Mitotic Abnormalities Induced by Nitrogen Mustard in Grasshopper Spermatocytes (Studies on Abnormal Nuclear Divisions, 5)[1]

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(*With 12 Text-figures*)

Following the discovery of the mutagenic power of mustard and some other chemically related substances, thanks to the brilliant works by Auerbach and Robson (1946) and some others, many investigations involving artificial induction of mutations by the treatment of nitrogen mustard have been carried out in Drosophila genetics. Owing to these findings, furthermore, mustard attracted the attention of cytologists, especially concerning the action of this drug on cell division. Gillette and Bodenstein (1946) have described the striking growth-inhibiting power of nitrogen mustard on the amphibian embryo, *Triturus trosus*. In *Tradescatia* exposed to mustard gas, various kinds of mitotic aberrations were observed by Darlington (1947). Novick and Sparrow (1949), in the growing root tips of *Allium cepa* treated with nitrogne mustard in aqueous solution at concentrations ranging from 0.0005% to 0.01% and for varying periods of time, reported that mitoses were usually eliminated within six hours, and that the percentage of aberrant anaphases gradually increased with time. All these works, however, deal with investigations using fixed material after treatment with the drug. It is highly desirable to observe the action of mustard through the course of cell division in a living condition. With this idea in mind, the author attempted the present study with grasshopper germ cells as material, the results of which will be reported in this paper.

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Meterial and Method: The present experiment was carried out with male germ cells of the grasshopper, *Podisma sapporense*. Nitrogen mustard which was dissolved in Ringer-Locke-Barta's solution at concentrations of 1%, 0.5% and 0.2% was used. The mustard solution thus prepared was injected in the abdomen of the grasshopper. About 30 minutes after treatment, the testes were taken out from the body, and germ cells from testicular follicles gently mounted on the cover-slip with an application of body fluid. With the material thus mounted the hanging drop method was adopted. Observation was made with the aid of a phase contrast microscope, at a temperature kept about 17–18°C. The behavior of the cell and chromosomes in response to the application of nitrogen mustard was observed in both the primary and secondary spermatocytes in living state.

The results of observations

The cytological abnormalities induced after treatment with nitrogen mustard are as follows:

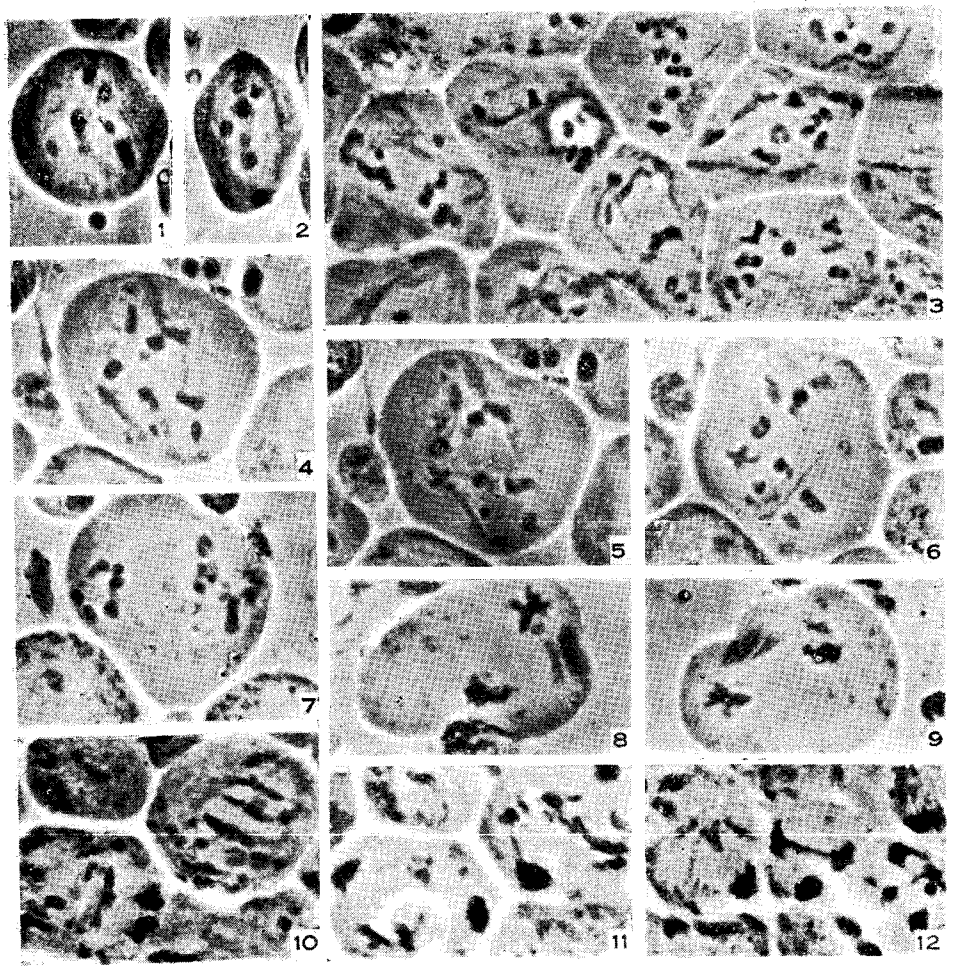
1. *Treatment with a* 1% *solution*: All spermatocytes were found in dead condition when observed. The chromosomes coagulate into irregular masses, and scatter in an irregular manner on the equatorial plate (Figs. 1, 2). The mitochondria and the spindle body were no clearly seen.

2. *Treatment with a* 0.5% *solution*: Remarkable abnormality in this group is the disturbance of the metaphase arrangement of chromosomes. The chromosomes showed various types of unusual arrangement irregularly scattering on the equatorial plate (Figs. 3–7). The arrangement and behavior of the mitochondria seem also to be considerably disturbed; sometimes the mitochondria were found abruptly breaking into the spindle body (Figs. 3–7). In some cases the drawing out of some bivalents in sticky condition was formed between the two ploes (Fig. 7). In most cases observed here, the cells failed to devide; this resulted in the formation of the bi-nucleate cell (Figs. 8, 9).

3. *Treatment with a* 0.2% *solution*: Stickiness of the chromosomes is the abnormality of common occurrence; they tended to stick together end-to-end or side-by-side (Figs. 10–12). Chromosome bridges at anaphase, the formation of chromosome fragments, the breaking down of inner chromonema spirals and the coalescence of chromosomes were produced due probably to stickiness of chromosomes (Figs. 10–12). The behavior of the mitochondria was also disturbed to a considerable extent; they coagulated into irregular masses during the period from metaphase to telophase instead of forming elongated bundles (Figs. 11, 12). The division of the cell body proceeded in many cases observed, though not completely, but in the extreme of nuclear abnormality the cellular division was prevented.

Remarks

Various mitotic abnormalities induced by nitrogen mustard in the spermato-



All are photomicrographs taken with the aid of "MIKAS". Magnification : ×650. Figs. 1–2. Coagulation and irregular scattering of chromosomes in primary spermatocytes after treatment with 1% nitrogen mustard solution. Figs. 3–9. The preimary spermatocytes after treatment with 0.5% nitrogen mustard solution. 3, irregular scattering of chromosomes. 4–6, successive stages in one and the same spermatocyte, showing the behavior of mitochondria. 4, 60 minutes after treatment. 5, 150 minutes after treatment. 6, breaking of the mitochondria into the spindle body ; 250 minutes after treatment. 7, drawing-out of bivalents between the poles; 280 minutes after treatment. 8–9, formation of the bi-nucleate cells ; about 200 –300 minutes after treatment. Figs. 10–12. Primary and secondary spermatocytes after treatment with 0.2% nitrogen mustard solution. 10–11, sticky chromosomes in the primary spermatocytes. 12, formation of chromoaome bridges and chromosome fragments.

cytes of *Podisma* have been described in the foregoing section. In the following, the results will be reviewed with some considerations.

In the treatment with 0.2% solution, the chromosomes alone were found affected by the drug; stickiness of chromosomes was specially conspicuous. Sticky chromosomes caused the chromosome bridges, the chromosome fragments, and the separation of chromosomes at telophase in most cases.

The exposure to 0.5% solution induced abnormalities of the chromosomes, mitochondria and spindle body. The abnormal arrangement of chromosomes at metaphase was remarkable. The evidence suggests that nitrogen mustard acts to injure the function of the spindle fiber or the kinetochor. At the same time, the behavior of the mitochondria becomes abnormal. Thus 0.5% solution of this drug causes spindle disturbance, and therefore, cell division remained incomplate resulting in the formation of the bi-nucleate cell in the majority of cases.

Under the influence of 1% solution, the chromatic material not only becomes pycnotic, but also both the behavior of mitochondria and the formation the the spindle body are disturbed. As a result the degeneration of the cell proceeds.

The spermatocytes of *Podisma* failed to complete division under the exposure to 0.2% solution of nitrogen mustard. At the same time the chromosomes showed marked stickiness. Darlington and Koller (1947) considered that stickiness and other related abnormalities are attributable to action of an excess of nucleic acid on the chromosomes. Novick and Sparrow (1949) stated that the mustards have an affinity for nucleo-proteins and may cause intranuclear precipitation. But the mechanism of the bridge-formation and of fragmentation of chromosomes remains still unknown.

In the 0.5% treatment of this experiment, the chromosomes invariably showed various types of irregular arrangement. The evidence seems to indicate that nitrogen mustard probably has an inhibiting effect on the spindle fiber or on the kinetochore.

In the present observations, the course of the fragment formation was successively traced through cell division. At first, the chromosomes become sticky. Then, sticky chromosomes form bridges stretching between the poles. The bridges were broken down into fragments in the course of telophase.

It is noteworthy that the division of the cell body remains always incomplete in the cell in which mitochondria became abnormal under the influence of nitrogen mustard. Viewed from this evidence, and considered from the results of experiments by Nakahara and Makino (1951) and Nakahara (1952), it is most probable that mitochondria take an important part in connection with the division of cell body.

Before finishing, it may not be out of place to state here the hydration and dehydration theory regarding the cause of abnormal mitosis postulated by Kuwada (1937). Shinke (1939), Sigenaga (1949) and Kano (1951), have dealt with mitotic abnormalities in both plants and animals based upon experimental

data obtained by subjecting cells to abnormally high or low temperatures, by treating them with various chemical drugs or some hypotonic or hypertonic media, or by exposing them to X-rays. The majority of the abnormal figures experimentally obtained by these authors are very much like those obtained in the present study. Kuwada (1937) and his followers, on the basis of their experiments, expressed the view that a change of water relation in the cell plays an important role in the production of mitotic abnormalities. Further, these experiments have shown that most of the mitotic abnormalities of common occurrence in nature or in experiments are regarded as being connected largely with the disturbance in normal water relation in the cells. In the light of these facts, the hydration-dehydration theory of abnormal mitosis seems to be significant for the case of the present experiment.

Here, it is very interesting and important that there is a striking similarity in a number of morphological characteristics, between the mitotic abnormalities observable in tumors, and those caused by artificial means, as pointed out by Makino and Yoshida (1951). It is therefore highly necessary to inquire into the nature and origin of these mitotic abnormalities experimentally induced for the elucidation of many cytological problems of tumors now not clear.

Summary

Mitotic abnormalities induced by nitrogen mustard in the spermatocytes of *Podisma sapporense* were observed in living condition with the aid of the phase contrast microscope.

The abnormalities observed here are as follows; stickiness and coalescence of chromosomes, chromosome bridge at anaphase, chromosomal fragmentation, deformation of chromosomes into irregular bodies, scattering or displacement of chromosomes at metaphase, breaking down of inner chromonema spirals, formation of bi nucleate cells, failure of spindle formation, irregular behavior of mitochondria during cell division.

Some considerations were offered on the cause of the appearance of mitotic abnormalities.

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