(9 + 2) flagellum of eukaryotic cells (*12*) must also contain cytochrome oxidase and other mitochondrial enzymes (*13*); that all cells with the "higher chromosomes" seen in classical mitosis (for example, red algae and ascomycetes) had a (9 + 2) flagellated ancestor and retain the relevant DNA of that "protoflagellum" (*4*) even if they lack visible (9 + 0) centrioles and basal bodies (*14*); that all eukaryotes potentially form the colchicine-sensitive protein of the microtubules (*15*); that all eukaryotic plant cells contain at least three different nonnuclear ("satellite") DNA's; and that steroid and flavonoid derivatives will be found only in relatively young sediments—much younger than those which first contain photosynthetically reduced carbon]. By challenging the students of the enormously diverse Thallophytes to find contradictions to the theory proposed here, perhaps some appropriately focused research will be stimulated. If it proves generally acceptable, the division of living organisms into four kingdoms proposed by Copeland (*16*) logically follows (Fig. 1 and Table 2).

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14 May 1968

Lysergic Acid Diethylamide: Mutagenic Effects in Drosophila

Abstract. *d-Lysergic acid diethylamide causes a significant increase in recessive lethal mutations in the X chromosome of* Drosophila *males after intraperitoneal injection of massive doses.*

An increase in chromosomal abnormalities has been detected cytologically in cultured leukocytes from *d*-lysergic acid diethylamide (LSD-25) users (*1*), as well as in leukocytes exposed in vitro to LSD-25 in doses ranging from 0.001 to 10 μg per milliliter of culture medium for 4, 24, and 48 hours (*2*). However, other workers detected no increase in chromosomal abnormalities in leukocyte cultures from humans exposed to recent heavy doses of LSD-25 (up to 150,000 μg) (*3*); nor could damage be detected in leukocytes of children receiving therapeutic doses of LSD (*4*). We now report on genetic damage in *Drosophila*, caused by LSD-25.

The testes of 75 virgin 6-day-old males of composition of *y* sc^{81} *In49* sc^{8}/sc^{8}*.Y.BS* were bathed (by intraperitoneal injection) in approximately 0.3 μl of a fresh solution containing 10 mg of LSD-25 per milliliter of saline (Sandoz, batch No. 43032). Since the average weight of a fly is 0.8 mg, this dose corresponds to about 4000 μg per gram of body weight and is roughly 2000 times that of the highest human dose referred to above. Only 15 of the males survived, and ten were fertile. They were mated individually to fresh, virgin females of genotype *y ct* (yellow body, cut wings) every 3 days for five broods. The offspring that hatched from brood 1 came from fully differentiated sperm cells at the time of the injection, and the later broods represented germ cells successively younger at the time of treatment. Thus the offspring from later broods come either from dividing gonial cells or germ cells in various stages of maturity during their exposure to LSD-25.

The sperm cells of each of the ten fertile males from brood 1 were tested for recessive lethals by crossing daughters of each male individually to their *y ct* brothers and looking for the absence of ct^{+} (noncut) sons. The number of sperm cells sampled from each male ranged from 28 to 47, with a mean of 37.8. Lethals were found among the progeny of six of the ten treated males, two each being found among the progeny of two of the males and one each among the other four. This distribution of lethals rules out the possibility that the lethals represent a cluster originating from a spontaneous lethal arising in the early germ track (in which case they would have all come from the same male). The distribution also indicates reasonable uniformity of mutagenic response of individual males to treatment. An overall recessive lethal mutation frequency in the X chromosome of 2.1 ± 0.7 percent was found for brood 1.

No flies were injected with saline alone because large-scale experiments in our laboratory had shown that injected saline solution has no mutagenic effect. The spontaneous lethal mutation frequency in the inbred stock furnishing the treated males was measured simultaneously, only one lethal being found among 2303 tested chromosomes.

Only mature sperm are appreciably affected by the drug so far as the production of recessive lethals is con-

Table 1. Recessive lethal mutations in the X chromosome and the loss of both markers from the Y chromosome among the offspring of *Drosophila* males according to the stage of maturity of the germ cells at the time of injection with LSD-25. The numbers in italics represent the overall mutation frequency (percent); that is, the ratio of the number of lethals to the number of chromosomes tested.

Brood	Time after injection (days)	Lethal tests			Loss of Y chromosome markers		
		Chromosomes tested	Lethals	Lethals (%)	Offspring examined (No.)	$y\,B^{+}$ (No.)	Males* (%)
1	3	378	8	2.12	808	2	0.25
2	6	238	1	0.88	530	1	.19
3	9	256	1	.39	795	1	.13
4	12	112	0	0	246	1	.41
5	15	119	0	0	471	0	0
6	18	96	0	0	209	0	0
Total		1,199	10	*0.84*	3,059	5	*0.16*
Spontaneous		2,303	1	*0.04*	23,249	9	*0.04*

* These males all proved to be sterile.

cerned (Table 1). The increase in the number of $y\ B^+$ sons indicates either simultaneous loss of the y^+ and B markers from the Y chromosome or the loss of the entire Y chromosome. The latter could be due to nondisjunction of the X and Y chromosomes at meiosis, but since in some cases the event occurred in mature sperm, some direct action on the Y chromosome, or on its centromere, is indicated [as postulated by Auerbach to account for a similar event after chemical treatment (*5*)]. All of the $y\ B^+$ males were sterile, which would be expected if they lacked the Y chromosome. Many would also be sterile if both markers had been removed by breakage that is followed by rejoining of the broken ends of the Y to form a ring, because the location of the breaks could be such as to exclude from the ring one or more of the genes for fertility. Other studies have shown that less than 5 percent of this class of males with losses of both markers are fertile. Whatever the mechanism, the continued appearance of these males through brood 4 indicates that cells undergoing spermatogenesis (and hence able to produce nondisjunctional sperm at meiosis), as well as mature sperm, are affected. However, after day 12 (broods 4 through 6), the cells would have been dividing mitotically, and nondisjunction does not occur during mitosis without treatment.

A 1:1 dilution of the original solution of this drug when injected into 100 males resulted in 35 survivors, of which 30 were fertile. Among 265 chromosomes tested from brood 1, two lethals were found (0.76 ± 0.53 percent), and none were found among 272 tested chromosomes from brood 2. Among 2910 offspring of the treated males, three $y\ B^+$ sons were found (0.10 ± 0.05 percent). Thus, although mutagenicity at this dilution is indicated, the frequency of mutation has markedly dropped.

The detection of eight lethals among 378 chromosomes in brood 1 gives a mutation frequency of 2.1 ± 0.7 percent. Since the total number of lethals found was small, a calculation based on a Poisson distribution and designed for analysis of small numbers derived from large populations (*6*) shows that this mutation frequency has a lower limit of 0.68 percent ($P = .01$), as compared with the upper limit of 0.32 percent ($P = .01$) of the control rate. Therefore we conclude that LSD-25 has caused a significant increase in the mutation frequency at this dosage.

The fact that mutations were produced only when a highly toxic and partially sterilizing dose was used does not alter the conclusion that the drug can attack the chromosomes and cause mutations. The experience of numerous workers has shown that many chemicals in doses of even higher toxicity have proved to be nonmutagenic (*7*).

The fact that in *Drosophila* LSD-25 produces recessive lethals is significant. At the molecular level, genetic changes are generally considered to be similar among eukaryotes. Penetrability, breakdown products, and reactions with other metabolites are some of the ways in which the two organisms may differ in their reaction to the drug. However, its administration by injection in these experiments makes it somewhat unlikely that breakdown occurred before the chromosomes were reached. Possibly, oxidation or other deterioration of the LSD-25 used in these experiments had occurred, but the sample was stored in the dark and seldom opened. Although negative results regarding mutagenicity of a particular agent are not conclusive proof of its lack of effect, positive results in a well-studied system such as *Drosophila*, even in a small-scale experiment, warrant testing for reproducibility by further investigation.

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29 April 1968; revised 31 July 1968

Trypanosome Transmitted by Phlebotomus: First Report from the Americas

Abstract. *Transmission studies carried out in the laboratory incriminated* Phlebotomus vexator occidentis *as a vector of a species of trypanosome that infects* Bufo boreas halophilus. *Toads free of parasites contracted the trypanosome after eating infected flies and after intraperitoneal inoculation of flagellates cultured from the hindgut of flies that had fed on infected toads. Discovery of this vector-host-parasite system in the Americas, and the localization of promastigote flagellates (leptomonads) in the hindgut of the vector, should assist in clarifying interpretative problems associated with infection of wild-caught flies in studies on leishmaniasis in the Americas and elsewhere.*

Previous studies of sandflies in California (*1*) revealed small percentages of *Phlebotomus vexator occidentis* infected with a flagellate of unknown origin. Only females that had taken a previous blood meal were infected. Concurrent studies showed that this phlebotomine normally fed only on anurans and reptiles (*1, 2*). An extended search for the source of infection has resulted in this first report of a *Phlebotomus*-transmitted trypanosome from the Americas, the first trypanosome to be reported from a toad in western North America.

The trypanosome found in the blood of the toad *Bufo boreas halophilus* proved infective for all of 38 *P. vexator occidentis* females fed on infected toads. As with most previously reported sandfly-transmitted trypanosomes (*3*), flagellates multiplied rapidly in the insect's midgut and then migrated to the hindgut, producing a "posterior station" infection. Trypanosome infections developed in noninfected *B. boreas* following (i) ingestion of infected sandflies and (ii) intraperitoneal inoculation of flagellates cultured from the hindgut of flies fed on infected toads. In the laboratory, unrestrained toads readily eat sandflies confined in the same cage. Both the western toad and *P. vexator occidentis* are nocturnally active and use burrows of the California ground squirrel, *Citellus beecheyi*, as diurnal resting sites.

All sandflies and infected toads were collected at a 10-hectare study area in Solano County, where 5 of 32 toads found between July and October 1967 were infected. Natural and experimental infections were diagnosed by wet mounts of peripheral blood and physiological saline; 0.05-ml samples of blood