EVIDENCE FOR DIFFERENTIAL RADIOSENSITIVITY RATHER THAN RECOVERY IN SPERM SAMPLES FROM X-IRRADIATED *DROSOPHILA MELANOGASTER* MALES

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IT is well established that sperm released the first day after the X-irradiation of 3 to 4 day old *Drosophila melanogaster* males shows a higher mutation frequency than second day sperm (for references see OSTER 1961 and MOSSIGE 1963). Two explanations for this effect have been offered: (1) there is some "recovery" of potential mutational damage during the one day storage period, or (2) the initial radiosensitivity of the sperm sampled during the first day after irradiation is higher than that of the second day. Evidence presented by previous workers as to the likelihood of either explanation has been conflicting (see OSTER 1961 and the discussion following this paper). The experiments reported here support the concept of a lower radiosensitivity of second day sperm rather than that of recovery of damage due to the one day storage period.

MATERIALS AND METHODS

D. melanogaster males, collected during a one day interval and aged 3 to 4 days, were irradiated with 3 kr X rays (150 kv, 6mm aluminum h.v.l., 500 r/min). They were then mated every 24 hours with a large excess of virgin females either immediately (Series 1, consisting of five separate experiments) or after storing them for one day without females (Series 2, consisting of seven separate experiments). In both series, a brood pattern of several one-day broods was obtained. The genotype of the P-males was *B*; that of the P-females γ *sc*⁵¹ *In49* *sc*⁸; *bw*; *st* (for explanations of gene symbols see BRIDGES and BREHME 1944). This dual-purpose stock, constructed by OSTER (1958) from similar stocks made by MULLER (1954), allows both recessive sex-linked lethals and 2;3 translocations to be scored from the progeny of the *same* irradiated males. All flies were cultured at $25 \pm \frac{1}{2}^{\circ}\text{C}$.

RESULTS AND DISCUSSION

The results are presented in Table 1 and in Figure 1. It is evident that the results from the separate experiments of the second day of Series 2, both for lethals and translocations, cannot be combined to a mean value because of the pronounced heterogeneity (the χ^2 -method gives a $P = 0.0009$ for lethals and a $P = 10^{-5}$ for translocations). The results from the other broods in Series 2 and from all broods of Series 1 are homogeneous. For both the lethal and translocation tests, the individual experiments of the second day in Series 2 can be classified into two groups: one which has about the same mutation frequency as the first day of Series 1 (both the translocation and lethal frequencies of Experiments

FIGURE 1.—The frequencies of sex-linked lethals and 2;3 translocations from daily broods after irradiating 3 to 4 day old *Drosophila* males with 3000r X rays. The males were mated either immediately after irradiation (Series 1) or after being kept for one day without females (Series 2). For the second day in Series 2 the separate experiments are represented individually (see text for explanation). Frequencies of spontaneous mutations have not been subtracted since they are negligibly low compared to the induced ones. Note that Series 2 starts with Day 2.

1, 6 and 7), and another group which resembles the second day of Series 1 (both the translocation and lethal frequencies of Experiments 2, 3 and 4 and the translocation frequency of Experiment 5). The lethal frequency of Experiment 5 is intermediate. These results are difficult to reconcile with the recovery hypothesis, which would predict a greater consistency between the mutation frequencies of the second day of Series 1 and 2, especially since the experimental conditions were carefully controlled in all of these experiments. It is doubtful that a recovery mechanism is acting in some experiments and not in others. The following explanation, which was suggested by MULLER (1963) when citing our unpublished results together with similar unpublished data by W. E. TRAUT, seems more likely. If we assume that in Series 2, spermatogenesis has proceeded during the one day waiting period, both the typical first and second day sperm are available for fertilization on the second mating-day. Then, experimental factors, which are difficult to control from one experiment to the other, such as the ratio of F_1 flies derived from either Day 1 sperm or Day 2 sperm, could lead to the heterogeneity obtained for Day 2 in Series 2. One wonders, however, why the mutation frequencies of most of the individual experiments of the second day of

TABLE 1

The percent frequencies of sex-linked lethals and 2;3 translocations from Drosophila males irradiated with 3000r X rays

	Day 1	Day 2	Day 3	Day 4	Day 5
<i>Sex-linked lethals</i>					
Series 1*					
Experiments					
1-5	9.6(301/3154)	4.9(131/2682)	6.9(137/1987)	11.8(87/735)	19.2(5/26)
Series 2					
Experiment					
1		9.7(45/466)	13.8(8/58)	18.2(4/22)	50.0(1/2)
2		4.9(25/515)	7.8(30/387)	14.3(18/126)	50.0(4/8)
3		4.7(26/554)	7.6(47/622)	18.2(32/176)	16.7(1/6)
4		5.5(29/530)			
5		7.6(66/864)	5.2(37/706)	12.6(24/190)	25.0(1/4)
6		9.7(9/93)	8.5(28/337)	10.2(22/216)	9.5(2/21)
7		9.3(79/849)	7.6(52/684)	11.8(28/237)	12.5(1/8)
Mean for Series 2†			7.2(202/2794)	13.2(128/967)	20.4(10/49)
<i>2;3 translocations</i>					
Series 1					
Experiments					
1-5	7.2(160/2213)	3.7(80/2195)	5.5(76/1390)	10.9(32/295)	26.7(4/15)
Series 2					
Experiment					
1		8.1(17/209)	20.8(5/24)‡	20.0(2/10)	
2		4.5(16/355)	3.4(7/203)	11.8(6/51)	16.7(1/6)
3		3.5(14/398)	5.5(24/438)	14.3(14/98)	0.0(0/1)
4		2.3(8/349)			
5		4.5(28/621)	3.1(18/588)	12.4(17/137)	
6		8.8(5/57)	5.3(14/262)	12.3(15/122)	27.2(3/11)
7		9.1(67/740)	4.8(22/460)	12.6(12/95)	25.0(1/4)
Mean for Series 2†			4.4(85/1951)	12.9(66/513)	22.7(5/22)

* The males were mated either immediately after irradiation (Series 1) or after being kept for one day without females (Series 2).

† Not calculated for Day 2 because of heterogeneity (see text).

‡ Day 3 of Series 2 (translocations) is homogeneous only after eliminating Experiment 1. The mean therefore has been calculated without this experiment.

Series 2 resemble either the first or the second day of Series 1 instead of being more intermediate. A further factor responsible for the high variability of the results from the second day of Series 2 might be variation in the amount of sperm ejaculated without copulation or in the resorption of unused sperm, as was suggested by LÜNING (1952). The progression of spermatogenesis during the one day waiting period is demonstrated by the fairly good agreement between the mutation frequencies of corresponding broods (see Figure 1), though the later broods are based on only small numbers of chromosomes tested as is typical for brood pattern experiments.

The mutation frequency of sperm X-rayed in the storage organs of *Drosophila* females does not change appreciably within four 4-day periods of egg laying after irradiation as was demonstrated by TRAUT (1962) as well as by OSTER (personal communication). This indicates a lack of recovery in stored mature sperm and further supports the differential sensitivity hypothesis.

Results from experiments by NORDBACK and AUERBACH (1957), performed in a similar way as ours, were tentatively interpreted differently, i.e. by the recovery hypothesis. We feel, however, as did these investigators themselves, that the number of separate experiments in this work and the amount of agreement between them was not large enough to allow this work to be considered as establishing that interpretation.

On the other hand the already mentioned experiments by TROUT (1964) and those of TROSKO (1964) led to the conclusion that differential radiosensitivity rather than recovery due to storing is the explanation for the low mutation frequency of the second day after irradiation. The possibility of sensitivity differences of sperm ejaculated a few days after irradiation had already been discussed by MULLER, HERSKOWITZ, ABRAHAMSON and OSTER (1954).

Causes of the proposed differential sensitivity between first and second day sperm samples are not yet clear. A factor contributing to this effect could be differential oxygenation of the corresponding sperm samples in different sections of the reproductive tract (OSTER 1961).

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SUMMARY

Sperm released the first day after the X-irradiation of 3 to 4 day old males shows a higher mutation frequency than the second day sperm. From a comparison of mutation frequencies of successive one day broods from males which were mated immediately after irradiation with those from males which had been stored for one day before they were mated, it seems likely that the difference between the mutation frequencies of the first and second day is caused by a relatively low initial radiosensitivity of the second day sperm rather than by recovery from radiation damage of the first day sperm owing to the one day storage period.

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