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Experimental analysis of the mechanism of cytomixis

1. Cytomixis in vegetative tissues

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INTRODUCTION

In the years 1958—1960 the author has studied the phenomenon of cytomixis (the displacement of nuclei or chromatin into the neighbouring cell through the cell wall) in the epidermis of onion scales and leaves and in the onion root meristem (*Allium cepa*). Results of these studies were published in 1960 (Tarkowska 1960). In the following years further studies were conducted on the experimental induction of cytomixis in order to establish the nature and direct causes of this curious phenomenon.

The phenomenon of cytomixis was studied in vegetative tissues and in pollen mother cells. The results concerning vegetative tissues are the subject of this paper. Data dealing with the pollen mother cells will be the subject of a separate paper in which the whole problem will be discussed and general conclusions will be presented.

MATERIALS AND METHODS

Leaf epidermis from *Ophiopogon* spp. and *Reineckea carnea* KTH (syn. *Liriope carnea* Salisb.) and staminal hairs of *Tradescantia* spp. where used as experimental material. For some studies also the root tips of onion (*Allium cepa* L.) were used.

In normal healthy plant tissues cytomixis is not to be found. Also it does not occur in normal, carefully fixed, undamaged material. However cytomictic patterns can readily be observed, and often have been observed, in material damaged by cutting, pressing, squashing etc.

On the basis of information from literature and from personal experience (Tarkowska 1960) it was assumed that the factors causing cytomixis are primarily injuries, and in particular mechanical injuries. Thus in this work the influence of mechanical factors was studied. The applied treatments had as an objective the induction of cytomixis in the experimental material, and the establishment whether and under what conditions a given treatment has an effect. Individual experiments were made on selected tissues which have been indicated in the description of the individual experiments. It was also checked

whether cytomixis can be induced by such factors as centrifugation, temperature shocks, and fixatives.

The epidermis of *Ophiopogon* and *Reineckea* leaves and the staminal hairs of *Tradescantia* were studied both living and fixed, whereas the root tips of onion only in the fixed condition.

The following treatments were applied:

1. The epidermis of *Ophiopogon* and *Reineckea* leaves were peeled off.
2. All the studied tissues (*Ophiopogon*, *Reineckea*, *Tradescantia*, *Allium*) were pressed or squashed. With *Ophiopogon*, *Reineckea* and *Tradescantia* also a micromanipulator was used.
3. The tissues of *Ophiopogon*, *Reineckea*, and *Allium* were stabbed with a sharp and with a blunt needle.
4. The tissues of *Ophiopogon*, *Reineckea* and *Allium* were cut with a sharp and with a blunt razor blade.
5. *Ophiopogon* and *Allium* were centrifuged.
6. Sudden temperature changes (temperature shocks) were applied to *Ophiopogon* and *Allium*.
7. The effect of a fixative was studied on *Ophiopogon*, *Reineckea* and *Allium*.

After applying the appropriate treatment the injured tissues of *Ophiopogon* and *Reineckea* were fixed in acetic-alcohol (1:3) or in a modified Navashin fixative (CrAF 0.5—1—20), then the epidermis was peeled off or cut off, stained with aceto-carmine or aceto-orcein (after acetic-alcohol), or with Heindenhain's iron hematoxyline, Ehrlich's hematoxyline or Feulgen reaction.

In order to expose the cytomictic patterns in the epidermal cells counter staining was employed, using a neutral red at pH 4 followed by a light green stains. In order to expose the plasmodesmata in these cells Meyer's pyoctanine staining method with Jungers (1930) modification was employed or when necessary the methods of Lambertz (1954) and Meeuse (1957) were used.

The injured onion root tips, 8—10 mm long, were fixed in the fixatives mentioned above, microtome sections, 7.5 μ and 10 μ, were taken and these were stained with iron hematoxyline, gentian violet and by the Feulgen method. The cytoplasm was slightly stained with light green. The mechanical injuries to onion root tips were made in the fixative (with the tip immersed) or in the air. The injured material was fixed immediately or after 5 min from the time of treatment.

In order to establish the influence of the fixative on the induction of cytomixis, control material was fixed in CrAF 0.5—1—20 and in acetic-alcohol 1:3. This last fixative was most suspected of causing cytomixis.

The behaviour of the nuclei in the cytomictic process was studied in the preparations stained in gentian violet or iron hematoxyline after a very strong differentiation, and also in anoptral contrast after the

Feulgen treatment. The observed results were checked using a method of selective staining of the nucleoli suggested by Semmens and Bhaduri (1941).

The viability of cells participating in the cytomictic process was studied by the same methods as used for the epidermis of onion scales (Tarkowska 1960).

The chemical composition of the cell walls was studied according to the generally applied methods of staining and microreactions. The observations were also made under polarized light.

For each tissue control material was also analysed. After a very delicate and carefull preparation it was fixed, dehydrated, cut and stained in the same way as the material that was subjected to the various mechanical treatments.

RESULTS

1. The peeling off of the epidermis of *Ophiopogon* and *Reineckea* leaves

The thick-walled epidermal cells of *Ophiopogon* and *Reineckea* leaves, with their numerous simple pit-pairs and very delicate pit-closing membranes constitute exceptionally good material for the study of cytomixis. These cells, measuring 100—190 μ in length and 20—26 μ in width, have pectin-cellulose cell walls with a predominance of pectic substances. Numerous pits (10—18 in a single cross-section), 1,5—7 μ in diameter, occur primarily in the longitudinal cell walls, perpendicular to the leaf surface (Plate I, photo. 1). The very delicate and elastic pit-closing membranes are supplied with numerous pores. Photograph 2, on Plate I shows pits seen from above. The epidermal cells are fixed firmly to the 2—4 layers of subepidermal cells which have thick walls with a predominance of cellulose (Plate I, photo 3). The epidermis can be easily removed with forceps. A rapid peeling off of the epidermis causes the formation of very numerous cytomictic patterns, very suitable for the analysis of this phenomenon.

In the study a special notice was taken of the viability of cells taking part in cytomixis. Information on the subject from literature is contradictory. Miehe (1901), Gavaudan (1936) and Tarkowska (1960) have described them as dead or dying cells; Su-Süan (1955a, b) on the other hand believes them to be alive, and completely normal. Using phase contrast and a dark field, plasmolysing and staining with neutral red and methylene blue it was found that the cells from which the nucleus extrudes show all indications of dying or death. The cells into which the nucleus intrudes are usually dead, or in the rare cases where they are still alive there are definite indications of degeneration. During the peeling off of the epidermis from *Ophiopogon* and *Reineckea*

leaves, most of the cells (80—90%) get killed, or seriously damaged, and among these cells there are numerous cytomictic patterns to be observed. In the living cells occuring singly, or in small groups, no cytomixis was found.

The amount of nuclear substance that becomes displaced is variable. Also the shape of the nucleus fragment that has passed through the cell wall can be variable. The displaced parts of the nucleus can have a regular shape of a "bud" (Plate II, photo 4), or they can form multishaped, often very interesting cytomictic patterns (Plate II, photos 5, 6 and 7). From these photographs it can be seen that chromatin can extend into long "threads" often passing through two, three or more cells. The chromatin threads from different nuclei can link up and a three dimentional tangle results. The microscopic picture of these patterns, on the counter stained preparations (cell walls red, nuclei green) is unusually impressive.

The direction of nuclei migration in the cells of a peeled off epidermis does not demonstrate any definite regularity. This no doubt is associated, among other things, with the structure of the cell wall. Its considerable thickness, numerous, deep and wide pits, and firm attachment to the thick-walled subepidermal cells, provides, on the rapid peeling off of the epidermis at a sharp angle to the leaf surface, considerable opportunities for the multidirectional action of sucking and pressing forces.

The interesting problem in the cytomictic phenomenon is the pathway of nuclear migration, the place in the cell wall through which the chromatin passes. There is no doubt that the site of chromatin transgression is to be found generally speaking in the pit-closing membranes. Using Meyer's method as described by Jungers (1930), and the methods used by Lambertz (1954) and Meeuse (1957) for the demonstration of plasmodesmata, numerous protoplasmic strands can be shown to be traversing the pit-closing membrane. (Plate II, photo 8). The microscopic pictures obtained permit the conclusion that chromatin migration occurs both through the whole pits (Plate II, photos 4—7) in which the pit closing membrane has been destroyed or through smaller perforations in the membrane caused by the injury. On the basis of some of the pictures it can be assumed that the migration of chromatin takes place through single pores in the pit membrane (Plate II, photos 5, 6).

2. Pressing or squashing

In an earlier paper (Tarkowska 1960) the cytomixis was described in the meristem of onion root apex, obtained by the squashing of root tips between two glass slides. This method of squashing was also employed with success for leaves of *Ophiopogon* and *Reineckea*.

A leaf fragment was placed delicately between two glass slides and pressed firmly, but in such a fashion as to obtain as far as possible an even distribution of pressing forces on the whole surface of the leaf. Then the leaf was fixed and the epidermis was removed delicately by cutting. On preparations made in these fashion cytomixis is not observed at all or only very rarely. The nuclei become homogenized, often elongate and thread-like, sometimes displaced towards the cell wall, but they do not pass through the pit-closing membranes.

Very different results were obtained when pressure was applied only to some, random regions of a living leaf. The treatment can be applied e.g. with a very blunt needle, without piercing the epidermis. The pressed regions can have any shape depending on the instrument with which pressure is applied e.g. a rod, or a ring etc. After the treatment and fixation of the material the epidermis was removed. The microscopic picture of the damaged part of the leaf is as follows: in the region where pressure was applied numerous migrating nuclei can be seen: "buds" and "threads", followed by homogenized but not migrating nuclei and further still perfectly normal nuclei.

Also interesting for the study are the staminal hairs of *Tradescantia*. Cytomictic patterns were readily obtained by squashing between a glass slide and a cover slip the young pubescent stamens followed by immediate fixing and later staining. It is also possible to obtain cytomixis by squashing straight in aceto-carmine or aceto-orcein, but it has to be done quickly in order to induce cytomixic before the fixation of the cells.

The migration of nuclei was observed both from one cell to the next (Plate III, photos 9—12) and through the outside cell wall of the staminal hair (Plate III, photo 12). The displaced nuclei, with their characteristic distribution of chromatin appear the same in both cases. In plate III, photo 11 an apical cell of a staminal hair is seen, devoid of a nucleus, whereas the cell below it has two nuclei. The upper nucleus, smaller, with the chromatin more dense, and staining intensively, was displaced from the upper cell. This is a clear demonstration of the fact, that the whole nucleus can migrate into an adjoining cell.

Pits in the cell wall of the staminal hairs have not been observed and it is difficult in direct observation to establish the pathway of the nucleus migration across the cell wall. Analysing the obtained microscopic pictures and the electron micrograph (included with the paper of Roelofsen and Houwink 1951) of a transverse cell wall of a *Tradescantia* staminal hair, it appears that the migration of chromatin takes place not only through the individual pores of plasmodesmata but also through larger perforations developing in the process of chromatin migration through the cell wall.

Very interesting data on cytomixis can be obtained using a micromanipulator for the treating of living cells of *Ophiopogon* and *Reineckea* leaf epidermis, and staminal hairs of *Tradescantia*. For the purpose a Zeiss sliding Péterfi type of micromanipulator was used. A glass needle was so constructed, that it was possible with its help to press individual cells without piercing them.

The pressed cell behaves like an elastic bubble, completely filled with a liquid. When the pressure at one end of the cell is increased (in a staminal hair of *Tradescantia*), the protoplasm together with the nucleus moves towards the other end, which becomes slightly swollen.

When the pressure is applied suddenly (a "hit") it is possible to cause the migration of a part of the protoplasm or even the nucleus, through the inner cell wall into the neighbouring cell of the staminal hair. The nucleus behaves, while it is still alive, as a very plastic and elastic body. However when the typical postmortal structures appear in the nucleus it becomes non plastic and very faintly elastic. Then it very easily brakes and falls into fragments. In the same way, by a sudden pressure, a "hit", it is possible to force the nucleus through the outside cell wall (Plate III, photo 12). This pattern results more rarely, however with sufficient experience it is possible to induce it at will. The appearance of the nucleus that has passed through the inner or through the ouside cell wall is the same. In both cases also some cytoplasm oozes out. The site of migration (perforation) is often very small. and it is not always possible to observe through a microscope. After such a treatment, the cell from which the nucleus has escaped always dies.

Treatment with the micromanipulator coupled with simultaneous observation is more easy when dealing with the epidermal cells of *Ophiopogon* and *Reineckea*. Thick cell walls of these cells are supplied with numerous simple it pairs. The pit-closing membranes are thin and very elastic but delicate and not durable. With the slightest pressure applied to any part of the cell the pit closing membranes react with immediate bulging. A sudden pressure results in a sudden bulging of the pit closing membranes in the direction of the neighbouring cells that are not under pressure. This indicates that artificially the pressure within the treated cell is increased and pressure difference results between this cell and its neighbours.

In this paper by inner cell pressure the author understands this pressure which, regardless of its origin, the protoplasm exerts on the cell wall.

A sudden release of the applied pressure causes an immediate bulging of every pit-closing membrane in an opposite direction. This indicated the elasticity of the cell walls and the nature of the operating forces. When pressure is applied to a cell direct pressing forces are acting.

and when the applied pressure is released, the suction forces come into operation. A greater sudden pressure, a "hit", and the keeping of the needle in such a position causes a desorganisation of the protoplasm and its migration through some of the pit-closing membranes into adjoining cells. Often it is possible to observe the whole pit-closing membrane falling out, however more often a stream of flowing cytoplasm is seen with the perforation remaining invisible. The flow of protoplasm takes place in the same fashion as when a dense liquid is flowing out under pressure through a small hole in a vessel.

From the pressed cell usually it is water that oozes out first, followed by cell contents nearest to the perforation. This water causes in the naighbouring cell a displacement of the protoplasm from the cell wall, very much like in plasmolysis, and a watery bright zone results in the cell. Together with the water flow a part of the protoplasm, including the nucleus or parts of its chromatin, can migrate, and remain surrounded with a watery zone.

The behaviour of the nucleus is interesting. It is moved together with the cytoplasm and plastids towards the perforation. If the perforation has developed close to the nucleus, then it falls into the perforation quickly and only a small amount of cytoplasm migrates before it. This migration is entirely passive, and its direction depends entirely on the position where pressure is applied and on the position where a perforation has developed. Through a small opening in the pit closing membrane the nucleus passes surprisingly easily. It is possible, without releasing the pressure to cause the displacement of the whole nucleus to the neighbouring cell. Having somewhat released the pressure it is possible to suck back the nucleus into the cell from which it has already left. This treatment however has to be performed rather quickly, because the migration of the nucleus is coupled with its degeneration. The whole operation, from the time the nucleus is first moved from its location in the protoplasm to the time of its passing through the perforation in a pit membrane has to be performed within about 15 seconds. During that time the nucleus is still very plastic and elastic, and it is then that various cytomictic patterns can develop. A nucleus, which has died while in the perforation of the pit membrane cannot be further moved, or brought back, because it very easily breakes up.

In plasmolysed cells, that is without turgor pressure, it is difficult to induce cytomixis. A pressure applied by the needle results primarily in the movement of the protoplasm towards the cell wall, and only later in its flow towards the perforations in the pit-closing membrane. Thus it is hardly surprising that in the strongly plasmolysed and squeezed root tissues cytomixis occurs only sporadically (T a r k o w s k a 1960).

The experiments conducted with the micromanipulator are instructive for many reasons. They show primarily that the phenomenon known as cytomixis can occur between cells with a large pressure difference. The pressure difference has to be such that some points on the cell wall do not sustain the strain and perforations develop, through which an equalisation of the pressure: results or a type of plasmoptysis. A pressure difference between the cells, and the character of the cell wall have a deciding influence here. This pressure difference can be caused both by a direct force, during pressing or squashing, or by a suction force acting during the return of the cells, particularily thick-walled cells, to their original state, or during the stretching of the cell walls effected when the epidermis is peeled off.

The first experiments releasing cytomixis in the epidermis of onion scales by its rapid peeling off, and in the root by its squashing (T a r k o w s k a 1960) have permitted the formulation of a hypothesis, that the phenomenon of cytomixis is caused by a difference in pressures between adjoining cells. The experiments with the help of a micromanipulator on the epidermis of *Ophiopogon* and *Reineckea* leaves, which is a particularily suitable material for the study of cytomixis, and on the staminal hairs of *Tradescantia*, have completely confirmed this hypothesis.

In the cells of epidermis peeled off with pincers or pressed, in the cells of squashed root, or in the mangled cells of staminal hairs, cytomixis was the result of the creation of sudden and considerable pressure differences between adjoining cells.

The experiments with the help of the micromanipulator have also shown that not every pressure difference will induce cytomixis. It has to be such a pressure that will injure the cells so as as to provide pathways for the equalisation of the pressure differences. These pathways are perforations, or groups of them, in the pit-closing membranes between the two cells in which the pressure difference has developed.

Thus it is to be expected that stimuli, which injure the tissue, but do not cause sudden and large pressure differences between neighbouring cells, will not induce cytomixis.

To check this suggestion and to confirm the above conclusions, further experiments were conducted on the epidermis of *Ophiopogon* and *Reineckea* leaves, and on the root tips of *Allium cepa*, consisting of stabbing and cutting of the tissues with sharp and blunt blades and needles. The experiments were always made on living material. After the treatment the material was fixed, epidermis was removed (*Ophiopogon* and *Reineckea*) or microtome sections were cut (onion root tips), and observations were made.

3. Stabbing with a sharp and a blunt needle

For the treatment usually a very fine and sharp glass needle was used, enabling the application of injury to a few cells only. The treatments were done very carefully, strictly in the direction of the needle length, and avoiding as far as possible the application of further pressures which could cause a strain and a pressure excess in the neighbouring cells. The obtained pictures are presented in plate IV, photo 13 for the leaf of *Ophiopogon* and on photos 14 and 15 for the onion root tip. The wounding caused by a very sharp and thin needle was not associated with cytomixis, even when there were several stabs of the needle close to each other (Plate IV, photo 15). On the presented photographs it can be readily seen that in the rows of cells lying next to the wounds, the nuclei have not even changed their position. The sporadically occuring migrating nuclei close to the wound, indicate that even in such a carefully conducted experiment, with this type of injury it is not possible to avoid the creation of greater pressure differences between adjoining cells.

When the needle used for the treatment was not sufficiently sharp then the cells surrounding the wound, as a result of the induced pressure, exhibit numerous cytomictic patterns. The blunter was the needle, the more damaged were the cells (shapeless mass), and the more cytomictic patterns it was possible to observe. Photo 16, Plate IV, shows fragments of a root tip stabbed with a needle whose point was deliberately blunted. The wound here is large, and the cytomictic figures are numerous.

Analogous experiments conducted with a blunt needle on the leaf epidermis of *Ophiopogon* and *Reineckea* have produced similar results.

From the above experiment it may be concluded that when operating with a very sharp and thin needle, a sudden piercing of the cells does not cause cytomixis. The stimulus acts too quickly and over a very small area. It does not induce sufficiently large pressure differences between neighbouring cells. The pierced cells have lost their turgor (a pressure resulting from osmosis), and a sudden pressure difference has developed between the living cells and the dead ones, however the turgor of the cells still alive in the studied material did not exceed the durability of the cell walls. It did not cause plasmoptysis, that is the expulsion of a portion of the protoplasm outside the cell. When a more or less blunt needle meets a greater resistance of the cell walls it causes mechanically an increase in pressure in some cells, which results in direct forcing of the contents towards adjoining cells not under pressure. When the tissue is eventually pierced the state of tension is suddenly released, and the elasticity of the cell walls, particularily in *Ophiopogon* and *Reineckea*, results in the formation of sucking forces operating in

an opposite direction. Both during the time of increased tension and at the time of its release, cell nuclei can be displaced across the cell wall.

4. Cutting with a sharp and with a blunt blade

For the experiments a pointed fragment of a very thin and sharp razor blade was used. Along a cut formed by the blade across the surface of an *Ophiopogon* leaf or of an onion root tip (Plate V, photo 17) it is either not possible to observe any cytomictic patterns or they occur very sporadically only.

A blunt blade causes during cutting a considerable pressure, or even the mashing of larger groups of cells. Such a treatment is accompanied by numerous cytomictic patterns, similarily as in the case of blunt needles.

When operating with a sharp and thin blade the results are analogous as when stabbing with a sharp thin needle, since the nature and the mode of action of the factor inflicting the injury are similar.

Results of the conducted experiments have confirmed the suggestion that a sufficient but necessary condition for the induction of cytomixis is the creation of a sudden but considerable pressure difference between neighbouring cells. The treatments which have induced large pressure differences (pressing, squashing, stabbing with a blunt needle, cutting with a blunt blade) have at the same time caused a state of tension at the cell walls, their injury at the weakest points and the flowing over of protoplasm leading towards an equalisation of the pressures. Thus only these types of treatments need to be taken under consideration when analysing the phenomenon of cytomixis.

Accepting the hypothesis that cytomixis is the result of a sudden equalisation of the pressure differences existing between neighbouring cells, one would expect that in a tissue whose turgor has been lowered, the number of migrating nuclei after injuring the tissue should be relatively less. In order to reduce the turgor pressure of onion root tips they were subjected to the influence of 20% or 40% sucrose for a period of 4 hours, after which the root tips were squashed and fixed. On the microtome sections of root tips subjected to the influence of 20% sucrose it was possible to observe a definite plasmolysis and a visible reduction in the number of cytomictic patterns. After 40% sucrose the cells were very strongly plasmolysed (concave plasmolysis), and migrating nuclei were found only very sporadically. The induction of cytomixis in such cells requires the action of greater forces than in cells with a normal turgor, which was confirmed on numerous occasions when operating with a micromanipulator on the epidermal cells of *Ophiopogon* leaves.

5. Centrifugation

Among the factors inducing cytomixis centrifugation has also been mentioned. Miehe (1901) and Jungers (1934) have centrifuged onion scales and epidermis from onion scales for periods from 30 min. to 1 hr. with a relative centrifugal force from 357 g to 3300 g, and they have not observed the migration of chromatin or whole nuclei across the cell wall. Miehe has sometimes seen nuclei with small bulges beyond the cell wall but he has not considered them of much importance.

However Andrews (1915) when centrifuging, staminal hairs of *Tradescantia* for 2 hours with a relative centrifugal force of about 1107 g has observed the existence of two or even three nuclei in some cells with the adjoining cells having no nuclei. However, the author has interpreted these observations as the result of the damaging of the material during the preparation of the material or during the centrifugation itself.

In the experiment conducted by the author lower portions of the leaves of *Ophiopogon* and *Reineckea* were taken, because in these tissues cytomixis is easiest to induce, and also the onion root tips. Durations of 15 min. and of 1 hr were used and the relative centrifugal forces of 1206 g and 4824 g. The centrifugation has caused the deformation of cell nuclei. They always became elongated and were always displaced in one direction, towards the centrifugal parts of the cell wall. When the centrifugal force was greater or the centrifugation time longer the deformations and displacements of the nuclei were more obvious. The nuclei were usually appressed to the cell wall or even pressed into the pits but only as far as the pit-closing membrane. Cytomictic patterns did not result, not counting the single, sporadically occuring, small "buds" appearing on the other sides of the cell wall. They could have developed during the preparation of the material for the experiment.

The negative result of this experiment was not surprising, since Beams and King (1935) when studying the roots of bean (*Phaseolus vulgaris*), even with the help of an ultracentrifuge (about 400,000 g for 15—20 min.) have not noticed the appearance of cytomictic patterns.

6. The effect of temperature

The following tests were tried. Cut root tips of onion, about 2 cm long, were transferred from water at room temperature to water at +2°C for 10 min. and others from the same culture also for 10 min. to water at +35°C. In two further tests a double temperature shock was applied. From room temperature the root tips were transferred to +2° for 10 min. and then directly into water at +35°C also for 10 min.

Other root tips were first placed into water at +35° and then into +2°C. All the above tests were repeated using shocks of 20 min. duration. Then the roots were fixed and analysed in squash and microtome preparations. On none of the samples were any cytomictic patterns observed.

A second series of tests was tried on growing onion roots. The roots cultured at room temperature were dipped into water at +35°C for 10 min., and then for another 10 min. into water at +2°C. Other onions cultured at room temperature were transferred for 10 min. into water at +35°C, then into water at +2°C for another 10 min., and finally back into water at +35°C for a further 10 min. After these treatments the onions were transferred to room temperature for 48 hrs. allowing the roots to grow. They were cut, fixed, and squash or microtome preparations were made. On these preparations apart of changes in the mitotic figures, characteristic for temperature shocks (the fusion of chromosomes, bridges, etc.) no cytomictic patterns were observed.

Similar treatment were applied to lower parts of *Ophiopogon* and *Reineckea* leaves. Various temperatures from 2°C to 40°C were tried for durations ranging from several seconds to 20 min. The temperature treatment was applied to the whole length of the leaf or to some sections of it only, expecting that cytomixis may occur at the junction of leaf parts subjected to different temperatures. These boundries of zones subjected to different temperatures were also studied in the root tips of onion, in the belief that the expansion of gases and liquids, or the possible increase in the rate of water uptake by hydrophilous colloids, under the influence of temperature, may cause an increase in the volume of the protoplasm and as a result a difference in pressures between neighbouring cells, which in turn could induce cytomixis.

Under none of the conditions described above were cytomictic patterns ever observed.

7. The influence of fixatives

In the literature there are references to fixatives as possible factors causing cytomixis (Woodworth 1929, 1931, Sparrow and Hammond 1947, Bopp-Hassenkamp 1959, Takats 1959). The greatest activity in this respect was reported (Takats) for a mixture of glacial acetic acid and 95% alcohol in the ratio 1:3. The reports of the above listed authors are based on the assumption that not all constituents of a fixative enter a cell at the same speed. Methanol and ethanol belong to compounds entering the cells particularily quickly. Such compounds, entering some cells earlier than others may cause their swelling or even plasmoptysis.

In order to check the above mentioned data, the following experiments were conducted. Control material of all the examined tissues was

prepared very carefully, fixed in the CrAF mixture, 0.5—1—20, a fixative that has been shown not to cause any disturbances, and in acetic alcohol, 1:3, the fixative most suspected of inducing cytomictic patterns.

In none of the preparations were any cytomictic patterns observed.

Also immediately after causing mechanical injuries that induce cytomixis the tissues were fixed in the two fixatives mentioned above to check whether the fixative has any influence on the number of cytomictic patterns. The expected increase in the number of cytomictic patterns was not observed in the material fixed in acetic-alcohol.

The results described in this paper refer to treatment consisting of pressing, squashing, stabbing with a needle and cutting with a blade, all in air followed by immediate fixation of the treated tissue. On another lot of samples the treatments were also done in air (after drying the tissue with filter paper), but the fixation was done 5 min. after the treatment. Also the treatments were done on samples completely immersed in the fixative, acetic-alcohol or CrAF.

The obtained results are not certain nor consistent, because the number of cytomictic patterns obtained in the same material and after the same treatment varies with the sample and is to a large extent accidental. It depends on the way in which the injury is inflicted, and that is to some extent independent of the experimenter. It appears however that the effect of the fixative together with the mechanical stimulus (the material immersed in the fixative for the treatment) results in a decrease of the number of displaced nuclei which is consistent with the observations of Takats (1959). However no differences were observed between the samples fixed immediately or 5 min. after the treatment.

The participation of nucleoli in the phenomenon of cytomixis

In an earlier paper (Tarkowska 1960) it has been stated that in the epidermal cells and in the meristematic cells of *Allium cepa* roots, the nucleoli are not displaced during cytomixis. The information on the subject in literature is contradictory and refers only to the participation of nucleoli in the cytomixis occuring in the pollen mother cells. Some investigators like Gates (1911), West and Lechmere (1915) and Vaarama (1941) believed that nucleoli do not participate in cytomixis. However Youngman (1931) reported that whole nucleoli can migrate across the cell wall in an undamaged form. A partial displacement of the nucleolus has also been reported by Digby (1909). Others, like Kattermann (1933), Yu Chih-Chen (1936), Sarvella (1958) and Kamra (1960) have seen a migrating nucleolus only sporadically, and therefore have not taken a definite view on the subject.

There is no mention in the literature about the behaviour of nucleoli during cytomixis in vegetative tissues.

In the latest studies the author has observed that during cytomixis nucleoli can be displaced partially or completely in all the studied tissues. To report such a conclusion it is however necessary to make special preparations. The preparations stained with gentian violet or iron hematoxyline have to be differentiated in such a way so that the condensed chromatin in the displaced part of the nucleus does not obscure the nucleolus. Good results are obtained after selective staining according to the method of Semmens and Bhaduri (1941). However the best results are obtained from microtome preparations after Feulgen staining counter stained with light green, and observed under anoptral contrast. Under such conditions the nucleoli give a light green illuminescence. Using all the above mentioned methods it was possible to establish beyond doubt that nucleoli participate in cytomixis in the meristematic cells of onion root (Plate V, photos 18, 19), in the epidermal cells of *Ophiopogon* and *Reineckea*, and in the staminal hairs of *Tradescantia*.

RESULTS AND CONCLUSIONS

The experiments reported in this paper have demonstrated, that cytomixis is induced only by some mechanical stimuli. These stimuli have to create sufficiently large pressure differences between neighbouring cells, followed by injury of these cells.

By pressure within the cells the author understands such pressure which regardless of its origin, the protoplasm exerts on the cell wall.

In the studied material no cytomictic patterns were observed under the influence of centrifugation, temperature shocks, and the action of fixatives.

Also cytomixis was not produced by stabbing the tissues with a very thin and sharp needle, or cutting it with a very sharp blade. Both these stimuli act suddenly over a very small area and do not create sufficiently large pressure differences between neighbouring cells. Thus cytomixis is not a reaction to every form of tissue injury, nor is it the result of every pressure difference between adjoining cells.

The conducted experiments, particularity those employing the micromanipulator, and the analysis of the cytomictic process, have demonstrated that the creation of a sudden, and sufficiently large pressure difference between neighbouring cells is a necessary condition for the phenomenon. This pressure difference has to induce the flow of protoplasm from the cells with higher internal perssure to those with lower. The flow of protoplasm, taking place through the plasmodesmata pores or through larger perforations in the pit-closing membranes leads

towards the equalisation of pressures. The equalisation results from the expulsion of protoplasm with the nucleus or its part beyond the cell with an excessive inner pressure.

The pressure exerted by the protoplasm on the cell wall, and the durability of this cell wall have a determining influence here. The space where the expelled cell contents finds itself can be either the enviroment of the cell, as in the case of the staminal hairs of *Tradescantia* (Plate III, photo 12), or the neighbouring cells in the same tissue, which have a lower internal pressure. In both cases, according to the suggestions of Küster (1956, 1958) this is a form of plasmoptysis.

The causes inducing cytomixis, and their results, permit the conclusion that it is an abnormal phenomenon.

The reported study was conducted at the Warsaw University, Department of Plant Anatomy and Cytology. I wish to express my sincere gratitude to professor dr J. Szuleta for having supervised this work and for valuable suggestions during its execution. Also I wish to thank professor dr. H. Teleżyński for his valuable advice.

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Explanation of plates

Plate I

Photo 1. Cells of upper epidermis of *Ophiopogon* leaf seen from above. Neutral red and methyl green. About × 1000

Photo 2. Pits with outlines of pores in the cell walls of longitudinal cells of *Ophiopogon* upper epidermis. Pyoctanine. About × 2000

Photo 3. Upper epidermis and subepidermal cells in a cross section of an *Ophiopogon* leaf. Ruthenium red. About × 500

Plate II

Photos 4—7. Various cytomictic patterns in the cells of upper epidermis peeled off an *Ophiopogon* leaf. On photos 5 and 6 it is possible to see chromatin passing through small perforations in the pit-closing membrane. Acetoorcein. Photos 4, 6 and 7 about × 1000, photo 5 about × 500

Photo 8. Plasmodesmata in the pit-closing membrane of a cell from the upper epidermis of an *Ophiopogon* leaf. Pyoctanine according to J u n g e r s (1954) method. About × 2000

Plate III

Cytomixis in the staminal hairs of *Tradescantia*. Acetoorcein. About × 500

Photo 9. The nucleus migrates into the neighbouring cell through a perforation in the cell wall

Photo 10. A nucleus entering a neighbouring cell simultaneously at two points

Photo 11. A complete nucleus has been displaced into the neighbouring cell

Photo 12. Nucleus of one cell migrates outside of the cell, and a nucleus of another cell enters the neighbouring one

Plate IV

Photo 13. A fragment of the upper epidermis of an *Ophiopogon* leaf stabbed with a very sharp and thin needle. No cytomictic patterns have developed. Acetic-alcohol, neutral red and methyl green. About × 500

Photos 14 and 15. Fragments of microtome sections of onion root tip, stabbed with a very sharp and thin needle. On photo 14 there are no cytomictic patterns, and on photo 15 they occur sporadically. CrAF, Gentian violet and light green. About × 250

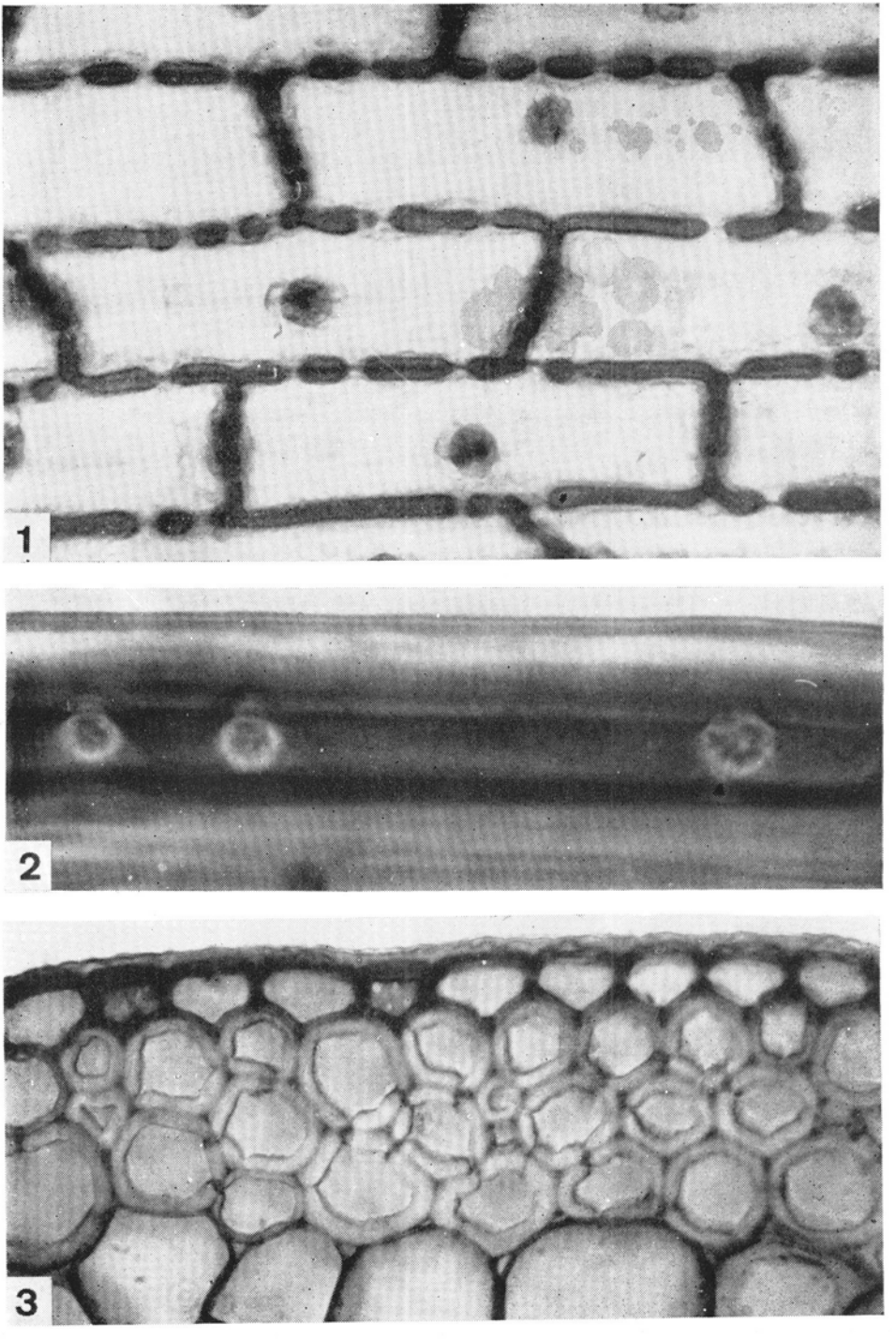
Photo 16. A fragment of a microtome section of an onion root tip stabbed with a blunt needle. Numerous cytomictic patterns are visible. CrAF, Gentian violet, and light green. About × 250

Plate V

Photo 17. A fragment of a microtome section of an onion root tip cut with a very sharp blade. No cytomixis has developed. CrAF, Gentian violet and light green. About × 250

Photos 18 and 19. Fragments of microtome sections of squashed onion roots. The migration of nucleoli during cytomixis. CrAF, Gentian violet. About × 1000

Plate I



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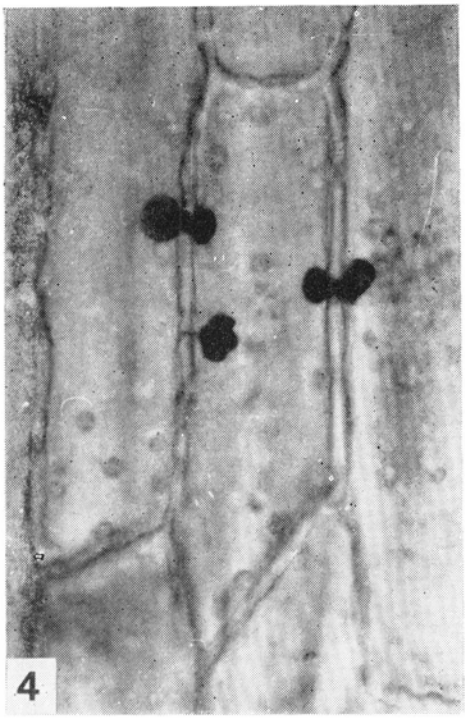
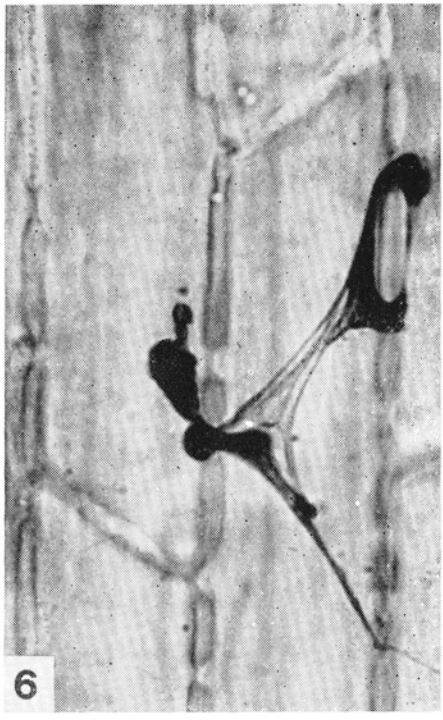
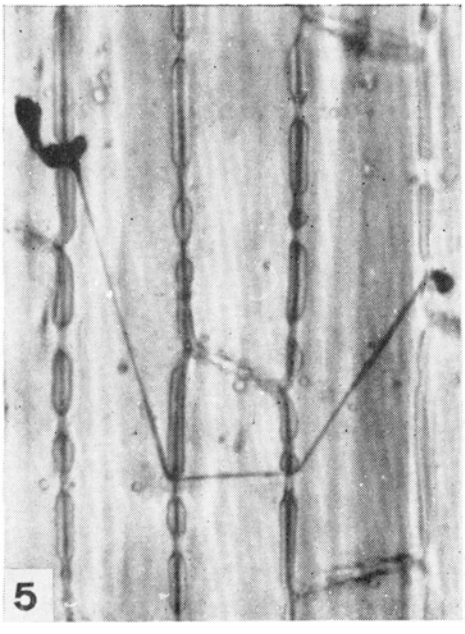






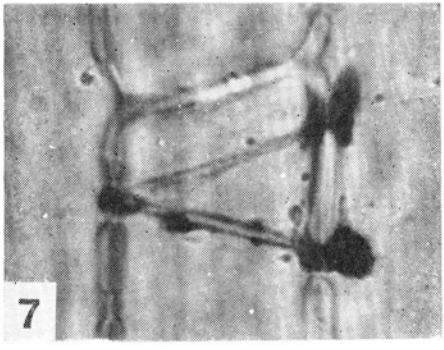
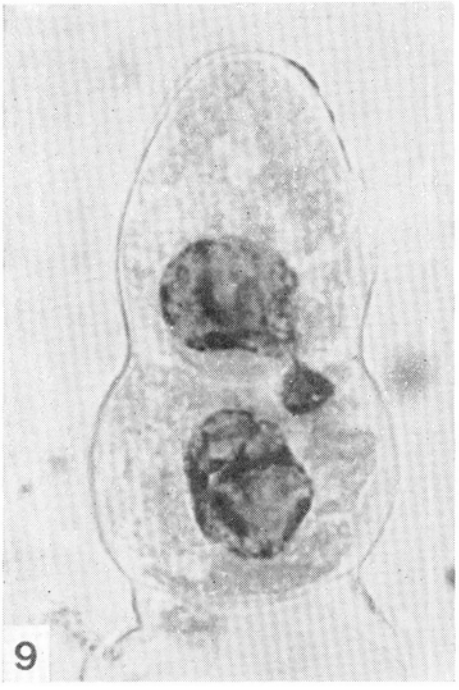
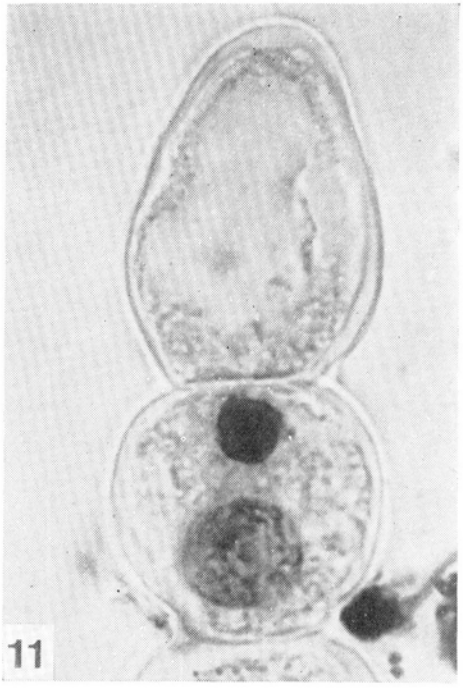
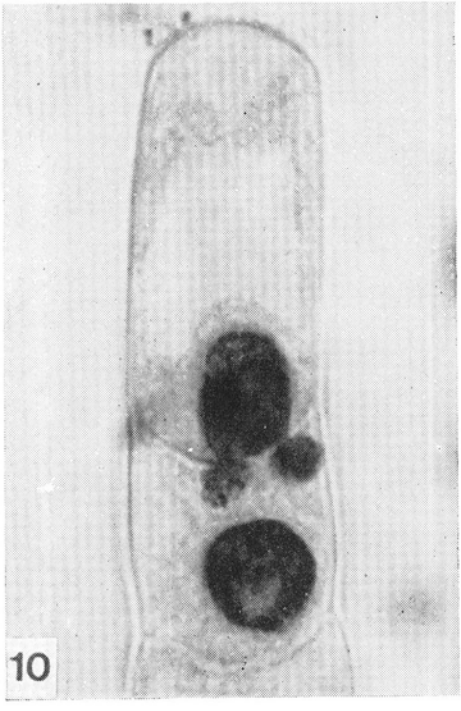


Plate III







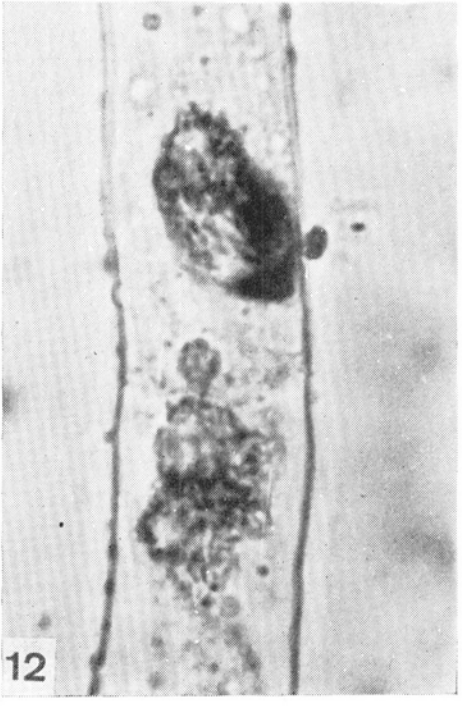


Plate IV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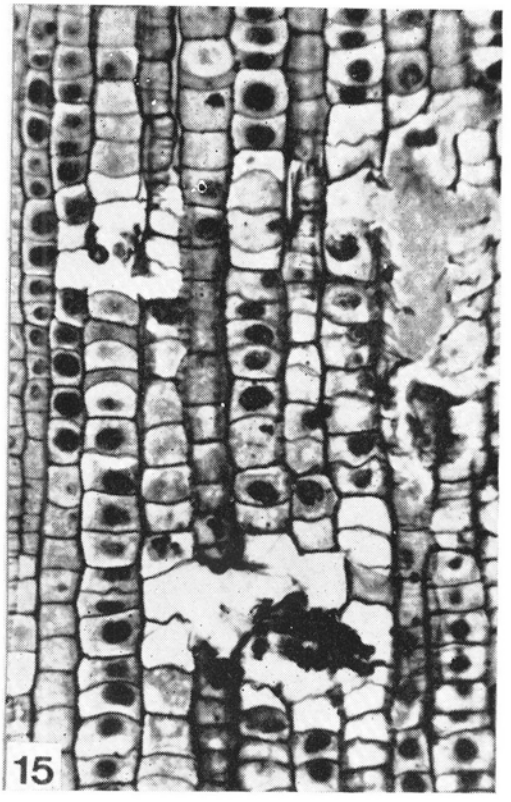
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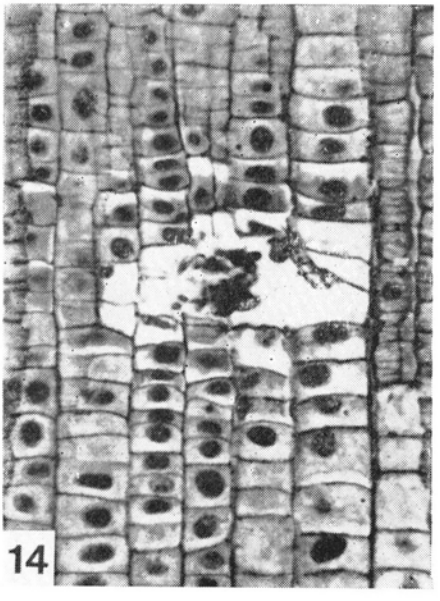
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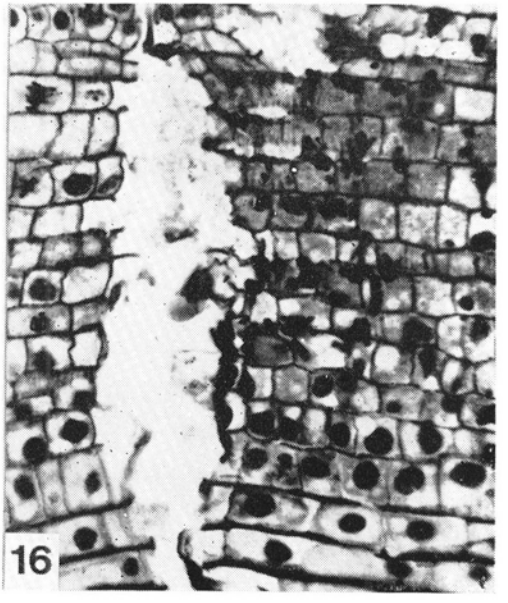
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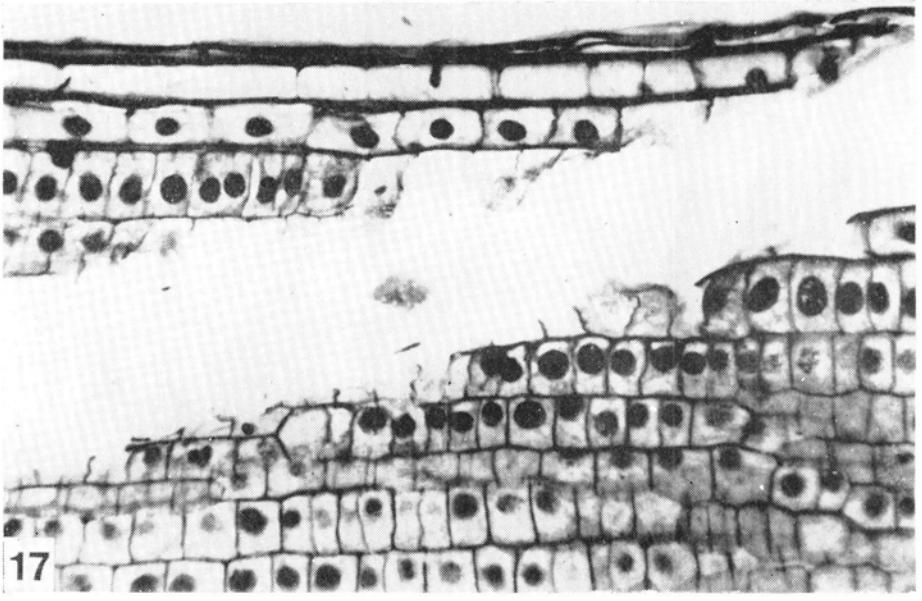
Plate V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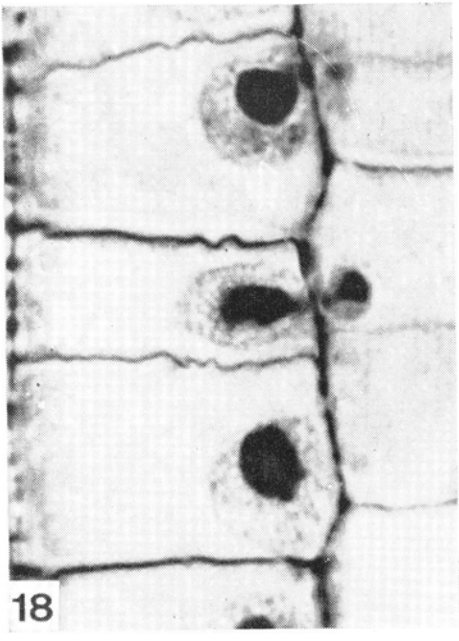
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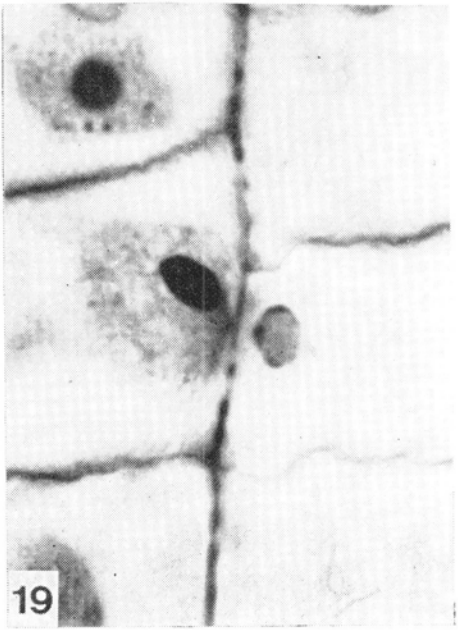
19

Eksperymentalna analiza mechanizmu cytomiksji. I.

Streszczenie

Zjawisko cytomiksji, czyli przenikania całego jądra komórkowego lub jego części przez błonę komórkową do sąsiedniej komórki, badano w skórce z liścia *Ophiopogon* i *Reineckea*, we włoskach z nitek pręcikowych trzykrotki (*Tradescantia*) oraz w komórkach wierzchołków korzeni cebuli (*Allium cepa*).

Cytomiksję wywoływano eksperymentalnie i przez analizę tego zjawiska badano jego mechanizm oraz ustalano bezpośrednie przyczyny.

Posługiwano się materiałem żywym i utrwalonym stosując następujące metody:

1. Ściągano (zrywano) skórkę z liści (*Ophiopogon* i *Reineckea*).
2. Uciskano lub zgniatano badany materiał (*Ophiopogon, Reineckea, Tradescantia, Allium*). Przy tych eksperymentach wykorzystano także mikromanipulator (*Ophiopogon, Reineckea, Tradescantia*).
3. Nakłuwano igłą ostrą i tępą (*Ophiopogon, Reineckea, Allium*).
4. Nacinano żyletką ostrą i tępą (*Ophiopogon, Reineckea, Allium*).
5. Wirowano (*Ophiopogon, Allium*).
6. Stosowano nagłe zmiany temperatury (*Ophiopogon, Allium*).
7. Badano wpływ utrwalacza (*Ophiopogon, Reineckea, Allium*).

Po dokonaniu odpowiedniego zabiegu uszkodzony materiał utrwalano w acetoalkoholu (1:3) lub w zmodyfikowanym utrwalaczu Nawaszyna (CrAF 0,5—1—20). Następnie, w przypadku liści *Ophiopogon* i *Reineckea*, ściągano lub ścinano skórkę, barwiono ją acetokarminem, acetoorceiną lub czerwienią obojętną przy pH 4 i bezpośrednio po tym zielenią jasną. Stosowano także barwienie hematoksyliną żelazistą Heidenhaina, hematoksyliną Ehrlicha lub wykonywano reakcję Feulgena.

Uszkodzone wierzchołki korzeni cebuli utrwalano podanymi wyżej utrwalaczami, sporządzano skrawki mikrotomowe grubości 7,5 lub 10 μ i barwiono je hematoksyliną żelazistą lub fioletem gencjanowym; stosowano także metodę Feulgena. Cytoplazmę we wszystkich przypadkach podbarwiano zielenią jasną. Zabiegi mechaniczne na wierzchołkach korzeni cebuli wykonywano albo w utrwalaczu (wierzchołek zanurzony), albo w powietrzu. W drugim przypadku uszkodzony materiał utrwalano bezpośrednio, lub po 5 minutach od chwili wykonania zabiegu.

Równolegle analizowano materiał kontrolny.

Dla stwierdzenia ewentualnego wpływu utrwalacza na powstawanie cytomiksji materiał kontrolny utrwalony w CrAF 0,5—1—20 lub acetoalkoholu 1:3. Ten ostatni był najbardziej podejrzany o powodowanie cytomiksji.

Zachowanie się jąderek w procesie cytomiksji badano na preparatach barwionych fioletem gencjanowym lub hematoksyliną żelazistą, albo po selektywnym barwieniu jąderka metodą podaną przez Semmens'a i Bhaduri (1941).

Żywotność komórek, w których wystąpiła cytomiksja stwierdzano metodami stosowanymi dla komórek skórki z łusek cebuli (Tarkowska 1960).

Skład chemiczny błon komórkowych badano stosując ogólnie używane metody barwienia i mikroreakcje. Przeprowadzano także obserwacje w świetle spolaryzowanym.

Bardzo ciekawych obserwacji dokonano na żywych komórkach skórki z liści *Ophiopogon* i *Reineckea* oraz na komórkach włosków z nitek pręcikowych *Tradescantia* przy wykorzystaniu mikromanipulatora (mikromanipulator ślizgowy Zeissa — Jena). Bezpośrednia obserwacja reakcji żywych komórek na stosowane bodźce mechaniczne ułatwiła wyjaśnienie różnych obrazów cytomiktycznych, obser-

wowanych w materiale utrwalonym po uprzednim uszkodzeniu, oraz samej natury zjawiska.

Wyżej wymieniony materiał (*Ophiopogon*, *Reineckea*, *Tradescantia*) a także mikromanipulator zostały przez autorkę po raz pierwszy wprowadzone do badania cytomiksji.

Nie stwierdzono wystąpienia figur cytomiktycznych pod wpływem wirowania, szoków temperatury ani pod wpływem działania utrwalaczy.

Wykonane w niniejszej pracy eksperymenty wykazały, że cytomiksję wyzwalają bodźce mechaniczne, powodujące znaczną różnicę ciśnień pomiędzy sąsiadującymi ze sobą komórkami.

Przez ciśnienie panujące w komórkach autorka rozumie to ciśnienie, które, niezależnie od jego pochodzenia, protoplast aktualnie wywiera na błonę komórkową.

Nie wyzwala cytomiksji w badanym materiale nakłuwanie tkanek bardzo cienką i ostrą igłą ani nacinanie bardzo ostrą żyletką. Oba te bodźce działają nagle na bardzo małą powierzchnię i nie wywołują dostatecznie dużej różnicy ciśnień pomiędzy sąsiadującymi ze sobą komórkami. Cytomiksja nie jest więc ani reakcją na każde uszkodzenie tkanki, ani następstwem każdej różnicy ciśnień pomiędzy przylegającymi do siebie komórkami.

Przeprowadzone eksperymenty, a szczególnie doświadczenia z mikromanipulatorem, i analiza przebiegu zjawiska wykazały, że koniecznym warunkiem wystąpienia cytomiksji jest zaistnienie nagłej, odpowiednio dużej różnicy ciśnień pomiędzy sąsiadującymi ze sobą komórkami. Różnica ta musi spowodować uszkodzenie komórek i przepływ protoplastu z komórek o dużym ciśnieniu do komórek o ciśnieniu mniejszym. Przepływ protoplastu, odbywający się poprzez pory plazmodesmów albo przez większe otwory w błonie zamykającej jamkę, prowadzi do wyrównania tej różnicy przez wyrzucenie części protoplastu z jądrem lub jego częścią poza obręb komórki o dużym ciśnieniu. Jąderko w całości lub częściowo jest przemieszczone razem z substancją jądrową.

Ciśnienie wywierane przez protoplast na błonę komórkową i wytrzymałość tej błony mają tu istotne znaczenie.

Terenem o mniejszym ciśnieniu, na który zostaje wyrzucona część zawartości komórki, może być albo otoczenie komórki (w przypadku włoska z nitki pręcikowej trzykrotki, fot. 12), albo, w obrębie tkanek, sąsiednie komórki o mniejszym ciśnieniu. I jeden i drugi przypadek, zgodnie z sugestiami Küstera (1956, 1958), jest swego rodzaju plazmoptyzą.

Wystąpienie cytomiksji można spowodować sztucznie zabiegami wywołującymi powstanie wystarczająco dużej różnicy ciśnień pomiędzy sąsiadającymi ze sobą komórkami. Zabiegami takimi, skutecznie stosowanymi w tej pracy, były: zdzieranie skórki, uciskanie i zgniatanie tkanek, kłucie ich tępą igłą oraz cięcie tępą żyletką.

Praca niniejsza została wykonana w Katedrze Anatomii i Cytologii Roślin Uniwersytetu Warszawskiego. Profesorowi dr J. Szulecie wyrażam serdeczne podziękowanie za kierownictwo pracą i cenne wskazówki udzielane w czasie prowadzenia badań. Profesorowi dr H. Teleżyńskiemu składam także podziękowanie za cenne rady.