Electrical forces in Mitosis. I.

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Introduction.

The electrical forces are important in the mechanism of cell division. Their base is the cytoplasm which is a complex system of large particles, protein molecules, free moving kations and anions. The phases of this system are in close contact with each other and their boundaries always show differences in their potential. The mode of action of electrical forces however is not satisfactory elucidated till now.

In 1873 Fol (from Wilson 1928) called attention to the similarity of the spindle apparatus to the system of iron particles in the electrical or magnetical field. From that time many authors based the hypotheses of the mechanism of mitosis on electric phenomena. Only few experiments were carried on to clair the mechanism of electrical forces in mitosis. Recent experiments were performed by Botta (1932), von Lehotzky (1936), Kamiya (1937) and Churney and Klein (1937). The mentioned authors with the exception of Kamiya expressed the opinion that the chromosomes have a negative whereas nuclear membrane a positive electrical charge.

To attack this problem from another side by using different method, which according to my knowledge has not been used till now, I examined the influence of a homogene electrostatic field upon the chromosome movement. To establish whether there is really any influence, it is necessary to investigate the anaphasic movement of the chromosomes. Thus the purpose of this study was:

1. to investigate the movement of the chromosomes in the anaphase.
2. To establish whether the chromosomes are electrically charged.
3. To establish whether the electrostatic field has any influence upon the chromosome movement in the mitosis.

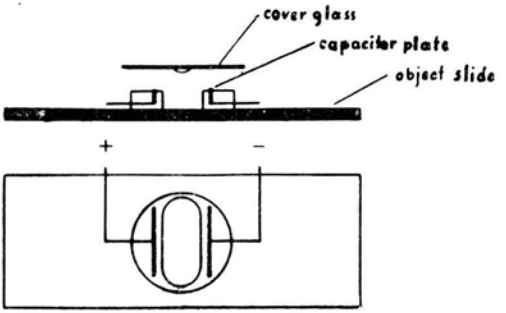
Material and methods.

Material and mounting. In the experiments young staminal hairs of a tetraploid race of *Tradescentia virginica* were used. The material originated from the Botanical Garden of the Cracow University. It is easy to obtain a suitable material from young buds. To obtain a material as uniform as possible, the stage of P. M. C.'s in the buds was always tested (B a r b e r's method 1939). Examined buds had 76% of the P. M. C.'s in the tetrad stage. It should be added however that the age of the hairs and the stage of the P. M. C.'s are only slightly correlated; the most important seems to be the shape of the cells. There are numerous mitoses in hairs of mediam age such as stated by B ě l a ř (1929). According to B ě l a ř's advice (1929) observations were executed in a hanging drop and much care was taken not to crush the hairs and to touch them as little as possible with preparations tools. In order to follow the normal course of mitosis in cells, the observations were started half an hour after the mounting of the hairs; on the other hand for the study of some pathological divisions the observations had to be started at once before the cells were allowed to return to aproximately normal conditions. The observation's medium was not 2% saccharose solution as used by B ě l a ř but liquid paraffin (T e l e ż y ń s k i 1930). Liquid paraffin dissolves at least five times as much oxygen as water and hairs may live in this medium during days and even weeks (K u w a d a and N a k a m u r a (1934). It is also very important that it is a bad electrical conductor.

All observations were carried out in 19,5—22,5° C.

Apparatus. A special generator for high voltage supply was constructed. Its most important elements were: a high voltage transformer, a high vacuum rectifying valve (RFC 5), two filtering capacitors (10 μ F) and a regulated potentiometrical output. The voltage could be changed almost gradually from O to 4150 V. The observations were executed in a flat capacitor of a diameter 10 mm (Text. fig. 1), the negative pole of which was always grounded.

The capacitor was fixed on an object slide. Hairs in a hanging drop of liquid paraffin were placed on a cover glass which was glued by a thin layer of vaseline to the capacitor. After finding a dividing cell the cover glass was adjusted in a suitable position. Special care was taken not to bring into immediate contact the drop of paraffin and the plate of the capacitor to avoid the drying out of cells, as the paraffin was removed almost completely in such cases.



Textfig. 1. Capacitor in which observations were executed; explanations in text.

Optics. L e i t z oil immersion lens 100 x N. A. 1,30 was used with Z e i s s'e s 10 x compensation eyepiece. Heat rays were absorbed by means of a water filter with a 20% solution of M o h r's salt (B ě l a ř 1929). In the observations in the electrostatic field the condensor was lowered down and the immersion lens lifted immediately after each measuring. The purpose of this was to prevent the disturbances of the electrostatic field caused by the vicinity of the condensor and the objective.

For measurement of the chromosome movement, A b b e's camera lucida was used. The positions of the kinetochores with regard to the equator were marked in short time intervals. This method used by R i s (1943) is quick and more exact than the photographic method (B a r b e r 1939).

Errors and graphs. (cf. p. 720). Errors in measurement vary in different cells as well as in different stages of mitosis. They are smaller in the late anaphase than in the early anaphase and early telophase. It is difficult however to estimate its absolute value. Additional sources of errors are: 1. changes in length of the cell which was observad in many cells during mitosis. 2. the difficulty of exact tracing of kinetochores in metaphase. In each cell the distances between the equator and each of the two chromosome

groups at anaphase have been plotted separately. This was done in order to estimate as exactly as possible whether the chromosomes move to the poles with the same speed and in the same time. Thus the graph for each cell is represented by two curves which may be more or less symmetrical. In addition, a third curve showing the distance between the kinetochores, representing a sum of the two former, has been plotted. It should be mentioned that only this last type of graphs is given by B a r b e r (1939) and R i s (1943) in their papers dealing with chromosome movement.

O b s e r v a t i o n s.

In the course of the present work 68 divisions without field action were observed and the analysis of 40 from among them is given in Table I. The remaining 18 cells were cells situated at a distance of 4—6 cells from the top of the hair and the cells with an oblique metaphase plate. In view of the fact that they were not always observed since the beginning of the anaphase, the observations have not been included in Table I. Besides that 12 cells with pathological divisions and 234 cells in the electrostatic field were observed.

Studies on mitosis in staminal hair celles of *Tradescantia* were carried on by many authors (B ě l a ř 1929, T e l e ż y ń s k i 1930 and others). The chromosome movement in anaphase studied on a limitated number of cells by B a r b e r (1939), gives a general idea about the course of the anaphase.

The present study deals exclusively with anaphase and telophase. Barber's data concerning mitosis differ from those obtained below; this can be due to the fact that probably different races of *Tradescantia virginica* were studied. In the present observations in the normal division the prophase lasted 1,5—2,5 h., the metaphase 5—18 min., the anaphase 13,5—26 min., the telophase till to the formation of the resting nuclei approximately half so long as the prophase. It is impossible to make an exact delimitation between the end of the anaphase and the beginning of the telophase; thus as the end of the anaphase is considered the moment in which the maximal distance between the kinetochores is reached. In B a r b e r's studies all the respective stages last longer in spite of similar external conditions (temperature, medium): the prophase lasts 3—4 h., metaphase 15—25 min., the anaphase about 25 min. Thus in the material of the present studies the cell division goes quicker on.

Normal divisions.

In the exact studies of the anaphase movement mechanical relations in the cell division were taken into considerations. According to B a r b e r (1939), metaphase passes rather abrupty in the anaphase; the kinetochores reach the max. velocity 1—2 min. after beginning of the anaphase movement. The earliest stages of the anaphase are very difficult to trace owing to the position of the kinetochores in respect to the chromosome limbs. As it is visible from Table I, there is no much connection between the lengh of the cell, the length of the kinetochores way, the time of the anaphase and the max. distance between the kinetochores. Sometimes the way of the kinetochores is much shorter in a long cell than in a short one. For instance in a cell 55 μ long the max. distance between the kinetochores was 27 μ; the same distance was passed by the chromosomes in a notably shorter cell (34 μ) and a still longer distance exeeding the former by 5,5 μ has been observed in a different cell of approximatively the same length (36,5 μ). Similarly the max. velocity of the kinetochores does not depend upon either the length of the kinetochore's way or the time of the anaphase. In some cells where the max. distance between the kinetochores was 30—32 μ, the time of the anaphase was 13,5—26 min. and the max. velocity 1,2—2,2 μ/min. The interesting fact that a greater velocity is not always connected with a shorter time may be explained as follows: a great velocity is of a relatively short duration and it is followed by a slower movement. B a r b e r (1939) expressed the opinion that the chromosome movement may be checked by the vacuoles occurring near the poles; according to my observations too, the stopping of the chromosomes caused by the vacuoles is plainly visible in cells situated at a distance from the top of the hair (5-th or 6-th cell). Unfortunately such cells divide very rarely. In such cells there may be two vacuoles on each end; at anaphase the vacuoles are pushed aside by the moving chromosomes. On the other hand, if only one large vacuole which occupies the whole width of the cell, is present at each end, the chromosomes are stopped in their movement as soon as the kinetochores approach the surface of the vacuole and only little pressure is exerted on such vacuole (Plate I fig. 13).

In cells situated near or at the top of the hair as a rule two or more vacuoles are observable at each end. In such cells the chromo-

TABLE I.

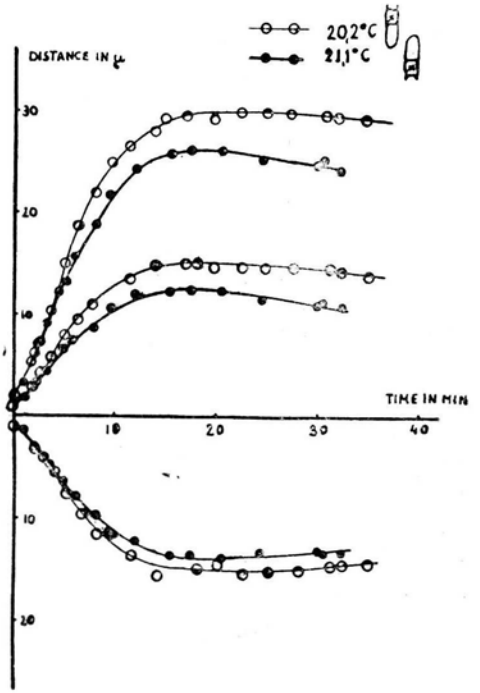
Normal division. 40 cells from 68 observed.

Position of cell in the hair	Cell dimensions in μ	Time of max. seperation in min.	Distacce of max. seperation in μ.	Max. velocity of kinetochores in μ/min.	Changes of position of nuclei in telophase		
					Hair top	Symmetrically or no change	Hair base
1	44 ×22	26	30,5	1,2	I		
1	66 ×20	25	42	1,7		I	
1	63 ×21	24,5	29	0,7	I		
2	40 ×19	24,5	27,5	0,8	I		
3	43 ×21	23	28,5	0,6		I	
1	50,5×21,5	23	31	1,9		I	
1	44 ×21,5	23	29	2,2	I		
1	49,5×22,5	22,5	32,5	2,1	I		
1	57 ×21,5	22	32	2,2	I		
3	55 ×22	21	31,5	2,0	I	I	
2	43,5×20,5	21	30,5	1,5		I	
1	53 ×20	21	31	1,6	I		
2	45 ×19	21	29	1,4	I		
1	47,5×19,5	20,5	29,5	1,8	I		
2	34 ×20	20	27	1,8		I	
3	36,5×22	20	32,5	1,5			I
1	50 ×18	20	32,5	2,1	I		
1	52 ×18	20	32	1,8	I		
2	40 ×21	20	30	1,7	I		
1	67 ×18,5	19	26	1,4	I		
1	49 ×21	19	30,5	1,2	I		
3	34,5×21,5	18,5	28,5	1,5	I	I	
2	39 ×21,5	18,5	29	2,2			
2	53 ×20	18,5	31,5	1,9	I		
1	66 ×19	18,5	32,5	1,8	I		
2	48 ×20	18	30,5	2,0	I		
2	49 ×18	18	32	1,9	I		
1	62 ×16	18	33,5	1,5	I		
1	53 ×17	18	37	1,9	I		
3	42 ×26	18	29,5	1,7	I		
1	60 ×19	18	33	1,9	I		
1	55,5×20	18	27	1,5	I		
3	39 ×22	17,5	29	1,7	I		
2	62 ×20	17,5	34	1.9	I		
1	45 ×20	17,5	29	1,5	I		
1	67 ×22	17	34,5	1,6	I		
3	42,5×22	15,5	33,5	2,2	I		
2	50 ×21,5	15	32	2,1			I
5	31,5×25	15	24	1,2	I		
1	39 ×20	13,5	29	1,9	I		

Numbers I, II, III, mean early middle and late stage

somes press the vacuole towards the longitudinal walls, thus, their movement is beeing only slowered in a slight degree.

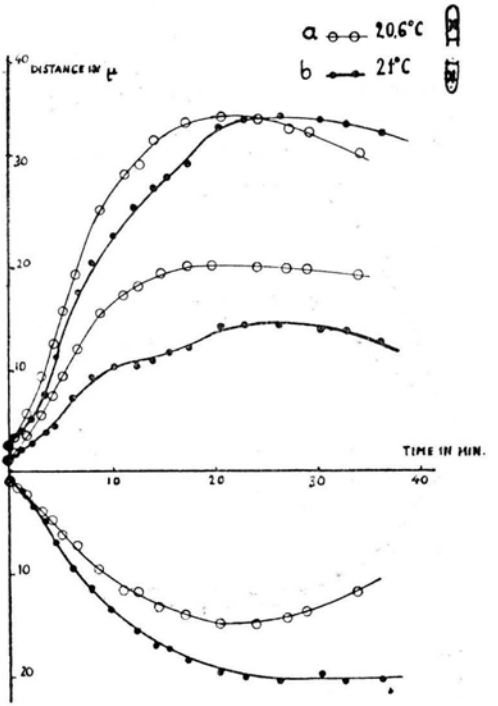
At telophase the chromosome groups are slightly pushed back towards the middle of the cell or alternatively to one of its ends. This change of position may be explained by three main factors: 1. contraction of the chromosomes in telophase (B ě l a ř 1929); 2. the formation and the developement of the phragmoplast which becomes shorter and broader (B a r b e r 1939); 3. the growth of the vacuoles and the formation of new ones, at the transversal walls of the cell and in the area of the phragmoplast (Plate I figs. 4—7). The pushing of telophasic nuclei is most frequently towards the top of the cell. It takes place in almost all top cells and also very often in the next few cells. A symmetrical pushing back of nuclei is found rarely and almost exclusively in cells situated farther from the top. In numerous cells (35—40%) a shortening of the cell length (0,5—8 μ) and a parallel increase of its width were observed between the metaphase and the telophase.



Textfig. 2. Graphs showing symmetrical division in two cells; one division symmetrical, the second almost completely symmetrical, (temperature and the position of the resp. cells are given).

With regard to the position of the metaphase plate, the mode of the chromosome separation and the displacement of the telophasic nuclei it is possible to distinguish roughly three modifications of cell divisions which are linked however by transitory forms.

1. *Symmetrical division.* Groups of the chromosomes in their movement to the poles have the same velocity and cease to move simultaneously. Graphs of the movement of the two chromosome groups are like a mirror reflections. (Textfig. 2). Such division is very rare and could be observed only in few cases (some %). Special conditions in the cell are necessary for the performance of such a division. The cell must be well filled with cytoplasm lacking larger vacuoles or having a symmetrically distribution of the later. Such conditions however are fulfilled very rarely, if occur at all, especially in the top cells. In farther cells they may be found probably sometimes.



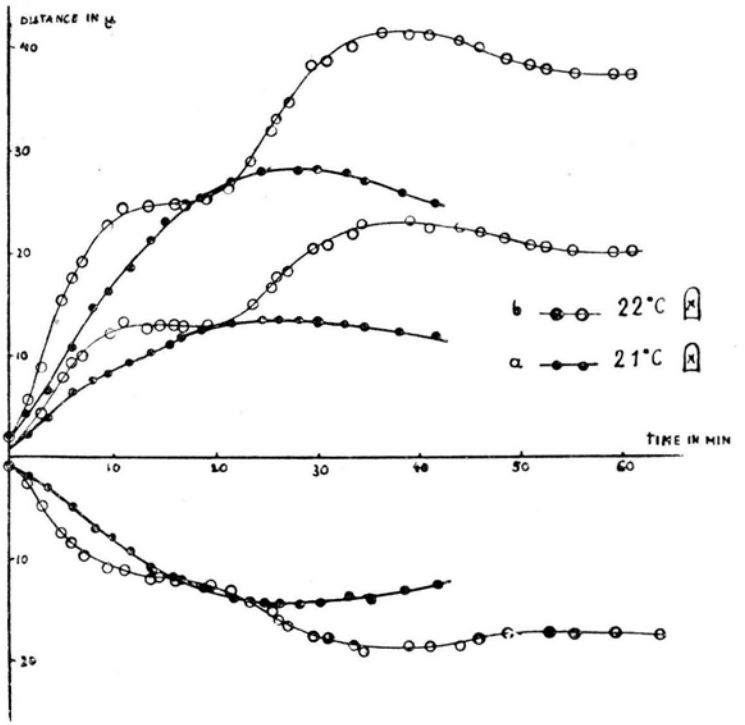
Textfig. 3. Graphs showing asymmetrical division in two cells; a. two groups of chromosomes stopped not synchronically, b. differences in kinetochor's velocity between two groups, (temperature and the position of the resp. cells in the hair are given).

2. *Asymmetrical division.* This type was observed in the majority (about 90% of the cells), especially in the top cells of the hair. In this respect two possibilities exist: 1) The two groups of chromosomes stop their movement to the poles in different time (Textfig. 3a). In extreme cases this could lead to unipolar division, which however has not been observed. 2. The velocity of the two chromosome groups is different in the same time (Textfig. 3b). The first possibility is usually fulfilled when the metaphase plate is placed nearer to one pole, whereas the second, when the vacuoles are distributed asymmetrically in respect to the metaphase plate. The two graphs plotted separately for the movement of each group of anaphase chromosomes, are asymmetrical in the above types of division; the shape of the third graph however representing their sum does not reveal often the details of the course of the anaphase; this is due to the fact that the irregularities in the movement of the two groups may interfere with each other. Thus the resultant curves for cells with distinct differences in anaphase movement may have similar shapes.

3. *Cell division with oblique metaphase plate.* In a few cells cells the metaphase plate is not approximately perpendiculary oriented but at an acute angle (till. 30°) to the longitudinal axis of the cell. Such cells were observed by B ě l a ř (1929). As a rule they are in very narrow cells with about 15—17 μ. In the limited space the metaphase plate which is relatively broad takes an oblique position (Bajer 1951 in the press). In the anaphase the kinetochores first approach the longitudinal cell walls and then they glide along the walls; in the subsequent stages the cell division follows one of the above described types (Textfig. 4a). In some cases (two observations), the chromosomes stop when they are near to the longitudinal cell walls and after a moment they continue their farther movement to the poles (Textfig. 4b).

Pathological division.

They could be observed in hairs immediately after their mounting. Usually they occur in wounded cells, or cells mechanically irritated; frequently in such hairs in which some cells are crushed. In all these cells often a reversible „Entmischung" (B ě l a ř 1930) takes place. The description of the appearance and the characteristic of such cells has been given already in his work. Cells with a strong „Entmischung" do not divide at all, in those with a weak



Textfig. 4. Graphs showing cell division with oblique metaphase plate; a. the kinetochores glide along the cell wall, b. the kinetochores stopped and after some time the anaphase movement is resumed, (temperature and the position of the resp. cells in the hair are given).

degree of „Entmischung", cell division continues but it lasts usually longer. It shows a high degree of asymmetry; this may be caused partly by better developed vacuoles. In most instances the mitosis does not go to the end and the cell dies, while in some cells the formation of a restitution nucleus was observed.

Cell division in the electrostatic field.

The influence of the electrostatic field was examined in two directions: the field lines act parallely or, alternatively perpendicularly to the lingitudinal axis of the cell. Very great differences between the action of the low field intensity (10—480 v/cm) and the

TABLE II

Field acting parallely to the longitudinal axis of the cell. 72 cells from 93 observed *.

Position of cell in hair	Intensity of electrostatic field	Cell dimensions in μ	Beginning of field action									Time of max. seperation in min.	Distance of max. seperaton in μ	Max. velocity μ/min.	Changes of position of nuclei in telophase			Pushing to	
			Prophase			Metaphase			Anaphase						Hair top	Symmetrically or no change	Hair base	+	—
			I	II	III	I	II	III	I	II	III								
1	10	60,5×19,5	I									17,5	33,5	1,8		I			
1	V/cm	50 ×20	I									19,5	31,5	1,7	I			I	
1		58 ×20	I									17	32,5	1,7	I			I	
1		52,5×20	I									19	27	1,6	I			I	
2		43,5×20,5		I								15	29,5	1,4	I				I
3		49,5×21		I								24	29	1,0	I				I
1		45 ×21			I							16	28	1,2	I			I	
3		47 ×20			I							18	25	1,1	I				I
1		50 ×21				I						21	33	1,5	I			I	I
1		52 ×20				I						20	34	2,1	I				
2		44,5×20					I					22	29,5	1,8			I		I
1		45 ×20,5					I					19,5	30	1,7	I			I	
1		42 ×20						I				20,5	31,5	2,0	I				I
1		43 ×21						I				19	26	1,3	I				I
1		55 ×17							I			18	26,5	0,9		I			
1		45 ×20							I			15	31'5	1,8	I				I
2		43,5×23								I		23	27	1,5		I			
1		67 ×21								I		22	28	1,7	I				I
2		48 ×20								I		20	32	1,8	I			I	
2		37 ×17								I		22	30	1,7		I			
3		44 ×19									I	19	26	0,6	I			I	
3		40 ×20									I	21	23,5	0,7			I		I
2		49 ×21									I	18,5	25	1,2	I			I	
2		50 ×20									I	17	29	1,4	I			I	
1	100	50 ×18	I									21	30	1,2	I				I
1	V/cm	60 ×19	I									21,5	32	1,2	I				I
2		38 ×20		I								23	29,5	1,4	I				I
1		45 ×21		I								20	31,5	1,2	I			I	
1		46 ×19			I							18	34,5	1,5	I			I	
2		40,5×20,5			I							16	31	1,6	I			I	
1		72 ×17				I						18	30	1,4	I				I
1		53 ×21				I						18	25	1,1	I			I	
1		58 ×10,5					I					18	27	1,3	I			I	
1		68 ×21,5					I					16,5	30,5	2,1	I			I	
1		56 ×18					I					17	31	1,8	I				I
2		35 ×21,5						I				22,5	26,5	0,8	I				
1		67 ×20						I				26	34	1,6	I			I	I
1		53 ×21,5							I			25	30	1,5	I				I
3		35 ×22,5							I			13,5	22	1,6		I			
2		36 ×23							I			20	29	1,6		I			
2		38 ×22							I			19,5	24,5	1,3	I				I
1		61 ×20								I		15	32,5	2,2	I			I	
2		53 ×18								I		19	29	2,8	I			I	
1		47 ×19								I		18,5	24	1,7	I				I
1		46 ×20									I	20	31	1,6	I			I	

TABLE II (continued)

Position of cell in hair	Intensity of electrostatic field	Cell dimensions in μ	Beginning of field action									Time of max. seperation in min.	Distance of max. seperation in μ	Max. velocity μ/min.	Changes of position of nuclei in telophase			Pushing to	
			Prophase			Metaphase			Anaphase						Hair top	Symmetrically or no change	Hair base	+	—
			I	II	III	I	II	III	I	II	III								
1		49 ×19										24	31	1,5	I				I
1	480	40 ×22	I									20	26	0,9	I				I
1	V/cm	38 ×21	I									19	29	1,3	I				I
2		29 ×24		I								15,5	25,5	1,0		I			
1		70 ×18		I								17	35	1,4	I			I	
1		72 ×16		I								21	33,5	1,3	I				I
2		37 ×25										20	27	1,5		I			
2		40 ×24			I							22,5	30,5	1,3			I		I
1		54 ×19,5			I							24	26	1.5	I			I	
1		50 ×20				I						20	28,5	1,7	I			I	
1		50 ×22,5				I						20	29,5	2,0	I			I	
2		54 ×26					I					24	28,5	1,7	I			I	
1		49 ×19					I					21,5	34	1,7	I				
1		49 ×19					I					22	24	1,2	I				I
3		58 ×19,5						I				16	32	1,6	I			I	
1		59 ×20						I				21	32	1,2	I			I	
2		42 ×21,5						I				26,5	22	1,3	I			I	
1		54 ×20,5							I			17,5	27	1,5	I			I	
1		65 ×18,5							I			19	33	1,8	I				I
2		56 ×18							I			15	24	2,0	I				I
2		57 ×19,5								I		22	28	1,6	I			I	
1		48 ×20								I		20	24	2,2		I			
1		52,5×18								I		25	28	0,7	I			I	
1		49 ×21								I		20,5	32	1,6	I			I	
1		48 ×20									I	24	28,5	1,0		I			
1		48 ×18									I	23	33	1,6	I			I	
1		49,5×20									I	18,5	29,5	1,4	I			I	
3		45 ×22									I	24,5	27	0,7			I		I

high field intensity ** (4150 v/cm) could be established. The results are given on Tables II, III, IV. The field action upon various cells involves cells in different stages ranging from early prophase to telophase; the numbers: I, II, III on the Tables indicate the early, middle and late stage.

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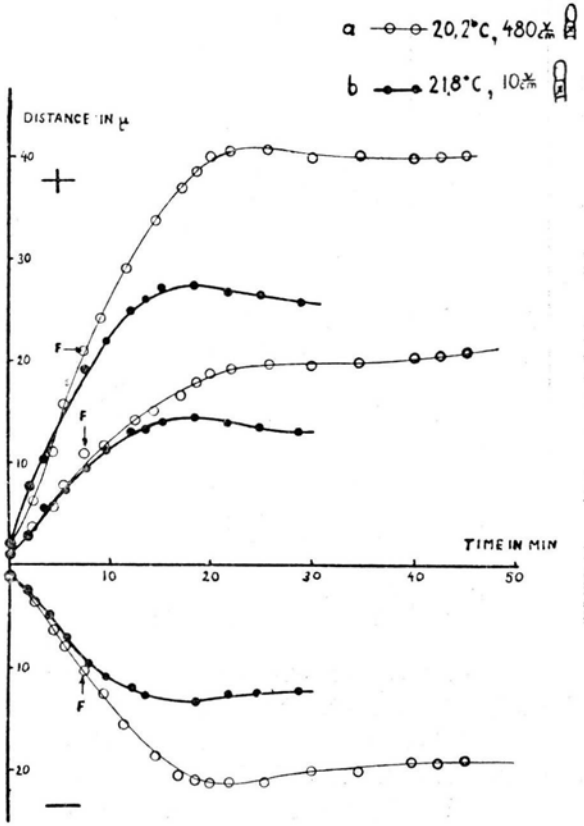
* Numbers I, II, III, mean early middle and late stage.

** During the work owing to the fuse of the part of the transformer's primary circuit, the voltage became probably much higher at least twice as high as before. It is impossible to precise in what a moment the quoted defect took place and only the lower electrostatic field intensities represented on the Table II are sure. The photos and graphs were also made with the defective transformer.

Influence of field intensity 10—480 v/cm

98 cells were observed in fields of such intensity. Numerous cells in that field as well as in higher ones were influenced parallely to the longitudinal axis of the cell since the anaphase. This was done with the purpose of investigating whether the chromosomes bear any electrical charge. If they are charged and the field lines act parallely to the direction of the chromosome movement the separating groups of chromosomes should have a greater or smaller velocity according to the sign of the electrical charge and the direction of the field, provided that the field is sufficiently strong. In fact neither slowering nor acceleration of the chromosome movement has been observed. (Textfig. 5). Von Lehotzky (1936)



Textfig. 5. Graphs showing cell division in low electrostatic field; F indicates the beginning of the field action; cell division is quite normal, (temperature and the position of resp. cells in the hair are given).

TABLE III

Field 3000 v/cm acting perpendicularly to the longitudinal axis of the cell. 25 cells from 54 observed *.

Position of cell in hair	Beginning of field action									Time of max. seperation in min.	Distance of max. seperation in μ	Max. velocity μ/min.	Changes of position of nuclei in telophase			
	Prophase			Metaphase			Anaphase						Hair top	Symmetrically or no change	Hair base	
	I	II	III	I	II	III	I	II	III							
3	I									32	29,5	1,3	I			
1	I									29,5	32,5	1,0	I			
2	I									28	27,5	1,4	I			
1	I									37	31	0,8	I			
1		I								28	28,5	1,7	I			
4		I								23	29,5	1,6	I			
1		I								22	29	1,9	I			
1		I								22	29,5	1,2	I			
2		I								26	31,5	1,1			I	
2		I								28	30,5	1,1	I			
1			I							22,5	30,5	1,3	I			
1				I						21,5	25,5	1,0	I			
2				I						21	29,5	1,8	I			
1				I						17	28	1,6	I			
1					I					20	25,5	0,9	I			
1					I					15,5	26	1,4	I			
1						I				21	30	1,6	I			
1						I				20	25,5	1,0	I			
2							I			22	28,5	1,7	I			
1							I			18	26,5	1,5	I			
1							I			17	29,5	2,0	I			
1								I		17	29	1,8	I			
1								I		21	28,5	1,4	I			
1									I	18	32	1,9	I			
1									I	20,5	31	1,6	I			

expressed the opinion that the chromosomes in the resting nuclei are not electrically indifferent. If this is true either the whole nucleus would be pushed to one pole or the chromosomes would alter their position inside the nuclei. Such phenomena however were not observed. As Table II shows in comparison with Table I, the pushing of telophasic nuclei occurs mostly from the base towards the

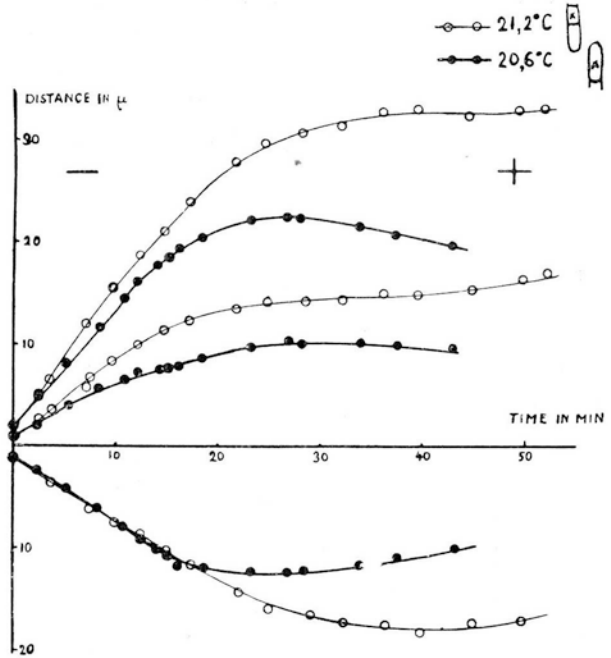
* Numbers I, II, III, on the Table mean early, middle and late stage.

top of the hair; irrespectively from the direction of the field. In numerous cells the field action began at prophase. Also after a prolongated field action no visible effect was observed. The cell division in the field is quite normal.

Similarly a field of the same intensity acting perpendicularly to the longitudinal axis of the cell had no effect on mitosis.

Influence of field intensity 3000 v/cm.

54 cells were observed in the field of such intensity. Also here the field parallel to the longitudinal axis (20 cells observed) did not



Textfig. 6. Graphs showing influence of 3000 v/cm parallel to longitudinal axis of the cell from early prophase (I); the anaphase is prolonged, (temperature and the position of resp. cells in the hair are given).

influence the mitosis. The effect however could be observed after the action directed perpendicularly to the longitudinal axis since the early stages of prophase, whereas the action started at metaphase and anaphase seemed to be without any effect. The only influence of such a field manifested itself in prolongation of the anaphase till 30 min. and more (Table III and Textfig. 6). It should be added that pathological symptoms were not observed in

TABLE

Field 4150 v/cm. 53 cells

Position of cell in hair	Beginning of field action												Time of max. separation in min.	Distance of max. separation in μ	Max. velocity μ/min.	Changes of position of nuclei in telophase			Pushing of nuclei to	
	Prophase			Metaphase			Anaphase			Telophase						Hair base	Symmetrically or no change	Hair base	—	+
		II	III	I	II	III	I	II	III	I	II	III								
	Field acting ‖ to the longitudinal axis of the cell.																			
1	I																			
1	I																			
2	I																			
2		I																		
2		I																		
2		I																		
1				I																
1				I																
1				I										22						
1					I															
2						I														
1							I						23	24	1,2					
1							I													
1								I					17,5	29	1,7					
2								I					17,5	28,5	1,6					
1								I												
1								I					40	18	0,2					
1									I				20	25,5	0,8	I				I
1									I				18	30	1,9	I				I
2									I				17	29	1,6		I			
1										I			16	26	1,8	I			I	
1	I	I											36	21	0,3					
2	I	I											18	25	1,4			I	I	
2			I				I						31	21	0,3					
1			I					I					29	20	0,5		I			
1					I	I							21	25	1,4					
2							I	I					35	24	0,3		I			
1								I					16,5	29	1,2	I				
1								I	I				19	30	1,3	I			I	
2								I	I				16,5	30	1,5				I	I

* Numbers I, II, III mean early, middle and late stage; P = prophase,

IV

from 82 observed *.

REMARKS
cell division stopped; cell died in P II
coagulation in P II; cytoplasm circulation maintained 3 h. later
coagulation in P II; cytoplasm circulation maintained 3 h. later
coagulation in P II; cytoplasm circulation maintained 3 h. later
coagulation in P III
coagulation in M I
coagulation in M
coagulation in M
coagulation in A II — III
coagulation in A I; cytoplasm circulation maintained 3 h. later
coagulation in A III; cytoplasm circulation maintained 3 h. later
no formation of nuclear membrane; retarded cell wall formation (20 min.)
coagulation in A II
no formation of nuclear membrane; retarded cell wall formation (20 min.)
cell wall formed after 25 min
coagulation in A II
coagulation in T I
no formation of nuclear membrane and cell wall
no formation of nuclear membrane and cell wall
no formation of nuclear membrane and cell wall
no formation of nuclear membrane; cell wall formed
field acting 3,5 h.
fied acting 1h.; cell wall formed after 3h. nuclear membrane after 3,5 h.
field acts 3h. T lasts 4h. 2h. after ceasing of field action death of the cell
field acts 3,5h. T lasts 4h. 3h. after ceasing of field action death of the cell
field acts 1h. nuclear membrane and cell wall formed after 25 min.
field acts 10 min. anaphase is prolonged; cell wall formed after 2,5h.
field acts 10 min.; normal division
field acts 1h. cell wall and nuclear membrane formed after 15 min.
field acts 1h. cell wall and nuclear membrane formed after 25 min.

M = metaphase, A = anaphase, T = telophase.

TABLE IV

Position of cell in hair	Beginning of field action												Time of max. separation in min.	Distace of max. seperation in μ	Max. velocity μ/min.	Changes of position of nucei in telophase			Pushing of nuclei to	
	Prophase			Metaphase			Anaphase			Telophase						Hair top	Symmetrically or no change	Hair base	—	+
	I	II	III	I	II	III	I	II	III	I	II	III								
	Field acting ⊥ to the longitudinal axis of the cell.																			
1	I																			
2	I																			
3	I																			
1		I																		
1			I																	
1			I																	
1				I																
2				I																
2					I															
1					I															
3						I														
1							I													
1								I												
1								I												
1								I												
2								I												
2									I				17	32	1,5					
1											I		19	30,5	1,7					
1				I		I							34	28	1,0					
1					I	I							27	29,5	1,2					
2							I	I					20	31	1,6					
1							I	I					16,5	30	1,8					
1						I	I						18,5	32	1,5					

such cells and even the slightest „Entmischung" could not be revealed. The vacuoles undergo quite normal changes; the refraction coefficient is quite normal.

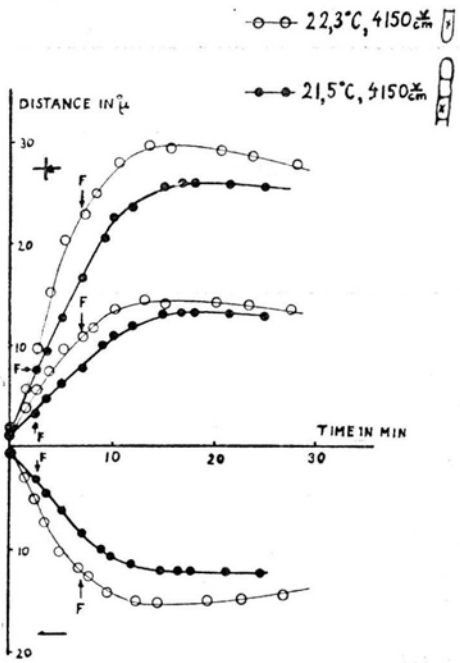
Influence of field intensity 4150 v/cm.

82 cells were observed in the field of this intensity. The results of observation of 53 among them are on the Table IV. A field acting parallely as well perpendicularly to the long axis of the cell was used. The influence of field manifested itself already after

(continned)

REMARKS
coagulation in P I
coagulation in P I
coagulation in P II
coagulation in P II
coagulation in P III
coagulation in P III
coagulation in M
coagulation in M
coagulation in M
coagulation in A I
coagulation in A III
no formation of nuclear membrane and cell wall
no formation of nuclear membrane and cell wall
no formation of nuclear membrane and cell wall
no formation of nuclear membrane and cell wall
no formation of nuclear membrane and cell wall
cell wall formed partially
nucear membrane and cell wall formed
field acts 30 min. formation of cell wall prolonged
field acts 15 min. formation of cell wall prolonged
normal cell division; no influence marked
normal cell division; no influence marked
normal cell division; no influence marked

several minutes; at the beginning cell division was slowered and then it was completely stopped. It should be emphasized that the action does not influence the actual stage but manifests itself in a later one. Thus the influence of the field since the anaphase causes changes in telophase, while that started at metaphase is responsible for disturbances at anaphase and so on. Stages of a longer duration however may be influenced at once e. g. early prophase. Individual cells shows differences in the effect of the field action; an irreversible „Entmischung" appears in most instances after 4—5 hours,



Textfig. 7. Graphs showing influence of 4150 v/cm perpedicular to longitudinal axis of the cell from anaphase; F indicates the beginning of the field action; completed anaphase, (temperature and the position of resp. cells in the hair are given).

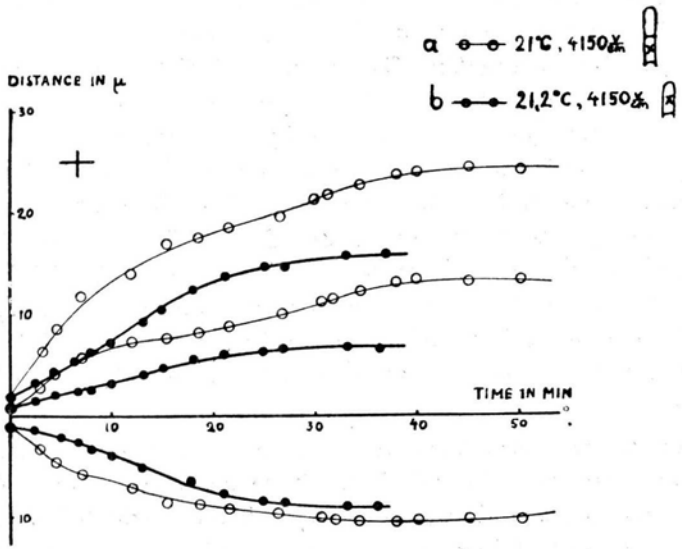
but in some cells it may be visible sooner (after 1,5—2,5 h.) or later (6—7 r.). The influence of the field since the late prophase may cause sometimes a complete stopping of the cell division in early anaphase and in others already in metakinese.

Numerous cells in different stages of mitosis were submited to the action of the field only for a short or longer period of time (10 min. — 3 h.). After some time such cells returned to normal conditions; differences however were found among them in recovery of their normal appearance, in the length of the anaphase as well as the formation of the cell wall and the nucleus membrane. Some examples are found on Table IV.

The effect on prophase. After an action of sveeral minutes the cell division stops, the refraction coefficient of the chromosomes increases and after 1—2 hours it decreases: then the chromosomes begin to swell (Plate II Figs. 15—16). In the late prophase after

swelling they are nearly twice as thick as normally (Plate II Figs. 17a—17b), similarly in metaphase. Owing to the decreasing of the refraction coefficient of the chromosomes and its slight increasing in the cytoplasm the chromosomes seem to be surrounded by a thin more refracting peculiar „coat". The chromosomes seem to be quite empty optically. After 3 h. in spite of their very strange appearence, they are usually in the stage of a reversible „Entmischung" and recover when the field ceases to act. The cell division continues but the formation of the cell wall is retarded.

The effect on metaphase and anaphase. If the field acts from metaphase there may by either no anaphase or it may last a very long time (30—40 min. instead of about 18 min.); the max. distance reached between the kinetochores is about 20 μ (normally about 30 μ); the max. velocity of the kinetochores is very small — usually below 0,5 μ/min. The telophase is of a very long duration (3—4 h.), it is abnormal, it becomes interrupted as the nuclear membrane and the cell wall fail to be formed (Plate II Figs. 19—20). This phenomenon is connected with the chromosome swelling. In most cases the chromosomes swell already in the late prophase: they are optically empty and are surrounded by a peculiar „coat".



Textfig. 8. Graphs showing influence of 4150 v/cm from: a. middle metaphase (II), b. early metaphase (I); the max. distance between kinetochores is short, as a result of field action the cells died, (temperature and position of resp. cells are given).

If the field acts from early anaphase (I) usually the chromosome movement is completely checked in the late anaphase (III), and the distance between the chromosome groups is shorter, usually below 30 μ. The swelling appears in the early telophase or even in the late anaphase. In the telophase the cell wall is formed later than normally (20—25 min. instead of 8—12 min.), or it is not build at all. In the phragmoplast too, changes in the refraction coefficient appear, and after some hours fine longitudinal lines may be discerned within it. Chromosomes do not restitute resting nuclei but swell and form irregular clumps and finally the cell perishes.

The influence on resting nuclei. In general the chromosomes at the time of mitosis undergo quicker changes than the resting nuclei. The changes in the resting nuclei render visible the nucleoli as well as the chromosomes in cells which have divided short time ago. The first changes are visible after 1,5—2,5 h. The structure of the nucleus becomes coarser and 4—8 nucleoli are visible (Plate II Figs. 23—25); normally the nucleoli are rather difficult to discern. The refraction coefficient of the nucleoli is similar to that in cells with „Entmischung" caused by mechanical factors; in the first case they are better visible. Around each nucleolus there is a free space. As a further effect the chromotin accumulates on the surface of the nucleus forming clumps connected with each other. Owing to the crowding of the chromatin after 4—6 h. of field action the nucleus gets the appearence of a ball with many irregular holes on its surface. The nucleus is surrounded by a very strongly refracting „coat" presumably the nuclear membrane. After 6—8 h. the resting nuclei either coagulate (Plate III Figs. 26—27), or their structure dissapears completely (Plate III Figs. 28—29). The nucleoli disappear later on; the nuclear membrane which was well visible before disappears also and the nucleus become optically empty. It is difficult to explain the difference in the behaviour of the nuclei which partially coagulate, while in others the structure becomes dissolved.

The changes occurring in the nuclei of the cells which divided short time ago are different from those described above. The first signs of changes are strings of some thickness which become more and more plainly visible and are placed like the chromosomes at telophase. These are chromosomes, the appearence of which is however quite different from that of the prophase chromosomes; they are shorter and thicker — and their appearence suggests that

their structure is at least double. The chromosomes seem not to swell but to coagulate after 4—6 h.

In cells which are more remote from the top of the hair changes appear later (4—6 h.). The structure of the nuclei becomes coarser and the nuclei coagulate. Only few nucleoli may be visible or they are not visible at all (Plate III Fig. 32).

In comparison with the above described phenomena, may be observed great differences in the field action on nuclei which are in „Entmischung". The structure of such nuclei is coarser than that of the normal ones and their refraction coefficient is greater (reversible „Entmischung" Plate I Fig. 11). If they are in irreversible „Entmischung" or if they are dead (Plate I Fig. 12), their structure can be strongly changed and be very coarse. In the field such cells may coagulate (Plate III, Fig. 31); their coagulation may be followed by a complete dissolving of all their structures exept the cell membrane (Plate III Figs. 33—34). The nucleus in this last case seems to be optically empty and frequently it is similar to a large vacuole.

Influence on the cytoplasm. After 3—6h. of field action the refraction coefficient of the cytoplasm increases. In the cytoplasm of dividing cells especially on the poles indistinct lines or strands may be observed sometimes. The circulation of the cytoplasm seems to be quite normal after 3h., then it becomes slowered and after 5—6h. finally it comes to a still stand. In cells without cytoplasm circulation the Brownian movement of its minute particles can be always observed. If the field ceases to act not too late, the circulation of the cytoplasm becomes resumed in numerous cells which return to their normal appearence after 20—40 min.; even cells in which the circulation of the cytoplasm has been stopped completely are able to recover. It is interesting to note that the cytoplasm regains its normal appearence remarkably quicker than the nuclei and the chromosomes.

Discussion.

According to recent investigations (Frey — Wyssling 1946), the cytoplasm should not be considered as a simple colloidal system, as its definite submicroscopical structure has been revealed. In spite of this fact it has many features of colloids. In the cytoplasm similar as in hydrosol acid particles are charged negatively, the basic particles positively (Wilson 1928, p. 186). In view of this fact it would seem probable that the chromosomes are negatively charged in agreement with the results of some authors (Botta 1932,

Churney and Klein 1937, von Lehotzky 1935, 1936). To attack this problem the mentioned authors used the cataphoresis. Churney and Klein think that in Sciara the chromosomes are charged negatively and the nuclear membrane and the sap positively.

Though the results of these authors agree, the method used by them can not be accepted without serious objections. The electric current passing through the cell causes various processes, the most important of which is the electrolysis. Quite different changes in the viscosity of the cytoplasm are found on two opposite cell walls. According to Greely: (Greely 1904 from Bersa und Weber 1922, p. 257) „about the anode and on the anodal side of the cell the protoplasm is coagulated... about the cathode and on the cathodal side of the cell the protoplasm is liquified". The changes of the viscosity can facilitate the pushing of the chromosomes in a definite direction, especially if they are electrically charged; it should be emphasised as a possible result of the treatment. Kamiya (1937), applied the same method to the staminal hairs of *Tradescantia*. He investigated very exactly the secondary effects of the electric current and did not arrive to the conclusion that the chromosomes are charged. Similarly the present author who investigated the cell division in homogene electrostatic field was unable to observe even the slightest facts which would suggest that the chromosomes in cell division as well as in resting nuclei were electrically charged. However though the chromosomes seem to be electrically indifferent in staminal hairs of *Tradescantia* it remains unknown whether they behave in the same way in other objects.

Electrical forces however must play in mitosis a prominent part, not considering the forces causing the chromosome movement (cf. Bajer and Hrynkiewicz 1950 in the press). This is shown by the influence of the electrostatic field directed perpendicularly to the longitudinal axis of the cell when acting from prophase (300 v/cm) and its existence only if the field acts from prophase (the moment in which the spindle apparatus is formed) indicates that the field does not influence the chromosomes but the spindle. The spindle apparatus is build mainly from long polypeptide chains orientated longitudinally. Probably its formation is partially disturbed, in consequence the anaphase is prolonged and the mechanism of chromosome movement is affected. The effect of a field action of high field intensity (4150 v/cm), leading to an entire stopping of mitosis after few minutes, shows that its mechanism becomes upset.

Very important for understanding of cell division is the examination of the mechanical relations in mitosis. It is difficult to say why in some cells the chromosomes stop very near the transversal walls while in others far from it. The chromosome movement may be stopped completely if one large vacuole is present. This suggests that the chromosomes can move only if a layer of cytoplasm outside the kinetochores is present. The force responsible for the chromosome movement should be remarkable if it is able to push aside the vacuoles and to change their shape. Since small vacuoles have a higher surface tension than the large ones it is probable that more work is necessary to displace the small valuoles (Plate I Figs 2—3), than to press in in a large (Plate I, Fig. 13). If this is true the forces causing the chromosome movement would act mainly from the poles of the cell.

Only in very rare instances the movement of two chromosome groups in the anaphase is exactly symmetrical; asymmetry occurs in most cases. It seems to be caused by differences in mechanical conditions on both ends of the spindle. In author's opinion two anaphasic groups of chromosomes are in their movement completely or almost completely independent from each other, thus each of them has its own movement apparatus. Possibly the best argument are irregularities observed especially in meiosis (B a j e r 1950 in the press). In *Cochlearia Tatrae* B o r b. growing in the Tatra mountains at 2311 m. a. s. l. a sudden fall of temperature in summer has induced far reaching disturbances in meiosis. The normal anaphase could not be observed oving to a high degree of desynchronisation of the individual chromosomes. The existance of two separate apparata would best explain the appearance of asymmetry.

The observed facts seem to give some support to one of the recent hypotheses of passive chromosome movement (S c h r a d e r 1946); possibly mainly to the strain theory (Zugfasertheorie) in its new from (S c h m i d t 1937, 1939), or the tactoid hypothesis — Ö s t e r g r e n (1949, 1950). They are not however in full agreement with the conceptions of R i s (1943). R i s maintains that in *Tradescantia* hairs the only factor causing the chromosome movement is represented by chromosomal fibers, whereas in numerous organism a prominent role is attributed also to the spindle body (Stemmkörper, B ě l a ř 1929). The pressing in in the large vacuole, if only a single one is present seems to indicate that the part of the spindle body should not be overlooked, if cytoplasm currents are not considered.

The present studies were carried out in June, July and August 1950 in the Institute of Plant Anatomy and Cytology of the Jagellonian University in Cracow. The author wishes to express his gratitude to Prof. Dr M. Skalińska the Head of the Institute and Prof. Dr H. Niewodniczański the Director of the Physical Institute for the possibility of its executing. I wish also to thank Prof. Dr W. Szafer Director of Botanical Garden for plant material. For the assistance in construction of the generator I am obliged to Dr Hrynkiewicz.

SUMMARY

In staminal hairs of *Tradescantia virginica* it is possible to observe three types of normal cell division:

1. Symmetrical division occurring only in few %. Two groups of anaphasic chromosomes separate in identical manner, symmetrically to each other.

2. Asymmetrical division occurring in about 90% of cells. The movement of the two groups of anaphasic chromosomes is not completely synchronized.

3. Cell division with an oblique metaphase plate — is very rare. Anaphase lasts much longer than usually.

Observations in homogene electrostatic field have shown:

1. There is neither acceleration nor checking of chromosome groups movement in the strong field acting parallely to the direction of chromosome movement.

2. There is no pushing of resting nuclei.

3. Pathological changes in the nuclei are independent from the direction of the field action.

The above observations permit to infer that the chromosomes of *Tradescantia virginica* are not electrically charged either in mitosis nor in resting nuclei.

Electrical forces play a prominent role in mitosis; the following results are in favour of this conclusion:

1. The electrostatic field affects the mechanism of chromosome movement and induces structural changes on chromosomes; the cell division stops several minutes after start of the action of a strong field, while the cytoplasm circulation is stopped after some hours.

2. After a cessation of shorter field action mitosis may continue but is disturbed sometimes.

PLATE I

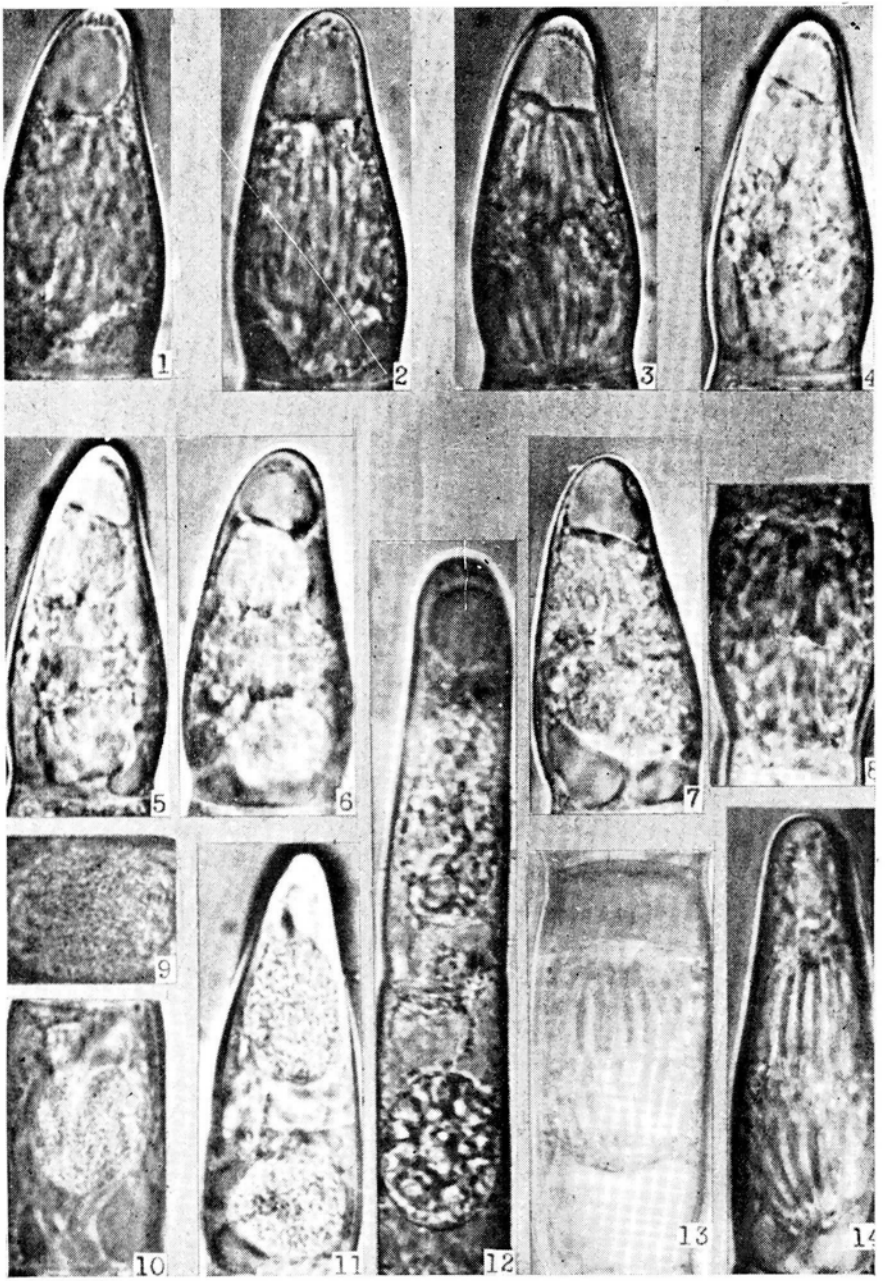
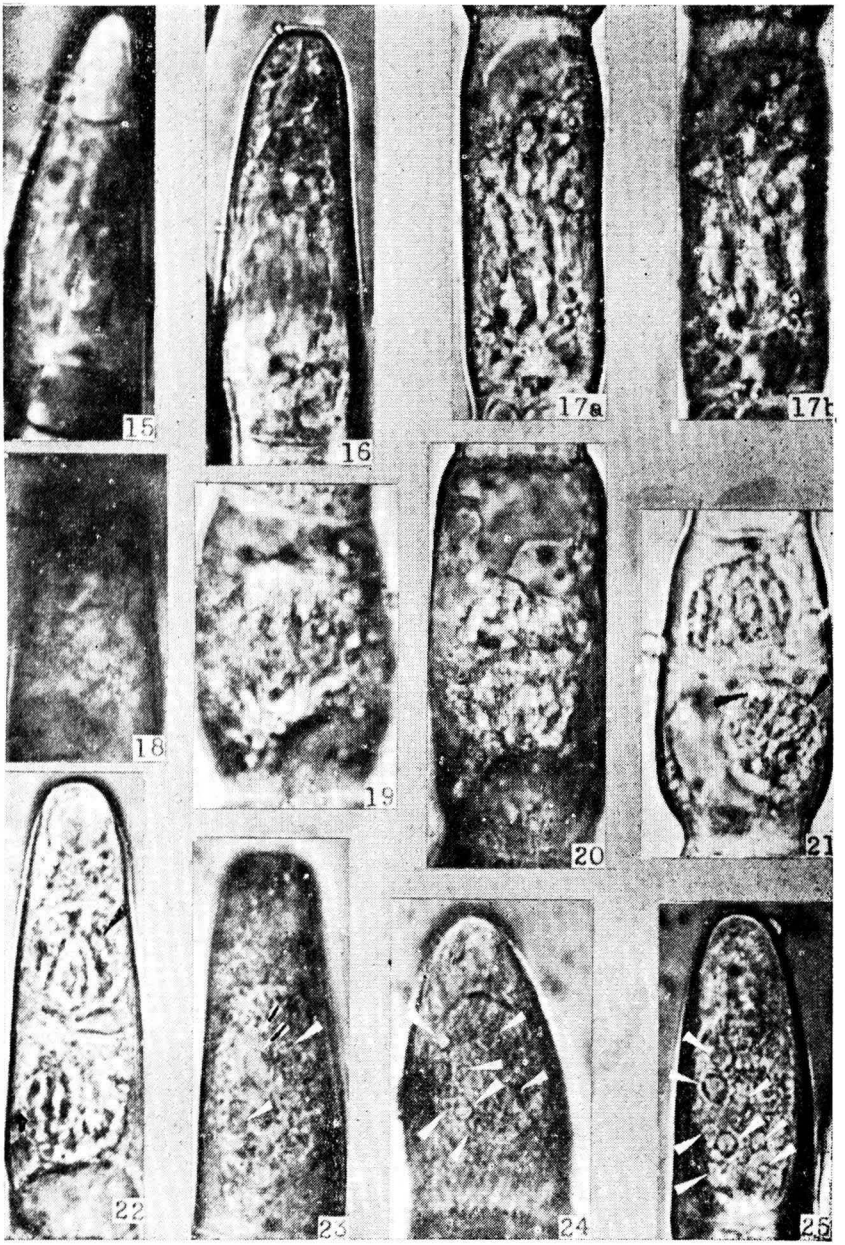


PLATE II



3. The action of the field may cause primarily reversible and later irreversible changes consisting in: the appearance of the chromosomes in late telophase and of nucleoli in resting nuclei.

Description of Plates

All microphotos were made with the aid of Zeiss's apochromate oil immersion 90x N. A. 1,30 and compensating eyepiece 10x and the M a k a m of L e i t z. Magnification 1100x.

PLATE I

Figs. 1—7. 7 successive stages of the same cell; 1. metaphase, 2 anaphase, 3. early telophase, 4—6 telophase, 7. resting nuclei nearly formed; mark the changes of the shape of the vacuoles.

Fig. 8. Metaphase.

Figs. 9—10. Normal resting nuclei.

Fig. 11. Nucleus with reversible „Entmischung"; the structure is coarser.

Fig. 12. Cells with irreverible „Entmischung" caused by mechanical factors (cells almost dead).

Fig. 13. Normal early telophase in the fifth cell from the top of the hair; chromosome's movement checked by two large vacuoles at the ends of the cell.

Fig. 14. Normal early telophase.

Plate II and III — action of the electrostatic field.

PLATE II

Figs. 15—16. Notably swollen chromosomes in middle prophase (II), just before the metakinesis. 4150 v/cm, 4,5 h.

Figs. 17a—17b. The same cell on different levels; swollen chromosomes in metakinesis. 4150 v/cm, 3 h.

Fig. 18. Strongly swollen chromosomes in metaphase. 4150 v/cm, 3 h.

Fig. 19. Swollen chromosomes in late anaphase (III); the distance between the chromosomes is short and the cell wall is not formed. 4150 v/cm, 4 h.

Fig. 20. Swollen chromosomes in early telophase; max. distance between the kinetochores is rather short; beginning of the formation of the cell wall, while the nucleus membrane fails to form. 4150 v/cm, 4 h.

Figs. 21—22. Chromosomes discernible in cells which divided short time ago 4150 v/cm, 2,5 h.

Figs. 23—25. Nucleoli discernible (indicated by arrows); each nucleolus is surrounded by a free space — best visible in Fig. 27: arrows with lines indicate the limits of the free space and the nucleolus. 4150 v/cm, 2,5 h.

PLATE III

Figs. 26—27. Resting nuclei after influence 4150 v/cm, 4,5 h.; arrows mark nucleoli; irreversible „Entmischung".

Fig. 28. Disappearing of the structure; nucleoli still visible. 4150 v/cm, 6 h.

Fig. 29. Complete disappearing of the structure. 4150 v/cm, 7 h.

Fig. 30. Cell with irreversible „Entmischung"; only slight changes observable after 4150 v/cm, 4 h.

Fig. 31. Cells with a slight reversible „Entmischung"; 4150 v/cm, 4,5 h. caused the death of the cell.

Fig. 32. Reversible „Entmsichung" in old cell which the cytoplasm circulation is completely stopped by the field. 4150 v/cm, 5 h.

Fig. 33. Disappearing of the structure in cells with „Entmischung". 4150 v/cm, 4,5 h.

Fig. 34. The structure of the nucleus disappeared completely (cell with „Entmischung"). 4150 v/cm, 5 h.

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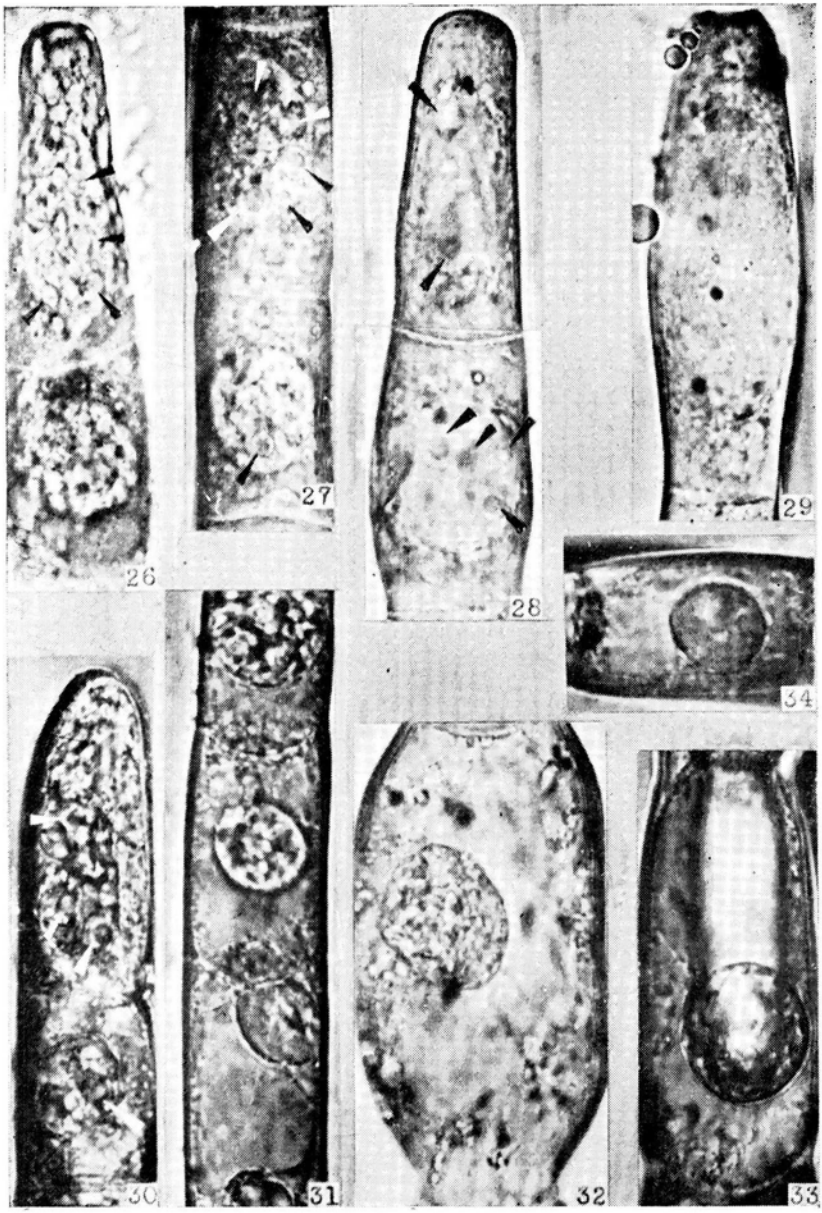
PLATE III



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