Phase Cinematography Studies on the Effects of Radiation and Chemicals on the Cell and the Chromosomes, I. Types of X-ray Induced Chromosome Abnormalities in Grasshopper Spermatocytes, with a Note on the Normal Course of the First Division as Control[1)]

By

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(With 11 Text-figures)

A great amount of work has been done on the effects of radiation and chemcials on the cell and the chromosomes, and many valuable contributions have been made to the analysis of the mechanism by which the cell and the chromosomes are damaged. Radiation is the most effective experimental agent of the death of cells, and therefore it is the chief means of cancer treatment. It is also the agent of all genetic and evolutionary changes in the fact that it causes alteration in the structure of chromosomes. The atomic radiation gives rise to changes of similar nature which are becoming most important because of their dangerous influence upon human beings. Now, it is obvious that the effect of radiation on chromosomes is a focal problem in biology.

Most of the previous work on the radiation effects on the chromosomes have been done on fixed and stained materials. Every sequence of the observed events in those studies was derived from different cells, and did not show the succesive series of changes which occur in the chromosomes. To supplement the knowledge from fixed material in the older literature, it is highly desirable and of fundamental importance to follow in the living condition the serial changes of cells and the irregular behaviors of chromosomes in response to radiation. Phase contrast microscopy has helped to a great extent in studying the dynamic cellular phenomena in the living stage. Recording radiation injury by the phase cinematographic method has gained increasing attention of workers with the use of tissue culture materials in recent years. Bloom, Zirkle and Uretz (1955) have made a

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brilliant contribution to the study of cell injury by radient energy using microbeams of protons and of ultraviolet. Recently Makino, Nakahara and Nakanishi (1957) recorded the behavior of the mitochondria in relation to cell division in grasshoppers following treatments by some chemicals. With the cooperation of specialists the dynamic analyses of the mechanism of injury to cells by radiation and by some chemicals have been undertaken with the use of cinematographic technique in this laboratory. The results of experiments obtained will be reported in a series of papers according to the accomplishment of investigations.

As the first undertaking in a series of studies, the irregular behaviors of chromosomes induced in grasshopper spermatocytes by exposing grasshoppers to X-rays were successively recorded by phase contrast 16 mm cinematography by Ohnuki. In the present communication are described in the form of a preliminary report the types of induced irregularities of chromosomes.

Sajiro Makino.

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Material and method. The larger part of this study was carried out with the first and second spermatocytes of the following two species of grasshoppers: *Podisma sapporense* and *Chrysochraon japonicus*. The supplementary data were obtained from *Chorthippns bicolor* and *Oxya yezoensis*. For observation, hanging-drop preparations were made following Makino and Nakahara (1955 a, b) and Makino and Nakanishi (1955). For expreiment with a dosage-rate of 17.8 r per minute, the X-ray tube was run at 140 kvp. and 3 ma. with a filtration of 0.3mm. Cu plus 0.5mm. Al at the focal distance of 23 cm.. Doses of 50 r, 100 r, 200 r, 300 r, 500 r, 600 r, and 800 r were given by the whole body exposure. The data presented in the following were mainly furnished by the experiments with 300 r dosage, since various types of abnormalities of chromosomes were observed most abundantly in this dose. Observations have been made at 4, 6, 8, 12, 18, 24, 36, 42, 50, and 63 hours after irradiation. All cine records were taken on Sakura Neopan SS 16 mm films, with a 25× dark contrast objective in combination with a "Zoomlens" as an eyepiece, at various magnifications ranging from 1× to 8×, with the aid of 'Mikro-Kino-Kamera' (Zeiss). The sequences were taken at 8 and 15 frames per minute at a room temperature of 22°–26°C, with the shutter speed at 2 seconds.

Observations

1. Control

As controls for the experiments to be dealt with in the following, the normal behavior of the chromosomes was serially followed in single spermatocytes of *Podisma sapporense* through the stages from diakinesis downward to the first telophase. Observations of a similar type were made by Nakahara (1952), Makino and Nakahara (1955), and Makino and Nakanishi (1955, 1956). The results of the present observations are in complete agreement with those of previous investigators.

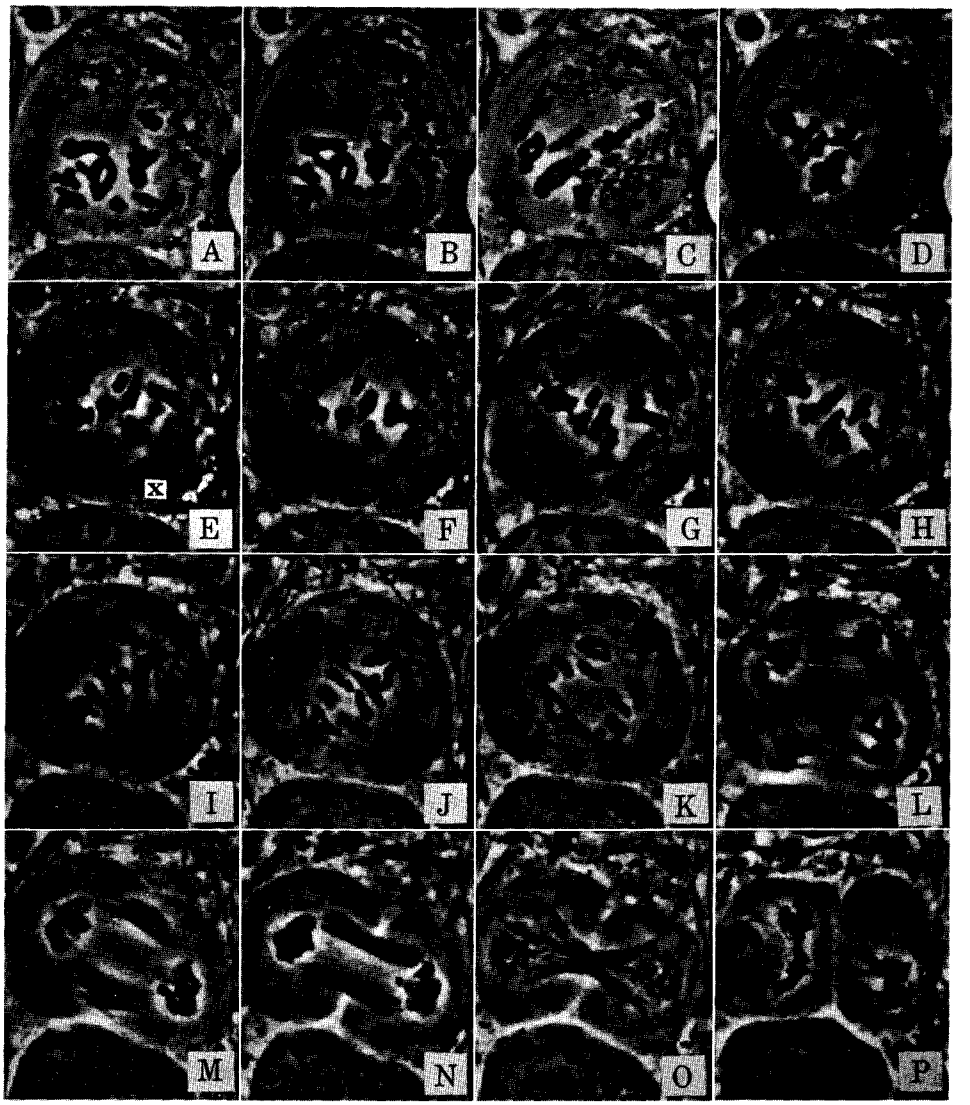

Fig. 1, A-P. Showing the successive stages of the first division in a living spermatocyte of *Podisma sapporense*, from diakinesis to the first telophase. From 16 mm cine film. Dark contrast, ×550. A, diakinesis, the nuclear membrane are slightly visible (taken 105' after preparation). B, the nuclear membrane becomes irregular in outline. C, as soon as the nuclear membrane disappears, the chromosomes make their arrangement along the spindle axis. D-G, the autosomes gradually migrate towards the equatorial plate. X shows X-chromosome. H, metaphase (240'). I, the onset of anaphase (345'). J-K, anaphase, the

autosomes migrate to the opposite poles. L, late anaphase, most chrosomomes reach the poles, while the mitochondria begin to form the bundles (365′). M, telophase, the mitochondrial bundles stretch like bridges between two daughter chromosome groups. N, the cleavage furrow is formed across the middle part of the mitochondrial bundles (395′). O, the cleavage furrow cuts the cell body into halves (405′). P, interphase, the daughter nuclei have been formed completely (525′).

The condensation and shortening of chromosomes proceed rather rapidly nearing the end of diakinesis within the nucleus which is well-expanded. The mitochondria which are short rod in appearance scatter in the cytoplasm surrounding the nuclear body with a movement like oscillation. Meanwhile the nuclear membrane becomes irregular in outline. As soon as the nuclear membrane becomes partially invisible, the chromosomes, now fully developed, disperse into the area which remains distinct because free from the mitochondria. The chromosomes, now being free in that distinct area, move as if pulled towards and into the equatorial area of the spindle body. The spindle body remains as a clear body which is free from the mitochondria and other cytoplasmic granules. The spindle fibers are entirely invisbile. Then the chromosomes have completed the arrangement on the equatorial plate. During metaphase, individual chromsomes show an oscillation in greater or less degree, as though pulled slightly towards one pole and then towards the other. Individual chromosomes at metaphase exhibit their well-defined bivalent structure. The chromosomes are not adherent to one another. The metaphase chromosomes remain without observed change for a considerable length of time. The spindle body is freely movable as a whole. The mitochondria lie adhering to the outer surface of the spindle body in a nearly longitudinal alignment.

With the onset of the chromosome separation at anaphase, the mitochondria take a definite orientation and lie with their long axes nearly parallel to the spindle body. They are filamentous in appearance, and collected into a few bundles. Simultaneously with the separation of the chromosomes, the mitochondrial bundles elongate along the spindle axis. The separation of chromsomes is generally synchronous. After the daughter chromosomes have reached the opposite poles at telophase, the cell body begins to elongate along with the spindle axis, and the mitochondrial bundles remain stretching like bridges between the two groups of daughter chromosomes. At each pole, the daughter chromosomes swell and the nuclear membrane appears and confines them. Within a short time, all visible traces of the chromosomes are lost from sight in the reconstructing telophase nuclei, whilst the cleavage furrow appears cutting the cell body into havles across just the middle part of mitochondrial bundles. After the cell division has been completed, the two daughter cells remain connected with the mitochondrial bridges for a considerably length of time. Figure 1, A to P, illustrate one example of a successive course of division ranging from diakinesis to the first telophase.

The normal course of the first division was also observed in the other species

of grasshopper, *Chrysochraon japonicus*, with results nearly similar, though not entirely, to those of *Podisma sapporense*. The above descriptions may be applied also to the case of *Chrysochraon*; the reader is referred to Figure 2, A to H which deals with important mitotic events only.

2. Types of mitotic irregularities induced by X-rays

Though X-ray irradiation applied tc grasshoppers induced abnormal mitoses and chromosome irregularities in a wide variation in details of types, the following effects were rather common and remarkable in dividing spermatocytes:

1) stickiness and clumping of chromosomes;
2) disturbance of spindle formation resulting in the formation of anuclear buds;
3) sticky bridges of chromosomes at anaphase;
4) formation of the chromosome bridges, interlocking and pseudo-multivalents;
5) fragmentation of chromosomes and the formaiion of micronuclei.

Almost all of thes abnormalities concern the chromosomes, or the spindle, or both: it is evident that they lead to degeneration and death of the cells.

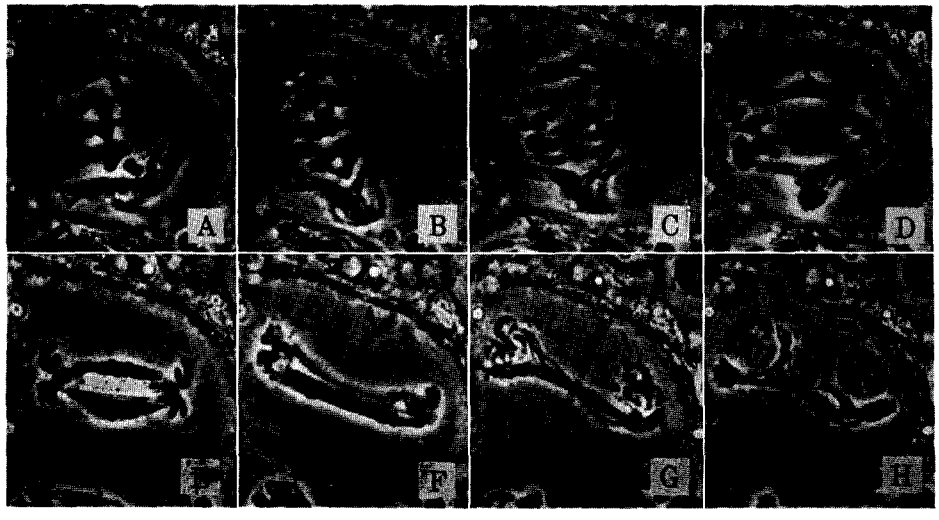

Fig. 2, A-H. Showing the successive stages of the first division in a living spermatocyte of *Chrysochraon japonicus*, from the first metaphase to telophase. From 16 mm cine film. Dark contrast, ×360-400. A, metaphse (150′). B, the onset of anaphase (170′). C, anaphase. D-E, late anaphase, the mitochondrial bundles stretch like bridges (190′-220′)' F, the onset of telophase (270′). G, the cleavage furrow cuts the cell body into halves (340′). H, interphase, daughter nuclei have been formed completely (450′).

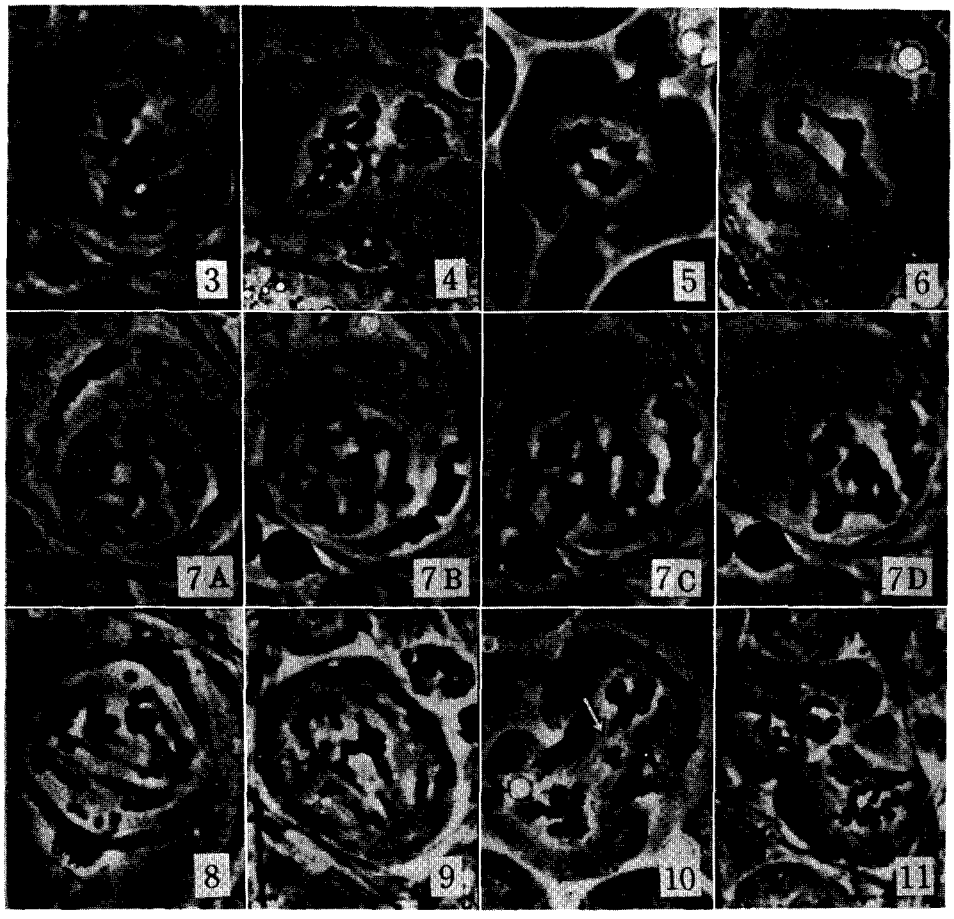

Fig. 3-11. Showing the various types of aberration of chromosomes induced by the X-ray irradiation. From 16 mm cine film. Dark contrast. 3, showing the stickiness of chromosomes in the less affected spermatocyte of *Chorthippus bicolor* which was exposed to 300 r of X-rays. ×840. 4, showing the aggregation of chromosomes in the highly affected spermatocyte of *Oxya yezoensis* which was exposed to 300 r of X-rays. ×650. 5, the formation of anulear buds in the first spermatocyte of *Podisma sapporense* which was exposed to 600 r of X-rays. ×650. 6, the sticky brgidges of chromosomes in the first spermatocyte of *Podisma sapporense* which was exposed to 300 r of X-rays. ×750. 7, A-D, showing the successive stages of the development of stickiness in the chromosomes of a spermatocyte, from the diplotene stage to the first metaphase. *Chorthippus bicolor* (exposed to 300 r). ×840. A-B, chromosomal elements are seemingly free stickiness. A, diplotene stage (100′). B, diakinesis. C-D, the chromosomes show a tendency to adhere with each other. C, just after the disappearance of the nuclear membrane (290′). D, prometaphase (320′). 8, a few chromosome bridges and many chromosome fragments observed at the first anaphase

of *Chrysocharon japonicus* which was exposed to 300 r. ×660. 9, a few huge chromosomal bodies at anaphase. *Podisma sapporense* (exposed to 300 r). ×650. 10, the slender chromosome bridges (arrow) and a few fragments at telophase. *Podisma sapporense* (exposed to 300 r). ×520. 11, the accessory micronuclei of daughter cells. *Podisma sapporense* (exposed to 300 r). ×520.

The X-ray radiation is very effective in producing the stickiness of chromosomes : almost all dividing cells show sticky chromosomes in greater or less degree at metaphase and anaphase when observed some ten minutes after irradiation with comparatively higher dosage. The stickiness of chromosomes was noted as a typical phenomenon of the physiological effects of X-rays by Lea (1946). In the less affected cells, two, three or more chromosomes stick with each other (Fig. 3), while those highly affected show the chromosomes aggregated into irregular masses (Fig. 4). In every case, the irregular condensation of chromosomes is remarkable. The reduction in size of chromosomes seems to be one of the common abnormalities induced by X-rays, it is seen with a variation of degree in almost all affected cells at any dosage under examination.

Under the effect of the usual dosages ranging from 200 r to 500 r, the spermatocytes show the mitochondria which scatter in the cytoplasm in rather regular manner with ordinary features. This suggests that X-ray irradiation may exert no influcence upon the morphology and behavior of mitochondria. In dividing cells the mitochondria generally remain unaffected, even in the case where the chromosomes show heavy stickiness. At anaphase, the chromosomes fail to migrate towards poles, probably due to the disturbance of spindle formation, and are clumped into a mass. But the mitochondria elongate into a few bundles and push out the cell surface at two points; this results in the formation of two small anuclear buds containing neither nuclear components nor chromosomal elements (Fig. 5).

In the cells in which the spindle mechanism seems to remain mostly unaffected, though not completely, the chromosomes migrate to the opposite poles at anaphase, and the sticky bridges are always formed between the daughter halves of chromosomes (Fig. 6). It is most probable that the bridges are resulted from stickiness of chromosomes. However, the mitochondria seem to be regular in behavior showing elongation into bundles, and the cleavage furrows incompletely cut the cell body into two parts of unequal size.

It was observed that under the influence of the usual dosages here examined (200 r to 500 r) the cells at diplotene and diakinesis contain the chromosomal elements which are seemingly free from stickiness (Fig. 7, A, B) : every chromosomal body is freely distributed within the nucleus showing a slight movement. Earlier or later than the disapperance of the nuclear membrane nearing the end of diakinesis, the chromosomes show a tendency to adhere with each other. Just after the disappearance of the nuclear membrane, two or three chromosomes are found

sticking together (Fig. 7, C, D).

The irregularities of the chromosomes observed 40 to 60 hours after irradiation will be dealt with next. The production of chromosome fragments, chromosome bridegs, interlocking between two or more elements, and pseudo-multivalents are of rather frequent occurrence in the materials observed about 40 hours after the 300 r exposure. Some spermatocytes furnished evidence that most chromosomes migrate to the opposite poles, while a few form slender chromosome bridges stretching between two or three elements, leaving a few fragments freely moving in the cytoplasm (Fig. 8). In the present study, the breakage and reunion analysis in relation to the formation of fragments has not been dealt with: its direct analysis is a task of the most difficulty in living material. Further, there are found in some cells a few huge chromosomal bodies of unusual shape; they are probably derived from the interlocking of two or more bivalents (Fig. 9). There are also irregular shaped chromosomal bodies which seem to be pseudo-multivalents comprised of two or three bivalents in close association. In most of the above cases, the cleavage furrow appears and cuts the cell body into incomplete halves, with slender chromosome bridges which remain between the daughter cells after division (Fig. 10).

The chromosome fragments vary in number and size, and drift about in the cytoplasm until anaphase without joining the equatorial plate. At anaphase they leave the other elements behind. They never show definite orientation, nor anaphase movement to poles. At telophase they are squeezed into one of the daughter cells by the cleavage furrow. Meanwhile they form the accessory micronuclei of varying sizes in the daughter cells (Fig. 11).

The above statements give only the types of mitotic abnormalities observed by phase cinematography in the living spermatocytes of some grasshoppers which were exposed to X-rays. Detailed analysis of the events is the subject of further studies now in progress.

Summary

The grasshoppers were exposed to 50 r, 100 r, 200 r, 300 r, 500 r, 600 r, and 800 r of X-rays, and the induced mitotic abnormalities were observed by means of the phase cinematographic technique. The present paper was confined to a brief description of the types of irregularities mainly produced in chromosomes, with a note on the normal course of the first division in grasshopper spermatocytes.

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