TEMPERATURE EFFECTS ON LETHAL MUTATION RATES OF HABROBRACON OOCYTES X-IRRADIATED IN FIRST MEIOTIC METAPHASE

ANNA R. WHITING

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, and Biology Division, University of Pennsylvania, Philadelphia, Pennsylvania

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A BRIEF note was published in 1940 (Whiting) concerning the influence of temperature on X-ray sensitivity of different meiotic stages in the Habrobracon oocyte. Unmated females were kept at 0°C, 13°C, 25°C, and 35°C for one hour before and one hour after irradiation. During a one half minute of exposure to 212r, they were at room temperature. For oocytes in first meiotic metaphase (metaphase I), mortality was lowest at 0°C, highest at 25°C and intermediate at 35°C. The consistent and extensive radiation data on metaphase I obtained since 1940 made it seem valuable to redesign and to repeat the experiment.

When Habrobracon females are X-irradiated, responses of their oocytes in prophase I can be distinguished from those in metaphase I (Whiting 1945). The latter were used in the experiments reported in this paper. LD 50 for the stage is about 400r. The nature of the dose-action curve as well as the absence of any significant differences in response of this curve to different experimental conditions (for example, 124 kv, 50 mev by Heidenthal, Clark and Gowen 1955; 100r/min X-rays, 200r/sec cathode rays by Heidenthal 1960; 6.6r/min, 800r/min by Lachance 1960; and fractionation by Whiting 1945) furnish evidence for one-hit X-ray-induced changes. Tetrad conditions at the time of irradiation and cytological observations on succeeding stages also make the one-hit explanation a plausible one.

Viable eggs laid by unmated females develop into haploid males. Those in which dominant or recessive lethal mutations acting on the embryo are induced (as happens in the majority of eggs) fail to hatch.

MATERIAL AND METHODS

Virgin females were X-irradiated and bred unmated. Response of oocytes irradiated in first meiotic metaphase was measured by egg hatchability.

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For preirradiation low temperature experiments, well-fed females were kept without food for 16 hours, the first eight at 30°C followed by eight in the freezing compartment of a refrigerator (-3°C to -5°C). For low temperature during irradiation, females were put into chilled gelatin capsules which were placed in a small plastic dish submerged in ice and salt water. For postirradiation low temperature experiments, the females immediately after irradiation were kept for 40 minutes in the freezing compartment. For high temperature, 30°C was substituted for the -3°C to -5°C treatment. Both groups were irradiated at the same time, the higher temperature capsules being placed above the lower or vice versa. A dual-tube self-rectifying unit with a simultaneous cross-firing technique was used. Secondary voltage was 182 kv; tube current on each tube, 25 mA; and rate, 722 rpm. Doses were 1263r and 1444r.

Hatchability of eggs from nonirradiated females kept at -5°C for 12 hours was 98.1 ± 0.93 percent, for those kept at 30°C, 97.4 ± 1.04 percent.

The use of low temperature is indicated by -; of high by +. The conditions before, during, or after irradiation are designated by using the symbols sequentially. Thus, in - - - experiments, low temperature was used throughout; in + - + experiments, during irradiation only.

**RESULTS**

Results are presented in Table 1. The difference in hatchability between total - - - and + + + in experiments at 1263r was 11.07 ± 1.77 percent; at 1444r, it was 8.95 ± 2.33 percent. Arranged in the order of decreasing survival at 1263r, the results were - - - , - + + , + - - , - - + , + + + , + - + ; for 1444r, - - - , + + - , + + + , + - + . It is apparent that, with reduction of low tem-

### Table 1

**Hatchability of unfertilized eggs X-irradiated in first meiotic metaphase.** The - sign represents -3°C to -5°C; + is 26°C to 30°C. First or + in a given series refers to temperature treatment before irradiation; second, during; and last, after.

<table>
<thead>
<tr>
<th>Dose (r)</th>
<th>Temperature</th>
<th>Hatchability</th>
<th>Hatchability</th>
<th>P</th>
<th>Approx. significance level of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Viable/ total)</td>
<td>(Percent viable)</td>
<td>Temperature</td>
<td>(Viable/ total)</td>
</tr>
<tr>
<td>1263</td>
<td>- - -</td>
<td>89/441 = 20.18</td>
<td>+ + +</td>
<td>43/351 = 12.25</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>+ - +</td>
<td>12/116 = 10.34</td>
<td>+ + +</td>
<td>12/124 = 9.67</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>- - +</td>
<td>41/247 = 16.59</td>
<td>+ + +</td>
<td>28/320 = 8.75</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>+ - -</td>
<td>47/297 = 15.82</td>
<td>+ + +</td>
<td>31/296 = 10.47</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>- - +</td>
<td>65/302 = 20.73</td>
<td>- - -</td>
<td>43/285 = 15.09</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>- - +</td>
<td>154/743 = 21.52*</td>
<td>+ + +</td>
<td>114/1091 = 10.45*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1444</td>
<td>- - +</td>
<td>33/246 = 13.41</td>
<td>+ + +</td>
<td>15/354 = 4.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>+ - +</td>
<td>5/151 = 3.31</td>
<td>+ + +</td>
<td>4/105 = 3.91</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>+ - -</td>
<td>14/199 = 7.03</td>
<td>+ + +</td>
<td>8/146 = 5.48</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>- - +</td>
<td>33/246 = 13.41*</td>
<td>+ + +</td>
<td>27/605 = 4.46*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Total of either - - - or + + + treatments.
temperature exposure, hatchability fell. There was no significant difference shown between \(-\ -\ +\) (15.09) and \(+\ -\ -\) (15.82). Cold treatment given during irradiation only, \(+\ -\ +\), increased hatchability slightly over \(+\ +\ +\) in one experiment (10.34 over 9.67), lowered it slightly in the other (3.31 and 3.91), neither difference being significant. In summary, the higher hatchability of \(-\ -\ -\) than of \(+\ +\ +\) results from protection by low temperature before and after irradiation while low temperature during exposure (1½–2 minutes) has no significant effect.

**DISCUSSION**

Many tests have been made on the relation of temperature differences to incidence of X-ray-induced changes in the cell, often with apparent inconsistencies in results. Those for cytogenetical effects reported before 1949 are summarized by Baker (1949) and before 1954 by Muller (1954). Baker noted that, in all the experiments reporting a positive effect of temperature during irradiation, the low temperature used varied from 0° to 4°C and the high from 20° to 32°C; in experiments indicating no effect, the two temperature ranges used were 5° to 10°C and 34° to 37°C. When attention was concentrated on the relation of oxygen to X-ray-induced injury (Thoday and Read 1947), it became clear that some effects which seem to be owing to temperature differences could be explained by oxygen changes in the cell.

That the oxygen effect is not wholly responsible for temperature-radiation experimental results has been clearly demonstrated (Giles, Beatty and Riley 1951). Inflorescences of Tradescantia were irradiated using (1) a constant percentage of oxygen at different temperatures, (2) a constant temperature with different percentages of oxygen, and (3) various temperatures in the absence of oxygen. In (1) damage was greater at low temperatures, in (2) greater at high oxygen concentration, and in (3) less at low temperatures. Result (3) demonstrates a temperature effect sensu stricto, it being the reverse of (1) when oxygen was present.

Therefore, temperature and oxygen independently can influence X-ray-induced chromosome change. When, during exposure to low temperature, oxygen is available, the low temperature effect may be counteracted by that of oxygen. X-ray-induced injury thereby would be increased. In animals, the effect of extended periods of temperature change would depend upon the availability of oxygen to the cells as well as temperature conditions. An insect exposed to very low temperature for a relatively long period might have little oxygen in the cell environment if spiracles are closed and tracheal action is inhibited. X-ray-induced injury thus would be reduced. This oxygen-temperature relation seems to explain the results obtained in experiments presented in this paper.

Metaphase I tetrads at the time of exposure to X-rays have almost completely terminalized and appear stretched between centromeres and the terminal chiasmata. They break in the "stretched" dyad regions with lateral fusion of broken ends of chromatids and this fusion prevents restitution of breaks. Later meiotic
stages furnish evidence of this behavior by the presence of double fragments in division I and bridges in division II. Tension may be responsible for high frequency of breaks, lateral fusion for prevention of recovery.

Metz and Bozeman (1940), in discussing the high sensitivity of anaphase I chromosomes of Sciara oocytes to X-rays, suggested that cells in which chromosomes are more actively moving or changing in form are more sensitive to X-rays than those in which chromosomes are relatively quiet. Tension was suggested (Whiting 1945) as a possible factor in sensitivity between metaphase I and prophase I (LD 50 about 400 and 12,000r respectively) in Habrobracon.

Sax (1943) found that centrifugation applied simultaneously with X-irradiation causes the broken ends of Tradescantia chromosomes to separate, thereby increasing injury. Sparrow, Moses and Dubow (1952) wrote, "Sensitivity appears to be related to the degree of chromosomal contraction." Wolff and Von Borstel (1954) observed increased aberration frequency after postirradiation centrifugation in Tradescantia microspores and in Vicia seed. Öster (1956) reviewed the evidence for greater sensitivity of spermatids than of mature spermatozoa in Drosophila. It has been suggested that the elongation of the spherical spermatid and chromosome movement in spermiogenesis might be the cause of the greater sensitivity. Öster believes that this explanation is disproved by an experiment in which he irradiated males with either a rod X chromosome or a ring-shaped X. He got no difference in incidence of sex-linked lethals although in meiosis after irradiation ring chromatids should be less sensitive to stress than rods.

Does oxygen increase chromosome movement and stress? Does low temperature reduce chromosome movement and stress?

One result obtained in the present study, difficult to reconcile with the theory of tension and the facts of terminal deletion and lateral fusion to explain the extreme sensitivity of metaphase I chromosomes, is the significant protection afforded by low temperature after irradiation. Breakage probably has occurred before exposure; thus, if restitution has taken place, low temperature must have prevented the lateral fusion of sister chromatids. Wolff and Luippold (1958) found that removal of oxygen or inhibition of respiration subsequent to irradiation inhibits rejoining. Either treatment allows breaks to remain open and capable of forming exchanges with breaks induced later. Does low temperature through inhibition of respiration prevent lateral fusion of broken ends of dyad chromatids and thereby permit restitution upon return to higher temperature? This seems plausible since a relative decrease in lateral fusion is a required factor for recovery or reduction of injury.

Facts which must be reconciled with the theory of stress, if it is to be maintained, are the direct relations between incidence of visible and of recessive lethal mutations with incidence of dominant lethals. After 1100r, recessive lethal rate for metaphase I is 15.2 percent, visible rate 4.35 percent and dominant lethal rate about 75 percent. On the other hand, for late prophase I, after 1100r, visible mutation rate is 0.995 percent and lethal rates are so low as to be difficult of
significant detection. If visible and some recessive lethal mutations are "point mutations," their rates should not be affected by movement or chromosome tension. However, the consistently greater sensitivity of stages in which chromosomes are under stress challenges the student of radiation effects to continue the search for a theory consistent with all facts.

SUMMARY

1. Unmated females of Habrobracon were X-irradiated with 1263 and 1444 r at high and low temperature before, during, and/or after irradiation.

2. Observed was a significant lowering of lethal changes induced in oocytes X-irradiated in first meiotic metaphase (LD 50 about 400 r) at low temperatures before and after exposure as measured by hatchability of unfertilized eggs.

3. Low temperature during irradiation (1½–2 minutes) did not significantly change survival rate although there is a suggestion of a tendency toward increased lethality.

4. Theories are discussed as to the cause or causes of differential sensitivity of cells and the relation of this sensitivity to oxygen and temperature.

LITERATURE CITED


