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# Phase Cinematography Studies on the Effects of Radiation and Chemicals on the Cell and the Chromosomes, IV. Behavior of a Single Chromosome of Grasshopper Spermatocytes Received Beta-Irradiation in Relation to Cell Division[1)]

By

**Yuh H. Nakanishi**

(Zoological Institute, Hokkaido University)

*(With 26 Text-figures)*

In three reports formerly published in this series, the irregular behavior of chromosomes induced by X-rays with the whole body exposure and by beta-rays applied to individual cells selected for certain stages has been described in grasshopper spermatocytes (Ohnuki 1958, Ohnuki and Makino 1960, Nakanishi 1959). Recently, the development of microbeam irradiations with a proton, an ultraviolet or alpha particles has rendered possible the select irradiation of small parts of single cells (cf., Zirkle and Bloom 1953, Uretz *et al.* 1954, Munro 1959, Davis and Smith 1957).

The present paper describes the results of beta-irradiation with the use of a microbeam apparatus in a hope to learn the behavior and function of a single chromosome of grasshopper spermatocytes which received beta-irradiation in a restricted part of the cell, with reference to cell division.

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**Material and method** : The experiments were carried out with first spermatocytes of the grasshopper, *Podisma sapporense*. Preparations for beta-microbeam irraditation were made based on a new hanging-drop method : spermatocytes were mounted in a mixture of heparinized rooster plasma, chick embryonic extract, double distilled water and the body fluid in a proportion of 1:3:2:1. Detailed data will be published elsewhere by the author.

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The detailed structure of the microbeam apparatus used here was described by Sugahara and Horikawa (1959), The beta source was a strontium 90 which was deposited in the inner vesicle of a microscopic objective after removal of lens. It was screwed into the revolving nosepiece of the microscope. Thus the beta source and the objectives for observations were set to have the same center. They are quickly interchanged by turning the revolving nosepiece. An apparatus for producing variable microaperture consisted of two adjustable slit made of silver, 1 mm in thickness (Figs. 1–2). The two jaws can be adjusted to any desired width by sliding horizontally by means of a turning ring (Fig. 2, t), leaving a micro-aperture of square cross section.

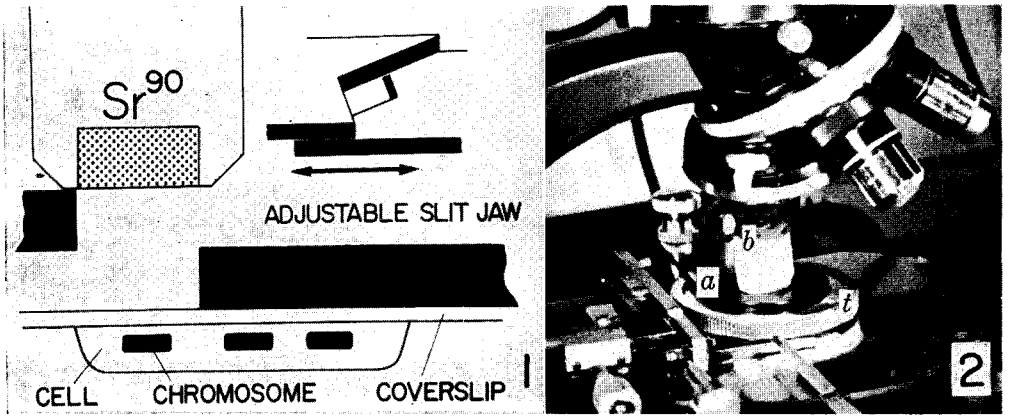
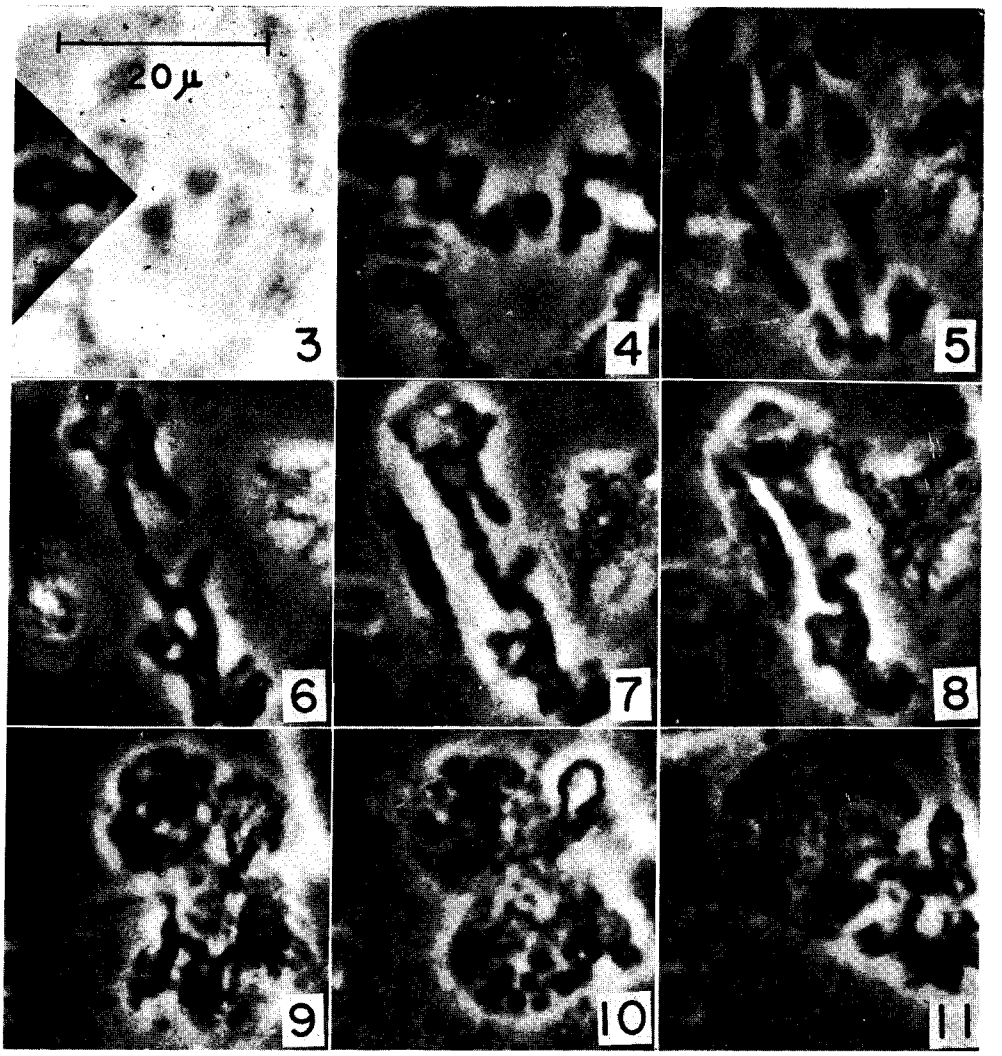


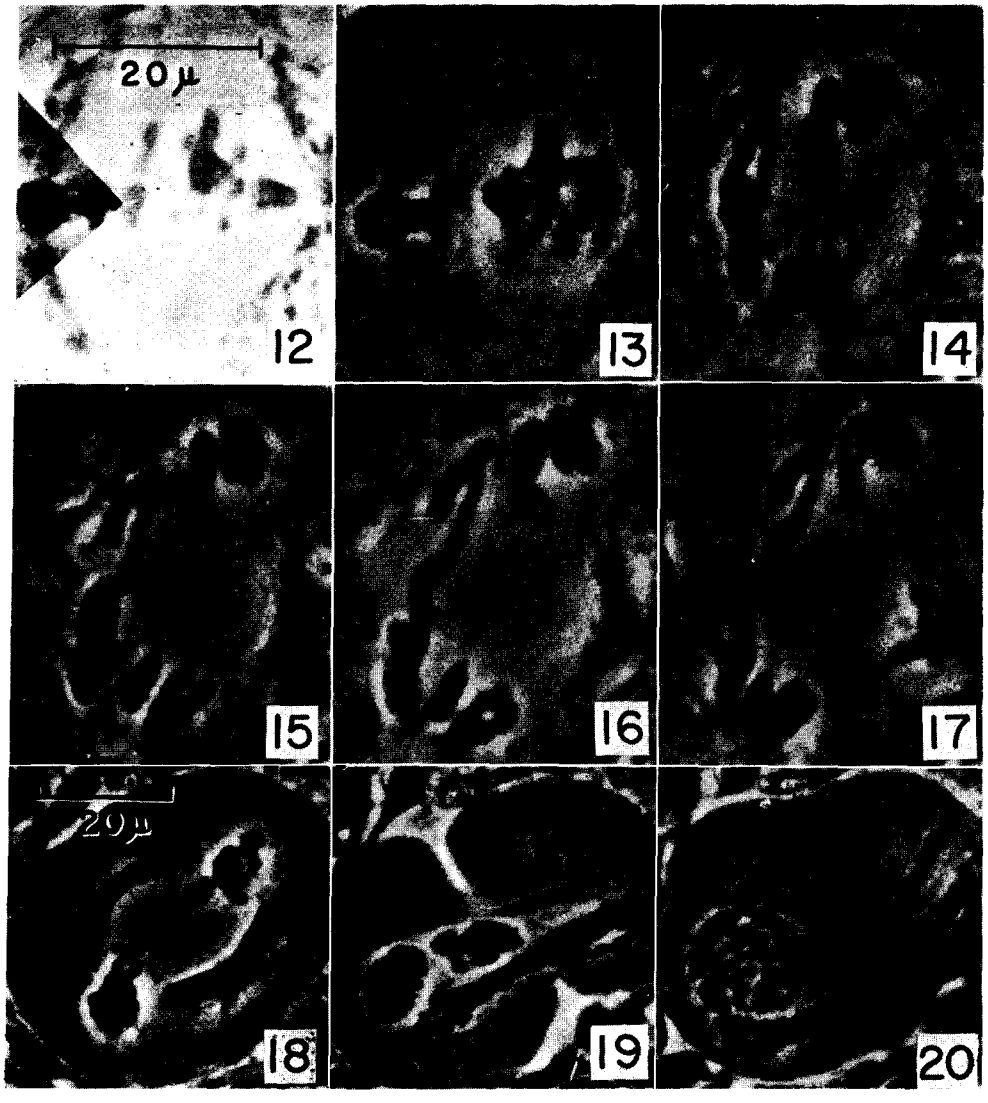

Fig. 1. Schematic diagram illustrating the procedure of beta-irradiation with a microbeam apparatus.

Fig. 2. Photograph showing the whole apparatus in the position of irradiation. b : beta source, a : apparatus for producing variable microaperture, and t : turning ring to adjust the aperture.

Because of geometrical requirements in practice, a spermatocyte at meta-phase-I was selected in which a large bivalent lay at the spindle contour being well apart from neighbouring chromosomes. The behavior of the chromosomes and mitochondria was recorded by the time-lapse camera for 15 minutes in most cases. Then the microbeam apparatus was placed in contact with the coverslip (Figs. 1–2). By direct observation of the cell through the aperture at 250× magnification, both the aperture of the adjustable slit jaws and the position of the cell were carefully adjusted so as to leave a restricted part in which a single bivalent for irradiation was presented and to shield all other parts of the cell (Fig. 1). After preliminary experiments with the use of various sizes of the aperture, 0.5 mm side length of the square aperture proved to be most suitable for this challenged experiment. Then the objective was rapidly raised and the beta source was




Figs. 3–11. Selected film frames from a cine record of successive stages showing the formation of a chromosome bridge and the reconstruction of a nucleus in a cell a restricted part of which received beta-irradiation (film no. SS-144-3). The scale is shown in Fig. 3. 3, just before irradiation, a part of the cell in which a single bivalent is present is confined with the aid of a microbeam apparatus, shielding all other parts of the cell (lightly printed). 4, immediately after irradiation. 5-7, anaphase, most chromosomes migrate to the opposite poles, while an affected chromosome forms a sticky bridge (taken 98', 110' and 130' after irradiation). 8–9, a cleavage furrow appears and acts to cut the cell body, but the formation of the cleavage furrow remains incomplete, leaving the chromosome bridge without its being cut off (133'–158'). 10, all the separated chromosomes clump into a single mass, together with the bridge, and a nuclear membrane appears around the chromosome mass (188'). 11, the reconstructed nucleus is sequeezed into one of the daughter cells, and it develops into a spherical nucleus (218').




brought downward to the surface of the aperture. The restricted area of the cell and a single chromosome were thus irradiated for 7 minutes to give approximately 1500 rads. Immediately after irradiation, the source was removed, the microscope was refocused, and only cells in which the chromosomes remained stable within the aperture were continued to be record. The film record was interrupted not more than 10 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 a 'Mikro-Kino-Kamera' (Zeiss). The sequences were taken at 4, 8 and 15 frames per minute at 22° to 26°C, with the shutter speed at 1 second. Figures 3–26 present selected film frames abstracted from the record (ED no. 60–1).

## Results of Observations

1. *Formation of a chromosome bridge and the reconstruction of a nucleus* : A part of a single metaphasic cell in which a bivalent was present was confined with the aid of a microbeam apparatus (see Fig. 3) and irradiated with beta-rays. About 90 minutes after irradiation, the cell entered into anaphasic stage : all the chromosomes began to separate synchronously and moved to the opposite poles. But, the affected chromosome formed a remarkable bridge, sticking together at its distal end (Figs. 5–7). The mitochondria even those lying in the irradiated area of the cell, were observed to be regular in behavior; after elongation they formed a few bundles. A cleavage furrow appeared and acted to cut the cell body into equal halves across the middle portion of mitochondrial bundles. But, the formation of the cleavage furrow remained incomplete, leaving the chromosome bridge without its being cut off (Figs. 8–9). Meanwhile all the separated chromosomes clumped into a single mass together with the bridge, and a nuclear membrane appeared around the chromosome mass, resulting in the formation of an hourglass-shaped nucleus (Fig. 10). With the lapse of time, the reconstructed nucleus was squeezed into one of the daughter cells probably by the function of the cleavage furrow. Later it developed into a spherical nucleus (Fig. 11). Similar results were obtained in four different cells after similar treatment.

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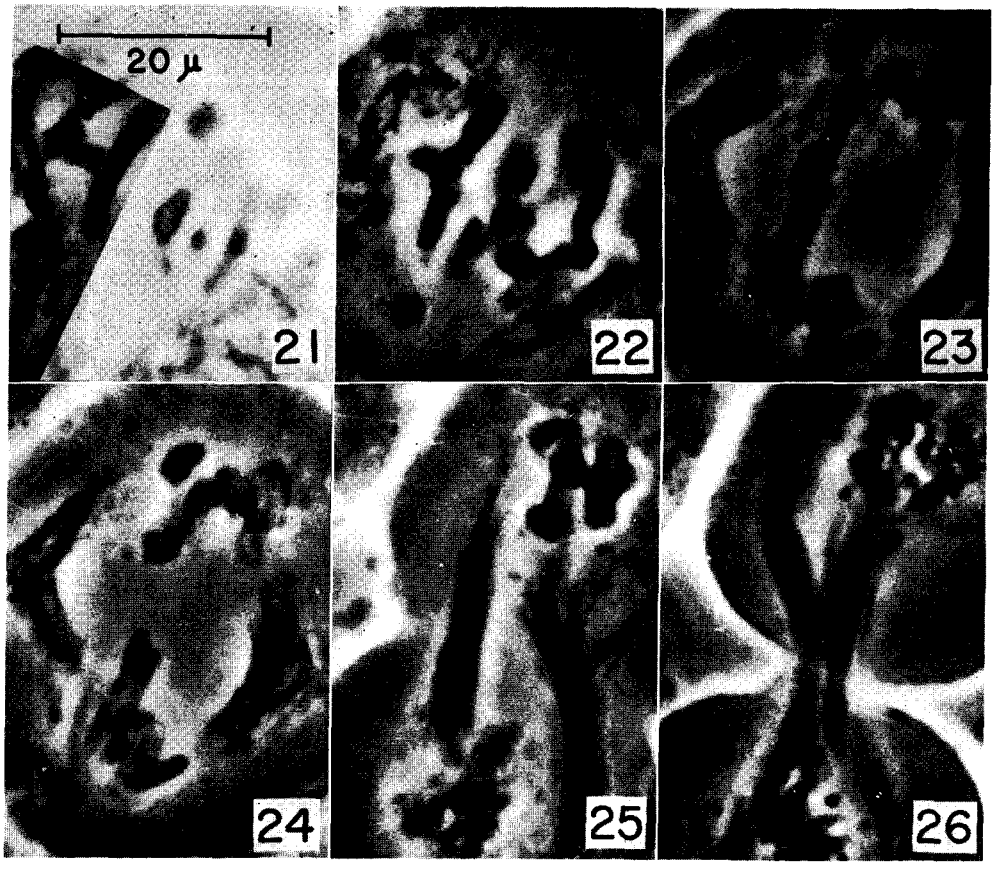
Figs. 12–20. Selected film frames from a cine record of successive stages showing the lagging of an affected chromosome and the reconstruction of a nucleus in a cell a restricted part of which received beta-irradiation (film nos. SS–146–6 and SS–147–1). The magnification for Figs. 12–17 is indicated by the scale on Fig. 12, and Figs. 18–20 as shown in Fig 18. 12, just before irradiation, a part of the cell in which a single bivalent is present is confined with the aid of a microbeam apparatus, shielding all other parts of the cell (lightly printed). 13, immediately after irradiation. 14–16, all the unaffected chromosomes complete their anaphase segregation, while an affected chromosome forms a sticky bridge (taken 130', 135' and 139' after irradiation). 17, the sister homologues forming the bridge become free from stickiness and join to the major chromosome groups at each pole (143'). 18, the chromosome group at each pole begins to move towards the equator each directing face to face the distal end of each homologue (159'). 19, the two chromosome groups nearly come together. A cleavage furrow appears but fails to constrict the chromosome mass (179'). 20, a single reconstructed nucleus is formed. The cell body becomes spherical in form (194').




Figs. 21–26. Selected film frames from a cine record of successive stages showing the lagging of an affected chromosome followed by regular cell division in a cell a restricted part of which received beta-irradiation (film no. SS–146–2). The scale is shown in Fig. 21. 21, just before irradiation, a part of the cell in which a single bivalent is present is confined with the aid of a microbeam apparatus, shielding all other parts of the cell (lightly printed). 22, the onset of anaphase (taken 19' after irradiation). 23, an affected chromosome remains stretching like a bridge between the unaffected daughter chromosome groups which have reached the opposite poles (32'). 24, the chromosome becomes free from its bridge-connection, and joins to the major chromosome groups (35'). 25–26, the cleavage furrow cuts the cell body, as well as the mitochondrial bundles into equal halves. The nuclear membrane is formed around each chromosome group, remaining trace of the laggard chromosome with a pointed outline in each (67'–97').

2. *Lagging of an affected chromosome and the reconstruction of a nucleus*: The above experiment has demonstrated that even the occurrence of a sole chromosome bridge prevents the development of the daughter nuclei at telophase and results in the formation of a reconstruction nucleus. Further striking evidence was provided in cells a part of which received beta-irradiation by a procedure as above described. The following descriptions were based on the observations of three treated spermatocytes which followed a similar pattern of abnormal division. About 110 minutes after irradiation, all the unaffected chromosomes completed their anaphase segregation. In contrast to the regular movement of the unaffected chromosomes, an affected chromosome formed a sticky bridge (Figs. 14–16). About 20 minutes later, however, the sister homologues forming the bridge became free from stickiness and moved slowly to the opposite poles. Then they joined to the major chromosome groups at each pole (Fig. 17). About 25 minutes were required for the anaphasic movement of the unaffected chromosomes (from separation to their arrival near the poles), whilst about 45 minutes for that of the affected chromosome. Shortly later, the chromosome group at each pole began to move rather rapidly towards the equator each directing face to face the distal end of each homologue (Fig. 18). About 20 minutes had lapsed when the two chromosome groups nearly came together into a mass (Fig. 19). In the course of the above movement, a cleavage furrow appeared across the elongated mitochondrial bundles, but failed to constrict the chromosome mass (Fig. 19). Meanwhile the chromosome mass migrated into one of the daughter cells just in a manner like a forcible entry. Then a nuclear membrane was developed around the chromosome mass resulting in the formation of a single reconstricted nucleus (Fig. 20). By this time, the mitochondria became free from bundles, and they were distributed in the cytoplasm in a rather normal manner. Later, the cell body, though it had been incompletely constricted into two parts, became apparently spherical in form (Fig. 20).

3. *Lagging of an affected chromosome with regular cell division*: In one of the cells which was irradiated at very late metaphase with the same manner as described above, the following evidence was presented (Figs. 21–26).

Approximately 19 minutes after irradiation, the cell entered into the anaphsic stage (Fig. 22). An affected chromosome remained stretching like a bridge between the unaffected daughter chromosomes which had reached the opposite poles (Fig. 23). About 15 minutes later, the affected chromosome suddenty became free from its bridge-connection, and joined to the major chromosome groups of the corresponding poles (Fig. 24). The nuclear membrane was formed around each chromosome group. But, the nucleus thus formed appeared not completely normal in outline, since it had remainder traces of the laggard chromosome with a pointed outline in each (Fig. 26). The cleavage furrow cut the cell body, as well as the mitochondrial bundles, into equal halves in an apparently regular manner (Figs. 25-26).

The data obtained in the three types of experiment as described above form the material for further quantitative analysis, which will be published elsewhere by the author.

## Discussion

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 investigations have been undertaken with interesting results (cf., Bloom *et al.* 1955, Izutsu 1959). More recently, it became possible to irradiate selected parts of dividing chick fibroblasts with alpha particles which came from polonium deposited on the tip of a micro-needle (Munro 1959), and also with an extremely fine pencil of alpha particles (Davis and Smith 1957). As far as the author is aware, however, no attempt has been made to irradiate selected and restricted parts of cells with beta-rays. In the present study, some experiments have been undertaken with the hope of determining the behavior and function of a single chromosome, which received beta-irradiation in a restricted part of the grasshopper spermatocytes, with regard to cell division.

Bloom *et al.* (1955), working with cultured newt cells following microbeam irradition with either a proton or an ultraviolet, demonstrated that the formation of anaphase hinges by suitable exposure of one side of the metaphase chromosome configuration resulted in the reconstruction of a single large nucleus. An apparently corresponding feature has been recorded by the present experiments that reconstructed nuclei are formed when the chromosomes fail to migrate to the poles due to chromsome stickiness, even though a single sticky bridge was formed (Figs. 3–11). In those cases, the chromosome bridge (or bridges) always joins together the daughter chromosome groups, and a single reconstructed nucleus is formed. The mechanism has however remained inexplicable regarding the reconstruction of a single nucleus in connection with the bridge-formation and lagging of a single chromosome which received beta-irradiation in a restricted part of the cell (Figs. 12–20), no adequate literature pertaining to this subject being accessible to the author, except for Hsu and Moorhead's observations (1956) on an untreated HeLa cell in culture. According to them, the chromosomes which had migrated regularly to the poles at anaphase moved back to the center of the cell, with the formation of a single nucleus. No discussion on this feature was given by them.

There is available evidence that the interzonal connections are nothing other than a sticky coating of the chromosomes which is stretched like mucilage between the daughter chromosomes as they move further and further apart (Schrader 1953). In microdissection studies of living neuroblasts of *Chortophaga*, Carlson (1952) proved the existence of the interzonal connections between the separated daughter chromosomes. In the spermatocytes of *Podisma sapporense* employed in the present experiments, the interzonal connections are not observable at least through phase-optic observations. However, there is a possibility that a very slender thread-like element, although invisible with pahse-optics, may connect the two sister chromosomes which have once formed a sticky bridge. Since the surface stickiness of

chromosomes might be strikingly increased in the irradiated elements, it is probable that the sticky surface may form a slender element like an interzonal connection. The author's further interest is attracted to the findings of Carlson (1946, 1952) which showed that the polar regions of the spindle had a much higher viscosity than did the interzonal region at late anaphase of *Chortophaga* neuroblasts. Similar results were also obtained by Bajer (1953) in endosperms of *Haemanthus katharinae*.

The discussions based on repeated projections of the cine records provided an impression that the coming together of the chromosomes after their telophase segregation may probably be taken place through the function of slender elements formed by the sticky surface of chromosomes, though no experimental attempt to analyse it has been made. The force or functioning of the spindle which is responsible for moving the chromosomes to the poles may come to an end at the time when the affected sister homologues join to the major chromosome groups at each pole, on account of an abnormaly prolonged anaphasic period (approximately twice as long as that of the control). Then the daughter chromosomes may be pulled by the contraction of slender elements connecting them, and drift from the polar regions having a high viscosity towards the equatorial area, probably in conjunction with cytoplasmic streaming (comp. Danielli 1951).

In view of a very unsatisfactory status of current knowledge as expressed in the foregoing discussion, the problem is far from being understood. For final understanding the evidence presented in this study, a detailed quantitative analysis of data is highly demanded with measurements of the chromosome movement as well as other geometrical changes of the cell in relation to the lapse of time after irradiation. Though further discussion should be postponed until the accomplishment of the work, it seems to the author on the basis of observed facts that the cell generally completes its division under the mutual control by the functioning of all cellular organelles other than of particular elements such as the spindle and aster.

## Summary

The behavior of a single chromosome of grasshopper spermatocytes received beta irradiation in restricted part of the cell was studied in relation to cell division by phase cinematography. The mitotic abnormalities induced are: 1) formation of a chromosome bridge and the reconstruction of a nucleus, 2) lagging of an affected chromosome and the reconstruction of a nucleus, and 3) lagging of an affected chromosome followed by regular cell division. Evidence was provided that the occurrence of a sole chromosome bridge, even though which had been once formed and became free from its-bridge connection, prevented the development of the daughter nucleus.

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