SHORT COMMUNICATION

STORAGE EFFECTS ON TRANSLOCATIONS AND DOMINANT LETHALS INDUCED BY ETHYL METHANESULFONATE (EMS) IN *DROSOPHILA MELANOGASTER*

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When *Drosophila* sperm treated with different doses of ethyl methanesulfonate (EMS) were stored in untreated females, changes in the yields of translocations and dominant lethals were examined. At high EMS doses yielding 25-33% X-linked recessive lethals, the frequencies of both translocations and dominant lethals increased markedly with increasing storage time. At low doses yielding 7-15% recessive lethals, however, no increase in their yields was detected after the storage of treated sperm, indicating that storage effects of EMS are dose dependent.

When *Drosophila* spermatozoa treated with chemical mutagens, such as triethylene melamine, ethyleneimine and diepoxybutane were stored in untreated females, the yields of translocations (Snyder 1963; Watson 1964, 1966; Ratnayake *et al.* 1967; Sram 1970a) and dominant lethals (Ratnayake 1968; Sram 1970b) increased strikingly, while those of X-linked recessive lethals (Snyder 1963; Watson 1964, 1966; Sram 1970a) increased only slightly or did not change. Ethyl methanesulfonate (EMS) has also been reported to show storage effects on translocations (Abrahamson *et al.* 1969) and dominant lethals (Sram 1970b). In these investigations, EMS was tested only at a single dose. We examined in the present studies whether the yields of translocations and dominant lethals induced by EMS at different doses are affected by the storage treatment. The data obtained show that both translocations and dominant lethals increase during storage only at high EMS doses.

Two-day-old *Drosophila melanogaster* males of wild type Or-R strain were injected with 0.2 μ l of EMS (Eastman Organic Chemicals, New York, U.S.A.) solution at concentrations of $2.6-10.5 \times 10^{-2}$ M in saline. The treated males were placed on a normal standard medium for one day before mating to females. The medium contained 1,100 ml water, 15 g agar, 70 g cornmeal, 15 g dry yeast, 35 ml treacle, and 5 ml of methyl-*p*-

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benzoate 5% solution in 75% alcohol as a fungicide.

For translocation tests, treated males were mated individually to 3 virgin females of strain 0-1 with the genotype *In(1)sc^{1L}sc^{8R}+dl-49, y; bw; st* (for the symbols, see Lindsley and Grell, 1968) on the standard medium for 3 days and then the males were discarded. The inseminated females were transferred to a fresh standard medium at a three-day interval to furnish three to five successive broods from inseminated females. F₁ males from each brood were individually backcrossed with 0-1 virgin females to detect translocations involving chromosome II, III, and Y in their F₂.

In the examination of dominant lethals, treated males were crossed with two-day-old Or-R females, at ratio of two males to one females, for one day on the standard medium and for another one day on the egg-collection unit (see Fig. 1). Then the males were discarded and the inseminated females were transferred successively to the egg-laying medium or the deficient medium. The egg-laying medium was prepared by boiling a mixture of 1,000 ml water, 20 g agar, 15 g dry yeast, 30 ml treacle, and 5 ml of methyl-*p*-benzoate 5% solution. A yeast suspension was smeared on the surface of medium, immediately before use, to furnish an additive nutrient for flies and to suppress the propagation of bacteria and fungi. The deficient medium, used for preventing egg-lying, was made of 4% agar and 2% sucrose. The eggs laid were counted immediately after the females were transferred to the fresh egg-laying medium, and after 24 h incubation the hatched eggs were scored. The females which yielded less than 5 hatched eggs were excluded from the data.

Fig. 1. An egg-collection unit used for the dominant lethal assays. (a) Vials were cut. (b) One end of cylindrical portion was covered by nylon gauze which was cemented to the wall, and the other end was sealed with a cotton plug after introducing an inseminated female. Medium was poured into the bottom portion of a cut vial. (c) The side of a cylinder with the nylon gauze was placed on medium contained in a cut vial and the two cut vials were fixed with a cellophane adhesive tape. The flies can deposit eggs on the medium through the nylon gauzes.

In order to estimate the effective dose of EMS, the frequency of X-linked recessive lethals was determined in the translocation or dominant lethal tests. Treated males were mated to 0-1 virgin females, and placed on a standard medium for three days. Resulting F_1 females were individually crossed with sibling males or M-5 males, and the X-linked recessive lethals were scored in F_2 cultures. EMS-induced X-linked recessive lethals were determined only in the first brood, since they are known to be unaffected by the storage treatment (Ikebuchi, in press).

Table 1 represents the data of translocations in the successive broods from inseminated females. At high EMS doses yielding 25% and 33% recessive lethals, the frequency of translocations increased markedly with increasing storage time. This confirms the report of Abrahamson *et al.* (1969). At low doses yielding 7-15% recessive lethals, however, an increase in the translocation yields after the storage of treated sperm was not detected in the present experiments on a limited scale, though a few translocations were detected in the early broods.

Table 1. Frequencies of translocations in the successive 3-day broods from inseminated females and X-linked recessive lethals in the first brood after treatment of *Drosophila* spermatozoa with EMS

Concentration ($\times 10^{-2}$ M)	% lethals	Frequencies of translocations (%)				
		Brood-1	Brood-2	Brood-3	Brood-4	Brood-5
2.6	7.06 ± 0.70 (98/1324)	— (0/1250)	0.09 ± 0.09 (1/1075)	— (0/870)	— (0/789)	— (0/447)
2.6	9.83 ± 1.31 (51/519)	— (0/812)	— (0/899)	— (0/662)	— (0/319)	N.T.*
5.2	15.21 ± 1.05 (179/1177)	0.09 ± 0.09 (1/1144)	0.12 ± 0.12 (1/825)	— (0/575)	N.T.*	N.T.*
7.0	24.89 ± 1.28 (286/1149)	0.19 ± 0.13 (2/1051)	1.03 ± 0.34 (9/871)	1.93 ± 0.53 (13/673)	N.T.*	N.T.*
10.5	32.93 ± 1.38 (380/1154)	0.20 ± 0.14 (2/992)	3.01 ± 0.62 (23/763)	7.20 ± 1.33 (27/375)	3.47 ± 1.53 (5/144)	8.82 ± 4.86 (3/34)

* Not tested.

That the storage effects depend on EMS doses was confirmed with the dominant lethal tests. As shown in Table 2, with increasing storage time the relative dominant lethal frequency increased markedly at higher doses (about 25% recessive lethals). This result confirms the report of Sram (1970b). At a low EMS dose yielding about 9% recessive lethals, an increase in the relative dominant lethal frequency was not found.

Auerbach (1949) and Slizynska (1973) proposed that the storage effects in *Drosophila* sperm are due to a delay, during storage, in a conversion of potential breaks to open breaks. In barley seeds, similarly to *Drosophila*, enhancing effects of storage on the yields of genetical damages induced by EMS (Gichner and Gaul 1971) and other chemicals (Garnina and Romanova 1970; Gichner *et al.* 1971; Gichner and Veleminsky 1972) were reported. The genetical damage yields are influenced by the moisture content of EMS-treated barley seeds during storage (Gichner and Gaul 1971). These

Table 2. The effect of storage on the frequency of dominant lethals induced by EMS in *Drosophila* sperm

Expt. No.	Conc. (x 10 ⁻² M)	% lethals	Days after treatment	Control		Treated		R.D.L.*
				No. of eggs laid	% hatched	No. of eggs laid	% hatched	
D-1	2.6	8.79±1.00 (71/808)	4	553	96.2	500	93.6	2.7
			5	313	97.4	377	95.0	2.5
			12	435	84.1	367	89.6	-6.5
			13	1,230	81.5	1,231	82.9	-1.7
D-2	7.0	24.89±1.28 (286/1149)	3	927	93.3	1,186	85.8	8.0
			4	1,148	94.3	1,587	81.9	13.1
			5	1,387	93.8	1,706	71.2	24.1
			12	265	69.4	403	26.3	62.1
			13	784	66.2	929	16.9	74.5
D-3	7.8	25.85±1.59 (197/762)	3	543	97.4	538	82.9	14.9
			4	299	97.3	152	92.8	4.6
			5	459	96.9	300	84.0	13.3
			7	590	94.7	287	70.7	25.3
			9	367	85.8	287	66.8	22.1
			10	1,119	89.5	997	59.4	33.6
			12	511	83.2	514	42.6	48.8
			13	734	77.1	409	32.3	58.1

* Relative Dominant Lethal Frequency = $100 - (ht/hc \times 100)$, where *ht* is % hatchability in the series of treated spermatozoa and *hc* is % hatchability in the control series.

findings are interpreted that repair processes are responsible for the modification of the storage effect. If, in *Drosophila* sperm, storage effects are lacked at the low EMS dose range, it may be suspected that the potential breaks induced by EMS are not converted to the true breaks due to the function of a repair system, which is inhibited by high doses of EMS. This assumption may also explain a highly upward-bending dose-response curve for EMS-induced translocations: A dose-exponent of about 3 was obtained (Ikebuchi, in press). However, the proposed model is only one way of interpreting the data and open to question for further experiments.

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