Differently Administered Colchicine Effects upon the Testis Cells of the Grasshopper, *Spathosternum pras niferum*

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It is an well known fact that colchicine, an alkaloid derived mainly from *Colchicum autumnale*, in contact with the dividing cells induces veritable changes in the chromosomes as well as in the spindle apparatus (see Eigsti and Dustin 1957). Since the discovery made by Blakeslee and Avery (1937) that polyploidy in plants could be induced by colchicine treatment, its use mainly by plant cytologists became extremely common for which Wellensieck (1939) described it as 'colchicine fad'. However, studies of colchicine effect on animal cells are relatively limited and the different lines of investigations may broadly be grouped in the following:—1) Attempts to induce polyploidy from the protozoan animals to mammals by various workers (see Eigsti and Dustin 1957). 2) Colchicine effect on animal tumour cells (Schairer 1940, Lettré *et al.* 1950, Klein 1951). 3) Colchicine pretreatment methods employed for analysis of mammalian (Hsu and Pomerat 1953, Ford 1961), and amphibian chromosomes (Manna and Bhunya unpublished). 4) Colchicine induced changes in the morphology of the cells and chromosomes (Yosida 1950, Gaulden and Carlson 1951) etc.

The present investigation was undertaken with a view to finding out the relative effectiveness of some known concentration of colchicine administered in two different ways, namely by injection and with food, into the body of the grasshopper, *Spathosternum pras niferum*. The studies made previously on the effect of colchicine in grasshopper cells were from either the testes immersed into colchicine solution after removal of the tissue from the body or injecting the chemical into the body (Yosida 1950), and from neuroblast culture (Gaulden and Carlson 1951).

Experimental procedure and methods

The control specimens of *S. pras niferum* were injected 0.02 ml. Ringer solution and the specimens were sacrificed at 20, 30, and 40 hour after injection. In the treated series, specimens were injected with 0.02 ml. of 0.1% colchicine prepared in Ringer solution and were sacrificed at the same intervals like those of control series (Table 1).

The control specimens of food administered series were fed with standard *Drosophila* food for about 20 hours and were sacrificed at 20, 30 and 40 hour after overnight feeding. In the treated materials of this series, the food was mixed with 0.1% colchicine solution and the specimens were fed and fixed just like the control series (Table 1).

Accurate determination of the amount of colchicine entered into the body of the treated specimens was not possible and it was still more uncertain in the food administered series. In the injection series casualties of specimens increased with the extension of post-treatment fixation time (Table 1). Since no casualty was observed in the control series, it was therefore, evident that the death was due to the toxic effect of colchicine.

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Cytological squash preparations were made from the dissected out testes following the technique of Smith (1943) modified by Manna (Unpublished). Some part of the materials was also cut 20 micra thick paraffin sections. Staining was mainly done with iron-alum haematoxylin.

Table 1. Details of experiments of the control and the treated specimens of *S. prasiniferum*

Types of Administration	Series	No. of specimens used in experiments		No. of specimens survived after treatment		Total specimens survived	Hour of fixation after treatment
		1st set	2nd set	1st set	2nd set		
INJECTION	Control (Ringer only)	3	3	3	3	6	20
		3	3	3	3	6	30
		3	3	3	3	6	40
	Treated (0.1% colch. in Ringer)	5	5	5	5	10	20
		5	5	5	4	9	30
		5	5	1	1	2	40
FOOD	Control (Drosophila food)	3	3	3	3	6	20
		3	3	3	3	6	30
		3	3	3	3	6	40
	Treated (0.1% col. in Drosophila food)	5	5	5	5	10	20
		5	5	5	5	10	30
		5	5	5	5	10	40

Observation

Qualitative study

Control series. In this series no perceptible changes from the normal meiotic behaviour of the chromosomes was observed in both the types of administrations. However, there seemed to be some general effect in the control series in comparison to normal individuals and that in the former the disposition of the chromosomes was relatively clear with some shortening effect. The general course of meiosis was agreeable to what has been recorded in this species by Asana, Makino and

Figs. 1-2. $\times 1100$. 1, spermatogonial metaphase of control specimen. 2, testis sheath cells showing C-mitotic effect. Figs. 1-12 are of *Spathosternum prasiniferum* drawn by Camera lucida.

Niiyama (1939). Spermatogonial metaphase complement consists of 23 acrocentric chromosomes, 22 autosomes and an X-chromosome (Fig. 1).

Treated series. Since all the types of abnormalities were present in materials injected or fed with 0.1% colchicine, the descriptions of the types of abnormalities would apply for both of them. The abnormalities could conveniently be put into four categories:—1) Mitotic changes, 2) changes in the meiotic prophase stages, 3) changes at metaphase I and at metaphase II, and 4) changes at first and second post-metaphase stages.

1) Mitotic changes:—Spermatogonial divisional stages were relatively rare. Among the small number of spermatogonial cells, sometimes C-mitotic metaphases were observed where the two chromatids of a chromosome lying parallel to each other and in some they were still attached at the centromere. In the testis sheath cell one type of effect was very conspicuous and that was clear disposition of the chromosome complement. The sheath cells in normal state are mostly endopolyploid in chromosome constitution. Due to the treatment sometimes these cells exhibited C-mitotic configuration (Fig. 2).

2) Changes in the meiotic prophase stage:—Pronounced disturbances were caused by colchicine administered by both ways. Multinucleate polyploid cells probably due to fusion of adjacent cells (Fig. 3) were more common than mononucleate polyploid cell (Fig. 4). The colchicine treatment impaired

Figs. 3-4. $\times 825$. 3, Multinucleate polyploid cell. 4, mononucleate polyploid diplotene stage.

upon the chiasma formation. The treated specimens had reduced chiasma frequency. Sometimes univalent chromosomes at late prophase stage were observed which might have originated due to either breaking down of chiasma or failure in forming a chiasma during early prophase stage. No breakage of chromosome was observed due to the colchicine treatment. However, in one instance the X-chromosome in a diplotene nucleus appeared to be broken.

The most noticeable effect of colchicine treatment was observed at the time of spindle formation particularly at the first spermatocyte prometaphase stage. The bivalents at this stage were haphazardly distributed because of the failure in the formation of the spindle. Most of the bivalents were forming small groups with interbivalent connections (Fig. 5).

3) Abnormalities in the first and second spermatocyte metaphase :—These stages were most vulnerable to colchicine effect. The chromosomes were relatively contracted. In the affected cells due to the destruction of the spindle at metaphase the bivalents were found to form small groups or they were uniformly distributed occupying the whole area of the cells (Fig. 6).

Deviation from the bipolar spindle to apolar and multipolar spindle (Fig. 7) were observed. Cells having multipolar spindle were polyploid in constitution. Second division metaphase spindle was also affected by the colchicine treatment and typical C-mitotic configuration was met with (Fig. 8). Ball (Fig. 9) or star shaped configurations were also observed. The chro-

Figs. 5-9. 5, prometaphase I with interbivalent connections. 6, metaphase I, bivalents scattered for the dissolution of spindle. 7, metaphase II with multipolar spindle. 8, metaphase II with C-mitotic effect. 9, metaphase II showing compact ball like appearance of the chromosomes.

mosomes were mostly much contracted in cells having haploid, diploid or polyploid constitution (Fig. 10).

4) Post-metaphase abnormalities :—Colchicine was found also to affect the spindle of post-metaphase stages. This was evident from the fact that the chromosomes after travelling some distance were found to be distributed haphazardly (Fig. 11). Colchicine seemed to affect the spindle gradually

because the chromosomal movement during separation was asynchronous due to which some chromosomes might lag behind (Fig. 12). Colchicine seemed to have the degeneration effect since some telophase nucleus was found to contain some pycnotic chromatin mass (Fig. 12).

Figs. 10-12. $\times 1100$. 10, polyploid metaphase II with condensed chromosomes. 11, failure of anaphase II for spindle dissolution. 12, telophase I, some chromosomes lagging behind and the nuclei forming pycnotic mass.

Quantitative study

In order to make a comparative study of the effect of known concentration of colchicine (0.1%) upon the testis cells, administered in two different ways, the frequency of four types of abnormalities e.g. C-mitosis, polyplid cells, polynucleate cells, and cells with destroyed spindle was determined (Table 2). The frequency was scored among 400 cells examined at that particular stage or stages of division for each type of abnormality at a particular hour of fixation.

An analysis of the abnormality data (Table 2) revealed some interesting facts. The frequency of C-mitotic cells in the tissues of the injected series dropped gradually with the enhanced hour of fixation e.g. 50% at 20 hour while 25.7% at 40 hour. A similar situation in the frequency of polyploid cells was observed in the food administered series. At 20 hour it was 41.5% which dropped down to 23.0% at 40 hour. Besides the above mentioned cases, the frequency of other abnormalities increased with the increment of the time of fixation. The reason for dropping the frequency with increased hour of fixation in the above mentioned cases was not known. However, it was interesting to note that with the advent of fixation hour in the injection

series while the frequency of C-mitotic cells dropped, the frequency of polyploid cells increased; and in the food administered series as the frequency of polyploid cells dropped, the frequency of C-mitotic cells increased. Since these two kinds of abnormalities are to some extent interrelated, there might have been some significance.

A general survey on the frequency or different types of abnormalities at all the different hours of fixation would reveal that almost in all cases the frequencies were relatively higher in food administered series than those

Table 2. Frequency distributions with their percentages of some types of abnormalities produced by 0.1% colchicine administered differently in *S. prasiniferum*

Types of abnormal	Mode of adminis	Frequency of abnormal cells among 400 cells at each fixation hour			Remarks
		20 hr.	30 hr.	40 hr.	
C-mitosis	Injection	200(50%)	174(43.5%)	130(25.7%)	Scored from spermatogonial metaphase II cells
	Food	183(45.7%)	290(72.5%)	260(65.0%)	
Polyploid cells	Injection	76(19.0%)	104(26.0%)	202(50.5%)	Scored from 1st and 2nd division metaphase cells
	Food	166(41.5%)	140(35.0%)	92(23.0%)	
Polynucleate cells	Injection	—	20(5.0%)	17 (4.2%)	Scored from 1st meiotic prophase cells
	Food	4(1.0%)	28(7.0%)	44(11.0%)	
Destroyed spindle	Injection	215(53.7%)	256(64.0%)	339(84.7%)	Scored from metaphase and post-metaphase cells
	Food	368(92.0%)	368(92.0%)	376(94.0%)	

Table 3. Relative frequencies of different abnormalities in two series of treatments among the same number of 1,200 cells examined for each type of abnormality (Three different hours of fixation have been combined)

Modes of administration	Types of abnormalities					
	C-mitosis	Polyploid	Polynucleate	Destroyed spindle	Total abnor. cells	Total cells studied
Injection	477	382	37	810	1,706	4,800
With food	733	398	76	1,062	2,269	4,800

of the injection series (Table 2). When the data of different hour of fixation are taken together (Table 3), the relatively higher effectiveness of the food administered series would be apparent. Among the same number of 4,800 cells examined in each type of administration, in the injection series the total number of abnormal cells was 1,706 as against 2,269 of the food administered series. Further, the frequency was found to be higher not only in the sum totals of the abnormalities but also in individual type as would be apparent from the data (Table 3).

It would appear from the present observations that in whichever way

the colchicine was administered, it was equally effective qualitatively but not quantitatively. Further, qualitatively the results were repeatable in both kinds of administrations but if that was true from the quantitative point of view, it had not been verified for the present.

Discussion

Relatively few studies have been made of the colchicine effect on meiotic chromosomes although the implication of such studies is quite evident. Barring a few lower forms, reproduction in animals takes place only by sexual means. Thus in order to induce polyploidy in bisexually reproducing animals the treatment of cells during gametogenesis would be one of the logical ways of approach. In grasshopper the effect of colchicine on the spermatocyte cells has been studied previously by Yosida (1950). He however mainly studied the effect of colchicine treatment on the dissected out testes. The present study reveals the effect of colchicine when the said tissue was in connection with the body. Further, Yosida reported that in his control specimens 28% cells were abnormal which in the present case is practically nil. Since the method of scoring the data between the two studies was different, a detailed comparison of the quantitative data is not possible. However 23.6% polyploid cells in 0.1% colchicine treated testes fixed at 20 hour after treatment (Yosida 1950) could only be compared with the present data which is 19%. On the whole it appears that the effect of colchicine has been observed to be more in the present study than that of Yosida. This fact suggests that the effect could be more in the present study because the tissue was *in vivo* condition after the treatment for a considerable time. The effect of colchicine is manifested more with the prolongation of post treatment fixation hour since the effect takes place at certain stages of division.

The significant finding of the present investigation is the differential effectiveness of some concentration of colchicine administered in two different ways. In the injection mode of administration the testes tissue comes more or less directly in contact with the chemical while in the food administered series the chemical must have reached to the testes tissue through intercommunications of different systems. These facts strengthen the point that the feeding method is more effective than the injection method. There is, however, one important fact which needs to be considered before one would want to be sure that feeding method is more effective than injection method among the two modes of administrations used in the present study. There is no way how to know the amount of colchicine solution consumed in 24 hours along with food administration series. Thus the higher effectiveness of the colchicine treatment along with food may in fact not be due to the more effectiveness but due to the accumulation of more amount of colchicine in 20 hours time than the amount received in one injection. However, if the effect is independent of quantity administered and dependent on concentration used,

the food administration method is then likely to be more effective. Some critical studies are to be taken to verify these points. Further, the present study indicates that colchicine remains effective after treatment for a considerable period and the effect becomes more with the prolongation of post-treatment period but how long this effect continues is to be studied later. Since colchicine produces some changes of permanent nature, the extension of the fixation period brings more abnormal cells.

If the susceptibility of meiotic and mitotic cells to colchicine effect is compared as this is possible in testis tissue, it seems from the present study that the spermatocyte cells are more affected than the spermatogonial cells. However, a conclusive statement could not be made since the frequency of spermatogonial cells was very low in comparison to the spermatocyte cells. If the fact is taken to be true, a question would arise as to the frequency of polyploid cells in spermatogonial and in spermatocyte cells. If the frequency of spermatogonial polyploid cells is lower than that of spermatocyte cells, how the latter could be present in higher frequency. An answer to this fact is that it is more likely that some percentage of polyploid cells in spermatocyte cells could have resulted from the fusion of adjacent cells. The failure of separation of the last spermatogonial chromosomes may also lead to the higher frequency of spermatocyte polyploid cells.

The effect of colchicine on the spindle is gradual for which it is likely to produce both polyploid and aneuploid cells. The effect on the cells could be generalized into two main types, namely (i) the effect on the cytoplasm as well as on the morphology of the chromosome and (ii) the effect on the spindle structure and probably on the centrosome. So far it is known that colchicine does not induce structural rearrangement of the chromosomes. The effect on the cytoplasm inhibits the spindle formation and causes dehydration to the cell. This effect accounts for the over-condensed condition of chromosomes and it may lead to fusion of cell-membranes resulting to polynucleate cell. The change of cytoplasm due to colchicine has been studied by Beams and King (1940). The dehydration effect also causes the chromosomes to contract to a considerable extent (Sigenaga 1945). How colchicine act on the spindle is a controversial issue. Ludford (1939), Bucher (1939), Sato (1939), and Sigenaga (1945) are of opinion that the chromosome scattering is due to the swelling of the spindle. Yosida (1950), however, considers that both swelling and contraction take place in the spindle structure due to colchicine effect. But unless the spindle is destroyed, there would be some difficulty in the origin of regular polyploid cell. Most worker are in agreement that it acts on the spindle in bringing its dissolution and also in its formation with which the present authors agree. However, the effect is gradual for which different types of abnormalities are encountered (Gaulden and Carlson 1951).

Summary

The effect of 0.1% colchicine administered by two different ways upon the testes cells of the grasshopper, *Spathosternum prasiniferum* has been studied.

The colchicine treatment produced different kinds of changes in the division apparatus as well as on the morphology of the chromosomes.

Qualitatively both the kinds of administrations produced similar types of abnormalities in the spermatogonial and spermatocyte cells. The different types of abnormalities observed have been described under four general headings:—1) mitotic changes—changes in the spermatogonial metaphase stage and in the testes sheath cells, 2) changes in the meiotic prophase stages—cytoplasmic fusion to form multinucleate cells, failure of synapsis, reduction of chiasmata, and failure of spindle formation etc., 3) changes in the first and second spermatocyte metaphase stage—C-mitotic metaphase at metaphase II, breaking down of spindle, star or ball metaphase, grouping of chromosome disposition, sticky connection, multipolar spindle etc., 4) post-metaphase abnormalities—unoriented chromosome disposition, asynchronous chromosomal division, lagging chromosomes, sticky chromosomes, pycnotic chromosomes etc.

Quantitatively the food administered series did show more effect in the cells than that of the injection series. The types of changes observed commonly in the treated series were not observed in the control series. The quantitative analysis of some types of abnormalities such as C-mitosis, polyploid cell, polynucleate cell and cell with destroyed spindle revealed that the frequency of abnormalities increased with the prolongation of post-treatment fixation hour except for the frequency of C-mitotic cells in injection series and polyploid cells in the food administered series.

The results have been discussed on the relative efficiency of different kinds of administration. Further, it has been revealed that the colchicine effect on the cells is enhanced with the extension of post-treatment fixation hour and the meiotic chromosomes seem to be more susceptible to colchicine effect.

Literature cited

- Asana, J. J., Makino, S. and Niiyama, H. 1939. A chromosomal survey of some Indian insects. II. A comparative study of the chromosomes in six species of Acridiade: Jap. J. Genet. 15: 251-260.
- Beams, H. W. and King, R. L. 1938. An experimental study on mitotic somatic cells of wheat. Biol. Bull. 75.
- Blakeslee, A. E. and Avery, A. G. 1937. Methods of inducing doubling of chromosomes in plants. Jour. Hered. 28: 393-411.
- Bucher, O. 1939. Zur Kenntnis der Mitose VI. Der Einfluß von Colchicin und Trypflavin auf den Wachstumsrhythmus und auf die Zellteilung in Fibrocytenkulturen. Z. Zellforsch. 29: 283-322.

- Eigsti, O. J. and Dustin, P. 1957. Colchicine in Agriculture, Medicine, Biology and Chemistry. The Iowa State College Press, Iowa, U.S.A.
- Ford, C. E. 1961. Methodology of chromosomal analysis in man. Nat. Can. Inst. Monograph 7: 105-121.
- Gaulden, M. E. and Carlson, J. G. 1951. Cytological effects of colchicine on grasshopper neuroblasts *in vitro* with special reference to the origin of spindle. Exp. Cell Res. 2: 416-433.
- Hsu, T. C. and Pomerat, C. M. 1953. Mammalian chromosomes *in vitro* III. On somatic aneuploidy. Journ. Morph. 93: 301-330.
- Klein, G. 1951. The production of ascites tumors in mice and their use in studies on some biological and chemical characteristics of neoplastic cells. Thesis: Stockholm, 33.
- Lettré, H., Krapp, R. and Ochenschlager, M. 1950. Wirkung des Colchicins und N-Methylcolchicamids auf die Mitose der Zellen des Mause-Ascites Tumors. Z. Krebsforsch. 57: 142-150.
- Ludford, R. J. 1936. The action of toxic substances upon the division of normal and malignant cells *in vitro* and *in vivo*. Arch. f. exper. Zellforsch. 18: 411-441.
- Satô, D. 1939. The effect of colchicine on meiosis in *Aloinae*. Bot. Mag., Tokyo 53: 200-207.
- Schairer, E. 1940. Der Einfluß des Colchicins auf den Mausasciteskrebs. Z. Krebsforsch. 50: 143-154.
- Sigenaga, M. 1945. Experimental studies of abnormal nuclear and cell division III. Observation with living cells of the effect of toxic substances. Cytologia 14: 98-121.
- Smith, S. G. 1943. Technique for the study of insect chromosomes. Can. Entom. 75: 21-34.
- Wellensiek, S. 1939. The newest fad, colchicine and its origin. Chron. Bot. 5: 15-17.
- Yosida, T. 1950. Some observations on abnormal nuclear division in grasshopper testes treated with colchicine. Jour. Fac. Hokkaido Univ. Ser. 6. Zool. 10: 61-70.
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