Leukocytes of Humans Exposed to Lysergic Acid Diethylamide: Lack of Chromosomal Damage

Abstract. *Leukocyte cultures from eight human subjects who had had recent exposure to large doses of lysergic acid diethylamide were examined for chromosome abnormalities. The number of abnormalities was not significantly greater than that in control cultures.*

Cohen and his co-workers have reported chromosomal damage in cultures of peripheral human leukocytes to which the psychotropic agent lysergic acid diethylamide (LSD) had been added at various concentrations for varying lengths of time (*1*). The leukocytes of a single patient previously treated with LSD were also studied, and the chromosome breakage in his cells was reported to be 12 percent, while that of a normal control was 3.7 percent. Because of our interest in chromosome aberrations accompanying various diseases and following treatment with various pharmaceuticals, we have observed chromosomal behavior following doses of LSD in vivo, and have compared it with that reported by Cohen.

Subjects claiming recent use of LSD, alone or in combination with other illegal drugs, were clients of a welfare agency in San Francisco. Histories and blood samples were obtained there. An extensive interview required divulgence of the claimed duration of illegal drug use, estimates of the size of each dose, and frequency of use. Each subject was questioned about his own exposure, and his parents' possible exposure, to dangerous drugs, toxic chemicals, radiation, and other agents thought to be responsible for chromosome aberration in vivo. The interviews revealed no exposure to agents known to be responsible for chromosome damage (Table 1). No pathophysiological effects other than chromosome damage to circulating leukocytes were studied; absence of such effects is not implied by our work.

We were entirely dependent on the subjects' own statements regarding drug use; thus the dosages, even if reported to the best of the subjects' knowledge, are uncertain. The integral doses (Table 1) are based on the subjects' recall of typical dose and frequency of use, and thus they are estimates of the total amounts of LSD taken. The smallest integral dose reported (5000 μg), is twice that of the previously reported patient (2500 μg) (*1*), the largest is 60 times as great. The estimates of dose may be in error, but there is no doubt that very large doses were used by these subjects. It is this heavy usage rather than the exact amount which is important here.

Our technique of leukocyte culture was similar to that used by Cohen and his co-workers. Two drops of blood were taken from a finger of each experimental subject and of a control subject with no history of drug use. The blood was allowed to drop into a culture tube containing 5 ml of medium (NCTC 109 with fetal bovine serum (15 percent), 2 percent phytohemagglutinin-M, 50 I.U. of heparin, 700 units of penicillin, and 0.4 mg of streptomycin sulphate). After 2 to 3 hours at room temperature the cultures were incubated at 37°C.

At 66 hours of culture, 0.5 μg of Velban was added to each tube to effect metaphase arrest. After 72 hours of culture the cells were harvested and placed in a hypotonic solution until swollen. They were then fixed in propionic acid (45 percent) in 0.1*N* HCl. Chromosome preparations were made as squashes (in 2 percent lacticpropionic orcein) to minimize chromosome loss and breaks which might occur with methods of air-drying. The preparations were examined microscopically under low power, and cells which had apparently diploid chromosome complements were selected for examination under oil immersion at 1500 diameters. All selected cells were included in the tabulations. An individual with no knowledge of the subject's drug histories scored the types and frequencies of chromosome abnormalities and the distribution of numbers of chromosomes per cell. Those chromosome abnormalities scored were gross structural changes which could be detected without detailed karyotype analysis. These included dicentrics, rings, acentric fragments, marked changes in size or arm ratio, and complex associations such as quadriradials, chromatid breaks, and chromatid gaps. A chromatid was considered to contain a break when it had a clear, nonstaining discontinuity. A gap was defined as a region with little or no stain, little or no apparent chromatin, but not subject to clear definition as a break. No attempt was made to assess minor aberrations (small deletions, small differences in arm ratio or size, and so forth) detectable only with detailed karyotype analysis. The significance of differences (for all classes of aberrations) between frequency or aberrations among drug users, between drug users and the controls, and between drug users and control values culled from the literature, were assessed with a *t*-test (*2*) and a 5 percent significance level. Using "small-number" methods we programmed our calculations on an electronic desk computer.

There are no significant differences in frequencies of chromosomal aberrations among the drug takers, between the drug takers and the previous or present controls, or between drug takers and a sample taken from the literature (Table 2). Thus, although we did not repeat Cohen's work in vitro we are unable to confirm in our subjects

Table 1. History of drug use by experimental subjects. Ha, previous heroin addiction; R, heroin (occasional); S, dexedrine, methedrine, benzedrine; D, DMT; M, marijuana.

Subject	LSD use		Latest LSD dose		Integral LSD dose (μg)	Other drugs
	Dose (μg)	Frequency	Days since	Dose (μg)		
♂ D1	500, 1000, 4000	1/wk, 9 mo	1	1000	40,000	S, D, H
♂ W2	About 300	50 times	6	300	15,000	S, D, H, M
♂ R3	1000–2000	100 times	5	1000	150,000	Ha, S
♀ M4	1000	25 times	1	4000	25,000	None
♀ L5	100–900	12 times	22	900	5,000	S
♂ W6	1000–4000	70 times	1	2000	140,000	S, H
♂ W10	250–2000	1 to 2/mo	1	1000	8,000	S
♂ G12	100–700	40 times	7	333	16,000	S, M

the findings of Cohen on his patient. Polyploidy and the distribution of chromosomes per cell in cultures of cells from drug users were not significantly increased above our control values (Table 3). The mitotic index of each culture was comparable to that seen in 150 cultures done earlier for routine karyotyping.

The rate of aberration found by Cohen *et al.* in control cultures is much higher than we find and higher than many reported previously in the literature. Schmickel (*3*) gives a total aberration rate of 1.21 percent in 1569 normal control cells. If the gaps (0.45 percent) are omitted (not used by Cohen *et al.*), the remaining aberrations (0.76 percent) of all sorts are much fewer than the breaks reported by Cohen *et al.* for their control subjects. If we allow for a reasonable number of "two-break" aberrations in their control data, reducing the "break" frequency to an aberration frequency, their control value is still significantly higher than that often reported [See Schmickel (*3*), who cites several other authors in this regard, and Mouriquand *et al.* (*4*)]. Cohen *et al.* did not report each type of aberration separately; instead they converted all aberrations to an "equivalent" number of breaks and then summed them. Therefore their data cannot be compared directly with ours. Six individuals were used by Cohen *et al.* for control purposes; a fortuitously high value in one subject is thus an improbable source of error. If some condition of culture or cell preparation was less than optimum, a high control value could have resulted. A synergistic relation between growth conditions in vitro and the presence of LSD may result in high frequencies of aberration.

Information on the distribution and fate of LSD in the human body is not available. However, Stoll *et al.* (*5*) have investigated the distribution and clearance of ^{14}C-LSD in mice. Analysis of his data indicates that his curves for clearance of radioactivity from the blood may be described as the sum of three simple exponential functions. The first two exponential functions probably represent distribution and mixing functions and have half times of about 1 minute and 23 minutes, respectively; in 30 minutes, the concentration in the blood is 25 percent of the uniform distribution value. Thereafter, the third exponential function of blood clearance has a half time of about 175 minutes, giving a mean time in the circulation of about 4 hours. Relative to chromosome breakage, the initial high concentrations of LSD may be of little importance. However, the third exponential of clearance from the blood has a long enough half time to yield a mean period of exposure, 4 hours, comparable to the exposure times used by Cohen *et al.*

Table 2. Frequency of chromosomal aberrations.

Chromatid gaps			Chromatid breaks			All other aberrations		
Number	Percentage	95% Confidence limits	Number	Percentage	95% Confidence limits	Number	Percentage	95% Confidence limits
			Control (1 subject, 112 cells)					
0	0.0	0.0–3.3	0	0.0	0.0–3.3	0	0.0	0.0–0.5
			Previous controls (18 subjects, 561 cells)					
4	0.7	0.3–1.8	1	0.2	0.0–1.0	1*	0.2	0.0–1.0
			Control (ref. 3, 1569 cells)					
7	0.4	0.2–0.9	11	0.7	0.4–1.3	1	0.1	0.0–0.4
			Experimental (8 subjects, 697 cells)					
3	0.4	0.1–1.3	0	0.0	0.0–0.5	0	0.0	0.0–3.3

* Quadriradial.

The organ which most actively concentrates LSD is the liver (*5*). The maximum concentration in this organ occurs at 20 minutes, when it is five times that of blood. At 2 hours the concentration is 3.5 times that in blood, and this ratio seems relatively constant thereafter. The LSD is cleared from the liver (and most other organs studied) with a half time, by our analysis, of about 1 hour. This probably represents degradation, since at 2 hours only 10 percent or less of the radioactivity in every organ studied was found to be unchanged LSD. We suggest that a similar pattern may hold for man; Kalbhen and Sargent have found that an analog of LSD, ^{82}Br-BOL (2-bromolysergic acid diethylamide), is concentrated in the liver and subsequently destroyed with a half time of 1 hour (*6*).

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 percent of this value, or 0.0004 μg/g. The maximum concentration of LSD in the liver is five times this value. However, the mean value is closer to 3.5 times that in blood, or 0.0012 μg/g.

Thus if degradation of LSD is not considered, the maximum effective concentration in the human body after a 100-μg dose of LSD would be found in the liver, and would be essentially the same concentration as the lowest used by Cohen *et al.* in their studies in vitro. For this level and duration of exposure (0.001 μg/ml; 4 hours) Cohen found that the frequency of chromosome "breaks" was no different from control values. We believe that Cohen's data implies that no chromosome breakage is to be expected in humans given doses of LSD of 100 μg. If degradation of LSD is considered, as found by Stoll *et al.*, and if the breakdown products are not active, then the effective concentration of unchanged LSD achieved in vivo would be one-tenth of the lowest amount used by Cohen *et al.* The lack of significant effect in our studies is in agreement with the expectation based on animal studies (*5*) and studies in vitro (*1*).

At higher concentrations (in vitro) than would be expected in humans given 100-μg doses of LSD, Cohen found significant increases in chromosome damage. All of our subjects had doses in excess of 100 μg; one subject

Table 3. Chromosome count distribution.

Chromosomes per cell (No.)	Cells (No.)	Percentage of total	95% Confidence limits
	Control (1 subject, 112 cells)		
40	0	0	0.0– 3.3
41	1	0.9	.2– 4.9
42	0	0	.0– 3.3
43	1	0.9	.2– 4.9
44	3	2.7	.9– 7.6
45	7	6.3	3.1–12.3
46	100	89.3	82.2–93.8
47	0	0	0.0– 3.3
4n	0	0	0– 3.3
	Experimental (8 subjects, 245 cells)		
40	2	0.8	0.2– 2.9
41	3	1.2	.4– 3.5
42	5	2.0	.9– 4.7
43	4	1.6	.6– 4.1
44	10	4.1	2.2– 7.3
45	20	8.2	5.3–12.3
46	200	81.6	76.3–86
47	0	0.0	0.0– 1.5
4n	1	.4	.1– 2.3

had a dose of 4000 μg only 1 day before our study. If the pertinent concentrations in liver and blood derived above are multiplied by 40, concentrations attained in vivo are similar to Cohen's values of 0.01 and 0.1 μg/ml. Even in this subject we have found no evidence of increased chromosome damage in peripheral blood leukocytes.

In this connection, it must be remembered that the dividing cells in peripheral blood cultures from normal humans are derived from mature, nondividing, circulating lymphocytes (*7*). At the time of blood collection these are almost exclusively in the G_1 or G_0 portion of the cell cycle (before DNA synthesis) (*8*, *9*). They are presumably in G_1 at the time of exposure in vivo to LSD. In Cohen's studies, the stated durations of exposure to the drug were sufficiently long to contain a portion of the DNA synthetic period, or of the stage immediately preceding mitosis (*8*). Therefore, if the effects of LSD in vivo relative to chromosome damage are restricted to stages of active synthesis or preparation for division, no chromosome damage would be expected in exposed circulating lymphocytes. Precursor cells of lymphocytes, or any other cells actively engaged in division processes, might still be affected. Without examination of other tissues of the body, this possibility cannot be ruled out.

Occasionally in squash preparations of drug users' cells, we found large cells with multiple micronuclei. Such cells were not found in control cultures. Cohen has suggested that high concentrations of LSD may be toxic to leukocyte cultures exposed to it for prolonged periods. These micronucleated cells might be the progeny of stem cells exposed to high concentrations of LSD in vivo. However, if such micronucleated cells result from previous chromosome damage, one would also expect to see some chromosome damage in cells reaching metaphase; this was not seen in our cultures.

We conclude from our work that LSD, in doses as high as 4000 μg has not been shown to damage the chromosomes of human peripheral blood lymphocytes in vivo. This conclusion is supported by observations of Petrakis (*10*). Other tissues of the body must be examined before ruling out the possibility of chromosomal damage to cells actively dividing in vivo.

Note added in proof: Since submission of this paper a comparable study supporting conclusions opposite to our own has been reported (*11*). Also, a paper has been called to our attention reporting a half time of LSD in vivo in human plasma essentially identical to that which we calculated from the mouse data (*12*).

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Anesthetization of Porpoises for Major Surgery

Abstract. *Comparison of three porpoises (*Tursiops truncatus *and* Lagenorhynchus obliquidens*) given nitrous oxide with 18 given halothane, with complete documentation of reflexes and comprehensive physiological monitoring, showed halothane to be a suitable anesthetic for major surgery while nitrous oxide was found to be inadequate. In addition, sodium thiopental administered intravenously was successfully used to facilitate intubation procedures. This development eliminated the need to intubate awake porpoises.*

The Atlantic bottlenosed porpoise (or dolphin) *Tursiops truncatus*, which has a well-developed brain (*1*) has impressed scientific investigators as being highly intelligent (*2*). Furthermore, these porpoises have a very sophisticated underwater sonar system (*3*) and highly sensitive hearing over a wide range of frequencies (*4*). The maintenance of comprehensive medical care for such valuable laboratory animals and our desire to study auditory physiology dictated the development of an anesthetic procedure suitable for major surgery.

Nagel, Morgane, and McFarland (*5*, *6*) have used 50 to 70 percent nitrous oxide (N_2O) supplemented with succinyldicholine for major surgery in the Atlantic bottlenosed porpoise *Tursiops truncatus*. Ridgway (*7*) has successfully used halothane to anesthetize the same animal.

Using the procedure recommended by Nagel, Morgane, and McFarland (*5*, *6*) we have anesthetized porpoises for surgery. Since the use of succinyldicholine and its attendant muscular relaxation preclude assessment of the depth of anesthesia by the use of reflex signs, we did not use it during our studies. Nitrous oxide was administered to three animals. To determine the degree of anesthesia, we observed the following reflexes: (i) eyelid reflex, contraction or closure of the eyelid induced by tapping on the inner canthus of the eye; (ii) corneal reflex, contraction of the eye muscles or lids when the cornea is touched; (iii) gag reflex, contraction of the throat muscles when the hand is inserted into the pharynx; (iv) tongue reflex, contraction or pulling away of the tongue when it is pulled forward; (v) anal reflex, reflex movements of any body parts when the anus is distended; (vi) swimming reflex, movements of the tail up and down in a swimming motion; (vii) pectoral scratch reflex, movements of the pectoral flippers in response to a pinprick or scratch of the chest or axillary region; (viii) blowhole reflex, movements of the blowhole when the finger is inserted into the nares of vestibular sacs; and (ix) vaginal or preputial reflex, movements of the vagina or penis or other body parts when the vagina or prepuce is distended by insertion of the fingers or other instruments.

In all three experiments with N_2O, we used a Bird Mark 9 respirator with porpoise apneustic-plateau control unit (operated as a controlled, open system), and intubation of the awake animal before administration of N_2O (*5*, *6*).

The first experiment with N_2O was performed on an adult *Lagenorhynchus obliquidens*. With intubation completed, the animal was administered 100 percent O_2 at five or six respirations per minute at a pressure of 20 mm-Hg (during respective apneustic plateaus)