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Some Observations on Abnormal Nuclear Divisions in Grasshopper Testes treated with Colchicine [1]

(Studies on Abnormal Nuclear Divisions, 2)

By

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(With 26 Text-figures)

Following the discovery made by Blakeslee and Avery (1937) dealing with artificial induction of polyploidy with application of alkaloid colchicine, an enormous number of works have been produced in this field in plant cytology; the literature may be referred to colchicine bibliography published by Eigsti and Dustin (1947). In animals, however, the reports in which colchicine has been applied as an agent for induction of polyploidy are rather meagre (Dunham 1939, Hirobe 1939, Keppel and Dawson 1939, Dunham and Banta 1940, Gelei and Csic 1940, Inaba 1941). Still further, very little have been known about the effects of colchicine upon chromosomes in animals. In the present investigation, colchicine treatment were tried on the testes of a grasshopper, *Podisma sapporoense*, with the hope of ascertaining what changes of the chromosomes are taken place in germ cells under the influence of this chemical.

The work has been carried out under the guidance of Professor Sajiro Makino to whom the author wishes to express his sincere obligation, for helpful suggestion and the revision of the manuscript.

Material and Method

The testes from nymphs of a grasshopper, *Podisma sapporoense* (Acrididae), were removed by vivisection and immersed in 0.01, 0.05 and 0.1 per cent solutions of colchicine prepared with Ringer's solution of the inverte-

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brate formula for a period of one, three, five, ten, twenty and thirty hours long. After the treatment the material was taken out of the solutions and fixed immediately in Allen-Bouin's solution. A part of the material was left for the control in each series and immersed in Ringer-solution of the invertebrate formula under the same condition as colchicine treatment. A series of 10 per cent saccharose solution in which the testes were left for thirty hours served also as control. The sections of the testes were prepared through the paraffin method as usual and stained after the Heidenhain's iron-haematoxylin method.

In addition to the above series of experiments, 0.2 cc of 0.01 per cent Ringer-colchicine solution was injected in the abdomen of six males and then the animals were reared for 100 hours. In this series the chromosomes were also examined in the same way as above.

Most of the present observations have been carried out on the chromosomes of the primary and secondary spermatocytes, except a few cases in which the spermatogonial divisions were observed.

Results of Observations

1) Treatment with a 0.01 per cent colchicine solution

The testes were exposed to a 0.01 per cent solution of colchicine for a period of three, five, ten, and twenty hours. They were fixed immediately after treatment. The results of the cytological investigation are as follows:

In the series of the three-hour-treatment, the testes contain both of the normal and abnormal divisions of the chromosomes in nearly equal number. As shown in Fig. 1, some of the spermatocytes proceed regularly in division, showing the normal number of chromosomes, while in Figs. 2 to 5 are shown several abnormal divisions of primary spermatocytes. The metaphase plate as shown in Fig. 2 contains a certain number of bivalent chromosomes with addition of a few elements which are dyad in appearance, while that indicated as Fig. 3 shows most of the dyad chromosomes together with a few of a bivalent form. Another abnormality is seen in the separation of chromosomes at anaphase, the number of chromosomes migrating to poles is unequal (Figs. 4 and 21). Unipolar (Figs. 5 and 21) and tripolar divisions (Fig. 21) make also their occasional appearance.

In the series of the five-hour-treatment, both normal and abnormal divisions are found, as occurred in the previous case, but the degree of abnormality is larger as compared with the former, being expanded to 62.0 per cent in occurrence. Most of the abnormality concern with irregular migration of daughter chromosomes at anaphase, as seen in Figs. 6 and 7.

Fig. 7 shows two cells after division, one of which contains only three elements.

The testes treated for ten hours show also both normal and abnormal divisions, in approximately similar proportion to the former case, namely the abnormal ones appearing in 65.7 per cent. In the majority of cases, the process of cell division proceeds to anaphase and here it is arrested (Fig. 22). It is noteworthy that the bivalent having the configuration of the so-called c-pair (Levan, 1938) makes its frequent appearance (Fig. 8).

There are observable, in the material treated for twenty hours, unequal migration of the chromosomes to poles, and tripolar and unipolar divisions. Occasional appearance of the diploid spermatocytes was also encountered in this series. Under the influence of this treatment the chromosomes tend to show a spiralized configration that seems to be a first sign of degeneration.

In fine, the effect of a 0.01 per cent solution seem to result in the induction of abnormal divisions, such as unequal migration of daughter chromosomes, and tripolar and unipolar divisions in shorter duration of treatment, while in longer exposure the polyploid cells are induced, and the chromosomes seem to carry a sign of degeneration.

2) *The treatment with a 0.05 per cent colchicine solution*

The observations were made immediately after one, five, twenty and thirty hours of treatments. In the material treated for one hour the induction of polyploid cells is most remarkable. Fig. 9 shows a primary spermatocyte containing a $2n$ number of chromosomes, all of them assuming the dyad form. Fig. 10 (Fig. 23) shows the same, including a $4n$ number, the chromosomes being of the monad form.

In the series of the ten-hour-experiment the formation of the polynucleated cells is very conspicuous. The size of the nuclei of the polynucleated cells is generally smaller than that of the normal ones. Such cells are usually provided with pycnotic nuclei (Fig. 24).

The effect of the twenty-hour-exposure, seems to be almost similar to the former case, but the appearance of the polynucleated cells is increased to 43.7 per cent. The configration including two nuclei which are connected with each other by bridges as shown in Fig. 11, makes often its appearance. This is regarded as the result of the incomplete separation of some chromosomes.

Under the treatment for thirty hours the germ cells entirely undergo degeneration. The testes are filled with the dead cells with pycnotic nuclei.

Figs. 1-8. Treatment with 0.01 percent colchicine. All figures show the cells of primary spermtaocytes. 1-5. Three-hour-treatment. 1, regular division. 2 and 3, process of formation of polyploid cells. 4, unequal migration of the chromosomes to the poles. 5, unipolar division. 6-7. Five-hour-treatment. 6, irregular migration of daughter chromosomes at anaphase. 7, two cells after the irregular migration of chromosomes. 8. Showing c-pairs in the treatment for a period of ten hours. **Figs. 9-11.** Treatment with 0.05 percent colchicine. 9 and 10, polyploid cells in the spermatocytes, after treatment of one hour. 9, $2n$ and 10, $4n$. 11, a cell including two nuclei connected with bridges, twenty hours exposure. **Figs. 12-17.** Treatment with 0.1 percent colchicine. 12 and 13, spiral configuration of chromosomes in primary spermatocytes. 14, spermatogonial division having a reduced number of chromosomes. 15, 16 and 17, polynucleated cells containing a certain number of micronuclei. **Figs. 18-20.** Showing stickiness of chromosomes due to injection of colchicine. 18-19, primary spermatocytes. 20, secondary spermatocyte.

3) The treatment with a 0.1 per cent colchicinesolution

The material was immersed in the solution for a period of five, twenty and thirty hours. As the influence of the fiveh-our-treatment, irregular migration of chromosomes to the poles, the induction of polyploid cells and appearance of the spiral configuration of chromosomes are remarkable, occurring in 54.5, 30.4 and 15.1 per cents respectively. Any normal divisions can be observed no longer in this series. Figs. 12, 13 and 25 show the chromosomes of the primary spermatocytes assuming the outline of the spiral configuration. Fig. 14 indicates the metaphase of the spermatogonial division, in which the number of chromosomes contained is highly reduced to about 15 in number.

In the material treated for twenty hours a number of cells are found in process of degeneration, as the chromosomes observed here take the outline of spiralization. They appear in the frequency of 64.7 per cent. The polynucleated cells and those containing a certain number of micronuclei as well make a frequent appearance (Figs. 15, 16 and 17).

In the case of the thirty-hour-treatment the germ cells entirely undergo degeneration, and there is no sign of the cell division.

4) Injection of colchicine solution

A 0.01 per cent solution of colchicine prepared in Ringer's solution was injected in the abdomen of six males, 0.2 cc in volume per individual, using an injection-syringe with a fine needle. Only one individual of them was survived. After having been reared for one hundred hours, the testes were fixed and examined cytologically.

The most conspicuous evidence observed herewith is the clumping together of the chromosmes leading to the difficulty of their separation. Figs. 18, 19 and 20 show stickiness of chromosomes which is remarkable in an at random fusion taking place either side-by-side or end-to-end in any chromosomes.

5) Observations in the control series

The testes were exposed to Ringer's solution of the invertebrate formula for a period of three, five, ten, twenty and thirty hours under the same conditon as with colchicine. In the case of the shorter duration of the experiment, most of the cells seem to be regular in appearance. But if the duration is longer, the irregular divisions become proportionally increased. The polyploid cells and irregular migration of the chromosomes to the poles occur in some cases. The spiral configuration of the chromosomes makes its occasional appearance in the material of the longer treatment.

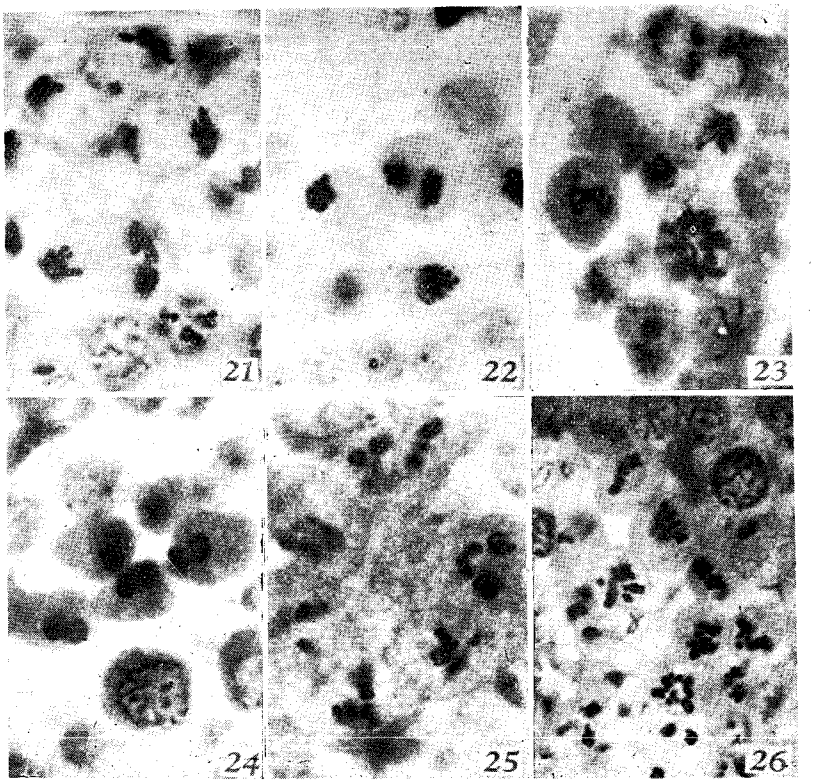
Another series of the control experiments was carried out with 10 per cent aq. solution of saccharose. The material was immersed in this solution for thirty hours. After observations it becomes evident that the process of cell division proceeds rather regularly and no abnormality is observable. To judge from this result, it is presumable that a 10 per cent solution of saccharose serves as an adequate culture medium for germ cells of grasshoppers, at least of *Podisma*.

Remarks

The field of investigations concerning the artificial induction of polyploidy with the application of colchicine is a current subject in plant cytology, and a great number of works have been contributed along this line (cf. Eigsti and Dustin 1947). To sum up the results of many authors so far published on the subject, it is to assume that the change in the process of cell division caused by colchicine is in the first place the inhibition of spindle formation, and that this inhibition gives rise to the form of cell with double chromosomes number. Wada (1939), relating to this problem, expressed the view that the primary effect is chiefly the change in surface tension of the atractoplasm. Recently, Sigenaga (1945) has observed with living petal cells of *Tradescantia* concerning the influence of toxic substances such as KCn, NaN_3, uretan, auramin, colchicine, acenaphten, cacodylate, etc., and pointed out that the polyploid and polynucletaed cells are frequent, owing to the effect of those chemical agents. Under the influence of colchicine, aggregation and scattering of chromosomes were also reported by Ludford (1936), Bucher (1939), and Sato (1939). Sigenaga (1945) is of the opinion that the chromosome scattering may probably be attributed to the swelling of the spindle. Beams and King (1938) and Yamaha and Ueda (1940) have stated that the cytoplasmic viscosity decreases under the influence of colchicine. In the study working with toxic chemicals upon living plant cells, Sigenaga (1945) gave the general conclusion that the effects of toxic chemicals are different in different chemicals and different concentration, but that there is a common effect among them to cause the coagulation or dehydration of chromosomes and spindles. He has added further that some chemical substances such as colchicine, acenaphten and cacodylate act on the spindle in two ways, dehydration on one hand and hydration on the other, and further that in these cases the intercelluler change in water-relation seems to be more complicated than in the case of other toxic chemicals.

The results of the present investigations clearly show that the influence of colchicine is different according to different concentrations and to different durations of exposure. The abnormal figures encountered in

the author's experiments closely resemble those reported by Sigenaga (1945) in the experiments with toxic chemicals as above cited. Though no decided statement has been made for the cause of nuclear abnormalities observed



Figs. 21-26. All are photomicrographs taken with Leitz-Makam under 400×. 21, unipolar, tripolar and unequal migration of chromosomes in 0.01 percent colchicine for three hours (primary spermatocytes). 22, showing a cell in nuclear division proceeding to anaphase and here arrested. 23, a cell corresponding with Fig. 10 including $4n$ number. 24, polynucleated cells provided with pycnotic nuclei (0.05 percent solution ten-hour-treatment). 25, spiral configuration of chromosomes in 0.1 percent colchicine for five hours. 26, a cell division of primary spermatocyte in the control experiment in Ringer's solution for twenty hours. Prof. Makino photo.

in the present study, the interpretation given by Sigenaga (Cytologia 14, p. 117, 1945) seems to be most favourable for the cases of the present investigation too. Sinke (1939) who has made an intimate study on the effects of

different temperatures on the cells has pointed out that the majority of abnormal figures produced under the influence of high temperatures resemble the degeneration figures caused by hypertonic solutions. The similar result was attained by Momma (1950) in germ cells of the grasshopper, *Podisma sapporoense* treated with high temperatures. In the experiment by transplanting testes of *Podisma* in female bodies, various abnormal figures, quite close to those obtained in the present investigation, have been reported to occur by the present author (Yosida, 1950). In the light of the findings obtained by Sigenaga and Sinke, it seems highly probable that occurrence of many mitotic abnormalities is closely associated with dehydration and hydration phenomena in the cell. Similar view has been expressed very recently by Nakamura and Makino (1950, the 1st report of this serial of study) n cryptorchid experiments of rats, with a consideration on the cause of artificial mitotic abnormalities.

Summary

The testes of the grasshopper, *Podisma sapporoense*, were immersed in 0.01, 0.05 and 0.1 per cent solutions of colchicine prepared with Ringer-solution of the invertebrate formula, for a period of one, three, five, ten, twenty and thirty hours. After the treatment they were taken from the solution and immediately preserved in fixative. The cytological observations were made on sections of the testes thus treated and prepared by the usual paraffin method.

Under the influence of colchicine treatment herein examined, many cytological abnormalities were encountered in male germ cells. Among them the remarkable ones are enumerated as below;

1. Abnormal divisions of chromosomes; namely, a) unequal distributions of chromosomes to the poles at anaphase, b) tripolar divisions, and c) unipolar divisions. 2. Polyploid cells; namely duplication of the chromosome number in the primary spermatocyte. 3. Polynucleated cells; namely a) binucleated cells and b) trinucleated cells. 4. The appearance of spiralized configulation in chromosomes.

In the case of the low concentration (0.01 per cent), the abnormalities relating to the division of chromosomes are remarkable; namely the unequal migration of chromosomes to the poles at anaphase, and polypolar and unipolar divisions are very frequent. In the case of 0.05 per cent solution, the induction of polyploid cells is striking, even in the short duration of exposure. In the medium of the high concentration (0.1 per cent) the death of the cells is caused even in the short duration of the experiment. Many cytological abnormalities leading to the degeneration of cells are aburdant. The results herein obtained are reviewed in the

annexed table (Table 1) by way of summary.

A tentative statement was made that the hydration-dehydration theory of abnormal mitosis seems to be significant for the case of the present investigation.

Table 1. Summary of the results obtained.

Conc. of colchicine	Hrs. treated / Types	1	3	5	10	20	Total
0.01 %	N.	%	50.0 %	38.0 %	36.3 %	27.2 %	40.4 %
	Abn.		46.1	62.0	65.7	18.1	49.2
	P.p	—	3.9	—	—	45.4	8.5
	P.n.		—	—	—	—	—
	Spr.		—	—	—	9.0	1.4
0.05 %	N.	36.8			22.2	18.7	27.2
	Abn.	36.8			33.3	12.5	27.2
	P.p.	26.4	—	—	11.2	6.4	15.9
	P.n.	—			33.3	18.7	13.6
	Spr.	—			—	43.7	15.9
0.1 %	N.			—		11.8	2.0
	Abn.			54.5		—	36.0
	P.p.	—	—	30.4	—	23.6	30.0
	P.n.			—		—	—
	Spr.			15.1		64.7	12.0
Control	N.		70.0	65.3	66.6	57.1	65.3
	Abn.		30.0	26.9	26.6	28.5	28.0
	P.p.	—	—	3.9	7.0	—	2.6
	P.n.		—	—	—	—	—
	Spr		—	3.9	—	14.2	4.0

Abbreviations used in the table are as follows: N=normal division of chromosomes. P.p.=polyploid cells. P.n.=polynucleated cells. Spr.=spiral configuration of chromosomes. Numerals denote the percentage calculated.

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