Drosophila melanogaster Treated with LSD: Absence of Mutation and Chromosome Breakage

Abstract. *Injection of lysergic acid diethylamide in* Drosophila melanogaster *males induced no mutations or chromosome breaks in premeiotic, meiotic, or postmeiotic sperm. Tests included those for sex-linked lethals, sex-linked visibles, a specific visible (dumpy), and translocations. Some implications of these experiments are discussed.*

Chromosome breakage, apparently induced by lysergic acid diethylamide, LSD 25, in human leukocytes was reported, in vitro, by Cohen *et al.* (*1*) and, in vivo, by Irwin and Egozcue (*2*). In these two reports there was no consistent relation of LSD concentration to breakage frequency. This was unusual because chemical mutagens in *Drosophila* do show a proportionality between concentration and mutation frequency (*3*). The frequency of breaks among the controls in the leukocytes studied by Cohen (34/925) is significantly lower than that obtained from Irwin's study (211/1800).

The unusual features of the dose-frequency response by the LSD-treated cells in Cohen's report prompted us to design a series of experiments on *Drosophila melanogaster* to test for chromosome breakage and mutation. The choice of *Drosophila* was based on our experience in studying mutagenesis with a variety of chemical mutagens: monofunctional quinacrine mustard (ICR-170) (*4–6*), nitrosomethylurea (NMU) (*7*), and ethyl methanesulfonate (EMS) (*3*). Fruit flies were the first organisms used to detect mutations induced by ionizing radiation (*8*) and by chemical mutagens (*9*). In none of our tests did we find any evidence that LSD was associated with chromosome breakage or mutations. Our tests were designed to assay the sensitivity to LSD for all stages of the male meiotic cycle.

We assayed for mutations with three techniques: the sc^{S1} *B InS* w^a sc^8 (Basc) sex-linked lethal test, which detects lethal mutations by an absence of a class of X chromosomes (*10*); the yf doubly attached-X (yf:=) sex-linked visible test, which detects any induced, visible mutation in the X chromosomes of the sons (*11*); and a specific visible test, *ed dp cl*, using a well-studied complex locus, dumpy, which detects all induced alleles of that specific locus (*4, 12*). In addition to the three tests for mutation, we employed a *bw; st* translocation test for breakage (*11*).

LSD was injected into males at three different concentrations: 1, 100, and 500 μg per milliliter of a tartrate (8.7 μg/ml) solution (for the 100-μg solution the tartrate was already present in the capsules; for the 1-μg and 500-μg concentrations, a stock solution was prepared with the same solution of tartrate—8.7 μg/ml). The injection techniques were identical to those used for analysis of dumpy mutations and sex-linked lethals. The males were transferred by an aspirator to new virgin females every 3 days, the procedure thereby establishing a minimum of five broods. These provided a test of post-meiotic (0 to 7 days), meiotic (8 to 10 days), and premeiotic (11 or more days) stages of spermatogenesis (*13*). This brooding analysis is particularly useful in the detection of mutations requiring one or more replications for incorporation of a mutagen; the post-meiotic cells might not be reactive to the agent, but the premeiotic broods could provide the assay for such late-arising mutations. The range of concentrations was based on the tissue-culture studies of Cohen (*1*) (0.001 μg/ml to 10 μg/ml) and the studies in vivo of Irwin (*2*) (200 μg to 500 μg of LSD per mean dose per subject). Our 100 μg/ml and 500 μg/ml series represent a much higher tissue saturation in *Drosophila* than in human tissue because sufficient LSD solution was injected to distend the abdomens of the flies. This would be equivalent to intraperitoneal injections of about 1 liter of the LSD concentration in each human subject. A concentration of 500 μg of LSD per milliliter is approximately that used for ICR-170 studies (0.5 to 1.0 mg/ml) which would induce approximately 10 percent of sex-linked lethals and 0.5 percent of dumpy mutations. Controls were injected with the stock tartrate solutions.

In all four series the males were Oregon R wild type (+). These males, 24 to 48 hours old, were injected abdominally with fine glass needles prepared from Pasteur pipettes heated over a microburner (*4*). Although flies may express some of the fluid injected at the site of puncture, tests with vital dyes showed clearly that the tissues of the fly are perfused rapidly after injection (*14*).

In the sex-linked lethal tests (Table 1), among 4786 progeny of the LSD series, a total of seven lethals and semi-lethals were found. There were two lethals among 3168 tested X chromosomes in the controls. The frequency of spontaneous mutation previously found for this Oregon R stock in our laboratory was 0.2 percent (*5*).

In the sex-linked visible test, all aberrant male phenotypes were recorded and tested with yf:= females. Only one mutant phenotype, a Minute bristle (*M*, about 50 different loci) disturbance associated with smaller body size and pale body color, was transmitted with high frequency. These Minute mutations usually arose as complete rather than mosaic or fractional phenotypes. The frequencies were 5/7944 for the control series; and 14/14,918 among the total LSD series. None of these LSD values differs significantly from that obtained in the control. Because the Minute mutant is a dominant, the appearance of F_1 Minutes tells nothing about the chromosome which bears it nor the sex from which it arose. This can be determined, however, from the F_2 progeny when Minute F_1 males are mated to yf:= females. If the Minute is autosomal (not sex-linked), at least half of the F_2 males will show normal bristles. Minutes are usually recessive lethal and it was thus expected and found that all of the transmitted male Minutes from the F_1 were autosomal. Among all of the other phenotypes (mostly fractional and mosaic wing mutations) only one mutant, in the 100-μg series, transmitted. This was a male with a trident or pentagon pattern on the thorax, which proved to be a recessive sex-linked mutant. The frequency of aberrant phenotypes among the F_1 males was: 47/7944 for this control; and 64/14,918 among the total LSD series. The lower frequency of aberrant phenotypes among the LSD series is not significant. The transmission of only one sex-linked mutant among the 18,296 F_1 males examined is not unexpected. Schalet (*15*) reported a frequency of about 1/90,000 or 1.1×10^{-5} as the average spontaneous mutation frequency for any one of 13 specific sex-linked mutants he studied.

For the dumpy series, only an injection of 500 μg of LSD per milliliter was used in this experiment. Among the F_1 progeny there were nine dumpy pheno-

types and 35,157 non-dumpy phenotypes. Control frequencies from the same Oregon R stock had been obtained by Carlson (6/31,154), Southin (8/27,426), and Jenkins (3/13,789) for a total spontaneous mutation frequency of 17/72,369 (*3, 6, 12*). Thus, in the LSD series in which 500 μg/ml were given, there was no difference from control expectations. By contrast, injections of EMS, NMU, or ICR-170 would have yielded frequencies of about 1 percent dumpy phenotypes in the F_1 (*7, 12*).

A very high percentage of the Minute mutations arose as complete, rather than mosaic, phenotypes (17 complete and 2 mosaics). By contrast, most of the dumpy mutations were mosaics (two complete and seven mosaic phenotypes). None of the nine dumpy mutants transmitted the dumpy phenotype to the F_2 progeny. Most of the fertile Minutes (11/16) transmitted to the F_2 generation. Schalet reported most spontaneously arising sex-linked visible mutations to be mosaic in origin, a characteristic also found for spontaneous dumpy mutants. Most of the Minute mutations arising spontaneously probably are derived from nondisjunction of the fourth chromosome (*16*) rather than from small deletions of chromatin in the larger autosomes. In radiation experiments, Minute mutations are frequently associated with small deletions of the chromatin (*17*).

The translocation test for breakage was negative for all of the LSD series—no translocations were found among a total of 4205 progeny tests. Spontaneous translocations are rare, their frequency being less than 10^{-4} (*18*). The absence of translocations places LSD in a class quite distinct from ionizing radiation which would have yielded 14.4 percent translocations in the *bw; st* test at a dose of 4000 roentgens (*19*); atmospheric mustard gas bubbled through water would have given 0.4 percent translocations in the *bw; st* test (*20*).

The absence of induced mutations (sex-linked or autosomal, visible or lethal) and the absence of induced chromosome breakage in *Drosophila* by LSD suggests that LSD, if it is a mutagen or radiomimetic agent in human chromosomes, is not a very powerful one. It is more probable, in view of reports that do not confirm leukocyte breakage (*21*) and from our results, that LSD induces neither mutations nor chromosome breaks in man. The initial report of positive results with LSD in human leukocytes or among LSD-users, we believe, was based on small samples of individuals and on inadequate controls (*22*).

The difficulties of proving an agent nonmutagenic are more severe than for obtaining positive results. It is unlikely but theoretically possible that LSD is enzymatically degraded in *Drosophila* but not in mammals. Alternatively, it might be argued that LSD can induce very low frequencies of mutations or breaks which are slightly above control rates. Our tests are not extensive enough to detect such low level muta-

Table 1. Mutational and breakage events among F_1 progeny of untreated and LSD-injected *Drosophila melanogaster* males, at intervals indicated.

Types	Mutations and breakages (No.)							
	1–3 days	4–6 days	7–9 days	10–12 days	13–15 days	16–18 days	To-tal	Fre-quency
			yf:= (control)					
Minutes	0	1	1	1	2		5	0.6
F_1 abnormal phenotypes	9	20	9	5	4		47	5.9
F_2 transmitted mutants	0	0	0	0	0		0	0.0
Normal F_1 males	1176	2875	1297	516	810		7944	
			yf:= (1 μg/ml)					
Minutes	0	0	3	0	0		3	0.8
F_1 abnormal phenotypes	4	0	6	0	11		11	2.8
F_2 transmitted mutants	0	0	0	0	0		0	0.0
Normal F_1 males	898	396	1349	221	530		3894	
			yf:= (100 μg/ml)					
Minutes	1	2	1		1		5	0.8
F_1 abnormal phenotypes	9	7	8		6		30	4.7
F_2 transmitted mutants	1	0	0		0		1	0.2
Normal F_1 males	1706	1780	1990		946		6422	
			yf:= (500 μg/ml)					
Minutes	1	3	1	0	1		6	1.3
F_1 abnormal phenotypes	5	10	5	2	1		23	5.0
F_2 transmitted phenotypes	0	0	0	0	0		0	
Normal F_1 males	1269	1166	605	1924	268		4602	
			ed dp cl *(500 μg/ml)*					
Mosaic F_1 dp phenotypes	2	2	1	2	0		7	0.2
Complete F_1 dp phenotypes	1	1	0	0	0		2	0.06
Normal F_1	8853	11205	4453	7428	3118		35157	
			Basc (control)					
Lethals	0	1	0	1	0		2	0.6
Semilethals	0	0	0	0	0		0	0.0
Nonlethals	674	723	669	604	498		3168	
			Basc (1 μg)					
Lethals	0	1	0		0		1	0.6
Semilethals	1	0	0		0		1	0.6
Nonlethals	415	480	395		344		1634	
			Basc (100 μg)					
Lethals	0	0	0		0		0	0.0
Semilethals	2	0	1		0		3	1.7
Nonlethals	496	494	435		367		1792	
			Basc (500 μg)					
Lethals	2	0	0		0		2	1.5
Semilethals	0	0	0		0		0	0.0
Nonlethals	410	343	342		265		1360	
			bw; st *(1 μg)*					
Translocations	0	0	0				0	0
Nontranslocations	376	314	442				1132	
			bw; st *(100 μg)*					
Translocations	0	0	0				0	0
Nontranslocations	475	468	353				1296	
			bw; st *(500 μg)*					
Translocations	0	0	0		0	0	0	0
Nontranslocations	461	417	414		273	212	1777	

genesis, but they do rule out any effect above a doubling of the spontaneous mutation frequency. To assay for such low-level effects, it would be more appropriate to test LSD with microbial systems (*Neurospora, Aspergillus, Saccharomyces,* bacteria) where populations of 10^7 or more treated cells have been assayed by selective techniques for antibiotic resistance, nutritional mutants, or reverse mutations (*11*).

It is our belief that the most satisfactory screening method for a definitive analysis of the genetic effects of LSD or other chemicals should be a three-step procedure: (i) mutagenesis tests with microbial organisms; (ii) mutagenesis and breakage tests with *Drosophila;* and (iii) cytological tests for breakage in small mammals (rats, hamsters, and so forth) whose genetic lineage can be rigorously controlled. The universality of the basic components of metabolism and the universality of the genetic coding machinery (*23*) make it unlikely that a dangerous mutagen would be missed by the three-step procedure we recommend. This procedure is similar to that recommended by Kaufmann and Schuler for pharmaceutical products (*24*). Because of the possible or proven genetic and teratogenic hazards associated with certain pharmaceuticals, antibiotics, preservatives, additives, and other chemical agents (*25*) we believe that the screening procedure we described should be used before these substances are made available to the public.

Our experiments in *Drosophila* do not substantiate the conclusion that LSD has any pronounced effect on genes and chromosomes. We recognize that high doses of LSD (varying from 100 μg to several thousand micrograms) induce strong psychological reactions in humans (*26*). For this reason we do not wish our results at the genetic level to be used as a justification for nonmedical use of LSD as a mind-altering chemical. We strongly recommend that the social debate on the uses and abuses of LSD be based on what is actually known, from rigorously controlled experiments, rather than from conjecture, insufficient sample size, isolated case histories lacking rigorous controls, and subjective individual experience.

DALE GRACE
ELOF AXEL CARLSON
Department of Zoology,
University of California, Los Angeles
PHILIP GOODMAN
School of Medicine
University of California, Los Angeles

References and Notes

1. M. Cohen, M. J. Marinello, N. Back, *Science* **155**, 1417 (1967).
2. S. Irwin and J. Egozcue, *ibid.* **157**, 313 (1967).
3. J. B. Jenkins, *Mutation Res.* **4**, 90 (1967).
4. E. A. Carlson and I. I. Oster, *Genetics* **47**, 561 (1962).
5. E. A. Carlson and J. L. Southin, *ibid.* **48**, 663 (1963).
6. J. L. Southin, *Mutation Res.* **3**, 54 (1966).
7. H. O. Corwin, thesis, University of California, Los Angeles (University Microfilms, Ann Arbor, Mich., 1966).
8. H. J. Muller, *Zeitschr. Indukt. Abstamm. Vererb. Suppl. I* (1928), p. 234.
9. C. Auerbach, *Proc. Roy. Soc. Edinburgh* **62**(2), 211 (1945).
10. ———, *Mutation,* part 1, *Methods* (Oliver and Boyd, Edinburgh, 1962); T. Alderson, *Nature* **207**, 164 (1965); O. G. Fahmy and M. J. Fahmy, *ibid.* **180**, 31 (1957); E. A. Carlson, *J. Theor. Biol.* **6**, 432 (1964); J. L. Epler, *Genetics* **54**, 31 (1966).
11. W. J. Burdette, Ed., *Methodology in Basic Genetics* (Holden-Day, San Francisco, 1963).
12. E. A. Carlson, *Genetics* **44**, 347 (1959); J. B. Jenkins, *ibid.* **47**, 783 (1967).
13. A. C. Chandley and A. J. Bateman, *Nature* **193**, 299 (1962).
14. The efficiency of this injection technique depends primarily on the quality of the needle used. In these experiments only those flies which visibly retained the injected solution, and were not otherwise injured, were used.
15. A. P. Schalet, thesis, Indiana University, Bloomington (University Microfilms, Ann Arbor, Mich., 1960).
16. C. B. Bridges, *Proc. Nat. Acad. Sci. U.S.* **7**, 186 (1921).
17. T. H. Morgan, C. B. Bridges, J. Schultz, *Carnegie Inst. Wash. Year Book* **32**, 298 (1933).
18. H. J. Muller, *Proc. Nat. Acad. Sci. U.S.* **14**, 714 (1928).
19. L. A. Snyder and I. I. Oster, *Mutation Res.* **1**, 437 (1964).
20. E. M. Sonbati and C. Auerbach, *Z. Vererb.* **91**, 253 (1960).
21. L. Bender and D. V. Siva Sankar, *Science* **159**, 749 (1968); W. D. Loughman, T. W. Sargent, D. M. Israelstam, *ibid.* **158**, 508 (1967).
22. A proper control would have taken into account the subject's history of all drugs taken and health records including diseases contracted and general health condition. Experimental and control subjects should be closely matched.
23. R. E. Marshall, C. T. Caskey, M. Nirenberg, *Science* **155**, 820 (1967).
24. B. N. Kaufmann and D. Schuler, *Genetic and Chromosomal Changes Produced by Drugs in Pharmacological Techniques in Drug Evaluation,* P. E. Siegler and J. H. Moyer, III, Eds. (Year Book, Chicago, 1967), vol. 2.
25. W. Ostertag, E. Duisberg, M. Sturmann, *Mutation Res.* **2**, 293 (1965); J. M. Krogh, *Acta Med. Scand.* **177**, 783 (1965); R. C. Nowell, *Exp. Cell Res.* **33**, 286 (1964); M. W. Shaw and M. M. Cohen, *Genetics* **51**, 181 (1965).
26. J. T. Ungerleider, D. D. Fisher, M. Fuller, *J. Amer. Med. Ass.* **197**, 389 (1966); D. B. Louria, in *LSD, Man and Society,* R. C. Debold and R. C. Leaf, Eds. (Wesleyan Univ. Press, Middletown, Conn., 1967).
27. We thank Patricia Girard for technical assistance and Patricia Mukai for clerical assistance. Supported by NSF GB 6595 to E.A.C. The LSD was supplied by the NIMH under drug No. IND 4102. The LSD for the 1-μg and 500-μg tests was Sandoz batch No. 53032; for the 100-μg tests, it was Sandoz batch 65002. D.G. is a predoctoral NASA fellow, and P.G. is a student in the School of Medicine, University of California, Los Angeles. Reprint requests should be addressed to E. A. Carlson, Biology Department, State University of New York, Stony Brook 11790.

8 April 1968; revised 18 June 1968 ■

Trophoblast Antigenicity Demonstrated by Altered Challenge Graft Survival

Abstract. *Adult C57BL/6J male mice received either a primary ectopic transplant or a primary and a challenge ectopic transplant of trophoblast tissue obtained from the ectoplacental cones of 7½-day-old C3H/HeJ × C3H/HeJ embryos. Gross and histologic examinations of these grafts at 5, 7, and 12 days of growth indicated that the challenge grafts were inhibited in growth; there were a smaller percentage of grossly successful grafts, smaller hemorrhagic reactions, fewer viable cells at all stages of growth, and better host containment of the colony. The evidence indicates that exposure to pure trophoblast alters an animal's subsequent reaction to grafts syngeneic with the original trophoblast and can best be explained as evidence of immunologic sensitization of the host.*

In considering the immunologic relationship between a mother and her fetus, one must determine whether the trophoblast can be recognized by the mother as antigenic. Previous work has suggested that the allogeneic histocompatibility antigen is not expressed on the trophoblast (*1*), although xenogeneic antigen may be present (*2*). The possibility that an antigen specific to trophoblast exists has been suggested for humans (*3*) but has not been confirmed in animal studies. We now report observations of the effects of ectopic trophoblast allografts on the growth of subsequent trophoblast transplants.

Female mice (C3H/HeJ from Bar Harbor, Maine) were killed 7½ days after mating with syngeneic males. The embryos were removed from the deciduomata, and the ectoplacental cones were separated from embryonic and decidual tissue. Each cone was transplanted by means of a 22-gauge spinal needle into the subcapsular area of a kidney of a C57BL/6J male.

This methodology was chosen for a number of reasons. Earlier observations indicated that embryonic parts can develop into trophoblast colonies derived from 2½-day-old fertilized ova. The presence of such embryonic elements suggested that immunologic activity could be stimulated by tissues other