Phase Cinematography Studies on the Effects of Radiation and Chemicals on the Cell and the Chromosomes, III. Immediate Effects of Beta-Irradiation, from a Strontium^{90} Source at Some Different Stages, on Meiosis of Grasshopper Spermatocytes[1)]

By

Yuh H. Nakanishi

(Zoological Institute, Hokkaido University)

(With 29 Text-figures)

Recently, Zirkle and Bloom (1953) and Uretz *et al.* (1954) have developed a new technique to irradiate selected small parts of living cultured cells of newt heart with either a proton or an ultraviolet beam. Using this technique several investigators have performed a series of experiments with interesting results (cf., Bloom *et al.* 1955, Izutsu 1959). In the two former reports of this series the X-ray induced irregular behavior of chromosomes has been described in grasshopper spermatocytes (Ohnuki 1958, Ohnuki and Makino, in press).

The present paper describes the results of irradiation on grasshopper germ-cells at some different stages of meiosis with the use of a strontium 90 plaque applicator, in a hope to learn the most radiosensitive stage when the chromosomes first began to show abnormal behavior.

The author wishes to express sincere gratitude to Professor Sajiro Makino for his direction and improvement of the manuscript. Further cordial thanks are offered to Professor N. Muta of the Department of Radiology, Sapporo Medical College for the use of a strontium 90 plaque applicator.

Material and method

The experiments were carried out with first and second spermatocytes of the grasshopper, *Podisma sapporense*. For observation, hanging-drop preparations were made following Makino and Nakahara (1955a, b) and Makino and Nakanishi (1955). The beta source was an Amersham strontium 90 plaque applicator, of which the nominal content was 20 mc (Nagai 1958). A spermatocyte in an

1) Contribution No. 460 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Supported by a grant from the Rockefeller Foundation to the National Institute of Genetics, Mishima, for research in Animal Genetics.

appropriate meiotic stage was selected, and the behavior of the chromosomes and mitochondria was recorded by the time-lapse camera for an adequate period, usually for 15 minutes. Then the objective and body tube of the microscope were removed and the strontium 90 plaque applicator was placed directly on the coverslip (Fig. 1). Thus the cell was irradiated for 4.5 minutes to give approximately 1000 rads. The dosage rate was 3.68 rads per second through the # 1 thinness coverslip. After irradiation the source was removed, the microscope refocused, and the cine record resumed.

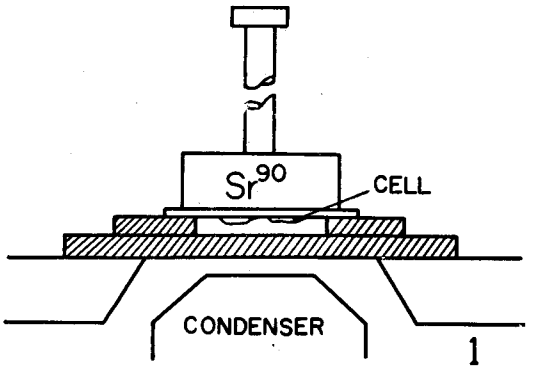


Fig. 1. Schematic diagram illustrating the method of beta-irradiation with a strontium 90 plaque applicator. For details see text.

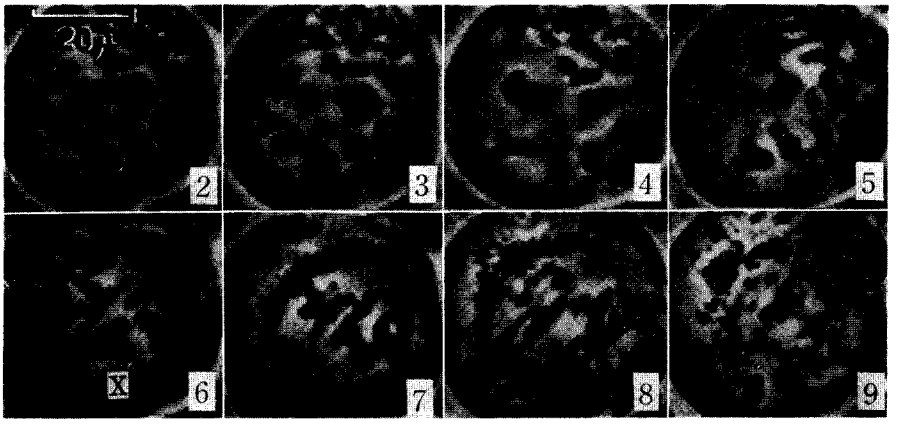
The film record was interrupted for not more than 7 minutes. All cine records were taken on Sakura Neopan SS 16 mm films, with a 25× dark contrast objective in combination with a "Zoomlens" as an eyepiece, at various magnifications ranging from 1× to 8×, with the aid of 'Mikro-Kino-Kamera' (Zeiss). The sequences were taken at 8 and 15 frames per minute at 22° to 26°C, with the shutter speed at 1 second. Figures 2 to 29 present selected film frames abstracted from the record (ED no. 59–2).

Observations

1. Results of irradiation at diakinesis

At diakinesis when the bivalent chromosomes were apparent within the nucleus (Fig. 2), the cell was irradiated with 1000 rads. After irradiation the chromosomal elements were seemingly free from stickiness: every body was freely distributed within the nucleus showing a slight movement or oscillation (Fig. 3). The condensation and shortening of bivalent chromosomes proceeded nearing the end of diakinesis within the nucleus which was well-expanded (Fig. 4). The mitochondria, short rod in appearance, were found scattered in the cytoplasm surrounding the nuclear body with a movement like oscillation. Meanwhile the nuclear membrane became partially invisible and the chromosomes, now fully

developed, dispersed into the area which remained distinct because free from the mitochondria (Fig. 5). The chromosomes, being now free in that distinct area, moved as if pulled towards and into the equatorial area of the spindle body. Then the chromosomes completed the arrangement on the equatorial plate (Fig. 6). The chromosomes in material under treatment required nearly the same length of time



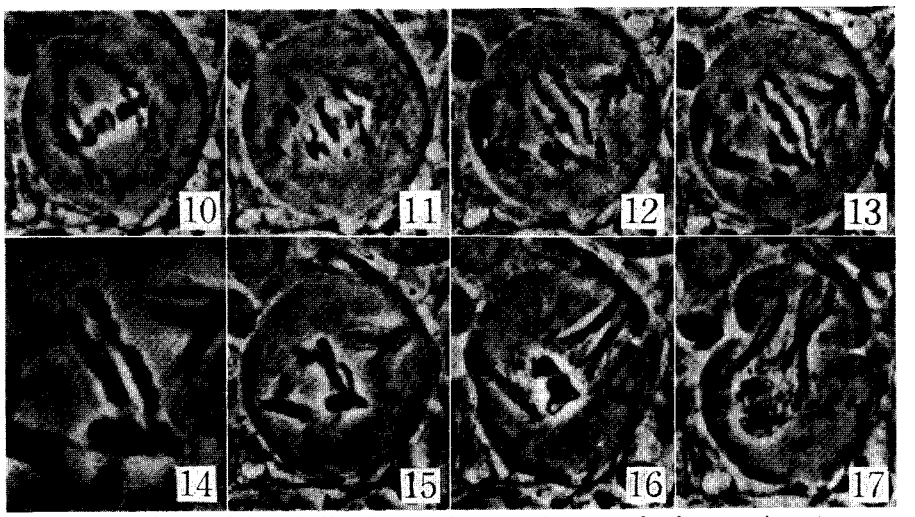
Figs. 2–9. Selected film frames from a cine record of successive stages showing stickiness of chromosomes in a cell irradiated at diakinesis (film no. SS-99-3 and SS–100–1). The scale is shown in Fig. 2. 2, diakinesis just before irradiation. 3, immediately after irradiation. The film record was interrupted for 7 minutes. 4, the nuclear membrane becomes irregular in outline (taken 11′30″ after irradiation). 5, as soon as the nuclear membrane disappears, the chromosomes make their arrangement along the spindle axis in a regular manner (22′10″). 6, the autosomes gradually migrate towards the equatorial plate. X : X-chromosome (67′30″). 7, metaphase (94′10″). 8, the centromeres of each bivalent are pulled strongly towards the opposite poles, but the bivalents did not separate at all (335′25″). 9, the chromosomes, as well as the cell, underwent disintegration (572′12″).

(approximately 130 minutes) from diakinesis to metaphase and were also nearly identical in behavior to those observed in the control material ; however they were somewhat hazier in outline than the treated ones. The formation and function of the spindle body also seemed to be regular as compared with that of the control, at least through phase-optic observations. During metaphase, individual chromosomes showed an oscillation in greater or less degree, as though pulled slightly towards one pole and then towards the other (Fig. 7). With the lapse of time nearing the anaphase the centromeres of each bivalent seemed to be strongly pulled towards the opposite poles, but the chromosomes failed to separate at all, probably due to a striking stickiness (Fig. 8). Cinematographic record taken at 8 and 15 frames per minute and projected at 24 frames per second showed that the stickiness of the chromosomes seemed to offer resistance against the poleward

pulling force of the chromosomal fibers which run between centromeres and poles. The mitochondria showed no remarkable visible change in appearance. Neither migration of the chromosomes to the poles, nor the formation of a cleavage furrow took place in irradiated cells, while, in the control cells, the duration of metaphase of the first spermatocytes was approximately five hours and a cleavage furrow appeared at about 20 to 40 minutes after the chromosomes had nearly reached the poles (Makino and Nakanishi 1955, Ohnuki 1958). For example, in an irradiated cell no chromosome separated at all for more than eight hours at the metaphasic stage. Finally the chromosomes, as well as the cell, underwent disintegration (Fig. 9).

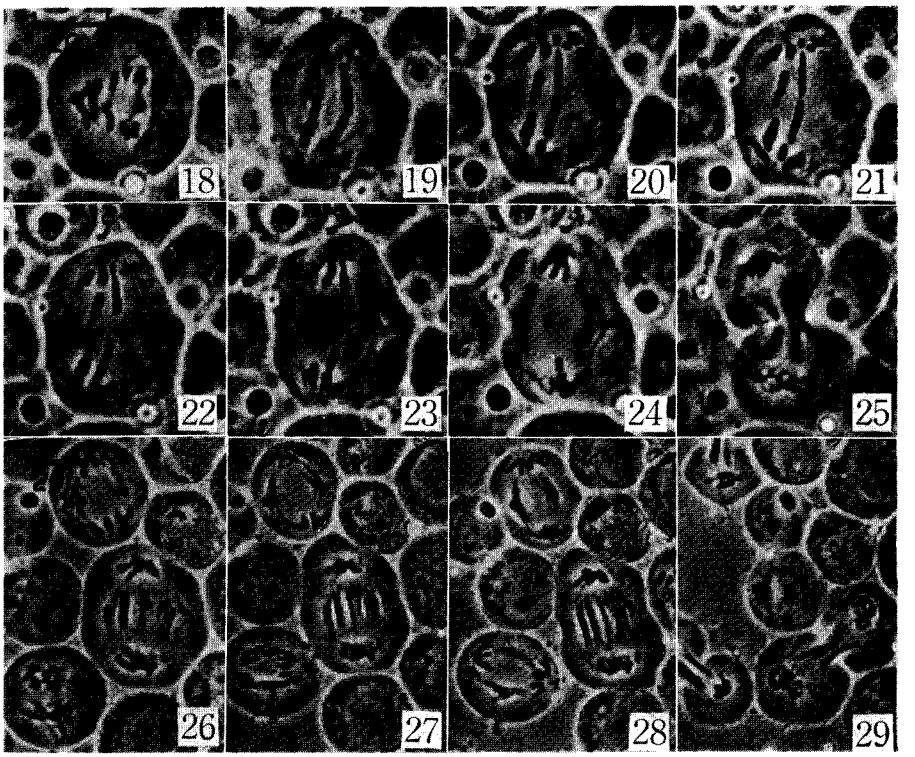
2. Results of irradiation at metaphase-I

In the majority of cells irradiated at metaphase with 1000 rads, the anaphasic migration of the chromosomes was disturbed in various degrees. The chromosomes migrated after separation, though not completely, to the opposite poles, but some of them formed two or three thick sticky bridges (Figs. 11–15). Shortly, all the chromosomes clumped into a single mass, and a nuclear membrane was formed around the mass, resulting in the formation of a reconstructed nucleus



Figs. 10–17. Selected film frames from a cine record of successive stages showing the formation of sticky bridges and an anuclear cytoplasmic mass in a cell irradiated at metaphase-I (film no. SS–87–1). The magnification for Figs. 10–13 and 15–17 is indicated by the scale on Fig. 10, and Fig. 14 as shown. 10, metaphase (taken 178′12″ after irradiation). 11, onset of anaphase (291′02″). 12–13, formation of sticky bridges (308′30″–332′48″). 14, showing the sticky bridges at higher magnification (367′26″). 15, all the chromosomes gradually aggregate into a mass (416′54″). 16–17, the mitochondrial bundles begin to push out the cell surface, resulting in the formation of an anuclear cytoplasmic mass (548′30″–724′41″).

(Figs. 16–17). In every case, however, the mitochondria seemed to remain unaffected. They lined up in the polar axis and elongated into a few bundles (Figs. 13–15). With further elongation the mitochondrial bundles began to push out the cell surface (Fig. 16). This resulted in the formation of an anuclear cytoplasmic



Figs. 18–25. Selected film frames from a cine record of successive stages showing the slight stickiness of chromosomes in a cell irradiated at anaphase-I (film no. SS–86–2). The scale is shown in Fig. 18. 18, just before irradiation, the chromosomes begin to migrate to the poles. 19, immediately after irradiation, two bivalents show sticky bridges. The film record was interrupted for 7 minutes. 20–23, the homologues become free from stickiness and join to the major chromosome groups (taken 5′20″, 6′20″, 7′50″ and 14′30″ after irradiation). 24–25, formation of a cleavage furrow (23′50″–32′05″).

Figs. 26–29. Selected film frames from a cine record of successive stages showing the completion of cell division in a regular manner in cells irradiated at telophase-I (larger cell) and -II (film no. SS–80–8). The magnification is in Fig. 26. 26, just before irradiation. 27, immediately after irradiation. The film reocrd was interrupted for 6 minutes. 28–29, the cleavage furrow cuts the cell body, as well as the mitochondrial bundles, into halves (taken 3′20″ and 27′30″ after irradiation).

mass. The constriction furrow appeared around them across just the middle part of elongated mitochondrial bundles, and shortly pinched off an anuclear bud from the mother cell (Fig. 17).

3. Results of irradiation at anaphase-I

The cells in the beginning of the anaphasic stage as seen in figures 18 to 25, in which some homologues had not been separated as yet, was irradiated with 1000 rads. Immediately after irradiation, two bivalents showed sticky bridges, probably due to their slight stickiness (Figs. 19–20). Shortly later the homologues, however, became free from stickiness and moved rapidly to the poles (Figs. 21–22). Then they joined to the major chromosome group at each pole (Figs. 23–24). The mitochondria seemed to be regular in behavior showing elongation into bundles, and the cleavage furrow cut the cell body, as well as the mitochondria bridges, into halves (Figs. 24–25).

4. Results of irradiation at telophase-I and -II

In the cells irradiated at the stage when the chromosomes had reached near the poles, the formation of daughter nuclei seemed to be unaffected; a cleavage furrow was formed at the end of telophase across the middle part of the mitochondrial bundles in quite such a regular manner as in the control (Figs. 26–29).

Discussion

The present phase cinematography study has recorded the results of beta-irradiation in some grasshopper spermatocytes selected at different stages of meiosis prior to irradiation. The mitotic abnormalities induced were: (A) the stickness of chromosomes and the disturbance of anaphasic migration to the poles when cells were irradiated at diakinesis; (B) the formation of chromosome bridges in cells irradiated at metaphase; (C) a slight delay of the anaphase movement of the chromosomes after the irradiation at early anaphase, and (D) the formation in a regular manner of the daughter nuclei and the cleavage furrow in cells when irradiated at telophase.

According to Lasnitski (1948), there is no striking difference in the proportion of degenerated avian fibroblasts *in vitro* when exposed to either X- or beta-rays at 1000 r. Working on the relative efficiencies of beta- and X-rays in lowering the mitotic count, she found that mitotic activity fell off more abruptly and rose more rapidly during recovery in the beta- than in the X-irradiated material.

The stickiness of chromosomes was common in the grasshopper spermatocytes when observed at rather early intervals after X-irradiation with comparatively higher dosages (Ohnuki 1958, Ohnuki and Makino, in press). Those workers suggested that the stickiness of chromosomes probably induced the disturbance of the spindle mechanism resulting in their anaphasic migration. From the results of the present observations, it seems apparent that the disturbance of anaphasic migration of the chromosomes to the poles in cells irradiated at diakinesis or metaphase may be caused by the stickiness of the chromosomes rather

than by destruction of the spindle mechanism, since in cells irradiated at diakinesis the chromosomes completed the equatorial arrangement in a regular manner, while in cells irradiated either at diakinesis or metaphase, the chromosomes failed to separate though strongly pulled to the poles, or formed sticky bridges at anaphase. Carlson (1941, 1954) reported that large doses of X-rays applied to dividing grasshopper neuroblasts showed no demonstrable effect on the structure or functioning of the spindle. Bloom *et al.* (1955), working with cultured newt cells, also observed that irradiation of the spindle with a proton beam produced no observable abnormalities in the spindle even through the exposures were 10,000 times as great as those that produced chromosome stickiness (bridges). Izutsu's observations (1959) on the grasshopper spermatocytes following ultraviolet microbeam irradiation showed that irradiation on a region of the chromosome not containing the kinetochore at the end of metaphase or early anaphase produced a chromosome bridge due to the stickiness at the irradiated site. The results of these observations would support the view that at anaphase the beta-rays produce stickiness of chromosomes which form bridges even though the spindle mechanism is not primarily destroyed.

Based on the results of direct observations of mitosis in living *Chortophaga* neuroblast following X-irradiation, Carlson (1941, 1950) has postulated that there exists a critical period between late and very late prophase. Neuroblast that had passed this stage at the time of treatment completed mitosis with little or no delay, if the dose was less than 250 r. Larger doses produced a delay that increased with the dose. He concluded that this seemed to result partly from chromosome "stickiness", which prolonged the anaphase separation of daughter chromosomes. Further, Carlson and Harrington (1955) reported that the chromosomes were more sensitive to the production of X-ray-induced "stickiness" at very late prophase and became progressively less sensitive toward anaphase. It has been shown by the present observations that stages arranged in a successively decreasing order of sensitivity as measured on the basis of the degree of the stickiness of chromosomes following beta-irradiation are diakinesis, metaphase and anaphase. No observable change was produced through irradiation at telophase.

The results of the present experiments have indicated that stickiness of chromosomes inhibits their migration at anaphase and results in formation of sticky bridges, while the elongation of mitochondria seems to remain unaffected leading to the formation of an anuclear cytoplasmic mass. A series of experimental studies with chemicals by Nakahara (1952), Makino and Nakanishi (1956), and Ohnuki (1956) demonstrated (A) that in untreated grasshopper spermatocytes the mitochondria elongated into bundles at telophase, stretching between the telophase nuclei, whilest a cleavage furrow was formed across the middle part of the mitochondrial bundles; (B) that the movement of the chromosomes to the poles was inhibited by caffeine, acriflavine, or sarkomycin, while the mitochondria regularly elongated into bundles and led to the formation of a cleavage furrow

resulting in the construction of two small anuclear buds from the mother cell; and (C) that in benzol- or phenol-treated material the chromosomes could move regularly to the poles after separation, whereas the mitochondria were inhibited from elongating, and the cleavage furrow was not formed at all.

A series of results presented suggests that mitochondrial elongation is closely associated with the formation of a cleavage furrow in cell division. It is interesting to know that the feature involving a similar function has been induced also by X-rays (Ohnuki 1958, Ohnuki and Makino, in press) as well as by beta-rays (this paper) in the grasshopper spermatocytes. Makino and Nakanishi (1956) have suggested that the elongation in turn causes sufficient tension to distort the cell wall, resulting in the subsequent formation of a cleavage furrow.

Summary

A phase cinematography study was undertaken to record the results of beta-irradiation from a strontium 90 source in some grasshopper spermatocytes selected at different stages of meiosis prior to irradiation. The mitotic abnormalities induced are: (A) stickiness of chromosomes and disturbance of anaphasic migration to the poles when cells were irradiated at diakinesis; (B) the formation of chromosome bridges in cells irradiated at metaphase; (C) a slight delay of the anaphase movement of the chromosomes after the irradiation at early anaphase, and (D) the formation in a regular manner of the daughter nuclei with the completion of the cleavage furrow in cells irradiated at telophase. It has been shown that the stages arranged in a successively decreasing order of sensitivity as measured on the basis of the degree of stickiness of chromosomes following beta-irradiation are diakinesis, metaphase and anaphase. No observable change was produced through irradiation at telophase.

Literature

Bloom, W., Zirkle, R. E. and Uretz, R. B. 1955. Irradiation of parts of individual cells, III. Effects of chromosomal and extrachromosomal irradiation on chromosome movements. Ann. New York Acad. Sci. 59 : 503–513.

Carlson, J.G. 1941. Effects of X-irradiation on grasshopper chromosomes. Cold Spring Harbor Symposia Quant. Biol. 9 : 104–111.

——— 1950. Effects of radiation on mitosis. J. Cellular Comp. Physiol. 35 (Suppl. 1) : 89–102.

——— 1954. Immediate effects on division, morphology, and viability of the cell. Radiation Biology. New York, McGraw-Hill Book Co. 1 : 763–824.

——— and Harrington, N.G. 1955. X-ray-induced "stickiness" of the chromosomes of the *Chortophaga* neuroblast in relation to dose and mitotic stage at treatment. Radiation Res. 2 : 84–90.

Izutsu, K. 1959. Irradiation of parts of single mitotic apparatus in grasshopper spermatocytes with an ultraviolet-microbeam. Mie Med. Jour. 9 : 15–29.

Makino, S. and Nakahara, H. 1955a. Behavior of the mitochondria in relation to the division of the anuclear cytoplasmic bud in grasshopper spermatocytes. Chromo-

soma 7 : 14–18.

——— and ———. 1955b. Study of cell division by phase microscopy. J. Hered. 46 : 245–251.

——— and Nakanishi, Y.H. 1955. A quantitative study on anaphase movement of chromosomes in living grasshopper spermatocytes. Chromosoma 7 : 439–450.

——— and ———, 1956. Behavior of the mitochondria in relation to cytoplasmic division in grasshopper spermatocytes. Chromosoma 8 : 212–220.

Nagai, J. 1958. Film dosimetry of a strontium 90 beta applicator. Nipp. Act. Radiol. 18 : 485–489.

Nakahara, H. 1952. Behavior of the mitochondria in cell division, with evidence concerning the kinetic function. Cytologia 17 : 168–178.

Ohnuki, Y. 1956. A phase microscopy study on the effect of sarkomycin upon the dividing spermatocytes of grasshoppers. Jap. J. Genet. 31 : 197–200.

——— 1958. 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. J. Fac. Sci. Hokkaido Univ. Ser. VI, 14 : 83–91.

——— and Makino, S. Phase cinematography studies on the effects of radiation and chemicals on the cell and the chromosomes, II. Formation of anuclear buds, continuation of chromosome stickiness and formation of an accessory nucleus in grasshopper spermatocytes following X-irradiation. Tex. Rept. Biol. Med. (in press).

Uretz, R.B., Bloom, W. and Zirkle, R.E. 1954. Irradiation of parts of individual cells, II. Effects of an ultraviolet microbeam focused on parts of chromosomes. Science 120 : 197–199.

Zirkle, R.E. and Bloom, W. 1953. Irradiation of parts of individual cells. Science 117 : 487–493.