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Sensitivity of *Artemia* Eggs to the γ-irradiation
VI. The Effect of Fractionated Irradiation * **

by

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Introduction

The large amount of works on fractionated irradiation have provided valuable informations on important problems in radiation biology such as lethal mechanism, recovery from radiation damage, radiation tolerance, *etc.* Most studies with fractionated irradiation have been carried out with organism in metabolic states. The results of these works naturally involved effects of metabolism which eventuated in changes of the radiosensitivity of organisms after being irradiated. It is needed, therefore, to perform experiments using organisms in non-metabolizing states, which provide evidences of basic importance to aproach the problem of fractionated irradiations.

The dry eggs of *Artemia* used as materials in a series of present works are suitable for analysis of the effect of fractionated irradiation on radiation damage because their metabolism is almost completely arrested under dry conditions (MURAMATSU, 1960). Moreover, as has been demonstrated, the after-effect of irradiation is restrained by keeping the irradiated eggs at dry ice temperature (approximately -10°C) (IWASAKI, 1965). So that, changes in the metabolic conditions during intervals of fractionated irradiations would be minimized, if occurring at all, under such conditions.

Methods

The present experiments were carried out in two series ; in the first series effects of prolongation of the interval between fractionated irradiations, and in the second series effects of changing the ratio of doses in fractionated irradiations were examined. Dry eggs of *Artemia salina*, with the water content of approximately 7.4%, were irradiated at room temperature with γ-rays at the

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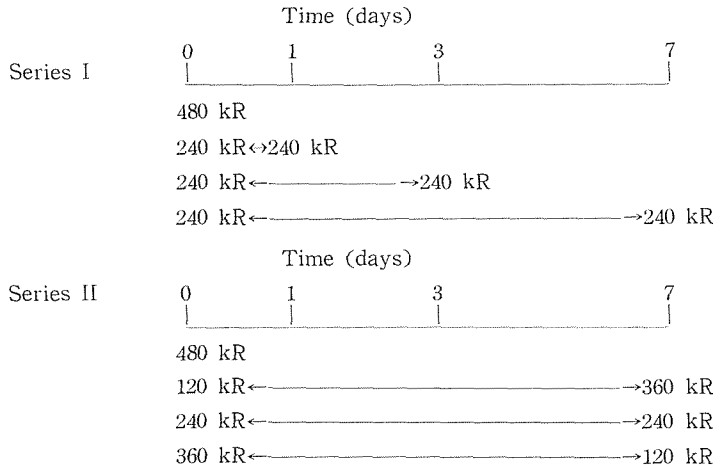
dose rate of 12 kR per minute. The procedure of irradiation was same as in the previous paper (IWASAKI, 1964a). In both seires of experiments the total dose was 480 kR. During intervals between irradiations the eggs were divided into two groups, one kept in a desiccator at 25°C and the other in a vessel with dry ice. The experimental schemes were as follows (cf. Fig. 1) :

Fig. 1. Schematic illustrations to show the irradiation schedules



Series I ; two separate irradiations with 240 kR each, at intervels of one, three and seven days, respectively.

Series II ; two separate irradiations with different doses at the ratio of 120 : 360 kR, 240:240 kR and 360:120 kR, respectively, the interval being one week.

Hatchability in each group of eggs was tested immediately before and after each irradiation. The eggs were soaked in salt solution at 27°C and hatchability was examined at the 72nd hour. It was 73.2% in unirradiated eggs, and this figure was converted to a standard or 100% hatchability of the eggs. An average hatchability was calculated from data obtained from three repetitions of experiments per series.

Results

1. Effects of changing time interval between successive irradiations

The control experiments showed that the hatchability of eggs immediately after irradiation decreased to 71.6% and 56.5% by a single irradiation with 240 kR and 480 kR, respectively. In both series of experiments there were remarkable differences in the effects of fractionation of irradiation between two groups of eggs, one kept at dry ice temperature and the other at 25°C during post-irradiation storage.

In the first series of experiments, in which the eggs were subjected twice to irradiation with 240 kR, the ultimate hatchabilities of eggs were 52.2%, 52.9 % and 55.3% with storage of one, three and seven days respectively when the eggs were kept at dry ice temperature during intervals between two irradiations.

Table 1. Hatchability changes of *Artemia* eggs caused by prolongation of the time interval between fractionated irradiations at different temperature

Time interval between irradiations (days)	Doses (kR)		Total dose (kR)	Hatchability (%) of eggs stored during interval at		Effectiveness of the second irradiation at	
	1st	2nd		dry ice temp.	25°C	dry ice temp.	25°C
—	240		240	71.6 ± 1.5		—	—
—	480		480	56.5 ± 1.6		—	—
1	240	240	480	52.2 ± 1.8 (73.3 ± 1.4)	40.3 ± 1.7 (73.0 ± 2.4)	71.2	55.2
3	240	240	480	52.9 ± 1.8 (72.7 ± 1.6)	25.4 ± 1.5 (56.6 ± 1.9)	72.8	44.9
7	240	240	480	55.3 ± 2.0 (70.2 ± 1.6)	4.7 ± 0.8 (13.8 ± 1.4)	78.8	34.1

Numbers in parentheses represent the hatchabilities of eggs measured immediately before the second exposure.

The results showed that the ultimate hatchabilities of fractionated irradiations remained at the same level with that of the single irradiation with 480 kR, irrespective of differences in the time length of intervals between two irradiations. To examine whether or not the after-effects of the first irradiation were included in these results, and to measure the resistivities of the eggs to the second irradiation at the same time, hatchabilities of the eggs were tested at the end of storage (immediately before the second irradiation). These figures are shown in parentheses in the Table 1. The hatchabilities were statistically equal to the hatchability after the first irradiation, being 73.3%, 72.7% and 70.2%, respectively. These facts indicated that the effectiveness of the second irradiation to the irradiated eggs was quite same with that of the first irradiation. In other words, the resistivity of the eggs was not changed by the post-irradiation storage at dry ice temperature. Taking the hatchabilities immediately before the second irradiation as the standards, the hatchabilities after the second irradaition were converted into 71.2%, 72.8% and 78.8%, respectively. These figures coincided with that after the single irradiation of 240 kR. This indicates the effectiveness of the second irradiation was not changed by the storage at dry ice temperature after the first irradiation.

In the second group, eggs were kept at 25°C during the interval. The

result is shown in Table 1. Quite in contrast to the first group, the effects of storage were striking and the hatchability decreased markedly as the time length of the interval was prolonged. By storing the irradiated eggs for one day at 25°C, the hatchability after the second irradiation fell to 40.3%, and successively to 25.4% and to 4.7% after three and seven days had passed. As revealed in the previous work (IWASAKI, 1964b), the hatchability of irradiated eggs successively decreased by keeping them at 25°C. However, the hatchability of eggs irradiated with 240 kR (71.6%) was not changed by the storage of one day, being 73.0 %, but decreased to 56.6% after three days and to 13.8% after seven days (Table 1). By taking these hatchabilities immediately before the second irradiation as the standards, the resistivities of these eggs against the second irradiation were 55.2%, 44.9% and 34.1%, respectively. This clearly indicated that the resistivity of eggs decreased markedly even in one day after irradiation, notwithstanding that the hatchability remained unchanged. But it also decreased by further prolongation of the post-irradiation storage.

2. Effects of changing dose ratio in fractionated irradiations

The eggs were irradiated with 480 kR fractionated into two doses at proportions of 120:360 kR, 240:240 kR and 360:120 kR, respectively. The time interval between the first and the second irradiation was fixed at one week, during which one group of the eggs was kept at dry ice temperature and the other at 25°C. The result obtained is shown in Table 2.

As has been demonstrated in the first series of experiment, lowering of hatchability was minimized by keeping the irradiated eggs at dry ice temperature during the interval of fractionated irradiations. In the control experiments the hatchability of eggs irradiated with 480 kR at one time was 49.1%, and as has been expected from the results obtained in the foregoing experiments there occurred no remarkable change in the ultimate hatchability by

Table 2. Hatchability changes of *Artemia* eggs caused by varying the dose ratio of the fractionated irradiations at different temperature

Doses in the first and second irradiations (kR)		Total dose (kR)	Time interval (days)	Ultimate hatchability (%) of eggs stored during interval at	
1st	2nd			dry ice temp.	25°C
480		480	—	49.1 ± 1.8	
120	360	480	7	48.4 ± 2.7	17.3 ± 1.6
240	240	480	7	55.3 ± 2.0	6.6 ± 1.9
360	120	480	7	51.2 ± 2.5	0

fractionation of irradiation at different dose ratios when the eggs were kept at dry ice temperature during the interval of seven days, the actual hatchabilities being 48.3%, 55.3% and 51.2% at dose ratios of 1:3, 1:1 and 3:1, respectively. Quite in contrast to these results, differences in the dose ratio strikingly affected the ultimate hatchability when the eggs were kept at 25°C instead of dry ice temperature. It decreased to 17.3%, 6.6% and 0%, at dose ratios of 1:3, 1:1 and 3:1, respectively. The results indicated that higher the dose ratio of the first irradiation against the second severer the lowering of the ultimate hatchability.

Discussion

Difficulties in approaching the problem of fractionated irradiation lie chiefly in the fact that the physiological state of organisms changes after irradiation and, therefore, the fractionation does not mean mere repetition of irradiation upon organisms in a constant physiological state. Changes in the metabolic states caused by irradiation may eventuate in some radiosensitivity changes. Diversity of results obtained hitherto in fractionation experiments would due not only to differences in the physiological state of materials but also to varieties of changes caused by preceding irradiation. Very little information is available on the influence of fractionated dose on the dormant materials measured in terms of growth rate. SCHOOLER *et al.* (1957) had performed the experiments on dormant barley seeds under the schemes nearly same with the present experiments. They indicated that no significant difference on seedling height existed between the single irradiation and the fractionated ones of X-rays.

The results of the present experiment clearly indicate that when dry eggs of *Artemia* are kept at dry ice temperature during the different intervals between the first and the second irradiation, the effectiveness of the first irradiation as measured by lowering of hatchability is nearly the same as that of the second irradiation and, accordingly, the effectiveness of the fractionated irradiations approximates to that of a single irradiation with the dose epual to the total dose of repeated irradiations. In the fractionated irradiations not only the prolongation of the post-irradiation storage, but changes of the dose ratios do not affect the effectiveness of irradiation as far as eggs are kept at dry ice temperature during storage. It probably means that damages induced by the first irradiation remain unchanged during storage. The author found in the preceding work of this series (IWASAKI, 1965) that the after-effects of irradiation were subsided by keeping the irradiated eggs at dry ice temperature. The results of the present experiments fall in the same line. It is concluded, therfore, that the effects of fractionation of irradiation are simply cumulative under these conditions.

Quite in contrast with these results, effects of irradiation are remarkably enhanced when the eggs are kept at 25°C after irradiation. By the second

irradiation the hatchability of eggs decreases markedly as compared to that after a single irradiation with an equal dose. It must be noticed, however, that the hatchability of irradiated eggs remains unchanged at least in one day even they are kept at 25°C, while the resistivity of eggs to the successive irradiation decreases from 71.6% (in the control) to 40.3%. This indicates that there exists no accurate correlation between lowering of the hatchability caused by irradiation and that of the resistivity. Therefore, it is suggested that radiation damages responsible for lowering of the resistivity cannot be identical with those affecting the hatchability.

Results of fractionated irradiations at different dose ratios fell in the same pattern with the effect of temperature difference during post-irradiation storage. By keeping the eggs at 25°C, the effects become quite different according to the dose ratio between two irradiations ; declination in the hatchability becomes sharper as the dose ratio of the first irradiation against the second increased. This relationship can easily be expected from the result of the foregoing experiments that post-irradiation storage of irradiated eggs resulted in a notable decrease in the hatchability and that the rate of decrease was much greater at a higher dose than at a lower one (IWASAKI, 1964b). In the present experiments, no indication of recovery from radiation damages is found.

Summary

Present paper deals with the effect of fractionation of γ-irradiation on the hatchability of dry eggs of *Artemia*.

The eggs were irradiated twice with 240 kR at intervals of one, three and seven days, respectively. When the eggs were stored at dry ice temperature after the first irradiation, the total effects of fractionated irradiations were nearly the same as those of a single irradiation with 480 kR. On the other hand, the irradiated eggs were stored at 25°C, the ultimate decrease in hatchability was much higher than that after a single irradiation with 480 kR. The effect increased as the time interval between two irradiations increased.

The eggs were subjected to irradiation with 480 kR fractionated into two doses at different ratios, 120:360kR, 240:240 kR and 360:120 kR, respectively. When the eggs were kept at dry ice temperature during one week between two irradiations, the ultimate hatchabilities always remained nearly at the level of that caused by a single irradiation with 480 kR, irrespective of differences in the dose ratio between two irradiations. At 25°C, on the contrary, the hatchability became lower as the dose ratio of the first irradiation to the second one increased.

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