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Chromosomal Damage in Human Leukocytes Induced by Lysergic Acid Diethylamide

Abstract. *Addition of lysergic acid diethylamide to cultured human leukocytes resulted in a marked increase of chromosomal abnormalities. The distribution of chromosome breaks deviated significantly from random, with an accumulation of aberrations in chromosome No. 1. Cytogenetic investigation of a patient extensively treated with this drug over a 4-year period for paranoid schizophrenia showed a similar increase in chromosomal damage.*

The induction of chromosomal abnormalities by various exogenous agents has been studied extensively (*1*, *2*). In addition, compounds with specific pharmacologic and chemotherapeutic value cause chromosome damage (*3*). The psychotomimetic agent lysergic acid diethylamide (LSD-25), when added to cultures of human peripheral leukocytes, produces a marked increase in the frequencies of chromosomal breaks and rearrangements compared to untreated cultures.

Chromosomal preparations were made from cultures of whole blood with a microtechnique and standard procedures (*4*). All cultures were incubated for 72 hours at 37°C, and colcemide (0.05 μg/ml) was added for the last 2 hours of culture to arrest cells at metaphase. Lysergic acid diethylamide was dissolved in sterile distilled water and added to the cultures in various concentrations (100, 50, 10, 1, 0.1, 0.01, and 0.001 μg/ml of culture) for different periods of exposure before harvest (48, 24, and 4 hours). Concentrations of 100 and 50 μg/ml caused cellular degeneration and suppressed mitosis so that the number of analyzable cells was insufficient. Leukocytes obtained from two healthy individuals (one male and one female) were treated with LSD-25 at final concentrations of 10, 1, 0.1, 0.01, 0.001 μg/ml for 48, 24, and 4 hours. Each concentration and exposure time was repeated twice. The controls consisted of untreated cultures from these two individuals as well as from four additional persons, two males and two females.

Several slides from each culture were prepared and coded by individuals who did not participate in the microscopic scoring of the cells. It was hoped that 25 metaphases per slide could be obtained to yield a total of 200 cells for each concentration and time period. However, in some of the treated cultures, we could not find this number of cells. Well-spread mitoses were selected under low magnification (× 250), and chromosomes were scored under oil-immersion phase-contrast microscopy (approximately × 1560). Once a cell was selected under low power, it was included in the study.

Abnormalities were scored as breaks only if a clear discontinuity of the chromatid was visible. Breaks were classified as "chromatid" if only one chromatid was affected and "isochromatid" if both sister chromatids were broken at the same location. Both of these types of abnormalities were scored as single breaks. Single fragments were included with chromatid breaks while "double" fragments were scored as isochromatid breaks. Dicentric chromosomes and "translocation" configurations were considered as containing two breaks. Attenuated, pale-staining chromosomal regions, other than the normal secondary constrictions, were scored separately as "gaps" but were not included in the calculation of breakage rates. Whenever possible, each break was assigned to a given identifiable chromosome or chromosome group according to the Denver classification (*5*).

Since there was no observable difference in the responses of the two individuals, the data for each treatment were pooled. Table 1 illustrates the distribution of chromosomal abnormalities observed for various exposure times and concentrations of the drug. At least a twofold increase in the rate of chromosomal breaks over the control rate was evident for all treatments (except 0.001 μg/ml for 4 hours). A relationship between dose and response existed; however, this appeared to be time dependent. The highest concentration (10 μg/ml) caused greater damage in shorter incubation times, an indication that the longer exposure may have caused cellular destruction. The same effect is also noted with a concentration of 1.0 μg/ml in the 48-hour treatment. Conversely, with 0.001 μg/ml, more chromosomal damage was evident at longer exposure times, while, with the 4-hour exposure with this dosage, a direct reduction in the number of chromosome breaks was observed.

Table 2 depicts the distribution of chromosome breaks among the various identifiable chromosomes or chromosome groups. The test of significance indicates a nonrandom distribution of breaks ($P < 0.001$), with a disproportionate accumulation of anomalies in chromosome No. 1. The array of expected values is based on random breakage per unit of chromatin as calculated from the Denver measurements (*5*). Studies of other agents inducing nonrandom breakage of human chromosomes have demonstrated "hot spots" in the heterochromatic regions of chromosome No. 1 [for example, the centromere and secondary constriction (*2*)]. Lysergic acid diethylamide also shows an apparent affinity for these chromosomal regions.

At most concentrations, the greatest damage was induced by 24- and 48-hour exposure periods. Although the

Table 1. Distribution of chromosomal breaks induced in cultured human leukocytes by various dosages of and times of exposure to LSD-25. Data are given as breaks per number of cells. Figures in parentheses denote breaks per cell.

Time before harvest (hours)	Dosage (μg/ml)				
	10	1	0.1	0.01	0.001
48	15/164 (0.091)	13/194 (0.067)	41/125 (0.328)	19/200 (0.095)	27/195 (0.138)
24	22/200 (0.110)	46/125 (0.368)	34/175 (0.194)	28/175 (0.160)	22/175 (0.216)
4	38/150 (0.253)	18/200 (0.090)	23/200 (0.115)	28/200 (0.140)	10/200 (0.050)
Control	34/925 = (0.037)				

Table 2. Distribution of chromosome breaks according to individually identifiable chromosomes or chromosome groups. There were 30 unidentifiable fragments and breaks. Data are given as numbers of breaks.

Distribution by chromosome group									Total
A1	A2	A3	B	C	D	E	F	G	
				Observed					
74	28	18	33	161	15	16	7	2	354
				Expected					
30.8	28.8	24.1	43.0	131.9	35.6	30.6	16.1	13.1	354.0
				*Chi-square test**					
60.6		1.5	2.3	6.4	11.9	7.0	5.1	9.4	104.2

* d.f. = 8; $P < .001$.

leukocyte system is not an absolutely synchronized cell population, a large proportion of the cells seen at metaphase after 72 hours of culture must have been in either the G_1 (before DNA synthesis) or S (DNA synthesis) period during these longer exposure times, while 4 hours before harvest the cells are in the G_2 period (after DNA synthesis) of the cell cycle. Since in most cases the lowest frequency of breaks was observed after this 4-hour exposure (except where the dose was 10 μg/ml), LSD-25 may cause chromosome breaks during the G_1 or S period of the cell cycle. Figure 1, a–d, illustrates typical chromosomal aberrations observed in vitro.

We also studied the leukocytes of one patient who had undergone extensive treatment with LSD-25 in conjunction with psychotherapy for paranoid schizophrenia. This patient is a 51-year-old male who, with the exception of his schizophrenia, is physically healthy with no history of malignancy, viral infection, or radiation treatment other than routine diagnostic procedures. From 30 September 1960 to 9 March 1966 he had a total of 15 treatments with LSD-25. The ingested dosages were 80, 100 (three times), 150, and 175 μg for the first six treatments, while the last nine treatments were with 200 μg. Leukocyte cultures were initiated for chromosomal preparations 8 months after the last treatment. There was no other known ingestion of drugs of any kind during this interval. The chromosome breakage rate of 200 cells in metaphase was 12 percent compared to the normal 3.7 percent. Figure 1e demonstrates some of the chromosomal anomalies seen in the patient. Of extreme interest is the one quadriradial formation observed between two No. 1 chromosomes. Such figures are seen in only extremely low frequencies in untreated, normal cultures but may be induced routinely by treatment of human leukocytes with mitomycin C (*6*). The genetic consequences of this phenomenon have been discussed (*7*). "Quadriradials" and increased chromosomal breakage also characterize the cytogenetic picture in two syndromes—Bloom's syndrome and Fanconi's anemia—caused by autosomal recessive genes (*8*). Such exchange figures also are frequently observed in tumor cells as well as cells that have undergone "malignant transformation" by the oncogenic virus SV_{40} (*9*). It is also of interest that patients with Bloom's syndrome and Fanconi's anemia demonstrate an increased frequency of developing neoplasia (*10*).

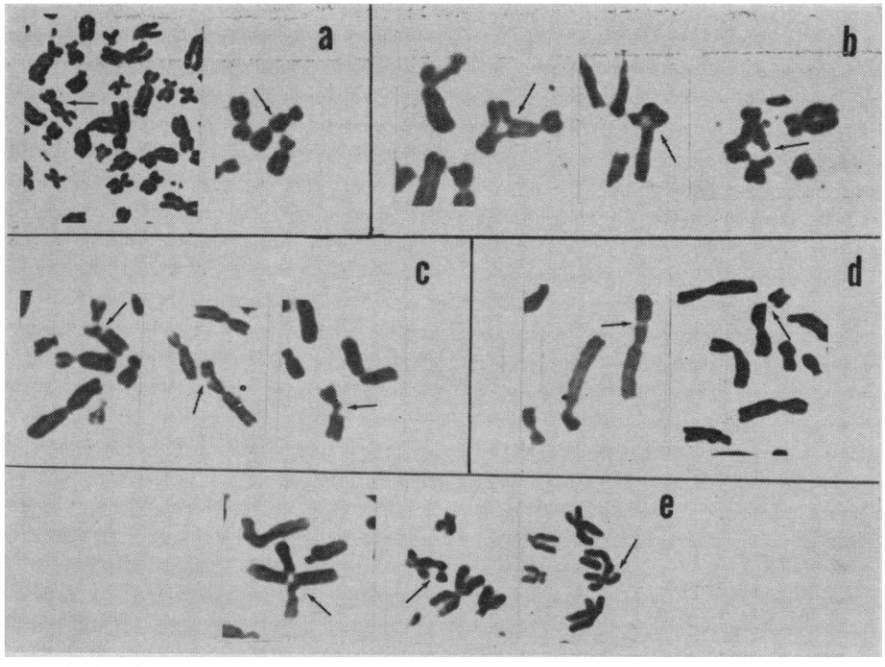

Fig. 1. Partial cells with various chromosomal abnormalities induced by LSD-25 (approximately × 2400). (a) Arrows indicate dicentric chromosomes seen in two cells. In the cell on left notice two double fragments and one single fragment. (b) Chromatid exchanges from three different cells. (c) Single chromatid breaks. (d) Isochromatid breaks. (e) Chromosomal anomalies in leukocytes of the patient treated with LSD-25 (quadriradial and two chromatid breaks).

Since the patient we studied had been treated for short periods of time with the tranquilizing drugs chlorpromazine (thorazine) and chlordiazepoxide (librium) before and during treatment with LSD, our cytological findings should be interpreted with caution. However, 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 (*11*).

The significance of these findings cannot yet be assessed fully. However, LSD-25 is apparently another agent which is capable of quickly producing chromosomal damage in vitro, perhaps in the first or second division of cultured leukocytes. Moreover, the observation of increased chromosomal damage in the patient suggests an additional long-term effect of the drug. Individuals accidentally exposed to irradiation (*12*), therapeutically irradiated (*13*), or treated with the chemotherapeutic agent 1,3-bis(2-chloroethyl)-1-nitrosourea (*14*) and then studied long after the initial exposure still manifest increased frequencies of chromosomal abnormalities. Such studies suggest two possible mechanisms of LSD action: (i) permanent damage to the stem cells that may give rise to sub-

sequent leukocytes, or (ii) damage in the G_1 period to long-lived lymphocytes, the damage not being observed as chromosomal abnormalities until mitosis. The latter may be the more likely hypothesis.

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Swimming Sea Anemones of Puget Sound: Swimming of Actinostola New Species in Response to Stomphia coccinea

Abstract. *Swimming as a response of the sea anemone* Actinostola *new species can be elicited as a result of contact with the submarginal surface of another swimming sea anemone* Stomphia coccinea. *However,* Stomphia *does not swim as a result of contact with* Actinostola. *In all other known respects, swimming is caused in both species by the same stimuli, including certain starfishes, a nudibranch, and electrical stimuli. No agent that causes* Actinostola *to swim has been detected in extracts, rubbings, or dried matter from* Stomphia.

Since the observation that the sea anemone *Stomphia coccinea* swims in response to certain starfishes (*1*), a number of other circumstances that cause swimming have been discovered and investigated. These are (i) electrical stimuli of the appropriate strength, number, and frequency (*1, 2*), (ii) extracts from the aboral surface of one of the active starfish, *Dermasterias imbricata* (*3*), and (iii) contact with a nudibranch *Aeolidia papillosa* (*4*).

The swimming sea anemones of Puget Sound were first regarded as members of a single species, *Stomphia coccinea.* However, it was noted that two groups exist, differing from each other in size and color, and investigators and collectors referred to these groups as "large" and "small" *Stomphia.* The latter corresponded with published descriptions of *S. coccinea* (*4, 5*). The former has now been identified as a new species of *Actinostola* (*6*). Besides differences in appearance and morphology, there are certain differences between the swimming movements of the two forms (*6, 7*). Both anemones belong to the large family Actinostolidae.

We have now discovered that the bigger swimming sea anemone, *Actinostola,* swims in response to contact with the smaller *Stomphia. Actinostola* has no such effect on *Stomphia,* nor does one *Stomphia* cause another *Stomphia* to swim. This response was obtained in more than 50 percent of matched trials (Table 1), though it seemed to be a more frequent occurrence in some animals than in others. In some respects, swimming of *Actinostola* is more easily evoked by *Stomphia* than by *Dermasterias* or *Aeolidia.* Thus *Stomphia* brought into contact with a single tentacle of *Actinostola* will frequently cause the latter to swim, but this rarely happens when one of the active starfishes is brought into contact with a single tentacle of *Actinostola.*

The surface just below the margin of the oral disc of *Stomphia* is most effective in causing *Actinostola* to swim. When ten animals were tested against the tentacles, the lower column, and the submarginal region of *Stomphia,* none swam in response to the tentacles, one swam on contact with the lower column, but nine of the ten *Actinostola* swam in response to contact with the submarginal region. Figure 1 shows a typical response under the most effective circumstances, namely when tentacles of *Actinostola* are in contact with the epidermis immediately above the marginal sphincter of a partly closed *Stomphia.*

Some features of the response have emerged from attempts to detect a chemical substance in *Stomphia* that causes swimming in *Actinostola.* Rubbing the active area of *Stomphia* with absorbent objects (such as pipe cleaners or swabs) does not pick up active materials as in the case of *Dermasterias* or *Aeolidia.* Sea-water and alcohol extracts and freeze-dried powders of *Stomphia* do not elicit swimming of *Actinostola* as corresponding preparations of *Dermasterias* and *Aeolidia* (*3, 4*) do. Nevertheless, since the two animals must be brought into contact if swimming is to occur, it is reasonable to suppose that *Actinostola* swims in response to some substance in *Stomphia* to which it is highly sensitive. The failure to detect activity in extracts and rubbings would suggest that this substance is present only in small quantities in the animal as a whole, and that perhaps it can be delivered locally at high concentrations when the two animals are brought into contact in the appropriate way.

The most obvious functional explanations for the swimming responses to certain starfishes, for example, as escape reactions, are not consistent with certain facts (*2, 8*). It is even more