Effects of X-rays on Division of *Paramecium aurelia* at Different Culture Ages[1)]

By

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(*With 2 Tables*)

Radiation effects in *Paramecium* have recently attracted the attention of many investigators in the filed of Protozoology, following significant work of Sonneborn (1947) and his colleagues. Several important papers have been published regarding the effects of X-rays on the growth rate, cell division and survival of paramecia and some other ciliates (Wichterman 1948, Powers and Shefner 1949, Kimball *et al.* 1952, Wichterman and Figge 1954, Powers 1955). According to Wichterman (1948), in *Paramecium bursaria* irradiation temporarily inhibits cell division but the animals recover normal division rate after a certain length of time. Kimball *et al.* (1952) found that the division delay was remarkable after irradiation. Wichterman and Figge (1954) observed that lethal dose of X-rays in *Paramecium caudatum* is about 340,000r which reflects a much higher radiation resistance than that of vertebrates or human beings; also they noted that some acceleration in division appears when the animals are exposed to dosages lower than LD50. But, little knowledge has been accumulated on the relation between the culture age of paramecia and the X-ray dosage.

In the present study, the authors aimed to analyze effects of comparatively low dosages of X-rays on the survival and division of *Paramecium aurelia* in various culture ages. Further attention has been extended to the effect of magnesium sulfate during irradiation, since the modification of X-ray injury by the treatment with the latter chemical has been reported by Park (1956) in *Hydra littoralis*.

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Material and methods

Paramecium aurelia based on a clone culture with lettuce infusion at room temperature (25±0.2°C) was used in the present study. This clone originated from the animals collected in the City of Sapporo.

The specimens, about 1000 in number, were separated into different culture dishes at 10 day intervals. Culture ages here under consideration are 3–7 days (A_1 group), 10–17 days (A_2 group) and 20–27 days (A_3 group), respectively. A_1 group contains the most vigorous specimens, and A_3 group the most inactive ones. In experiment, each of the cultures in a small glass dish containing about 3 cc. culture medium with high density of organisms was X-rayed simultaneously with the others.

Irradiation conditions were 60KV., 16mA., 1908r per minute; a water-cooled Coolidge tube was set at the distance of 10 cm from the dishes. The dosage, 100,000r, was exclusively used throughout these experiments.

Observations

1. Effects of X-rays on cell division of *Paramecium aurelia* at different culture ages

Powers and Shefner (1949) starved *Paramecium aurelia* in a bacteria-free salt solution for 12 to 14 hours before X-ray exposure in order to eliminate the different responses to different stages of the division cycle. But, their experiments paid no particular attention to the culture age of the organism. In the present experiment, three different culture ages, viz. 3–7 days (A_1), 10–17 days (A_2) and 20–27 days (A_3), were considered. After irradiation, a single organism from each culture was isolated into a separate dish. Observations were made on the number of organisms after fission in each culture dish at intervals of 24 hours after irradiation for successive four days.

In Table 1, the total number of paramecia based on four-day-observations in each age is presented. Numerals indicated in the table show the average based on five repeated experiments.

Table 1. Effects of X-rays on division of *Paramecium aurelia*. After irradiation, a single organism from each culture age was isolated, and the number of paramecia after fission was observed at intervals of 24 hours for successive four days. The numerals show the average numbers of organisms.

	Age \ Hour	24	48	72	96
X-rayed	A_1' (3–7 days)	4.5	43.7	170.4	434.1
	A_2' (10–17 days)	2.6	30.2	41.0	57.6
	A_3' (20–27 days)	2.8	42.5	126.2	330.3
Non-X-rayed (Control)	A_1 (3–7 days)	4.0	29.4	78.0	251.0
	A_2 (10–17 days)	2.5	22.2	64.0	226.0
	A_3 (20–27 days)	2.5	19.0	42.2	107.3

By reference to Table 1, it becomes evident that the number of specimens in three irradiated groups (A_1', A_2' and A_3') approximates to that of control groups at the examination 24 hours after exposure. In the observation at 48 hours, the A_2' group shows a sudden delay of division. With the passage of time, a delay in division was remarkably noted as seen in the examinations at 72 and 96 hours. Therefore, a delay of division occurs strikingly with the passage of time, showing a considerable decrease in number of organisms. This seems to imply that the delay of division may be due to the injury caused by X-rays. On the other hand, A_1' and A_3' groups were not affected by X-rays; they show moderate increases in number of organisms. Mention should be made that in the A_2' group the occurrence of individuals of unusually small size was noted. It was difficult to culture these small organisms.

2. Role of $MgSO_4$ in irradiation of *Paramecium aurelia*

It was experimentally shown that magnesium sulfate serves to prevent X-ray injury in certain organisms. With the purpose to test the X-ray injury as observed in the A_2' group, the following experiment was undertaken. Magnesium sulfate at 5.0×10^{-4}M concentration was added to the culture medium containing high density of organisms in nearly equal volume. Under the same condition as that of Table 1, the dishes were exposed to 100,000r of X-rays. As controls other dishes containing $MgSO_4$-free medium were also irradiated. The results are shown in Table 2. The A_1'' and A_3'' groups show no remarkable X-ray injury indicating

Table 2. Effects of X-rays on division of *Paramecium aurelia*. The organisms were single-cultured in the medium containing $MgSO_4$, and the number of organisms was observed at intervals of 24 hours for successive four days (for detail, see text).

Age \ Hour	24	48	72	96
A_1'' (3-7 days)	4.2	30.6	100.8	449.1
A_2'' (10-17 days)	3.6	16.9	70.3	251.4
A_3'' (20-27 days)	2.7	12.0	55.5	149.4

an acceleration in division rate with time (Table 2). Noticeable is the fact observed in the A_2'' group: the organisms of this group show a rather regular growth rate with a moderate acceleration in division. From the results of the above experiment, the conclusion may be reasonable that magnesium sulfate may serve to prevent the X-ray injury in *Paramecium*.

Discussion

Most investigators who dealt with irradiation effects in *Paramecium* have given no special consideration to the life cycle of organisms. Since Sonneborn

(1954) called attention to the problem of senescence and rejuvenescence in *Paramecium aurelia*, the significance of aging has become important in various fields of experiments. In the present study the authors have paid some attention to the relation between the aging and the effect of X-rays.

Generally, it has been known in *Paramecium* and in other ciliates that they are able to survive even at very high dosages of X-rays. According to Wichterman and Figge (1954) the LD50 dose on *Paramecium caudatum* is about 340,000 r. It is noteworthy that this dosage is much more about 850 times that for human beings or other vertebrates. Dosage used in this study is much lower than LD50. In this dosage the suppression of division was found in the A_2' group, when the aging of organisms was considered. Wichterman and Figge (1954) observed the acceleration in division in *Paramecium* at dosage of X-rays lower than LD50.

A question arises whether the delay in division as observed in the A_2' group is due to the suppression of division, or the increase of dead cells. The senior author has made an experiment of daily isolation after irradiation; the results of the experiment show that the division rates are rather identical in the A_1', A_2', A_3' and control groups. From this evidence it is probable that division delay in the A_2' group is not due to the suppression of division.

Next, the death of cells should be considered in the A_2' group. Powers (1955) reported that the postirradiation temperature affects the survival of paramecia. But, in the present experiments the postirradiation temperature was set at a rather constant degree.

Sonneborn (1954) informed that autogamy which occurs following non-viability may lead to death at once or after several fissions. The present authors observed, however, that the first occurrence of autogamy was the sixth day after X-ray exposure. It is then evident that autogamy did not lead to death of cells in the present experiment.

Consideration should be extended to the culture age. The fission rate is very high in the A_1 group, while the A_2 group shows a little or no decrease in fission rate. In the A_3 group the fission rate shows a progressive decrease. In view of these facts it is apparent that the most vigorous group, A_1, and the most inactive group, A_3, undergo a little or no injury by X-rays, whereas the A_2 group is remarkably affected by X-rays.

Based on the sexual maturity, the three culture ages dealt with in this study are considered as follows: the culture age of the A_1 group corresponds to the stage of sexual immaturity, and the age of the A_2 group seems to be the stage of sexual maturity since the conjugation readily takes place between the mating types. The age of the A_3 group may be the stage of decline. It is interesting to know that the A_2 group which is most sensitive to X-rays is in the stage of sexual maturity. In other words, the greatest sensitivity to X-rays in *Paramecium aurelia* may occur in the stage of conjugation, even in the dosage lower than LD 50.

Summary

The effects of X-rays were investigated in *Paramecium aurelia* with special consideration of different culture ages at the dosage of 100,000r.

From the results of the present experiments, the conclusion may be drawn that the paramecia were strongly affected and led to death at the age of sexual maturity in which conjugation takes place.

Additional experiment revealed that magnesium sulfate acts to prevent the injury effect of X-rays.

References

Kimball, R.F.,R.P. Geckler and N. Gaither 1952. Division delay by radiation and nitrogen mustard in *Paramecium*. J. Cellular Comp. Physiol., 40 : 427-459.

Park, H.D. 1956. Modification of X-ray injury to *Hydra littoralis* by post irradiation treatment with magnesium sulfate and glutathione. Biol. Bull., 111 : 240-247.

Powers, E.L. 1955. Radiation effects in *Paramecium*. Ann. N. Y. Acad. Sci., 59 : 619-637.

Powers, E.L. and D. Shefner 1949. Effects of high dosages of X-rays on *Paramecium aurelia*. Agronne National Laboratory Report, 4333 : 127-134.

Sonneborn, T.M. 1947. Recent advances in the genetics of *Paramecium* and *Euplotes*. A advances in Genetics, 1 : 263-358.

———— 1954. The relation of autogamy to senescence and rejuvenescence in *Paramecium aurelia*. J. Protozool., 1 : 38-53.

Wichterman, R. 1948. The biological effects of X-rays on mating types and conjugation of *Paramecium bursaria*. Biol. Bull., 94 : 113-127.

Wicherman, R. and F.H.J. Figge 1954. Lethality and the biological effects of X-rays in *Paramecium* : Radiation resistance and its variability. Biol. Bull., 106 : 253-263.