Experimental analysis of the mechanism of cytomixis

II. Cytomixis in the pollen mother cells of the lily—*Lilium candidum* L.

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I. INTRODUCTION

For the analysis of the cytomixis phenomenon only studies which are experimental in nature are important. Such studies were conducted by Levitsky (1928), Woodworth (1931) and Takats (1959).

Levitsky cut transversely or longitudinally the tips of *Plantago* anthers, and after squashing them in acetocarmine, found the migration of chromosomes or whole nuclei across the cell walls. Woodworth studied meiosis in the *Betulaceae* and found that when he fixed whole inflorescences no cytomixis occurred, yet when he cut or squashed them before fixation numerous cytomictic figures occurred. The experiments of Takats (1959) have shown that any damage done to anthers of *Allium cepa* and *Lilium longiflorum* resulting in the rupture of the pollen chamber before fixation can induce cytomixis.

A separate group of papers deals with descriptions of typical cytomictic figures though the authors of these papers consider the described phenomena as showing only a resemblance to cytomixis or even do not associate them with it. Publications of Sparrow and Hammond (1947) and Copper (1952) can be included here.

Sparrow and Hammond tried to prove the migration of DNA in the form of Feulgen positive granules from the nucleus to the cytoplasm of the same cell in the microsporocyte of *Lilium henryi*, *L. longiflorum* and others. They have described typical cytomictic patterns, however the term "cytomixis" was used by the authors only once and even there in brackets.

Cooper (1952) claims that during meiosis DNA is exuded from the tapetum to the microsporocytes. The author considers this process as normal, metabolic turnover associated with the development of the microsporocytes.

The paper of Takats (1959) mentioned above is a reply to Cooper's interpretation. Takats has found that the chromatin which is extruded into the cytoplasm of the microsporocytes comes from the nuclei of the microsporocytes themselves and not from the tapetum. Results of further investigations into the presumed transfer of DNA from the tapetum to the microsporocytes were published by Takats in 1962. In these studies anthers of *Lilium longiflorum* were used. In the growing

medium labelled thymidine (H^3 -thymidine) was used, which was introduced into the tapetum, where it very markedly was incorporated into the nuclei. The experiment has shown that the labelled thymidine did not appear in the chromatin of the microspores after the desorganisation of the tapetum. Takats concluded that DNA from the tapetum is not transferred into the chromatin of the tetrads.

During the studies on cytomixis in vegetative tissues (Tarkowska 1960, 1965) it was found that the cause of chromatin or whole nuclei migrating across cell walls is to be found in the mechanical injuries, that result in sudden, yet considerable, pressure differences between neighbouring cells. In this paper methods were used which proved successful with the vegetative tissues, both those which induced, cytomixis and those which did not. The purpose of the study was to investigate whether the type of tissue used affects the occurrence of cytomixis and whether the character of the phenomenon in the sporocyte tissue is the same as in the vegetative tissues.

II. MATERIALS AND METHODS

As experimental objects microsporocytes of *Lilium candidum* L., in various stages of development starting from before the onset of meiosis to its completion, were used.

The following methods were employed.

1. Squeezing or squashing of the anthers.
2. Stabbing with a sharp and a blunt needle.
3. Cutting with a sharp and a blunt razor blade.
4. The influence of the fixatives was studied.

Always complete anthers were used for the studies, and also complete anthers were fixed (without cutting into parts). The damage to the anthers was performed either in the fixative or in the air. The injured anthers were fixed immediately or after a time lapse of 5 min. following the treatments, as was done on the onion root tips (Tarkowska 1965). The fixatives used were CrAF 0.5—1—20 and acetic alcohol 1:3. In CrAF the anthers were fixed for 24 hrs. and in acetic alcohol for 4 hrs. Microtome sections 10 to 12 μ thick were stained as the onion root tips (Tarkowska 1965).

The participation of the nucleoli in the process of cytomixis and the vitality of the cells participating in the phenomenon were studied by the same method as in the cells of the onion leaf epidermis and onion root tips (Tarkowska 1960, 1965).

The chemical composition of the microsporocyte cell walls was studied by the generally known and used staining methods and histochemical tests for pectic substances and cellulose. Observations were also made under polarized light. For callose staining aniline blue and resorcin blue was used. The fluorescence method of Arens (1949) checked and improved by Currier and Strugger (1956) and by Currier (1957) was also used.

III. RESULTS

A. A description of the microsporocyte tissue in the lily — *Lilium candidum* L.

The multiwalled microsporocytes of *Lilium candidum*, with thin primary walls, form a compact mass in the first stages of prophase I. This state continues until pachytene. From pachytene onwards the microsporocytes begin to round off slightly, and small intercellular spaces form between them. This is accompanied, at first very slowly, by a thickening of the cell walls. The so-called special wall begins to form. During the further division stages of the nucleus the cells dissociate even further, and finally split altogether. At this time they are already oval with the characteristic wall thickening.

The above mentioned changes in the shape and content of the sporocyte tissues are associated with changes in the chemical composition of the cell walls.

There is nothing surprising in the fact that the thickening of the cell walls in the microsporocytes, that is the formation of the special wall that has originated in pachytene, is accomplished through the laying of callose layers by the protoplast. However the chemical composition of the primary walls of these cells has not been consistently identified as yet. The studies of Digby (1909), West and Lechmere (1915), Vaarama (1941) and Waterkeyn (1962) lead to divergent conclusions.

All the observations made by the author concerning the chemistry of microsporocyte cell walls in lily, during the first reduction division, permit the conclusion that before meiosis and during its most early stages the content of cellulose gradually decreases so that at the beginning of pachytene there are only traces of it. During diplotene and metaphase I all the tests for cellulose tried gave negative results. From pachytene onwards on the inside of the primary wall callose begins to accumulate which forms the so called special wall. The accumulation of callose is at first fragmentary and entirely accidental. It begins either in cell corners or in central parts of the cell wall. Further accumulation of callose leads to a rounding off of the lumen of the microsporocyte.

The tapetum in *Lilium candidum* is built of cells elongated in the radial direction. These cells have many vacuoles, easily dissociate from the walls of the anther chambers or from the microsporocytes, and also easily desorganize. The chemical composition of the walls of the tapetal cells undergoes changes during the development of the microsporocytes. At first the cell walls are pectic with an admixture of cellulose (as the microsporocytes), however already during leptotene they do not contain cellulose in the primary radial walls and external walls adjoining other cells, however in internal walls adjoining microsporocytes the cellulose persists until the end of pachytene.

B. Experiments causing cytomixis

1. The squeezing of anthers.

The squeezing of anthers was done between two microscopic slides. This type of injury gives the greatest number of the most effective cytotoxic patterns, simi-

larly as was the case when onion root tips were squashed (Tarkowska 1960, 1965). However in the microsporocytes the displaced chromatin always has the shape of granules. Filiform figures were not observed.

The displacement of chromatin appears to be multidirectional, however a closer analysis of serial cross sections permits the localization of the damaged area, the wound, and the conclusion that the migration of nuclei usually takes place towards that area. Photos 1 and 2 (Plate I) represent fragments of squeezed anthers with cytotoxic figures visible (the wound is not visible). The forces operate in all directions, and therefore the resultant pressure differences on all sides of a cell explain the "exchange" of chromatin between two cells, as can be seen in photo 3 (Plate I).

In this type of damage, it is sometimes possible to observe in the microsporocytes both the granules which are joined with the nucleus from which they are derived, and the chromatic substance in the form of apparently isolated granules. These are granules seen from the top or from the bottom, originating from cells either above or below the cell on the microscopic slide. Some of these granules have a very definite structure —Plate I, photo 4).

2. Stabbing with a sharp or a blunt needle.

When anthers were pricked with a sharp and thin glass needle then around the inflicted wound, as well as in the whole pollen sac usually no cytotoxic figures were observable (Plate I, photos 5 and 6). In the few instances where cytotoxic was seen this was probably the result of the treatment being performed insufficiently precisely.

When for the treatment a thicker or slightly blunted needle was used then numerous cytotoxic figures were always observable (Plate II, photo 7).

3. The cutting with a sharp and a blunt razor blade.

Photos 8 and 9 (Plate II) demonstrate pollen chambers cut across with a sharp thin razor blade. In no place has cytotoxic occurred. Even the nuclei were not displaced in their cells.

A blunt and thick razor blade causes considerable damage and many cytotoxic patterns. However during this treatment very often the whole pollen chamber is damaged, and the resultant mass of squashed cells is unsuited for analysis.

The results obtained with a sharp and a blunt needle, and also with a sharp and blunt razor blade are entirely in agreement with the results obtained after these treatments were applied to onion root tips (Tarkowska 1965). They confirm the belief that also in the microsporocytes a necessary and sufficient condition for the occurrence of cytotoxic is the creation between adjoining cells of a sudden and substantial pressure difference. This difference causes the migration of the protoplast from the cells with the higher pressure to those with the lower pressure. Thus very sharp instruments (needle, blade) which cause a sharp, but very localized damage to the tissue, do not induce a sufficiently large pressure on the cell walls to liberate cytotoxic.

4. Influence of the fixative.

The results obtained with the sporocyte tissues of lily are in agreement with the results obtained for the meristematic tissues of onion roots (Tarkowska 1965). The fixatives used on the studied sporocyte tissue do not cause cytomixis, nor do they affect the number of cytomictic patterns obtained by mechanical injuries.

IV. DISCUSSION

The pathway of chromatin migration from one cell to the other is not longer disputable. Generally speaking cytomixis takes place through pits, along the plasmodesmata. These can be individual plasmodesmatal pores as well as larger openings formed by a partial or complete rupture of the pit closing membrane.

The existence of plasmodesmatal connections between cells is a condition for the occurrence of cytomixis. The existence of such connections between all the studied vegetative cells as well as between the microsporocytes (Waterkeyn 1962) is unquestionable. However plasmodesmatal connections were not found between the microsporocytes and the tapetal cells. This could explain the fact that none of the investigators including the author was able to establish in a manner that would be convincing the migration of nuclei from the tapetum to the microsporocytes nor in the opposite direction. Cytomixis between neighbouring tapetal cells was sporadically observed by the author (Plate II, photo 10). Such individual cytomictic patterns in the tapetum were also mentioned by Vaarama (1941) and Takats (1959).

The way of migration of the nuclear substance across the cell wall influences the structure of the chromatin. Tarkowska (1960, 1965) has suggested that a homogenous, compact structureless mass of chromatin found on the other side of the cell wall was squeezed through individual plasmodesmatal pores. However chromatin which maintains its structure has crossed the cell wall through larger perforations in the pit closing membrane. This relationship holds true for all the materials studied. The microphotographs included, represent microsporocytes of lily with structureless granules of homogenous chromatin (Plate III, photos 14, 16; Plate IV, photos 17, 20–22), chromatin with well preserved structures (Plate I, photo 4; Plate III, photos 12, 15; Plate IV, photo 19), and sometimes even the structure of displaced individual chromosomes (Plate V, photos 23, 24).

The chromatin substance, displaced across microsporocyte wall in *Lilium candidum*, is usually globular. The amount of displaced chromatin can be very variable. Usually there is only one granule (Plate II, photo 11; Plate III, photo 12; Plate IV, photos 17, 18, 21) or a few granules only (Plate IV, photos 19, 20). In very few cases, during leptotene to pachytene, displacement of larger parts of the nucleus were observable (Plate III, photo 14). In photograph 13 (Plate III) the greatest amount of displaced chromatin that was observed in microsporocytes of lily is presented.

The displaced granules can become localized either in one neighbouring cell

(Plate III, photos 12, 13; Plate IV, photos 17–22; Plate V, photos 23–26) or in two adjoining cells (Plate III, photo 14). The chromatin can be displaced from the same nucleus also into two cells in the opposite directions (Plate III, photos 15, 16). This last instance often causes an elongation and deformation of the nucleus.

Cytomixis can take place regardless of the positioning of the nucleus in the cell. During an experiment usually the whole nucleus is displaced towards the cell wall, however in some cases the nucleus does not change its normal place, and only a thin thread of chromatin, often as thick as a chromosome moves towards the cell wall and migrates through it (Plate V, photos 23, 24),

The displaced chromatin granules are always connected with the nucleus from which they originate. The extrusions of chromatin, observable in the cytoplasm and apparently without any connection to a nucleus, are granules which have migrated from cells positioned above or below. Presumably this is the explanation of the figures described by West and Lechmere (1915), and the chromatin extrusions which Sparrow and Hammond (1947) have described as loose chromatin granules, occurring in the cytoplasm of microsporocytes of many plants.

Digby (1909) and West and Lechmere (1915) point out the fact that chromatin granules which have migrated into other cells are usually surrounded by a clear zone. West and Lechmere believe that this zone is separated from the cytoplasm by a membrane which has developed from cytoplasmatic strands.

The presence of lighter zones around the displaced chromatin was observed by the author on most of the preparations. The size of the zone itself can be very different. In lily this is well seen on the microphotographs (Plate III, photos 14–16; Plate IV, photo 18).

Experiments with the micromanipulator, that were performed on the cells of *Ophiopogon* leaf epidermis (Tarkowska 1965) explain the mechanism of formation of these clear zones. It can be assumed that the zones in the microsporocytes form in the same fashion. According to the same reasoning they could have developed as a result of suction pressures. Presumably plasmalemma, which now separates the clear, watery zone from the displaced cytoplasm, forms this membrane, which was mentioned by West and Lechmere (1915).

The next problem that emerges from the analysis of cytomixis, is the viability of the cells with an increased and a decreased amount of chromatin. This topic with respect to vegetative tissues was discussed in the earlier publications (Tarkowska 1960, 1965). A study of the viability of microsporocytes engaged in cytomixis confirmed the conclusions made for vegetative tissues.

It appears that cells from which chromatin migrates demonstrate all the symptoms of dying and death. Cells to which chromatin has intruded are usually also dead or in very few instances still alive, yet with obvious signs of desorganization.

Studies pertaining to the phases and stages of meiosis, during which the migration of chromatic substances is possible, constitute one of the more important subjects dealt with in the literature on cytomixis. It has been commonly stated that cytomixis occurs only during certain stages of the meiotic divisions.

During prophase I cytomixis has been observed commonly and in large quanti-

ties. It occurs most frequently during the leptotene-pachytene stages, and from diplotene onwards a gradual decrease of cytomictic figures can be noted. During anaphase I — telophase I cytomixis is only a sporadic phenomenon. Only Levitsky (1928), Stebbins (1932) and Gorczyński (1934) have observed it in the second meiotic division.

In *Lilium candidum* the author has observed cytomixis before meiosis, in prophase I and in metaphase I. Before meiosis the displacement of chromatin is limited to few occurrences, it increases substantially in preleptotene, and in leptotene it is already a mass phenomenon. The maximum frequency of cytomictic figures occurring was observed by the author in zygotene and in very early pachytene. Towards the end of pachytene the frequency sharply declines, and in early diplotene is very low. In late diplotene and in metaphase I cytomixis occurs, only very sporadically. In later phases of meiosis the author has not observed any cytomictic figures. On photographs 17–22 (Plate IV) a displaced chromatin in prophase I and metaphase I are presented: in photo 17 — before the beginning of meiosis, photo 18 — preleptotene, photo 19 — leptotene, photo 20 — zygotene, photo 21 — diplotene, photo 22 — prometaphase.

The gradual increase in cytomictic patterns and then its sudden decline, indicates a relation with the already described structure of the sporocyte tissue. The great compactness of microsporocytes, thin cell walls and numerous plasmodesmatal connections during the period from leptotene to pachytene, create the most favourable conditions for the displacement of chromatin. It is hardly surprising therefore that some research workers have observed the phenomenon only during this period.

On the basis of the theory that pressure differences are the direct cause of cytomixis, it would appear that microsporocytes in these phases of division when cytomixis is most frequent, would be characterised by a higher osmotic pressure than during stages when the displacement of nuclei is less common. In view of the lack of information in the literature on this subject, the following experiment was performed. Anthers, in various stages from leptotene to metaphase I were subjected for 4 hours to the action of 2%, 10%, 20% sucrose and thereafter they were fixed. On microtome sections the limiting plasmolitic values were sought for the different phases of the meiotic division.

From the results obtained it appears, that the osmotic value of the cell sap during different meiotic phases tends to change. In the period from preleptotene to zygotene the osmotic value gradually increases reaching a maximum during the transition from zygotene to pachytene. Already in pachytene it begins to decline and from diplotene onwards it continues to decline rapidly.

These results are in total agreement with the frequency of cytomixis during meiosis. This would be a further confirmation of the hypothesis that cytomixis is dependent among others on the pressure existing within cells. The greater the pressure, the more easy it is to cause pressure differences while damaging the cells and therefore the greater number of cytomictic patterns will result.

The inclusion of nucleoli in the cytomictic phenomenon has been observed

in the microsporocytes of lily during leptotene — pachytene. The photos 25 and 26 (Plate V) demonstrate the displacement of nucleoli. The migration of nucleoli is best observed on microscopic preparations after Feulgen staining, counter-stained with light green and examined under anoptical contrast. Under these conditions the nucleoli shine pale green, and on the photographs can be seen as larger bright spots.

The various causes of cytomixis as presented in the literature require a more detailed discussion. They can be divided into two groups.

1. Some authors have considered cytomixis as a normal phenomenon occurring in a healthy cell. This view was presented by Digby (1909), West and Lechmère (1915), Sparrow and Hammond (1947), Cooper (1952), Su-Süan (1955 a, b) and Cheng (1955).

With this group of authors one should include also those, who supposed that cytomixis in microsporocytes is a way of forming polyploids. Such suggestions were made by Levitsky (1928), Kattermann (1933), Vaarama (1941), Mendes and Rijo (1951) and Sarvella (1958).

2. Most research workers have considered cytomixis as an abnormal pathological phenomenon, giving various explanations of its cause.

a) Premeiotic aberrations were envisaged by Dufrénoy and Dusseau (1939), Müntzing and Prakken (1941), Mendes and Rijo (1951) and Sarvella (1958).

b) Degeneration was suggested as the cause by Gates (1911, 1920), Fraser (1914), Gates and Rees (1921), de Litardière (1925) and Gorczyński (1934).

c) The effect of fixatives was considered by Erlanson (1929), Woodworth (1931), Sparrow and Hammond (1947), Bopp-Hassenkamp (1959) and Takats (1959).

d) Mechanical damage has been suggested by Miehe (1901), Schweidler (1905), Schürhoff (1906), Levitsky (1928), Kattermann (1933), Courtine (1939), Takats (1959) and Kamra (1960).

e) Action of stimuli that create a considerable pressure difference between neighbouring cells was viewed as the cause by Gates (1911), Vaarama (1941), Takats (1959) and Tarkowska (1960, 1965).

f) Finally it has also been suggested that centrifugation and temperature shocks may cause cytomixis.

The studies conducted by the author permit a reassessment of the above presented views in light of the new data obtained.

The view that cytomixis is a normal phenomenon taking place in healthy, normally functioning cells is most objectionable.

This view is strongly proposed in the paper of Cooper (1952). However it is difficult to agree with his conclusions and interpretations. In the early phases of meiosis Cooper has reported numerous Feulgen positive granules emitted by the nuclei of tapetal cells, and then transferred across the cell wall into the pollen chamber.

During the studies conducted by the author on *Lilium candidum* and also during numerous observations on preparations demonstrating the development of anthers and microsporocytes in various plants, never have similar chromatin bodies been

seen. On the other hand the transfer of Feulgen positive bodies from the pollen chamber to the microsporocytes as described by Cooper should be considered as typical cytomixis between microsporocytes, or between microsporocytes and the intercellular spaces, and thus as migration of chromatin in the opposite direction than has been suggested by Cooper.

It needs to be stressed that thousands of preparations studied by the author both in vegetative tissues and in anthers, have yielded no evidence for the suggestion that cytomixis is a normal phenomenon, taking place in healthy cells.

Thus cytomixis has to be considered as an abnormal phenomenon, pathological in nature.

Of all the factors believed to be capable of causing cytomixis, a special note needs to be made of the mechanical damages and stimuli creating pressure differences. Only these causes have found confirmation in the studies conducted by the author (Tarkowska 1960, 1965) and in experimental papers of Mische (1901) on vegetative tissues and those of Levitsky (1928), Woodworth (1921) and Takats (1959).

Takats considers it as certain that the extrusions from microsporocytes are formed during the preparation of the material before fixation. He believes that the wounding of tissues creates shocks in the osmotic pressure and that these aberrations in the osmotic balance result in the displacement of nuclei.

Mechanical injuries were presumably the cause of cytomixis in the studies of Vaarama (1941) and Su-Süan (1955 a), although the authors did not take these factors into account.

Vaarama was fixing whole buds of *Sagittaria natans*, however he was tearing them off with pincette. Also he removed root tips with pincette. In his paper he has mentioned that in the few damaged pollen chambers he has observed a greater number of displaced nuclei. However Vaarama did not expect that this treatment could have induced cytomixis.

The experiments conducted on microsporocytes of lily confirmed the hypothesis on the causes of cytomixis induction presented as a result of studies on cytomixis in vegetative tissues (Tarkowska 1960, 1965). The cause of cytomixis has to be attributed to these factors which create a sudden and sufficiently large pressure difference between neighbouring cells. This pressure difference has to injure the cells and induce a transfer of the protoplast from cells with a high pressure to those with a lower one. The movement of the protoplast through the plasmodesmatal pores or through larger perforations in the pit closing membrane leads to an equalization of this difference. The closer to the point of transfer of the protoplast is the nucleus, the more readily it will be displaced.

It can be assumed that in all the studies where cytomixis was observed but was not experimentally induced, it was caused during the careless preparation of the material for fixation. Even when the whole anthers or the whole buds were fixed, when these were removed from the plants and transferred into the fixative with pincette, conditions could have been created for the liberation of cytomixis. The probability of inducing nuclei displacement is increased when the material is cut

into smaller pieces for better fixation. Unless particular care is taken during the preparation before fixation of the material, small injuries are almost impossible to avoid.

In the light of these remarks the observations by some authors of rare or casual occurrences of cytomictic patterns take on a different meaning (Kattermann 1933, Müntzing and Prakken 1941, Vaarama 1941).

V. CONCLUSIONS

Results of experimental studies of cytomixis in vegetative tissues (Tarkowska 1960, 1965) and in microsporocytes of the lily (*Lilium candidum*) permit the formulation of the following conclusions:

1. The term "cytomixis" needs to be employed both in the case of pollen mother cells and in all vegetative tissues, meaning an extrusion of a part of the protoplast with a nucleus or its part from one cell, across the cell wall, into the neighbouring cell.

2. It was possible to induce the phenomenon of cytomixis experimentally only by some mechanical stimuli applied in a particular way.

Cytomixis was not induced in the studied material by such stimuli as fixatives, temperature shocks and centrifugation.

3. Cytomixis was induced in the epidermis of *Ophiopogon* and *Reineckea* leaves, in onion root tips, in staminal hairs of *Tradescantia* and in the microsporocytes of the lily with the help of mechanical treatments causing sudden and sufficiently large pressure differences between neighbouring cells. These treatments were: the sudden peeling off of the epidermis, which causes a stretching of cell walls and the action of suction pressures, and also the sudden squeezing, squashing and piercing of the tissues with a blunt needle or the cutting with a blunt blade, which induce the action of direct pressures. Suction or direct pressures caused mechanically are the immediate causes of the pressure differences that cause the migration of the protoplasts.

4. Other mechanical treatments such as stabbing with a sharp thin needle, or cutting with a thin and sharp razor blade did not cause cytomixis. These stimuli act on a very small surface, and the pressure differences induced by them between the neighbouring cells are insufficient to cause the migration of protoplasts and the formation of cytomictic figures.

5. In the epidermis peeled off the leaves of *Ophiopogon* and *Reineckea*, cytomixis is a very frequent phenomenon and occurs on the whole surface of the sample. In the squashed root and anther cytomixis occurs around every crack forming as a result of rupture of the tissues caused by the sudden pressure.

In the peeled epidermis the migration of nuclei is apparently multidirectional whereas in the squashed roots and anthers the migration is generally directed towards the wound, the place where a sudden drop of pressure was created.

6. Cytomixis is an instantaneous phenomenon and takes place in cells which are still alive.

Cells deprived of a part of their chromatin are dead, or rapidly dying, whereas the cells into which chromatin has intruded, remain seriously damaged and usually also die.

Thus cytomixis can not lead to the formation of polyploids.

7. The nuclei migrate into the neighbouring cells through pits in the cell walls. It seems that in all the studied cells the migration took place both through the plasmodesmata pores, and through larger perforations caused by the rupturing of the pitclosing membrane. Thus the existence of plasmatic connections is a condition for the occurrence of cytomixis.

In the anthers of *Lilium candidum* no plasmodesmata could be found between the tapetal cells and the microsporocytes, nor was it possible to demonstrate the occurrence of cytomixis between these cells.

8. The chromatin having passed through the pit, either forms a compact homogenous mass, or maintains a structure normal for the nucleus from which it originates. Presumably the structure of the displaced chromatin depends on the size of the opening through which it has migrated. On passing through the small plasmodesmata pores it probably becomes homogenous and compact, while on passing through larger perforations in the pit closing membrane it can maintain its original structure.

9. Both parts of nuclei and whole nuclei can migrate into neighbouring cells.

In the microsporocytes of *Lilium candidum* and in the staminal hairs of *Tradescantia* the displaced parts of nuclei are always granular. In the meristematic tissues of the *Allium cepa* roots, the extruded chromatin usually forms granules, but sometimes the nucleus can become subjected to a strong elongation and becomes filiform. In the epidermal cells of *Ophiopogon* and *Reineckea* the cytomictic figures form, besides granules, an unexpected tangled mass of filiform structures caused by the stretching of chromatin.

10. Nucleoli also take part in cytomixis. They are displaced across the cell walls either whole or in part, together with the chromatin of the cell nucleus.

11. In vegetative tissues both resting nuclei and nuclei in all phases of mitosis (displacement of the chromosomes) undergo cytomixis.

In the microsporocytes of *Lilium candidum* cytomixis was observed from the pre-meiotic phase onwards till metaphase I inclusive. Before meiosis and in preleptotene cytomixis is rare. The number of cytomictic patterns gradually increases in leptotene and zygotene and towards the end of zygotene and early pachytene it reaches its maximum. From that time onwards the number of cytomictic patterns observable markedly declines. In late diplotene and metaphase I cytomixis occurs only sporadically.

12. Mechanical treatments, that cause cytomixis when tried on plasmolysed onion roots and experiments on the limiting plasmolysis in microsporocytes of lily, indicate the role played by the pressure that protoplast exerts on the cell wall.

In plasmolysed, and then squashed roots cytomixis occurs rarely or does not occur at all.

The osmotic value of the cell sap in the microsporocytes changes with the various stages of meiosis. It was established that the frequency of cytomixis first increases and then fall parallel to changes in the osmotic pressure.

13. Taking into account the fact that cytomixis is caused by considerable pressure differences between neighbouring cells, that suddenly equalize the pressure differences, and that a part of protoplast migrates out of the cell, the phenomenon of cytomixis has to be considered, according to the suggestions of Küster (1956, 1958), as a form of plasmotysis.

14. An analysis of the causes inducing cytomixis and its consequences, permits the conclusion that it is an abnormal phenomenon, pathological in nature.

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EXPLANATION OF PLATES

All the microphotographs represent fragments of microtome cross sections of *Lilium candidum* L. anthers

Photographs 1—24 represent material fixed in CrAF 0.5:1:20 stained with Heindenhein's iron hematoxyline and light green. Photos 25 and 26 represent materials fixed as above and stained with Feulgen and light green, under anoptral contrast

Plate I

Photo 1. Numerous multidirectional cytomictic patterns in a squashed pollen chamber, $\times$ ca 250

Photo 2. As in photo 1, $\times$ ca 500.

Photo 3. An exchange of chromatin substance between neighbouring cells. Fragment of a squashed pollen chamber, $\times$ ca 1000

Photo 4. Drops of displaced chromatin as seen from above. Structure well seen. A fragment of a squashed pollen chamber, $\times$ ca 1000

Photos 5 and 6. Pollen chambers after stabbing with a very thin and sharp glass needle. No cytomictic patterns, $\times$ ca 250.

Plate II

Photo 7. Pollen chamber pierced with a blunt needle. Numerous cytomictic patterns can be seen, $\times$ ca 250

Photos 8 and 9. Pollen chambers carved with a sharp thin razor blade. No cytomictic patterns, $\times$ ca 250

Photo 10. Cytomixis in the tapetal cells. A fragment of a squashed pollen chamber, $\times$ ca 1000

Photo 11. Displacement of chromatin from a nucleus far removed from the cell wall. During the treatment (squashing) the nucleus did not change its position, $\times$ ca 1000

Plate III

All the microphotographs represent fragments of a squashed pollen chamber, $\times$ ca 1000

Photo 12. A typical, most commonly found cytomictic pattern — displaced chromatin in the form of a granule

Photo 13. A large quantity of displaced chromatin. A well preserved structure of the displaced chromatin surrounded by a clear zone is visible

Photo 14. Chromatin from one nucleus was displaced to two neighbouring cells. Clear zones surrounding the displaced chromatin are visible

Photos 15 and 16. Chromatin from one nucleus was displaced in two opposite directions. The nuclei became deformed, elongated. Clear zones visible. In photo 15 the structure of the displaced part of nucleus well preserved

Plate IV

All the microphotographs represent fragments of a squashed pollen chamber, $\times$ ca 1000

Photo 17. Chromatin displaced from a nucleus before the beginning of meiosis

Photo 18. Chromatin displaced during preleptotene

Photo 19. Chromatin displaced during leptotene

Photo 20. Chromatin displaced during zygotene

Photo 21. Chromatin displaced during diplotene

Photo 22. Chromatin displaced during prometaphase

Plate V

All the microphotographs represent fragments of a squashed pollen chamber, $\times$ ca 1000

Photos 23 and 24. Individual chromosomes after migration across the cell wall have maintained their original structure

Photos 25 and 26. Displacement of nucleoli during cytomixis. The nucleolar substance is visible as larger clear zones

*Eksperymentalna analiza mechanizmu cytomiksji**II. Cytomiksja w komórkach macierzystych ziaren pyłku lili — Lilium candidum L.*

Streszczenie

Przedmiotem badań były mikrosporocyty *Lilium candidum* L. w okresie przed rozpoczęciem mejozy i w czasie jej przebiegu.

Celem pracy było sprawdzenie, czy wystąpienie cytomiksji nie zależy od materiału użytego do badań i czy natura zjawiska w tkance sporocytowej jest taka sama, jak w tkankach wegetatywnych.

W pracy tej zastosowano metody wypróbowane i stosowane dla tkanek wegetatywnych (Tarkowska 1965) zarówno te, które wywoływały cytomiksję, jak i niektóre z tych, które nie powodowały jej wystąpienia. Stosowano następujące metody:

- 1 uciskano lub zgniatano pylniki,
- 2 nakłuwano igłą ostrą lub tępą,
- 3 nacinano żyłką ostrą lub tępą,
- 4 badano wpływ utrwalcza.

Na pylnikach przeprowadzano wyżej wymienione zabiegi, następnie pylniki utrwalano i stosowano barwienia, jak dla wierzchołków korzeni cebuli (*Allium cepa* L.). Udział jąderka w procesie cytomiksji i żywotność komórek uczestniczących w tym zjawisku stwierdzano metodami opisanymi w poprzednich publikacjach (Tarkowska 1960, 1965).

Skład chemiczny błon komórkowych mikrosporocytów badano stosując powszechnie znane i używane metody barwienia i mikroreakcje na substancje pektynowe i celulozę. Przeprowadzano także obserwacje w świetle spolaryzowanym. Przy wykrywaniu kallozy posługiwano się barwieniem błękitem anilinowym i błękitem rezorcynowym oraz, wykorzystując zjawisko fluorescencji, stosowano metodę Arens'a (1949) sprawdzoną i udoskonaloną przez Currier i Strugger'a (1956) oraz przez Currier (1957).

Wyniki badań eksperymentalnych, odnoszące się do zjawiska cytomiksji w tkankach wegetatywnych (Tarkowska 1960, 1965), a także w mikrosporocytach *Lilium candidum*, będące przedmiotem niniejszej publikacji, pozwalają na sformułowanie następujących wniosków:

1. Termin „cytomiksja” należy stosować zarówno do komórek macierzystych ziaren pyłku, jak i do wszelkich komórek wegetatywnych, określając nim wyrzucanie części protoplastu z jądrem lub jego częścią z jednej komórki, poprzez błonę komórkową, do komórki sąsiedniej.

2. Eksperymentalnie zjawisko cytomiksji udawało się dowolnie wywoływać tylko niektórymi i odpowiednio stosowanymi bodźcami mechanicznymi.

Nie wyzwały cytomiksji w badanym materiale takie bodźce jak: utrwalcze, szoki temperatury i wirowanie.

3. Cytomiksję wywołano w skórcze z liści *Ophiopogon* u *Reineckea*, w wierzchołkach korzeni cebuli, we włoskach z nitek pręcikowych trzykrotki i w mikrosporocytach lili, przy pomocy zabiegów mechanicznych powodujących powstanie nagłej i odpowiednio dużej różnicy ciśnień pomiędzy sąsiadującymi ze sobą komórkami. Zabiegami takimi były: gwałtowne zrywanie skórki, powodujące rozciąganie błon komórkowych i działanie sił ssących, oraz nagłe uciskanie, zgniatanie i nakłuwanie tkanek tępą igłą lub nacinanie tępą żyłką, powodujące działanie sił tłoczących. Siły ssące lub tłoczące, wywołane mechanicznie, są bezpośrednio przyczyną różnicy ciśnień i przemieszczeń protoplastów.

4. Inne zabiegi mechaniczne jak: nakłuwanie cienką i ostrą igłą, nacinanie bardzo cienką i ostrą żyłką nie powodowały w badanym materiale wystąpienia cytomiksji. Bodźce te działały na bardzo małą powierzchnię, a wytworzona przez nie różnica ciśnień pomiędzy sąsiadującymi komórkami jest niewystarczająca, aby wywołać przepływ protoplastów i wystąpienie figur cytomiktycznych.

5. W ścigniętej skórcie z liści *Ophiopogon* i *Reineckea* cytomijsja jest zjawiskiem masowym i występuje na całej powierzchni skrawka. W zgniecionym korzeniu i pylniku cytomijsja występuje wokół każdej szczeliny powstałej w wyniku rozerwania tkanki pod wpływem nagłego ucisku.

W ścigniętej skórcie przenikanie jąder jest pozornie bezkierunkowe, w zgniecionych korzeniach i pylnikach przeważnie skierowane ku ranie — miejscu nagłego spadku ciśnienia.

6. Cytomijsja jest zjawiskiem błyskawicznym i odbywa się w komórkach jeszcze żywych. Komórki pozbawione części chromatyny są martwe lub szybko zamierają, zaś komórki, do których chromatyna przechodzi, zostają silnie uszkodzone i zwykle także zamierają.

Cytomijsja nie może więc prowadzić do powstawania poliploidów.

7. Jądra przenikają do sąsiedniej komórki poprzez jamki w błonach komórkowych. Wydaje się, że we wszystkich badanych komórkach przemieszczenie to odbywa się zarówno przez pory plazmodesmów, jak i przez większe otwory, powstałe przez rozerwanie błony zamykającej jamkę. Warunkiem wystąpienia cytomijsji jest zatem obecność połączeń plazmatycznych.

W pylnikach *Lilium candidum* nie stwierdzono występowania plazmodesmów pomiędzy komórkami tapetum a mikrosporocytami, nie obserwowano też cytomijsji pomiędzy tymi komórkami.

8. Chromatyna po przejściu przez jamkę albo tworzy zbitą, jednorodną masę, albo zachowuje strukturę właściwą dla jądra komórkowego, z którego pochodzi. Prawdopodobnie struktura przemieszczanej chromatyny zależy od wielkości otworu, przez który została przemieszczona. Przypuszczalnie po przeciśnięciu przez małe pory plazmodesmów chromatyna staje się jednorodna i zbita, przemieszczona zaś przez większe otwory w dnie jamki może zachować strukturę wyjściową.

9. Do sąsiednich komórek mogą przenikać części jąder lub całe jądra komórkowe.

Przemieszczone części jąder mają zwykle postać kuleczek.

10. Jąderka biorą udział w procesie cytomijsji; są przemieszczane przez błony komórkowe częściowo lub w całości, razem z chromatyną jądra komórkowego.

11. W tkankach wegetatywnych cytomijsji podlegają zarówno jądra spoczynkowe, jak i jądra we wszystkich stadiach mitozy (przemieszczanie chromosomów).

W mikrosporocytach lilii cytomijsję stwierdzono od stadium przedmeiotycznego do metafazy I włącznie. Przed mejozą i w preleptotenie cytomijsja występuje rzadko, ilość figur cytomiiktycznych stopniowo wzrasta w leptotenie i zygotenie, a przy końcu zygotenu i na początku pachytenu osiąga maksimum. Od tego stadium ilość występujących figur wyraźnie maleje; w późnym diplotenie i metafazie I cytomijsja jest już zjawiskiem sporadycznym.

12. Zabiegi mechaniczne, wyzwalające cytomijsję, wykonane na splazmolizowanych korzeniach cebuli, oraz doświadczenia z plazmolizą graniczną w mikrosporocytach lilii wskazują na istotną rolę wielkości ciśnienia, jakie protoplast aktualnie wywiera na błonę komórkową.

W splazmolizowanych, a później zgniecionych korzeniach cytomijsja jest zjawiskiem sporadycznym lub zupełnie nie występuje.

Wartość osmotyczna soku komórkowego w mikrosporocytach ulega zmianie w poszczególnych stadiach mejozy. Stwierdzono, że ilość otrzymywanych figur cytomiiktycznych (por. p. 11 wniosków) wzrasta wraz ze wzrostem wartości ciśnienia osmotycznego, zaś spadkowi ciśnienia towarzyszy spadek ilości występujących cytomijsji.

13. Biorąc pod uwagę powodujące cytomijsję działanie znacznej różnicy ciśnień pomiędzy sąsiadującymi ze sobą komórkami, nagle powstanie dróg prowadzących do wyrównania tej różnicy, oraz fakt przeciskania części protoplastu poza komórkę, zjawisko cytomijsji, zgodnie z sugestiami Küster'a (1956, 1958), można traktować jako swego rodzaju plazmoptyzę.

14. Analiza przyczyn wywołujących cytomijsję oraz jej skutki, pozwalają stwierdzić, że jest to zjawisko anormalne, o charakterze patologicznym.

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