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Abstract

1. The unsaturated fatty acid fraction (OX) from the liver of irradiated rabbits contains substance which has the same effects as X-ray irradiation on the testicular cells. 2. This substance introduced intravenously causes the degeneration of the germinal cells with the formation of giant cells or multi-nucleated cells and the mitotic abnormalities. 3. The DNA content of the cell also shows the changes exactly identical with that seen after X-ray irradiation. 4. From these results we conclude that the X-ray injury will be mainly due to the production of some toxic substance which is found in the unsaturated fatty acid fraction and severely affects the cells in mitosis and DNA metabolism.

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MORPHOLOGIC CHANGE AND THE DNA CONTENTS OF THE TESTICULAR CELL OF RABBITS TREATED WITH THE FATTY ACID EXTRACTED FROM THE IRRADIATED ANIMALS

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Accompaning the development of the therapeutic application of X-rays, many serious problems of the side effects and accidents have arisen, but the true mechanism of the injury by X-rays is still uncertain, though several ways of interpretation for the mechanism have been proposed on the basis of the theoretical studies and experimental works. Concerning the biological effects of X-rays, ALBERS-SCHÖNBERG[1] was first to reveal the injury of testicles in 1903, pointing that the castration can be induced by irradiating with X-rays and then REYNAUD and BLANC[2] gave a report about the morphologic changes in the epithelium of seminiferous tubles. At that time it had been uncertain what is the mechanism of the severe injury of the testicular cells leading to the castration. In 1903, ALBERS-SCHÖNBERG[1] found that the injury of the germinal cells is due to the high sensitivity of the dividing cells to X-rays through his experimental studies on dogs. Since then, many studies have been conducted reconfirming this fact, however, it appears that there is hardly any report in eludicating what factor actually induces such damage as the degeneration of the cells in mitotic stage selectively. LEA[18] and WEISS[16,17] reported that by the oxidative radicals produced by the decomposition of water the SH groups of the SH enzymes and others are attacked and this is the main source of the damage of the cells. MAZIA and DAN[15] showed that the SH groups play an important role in the spindle formation through their studies on the isolated spindle of the dividing sea urchin eggs. These findings seem to explain reasonably well the high sensitivity of the dividing cells to X-rays. However, our experiment on blood proved that the

damage of the cell becomes rather severe some period after the irradiation of animal, suggesting that the injury is likely to be induced mainly by some toxic substance produced secondarily by the irradiation than the momentary attack solely by the decomposing products of water. Therefore, we have attempted to observe whether or not the substance extracted (OX) from the organs of irradiated animals can induce the change similar or identical with those observable in the case of X-ray irradiation and have successfully obtained the substance having almost the same effect on the cells as that of X-ray irradiation. In this paper the morphologic changes and the changes in contents of deoxyribonucleic acid (DNA) in testicular cells after the introduction of the unsaturated fatty acid fractions obtained from the liver of irradiated animals are reported.

MATERIALS AND METHODS

For the observation of the testicular cells 30 adult male rabbits served as materials. These animals were divided into two groups, 15 animals in each and introduced intravenously with the solution of the unsaturated fatty acid fraction extracted from the liver of the irradiated animals. The methods used for the extraction of the unsaturated fatty acid fraction from the liver is the same as that reported in the previous paper. The oily substance derived from the each 10 g of the dried powder of the liver was dissolved in 20 cc of or 40 cc of the physiologic saline solution and introduced intravenously, 2 cc per kg of body weight daily for 10 to 14 days, the solutions of higher concentration for those of the first group and the dilute solution for another group.

On the day after the last injection the animals were sacrifised by exanguination from carotic artery.

The testicules excised immediately after the death were cut into two parts; one for the imprinting of the cell and the other for the preparation of the tissue sections. Two imprinted prepartions were made in each sample; one for the morphologic observation of the cell and another for the estimation of DNA contents in each cell. For the morphologic observation of the cell the imprints were dried, fixed with methanol, stained with Giemsa staining and observed under the light microscope. For the estimation of DNA contents the imprinted cells were dried, fixed with the method devised by MARRIAN[12] and stained with Feulgen reaction with the method devised by SHIBATANI[14] and the contents of DNA in each cell were estimated by the microspectrophotometry devised by SENO and UTSUMI[13]. Another half of the testicules were fixed with 10% formol, the sections

were prepared by the routine method and the morphologic structures of the tissues were observed by staining with hematoxylin-eosin.

OBSERVATIONS AND RESULTS

The histological observations on the sectoins of the testis of the rabbits given intravenous injections of the OX-extract revealed that the seminiferous tubles are severely atrophied losing most of the germinal cells, and the basal membranes show irregular undulation. The cells found in the seminal tubules are atrophied or irregular in shape with some giant cells. In the walls of the tubules Sertoli cells are found to be normal morphologically. Hardly any formation of mature spermatozoa can be observed in the seminiferous tubles.

These changes appear more severely in those animals loaded with the solution of higher concentration than in those with lower concentration. Observations on the imprinted cells stained with Giemsa revealed that the nuclei of the germinal cells are extremely irregular both in shape and staining. Among the picnotic or swollen cells a number of large mononuclear- and polynuclear giant cells are found with abnormal mitotic pictures. The lobules of nulcei of some giant cells are connected by thin fibrous material with each other suggesting the cessation of the cell division at metaphase and succesive development (Plate B). On the other hand, in the testicules of the animals injected with the substance from the liver of non-irradiated rabbits, intercellular space in the tubles become somewhat less compact as shown in Plate A-4, but the observation on the imprinted cells proved that all series of the cells from spermatogonia and spermatozoa are kept in nearly the normal condition with the normal mitotic figures, no giant cells nor degeneration. These morphological findings in the testicules of rabbits treated with the substance from the irradiated animal are closely resemble to those found in the testis of irradiated rabbits.

The DNA contents have been measured by the methods above mentioned with the imprinted cells of testis tissues selecting the mononuclear cells having round nuclei. The cells from the rabbits injected with the extracts from irradiated rabbits showed a markedly irregular distribution in the contents of DNA and the appearance of those of pentaploid and octaploid cells, suggesting the disturbance in DNA metabolism. The irregularity in distribution becomes marked in the heavily loaded animals (Fig. 1. C. D. E.). Almost the same tendency in the disturbance of DNA metabolism have been observed on the cells from the irradiated animals

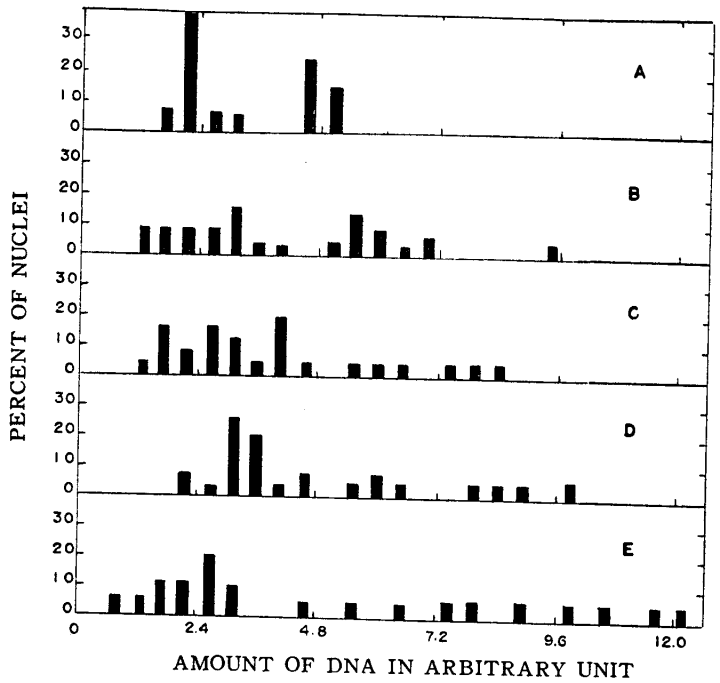

Fig. 1.

A. Control
B. Rabbits irradiated with 3000r X-rays, after 24 hours
C. 1.5 cc/kg extract (fourfold dilution) injected for 14 days
D. 1.5 cc/kg extract (twofold dilution) injected for 10 days
E. 1.5 cc/kg extract (twofold dilution) injected for 14 days

A. DNA distribution in the cell of rabbit testis of the control
B. DNA distribut. in the cell of rabbit testis 24 hr after irradiation of 3000r X-rays
C. DNA distribut. in the cell of rabbit testis injected daily dose of 1.5 cc/kg extract (fourfold dilution) for 14 days
D. ibido (twofold dilution) for 10 days
E. ibido ibido for 14 days

(Fig. 1. B.). But in the cells from the control group injected with the extract from untreated animals there can be observed two peaks of haploid and diploid in the distribution in DNA contents (Fig. 1. A).

These facts indicate that the substance interferes profoundly with the metabolism of DNA and causes the cell death or abnormal mitosis and has a quite similar effect as that of X-ray irradiation.

DISCUSSON

The results of our experiment are sufficient to show that the very effects of X-rays on the dividing cells and the DNA metabolism will be mainly due to the action of some substance produced secondarily in the tissue by X-ray irradiation and to be contained in the unsaturated fatty acid fraction from liver of irradiated animals.

The pictures also showed that the cells affected by X-rays or by the substance (OX) seem to keep the life for some period and continue the process of cell division, forming the abnormal giant cells, if the damage of the cell is not so severe. This fact indicates that the attack of the SH- groups would not be the main factor of the X-rays injury, because many respiratory enzymes are of SH-enzyme, and if the-SH groups were attacked momentarily by the decomposision of the water as claimed by Lea then the cells would be degenerated from the onset by the disturbance of the very metabolism, and some cells might be restored by the reducing mechanism, and acquire the normal function for mitosis, as is generally the case of the -SH inhibititory substance. Moreover another experiment on the substance which we have found in the unsaturated fatty acid fraction proved that this substance does not act to reduce the respiration of bacteria but disturbs the nucleic acid metabolism. About the fact that the effects of X-ray irradiation become marked and continue for a long period, which can not be explained by the momentary demolision of SH group, there are many reports in the past. Already in 1924 SCHINZ-SLOTOPLSHY[5,6] reported that the changes in the seminiferous tubules occur with the lapse of time after X-ray irradiation, describing that the epithelium of seminal tubules is eroded in proportion to the dosage of X-rays.

In 1926 TSUZUKI[7] stated that within 24 hours after the irradiation of 12 % HED changes in the nuclei of spermiocytes can be observed and after the seventy-two hours some restoration can be recognized in spermospores but after that the degeneration proceeds and after ninety-six hours the epithelium of semiferous tubules is extensively invaded. SCHINZ[5] states that among the epithelial cells of the seminiferous tubules spermospores are most liable and most readily to be destroyed by X-rays, followed by spermiocytes and daughter cells, and the changes in the latter group occur one or two weeks later than in spermospores.

The fact that the dividing cells are most susceptibe to X-rays in also contradictory to the hypothesis of Lea and others, because on the experiment of the sea urchin eggs the dividing cells have been proved to be not

so sensitive to the oxygen deficiency, the supression of respiration. TAKAHASHI[20] reported that no changes at all can be recognized on Sertoli cells when the testis are irradiated with 300r X-rays the irradiation affects most strongly on those cells undergoing cell division; and the majority of later experiments also show that the cells in the process of cell division undergo specifically strong changes.

On the other hand, our experiment showed the introduction of the substance from the irradiated animals proved to induce almost the same change by X-ray irradiation, attacking specifically the dividing germinal cells but Sertoli cells survive showing no marked morphologic change.

EULER[8] in his experiment on the nucleic acid synthesis of JENSEN's sarcoma states that with the irradiation of 450r X-rays the DNA synthesis is inhibited down to one half. PAIGN[9] and KAUFMANN[9] in their study on the nucleic acid content of the liver in the mice irradiated the whole body with 600r reported that both DNA and RNA tend to decrease similarly at the early stage and by 12 hours these values return to the normal level, and then by 18 hours both decrease markedly.

But the results obtained by actually tracing the DNA contents in each cell show the marked irregularity in contents per cell. The data which point to the decrease in DNA by X-rays, show also the decrease of the cells in number. As above mentioned, the data of DNA contents obtained on the cells from the animals treated with the substance give almost the same results as those of the irradiated animals[10,11]. Therefore, in the point of DNA metabolism the action of this substance completely coincides with that seen after X-ray irradiation. Recently, WILBER[19] succeeded in isolating the similar substance from the sea urchin eggs irradiated with ultraviolet rays, which has the severe effect on the dividing cell inducing the abnormality in mitosis.

From these facts it is resonably possible to assume that the main factor of the X-ray injury will be due to the production of some substance derived from the unsaturated fatty acid fraction.

SUMMARY

1. The unsaturated fatty acid fraction (OX) from the liver of irradiated rabbits contains substance which has the same effects as X-ray irradiation on the testicular cells.

2. This substance introduced intravenously causes the degeneration of the germinal cells with the formation of giant cells or multi-nucleated cells and the mitotic abnormalities.

3. The DNA content of the cell also shows the changes exactly identical with that seen after X-ray irradiation.

4. From these results we conclude that the X-ray injury will be mainly due to the production of some toxic substance which is found in the unsaturated fatty acid fraction and severely affects the cells in mitosis and DNA metabolism.

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REEERENCES

1. Abers-Schönberg. Ueber eine unbekante Wirkung der Röntgenstrahlen auf der Organismus. Münchener med. Wochenschrift. **50**, 1859, 1903
2. REYAUD u. BLANC : Action des rayons X aur les diverses générations de la lignée spermatique Extrême sensibilité des Spermatogonies a Rayons. C. r. Soc. Biol. **61**, 163, 390, 652 & 731, 1946
3. YAMAMOTO, M. and M. OFUJI : Morphologic change of YOSHIDA sarcoma cellls and coelothelioma cells after exposing to the cell toxin from X-ray irradiated animal, in press.
4. SENO, S. : The structure and the function of reticulocyte. Acta Hem. Jap. **21**, 351, 1958
5. SCHINZ u. SLOTOPOLSKY. : Bemerkungen über Entwicklung und Pathologie des Hodens. Virchow's Archiv f. Path. Anat. **253**, 413, 1924
6. SCHINZ u. SLOTOPOLSKY. : Experimentelle und histologische Untersuchungen an Hoden. Deutsch. Ztschr. f. Chir. **188**, 76, 1924
7. TSUZUKI, M. : Experimental studies on the biological action of hard roentgen rays. Amer. J. Roentogenol. **16**, 134, 1926
8. EULER, H. V. : Influence of roentgen rays on isolated cell nuclei. Acta Radiol. **27**, 269, 1946
9. PAIG, K. and B. N. KAUFMANN. : Effects of X-irradiation on amount and composition of nucleic acids in liver. J. Cell. Comp. Physiol. **42**, 163, 1953
10. COLE, L. J., MAURICE, F. and M. E. ELLIS. : Protection of mice against X-irradiation by spleen homogenates administrated after exposure. Proc. Soc. Exp. Biol. & Med. **80**, 112, 1952
11. COLE, L. J., ELLIS, M. : Spleen desoxyribonucleic acid content as index of recovery in X-irradiated mice treated with spleen homogenate. Cancer Res. **14**, 738, 1954
12. MARRIAN, R. W. : Standard chemical fixations as a basic for quantitative investigation of substances other than DNA. J. Histochem. Cytochem. **6**, 43, 1958
13. UTSUMI, K. : Quantitative studies of nucleic acids in the cell with the microspectrophotometer. 1. The critique and improvement of the spectrophotometry. Acta Med. Okayama **13**, 1, 1959
14. SHIBATANI, A. and NAORA, H. : Feulgen reaction and quantitative cytochemistry of desoxyribonucleic acid. Biochem. et Biophys. Acta **12**, 512, 1953
15. MAZIA, D. and DAN, K. : The isolation and biochemical characterization of the mitotic apparatus of dividing cells. Proc. Nat. Ac. Sc, **38**, 826, 1952
16. WEISS, J, : Radiation and living cell. Nature, **157**, 738, 1946
17. WEISS, J. : Radiochemistry of aqueous solutions. Nature **153**, 748, 1944

18. LEA, D. E. : Actions of radiations on living cells. The Macmillan Company, New York. 19. K. M. WILBUR. Inhibition of cell division by ultraviolet irradiated unsturated fatty acid. Experimental Research 13, 503, 1957
20. TAKAHASHI, S. : Damage of the testicular cells by X-ray irradiation. Nippon Acta Radiologica 7, 849, 1950
21. YAMAMOTO, M. : Toxic substance found after X-ray irradiation. Symposia Cell. Chem. 9, 141, 1959

EXPLANATION FOR PLATES

1. Rabbit testis (untreated) 2. Rabbit testis injected with extract for 14 days.
3. The same as (2) and (4).

1. Stamped specimen of the untreated rabbit testis
2. Stamped specimen of rabbit testis injected with extract for 14 days, showing giant cells as well as the swelling of chromosomes
3—7. Magnified pictures of individual cells in (2), showing multinucleated giant cells and swollen and enlarged chromosomes.
8—11. The mitosis of giant cell is not complete so that there are odd-numbered nuclei. Chromosomes lying between nuclei connect each of these nuclei, showing a centrical connection.

Plate 1

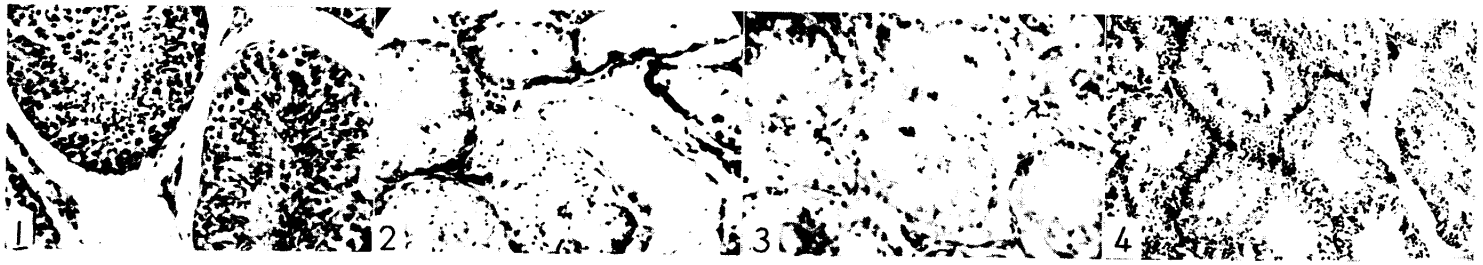

Plate 2