Chromosomal Abnormalities in Leukocytes from LSD-25 Users

Abstract. *A significant increase of chromosomal abnormalities was found in leukocytes from LSD-25 users (six out of eight) compared to nonuser controls (one out of nine). The two LSD-25 users showing no damage reported the lowest estimated average dose. The subjects whose cells showed damage were tested between 1 day and 6 months after their last LSD-25 dose.*

The addition of LSD-25 to cultured human leukocytes produces increased chromosomal abnormalities. Similar damage was observed in a schizophrenic patient tested 8 months after he had received 15 treatments with LSD-25 over a 4-year period (*1*). The present study was undertaken to validate these observations among users of LSD-25.

Experimental subjects were selected at random from a volunteer group of LSD-25 users and a cross section of volunteer nonusers. A 5-ml sample of blood was drawn from eight LSD-25 users (ranging from an estimated 4 to 200 doses per individual) and from nine control subjects that had not taken the drug. The samples were placed in heparinized tubes, randomly assigned numbers, and processed for chromosomal assessment on a blind basis. Chromosome preparations were made with a standard procedure (*2*) from cultures of the blood incubated at 37°C for 72 hours. Colcemid (0.5 μg/ml) was added for the last 2 hours of incubation to arrest the cells in metaphase; afterward, the cells were stained with bleu de Unna. Well-spread metaphase figures were selected under low magnification (× 125), and the chromosomes were classified under oil immersion (× 1250). Once a cell was selected under low power, it was included in the study; 200 cells were classified per subject.

Determination of "breaks" was based on clear discontinuity of the chromatids. Breaks were classified as "chromtid" when one chromatid was affected and "isochromatid" when both sister chromatids were broken at the same point. Single fragment breaks were classified as chromatid breaks and double fragments as isochromatid breaks. A small, Philadelphia-like chromosome (Ph_1) found in some LSD-25 users also was classified as an isochromatid break; dicentric chromosomes and exchange figures were classified as containing two breaks. (The exchange figures were considered as only one abnormality in calculating the percentage of abnormal cells.)

The data, summarized in Tables 1 and 2, show a significant increase in cell abnormalities and breaks in one out of the nine control subjects and in six of the eight LSD-25 users ($P < .05$). The one control subject showing a borderline increase in breaks had received seven diagnostic x-rays of the head and neck 1 to 2 months before the sampling. The control subject with a significant increase in breaks had received an estimated ten therapeutic dermal treatments of x-rays for acne 22 years earlier. None of the LSD-25 users received therapeutic radiation treatments or diagnostic x-rays within several months of sampling. Some, however, had also taken several doses of cannabis (mescaline or peyote), psilocybin, or dimethyltryptamine, or combinations of the three. Therapeutic irradiation can cause increased chromosomal abnormalities that may last for the life of the individual (*3*).

The mean estimated dose of LSD-25 taken was between 200 to 600 μg, with a peak dose intake ranging from 400 to 2800 μg. The percentage of abnormalities recorded, however, seemed unrelated to the number of doses or magnitude of the doses taken. Noteworthy, however, was the fact that the two LSD-25 users exhibiting a normal number of breaks estimated their average dose intake to be 200 μg; the mean dose intake of those exhibiting signif-

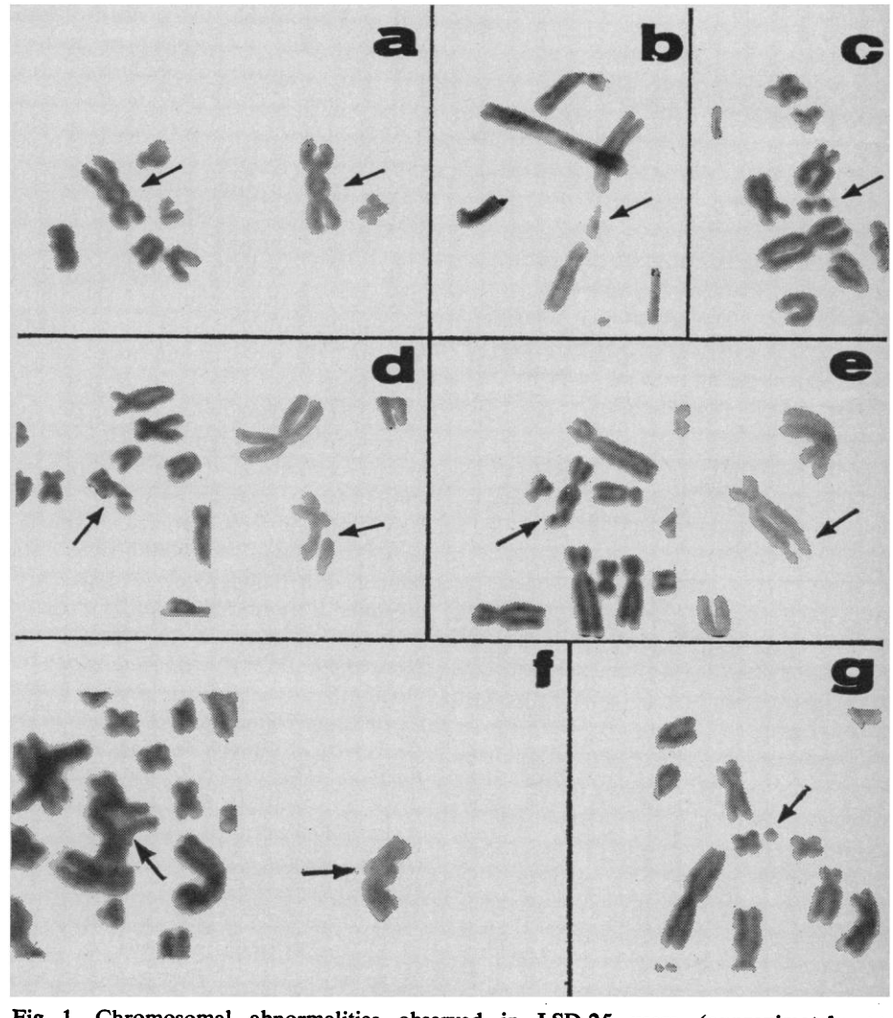

Fig. 1. Chromosomal abnormalities observed in LSD-25 users (approximately × 2500). Arrows indicate (a) dicentric chromosomes, (b) break and gap in a No. 1 chromosome, (c) double fragment, (d) single chromatid breaks, (e) isochromatid breaks, (f) quadriradials, and (g) Ph_1-like chromosome.

icantly increased abnormalities was 300 μg or more. The last dose of LSD-25 taken by six of the eight users before sampling was betweeen 1 to 30 days; two of the subjects exhibiting abnormalities took theirs 3 and 6 months earlier, respectively. One of the LSD-25 subjects exhibiting abnormalities had had only four doses of approximately 300 μg each.

Table 1. Number and type of chromosomal breaks in controls and LSD-25 users. Data are given as total number of breaks in 200 cells classified.

Case No.	Breaks (No.)		Exchange figures	Chromosomes	
	Chromatid	Isochromatid		Dicentric	Rings
		Controls			
12-F	9	3			1
14-M	13	1			
4-M	14	3			
16-M	16	3			
11-F	17	3			
3-M	18	3			
8-M	20	1		3	
6-F	29	4			
13-M	38	12			
		LSD-25			
1-M	15	5		2	
7-M	21	2	1		
17-M	35	7			
10-M	39	5*		1	
20-M	36	10	2	1	
15-M	42	8			
5-M	58	5			
9-M	61	9*	1	2	

* Ph_1-like chromosomes.

Table 2. Distribution of abnormal cells and chromosomal breaks in controls and LSD-25 users as related to number of doses and dosage. A total of 200 cells were classified for each subject. $P < .05$ for abnormal cells and breaks.

Case No.	Doses (No.)	Estimated dose (μg)		Last dose (days)	Abnormal cells (%)	Breaks (%)
		Mean	Peak			
			LSD-25			
1-M	10	200	800	14	11.0	12.0
7-M	100	200	500	14	12.0	12.5
17-M	4	300	400	30	21.0	21.0
10-M	50	300	1500	180	21.0	23.0
20-M	50	500	1500	3	24.5	26.0
15-M	110	350	600	90	25.0	25.0
5-M	25	400	500	21	30.0	31.5
9-M	200	350	2800	1	34.0	38.0
			Controls			
12-F					6.5	7.0
14-M					7.0	7.0
4-M					8.0	8.5
16-M					9.5	9.5
11-F					9.5	10.0
3-M					10.0	10.5
8-M					11.5	13.5
6-F					14.0	16.5*
13-M					24.0	25.0†

* Recent diagnostic x-ray. † Past x-ray therapy.

Figure 1 illustrates some of the abnormalities seen among the LSD-25 users. Of special interest were the quadriradial formations (three cases); these occur rarely in untreated, normal cultures (*4*) but can be induced in human leukocytes by the addition of mitomycin (*5*). Quadriradials and increased chromosomal breaks also are characteristic of the cytogenetic picture of Fanconi's anemia and Bloom's syndrome (*6*), both caused by recessive autosomal genes and accompanied by an increased incidence of neoplasia (*7*). They are also seen in tumor cells or cells transformed by the oncogenic virus SV40 (*8*). The genetic consequences of the quadriradial figures have been generally discussed (*4, 9*).

Also of much interest is the Ph_1-like chromosome observed in LSD-25 users 9 and 10. The Ph_1 chromosome, a deleted G-group autosome, is characteristic of chronic myelogenous leukemia (*10*) and is thought to be absent from lymphoid cells but present in neutrophil and erythrocyte precursors and possibly megakaryocytes of the bone marrow (*11*). All cells showing the Ph_1-like chromosome were karyotyped; in every instance, only three normal G-group chromosomes plus the Ph_1-like chromosome were found.

It is still too early to assess the significance of these findings. The chromosomal abormalities in human lymphocytes, for the moment, appear to be induced by as little as four doses of LSD-25 exceeding an estimated 200 μg. It remains to be determined also whether the chromosomal abnormalities result from permanent damage to the stem cells, or from damage in the G_1 period to long-lived lymphocytes, the damage not being recognized as chromosomal abnormalities until mitosis.

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2-Dicyanomethylene-1,3-Indanedione: A New Electron Acceptor

Abstract. *The electron affinity of 2-dicyanomethylene-1,3-indanedione (I) (1.33 ev), as computed from charge-transfer spectral analysis and from polarographic measurements of half-wave reduction potential, shows that the incorporation of the 2-dicyanomethylene moiety in 1,2,3-indanetrione (II) enhances its π-acid character. The electron spin resonance spectrum of the anion radical of I, generated electrolytically in solution, shows hyperfine structure that is due to two nitrogen atoms and four equivalent hydrogen atoms.*

The acceptor strength of a π-electron system is based on the electron-withdrawing power, number, and location of the substituents as well as on the extent of conjugation. The π-acid strength and electron affinity of an acceptor change in a predictable manner when one or more of the above factors are systematically varied (*1*). The suitable combination of two acceptor moieties within one molecular framework could possibly lead to an acceptor which might possess a greater electron affinity than molecules containing only one of the parent moieties (*2*).

We studied the electron affinity and π-acid property of a new electron ac-