Influence of hydration and dehydration on mitosis II

by

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INTRODUCTION

The results of the influence of different ions on viscosity changes of cytoplasm are contradictory. The same ion according to different authors dimnishes or increases the viscosity coefficient of cytoplasm. Heilbrunn (1937) thinks that K and Na ions increase the viscosity coefficient of cytoplasm while Ca and Mg ions dimnish it, though other writers are of a different opinion.

One of the methods of examination of viscosity changes is centrifugation. The influence of centrifugal force on cell division in different objects have been examined, but observations were made mainly on fixed material, and cell division was not observed afterwards in vivo (Andrews 1915, Schaede 1930, Schrader 1934, Beams and King 1936 and others). In the present study the author was interested in cell division in live cells after centrifuging and her aim was to investigate the influence:

1. of K and Ca ions on viscosity changes of cytoplasm,
2. of centrifugation on mitosis in dependence of cytoplasm viscosity.

MATERIAL AND METHODS

Staminal hair cells of *Tradescantia virginica* were used (second half of June, July and September 1951). Young inflorescences were put into a test tube containing kalium nitrate (0,4%, 0,8%), calcium nitrate (0,8%) or sacharose solution (2%). Buds were placed in two positions, so that in one position the centrifugal force acted perpendicularly to the axis of the hairs, and in the other parallely to it. During centrifuging buds could not change their position. The time of centrifugation was 2—60 mins. at 5000 and 6000 revolutions/min.

and centrifugal force approximately 3870 x g and 5620 x g respectively. Immediately after centrifugation staminal hairs were prepared (within 3—6 mins.) and observed in a hanging drop of the solution in which they were centrifugated.

The velocity of displacement of nuclei under the action of centrifugal force in each solution was estimated by counting at short intervals the percentage of displaced nuclei. The curve of nuclei return to normal state was obtained in the same way. The oscillation of nuclei during their return to normal position was estimated by counting at short intervals the number of displaced nuclei. and of those in the center and near the centripetal cell walls. The curves of chromosome movement for two groups of anaphase chromosomes were obtained from measurements of the distance between anaphase chromosomes and the plane of the metaphase plate. The curves for the two groups of anaphase chromosomes and the curve resulting were drawn in on the same graph (method used in the previous work (M o l é - B a j e r 1950)).

Photomicrographs were made with the aid of a „Lumipan" Zeiss microscope with apochromate oil immersion objective 60x, N. A. 1,0, apochromate 20x, N. A. 0,17 and a 7x compensating eyepiece.

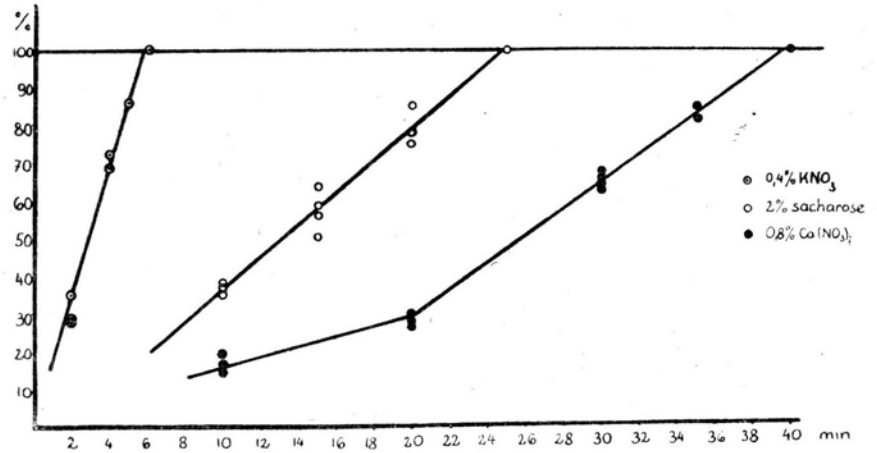
In order to observe hair cells of approximately the same age, only four top cells were considered.

OBSERVATIONS

The curves of dependence between the number of nuclei displaced onto the centrifugal walls and the time, were drawn for the revolving speed of 5000 revolutions/min. (Textfig. 1).

In sugar solution the time necessary for displacing all nuclei on centrifugal cell walls is 25 mins., in 0,4% kalium nitrate 6 mins., in 0,8% kalium nitrate less than 2 mins. and in 0,8% calcium nitrate approximately 45 mins. The exact moment at which the nuclei begin to be displaced, could not to be found in any of the solutions because after a very short time of centrifuging some of the nuclei are found near the centrifugal cell walls.

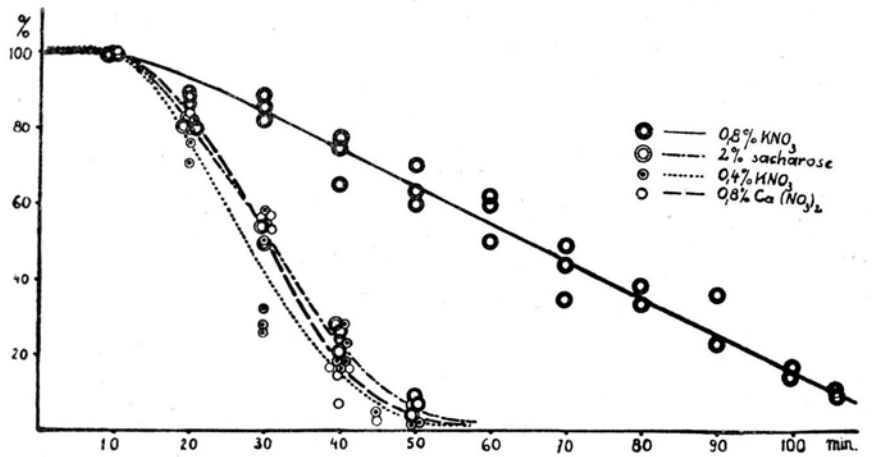
The shapes of curves of nuclei displacement in 0,4% kalium nitrate and 2% sacharose solutions are very similar. The number of nuclei near centrifugal walls is directly proportional to the duration of centrifuging. The curve of displacement in 0,8% calcium nitrate is somewhat different, as it breakes at the 20-th minute. In 0,8% kalium nitrate the nuclei are so quickly thrown on the centrifugal cell walls that is was not possible to make the countings necessary for



Textfig. 1. The percentage of nuclei displaced during centrifuging in 2% sacharose, 0,4% kalium nitrate 0,8% calcium nitrate solutions, plotted aguinst time of centrifuging. Each point calculated from 200 cells.

drawing the curve. In solution of kalium nitrate, the time necessary to move the nuclei is much shorter, and in calcium nitrate longer, than the time needed in sugar solution.

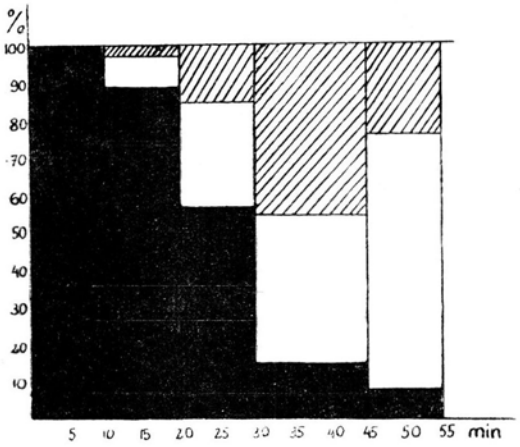
It appears from Text fig. 2 that the duration of the return of the nuclei to normal position (approximately the center of the cell) and also the shapes of the return curves in solutions of sacharose, 0,8% calcium nitrate, and 0,4% kalium nitrate are the same (approximately



Textfig. 2. The return to normal position of nuclei displaced during centrifuging in sacharose, kalium nitrate and calcium nitrate solutions plotted against time. The return is very similar in all solution except in 0,8% kalium nitrate where it lasts much longer. Each point calculated from 200 cells.

45 mins.). In 0,8% kalium nitrate solution the return is much longer (approximately 120 mins.) and some of the nuclei never return to normal position.

It was noticed that in 2% sacharose, some time after centrifuging was ended, the nuclei are not in the center of the cell, but near the centripetal cell walls, i. e. the cell walls opposite to the direction of the acting force. The number of such nuclei changes in the course of observation, which is shown in Textfig. 3. The percentage of nuclei

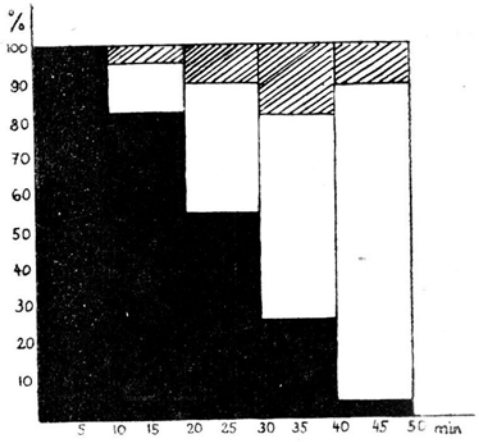


Textfig. 3. The percentage of nuclei near the centrifugal cell walls, in the center of the cell, and near centripetal cell walls, aftr centrifuging in 2% sacharose, plotted against time. Black-nuclei near centrifugal, shaded — near centripetal cell walls, white — in the center of the cell. Graph plotted from observations of 800 cells.

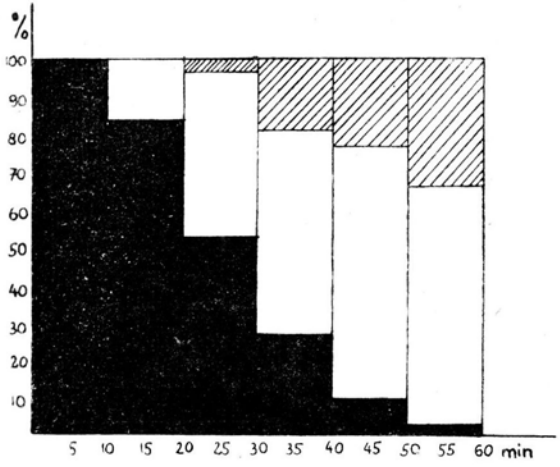
near centripetal and centrifugal cell walls, and in the middle part of the cell was calculated from countings at 5 mins. intervals. The number of nuclei near centrifugal walls dimnishes gradually, while the number of those in normal position (approximately the center of the cell) and near centripetal walls increases. The number of nuclei near centripetal walls begins to increase, 10 mins. after the centrifuge was stopped, reaches its maximum after 30—45 mins., to dimnish afterwards.

When observing the return of nuclei to normal position it often was noticed that some of the nuclei move not only to the center of the cell (normal position) but also farther to the opposite cell wall. After reaching the centripetal wall they move towards the center and sometimes even to the centrifugal wall. This oscillation could be re-

peated twice. The oscillating movement is variable in different cells and some of the nuclei may never reach the opposite cell wall. Some examples are given in Textfig. 7. The return of nuclei to normal position in 0,4% kalium nitrate and 0,8% calcium nitrate solutions is very similar (Textfigs. 4, 5).



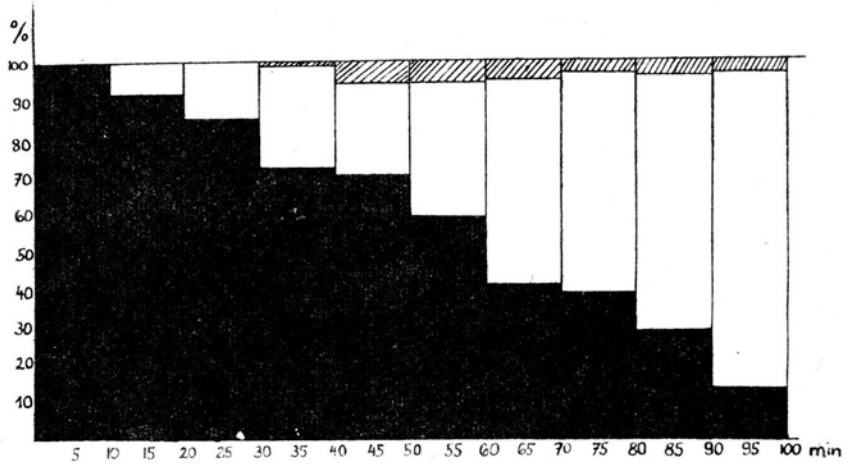
Textfig. 4. The percentage of nuclei near the centrifugal cell walls, in the center of the cell, and near centripetal cell walls, afer centrifuging in 0,4% kalium nitrate solution, plotted against time. Black — nuclei near centrifugal, shaded — near centripetal cell walls, white — in the center of the cell. Graph made from observations of 800 cells.



Textfig. 5. The percentage of nuclei near the centrifugal cell walls in the center of the cell, and centripetal cell walls, after centrifuging in 0,8% kalium nitrate solution, plotted aganist time. Black — nuclei near centrifugal shaded — near centripetal cell walls, white — in the center of the cell. Graph made from observations of 800 cells.

In 0,4% kaluim nitrate solution the percentage of nuclei which reach the centripetal walls is not so great as in 2% sacharose, and the curve reaches its maximum after 30—40 mins. In 0,8% calcium nitrate solution the maximum is reached somewhat later.

The return of nuclei in 0.8% kalium nitrate solution differs from what was described above. In the course of observations a small number of nuclei can be seen near the centripetal cell walls, and their number is approximately constant, and so small that it need not be considered. (Textfig. 6).



Textfig. 6. The percentage of nuclei near the centrifugal cell walls in the center of the cell, and near centripetal cell walls, after centrifuging in 0,8% calcium nitrate solution, plotted against time. Black — nuclei near centrifugal, shaded — near centripetal cell walls, white — in the center of the cell. Graph made from observation of 300 cells.

In centrifugated hairs the course of cell division was observed, and especially the course of anaphase.

It was found that after centrifugation in 0,4% kalium nitrate, 0,8% calcium nitrate and 2% sacharose, mitosis is similar in all three cases and differences occur only in the duration of its different phases.

If during centrifuging the prophase nucleus is displaced towards the centrifugal wall, the course of mitosis continues usually without disturbances, and the time of anaphase is quite normal. In some cases however centrifugated nuclei did not continue their division, but returned finally to the state of resting nuclei. This seems to happen only in the case of a definite stage of early prophase: however, in vivo this stages is very difficult to establish.

TABLE I* 48 cells from 125 observed

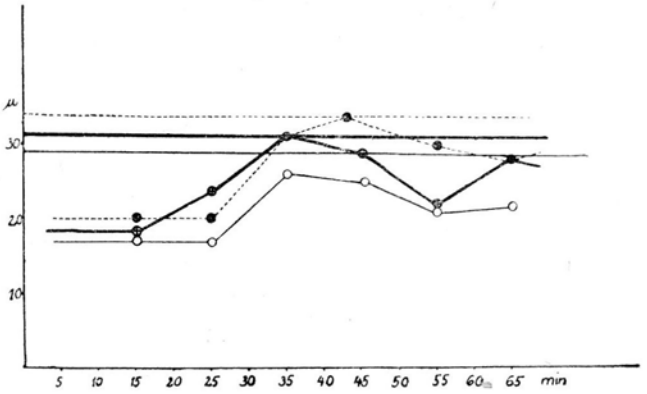
Revolutions/min.	Time of centrifuging in mins.	Position of cell in hair	Stage of cell	Max. separation in anaphase in μ			Time of anaphase in mins.
				centrifugal pole	centripetal pole	total way	
			2% sacharose				
5000	30	1	Proph.	12,5	13,5	26	15,5
,,	30	2	,,	15	15	30	17
,,	30	1	,,	16,5	16	32,5	17
,,	30	1	Metakin.	15	19	34	17,5
,,	80	2	,,	16	18	34	18
,,	30	1	Metaph.	14	20	34	16
,,	30	1	,,	13	20	33	17
,,	30	2	,,	12	18	30	18
,,	30	2	,,	14	22	36	20
			0,4% kalium nitrate				
5000	6	1	Proph.	15	16	31	8
,,	6	2	,,	15	16	31	10
,,	6	1	,,	16	16	32	11
,,	30	1	,,	16	16	32	11
,,	6	1	,,	18	18	36	12,5
,,	6	2	,,	16	17	33	13
,,	6	1	,,	15	15	30	14
,,	6	2	,,	15,5	17,5	33	16
,,	6	1	Metakin.	11	24	35	8
,,	30	1	,,	15	20	35	11
,,	30	1	,,	16,5	17,5	34	11,5
,,	30	1	,,	11	22	33	12
,,	6	1	,,	17	17	34	12,5
,,	30	1	,,	12	15	27	14,5
,,	30	1	,,	14	16	30	16
,,	30	1	,,	14	19	33	19
,,	30	2	,,	11	17	32	19
,,	30	2	,,	12	16	28	23
,,	30	1	Metaph.			30	9
,,	30	1	,,	13	16	29	10
,,	30	1	,,	10	19	29	11
,,	6	2	,,	13	23	36	11
,,	30	1	,,	8	24	32	18
6000	15	2	Proph.	14,5	14	28,5	17
,,	15	1	,,	17	17	34	20
,,	15	1	Metakin.	8	25	33	18
,,	15	2	Metaph.	8	26	34	18
,,	15	1	,,	12	19,5	31,5	18
			0,8% calcium nitrate				
5000	45	1	Proph.	16	22	38	23
,,	45	1	,,	14	17	30	23
,,	60	1	Metakin.	14	17	31	20
,,	60	1	,,	14	19	33	23
,,	45	2	Metaph.	14	16	30	20
,,	45	2	,,	17	19	36	21
,,	45	2	,,	13	18	31	22
,,	60	2	,,	12	19	33	23
,,	60	3	,,	12	18	30	25
,,	60	1	,,	13	18	31	21

*) Cells centrifugated in anaphase are not included as it is difficult to distinguish the anaphase after centrifuging.

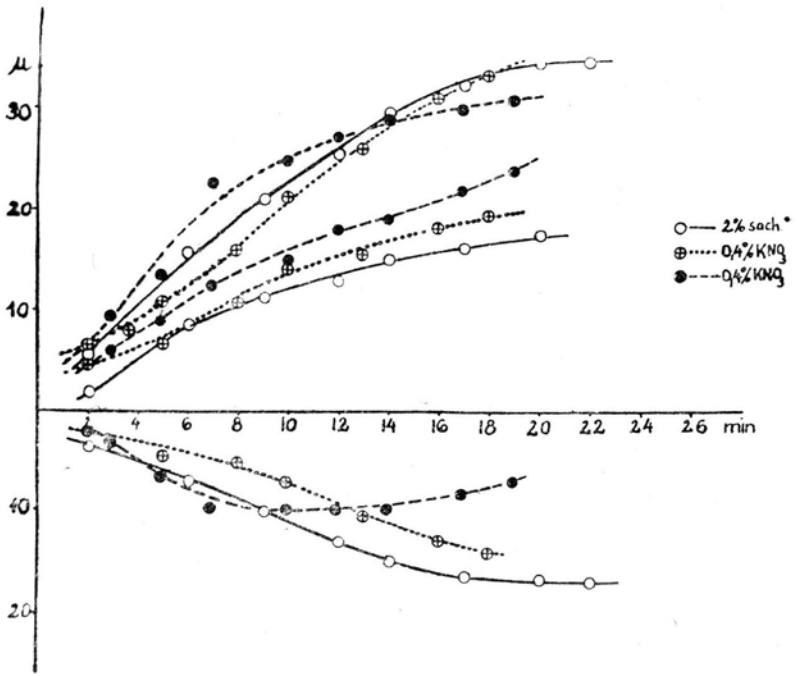
Also no disturbances were found, when dividing cells were centrifugated in metakinensis. The time of anaphase is normal or slightly prolonged, and depends upon the solution in which the nuclei were centrifugated.

All above observations refer to the top cells as well as to the lower ones.

In cells centrifugated in metaphase, and early or advanced anaphase characteristic changes were noted. All the division apparatus (the spindle and chromosomes) is pressed to centrifugal cell wall, and flattened and the plate seems to be broader than usual. Metaphase lasts longer, and the later course of mitosis differs from normal. The metaphase plate is placed obliquely, and the centrifugal chromosome group in anaphase is pressed in one corner of the cell, and therefore it cannot move. The opposite chromosome group (centripetal) moves quickly and farther than usual. It is interesting to note that the maximum distance of separation of chromosomes is the same as in normal division (cf. Table I). During anaphase chromosome groups change their position, and late anaphase resambles the anaphase in not centrifugated cells (cf. Table I, Figs. 4—11). The course of such anaphase is well illustrated by the curves in Textfig. 8. The ways of two anaphase chromosome groups differ, and the curve is flattened, whereas the resultant one is similar to that in not centrifugated cells. Late anaphase and telophase is in such cells normal, and cell walls are formed perpendicularly to the long axis of the cells.



Textfig. 7. The movements of nuclei after centrifuging in 0,8% calcium nitrate plotted against time in 3 cells (dotted and continuous thin and thick lines). Centripetal cell walls of cells observed (centrifugal cell walls are abscissa). Points marked not from the center of the nucleus, but from its centripetal end. Lines parallel to ordinate -- ends of cells.



Textfig. 8. Chromosome movements in anaphase in a not centrifugated cell in 2% sacharose solution, and after centrifuging in 0,4% kalium nitrate solution.

Such disturbances were found usually in lower cells, contrary to what was found in top cells in which the mechanical conditions are very suitable and cell division is in most cases normal.

In some cases the obliquely placed metaphase plate changes its position to perpendicular to the longer axis of the cell — then the anaphase develops normally but is prolonged.

It was observed that in anaphase some chromosomes were sometimes lagging, and the formation of cell wall was disturbed, while in older cells mitosis was not continued.

In certifugated cells, during late anaphase or telephase, two chromosome groups are displaced toward the centrifugal wall, one group close to the other. After a short time however the two groups reach their normal position and cell walls are formed.

DISCUSSION

In a previous paper (M o l è — B a j e r 1950) the influence of different concentrations of kalium nitrate and calcium nitrate on mitosis was studied. It was confirmed that in kalium nitrate solutions

the duration of anaphase is shortened or prolonged in comparison to sugar solutions. In concentrations below 0,4% of kalium nitrate solution the anaphase is shortened; in concentrations from 0.5% to 0,8% prolonged, and above 0,8% all mitoses are irregular and the durations of anaphases very varied. To explain these facts it was suggested that the structure of the spindle is loosened as a consequence of its liquefaction, and finally destroyed. This process at first accelerates the movement of anaphase chromosomes (3–4 times), and then makes it impossible.

The purpose of this paper was to verify these hypotheses and to study the viscosity changes caused by kalium nitrate and calcium nitrate solutions.

Observations on certifugated cells show that the action of K ions liquefies and of Ca ions hardens the cytoplasm. The curves illustrating the displacement of nuclei to centrifugal cell walls indicate that this process is proportional to the time of centrifugation. The displacement of other cell components, i. e. chloroplasts, during centrifugation is very similar (Zurzycka and Zurzycki 1951).

The results of observations on the return of nuclei to normal position are very unexpected. The duration and also the course of the return in 2% sacharose, 0,4% kalium nitrate, and 0,8% calcium nitrate solutions is approximately the same in these three cases. This suggets that the time of return is not dependent on the viscosity of cytoplasm, which conclusion seems rather strange. Similar facts were reported by Timmel (1927) in his observations on the return of centrifugated chloroplasts in water and kalium nitrate solutions.

The return of nuclei in 0,8% kalium nitrate is different. It lasis longer, is less regular and a considerable number of nuclei do not return to normal position. These facts resemble those occurring during anaphase in 0,8% kalium nitrate, and may be also explained by the strong liquefaction of cytoplasm as a result of K ion action. The movement in such cytoplasm is much more difficult if at all possible.

When camparing the curves from Textfigs.: 3, 4, 5 and 6, it is evident that the number of nuclei which reach centripetal walls during their return to normal position increases to a maximum, and then dimnishes (2% sacharose, 0,4% kalium nitrate). In the sugar solution this maximum is higher than in 0,4% kalium nitrate. In 0,8% kalium nitrate solution the number of nuclei near to the centripetal cell walls is low and approximately constant. This seems to indicate that the cytoplasm elasticity is dimnished in kalium nitrate solution.

which is most probably caused by the loosening of cytoplasm structure. This is in agreement with N o r t h e n's (1939) observations on the action of different ions on cytoplasm elasticity in *Spirogyra*.

These facts confirm fully the hypotheses of the previous work (M o l è — B a j e r 1950).

The revolving speed of the centrifuge used was to small to cause any serious disturbances in cell division. Such disturbances were observed usually when experimenting with an ultracentifuge of which the force is 150 000 x g or more (B e a m s a n d K i n g 1936).

Some of the few disturbances noticed (the curving of the spindle, lagging of chromosomes in anphase and so on) are similar to those described by S c h a e d e (1930), S c h r a d e r (1934) and others.

It is evident from the curves of chromosome movement in anaphase in centrifugated and not centrifugated cells, that the way of centrifugal chromosome group in anaphase is shorter than the way of the opposite one. The maximal seperation of the two chromosome groups in anaphase is the same as in not centrifugated cells. This indicates that centrifuging did not destroy the submicroscopical structure of the spindle.

The experimental data are unsufficient to explain the differences in the distances that the two chromosome groups move through in anaphase. It seems probable that this is caused by the return of the spindle or perhaps a halfspindle to normal shape, after being squeezed during centrifugation.

The results of the present investigation fully support the opinoins of other writers on the stiffnes and uniformity of behaviour of the spindle.

As a result of centrifuging anaphase and metaphase are sometimes slightly prolonged (cf. Table I). Similar facts were noticed by S c h r a d e r (1934) in his experiments on certifuging of Crustacea.

SUMMARY

Flower buds of *Tradescantia virginica* were centrifugated for 20—60 mins. at 5000 and 6000 revolutions/min. and forces approximately 3870 and 5620 x gravitation respectivly, in 2% sacharose, 0,4% and 0,8% kalium nitrate, and 0,8% calcium nitrate.

1. The time necessary to displace the nuclei in 0,4% kalium nitrate solution is shorter, and in 0,8% calcium nitrate longer, than in 2% sugar solution. This indicates that kalium nitrate solutions decrease, while calcium nitrate increase, cytoplasm viscosity. In 0,8% kalium nitrate the viscosity coefficient of cytoplasm decreases exceptionally.
2. The return time of centrifugated nuclei to normal position is similar in 0,4% kalium nitrate, 2% sacharose, and 0,3% calcium nitrate solutions. In 0,8% kalium nitrate it is much longer and often irregular. From facts obtained conclusions concerning the liquefaction and dystroying of cytoplasm structure under the action of K ions were drawn.
3. The revolving speed used for centrifuging was to small to cause any serious disturbances in cell division.

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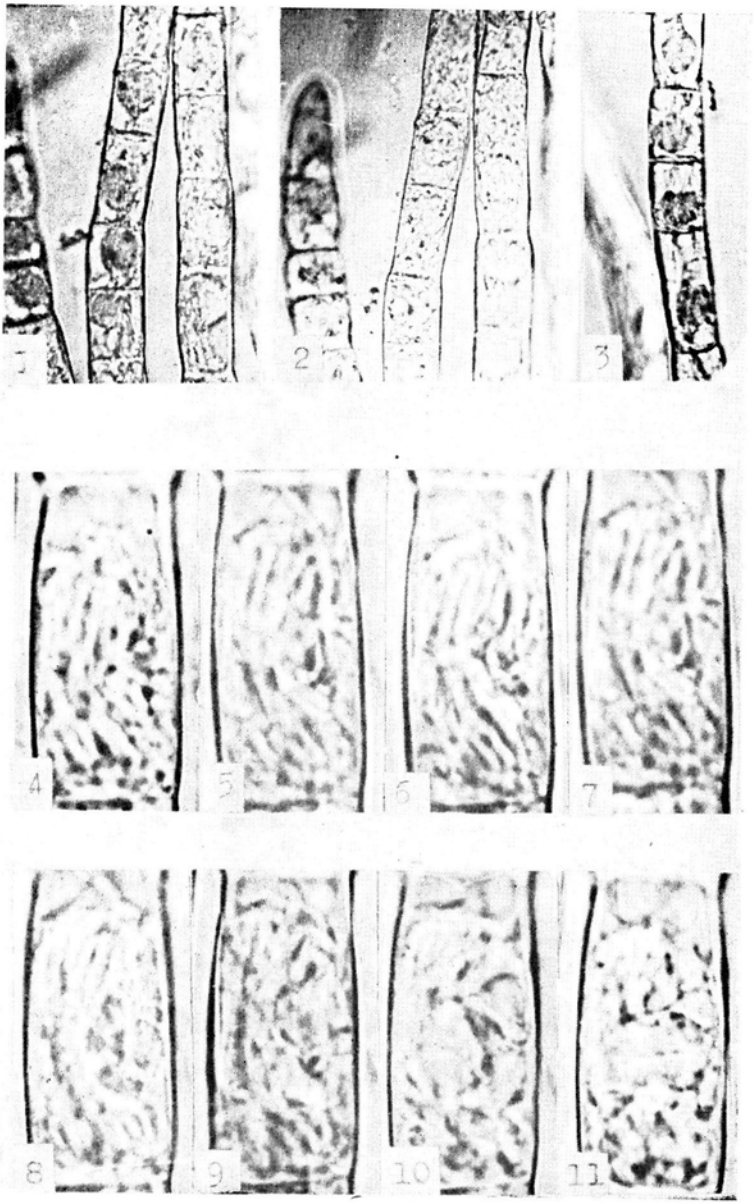

EXPLANATION OF TABLE I

Fig. 1. Not centrifugated staminal hairs of *Tradescantia virginica*

Fig. 2. Staminal hairs with displaced nuclei as a result of centrifuging. The top side — centrifugal pole.

Fig. 3. Return of nuclei to normal position after centrifuging in 0,8% calcium nitrate solution. Two nuclei moved to centripetal cell walls. The top side centrifugal pole.

Figs. 4—11. Anaphase and telephase in centrifugated cell in 0,8% calcium nitrate at 6000 revolutions/min. during 45 mins. The bending of the spindle is visible. The top side centripetal pole.