EFFECT ON GENETIC DAMAGE OF POSTTREATMENTS GIVEN X-RAYED DROSOPHILA MALES

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A postirradiation decrease in the amount of genetic damage recovered from sperm led BAKER and VON HALLE (1953) to postulate that some genetic recovery occurred when the sperm remained in the male for 24 hours before mating. BONNIER (1954) and LÜNING (1954) had observed an increase in scored genetic damage from sperm irradiated in females as compared with those irradiated in the males. It has been suggested by AUERBACH (see OSTER 1961) that a certain amount of recovery occurs in the male during the first day after irradiation, full recovery in spermatozoa stored in the males one day after irradiation, and no recovery at all in inseminated females. BONNIER suggested that this difference in the measured radiation-induced damage in sperm was possibly due to differences in oxygen concentrations, differences in chemical protection or to systems of metastable energy levels.

It is well known that the relative amount of oxygen present at the time of X-irradiation affects the measured genetic damage. An oxygen gradient may exist in the testes which is responsible for the observation that the sperm released first are the most sensitive to the X-irradiation. Because the mutagenic effect of neutrons is much less sensitive than X rays to changes in oxygen concentration, irradiation with neutrons would be expected to eliminate the observed difference in the genetic damage observed from sperm used on the first day and that used on the second. As expected, BAKER and VON HALLE (1954), LÜNING and JONSSON (1956), OSTER (1961), and ALEXANDER (1962) have failed to find a difference in the measured radiation-induced damage between the first- and second-day sperm batches when the sperm were irradiated in air with neutrons. Under these circumstances, no detectable recovery from genetic damages occurs.

Other studies have shown that the mutation rate obtained from rayed males is modifiable [(see discussion paragraph 3; HERSKOWITZ]. Mixing of clones of male germ cells of different “sensitivities” may occur prior to ejaculation (MULLER, HERSKOWITZ, ABRAHAMSON and OSTER 1954), rather than involving a recovery phenomenon.

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The experiments presented here were designed to study the postirradiation effect on mutation rate of variables such as storage of sperm in males and females, cold temperature, physiological condition of the female, and sperm loss. To eliminate any indirect effect of the irradiation on the female, only the males were irradiated. Sex-linked lethal mutation rates, relative egg-hatches and progeny numbers were studied. The results are discussed relative to the bearing on the hypothesis of recovery from genetic damage.

METHODS AND MATERIALS

Two stocks of *Drosophila melanogaster*, Oregon-R (OR) wild-type strain and *Basc* X chromosome tester-strain (Muller-5), were used exclusively. In all experiments, virgin OR males were aged three days on a complete nutrient medium (modified after CARPENTER 1950) prior to treatment. OR males were exposed to 0 (control) or to 2760 r X rays in each experiment. In the sex-linked lethal Experiments 1 and 2, whenever the *Basc* females were stored after insemination, they were stored as virgins on OFFERMANN and SCHMIDT's (1936) minimal medium for seven days prior to mating, either at room temperature (Experiment 1) or at 10°C (Experiment 2). This was done to reduce ovarian development and eliminate egg-laying during the mating and subsequent storage period (TROSKO and MYSZESWRKI 1963). In Experiments 3 and 4, where some males were stored after irradiation, virgin *Basc* females were aged only two days on a nutrient medium prior to mating.

For Experiments 1 and 2, flies were mass-mated on OFFERMANN and SCHMIDT's minimal medium at room temperature. After 24 hours, males were discarded and females were divided into two groups. One group (approximately 100 to 200 females) was placed on complete nutrient medium (25 females/bottle) and transferred every two days until all sperm were depleted. The other group (approximately 300 to 600 females) was stored for two weeks at 10°C on the medium of OFFERMANN and SCHMIDT. At the end of this storage period, they were placed on nutrient medium and treated in the same way as the nonstored group. All *F₁* females were tested for recessive sex-linked lethals by individually mating them to *Basc* males. *F₁* cultures containing no wild-type males were scored as lethal only if a minimum of ten *Basc* male progeny were present. In doubtful cases, appropriate *F₁* females were retested.

The males of Experiments 3 and 4 were divided after irradiation into three groups. One group of males was mated immediately. The second and third groups were stored for 24 hours without females at 23° and 10°C respectively before mating. Males of each group were individually mated to three virgin *Basc* females for 24 hours (providing sperm batch 1) and then transferred to a second set of three virgin *Basc* females (providing sperm batch 2). After the second mating of 24 hours, the males were discarded. In Experiment 3, the females were then transferred to new vials every two days until no more fertile eggs were laid. All *F₁* females were tested for sex-linked recessive lethals. In the egg mortality study (Experiment 4), the inseminated *Basc* females were placed individually in vials which were inverted on a petri dish containing nutrient medium (charcoal added) for 24 hours and transferred once to another petri dish for an additional 24 hours. Egg hatch was scored 30 to 34 hours after removal of the females from the medium. In Experiments 3 and 4, males that were held at 10°C were placed at this temperature within 10 minutes after irradiation and were mated immediately upon removal from the cold temperature after 24 hours storage. The flies were irradiated in plastic vials at room temperature with a General Electric Maximar-250, operating at 250 kv, 15 ma, with a .50 mm copper filter, giving an average dose rate of 150 r/minute. Ordinarily, the room temperature was 22 ± 1°C.

RESULTS

The results of storing irradiated sperm in prestarved (held on OFFERMANN and SCHMIDT's minimal medium for seven days at room temperature) cold fe-
Experiment 1. Comparison of the number of radiation-induced sex-linked lethal mutations in nonstored sperm and in sperm cold-stored for 14 days

<table>
<thead>
<tr>
<th>Set</th>
<th>Nonstored</th>
<th>Cold-stored</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lethals</td>
<td>Percent</td>
<td>Lethals</td>
<td>Percent</td>
</tr>
<tr>
<td></td>
<td>Normals</td>
<td>lethals</td>
<td>Normals</td>
<td>lethals</td>
</tr>
<tr>
<td>A</td>
<td>297</td>
<td>9.43</td>
<td>44</td>
<td>11.28</td>
</tr>
<tr>
<td></td>
<td>2851</td>
<td></td>
<td>346</td>
<td></td>
</tr>
<tr>
<td></td>
<td>91</td>
<td></td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1008</td>
<td>8.28</td>
<td>284</td>
<td>11.52</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1036</td>
<td>8.32</td>
<td>519</td>
<td>10.98</td>
</tr>
<tr>
<td></td>
<td>252</td>
<td></td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2722</td>
<td>8.47</td>
<td>1276</td>
<td>10.39</td>
</tr>
<tr>
<td></td>
<td>225</td>
<td></td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2320</td>
<td>8.84</td>
<td>1611</td>
<td>9.19</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>F§</td>
<td>1533</td>
<td>0.26</td>
<td>1395</td>
<td>0.29</td>
</tr>
</tbody>
</table>

* 2700 r irradiation.
† Females prestored on minimal medium seven days prior to mating at room temperature.
‡ \( 2 \times 2 \) chi-square contingency tests (1 degree of freedom).
§ Control set, nonirradiated sperm.

males are presented in Table 1. There was no statistically significant change after storage in the lethals scored for any individual experimental run. However, since there was a consistent increase in lethal frequency after storage in each of the five experiments, the combined probabilities of each experiment were tested (Fisher 1950). A chi-square value of 19.37 with 12 degrees of freedom (.05 > P > .025) was obtained. This shows a significant increase in lethal frequency correlated with the prestarved-cold-storage treatment.

It is possible that this result is due to the cold temperature, aging of the sperm, starvation treatment of the female and sperm loss, alone or in some combination. To test whether the cold temperature or aging might be responsible for the increase of sex-linked lethals, all Basc females were prestored for seven days at 10°C on minimal medium immediately after eclosion and before mating (Trosko and Myczewski 1963). This treatment permitted the stored inseminated females to have a comparable fecundity to that of the nonstored females in Experiment 1. Results on lethal rate of storing sperm for different lengths of time in such pre-stored females are summarized in Table 2.

A chi-square test for heterogeneity between the three treatments in Experiment 2 resulted in a value of 0.11, with 2 degrees of freedom (.95 > P > .90). These results show that 12 days of postirradiation storage of the inseminated females at 10°C does not modify the sex-linked mutation rate. The only obvious difference between the experiments reported in Table 1 and Table 2 is the pre-treatment of the Basc females prior to mating and the degree of fecundity after storage. This is concluded from the observation that nonstored and stored Basc
TABLE 2

Experiment 2. Results of cold-storing irradiated sperm in cold-prestored females

<table>
<thead>
<tr>
<th>Set</th>
<th>Length of storage</th>
<th>Sex-linked lethals/Normal</th>
<th>Percent lethal</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>0 days</td>
<td>143/1733</td>
<td>7.62</td>
</tr>
<tr>
<td>H</td>
<td>1 day</td>
<td>213/2508</td>
<td>7.83</td>
</tr>
<tr>
<td>I</td>
<td>12 days</td>
<td>239/2795</td>
<td>7.88</td>
</tr>
</tbody>
</table>

* Males irradiated at 2760r.
† Nonirradiated females prestored on minimal medium at 10°C seven days prior to mating.

females of Experiment 1 had an average of 28 and 12 progeny respectively, while the nonstored and stored females of Experiment 2 had an average of 36 and 33, respectively.

The effects of postirradiation cold-storage of OR males are summarized in Tables 3 and 4. Comparisons can be made between the first- and second-day sperm batches and between the total damage in the first two sperm batches of the nonstored males (NS), males stored at room temperature (SRT) and males stored at 10°C (SCT). Both sex-linked lethals and egg hatch were studied. Table 3 summarizes the results of the study on sex-linked lethals.

The results of this experiment show a significant reduction in the sex-linked lethal rate associated with the second sperm batch over that of the first sperm batch of NS and SCT males. Chi-square values of 15.5 and 7.7 (P < .005; P < .01, with 1 degree of freedom) were obtained when a comparison was made between first- and second-day damage of NS and SCT males respectively. There was no corresponding significant reduction of sex-linked lethals associated with the second sperm batch of SRT males. A chi-square value of 0.12 (1 degree of freedom; 0.75 > P > .50) was obtained for the first- and second-day comparison of SRT males.

When the value for the sex-linked lethals scored for both first and second sperm batches of the three groups are compared, a chi-square of 0.55 is obtained (2 degrees of freedom; .90 > P > .75). This suggests that there is no heterogeneity in the results due to the type of postirradiation treatment to the males.

When the sex-linked lethals of the first sperm batch of the NS males are compared to the first sperm batch of the SRT males, there is a reduction, although not statistically significant (chi-square = 2.82; .10 > P > .05, with 1 degree of

TABLE 3

Experiment 3. Effect on the recovery of sex-linked lethal mutations of storing males after 2760r X-ray dose

<table>
<thead>
<tr>
<th>Storage conditions prior to mating</th>
<th>First sperm batch</th>
<th>Second sperm batch</th>
<th>Percent lethal, both batches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lethals/Normals</td>
<td>Percent lethal</td>
<td>Lethals/Normals</td>
</tr>
<tr>
<td>Not stored</td>
<td>138/1616</td>
<td>7.87</td>
<td>69/1459</td>
</tr>
<tr>
<td>24 hr at 22°C</td>
<td>54/835</td>
<td>6.07</td>
<td>36/601</td>
</tr>
<tr>
<td>24 hr at 10°C</td>
<td>146/1794</td>
<td>7.53</td>
<td>96/1717</td>
</tr>
</tbody>
</table>
DIFFERENTIAL RADIATION SENSITIVITY

TABLE 4

<table>
<thead>
<tr>
<th>Set</th>
<th>First sperm batch</th>
<th>Second sperm batch</th>
<th>Percent hatch, both batches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hatched/total</td>
<td>Percent hatch</td>
<td>Hatched/total</td>
</tr>
<tr>
<td>Control</td>
<td>365/399</td>
<td>91.5</td>
<td>582/643</td>
</tr>
<tr>
<td>NS</td>
<td>289/556</td>
<td>51.4</td>
<td>380/627</td>
</tr>
<tr>
<td>SRT</td>
<td>377/718</td>
<td>52.5</td>
<td>865/1466</td>
</tr>
<tr>
<td>SCT</td>
<td>425/771</td>
<td>55.1</td>
<td>396/627</td>
</tr>
</tbody>
</table>

The increase of egg hatch was associated with the second sperm batch of NS, SRT and SCT males. This increase was statistically significant in every case. Chi-square values of 9.4, 8.3 and 9.2, with 1 degree of freedom, were obtained when the first- and second-day sperm batches were compared. There was also an increase in egg hatch, although not significant, in the first sperm batch of males stored at room temperature for a day when compared to the first sperm batch of NS males.

As with the sex-linked lethal experiment, recovery in terms of egg hatch associated with the second sperm batch of the SRT males was not as great as one might expect if recovery occurred in the postirradiation-stored males.

A Student’s t test, comparing the total egg hatch for NS and SRT males gave a value of 0.48 (18 degrees of freedom; .35 > P > .30). These results do not support the idea that genetic recovery occurs if the irradiated sperm can remain in the male for some time. A t test was also used to compare the total egg hatch of NS and SCT males, and a value of 0.66 was obtained (17 degrees of freedom; .30 > P > .25). Again, as in the sex-linked lethal study, there seemed to be no modification of the damage correlated with the type of posttreatment to the males used in this experiment.

DISCUSSION

The higher frequency of sex-linked lethals after cold-storage of X-rayed sperm in prestarved females might be the result of several possible postirradiation phenomena. Because NOVITSKI (1949) found that postirradiation cold shocks to inseminated females resulted in higher lethal mutation rates than when they were posttreated at room temperature, it was thought that the cold temperature (10°C) in Experiment 1 (prestarved females) was responsible for the higher frequency of lethals after storage. It has been suggested in the reports of ABRAHAMSON and TELPER (1956) and OSTER (1961) that no recovery of radiation-induced damage occurs in the mature sperm when it is in the female. Hence, the cold temperature...
posttreatment would not be expected to inhibit a recovery process, but might enhance the damage via some other process. Experiment 2 (nonprestarved females) has definitely shown that the cold temperature posttreatment and aging, per se, were not responsible for the observed higher frequency of sex-linked lethals. Also it serves to demonstrate that no demonstrable recovery occurred during the storage period.

The manner of posttreatment of the Basf females prior to mating in Experiments 1 (prestarved females) and 2 (nonprestarved females) might have influenced the frequency of lethals scored after storage. Females were either starved (Experiment 1) or pre-cold stored (Experiment 2) prior to mating in order to inhibit their egg-laying, during and subsequent to the mating period. In Experiment 1, the stored females were far less fecund than the nonstored females. In fact, the nonstored females of Experiment 1 were less fecund than the nonstored females of Experiment 2. Stored females of Experiment 2, however, did not exhibit a reduction in progeny numbers, probably because they were not starved to the same degree as the females of Experiment 1. The premating starvation treatment of the females affected the relative progeny numbers, which may have, in turn, affected the frequency of scored lethals.

There is evidence that the environment of the sperm in the female might affect the fate of breaks produced in the paternal chromosomes. It was shown by Bonnier and Lüning (in Muller 1954) and Bonnier (1954) that by aging or irradiating eggs, the manner of reunion of broken chromosome pieces brought in by the sperm can be affected. Hildreth and Carson (1957) showed that the frequency of spontaneous lethals in the sperm of wild-type D. melanogaster was influenced by the type of females that the males inseminated. One of Hildreth and Carson's explanations was that the different types of females might have a differential influence on the "healing" of lethals that originated in the sperm before mating. Herskowitz (1958, 1963) has reported that the physiological condition of the female will influence the gross chromosomal mutation frequency scored from X-rayed sperm. He postulates that the mutational enhancement by undernourishment of the females is associated with some effect upon the rejoining of broken chromosome ends. The data of Experiment 1 (prestarved females) would lend support to Herskowitz's observation that undernourishment enhances some radiation-induced damage. Since the females of Experiment 2 were kept at a lower metabolic rate by the cold temperature, they were less undernourished than the females of Experiment 1. This may explain why no increase of lethals was found in Experiment 2 after storage. It was also noticed that the nonstored females of Experiment 1 (prestarved) had slightly higher mutation rates than the nonstored females of Experiment 2 (nonprestarved). Although these experiments were run at different times, this difference may be due to differences during the irradiation treatments. However, it is felt that calibrations were accurate for all the experimental runs.

Another hypothesis, although probably less likely, would suggest that the increase in lethals after storage was related to a reduction in fecundity which was noticed in the stored females of Experiment 1. It has been shown that X-radiation
may influence the sperm's ability to survive a postcopulatory migration from the vagina to the ventral receptacle (YANDERS 1959b) or that it might cause some inactivation of sperm (LEFEVRE and JONSSON 1962). Also, LINDSLEY, EDINGTON and VON HALLE (1959) have shown that there is a relationship of the genetic constitution of the sperm to their sensitivity to X-radiation. These observations may be interpreted as indicating that the measured radiation damage can be influenced by differential survival of the irradiated sperm. Since the sperm in the stored females of Experiment 1 (prestarved) were less able to survive the storage than the sperm in the stored females of Experiment 2 (nonprestarved), it might be possible that ability of the sperm to survive the storage period in the undernourished females was related to their radiation sensitivity.

To test the hypothesis of postirradiation recovery, irradiated males were stored at room temperature and 10°C. BAKER and VON HALLE (1953), LÜNING (1954), TELFER and ABRAHAMSON (1954), NORDBACK and AUERBACK (1956) and YANDERS (1959a) have reported that a reduction in genetic damage occurs in sperm the second day after X-ray treatments in air. In the present tests (Experiments 3 and 4), the SRT males' first sperm batch served as a control, and a reduction of the damage was noted in their first sperm batch when compared to the NS males' first sperm batch. One might expect, if recovery did occur in the SRT males' sperm, a reflection of this recovery in the second sperm batch of the SRT males. This was not the case in either the sex-linked or egg mortality experiments when the total damage for the two sperm batches of the SRT males are compared to that of the NS males combined two sperm batches. This observation leads this investigator to believe that the mature sperm at the time of irradiation exhibit a differential sensitivity gradient, possibly due to the age of the sperm (LEFEVRE 1963) or to an oxygen effect as discussed by BONNIER (1954), LÜNING (1961) and OSTER (1961). If the irradiated males are stored a day before mating, new sperm being produced and entering the seminal vesicle causes the already-present sperm to be mixed with regard to their initial radiation sensitivity.

On this view, in the NS males, the most sensitive sperm would be used first, and the least sensitive, second, and this effect would be mistakenly interpreted a recovery. Also, if the less sensitive sperm mixes with the more sensitive during postirradiation storage, the observed effect would be a reduction in the damage in the first sperm batch. Supporting this notion of mixing are the results of the SCT males in Experiments 3 and 4. The 10°C storage would be expected to stop the development of the new spermatozoa and even retard a mixing of the irradiated sperm during this postirradiation storage. Therefore, the SCT males' first sperm batch would have included the most sensitive sperm on its first day of mating, as did the NS males. The results are consistent with the mixing hypothesis. The shortcoming of this sperm-mixing hypothesis is that it is not known to what degree, if any, mature sperm are stored or lost during a nonmating storage period. Mossige (1955) has shown, however, that the sperm are stored in males when they are kept without females at least for a few days after emerging.

The absence of a difference after neutron irradiation in air in the measured genetic damage between the first- and second-day sperm batch and between
sperm irradiated in males or females led OSTER (1961) to postulate that differential radiation sensitivity is responsible for the forementioned differences after X-irradiation of spermatozoa. The data presented here are also interpreted as indicating a differential sensitivity of the spermatozoa to X-irradiation, and it is concluded that no detectable postirradiation recovery occurs in Drosophila spermatozoa.

I express my appreciation to Dr. A. F. Yanders for discussing the research, and reading the manuscript. I am grateful to Dr. U. V. Mostosky of the Department of Surgery and Medicine, Michigan State University, for providing X-ray treatments, and to Dr. P. J. Clark for statistical advice.

SUMMARY

An increase in sex-linked lethal mutation rate after cold storage of irradiated sperm was noted when nonirradiated females were starved prior and subsequent to mating. Cold and aging during the storage of the irradiated sperm did not, per se, contribute to the increase of sex-linked lethals. At the same time, these data supplement the findings of others that no change in radiation-induced damage to sperm occurs when the irradiated sperm are or are not stored in the female.

The evidence from postirradiation storage of the male at room temperature and at 10°C does not support a hypothesis for a recovery mechanism occurring in the sperm. The rates of sex-linked lethals and of egg mortality, associated with the first sperm batch, decreased after 24 hours postirradiation storage of the males at room temperature. The rates of the second sperm batch (sperm used 48 hours after irradiation) of the latter males did not exhibit a decrease as might be expected if a recovery process exists. Furthermore, postirradiation storage of the males at 10°C did not modify the rates of sex-linked lethal mutation or egg mortality. The data are interpreted to indicate that the population of sperm at the time of irradiation is heterogeneous and compartmentalized with respect to radiation sensitivity, and that mixing of these sperm can occur in the testes during postirradiation storage at room temperature.

LITERATURE CITED


Differential radiation sensitivity


