Cine-Micrographic studies on mitosis in endosperm. I.

Badania kinematograficzne nad mitozą w endospermie. I.

by

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STRESZCZENIE — SUMMARY

W badaniach nad mechanizmem mitozy u roślin metoda kinematograficzna była użyta zaledwie kilkakrotnie. Wydaje się to dziwne, ponieważ metoda filmowania w zwolnionym tempie pozwala szczególnie dobrze analizować powolne ruchy i przemieszczenia, często nie· do stwierdzenia przy bezpośredniej obserwacji. Autor zastosował tę metodę do badań nad mitozą w endospermie *Haemanthus katharinae*. Metoda badań używana w poprzednich pracach została udoskonalona i udało się przedłużyć czas życia komórek po wypreparowaniu, a także eksperymentalnie rozpłaszczyć żywe wrzeciono mitotyczne prawie do grubości chromozomów, przy czym mitoza jest kontynuowana w tych warunkach od wczesnej profazy do telofazy, a następnie jąder spoczynkowych.

Celem pracy było stwierdzenie *kiedy rozpoczyna się rola kinetochorów w profazie*, zbadanie ruchu chromozomów w metakinezie i czynników, wpływających na ten ruch w czasie mitozy. Przy pomocy metody filmowej zostały wykonane obserwacje nad tworzeniem się wrzeciona i przemieszczaniem się chromozomów w profazie. Stwierdzono, że najpierw chromozomy są powoli przesuwane w kierunku środka wrzeciona i w wyniku tego procesu trwającego zazwyczaj ponad 30 min. tworzą one luźny kłębek w środku wrzeciona. Wtedy w wyniku krótkotrwałego procesu, nie trwającego dłużej jak 2—4 min. są silnie zciśnięte, przy czym siła powodująca ten proces działa od bieguna w kierunku równika. To stadium znaleziono ponadto u ponad 20 roślin badanych *in vivo*, a także na trwałych preparatach (u wszystkich badanych pod tym aspektem), najprawdopodobniej występuje więc u wszystkich roślin, nadano więc mu nazwę: *stadium kontrakcji mitotycznej*. Po zakończeniu tego krótkotrwałego stadium objawia się po raz pierwszy w mitozie kierująca rola kinetochoru i zapewne w tym stadium *następuje połączenie kinetochorów z wrzecionem*. Stadium kontrakcji jest drugim, oprócz początku anafazy, stadium, które można w mitozie ściśle umieścić w czasie. Jest to stadium zakończenia bardzo ważnego etapu mitozy: tworzenia wrzeciona. Obserwacje wykazały, że *w metakinezie działają dwie siły: jedna skierowana od środka wrzeciona do biegunów i powodująca rozprostowywanie się ramion chromozomów, druga skierowana do równika i powodująca ruch kinetochorów w kierunku równika i tworzenie się*

płytki metafazowej. Te dwie siły działają równocześnie, a pierwsza także jeszcze w metafazie. W czasie metakinezy obserwowano kilkakrotnie dziwny i trudny do wytłumaczenia fakt: ruch kinetochoru do bieguna. Odległość przebyta przez taki kinetochor jest mała i następnie kinetochor wędruje normalnie do płytki. Obserwacja metafazy i anafazy w rozpłaszczonych komórkach wskazuje, że jakkolwiek *istnieje silna tendencja do ustawienia kinetochorów w jednej linii* (można przyjąć, że podział w rozpłaszczonych komórkach jest w płaszczyźnie dwuwymiarowej, mamy więc do czynienia z linią nie zaś płaszczyzną), *nie jest to sine qua non dla anafazy. Od ustawienia kinetochorów zależy kierunek ruchu chromozomów i biegunowość podziału* i ustawienie kinetochorów w jednej linii powoduje podział dwubiegunowy, czyli normalny, w formie litery T lub Y trójbiegunowy, w formie krzyża, czterobiegunowy. To wskazuje, że *kierunek ruchu chromozomów jest wynikiem orientacji kinetochorów.* W kilku wypadkach obserwowano w komórkach rozpłaszczonych zaburzenia przypominające typ *Sciara* wzg. zaburzenia typu mejotycznego mieszańców roślinnych. Wg. autora te zaburzenia obserwowane po raz pierwszy w mitozie roślin są wywołane najprawdopodobniej wyłącznie warunkami mechanicznymi. Są duże trudności w wytłumaczeniu powyższych zaburzeń na podstawie współczesnych hipotez mechanizmu mitozy, ponieważ wskazują one że 1. *chromozom może być przesuwany razem z aparatem* (mechanizmem), *który go porusza i przy tym mechanizm ten nie zatraca zdolności działania,* 2. *mechanizm mitozy i zaburzeń mejotycznych jest ten sam.*

INTRODUCTION

It is astonishing that if the literature on mitosis in plants is reviewed few papers describing cine-micrographic methods will be found. S c h n e i d e r's (1938) and B a r b e r' s (1939) cine-micrographic studies in *Tradescantia* staminal hairs, which are unsuitable for such studies, are probably the most known. However cine-micrography has great advantages as is evident from studies on mitosis in animal tissues (M ö l l e n d o r f and O s t r o u c h 1939, H u g h e s and S w a n n 1948, H u g h e s 1950 and many others). Acceleration is of special importance in all studies on chromosome movements, especially in the analysis of such stages as late prophase, metakinesis and the formation of the metaphase plate. In these stages chromosome movements are most difficult to analyse exactly, and only a few attempts on fixed and living material have been reported. Numerous questions concerning these stages, as well as later ones, have not yet been answered. We know that the kinetochores play an important role in the normal course of mitosis and that their activity ends in late anaphase, but so far no information has been advanced on the beginning of their activity. Also the behaviour of chromosome arms has not been sufficiently studied. In consequence most hypotheses of mitosis deal mainly with anaphase and are exceptionally unsatisfactory in explaining the metaphase plate stabilisation (cf. review of S c h r a d e r 1953). The analysis of mitosis up to metaphase is one of the main aims of this paper and the following questions are mainly dealt with:

1. *At what stage of mitosis does the activity of kinetochores begin, i. e. when do they begin to guide the chromosomes?*

2. *What are the movements of chromosomes in metakinesis?*

3. *Is the arrangement of the kinetochores in one plane necessary in metaphase?*

4. *What are the factors on which the direction of anaphase chromosome movements depends?*

For experiments cells of normal dimensions, and flattened experimentally to approximately 6 μ were used.

MATERIAL AND METHODS

Endosperm of *Haemanthus katharinae* B a k. was used. Detailed notes on this material and the characteristic traits of normal and injured cells are to be found in my previous paper (B a j e r 1953 b).

All observations were made in a normal hanging drop on agar with glucose, in pure oxygen and a specially adopted chamber (Fig. 1).
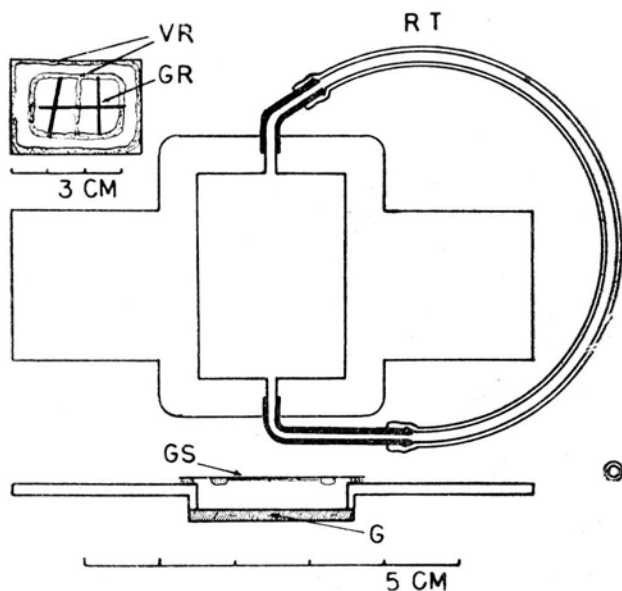

Fig. 1. O x y g e n c h a m b e r. Upper left corner: glass square with two vaseline rectangles (VR), the inner one surrounds the liquid with endosperm cells, the outer one fixes the glass to the chamber. GR — glass rods. The glass square (uper left corner) and the chamber are not drawn to scale. In the lower drawing the way to fix the glass square (GS) to the chamber is shown. To blow out the air and to fill the chamber with oxygen the rubber tube (RT) is removed from one side and joined with the tube supplying oxygen, it is then replaced and shuts the chamber.

Technical notes. An embryo sac at the most suitable stage of development is chosen and after cutting off its upper part a small drop of completely transparent liquid is pressed out and put aside. This liquid contains no cells. The remaining contents of the sac are then gently pressed out on a cover slip (24 × × 32 mm, No 0) covered beforehand with a 0,4% agar and 3,5% glucose solution. A vaseline ring is drawn on the glass round the agar, though it is more convenient to draw two vaseline rectangles (10 × 15 mm) before the agar is smeared. The layer of endosperm with the liquid surrounding it must be very thin. As the drops of liquid with endosperm are fairly large it is very convenient, in order to obtain uniformly thin layers, to place crosswise very thin (0,2 mm diameter) glass rods or a small quantity of glass wool in the pressed out drops. The cover slip is placed in a special chamber (Fig. 1) and about 20 ml of oxygen are blown through gently to remove the air. The lower glass of the chamber should to be covered on the inside with soap of the kind divers use to prevent condensation of water vapour. An inverted microscope is very convenient for observation, and if this type is not used, the chamber after the preparation of endosperm should be placed upside-down for about 15 mins to allow the cells to adhere to the agar. After some experience the preparations are so thin that an immersion lens can be used without difficulty. When this method is used, and when the preparations are well done, all uninjured, cells continue mitosis from early prophase to telophase, numerous cells enter mitosis after preparation and mitosis may be observed for 15 hrs or more. Also even 27 hrs after preparation normal mitosis (metaphase, anaphase and telophase) was observed. In experiments where this method is applied the thickness of the drops is of the greatest importance. In large drops the penetration of oxygen is not sufficient, and in consequence most cells die in prophase, though most mitoses in metakinesis are continued to telophase. B u l l o u g h (1952) reports that in animal tissues (mice) during prophase a free supply of oxygen is necessary. This is also the case in Haemanthus, though in the endosperm of numerous other plants (B a j e r and M o l è - B a j e r 1954) as well as in some animal (rat) tissues (R o o s e n - R u n g e 1953) oxygen is not necessary during prophase.

Flattening of the spindles. When the drop is very thin numerous cells and spindles, especially those on the outer parts of the agar, flatten to approximately 6 μ, whereas the normal diameter of the spindle is approximately 20 μ and the thickness of metaphase chromosomes approximately 4 μ. The speed at which the spindles are flattened is important which will be explained further (cf. observations). It is rather difficult to make preparations well; when too much liquid is placed on the glass square, cells do not flatten and when too little, most of them die quickly. When everything is done correctly, several flattened cells may be observed in each preparation. Numerous flattened cells continue mitosis from very early prophase and probably enter mitosis after preparation. Divisions can be observed for more than 20 hrs.

Zeiss phase contrast equipment and time lapse cine micrography was used. The rate of records was 6 per minute, thus the rate during projections (16 micrographies per second) was accelerated 160 times. A 16 mm cine camera was used. Cells were filmed from prophase to telophase, though in most cases one stage only was recorded, for instance: prophase to contraction stage, prophase to metakinesis, metaphase or anaphase. The different stages of mitosis in 56 filmed cells were analysed exactly, and special stress was laid on metakinesis. Also numerous cells were not filmed and measurements in these cells were made with the use of a drawing prism. Thus during this work about 500 chosen cells were observed very distinctly.

OBSERVATIONS

The course of normal mitosis in the endosperm of *Haemanthus* has been described previously (B a j e r 1953a, b). In the present paper only new facts and those not described sufficiently will be given.

M i t o s i s i n f l a t t e n e d c e l l s. Mitosis is continued in all observed cases in the cells which flatten slowly, i. e. during 1—2 hours or more after preparation. The thickness of such cells is approximately 6 μ, while the thickness of metaphase choromosomes is approximately 4 μ, and the diameter of a normal spindle is more than 20 μ. The arrangement of chromosomes is similar to that observed by H u g h e s (1952) in tissue cultures treated with solutions of abnormal tonicity. However, it is necessary to add that the spindle is not destroyed, which H u g h e s (1952) observed to be the result of hypotonic solutions, though often the chromosome arrangements are similar to those described by H s u and P o m e r a t (1953 a, b).

It is astonishing that in such flattened spindles in *Haemanthus* mitosis is usually normal, and cell walls and resting nuclei are formed, sometimes even several hours after preparation. All mitotic stages, are similar to those in a normal spindle, though chromosomes lie often in one plane only and very seldom their arms overlap. Microphotos (Figs. 10, 11, 18) illustrate such cells. In my opinion disturbances of mitosis occurring in the flattened cells are caused by the thinness of the cells. They will be described more fully further on. The course of mitosis in flattened spindles, in spite of differences in mechanical conditions, may be compared to that in normal ones, though the different stages may be prolonged (cf. Table I). Normal mitosis in such cells will not be described here. It is difficult to decide whether the cytoplasm of such cells is quite normal. The structure of the cytoplasm, the presence of chondriosomes and the refraction coefficient indicate that it is normal, and on the contrary the presence of vacuoles and the formation of splits are symptoms of an abnormal state. This question cannot be solved satisfactorily at present.

Mitosis is usually interrupted in cells which are flattened too quickly. Usually such cells are thinner than 6 μ, their refraction coefficient is much lower than in normal cells, their chromosomes swell, and neither the chromosomes nor the cytoplasm have any structure which is indicated by the vigorous Brownian movements. The decrease of the refraction coefficient is the best sign of the approaching death of a cell.

N o t e s o n c h r o m o s o m e s t r u c t u r e. Flattened cells are very suitable for studies of chromosome structure. As the chromosomes are large and are arranged in one layer there is often no difficulty in

TABLE I

Duration of different stages of mitosis in *Haemanthus katharinae* (in mins)

No of cell	Eearly prophase to contraction stage	Metakinesis and metaphase	Anaphase to max. separation of chromosomes
Not flattened cells			
593	210	85	40
595	240	60	40
597	180	70	45
681	180	40	30
682	240	45	40
683	240	70	40
684	300	90	45
685	300	65	40
686	270	100	35
687	360	70	30
Flattened cells			
888	180	85	65
889	180	100	35
890	180	130	55
892	240	75	50
893	180	100	60
894	240	120	60
900	210	100	35
901	180	80	40
930	240	130	40
932	180	60	40

observing distinctly the same chromosome from early prophase to late telophase. In early prophase the chromosome structure is much more distinct than later in this stage. From very early prophase the chromosome structure is at least double but the two chromatids are not closely united till metakinesis. Process of quick disjunction of chromatids in metaphase just before anaphase begins is clearly visible on an accelerated motion picture. The distinctly quadruple structure of chromosomes becomes visible in metakinesis. Prophase chromosomes have often „wooly out-

lines", i e. lumpbrush appearance. This semblance of chromosomes has been described by numerous recent writers (H u s k i n s 1948, G a- l i n s k y 1949, M o t a 1952). My observations of kinetochores con- firm L i m a d e F a r i a's (1949) suggestion that the kinetochore consists of two slightly separated granules. At the beginning of anaphase, or in late metaphase a similar structure of kinetochores can be observed (Fig. 19 B).

S p i n d l e   f o r m a t i o n   a n d   m i t o t i c   c o n t r a c t i o n s t a g e. In most endosperms studied till now (cf. B a j e r and M o- l è - B a j e r 1954, in press) in the first stage of spindle formation a clear usually structureless space appears round the prophase nucleus. This space, first described by J u n g e r s (1930) in fixed *Iris* endosperm cells, forms very slowly and motion pictures add no new data to what is already known. On the other hand changes in the arrangement of chro- mosomes are rather astonishing. In flattened and in normal cells the changes of chromosome arrangements are alike. As prophase continues the chromosomes, so far displaced regularly throughout the nucleus, are very slowly pressed together and pushed to the middle of the spindle. They form a regular *loose ball* in the centre of a clear space which in later stages develops into a mitotic spindle. The fact that direction of chromosome movement in this process lasting more than 30 minutes in-
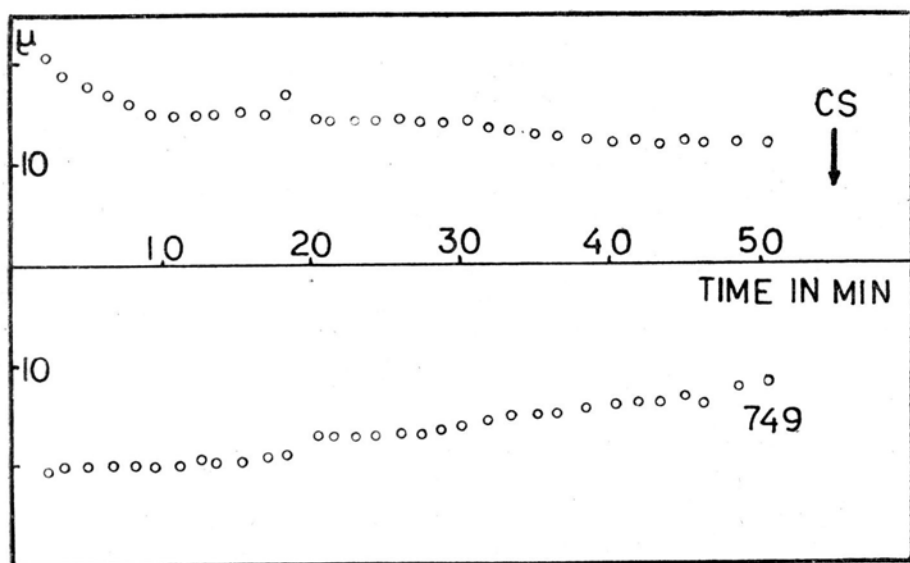

Fig. 2. C h a n g e s   i n   s p a c e   o c c u p i e d   b y   c h r o m o s o m e s i n   p r o p h a s e. Diminishing of the diamiter of the nucleus in prophase along the axis of the future spindle. Arrow (CS) marks beginning of contraction stage. The position of abscissa is chosen arbitrarily Cf. the curve in Fig. 3. Cell No. 769.

dicates that the acting forces are directed from the poles or from the two
opposite sides of the prophase nucleus. Curves in Fig. 2 show how the
diameter of the nucleus decreases. The nuclear wall disappears during
this loose ball stage, but as a rule there are still one or more nucleoli
present. After the more or less regular ball is formed the motion picture
snows no changes in the chromosome arrangement for at least 10—15
minutes. Then a curious stage lasting 2—3 minutes begins. During this
stage the chromosomes are more or less strongly pressed from the direc-
tion of the spindle poles. One receives a *strong impression* from the mo-
tion picture *that a purely mechanical force is then acting.* After this the
chromosome arms become all tangled and interwined and in this state
it is very difficult to trace the shape of all chromosomes. In flattened
spindles this process also takes place, though not so distinctly. The curves
in Fig. 3 and Fig. 4 C best illustrate the stage just described. It usually
lasts less than 4 minutes, and is difficult to analyse with methods other
than cine micrography. This stage has been observed in all cells studied
in prophase, though not in all the cells is the space occupied by chromo-
somes contracted in the same extents. As I have found this stage in nume-
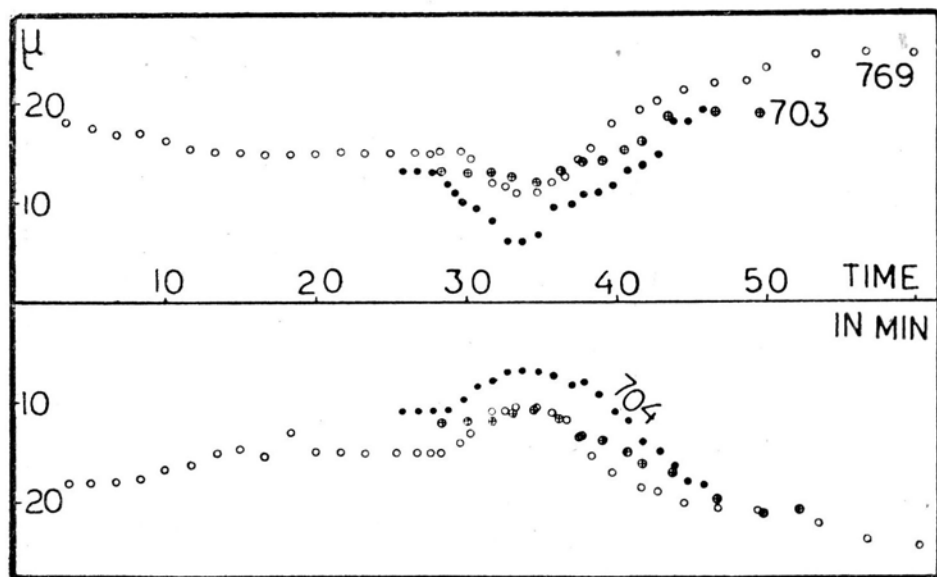

Fig. 3. C o n t r a c t i o n   s t a g e. The changes of nucleus diameter in con-
traction stage and beginning of metakinesis. The differences of space occupied by
chromosomes along the long axis of the future spindle. In cell No 703 the shrinking
is smallest of all observed. During the contraction stage the shirinking of the nu-
cleus takes place, the diameter of the nucleus increases when chromosome arms
move to the poles. In cell No 769 the shrinking of the nucleus in contraction stage
is considerable. The position of abcissa is chosen arbitrarily.

rous plants (cf. discussion), and as it is probably to be found in most plants I have called it the *mitotic contraction stage.*

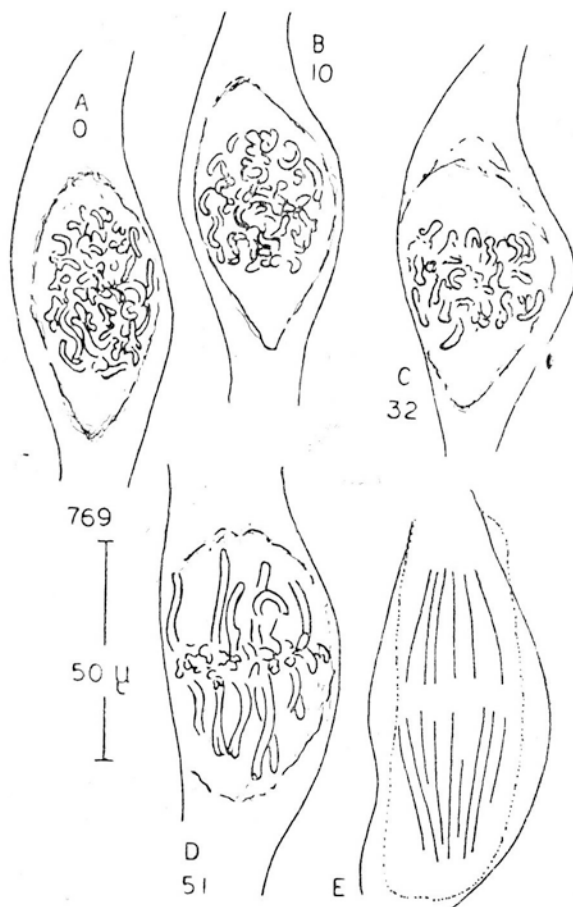M e t a k i n e s i s   a n d   m e t a p h a s e. Immediately after the chromosomes are pushed to the middle of the spindle two contrary for-

Fig 4. C h a n g e s   i n   c h r o m o s o m e   a r r a n g e m e n t   a n d   l i n e s   t r a c e d   b y   k i n e t o c h o r e s   i n   a n a p h a s e. A — prophase chromosomes spread evenly throughout the nucleus, C — contraction stage, D — early metaphase, E — lines of kinetochores movement in anaphase, the outlines of the cell in late anaphase are dotted in. Drawing are done from cine micrographs. Numbers — time in mins.

ces begin to act simultaneously *one directed to the poles and the other to the middle of the spindle* (cf. discussion). Metakinesis begins and chromosome arms — in large *Haemauthus* chromosomes they are about 20 μ long — bent irregularly in the contraction stage, begin to stretch out toward the poles, while the kinetochores move simultaneously toward the

middle of the spindle. These are the most characteristic features of metakinesis. In all not flattened cells the ends of chromosome arms move at the rate of 1—1,4 μ per minute (cf. Table II). The curves in Figs. 3 and 5 illustrate the course of metakinesis. The straightening of chromosome arms lasts usually not more than 20 minutes. While stretching out the arms are often waved to and fro and some resemble slowly moving fish or a rope placed in a stream of water. This is also observed in metaphase
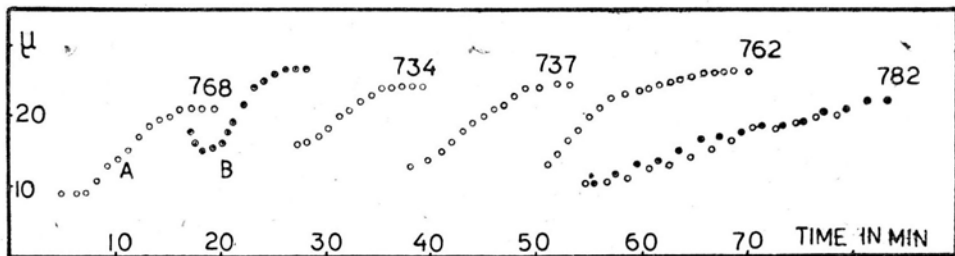

Fig. 5. M o v e m e n t s   o f   c h r o m o s o m e   a r m   e n d s   i n   m e t a-
k i n e s i s   i n   d i f f e r e n t   c e l l s   (Nos of cells are given). In cell No 768 the movements of the ends of two chromosomes was marked, measurements on the end of chromosome B began before contraction stage. The differences in the rapidity of chromosome ends are small. In cell No 782 the movement marked for two chromosome ends, was remarkably slow. The position of abscissa is chosen arbitrarily.

and in flattened cells. Ultimately the chromosomes have a tendency to arrange themselves parallelly to the spindle length (Östergren's 1949 factor δ).

In very rare cases single kinetochores moving toward the poles may be seen. It is very difficult to distinguish the bent ends of chromosome arms from small chromosomes, as in *Haemanthus* the set is composed of both long and short chromosomes. This fact has made it necessary to eliminate all observations doubtful in this respect, but even so at least 4 cases are certain. Microphotos (Fig. 17) show a chromosome with its kinetochore directed to the pole when moving towards it and away from it when it returns to the plate. The curve of the movement of this chromosome and another one in a flattened cell are given in Fig. 6. The movement is jerky which means that the acting forces are not in equilibrium and the fact that the chromosome changes its position so that its arms are parallel to the long spindle axis and they are waving indicates that the chromosome is subject to forces, one directed to the pole and the other directed to the plate. Some observations seem to indicate that though some chromosomes are distinctly pulled toward the poles, to most of them a sudden jerk toward the pole is given. This, however, is not absolutely proved.

T A B L E II

Comparison of the velocities in the mitotic spindle μ/min

			Velocities in μ/min															mean of mean and mean of max
Metakinesis — movement to the poles	kinetochores	mean	0,36*	0,35*	0,32	0,28												—
		max	1,0	—	0,4	0,6												—
	ends of chromosome arms	mean	2	1,6	1,2	1,2	1,1	1	1	0,9	0,9	0,9	0,8	0,7	0,7	0,5	0,4	1,0
		max	2	2,5	1,3	2,5	1,5	2,0	1,1	1,1	1,4	1,1	1,0	1,2	1,7	1,6	1,0 0,5	1,4
Anaphase	movement of kinetochores to equator	mean	0,6	0,4	0,2	0,2	0,2											0,3
		max	1,1	0,5	0,3	0,2	0,2											0,4
Anaphase	movement of kinetochores to the poles	mean	0,9	0,9	0,8	0,7	0,7	0,7	0,7										0,7
		max	1,0	1,0	0,8	1,2	0,8	1,0	1,0										0,9
Prophase	formation of loose ball	mean	0,3	0,2	0,2	0,2	0,1											0,2
		max	0,4	0,5	0,7	0,2	0,2											0,4
Contraction stage	movement to the equator	mean	2,5	1,7	1,7	1,0	1,0	1,0	0,8	0,7								1,3
		max	2,5	1,8	1,7	1,8	1,5	1,3	0,7										1,4
Anaphase	elongation of the spindle i. e. pushing body action	mean	0,5	0,5	0,4	0,4	0,3	0,3											0,4
		max	0,4	0,7	0,4	0,4	1,0	0,3	0,3										0,6

* Movement in flattened cells
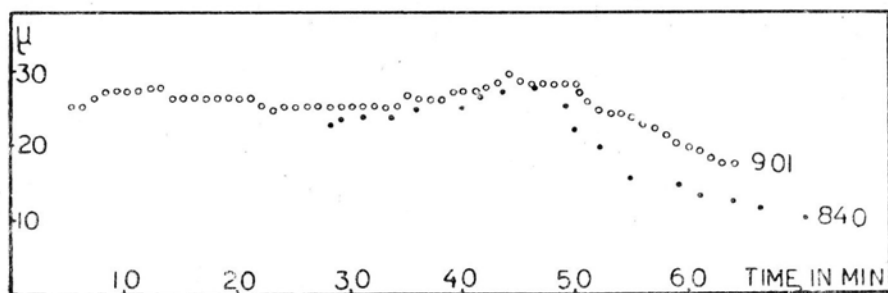
Fig 6. M o v e m e n t o f k i n e t o c h o r e to the p o l e and its r e t u r n to the e q u a t o r in m e t a k i n e s i s. The poleward movement is slow jerky and interrupted. Movement to the equator resembles the movement in cell No 850. The position of abscissa is chosen arbitrarily.

As a result of the contraction stage and the poleward movement of chromosome arms most kinetochores are directed to the equator of the cell. They begin to *move toward the equator at the same time as the chromosome arms stretch out*. It is remarkable that the kinetochores move towards the plate at the rate of 1/3—1/4 of the rapidity of chromosome ends to the pole (cf. Fig. 7 and Table II).

At metaphase in normal spindles the kinetochores lie in one plane, while in flattened spindles this is often not the case. When the spindles are very thin (6 μ) the kinetochores may be either arranged in two or more rows or from a half-circle or a Y (Figs. 10, 11 and 19 respectivly). When the kinetochores are not arranged in one line in metaphase, the metaphase lasts much longer and numerous attempts to form a normal metaphase plate are to be observed. It is the lack of space, i. e. solely *mecha-*
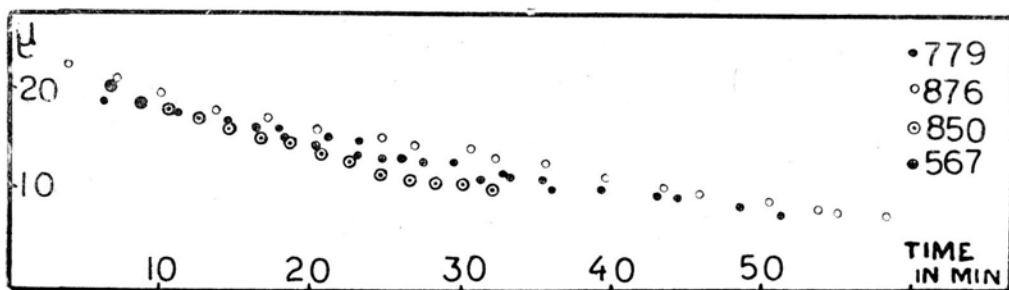

Fig. 7. M o v e m e n t s o f k i n e t o c h o r e s to the e q u a t o r. Note the very slow movement in this stage in comparison to anaphase. The velocity of kinetochores differed slightly in different cases, the quickest kinetochores is seen in cell No 850 which resembles that in Fig. 6. The position of abscissa is chosen arbitrarily.
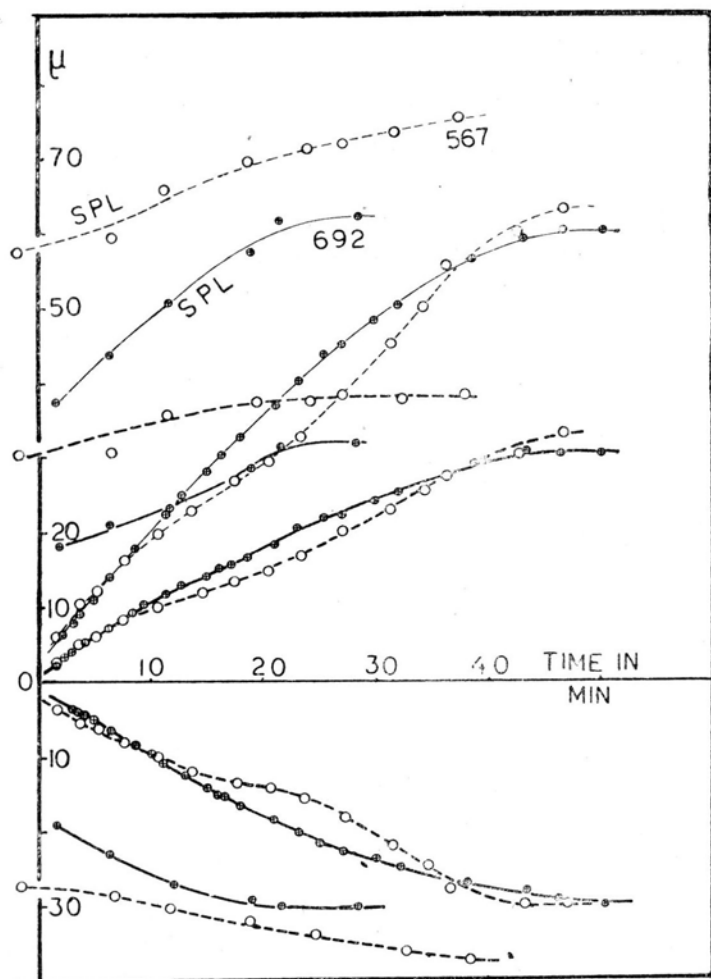
Fig. 8. Curves for the separation of daughter chromo-
somes in anaphase and spindle elongation (SPL).
Curves for two chromosomes and the resultant ones. Note the changes in the course
of the resultant curves.

nical conditions which *prevent the kinetochores arranging themselves
in a line*. Observations in cells of different thickness confirm this state-
ment. When the metaphase plate is Y shaped three polar anaphase re-
sults, and when it is shaped in a cross four polar anaphase follows. The
different arrangement of chromosomes in the plate of flattened cells
depends on how quickly and to what thickness cells are flattened. The
flattening of cells may begin 2—5 or more hours before metaphase, when
the cells are still in prophase or in later stages. Multipolar mitoses are

much more frequent when flattening begins in prophase than when it begins in metakinesis, though in the first case normal two polar mitoses also occur. However, in all cases multipolar mitoses are rare.

Motion pictures show also that in metaphase the spindles shorten and simultaneously their thickness increases. In different cases the shortening of the spindle length is not the same. The spindles are shortest at the beginning of anaphase. Fig. 17 M — O illustrate this process. Flattened cells have not been measured in this respect.

A n a p h a s e. In this stage my attention has been specially directed to the lines the moving kinetochores trace and to the pushing body action. The lines traced by the kinetochores are given in Figs. 4, 12 and 13. From this point of view the analysis of normal and flattened cells has been undertaken. The purpose of such an analysis has been to establish whether the lines kinetochores trace are directed to one point or rather to an area. It is evident that as anaphase continues the chromosomes become more and more crowded. At the beginning of anaphase the kinetochores move along parallel lines, then they deviate toward the poles. The deviations in direction of the chromosomes from the parallels in not flattened cells become well visible when we prolong the lines. The prolonged lines of kinetochore movements often cut outside the cell. At late anaphase chromosome ways again become parallel. In this late stage the movement of chromosomes is caused by the pushing body action.

Observations made during the present work on changes in the shape of the spindle during metaphase and anaphase support the results described in the previous papers (B a j e r 1953b, c).

Pushing body action is best illustrated by Fig. 9. Cine micrographic analysis shows that not flattened cells are usually shoved in one direction as a result of pushing body action. As a result curves illustrating the movements of two chromosome groups are very seldom symmetrical. The two chromosome groups usually move symmetrically when the spindle is surrounded by a sufficiently large amount of cytoplasm. When the Brownian movements are recorded on motion film it is seen that in the pushing body small particles usually oscillate irregularly. In some cases however, the particles move in one direction only i. e. to one of the poles, and follow the movement of the chromosomes. The particles during their movement to the pole do not oscillate to the sides.

In flattened cells the large area of the touching plane with which cells are fixed to the agar offers a strong resistance to the pushing body action mainly at the poles, and prevents the elongation of the spindle. in consequence the pushing body action is directed to the part of the spindle which offers least resistance. It may act in one or two directions
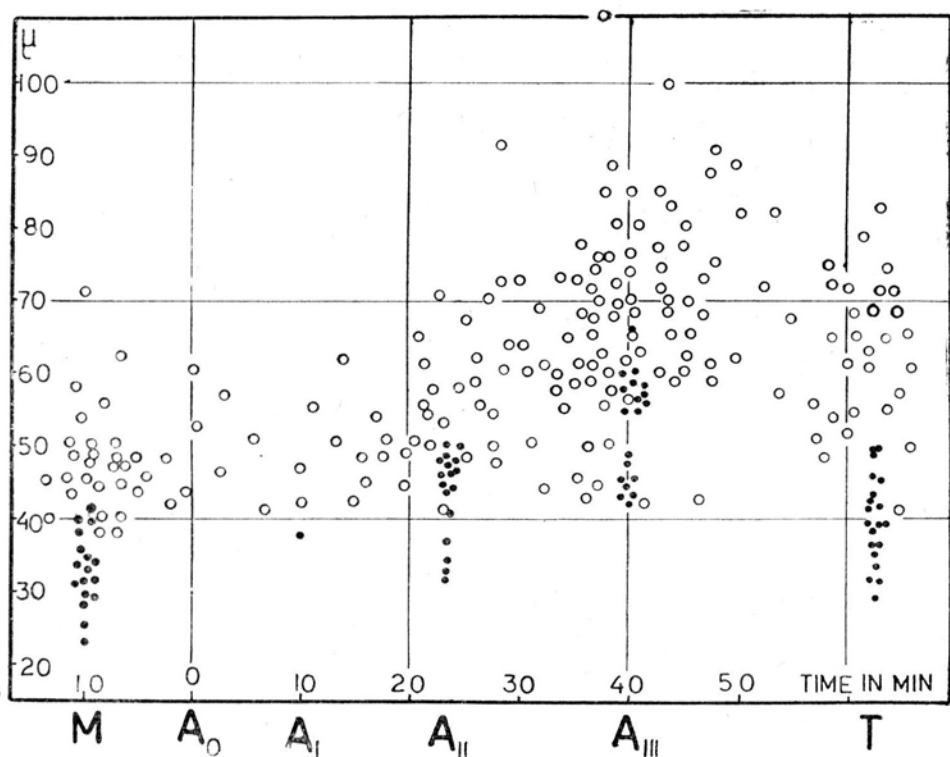
Fig. 9. C h a n g e s i n t h e s p i n d l e l e n g t h i n a n a p h a s e —
p u s h i n g b o d y a c t i o n. Only one measurement was made in each re-
gularly dividing cell (white circles), cells not flattened. M — metaphase, A_0 —
anaphase beginning, $A_{I, II, III}$, early middle and late anaphase respectively.
Beginning of anaphase considered as O time. Black circles: measurements in fixed
C a r n o y fluid cells, imbeded in paraffine and cut in the ussual manner. Mea-
surements made in unstained cells with the aid of phase contrast illumination. As
the time in these cells could not be measured, the cells have been ranged in time
after comparison with living cells. All fixed cells are from old endosperm while
the living ones are, in most cases, young. The age of the cells and contraction due
to the action of fixative fluid have shortened the distances.

and causes the bending of long chromosome arms. The arms of daughter
chromosome may not be bent simultaneously.

Observations on anaphase and telophase in multipolar mitosis throw
some light on primary cell wall formation. It has been found that the
cell wall originates in all places where chromosomes have passed inde-
pendently from the number of chromosome groups. In the first stages
the primary cell wall is similar to the arrangement of kinetochores in the
metaphase plate. Then gradually the cell wall grows between the daug-
hter nuclei.
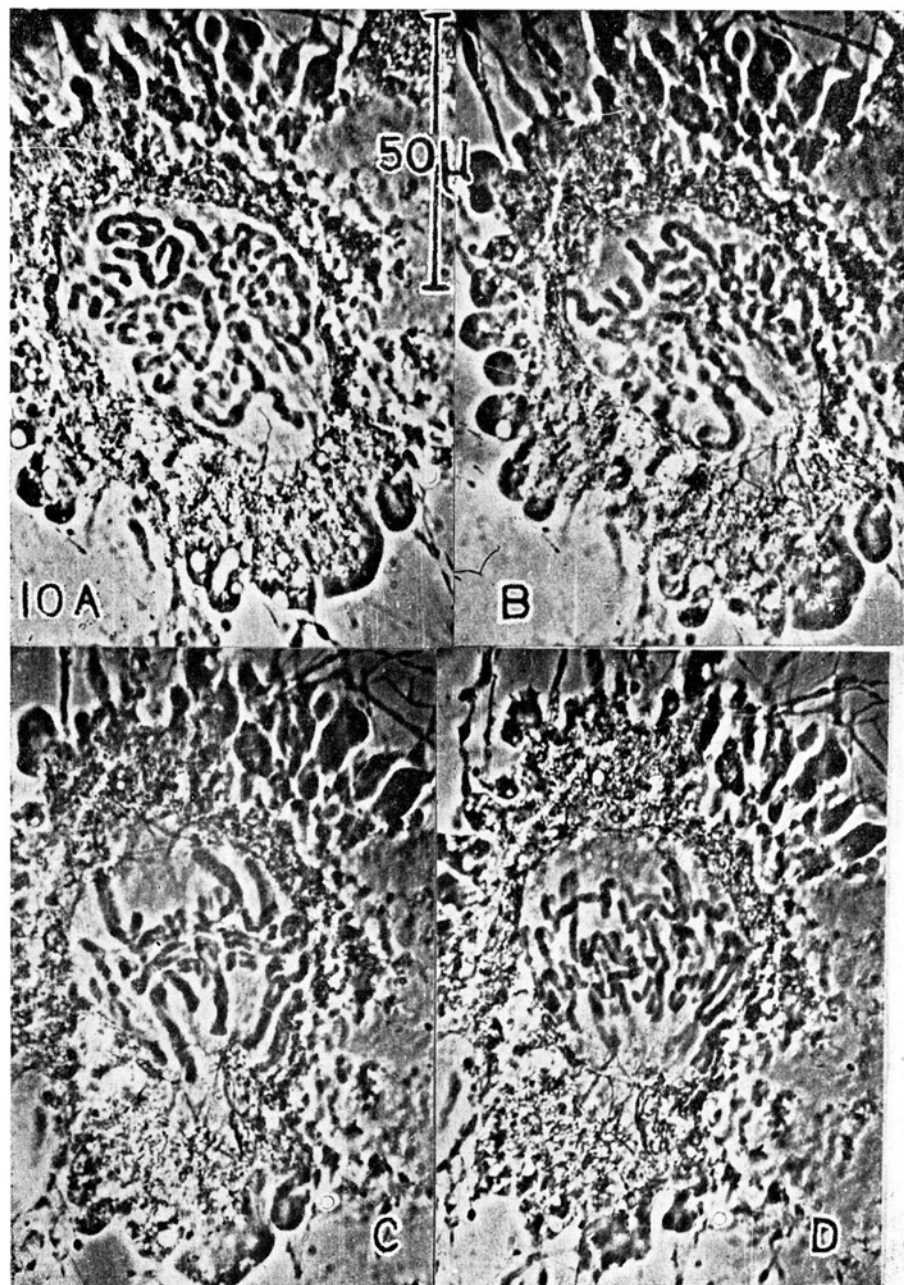
Figs. 10—11. Flattened cells. Fig. 10. A — late prophase just before metakinesis, B — metakinesis, C — metaphase, kinetochores are arranged not in one plane but in several layers one on the other, D — anaphase, E — resting nuclei. B — 23,5 mins after A, C — 50 mins after A, D — 2 hrs after A, E — 11 hrs after A. Cell No 888
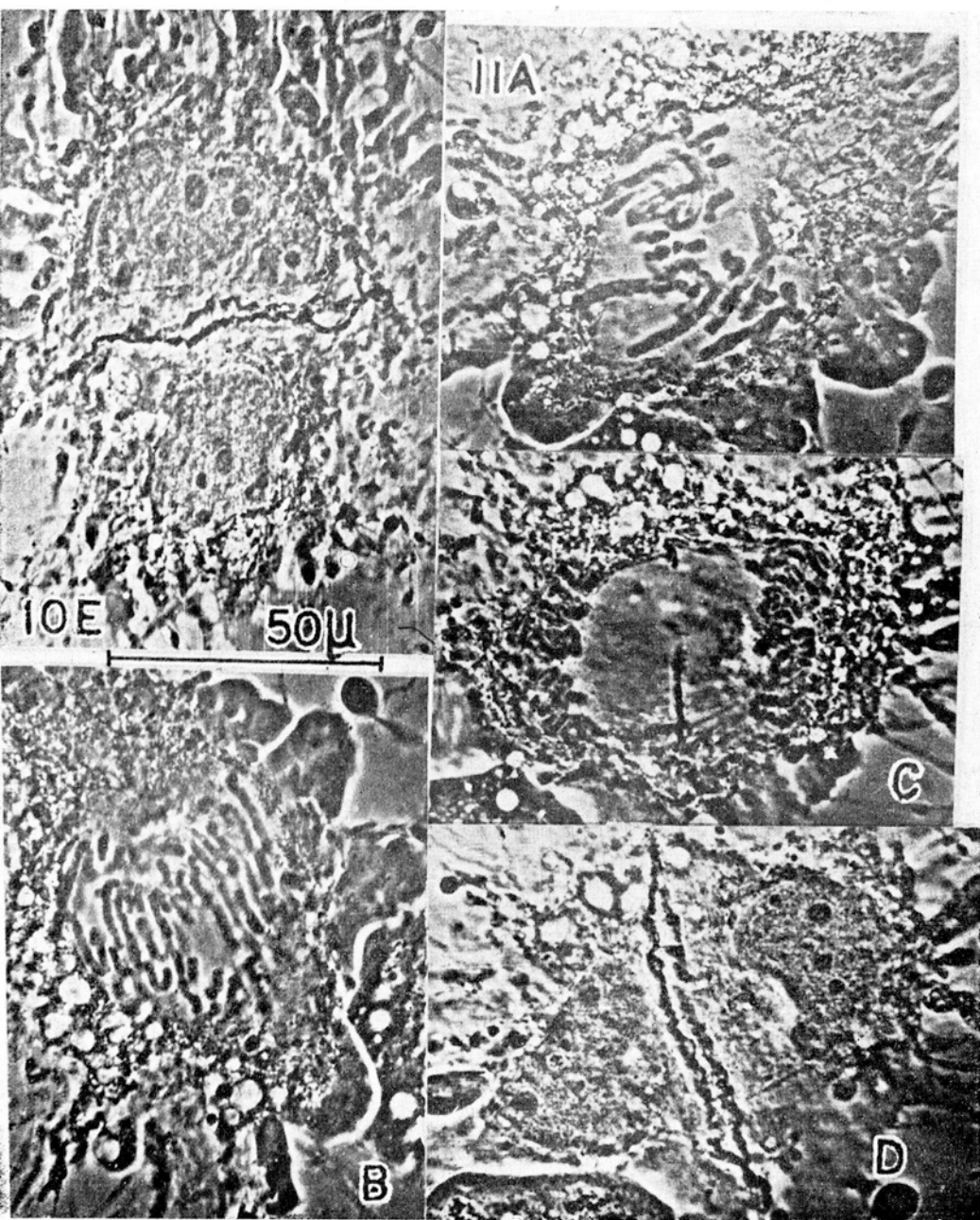
Figs. 10-11. Flattened cells. Fig. 11. A — metaphase with kinetochores arranged in half circle, nevertheless anaphase is normal, C — beginning of cell wall formation D — resting nuclei, B — 52,5 mins after A, C — 1 hr 17,5 mins after A, D — 10 hrs after A. Cell No 889.

During the present investigations also the action of hyper- and hypotonic solutions has been observed. The results do not fully support B ĕ l a ř' s (1929) observations. Hypertonic solutions cause a prolongation of the anaphase separation i. e. pushing body action. In hypotonic solutions the pushing body action is usually directed not to poles but to the sides of the spindle and chromosome arm also stretch out sideways. It is not known to what extent the stretching of chromosome arms is caused by the swelling of the spindle, which results from the hypotony of the solutions. Strong hypertony causes an increase of the refraction coefficient, plasmolysis of the cell, and stops the course of mitosis. High concentrations cause the death of the cell. Hypotonic solutions lessen the refraction coefficient of the cytoplasm, destroying its structure and diminish its viscosity coefficient which is shown by the more vigorous Brownian movements in strong hydrated cytoplasm.
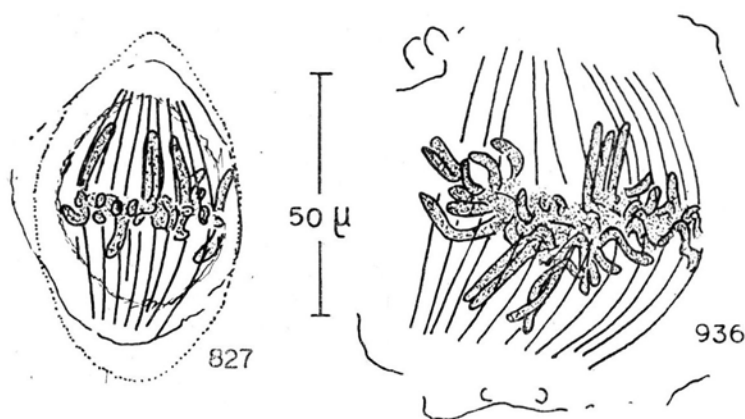

Fig. 12. L i n e s o f k i n e t o c h o r e s m o v e m e n t i n n o r m a l (No 827) a n d f l a t t e n e d (No 936) c e l l s. In cell No 827 the outlines of the spindle and of the cell in metaphase and late anaphase are drawn in continuous and dotted lines respectively. When the lines traced by kinetochores are prolonged they interset in metaphase and in anaphase outside the cell, this is also the case in flattened cells.

D i s t u r b a n c e s i n m i t o s i s. Only disturbances in chromosome movements will be considered. Disturbances in the formation of the metaphase plate and the resulting multipolar mitosis have already been described. Now two other types of disturbanes will be reported.

a. M i t o s i s r e s e m b l i n g t h e *Sciara* t y p e. Anaphase resembling that of *Sciara* and *Micromalthus* meiosis described by M e t z (1926) and S c o t t (1936) may be found in quickly flattened cells of *Haemanthus* endosperm. Usually such cells die in telophase but sometimes resting nuclei are found. Figs. 14 and 21 represent such anaphases. When in these figures the lines traced by the kinetochores are prolongated, it becomes visible that there is *one pole* only. To this one pole
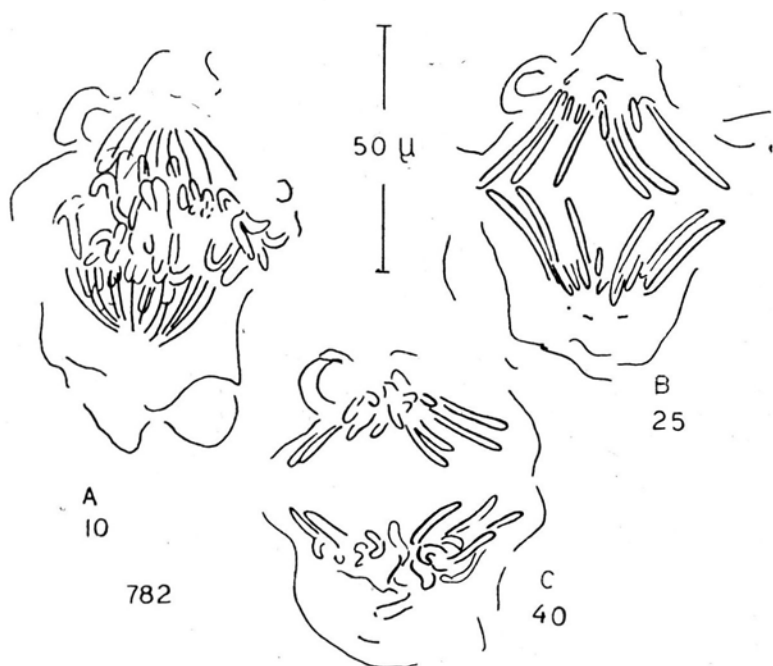
Fig. 13. Action of pushing body in flattened cell and lines traced by kinetochores. Time is measured in mins from the beginning of anaphase and is given in the Fig. In B and C it can be seen that pushing body acts to the sides of the cell. Drawings from cine micrographs.

moves one group of the chromosomes. *The second group of daughter chromosomes moves on lines radiating from this pole.* It is most astonishing that numerous chromosomes move with the arms first and the kinetochores follow them, and not the other way round which would be normal. Such mitoses are very rare and their appearance is most amazing. On Fig. 21 chromosome arms bent after they have been forced into the cytoplasm can be seen. The appearance and shape of chromosome indicate that they are under the influence of a force acting in the same direction as their movement. These disturbances are found in flattened cells of different thickness. The cell in Fig. 21 is less than 6 μ thick, and the one in Fig. 14 more than 7 μ.

b. Disturbances of the meiotic types. Fig. 20 illustrates a cell with disturbances resembling the disturbances reported in numerous plant hybrids. The thickness of this cell is less than 5 μ and its chromosomes are placed in a single layer. In anaphase *chromosomes move toward the poles independently of one an other and their movement is not synchronised.* The movement of these single chromosomes is remarkably slow, and its rapidity may be 10 times slower than in normal

anaphase. From two daughter chromosomes only on may move at a time, and while one moves the other remains motionless. In Fig. 20 chromosomes marked with an arrow behave very strangely: two daughter chromosomes are pushed to one of the poles by a third moving one, and during the process of the pushing to the poles of two daughter chromosomes they separate. The process of this pushing is different from pushing body action. The separation of these daughter chromosomes is the consequence of the action of forces acting on their kinetochores and pulling them to the poles. This is shown not only by the increasing distances between the daughter kinetochores but also by changes in the shape of all three chromosomes. Disturbances of this kind are found very seldom and only in very thin cells.

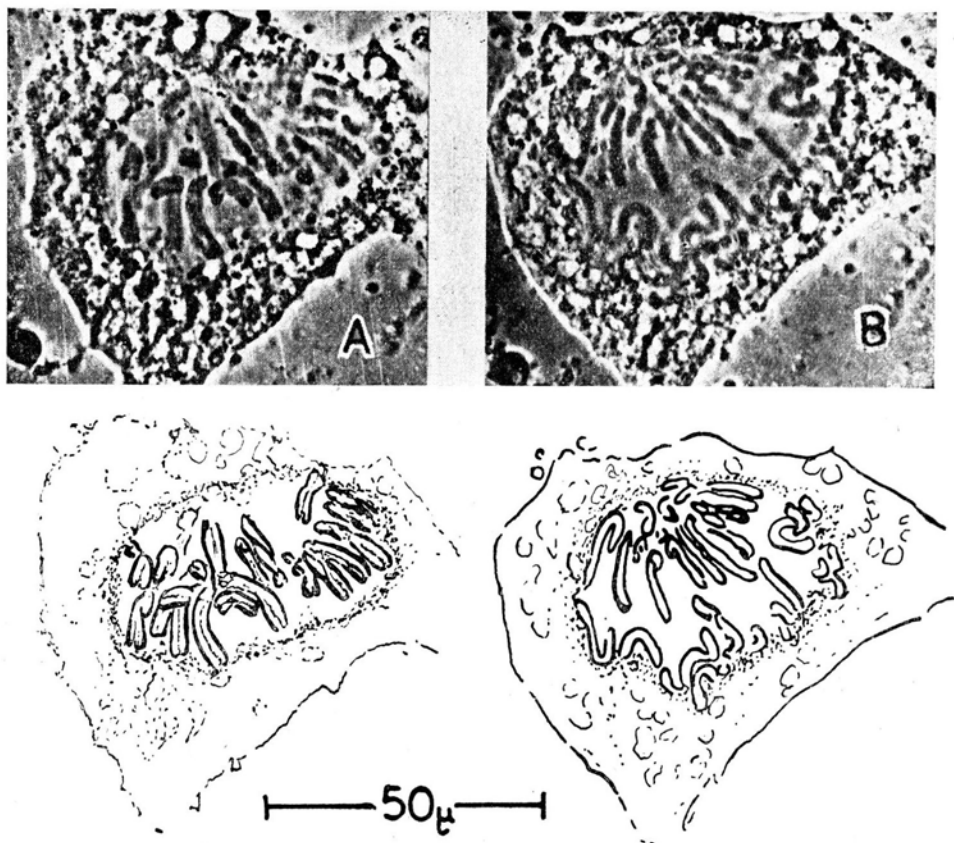
Fig 14. M i t o s i s r e s e m b l i n g t h a t i n *Sciara*, i. e. m o n o c e n-
t r i c m i t o s i s. A — metaphase, B — anaphase. The lines of kinetochore movement intersect in the upper pole and the lower pole seems to be dispersed or not existant. Some chromosomes move with arms first. B — 18 mins after A. Cell No 902.
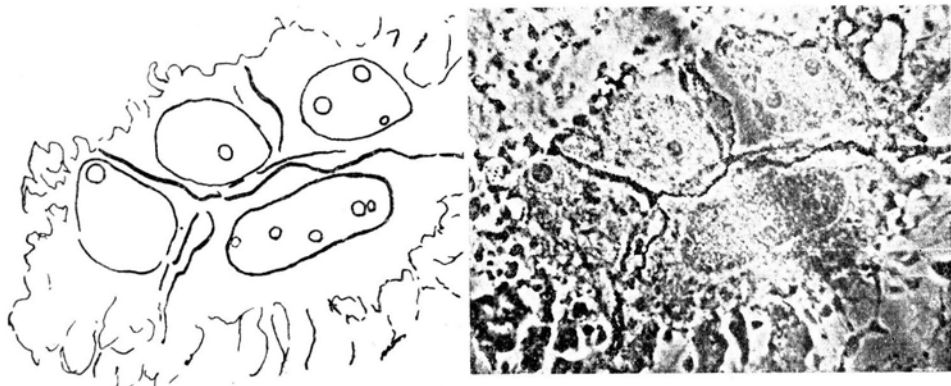
Fig. 15. F o u r   d a u g h t e r   n u c l e i   i n   f l a t t e n e d   c e l l   after, either four polar mitosis, or mitosis resembling that in *Sciara*. 10 hrs after preparation.

DISCUSSION

The facts observed in the course of the present work will now be compared with the reports of other writers and confronted with the current hypotheses of mitosis mechanism. The author wishes to stress, however, that it is not his aim to support or criticise any of the mitosis hypothesis, but to point out some difficulties in explaining the chromosome movement.

In view of the astonishing fact that mitosis is continued in the strange conditions in flattened cells this method appears to be of importance for future studies on the understanding of the mechanism of mitosis. The question should be here raised whether the disturbances previously described and found sometimes in flattened cells — i. e. multi- or monopolar mitosis or disturbances resembling those in meiosis — are caused by pathological changes on the submicroscopic level or by the strange purely mechanical conditions. In flattened cells mitosis may be almost considered as if it were taking place in one plane, that is in two dimensions only, and observations show that disturbances depend on how quickly and how much the cells are flattened. For those reasons it is the author's opinion that disturbances in flattened cells involve no new mechanism and are caused by mechanical conditions.

S t a g e   o f   m i t o t i c   c o n t r a c t i o n.   From the data obtained the existence of the contraction stage becomes evident. Observations on this stage and on the beginning of metakinesis show that *the guiding role of kinetochores in chromosome movements during mitosis first manifests itself in the stage of mitotic contraction. In this stage the interaction of the spindle and chromosomes begins.*

For some unknown reason the contraction stage and its important role during mitosis is usually overlooked by most writers. However, both S c h a e d e (1929 a, b) and S c h n e i d e r (1938) describe it, but not quite correctly. S c h a e d e (1929 a) writes: „Ist die Spindel vollkommen fertig, so besuchen sich die Chromosomen nach ihren Zentrum, und hier einen rundlichen Knäuel zu bilden, der recht dicht sein kann. Während dieser Wanderung behält die Gesamtheit der Chromosomen mit nur geringen Abweichungen die Form des rundlichen Körpers, den sie bei Auflösung der Kernmemebran bilden, und der demnach nur in Umfang abzunimmt, indem die Chromosomen sich einander nähren". The last part of this statement is not correct, and it seems that Schaede noticed only the slow shrinking of the nucleus and the formation of a „loose ball" and not the contraction stage. S c h n e i d e r (1938) describes this stage more fully in his paper on cine micrographic studies in *Tradescantia* staminal hairs. He reports that at the beginning of metakinesis..., ein deutlich erkennbares, eigenartig rasches Zuzammenzucken des ganzen Kernes" may be observed and „Verkleinerung des Kernes nur einer scheinbaren Kontraktion entspricht". Also W a d a (1950) on the basis of direct observations in *Tracescania* cells writes that ...„at the time of spindle formation may generate forces or pressures in the direction from the pole to the equator of the nucleus. Therefore all the chromosomes... are pushed toward the equator". The methods used by both S c h a e d e (1929 a, b) and W a d a (1950) made it impossible to distinguish the separate nature of the two processes: 1. the slow formation of the loose chromosome ball, 2. the contraction stage lasting a few minutes only. It is also probable that this stage is not characteristic in staminal hairs, though the author found it in root tips of: *Tradescantia*, *Allium*, *Vicia*, *Agrostemma*, *Lupinus* and some other material. The contraction stage was also noticed in *in vivo* studies on *Hymenophyllum* (B a j e r and M o l è - B a j e r 1953) and in the endosperm of more than 15 genera (B a j e r and M o l è - B a j e r 1954). It seem likely that the contraction stage is to be found in most, if not in all, plants.

M e t a k i n e s i s. Three processes will be discussed: 1. the poleward movement of chromosome arm ends, 2. the movement of kinetochores to the equator, 3. the movement of kinetochores to the poles.

1. To gain some knowledge as to the nature of the forces causing the poleward movement of the chromosome arms, curves for the movement of long chromosome arm ends have been plotted and exact measurements have been made. It appeared that the process of stretching of all analysed chromosome arms is similar in several respects: a) chromosome ends in the first part of their way move with a constant mean rapidity when the distance is sufficiently long, b) they stretch as much as possible,

and c) the way this movement stops. The above suggests the following conclusion: *the poleward force causing the poleward movement of chromosome arm ends acts with equal strength throughout all the movement.* This force is nothing else than the γ and the δ factors of Ö s t e r g r e n (1949) i. e. „the tendency of spindle to arrange rod shape bodies in parrallel to spindle fibers", and the „tendency of the spindle to extrude bodies out of it". This force is also active in metaphase and, which is strange, in flattened cells also. Changes in chromosome structure cannot alone explain the straightening of the chromosome arms, though these changes may play some part in these processes. The elimination of persisting nucleoli in metaphase (Y a m a h a and S i n o t o 1925, Z i r c l e 1928, S c h a e d e 1929 b and others) is the consequence of its action. The nature of this force is not known though attempts to explain it have been made. It has sometimes been explained that the elimination of nucleoli is the consequence of currents inside the spindle (above cited authors) or the growing of the spindle fibres to the poles (B a j e r 1953 c). The conclusion drawn seems to be in agreement with these two hypotheses. Some other explanations of the poleward force, such as those referring the action of the force to changes in chromosome structure, cannot alone explain such movement.

2. The observations here reported support S c h a e d e's (1929 a) statement that in metakinesis two forces exist: *one acting to the poles, the other to the equator of the spindle. These two forces act simultaneously.* The second force causes the movement of kinetochores to the equator. The characteristic feature of this movement is that it is very slow. It is approximately twice as slow as the anaphase movement. This suggests that the effective force which causes the movement is in metakinesis of the same order as in anaphase (in metakinesis chromosomes are twice as big as in anaphase), when the pushing body does not act.

3. The poleward movement of kinetochores is extremely rare. Curves plotted for this movement seem to indicate that the kinetochores are violently pulled in two opposite directions, which suggests that the equilibrium of the acting forces in disturbed. Similar pulls are reported by H u g h e s and S w a n n (1948) in metaphase of chick tissue culture, and have been observed by the present author in his cinematographic studies of abnormal cells of *Iris* endosperm. It is however, necessary to stress that cine-micrographic studies on mitosis in numerous endosperms with chromosomes of different dimensions *(Asparagus, Iris, Leucoium)* do not show the oscillations of individual chromosomes in metaphase of the normal cells. In the poleward movement observed in metakinesis of *Haemanthus* the kinetochore seems to play an important role. The mechanism causing this movement is probably different from that which

càuses the stretching out of chromosome arms as their rapidities and durations differ.

The movement of kinetochores to the poles at metakinesis of meiosis is well known in some animals as the pre-metaphase stretch described by H u g h e s - S c h r a d e r (1943, 1947). However though the mechanism of the poleward movements of kinetochores in plants and animals may be similar, the mechanism of the backward movements is different, because in animals it is caused by changes in chromosome structure (chromosome contraction).

M e t a p h a s e   p l a t e. In metaphase the kinetochores tend or arrange themselves in one plane even in the difficult mechanical conditions. However this is not necessary for the normal course of anaphase, which is shown by observations in flattened cells. These facts support Ö s t e r g r e n's (1951) statement: ...,,that an equatorial arrangement is by no means in itself a necessary condition that must be fulfilled if mitosis (meiosis) shall perform its primary purpose of distributing the daughter (partner) chromosomes". Observations on metaphases in flattened cells with different kinetochore arrangements allow one to draw the following conclusion: *two, three, or more polar mitosis is dependent on the shape of the metaphase plate i. e. on the arrangement of kinetochores in the plate which may be in one line, Y-, or cross shaped*. This indicates that *the arrangement (orientation) of kinetochores governs the regularity of mitosis, and* that *chromosomes move in the direction in which their kinetochores are directed*. This indicates that the process of kinetochore orientation is most important in mitosis. The importance of analogic process in meiosis was pointed out by Ö s t e r g r e n (1951). S c h r a d e r's (1935) observations on the behaviour of long chromosomes lead him to assume that the kinotochores participate in the formation of the spindle, which seems to support the conclusions drawn here from observations of *Haemanthus*.

A n a p h a s e. The asymmetrical movement of daughter chromosome groups in anaphase and its causes have been described previously (B a j e r and M o l è - B a j e r 1954). Now the following problems will be discussed:

1. the existence of the centres on spindle poles and *Sciara* like disturbances,

2. disturbances in anaphase resembling meiosis in hybrids,

3. pushing body action.

1. It is generally accepted that in normal mitosis in animals the lines of chromosome movement intersect at one point, at the spindle pole. This problem is much more complicated in plants. In normal not flattened cells the prolonged lines of chromosome movement (before the pushing

body causes the parellel movement of chromosomes) intersect usually at the spindle pole. On the other hand lines traced by chromosomes in flattened cells may be more or less dispersed though also in these cells they are usually slightly convergent. This indicates that there is a tendency for the lines of forces causing the chromosome movements to converge at the poles or at one pole, which in some cases may even lead to mitosis similar to that in *Sciara* (monocentric mitosis). In this last case, however, the influence of the pushing body is important. In mitosis of the *Sciara* type it seems that either one of the poles is missing or it is very dispersed, while the existence of the opposite pole is obvious. *The movement of chromosomes with the kinotochores following the arms is in Haemanthus the result of mechanical conditions.* S a x and O' M a r a (1941) write that in pollen tubes the chromosome arms often precede the kinetochores, and they conclude that such movement, though similar to *Sciara*, is caused by conditions of space. Observations on flattened cells of *Haemanthus* also indicate that it is not necessary to assume that a new mechanism is involved in such movements i. e. autonomous movement of chromosomes.

2. The chromosomes move to the poles separately and their movements are not synchronised because the cell is very thin (mechanical conditions). It may be concluded that: *each anaphase chromosome has its own movement mechanism which may act independently of the others and that a chromosome with its moving mechanism may be shoved or pushed about in the spindle.* If it is correct to assume that in flattened cells there is no new mechanism and the mechanism of *Sciara* like division and meiosis like disturbances in *Taemanthus* is the same as in *Sciara*, it becomes very difficult to explain the observed facts on the basis of traction fibres, their fixation to the poles and so on, though it is evident that the force causing the chromosome movement in anaphase is attached to the kinotochore.

3. The present observations on the action of the pushing body are not in full agreement with B ě l a ř' s (1929) studies, though they suport a number of his results (cf. text).

The bending of chromosome arms in one direction as seen in photographic records and the action of hypotonic solutions are the best proof of the pushing body action in flattened cells and it indicates that it acts in the direction of the least resistance. In flattened spindles the curving of daughter chromosome arms is a result of the pushing body action in flattened cells and it indicates that it acts in the direction of the least resistance. In flattened spindles the curving of daughter chromosome arms as a result of the pushing body action, is not simultaneous. it may begin

from the centre of the pushing body and sometimes the ends of the daughter chromosome arms do not touch at all, which does not support the hypothesis according to which interzonal connections elongate and are the nature of a pushing body action (C a r l s o n 1952). The curving of chromosome arms is also reported by H e i t z (1943) in root tips of different plants and is explained by him as the consequence of pushing body action.

The direction of Brownian movements in the pushing body changes constantly but some rare particles move without oscillations in the same direction as the anaphase chromosomes. This suggests the existence of currents or the wave of submicroscopical changes, i. e. changes in hydration (W a s s e r m a n 1929, cited from S c h r a d e r 1953). However, such particles are very rare and have not been observed in detailed studies on Brownian movements in the mitotic spindle (B a j e r 1953 a). The possibility that there are currents in the spindle was announced long ago by S p e c k (according to S c h r a d e r 1953) and confirmed by S c h a e d e (1929 a, b), S c h n e i d e r (1938, 1939) and some other writers. Lately, P f e i f e r (1952) has called attention to this hypothesis and its significance for the understanding of the mechanism of mitosis. He writes that: ....,,eine Erklärung der Chromosomenbewegung durch Strömungsvorgänge .... aus heuristischen Gründen mehr als andere Deutungen .... beachtenswert" is. However, important arguments have been advanced against such a hypothesis. So far no convincing data proving the existence of currents in the spindle have been advanced and this hypothesis has not been generally accepted.

Although the facts reported by numerous writers support some of the hypotheses of mitosis, it is the opinion of the author that the experimental data on the mechanism of mitosis are at present not sufficient either to prove conclusively any of the now existent hypotheses of mitosis, or to build a new one.

SUMMARY

1. Time lapse cine micrography and phase contrast illumination were used for the studies on mitosis in endosperm of *Haemanthus katharinae*.

A method which makes possible observations from early prophase to resting nuclei is described in detail. Also detailed information is given

on a method of flattening spindles to approximately 7 μ whereas the thickness of chromosomes is approximately 4 μ. Mitosis in flattened spindles was observed from very early prophase to resting nuclei. The methods described are quick, easy and may be used for experimental purposes.

2. The formation of the spindle and changes in chromosome arrangement were observed and it was found that in prophase the chromosomes are at first slowly pushed to the centre of the nucleus and then abruptly compressed. The compression of chromosomes was found to be of general occurrence and it was called the mitotic contraction stage. This stage is very short and lasts 2 — 4 mins, during which the guiding role of kinetochore in chromosome movements and the interaction of chromosomes and the spindle begin. The contraction stage similarly to the of beginning of anaphase is strictly limited and the moment when it begins may be defined exactly. The formation of the spindle, most important in the course of mitosis, ends before the contraction stage begins, though it does not mean that structural changes in the spindle may not occur also after the contraction stage.

3. It has been proved that two forces act in metakinesis: one is directed from the centre of the spindle to the poles causing the straightening of chromosome arms and their movement to the poles, and the other is directed to the equator and causes the movement of the chromosomes to the plate and the formation of the metaphase plate. The first force is also active in metaphase. In some very rare cases a very queer poleward movement of kinetochores has been observed in metakinesis.

4. The observations of metaphases and anaphases in flattened cells show that though the kinetochores have a tendency to arrange themselves in a line (in cells with normal dimensions they form the plate) this is not a necessary condition for anaphase to take place. The arrangement of chromosomes, i. e. the kinetochore orientation, governs the direction of chromosome movement and on it depends whether mitosis will be two- three- or four polar. This indicates that the direction of chromosome movement is the result of how kinetochores are orientated.

5. In some rare cases disturbances resembling meiosis in *Sciara* or some irregular meiosis in plant hybrids were found. It is the author's opinion that these disturbances are caused only by the unusual mechanical conditions in flattened cells. Their significance for the understanding of some problems in mitosis is discussed.

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EXPLANATION OF PLATES

Plate I

Fig. 16 Straightening of chromosome arms during metaphase plate formation, A — several mins after contraction stage, chromosomes are gathered in the middle of the nucleus, some arms begin to move toward the poles. Changes in the arrangement of the arms are visible in D — F. B — 3 mins after A, C — 11 mins after A, D — 47 mins after A, E — 1 hr 7 mins after A, F — 1 hr 70 mins. after A. Cell No 811.

Fig. 17. Movement of a chromosome to the pole, its return to the equator and movement of two sister chromosomes in anaphase. A and B — prophase with nucleolus, the long axis of the nucleus is much shorter than in A. C — several mins. after contraction stage. D — F movement of the chromosome to the pole, H — L and M (Plate II) return of this chromosome to the equator with kinetochore directed toward the equator: note that simultaneously chromosome arms straighten. The chromosome marked with an arrow (C) is probably the same which moves to the pole in the next figures. The graph of the poleward and return movement of the chromosome is given in Fig. 6. N — metaphase. O — early anaphase, note that the length of the spindle is much shorter than in the earlier stages and when it elongates in

anaphase. O — T the movement of small daughter chromosomes in anaphase. U — beginning of cell wall formation. Cell No 840. B — 47 mins. after A, C — 54,5 mins. after A, — D — 1 hr 12 mins. after A, E — 1 hr 17 mins, after A, F — 1 hr 29 mins. after A, G — 1 hr 35 mins. after A, H — 1 hr 37,5 mins. after A, I — 1 hr 41 mins. after A, K — 1 hr 43 mins. after A, L — 1 hr — 46 mins. after A, M — 2 hrs after A, N — 2 hrs 5 mins. after A, O — 3 hrs 51,5 mins. after A, P — 3 hrs 58 mins. after A, R — 4 hrs 24 mins. after A, S — S 4 hrs 32 mins. after A, T — 5 hrs after A, 5 hrs 12 mins. after A. Cell No 840.

Plate II.

Fig. 17. Description in Plate I.
Fig. 18. Prophase and beginning of metakinesis in flattened cell. In A the (at least) double structure of chromosomes and their lumpbrush structure or more exactly their woolly outlines are visible. The structure is less visible in B then in C. The coiling of chromatids one around the other is seen in B and C, nucleolus is visible in A — B and disappears in C, B — 1 hr 50 mins. after A, C — 2 hrs 17 mins, after A
Cell No 895.

Plate III

Fig. 19. Three polar anaphase in flattened cell. A — metakinesis, B — metaphase just before anaphase begins, kinetochores are arranged in a Y shape, C — D anaphase. E — cell wall formation, E — three resting nuclei, one much smaller than the other two. V — 47,5 mins. after A, C — 54 mins. after A, D — 1 hr 9 mins. after A, E — 2 hrs 25 mins. after A, F — 10 hrs after A. Cell No 894.

Plate IV

Fig. 20. Anaphase disturbances resembling irregular meiosis in some plant hybrids. When A, B, and C are compared it can be seen that: 1) daughter chromosomes do not move simultaneously because of the lack of space, 2) different chromosomes move separately and not synchronously, 3) chromosomes no dot move in straight lines, 4) chromosomes may be shoved slightly to one side of the spindle together with their movement mechanisms (long chromosomes in the upper right part of the spindle). The guiding role of kinetochore is discernable. D — single chromosomes scattered in the cytoplasm, cell probably dead. B — 1 hr mins. after A, C — 1 hr 30 mins. after A, D — 10 hrs after A. Cell No 925.

PLATE I

PLATE II

50 μ

PLATE III

PLATE IV