Effects of Radiation on the Growth of *Euglena* Cells

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Cells were irradiated in distilled water by γ-ray doses of 1 to 3×10^5 r for 30 to 90 minutes and by neutron units of 4.1×10^7 to 5.4×10^{12} n/cm^2 for 10 to 90 minutes. No apparent difference was observed between both effects of γ-rays and neutrons on the growth of the cells. Many cells survived these irradiations. But, some remarkable effects were observed on the growth, the death and the albino mutation rates of the irradiated cells. And the degree of these effects was in proportion to the irradiation dose in most cases.

Cells immediately after irradiation and their posterity after several generations reach the stationary phase of the culture at 10 to 25% lower cell concentration than the non-irradiated cells. For a lethal dose, LD-50 (11-13) of *Euglena* cells required about 2 to 3×10^5 r of γ-rays for 60 to 90 minutes and about 3.6×10^{12} n/cm^2/60 min. of neutrons.

The rapidly growing cells were generally more radioresistant than the cells of stationary culture. Not immediately after, but several generations from both irradiations, a little difference of cell size was observed between irradiated and non-irradiated cells.

Albino mutants of the cells are easily induced by γ and neutron irradiations.

INTRODUCTION

There are a few investigations on the effect of irradiation on *Euglena* cells. One has reported some effects of X, ultraviolent and neutron irradiations, the other no apparent effect even by a fairly high dose of X-rays.[2,5,7,13] Excepting that the ultraviolet at 265 mμ is a most effective killing wavelength for *Euglena*[6], the tendency of the effects of other radiations is similar one to another. And a common result through these studies is that *Euglena* cells are fairly radioresistant. Therefore, it is already an obvious fact through many previous studies that any changes found are more or less in the many living cells exposed to any radiations.

The purpose of the present work is to obtain more information on the growth, the radiosensitivity and other information on *Euglena* cells by the irradiation with some considerable high doses of γ-rays and neutrons.

MATERIAL AND METHODS

Material used on the present work was *Euglena gracilis var. bacillaris.*

The cells were gathered centrifugally from logarithmic and stationary phases of the culture and washed twice with distilled water and then suspended again in 800 ml of fresh distilled water. A half volume of this cell suspension was

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treated as the control for the checking of experimental results and the other half was exposed to the radiations. Each cell suspension was put into a flask and closed by a gum stopper.

γ-irradiation was carried out by the ^{60}Co γ-rays irradiation facility of the Institute for Chemical Research of Kyoto University. Neutron irradiations were in small doses by using the cyclotron of the same Institute above stated and in high doses by the JRR-1 of the Japan Atomic Energy Research Institute.

Immediately after the irradiation, the cell suspension was transferred separately into a fresh culture medium of 10 flasks and incubated under a continuous illumination at 30°C, until they were needed for the observation.

The components of culture medium were as follows: pepton 1.7 g, glucose 1.7 g, citric acid 1.0 g, magnesium sulfate 0.2 g and monopotassium phosphate 0.5 g in 1500 ml of distilled water.

The estimation of cell growth was made by counting the cells in about 50 drops of the culture medium diluted with a constant volume of water under a microscope. Capacity of one drop was 0.005 ml. The dead cells were observed by the staining with methylene blue. The error of death rate obtained by this staining was corrected through the single cell culture and the hanging drop culture. That is, by methylene blue staining, the death rate has a rising tendency of about 4 to 6% compared with the other methods.

RESULTS

1) Irradiation Dose and Growth Rate

The higher the irradiation dose was, the less the growth rates of irradiated cells decreased as is shown in Fig. 1. And it was observed that there is a proportional relation between the irradiation dose and the growth rate. That is, the growth rate decreased about 20% to about 10 fold doses of neutrons during 5 days culture after the irradiation. Although it is only a little, the growth rates under small dose irradiations were higher than that of the non-irradiated cells during 1 to 2 days after the irradiation.

2) Recovery of Growth

When the irradiated cells were transferred into another culture medium at some regular intervals of incubation time from the culture after irradiation and incubated, the growth of irradiated cells was recovered in direct proportion to the incubation time (Fig. 2).

The recovery of growth was a little higher in the cells of logarithmic phase than in that of stationary phase. But the cells transferred after 24 hours incubation showed a fairly same recovery in the cases of both phases (Fig. 2, B). And the growth rate of (B) shows the final growth rate of irradiated cells.

3) Cell Concentration of Stationary Phase

It was found that most irradiated cells have not the same cell concentration as the non-irradiated cells at the stationary phase of culture for at least several generations after irradiation. And this concentration decreased according to the

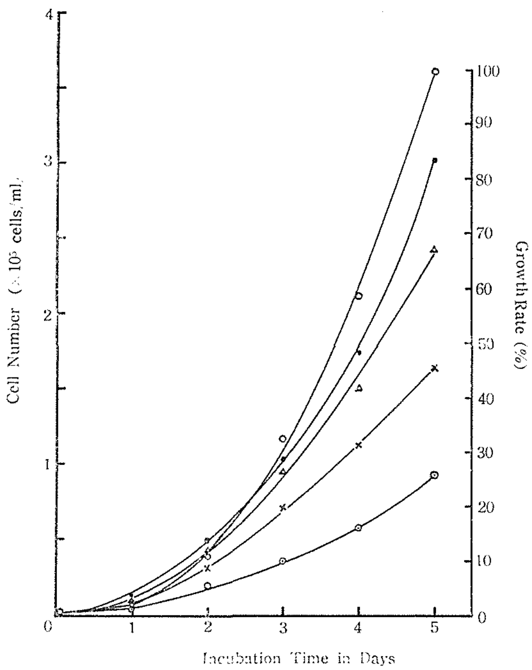

Fig. 1. Relation of the irradiation dose of neutron to the growth rate.

About 10 flasks holding the cell suspension of logarithmic phase of culture, were put at the measured places of the radiation doses around the cyclotron and exposed to neutron bombardment for 10 to 90 minutes.

○……Non-irradiated cells ●……4.1×10^{7} n/cm²/10 min.
△……7.8×10^{9} n/cm²/10 min. ×……7.4×10^{10} n/cm²/57 min.
◎……9.7×10^{11} n/cm²/90 min.

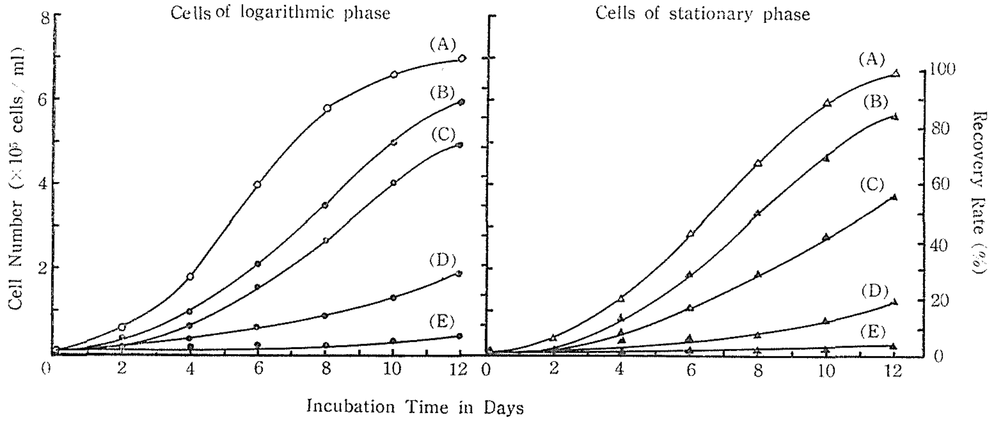

Fig. 2. Recovery rate of growth of the cells exposed to γ-rays.

Cells of logarithmic and stationary phase were irradiated by the γ-rays dose of 1×10^{5} r/30 min. and transferred into a fresh culture medium immediately after the irradiation and incubated. This is the initial culture (E). After 6 (D), 12 (C) and 24 (B) hours incubation after the irradiation, a part of the initial culture was transferred into each fresh medium and incubated under the same condition already described. (A) is the growth curve of the non-irradiated cells. Irradiated cells of logarithmic (—●—), stationary (—▲—) phases and non-irradiated cells of logarithmic (—○—), stationary (—△—) phases were observed.

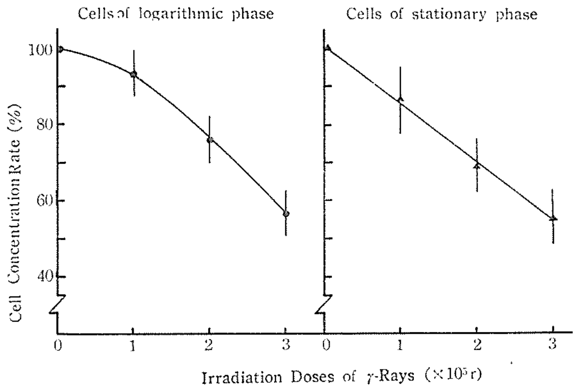

Fig. 3. Irradiation dose and cell concentration of the stationary phase.
Three cell suspensions of logarithmic (—●—) and stationary (—▲—) phases were irradiated by the γ-rays doses of 1, 2 and 3×10^5 r for 30, 60 and 90 minutes and then incubated for about 2 weeks.

increase of irradiation dosage. In the case of the irradiated cells of logarithmic phase, the concentration decreased in a sigmoidal curve, but in the case of irradiated cells of stationary phase, it decreased in a straight line.

4) **Growth of the Posterity of Irradiated Cells**

a) **Survived green cell.** Many irradiated cells yet remained as photosynthetic green cells after the irradiation. And some of them were also observed as pale green cells under a microscope. But, the pale green cells could not separate and be cultured.

In the present work, to make several populations of survived green cells by a single cell culture was successful. Most of these green cells fell in their growth rates behind the non-irradiated green cells. They reached the stationary phase of the culture at about 75 to 90% of the cell concentration of non-irradiated cells (Fig. 4,B). Several generations have passed, while these cell populations being made from a single cell and observed. Therefore, it seems that these cells were changed into a different physiological type from the non-irradiated green cells. The growth of non-irradiated green cells was also observed by the single cell culture (Fig. 4,A). But, so far as the cells are prepared from the same growth phase of culture and incubated in the same condition, little difference of the growth rate was observed among several populations of non-irradiated cells.

b) **Albino mutant.** Chlorophyll-less albino mutants of *Euglena* cells are easily induced by these γ and neutron irradiations. These albino mutants were separated by the single cell culture. And it was found that these cell populations could be separated roughly into two groups, one has a large growth rate (Fig. 4,C) and the other a small (Fig. 4,D). The growth rate of the former was nearly the same or a little smaller compared with that of survived green cells (Fig. 4,B). The cell concentration of the stationary phase was about 65 to 70% of the non-irradiated green cells. The latter was remarkably inferior

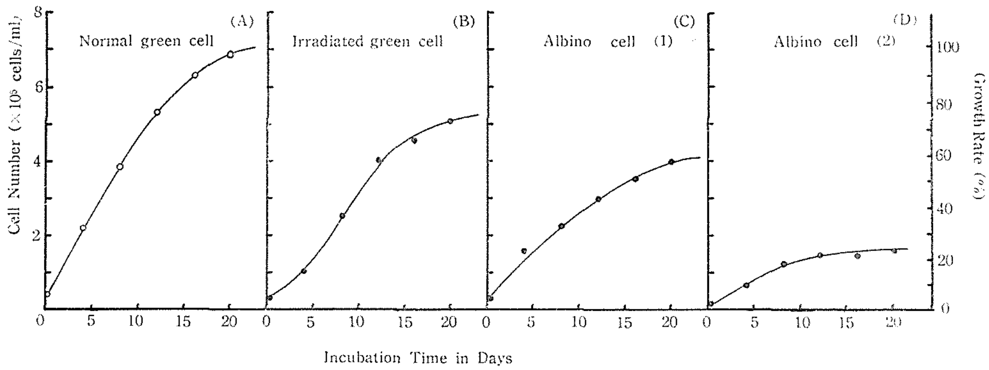

Fig. 4. Growth of the posterity of *Euglena* cells exposed to neutron bombardment. The irradiation dose was 1.8×10^{12} n/cm²/30 minutes. Each cell was separated by the single cell culture and incubated, until the cell population increased. Non-irradiated green cell (—○—), irradiated cell (—●—).

to the growth rates of any other cells. They increased in number so slowly. Their growth rate was only about 25% of the non-irradiated green cells on 12 days culture. And also it was observed by a microscope that about 50 to 60% of these cells were inactive in their behaviour in the culture medium.

5) Death Rate

No dead cell induced by the irradiation was observed during 1 to 2 days culture after the irradiation. But after that, the dead cells appeared in a considerable high degree (Figs. 5,A and 6,A). The death rate of irradiated cells increased during 7 to 11 days after the irradiation and reached to a maximum and then decreased in inverse proportion to the growth of survived cells. This suggests that the dead cells reach a constant number on nearly the same day as the maximal death rate was reached. When the cells were old (Fig. 5, A) and the irradiation dose was high (Fig. 6,A), the day of the maximal death rate was later. After the day of the maximal death rate, the death rate decreased

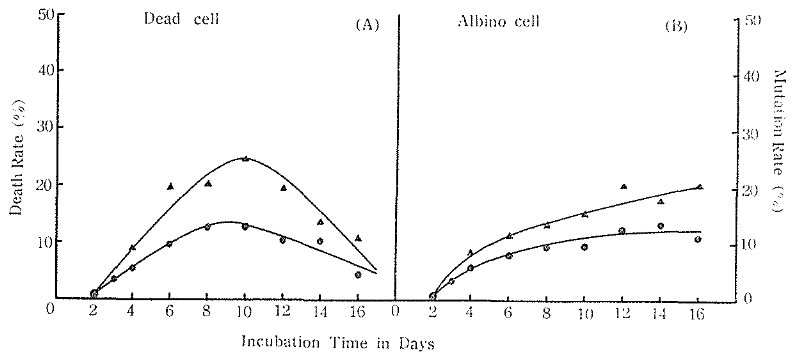

Fig. 5. Variations of death and albino mutation rates of *Euglena* cells exposed to γ-rays.

Cells of logarithmic (—●—) and stationary (—▲—) phases were irradiated by the γ-rays dose of 1×10^5 r. The cell concentration used to the irradiation was about 1×10^6 cells/ml.

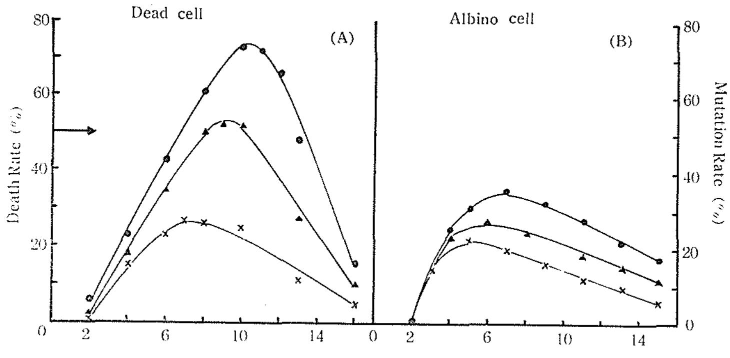

Fig. 6. Variations of death and albino mutation rates of *Euglena* cells exposed to neutron.

Cells of late logarithmic phase was used. Irradiation units of neutron were 1.8×10^{12} n/cm²/30 min (—×—), 3.6×10^{12} n/cm²/60 min. (—▲—) and 5.4×10^{12} n/cm²/90 min. (—●—).

according to the incubation days.

6) Lethal dose of *Euglena* cells

The death rate of each irradiated cell shows generally a different value by the cell age and the incubation days after irradiation, as is stated on paragraph 5. Therefore, it seems to be suitable to observe the maximal death rate on the culture after irradiation. Each maximal death rate of irradiated cells appears on abount the 8th to the 10th day of incubation after the γ-irradiation of 1×10^5 r, on about the 10th to the 12th day by 2×10^5 r and on about the 12th to the 14th day by 3×10^5 r. From Fig. 7, LD-50 of *Euglena* cells cultured for 12 days after the irradiation seems to require a γ-ray dose of about 2.5×10^5 r for 30 to

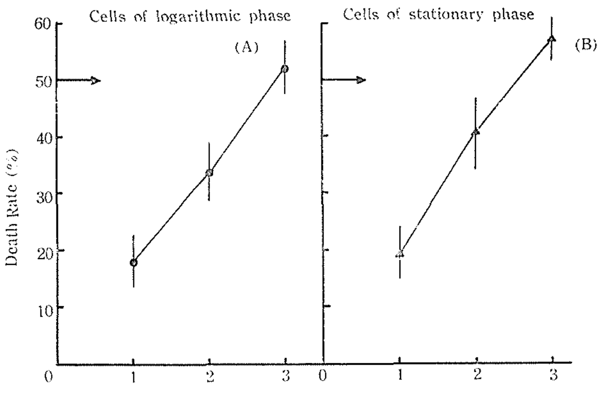

Fig. 7. LD-50 (11-13) of *Euglena* cells.

Cells of logarithmic (—●—) and stationary (—▲—) phases were irradiated by three different γ-rays doses of 1, 2 and 3×10^5 r for 30, 60 and 90 minutes. LD-50 is the lethal dose for 50% death ratio of irradiated cells. (11-13) means the incubation days after irradiation.

60 minutes. The logarithmic growing cells were a little more resistant to the irradiation than the cells of the stationary phase.

In the case of neutron irradiation, LD-50 was about 3.6×10^{12} n/cm^2/60 minutes (Fig. 6, A).

7) Mutation Ratio

Some of the *Euglena* cells are changed easily to the albino mutant by the irradiation. The number of the albino cells produced from a constant number of irradiated cells was nearly the same on both cells of logarithmic and stationary phases. But, the mutation ratio of albino cells of both phases differs. That is, the irradiated cells of logarithmic phase begin to increase in number before long after the irradiation. Consequently, the ratio of albino cells to the total irradiated cells becomes small according to the progress of culture. For this reason, the mutation ratio of albino cells was about 5 to 8% less on the irradiated cells of the logarithmic phase than was the case of the stationary phase. Albino cells carry out the cell division by themselves and increase in number. Therefore, as is shown in Fig. 5,B, the ratio of albino cells to the total irradiated cells increases gradually according to the incubation days. But, the growth of albino cells was generally delayed compaired with other non-irradiated and survived green cells. Therefore, the ratio of albino cells decreased at the end of the culture in most cases (Fig. 6,B).

8) Size of Irradiated Cells

Many sluggish and spherical cells were observed in the culture immediately after γ and neutron irradiations. But a significant difference of cell size was not recognized between irradiated and non-irradiated cells in this case. Not immediately after the irradiation, but after several generations from the irradiation, a little difference of cell size was observed between both cells as above stated (Table 1). This difference was significant at the 5% level. The size of

Table 1. Cell size after several generations from neutron irradiation*.

Non-irradiated green cell	Survived green cell	Albino cell (1)	Albino cell (2)
59.4×9.9	52.8×9.0	46.2×8.6	39.6×8.6
±(6.6×1.8)	±(10.4×2.5)	±(7.8×2.1)	±(10.1×2.5)

* Irradiation unit was 1.8×10^{12} n/cm^2/30 min. of neutron. Survived green cell means the green cells which survived the irradiation. Albino cell means the albino mutant induced by the irradiation. The first number shows cell length, the latter cell width at μ. All cells were inoculated by a single cell culture and observed.

survived green cells was a little more variable than that of non-irradiated cells. Albino cells were fairly smaller than non-irradiated and survived green cells. But the size of the albino cells (1) which have a large growth rate was considerable uniform. It was variable in albino cells (2).

DISCUSSION

Results of many investigations have been reported concerning the effects of radiation on many sorts of living cells. But a little data is available on the radiation of *Euglena* cells. *Euglena* cells are surely radioresistant to a considerable high dose of radiation[5,8]. But after all, they are effected by other things, for example: cell death, growth delay and mutation. *Euglena* cells used to produce easily their albino mutants by irradiation[7]. And it was observed that some of the albino mutants appear with some physiological differences in the present work.

Excepting that the ultraviolet at 265 mμ is the most effective killing wavelength for *Euglena* cells[6], there seems to be no specific effect through other X, γ and neutron irradiations[2,3,5,7,13]. In most cases in the present work, the degree of the effects is in proportion to the irradiation dose and also seems to relate to the age of the cells used as material, the preparation of materials for irradiation and the treatment after irradiation.

X-ray doses of 1 or 1.8×10^5 r and a small unit of neutron were applied to *Euglena* cells with little apparent effect, but the general growth of the culture was considerable delayed[5,13]. But in the present work, it was observed that γ-ray doses of 1 to 3×10^5 r and neutron units of 4.1×10^7 to 5.4×10^{12} had some remarkable effects on the growth rate, the albino mutation, etc..

Wichterman[13] has reported that, for a lethal dose, LD-50 of *Euglena* cells required 3.2×10^4 r of X-rays for 24 hours. But in the present work, the lethal dose was fairly high; between 2 and 3×10^5 r of γ-rays for 60 to 90 minutes and about 3.6×10^{12} units of neutron for 60 minutes was necessary. Of course, little or no effect of irradiation on living cells seems to be due to the irradiation dose and to the sort of radiation[6,9,14]. But, this may be also due to the objects of observation concerning the effect and to the preparation of materials for irradiation.

Some investigations have reported that rapidly growing cells and tissues are generally more radiosensitive than the slow- and non-growing cells and tissues[1,4,15]. From the present work, it was found that the rapidly growing cells were more radioresistant than the slow growing cells of the stationary culture. On the other hand, the author had some evidences that the rapidly growing cells are surely radiosensitive on the effect of ribonucleic acid content of *Euglena* cells, but many of these rapidly growing cells survived the γ and neutron irradiations with a low death rate in the end. On this subject, the author will soon report in detail.

Aebersold et al.[10,11,12] had reported that one unit of neutron corresponds to 2.5 r of X-rays on the effect of chromosome aberration. In the present work, it was observed that 1.8×10^{12} units of neutrons correspond approximately to about 3×10^5 r of γ-rays on the effects of cell death, albino mutation, *etc.* But the problem is that neutron radiation is always accompanied by a small dose of γ-rays. Therefore, all effects induced by the neutron irradiation of the present work do not come from only neutron bombardment.

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