Effects of the Moisture Content on γ-Ray Sensitivity in *Artemia* Eggs

Tamiko IWASAKI*

Zoological Institute, Faculty of Science, Kyoto University

(*Received August 10, 1959*)

Artemia eggs of various moisture contents were irradiated with γ-rays and then soaked in 2% NaCl solution at 27°C. After 72 hours hatchability was measured. On the basis of the measurement, the radiosensitivity of the eggs increased successively with an increase of moisture content.

The eggs once moistened prior to irradiation were redesiccated to the initial level of moisture content. Their sensitivity was shown to be almost at the same level of the initial one.

To test the influence of temperature, the eggs were infiltrated with moisture at 0°C and 25°C and then were irradiated at low or high temperature, but there was no remarkable difference in their sensitivity. However, the sensitivity of eggs irradiated at high temperature was found much higher than those irradiated at low temperature.

INTRODUCTION

It has long been emphasized that desiccation favours the radioresistance of biological systems. However, even highly dried materials such as bacteria[1] are inevitably affected by radiation. The dependence of sensitivity on moisture content has been demonstrated in spores[2], seeds[3], seedlings[4,5], tumor cells and isolated tissues[6,7]. Most of the investigations reported had for their basis a hypothesis that part of the biological effects of radiation are derived from radicals formed in the aqueous phase. It was surmised that the increased radiosensitivity of the biological materials with high moisture content would result largely from the frequency and nature of the radical species formed along tracks of ionizing electrons.

Recently, papers by Caldecott[8,9,10,11], Konzak[12], Ehrenberg[13], who worked with barley, showed that the influence of moisture content on the radiosensitivity of seeds might be much more complex than the participation of radicals in the initiation of the biological damage. The author also obtained a similar result with *Artemia* eggs by X-ray irradiation[14,15].

In the present report, both the influence of moisture content and temperature on the γ-ray sensitivity of *Artemia* eggs, are dealt with in comparison with the case of X-ray irradiation.

MATERIAL AND METHOD

Artemia is a branchiopod Crustacea which has a widespread distribution in

* 岩崎民子

salt pools and salt lakes of high salinities. It produces eggs provided with a thick shell, which remain dormant and are capable of withstanding prolonged desiccation. These eggs survive ten years or more under desiccated laboratory condition and when soaked in salt water of an appropriate salinity they resume to develop and larvae emerge from their shells.

The eggs of *Artemia* are especially well adapted to an investigation upon the relationship between moisture content and radiosensitivity, for, like seeds, the moisture content can be varied easily over a wide range without altering the viability of the eggs.

The moisture content of the dried eggs was 5.8%, and it was regulated at various degrees up to 54.0% by storing them for 24 hours at room temperature(25°C) in a glass chamber, in which the vapour pressure was changed in equilibrium with different concentrations of H_2SO_4.

Under the present experimental condition, the eggs were hatched in 24-36 hours in 2% NaCl solution at 27°C. Since hatching be delayed in irradiated eggs, the hatchability was measured at end of the 72nd hour after soaking eggs in salt water. Although the hatchability of the unirradiated eggs remains 73.2% on an average, and this was converted to be standard or 100% of hatchability of the unirradiated eggs, applying this to that of the irradiated eggs.

The eggs used for material in the present experiment were secured in Aquarium Society INC. of San Francisco in 1956, and were stored in a desiccator.

The experiments were carried out using the γ-ray irradiation facility in the Institute for Chemical Research, Kyoto University. The radiation from Cobalt-60 was composed of two γ-rays of 1.17 and 1.33 MeV energies, the target-object distance was 8.5cm. Under such a condition the intensity was approximately 60r/sec measured by Fricke dosimeter and the material was exposed to the γ-rays for 2 hours.

REMARKS

1) Relationship between Moisture Content of Eggs and Radiosensitivity

Artemia eggs of various moisture contents were irradiated with γ-rays for 2 hours at room temperature (25°C). The results obtained are illustrated in Table-1. As denoted by hatchability, the sensitivity of the eggs increased successively with an increase of the moisture content. This is quite in harmony with the well established opinion that living materials with a "high" moisture content are more sensitive to ionizing radiations than those with a "low" moisture content. In the previous work with X-ray irradiation (120.000r), *Artemia* eggs showed a temporary decrease of sensitivity at 16% of moisture content and it increased successively as moisture content increased beyond this level. This result accords with those of recent works by Ehrenberg and others with barley in respect that the radiosensitivity of the seeds decreases as moisture content increases up to 16.4%. They concluded that there does not exist a 1 : 1 relationship between the sensitivity of the seed to X-rays and the moisture content, that the sensitivity of the seed is not a simple function

of the quantitative interaction of similar radical species with biological structures of constant sensitivity, and that the moisture content itself appeares, at least in certain limits, more in setting the pace of physiological development than in supplying radicals.

Temporary decrease of X-ray sensitivity in *Artemia* eggs may be interpreted similarly. And if this be correct, the dissimilarity between the effect of X-ray irradiation and that of γ-ray irradiation will indicate a difference in physical behavior in biological materials. However, difference in radiation dose exist, and the energy existing in two experiments cannot be neglected to attain a conclusive explanation.

Table 1. Effect of moisture content of *Artemia* eggs.

	Initial weight of eggs. (mg)	At equilibrium. (mg)	Moisture content. (%)	Hatchability (%)	
				Unredesiccated	Redesiccated
Desiccated	100.0	100.0	5.8	57.4±3.5	—
33% H_2SO_4	100.0	105.0	11.1	51.6±2.9	58.1±2.5
23% H_2SO_4	100.0	110.0	16.4	41.1±2.9	60.2±2.9
13% H_2SO_4	100.0	126.5	33.9	23.8±2.5	60.9±3.3
7% H_2SO_4	100.0	139.5	47.6	11.5±1.9	54.7±3.1
3% H_2SO_4	100.0	145.5	54.0	5.7±1.3	57.4±2.9

2) Effect of Redesiccation

Increase of the moisture content may induce more or less physiological alteration of dormant eggs. In the present experiment, however, the time needed for hatching was not noticeably changed regardless of increase in the moisture content of eggs before being soaked into salt water.

In the previous work[15], *Artemia* eggs once moistened were redesiccated to the initial level of moisture content (5.8%) and then irradiated with X-rays. The radiosensitivity of these eggs was found to be almost at the same level of the initial state, i.e. the initial sensitivity of dried condition once changed by moisture infiltration was regained by redesiccation. The effect of γ-rays upon redesiccated eggs was tested. Results obtained are shown in the last column of Table-1. It is quite similar to that of X-rays. Many factors may be participating in this phenomenon, but this indicates that the presence of additional water plays the major role in increasing the radiosensitivity of the living system in producing radicals by ionizing radiations.

3) Influence of Temperature

The fact that the radiosensitivity of *Artemia* eggs rises as the moisture content increases, seems to favour the view that the effect of ionizing radiations upon living materials is due to radicals formed in the aqueous phase. However, there could be a possibility that changes in sensitivity were resulted

from changes in the physiological condition of eggs caused by moisture infiltration but not from changes in some physical nature. In other words, the metabolism of eggs would become more active as moisture content increases and the eggs rendered more sensitive to radiations in consequence.

If such is case with Artemia eggs, an exposure to different temperatures prior to irradiation would cause different effects upon radiosensitivity of eggs, because the metabolic activity of eggs becomes high as temperature rises under the existence of any amount of moisture. On the contrary, if changes in the sensitivity were caused chiefly by changes in some factors of physical nature, an exposure to different temperatures at the time of irradiation would give rise to different results.

To test the influence of a temperature change upon the radiosensitivity of *Artemia* eggs, materials were infiltrated with moisture at 0°C and 25°C respectively. Each group of eggs was divided into two, of which one was irradiated at a low temperature (approximately −10°C) using dry ice and the other at 25°C. Then, the hatchability of these four groups of eggs was examined after soaking into salt water at 27°C. It has been reported that both the hatchability and the time rate of hatching of dried *Artemia* eggs were not impaired by subjecting the eggs suddenly to liquid air (−190°C) for 7 and on another instance for 24 hours respectively[16]. Exposure to −10°C in the present experiment, therefore, showed that it exerted no effect upon the development of the eggs.

Table 2. The influence of moisture content of *Artemia* eggs with temperature treatment.

	Irradiated at			
	Low temperature		High temperature	
	Stored at		Stored at	
	0°C	25°C	0°C	25°C
Desiccated	71.4±1.8	72.5±1.7	65.1±1.9	59.6±2.2
33% H_2SO_4	72.7±1.6	74.5±1.6	56.0±2.0	60.4±1.8
13% H_2SO_4	62.2±2.2	63.9±2.2	20.4±1.6	16.4±1.2
7% H_2SO_4	57.0±1.8	57.5±1.7	11.1±1.2	14.3±1.2

As shown in Table-2, there was no remarkable difference in sensitivity between two groups of eggs which were subjected to different temperatures during moisture infiltration. On the contrary, the sensitivity of eggs irradiated at 25°C was found much higher than those irradiated at lower temperatures regardless of difference in temperature of moisture infiltration. In each series of these experiments the relation between the moisture content and sensitivity of eggs were not altered. It is difficult to give a conclusive explanation to these phenomena at present, but it reminds us the opinions that chemical

bonds are more resistant, or tend to be reconstituted more easily at lower temperatures than at higher temperatures, and that transfer of energy inside molecule is also temperature-dependent.

These results, together with those obtained in the foregoing experiment, might suggest that the main factors operating to raise the radiosensitivity of moistened eggs are of physical nature.

ACKNOWLEDGMENTS

Special thanks are due to Prof. K. Nakamura of Kyoto University for his continued encouragement and guidance, and for the provision of the first experimental source. I wish also to express my deep appreciation to Prof. S. Shimizu and Mr. Y. Nakayama at the Institute for Chemical Research, Kyoto University, who have carried out the irradiation of the Cobalt-60 γ-rays.

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