Incorporation of ^{32}P into *Euglena* Cells Exposed to γ-Radiation

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After *Euglena* cells were irradiated with γ-ray dose of 1×10^5 r, the cells were cultured for each time of 1, 6, 12 and 24 hours in culture medium containing 0.1 μc ^{32}P per ml. Cell fractionation was carried out by a modified Schmidt and Thannhauser's method. Incorporation of ^{32}P was observed on each of acid soluble, lipid, DNA, RNA and phosphoproteid fractions obtained by this method.

From the present experiments, following tentative conclusions are obtained; incorporation of ^{32}P into nucleic acids and other phosphorus containing substances stated above are inhibited by the irradiation of γ-rays. The results also show γ-irradiation markedly inhibits an increase of the cell population.

INTRODUCTION

Many investigations concerning the action of ionizing radiation on phosphorus metabolism in animals were reported (Kelly *et al.*[1], 1955; Smellie *et al.*[2], 1955). In these experiments, X-rays have long been used, and more recently γ-radiation from ^{60}Co has been widely employed. In the studies on changes of the incorporation rate of ^{32}P into nucleic acids caused by the radiation, radiation doses of $10^2\sim10^4$r levels have been commonly used.

In the present experiments, ^{32}P incorporation into several phosphorus containing substances in *Euglena* cells exposed to a larger dose, 10^5 r level of γ-irradiation was observed. Analysis of phosphorus containing substances, contained in *Euglena* cells, was carried out by a modified Schmidt and Thannhauser's method (Sugiyama, Shinke and Ishida,[3] 1954).

This paper gives some preliminary results obtained from the present experiments.

MATERIAL AND METHOD

Small parts of stock culture of bacteria-free strain of *Euglena glacilis* were inoculated in flasks containing 1500 ml. of culture medium without ^{32}P. Constituents of the medium are : pepton, 1.1 g ; glucose, 1.1 g ; citric acid, 0.7 g ; $MgSO_4$, 0.13 g ; KH_2PO_4, 0.33 g in 1000 ml. of distilled water.

Euglena cells were cultured under illumination at 30°C. When the cells were cultured in these conditions, the cell population showed lag-phase after about 7 days from inoculation. The cells for analysis were collected from "5 days culture" by centrifugation. The cells collected were washed repeatedly

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with distilled water. Then *Euglena* cells were suspended in a bottle containing 400 ml. of distilled water and exposed to γ-rays from ^{60}Co for 30 minutes. Dose of γ-radiation was 1×10^5 r. After irradiation, the cell suspension was centrifuged, and cells precipitated were suspended in culture medium containing 0.1 μc ^{32}P per ml. in four flasks. The cells in flasks were incubated at 30°C in the light.

Samples of cells for analysis were collected respectively from 1, 6, 12 and 24 hour cultures in this medium. Non-irradiated cells, cultured in ^{32}P containing media in four other flasks as well as irradiated cells, were used as controls.

Amount of phosphorus in each fraction, obtained by a modified Schmidt and Thannhauser's method, was determined by phosphovanado-molybdate method (Sugiyama, Shinke and Ishida,[3] 1954), after organic phosphorus were converted into inorganic phosphates by procedure of combustion with perchloric acid (King,[4] 1932). Radioactivity of ^{32}P on each fraction was measured by an apparatus using G.M.-tube. Specific activity was represented as the rate of total counting number to total phosphorus in each fraction.

RESULTS AND CONCLUSION

Effect of γ-radiation on growth curve for cell population is shown in Figure

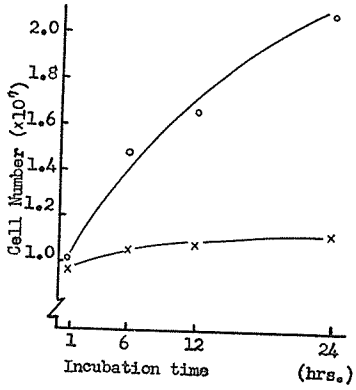

Fig. 1. Growth curves of γ-irradiated (-×-) and non-irradiated (-○-) *Euglena* cells.

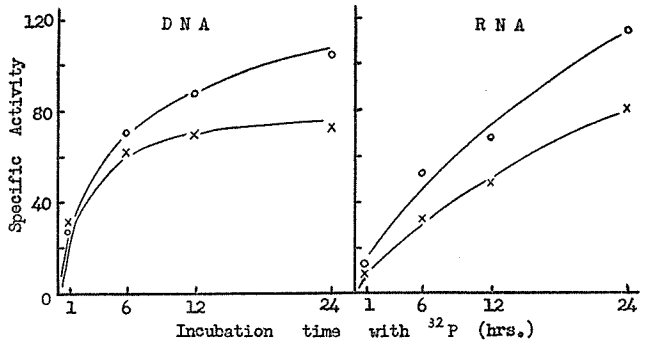

Fig. 2. Incroporation of ^{32}P into DNA and RNA in γ-irradiated (-×-) and non-irradiated (-○-) *Euglena* cells.

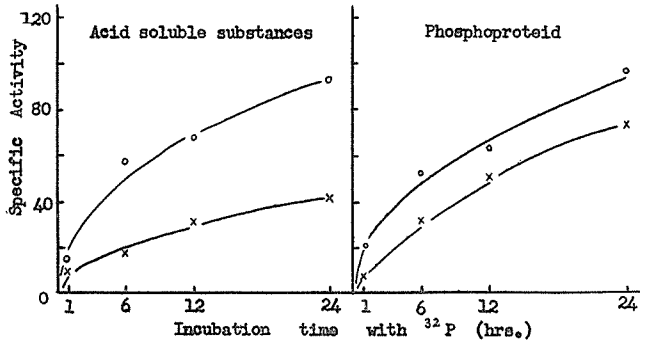

Fig. 3. Incorporation of ^{32}P into acid soluble substances and phosphoproted in γ-irradiated (-×-) and non-irradiated (-○-) *Euglena* cells.

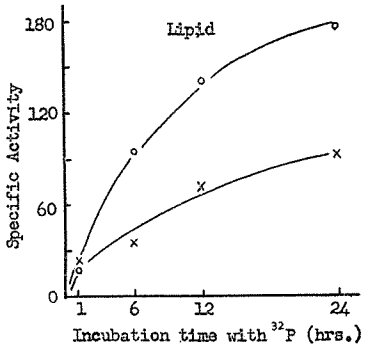

Fig. 4. Incorporation of ^{32}P into phospholipid in irradiated (-×-) and non-irradiated (-○-) *Euglena* cells.

1. In this figure, it is seen that the growth of cell population is markedly inhibited by irradiation of γ-ray dose of 1×10^5 r, that is, cell population of irradiated cells does not show rapid increase, at least during 24 hours after γ-irradiation. In the control cultures, however, the cell population has increased about twice during 24 hours.

Figure 2 shows the rate of incorporation of ^{32}P into nucleic acids. In this figure, it is seen that the specific activities of DNA and RNA in irradiated cells are, in general, lower than those of control cells. Speed of incorporation of ^{32}P into DNA of irradiated cells is very slow.

Figures 3 and 4 show the rate of incorporation of ^{32}P into acid soluble, phosphoproteid and lipid fractions. Incorporation into these fractions of irradiated cells is less than that of control cells.

It is not possible to conclude from the present experiments anything about the mechanism of action by γ-radiation on phosphorus metabolism. It is, however, assumed that γ-radiation exerts to produce an appreciable effects on the nucleic acid metabolism, and that the growth of cell population is markedly inhibited as a result of those effects by the irradiation.

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