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Abstract

The unsaturated fatty acid fraction extracted from the liver of rabbit irradiated with X-rays exerts a strong cytotoxic effect on human coelothelioma cells and Yoshida sarcoma cells both in vitro and in vivo. The cell damage seems to initiate at the nucleus, finally leading to the complete cytolysis. The inhibiting effect of this substance on the mitosis of Yoshida sarcoma cells can be observed, especially marked from prophase up to metaphase giving almost the same results obtained after X-ray irradiation. From these results and the observations reported by several authors on the cell damage by X-ray irradiation, we should call special attention to the fact that the essential mechanism of X-ray irradiation can be attributed to the cell toxin produced after the irradiation.

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**MORPHOLOGIC CHANGE OF YOSIDA SARCOMA CELLS
AND COELOTHELIOMA CELLS AFTER EXPOSING
TO THE CELL TOXIN FROM X-RAY-IRRADIATED
ANIMAL**

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The radiotherapy is at present one of the most promising way for the treatment of malignant tumors. PERTHES' experiments¹⁻³ on the influences of X-rays on the chromosomes of skin cancer cells and epithelial cells of rabbit, HERTWIG's experiments⁵ with eggs and spermatozoa of frog, in which he perceived the inhibition of cell division by X-ray irradiation, and LACASSAGNE and MONAD's findings⁴ that the irradiation arrests the cell division in canine sarcoma, all have given a theoretical basis for the treatment of malignant tumors with X-rays. However, it is still uncertain what mechanism arrests the cell division. We have recently come to know that the X-ray irradiation produces a certain kind of cell toxin in the body. Therefore, with the supposition that this substance may be correlated to the therapeutic mechanism of X-ray irradiation on malignant tumors, we observed the influences of this cell toxin on Yoshida sarcoma cells and coelothelioma cells *in vitro* as well as *in vivo*.

MATERIALS AND METHODS

Unsaturated fatty acid fraction is extracted from the liver of normal and of the irradiated rabbits 24 hours after the irradiation over the whole body with 3,000r of X-rays assisted by SENO. The fraction is made soluble combining with Na-radical and then used for the experiment as the physiological saline solution, that is, 40 cc of aseptic physiological saline

solution is added to sodium salt of the unsaturated fatty acid acquired from 10 g pulverized dry liver. This solution is added to the medium of tissue culture.

The tissue culture of Yoshida sarcoma has been carried out using the ascites from the rats 4 days after tumor transplantation, by the roller-tube method in the medium composed of 44 per cent HANKS' solution, 19 per cent chick embryo juice, 29 per cent horse serum, 8 per cent Yoshida sarcoma ascites, 0.8 mg/cc RNA and 0.8 γ /cc vitamin B₁₂. Furthermore, in order to avoid the lag phase which occurs 24 hours after the start of culture, the freshly prepared medium is used. The medium is kept 24 hours in the refrigerated room, and the supernatant filtered through Seitz filter No. 85 B is used for the experiment. For the culture rectangular glass plates are placed in the roller tubes to promote the growth of fibroblasts, which will help the growth of tumor cells. One to three droplets of fresh ascites are added to 2 cc medium adjusting the initial cell number of sarcoma cells approximately to 1000-3000/mm³ and cultured for 24 hours. For the culture of coelothelioma cells the medium composed of 70 per cent HANKS' solution, 15 per cent chick embryo juice, 15 per cent patient serum, 2 mg/cc RNA and 2 γ /cc vitamin B₁₂ is used. The ascites of patient is added to the medium and the cell count is adjusted to 1000—3000/mm³ as in the case of the Yoshida sarcoma culture. Since the lag phase is not so marked in this instance, the medium is used without cooling and filtration. Other processes in the culture are entirely the same as in the case of the culture of Yoshida sarcoma.

In one series of experiment 15 culture tubes are used, dividing into 3 groups, 5 in each. Each tube contains 2 cc medium. For one group 0.1 cc of unsaturated fatty acid solution from the irradiated animals is added to each, for the second group 0.1 cc of that from normal rabbit and the third group without addition. After culturing 6—12 hours at 37°C a drop of the culture fluid is placed on a slide, covered with coverglass sealing with paraffin and observed under phase-contrast microscope.

For the experiment *in vivo* 20 hybrid adult male albino rats were used, dividing into 4 groups, 5 in each. In all these animals 0.5 cc of Yoshida sarcoma ascites is transplanted into the peritoneal cavity. Six days later 0.5 cc of the unsaturated fatty acid solution is introduced intraperitoneally to 5 animals, 0.5 cc of the fatty acid solution twice in concentration to other 5 animals and the remaining 5 animals are left without injection. The last group is irradiated whole body with 300 r of X-rays without injection. After the introduction of the fatty acid solution, observations have been carried on the change of tumor cell counts, number of the cells undergoing

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mitosis, and of cells at each phase of mitosis, at the period of 1, 3, 6, 9, 12 and 24 hours. On the method of counting cell number refer to the previous report.

EXPERIMENTAL RESULTS

a. Morphological changes of tumor cells in culture :

Yoshida sarcoma cells in the culture media containing no fatty acid fraction show no pseudopodial projection. The cytoplasm contains a relatively small number of the distinct and strongly refractive lipoid granules. At the nuclear indentation the Golgi apparatus can be observed, surrounded by thread-like mitochondria. Most of the nuclei are kidney-shaped and have the clear nuclear membrane, in which generally two nucleoli of different size can be seen (Plate I. 1).

In the case added with the unsaturated fatty acid fraction from irradiated animals, the cell margin becomes irregular, ruptured, and some cells show the discharge of the nucleus. In some cells the cell body undergoes swelling degeneration, and some of them are disintegrated and nuclei are likewise disintegrated with a subsequent rapid decrease in the number of cells. No cell division can be seen (Plate I. 2, 3, 4).

While in the control in which unsaturated fatty acid fraction from the liver of non-irradiated rabbit is added, the cell division is rather enhanced, a 40% increase comparing to those cultured without addition of fatty acid.

The cultured human coelothelioma cells have clear cytoplasm and extremely minute filamentous mitochondria arranged in a concentric circle around the nucleus but no vacuole (Plate II. 2). The nucleus has distinct membrane with two or several nucleoli different in size (Plate II. 1). In the culture where the unsaturated fatty acid fraction from the liver of the irradiated rabbit is added, the cell body is swollen, the vacuolization appears in cytoplasm and the nucleus becomes pyknotic or swollen and finally disintegrated similarly as in the case of Yoshida sarcoma cell. (Plate II. 3—4).

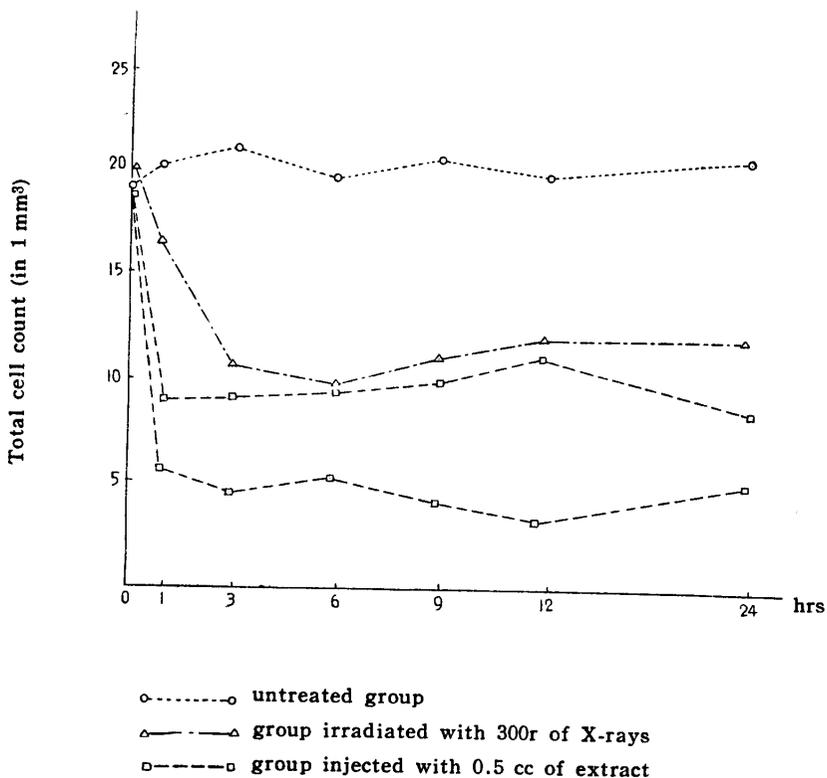
In both cases of Yoshida sarcoma and coelothelioma cells the unsaturated fatty acid fraction from the rabbit irradiated with X-rays brought about the pyknosis or swelling and disintegration of the nucleus at first and subsequent swelling of the cytoplasm, ultimately resulting in the complete cytolysis.

b. Observations on Yoshida sarcoma cells *in vivo* :

Observations at 4th day of implantation proved that the cell number in ascites shows actually no change within 24 hours (Fig. 1).

After the intraperitoneal injection of the extract from irradiated animals the number of sarcoma cells is markedly decreased. Namely, the original count amounting to about 200,000/mm³ is decreased to about 50,000/mm³ after one hour; and down to 40,000/mm³ after three hours, but some cases show a minimum as low as around 30,000/mm³. Even 24 hours afterwards the count does not show much increase with the mean value of 60,000/mm³ (Fig. 1).

Fig. 1. Changes occurring in the number of tumor cells, mean values of 5 cases in each



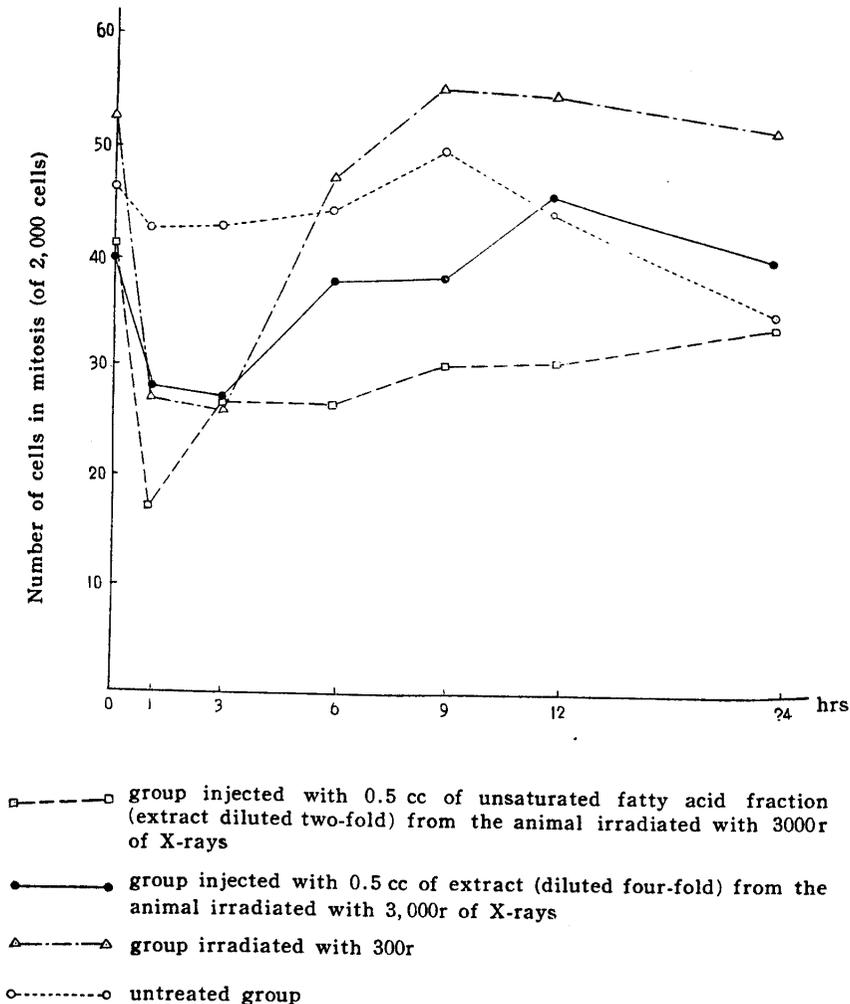
The number of cells undergoing mitosis also shows a marked decrease already one hour after the intraperitoneal injection of the extract, showing some recovering tendency after 6 hours. But in the case injected with the extract at a higher concentration the number of cells undergoing mitosis does not return to the pre-injection level even after 24 hours (Fig. 2).

In the animals irradiated on the whole body with 300 r of X-rays the

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number of cells undergoing cell division decreases temporarily showing almost the same tendency as in the cases injected the extract, sudden decrease after irradiation and the recovery after six hours (Fig. 2).

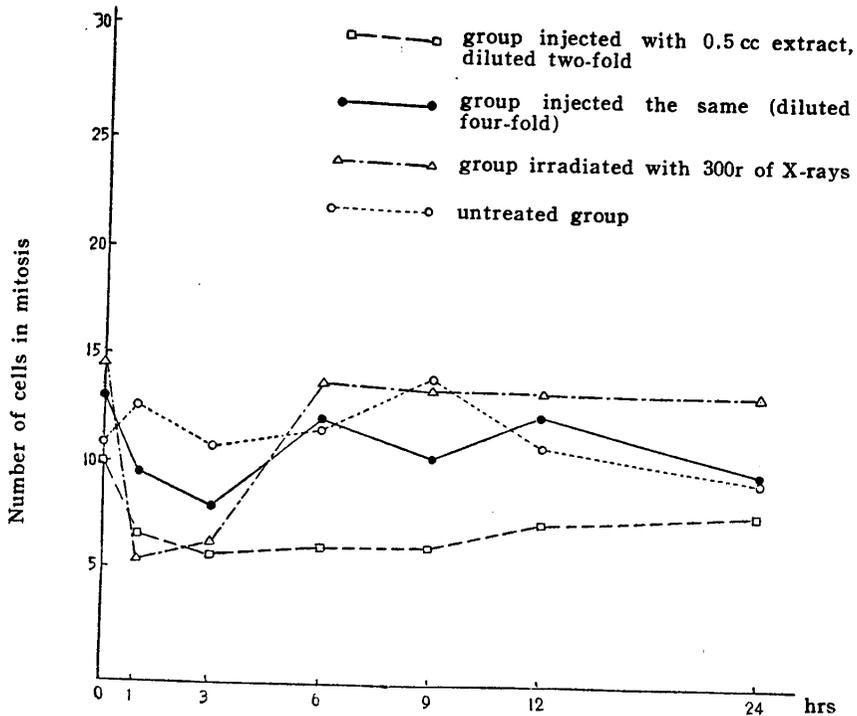
Fig. 2. Changes in the number of cells undergoing mitosis, mean values from 5 cases in each



The changes in the cell count at each phase of mitosis are shown in Figs. 3—6. Namely, in any phase of mitosis this substance acts as to decrease the number, with the most marked effect at metaphase. The

number of the cells in prophase decreases markedly one hour after the injection, showing the maximum inhibition three hours afterwards.

Fig. 3. Changes in the number of cells in prophase



Thereafter, a recovering tendency is seen, complete recovery after 6 hours in the cases injected the extract in a low concentration, and a delayed recovery in the case injected the extract in a high concentration, not reaching the preinjection level even after 24 hours.

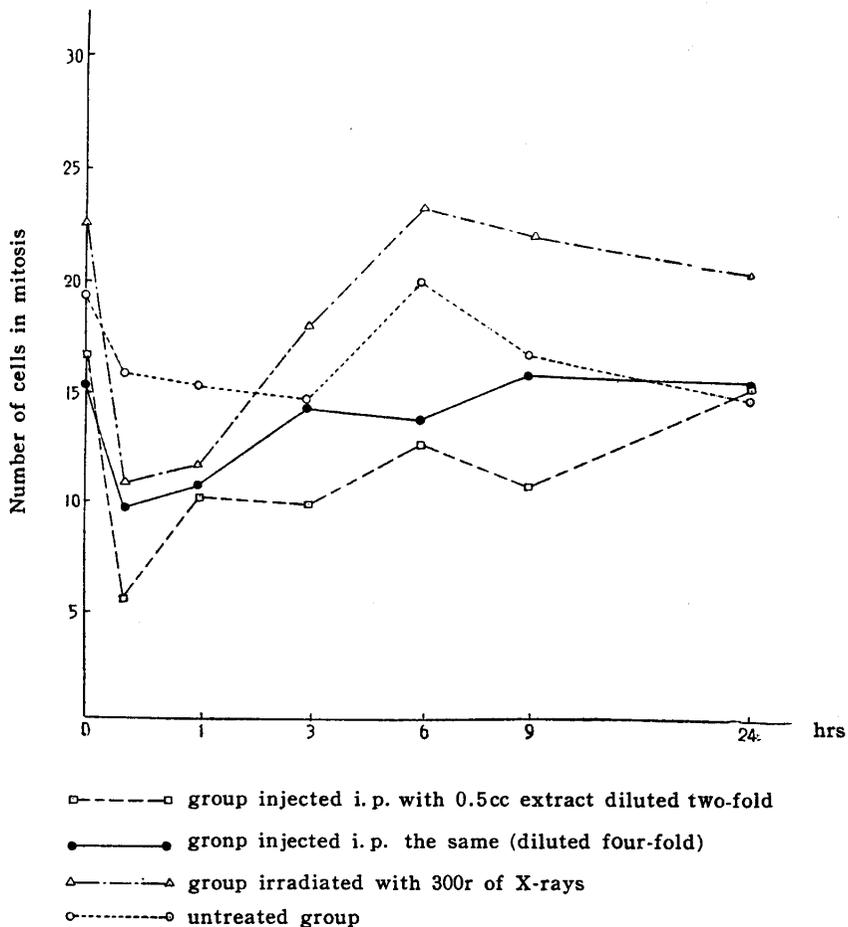
In the animals irradiated with 300 r of X-ray the cell count in each mitotic phase also falls markedly one hour after the irradiation recovering after 6 hours, similar in the cases injected with diluted extract. Similar trends can be observed in metaphase and anaphase, but great fluctuations can be noticed in metaphase (Fig. 4, 5, 6).

DISCUSSION

Ever since the observations of by LACASSAGNE and MONAD a great number of studies on the effects of X-ray on tumor cells have been publi-

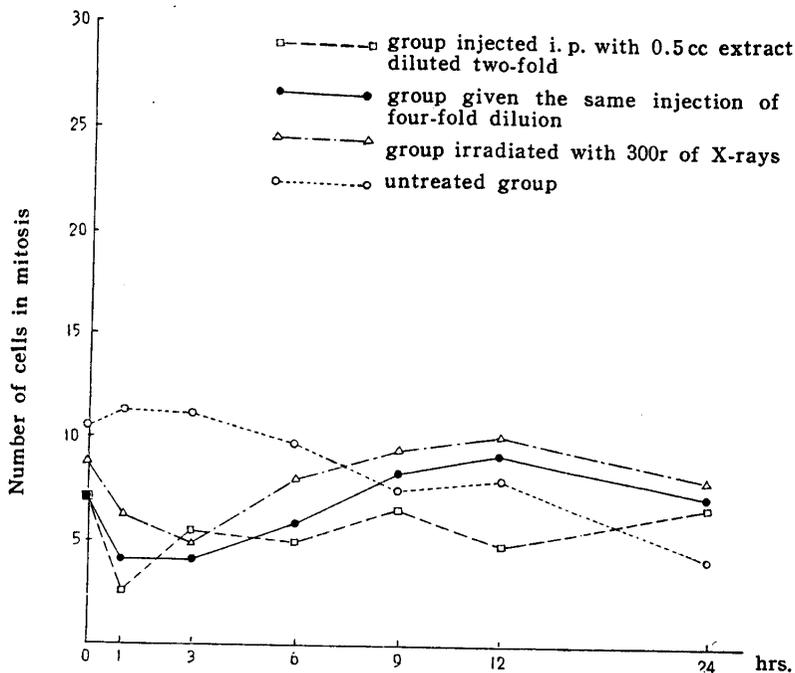
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Fig. 4. Changes in the number of cells in metaphase



shed^{4,8,9,10,11,12}. All of them prove the inhibitory effect of X-rays on the growth of tumors. Concerning the Yoshida sarcoma cells, KIKA⁷ et al.^{13,20,21,22,23,24} observed a marked decrease in the number of the cells undergoing cell division immediately after X-ray irradiation but recovery after a certain period of time. They believe that cell damage induced by X-rays is mainly due to the inhibition of the cell multiplication, claiming that X-rays exerts its most striking effect on the stage of premitosis. NOTE¹³ claims that the dose of 46 r acts as a stimulant on the mitosis of Yoshida sarcoma cells activating cell multiplication vigorously, but the irradiation with 92 r does not act as to stimulate nor to inhibit, while the irradiation with 184 r acts as to inhibit and arrest the cell multiplication.

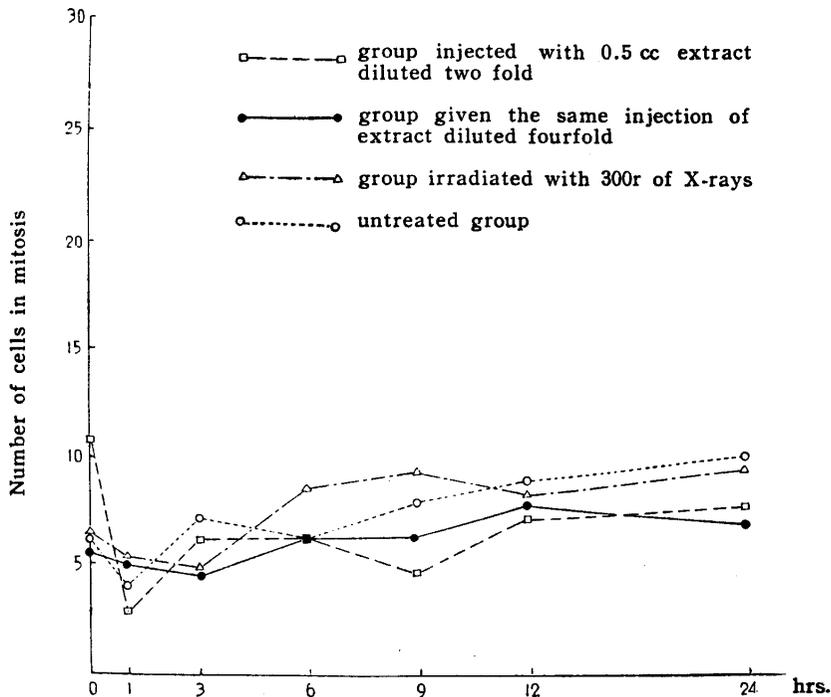
Fig. 5. Changes in the number of cells in anaphase



MASUDA¹⁶ investigated the changes on each mitotic phase of Yoshida sarcoma after the irradiation with 100r, 200r, 300r, and 500r of X-rays. He finds with all these doses the decrease in the number of cells at mitosis; the most marked in prophase followed by metaphase and anaphase, and the smallest in telophase. Recovering period to the preirradiation level in number is longest in prophase followed by metaphase, anaphase and telophase in the order mentioned. LEA¹⁴ states from his observation on that the X-ray irradiation with doses of 200—300r inhibits the cell division only temporarily acting at the stage of predivision but those at a mitotic state is not arrested contending that the effect of X-ray irradiation is greatest on those cells in prophase where the nuclear membrane is not yet burst open. Concerning the dose of X-rays, KIKI states that the inhibitory effect of X-ray irradiation on multiplication of Yoshida sarcoma cells can be recognized by giving 50r, while IKKATAI¹⁵ says from his observation on Yoshida sarcoma that the cell multiplication is markedly inhibited already by the doses of 25r showing the inhibition of mitosis up to 24 hours after the irradiation with 20 r or 50r, up to 48 hours in the case with 250 r. In

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Fig. 6. Changes in number of cells in telophase



his experiment the absolute number of the sarcoma cells decreased after the irradiation with 25 r but recovered after 48 hours, reaching the same level as that in the non-irradiated controls, delayed recovery up to 72 hours with 50r, and up to 96 hours with 250r. As can be understood from the above mentioned reports, the most striking biological effect of X-rays is no doubt at the mitotic or premitotic stage, though there is some differences of opinion in the effective dosage of X-rays and the mitotic stage of the cells. In our experiment of the whole body irradiation with 300r the temporary decrease in the tumor cell number is seen already one hour after the irradiation. The analytical observation proved that this decrease is mainly due to the destruction of the cells at mitosis, but differently from the observations of LEA, KIKI and others, the severest cell damage is observed at the stage of prophase and metaphase and yet in the whole course of mitosis the cell damage has been observed to some extent. From the point of DNA synthesis in the mitotic cycle, in which the active synthesis can generally

be seen in interphase²⁵, and the DNA synthesis is disturbed by X-ray irradiation¹⁷, it may be supposed reasonably that the cells in the premitotic stage is most severely damaged. But as generally accepted there is a possibility that the cells in mitosis may be damaged by break-down of the chromosomes¹⁸ directly by the action of X-rays, and these effects also might act as to reduce the number of the cells in mitosis.

But there is another possibility that some substance, which acts as to disturb the mitotic activity of the cell, is produced by irradiation. WILBER¹⁹ succeeded in extracting lipids from ultraviolet-irradiated sea urchin eggs, which acts as to inhibit the cell division of sea urchin eggs. Our experiment also proved that some substance, which inhibits the nuclear activity and causes the subsequent cell damage, is produced by X-ray irradiation, too. This substance can be extracted with the unsaturated fatty acid fraction and proved that the processes of cell damage by this substance are very similar to those seen after X-ray irradiation both in morphologic and numerical changes of ascitic cells of Yoshida sarcoma, as can be understood from the data presented by several authors and by those mentioned above. These results suggest that the essential mechanism of cell damage by X-ray irradiation is caused indirectly by the toxic substance produced after irradiation differing from the general supposition in which the attack of X-rays is laid on the inactivation of SH groups^{26,27,28} and the direct disrupting effect on chromosomes²⁹. The prolonged or delayed effects of X-ray irradiation will be explained by the effects of this toxic substance, which is lipid soluble and may be retained in the body fairly a long period. The prolonged or delayed effect presented by X-ray irradiation might not be explained by the inactivation of SH group or SH-enzyme nor by the direct destruction of chromosomes, as it is supposed that such an effect is considered to be a temporary one and the damages recover soon.

CONCLUSION

The unsaturated fatty acid fraction extracted from the liver of rabbit irradiated with X-rays exerts a strong cytotoxic effect on human coelothelioma cells and Yoshida sarcoma cells both *in vitro* and *in vivo*.

The cell damage seems to initiate at the nucleus, finally leading to the complete cytolysis. The inhibiting effect of this substance on the mitosis of Yoshida sarcoma cells can be observed, especially marked from prophase up to metaphase giving almost the same results obtained after X-ray irradiation. From these results and the observations reported by several

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authors on the cell damage by X-ray irradiation, we should call special attention to the fact that the essential mechanism of X-ray irradiation can be attributed to the cell toxin produced after the irradiation.

REFERENCES

1. BOHN, G. : Influence des rayons du radium sur les animaux en voie de croissance. C. r. Acad. Sci. 136, 1012, 1903
2. BOHN, G. : Influence des rayons du radium sur les oeufs vierges et fécondes, et sur les premiers stades du développement. C. r. Acad. Sci. 136, 1085, 1903
3. PERTHES, G. : Ueber den einfluss der Röntgenstrahlen auf epitheliale Gewebe, insbesondere auf das Carcinom. Arch. f. klin. Chir. 71, 955—1000, 1903
4. Lacassagne, A. & Monod, O. : Les caryocytocinèses atypiques provoquées dans les cellules cancéreuses par les rayons X et leur rôle dans la régression des tumeurs malignes irritables. Arch. franc. de Path. gén. et expér. 1, 1—32, 1922
5. HERTWIG, P. : Das Radiumexperiment in der Radiologie. Strahlenther. 11, 821, 1920
6. TANIMOTO, J. : Nippon Acta Radiologica. Tome 19, Fasc. 8.
7. KIKI, T. : The effect of X-rays on the mitosis of Yoshida sarcoma. Nippon Acta Radiologica. Tomes XII, Fasc. VIII, P. 8, 1952
8. Mottram, SCOTT u. RAYS, : Early change in nucleus of cells of tumors following exposure to β -radiation. Brit. J. Radiol. 32, 61, 1927
9. STOEL, : Zur genaueren Kenntniss der Röntgenwirkung auf Krebsgeschwülste. (Ueber einen röntgenbestrahlten Basalzellkrebs der Haut.) Strahlentherapie 24, 607, 1927
10. HAMPERL u. SCHWARZ, : Ueber die Wirkung der Radiumstrahlen auf den experimentellen Teerkrebs sowie auf seine Entwicklungsstadien. Ztschr. f. Krebsforsch. 26, 386, 1928
11. SEULBERGER, SCHMIDT u. KRÖNIG, : Röntgenbiologische Untersuchungen an Carcinoma ; Cytologische Studien an oberflächlichen und tiefgreifenden menschlichen Carcinoma. Strahlentherapie 31, 467, 1929
12. GAMBAROW, : Zur Frage der histologischen Veränderungen des Karzinomgewebes nach Strahlenbehandlung. Strahlentherapie. 41, 531, 1931
13. NOTE, N. : On the effect of X-rays on Yoshida sarcoma. Nippon Acta Radiologica. Tomus XII, Fasc. II, P. 65, 1952
14. LEA, : Actions of radiations on living cells. (1947). The Macmillan Company, New York.
15. IKKATAI, T. : Proliferation of the Yoshida sarcoma. Nippon Acta Radiologica. Tomus XVII, Fasc. 1. P. 6, 1957
16. MASUDA, T. : On the effect of X-rays on the mitosis of ascites hepatoma and also on the life-span of transplants rats. Nippon Acta Radiologica. Tomus XVIII Fasc. III, P. 374. 1958
17. PELC, S.R. & Howard, A. : Techniques of autoradiography and application of stripping-film method to problems of nuclear metabolism. Brit. Med. Bull. 8, 132, 1952
18. BERGONIÉ, J. & TRIBONDEAU, L. : De la résistance thermique on coefficient d'utilité des vêtements confectinés ; méthode et instrument de mesure. C. r. Soc. Biol. 57, 400, 1904. Action des rayons x sur le testicule du rat blanc. 8, XI, 1904
19. WILBAR, K.M. : Inhibition of cell division by ultraviolet irradiated unsaturated fatty acid. Experimental Research 13, 903, 1957

20. ONO, T. : Studies on radio-biological indirect function with Yoshida' sarcoma. Nippon Acta Radiologica. Tomus XII. Fasc V. P.6, 1952
21. KANEDA, K. : Effects of the radiation upon the dividing cells in vitro. Nippon Acta Radiologica. Tomus XIII Fasc. IX. P. 531, 1953
22. KUSAZUMI, T. : On the effects of X-rays on successive transplantation of Yoshida's sarcoma. Nippon Acta Radiologica Tomus XIII. Fasc. IX. P. 574, 1953
23. KUSAZUMI, T. : On the effects of X-rays on successive transplantation of Yoshida's sarcoma. Nippon Acta Radiologica Tomus XIII. Fasc. X. P. 621, 1954
24. OBARA, J. : The influence of X-ray irradiation on the process of mitosis of the Yoshida sarcoma cell. Nippon Acta Radiologica Tomus XV. Fasc. VII. P. 624, 1955
25. UTSUMI, K. : Quantitative studies of nucleic acids in the cell with the microspectrophotometer. III Nucleic acid contents in the cancer cells. Acta Med. Okayama, 13, 4, in press
26. BARRON, E. S. G. and SEKI, S. L. : Studies on mechanism of action of ionizing radiation, cellular respiration, cell division, and ionizing radiation. J. Gen. Physiol. 35, 865—871, 1952
27. BARRON, E. S. G. and DICKMAN, S. : Studies on mechanism of action of ionizing radiations ; inhibition of sulfhydryl enzymes by alpha, beta and gamma rays. J. Gen. Physiol. 32, 595—605, 1949
28. BARRON, E. S. G. : Thiol groups of biological importance. Advances Enzymol. 11, 201—266, 1951
29. YOSHIDA, T. H., T. TABATA, T. TAKAHASHI and H. HIRMI : Effects of X-rays on cell nuclei of ascites tumors, with special regards to the chromosome breaks and reunion. Symposia Cell. Chem. 10, in press

EXPLAMATION FOR PLATES

Plate 1. Yoshida sarcoma cells 24 hours after the culture

- Fig. 1. Cells cultured in the medium without addition of the extract. The margin of the cell is smooth and without any pseudopodial projections, and cytoplasm is relatively clear and contains scatteringly lipoid granules with strong refraction. The nucleus is distinct and likewise nucleoli are clear and of various size. Mitotic picture can be seen on the upper right corner.
- Fig. 2. Cells cultured in the medium loaded with 0.1 cc extract. Cell bodies are greatly swollen, nuclei are pyknotic and lipoid granules are greatly increased in size.
- Fig. 3. Another slide in the same experiment as in Fig. 2. A cell just before the complete cytolysis is seen.
- Fig. 4. The same experiment as in Fig. 2. Bleb formation of cytoplasm.

Plate 2. Coelothelioma cells cultured 6 hours.

- Figs. 1 and 2. Cell cultured without addition of the extract. The cell body has a smooth and regular margin with some needle-like projections. Cytoplasm is clear and contains only a few granules. Numerous small mitochondria concentrated around the nucleus can be seen. The nuclear membrane and nucleoli are both distinct.
- Figs. 3 and 4. Cells cultured in the medium loaded with 0.1 cc extract. The cells completely vacuolated and swollen are seen in Fig. 3. Compressed nucleus can still be seen. In Fig. 4 a cell lost a part of cytoplasm with the end gelatinized and irregular margin is demonstrated. The nuclear membrane is not clear.

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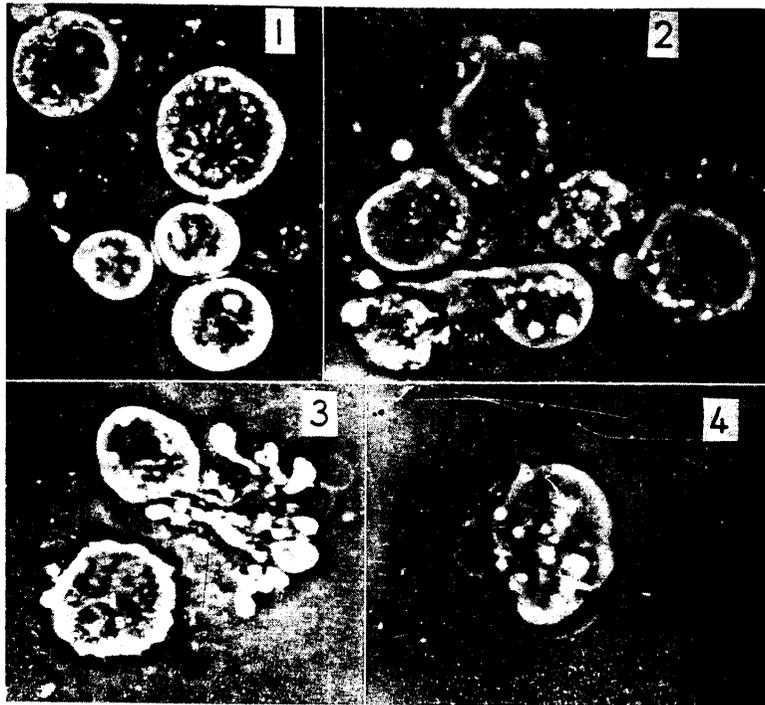


Plate 1.



Plate 2.