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Author(s)	OKADA, Tadashi A.; KAWAMURA, Fumio
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Studies on the Cytoplasmic Granules in Tumor Cells of the MTK-sarcoma, IV. Morphological Effect of X-rays on the Mitochondria¹⁾²⁾

By

Tadashi A. Okada and Fumio Kawamura

(Zoological Institute) (Department of Medical Radiology)
(Hokkaido University) (Hokkaido University)

(With 7 Text-figures)

It has recently been shown through biochemical studies that a substantial decrease in enzymatic activity of cells occurs following irradiation of X-ray (van Bekkum 1954, Barron 1955). There are reports which indicate that X-rays also affect the division rate in cells. Further it is stated that the mitochondria appear to be the locus of action of both the Krebs' tricarboxylic acid system and the cytochrom system, a center of respiration. Alterations in the number of mitochondria may supply important implications in relation to cell physiology. The present study was undertaken to obtain some information as to whether the fall in respiration rate following X-irradiation represents as change in morphological feature of mitochondria or merely a loss of respiration rate. Comparisons were made of the morphological features of mitochondria between the tumor cells of non-treated rat ascites tumor and those of an ascites tumor after irradiation.

The authors wish to tender their sincere thanks to Professor Sajiro Makino for his supervision. Further cordial thanks are offered to Mr. T. Tanaka and Mrs. K. Kano for their valuable advices and criticism. It should be mentioned here that the microscopical observations were performed by both authors and the arrangement of the data in the paper with their descriptions were prepared by T. A. O.

Material and methods

A rat ascites tumor, MTK-sarcoma III was used for examination of the effect of X-ray on the mitochondria. The ascites tumor was serially transmitted in pure bred Wistar albinos (*Rattus norvegicus*), weighing 80-100 g. The experimental procedure is as follows:

1. Whole body irradiation was made on tumor-bearing rats on the 3rd to 4th day after the transfer of the tumor, and then ascites tumor was examined for mitochondria by phase microscopy (*in vivo*).

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2. The ascites tumor prepared on the slide was subjected to X-irradiation and then examined for mitochondria by phase microscopy (*in vitro*).

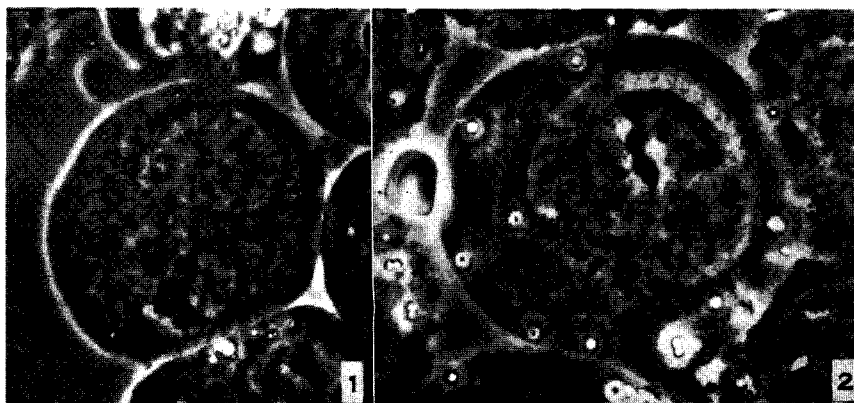
In the case of procedure (1), the preparations were made from a droplet of the tumor ascites obtained by abdominal puncture at appropriate intervals, beginning with 1 hour and ending with 13 hours after irradiation. At every one hour after irradiation, sampling was made from both untreated control tumor rats and treated ones. The preparations colored with acetic dahlia were also studied for comparison. In the case of (2), the slides prepared according to the ordinary drop method were irradiated *in vitro* and then observed continuously. More than 30 tumor-bearing animals were employed in the experiments described in this paper.

Observations

1. Irradiation *in vivo*.

X-rays were irradiated in doses at 50r, 200r, 500r, and 1000r. A single irradiation was given to each tumor-bearing rat on the 3rd to 4th day after transfer at 50r, 200r, 500r, and 1000r at room temperature (22°C). Tumor samples were removed from the treated animals at every 1 hour interval. The observation was aimed to discover the morphological change of the mitochondria of tumor cells in response to the X-rays. In addition, the frequency of the mitotic tumor cells was observed with the smear preparations sampled from the same tumor and stained with acetic dahlia.

Before 50r exposure, the mitochondria of tumor cells were filamentous or rod-like in form, dot-like ones being very rare in occurrence (Fig. 1). Such tumor cells with filamentous or rod-like mitochondria appeared in large number (65.9%). About 30 minutes after irradiation, the decrease in number of tumor cells with



Figs. 1-2. Photomicrographs of tumor cells taken with phase microscope (*Olympus*). $\times 1500$. 1, filamentous mitochondria in a tumor cell before X-irradiation. 2, dot-like mitochondria in a tumor cell after the whole body irradiation with X-rays.

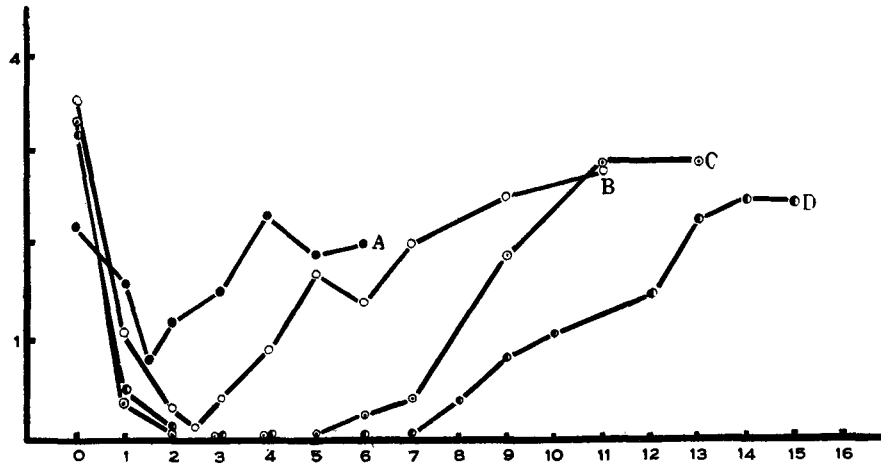


Fig. 3. Graphical representation showing the change of appearance of mitotic tumor cells after X-irradiation in the MTK-sarcoma III. A, 50r, B, 200r, C, 500r and D, 1000r.

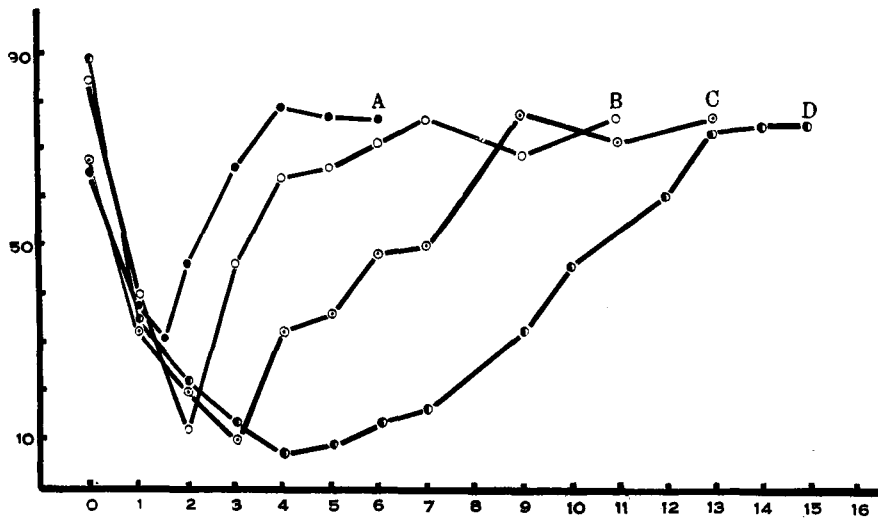


Fig. 4. Graphical representation showing the change of appearance of tumor cells with filamentous mitochondria after X-irradiation in the MTK-sarcoma III. A, 50r, B, 200r, C, 500r and D, 1000r.

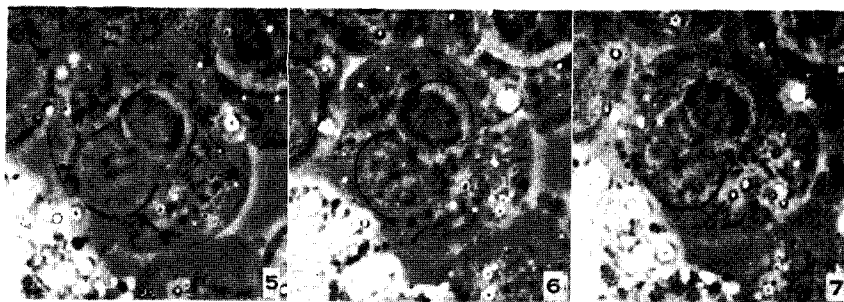
filamentous or rod-like mitochondria had occurred with the subsequent increase of tumor cells with dot-like mitochondria. The shape and size of tumor cells remained unchanged, while the mitochondria changed in form from filament to dot (Fig. 2). The frequency in occurrence of the tumor cells with dot-like mitochondria gradually increased. They showed the highest frequency at about 1.5 hours after irradiation. From 3 to 4 hours after irradiation, the tumor ascites showed many cells containing filamentous mitochondria which were arranged around the nucleus in a radial manner. Afterwards, the cells containing filamentous mitochondria increased in number with time. On the other hand, the mitotic frequency of tumor cells showed 2.2% before treatment and then gradually decreased showing 0.8% at 1.5 hours after treatment. Thereafter, tumor cells began to recover with the mitotic frequency at 2.3% about 3.3 hours after treatment.

The effects of X-rays at 200r, 500r and 1000r upon the mitochondria are almost similar to those at 50r exposure. But, with the increase of the dosage, the occurrence of tumor cells containing filamentous mitochondria showed decrease; namely, the frequency of the tumor cells was 11.5% for 200r, 9.5% for 500r and 6.5% for 1000r. Moreover, the time required for the appearance of the lowest frequency value of tumor cells with filamentous mitochondria differs by the X-rays dosage being 1.5 hours for 50r, 2 hours for 200r, 3 hours for 500r and 4 hours for 1000r. Graphs (Figs. 3 and 4) illustrate the above features clearly.

2. Irradiation *in vitro*.

The preparations made by the ordinary drop method were given a single dosage at 500r of X-rays *in vitro*. In addition some preparations were irradiated three times at 500r at intervals of 5 minutes at 22°C (room temperature). It takes 20 minutes to observe one set of the experiments.

The mitochondria in the tumor cells immediately after preparation exhibited



Figs. 5-7. Photomicrographs of tumor cells taken with phase microscope (*Olympus*), showing the successive series of a behaviour of mitochondria after X-irradiation at 500r *in vitro*. $\times 1500$. 5, filamentous mitochondria in a tumor cell before treatment. 6, mitochondria without changing form; 5 minutes after treatment. 7, mitochondria still are filament in form, 20 minutes after treatment.

a filamentous appearance (Fig. 5). After treatment in single or successive irradiations, the mitochondria remained unchanged in shape, size and number (Figs. 6 and 7). It appeared that the damaging activity of X-ray to mitochondria *in vitro* may be less intensive than *in vivo*. But practically, it is difficult to observe tumor cells under a living state for a long period of time through this ordinary drop method. The comparison between *in vivo* and *in vitro* sensitivity of mitochondria to X-rays is therefore left for further investigation.

Discussion

X-rays and other ionizing radiations are known as efficient inhibitors of cellular metabolism, cell division or metabolic activity; they affect both the malignant cells and normal tissue. Muta (1950) observed the effect of X-irradiation on the cellular activity of tumor cells in rat ascites sarcoma *in vivo* showing that X-irradiation temporarily prevents cells from entering mitosis. The chromosomes in the dividing cells remain unchanged in shape, size and number, but mitotic frequency of tumor cells decreases. Canti and Spear (1929), on the effect of γ -rays on chick tissue in culture, reported that treated chick tissue showed retardation of mitosis, resulting in an initial diminution of mitotic counts. Recent biochemical studies indicate that a substantial decrease in respiration rate of cells occurs following irradiation of X-ray. Barron (1955) reported that X-irradiation of fertilized sea urchin eggs with 1000r inhibited respiration and retarded cleavage. These reported facts deal with the cytochemistry of the nucleus and cytoplasm without being concerned with the effect of irradiation on mitochondria. The visible effect of X-irradiation on tumor cells as observed by phase microscopy appears to follow a pattern similar to the mitotic events as described by Muta (1950).

It has been shown in the present study that X-irradiation affects the cells as a whole exerting visible influence on the mitochondria. Mitochondria change in form from filament to dot-shape. At that time, mitotic tumor cells show the lowest frequency in occurrence, and resting tumor cells remain unchanged in their morphological features. From the results presented it appears that the cells are inhibited from entering mitosis due to the reduction of functional or metabolic activity through irradiation. Barron (1955), working on the effect of X-irradiation in the sea urchin eggs, indicated that functional activity of cells decreased, followed by cell cleavage retardation. Further, Bekkum et al. (1954) investigating the changes of enzymatic activity in the rat's spleen after X-irradiation showed that metabolic (enzymatic) activity of cells decreased and that the decreased activity after irradiation preceded mitochondrial deformation. Okada and Nakahara (1956) observed that in rat ascites tumors there is a parallel correlation between the occurrence of tumor cells with filamentous mitochondria and that of the mitotic cells. They concluded that the cells with filamentous mitochondria were of high metabolic activity and that the dot-like mitochondria being in process of disintegration possessed lower metabolic activity. In the present status of research it seems quite reasonable to assume that the decrease in cell activity after irradiation is a result of some impairment of mitochondria

metabolism.

Summary

This study deals with the effect of X-rays on mitochondria in tumor cells of a rat ascites tumor (MTK-sarcoma III) in the living state, observed by phase microscopy. By the application *in vivo* of X-irradiation at 50r, 200r, 500r and 1000r to the tumor-bearing animals, the temporary suppression of tumor growth was induced to a greater or less degree in experimental animals as a result of inhibition of metabolic activity in a large number of tumor cells. On the other hand, the preparations of the ascites tumor made according to the ordinary drop method were irradiated in single dose at 500r, or irradiated three times at 500r at 5 minutes intervals *in vitro*. In these experiments, however, the mitochondria remained unchanged in shape, size and number. It is apparent that the damaging effects of X-rays upon the mitochondria may be less intensive *in vitro* than *in vivo*.

The evidence presented seems to indicate that the decrease in cellular activity occurred after X-irradiation is a result of some impairment of mitochondrial metabolism.

It is a pleasure to dedicate this article to professor Tohru Uchida in celebration of his 60th birthday.

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