Effects of Ionizing Radiation on the Early Development of Oryzias Eggs

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Continuous irradiation/Fish embryo/Chromosome aberration

The chromosome aberrations (chromosome bridges) were observed at the blastula stage of Oryzias eggs treated in the following three manners, 1) incubated in HTO (0-10 Ci/1) and in 90Sr-90Y (0-1,000 μCi/1), 2) irradiated continuously with γ-rays (0-74 rad/h), 3) given single doses of X-rays (0-500 R). All the treatments were initiated immediately after fertilization (at the one-cell stage) and the continuous irradiation time was eight hours at 26°C. The frequencies of chromosome bridges increased significantly when the eggs were treated with solutions of concentrations higher than 0.5 Ci/1 (>19 rad) for HTO and 100 μCi/1 (>58 rad) for 90Sr-90Y, or were irradiated with γ-rays of 78 rad or more, or were given single doses more than 100 R of X-rays. Comparing the continuous irradiation experiments, it is considered that β-rays from tritium are more effective than the other radiations in inducing chromosome aberrations. Hatchability of Oryzias eggs irradiated with X-rays at the one-cell stage was checked and the dose required for 50% killing of embryos was estimated at 360 R.

INTRODUCTION

The effects of ionizing radiation on aquatic organisms have been extensively investigated, and were well reviewed by Templeton et al., Chipman, Blaylock and Trabalka, and also in IAEA Technical Reports Series No. 172. In viewing of such literature it can not be stated that enough attention has been paid to the cytogenetical aspects of radiation effects on fish eggs.

Pankova investigated the chromosome damage of loach's embryos (Misgurnus fossilis) at the middle blastula, late blastula and gastrula stages. The embryos were irradiated at the stage of four blastomeres with 1,000 R of X-rays, and it was found that more than 70% of anaphases at all the three stages had some chromosome aberrations, among which chromosome bridges were by far more frequent.

In the present study, the chromosome bridge was selected as an index of the radiation damage to the eggs of the freshwater teleost, Oryzias latipes, because it can
be easily seen under a microscope, while the other anomalies such as fragmentation and multipolar division are generally difficult and infrequent to detect.

Hatchability of Oryzias eggs irradiated with X-rays at the one-cell stage was checked because of a scarcity of the data of this kind.

MATERIALS AND METHODS

The inbred strain of an orange-red variety of the medaka, Oryzias latipes, maintained in our institute was used as parental fish. The male and female fishes of this species were separated in the afternoon of Day 1, and in the early morning of Day 2 (the next day) they were mated by putting together in the same tank for one hour. The fertilized eggs laid during that period were harvested for synchronized development. Immediately after being harvested, the eggs were exposed to three different manners of irradiation. First, they were immersed in tritiated water of 0.1 to 10 Ci/l, or in $^{90}$Sr-$^{90}$Y solutions ranging from 10 to 1,000 μCi/l. Secondly, the eggs were irradiated continuously with $^{60}$Co γ-rays at dose rates of 9.8-74 rad/h. Thirdly, they were given single doses of 200 kVp X-rays from 25 to 500 R at a dose rate of 100 R/min. All the treatments were initiated at the one-cell stage and the eggs were kept at 26°C throughout the experiments. Eight hours after the commencement of irradiation, squash preparation procedures were applied to the treated eggs on a slide glass, and the embryonal cells were fixed and stained with 45% aceto-orcein solution. The fixation was made at the blastula stage of the development. In the present experiments, only the chromosome bridge was selected as an index of the radiation damage for the above-mentioned reasons. The frequency (F) of the cells containing chromosome bridge(s) was calculated by the following equation:

$$F = \frac{\text{No. of the Cells with Chromosome Bridge(s)}}{\text{No. of Cells Observed at Ana- and Telo-Phases}}$$

In order to estimate the absorbed dose, the eggs were incubated in $^{90}$Sr-$^{90}$Y of 10 μCi/l, or in tritiated water of 0.1 Ci/l at 26°C for the same period (8 hours) with the chromosome experiments. Incubated eggs were separated into groups of 5, 10 and 20 eggs for the measurement of $^{90}$Sr-$^{90}$Y incorporated into the eggs, and those samples were ashed at 200°C in an electric oven. The activities of $^{90}$Sr-$^{90}$Y were measured with a coincidence type β-ray spectrometer. The β-ray spectrum was measured in logarithmic scale, so the counts in the logarithmic spectrum made it possible to identify the activity of $^{90}$Sr and $^{90}$Y. The absorbed doses in an egg of the medaka kept in water with a known activity of $^{90}$Sr-$^{90}$Y were determined with a method proposed by Spiers, assuming that the diameter and weight of the egg were 0.1 cm and 1 mg, respectively, and the maximum β-particle ranges in water were 0.0422 cm for $^{90}$Sr and 0.308 cm for $^{90}$Y. The geometrical factors of the egg, G, calculated by this method were 0.386 for $^{90}$Sr β-rays and 0.928 for $^{90}$Y β-rays. An egg is given the dose from the activities of $^{90}$Sr and $^{90}$Y both in egg itself and in water. The absorbed doses were calculated by
the following equation:

\[ D_1 = N_1 \frac{\bar{E}k(1-G)}{100} W \] (in egg itself)

\[ D_2 = N_2 \frac{\bar{E}kG}{100} \] (in water)

where:

- \( N_1 \) = the activity of \(^{90}\)Sr or \(^{90}\)Y in an egg (total number of disintegration throughout the experimental period)
- \( N_2 \) = the activity of \(^{90}\)Sr or \(^{90}\)Y in unit weight of water
- \( \bar{E} \) = the average energy of \( \beta \)-rays, 0.182 MeV for \(^{90}\)Sr and 0.761 MeV for \(^{90}\)Y
- \( k = 1.602 \times 10^{-12} \) (erg/MeV)
- \( G \) = the geometric factor
- \( W \) = the weight of an egg

For tritium measurement, 1, 5 and 10 eggs were put into vials with liquid scintillator. The activity of tritium in the eggs was measured with a liquid scintillation counter (Mark 1, Nuclear Chicago Co.). The average amount of tritium in an egg was calculated from these data. The absorbed dose was calculated by the following equation:

\[ D = N\bar{E}k/100 \] W

where:

- \( N \) = the activity of \(^3\)H in an egg (total number of disintegration throughout the experimental period)
- \( \bar{E} \) = the average energy of \(^3\)H \( \beta \)-rays (=0.569 keV)
- \( k = 1.602 \times 10^{-9} \) (erg/keV)
- \( W \) = the weight of an egg

The dose absorbed in an egg of the medaka was calculated under the following assumptions; (1) the radionuclide incorporated was distributed homogeniously within an egg and (2) the radionuclide was incorporated linearly into the egg from the commencement of immersion.

Hatchability of the eggs, which were collected in the same way as in the chromosome experiments and were given various single doses of X-rays at the one-cell stage, was checked, and the dose needed for 50% killing of embryos was estimated.

**RESULTS**

1) Calculation of the absorbed doses

The accumulated doses of the egg kept in water with a known activity for eight hours are shown in Table 1. The incorporated strontium-90 was the main source of the absorbed dose of the egg kept in solution of \(^{90}\)Sr-\(^{90}\)Y. Contribution of \( \beta \)-rays from tritium in water was negligible because of their extremely short range.

2) Observation of the chromosome bridges

A normal anaphase figure is show in Fig. 1 and in Fig. 2, a picture of a cell with chromosome bridges found in the eggs incubated in HTO of 0.1 Ci/l. The frequencies of cells with chromosome bridge(s) in the controls varied from 0.40% to 0.66% and
Table 1. Estimated Doses* to an *Oryzias* egg from $^{90}$Sr,$^{90}$Y and HTO

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Estimated Dose (rad) From egg itself</th>
<th>Estimated Dose (rad) From water</th>
<th>Total Dose (rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{90}$Sr-$^{90}$Y</td>
<td>$^{90}$Sr 5.1</td>
<td>$^{90}$Y 0.6</td>
<td>$^{90}$Sr 0.005</td>
</tr>
<tr>
<td>HTO 10 $\mu$Ci/1</td>
<td>3.7</td>
<td>Nil</td>
<td>3.7</td>
</tr>
<tr>
<td>HTO 0.1 Ci/1</td>
<td>3.7</td>
<td>Nil</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* Accumulated doses for eight hours

Fig. 1 A normal anaphase at the blastula stage of an *Oryzias* egg. ($\times$1,200)

Fig. 2. A typical picture of chromosome bridges found in an *Oryzias* egg exposed to 0.1 Ci/1 of HTO for eight hours after fertilization. ($\times$1,200)
there was no statistically significant difference among them. So, the composite control
value of the four, 0.56%, is shown in the tables.

a) Tritiated water

The concentrations of the tritiated water used were 0, 0.1, 0.5, 1, 5 and 10 Ci/l. The results are shown in Table 2. A general trend was that aberrant mitoses were increased with the increase in the radioactivity, and at the concentrations of 0.5 Ci/l and above, the frequencies of the aberrant mitoses were found to be significantly higher than the control.

b) 90Sr-90Y

The results on 90Sr-90Y exposure are shown in Table 3. The frequency of chromosome bridge formation was found to increase with increasing concentrations, and in the groups with the concentrations more than 100 μCi/l, the radiation was effective significantly.

c) Continuous γ-irradiation

In the continuous irradiation experiments with γ-rays, a significant increase in the

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**Table 2.**

Frequency of Cells with Chromosome Bridge(s) Induced in HTO

<table>
<thead>
<tr>
<th>Concentration (Ci/l)</th>
<th>Estimated Dose† (rad)</th>
<th>Mitoses (Ana-Telo)</th>
<th>Cells with Chromosome Bridge(s) No.</th>
<th>Percent ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>6078</td>
<td>34</td>
<td>0.56 ± 0.10</td>
</tr>
<tr>
<td>0.1</td>
<td>3.7</td>
<td>2214</td>
<td>20</td>
<td>0.90 ± 0.20</td>
</tr>
<tr>
<td>0.5</td>
<td>18.5</td>
<td>1309</td>
<td>16</td>
<td>1.22 ± 0.31*</td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>2115</td>
<td>22</td>
<td>1.04 ± 0.22*</td>
</tr>
<tr>
<td>5</td>
<td>185</td>
<td>1660</td>
<td>43</td>
<td>2.59 ± 0.39*</td>
</tr>
<tr>
<td>10</td>
<td>370</td>
<td>1565</td>
<td>43</td>
<td>2.75 ± 0.42*</td>
</tr>
</tbody>
</table>

† The estimated dose at 0.1 Ci/l is from Table 1 and the other doses are calculated from this value under the assumption that the absorbed dose is proportional to the concentration.

* Significant compared to the control (p<0.05).

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**Table 3.**

Frequency of Cells with Chromosome Bridge(s) Induced in 90Sr-90Y Solution

<table>
<thead>
<tr>
<th>Concentration (μCi/l)</th>
<th>Estimated Dose† (rad)</th>
<th>Mitoses (Ana-Telo)</th>
<th>Cells with Chromosome Bridge(s) No.</th>
<th>Percent ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>6078</td>
<td>34</td>
<td>0.56 ± 0.10</td>
</tr>
<tr>
<td>10</td>
<td>5.8</td>
<td>1905</td>
<td>18</td>
<td>0.94 ± 0.22</td>
</tr>
<tr>
<td>100</td>
<td>58</td>
<td>2439</td>
<td>30</td>
<td>1.23 ± 0.22*</td>
</tr>
<tr>
<td>1000</td>
<td>580</td>
<td>2158</td>
<td>41</td>
<td>1.90 ± 0.30*</td>
</tr>
</tbody>
</table>

† The estimated dose at 10 μCi/l is from Table 1 and the other doses are calculated from this value under the assumption that the absorbed dose is proportional to the concentration.

* Significant compared to the control (p<0.05)
d) Acute X-ray irradiation

The results are shown in Table 5. The embryonal cells irradiated with various doses of X-rays at the one-cell stage showed a rapid increase in the frequency of aberrant mitoses as the radiation doses increased. The radiation doses with 100 R and above yielded the significantly high frequencies of the cells with chromosome bridge(s) compared with the control.

3) Hatchability test

Figure 3 shows a sharp decrease of the hatchability of embryos irradiated at the one-cell stage with increasing doses of X-rays. Each point is the average of the three series of the experiments and the straight line is a regression line calculated by the least square method. From this line the dose required for 50% killing of embryos was estimated at 360 R.

![Graph showing relationship on probit scale between hatchability and doses of X-rays.](image)

Fig. 3. Relationship on probit scale between hatchability and doses of X-rays.
DISCUSSION

The anaphase method, in which chromosome bridges are found more frequently than other anomalies such as fragments and multipolar mitoses, has been used as an index of radiation effects to fish embryos by several Russian scientists. As Kligerman has pointed out, there is a limit to applying this method to the study of radiation damage to aquatic organisms, but it is supposed to be the best known means of investigating the cytogenetic effects of radiation on the eggs of the medaka, *Oryzias latipes*, which has long been used in the field of radiation biology, and has the diploid complement of 2n=48, consisting of small chromosomes. In the present experiments, only the chromosome bridge was selected as a practical index of the radiation damage to *Oryzias* eggs. The control value, 0.56%, which is the composite value of the four, is lower than those in other reports. The fish and the fixation time after fertilization were different from each other, though. The minimum concentration at which significant increase in the frequency of aberrant mitoses could be detected were 0.5 Ci/l for tritium and 100 μCi/l for *Sr-Y*. In the continuous irradiation experiments with

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**Table 4.**
Frequency of Cells with Chromosome Bridge(s) Induced by γ-rays

<table>
<thead>
<tr>
<th>Dose (rad)</th>
<th>Mitoses (Ana-Telo)</th>
<th>Cells with Chromosome Bridge(s)</th>
<th>Percent ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6078</td>
<td>34</td>
<td>0.56±0.10</td>
</tr>
<tr>
<td>78</td>
<td>1398</td>
<td>17</td>
<td>1.22±0.29*</td>
</tr>
<tr>
<td>148</td>
<td>1582</td>
<td>16</td>
<td>1.01±0.23*</td>
</tr>
<tr>
<td>263</td>
<td>1940</td>
<td>27</td>
<td>1.39±0.27*</td>
</tr>
<tr>
<td>378</td>
<td>1357</td>
<td>29</td>
<td>2.14±0.40*</td>
</tr>
<tr>
<td>591</td>
<td>1548</td>
<td>42</td>
<td>2.71±0.42*</td>
</tr>
</tbody>
</table>

* Significant compared to the control (p<0.05)

**Table 5.**
Frequency of Cells with Chromosome Bridge(s) Induced by X-Irradiation

<table>
<thead>
<tr>
<th>Dose (R)</th>
<th>Mitoses (Ana-Telo)</th>
<th>Cells with Chromosome Bridge(s)</th>
<th>Percent ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6078</td>
<td>34</td>
<td>0.56±0.10</td>
</tr>
<tr>
<td>25</td>
<td>1945</td>
<td>13</td>
<td>0.67±0.18</td>
</tr>
<tr>
<td>100</td>
<td>1352</td>
<td>18</td>
<td>1.33±0.31*</td>
</tr>
<tr>
<td>200</td>
<td>654</td>
<td>13</td>
<td>1.99±0.55*</td>
</tr>
<tr>
<td>300</td>
<td>1309</td>
<td>37</td>
<td>2.83±0.46*</td>
</tr>
<tr>
<td>400</td>
<td>1244</td>
<td>56</td>
<td>4.50±0.60*</td>
</tr>
<tr>
<td>500</td>
<td>1608</td>
<td>82</td>
<td>5.10±0.56*</td>
</tr>
</tbody>
</table>

* Significant compared to the control (p<0.05)
γ-rays, a significant increase in the frequency of aberrant mitoses was observed at the accumulated dose of as low as 78 rad eight hours after the commencement of irradiation. In the hatchability experiments, no significant effects were detected up to the concentrations of 1 Ci/l for tritium and 100 μCi/l for 90Sr-90Y in terms of hatchability and frequency of abnormal larvae. In continuous irradiation experiments the hatchability was not affected even with a total dose of 9,000 R at a dose rate of 1,000 R/day. Therefore, the chromosome bridge is found to be more sensitive and useful for the study of radiation damage to fish eggs than hatchability and frequency of abnormal larvae. The embryonal cells irradiated with various doses of X-rays at the one-cell stage showed a rapid increase in the frequency of aberrant mitoses as the radiation dose increased. The doses with 100 R and above yielded significantly high frequencies of the cells with chromosome bridge(s) compared to the control. If the increase of the frequency was assumed by extrapolating the present data linearly up to 1,000 R, it would be about 12%. This value is much lower than the data by Pankova, which were over 70%.

It is known that the chromosome bridge arises as a result of chromosome exchange or stickiness. As some Russian scientists pointed out, the chromosome bridge may appear directly after irradiation and be transmitted from cell to cell for a long time through the "breakage-fusion-bridge" cycle. Ejima et al. also reported that when sea urchin eggs were inseminated with UV-irradiated sperm, chromosome bridges appeared at the anaphase of the first mitosis and reappeared at the 4-cell stage.

Although the possibility of the chromosome bridge occurring as a latent damage can not be excluded, inheritable stickiness may account for a part of chromosome bridge formation after an acute X-irradiation at the one-cell stage.

As for the hatchability test, it is known by comparing the present data with those of Hyodo et al. that the one-cell stage is more radiosensitive than the later stages. From these X-irradiation experiments it can be stated that even a relatively small dose when given at the one-cell stage is significantly effective for the inactivation of hatching as well as for the induction of chromosome bridges. However, it must be further investigated whether chromosomal aberrations at early developmental stages could directly lead to the embryonal death.

ACKNOWLEDGEMENT

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REFERENCES