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Author(s)	MOMMA, Eizi
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Anomalous Divisions of the Grasshopper Germ-cells Induced by the Treatment with α- or β- Peltatin[1] (Studies on Abnormal Nuclear Divisions, 8)

By

Eizi Momma

(Zoological Institute, Hokkaido University)

(*With 2 Plates*)

Abnormal cell divisions artificially induced by various means have been investigated by many workers. Kuwada (1937), Shinke (1937, '39, '41) and Shigenaga (1937, '44, '45, '49) extensively studied such abnormalities of artificially induced cell division. They pointed out that these abnormalities are closely associated with hydration and dehydration phenomena of cells.

Recently, podophyllin has attracted the attention of cytologists on account of its effect as a miotic poison (Sullivan and Wechsler 1947, Cornman and Cornman 1951). More recently, the effects of this drug on growth processes of tumors have been studied by some investigators (cf. Makino and Tanaka 1953). This background aroused an interest in studying the effect of this drug on germ cells of the grasshopper. Following the method of Nakahara (1952), the entire course of cell division of the grasshopper germ cells was traced in the living state using a phase-contrast microscope, under both normal and experimental conditions. The present study is concerned with the action of α- and β-peltatin, derivatives of podophyllin, on cell division of the grasshopper spermatocytes through the course of maturation division in the living state.

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Material and Method

Young nymphs of the common grasshopper, *Podisma sapporense* Shiraki, were employed as material for this study. For observations, the hanging-drop

1) Contribution No. 301 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

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method recommended by Nakahara (for detail, see Nakahara 1952) was adopted in order to follow the course of cell division in the living germ cells. Both α- and β-peltatin[1] were used at the concentrations of 0.02 per cent. To prepare 0.02. per cent solution of α- or β-peltatin, 1 mg of α- or β-peltatin crystals were first dissolved in 0.01 cc of 1 N NaOH, and then 5 cc of Ringer-Locke -Barta solution were added. The grasshoppers received 0.05 cc of 0.02 per cent solution per individual in both cases by intra-abdominal injection. The animals were left after injection for 60 to 120 minutes. Then the testes were taken out from the body ; the germ cells were removed from testicular follicles, and mounted with their own body fluid. Hanging-drop preparations were made according to Nakahara (1952). For control, Ringer-Rocke-Barta solutions alone and those mixed with 1 N NaOH were injected and observed in the same way. For study, the phase-contrast microscope was exclusively usd at room temperatures ranging from 20° C to 26° C. The scheme of the experiments is summarized in Table 1.

Table 1. Scheme of experiments.

Series	Solutions used	pH	Timelapse after injection (in hrs.)
Experiments			
E–1	0.02 per cent solution of α-peltatin	8.4	2.0
E–2	0.02 per cent solution of β-peltatin	8.4	1.5
Controls			
C–1	Ringer-Rocke-Barta sol.	8.0	1.5
C–2	Ringes-Rocke-Barta sol. 5cc + NaOH (1N) 0.01cc	8.4	1.5
C–3	Ringer-Rocke-Barta sol. 5cc + NaOH (1N) 0.02cc	>9.2	1.0-2.0

Observations

The observations to be described below deal exclusively with the primary spermatocyte division ; the course of division was traced through successive stages in a single cell from metaphase to telophase, with special regard to the behavior of the mitochondria and chromosomes.

E-1. Experiments with α-peltatin solution : Hanging-drop preparations were made from the testicular material two hours after injection of the drug. The process of division of the primary spermatocytes was disturbed in a considerable degree (Figs. 1–3, 4–8). Metaphase figures of the first division were unobtainable

1) The drugs, α-and β-peltatin, were supplied through the courtesy of Dr. M. J. Shear of the National Cancer Institute, Bethesda, Maryland, U. S. A. They were sent to Dr. Makino with kind suggestions for their use. Here the author wishes to express his cordial thanks to Dr. Shear.

in this series.

In the telophase stage, the migration of the chromosomes seemed to proceed in a regular course. But, the mitochondria were found to be irregular in their appearance and behavior. The mitochondiral elements united to form a compact bundle, highly different in feature from the normal one which is rather cylindrical in form (Figs. 1, 4). The end of the mitochondrial bundle showed a projection at one side of the telophase nucleus (Fig. 1). Occasionally it surrounded the daughter nucleus (Fig. 4). The daughter chromosomes, after migration in group to the opposite poles, came close together resulting in the formation of the restitution-nucleus, or produced a bi-nucleate cell due to failure of the formation of the cleavage furrow (Figs. 1–3, 4–8). In addition to these abnormalities, the amorphous aggulutinations of the chromosomes into different bodies together with irregular mitochondrial masses were observed, though not frequently (Fig. 9).

E-2. Experiments with β-peitatin solution: The following observations were made 90 minutes after the injection of the drug. A few cells at the first metaphase followed the regular course of division, dividing into daughter cells (Figs. 10–15). Most cells however, exhibited abnormality during the course of division. Remarkable abnormalities observed here were the formation of heteroploid cells (Figs. 16–22) and unravelling of the chromonemata (Figs. 23–25). The course of the formation of heteroploid cells was as follows: One of the daughter groups of chromosomes remained lying on the way of the spindle along the cylindrical bundle of the mitochondria (Fig. 16). Then, the cleavage furrow was formed across the middle part of the mitochondrial bundle dividing this daughter chromosome group into two parts (Fig. 18). As a result, one daughter cell thus produced receives a part of the daughter chromosomes and the other cell contains a complete set of the daughter chromosomes together with a few elements from another set (Figs. 19–20). Each part of the chromosome group was enclosed by the nuclear membrane, and one large and one small nucleus having unequal chromsomes were produced. Then these two nuceli came together into one body (Fig. 22).

The unravelling of the chromonemata was observed in the first metaphase chromosomes with on extremely deformed outline (Figs. 23–25). Regardless of this abnormality the behavior of the mitochondria seemed to be nearly regnlar (Fig. 25).

3. Control series (C-1, C-2 and C-3): Three control series were examined in this study (Table 1).

C-1: Injection with Ringer-Rocke-Barta solution was made in the same way as in the experiments. The observations made 90 minutes after injection revealed that the course of cell division in the spermatocytes proceeded regularly, showing a number of normally dividing figures, and resulting in the formation of normal daughter cells (Figs. 26–31). A single case of a tri-nucleate cell formation was observed (Fig. 32).

C–2. Injection of 1 N NaOH dissolved in Ringer-Rocke-Barta solution (see Table 1) was made. The observations made 90 minutes after injection revealed a number of normal divisions going on from metaphase to anaphase of the primary spermatocytes. A few abnormalities scuh as the formation of bi-nucleate cells (Figs. 33, 34), and of the anucleated cytoplasmic bud (Fig. 35) were observed.

C–3. Injection was made of the same solution as the above but with a higher pH-value (see Table 1). The material for this study was taken at 60 to 120 minutes after injection.

For the most part, the germ cells were found to follow the regular course of division, except a few abnormalities in the behavior of the mitochondria (Figs. 36–41, 42). At anaphase of the first division, the mitochondria distribute unevenly on the surface of the spindle body (Fig. 37). They migrate arund the equatorial plate at telophase, and aggregate into several irregular bodies (Fig. 38). Then, the formation of an incomplete cleavage furrow takes place at the part of the mitochondrial masses (Fig. 40). But the furrow disappears soon after (Fig. 41), resulting in the formation of the bi-nucleate cell. Figures 42 and 43 show the abnormal features of the mitochondria observed at telophase of the primary and the secondary spermatocytes.

Considerations

The results of the observations as described above indicated that germ cells of the grasshopper displayed various kinds of mitotic abnormalities in different degrees as a result of the treatment with α- and β-peltatin. On the whole, the effects of the drug either α- or β-peltatin on the behavior of the chromosomes and the mitochondria are nearly similar. Remarkable abnormalities are the formation of bi-nucleate and heteroploid cells.

Makino and Tanaka (1953), in their work on the effect of podophyllin upon the tumor cells of ascites sarcomas of rats, reported that the chromosomes were condensed into irregular bodies, and stuck together, forming several pyconotic clumps scattered throughout the cytoplasm. Such an effect as described by them was not observed in the present study, so far as the concentrations of the solution used are concerned.

As given in the foregoing descriptions, the formation of bi-nucleate and heteroploid cells was found as a remarkable ab-normality in the present experiments. In the formation of these abnormal cells, the behavior of the mitochondria was found regular in most cases, while irregular behavior of the mitochondria occurred in a few instances. In the case of regular behavior of the mitochondria the formation of the cleavage furrow took place in the usnal manner. In the cells which showed irregular behavior of the mitochondria, the formation of the cleavage furrow was incomplete or entirely lacking.

Nakahara (1952), in his work on the effect of caffein and acriflavine on germ cells of the grasshopper, reached the conclusion that the mitochondria take a significant part in the formation of the cleavage furrow in cell division. The results of the present investigations lead to a similar conclusion: that the formation of the cleavage furrow at telophase takes place in close connection with the characteristic behavior of the mitochondria, regardless of the behavior of the chromosomes during cell division.

The study of Cornman and Cornman (1951) with marine eggs showed that the phenomenal potency of podophyllin and podophyllotoxin as mitotic poisons points to mitotic disruption as their mechanism of action, and that the destructive effects appear to exceed that explainable solely as a result of mitotic effects.

Summary

The effect of α- and β-peltatin, derivatives of podophyllin, on germ cells of the grasshopper (*Podisma sapporense*), in fresh condition was studied with the use of a phase-contrast micorscope. Remarkable abnormalities which occurred are the formation of the bi-nucleate and heteroploid cells. The course of the formation of these abnormal cells was successively traced in single cells, with particular attention to the behavior of the chromosomes and mitochondria. The behavior of the mitochondria was found regular in most cases, but a few cases were observed showing irregular behavior of the mitochondria. The formation of the cleavage furrow took place always in a regular manner in the case in which the behavior of the mitochondria was regular, while in the cells which showed the irregularity of the mitochondria, the formation of the cleavage furrow was incomplete or entirely lacking. The evidence suggests that the formation of the cleavage furrow at telophase takes place in close relationship to the behavior of the mitochondria.

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Explanation of Plates IX and X

Photomicrographs, from the hanging-drop preparations of living grasshopper spermatocytes. Magnification : ca. × 600.

Figs. 1–9. From the experiments with *a*-peltatin. Figs. 10–25. From the experiments with *β*-peltatin. Figs. 26–32. Control experiment, C–1 : Figs. 33–35. Control experiment, C–2. Figs. 36–43. Control experiment, C–3.

Figs. 1–3. Successive stages showing the abnormal behavior of the mitochondria at telophase.

Figs. 4–8. Successive stages showing the abnormal behavior of the mitochondria, and the separation of chromosomes into two daughter groups at telophase.

Fig. 9. Abnormal cell including some chromosome masses together with the mitochondrial bodies ot telophase.

Figs. 10–15. Successive stages showing irregular cytoplasmic movement during from metaphase to telophase.

Figs. 16–22. Successive stages in the formation of heteroploid cells during from telopase, resulting in the production of asymmetrical cells.

Figs. 23–25. Successive stages showing deformation of chromsomes at metaphase.

Figs. 26–31. Successive stages of regular division from metaphase to telophase showing the formation of two daughter cells.

Fig. 32. Abnormal cell including three nuclei at telophase.

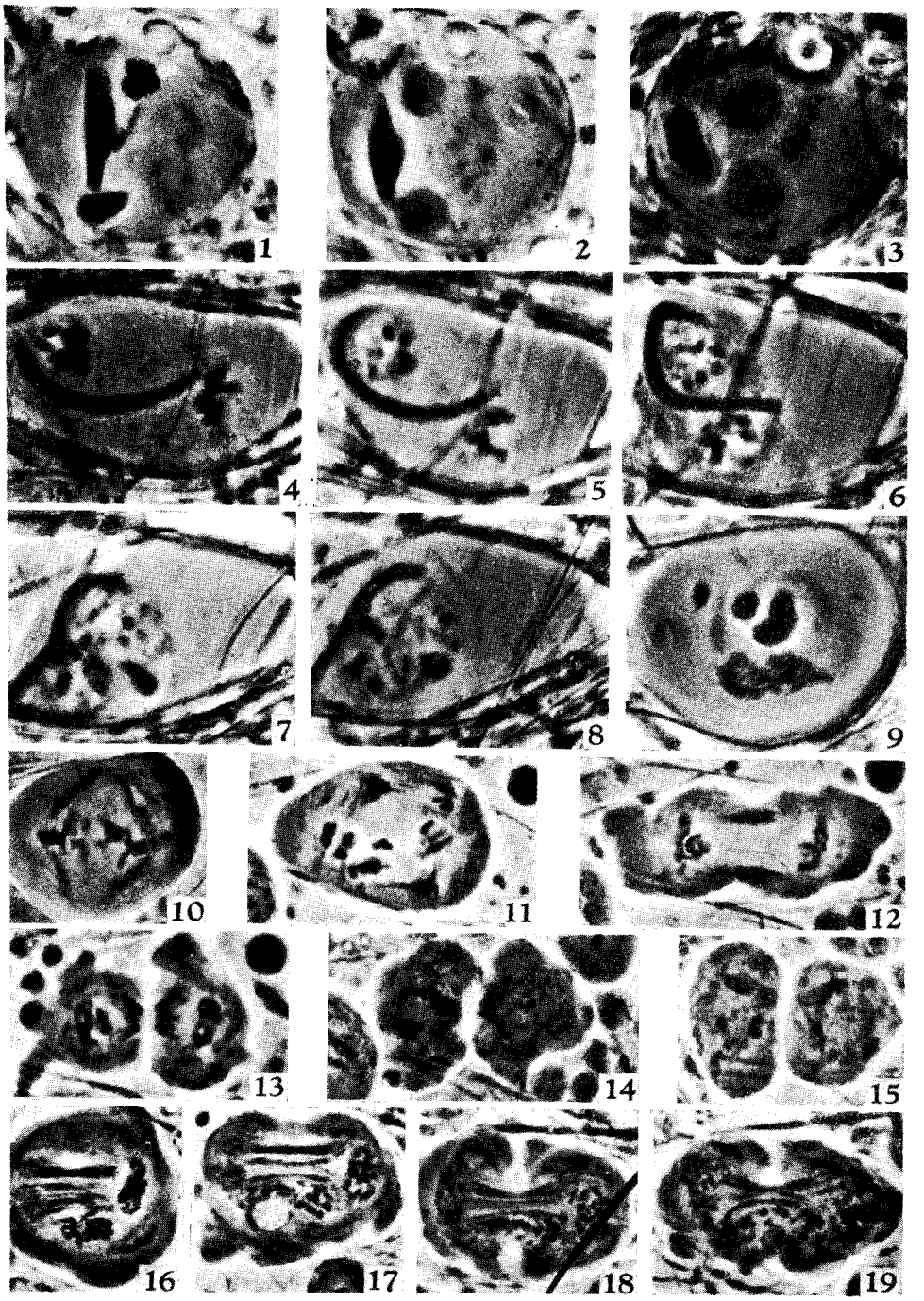
Figs. 33–34. Abnormal cells including two nuclei at telophase.

Fig. 35. A set of daughter cells including two nuclei and anuclear body.

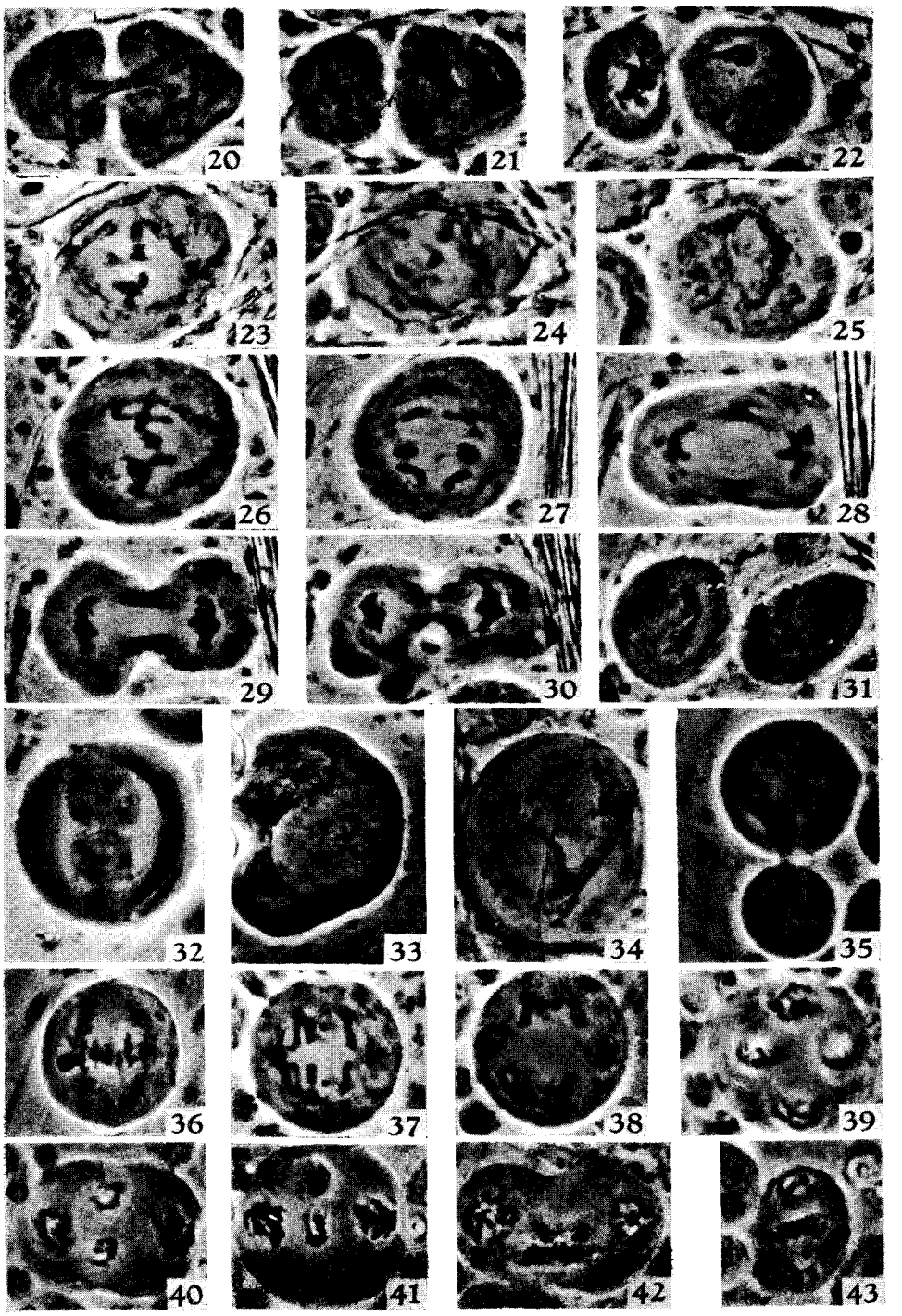
Figs. 36–41. Successive stages showing the formation of bi-nucleate cell caused by irregular behavior of the mitochondria.

Fig. 42. Abnormal cell including anomalous mitochondria at telophase.

Fig. 43. Abnormal cell including anomalous mitochondria at telophase of the secondary spermatocyte.



E. Momma: Effect of α- and *β-peltatin on Grasshopper Germ Cells*



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