Effect of Inorganic Salts on Cell Division I. Production of Meiotic Abnormalities in PMCs of *Tradescantia* by NaCl*

By

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In the past three decades, studies on the influence of various chemical and physical agents on cell division have been accumulated and the approaches have been made to various problems on the mechanism of cell division and the behavior of the structural elements of cells by analyzing abnormalities induced by these agents.

It is well known that inorganic salts widely distributing in the environment are harmful for the growth of plants, when they are contained in soil at high concentration. It has been considered that impairment of the growth by the salts is mainly attributable to inhibition of water absorption in roots of plant, resulting from unequal absorption of the cations and anions dissociated from salts. Accordingly, it will be important and attractive to know how the ions dissociated from the salts have influence on cell division.

As regards the influence of various inorganic salts upon cell division, the reports that they may induce various types of mitotic abnormalities have been made by several authors (YAMAHA, '27; SHIGENAGA, '44; LEVAN, '45; VON ROSEN, '54; KAUFMANN & MCDONALD, '56; HINDMARSH, '59). YAMAHA ('27) observed with *Pisum sativum* root tips and other materials that a number of metal salts possess an ability to produce chromosome breakage. SHIGENAGA ('44) reported that neutral and heavy metal salts induce di-diploid nuclei and bi-nucleate cells in the living material, young leaf epidermis and petal cells of *Tradescantia reflexa*. The cytological changes were also observed by LEVAN ('45) in the meristematic cells of onion root tips when treated with a number of inorganic salts, mostly nitrates. Recently, chromosome aberrations were brought about by inorganic salt treatments in the comprehensive experiments to investigate the influence of various elements of the periodic system on

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mitotic process of several plants (VON ROSEN, '54). HINDMARSH ('59) recognized several mitotic abnormalities except chromosome fragmentation in the meristematic cells of onion root tips treated with KCl and phosphate buffer solutions.

However, disregarding the studies of these authors as introduced above, unfortunately, a unified view has hardly been found among their interpretations on the subject that what action of the salts causes production of the mitotic abnormalities. Furthermore, no evidence about the effect of salts upon meiotic division has been reported at all. The present work, therefore, has been carried out with the aim to investigate the effect of Na^+ and Cl^- dissociated from NaCl, one of neutral salts, on meiotic division, which may be available in comparison with that on mitotic division.

Materials and Methods

Three clonal materials of diploid *Tradescantia paludosa* ($2n=12$) and one of a tetraploid *Tradescantia* ($2n=24$) were used in the present experiment. The salt solutions were prepared with analytically pure sodium chloride (NaCl) and deionized water. The concentrations employed ranged from 1 M to 0.025 M, i.e. 1, 0.5, 0.2, 0.1, 0.05 and 0.025 M. NaCl solutions were not buffered, because the purpose of the present study is to investigate the effect of the ions dissociated from NaCl in the absence of other ions. The pH's of the solutions were about 6.2.

The stalks with young inflorescences were cut about 15 cm. from their tops and the cut ends were kept in each NaCl solution at room temperature for 44 to 46 hours[1] before fixation. The old NaCl solutions were renewed at intervals of 12 hours. In order to investigate the osmotic influence of NaCl solution on PMCs, inflorescences of diploid material were treated with the aqueous solution of sucrose, isotonic to[2] each concentration of NaCl solution, by the same method as described above. The plants without any treatments were used as the control.

After treatments PMCs pushed out on a slide glass were fixed and stained with acetocarmine and cells were flattened by gentle pressure on a coverglass. One thousand to 1500 PMCs were examined at first anaphase, dyad stage, second anaphase and tetrad stage respectively from each treatment group and the control.

Observations and Results

Both the stems and the leaves of plants treated with high concentration

1) SAX ('37) has reported that the first meiotic division of PMCs in *Tradescantia* occurs at 18°C. about five days after the chromosomes begin to pair. Furthermore, TAYLOR ('49) has observed that excised anthers grown in culture at 25°C. take about 42 hrs. to reach pachytene stage from mid-leptotene. Judging from these evidences, it will be presumed that in the present investigation PMCs which were observed at first ana-telophase and second ana-telophase would be at mid-prophase and late porphase at initiation of the treatments, respectively.

2) The van't Hoff coefficient was employed as isotonic coefficient on preparing sucrose solution isotonic to NaCl solution. The van't Hoff coefficient of NaCl is 1.84.

of NaCl, 1 M and 0.5 M, extremely wilted within several tens of minutes to hours after the beginning of the treatments. They also perfectly wilted in 0.2 M treatment within 44 hrs. The result shows that these three concentrations of NaCl are hypertonic to cell fluid of these plants. Therefore, all the observations of meiotic division in the NaCl-treated materials were carried out at the concentrations below 0.1 M.

The various types of abnormalities of meiotic division were remarkably observed in the NaCl-treated materials at the stages from first metaphase to tetrad; very frequent were lagging chromosomes, chromatin bridges and spindle disturbance at the stages from anaphase to early telophase of the first and second meiotic division and the formation of micronuclei at interphase and tetrad stage. In the case of the diploid material the frequency of aberrant

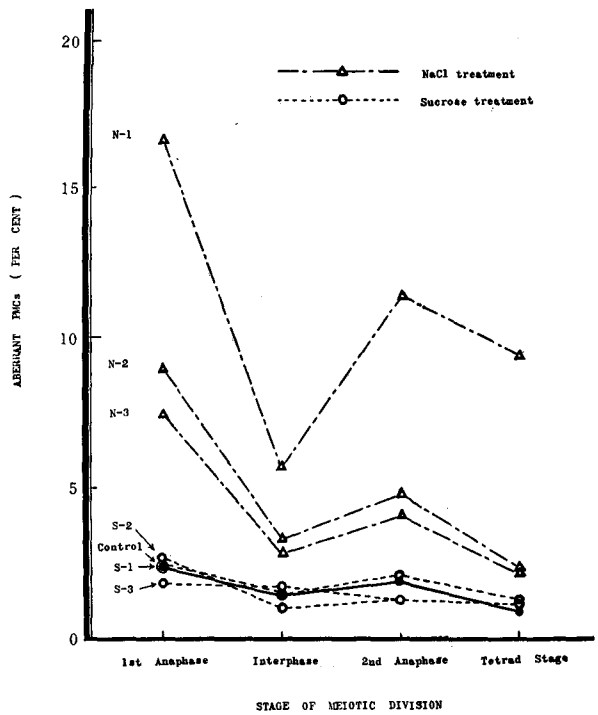


Fig. 1. Effect of NaCl at various concentrations on the occurrence of aberrant PMCs at the various stages in the meiotic division of *T. paludosa.*

N-1=0.1M NaCl, N-2=0.05M NaCl, N-3=0.025M NaCl. S-1, S-2 and S-3 represent sucrose solutions isotonic to N-1, N-2 and N-3, respectively.

PMCs is, as seen in Fig. 1, represented to be remarkably higher in the NaCl-treated groups than in any sucrose-treated ones or in the control, and it is also clear that there is no significant difference in the frequency of aberrant PMCs between the sucrose-treated groups and the control. It is conceivable that the frequency of aberrant PMCs generally increases with the rise of NaCl concentration. In the tetraploid material meiotic abnormalities occurred frequently even in the control plants, probably due to its cytological instability, but the similar results to those obtained with the diploid material were also brought about in the case of the NaCl-treatments. These results will lead us to a conclusion that the aqueous sucrose solution isotonic to each of three different concentrations of NaCl solution does not disturb the normal course of meiosis, suggesting that the NaCl-induced meiotic abnormalities can not be attributable to the osmotic dehydration.

It should be emphasized here that the frequency of aberrant PMCs was little variable among inflorescens of the same clone in both the diploid and the tetraploid.

1) *Lagging chromosomes and micronuclei*

At first anaphase, one or two half-bivalent laggards were observed in both the diploid (Pl. I, Fig. 4) and the tetraploid but sometimes three to five laggards in the tetraploid. Two half-univalent laggards which seem to have resulted from precocious division of a half-bivalent were often present in the diploid material treated with NaCl (Pl. I, Fig. 5). It is suggested that a function of kinetochore was injured by the salt treatment. At second anaphase, most of lagging chromosomes were of chromatid in type and one to two laggards were frequently observed (Pl. I, Fig. 10) and very rarely a half-bivalent laggard was met with. In regard to the types of lagging chromosomes there was seen no difference among the NaCl-treated materials, the sucrose-treated ones and the control.

The number of micronuclei in a PMC at dyad or tetrad stage was one or two in the diploid and tetraploid (Pl. I, Fig. 8). Only a few PMCs included three to five micronuclei. It might be possibly assumed that most of the micronuclei which were observed at dyad and tetrad stages were thought to have resulted from the lagging chromosomes at first and second anaphases respectively, because no evidence of chromosome fragmentation was obtained at both anaphases of meiosis. As these lagging chromosomes and micronuclei are supposed due to principally the common cause, their frequencies are summarized together in Fig. 2. Each of the frequencies of lagging chromosomes and micronuclei is, as shown in Fig. 2, surely hightened by three NaCl-treat-

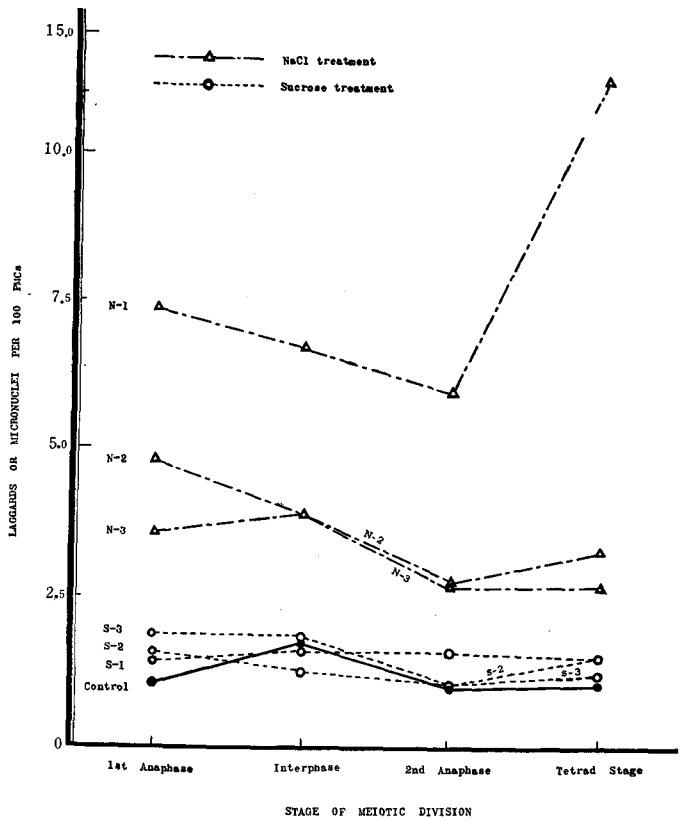


Fig. 2. Effect of NaCl at various concentrations on the occurrence of laggards (at AI & AII) and micronuclei (at interphase & tetrad stage) in the meiotic division of *T. paludosa.*

N-1=0,1M NaCl, N-2=0.05M NaCl, N-3=0.025M NaCl.
S-1, S-2 and S-3 represent sucrose solutions isotonic to N-1, N-2 and N-3, respectively.

ments. Figure 2 reveals too that there is no appreciable difference in the frequency of both the abnormalities between three sucrose-treated groups and the control.

2) *Spindle disturbance*

In the NaCl-treated materials, almost all of chromosomes at first and second anaphase were very often observed lying scattered at random within the cells, the kinetochores appearing to lose their direction toward the pole (Pl. I, Figs. 9 & 13). The causal origin of this abnormality may be thought

to lie in spindle disturbance. The abnormality has potentiality to form such multinucleate cells at telophase as seen in Fig. 5 and Plate I, Fig. 12. Besides, tripolar separation was frequently found at first anaphase after NaCl-treatment (Pl. I, Fig. 3). This type of abnormality is more conspicuous in the tetraploid than in the diploid, particularly at first anaphase.

Since the spindle disturbance was rather variable in its degree, it was sometimes difficult to decide whether such an abnormality certainly occurred or not. Consequently, only the cells in which spindle seemed remarkably to be disturbed was recorded. In Fig. 3, no difference is here found in frequency of the abnormalities between the control and the sucrose-treated groups. It is apparent in Figs. 3 and 4 that the NaCl-treatments enhance the occurrence of spindle disturbance in both the diploid and the tetraploid. Therfore, it will be certainly surmised that the devision apparatus such as spindle and kinetochore are affected by some action of NaCl.

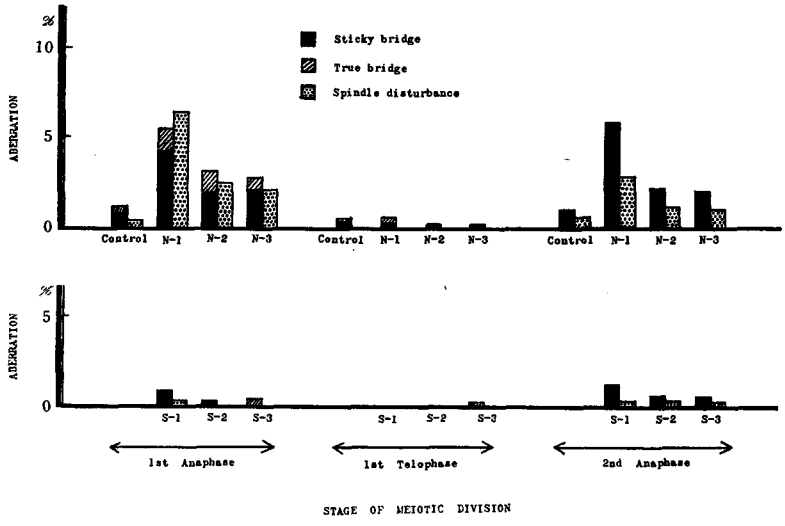


Fig. 3. Graphs showing the frequency of cells with chromatid bridges and spindle disturbance at the various stages in the meiotic division of *T. paludosa* treated with NaCl or sucrose.
N-1=0.1M NaCl, N-2=0.05M NaCl, N-3=0.025M NaCl.
S-1, S-2 and S-3 represent sucrose solutions isotonic to N-1, N-2 and N-3, respectively.

3) *Chromatin bridges*

The chromatin bridges accompanying either with acentric fragments or without them were observed at ana- or telophase (Pl. I, Figs. 6, 7 & 14). The

former may be called "true bridge" because they result from the brekage and reunion of chromosomes, and the latter "sticky bridge" because it is supposed that they are derived from stickiness of chromatin substance. However, HAQUE ('53) has reported that sister-reunion bridges without any acentric fragments were often observed at second anaphase in pollen mother cells of *Tradescantia* irradiated with the low dose of x-rays (18 *r*) and concluded the abnormality

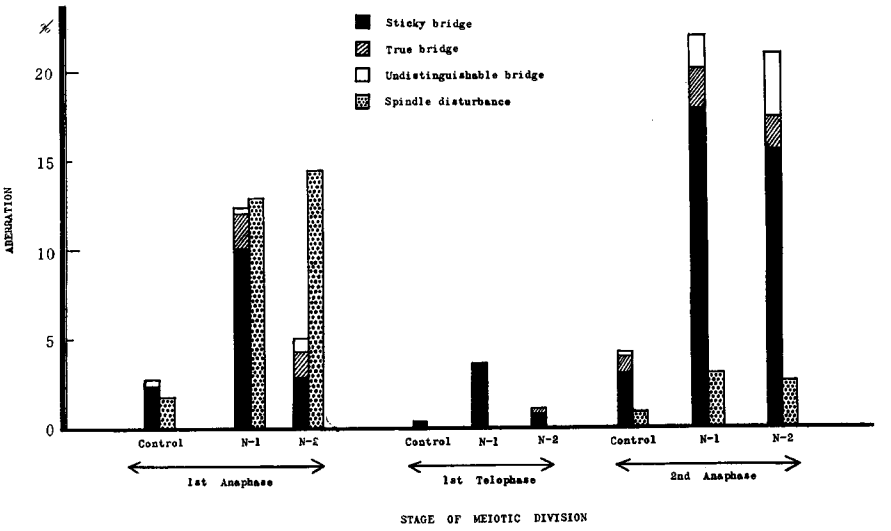
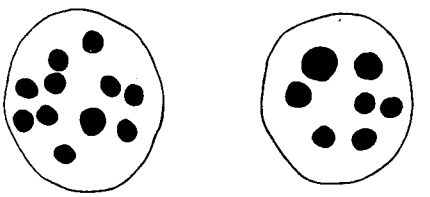


Fig. 4. Graph showing the frequency of cells with chromatid bridges and spindle disturbance at various stages in the meiotic division of tetraploid *Tradescantia* sp. treated with NaCl.
N-1=0.1M NaCl, N-2=0.05M NaCl



a b

Fig. 5 a and **b**. Multinuclei seen at TI in *Tradescantia paludosa* pollen mother cells after treatment with NaCl.

a, 0.05 M NaCl treatment. Each of the small nuclei must be composed of one half bivalent chromosomes. ×ca. 580.

b, 0.1 M NaCl treatment. 7 nuclei of unequal size. ×ca. 580.

apparently to be due to reunion of the two unbroken ends of the sister chromatids. The sister-reunion bridges such as he has proposed were rarely seen particularly at second ana- or telophase in the present investigation, but since it was in many cases difficult to distinguish the sticky bridges from the sister-reunion bridges, the latter was, for convenience sake, recorded together as "sticky bridge".

The results obtained in the diploid material are summalyzed in Fig. 3. The frequency of the true bridges at first anaphase is about two- or threefold higher in the case of three NaCl treatments than in that of the sucrose or the control ones but at second anaphase this type of abnormality was not recorded at all in the control and all the treated groups except a few in the S-1 group. In the NaCl-treated materials, the sticky bridges are more frequently observed throughout first and second divisions than the true bridges, and then no clear-cut difference is found in the frequency of PMCs with the sticky bridges between first anaphase and second anaphase (Fig. 3). However, the degree of stickiness of chromatin substance in the sticky bridges is more prominant in the materials treated with NaCl and is more remarkable at second anaphase than at first anaphase (Pl. I, Fig. 11).

In the tetraploid material, as shown in Fig. 4, similar results were obtained with the exception that the true bridges are observed also at second anaphase and the frequency of PMCs with the sticky bridges is higher at second anaphase than at first anaphase.

A bundle-like bridges of chromatin substance were very often observed at second anaphase of the tetraploid material treated with NaCl (Pl. I, Fig. 15). It seems certainly that the bundle-like bridges are due to extreme stickiness of chromatin substance. In almost all of the cells with such an abnormality, all the anaphase chromosomes form themselves into clots due to extreme adhesion of chromosome body.

The above results will probably indicate that NaCl makes chromosome structure labile to produce chromosome breakage and enhances the viscosity of constituting substance of chromosomes.

4) *Other abnormalities*

The conventional figure at first metaphase, as in the control material and the sucrose-treated one, was rare in the NaCl-treated material. Bivalent chromosomes in the control are daughnut-shaped and segregated normally at the beginning of first anaphase, while bivalent chromosomes in the NaCl-treated cells often clumped to result in tight mass. Such clumping chromosomes at metaphase would hardly segregate at anaphase or completely not, resulting in

TABLE 1. Frequency of PMCs with unequal 5:7 segregation of half-bivalent chromosomes at first anaphase of meiosis in *Tradescantia paludosa* treated with NaCl or sucrose.

Treatment		No. of cells observed	No. of cells with unequal segregation of chromosomes	%
Control		302	1	0.4
NaCl	N-1	614	19	3.1
	N-2	451	19	4.2
	N-3	492	11	2.2
Sucrose	S-1	421	3	0.6
	S-2	508	0	0.0
	S-3	350	1	0.3

N-1=0.1 M NaCl, N-2=0.05 M NaCl, N-3=0.025 M NaCl.
S-1, S-2 and S-3 represent isotonic solutions of sucrose to N-1, N-2 and N-3, respectively.

the formation of diploid nucleus at late telophase.

Univalent chromosomes were present at first metaphase in the control of diploid material the frequency of the univalent was about 0.8 per cent. In N-1 group of diploid material, the frequency of the univalent was about 1.3 per cent.

Unequal 7 : 5 segregation of anaphase chromosomes was often observed at first anaphase in the diploid material treated with NaCl (Pl. I, Fig. 2). The frequency of this type of abnormality, as shown in Table 1, is significantly high in each group of the NaCl treatments compared with the sucrose treatments or the control. Non-disjunction of bivalent chromosomes may be considered as the most reasonable factor responsible for the abnormal anaphase separation.

Discussion

The meiotic abnormalities observed in the present investigation in PMCs of *Tradescantia* treated with NaCl may be classified into three large groups, based on the nature of the abnormalities: the first group includes lagging chromosomes, micronuclei, spindle disturbance and unequal segregation (=non-disjunction) which are presumed due to impairment of division apparatus; the second, sticky bridges and bundle-like chromatin bridges which are in close contact with viscosity of constituting substance of chromosome body; and the last, true bridges derived from structural change of chromonema proper.

It must be noticeable that true bridges are increased by NaCl treatment, suggesting that the breakage and reunion of chromosomes is easy to occur by the salt-treatment. The report has been made by several authors that sticky bridges are induced in meristematic cells of roots treated with various inorganic salts (LEVAN, '45 ; VON ROSEN, '54). In the present investigation also one of the prominent abnormalities after treatment with NaCl was stickiness of chromosomes, manifested mainly by the formation of anaphase bridges. Furthermore, spindle impairment has been reported in dividing cells treated with various inorganic salts by some investigators (LEVAN, l. c.; VON ROSEN, l. c.; HINDMARSH, '59). SHIGENAGA ('44) reported in his investigation employing NaCl that in a 0.5 M solution di-diploid nuclei and bi-nucleate cells are easily formed. Both of them were formed in a 0.1 M solution too and in a 0.05 M solution mitosis can proceed normally. He has suggested that the factors inducing these abnormalities is principally denaturalization of spindle and phragmoplast due to osmotic action when the concentration of the salt solution is high enough to cause dehydration of cell plasm, and furthermore, proposed that, in addition to the osmotic action, alkali metal salt of median concentration, and of high concentration too, possibly has an injurious action which may play some role in the formation of the abnormal nuclei and cells.

It will be apparent that various abnormalities in the present experiment are not due to osmotic dehydration of cell plasm, based on the fact that the sucrose treatments could not induce the abnormalities different from the control in frequency and in type. The evidence mentioned above will also be supported by the report that in culturing PMCs of *Tradescantia paludosa*, optimum sucrose concentration was about 0.22 M (7.5 g/100 ml, theoretically isotonic to 0.12 M solution of NaCl) (SHIMAKURA, '36). It, therefore, must be presumed that the various abnormalities including "true bridges" observed in the present investigation were induced by a mechanism different from the osmotic dehydration of cell plasm.

In considering the influence of NaCl on dividing cells, the biological action of the cation (Na-ions) and anion (Cl-ions) dissociated from NaCl should separately be taken into consideration as well as an injurious action of the salt molecule. It has generally been recognized that Na-ion has an ability to enhance the permiability of protoplasm membrane of plant cells. As a result, intra-cellular substance a little flow out of cells in existence of Na-ion. Consequently, Na-ions will directly induce the physiological alteration of cells. On the other hand, protoplasm membrane of plant cells carries an ability to pass ions selectively into cells, i.e. selective permiability ; for example, K-ion or Cl-ion may be absorbed and accumulated within the cells more easily than Na-,

Br- and NO_3-ions. Therefore, if cells are immersed in the solution of NaCl, unequal absorption of Na-ions and Cl-ions into the cells will necessarily take place. LUNDEGÅRDH ('40) who measured the ratio of absorption of various cations and anions into wheat roots when immersed in various inorganic salt solution has reported that Cl-ions are quadruply superior in absorption into the cells to Na-ions in the case of NaCl.

It will be reasonable to assume that unequal absorption of the ions takes place also in PMCs of *Tradescantia* employed in the present experiment though any direct measurment on absorption of both the ions was not carried out. Therefore, it is likely that ionic unbalance of cells due to the unequal absorption of the ions, influences the normal course of meiosis and the structural elements of the cells.

The evidence that ionic alteration within cells exerts an effect on dividing cells has been reported by KAUFMANN and MCDONALD in the experiment employing a chelating agent, ethylenediaminetetraacetic acid (EDTA), which chelates divalent or trivalent cation (MCDONALD & KAUFMANN, '57 ; KAUFMANN & MCDONALD, '59). They have led to the conclusion that EDTA induces the all the types of mitotic abnormalities including breakage of chromosomes through the alteration of ionic enviornment in cells. They has also obtained similar results with 0.002 M $CaCl_2$ and $MgCl_2$ (KAUFMANN & MCDONALD, '56). Recently numerous studies have focussed attention on the possible role of divalent and trivalent cations in maintaining chromosomal integrity (MAZIA, '54 ; STEFFENSEN, '55, '57, '59 ; KIHLMAN, '57, '59), suggesting that these cations form chelate bonds with terminal phosphate groups between different DNA "species" along the chromosomes (STEFFENSEN, '57). KIRBY ('56) has reported the existence of metallic bonds linking DNA to protein in cell nuclei of mammalian tissues.

If the alteration of ionic environment of cells which is induced by the predominant absorption of Cl-ions over Na-ions makes the metallic bonds unstable,— because chelate bonds formed by metals between DNA "species" or metalic bonds between protein and DNA have relatively low energy when compared to covalent bonds,— then the structural changes of chromosomes may become more feasible to occur. Under such a condition of ionic unbalance within cells, the induction of other cytological abnormalities, of course, will be more promoted, though the precise mechanism remains to be solved.

The plausible assumption mentioned above will be also supported by the results obtained from the treatment of *Paris verticillata* (=*Paris hexaphylla*) with several concentrations of NaCl, KCl and $CaCl_2$, which showed that the salts induce chromosome fragmentation with considerable frequency at meiosis

in addition to the other abnormalities (MATSUURA & IWABUCHI, '62).

Summary

1) In the present experiment, effect of NaCl upon the division of pollen mother cells of diploid *Tradescantia paludosa* ($2n=12$) and tetraploid *Tradescantia* sp. ($2n=24$) was investigated.

2) Concentrations of aqueous solution of NaCl used were 1 M, 0.5 M, 0.2 M, 0.1 M, 0.05 M and 0.025 M, but since dehydration effect on cell plasm was very conspicuous at the first three concentrations, the observation of meiotic division in the NaCl-treated material was carried out at the last three concentrations.

3) In the material treated with NaCl, several types of meiotic abnormalities were obtained at several stages of meiosis; very frequent are lagging chromosomes, micronuclei, spindle disturbance and chromatin bridges which were classified into "true bridges" and "sticky bridges".

4) The frequency of the aberrant PMCs was higher in the NaCl-treated material than in the sucrose-treated one or in the control.

5) The aqueous solution of sucrose, isotonic to each of three different concentrations of NaCl, did not disturb the normal cource of meiosis at all, suggesting that the NaCl-induced abnormalities can not be attributable to the osmotic dehydration.

6) It is likely that the ionic alteration within cells resulting from predominant absorption of Cl-ions over Na-ions is responsible for production of abnormalities.

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Explanation of Plate I

Figs. 1–12. Representing abnormalities at several stages of meiosis in PMCs of *Tradescantia paludosa* following NaCl treatments.

Fig. 1. Occurrence of univalent chromosomes at 1st metaphase after treatment with 0.05 M NaCl.

Fig. 2. Unequal separation of 1st anaphase chromosomes after 0.1 M NaCl treatment.

Fig. 3. Tripolar separation of 1st anaphase chromosomes, treated with 0.025 M NaCl.

Fig. 4. Two univalent laggards at 1st late anaphase after 0.05 M NaCl treatment.

Fig. 5. Precocious division of a univalent laggard into a pair of daughter chromosomes at 1st anaphase after 0.05 M NaCl treatment.

Fig. 6. A true bridge with an acentric fragment of 1st anaphase chromosomes after treatment with 0.1 M NaCl.

Fig. 7. A sticky bridge without any fragment of 1st anaphase chromosomes after treatment with 0.05 M NaCl.

Fig. 8. Micronuclei, derived from lagging chromosomes, at interphase after 0.1 M NaCl treatment.

Fig. 9. Spindle disturbance resulting in scattering of the chromosomes within a cell at 2nd anaphase after treatment with 0.1 M NaCl.

Fig. 10. A lagging chromosome at 2nd anaphase after 0.05 M NaCl treatment.

Fig. 11. Sticky bridges of the chromosomes at 2nd ana- and telophase, treated with 0.05 M NaCl. Heavy stickiness of the chromosomes is seen in the lower left daughter cell.

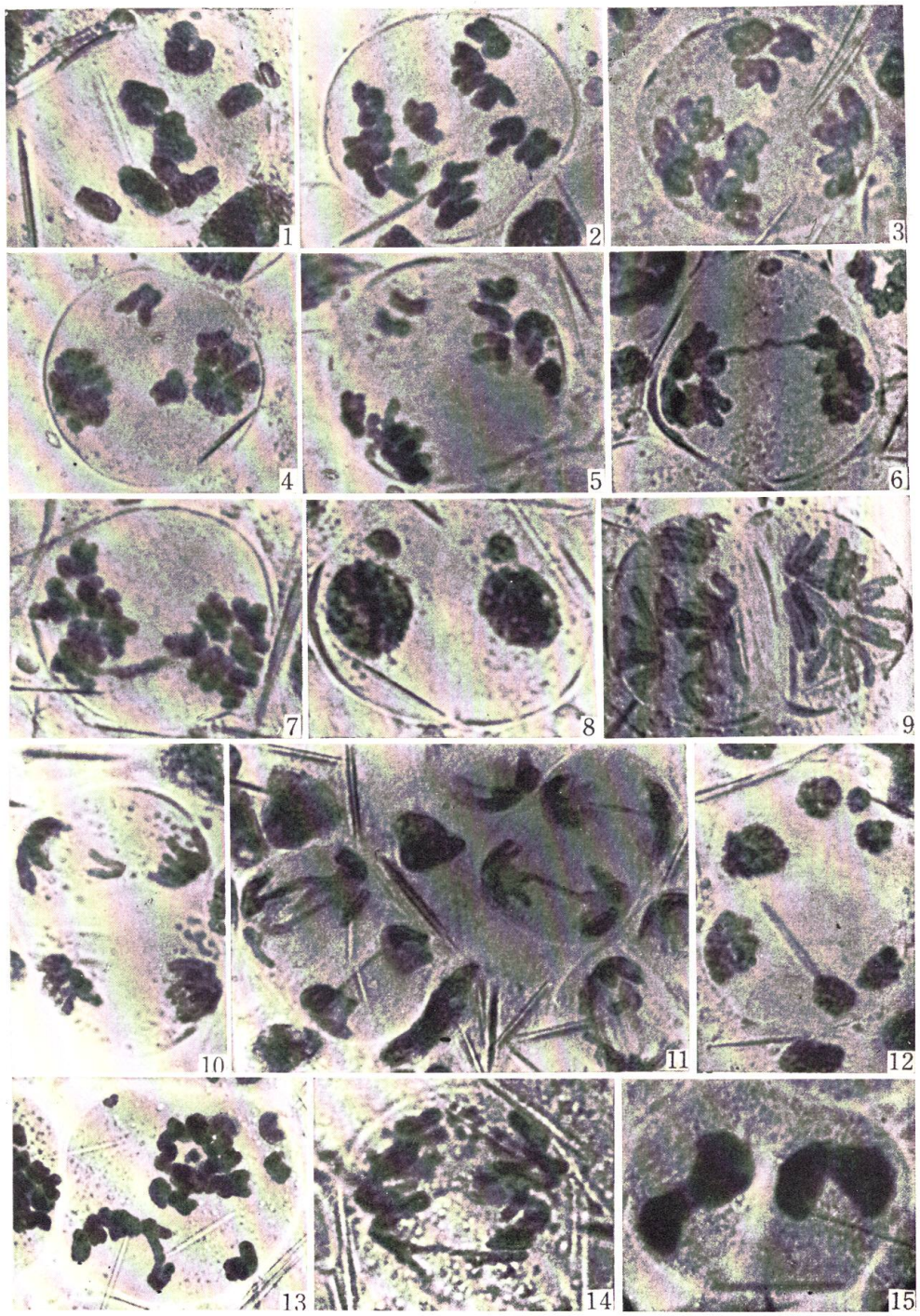
Fig. 12. Abnormally separate nuclei at 2nd telophase, derived from the disturbance of spindle or of bipolarity, after treatment with 0.05 M NaCl.

Figs. 13–15. Representing abnormal division in PMCs of tetraploid *Tradescantia* sp. following NaCl treatments.

Fig. 13. Abnormally separation of 1st anaphase chromosomes, due to the disturbance of spindle or of bipolarity, after treatment with 0.1 M NaCl.

Fig. 14. Sticky bridge of 1st anaphase chromosomes, treated with 0.05 M NaCl.

Fig. 15. Bundle-like bridge of chromatin material of 2nd anaphase chromosomes after 0.1 M NaCl treatment.



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