In:Psychopharmacology. A review of progress 1957-1967.- LSD 2054
Proc.6th Annual Meeting, Amer.Coll.Neuropsychopharmacol.,
San Juan/Puerto Rico, Dec.12-15, 1967, Ed.:Daniel H.Efron
Washington, U.S.Gov.Print.Off.1968,(Public Health Serv.
Public.No.1836).pp.1247-1252

LSD and Human Chromosomes[1]

Lissy F. Jarvik, M.D., PhD. Takashi Kato, M.D. Barbara Saunders, M.A. and Emelia Moralishvili, M.A.

Department of Medical Genetics, New York State Psychiatric Institute, College of Physicians and Surgeons, Columbia University, New York

NINE months ago there appeared the first publication (Cohen et al, 1967b) attributing to lysergic acid diethylamide (LSD) the ability to damage human chromosomes not only upon addition, *in vitro,* to cultures of peripheral blood leukocytes, but equally, upon ingestion, *in vivo,* of relatively low psychedelic dosages (maximum 200 mcg). The grave implications of this report are not restricted to the LSD "users" themselves—estimated to number thousands in this country alone—who may have to face an increased risk of developing malignant neoplastic disease, but invade the realm of social conscience with the prospect of malformation and genetic illness for untold future generations. Clearly, accumulation of evidence by independent investigators for or against the deleterious effects of LSD is urgently needed.

Indeed, four separate studies have already ascribed increasing fetal mortality and morbidity to LSD administered early in pregnancy. Susceptible organisms include the rat (Alexander et al., 1967), the hamster (Geber, 1967), the mouse (Auerbach and Rugowski, 1967) and, possibly, also man (Zellweger et al., 1967).

Two other independent laboratories examined the chromosomes in cultured leukocytes of persons who had ingested LSD. One team of investigators (Irwin and Egozcue, 1967) observed a definite increase in abnormalities, while the other (Loughman et al, 1967) failed to detect any comparable chromosomal damage. Meanwhile, an expanded report was published by Cohen and collaborators (1967a) confirming the original observations.

The present study, too, represents an attempt to replicate some of the *in vitro* experiments of Cohen's group. Our technique differed primarily in the use of venous rather than fingertip blood. In accordance with modifications of the method described by Moorhead et al. (1960), we drew 20–30 ml. of the donor's blood into a heparinized (Organon) sterile disposable syringe and allowed it to sediment at 37°C for one to two hours. Enough of the supernatant leukocyte-rich plasma was added to each sterile disposable culture flask (Falcon) containing 8 ml. of growth medium—80% Eagle's Minimum Essential Medium with additional l-glutamine, 20% calf serum, 800 units penicillin, 800 mcg of streptomycin, 2.0 mcg of fungizone (GIBCO—antibiotic and antimycotic mixture × 100) and 0.2 ml. of phytohemagglutinin M (Difco)—to give a final concentration of 700,000 to 1,000,000 leukocytes per ml. Cultures were then incubated at 37° for 68 hours, at which time one of the following pharmacologic agents was added to the culture flasks, following the retention of two-drug-free flasks from each blood donor: (1) Lysergic acid diethylamide, obtained[2] in solution of 0.1 mg/ml (lysergic acid diethylamide tartrate 0.1 mg., tartaric acid 0.0087 mg., sodium chloride 7.0 mg. and water for injection to make 1 ml.) and diluted in sterile distilled water; (2) lysergic acid hydroxyisopropylamide-ergonovine maleate, U.S.P. (Ergotrate Maleate, Lilly)—obtained in commercial solution 0.2 mg/ml. (with 0.1% ethyl lactate and 0.1% lactic acid) and diluted in sterile distilled water; (3) acetylsalicylic acid-aspirin, U.S.P.—obtained as a powder and dissolved in sterile distilled water; (4) Streptonigrin (Pfizer) obtained[3] as a powder, dissolved in acetone and diluted in sterile distilled water. All dilutions used were such that addition of 0.1 ml. of the drug solution produced the

Acknowledgment: The technical assistance of Susan Verbo is hereby gratefully acknowledged.

[1] Supported in part by the U. S. Public Health Service, General Research Support Grant No. FR 05650-01.

[2] The generous cooperation of Drs. Daniel Efron and John A. Scigliano, National Institutes for Mental Health, Bethesda, Md., is hereby greatfully acknowledged.

[3] The courteous generosity of Dr. Jane Wright, Associate Dean, New York Medical College, in giving us the drug enabled us to perform this part of the experiment.

desired concentration in the culture flask. After admixture of drugs, the cultures were returned to the incubator for another two hours. Then, 2 mcg of Colcemid (CIBA) were added to each flask and the cultures incubated for two hours more. Following a total of 72 hours in culture, cells were harvested, placed in distilled water, fixed in freshly prepared methanol-acetic acid fixative, flame dried and stained with Giesma. Slides were scanned microscopically under low power (x150) and cells which appeared to be suitable for karyotyping were examined under oil immersion (x1,500). With rare exceptions, cells selected under low power were analyzed and included in the tabulations.

All slides were coded and analysis took place without knowledge of the experimental condition, i.e., type and amount of drug, if any, added to the culture. Each code was broken only after results had been recorded for slides made from all culture flasks for a given individual. In analyzing the metaphases, a chromosome count was made and emphasis was placed on identifying gross structural abnormalities of the chromosomes, specifically dicentrics, ring chromosomes, quadriradials, Philadelphia chromosomes, acentric fragments, chromatid and isochromatid breaks, chromatid and isochromatid gaps. Clear discontinuity in both chromatids of a single chromosome was classified as an *isochromatid* break. A clear discontinuity in but one chromatid was defined as a *chromatid* break. A chromosome with two discernible centromeres, distinguished from a simple crossing of arms by the absence of a darker area at the cross point, was classified as a *dicentric* chromosome. *Fusion* was defined as joining of one or both chromatid ends, without overlapping, in a tandem manner to another chromosome. An attenuated region, other than a normal secondary constriction, was classified as a *gap* and not scored as a break. Examples of some chromosomal abnormalities are shown in Fig. 1.

When the results of our pilot study are compared with those published to date (Table 1), the 4.7% breaks observed by us in 11 persons without a history of LSD consumption fall between the extremes of 10.3% (Irwin and Egozcue, 1967) and 0.4% (Loughman et al., 1967), corresponding fairly well to the 3.9% and 3.8% breaks found by Cohen and collaborators (1967) in non-drug controls.[4] Our subjects were more heterogeneous in age, ranging from 22 to 61 years and included, in addition to paid volunteers, a number of persons seeking genetic counseling.

Regardless of subject differences our *in vitro* experiments essentially reproduced the published results—at least for the specific conditions reported today. Cohen et al., (1967a) exposed cultural leukocytes to five doses of LSD, ranging from 10.0 mcg/ml down to 0.001 mcg/ml for periods of 48, 24 and 4 hours each. Under all 15 experimental conditions a marked increase in chromosomal abnormalities (consisting

[4] After preparation of the present report the data of Lubs and Samuelson (1967) became available. In this study, specifically designed to examine chromosomal breaks in cultured lymphocytes of healthy adults, 3,720 cells from 23 cultures of ten persons aged 20 to 38 years were examined and 7.4% breaks found with a scoring system that was so similar to Cohen's as to permit direct comparison. The frequency of breaks ranged from 1% to 28% with a median of 7%.
Corresponding data from Court Brown, et al. (1966) when recomputed according to Cohen's scoring system are 0.4% for 417 subjects ranging in age from 15 to over 75 years.

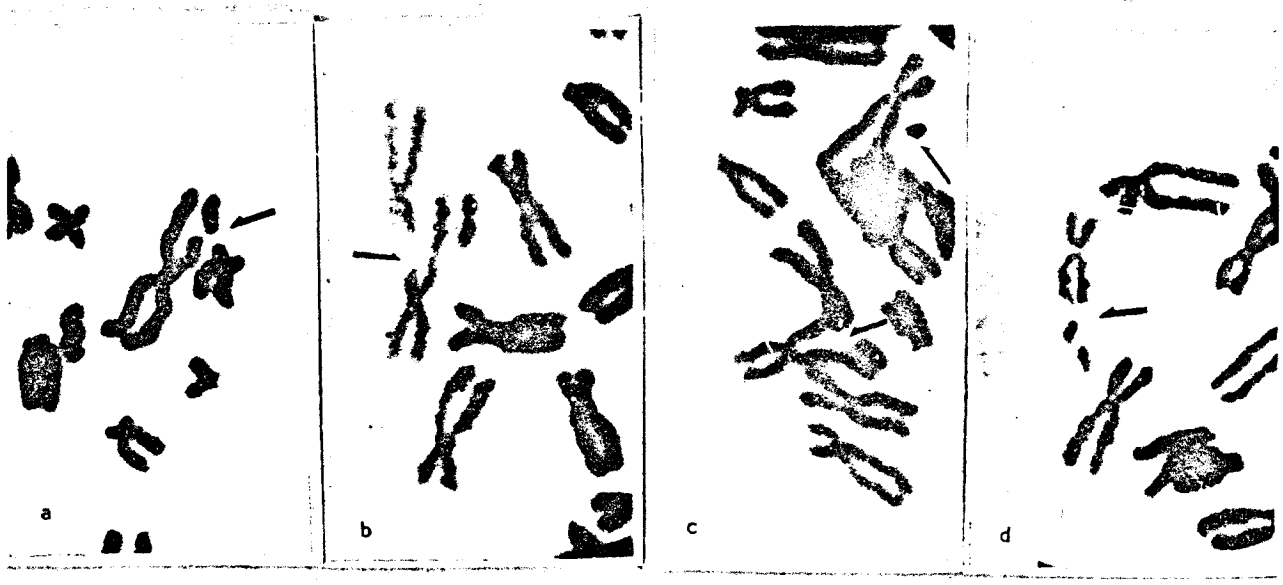


FIG. 1.—Chromatid breaks (a, b and c), isochromatid break (d) and acentric fragment (c).

mostly of breaks) over the control mean of 3.9% was observed by these investigators. Induction of breaks appeared to be independent of dosage and duration of exposure to LSD, the lowest percent of breaks (7.7%) occurring after 48 hours in 1.0 mcg/ml, while one of the highest frequencies (15.2%) was recorded for a culture exposed for 4 hours to 0.01 mcg/ml.

The frequency of breaks (10.0%) observed in our experiments falls within the range described by Cohen et al. (7.7%–17.5%) though toward the lower end of it. Far more important than the precise percentage, particularly when comparing results from different laboratories, is the fact that we were able to confirm an increase in chromosomal breaks associated with the *in vitro* exposure of cultured leukocytes to LSD (0.01 mcg/ml for 4 hours in our experiment).

Following the lead of previous investigators we have presented the combined results obtained from eight subjects. We do not have enough data as yet to undertake a case by case analysis, but there were two cases who did *not* respond to the addition of LSD with an increase in breaks. In one case, the respective counts were one break in 30 metaphases for the drug-free culture and no breaks in 30 metaphases for the LSD culture. In the other case, the drug-free culture showed 9% and the LSD culture 8% breaks (100 metaphases counted for each). Further investigation of possible individual differences in susceptibility to chromosome damage following exposure to LSD is indicated.

Such individual differences may be responsible for the divergent *in vivo* results obtained to date (Table 1). Cohen and collaborators found 13.2% breaks in 18 patients who had ingested LSD compared with 3.8% in 12 normal "drug-free" controls. Practically no overlap occurred between the two groups, LSD "users" showing from 5.3% to 25.1% breaks, controls from 2.0% to 5.5% (except for two controls with 14% and 21% breaks, respectively, who developed a viral infection within 24 hours after donating the blood sample and who were, therefore, excluded from the calculations). Irwin and Egozcue (1967) observed 23.6% breaks in the cultured leukocytes of eight LSD "users" and 10.3% in eight controls (omitting one control with 25% breaks because of a past history of therapeutic radiation). Though the frequency of abnormalities in Irwin and Egozcue's study exceeds that observed by Cohen and collaborators both for controls (former's lowest exceeds latter's highest) and for LSD "users", the intergroup differences clearly prevail and again there is very little overlap.

By contrast, Loughman et al. (1967), who also cultured leukocytes of eight persons with a history of LSD ingestion, failed to find a single chromosome break in the 697 cells examined. Their values for 19 control cases were likewise low (0.4% breaks if abnormalities are calculated according to the method of Cohen et al.). The reasons for the contradictory results are not the obvious ones of differences in dosages, duration of use, time elapsed since last dose, or intake of additional drugs. Even though LSD doses were estimated by the subjects, the California (Loughman et al.), Oregon (Irwin and Egozcue) and New York (Cohen et al.) groups seem to have taken comparable amounts. Nearly all of the persons using LSD had also taken other drugs including opiates, hallucinogens, marihuana, barbiturates, amphetamines and phenothiazines.

The present authors had an opportunity to culture the peripheral leukocytes of a 19-year-old female college student who had taken nine "trips" during the five months preceding the donation of the blood sample. Her doses of LSD increased from 100 to 1,000 mcg and the last three, which were taken within a month of the cell culture, were estimated by her as between 500 and 1,000 mcg. The last dose of LSD was taken approximately two weeks prior to obtaining the blood sample. In addition to LSD, the girl had

TABLE 1—*Chromosome breaks per cell (in percent)*

	without LSD			with LSD			
Investigators	N	% Breaks	Range	N	% Breaks	Range	
Cohen et. al.	6	3.9	?	6	15.2	7.7-17.5	in vitro
Present study	8	5.2	0-9.0	8	10.2	0-15.0	****
Cohen et. al.	12*	3.8	2.0-5.5	18	13.2	5.3-25.1	
Irwin and Egozcue	8**	10.3	7.0-16.5	8	23.6	12.0-38.0	in vivo
Loughman et al.	19	0.4	?	8	0	0	
Present study	11***	4.7	0-9.0	1	1.0	—	

*2 persons omitted because of viral infection (14% and 21% breaks)
**1 person omitted because of history of radiotherapy (25% breaks)
***2 persons omitted because of possible drug use (18.5% and 24% breaks)
****See text for details

310-723 O - 69 - 80

also taken amphetamine, methedrine and marihuana. We examined 101 metaphases and found only a single break. The frequency of 1% is more in line with the data of Lougman et al. (1967) than with those of the other teams. One or more cases of this type, however, will not assist appreciably in the solution of the dilemma. Too many questions remain unanswered.

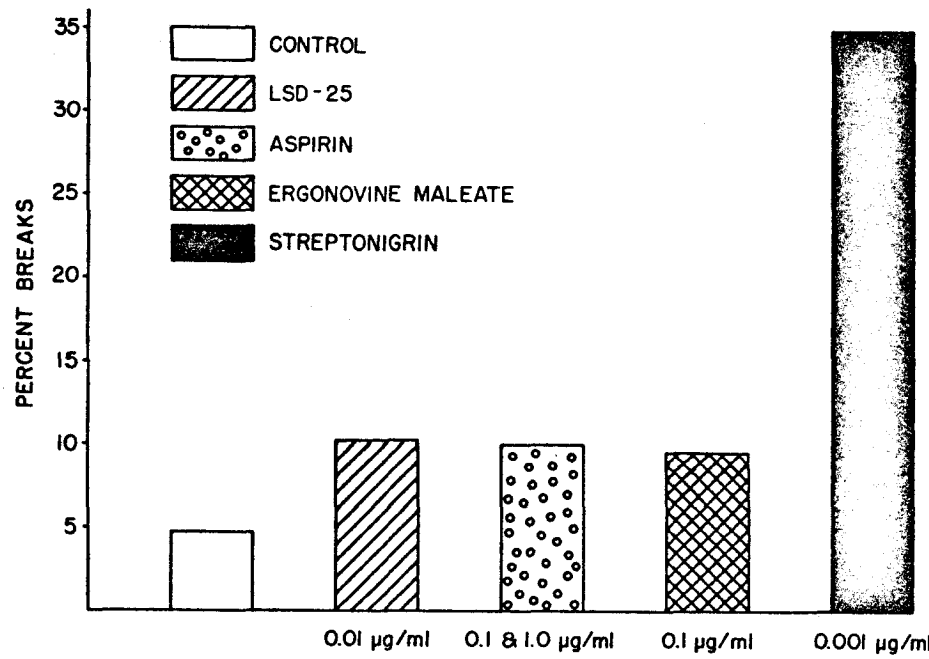
A more fruitful approach to the problem consists of the comparison of leukocytes cultured from the same patient before, during and after treatment with known doses of LSD. Such a study could demonstrate whether, following a given dose of LSD, measurable chromosome breakage is induced in the peripheral leukocytes of some, all, or none of the subjects. If induced, whether chromosome damage persists, and, if persisting, for how long and in what proportion of persons.

Further, leukocyte cultures of patients to whom known doses of LSD were administered many years ago would provide useful information concerning long-term *in vivo* effects; information for which we would have to wait a comparable period of time were we to start today.

As mentioned earlier, persons using LSD often use other drugs as well. Thus, the patient first reported by Cohen et al. (1967b) had been treated with chlorpromazine and chlordiazepoxide as well as LSD but, according to an unpublished study cited by the authors, "screening of chromosomes from 35 schizophrenic patients, some of whom were treated with these tranquilizers in a double blind study, revealed no increase in the frequency of chromosome breakage over that in untreated individuals". In their latest study Cohen et al. (1967a) report leukocyte cultures of two patients, each of whom had taken chlorpromazine, 200 mg. per day, for three and four weeks respectively, until the day of blood donation, without other drug history. Their leukocyte cultures showed 13.7% and 17.4% breaks, respectively, compared to the control value of 3.8%. The present authors recently observed 7% breaks in the cultured leukocytes of a 30-year-old housewife with the following six months history of chlorpromazine use: three week build up to 3,000 mg/day, on which she was maintained for two months, followed by gradual reduction for the next three months to 300 mg. per day, which is said to have been discontinued completely at the end of the six months period—2½ years prior to cell culture. Except for amphetamines, no other drugs were regularly used by this woman.

Another patient had received various phenothiazines, including chlorpromazine (Thorazine), trifluoperazine (Stelazine) and thioridazine (Mellaril) as well as the thioxanthene, chlorprothixene (Taractan) over a period of three years terminating two years prior to the donation of the blood sample and showed only 2% breaks.

The chromosome breaking ability of chlorpromazine has recently been demonstrated in culture of human fibroblasts with concentrations corresponding



CHROMOSOME BREAKS IN CULTURED LEUKOCYTES: 4 HOURS' EXPOSURE

FIG. 2

to commonly used clinical dosages (Abdullah and Miller, in press).

The *in vitro* effects of the four drugs used in our experiments are shown in Fig. 2. The chromosome damage produced by streptonigrin, an antibiotic and antitumor agent, well-known to induce chromosome damage, corresponds to that observed by others in cultures of human leukocytes. Thus, we obtained 35.0% breaks with a dose of 0.001 mcg/ml for four hours' exposure, while Cohen et al. (1963) for the same dose and six hours' exposure obtained 30.0% breaks. LSD, aspirin and ergonovine maleate all produced breaks in excess of control values, but not nearly as frequently as stretptonigrin. The respective percentages are: 10.2% for LSD, 10.0% for aspirin, and 9.6% for ergonovine maleate. The ratios of breaks per cell differ significantly from control ratio at better than 1% level of confidence for LSD and for aspirin ($Z = 3.26$) and better than the 5% level for ergonovine maleate ($Z = 2.10$).

Agents capable of producing chromosomal damage similar to the type observed in the present experiments, have long been regarded as detrimental to cells and the organisms (Kihlman, 1966). Best known among such agents are X-rays, viruses, and a variety of chemical mutagens, most of the latter showing carcinogenic as well as carcinostatic properties (Auerbach, 1967). Unlike the well-known dangers associated with a compound such as nitrogen mustard, aspirin has been considered a relatively harmless drug and is used on a scale many times exceeding that of LSD.

Provided results of the preliminary experiments reported here can be repeated, then it behooves us to undertake a reappraisal of the potential hazards of various pharmacologic agents. Aspirin will be but one of the several commonly used compounds requiring evaluation. Caffeine, long known as a mutagen to geneticists, likewise appears to be capable of producing chromosome damage to human leukocytes (Ostertag, 1966). Incidentally, the concentration of LSD used in our experiments (0.01 mcg/ml) approximates the blood level one to four hours after the ingestion of 1,000 mcg of LSD (Loughman et al., 1967). The concentration of aspirin (0.1 and 1.0 mcg/ml—dosages were combined since the frequency of breaks was similar in both) is considerably *below* the therapeutic blood level of 100–120 mcg/ml, two hours after the ingestion of four aspirin tablets (2.0 g).

Clearly, *in vitro* findings cannot be applied *in vivo* without careful further study. First, chromosome studies of pheripheral leukocytes are all *in vitro* studies. Even if we examine the cells of a person who has ingested a given drug, or of a child whose mother has a history of drug use during pregnancy, we are looking at cells that are the result of at least one cell division in the test tube. Second, compounds that are noxious when added in a given concentration to an *in vitro* system, need not have the same effect *in vivo*. The intact organism, unlike the test tube, has evolved a variety of mechanisms for detoxification. Third, peripheral leukocytes, like many other mammalian cells, are expendable. Any infection readily demonstrates the capacity of the human organism to eliminate damaged cells. In all likelihood it is the rare cell with chromosomal damage that is successful in establishing a new cell line. Similarly, damaged gametic cells have a drastically reduced probability of fertilization and subsequent production of viable offspring.

If a sufficiently large number of abnormal cells are produced, however, the change of similarly defective cellular progeny increases. It is conceivable that substances as widely used as caffeine and aspirin could be responsible for a considerable proportion of the carcinogenic and teratogenic exposure in our culture. Because of the ubiquity of these compounds in our society, it would be difficult, if not impossible, to get accurate histories of consumption which could be correlated, years later, with the appearance of neoplastic processes or, months later, with the birth of a malformed child.

In contrast to the dramatic discovery of the teratogenecity of thalidomide, anoxia (Ingalls et al., 1952), and cortisone (Fraser et al., 1954) have long been known to produce congenital malformations and reserpine (Sobel 1960, Limborgh and Meenen, 1963) has been implicated as well. Given the right circumstances, the proper dose and the appropriate time in gestation, it is expected that nearly any compound will be found capable of inducing fetal death, congenital abnormality or postnatal morbidity.

Controlled studies would require the examination of chrosomes prior to, during and after the administration of known amounts of these substances to a large enough number of individuals to provide generalized results. Meanwhile, it might be valuable to examine the chromosomes, obstetrical and oncological histories of patients with rheumatoid arthritis or similar diseases who have taken high doses of aspirin for long periods of time and to collect a group of coffee or coca cola addicts.

Further research is needed to elucidate the mechanisms of action of the various chromosome breaking agents. At this time the choice is almost unlimited including "free radical formation due to ionizing radiation, 'incorporation of nucleotide analogues into DNA', cross-linking of the DNA molecule by agents such as mitomycin C^{15}, faulty ATP and protein synthesis needed for repair; and release of lysosomal DNAase and cathepsins by agents incorporated into lysosomes" (Cohen et al. 1967a). Several different mechanisms undoubtedly are responsible for the

actions of different agents. Streptonigrin, for example produces increased breaks with increased concentration and increased exposure time, while to date no such relationship has been established for the LSD or aspirin. Similarly, the frequency of breaks was considerably higher for streptonigrin than it was for LSD, aspirin or ergonovine maleate. Streptonigrin produced far more breaks than gaps while for LSD, ergonovine maleate and aspirin, the number of gaps was as large as the number of breaks found. The same was true of the control slides and, apparently, was also observed in the caffeine study (Ostertag, 1966).

Regardless of technical difficulties and lack of information on mechanisms of action, it is of the utmost importance to establish whether or not the detrimental effects observed *in vitro* do occur *in vivo*. In this instance applied and basic research should be performed simultaneously and a number of laboratories will have to be involved. Present techniques of chrosome analysis are tedious and time consuming; only by combining the manpower resources of diverse research teams is it likely that definitive answers can be provided to the questions urgently demanding them.

The potential danger to society in terms of carcinogenesis and teratogenesis is such that further investigation, using both basic and clinical studies, should be undertaken promptly.

REFERENCES

ABDULLAH, S. and MILLER, O. J. Effect of drugs on nucleic acid synthesis and cell division in vitro. *Dis. Nerv. System* (in press)

ALEXANDER, C. J., MILES, B. E., GOLD, C. M. and ALEXANDER, R. B. LSD: Injection early in pregnancy produces abnormalities in offspring of rats. *Science, 157*:459–460, 1967.

AUERBACH, C. The chemical production of mutations. *Science, 158*:1141–1147, 1967.

AUERBACH, R. and RUGOWSKI, J. A. Lysergic acid diethylamide: effect on embryos. *Science, 157*:1325–1326, 1967.

COURT BROWN, W. M., BUCKTON, K. E., JACOBS, P. A., TOUGH, I. M., KUENSSBERG, E. V., and KNOX, J. D. E: *Chromosome Studies on Adults*, Eugenics Laboratory Memoirs XLII, published for the Galton Laboratory, University College London by the Cambridge University Press, London and New York, 1966.

COHEN, M. M., SHAW, M. W. and CRAIG, A. P. The effects of streptonigrin on cultured human leukocytes. *Proc. Nat. Acad. Sci., 50*:16–24, 1963.

COHEN, M. M., HIRSCHHORN, K. and FROSH, W. A. In vivo and in vitro chromosomal damage induced by LSD-25. *New Eng. J. Med., 277*:1043–1049, 1967a.

COHEN, M. M., MARINELLO, M. J. and BACK, N. Chromosomal damage in human leukocytes induced by lysergic acid diethylamide. *Science, 155*:1417–1419, 1967b.

FRASER, F. C., KALTER, H., WALKER, B. E., and FAINSTAT, T. D. The experimental production of cleft palate with cortisone and other hormones. *J. Cell. Comp. Psysiol.* (Suppl. 1), *43*:237–259, 1954.

GEBER, W. F. Congenital malformations induced by mescaline, lysergic acid diethylamide and bromlysergic acid in the hamster. *Science, 158*:265–266, 1967.

INGALLS, T. H., CURLEY, F. J., and PRINDLE, R. A. Experimental production of congenital anomalies; timing and degree of anoxia as factors causing fetal deaths and congenital anomalies in the mouse. *New Eng. J. Med., 247*: 758–768, 1952.

IRWIN, S. and EGOZCUE, J. Chromosomal abnormalities in leukocytes from LSD-25 users. *Science, 157*:313–314, 1967.

KIHLMAN, B. A. *Actions of Chemicals on Dividing Cells.* Prentice-Hall, Englewood Cliffs, N. J., 1966.

LIMBORGH, J. VAN and MEENEN, F. VAN. Effect of reserpine on rate of mortality and growth of duck embryos. *Nature* (London) *197*:615–616, 1963.

LOUGHMAN, W. D., SARGENT, T. W. and ISRAELSTAM, D. M. Leukocytes of humans exposed to lysergic acid diethylamide: lack of chromosomal damage. *Science, 158*:508–510, 1967.

LUBS, H. A. and SAMUELSON, J. Chromosome abnormalities in lymphocytes from normal human subjects. *Cytogenetics, 6*:402–411, 1967.

MOORHEAD, P. S., NOWELL, P. C., MELLMAN, W. J., BATTIPS, D. M., and HUNGERFORD, D. A. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res., 20*:613–616, 1960.

OSTERTAG, W. Koffein- und theophyllinmutagenese bei zell- und leukozytenkulturen des menschen. *Mutation Res., 3*: 249–267, 1966.

SOBEL, D., Fetal damage due to ECT, insulin coma, chlorpromazine or reserpine. *Arch. Gen. Psychiat., 2*:602–611, 1960.

ZELLWEGER, H., MCDONALD, J. S. and ABBO, G. Is lysergic acid diethylamide a teratogen? *Lancet, 11*:1066–1068, 1967.