Cytological Studies of Tumors, XXXIV. Effect of X-Irradiation on Yoshida Sarcoma Cells with Special Regard to Chromosome Breaks[1)]

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(With 4 Text-figures)

A great amount of work has been done upon the effects of radiation on cells of various kinds of plants and animals. There are a number of valuable reports published on chromosome breakages. However, the studies of radiation effects on tumor chromosomes are very limited (Koller and Casarini 1952, Conger 1956, Tabata *et al.* 1959), many important problems having been left unexplored. Particularly, there is comment of fundamental importance (a) that the break occurs at random in the chromosomes, or (b) that certain definite points are specially liable to the break. A considerable number of studies have been undertaken on this question making use of several kinds of plants and animals with multifarious results (cf., Giles 1954, Kaufmann 1954, Lea 1955).

Formerly the present author reported some preliminary results on the localization of chromosome breaks in tumor cells of the Yoshida sarcoma of rats following X-irradiation, with a note on the radiosensitivity of different types of chromosomes (Seto 1960). The present article gives some detailed account of the distribution of simple breaks occurring in certain chromosomes of the Yoshida sarcoma.

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Material and methods: The Yoshida sarcoma which provided the material for this study was obtained from a transfer line maintained at the Medical Institute of Sasaki Foundation, Tokyo, in October 1958, and has been serially transferred in highly purebred rats, (W/Ma). For observation of the chromosomes, a water pretreatment squash method was employed advantageously (Makino 1957).

Preliminary examinations revealed that the tumor ascites three days after transplantation would be most suitable for irradiation and for observation of the chromosomes because of the prevalence of mitotic figures. On the third day after transfer of the tumor, tumor

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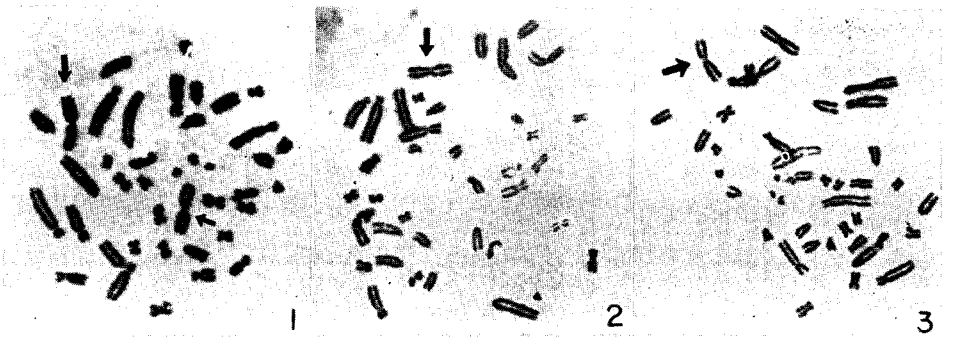
bearing rats were irradiated with whole body exposure at doses of 500 r and 1000 r.

A dosage-rate of 35.4 r per minute was obtained as follows: the X-ray tube was run at 180 Kvp and 6 mA with a filtration of 0.5 mm Cu plus 1.0 mm Al at 30 cm focal distance. Samplings were made at 12 and 24 hours after X-irradiation.

Observations

1. *Morphology of the chromosomes of untreated Yoshida sarcoma cells*: Prior to inquiring into the radiation effect on the chromosomes of the Yoshida sarcoma, it is important to have exact knowledge of the chromosomes of untreated tumor cells. The chromosome number of stem cells of the Yoshida sarcoma was found to be 40 with a frequency as high as approximately 80 per cent at the present status. An excellent metaphase example is shown in Fig. 1. According to the system offered by Tjio and Levan (1956), the chromosomes were classified into three morphological groups; M chromosome with a median or submedian centromere,



Figs. 1–3. Photomicrographs of metaphase chromosomes of Yoshida sarcoma cells (water-pretreatment, acetic dahlia). × 800. 1, unirradiated, 40 chromosomes. Arrows indicate large M chromosomes. 2, irradiated with 500 r. 3, irradiated with 1000 r. Arrows indicate simple chromosome-breaks in a marker chromosome.

S chromosome with a subterminal centromere, and T chromosome with a nearly terminal centromere. Among the M chromosomes, two elements are outstandingly large in size. Those two large M chromosomes were unequal in size; the average length measured on the base of 30 metaphase plates is 6.4 μ for the large M chromosome and 5.8 μ for the smaller M. It is then evident that the chromosomes of the Yoshida sarcoma observed in the present study are of similar type in both number and morphology to those of subline B with which Makino and Sasaki dealt (1958).

2. *Effects of X-irradiation*: As mentioned above, the stem cells of the Yoshida sarcoma are characterized in each by having two large M chromosomes. These two M elements are outstanding as marker chromosomes because of their large size and remarkable feature. Usually, the division of tumor cells was in-

hibited for 6 to 9 hours from the time just after irradiation. With a recovery of proliferation activity, metaphasic chromosomes manifest various types of abnormalities in their structure. With the passage of time after irradiation, the frequency of chromosomal aberrations increased and showed a peak at 24 hours in every dosage.

The frequency of simple breaks was observed in the marker chromosomes: it was found to be higher in 12 hours' samples than 24 hours' samples. The average frequency value from 12 hours' samples was 20.6 per cent at 500 r and 26.9 per cent at 1000 r, while that from 24 hours' samples was 9.3 per cent at 500 r and 13.9 per cent at 1000 r (Figs. 2–3). In the following described observations, data pertaining to simple chromosome breaks were based on 12 hours' samples.

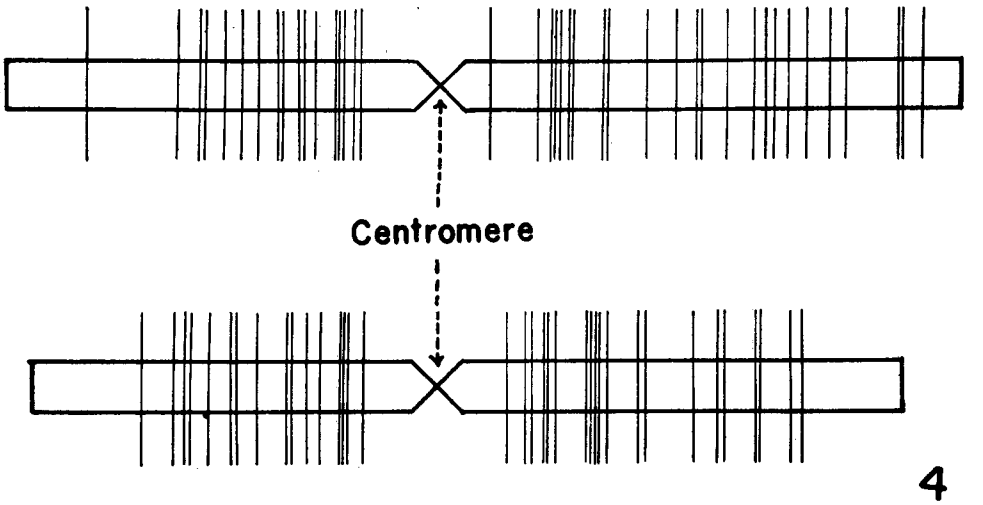


Fig. 4. Distribution of chromosome-breaks in marker chromosomes induced by X-ray irradiation.

The position of breaks was examined in the chromosomes in which single breaks have taken place in one of the sister chromatids. The data obtained were based on observations of more than one hundred marker chromosomes including two types. The results of observations are given in Fig. 4. It is evident from Fig. 4 that in the two types of marker chromosomes the positions of observed chromatid breaks are frequent on the arm at a distance about one-fourth of the arm length from the centromere. In the light of the above data it may be reasonable to conclude that the chromosome breaks are not caused by irradiation to occur at random, so far as the marker chromosomes here considered are concerned.

Discussion

The results of the present study give evidence that chromosome breaks induced by X-irradiation show a frequent distribution on the arm at about a distance of one-fourth the arm length from the centromere, so far as concerns the two types of marker chromosomes of the Yoshida sarcoma cells.

A considerable number of papers have been published which comment that chromosome-breaks are induced at random, or else in certain limited loci under the influence of radiation, such comment is based upon work with various kinds of plants and animals with multifarious results (Lewitsky and Sizova 1935, Bauer *et al.* 1938, Sax and Mather 1939, Muller 1940, Sax 1942, Kaufmann 1946, Camara *et al.* 1950, Revell 1953, Tabata *et al.* 1959, and some others). According to Revell (1953), simple breaks induced by X-rays showed a rather random distribution except that there were a few in the heterochromatic regions in the root-tips of *Vicia*. On the other hand, Sax and Mather (1939) reported that the distribution of chromosome breaks in *Tradescantia* microspores was not random for various loci of the chromosome arms: aberrations occurred more frequently near the centromere than in the distal loci of the chromosome arms. A frequent distribution of X-ray induced breaks in the proximal regions of the chromosome arms was also reported in *Crepis* by Lewitsky and Sizova (1935). Working with tumor cells of the Yoshida sarcoma, Tabata *et al.* (1959) reported that most chromosome breaks were located in the middle part of the chromosome arms.

Lea (1955) postulated that simple breaks which were observed at rather late durations after irradiation did not represent the total number of breaks primarily produced: only the residual elements were observable after some of the primary breaks had taken part in rearrangements and many others had joined a pair of breakage ends to re-form the original chromosome. Bauer *et al.* (1938) investigated salivary-gland chromosomes of *Drosophila* following X-irradiation, and reported that the frequency of location of breaks was higher in the heterochromatic region than in the euchromatic region. They stated that: the cause of higher frequency in the heterochromatic region may be due to (1) an inherent property of that region to break more easily than the euchromatic part, or (2) a different coiling state of the chromonemata in the heterochromatic region. According to Sax (1940), the frequency of chromosome breaks observed per unit length in *Tradescantia* micrspores in response to X-rays was only one-ninth as great in acentric fragments as in centric chromosomes. The explanation presented was that, since the centromere was largely responsible for movement of the chromosome during cell division, the strains would be less in an acentric fragment than in a centric chromosome, thus making restitution more probable.

Although, referring to the above statements made by certain previous authors, some available suggestions have been given to explain the cause of non-random occurrence of breaks as observed in the present material, the question has remained whether the localization of the breaks is due to some fundamental

structural difference of chromosome parts, or to the difference of a restitution factor (or factors).

Summary

The Yoshida sarcoma, a rat ascites tumor, was irradiated with X-rays with whole body exposure at dosages of 500 r and 1000 r. The positions of simple chromosome breaks were observed in two types of marker chromosomes. It was found that frequent breaks occurred in the arm at or near a distance about one-fourth of the arm length from the centromere, so far as marker chromosomes here considered were concerned.

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