CYTOGENETIC EFFECTS OF IONIZING RADIATION ON THE EARLY DEVELOPMENT OF *ORYZIAS* EGGS

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Although the effects of ionizing radiation on aquatic organisms have been extensively investigated (IAEA, 1976), insufficient attention has been paid to the cytogenetic aspects of radiation effects on fish eggs. 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 the effect of radiation on fish embryos by several Russian scientists (Pankova, 1965; Tsytsugina, 1973). As Kligerman (1979) has pointed out, there is a limit to the application of this method in the study of radiation damage to aquatic organisms. Probably, however, the anaphase method is currently the best known means of investigating the cytogenetic effects of radiation on *Oryzias* eggs (Suyama et al., 1980, 1981). In the present report, the chromosome bridges and micronuclei are used as indices of the radiation damage to the eggs of medaka.

An Inbred strain of an orange-red variety of the medaka (*Oryzias latipes*) maintained in our Institute was used as parental fish. The procedures for collection of fertilized eggs for synchronized development and for preparation of the microscopic slides are given schematically in Fig. 1. Immediately after being harvested, the eggs were exposed to tritiated water (0.1, 0.5, 1.5 and 10Ci/l), ^{90}Sr-^{90}Y solution (10, 100 and 1,000μCi/l) and ^{60}Co gamma-rays (9.8, 18.5, 32.8, 44.8 and 73.9 rad/h). All treatments were initiated at the one-cell stage, and the eggs were kept at 26°C throughout the experiments.

Microscopic slides were prepared as illustrated in Fig. 1. For observation of chromosome bridges, 1 to 4 eggs were squashed on a slide glass, and the embryonal cells were fixed and stained with 45% aceto-orcein solution. For observation of micronuclei, about 100 eggs were squashed in a solution of Ca^{2+}-free phosphate-buffered saline. The embryonal cells were collected and treated with hypotonic saline, fixed with Carnoy's solution, and stained with Giemsa.

The results obtained in these experiments

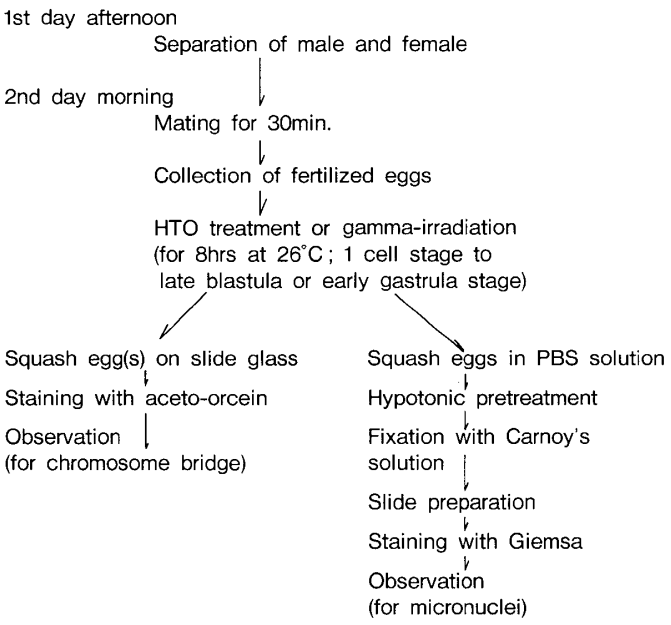

Fig. 1. Schematic illustration of the procedure for collection of fertilized eggs and for preparation of microscopic slide.

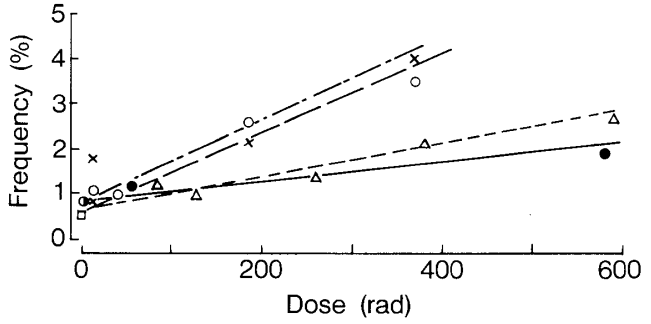

Fig. 2. Frequency of the chromosome bridges in embryonal cells of medaka treated with tritriated water (○-○), ^{90}Sr-^{90}Y solution (●-●) and irradiated continuously with gamma-rays (△-△), together with frequency of the micronuclei (×－-－×) treated with tritiated water.

are shown in Fig. 2. The frequency of chromosome bridges in the embryonal cells irradiated continuously with beta-rays from tritium or ^{90}Sr-^{90}Y, and gamma-rays from ^{60}Co increased with increasing doses. There were no significant difference in the frequency of chromosome bridge between treatment with ^{90}Sr-^{90}Y solution and irradiation with gamma-rays from ^{60}Co. However, higher frequencies of aberrations were obtained in the group treated with tritiated water. The minimum doses for eight hours at which significant increases in the frequency of aberrant mitoses could be detected were 18.5 rad for tritium (0.5 Ci/l), 58 rad for ^{90}Sr-^{90}Y (100μCi/l) and 78 rad for continuous gamma-ray irradiation from ^{60}Co.

Micronuclei are formed in the cytoplasm from fragments of chromosomes left behind at anaphase. The micronucleus test has become a screening procedure for the detection of structural chromosome aberrations induced *in vivo* by various stimulants. As shown in Fig. 2, the frequency of micronuclei in the embryonal cells of medaka treated with tritiated water was increased with increasing doses in a pattern similar to that observed for chromosome bridges. It is generally known that chromosome bridges arise as a result of chromosome exchange or stickiness. The results obtained here suggest that radiation induces the formation of chromosome bridge, as a result of chromosome exchange.

In hatchability experiments, no significant effects were detected up to a concentration of 1 Ci/l (1,700 rad of accumulated dose for 10 days) for ^{3}H, 100μCi/l (724 rad for 10 days) for ^{90}Sr-^{90}Y and 9,000 rad of gamma-rays from ^{60}Co for 10 days (Ichikawa et al., 1977). Therefore, chromosome bridge formation was a more sensitive and useful method for detecting radiation damage to fish eggs than hatchability.

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