Endosperm – a valuable material for experimental studies of mitosis in vivo

by

A. BAJER

Laboratory of Plant Physiology Jagellonian University, Kraków

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Introduction

One of the greatest difficulties in experimental studies on the physiology of cell division is the lack of suitable material. Plant cytology has no such method as animal tissue culture, a method very suitable for all systematic studies. Plant tissue culture is useless for experimental studies of a cell in vivo. Due to this, though it is possible to observe mitosis in vivo in numerous animals (all the higher ones), it was so far done successfully in several plants only.

For this purpose it is important to find such plant material, which would be more universal, and would make possible the study of cells in vivo of numerous common plants.

It is very probable that endosperm will furnish such material, and it seems strange that so far it was no systematically studied in vivo, though it was mentioned in elder literature (Strasburger-Koernicke 1921 p. 677). Recently only Geitler (1949) mentions endosperm of two plants as a suitable material for this purpose.

Endosperm is found in all angiosperms and two types of its development are known: 1. cell walls are formed in the earliest stages and 2. cell walls are formed in late stages. In the later case endosperm will be often suitable for experimental analysis of cell division.

In the course of the present work the writer experimented with endosperm of *Haemanthus* * the mitosis of which is very distinctly visible. The purpose of this work was to find a method of handling material and to establish whether it is adequate for studies of mitosis in vivo.

Material and methods

Haemanthus Katharinae Bak. from the Botanical Garden of the Jagellonian University was used as material. This species flowers from mid July till mid September. Some weeks after pollination embryo sacs reach a stage appropriate for experimental treatment in vivo (embryo sacs have 5 — 7 mm length). Embryo sacs may be prepared out of the ovary, or the ovary may be opened in its upper part. Then the upper part of the embryo sac is cut off, and the endosperm carefully pressed out together with liquid surrounding it. Embryo sacs containing big embryos are not suitable, as most of the endosperm has formed cells with cellulose walls.

Different mediums and nutrients were experimented with, and observations were done both in a normal hanging drop and in Gautheret's drop (Gautheret 1942).

Cells on clean glass squares die within few minutes. Cytoplasm begins to creep till bare nuclei or mitotic spindles surrounded by a small quantity of coagulated cytoplasm remain. The same results are observed when the glass squares are coverd with a very thin layer of vaseline, paraffine oil, collodion (a method similar to the one used in studies with electron microscope), or surrounded by liquid paraffine. It was found that a sugar (sacharose, or glucose) solution with agar was the only suitable medium. Differences in 1 percentage of sacharose and 0,1 percentage of agar concentrations have a considerable influence on cells and on the normal course of mitosis, which is continued best in 0,5 percentage of agar and 6 — 7 percentage sacharose (3 — 3,5 percentage glucose) concentrations only. In this medium most cells (often more than 95 percent) continued to divide usually even 6 — 8 h after preparation, and cells had a normal apperance during 10 — 14 h.

If Gautheret's drop was applied the following method for mounting material was used. A warm agar solution was spread in a very thin layer on two glass squares (32 × 24 and 22 × 22 mm); with

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* Prof. Dr H. Teleżyński experimented on endosperm of *Haemanthus* in vivo long before World War II, but he did not publish his results till now. The author wishes to express here the most sincere gratitude for his generosity in suggesting endosperm of *Haemanthus* as material, and also for his valuable advice.

a pipette a vaseline ring was made on agar on the larger of the two glass squares (it is better to make the ring on a clean glass square before spreading agar on it). Endosperm was pressed out of the embryo sac direct on the larger glass and covered then with the smaller one. The edges of the smaller glass square were made tight with liquid paraffine. 20 minutes after the larger glass square was mounted on a thin brass plate with special slit.

A normal hanging drop, when used, should be surrounded by a vaseline and should be fairly small (drops for phase microscope should be flat).

Endosperm thus prepared should be left lying quietly for at last 20 minutes to allow cells to affix themselves to agar.

After some practice it is possible to make the agar layers so thin that an immersion lens with an eyepiece 30x may be used without difficulty.

In this work Zeiss phase contrast equipment was used. 90x immersion lens, 20x objective, and eyepieces 5x and 10x respectivly, for microphotos were used. During observations heat rays were eliminated by a 20% Mohr's salt water solution.

Observations.

This paper deals mainly with critical observations on the material. Cells from young endosperm have no cellulose walls, which make many studies on plant cell so difficult. Cells placed on agar begin immediately to change shape and spread out cytoplasmic outpushings. This process is usually slow, but is very marked during division (cf. Plate I, Fig. 1), though in some cases the change of shape is noticeable after several minutes. Outpushings form quickest immediately after preparation and some hours after cells become gradually spherical. The creeping of endosperm is much better observed in young than in old endosperm; in the later case most cells after preparation have a spherical shape.

Endosperm cells of *Haemanthus* are large, young ones have 75—100 μ length and old ones 30—60 μ length. In young endosperm nuclei measure 20—40μ in diameter while in old endosperm they are much smaller.

It is not difficult to distinguish young endosperm from an elder one. In young endosperm chondriosomes look like thin long threads, and in old endosperm numerous starch grains are visible. In some cases these starch grains make the observations difficult.

It is very important to distinguish normal cells from dead cells or cells in demixing („Entmischung" of B ě l a ř 1930). Some of the cells

get injured during preparation and they either coagulate (they can then be distinguished at once) or have a higher refraction index. This is easily noticeable if the central ring of a phase contrast condensor is used or an objective with higher magnification (i. e. objective 20x central ring for objective 40x). The refraction index indicates best the normal or abnormal stage of the cell. There are also 3 other indicatives of the normality of the cell: 1. the appearance of the chondriosomes, 2. observation of Brownian movement, 3. the shape of vacuoles. The change of shape of chondriosomes from thread like may be twofold: either they form loops or small highly refracting balls; often at two poles the walls of these balls are thicker and have a higher refraction index than the rest of the ball. These changes and also the grouping of chondriosomes at the poles of the cell (Plate II, Fig. 4) indicate in most cases that the cell is not in a normal stage .

As cells are large, observations of Brownian movements are possible in the different parts of the cell. In perfectly normal cells vigorous Brownian movements were observed in the pushing body („Stemmkörper" of B ě l a ř 1929a) only. The intensity of Brownian movement indicates the degree in which the cytoplasm has a structure. In demixed cells the Brownian movement is very vigorous and cytoplasm is liquefied. The destroying of cytoplasm structure and liquefaction of cytoplasm begins at the edges of the cell and spreads to the nucleus.

In quite normal cells vacuoles are very small, or there are none. In cells in demixing vacuoles are often present (Plate II, Fig. 7).

It is necessary to stress however that the best sign of demixing is the refraction index, and the others described are not always reliable.

Cells in prophase with marked demixing have often the nuclear membrane removed from the nucleus which is not ball shaped but folded (Plate II, Fig. 5).

Reversible demixing such as observed by B ě l a ř (1930) in consequence of mechanical stimuli is seldom observed (demixing is usually irreversible) and found more often in normal hanging drop than in G a u t h e r e t's one. This may indicate that oxygen is neccessary for reversible demixing.

In normal cells the course of cell division may be traced in detail. In G a u t h e r e t's hanging drop most of the cells die in prophase, in the normal hanging drop the percentage of cells which enter metaphase is much higher. This is probably due to the lack of oxygen the presence of which, according to the B o l l o u g h's (1950) observations made on animal material, is necessary in prophase. In G a u t h e r e t's hanging

drop it also was noted that some cells in prophase retreat to resting nuclei (Plate II, Fig. 8). Normal anaphases are found also in G a u t h e r e t ' s drop often even 12 h after preparation (if the cell is not constantly lighted), and as metaphase is short it is probable that light or some other unknown factors have harmful influence on prophase. In spite of what was said above mitosis from prophase to resting nuclei was observed in numerous cells.

Beginning from metakinesis cells may be observed without difficulty. Chromosomes in prophase are very plainly visible as well as in the further stages. A nuclear membrane is well visible in the earlier stages while in the later ones, a membrane such as was found by W a d a (1950) in staminal hairs of *Tradescantia* and in *Osmunda* (W a d a 1941) and such as probably envelopes the mitotic spindle in *Hymenophyllum* (B a j e r and M o l è - B a j e r 1952), cannot be seen in *Haemanthus*. Observations on Brownian movement (B a j e r 1952; particles invade the mitotic spindle from cytoplasm) seem to indicate that such a membrane does not exist.

Before metakinesis a similar stage as in *Hymenophyllum* was observed: chromosomes are crowded in the center of the spindle (Plate II, Fig. 6). It seems probable that in this stage kinetochores begin their activity (possibly they affix themselves to the spindle fibers, or begin to form them, or act in some other way). After this stage metakinesis, the mechanism of which consists in fiber action, begins and thus confirms the above hypothesis.

Metakinesis and metaphase last 1—2 h, and similarly as in other cases it is here difficult to establish strictly the end and the beginning of these two stages. The kinetochores are placed in one plane during not more than 1/2 h. In some cells in metaphase persisting nucleoli were found in the plate or at the spindle poles. Anaphase lasts 25—50 mins. and often the observations of two sister kinetochores (a kinetochore has the appearance of a cup with a small swelling on one edge cf. Plate III, Fig. 9d) is possible from the very beginning till late anaphase.

In most young endosperm cells (also in old ones but in a lesser degree, than in young ones) considerable action of pushing body („Stemmkörper" of B ě l a ř 1929a) is noticeable and the whole cell elongates (Plate III, Fig. 9).

Telophase is short — it lasts less than 2 hours and the formation of resting nuclei may be observed in detail. The formation of cell wall is very interesting. It begins to form from the edges of a usually not fully developed phragmoplast (it forms a ring which grows towards its center). However normal cell wall formation was also observed in few cases.

Discussion

In my published and unpublished studies I have observed so far mitosis in vivo in following plants: staminal hairs and petals of *Tradescantia virginica* and related species *(Rhoeo discolor, Tinantia fugax)*, young leaves of some grasses (mitosis without demixing is here difficult to observe), leaves of water plants: *Vallisneria* and *Elodea* and leaves of fern *Hymenophyllum*. From all these plants the course of mitosis is best visible in *Hymenophyllum* and may be observed under the microscope even during 2 days in the same leaf. Contrary to all other material demixing is very seldom observed in this plant. But *Hymenophyllum* is not suitable for systematic physiological studies in vivo, as it has a very thick cuticule layer. From all mentioned plants staminal hairs of *Tradescantia* seem to be the most suitable for such studies (cf. W a d a 1940 — colchicine action, M o l è - B a j e r 1951 — K and Ca ions action), though it cannot be compared in this respect to material without cellulose walls such as animal cells.

The endosperm of *Haemanthus* and probably of numerous other plants has important advantages, as it has no cellulose cell walls, which make easier studies both micrurgical as in polarised light. Cellulose walls cannot influence here the length of the chromosome way in anaphase, which length may be experimentally considerably prolonged in some plants by shock high temperature action (B a j e r and M o l è - B a j e r 1952). The penetration by different substances meets the same difficulty, i. e. a semipermeable membrane, as in animal cells in vitro. In endosperm of *Haemanthus* plasmolysis begins immediately while in stanimal hairs of *Tradescantia* it does not begin till several mins. after hypertonic medium is applied (B ě l a ř 1929b). In *Hymenophyllum* even 2 mol sacharose solution during 6 h does not cause visible effect in this respect.

The course of cell division is here very well visible and this is probably one of the best of all plant and animal material observed so far. Mitosis in endosperm of *Haemanthus* resembles mitosis in animals because of the pushing body action. I found this the first fully convincing proof of pushing body action in normal plant mitosis.

The main deffect of *Haemanthus* endosperm is that the normal course of mitosis can be observed only during a short time after preparation (usually 6h) and that most cells die in prophases (which is not sufficiently explained); though even this difficulty will probably be surpassed. If this is achieved endosperm will be probably the best plant material for systematic studies of physiological problems of mitosis similiar, to those carried

out on animal tissue culture by H u g h e s (1950, 1951, 1952). Such systematic studies are according to S c h r a d e r (1951) very important for the full understanding of the mechanism of mitosis.

SUMMARY

Endosperm of *Haemanthus Katharinae Bak.* was used for studies in vivo. Cell division is continued only in one of all the mediums used, i .e. in agar and sugar water solution. Methods are described in detail.

The course of normal division from metakinesis to resting nuclei may be observed without difficulty and very plainly (i. e. the movement of particular chromosome may be traced without difficulty). Action of pushing body is very distinct from very early or middle stages of anaphase and the whole cell elongates. New cell walls begin usually to form not in the centre but at the edges of a not properly developed phragmoplast.

As these cells have no cellulose walls, which are usually covered with a cutin layer, endosperm is probably a very suitable material for physiological studies of mitosis in plants.

Though difficulties in such studies on endosperm (i. e. numerous cells are in demixing) are serious at present, endosperm is suggested as a material for experimental studies in vivo on a large scale.

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EXPLANATION OF PLATES

Magnifications of all Figs. approximately 800 × except Figs. 5, 6 which are approximately magnified 260 ×. Phase contract microscope. All cells except in Figs. 5, 6 are in Gauthere t's hangnig drop.

Plate I

Fig. 1a—d. Early metakinesis and telophase in the same cell. Note the elongation of the cell and pseudopodia-like outpushings. (b) 46 mins. after (a), (c) 1h and 09 mins. after (a), (d) 3h and 57 mins. after (a).

Fig. 2. Cells with resting nuclei approximately 8h after preparation. Amoeba-like pseudopodia scarce.

Plate II

Fig. 3. Cells in prophase 30 mins. after preparation.

Fig. 4. Cell in demixing. Chondriosomes having the shape of strong refracting balls are on two poles of the cell only (cf. text). In cytoplasm no structure is visible.

Fig. 5. Cell in demixing. Nuclear membrane and the chromosomes do not touch.

Fig. 6. Stage after which metakinesis begins; chromosomes crowded in the center of the spindle (cf. text). Long chondriosomes in the cytoplasm.

Fig. 7. Cell in demixing. Changed chondriosomes, large vacuoles, structurless cytoplasm.

Fig. 8. Return of cell from prophase to resting nucleus: (b) 10h 32 mins. after (a).

Plate III

Fig. 9,a-m. Cell from not very young endosperm. On the poles of the cell starch grains. Action of pushing body — whole cell elongates, a—b-metaphase; b — note the square shape chromosome optical cross sections. d — two sister chromosomes are indicated by arrow; kinetochores — especially in the upper chromosome — are visible, h — begining of phragmoplast formation, cell returns to previous length i—k — formation of cell wall. (b) 17 mins. after (a), (c) 27 mins. after (a), (d) 30 mins. after (a), (e) 39 mins. after (a), (f) 54 mins. after (a), (g) 1h 04 mins after (a), (h) 1h 17 mins. fater (a), (i) 1 h 32 mins. after (a), (k) 1h 40 mins. after (a), (l) 2h 36 mins. after (a), (m) 9h after (a).

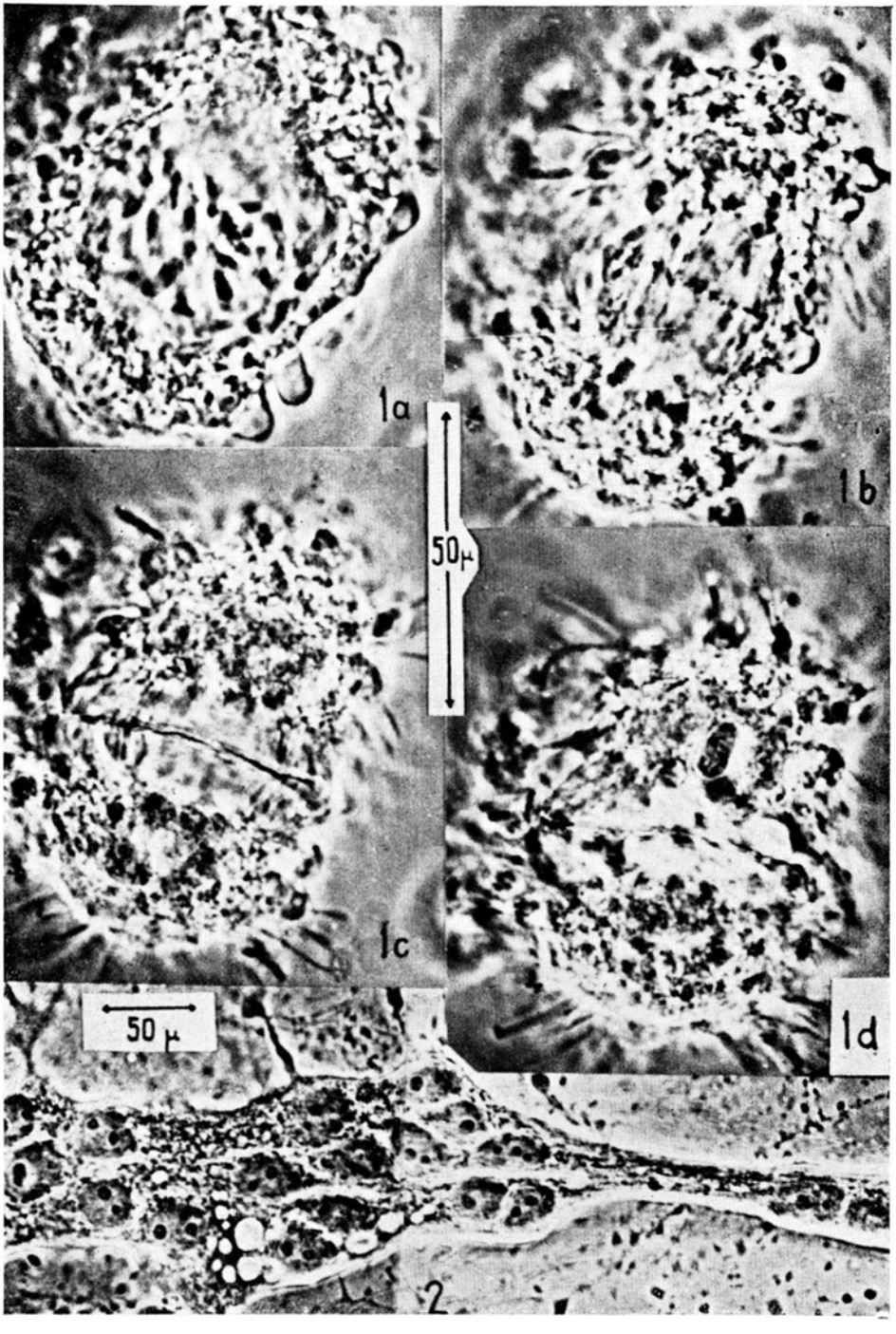

Plate I

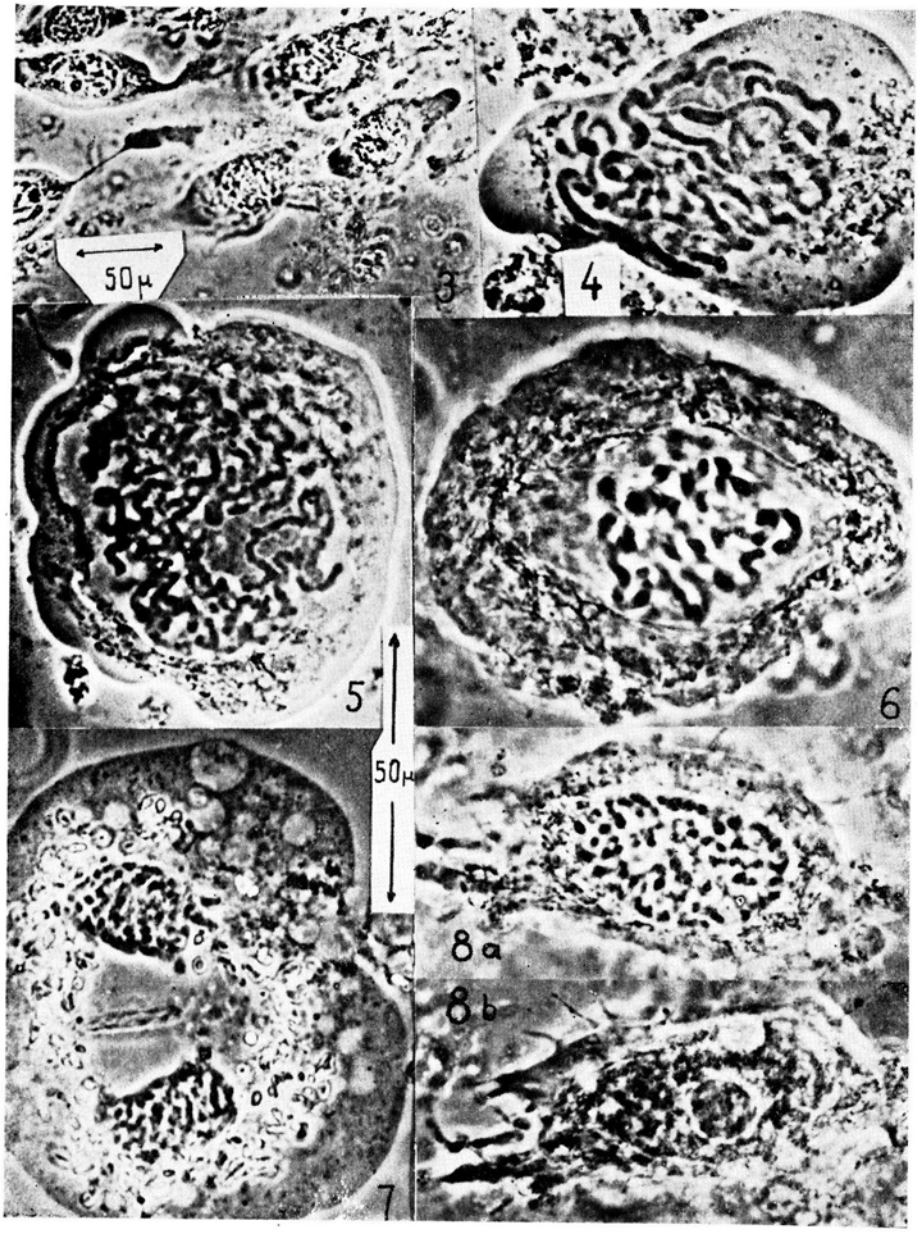

Plate II

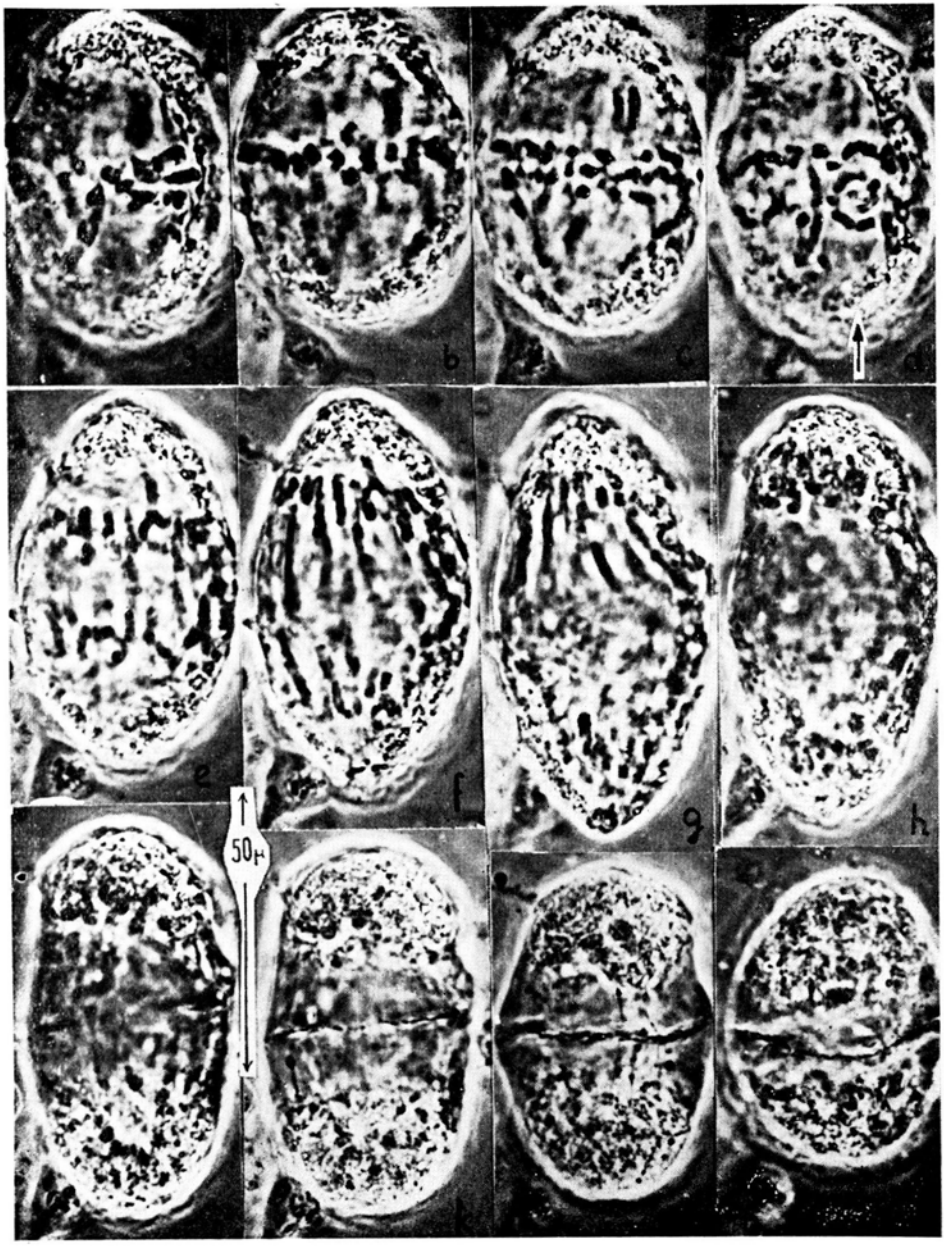

Plate III