Comparison of the Radiosensitivity of Dry Dormant Eggs, Gemmules, and Statoblasts of Three Invertebrate Forms

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Radiosensitivities of the special structures (reproductive organs) of various invertebrate animals were examined. The doses of 50% hatch were about 5 kR for statoblasts of the bryozoa, Pectinatella magnifica; 70 kR for gemmules of the freshwater sponge, Eunapius fragilis; 130 kR for gemmules of the freshwater sponge, Ephydatia fluviatilis; 180 kR for dry eggs of the rotifer, Brachionus plicatilis, and 350 kR for dry eggs of Artemia salina. The results showed that Artemia eggs were the most radioresistant reproductive organ among the materials examined.

INTRODUCTION

It is well known that the dry dormant eggs of the brine shrimp Artemia salina, have a tolerance to extreme environmental conditions, such as low and high temperatures and a vacuum, and they show a high resistance to ionizing radiation). Accordingly, Artemia eggs are very useful materials for the radiation biology; for instance, the relationship between the amount of radiation-induced free radicals in cells and the biological effects has been analyzed using this system.

Besides the Artemia eggs, dormant eggs, gemmules, and statoblasts are known as the reproductive organs in various freshwater invertebrate animals (Table 1). They function as the structures not only for reproduction but also for withstanding extreme environmental condi-

| Irradiated organ | Phylum (general name)           | Species                          |
|------------------|---------------------------------|----------------------------------|
| Gemmules         | Porifera (sponges)              | Ephydatia fluviatilis, Eunapius fragilis |
| Dormant eggs     | Trochelminthes (rotifers)       | Brachionus plicatilis            |
| Statoblasts      | Prospopygii (bryozoa)           | Pectinatella magnifica           |
| Dry eggs         | Arthropoda (crustacean)         | Artemia salina (brine shrimp)    |
tions. The aim of the present work is to compare the radiosensitivity of these animals and to explore suitable animal materials for the irradiation experiments under vacuum conditions for the space biology, synchrotron biology, etc.

MATERIALS AND METHODS

All the materials were irradiated at the “dormant” stage at the room temperature with $^{137}$Cs source as the Research Center for Nuclear Science and Engineering, University of Tokyo. The dose rate was 2400 R/min except for the statoblasts exposed at 250 R/min. Just before the irradiation under the hydrated conditions, most materials were transferred from a stock culture (stored at 4°C) into the each of the incubation medium which gave the highest hatchability in the control groups of each organism. After the irradiation, they were incubated at 26 ± 2°C in a small plastic dish without aeration and observed daily under binocular microscope to examine the hatchability.

2.1. Freshwater sponges

The gemmules of two species of sponge were collected at the Yokotone River in Ibaraki Prefecture, Japan, by Dr. Watanabe and her students of Ochanomizu Women’s University. The gemmules of *Ephydatia* were spherical or egg in shape, about 0.4 mm in diameter and light brown or dark orange in color. Several hundred or several thousand resting cells, thesocytes, are enclosed in a hard collagen shell (Fig. 1). The gemmules of *Eunapius* were taken to pieces before the irradiation because they were connected by a collagen layer (Fig. 1). Incubation was performed in the mineral medium composed of 1.00 mM CaCl$_2$·2H$_2$O, 0.50 mM MgSO$_4$·7H$_2$O, 0.25 mM Na$_2$SiO$_3$·9H$_2$O, 0.05 mM NaHCO$_3$, and 0.05 mM KCl and adjusted the pH to 7.0 by HCl. About 200 gemmules were irradiated for the each dose group.

![Fig. 1. Illustrations of two species of freshwater sponges. g, gemmules; gs, gemmoscleres; m, micropyle; s, shell; t, thesocytes.](image-url)
2.2. Rotifer

Eggs of rotifer, *Brachionus plicatilis*, were kindly supplied by Dr. Hino of the University of Tokyo. The eggs were dormant and strong enough to suffer dry conditions. They were egg-shaped, about 0.1 mm in diameter and orange in color, enclosed by a transparent, membranous shell (Fig. 2). The incubation medium for the rotifer was 1/2-fold-diluted sea water (sea water:distilled water 1:1, v/v). For the irradiation experiments under the dry conditions, the eggs were stored in a refrigerator without the medium for more than 1 week to dry them up completely before irradiation, and then they were soaked in 1/2-fold diluted sea water to develop them. About one thousand dried eggs were irradiated in each dose group of the experiment under the dry conditions. For the irradiation experiments under the hydrated conditions, about 500 eggs were used for the each dose-group experiment.

![Fig. 2. Dormant eggs of the rotifer, Brachionus plicatilis (in sea water) (x33).](image)

2.3. Freshwater byozoa

Statoblasts of the freshwater bryozoa, *Pectinatella magnifica*, were kindly given by Dr. Oda of St. Paul's University. The statoblast is a squarish circle in shape, about 1 mm in diameter and dark brown in color. From 11 to 22 spines with hookd extended out from the entire border of its dorsal valve (Fig. 3a). If a normal statoblast, awakened from dormancy, was incubated in tap water at 26 ± 2°C, it began to germinate; the valves split, and the germinal material appeared between them within 2 days (Fig. 3b). Then the first young polypide, bearing a tentacular crown with short tentacles, evaginated from a germinal mass 3 days after the incubation (Fig. 3c). Five days after the incubation, the second polypide evaginated by budding (Fig. 3d).

In irradiation experiments, about 60 statoblasts were exposed in each dose group.

2.4. Brine shrimp

The dry eggs of *Artemia salina* were obtained from Tetra Werke, W. Germany. The eggs were irradiated under a dry conditions and then soaked in a 2% NaCl solution. About 2000 eggs were examined in each dose group.
RESULTS

Gemmules of the freshwater sponge

Figure 4 shows the delay in hatching of irradiated gemmules of *Ephydatia fluviatilis*. In the unirradiated control, at first, histoblasts differentiated from the thesocytes, and migrated out from the micropyle of the shell onto their external surface and then onto the surface of the plastic dish about 2 days after the incubation. We call this stage as "hatch". If the gemmules were irradiated with 32 kR or 128 kR of γ-rays, the hatching was delayed and the incubation times for 50% hatch were about 3 days and 4.5 days, respectively. Microscopical observation showed that in the 32 kR-irradiated groups no choanocyte develop from archaeocytes, although almost all other kinds of cells appeared. In the groups irradiated with 128 kR, only abnormal histoblasts rich in vitelline platelets and some pieces of spicule could be seen.

Figure 5 shows the relative hatchabilities of the gemmules of the two species irradiated with various doses of γ-rays. The hatchability in the unirradiated controls (*Ephydatia*: 96 ± 1.8%; *Eunapius*: 44 ± 2.0%) is set at 1.0 in this figure. All the materials were examined at 12th day after the incubation, when none of them seemed to develop any more under those conditions without nutrition. From the Fig. 5 results, the dose giving 50% hatch in *Ephydatia* was found to be about 130 kR, while that in *Eunapius* was found to be about 70 kR.
**Fig. 4.** Time course of hatch in gemmules unirradiated and irradiated with various doses of γ-rays (*Ephydatia fluviatilis*).

**Fig. 5.** Dose-effect relationship between γ-ray dose and relative hatchability in two species of sponge.

**Eggs of the rotifer**

In the unirradiated controls, both wet and dried eggs began to hatch within 2 days after the incubation. The hatchabilities were 59 ± 2.4% and 16 ± 4.0%, respectively. The dose-effect relationship in the rotifer eggs are shown in Figure 6. The hatchability were examined 5 days after the incubation of the eggs. From the results, the dose of 50% hatch in the eggs irradiated in water was found to be about 125 kR, while that in the eggs irradiated under the dry condi-
The shoulder of the dose-effect curve for the eggs irradiated in water was clearer than that for the eggs irradiated under dry conditions.

Fig. 6. Dose-effect relationship between γ-ray dose and relative hatchability in the rotifer eggs and the *Artemia* eggs. •, Rotifer eggs irradiated in water; ○, Rotifer eggs irradiated under dry conditions; □, *Artemia* eggs irradiated under dry conditions.

Statoblasts of freshwater bryozoa

Figure 7 shows the relationship between the γ-ray dose and the relative hatchability in statoblasts of bryozoa.

Fig. 7. Dose-effect relationship between γ-ray dose and relative hatchability in statoblasts of bryozoa.

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the stoblasts. The data are based on the rate of evagination (formation of tentacles) at 12th
day after incubation. The hatchability in the control group was 87 ± 7.1%. When a dose of
more than 7 kR of γ-rays was delivered, evagination was completely inhibited. However,
germination (valve splitting) could be observed at higher doses (>10 kR). It was noting that
the second polypides always developed when the first ones were formed.

*Dry eggs of brine shrimp*

Dose-effect relationship in *Artemia* eggs is shown in Figure 6. In the unirradiated control
(the hatchability was 40 ± 1.5%), the shell of the eggs cracked open and the swimming nauplii
hatched within 2 days after the incubation. The curve indicated that the dose of the 50%
hatch of *Artemia* was about 350 kR. This dose was lower than that obtained by Iwasaki1, but
markedly higher than those of the gemmules, the dormant eggs of the rotifer and the stato-
blasts.

**DISCUSSION**

The results obtained in the present experiments showed that the radiosensitivity of the
organisms, as measured by the relative hatchability, was in the following decreasing order:
(1) the stoblasts (about 5 kR for a 50% hatch dose) > (2) gemmules of the sponge, *Eunapius*
(70 kR) > (3) eggs of the rotifer irradiated in water (125 kR) = (3) gemmules of the sponge,
*Ephydatia* (130 kR) > (4) eggs of the rotifer irradiated under dry conditions (180 kR) > (5)
Dry *Artemia* eggs (350 kR). All the materials used were more or less in the dormant or low
metabolic state, and it is probable that the repair ability from damages by radiation is not so
efficient. The stoblasts of bryozoa showed extremely high radiosensitivity compared with
the other materials examined. One of the reasons for the extremely high radiosensitivity in
stoblasts of bryozoa seem to be due to the requirement of a more complicated process in
development for the evagination. Oda reported on the radiosensitivity of the bryozoa, *Lopho-
pedella carteri*; although no studies on the hatchability has been carried out in his report, this
species also showed a high radiosensitivity11). It is known that the gemmules of the sponge
*Eunapius* are diapausing12), whereas those of *Ephydatia* are non-diapausing13-14). Contrary to
our expectations, however, the present results showed that the gemmules of *Ephydatia* were
more resistant to γ-rays than those of *Eunapius*. According to Iwasaki15), *Artemia* eggs in a
wet state were more radiosensitive than those in the dried state; that is, hatchability was re-
duced to 76.7 ± 2.7% and 14.8 ± 1.7% by irradiation with 100 kR and 200 kR, respectively
(0°C, in 2% NaCl solution). From the above results, it can be said that he radiosensitivity of
*Ephydatia* and that of wet *Artemia* eggs is almost identical as long as the hatchability is con-
cerned. The radiosensitivity of the dry eggs of *Artemia* was the lowest, perhaps because of their
characteristic feature in development in which the blastulae (the irradiated stage) develop into
free swimming nauplii without a single-cell proliferation16), as well as because of the dehydrated
conditions in the eggs (about 7% free water content17). Therefore, it is concluded that *Artemia*
eggs are the most useful material for the radiobiological experiments under extreme conditions,
including a space biology.
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