [61]. Srám [135] injected day-old males with 150 or 500 μg LSD/ml who were then mated. No sex-linked recessive lethals were observed but there was a significant increase in non-disjunction and loss of the sex chromosomes. Possible explanations for this discrepancy may be a threshold effect of the drug. Using similar treatment regimens, Vann [150], Tobin and Tobin [145] and Markowitz, Brosseau and Markowitz [98] also failed to demonstrate an increase in mutation rates after feeding LSD to male larvae. However, Vann [150] noted that oral ingestion was a less-efficient mode of drug administration than injection. Nonetheless, a threshold effect was supported since at the higher LSD concentrations a significant increase in mutations was observed [98,150].

Muñoz and Barnett [104] and Barnett and Muñoz [13] observed the mutagenic effect of LSD only in the progeny of treated males who were mated immediately after exposure. There was no increase in mutations when mating was delayed for 24 h. Using egg laying capacity and egg to adult viability and sex ratio as test parameters, Kastritsis and Jacob-Stocker [84] found no effect of the injection of LSD into third instar larvae of *D. pseudoobscura*. On the other hand, bromolysergic acid diethylamine lowered egg-laying capacity and egg to adult survival, while *D*-lysergic acid decreased egg-laying capacity only.

(2) Mice

Using the dominant lethal test in mice, Srám et al. [137,138] found no sig-

nificant increase in mutation after intraperitoneal injection of LSD into either sex. The frequency of induced dominant lethals was dose—dependent, and, in males, also related to the stage of spermatogenesis showing a threshold of 1 mg/kg LSD.

(3) Microorganisms

Zetterberg [160] studied the ascomycete *Ophiostoma multiannulatum* for both forward and back mutations following LSD exposure. No effect was observed with respect to forward mutation. Regarding back mutation, the result was less clear since a great fluctuation existed among the controls in reversion frequency. The author concluded that LSD is non-mutagenic in this test organism.

A significant rise in mutation rate in *E. coli* after treatment with LSD was observed [151]. The dose—response curve was found to be linear with an increase of mutation rate over the spontaneous level of 0.001% per 0.1 μg/ml added to the culture medium. A threshold effect, if it exists, lies at a concentration below 0.1 μg LSD/ml. In addition, LSD treatment seemed to increase the generation time.

(4) Plants

Seeds of *Arabidopsis thalion* were soaked in LSD solutions of 1, 10 and 10[illegible] g/ml for 24 h or a double soaking treatment using various concentrations [95]. For positive controls, seeds exposed to 1% EMS solutions or 10 kR X-irradiation were used. The parameter used to assess these treatments was the induction of chlorophyll mutations. No mutations were observed in the progenies of 165 LSD-treated plants while they were seen following the other two treatments.

Teratogenicity

(1) Humans

Several infants that were exposed to LSD in utero or prior to and/or during their mothers' pregnancies were born with congenital anomalies. In almost all cases additional drugs were also ingested during gestation. Zellweger, McDonald and Abbo [158] reported a girl born with unilateral fibular aplastic syndrome whose mother took LSD on day 25 and 3 doses between days 45 and 98 of pregnancy. Since the second dose was taken during the critical period for the production of deformities of the leg, the authors suggested a causal relationship. Additional limb anomalies were reported among other children exposed to LSD in utero. Hecht et al. [67] report a child born with right terminal transverse acheiria (absence of the hand). Carakushansky, Neu and Gardner [27] described a girl born with webbing of the fingers of the right hand and foot. In addition, the left hand showed absent nails and shortened 4th and 5th figers while the left foot had talipes equinovarus. Assemany, Neu and Gardner [9] reported another infant with amputation deformities of the right third finger and left third toe. Jeanbart and Berard [80] describe an infant with bilateral clubfoot, short humeri and bilateral aplasia of the fingers. Shapiro and Kerr [123] described two children born to mothers whose husbands only were

LSD users and who suffered from tibial and fibial aplasia or hypoplasia. Blanc et al. [19] noted the similarity of these limb deformities to those discerned in the amniotic band syndrome associated with premature rupture or separation of placental membranes. In a review of 148 pregnancies following LSD ingestion, Bro-Rasmussen, Jensen and Sorensen [24] did not find any evidence for the amniotic band syndrome among these children. Eller and Morton [47] described a case of spondylothoracic dysplasia but noted that this may have arisen on a genetic background, since 6 similarly affected children have been reported. All of these propositi were of Puerto Rican origin and 4 of them were products of consanguineous matings, thus suggesting a possible autosomal recessive mode of inheritance. In addition, single cases of central nervous system, visceral and ocular anomalies have been reported [8,21,58,68,75].

A much larger number of apparently normal children have been born after similar in utero exposure to LSD and other drugs [1,31,33,46,69,120,139,155]. McGlothlin, Sparkes and Arnold [101] monitored the outcome of 148 pregnancies following medical or illicit LSD administration, either during pregnancy (12 mothers) or prior to conception only (136 mothers). Of the 12 pregnancies exposed during gestation, 6 terminated in spontaneous abortion; however, it must be pointed out that 5 of these were in the same woman. No congenital anomalies were found among the 6 liveborn infants. Among those pregnancies exposed to LSD only prior to conception, the frequencies of spontaneous abortion, premature births and birth defects were within normal limits. It may be of interest that spontaneous abortions occurred significantly more frequently if the mother or both parents used the drug than if there was paternal use only. Additionally, abortion was more frequent in the "illicit user" group compared to medically treated patients (37% vs. 15%). However, this comparison includes the above mentioned woman with 5 abortions. 14 children possessed congenital anomalies of various types. Although this figure may seem increased at first glance, in 7 instances there was a positive family history for similar findings, in two others a genetic basis for the condition was known, and in one other the symptoms fit the rubella syndrome. Berlin and Jacobson [16] and Jacobson and Berlin [75] reported a total of 148 pregancies in 140 women and their spouses who used illicit LSD prior to or during pregnancy. In 10 cases, paternal use only was acknowledged. There were 65 abortions — 53 therapeutic and 13 spontaneous. 14 of the therapeutically aborted embryos were examined and 4 showed severe midline fusion defects. In the spontaneous abortion group, 7 of 12 fetuses were studied and 4 possessed encephaloceles and failure of skeletal development of the extremities. Of the 83 live births there were 3 cases of myelomenengocele, one of hydrocephalus, one case of bilateral pedal amputation and one child with congenital heart defect and growth retardation.

(2) Animal studies

In view of the lack of consistent results among the reports of human teratogenesis associated with LSD, it may have been hoped that better controlled animal studies would yield more precise answers. However, conflicting results also exist in these studies with respect to practically every species of experimental animal investigated.

(a) Rats

The first report of animal teratogenesis was published by Alexander et al. [4] who injected a single dose of 5 μg/kg (calculated to correspond to the average hallucinogenic dose in humans) into pregnant Wistar rats on the 4th day of gestation. An increase in fetal mortality, abortion, stunting and stillbirths was observed — with no congenital malformations. All matched control litters were normal and yielded 11—16 offspring per pregnancy. Identical LSD treatment, later in pregnancy (day 8—13), had no obvious effect on the offspring. Several years later this same group repeated their earlier experiments on a larger group of animals [3]. A total of 55 pregnancies was treated with either oral [29] or subcutaneously injected [26] LSD and a total of 521 offspring examined. Damage to litters was 3—4 times higher than in controls. In some of the stillborn fetuses and liveborn offspring, visceral anomalies were observed but no particular focus of toxicity or teratogenicity could be found. The proportion of deaths during gestation, abortions, resorptions, runting, offspring stilbirths and rate of mortality was increased. However, no unusual number of specific deformities was seen nor was any particular organogenetic period more sensitive. Treatment during the first 7 days was harmful but later in pregnancy was ineffective. The LSD effects appeared to be dose-related. Interestingly, LSD effects apparently persisted to the second generation. Some offspring of rats treated early in pregnancy were fertile but a significant number bore defective litters. The damage in the third generation was negligible when only one p: ıt was derived from an LSD-treated mother but was more severe when both parents were derived in this way (70% of total pregnancies showing abnormalities). In a preliminary study, Nosal [110] noted a dose—response relationship in the changes observed in Sprague-Dawley rats and found that there was a specific critical period in the fetal development when LSD was teratogenic. Warkany and Takacs [154], also using pregnant Wistar rats, did not confirm the above studies. Single doses of LSD were administered interperitoneally or orally on day 7, 8, or 9 of gestation or in multiple doses from day 7—12. Total dosage in individual rats ranged from 1.5 to 300 μg. 55 mothers were treated, 47 of whom were sacrificed on day 21 and 4 allowed to continue to term while 4 litters were completely resorbed. In this series the number and types of abnormalities observed did not differ from those normally expected in this strain of animal. Similar negative results in various strains of rats, using slight modifications of the treatment regimen have been reported [14,64,105,117, 121,122,148,149].

(b) Mice

The initial report of teratogenecity in this species was published by Auerbach and Rugowski [10]. A single intraperitoneal injection of LSD into pregn mice before day 7 of gestation led to a 57% incidence of grossly abnormal embryos. It is of interest that at this time neural structures are being formed and that most of the abnormalities observed were characteristic brain defects, as well as malformations of the lower jaw, position of the eye and facial contours. LSD administration on days 8 or 9 of gestation was not teratogenic. Hanaway [65] injected doses of LSD (5 μg/kg) intraperitoneally into pregnant Swiss-Webster mice on gestational day 6, 7, 8 or 9 and observed subcapsular

lens defects which appeared time-dependent. Administration of LSD on days 4 or 5 did not cause abnormalities, however, on day 6, 81% abnormal lenses were observed; day 7, 65%; day 8, 55% and day 9, 79%. These ocular results were confirmed in a duplicate experiment performed one year later; however, congenital defects of the central nervous system were not observed. DiPaolo, Givelber and Erwin [40] attempting to repeat Auerbach and Rugowski's experiments [10] used the identical treatment regimen on two different strains of mice. The observed abnormalities and their frequencies in the treated animals were entirely within those normally expected for these strains respectively. Similarly, Roux, Dupuis and Aubry [117] using repeated subcutaneous doses of LSD (5, 50 or 500 μg/kg) from days 6—10 or 6—14 of pregnancy did not observe any significant increase in fetal mortality or mean weight of treated fetuses; nor were any external malformations observed. In the group of animals treated with 500 μg/kg per day from 6—14, 50 fetuses were dissected and no abnormalities of the thoracic or abdominal viscera were observed.

(c) Hamsters

Geber [57], in an investigation of the teratogenic effects of LSD, its bromo-derivative and mescaline, injected pregnant hamsters subcuteneously (doses of 0.0008—0.24 mg/kg) on the 8th day of pregnancy. At day 12, the animals were sacrificed and the fetuses evaluated for viability and developmental status. All three drugs produced a reduction in litter size, increase in resorptions and fetal mortality. No congenital anomalies were observed among the fetuses of controls (saline-injected) but between 5—8% abnormalities were seen in LSD-treated animals. The malformations noted were mainly CNS defects such as exencephaly, spina bifida, intraparietal meningocele, myelocoele, edema along the spinal axes and localized hemorrhages of the brain. Approximately 10% of abnormal fetuses had more than one abnormality and several had 4 or 5. There was no apparent relationship between the dose administered and percentage of congenital malformation in the fetuses. DiPaolo, Givelber and Erwin [40] injected doses of 100, 200 or 300 μg of LSD on days 6, 7 or 8 of pregnancy, into Syrian hamsters which responded to a wide variety of teratogens. In one case, repeated doses of 100 μg were used on days 7—9. All pregnancies were terminated on day 14 and from 171 implantation sites, 168 live fetuses were obtained. There were 3 resorptions. These treated fetuses did not vary significantly in any parameter examined from the control litters. Roux, Dupuis and Aubry [117] also used multiple LSD doses (either 50 μg/kg or 500 μg/kg) from gestational day 7—13 or 4—12 in hamsters. A total of 189 fetuses were examined on day 15 of gestation. The mean mortality and mean number of live young per litter of LSD-treated mothers were comparable to untreated animals. In the group treated with the highest doses dissected fetuses showed no visceral malformations.

(d) Rabbits

A comparison of LSD (20 or 100 μg/kg) with the teratogen thalidomide (150 mg/kg) was performed on New Zealand white rabbits by Fabro and Sieber [51]. Animals were treated with aqueous solutions of the drugs at different intervals between days 4 and 12 of gestation. The mothers were sacrificed on

day 28. Thalidomide demonstrated its known effects but LSD-treated does did not differ from controls in number of implantations, resorptions and average weight of offspring. No gross malformations were detected.

(e) Chickens

Administration of LSD (50 or 100 μg) to chick embryos cultured in vitro during stages 8 to 12 of gestation did not increase mortality or abnormally decrease growth [66]. However, there was a retardation of neural tube fusion as well as failure of neural tube closure both in the brain and spinal cord. Disturbances were also observed in segmentation of the paraxial mesoderm with a significant reduction in the number of somites per embryo. No direct dose—response was found. Similar results were obtained by Messier [103] who exposed chick embryos in vitro to LSD concentrations of 0.0005, 0.005, 0.5 and 2.5 μg/ml. Retardation of mesoderm segmentation into somites and failure of neural tube fusion were observed in animals treated with the two highest concentrations. Electron microscopy revealed alterations in nucleolar components and mitochondria. A dose—response was suggested since fewer effects were noted at 0.015 and 0.05 μg/ml while no effects at all were induced by the lowest concentration.

(f) Sea urchins

Hoffler [88] tested LSD plus mescaline and three phenothiazines on three species of sea urchins. Each drug appeared to act during differentiation but with apparently different mechanisms leading to different anomalies. LSD administered during gastrulation retarded the growth of the exoskeleton.

Perhaps some of the above conflicting results may be explained by differences in transplacental transfer and tissue distribution of the drug. This problem was investigated by Idänpään-Heikkilä et al. [71—73] in hamsters and mice. ^{14}C-labeled LSD administered intravenously passed within a few minutes from the blood to tissues, localizing in the brain, adrenals, hypophysis, kidneys, liver and lungs of mice. Almost immediate excretion into the bile was noted. In the early stages of pregnancy (days 5—6), 2.5% of the radioactivity crossed the placental barrier in 5 min; 70% of this activity was unchanged labeled LSD. In later stages of pregnancy (days 15—16), only 1/5 of the radioactivity passed in the same time period. Distribution in fetal tissues was similar to that seen in the adult animals. The placenta was found to accumulate 1.5—2.0 times the amount of radioactivity than was observed in maternal plasma. Almost identical results were obtained with hamsters indicating that LSD easily penetrates the young fetus but the placenta seems to impede drug transfer during the last week of pregnancy.

Oncogenicity

On the basis of the structural chromosome damage observed and by analogy to inherited syndromes manifesting spontaneous chromosome damage as well as the appearance of a Philadelphia-like chromosome, several authors postulated a possible oncogenic effect of LSD [31,45,74]. However, only three cases of leukemia associated with LSD usage have been published. Grossbard et al.

[63] described a patient with acute myelocytic leukemia after having taken multiple doses of illicit LSD. Peripheral-blood lymphocytes, with and without phytohemagglutin stimulation, were studied prior to the institution of chemotherapy. In all 35 cells investigated following culture for 24 h without PHA, a "Ph^1-like" chromosome was found. This marker was not observed in normal 72-h PHA-stimulated lymphocytes. In a patient who had been treated many times with LSD for a depressive state, Garson and Robson [55] reported acute lymphoblastic leukemia. Cytogenetic studies of both bone-marrow cells as well as PHA-stimulated lymphocytes revealed multiple breaks and hyperdiploid cells. However, it should be pointed out that two of the proposita's sibs died of other types of malignancies and therefore a familial predisposition to cancer cannot be ruled out. Another case of acute lymphoblastoid leukemia in a LSD user was described by Sohn and Boggs [133]. Cytogenetic analysis of PHA-stimulated lymphocytes revealed a modal number of 48 chromosomes, the extra elements apparently being X chromosomes. Therefore, the patient most likely represented 48,XXXY Klinfelter's syndrome. The authors point out other cases of Klinfelter's syndrome in which a concomitant hematological malignancy is also present without LSD ingestion. Based on only these cases, any attempt to establish a causal relationship between LSD and malignancy is unfounded and the above reports most likely represent a chance phenomenon.

Discussion

The observations presented above clearly indicate that practically every question originally posed by the investigators studying various effects of LSD still remains unanswered. In the past 10 years, the discordant results obtained have not been adequately explained, although possible causes for their occurrence have been posited in previous reviews of this topic [41,90,100,130,136]. Our intention is not to construct a "score card" which will tabulate the number of "positive" or "negative" publications on a given aspect of this topic, but rather to point out some of the possible sources of variability which may contribute to the observed discrepancies. These considerations pertain not only to LSD but may be expanded to include the assessment of effects of any exogenous agent.

Much of the difficulty arises due to lack of similarity and, therefore, lack of comparability among the various studies performed. Attempting to draw conclusions from such a diverse body of data often leads to generalization and oversimplification. For example, the experiments assessing clastogenicity of LSD show an amazing spectrum of experimental designs and in many cases these do not fulfill even the minimal criteria that could lead to clear-cut interpretation of the data. As a result, diametrically opposite conclusions may be extracted from identical data by reanalysis using other statistical methods. This has been achieved several times with regard to LSD-induced chromosome damage [87,91,97,129,134,156]. Such a controversy perhaps results partially from differences in original experimental design and lack of attention to critical points such as: (1) adequate controls; (2) replicate cultures; (3) "double blind" experimentation; (4) a standardized system of scoring chromosome abnormalities; and (5) proper statistical evaluation (sample size, interactions, confound-

ing effects, etc.). These considerations with respect to chromosome-breakage experiments, have been previously reviewed [36] and should be controllable by proper planning of in vitro experiments.

However, even in this regard, discrepancies exist. Sparkes, Melnyk and Bozzetti [134] have suggested that perhaps variations in the tissue culture medium and possibly laboratory techniques in culture, harvest and slide preparation may account for differences in the results, since those laboratories obtaining higher rates of chromosome damage used "less complete" medium. This point may be important since several lines of investigation indicate the possible importance of various tissue-culture medium components in the induction of chromosome breakage [50,54,112,141]. Although the concensus seems to indicate in vitro clastogenicity of LSD, a rather disturbing observation is the almost total lack of correlation between drug dosages and chromosome damage. Although a multitude of agents have been assessed for clastogenicity, it is very difficult to find examples of those demonstrating no clear dose–response curve. This phenomenon was observed within individual studies and therefore is not related to interinvestigation comparisons. It remains unexplained and quite puzzling.

Another contribution to variability may be minor differences in various laboratory techniques which are, indeed, uncontrolled since they are not definable as discrete steps in the investigative procedure. For example, control ra[illegible] for chromosome damage vary significantly (and consistently) between labs [11,20,23,28,30,39,89,93,113], to say nothing of interindividual fluctuation and intraindividual differences when subjects are examined at different times. Although not well understood at all, such factors may be responsible for the observations that in some studies the highest level of chromosome breakage (significantly higher than controls) is much lower than the "normal" control rates of other studies performed under essentially identical conditions. Although, as a first approximation of the activity of a putative clastogen, in vitro studies may seem the best approach, they contain pitfalls which are difficult to assess because of a lack of complete understanding of the system. Additionally, a major drawback of such experimentation is limitation in its extrapolation to in vivo conditions. Although several new approaches have attempted to combine and facilitate in vitro studies with an in vivo component (e.g., host-mediated assay, dominant-lethal tests, etc.), direct in vivo studies are likely to yield the most informative data.

With regard to LSD, most of the in vivo studies performed represent a collection of investigations which suffer from all the technical and design deficiencies presented above, confounded by biological effects upon which very little "control" effort was expended. The vast majority of these reports consisted of o[illegible]rvations on users of many illicit drugs, among them LSD. The dosages ingested are at best "guestimates" due to the impurity of the "street" LSD used. In addition, concomitant exposure to a multitude of pharmacologically active agents, some with clastogenic capabilities, is not adequately considered. The contribution of these other drugs, either single or synergistically with LSD, to the overall picture of chromosome damage is unknown. An additional factor which was not adequately controlled and may play a role is the medical and nutritional status of the individuals examined and their exposure to pos-

sible viral infections, both of which are capable of inducing transient chromosome damage [7,12,17,48,62,81,99,102,119,142]. An attempt to overcome some of these obvious difficulties were those studies using patients being treated with pure LSD under controlled conditions. However, even in such "optimum" circumstances, agreement among the investigations was not achieved, although all the results are almost completely negative.

It is difficult to reach general conclusions from the results of animal studies on the possible teratogenicity of LSD owing to those experimental factors already enumerated above. Differences in animals (species and strains), dosages used, exposures at different gestational periods and various parameters estimating teratogenesis are too great to allow for comparisons. However, as far as human teratogenesis is concerned, LSD exposure was sufficiently widespread and a sufficient period of time has now elapsed in the proper age group for any such effect, if it exists, to have been expressed. Those few reported cases of malformed children born to LSD users may merely be coincidental and may fall within the expected population frequencies for those abnormalities observed. On the other hand, sufficient time has not elapsed to assess the possible effects of LSD with respect to carcinogenicity and mutagenicity but based on the available data such results are not seriously anticipated.

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