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Authors
Dishotsky, Norman I, M.D.
Loughman, William D, M.S.
Mogar, Robert E, Ph.D.
et al.

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Norman I. Dishotsky, M. D.
Research Department
Mendocino State Hospital

William D. Loughman, M.S.
Donner Laboratory
University of California

Robert E. Mogar, Ph.D.
Psychology Department
Mendocino State Hospital
Professor of Psychology
San Francisco State College

Wendell R. Lipscomb, M. D.
Chief of Research
Mendocino State Hospital

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LSD USE AND GENETIC DAMAGE:

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Is LSD a chromosome damaging agent with carcinogenic, mutagenic, and teratogenic properties in man?

Norman I. Dishotsky, William D. Loughman, Robert E. Mogar,

Wendell R. Lipscomb

Chromosome damage in human white blood cells, leukemia, malformed infants, and animal mutations have been reported subsequent to LSD exposure. The grave implications for the biologic fitness of future generations and the fear and doubt aroused in millions of people who have experimented with this drug remain unresolved, in spite of vast expenditures of scientific energies. In the past 3.5 years 66 studies and case reports directly related to this issue have been published. We have undertaken a critical review of these studies in order to clarify what is presently known. Problems will be identified that require further research. The questions we attempt to resolve are: Is LSD a chromosome breaking agent? Is it a carcinogen, a mutagen, or a teratogen in man?

Chromosome Studies In Vitro

Cohen and associates (1) added LSD to cultured human leucocytes in five concentrations ranging from 0.001-10.0 µg LSD/ml for 4, 24, and 48 hours each. The breakage rate for treated cells was at least twice the control rate for all treatments except the lowest concentration and time [0.001 µg LSD/ml for 4 hours], where no difference existed between treated and control cells. The frequency of aberrations, however, was not simply related to dosage or duration of exposure.

Loughman et al (2) emphasized that it is precisely the lowest concentration and duration of exposure that most closely approximates the expected concentration in blood, liver and other organs after a 100 µg LSD dose ingested by a 70 kg man. For example, the half time of LSD in plasma is approximately 175 minutes in mice (3) and man (4), giving a mean circulation time of about 4 hours. A 100 µg dose of LSD, uniformly distributed in a 70 kg man will yield a uniform concentration of 0.0014 µg/g. The expected concentration in blood after 30 minutes is 25 per cent of this value, or 0.004 µg/g. If the metabolic degradation of LSD is considered (3), then the effective in vivo concentration of unchanged LSD would be approximately .0001 µg/ml -- a concentration used only by Kato and Jarvik (5), at which no increase in breakage was found.

In vitro experiments on a variety of cell types have been reported by 8 teams of investigators [see Table 1]. Chromosome damage has been reported (1,5-9), although not consistently (10-12) at concentrations and durations of exposure greater than .001 µg LSD/ml for 4 hours. In a second study Cohen et al (6) found significantly elevated break rates at this lowest concentration and duration of exposure. Their finding has not been confirmed by Tjio (8) in an unpublished pilot study.

Interpretation of the significance of chromosome change in vitro requires the following considerations:

(i). All of the study performed on cultured lymphocytes used a modification of a technique described by Moorehead et al (13), in which phytohemagglutinin stimulates lymphocytes to enter the reproductive cell cycle. In the normal in vivo state small lymphocytes are in the G_1 or pre-DNA synthetic phase. They do not grow, or divide or enter the cell cycle (14-16). Thus, in the in vitro studies lymphocytes are exposed to chemical agents during stages of the cell cycle, including DNA synthesis, which do not normally occur in these cells in the body. Damage to a lymphocyte occuring in the G_1 phase generally will not manifest itself as a chromatid-type change in a

subsequent division (15,17). Most, if not all, chromatid-type changes are initiated by technical procedures (15). The great majority of lesions reported in most in vitro and in vivo studies were chromatid-type; therefore, the findings of elevated chromosomal break rates in lymphocytes exposed to LSD in vitro must be interpreted with great caution.

(ii). This need for caution is well illustrated by the large numbers of conditions and substances in common usage reported to induce chromosomal breakage in vitro such as: changes in osmotic pressure, changes in temperature, changes in oxygen levels, the nucleotides, antibiotics, benzene, caffeine, calcium and magnesium deficiencies, chloroform, DDT, hydrogen peroxide, mercury compounds, morphine, plant pigments, salicylates, theobromine, theophylline, vegetable oils, fats and essences, vitamins, water unless twice distilled, and many others (18). A current example may be derived from the study of Kato and Jarvik (5) who examined the comparative breakage rates induced by LSD, acetyl salicylatic acid [Aspirin], and ergonovine maleate, a widely used oxytocic. Each compound doubled the control rate and appeared equally effective in producing breaks [10.2, 9.6, 10.0 per cent, respectively]. Aspirin-induced chromosome breakage in vitro has been supported by one study (19), but not by another (20) in which both in vitro and in vivo experiments were performed.

(iii). The intact human organism differs markedly from the in vitro test tube in its ability to detoxify and excrete noxious compounds. Compounds that are toxic in vitro do not necessarily have the same effect in vivo.

Insert Table 1

In summary, no consistent dose-response correlation has been reported. Generally, damage occurred with high concentrations and/or prolonged exposures which could not be achieved in humans with reasonable doses. The magnitude of breakage, when found, was within the range induced by many agents in common usage. We believe that the special

nature of the in vitro test system, the several negative reports, and the absence of excretory and detoxification mechanisms all suggest that the in vitro results would inadequately predict the effects of LSD exposure in man.

Chromosomal Studies In Vivo

Does ingestion of LSD by humans produce chromosome damage? If so, is the damage related to carcinogenesis or genetic damage in subsequent generations? Viewed singly, many of the 21 in vivo reports from 17 laboratories contain both major deficiencies and minor shortcomings. As a result, individual reports have been contradictory and at best inconclusive. However, consistent trends do emerge from the evidence examined comprehensively.

Two types of experimental design were employed: In 11 of the studies LSD groups consisted of individuals exposed to known quantities of pure LSD in experimental settings. In 14, subjects were exposed to illicit substances of unknown composition and potency alleged to be LSD. The significance of distinguishing between medically supervised pure LSD-treated groups in contrast to illicit LSD-exposed groups was recently suggested by several reports that showed marked discrepancies between alleged and actual composition and potency of illicit drugs (21-24).

A total of 310 subjects have been studied [see Tables 2-4]. Of these, 126 were treated with pure LSD; the other 184 were exposed to illicit "alleged" LSD. Eighteen of 126 [14.29 per cent] of the subjects in the pure LSD group had chromosome aberration frequencies elevated above mean control rates. In contrast, 89 of 184 [48.9 per cent] of the subjects in the illicit LSD group had elevated aberration frequencies. The frequency of individuals with chromosome damage reported among illicit drug users is more than triple that associated with use of pharmacologically pure LSD. Of all the subjects reported to have chromosome damage, only 16.67 per cent [18/108] were treated with pure LSD.

Insert Table 2

Illicit LSD and Chromosome Damage

Irwin and Egozcue (25,26) reported the initial findings of chromosomal damage in illicit LSD users. In the first (25) of two reports, illicit LSD users had a 23.4 per cent mean breakage rate, nearly double the 11.0 per cent mean breakage rate in drug free controls. Only 2 of the 8 users cited did not have elevated breakage rates. In a subsequent more extensive study (26) the same authors reported a 18.76 per cent mean breakage rate in 46 illicit LSD users [range 8-45 per cent] which was double the 9.03 per cent rate in control cells. Only 3 of the 46 users did not have a breakage rate elevated above the mean control rate. Of 4 children exposed *in utero*, 1 did not have elevated breakage rates. The authors emphasized the long-lasting effect [up to 2 years] suggested by these findings. There was no evidence of ill health or physical malformation in any of the children.

These findings were supported by Cohen *et al* (6) who reported that 18 illicit LSD-exposed subjects had an elevated chromosome breakage rate which was triple the 3.8 per cent mean control level. All illicit LSD-exposed subjects had breakage rates elevated above the mean control level.

Cohen *et al* (27) reported that 13 of the 14 illicit LSD-exposed adults had chromosome breakage rates elevated above mean control values. Nine children exposed to illicit LSD *in utero* had a 9.2 per cent mean breakage rate compared to 4.0 per cent in 4 children whose mothers used illicit LSD prior to but not during pregnancy. The control rate was one per cent. Here, too, the authors emphasized the apparent long-lasting effect suggested by the findings. All but 2 children were exposed to other drugs during pregnancy, all were in good health and exhibited no birth defects.

Nielsen et al (28) found that 10 subjects exposed to illicit LSD had a mean breakage rate [2.5 per cent] elevated above controls [0.2 per cent]. However, the 2.5 per cent rate was below the control rates of the other positive studies, and the number of subjects with elevated breakage rates was not given. In all of the positive studies there was no correlation between dose, time since last exposure, number of exposures, or frequency of aberration.

Nine separate teams of investigators have been unable to support the positive findings (2,29-36). [See Table 2.] In three studies (30-32) karyotypes were constructed to detect the presence of translocations, deletions, Ph^1-like chromosomes, and other morphological changes. None were found. In another report (33) autoradiographic studies of cells from 20 exposed individuals showed no difference in labeling pattern from controls.

Several of the negative studies have attempted to resolve the discrepancies between positive and negative reports by criticizing the control breakage of Cohen et al [3.8 per cent] and Irwin and Egozcue [11.9 and 9.03 per cent] for being unusually high (2,5,29,51). Extensive studies of normal populations have provided both low and high control rates (17,37-40). [See Table 3.] It was suggested that the high control values may have arisen from sub-optimal conditions of culture (2), less-than-complete media (29), viral contamination of culture (5), or artifacts arising from micromethods of culture (51). In the studies of Cohen et al and Irwin and Egozcue most of the aberrations described were chromatid-type changes. Chromosome and chromatid-type changes were not reported separately, but were combined and then converted to "equivalent numbers of breaks". Combining chromatid and chromosome aberrations into a single index obscures the distinction between real chromosome damage occurring in vivo and spurious damage arising in the course of cell culture.

All of these effects should distribute randomly between illicit LSD-exposed and control groups, but they do not. Therefore, these factors do not explain the non-

random significantly elevated breakage rates in 80 of 86 illicit LSD-exposed subjects studied by Cohen et al and Irwin and Egozcue.

The studies reviewed above employed subjects using illicit LSD. As a result, the experimenters relied upon the subjects' recall and reliability to establish patterns of drug use. Dosage, frequency, purity, total number of exposures, and interval since last exposure were crude undocumented estimations.

We believe impurities were very likely to have been present in the drugs used by these individuals. Marshman and Gibbins found wide discrepancies between the alleged and actual composition of illicit drugs (21). Only 54 per cent of 57 samples alleged to contain LSD actually contained this drug in a relatively pure form. The remainder contained a large proportion of impurities or no LSD at all. The Federal Bureau of Narcotics and Dangerous Drugs Laboratory in San Francisco has found DOM [4-methyl-2,5-dimethoxyamphetamine, or STP] and PCP [phenylcyclohexyl piperidine] either substituted entirely for LSD or added to samples containing LSD (22).

Krippner (23) analyzed 12 tablets alleged to contain 250 µg LSD. One contained no LSD; most contained very small quantities [2, 7 26 µg]; some contained more DOM than LSD; only two contained more than 150 µg LSD. Discrepancies of comparable magnitude in illicit LSD samples were also reported by Cheek and Joffee (24).

These findings suggest that the estimated LSD dosages obtained from interview data of subjects using illicit LSD were highly unreliable and must be interpreted with extreme caution. Nearly all the subjects used and/or abused drugs other than LSD. These included alcohol, amphetamine, antihistamines, aspirin, barbiturates, cannabis, cocaine, diethyltryptamine, dimethyltryptamine, DOM, heroin and all other varieties of opiates, mescaline and the methylenedioxy amphetamines [MDA, MMDA], nicotine, peyote, phenothiazines, psilocybin, and ritalin.

The role which might be played by other chemical agents was illustrated in the study of Cohen et al (6). In a group of 6 individuals ingesting drugs other than

illicit LSD [chlorpromazine, amphetamine, barbiturates, cannabis, and diphenhydramine] the mean breakage rate of 12.6 per cent was not significantly different from the 13.2 per cent value in the 18 subjects exposed to illicit LSD with other drugs.

Chromosome breakage rates elevated above mean control rates were found in 89 of the 184 adults [less than half] exposed to illicit LSD. Of these, detailed drug histories were available on 77 subjects, 43 of whom [56 per cent] used amphetamine. In the experience of the present authors, abusers of amphetamine are the most physically debilitated segment of the drug abuse subculture. Of all illicit drugs, methamphetamine is reputed by users to be especially contaminated and toxic, an impression which has been substantiated. Three solvent residues -- benzene, ether, and chloroform -- used in the manufacture of methamphetamine, as well as dangerous unreacted raw materials such as mercury, phenyl-2-propanone, methylamine, etc., have been found in illicit samples of this drug (22). Material sold as liquid methamphetamine was found to contain human urine and a toxic floor cleaner(21).

We believe the original sample populations were inadequately described as "LSD users". In fact, most subjects were multiple drug users, abusers, or addicts exposed to toxic substances of unknown composition and potency. Given the unreliability of self-report and the unpredictable composition of illicit drugs, it is unlikely that all investigators could have sampled comparable drug abuse populations.

Smith and Rose (41) have reported very high rates of hepatitis and other viral illness in a drug abuse population in San Francisco. The role of viral infection, reported to induce chromosome damage (42,43), was not independently assessed in the positive studies. The role of malnutrition, a predisposing factor to infection and a common condition among amphetamine abusers, remains to be evaluated.

The numerous methodologic questions which have been raised here certainly qualify, but do not completely dismiss the unusually high aberration frequencies reported by three separate teams of investigators. It could well be that multiple

factors such as chronic excessive exposure to illicit chemical agents, the presence of toxic contaminants, the intravenous route of administration, and the physical debility of many drug abusers would result in chromosome breakage to the extent reported. However, all of the drug abuse subjects shown to have elevated chromosome breakage rates [90 of 184] were in the early reports of three laboratories. Nine independent teams of investigators studying similar individuals not only failed to substantiate these earlier findings but were able to demonstrate only a single instance (31) of chromosome damage beyond that present in the general population. We conclude that positive results, when found, are related to the more general effects of drug abuse, and not as initially reported to LSD use.

Pure LSD and Chromosome Damage

In the chromosomal studies of pure LSD users, the potency, purity and frequency of exposure to LSD were definitely established independently of the subject's presumed knowledge or recalled estimates. The interval between exposure and blood sampling was under direct experimental control. For these reasons, the studies of pure LSD users provide the most reliable evidence concerning the questions: Does LSD damage human chromosomes *in vivo* and, if so, is this damage of a transitory or relatively permanent nature?

The studies of pure LSD users can be divided in two classes: Most compared a post-LSD exposure group to a non-LSD or drug free control group. For convenience and clarity of description, this experimental approach will be referred to as the "post exposure design", which is to be distinguished from the *before and after* approach, or the "pre-post design", in which each subject is used as his own control.

Chromosome Studies After LSD Treatment

Chromosomal damage in human white blood cells after treatment with pure LSD was initially reported by Cohen *et al* (1). In this investigation chromosome damage

[a chromatid exchange figure and a significantly elevated breakage rate] was observed in one paranoid schizophrenic individual treated 15 times with 80-200 µg LSD over a period of 5 years. The authors suggested that chromosome damage found 8 months after the last LSD treatment was analogous to the chromosome damage of long duration induced by radiation and associated with leukemia (44-46). However, they also suggested caution in interpretation of their findings since the patient had been treated with the phenothiazine chlorpromazine and chlordiazepoxide until 8 months prior to the study.

A review of the chromosomal studies of post-exposure design revealed that all subjects reported to have an elevated chromosome breakage rate [12 of 70 -- 17.1 per cent] were in the studies of two laboratories (1,28,47,48). Five independent teams of investigators (7,29,35,49-51) not only failed to confirm these earlier reports but were able to demonstrate only one doubtful instance of chromosomal damage beyond that present in the general population (50). [See Table 3.]

Insert Table 3

By far the largest number of pure LSD treated subjects with chromosome damage were reported by Nielsen et al. In their first report these authors find "no correlation between any specific drug and the frequency of gaps, breaks and hyperdiploid cells".(28) In a subsequent publication (47) Nielsen et al regroup their original data [same subject, cells, and aberrations] and conclude that LSD induced chromosome damage. Five aberrations initially tabulated in a phenothiazine treated group were redistributed in 4 groups of 10 individuals arranged by sex and age. This redistribution transforms a homogeneous group based on drug exposure into smaller, less meaningful groups based on sex and age. In addition, Tjio has emphasized that the number of cells analyzed [71.6 per LSD subject and 34.4 per control on the average]

was insufficient to determine reliable break rates (51). This variable is particularly relevant due to the small number of lesions identified. Three of the 5 LSD subjects had no aberrations. Two subjects accounted for the 6 breaks found. The 1.7 per cent breakage rate is well within the values reported for the general population [see Table 3].

In a second study by Nielsen et al (48) the methods used to count and analyze data were atypical. For example: (i). In tabulating exchange figures and fragments, the pure LSD subjects were combined with subjects exposed to illicit LSD, making intergroup comparisons impossible. (ii). Dicentrics, rings, acentric fragments and centric fragments were scored separately. Damage that produces dicentrics and rings simultaneously produces acentric fragments. Therefore, the aberration rate may have been inflated, since two-thirds of the lesions found in the combined LSD group were acentric fragments. (iii). As few as 2 or 3 patients with markedly elevated breakage rates could account for all the breakage found. This and other conclusions must remain speculation since the actual number of treated patients with breakage rates above controls was not given.

In summary, 82.9 per cent [58 of the 70] of the subjects studied after treatment with pure LSD did not have chromosome damage. Due to incomplete data on 9 of the remaining 12 subjects, it is not possible to compute a precise percent of subjects with elevated breakage rates. All but one were reported by a single team of investigators (28,47,48). In light of the procedural shortcomings, incomplete data, questionable data re-analysis and low breakage rates in their reports, we conclude that there is no definite evidence from the post-treatment studies that pure LSD causes chromosome damage.

Before and After LSD Use

Tjio et al (51) reported a carefully designed and executed study in which over 22,500 cells from 37 patients were examined before and after treatment with LSD.

The number of cells observed was more than double the total combined number of cells observed in all other studies of pure LSD users. The effects of both single and multiple treatment were examined. In the study on the effects of a single treatment with 50-450 µg LSD, pre-exposure baseline breakage rates were established 1-66 days prior to treatment. Post-exposure rates were established 1-10 days after treatment. The mean difference for 32 subjects was +1.6 per cent. There was no relationship between dose and amount of breakage. The low dose group had a greater pre-post difference than the high dose group. There was no significant difference between before-and-after-LSD breakage rates in either high or low dose group. Another group was composed of 5 persons who had taken illicit LSD from 4-36 times prior to the study. In this series the investigators took blood cultures for 7-10 consecutive days before, during, and after treatment with pure LSD [1 µg/kg and 2 µg/kg] 2 or 3 times. Again, there were no significant pre-post differences.

These findings have received support from two other pre-post studies (7,52). Of the 53 individuals who ingested pure LSD in these 3 studies, 50 [94.3 per cent] showed no increase in breakage rates. [See Table 4.]

In contrast, Hungerford et al (53) observed a pattern of chromosome aberration increase following each of 3 intravenous LSD treatments. This is the only study utilizing intravenous administration. The elevated aberration rate was small in 2 of the 3 subjects; however, dicentric and multiradials appeared only after treatment and acentric fragments appeared more frequently after treatment. Blood samples taken 1-6 months after the final dose showed a return to pre-LSD baseline levels.

Transitory effects were also reported after multiple high dose subcutaneous injections in Rhesus monkeys (54). Statistical evaluation of these findings were not provided. Our analysis reveals that there were no statistically significant positive effects.

Insert Table 4

In summary, only 6 of the 56 patients [10.7 per cent] studied before and after treatment with pure LSD had elevated breakage rates; of these, 3 received LSD intravenously, one had a viral infection (51). One individual was not available for follow-up determinations (51), but the remaining 5 demonstrated a complete return to pretreatment breakage levels, indicating a reversibility of effect and, as suggested by Hungerford, a mechanism for repair or elimination of aberrations.

89.3 per cent of the subjects in the pre-post studies did not have chromosome damage. This confirms the conclusions of 5 of 7 laboratories employing the post-exposure design. We conclude that the ingestion of moderate doses of pure LSD does not break human chromosomes.

Is LSD a Carcinogen?

Cohen et al(1) first suggested the carcinogenic potential of LSD. Their speculation was based on the finding of a type of chromosome exchange figure, the quadriradial, and a markedly elevated breakage frequency. Such findings partly characterize three inherited disorders considered to have a high incidence of leukemia and other neoplasia (55). The cause of the chromosomal lesions in these disorders is not known, nor is it known whether they have any relation to subsequent neoplastic development. Moreover, there are many chromosome breaking agents which do not cause leukemia and the two may be quite distinct effects. No cause and effect relationship was demonstrated and none is known. Quadriradials and other rearrangement figures have been found in white blood cells of normal individuals (2,38,39,56).

These authors also suggested that the broken and rearranged chromosomes found in one patient 8 months after the last LSD treatment was analogous to the chromosome damage of long duration induced by radiation and associated with leukemia. The finding of long-term damage has been supported by 3 studies (25-27) which were retrospective. In two pre-post studies (51,53) occasional damage, when found, without exception was transitory, suggesting a reversibility of effect unlike that associated with radiation.

Supporting the carcinogenic hypothesis, Irwin and Egozcue (25,26) reported that 9 illicit LSD subjects had centric fragments resembling the Ph^1 chromosome often associated with chronic granulocytic leukemia. Grossbard (57) reported a Ph^1-like chromosome in all 35 peripheral leukocytes examined from an individual using illicit LSD and other illicit drugs who had acute leukemia. There was no other indication of chromosome damage in peripheral cells. We are not aware of other reports of leukemia in illicit LSD users and we do not find other reports of a Ph^1-like chromosome in pure or illicit LSD-exposed subjects. The Ph^1-like chromosome was found in peripheral leukocytes in both reports. However, in C.G.L. the Ph^1 chromosome is only characteristic of myeloid and erythroid cells which normally do not divide in peripheral blood. Hungerford, who along with Nowell (58) initially described this lesion, writes, "A chromosome compatible with the Ph^1 would have to be observed in blood cells other than lymphocytes to be relevant to the question of chronic granulocytic leukemia." (59), and ".... in the absence of appropriate retrospective data concerning cancer patients and data concerning carcinogenic effects of LSD in experimental animals, the suggestion remains highly conjectural."(53)

There are 2 reports of leukemia in individuals treated with pure LSD. In one case there was a ".... remarkable incidence of childhood malignancies ... strongly suggestive of a familial predisposition to malignant disease"(60). In the second, no details were given (61).

Information relating LSD to leukemia is rare; to date there appears to be no definite evidence that LSD is a carcinogen.

Is LSD a Mutagen?

Mutagenesis has been widely studied in Drosophilia. Radiation and chemically induced mutations were initially detected in this organism (62,63).

Grace and Carlson (64) performed genetic tests of LSD for sex-linked lethals [a technique uniquely sensitive to mutagenesis], sex-linked recessive visibles, translocations, and a specific visible. Treated flies received intra-abdominal

injections of solutions with concentrations of 1-500 µg LSD/ml. No significant differences were found between treated and control populations. The absence of translocations places LSD in a class quite distinct from ionizing radiation or potent chemical mutagens. The authors concluded, "LSD, if it is a mutagen or radiomimetic agent in human chromosomes, ... it is not a very powerful one. It is more probable that LSD induces neither mutation nor chromosome breaks in man."(64)

These findings have been supported by 2 other Drosophilia studies at concentration ranges of 0.28-100 µg LSD/ml (65,66). A study using a different standard genetic system (the ascomycite Ophistoma multiannulatum) exposed cells to 20-50 µg LSD/ml. There was no difference between treated and control cells (67).

When Drosophilia were injected with 2,000-10,000 µg LSD/ml significant increases in lethal mutations were found (66,68). The evidence of no effect from 0.28-500 µg LSD/ml and a definite effect from 2,000-10,000 µg LSD/ml is consistent with a threshold dose response (69), or a sigmoid dose-effect relationship.

If LSD was ingested, doses 18 times greater [420 µg/g body weight] than those injected were required to induce lethal mutations (66). Positive mutagenic effects were obtained by injection only at or above 470 µg LSD/g. On a body-weight basis this would be roughly equivalent to a 32.9 million µg dose of LSD in a 70 kg man.

The evidence from Drosophilia and fungi strongly suggests that LSD is a weak mutagen effective only in extremely high doses and it is unlikely to be mutagenic in any concentration used by human subjects.

LSD and DNA

Early reports of an LSD-chromosome interaction suggested the need for direct observation of the effect of LSD on DNA. Yielding and Sterglanz (70) performed spectrophotometric studies on the binding to calf thymus DNA of the active and inactive optical isomers of LSD and a hallucinogenically inactive analogue [D_1L-2-Br-LSD]. They noted similar patterns of optical absorption. The authors suggested that binding to DNA might be a general property of this group of compounds. Binding did not take place with yeast-RNA or non-helical DNA, suggesting that binding is specific for

helical DNA. They interpreted their data as showing that each nucleotide residue in helical DNA is a potential binding site.

Conformation of macromolecules and conformational alteration have been extensively studied by circular dichroism [unequal absorption of circularly polarized light]. Wagner (71) described LSD as a planar, cationic, aromatic molecule that has the molecular characteristics necessary for interaction with the phosphate anions and for stacking between the bases of the DNA helix. He reported circular dichroism experiments which suggested that the specific mechanism of action of LSD on DNA was a direct interaction, by intercalation of LSD within the DNA helix, causing conformational changes which appeared unlikely to result in a decrease of internal stability sufficient to cause breakage of chromosomes. Smythies and Antun (72) performed similar experiments which supported the hypothesis that LSD binds to nucleic acids by intercalation.

We agree that the LSD-DNA interaction is inadequate to explain the claimed chromosome breaking potential of LSD. However, the evidence of LSD intercalation into the DNA helix seems to provide the physical mechanism for the high dose mutagenic effects reviewed above.

Is LSD a Teratogen?

It has been postulated that LSD may be a potential cause of congenital malformations, fetal wastage and germinal chromosome mutation. Data from humans and primates is scanty; the major body of information has been obtained from 14 rodent studies.

Auerbach and Rugowski (73) reported that low doses of LSD administered early in gestation [0.05-1.0 µg, days 6-7] induced a high rate of embryonic malformations in several strains of mice. CNS abnormalities were common, but there was no observable effect of LSD exposure occurring later than day 7 of gestation.

In a second study (74) with a different strain of mice at higher doses, a high incidence of lens abnormalities was reported, although malformations of CNS were not

found even on histologic examination. In a third study (12) the frequency of malformed embryos in a different strain was 19 per cent compared to 10 per cent in controls [at doses 25-1,000 times the usual human dose], suggestive of a potentiation of individual threshold differences. In this study another strain that received equivalent doses showed no teratogenic effect, suggesting strain specificity. In a fourth study (75) no abortifacient, teratogenic, or embryonic growth depressing effects were observed even at enormous doses in 521 offspring.

An autoradiographic study (76) on pregnant mice showed that ^{14}C-LSD easily diffused and penetrated cell membranes and rapidly crossed the placental barrier and entered the fetal circulation. Early in pregnancy 2.3 per cent of the ^{14}C-LSD traversed the placenta within 5 minutes compared to only 0.5 per cent late in gestation.

It has been suggested that a teratogenic effect might occur through direct chromsomal damage to germ cells (6). Such damage could only be ascertained by direct observation of germinal cells from gonadal biopsy. A study (77) on meiotic chromosomes of male mice revealed a small number of breaks, gaps, and fragments in treated animals -- statistically not significant -- at massive doses [1-8,000 µg LSD]. Strongly positive indications of male meiotic damage were seen in another study at doses [25 µg/kg] that were 6-8 times the usual human dose on a body weight basis (78). Both of these studies examined male meiosis only. A third study applying both acute [27-30 µg/LSD) and chronic exposures [0.1-5.0 µg LSD/day for 8-31 days] to males and females failed to find any evidence of structural change in meiotic chromosomes or impairment of meiotic activity (79). Yet the doses used were 90-fold the level shown to be teratogenic for several strains of mice (73).

Insert Table 5

Stunted and stillborn offspring were reported (80) in Wistar rats following a single subcutaneous dose [5 µg LSD/kg] early in gestation [4th day], but not late in pregnancy. This study was performed on only 10 mothers with 85 offspring. There are 4 other studies (75,81-83) on Wistar rats which involve: Very large numbers of offspring [approximately 1500]; extremely high doses [100-6,000 µg]; meticulous repeats [same dose, day, species, and route of administration]; histologic examination; maze running, shock avoidance and food competition (82,83). All report negative results. [See Table 5.]

A study (84) in which hamsters received a wide range of doses of LSD [0.84-240 µg] on the 8th day of pregnancy revealed 5-8 per cent malformations of the brain, spinal cord, liver and other organs. Some fetuses had several malformations and the actual percentage of malformed fetuses was not specified. No correlation existed between dose and number of malformations. These findings have not been confirmed by 2 other investigators (12,75) using higher doses [10-500 µg/kg]. One author (12) suggested that in the case of a truly positive teratogenic effect a dose-response relationship and an increase in abnormalities much higher than 5-8 per cent [average of less than 1 per litter] would be expected.

A study in rabbits (85) demonstrated no teratogenic effect after doses of 20 and 100 µg LSD/kg on days 4-12 of gestation.

An overall view of the rodent studies indicates a wide range of individual, strain, and species susceptibility to the effects of LSD. The effect, when found, was at a highly specific time early in gestation. No effect was reported with exposures occurring late in pregnancy. Cohen et al (27) have suggested that if a similar critical period exists early in human pregnancy the result would be abortions in the majority of instances, rather than live-born malformed infants.

Rodent studies are indications of what might possibly occur in humans; however, the fetal growth and development in these species is markedly different from man.

For example, human and rodent placentation differ in the degree of intimacy between fetal and maternal circulations. In the higher rodents is found the nearest approach to actual intermingling of the blood of the two circulations [the hemoendothelial placenta]. In humans there are chorionic villi [hemochorial placenta] which in rodents reduce to bare blood vessels whose endothelial walls alone separate the fetal blood from the maternal sinuses (86). For this reason alone, rodents are more sensitive than humans to the teratogenic potential of any given substance. Auerbach considers direct "... extrapolation from mice to man to be hazardous".(87)

Kato et al (54) reported the effects of multiple [4-11] subcutaneous injections of LSD in pregnant Rhesus monkeys. Single doses of 0.125-1.0 mg/kg [total dose: 0.875-9.0 mg/kg] were administered to 4 monkeys starting at months 3-4 of gestation. Of the 4 treated animals, one delivered a normal infant, 2 were stillborn with facial deformities, and one died at one month. Two control animals delivered normal offspring. The lowest dose exceeded by 100-fold the usual experimental dose in man. The authors did not believe this preliminary data warranted conclusions.

The information from man is meager. Three studies reported that ingestion of illicit LSD by pregnant women resulted in elevated numbers of chromosome breaks in their offspring (26,27,31). Of the 14 children exposed to illicit LSD in utero, 10 had elevated breakage rates. The one child studied serially demonstrated a return to normal chromosome breakage rate (31). We have suggested that these findings are relevant to the effects of drug abuse in general rather than to the effects of pure LSD. All of these children were in good health and had no birth defects.

There are 5 cases of malformed infants born to women using illicit LSD prior to or during pregnancy. Zellweger et al (88) reported the birth of an infant with unilateral fibular aplastic syndrome to a woman who used illicit LSD 4 times during the first trimester. Hecht et al (89) reported an infant with a malformed arm born to a woman who had been exposed to illicit LSD, cannabis, and several anti-emetic drugs

during the first trimester. Carakushansky et al (90) reported an infant with a terminal transverse deficit of portions of fingers of the left hand and syndactyly of the right hand whose mother was exposed to illicit LSD and cannabis during pregnancy. Hsu et al (91) reported an infant girl born with trisomy-13 to parents who used illicit LSD prior to but not during pregnancy. These authors suggested that LSD could have damaged the germ cells prior to pregnancy. However, the mother was using cannabis, barbiturates, and amphetamines during pregnancy. We suggest that the transplacental effects of these compounds cannot be discounted. Eller and Morton (92) reported an infant with severe deformities born to a woman who had ingested illicit LSD once near the time of conception. An estrogen and medroxyprogesterone were ingested in the first trimester. The authors stated that previously described infants with this combination of anomalies, referred to as spondylothoracic dysplasia, were usually the result of consanguinous marriages, and the cause probably involves an autosomal recessive mode of inheritance.

In all 5 reported cases the drug was illicit. To date there is no report of congenital malformations in human offspring exposed to pure LSD.

McGlothlin et al (93) reported frequencies of spontaneous abortions, premature births, and children with birth defects in 148 pregnancies when one or both parents were exposed to pure or pure and illicit LSD. The only increased risk observed was in spontaneous abortions in the pure plus illicit LSD group [37 per cent] compared to the pure LSD group [15 per cent] and the general population [20 per cent]. Only 12 pregnancies involved ingestion of LSD [pure vs. illicit not specified] during gestation; 6 of these ended in abortions. The authors note that one woman accounted for 5 of the 10 abortions in the pure plus illicit group and 5 of the 6 abortions involving exposure during pregnancy; she had one abortion prior to LSD use. If this subject is deleted from the calculations, the spontaneous abortion rate is 24 per cent in the pure plus illicit group and 14.4 per cent for exposure during pregnancy.

Hulten et al (31) performed a gonadal biopsy and reported no evidence of an increased rate of meiotic chromosome aberrations in a male 6 months after the last ingestion of a very large amount of illicit LSD and other drugs. This is the only study of human or primate germ cells exposed to LSD.

In summary, then, a teratogenic effect has been reported in hamsters, rats and mice, with confirmation only in mice. The information from lower primates, although preliminary, is suggestive of a teratogenic effect and deserves further investigation. Case reports of malformed children born to illicit LSD users are rare; although there is some indication of an increased risk of spontaneous abortion. There is no evidence that pure LSD causes birth defects or fetal wastage in man.

Summary and Conclusion

Of 9 in vitro studies, 6 have reported some degree of induced chromosomal breakage after exposure to LSD; 3 reports failed to confirm these results. The damage, when found, was generally of the chromatid type, arising during or after DNA synthesis. This damage, with one exception, was the result of concentrations of drug and durations of exposure which could not be achieved in humans with reasonable dosages. There did not appear to be a dose-response relationship. The magnitude of damage, when found, was in the range encompassing the effects of many commonly used substances. The absence in vitro of excretory and detoxifying systems present in vivo, and several negative reports, cast doubt on the relevance of in vitro results.

In 21 in vivo chromosomal studies a total of 310 subjects were reported. Of these, 126 were treated with pure LSD; the other 184 were exposed to illicit "alleged" LSD. Only 18 of 126 [14.29 per cent] of the subjects in the pure LSD group were reported to have chromosome aberration frequencies above mean control rates. In contrast, 89 of 184 [48.9 per cent] of the subjects in the illicit LSD group had elevated aberration frequencies. Of all the subjects reported to have chromosome damage, only 18 of 108 [16.67 per cent] were exposed to pure LSD. The frequency of individuals

with chromosomal damage reported among illicit drug users was nearly triple that associated with the use of pharmacologically pure LSD. We conclude that chromosome damage, when found, was related to the more general effects of drug abuse and not, as initially reported, to LSD use. We believe that pure LSD ingested in moderate dosages does not produce chromosome damage detectable by presently available methods.

The suggested carcinogenic potential of LSD has been a cause of great concern. However, no significant work has been reported to date. No cause and effect relationship and no increase in the incidence of neoplasia among LSD users has been demonstrated. Case reports [3 in 3.5 years] of leukemia and other neoplasia in this population are exceedingly rare.

The results of early chromosome studies suggested that true genetic damage might be a consequence of LSD exposure. The comprehensive evidence from Drosophilia indicates no mutagenic effect from 0.28-500 µg LSD/ml and a definite mutagenic effect from 2,000-10,000 µg LSD/ml; this is consistent with a threshold response or a sigmoid dose-effect relationship. We believe that LSD is, in fact, a weak mutagen, effective only in extremely high doses; it is unlikely to be mutagenic in any concentration used by human subjects.

Circular dichroism experiments suggested that the specific mechanism of action of LSD on DNA was a direct interaction. Intercalation of LSD within the DNA helix caused conformational changes. These are unlikely to result in a decrease of internal stability sufficient to cause breakage of chromosomes, but may be the physical basis of the weak mutagenicity.

Early chromosomal studies implicated LSD as a potential cause of congenital malformations, fetal wastage and germinal chromosome damage. First reports of a teratogenic effect in hamsters and rats have not been confirmed. A review of 14 rodent studies indicated a wide range of individual, strain, and species susceptibility to the effects of LSD. The applicability of such investigations to man is doubtful.

Information from lower primates is regrettably meager. In a study of human pregnancies, those exposed to illicit LSD had an elevated rate of spontaneous abortions. There is no reported instance of a malformed child born to a woman ingesting pure LSD; there are 5 such cases associated with exposure to illicit LSD. Given the high frequency of unexplained "spontaneous" birth defects, the rare occurrence of malformed infants born to women who used illicit LSD may be coincidental. There is no evidence that pure LSD is teratogenic in man. Nevertheless, in light of so many unexplained birth defects, the use of any drug during pregnancy requires that its potential benefits significantly outweigh its potential hazards.

From our own work and from a thorough review of the literature, we believe that pure LSD ingested in moderate doses does not damage chromosomes in vivo, does not cause detectable genetic damage, and is not a teratogen or a carcinogen in man. Within the bounds of these parameters, therefore, we suggest that, other than pregnancy, there is no present contraindication to the continued controlled experimental use of LSD.

TABLE 1

CHROMOSOMAL STUDIES: IN VITRO

Study	Cell Type	LSD (ug)	Exposure Duration (hours)	Treated Cells (No.)	Treated Cells % Breaks	Control Cells % Breaks	Control Cells (No.)
1 (+)	Lymphocyte	0.001-10	4-48	2678	6.7-36.8	3.7	925
5 (+)	Lymphocyte	0.01	4-24	598	10.2-12.0	5.0	100
6 (+)	Lymphocyte	0.001-10	4-48	8725	7.7-17.5	3.9	1680
7 (+)	Lymphocyte	1.0	24	1010	4.0-18.7	0.0-15.1	1094
8 (+)	Lymphocyte	0.001-10	48-72	2750	10.8-72.4	5.2-7.6	500
9 (+)	Barley Root	25	4-8	791	37.7-56.2	0.0-1.6	470
1 (-)	Lymphocyte	0.001	4	200	5.0	3.7	925
5 (-)	Lymphocyte	0.0001-1.0	4-24	600	2.0-5.0	5.0	100
8 (-)	Lymphocyte	0.001-1.0	4	1250	4.8-8.4		
		0.1-10	48	750	8.0-9.6	5.2-7.6	500
		1.0	64	250	8.8		
10 (-)	Lymphocyte	0.01-0.1	48	453	7.2-8.3	4.3	184
11 (-)a	Lymphocyte	10.0	24-48	372	0.3	0.0	329
(-)b	Hamster Fibro-blast	10-20	2-48	850	0.1	0.0	400
(-)c	Vicia Faba	1.0-50	2-24	300	0.0	...	...
12 (-)	Hamster Embryo	0.9-45	24-72	274	0.0	...	...

In Vitro Chromosome studies are designated by footnote reference number. They are arranged according to reported results: (+) Chromosomal breakage rate of LSD treated cells significantly elevated above control rate, or (-) no significant elevation of breakage rate. Some studies are presented twice in order to describe conditions of both negative and positive findings.

TABLE 2

CHROMOSOMAL STUDIES OF ILLICIT LSD USERS
POST-EXPOSURE DESIGN

	Illicit LSD Groups			Controls			
Study	N	Cells(No.)	% Breaks	N	Cells(No.)	% Breaks	Interval Blood Sample Taken After Last LSD Exposure (months)
2 (-)	8	697	0.0	19	673	0.2	.033-0.75
35 (-)	14	1412	0.36	8	805	0.63	0.25-47
30 (-)	17	595	1.01	8	280	0.72	0.25-6
32 (-)	14	1284	0.76	10	1018	0.79	.002-3
29 (-)	4	937	1.4	4	950	1.5	0.25-6
34 (-)	3	...	1-4.0	11	...	4.7	...
36 (-)	5	...	wnl	..	...	...	...
31 (-)	3	350	wnl	4	...	...	5-7
33 (-)	20	...	wnl	..	...	...	...
48 (+)	10	635	2.5	41	1584	0.28	...
27 (+)	14	...	7.53	9	...	1.20	0.25-30
6 (+)	18	4282	13.2	12	2674	3.8	0.5-8
26 (+)	46	9140	18.76	14	2800	9.03	.033-12
25 (+)	8	1600	23.4	9	1800	11.9	.033-6

Studies employing the post-exposure experimental design compared groups exposed to illicit LSD and other black market drugs to unexposed controls. Children exposed in utero are not included in Tables. Studies are designated by footnote reference number. They are arranged according to positive (+) or negative (-) findings; and then in order of increasing percent control breakage rate.

wnl - chromosome breakage rate within normal limits - percent not given
... - data not given
N - number of subjects

Study: 35(-) - chlorpromazine control group: N-2, cells(No.)-200, % breaks-1.5.
29(-) - includes combined results of 2 independent laboratories.
32(-) - also includes a cannibis exposed group: N-9, cells(No.)-816, % breaks-0.86.
27(+) - does not include 3 adults previously reported in study (6).
6(+) - control breakage rate does not include two subjects retrospectively eliminated due to viral infection. Breakage rates were 31.0 and 14.0 percent. Also includes a non-LSD, drug-using control group: N-6, cells(No.)-1361, % breaks-12.6.

TABLE 3

CHROMOSOMAL STUDIES OF PURE LSD USERS
POST-EXPOSURE DESIGN

Study	Pure LSD Groups N	Cells(No.)	% Breaks	Controls N	Cells(No.)	% Breaks	Interval Blood Sample Taken After Last LSD Exposure (months)
35 (-)	5	500	0.40	8	805	0.63	...
50 (-)	22	2200	0.86	32	3200	0.66	24-48
29 (-)	4	914	1.4	4	950	1.5	1-60
49 (-)	5	50	<2.0	5	50	<2.0	20-48
51 (-)	8	1646	2.79	2	400	2.65	2.2-14.6 9-163
7 (-)	11	1094	7.36	13	1300	7.0	0.33-96
28,47 (±)	5	358	1.70	23	802	0.00	6-38
48 (+)	9	603	4.30	30	1030	0.2	...
				11	554	0.7	...
51 (+)	1	200	12.00	6	925	3.7	8
53 (+)	1	300	3.5	3	900	1.2	...
37 (X)	...	...	...	20	1810	0.0	...
38 (X)	...	...	...	417	12,400	0.4	...
17 (X)	...	...	...	11	1,569	0.7	...
40 (X)	...	...	...	171	10,393	1.7	...
39 (X)	...	...	...	10	3,720	7.4	...

Studies employing the post-exposure experimental design compared groups treated with pure LSD to unexposed controls. Studies are designated by footnote reference number. They are arranged according to positive (+) or negative (-) findings; and then in order of increasing percent control breakage rates. Control breakage rates from 5 non-LSD chromosome studies (X) are given (17, 37-40).

Study: (50) pure LSD group does not include 1 subject with 9 percent breakage rate excluded by reason of being widely deviant.
(29) includes combined results of 2 independent laboratories.
(28) includes a group treated with psychotropic medications: N-17, Cells(No.)-510, % breaks-1.0.
(47) control group contains 40 subjects (23 drug-free controls and 17 patients treated with psychotropic medication); Cells(No.)-954, % breaks-0.4.
(48) also includes a phenothiazine treated group: N-28, Cells(No.)-1841, % breaks-1-4.5.
(53) subject not included in calculations due to prior extensive therapeutic radiation.

TABLE 4

CHROMOSOMAL STUDIES OF PURE LSD USERS
PRE-POST DESIGN

Study	N	Cells(No.)	Pre-LSD % Breaks	Post-LSD % Breaks	Pre-Post Difference	Interval Blood Sample Taken After Last LSD Exposure (hours)	Single LSD Dose (ug)
7 (-)	10	1000	5.7	4.7	-0.80	24	200-600
52 (-)	6	...	0.0	0.0	0.0	...	...
51 (-)	5	7600	2.81	3.57	+0.76	0.5-48	100-150
	32	14,984	4.28	5.91	+1.63	24-240	50-450
53 (+)	3	1,725	1.0	4.78	+3.78	1-336	187-200

In the studies employing the pre-post experimental design each subject was tested before and after treatment with pure LSD. Studies are arranged in order of increasing pre-post differences in breakage rates. Except for (53) differences were not statistically significant. Most subjects received single treatments, 5 subjects from (51) and 3 from (53) received up to three treatments. Administration of LSD was oral except in (53) where it was intravenous. Of the six subjects with significantly elevated breakage rate, five were tested again two weeks to 7.67 months after last treatment. In all five cases breakage rate returned to pre-LSD baseline.

TABLE 5

TERATOGENESES: RODENT STUDIES

-Study -Result -Animal	LSD ug/kg	Day	Treated Animals Off/Lit	Mal	Percent Dead	Runts	Controls Off/Lit	Mal	Percent Dead	Runts
73 (+) M	0.05-1.0	6-7	158/20	57	...	...	64/9	7.8	...	...
74 (+) M	5	6-9	120/18	62.5	...	...	241/28	0.0	...	...
12 (+) M	0.5-30	6-7	79/14	24	3.8	...	66/10	3.3	...	...
12 (-) M	0.5-30	6-7	167/22	0.0	...	...	58/6	0.0	...	...
75 (-) M	5-500	4-13	522/67	0.0	20.7	...	70/10	0.0	35.7	...
80 (+) R	5	4	64/10	0.0	29.6	4.7	130/10	0.0	0.0	0.0
80 (-) R	5	8-10	51/5	0.0	0.0	0.0	65/5	0.0	0.0	0.0
81 (-) R	1.5-300	4-12	887/89	0.5	5.9	0.0	...	1.0	...	...
82 (-) R	2.5-10	4	666/49	0.0	0.2	1.2	390/29	0.0	0.5	0.5
83 (-) R	$2\text{-}6 \times 10^3$	4	594/52	0.0	3.4	0.5	365/29	0.0	0.8	0.3
75 (-) R	5-500	4-13	1003/98	1.0	14.8	...	203/20	0.0	25.7	...
84 (+) H	0.84-240	8	378/37	5-8*	11.7	8.5	300/25	0.0	1.0	1.0
12 (-) H	10-300	6-9	168/14	0.6	...	...	...	...	...	...
75 (-) H	50-500	4-13	189/22	0.0	31.2	...	170/18	0.0	25.9	...
85 (-) Rab	100-300	4-12	123/14	0.0	4.1	...	45/6	0.0	6.7	...

Studies are arranged according to species: M - mice, R - Wistar rats, H - hamsters, Rab - New Zealand White Rabbit; and then, in order of positive (+) or negative (-) results.

Study: (73) - animals treated on days 8-9 had normal offspring.
(74) - 58-81% lens abnormalities, but no CNS anomalies. Offspring of animals treated on days 4-5 were normal.
(12) - data on controls not given but treated animals did not vary significantly from untreated.
(84) - 5-8% malformations.* Total number of malformed animals not given. Also includes 8-14% resorptions in treated vs 2% in untreated animals.
(81) - Control data not given but treated animals did not vary significantly from controls, i.e., 5.9% resorption rate for treated animals is within normal.

Dead: stillborn or resorption; Off/Lit: Offspring per litter; Mal: malformed animals.

The data was not presented in a uniform manner by all studies. Conversion from number of animals to percentage of animals possibly entailed minor distortions but did facilitate comparisons between studies.

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