Influence of hydration and dehydration on mitosis I.

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wpłynęło 23. III. 1951.

Introduction

In numerous hypotheses concerning cell division (Schrader 1946) viscosity phenomena play an important role. To elucidate this problem however few experiments were carried out. Barber (1939) after observing cell division in different temperatures, demonstrated that there is no proportionality between the velocity of chromosome movement in anaphase and the viscosity changes, and concluded that the changes of viscosity have no important influence on the course of anaphase.

Möllendorf (1937, 1938, 1938 a) induced changes in viscosity of chick embryo cells with liquefying (KBr, KCl, KJ) and hardening (Na_2So_4, K_3SO_4) salts and examined the time of different stages of mitosis. He demonstrated that the activity of the salts either shortens some stages and prolongs others or prolongs all of them.

No investigations similar to Möllendorf's work were undertaken on plant material, and those done by Möllendorf do not show mechanism of the increase or decrease in hydration and its influence on the time of different stages of mitosis.

The purpose of the present work was to find out, whether changes in hydration:

1. influence the time of anaphase,
2. shorten or lengthen the chromosome seperation,
3. cause morphologic changes in cell division.

KNO_3 and $Ca(NO_3)_2$ were used as hydrating and dehydrating agents.

Material and methods.

Staminal hair cells *of Tradescantia virginica* (tetraploid race) from the Botanical Garden of the Jagellonian University were used. Preparations were made according to B ě l a ř (1929). Cell division was observed in sugar (sacharose), KNO_3 and $Ca(NO_3)_2$ solutions. To avoid cells with demixing of cytoplasm („Entmischung" B ě l a ř 1930) observations were started 30 min after preparation. The osmotic pressure of all solutions used — exept the 1, 1,2, and 1,6% solutions of KNO_3 — were the same and equaled the pressure of a 2% water solution of sacharose. It is only in the case of the 1,6% KNO_3 solution that the osmotic pressure exceeded this value slightly. In the case of weak concentrations the solution was strengthen with a suitable quantity of sacharose.

Observations were done at temperatures 19,5° to 22° C. Measurements were done with a Zeiss drawing prism, and from the drawings graphs the chromosome movements were ploted. On the graph the curves of each chromosome group and also the curve of the distance between the two chromosome groups were ploted against time according to a method used by B a j e r (1950).

Observations were made on approximately 300 dividing cells. In the Tables however only those cells, in which the division suggested no doubt, were taken into consideration, though others did not seem to disagree with the resuits obtained.

The error in measurements varied in different cells, and might have been caused partly by the difficulties in estimating exactly the level of the equatorial plate in metaphase.

Observations.

Observations of mitosis in staminal hair cells of *Tradescantia virginica* were done both on living and on fixed material by numerous authors (B ě l a ř 1929, T e l e ż y ń s k i 1930, S c h n e i d e r 1938 and others) and conclusions drawn from results obtained by them often differ considerably.

Observations in sacharose solution.

After formation of chromosomes in prophase, kinetochores of all chromosomes are placed on one side and near each other (B ě l a ř 1929). In metakinesis kinetochores move toward the equatorial plate. The movement is rapid and it is difficult to point out exactly, the moment of the beginning of the metakinesis, the end of metakinesis, and the beginning of the metaphase. As it is not known whether in staminal hair cells of *Tradescantia virginica* there is a normal metaphase plate (S c h n e i d e r 1938), it is probable that in metaphase kinetochores are not arranged in one plane. It seems that in metaphase all the plate and also the individual chromosomes oscillate irregularly. Plate oscillation was confirmed by H u g h e s and S w a n n (1948) in chick tissue culture.

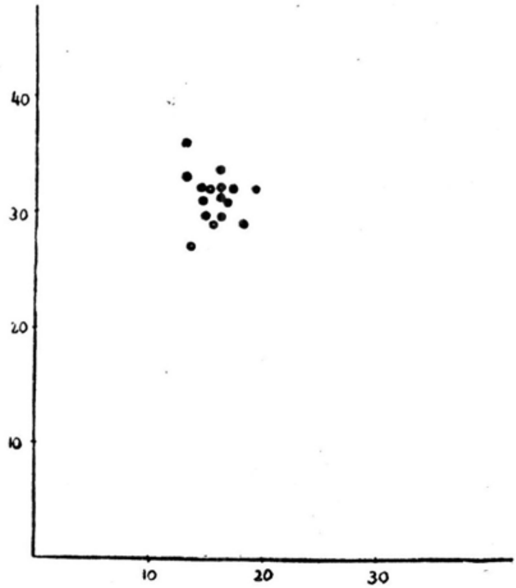
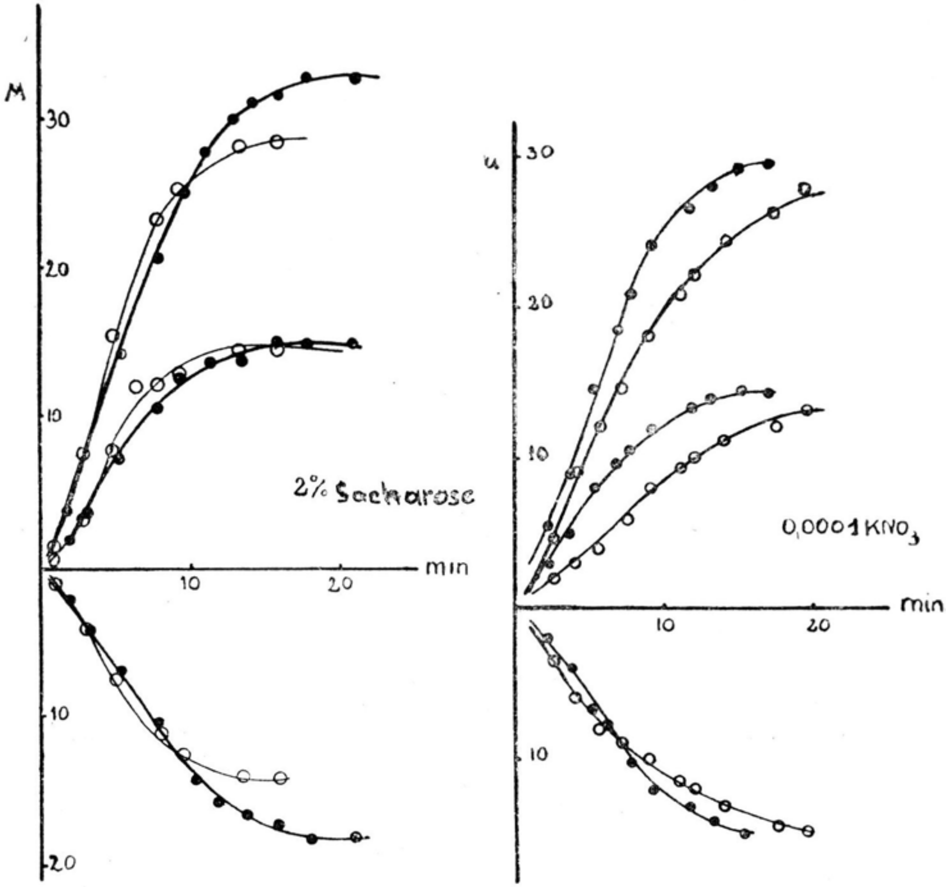


Fig. 1. The dependence between distance travelled by anaphase chromosomes (the maximal distance between the kinetochores of two chromosome groups) and time of anaphase in 16 cells. Abcissa — time in nim., ordinate-distance in μ.

The transition between metaphase and anaphase is abrupt (B a r b e r 1939, B a j e r 1950) and observations of first stages of anaphase are very difficult. In the first stages of anaphase the movement of two groups of chromosomes is very rarely synchronised, which is the consequence of the irregular arrangement of chromosomes in the metaphase plate and the fact, that not all chromosomes in the plate begin to move simultaneously (B a j e r 1951). Later stages of anaphase are much better visible. During the anaphase the kinetochores move to the poles and the arms of individual chromosomes come near each other, in consequence the width of all anaphase chromosome groups dimnishes.

The time of different stages of division in sugar solution is similar to the results obtained in liquid paraffine by Bajer (1950); no prolongation or shortening of some of the stages was confirmed. Contrary facts were observed by Möllendorf (1938) in culture of chick fibroblasts, where a sugar solution prolongates all stages approximately in the same degree.



Figs. 2.—3. Graphs of chromosome movement in 2% sacharose solution and 0,0001% KNO^3. Normal duration of anaphase. Graphs for two cells.

The time of each stage of cell division in liquid paraffine and in sugar solution is not the same in all cells but differs considerably. The limits of this oscillation are the same in both cases and are characteristic for normal cell division (Table I).

In sugar solution as well as in KNO_3 and $Ca(NO_3)_2$ solutions there is no dependence between the time of the anaphase and the maximum seperation of the chromosomes. The lack of this dependence is evident from Figs. 1 and 11.

According to Möllendorf in chick fibroblasts metaphase occurs approximately in the middle of the division. The time of prophase and of resting nucleus formation in telophase is the same. This indicates that the time necessary for a cell to develop from the structure characteristic for interphase, to the structure in mitosis is the same as the time of transition from mitosis to interphase. In *Tradescantia virginica* the time of prophase is much longer than the time of resting nucleus formation. The prophase in staminal hair cells lasts 1,5—3 h while the return to resting nucleur from the moment of cell wall formation lasts approximately half as long. The cell wall is formed within 6—12 mins after the end of anaphase.

Observations in KNO_3 solutions.

To study the influence of hydration change on the time of different stages of cell division and especially on the chromosome movement in anaphase, the course of mitosis was observed in different concentrations of KNO_3. The following concentrations were used: 0,0001; 0,001; 0,01; 0,1; 0,2; 0,3; 0,4; 0,5; 0,6; 0,7; 0,8; 1; 1,2; 1,6%.

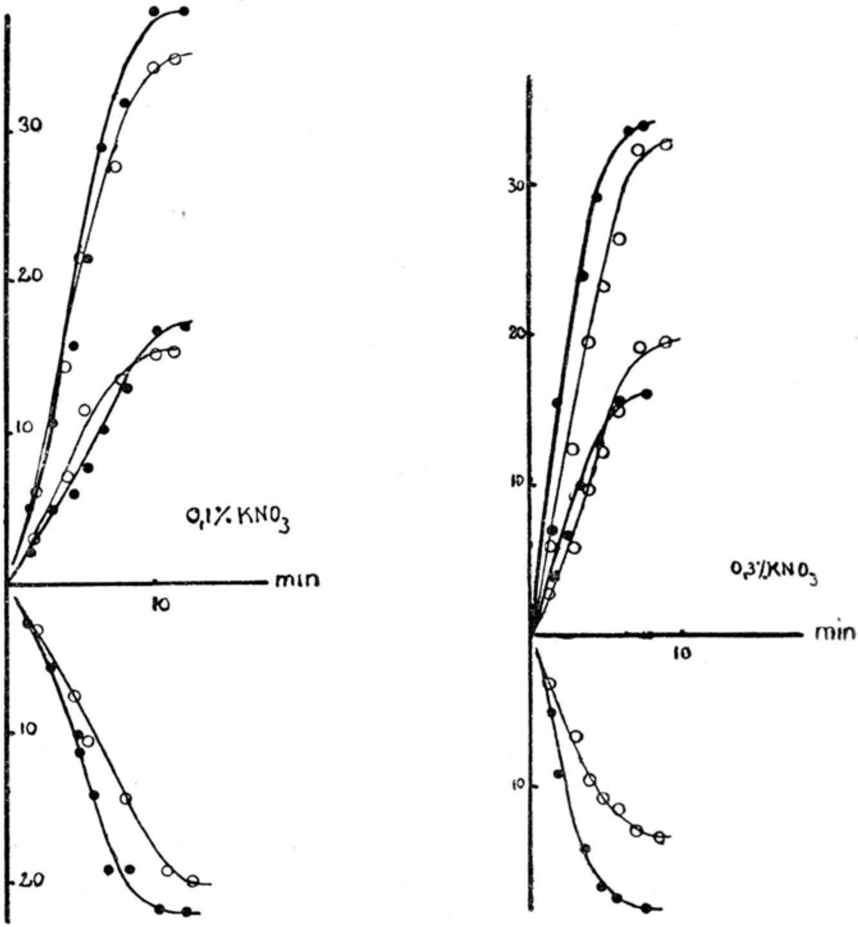
Beginning from 0,1% concentration the refraction coefficient of the cytoplasm decreases, while for chromosomes — in dependence on the stage of mitosis — the coefficient decreases or does not change.

In 0,0001 to 0,5% solutions the cell division is normal. In all concentrations of KNO_3 different degrees of vacuolisation were found. New vacuoles are formed and old ones become larger. The formation of vacuoles was observed in some cases in Bělař's „Stemmkörper" (Bělař 1929) in anaphase.

In a given concentration the duration of different stages of mitosis undergoes characteristic oscillations. The exact data for anaphase are found in Table I.

Influence of K ions on the course of division is marked in very diluted solutions. The 0,0001% solution however does not cause any change in the duration of different stages of division. The course of anaphase which was observed most carefully, is normal without even the slightest disturbances, and the time of anaphase is practically the same as in sugar solution (Fig. 3, Table I).

The influence of 0,0001% concentration of KNO_3 is as follows: 1) the duration of anaphase is shorter, 2) the degree in which the anaphase duration oscillates dimnishes (Table I); the duration of other stages of mitosis most probably does not change.



Figs. 4—5. Graphs of chromosome movement. In 0,1% KNO_3 time of anaphase shortened, in 0,3% KNO_3 shortest anaphase. Graphs for two cells.

In 0,01% solution the time of anaphase is approximately the same as in the case previously described (Table I). It seems however that the metaphases in this concentration are much more numerous than in other solutions. It is not caused by the apparent increase in cell divisions often observed as the consequence of action of factors which check mitosis. Such facts were observed in chick embryo

TABLE I

	Position of cell in hair	Time of metaphase	Time of anaphase in min.	The max. distance between kinetochores in anaphase in μ
2% sacharose solution	1	normal	19,5	35
	2	,,	17	34
	1	,,	18,5	32
	1	,,	19	35
	3	,,	14	31
	2	,,	16,5	29
	3	,,	18	36
	4	,,	18,5	34
	1	,,	16	30
	3	,,	19	37
0,0001% KNO_3 solution				
	2	,,	15	34
	2	,,	16,5	33
	1	,,	16,5	44
	2	,,	17,5	31
	2	,,	17	34
	3	,,	17,5	32
	1	,,	18	29
	1	,,	18,5	30
	1	,,	20	33
	2	,,	21,5	25
0,001% KNO_3 solution				
	1	,,	13,5	32
	2	,,	14	34
	1	,,	14	39
	4	,,	1,5	33
	1	,,	15	32
	1	,,	15	29
	2	,,	15,5	31
	2	,,	15,5	31
	3	,,	16	34
	1	,,	18	33
0,01% KNO_3 solution				
	1	,,	13	33
	2	,,	13,5	32
	2	,,	13,5	27
	1	,,	14,5	32
	1	,,	14,5	31
	1	,,	15	32
	1	,,	15	29,5
	3	,,	15,5	29
	2	,,	15,5	31,5
	1	,,	16	32
	1	,,	16	29,5
	2	,,	16	36
	2	,,	16	31
	1	,,	18	29
	1	,,	19	32

	Position of cell in hair	Time of metaphase	Time of anaphase in min.	The max. distance between kinetochores in anaphase in μ
0,1% KNO_3 solution	1	normal	7,5	36
	1	,,	8,5	35
	1	,,	8,5	32
	3	,,	9,5	37
	1	,,	8,5	32
	2	,,	10	33
	2	,,	10	35
	1	,,	12	36
	3	,,	12	30
	1	,,	12,5	34
0,2% KNO_3 solution				
	1	,,	6,5	32
	1	,,	7	35
	2	,,	7,5	31,5
	1	,,	8,5	34
	2	,,	9	35
	2	,,	9,5	24
	1	,,	10	34
	2	,,	11	32
	1	,,	11	34
	3	,,	11	35
	1	,,	11	34
0,3% KNO_3 solution				
	1	,,	6	32
	2	,,	6,5	30
	1	,,	6,5	30
	2	,,	7	36
	1	,,	8	30
	1	,,	8	32
	1	,,	9	34
	3	,,	9,5	30
	3	,,	11	26
	2	,,	12	32
0,4% KNO_3 solution				
	1	,,	6,5	35
	3	,,	7,5	25
	1	,,	8	31
	2	,,	8	31
	1	,,	9	27
	2	,,	9,5	32
	1	,,	10,5	30
	1	,,	11	30
	2	,,	11	34
	1	,,	8,5	28
0,5% KNO_3 solution				
	2	Prolongated	12	28
	3	,,	13	26
	3	,,	14	31
	1	,,	14	34
	1	,,	15	36
	1	,,	15	35
	1	,,	16	31
	1	,,	16	35
	1	,,	17	30
	1	,,	19	34

	Position of cell in hair	Time of metaphase	Time of anaphase in min.	The max. distance between kinetochores in anaphase in μ
0,6% KNO_3 solution	2	Prolongated	14	38
	1	,,	15,5	32
	1	,,	15,5	34
	1	,,	16	38
	1	,,	17	35
	1	,,	17	36
	2	,,	17,5	31
	2	,,	18,5	31
	1	,,	22	35
0,7% KNO_3 solution	2	,,	15,5	25
	2	,,	17	30
	1	,,	17	32
	1	,,	17	35
	1	,,	18	32
	1	,,	18	29,5
	1	,,	18	30
	2	,,	19,5	30
	2	,,	20	31
0,8% KNO_3 solution				
	2	,,	17	25
	2	,,	18	31
	3	,,	18	36
	1	,,	23	33
	1	,,	24	30
	1	,,	25,5	31
	1	,,	26	32
	1	,,	27	34
	1	,,	28	26
	1	,,	32	34
	1	,,	33	31
	3	,,	45	30
	1	,,	60	30
	1	,,	150	30

tissue culture by M ö l l e n d o r f as a result of butanol action. In the case of *Tradescantia* it seems probable that this concentration is especially suitable — i. e. optimal for the cell division. S p e k (1923 from M ö l l e n d o r f 1937) observed similar facts in *Paramecium* as the result of the action of potassium salts.

In concentration 0,1 and 0,2% the duration of anaphase further dimnishes and anaphase lasts a shorter time than in sugar solution.

The shortening of anaphase duration and the consequent acceleration of chromosome movement reaches its maximum value in 0,3 and 0,4% of KNO_3 solutions. The shortest time of anaphase observed is 5 to 6 min.; this is 3—4 times less than in a 2% sacharose solution (cf. Fig. 5 and Table I).

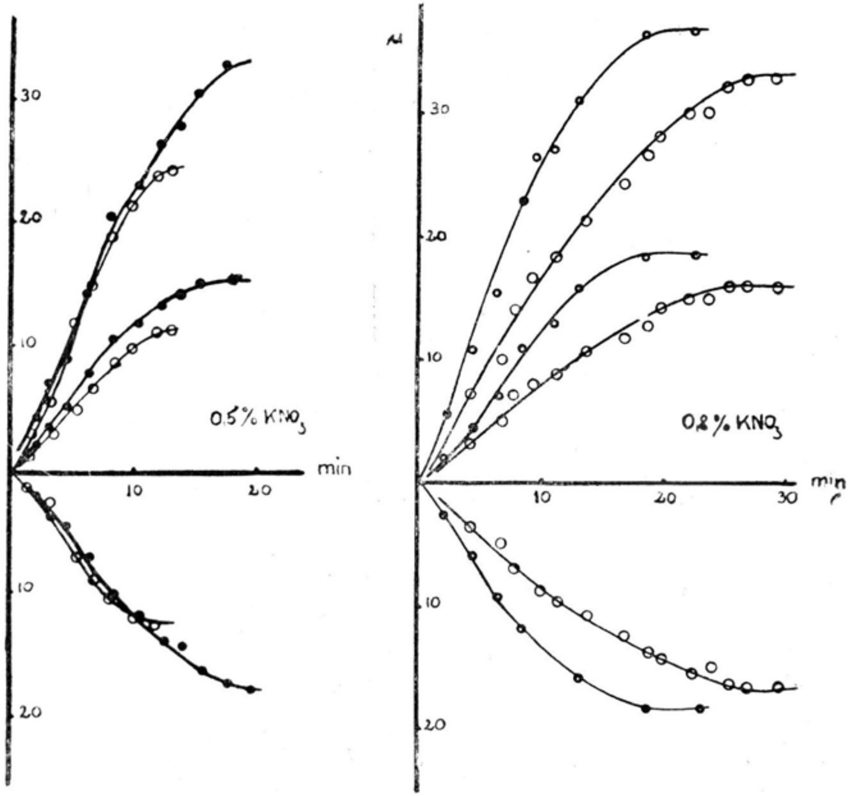
In the case of increasing concentrations described here, changes in chromosome movement were noticeable. In a 2% sugar solution kinotechores reach within 1—2 min. after anaphase begins their maximal velocity, here on the other hand, it seems that at the very beginning of anaphase the velocity has its maximum value (Figs. 4—5). Similarly in the case of rapidly moving small chromosomes (4 μ /min. the diameter approximately 1 μ) as was observed by H u g h e s and S w a n n (1948) in chick embryo tissue culture — it is at the very beginning of anaphase that chromosomes reach their maximal velocity.

Beginning on the 0,5% solution of KNO_3 these relations change. In comparision to previous solutions anaphase is prolonged, though shortened if compared to anaphase in a 2% sugar solution. The values of anaphase durations are similar to the shorter durations in 2% sugar solution. In this concentration metaphase is also prolonged. These two facts and the much stronger vacuolisation of the cell indicate that this concentration of KNO_3 is not a suitable medium. In this and in stronger concentrations returns from prophase to resting nucleus were also observed. The chromosome movement is shown in Fig. 6, the times of anaphases in Table I.

In 0,6% solution of KNO_3 the duration of anaphase is approximately the same as in mitosis in sugar solutions, and in the 0,7% one the time was prolonged (Table I).

The 0,8% concentration of KNO_3 prolongates metaphases as well as anaphases. The duration of anaphase is usually longer than in normal divisions (Table I, Fig. 7). Numerous prophase nuclei return to the resting stage and the time of anaphase in this concentration depends approximately on the time of action of this solution. The longer the cell is in solution, the longer is the course of anaphase.

The dependence of the time of anaphase from the different solutions of KNO_3 is represented in Fig. 12.



Figs. 6—7. Graphs of chromosome movement. In 0,5% KNO_3 the time of anaphase is prolongated as compared to Fig. 5; the 0,8% KNO_3 solution causes remarkable prolongation of anaphase. Graphs for two cells.

In 1% solution of KNO_3 almost all prophases and metakineses return to the state of resting nuclei. In some cases the first stages of division were considerably retarded which was followed by the checking of division and oblitering of chromosome shapes. Also some chromosomes were often lost or lagging. Similar facts were observed by Möllendorf (1938) as a result of hyper or hypotonic medium, and were explained by him as the result of disturbances of the division mechanism. In 1% sugar solution in *Tradescantia* other disturbances such as arresting of some chromosomes by the cell wall and formation of three instead of one cell wall were observed. In the latter case two of the walls were quickly resorbed. W a d a (1934) observed similar disturbances in his micrurgical experiments on the staminal hair cells of *Tradescantia*.

Cells observed in 1,2% KNO_3 solution show slow cytoplasm cyclosis. Refraction coefficients of cytoplasm and chromosomes dimnish, and strong vacuolisation of cells occurs. All mitoses stop after some time an the swelling of chromosomes and cytoplasm follows, until the chromosomes disappear.

The 1,6% concentration of KNO_3 causes quickly mortal changes in cells. Some cells undergo the first kind of demixing which was observed by B ě l a ř (1930) in cells injured by mechanical factors or hypertonosy. The structures of nuclei vanish and a change of the refraction coefficient of cytoplasm is observed. This type of demixing often took place in very young cells. In most cells coagulation of nuclei and of chromosomes, the lack of formation of cell walls in telephase and stopping of cytoplasm streaming could be noticed. It is the second kind of demixing described by B ě l a ř (1930). Similar facts were noticed by M ö l l e n d o r f (1938) in chick tissue culture as a result of hyper or hypotonic medium. In my observations two kinds of demixing are caused by the same factor, i. e. the intermicellar removing of water in consequence of action of the hypertonic medium.

The cytoplasm cyclosis may indicate to some extent whether the cell is in a normal state. The streaming becomes quicker, when the concentration of KNO_3 is strengthened up to a point after which it slows down and stops altogether in strong concentrations.

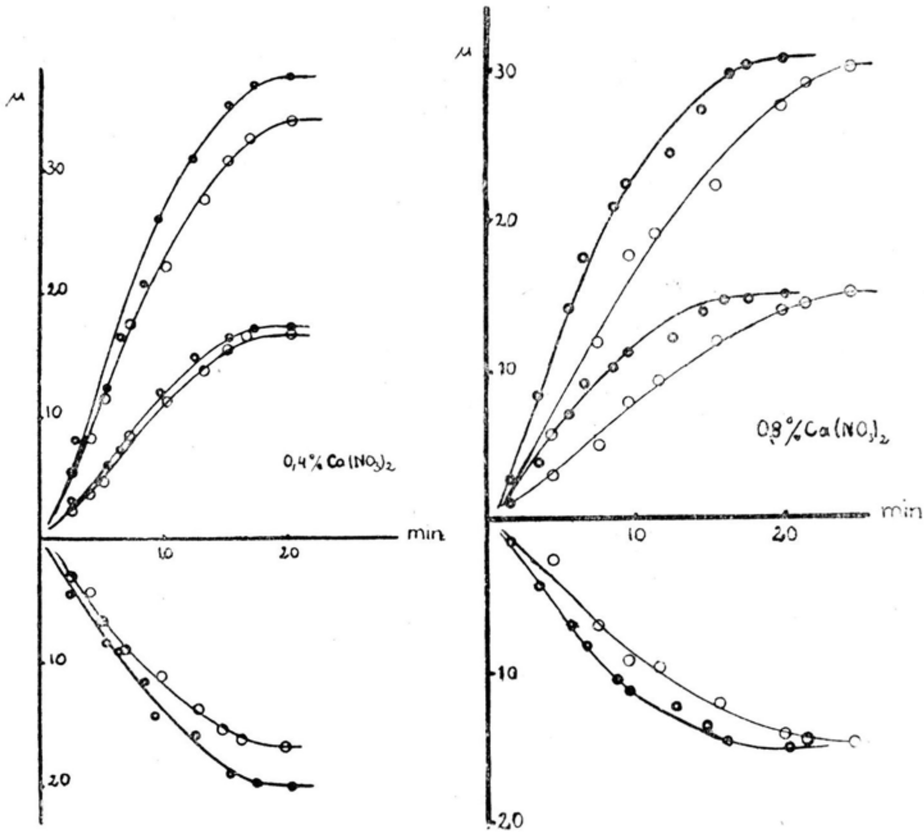
O b s e r v a t i o n s i n $Ca(NO_3)_2$ s o l u t i o n s.

The following concentrations were used: 0,2, 0,4, 0,8, 1, 1,3%. In this case as in the case of KNO_3 the stage of division and the time of acting of the solution were considered.

The changes caused by the action of $Ca(NO_3)_2$ are discernible in dilute (0,2 and 0,4%) solutions and become plainly visible in concentrated ones. In a medium in which Ca ions are present the refraction coefficient of cytoplasm and chromosomes increases and the refraction of chromosomes increases less thant that of cytoplasm. Usually the cytoplasm is more vitrous than in sugar solution, and both the nucleus and the cytoplasm structure becomes better visible. Cytoplasm circulation slows down when the concentration increases.

TABLE II

	Position of cell in hair	Time of anaphase in min.	The max. distance between kinetochores in anaphase in μ
0,1% $Ca(NO_3)_2$ solution	3	16	30
	1	17	36
	1	17	32
	1	17,5	32
	2	18	29
	1	18	32
	1	19	31
	1	20,5	34
	3	20,5	32
	1	21	32
0,4% $Ca(NO_3)_2$ solution	2	16	32
	3	16,5	34
	1	16,5	30
	1	17	34
	2	18	33
	1	19,5	29
	1	20,5	30
	1	20,5	32
	1	21	30
0,8% $Ca(NO_3)_2$ solution	3	17	30
	1	17,5	30
	1	17,5	34
	1	18	30
	1	18	32
	1	18	31
	2	18	32
	2	19	30
	2	19	29
	1	20,5	40
	1	21	32
	3	22	28
	1	23	33
	2	26	30
	1	26	32
	1	26	31
	1	26,5	33
	1	26,5	29
1% $Ca(NO_3)_2$ solution	3	18	35
	1	18	32
	1	18	33
	1	19,5	30
	2	20	30
	1	20	29
	1	23,5	32
	1	23,5	30
	1	24,5	34
	1	25,5	32
	3	19	32
1,3% $Ca(NO_3)_2$ solution	1	19	33
	1	20	31
	1	25,5	30
	2	30,5	40



Figs. 8—9. Graphs of chromosome movement in 0,4 and 0,8% $Ca(NO_3)_2$. 8 — the velocity of the chromosomes similar as in sugar solution. 9 — anaphases prolongated. Graphs for two cells.

In $Ca(NO_3)_2$ solutions most markedly in 0,8% concentration the formation of „Polkappen" was observed. Just before metakinesis kinetochores are on one side of the nucleus, and Polkappen form after the desappearing of nucleus membrane (this moment is very difficult to observe). On two sides of the nucleus two half moon spaces are visible; they are Polkappen (B ě l a ř 1929) with the lesser refraction coefficient than the cytoplasm and nucleus. After their formation they grow quickly in the direction of the poles and at the same time they become fainter. This is the way in which the spindle originates. The difficulties in precising the moment of the desappearing of nuclear membrane does not allow to confirm whether or not in their formation nuclear sap takes place. Metakinesis begins after the formation of Polkappen, and according to Bělař (1929) they are the spaces left by chromosomes moving to the metaphase plate. Most probably however metakinesis is accomplished after at least partial formation of the spindle (Hughes — Schrader 1943, Schrader 1947).

In anaphase in the 0,8% solution of $Ca(NO_3)_2$ the half-spindles are well visible (as a negative). It is necessary to stress that as a rule the spindle is sometimes visible but only in polarised light and after slight dehydration (Schmidt 1937, 1939). In this concentration in late anaphase it was often observed that kinetochores are semicircullarly arranged — concave sides toward the poles. Durations of anaphases are usually similar to those in sugar solution, or even longer (Table II, Fig. k).

In 1% solution the cell division does not differ much from the preceding one. In 1,3% concentration of $Ca(NO_3)_2$ most cells in metakineses and prophases return to the resting stage. Out of 23 cells 17 returned to the resting stage and only in 5 cells mitosis was noted. The time of anaphase does not differ much in comparison to the time in the previous solution. In exceptional cases however cell division with anaphase lasting 30 mins. was observed.

Only strong concentrations of $Ca(NO_3)_2$ solutions affect the cell division. Its prolongating effect on cytoplasm is slight, whereas the changes in division apparatus are very marked. The time of telophase and of cell wall formation does not change.

The action of KNO_3 is much stronger than that of $Ca(NO_3)_2$. Control solutions of KCl and $CaCl_2$ were used to examine the degree in which salts used depend on the cations and anions. The prolongating and shortening action of these salts is the same as the action of nitrates.

Discussion.

Completing data concerning the degree of hydration were obtained from various works on mitosis in plants and animals (Bělař 1929, Wada 1934, Möllendorf 1937, 1938, 1938a, Möllendorf and Ostrouch 1939, Schneider 1938, Schmidt 1937, 1939, Caspersson 1939, 1940 and others).

It appears that all mitosis is characterised by dehydration and hydration processes. All of the water involved in these processes is not derived from within the cell, and its quantity in different parts of the cell changes (Wasserman 1938).

In interphase the viscosity coefficient of cytoplasm is greater than in mitosis and chromosomes are strongly hydrated. In prophase the process of hydration of cytoplasm causes a decrease of the viscosity coefficient. At the same time a part of cytoplasmic water is absorbed by the nucleus, the volume of the nucleus increases and a part of the cytoplasm near it is hydrated (Wasserman 1938). Quantitative studies on volume change of prophase nucleus were made by Schrader (1947). It seems probable that the water absorption by the nucleus is related to the formation of the chromosomes and migration of nucleic acids from the cytoplasm to nucleus (Caspersson 1939, 1940). The processes of chromosome formation are connected with dehydration (Kuwada 1937, Kuwada, Shinke and Nakazawa 1938). „Teilungsraum" (Möllendorf 1938) — i. e. the space in which the formation of the spindle takes place — is hydrated by the water from chromosomes. In a cell two spaces: „Teilungsraum" with low viscosity coefficient and cytoplasm with a higher viscosity coefficient are formed (Möllendorf 1938,a,b).

The next process is the formation of the spindle. In plants the formation of spindle is preceeded by the formation of „Polkappen". In these places the secretion of liquid is presumed. Robyns (after Schneider 1938) maintains that the „Polkappen" are necessary for the formation of the spindle. The spindle is formed of liquid material, though it is an elastic structural gel (Frey — Wyssling 1946). In the process of its formation it becomes rigid; this is acomplished by the change of viscosity of the original substance. This change starts at the pole before reaching the middle of the cell, and resembles the crystalisation, with poles as cristalisation centers (Wasserman 1938, Schmidt 1937a). Numerous authors maintain that the spindle is of tactoid structure, which seems very probable (Östergren 1950).

As „Stemmkörper" is more liquid than the spindle, hydration must take place in anaphase. Some authors (Wasserman 1938) think that the process of hydration of „Stemmkörper" is connected with the destroying of spindle structure. The hydration process begins at the equator and then moves towards the poles. At the same time the viscosity coefficient of cytoplasm increases from metaphase to telophase and reaches its maximum value in interkinesis. In telophase processes of hydration and dehydration cause the protoplasm to have an equal viscosity coefficient.

These facts help to explain the influence of K and Ca ions. Unfortunately no method of quick and manifold measurement of viscosity coefficient of cytoplasm in the same cell so far is known. In my opinion those salt solutions, of which the hydrating and dehydrating influence on cytoplasm is known, cause viscosity changes in cytoplasm and in all division apparatus. In this process other changes — though their influence is small — may be caused. The change of viscosity coefficient as a result of salt action was proved by centrifugation (H e i l b r u n 1932), B r o w n movements (M ö l l e n d o r f 1937), and by the ability of fusion of separate cytoplasm parts (L o r e y 1929).

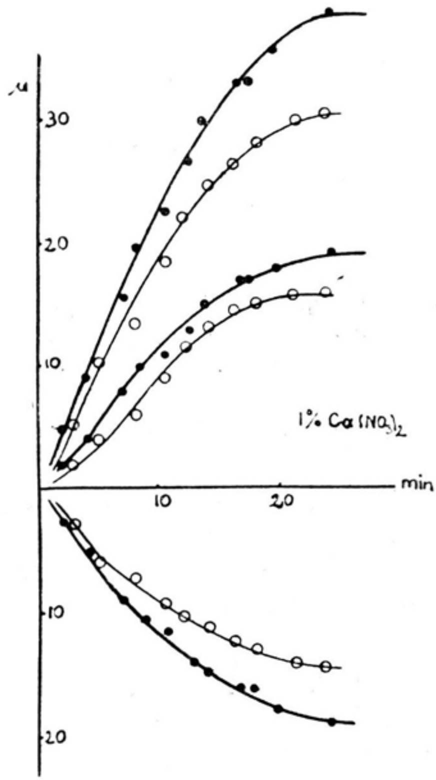


Fig. 10. Graphs of chromosome movement in 1% $Ca(NO_3)_2$ Anaphase considerably prolongated. Graphs for two cells.

According to F r e y — W y s s l i n g (1946) the elements of the first order (Li,Na,K a.s.o.) do not join with cytoplasm in stabile compounds, but only heteropolar bonds are formed, and they regulate the degree of hydration. The swelling of the cytoplasm is in a great extent dependant on the ions of Hofmeister series. Each cell is in a balanced stage, and when external conditions change, they cause the change of the physical constants of the cell. As K,Ca and Cl play an important role in cells it is characteristic that cells are able to withstand much stronger concentrations of these substances than of other. These elements cause neither death nor pathological changes in concentrations which in the case other elements cause strong demixing or the dying of the cell. If in a medium the amount of one of these elements is excessive, the equilibrium changes. The formation of the spindle depends on the hydration process of the cytoplasm.

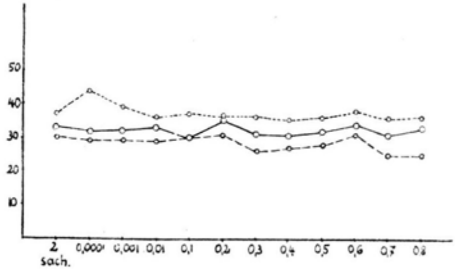


Fig. 11. Dependance of length of chromosome separation in anaphase in 2% sacharose and different KNO_3 concentrations. Concentration change does not influence the distance of chromosome separation. Dotted line — maximal value, continual line — mean value, marked line — minimal value. To the left of 0,1 logarithmic scale, to the right normal. Abcissa sacharose and KNO_3 concentrations, ordinate maximal separation of chromosomes in μ.

Prolongation of metaphase or more exactly of metakinesis in chick fibroblasts is explained by Möllendorf (1937) as being a result of the lack of the spindle formation. In my opinion in this case the action of the spindle becomes impossible. The studies of Hughes and Swann (1948) indicate that there are numerous tries of anaphase and only the last of them is successful. The facts observed in stronger concentrations of KNO_3 in *Tradescantia* may be explained similarly. Strong weaknes of the division mechanism causes the prolongation of metaphase as a consequence of numerous and prolonged tries of anaphase. It is difficult to establish whether this is the prolongation of metakinesis or the metaphase; it seems however that the reason lies in more serious changes in cytoplasm structure than the shortening of the time of anaphase. The present work indicates that anaphase is especially susceptible to the changes of hydration caused by potassium. The shortening of the time of anaphase is the result of acceleration of movement mechanism; the velocity of chromosomes increases and the maximal seperation does not change (Fig. 11). The strengthening of concentrations shortens anaphase and strong concentrations stop it alltogether. The changes of time in dependence of different concentrations of KNO_3 are shown in Fig. 12.

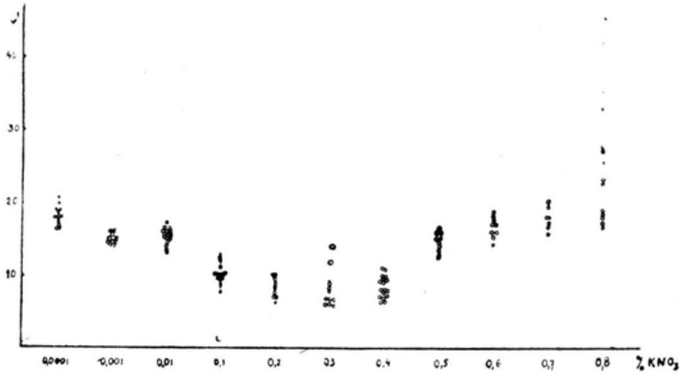


Fig. 12. Dependence of time of anaphase on KNO_3 concentrations. To the left of L logarithmic scale, to the right normal. Changes of time of anaphase

Most probably the prolongation of anaphase in strong concentrations of KNO_3 is caused by pathological changes, strong loosening or even partial destroying of the spindle structure. As a result of liquefying the spindle is not able to play its role in anaphase. If it is assumed that the structure of the spindle is tactoidal, the changes in chromosome movement may be explained by F r e y — W y s s l i n g's theory of submicroscopic structure of cytoplasm. According to the tactoid hypothesis the spindle is build of long polypeptide chains. Between the groups of polypeptide chains of gel — tactoids, there is a substance with liquid properties.

The basis of movement is always the structural gel. When the concentrations increase the viscosity coefficient between tactoids decreases, the viscosity resistance dimnishes and the movement is accelerated, which is due to the normal action of movement mechanism. Strong liquefaction also loosens polypeptide chains — i. e. the fibers causing the chromosome movement (C o r n m a n n 1944) — the movement apparatus is affected and the movement is retarded. In very strong concentrations of KNO_3 (1, 2, 1,6%) the structure is destroyed and the movement stops. It is well known that when the viscosity coefficient dimnishes considerably the structure of the cytoplasm is destroyed and all movement stops. For instance under action of high pressure the viscosity coefficient dimnishes and „in liquefied cytoplasm all plasma flow has stopped not only the creeping motion of amoeba cell but also the rotation in Elodea cells" (F r e y — W y s s l i n g. 1946 p. 119). Cytoplasm being a sol and having no structure, is not able to move, as structure according to F r e y — W y s s l i n g is the physical basis of the movement. It is probable that in the case of chromosome movement highly liquefied cytoplasm causes the breaking of bonds and stops all movement. In the light of these considerations Fig. 12 also represents the degree of plasma desorganisation.

Contrary to the K ions the influence of Ca ions is dehydrating, and in stronger concentrations of $Ca(NO_3)_2$ the Ca ions prolongue the anaphase and finally stop it altogether.

In normal conditions in cytoplasm K and Ca ions are in equilibrium and act as regulators. If there is a lack of one of these elements, disturbances in cells are observed. For instance the lack of potassium in marine animal eggs causes disturbances in the course of anaphase.

S U M M A R Y.

1. The influence of different concentrations of KNO_3 (0,0001 to 1,6%) on the time of anaphase was examined. In dilute solutions the time of anaphase is shortened (min. time in 0,3% KNO_3) and in concentrated solutions (to 0,8%) it is prolonged.

2. The shortening of the time is a result of an accelerated chromosome movement while the maximal separation in anaphase does not change.

3. In solutions of KNO_3 the refraction coefficient of cytoplasm and chromosomes decreases. In stronger concentrations numerous prophases and metakineses return to resting nuclei.

4. The influence of $Ca(NO_3)_2$ in 0,2 to 1,3% solutions was examined. In the 0,8% concentration and in stronger ones the anaphase is prolonged.

5. In $Ca(NO_3)_2$ solution the refraction coefficient of cytoplasm and of chromosomes increases. „Polkappen" and the spindle are often observable. In the 1,3% solution most of the prophases and metakineses return to resting nucleus.

6. The dependence of the chromosome movement on the degree of hydration of the cytoplasm by K and Ca ions indicates that changes occur in the submicroscopical structure of the spindle.

This work was carried out in the Institute of Plant Physiology of the Jagellonian University in Cracov. In conclusion I wish to thank the Head of the Institute Prof. Dr F. Górski for enabling me to carry out this investigation. I am also very sincerely gratefull to Prof. Dr H. Teleżyński, the Director of the Institute of Plant Anatomy and Cytology of Wrocław University for his most valuable help and criticism.

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