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Influence of extreme temperatures on mitosis in vivo I. *Hymenophyllum* *

by

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One of the most important problems in investigations on cell division mechanism in undoubtedly, as is pointed out by S c h r a d e r (1951), the problem of submicroscopic structure of the spindle. Some informations on this structure can be obtained from investigations of cell division of live cells in conditions in which the spindle itself and the movements of chromosomes undergo different changes the nature of which is partly pathological. These changes can be for instance such as the breaking up of some kinds of bonds, stiffening some elements in spindle structure, disturbances in chromosome movements and so on. The stimuli causing these changes are very varied, as for instance changes in temperature, action of such substances as colchicine, chloral hydrate etc.

B a r b e r (1939) investigated the influence of temperature of the rapidity of chromosome movements in staminal hairs of *Tradescantia virginica* within normal temperatures. R i s (1949) experimented on grasshopper with extreme temperatures, but he was mainly interested in the mechanism of chromosome movements and paid little attention to changes occurring in the spindle.

In the present investigations the shock action of extreme temperatures (from —16° to 0° and from + 38° to + 45°C) on cell division in plant cell was examined. Material with well visible in vivo

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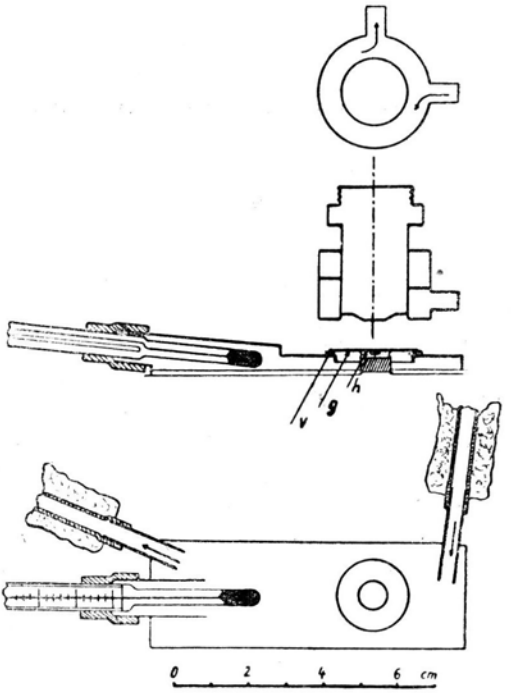
mitotic spindle was chosen and the aim of the investigaiton was to observe the action of shock temperature changes from the polar cap stage to telophase, particularly in metaphase and then on chromosome movements in anaphase.



Textfig. 1. Heated or cooled microscope table on which observations were carried out. Thermometer fixed with the aid of rubber link. Rubber tubes well isolated. h — G a u t h e r e t's hanging drop, g — glass square, v — vaseline ring. Above is microscope immersion lens with water jacket.

M a t e r i a l a n d m e t h o d s

M a t e r i a l. *Hymenophyllum tunbridgense Sm.* from the Botanical Garden of the Jagellonian University was used as material. The leaves of this fern are composed of one layer of cells and the course of mitosis is well visible in it. H e i t z (1942) was the first to observe mitosis in vivo in this plant. He did not however describe the course of mitosis in detail and suggest *Hymenophyllum* as material suitable for investigations. Young cut off leaves were kept, as advised by H e i t z, in a Petri dish lined with water saturated blotting paper. Leaves so preserved can be used for several days.

M e t h o d s. A special microscope table (Textfig. 1) was constructed for observations. On this table observations during the sudden and rapid temperature changes were possible.

G a u t h e r e t's hanging drop was used. The temperature could be lowered to — 16° and raised + 45°C within less than one minute and so the changes in temperature came as sudden shocks. During observations the objective of the microscope was warmed or cooled constantly. Rubber tubes leading water or water salt solution to the microscope table were well isolated. For cooling a mixture of ice and salt (NaCl) were used. The vessel in which the cooling mixture was kept was thermically well isolated. The cooling mixture used allowed to lower the temperature of the microscope table to —16°C without difficulty. By regulating the rapidity of the flow of the cooling mixture through the microscope table the temperature in it could be easily regulated within limits of —16°C to +5°C with an accurancy of ±0,5°C. (temperature of hanging drop might be somewhat higher due to objective lens). The high temperatures were obtained with the aid of H ö p l e r's (Type N) ultrathermostat and then the changes of temperature did not exceed 0,1°C. The temperature of the G a u t h e r e t's hanging drop during shocks was controled at 45°C with the aid of thermocouple and the differences were less than ± 0.5°C.

The medium used to observe the course of mitosis was liquid paraffine and not water' solutions and so the effects of water freezing were eliminated.

The microscope used was L u m i p a n Z e i s s. Observations and microphotos were done with a 60x N. A. 10 apochromate immersion lens and 10x and 15x compensating eyepieces.

The drawings were made in the course of obserwations with a Z e i s s drawing prism. From markings of the chromosome distance, made with a Z e i s s drawing prism graphs were plotted. On the graphs the ways of the two anaphase chromosome groups were plotted and the resultant ones.

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

At the ends of young leaves cell divisions are numerous, though they are best visible in somewhat older cells, which are placed a few cells away from the margin of the leaf. In *Hymenophyllum* it is easy to find mitosis at any time of the day and night and there is no noticeable periodicity in its occurrence in the course of the day which was noted by P e k a r e k (1932) in rhizoids of *Chara* and also by W a d a (1944). The number of mitoses diminishes several days after the leaf is cut off which is in accordance with H e i t z ' s (1942) observations. Cell division can be observed during 3-4 days and even 5 days after the leaf was cut off. Mitoses are most numerous on the second and third day, on the fourth day they are fewer and on the sixth it is very difficult to find them. The course of cell division is the same on the first day as it is several days after the leaf was cut off and on the 5-th day no pathological changes in mitosis were noted. Mitosis can also be observed during at least 36 h without changes in leaf strips placed in a hanging drop of liquid paraffine.

Such demixing as observed by B ě l a ř (1930) in consequence of mechanical injury was noted only very few times. Mechanical, stimuli if not very strong, do not cause demixing which is contrary to what was observed in staminal hairs of *Tradescantia* and most other material.

Course of division at 20°C

The resting nuclei are well visible and their diameter is 15-17 μ, their structure is the same as, and even better visible, than in staminal hairs of *Tradescantia*. In prophase this structure becomes more and more visible until chromosomes can be distinguished. They are long thin and numerous (according to L i t a r d i è r e (1921) 2n = 24). The formation of the spindle can be observed exceptionally distinctly since early prophase. As the result of the formation of the spindle and of the phototactic movements caused by the illumination, the chloroplasts move mostly to the side walls of the cell and usually do not interfere during the observations of the spindle. Often however the chloroplasts are grouped at the spindle poles and then poles are not well visible. It seems probable that in *Hymenophyllum* the whole spindle is enveloped by a membrane which encloses it in prophase when the polar caps are formed, in metaphase, and in anaphase. The shape of the spindle is very distinctly marked because of the chloroplasts grouping round it and also because of the vacuoles adhering often to it. However even in these cases when neither chloroplasts nor vacuoles are in close proximity of the spindle, the membrane is often well visible. The spindle is here seen not as a negative as happens in many zoological objects in vivo (*Stenobothrus*, B ě l a ř 1929 a, snails *Cepea hortensis*, *Helix pomatia*, B a j e r 1951 unpubl.) where the spindle is outlined on the background of chondriosomes grouped round it, but as a body separated from the cytoplasm by the membrane behind which no chondrisomes could be found. It is necessary to add that in *Haemanthus* where mitotic spindle is large (to 70 μ) and all details of mitosis are well visible such membrane seems not to exist (B a j e r 1952)

Division was observed at 20 ± 0,5°C and also several divisions in lower temperatures were noticed (+5° and +7°C). In low temperature the membrane surrounding the spindle is far more distinct than in higher ones, which was already noted by W a d a (1950). The refraction coefficient of the spindle seems to be somewhat different than the refraction coefficient of the cytoplasm.

The polar caps are quickly formed (usualy in less than in 30 mins.) and can be more or less elongated and pointed. Their poles are either pionted or more or less rounded. In this stage however the spindle is far more elongated than in metakinesis and in metaphase. It can often be seen that there are several attempts to form the polar caps and the formation of both polar caps is often not simultaneous. It happens sometimes that at one end of the nuc-

leus a small polar cap is being formed while at the other pole there is not polar cap yet, or it is being formed not exactly on the opposite side of the nucleus but a little to one side of it. Small polar caps disappear sometimes, and then are formed on another place or after some time in the same place again. It is hard to establish the exact moment at which prophase ends and metakinesis begins and so it is difficult to estimate exactly the duration of prophase, however this stage does not last very long (approximately 2-4 h) in comparison to other stages. In late prophase just before metakinesis, when the spindle is partly formed, a stage can often be noticed in which the chromosomes are very crowded and most of them are near the centre rather than in the outer part of the nucleus. This stage resembles the contraction stage found usually in animals (cf. S c h r a d e r 1946). It is only some time after this stage that metakinesis occurs. It is impossible to establish exactly the beginning of metakinesis and it also is very difficult to distinguish late metakinesis from metaphase. The duration of metakinesis and metaphase is together 1 to 1,5 h. Before anaphase the metaphase plate is very regular and the kinetochores seem to lie exactly in one plane. This very regular plate is visible during 10—30 mins. before anaphase begins however, not less than 20 mins. earlier, the plate is already regular.

The length of the spindle is shorter in metaphase than in metakinesis. The beginning of anaphase is easy to establish, which is the opposite to what is found for instance in staminal hairs of *Tradescantia*. It is well visible that in anphase the chromosomes are thiner than in metaphase. This enables to distinguish immediately the anaphase from all the remaining stages, and to start observations from its very beginning. In the plane of the plate the separation of each chromosome is well visible and their movements can be observed in detail. It seems that the maximum velocity of kinetochores is reached almost immediately and not as in the case of *Tradescantia* after some time. Only very occasionally all the chromosomes begin to move simultaneously, usually the movement begins at one or two points of the plate, and spreads gradually to the whole plate. This kind of anaphase beginning was observed also on fixed material mainly in the case of short, small and numerous chromosomes and was often noted in vivo in snails (B a j e r 1951 unpubl.). During anaphase in *Hymenophyllum* some chromosomes seem to change their velocity and either precede or are retarded. In most cases a few arms precede kinetochores in their movement

toward the pole. Such cases can be found in most mitoses and depend on the size and number of chromosomes (cf. B a j e r 1951). The maximum separation of chromosomes lasts 20-30 mins. In different cells the course of anaphase is very similar (the velocity of movement, the time when the velocity change and distance passed cf. Table I). The changes of velocity in time i. e. the curves of chro-

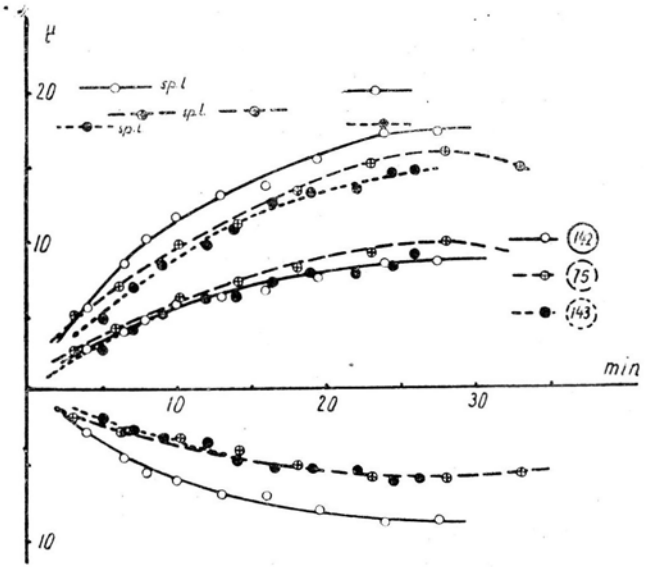
TABLE I

Cell division at 20°C. 13 celles from 35 observed

No of cell	Duration in mins.		Distance of max. seperation of chromosomes in μ	Spindle length in μ		Notes
	metakinesis and metaphase	anaphase		metakinesis	metaphase	
100	48	18	17	25	23	
101	—	18	20	—	22	
144	—	18	16	—	18	
146	—	20	16	—	18	
148	60	22	19	23	21	
145a	—	22	16	—	18	
140	50	22,5	15	19	17	formation of polar caps not synchronised
141	more than 40	23	19	24	20	
147	„	24	17	—	19	
142	more than 55	24	19	—	23	
143	—	25	15	—	17	
143a	—	25	17	—	18	

mosome movement (Textfig. 2) are far more in a mutual agreement than in the case of *Tradescantia virginica* (B a j e r 1950). Similarly to what was found in *Tradescantia* two dictinct types of movements of the two anaphase chromosome groups were noted. The first type i. e. the one, in which there is absolute symmetry and the two groups of chromosomes move exactly in the same manner, is met very seldom, and the other in which chromosome movement is more or less asymmetrical (the curves of 2 groups of anaphase chromosomes are not inverted reflections of each other) can be found in most cases. Nearly symmetric and asymmetric curves are given in Textfig. 2. During the whole anaphase the length of the spindle does not increase and the differences in measurement do not

exceed 0,5 μ and lie within the limits of permissible error. In telophase the nuclei are often moved a little towards the newly formed cell membrane and so the separation of chromosomes in late telophase is shorter than in late anaphase. When anaphase ends the formation of the phragmoplast begins. The phragmoplast has a mem-



Textfig. 2. Graphs of chromosome movement at 20 ± 0,5°C. sp. l. — spindle length. No of cell in ring. Cell No 142 — asymmetrical movement of two anaphase chromosome groups, less asymmetrical in No 75, almost symmetrical in No 143. The upper curves of three chromosome groups very similar — resultant curves differ considerably. Distance of max. separation of chromosomes shorter than spindle length.

brane; the existance of this membrane is perfectly evident and it is best visible in low temperatures. The new cell wall formed within the phragmoplast spreads toward its sides and than it can be seen how the newly formed cell wall pushes on the membrane of phragmoplast. In the place where it is tuched by the newly formed cell wall the phragmoplast membrane swells and the sides of the phragmoplast are as if pushed towards the cell walls (Plate I, Figs. 12-15, Textfig. 4). It often happens that the vacuoles which are between the phragmoplast and the cell wall get into the way of this movement, however in such cases the vacuoles are broken in two parts. After the new cell wall is formed there takes place a strong vacuoli-

sation of the phragmoplast, though it often happens that vacuolisation occurs ealier i. e. in late anaphase. The formation of the cell wall is very difficult then, but no disturbances while it forms were noticed.

Influence of low temperature

Shock changes of temperature from + 20°C to —5° and —16°C were used and the low temperatures were applied during from 10 mins. to 1,5 h.

Even the lowest of these temperatures, if its action does not much exceed 1,5 h does not kill the cell, moreover it seems that the harmful effects of such a shock are not serious and within a short time after the cooling is interrupted, both phototactic movements of chloroplasts and cell division itself come back to normal. Also new prophases were found soon after the leaf came back to normal temperature. The above is true even, if a leaf is subjected 4-5 and more times at 1-2 h intervals, to low temperature during 30 mins. New prophases could be found not only soon after low temperature action was interrupted but also the next day.

Under the action of low temperature some changes take place within the cell. Some of these changes are idependent of the stage of mitosis and are observed in all cells; they consist mainly in a sudden and strong vacuolisation of cytoplasm (mainly the old vacoules become larger), and also in an increase in the visibility of the membrane of the spindle in all stages.

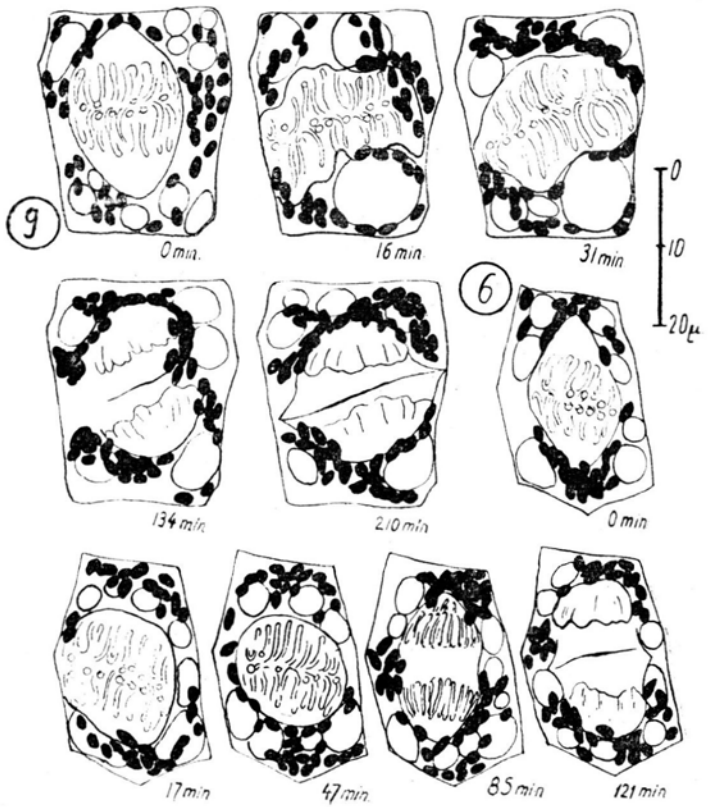
Shock changes to the higher of the low temperatures (+5°, 0°C) have no visible influence on the course of different mitosis stages. Division is not stopped and the chromosome movement in anaphase is more or less normal, but of course all processes are slowed. It is not till the low temperatures (—3° to —16°C) are attained that visible changes in chromosome movements and the morphology of the spindle take place. It is important however to stress that these changes are temporary and reversible. —7° and —8°C were the temperatures used most often.

Influence on early prophase. If a cell is subjected to the action of low temperature when it is in the early prophase no other changes besides those described previously are observed. When the cell comes back to normal temperature the course of division is quite normal though it seems that the duration of prophase is slightly prolonged.

Influence on prophase during the polar caps formation. Low temperature causes at this stage a partial disappearance of polar caps. When the cooling ceases they are formed again and division develops as usual.

Influence on metakinesis and metaphase. The influence of low temperature on cells in both these stages of mitosis is very similar and although the different cells react differently to cooling, two main kinds of changes can be noticed at both stages.

a. Soon after cooling begins and simultaneously to the increasing vacuolisation of the cell there occurs a fairly quick rounding of the spindle, which so far was more or less elongated. This process is rapid and within several minutes the spindle becomes spherical, or nearly spherical. The spindle resembles the resting nucleus with a fully formed metaphase plate (Textfigs. 3-4). Often the spindle membrane is dented and ruffled by vacuoles (Textfig. 3) and often

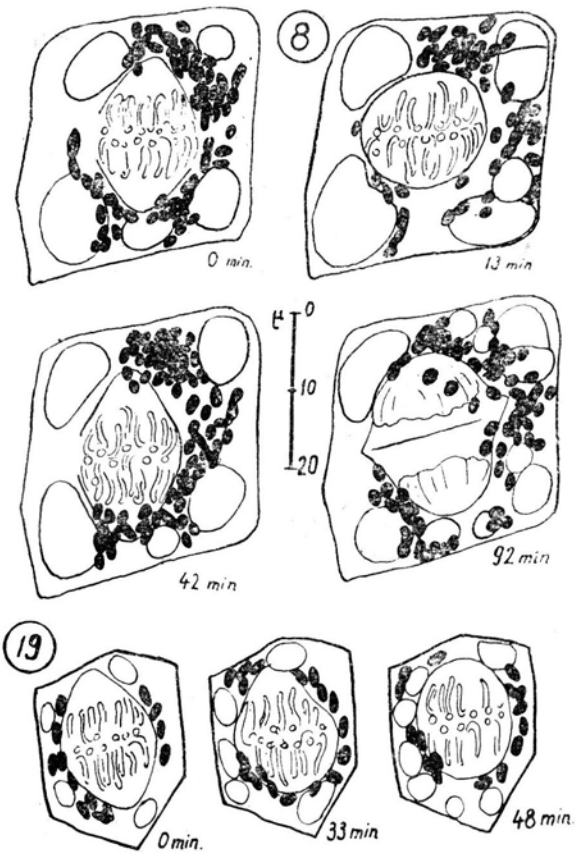


Textfig. 3. Influence of low temperature on spindle morphology. In rings No of cells. Cell No 9, temperature —8°C acts during 3 to 33 mins. Spindle is deformated by outgrawing vacuoles. After return to normal temperature spindle is reconstructed and the course of cell division is normal. Cell No 6 temperature —5 to —8°C act from 2 mins. to 35 mins.

their growth also displaces the whole division figure. During all those changes the metaphase plate is usually not much deformed. On the other hand it sems that in metakinesis there occur greater disturbances in the displacement of chromosomes and in this stage the process of the spindle becoming spherical is less pronounced.

If the structural changes in the spindle do not occur soon after the action of cooling begins a subsequent even very prolongated cooling will not usually induce this process to develop further.

b. In the second kind of changes the rounding of the spindle poles under the action of temperature is only slight, sometimes ho-



Textfig. 4. Influence of low temperature on spindle morphology. In rings No of cells. Cell No 8, temperature —5°C acts during 3 mins. to 33 mins. Spindle rounded and metaphase plate nearly regular. After return to 20°C the spindle is reconstructed and cell division is normal. Well visible membrane on phragmoplast. Cell No 19, low temperature (—7°C) slightly influenced the spindle. Spindle slightly rounded.

wever if the cooling is repeated two or three times the spindle becomes more rounded than after the first cooling.

Those differences in behaviour are undoubtedly caused by the different resistance of each cell. It seems however that the mechanical conditions in the particular cells and also the exact moment at which cooling begins (early or late metaphase) have some influence here.

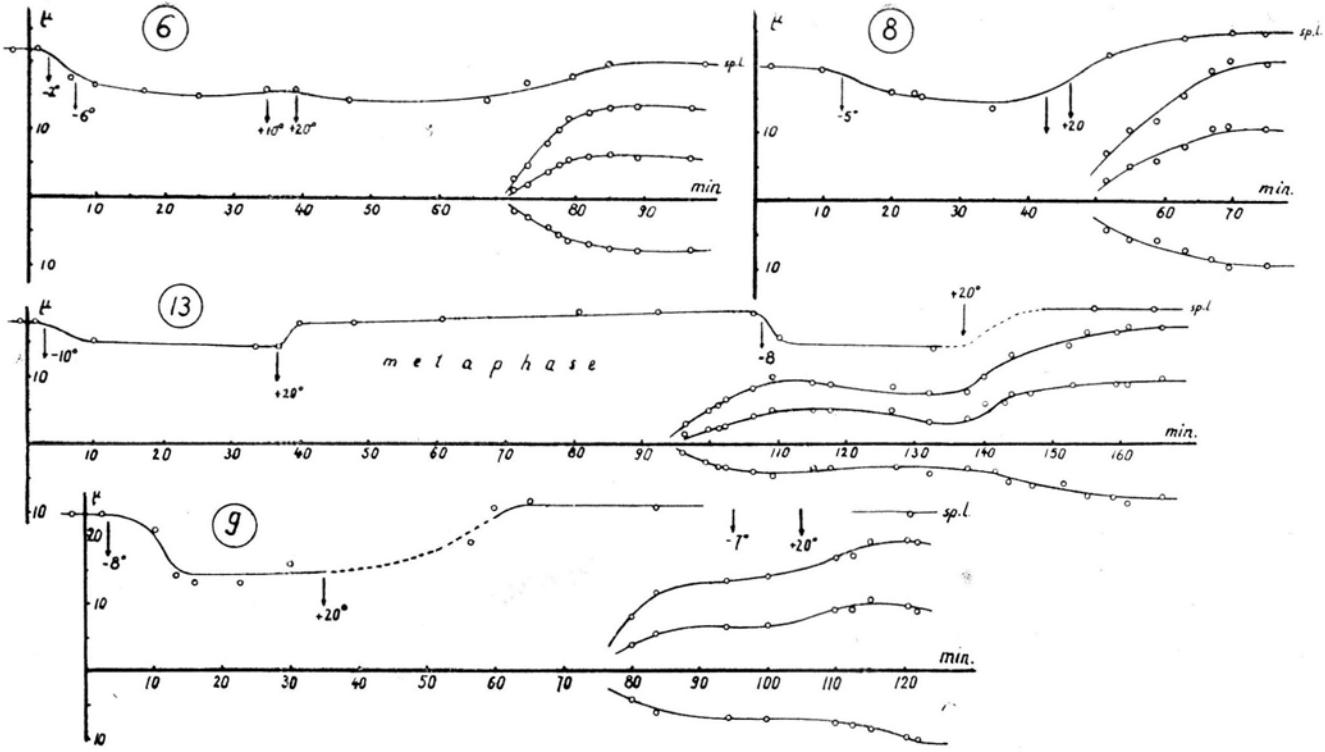
In both kinds of reactions described above, the chromosome movement is made impossible while cooling continues. After the return to normal temperature (in case a) the spindle is slowly reconstructed. It seems to be formed anew and the proof of this is found in that its new length is often different, longer or shorter, than it was before. While the rounding of the spindle and the desappearance of the half spindles progress rapidly their reconstruction is much slower (it lasts 30-60 mins. that is often ten times longer). Some time after the spindle is reconstructed the metaphase passes to anaphase.

In the second case (b) this reconstruction is very rapid and often several minutes after return to normal temperature anaphase begins.

Influence on anaphase and telophase. The changes caused by low temperature in early and middle anaphase are very similar to what was described in metaphase and metakinesis. Here also half spindles become more or less rounded and the whole spindle can be displaced by vacuoles. The chromosome movement is stopped altogether and even sometimes the chromosomes are pushed back a little (cf. Textfig. 5). After the spindle is reconstructed the chromosome movement is quite normal and its velocity is approximately the same as it is in normal division. Also the further course of mitosis is quite normal.

Low temperatures have less influence on late anaphase and telophase than on previous stages. The sequence of changes occurring in chromosomes is delaid but otherwise developes regularly. However the formation of the cell wall is made more difficult which is mainly due to mechanical reasons (vacuoles forced into and formed inside the pragmoplast). The cell wall which is being formed must push them aside, and this process is lengthy (1-2 h).

As it was previously mentioned the membrane of the spindle is very well visible in low temperatures which enables a very good observation of it being pushed aside and bulged out by the newly forming cell wall.



Textfig. 5. Influence of low temperature on spindle length and chromosome movement in anaphase. In rings No of cells. sp. l. — spindle length, dotted line indicates probable spindle length, interruptions (cell No 9) indicate not measured spindle length. Arrows indicate the time at which the temperature was changed. Cells No 6, 8, normal anaphase after low temperature action in metakinesis (No 6) and metaphase (No 8). Cell No 13, influence of low temperature in metakinesis and anaphase, in anaphase chromosomes are pushed in the direction of the metaphase plate. Cell No 9, influence on metaphase and anaphase.

TABLE II

Low temperature action. 11 cells from 45 observed*.

No of cell	Spindle length in µ at temperature 20° C: metakinesis	Spindle length in µ at temperature 20° C: metaphase	Spindle length in µ at temperature 20° C: anaphase	Low temperature action: stage of cell division	Low temperature action: temperature °C	Low temperature action: duration in mins.	Distance of max. separation of chromosomes in µ	Duration in mins.: metaphase	Duration in mins.: anaphase	Notes
9	—	—	20,5	$P_{I,II}$	—7	60	19	25	30	cell division after 2 low temp. shocks
11	22.5	22,5	24	$P_{I,II}$ $M_{I,II}$	—5, —10	60 90	21	150—170	28	sp. l. in low temp. in M_I approx. 14 µ
12a	—	—	20	$P_{I,II}$ $M_{I,II}$	— 8	60	17	150—170	20	sp. l. in low temp. in M_I - 17 µ, in M_{II}, 14 µ (rounded)
12b	—	—		$P_{I,II}$ $Mk_{I,III}$	— 8	30	15	25	19	sp. l. after low temp. in Mk_I, 16 µ (elipsoid), reconstruction 45 mins, in Mk_{II} almost spherical
13	18	17,5	15	Mk_{III} A_{II}	— 7	30 30	17	50	65	sp. l. after low temp. in Mk^{III}, 15 µ, in A_{II} 15 µ.
109	—	—	23	M	— 3	30	19	60	—	sp. l. in low temp. 18 µ
115	—	20	—	M	— 5	50	19	90	—	sp. l. in low temp. 18 µ
6	—	22,5	20	M_{II}	— 7	30	14	85	17	sp. l. after low temp. 15 µ, reconstruction at least 30 mins.
8	—	20	25	M_{III}	— 5	30	20	65	27	sp. l. in low temp. 15 µ
9	—	23	25	M_{III} A_I	— 7	30 10	20	75	40	sp. l. in low temp. in A_I, 15 µ
32	—	—	23	A_{II}	— 5	30	19	—	65	sp. l. in low temp. 17 µ

*P — prophase
Mk — metakinesis
A — anaphase
I,II,III early, middle, late
sp. l. — spindle length

Influence of repeated cooling. The effect of lowering temperature repeatedly are the same every time. The changes observed are similar when temperature is lowered the first time to what they are when it is lowered several times, the only exception is that the rounding of the spindle is usually more marked when cooling is repeated (cf. Table II). This corroborates Ris'es (1949) experiments who obtained a repeated disappearance and formation of the spindle in one and the same grasshopper cell.

Influence of high temperature

The temperatures used were from +38° to +45°C, in most cases +43° to +45°C; temperatures lower than 42°C were raised above 42°C during observations. The time during which the high temperatures were applied was 10-120 mins. (cf. Table III). These temperatures do not kill the cells if they act on it for a short time, but they are usually deadly to it, if applied for 60 mins. (+45°C). In all cells within the first few minutes that high temperature is applied vacuolisation takes place, the existing vacuoles grow larger and new ones are formed. Vacuolisation begins immediately the high temperature begins to act and once the big vacuoles are formed, they do not grow much larger. The refraction coefficient of chromosomes increases but due to changes which take place within the spindle the observation of chromosomes is made more difficult. The spindle membrane is less distinctly visible than in low and normal temperatures, or it is not discernible.

Influence on prophase in stage of polar cap formation. Within the first few minutes the polar caps elongate and become more pointed, this change is however not very marked. As a result of the high temperature cell division is interrupted for a long time, i. e. for at least 6 h. The spindle is then reconstructed and cell division continues as usual.

Influence on metakinesis and metaphase. The elongation of the half spindles is now far more visible and is greater than in prophase and also greater in metaphase than in metakinesis. Most observations done, concern metaphase. Changes inside the spindle begin within the first few minutes after high temperature is applied. The spindle elongates by 1/5 to 1/2 of its initial length, and at the same time the spindle poles become less clearly visible. The spindles not only elongate but also their points become suddenly much sharper, while simultaneously long folds

TABLE III

High temperature action. 27 cells from 75 observed*.

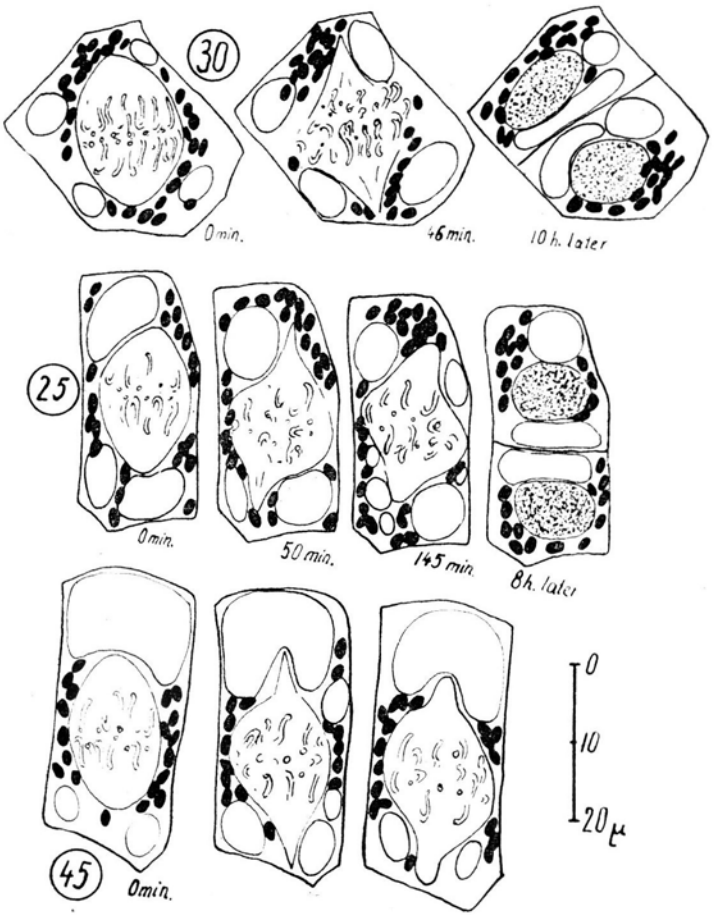
No of cell	High temperature action: stage of cell division	High temperature action: temperature in °C	High temperature action: duration in mins.	Spind. length in µ: in 20°C	Spind. length in µ: in high temp.	Distance of max. separation of chromosomes in µ	Cell lenght in µ	Notes
123	Mk	43	95	22	32	—	—	
124	Mk	43	95	20	23	—	—	after 12 h cell not yet divided
43	M	44	20	16	24	22	—	
20	M	42	30	23	35	—	—	after 8 h new cell wall was found
35	M	45	10	19	29	—	—	,, ,,
17	M	44	24	16	27	—	—	,, ,,
106	M	38	60	20	25	—	—	
73	A early (2)	41	25	18	28	—	—	anaphase not continued
121	A early (2)	43	35	—	28	—	—	,, ,,
16	A early (3)	42,5	24	—	28	—	—	,, ,,
132	A early	45	60	21	32	—	—	,, ,,
51	A middle (6)	45	29	22	—	23	—	
50	A middle (7)	45	20	—	—	22	—	
54	A middle (7)	45	30	18	25	11	—	
45	A middle (10)	44	20	20	29	16	—	
44	A middle (10)	43	30	21	40	33	36	spindle bent
69	A middle (11)	38	30	22	25	20	—	,, ,,
68	A middle (12)	38	30	19	23	20	20	
129	A middle	44	30	20	23	16	—	
131	A middle	45	60	19	27	15	—	
127	A late (14)	42	26	25	32	27	35	
71	A late (14)	45	30	19	27	25	28	
66	A late (14)	45	36	21,5	27	24	24	,, ,,
65	A late (15)	45	30	22	37	29	41	,, ,,
27	A late (18)	45	10	22	30	28	32	after 8 h new cell wall was found
126	A late	45	60	19	30	25	29	spindle bent
72	A late	41	25	19	27	24	30	

*Mk — metakinesis

M — metaphase

A — anaphase, time in mins. from anaphase beginning given in brackets

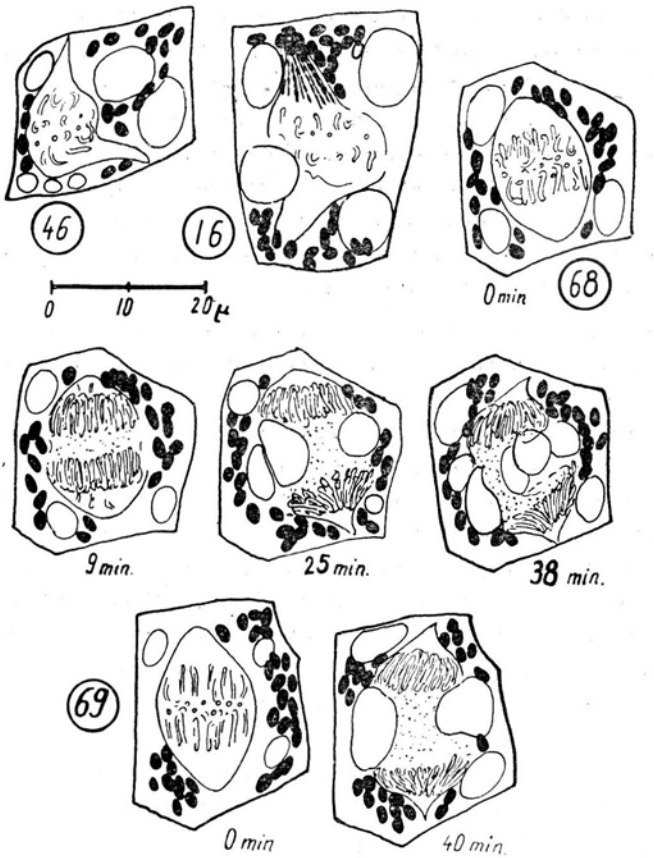
appear on their surface, i. e. the cross section is now not as usually a circle or an elipse but an irregular many-armed star. The elongating half spindles are stiff which is well visible in cells N o s. 45 and 46. In cell No 45 the sharp elongating spindle is pushed into



Textfig. 6. Influence of high temperature on spindle morphology in metaphase. Elongation of the spindle, sharpening of spindle poles, deformation of metaphase plate. In cell No 45 the pointed half spindle dents the vacuole.

and dents a vacuole and the spindle is not bent at all. In cell No. 46 the elongating spindle met with cell walls and was bent as a result of the resistance. Extreme elongation of the spindle was also observed; then the spindle become S shaped. It is important to stress that as a result of high temperature the cytoplasm surrounding the spindle also become streaky which makes the exact measuring of the

spindle length very difficult. Often after or during the action of high temperature long fibers running from the meatphase plate to the poles seem to appear inside the spindle. The fibers are arranged convergently to the poles, they are very thin, and their thickness is within the limits of revolving power of the microscope and does not surpass 1/3 μ. They are long but their structure is not uniform but is composed of regular elongated spindles the length of which is 0,7 to 0,9 μ (Textfig. 7). Such structure of the fibers has been found also on fixed material in *Lupinus* and *Haemanthus* (B a j e r 1952 unpubl.).

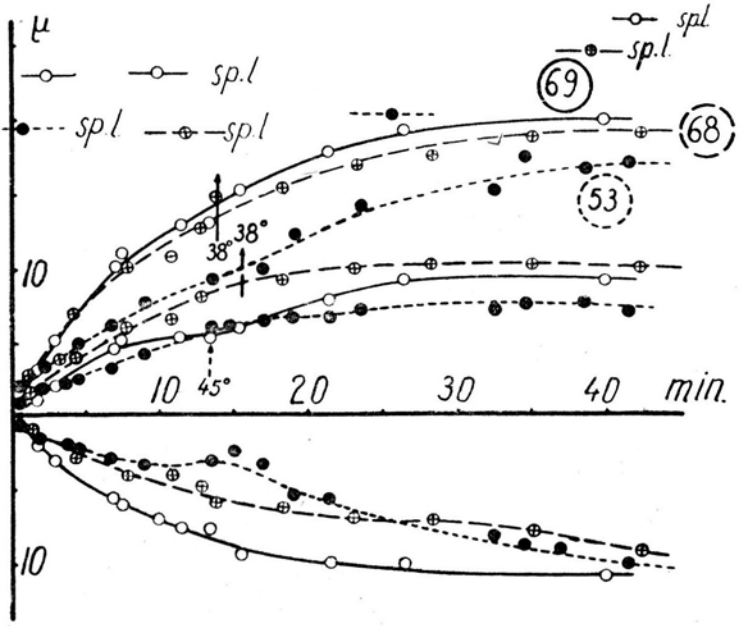


Textfig. 7. Influence of high temperature on metaphase and anaphase. In rings No of cells. Cell No 46, half spindles are bent. Cell No 16, reconstruction of the spindle, fibers of which structure is not uniform are visible. Cells No 68, 69, influence of high temperature on middle anaphase. Chromosome move but their max. distance is not prolongated (cf. Textfig. 8).

Simultaneously to the changes of the spindle the metaphase plate is deformed and crumpled. The deformation is such, that after the action of high temperature during some time the chromosomes are crowded and arranged so irregularly, that the metaphase plate cannot be distinguished. The greatest disturbances are probably caused when high temperature is applied in early anaphase.

Several hours after the action of high temperature ceases the spindle returns to its normal conditions. The first sign of this return can be seen in the rounding of spindle poles (Textfig. 7). Next a more or less regular spindle is formed. The reconstruction of the spindle lasts at least 6 but usually 8-12 h. After the spindle reconstruction anaphase is usually very irregular. It can be seen how the chromosomes move toward the poles, but it was not possible to follow through the movements of two groups and it seems that each chromosome mostly travels independently of the others and that their movements are not synchronised. More than 30 already divided cells were observed after the action of high temperature but anaphase was observed only in 9 of them. However not in one case the division observed was such that the curve of chromosome movement could be plotted from it.

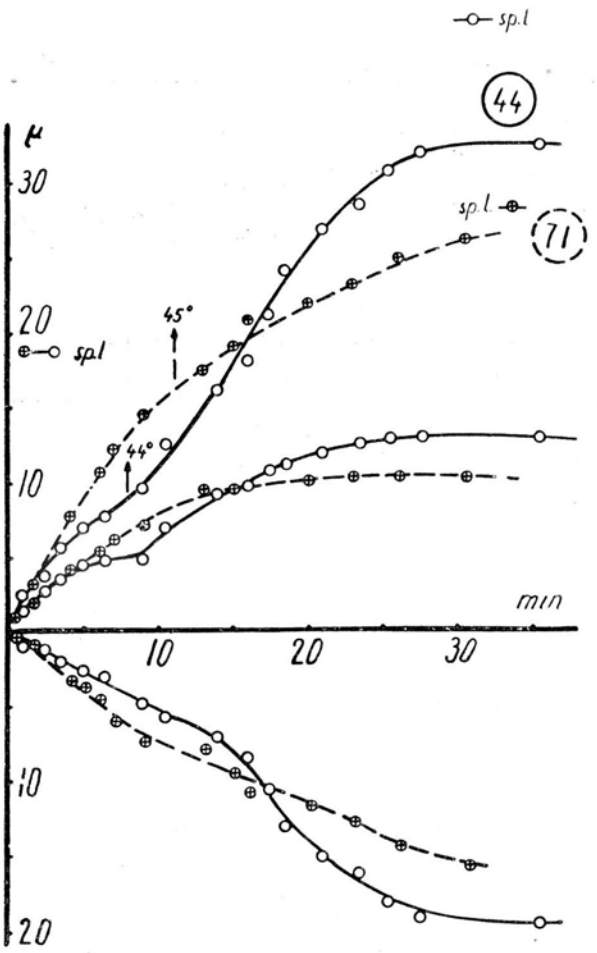
Influence on anaphase and telophase. The influence of a shock change to high temperature on anaphase depends on the stage of anaphase at which the shock is applied (early, middle, late anaphase). As it was mentioned previously, the influence of high temperature on early anaphase (within 2-4 mins. after it begins is very similar to the influence on metaphase. The influence on middle anaphase can be of two kinds (the same as in the case with early and late anaphase) and the different cells respond differently. As the result of heat the cell is suddenly vacuolised, the half spindles elongate and in most cases the chromosome movement continues. The way of chromosome movement is usually shorter than the length of the spindle before the temperature is raised. In late anaphase the poles of the spindle very often touch the cell walls (Textfig. 7), and in later stages the whole spindle can be bent. This bend is usually in the pushing body while the two anaphase chromosome groups are flattened. This suggests that there is a force which acts from the centre of the pushing body. Anaphase is not much prolonged (not more than 5-10 mins.). However in some very rare cases, far less common than those described above, not only does anaphase continue but the chromosomes continue on their way, (contrary to what they do in normal division at 20°C)



Textfig. 8. Influence of high temperature on middle anaphase. Graphs of chromosome movement. sp. l. — spindle length. In rings No of cells. Arrows indicate the beginning of high temperature action. Anaphase is continued, graphs are similar to that in normal division, exept in cell No 53 where lower chromosome group is retarded. Max. separation of chromosomes is shorter than spindle length before high temperature action.

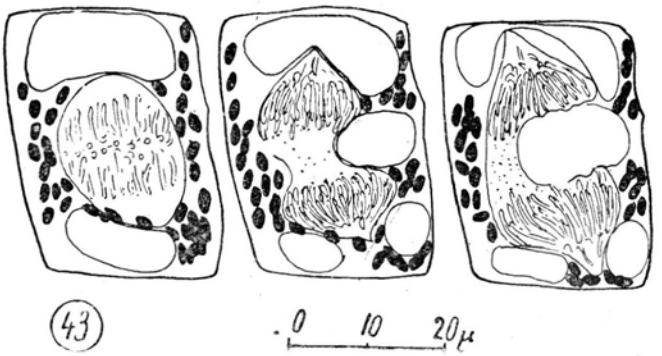
and move further on even after travelling through the distance somewhat shorter than the length of the spindle. They are then displaced together with the half spindles which are thin and pointed. There are two possible developements of the above: 1. The chromosomes do not change their position within the half spindles and so the half spindles do not shorten, but on the contrary elongate all through the duration of the high temperature action; 2. Here on one hand the anaphase chromosome groups are pushed apart and on the other they move within the half spindles (this can be seen in cell No 44, Textfig. 9) and there results the shortening of the half spindles. The two developements of this movement are illustrated in Textfig. 9 (cells N o s 44 and 71). The acceleration of the chromosome motion causes bends in the curve of the chromosome movement (cell No. 44). In the second case the curves resemble the curves of chromosome movement in normal division. It should be stressed here that the duration of anaphase does not differ much from normal

i. e. not very much prolonged. If the temperature is raised in middle anaphase the separation of chromosomes further away than the normal spindle length is rare, and in the greatest number of cases the effect is such as already described (cells N o s. 53, 68, 69). If the temperature is raised in late anaphase, 14 or more minutes after anaphase begins, there occurs in most cases the pushing apart of the two chromosome groups similar to what was observed in cells N o s. 44 and 71. The acceleration of the chromosome



Textfig. 9. Graphs of chromosome separation in consequence of high temperature action on middle anaphase. No of cells in rings. Arrows indicate the beginning of high temperature action. Pushing body elongates and chromosome move in each halfspindle. Spindle elongates during anaphase.

movement does not begin immediately the high temperature is applied but some minutes later. The anaphase is usually prolonged 10—20 mins. It seems that in all cases observed the movement was stopped by the poles of half spindles reaching cell walls. and it seems also that if this obstacle were not in the way of half spindles, the chromosomes would continue to move on. This is indicated by the flattening of chromosome groups just as if a force was acting on them from direction of the pushing body. This force can also bend the half spindles and the pushing body (Table III, Figs. 30-31).



Textfig. 10. Influence of high temperature (45°C). on late anaphase. Cell in metaphase stage at 20°C. Elongation and sharpening of the spindle and separation of chromosomes. Max. separation of chromosomes much longer than spindle length in normal temperature.

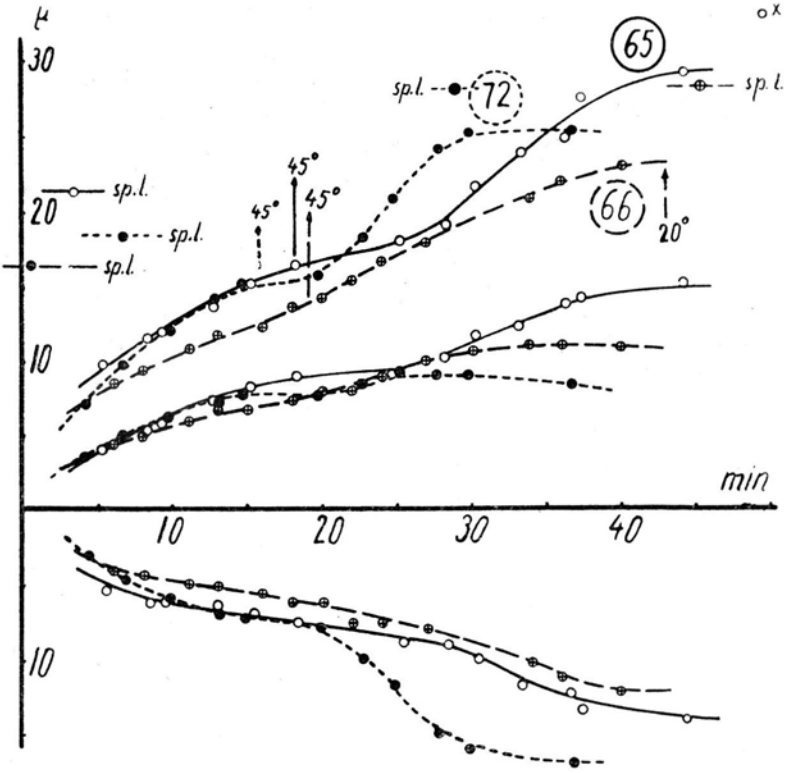
After the action of high temperature is interrupted telophase lasts a very long time (far more than 4 h), but in most cases it is achieved. The formation of cell walls is here difficult and lasts a long time. The difficulties are mainly due to the extensive vacuolisation around and inside the pushing body, nevertheless the cell wall is formed normally, though very slowly. In many cases the membrane on the phragmoplast is well visible.

Action of high and low temperatures

The influence of succussive opposite extreme temperatures on cell division was investigated. Both alternatives i. e. first low and then high temperature, and first high and then low temperature, were experimented with. Time of duration of the changed temperatures was 10-60 mins.

a. Influence of first high and then low temperature. If under the influence of high temperature the above described changes take place, the action of the extreme low temperature even applied for a long time, will not alter the previous changes.

b. Influence of first low and then high temperature. After applying high temperature on a cell in which the spindle was rounded under the influence of cooling, the effects of low temperature disappear very quickly. The spindle soon elongates becomes pointed, and resembles greatly the spindle which is formed under the action of high temperature only. It seems that the time in which this shape of the spindle is obtained is longer



Textfig. 11. Influence of high temperature on late anaphase. sp. l. — spindle length. In point X sp. 1. of cell No 65 is 50 μ. In rings No of cells. Arrows indicate the beginning of high temperature action. Some minutes after beginning of high temperature action pushing body begins to elongate and in consequence the distance of chromosome separation increases. Most plainly it is visible in cells No 65 and 72.

and the spindle itself somewhat shorter than when only a high temperature was applied. Exact measurements however were not undertaken.

Discussion

In investigations on the mechanism of mitosis the role and significance of the very important element — the nuclear membrane was so far unsufficiently stressed.

Investigations carried out on fixed material seemed to indicate that the nuclear membrane disappeared during prophase and the first stages of the spindle formation, the polar caps, are formed in cytoplasm. This course of the process was in fact thought to be proved.

However investigations on plant material *in vivo* did not verify these results. W a d a ' s (1934) micrurgical experiments on mitosis in staminal hairs of *Tradescentia* seem to indicate that the membrane persists throughout mitosis, and the polar caps are formed inside an enlarged nucleus. W a d a ' s further investigations on cell division in *Osmuda* (1941) and specially his experimental and analitical work on mitosis *in vivo* in *Tradescantia* (W a d a 1950), show the persistance of the membrane on the mitotic spindle throughout mitosis. This membrane is of same kind as the membrane that on the two phase boundiers and when well fixed it is not noticeable in an electron microscope (R o z s a and W y c k o f f 1949). Its invisibility on the spindle in fixed material is no argument against its existence, as these kinds of membranes are destroyed during fixation (e. g. tonoplast on vacuoles), nevertheless there is no reason to doubt their existence.

This kind of membrane undoubtedly exists in *Hymenophyllum* and is well visible in normal temperatures and still better in low ones. This would indicate its partly lipoid nature. Such a membrane exists probably also on the spindles of some annimals. Its existance is suggested by the drawing and microphotos in B ě l a ř's (1929a) paper. It can be noticed that during division of primary spermatocytes of snailes the action of a mechanical stimulus causes the spindle to become quite spherical, within a few minutes (2-5 mins.), the spindle is then limited with a well visible membrane (B a j e r 1951 unpubl.). However these facts are no proof that such a membrane exists in most other material.

The assertion of the existence of a membrane is a great help in the understanding of the facts described in the present paper.

It has ben found that when during division the temperature is lowered rapidly a few °C below 0° the whole spindle become quickly rounded and the half spindles shorten while the metaphase plate is very slightly deformed. Similar rounding was noted by W a d a (1938) under the action of butyl alcohol vapours, though in this case the arrangement of chromosomes in metaphase is strongly disturbed. W a d a explains these facts by changes of viscosity of cytoplasm which supposedly governs the shape of the spindle. The different character of changes observed in the course of present investigations is indicated among other things by the different behaviour of the metaphase plate.

There are many factors responsible for the changes occurring in *Hymenophyllum,* though two main of them can be distinguished: in the first group would be those changes, which occur inside the spindle and in the other changes occurring outside i. e. a considerable growth of vacuoles. It seems however in spite of some appearances that the growth of vacuoles has only a secondary effect on the rounding and contraction of the spindle. It is indicated by, a) the different appearance of the spindle observed by W a d a (1938) when it contracted under the influence of the growing vacuoles when ether or chloroform vapour were acting on it, b) the regular plate in low temperature, c) elongation of spindle in high temperature in spite of the growth of vacuoles.

According to two hypotheses of spindle structure i. e. the polypeptide chain (S c h m i d t 1939, W a d a 1950, S w a n n 1951a, b) and the tactoid hypothesis (Ö s t e r g r e n 1949, 1950), it is composed of polypeptide chains. Their structure is probably between fibrillar and globular proteins (framework and reserve proteins). The capacity of polypeptide chains to change their shape (coil, bend, strighten, and shorten) is according to F r e y - W y s s l i n g (1947 a, b) the main characteristic of living cytoplasm.

It seems that the rounding of the spindle under the influence of low temperatures, can partly be caused by at least partial coiling of the polypeptide chains, while simultaneously some of the bonds are broken, and consequenly the structral viscosity of the spindle is lessened. As a result the role of the surface tension becomes more important and the spindle assumes a more or less spherical shape. Changes in cytoplasm and probable ones in the spindle membrane also, have an effect on the shape of the spindle.

The regular metaphase plate indicates that changes of polypeptide chains (such as bending, coiling and etc.) begin at the poles

and move towards the metaphase plate and not away from it. This is also indicated by observations during shocks in anaphase: the chromosomes are pushed back a little (towards the equator of the spindle) which means that bonds joining the kinetochores to the poles are loosened. Usually these bonds are extremly strong which is best indicated in B e a m s ' and K i n g ' s (1936) experiments with an ultracentrifuge.

The changes which take place in *Hymenophyllum* under the influence of low temperatures seem to be in agreement with the observations on the curve of the outline of the spindle drawn from fixed material at normal temperature (E h r e n b e r g 1946). According to E h r e n b e r g when temperature is lowered the curve of the outline of the spindle becomes more curved and the length of the spindle shortens.

The influence of high temperature on metakinesis, metaphase and early anaphase can be explained by the uncoiling or strightening of the polypeptide chains (partly denaturation) while simultaneously the spindle is strongly dehydrated. In high temperatures some kinds of bonds are broken up. i. e. sensitive to temperature homopolar cohesive bonds between the lipophylic groups, some of the hydrogen bonds, which undoubtedly facilitates the uncoiling or strightening of chains. On the other hand the new arrangement of molecules and their mutual dislocation can lead to the formation of closer and stiffer bonds which makes the breaking up of the structure difficult.

Denaturation of each of the chromosome fibers can be different and the results are the disturbances in the metaphase plate. The fibers of the spindle are fairly stiff and pointed poles of the spindle can be pushed into vacuoles. In normal division spindle fibers are certainly in some cases stiff and can not only pull but even push the chromosomes. Such cases were observed by S c h r a d e r (1947) in normal division in *Mecistorhinus* and *Brachystetus*.

The process of the elongation of the spindle is reversible, though the return to normal conditions lasts a long time (at least 8 times longer than after low temperature). This would corroborate F r e y-W y s s l i n g's (1947a, b) supposition that if the fibrillar proteins change into globular ones, there would to take place the breaking up of the protein molecules which has not so far been found in vitro. Undoubtedly changes in the spindle structure under the influence of temperature are much more complicated than described by F r e y - W y s s l i n g, nevertheless the nature of the changes

which the spindle proteins undergo can be similar (e. g. the change to normal stage of denaturised proteins by high temperatures is difficult and long lasting as the framework of the spindle must be in some way broken up).

A process difficult to explain is the prolongation of the way that the chromosomes move through, if the high temperature is applied in late or sometimes middle anaphase. In early anaphase this fact does not occur. This can indicate that in normal division in anaphase, changes which take place, must reach a certain stage of development before high temperature causes this process. Measurements of the spindle length and the distance of the separation of the chromosomes indicate that the chromosomes under the action of the high temperature move not only because of a force pulling them to the poles, but also because of one pushing them apart.

In normal division in most plants the spindle length in anaphase seems to undergo no or very slight changes, this is the case both in *Tradescantia* and *Hymenophyllum* mitosis. However elongations similar to those observed by H u g h e s and S w a n n (1942) in chick tissue culture, were found in *Haemanthus* (B a j e r 1952). An elongation however does take place in animals and B ě l a ř (1929a) from his observations on *Stenobothrus* suggested the pushing body (Stemmkörper) hypothesis.

B ě l a ř (1929 b) states that the elongation occurs also in plants. R i s (1943) on the basis of the measurements done in B ě l a ř's (1929 b), work thinks that the spindle does not elongate in plants. It should be pointed out that in B ě l a ř's object, i. e. staminal hairs of *Tradescantia*, the spindle in not normally visible (it can be seen in lower temperatures c. f. W a d a 1950). From measurements done from B ě l a ř's (1929 b) drawings (Figs. 13, 15, 17, 19) and microphotos it appears that the elongation observed by him is either very small (Figs. 17 and 19) and is only as the result of hypertonic medium, or does not take place (Fig. 13). In some cases it can appear as if the spindle was elongated though that is not the case, this happens in cells with the obliquely placed metaphase plate (Fig. 15), or when the arms move before the kinetochores (Table III, Figs. 11-21).

Statistical measurements of the length of the spindle in metaphase and different stages of anaphase in different plants (B a j e r 1951) indicate that a very small elongation of the spindle in anaphase takes place. Observations on *Hymenophyllum* are in agreement with the above. Observations of cell division in older cells (4-6 from the top) in staminal hairs of *Tradescantia* (B a j e r 1950) can suggest that the pushing body is active (in these cells the spindle seems to dent into the vacuole during anaphase). However this can here be explained by, that the ends of the spindle move apart during the

course of anaphase and as the cells are narrow, part of the cytoplasm is pushed into the vacuoles. The elongation of the spindle in the plant *Impatiens* in pathological meiosis was observed by S m i t h (1934) but in this case it also does not seem that it was the action of the pushing body. As far as it can be ascertained there is no good evidence in the literature of the subject proving the action of the pushing body in plants during normal mitosis (but *Haemanthus* endosperm, B a j e r 1952). A very good argument against this hypothesis is the so far overlooked fact that in plants the two chromosome groups do not move in anaphase synchronically and symmetrically (less than 5%) and this could be expected if there was a force acting from the metaphase plate and if the cytoplasm around the spindle was uniform which seems to take place very often.

In *Hymenophylum* when the spindle elongates during high temperature action, the anaphase chromosome groups are bent or flattened. It results from this, that the force causing this movement acts from the center of the pushing body, and the separation would be far greater if there were no cell walls.

Hydration and the consequent increase in volume of the pushing body proteins offers a simple explanation of the observed elongation of the spindle. L e c o m t e d u N o ü y (1945) states that the proteins of blood serum are strongly hydrated in high temperatures (e. g. 900 times) and as a result the volume of the molecules increases 4 times (pp. 81-86). The increase in volume of the pushing body in *Stenobothrus* was also observed by B ě l a ř (1929 a) in hypertonic solutions. From observations on *Hymenophyllum* it is impossible to state whether the increase in volume of the pushing body does occur, though there is no proof to the contrary. It seems that if there is an increase of the volume it is very slight. Instead of the increase in volume a change in the arrangement of molecules can probably cause the elongation of the spindle. As the spindle elongates, and also in the course of normal mitosis, the pushing body becomes very thin mainly in its center. Within the pushing body itself new vacuoles appear and grow all the time the high temperature lasts. These vacuoles and also the growing ones from the cytoplasm sometimes bend the pushing body and can press a little on the chromosomes. This however can have only a secondary effect. If active elongation of the pushing body existing in animals may be compared to that induced in *Hymenophyllum* then perhaps the mechanism of spindle elongation in *Hymenophyllum* is the same as in animals. It must be stated however that the explanation of the pushing body mechanism which appears in *Hymenophyllum* under the influence of high temperature is very difficult and cannot be satisfactory now.

Acknowledgment

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SUMMARY

The influence of shock changes to low (0° to —16°C) and to high (+38° to +45°C) temperatures on mitosis *in vivo* in *Hymenophyllum tunbridgense Sm.* were investigated. Change temperatures were applied for 10-120 mins.

1. In normal mitosis the spindle is usually well visible and it can be seen that it has a membrane. The membrane is on the half spindles, on the pushing body (Stemmkörper of Běla ř 1929) and also on the phragmoplast. This membrane is of the kind of phases boundary and is best visible in low temperatures.

2. The length of the spindle diminishes from the stage of the polar cap formation till anaphase and in anaphase it remains constant.

3. The chromosome are well visible and the metaphase plate is remarkably regular. Anaphase is very distinct and it is easy to establish its beginning and to observe the movement of each of the kinetochores.

4. In low temperatures (—4° to — 16°C) occur:

a. strong vacuolisation of cytoplasm, pushing body and phragmoplast.

b. Rounding and contraction of the spindle though the metaphase plate is not deformed.

c. Complete interruption of mitosis.

After return to normal temperature (+20°C) mitosis is continued and its course is quite normal. Similar changes may be repeated in the same cell several times.

5. In high temperature (+38° to +45°C) occur:

a. Vacuolisation of cytoplasm;

b. Elongation, thinning and sharpening of half spindles and a simultaneous strong deformation of the metaphase plate,

c. Increase of chromosome seperation (to twice the original

separation) if the high temperature action begins in late and sometimes in middle anaphase. In this case two factors responsible for chromosome movement can be active: pushing apart (action of pushing body), pulling to the poles (shortening of the half spindles), or one of them. Dependent on this is the shape of the chromosome movement curve. The action of high temperature since middle anaphase does usually cause the elongation of chromosome separation in comparison to normal separation. In these last cases the course of anaphase is similar to that found in most animals.

After the return to normal temperature the spindle is reconstructed (if high temperature is applied in prophase, or in metaphase) and the course fo cell division is normal. The reconstruction lasts a long time (6-8 h).

After the action of high temperature in anaphase the formation of the cell wall is very retarded (it lasts 4 times longer i. e. 2-4 h).

6. The results of investigations on submicroscopical structure of the spindle and cytoplasm were discussed and an attempt was made to explain some of changes observed.

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EXPLANATION OF PLATES

Magnification of all microphotos approximately 1000×

Plate I

Figs. 1—11. The same cell division at 20°C. Cell No 103.

1. Prophase (0 min.) in stage of polar caps.
2. Very early metakinesis (23 mins.).
3. Metaphase, plate is very regular, almost all kinetochores are in one plane (108 mins.).
4. The very beginning of anaphase. On the left side of the plate chromosomes begin to move ,while on the right they lie still in the plane of the equatorial plate (124 mins.).

5—6. Early (138 mins.) and middle anaphase (142 mins.). The spindle is well visible. Chromosomes are thinner than in metaphase.

7. Late anaphase (149 mins.).
8. Early telophase (157 mins.).

8—10. Cell wall partly formed in phragmoplast (172, 197 mins.).

11. Late telophase, vacuolisation of the phragmoplast. (210 mins.).

Figs. 12—13. Two stages of telophase with membrane visible on the phragmoplast.

Fig. 14. Late anaphase with membrane visible on the pushing body.

Fig. 15. Late telophase, membrane on the phragmoplast. The membrane is swollen on the right by new a cell wall.

Plate II

Figs. 16—21. The same cell, cell division after low (—7°C) temperature action in time 0—35 mins. Cell No 115.

16—17. Rounded spindle, on both sides of the spindle large vacuoles, regular metaphase plate, (40 and 42 mins.), two planes.

18. Spindle almost reconstructed (69 mins.).

19—20. Anaphase after reconstruction of the spindle (90, 91 mins.).

21. Telophase, formation of new cell wall in phragmoplast.

Figs. 22—24. The same cell. (No 115).

22. Metaphase at + 20°C (0 min.).
23. Cell in temperature —7°C, spindle slightly rounded, strong vacuolisation of the cell (8 min.).
24. Cell after high temperature action (88 mins.) elongated and pointed spindle.

Low temperature acts 0—60 mins. high (+ 42°C) begins to act at 60 mins.

Figs. 25—27. Elongation of the spindle in metakinesis as the consequence of high temperature (42°C) action. High temperature acts 18—69 mins. 25 — cell at 20°C (0 min.), 26—19 mins., 27 — 59 mins.

Plate III

Figs. 28—31. Elongation of half spindles and pushing body in consequence of high temperature (+ 45°C) action ,temperature acts 0—23 min.

28. Early anaphase before high temperature action (0 min.).
29. Elongation and pointing of the spindle, enlarging vacuoles (11 min.).

30—31. Bending of the spindle (29, 80 mins.).

Figs. 32—37 and 38—43. Influence of high temperature (+ 45°C) on late anaphase. Lengthening of anaphase separation of chromosomes. Flattening of anaphase chromosome groups very well visible in 34, 41, 42. 32 (0 min.), 39 (17 min.), 34 (21 min.), 35 (28 mins.), 26 (46 mins.), 27 (60 mins.), high temperature acts 14—40 mins. 38 (0 min.), 39 (13 mins.), 40 (18 mins.), 41 (27 mins.), 42 (45 mins.), 43 (46 mins.), high temperature acts 8—34 mins.

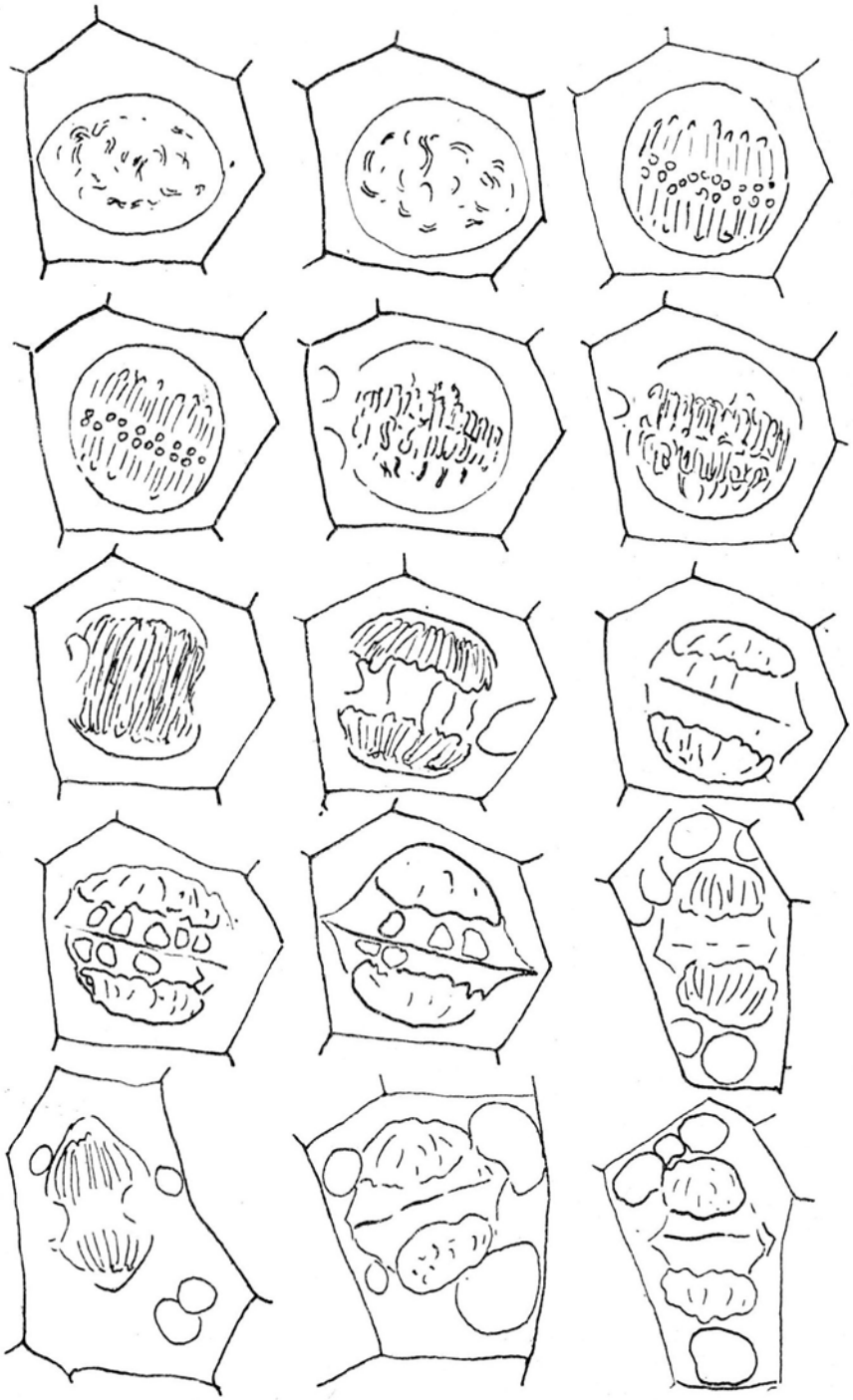

Plate I.

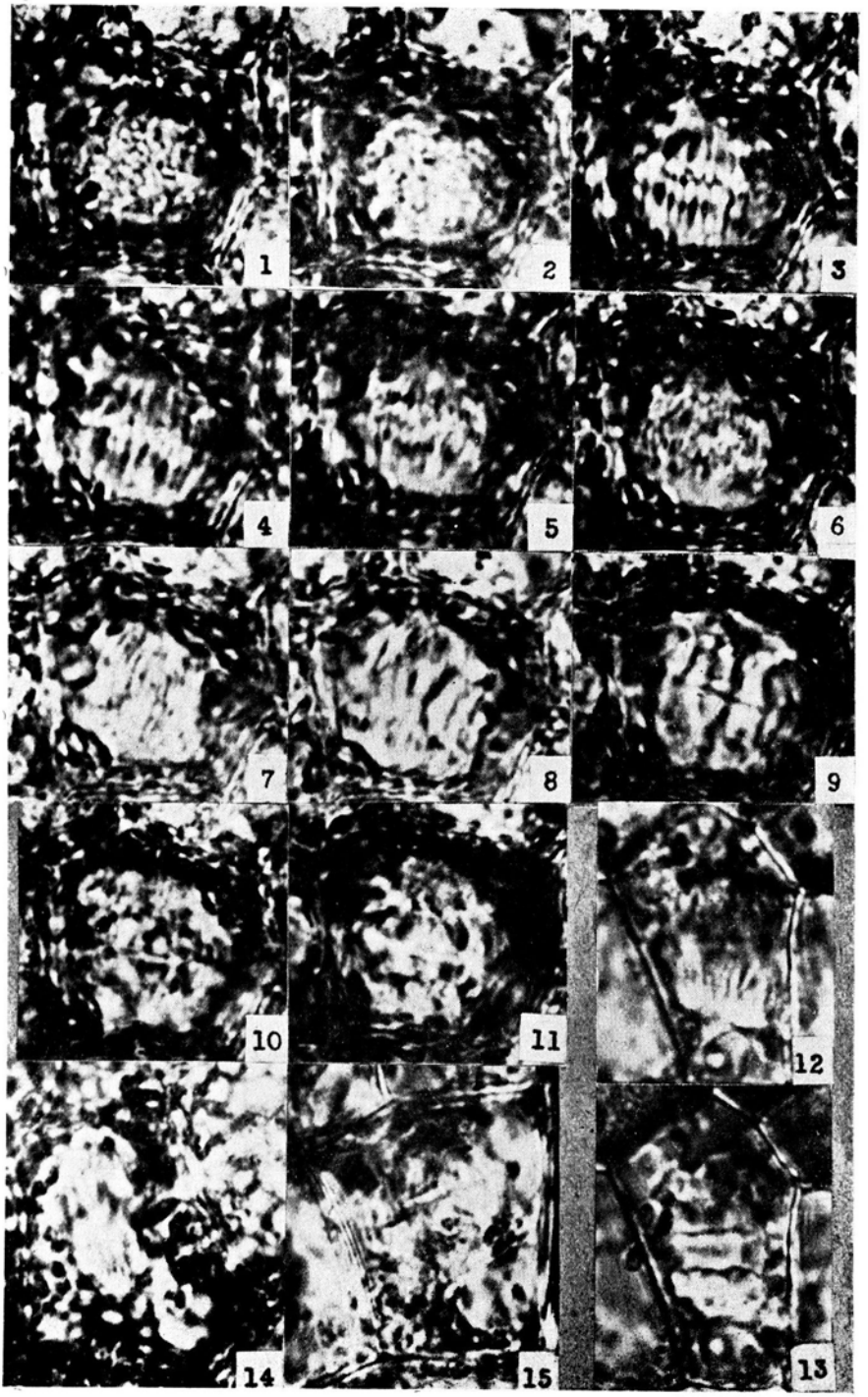

Plate I.

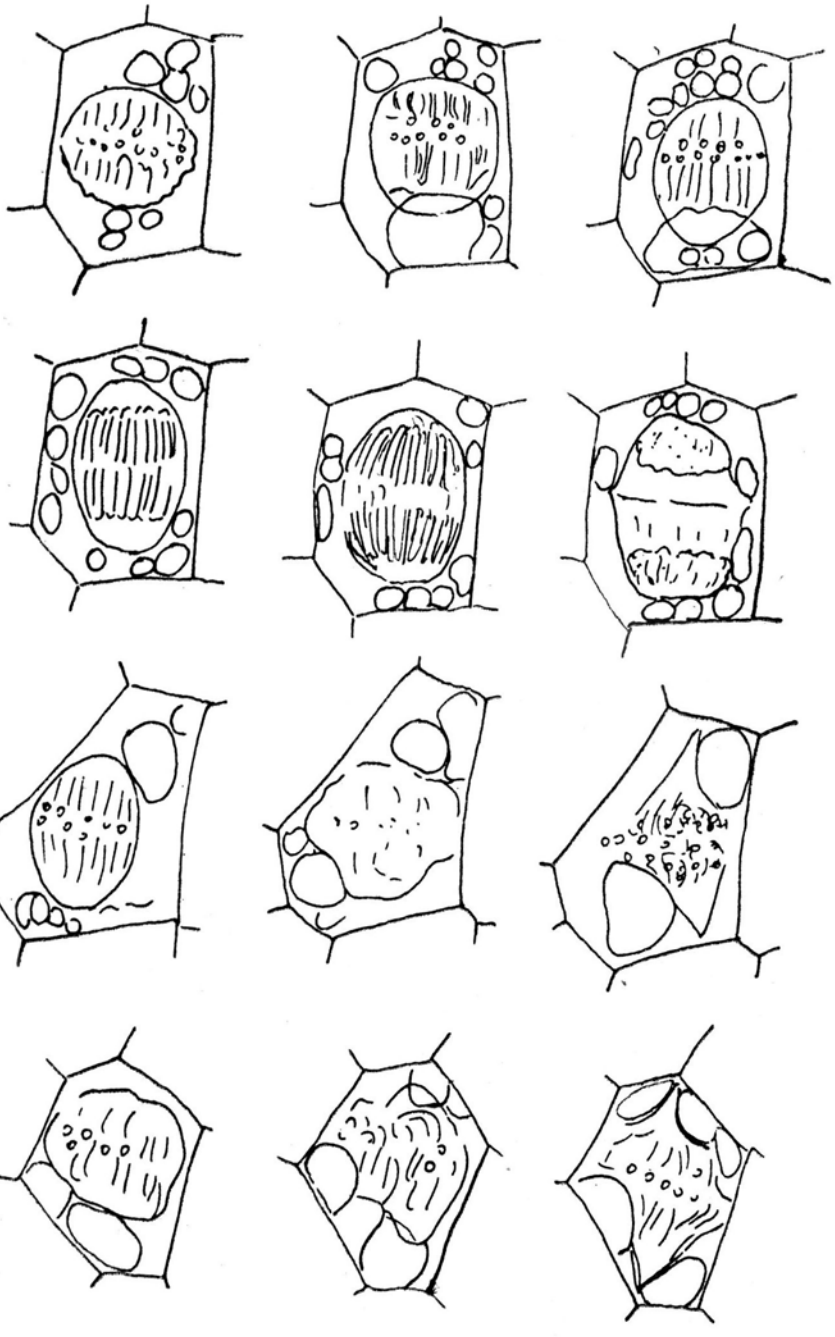

Plate II.

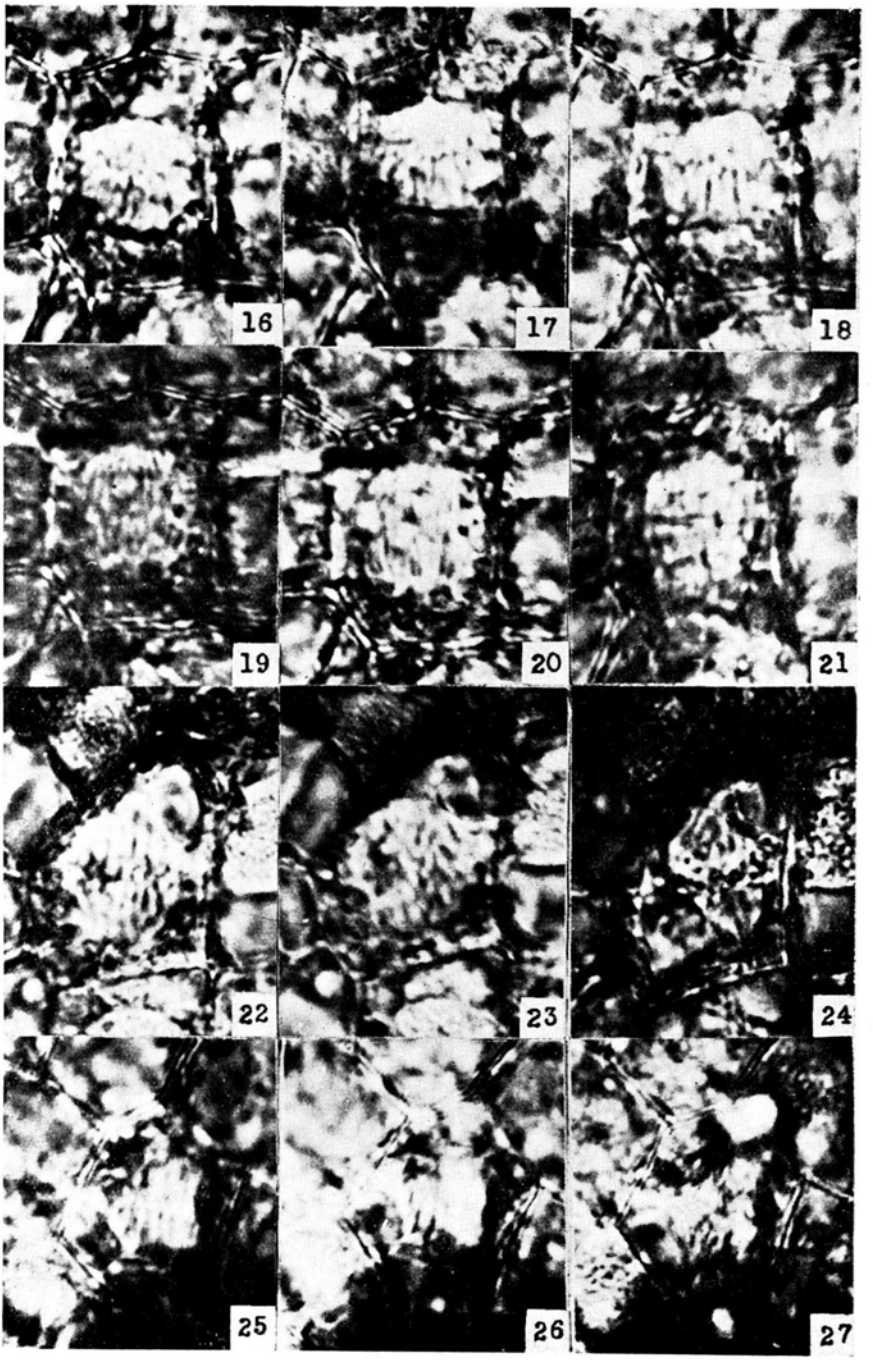

Plate II.

Plate III.

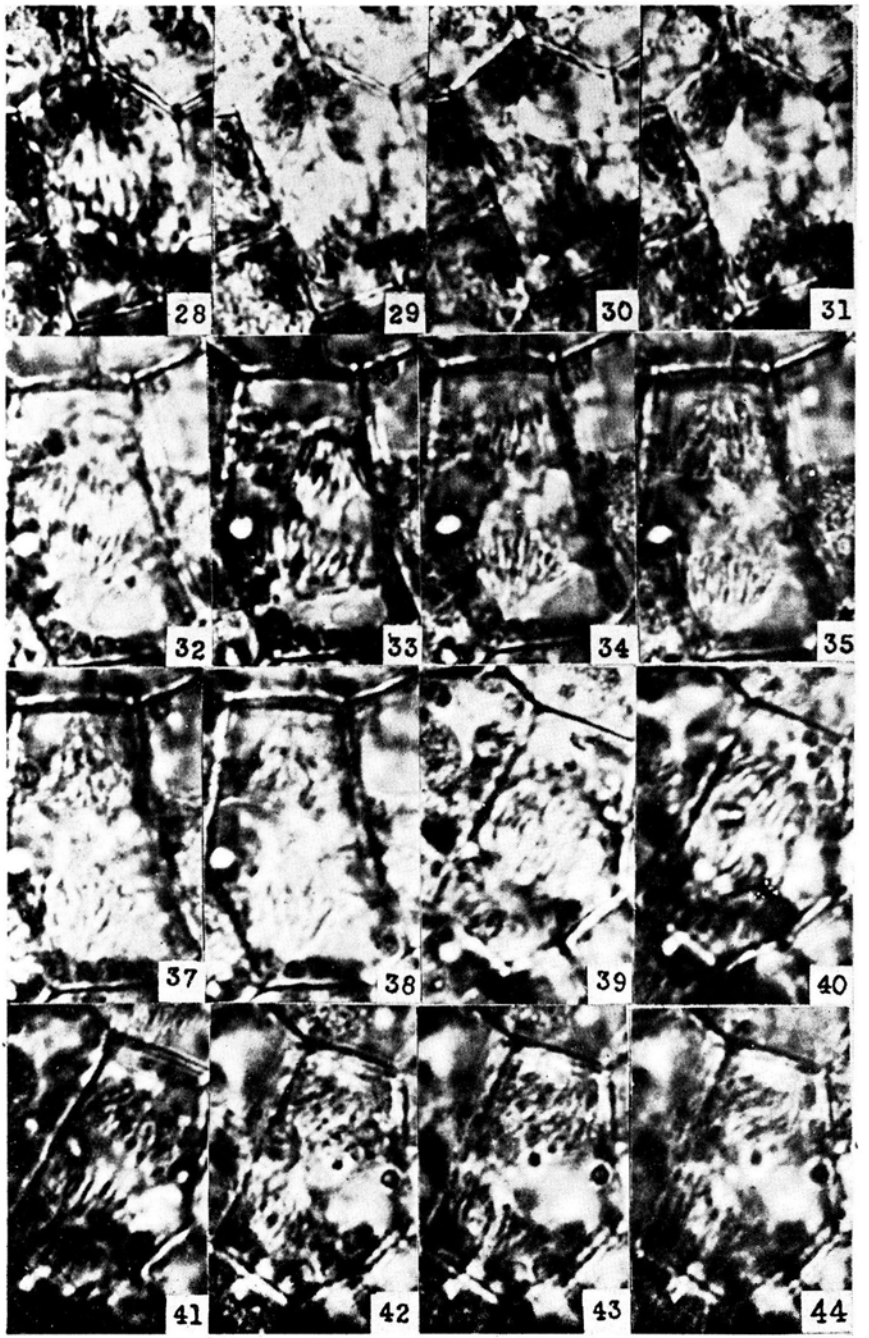

Plate III.