Endosperm, material for study on the physiology of cell division

ENDOSPERM: MATERIAŁ DO BADAŃ NAD FIZJOLOGIĄ PODZIAŁU KOMÓRKI

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STRESZCZENIE

Mimo licznych badań, dotychczas jest znanych bardzo mało obiektów odpowiednich do badań in vivo nad mitozą i pokrewnymi problemami. Ważne więc jest znalezienie materiału lub metody bardziej ogólnej, tzn. udostępnienie do badań in

vivo większej ilości roślin. Takim materiałem może być endosperma. Zalety endospermy, jako materiału do badań in vivo były omówione poprzednio (B a j e r 1953b). W celu stwierdzenia czy bielmo większej ilości roślin nadaje się do badań i w jakim stopniu, wykonane zostały badania nad endospermą jedno i dwuliściennych, przy czym szczególną uwagę zwrócono na jednoliścienne. W czasie pracy przebadano porównawczo mitozę in vivo u około 20 rodzajów.

Używano metody opisanej dokładnie w poprzedniej pracy (B a j e r 1953b). Metoda ta jest zadawalniająca do obserwacji podziału od metakinezy do telofazy u prawie wszystkich roślin, natomiast u części obiektów profazy nie są kontynuowane w tych warunkach. Metoda ta może być jednak znacznie udoskonalona. Najprawdopodobniej dostarczenie tlenu, który wydaje się niezbędnym do normalnego rozwoju profazy umożliwi u takich roślin obserwację mitozy od wczesnej profazy do jąder spoczynkowych. Wskazuje na to endosperma *Haemanthus*, w którym po dostarczeniu tlenu normalna mitoza jest kontynuowana co najmniej przez 24 godz. (B a j e r 1954). Wśród badanych roślin u *Iris*, *Leucojum*, *Zephyranthes* i *Pisum* ponad 90% komórek w warunkach beztlenowych kontynuowało mitozę od wczesnej profazy do telofazy bez śladów zaburzeń. Powyższe obiekty uważane są za najłatwiejsze z przebadanych. Ponad to łatwymi są: *Ornithogalum*, *Asparagus*, *Agapanthus*, *Haemanthus*, *Helianthus*, natomiast niezwykle trudnymi: *Lupinus*, *Veratrum*, *Vicia* i *Borago*. Jedną z głównych trudności w tych ostatnich obiektach jest znalezienie mitozy. W pracy podano wykresy ruchu chromosomów w anafazie oraz Tabele ilustrujące przebieg mitozy w różnych obiektach. Okazało się, że czas anafazy — stadium, które było najdokładniej obserwowane, jest tym krótszy im mniejsze są chromosomy. We wszystkich obserwowanych obiektach znaleziono różny stopień działania „Stemmkörper“.

Jednoliścienne okazały się materiałem lepszym i łatwiejszym niż dwuliścienne, jakkolwiek niektóre obiekty z tych ostatnich, nie są trudne (np. *Pisum* i *Helianthus*) i nadają się do bardziej szczegółowych badań. W pracy podano bardzo dokładnie uwagi metodyczne, oraz podano wady i zalety endospermy. Autorzy uważają, że otrzymane wyniki uprawniają do wysunięcia wniosku, że endosperma jest najlepszym materiałem do badań in vivo mitozy u roślin i zasługuje na używanie go do badań na większą skalę.

INTRODUCTION

The plants in which mitosis in vivo can be observed are very few. B e c k e r, (1938) and R o s k i n (1951) mention about 20 species, but even so, most of these species are difficult to prepare and their course of cell division is hardly visible. Also the results obtained with some species are not necessarily valid in the case of most species. E. g. the nuclear membrane which persists throughout mitosis in *Osmunda* and *Tradescantia* (W a d a 1942, 1950), seems also to appear in *Hymenophyllum* (B a j e r and M o l è - B a j e r 1953), but has not been found in about 30 species of those listed in Table I. To make possible detailed study the material chosen should have adequate properties, such as e. g.: convenient dimensions of the chromosomes (for studies on the chromosome structure chromosomes should be large), suitable distance of chromosome se-

paration, or duration of mitosis. Also for studies of chondriosomes, primary cell wall formation or nucleoli behaviour, special material must be chosen. In our opinion the endosperm is the material most suitable for many types of experimental studies. It is of great importance in quantitative studies on the influence of different chemical and physical factors on the course of cell division, as in one preparation as many as 50—300 mitoses can be observed. Experiments with the endosperm of *Haemanthus* (B a j e r 1953, a, b, 1954) have shown the great advantages of the endosperm as material for investigations. Also these investigations have been undertaken to demonstrate the endosperm as material for studies *in vivo*. Therefore our aim has been to:

1. study *in vivo* the properties of the endosperm from numerous species (mono and dicotyledons),
2. elaborate simple methods adequate for any kind of material,
3. make preliminary comparative observations on the course of mitosis in the endosperm of different species.

MATERIAL AND METHODS

M a t e r i a l. Detailed notes on material are given together with the results of observations on each species. All the material studied was obtained from the Botanical Garden and Plant Breeding Institute of the Jagellonian University. In Table I the investigated species together with some of their properties are listed.

M e t h o d s. The method used is the same as that described in detail in a previous paper (Bajer 1953): a vaseline ring is drawn on a ungreased glass square, the area within ring is smeared with an agar and glucose solution, then the endosperm with the liquid surrounding it is pressed on, and the whole is covered with a second glass also smeared with agar and glucose; the edges of the two glasses are made secure with liquid paraffine. This simple method, very quick and convenient, is suitable for all preliminary and many detailed studies. In the case of *Haemanthus*, studied previously, and some other species (cf. below) division often stops at prophase and cells die. This is probably caused by the lack of oxygen, which according to B u l l o u g h's (1952) studies is necessary in prophase. It has been found in the case of *Haemanthus* (B a j e r 1954) that, if the normal hanging drop is used (the drop must be very flat and thin) and the drop is placed in pure oxygen, mitosis develops even from very early prophase in more than 90 per cent of cells, and cell division may have a normal course as late as 24 hrs. after preparation. It is most probable that similar results will be obtained with this improved method in the case of those plants described here in which division is interrupted at prophase. The general rules for distinguishing the cells injured during preparation are to be found in an earlier paper (B a j e r 1953b).

Most graphs of the chromosome movement are plotted in the manner described in detail in a previous paper (B a j e r 1950), and some are plotted from cine-micrographies or microphotos.

TABLE I

List of investigated species. Material valuable and easy to handle marked by *, ? — the concentration was not established. Chromosome numbers according to *Darlington* and *Janaki Ammal* (1945)

Name of species	Chromosome number (2x)	Agar with glucose concentration in %	Suitability for experiments		
			Approximate date	Number of weeks after flowering	Number of weeks it lasts
* <i>Ornithogalum umbellatum</i>	27	0,5; 3	10 May	2—3	4
* <i>Ornithogalum nutans</i>	30	?	15 May	2—3	4
<i>Veratrum nigrum</i>	64	0,5; 3	20 Aug.	3	4
<i>Veratrum album</i>	?	?	10 Aug.	3	4
<i>Funckia subcordata</i>	60	0,5; 3	25 Jul.	4	5
* <i>Asparagus pseudoscaber</i>	?	0,5; 4	1 Jun.	3—4	5
* <i>Asparagus officinalis</i>	20	?	1 Jul.	3—4	5
<i>Agapanthus umbellatus</i>	34	0,5; 3	1 Aug.	3—4	3
* <i>Leucojum aestivum</i>	22	0,5; 3	15 Jun.	5—6	3
* <i>Zephyranthes candida</i>	38	0,4; 3,5	1 Sept.	3	4
* <i>Haemanthus katherinae</i>	18	0,4; 3,5 0,5; 3,5	1 Aug.	3—4	8
* <i>Haemanthus puniceus</i>	?	0,4; 3 0,5; 3	25 Jul.	3—4	3
* <i>Haemanthus albiflos</i>	16	?	25 Aug.	3—4	3
<i>Narcissus poeticus</i>	14, 21, 28	?	15 Jun.	3—4	4
* <i>Clivia miniata</i>	22	?	Feb., Aug.	3—4	4
* <i>Clivia nobilis</i>	22	?	Feb., Aug.	3—4	4
* <i>Iris xiphioides</i>	32	0,5; 3,5	20 Jun.	4	3
<i>Iris prismatica</i>	42	?	15 Jun.	3—4	3
* <i>Iris pumila</i>	32, 36	?	25 May	3—4	3
<i>Iris sibirica</i>	?	0,5; 3	15 Jun.	3—4	3
<i>Iris germanica</i>	44	?	15 Jun.	3—4	3
<i>Iris florentina</i>	24	?	25 Jun.	3—4	3
* <i>Iris aphylla</i>	24, 48	0,5; 3,5	25 May	3—4	3
<i>Iris interregna</i>	?	0,5; 3-3,5	10 Jun.	3—4	2
<i>Secale cereale</i>	14	?	15 Jun.	few days	few days
<i>Peonia officinalis</i>	10, 20	?	1 Jun.	3—4	3
* <i>Pisum sativum</i>	14	0,5; 4,5 0,5; 5	1 Jun.	3—3	10
<i>Vicia faba</i>	14	0,5; 5,5	1 Jun.	3	10
<i>Lupinus polyphyllus</i>	48	0,5; 4	1 Jun.	3—4	12
<i>Borago officinalis</i>	16	0,5; 3	1 Aug.	3	4
* <i>Helianthus annuus</i>	34	0,5; 4	25 Jul.	4	8

NOTES ON HANDLING THE ENDOSPERM IN VIVO

1. The phase contrast illumination in inversion microscope is most convenient.
2. Ungreased cover slips should be used. The agar with glucose used for smearing the glass should not be too warm so as not to melt the vaseline ring.
3. Usually 0,4—0,5 per cent agar and 3—6 per cent glucose concentrations are used. Stronger agar concentrations cause the liquification of cytoplasm and of mitotic spindles. The endosperm cells of some species are sensitive to 0,5 per cent differences of the concentration of glucose and mitosis is continued only within sharply defined limits of concentrations. In endosperm of other species mitosis may also develop in pure 0,4—0.5 per cent agar solution (without glucose). The supply of oxygen seems to diminish the sensitivity of cells to light and mechanical injuries.
4. Hypotonic agar-glucose solutions usually cause: decrease in the refraction index in chromosomes, liquefaction of cytoplasm, destruction of the spindle (vigorous Brownian movements), formation of vacuoles, as well as the swelling and disappearance of chondriosomes.
5. The hypertonic agar-glucose solutions usually cause: increase in the refraction coefficient in chromosomes, stiffening of the spindle, interruption in the formation of cytoplasmic amoeba-like protrusions (in young endosperm and often cell coagulation).
6. In most cases a well formed embryo indicates that the endosperm is too old. In monocotyledons the endosperm is suitable for study when in the embryo sac there is either no embryo or when embryos are very small; in dicotyledons the suitability of endosperm is indicated by the presence of small cotyledons.
7. Young endosperm is more suitable for experiments.
 - a) The characteristic features of young endosperm are: amoeba-like protrusions form vigorously, the chondriosomes are well visible and the starch grains are either small or there are none. Amoeba-like protrusions are formed most vigorously in prophase and in telophase while during metaphase and anaphase such cells are often rounded. The too young endosperm is flaky, mitoses are few and are highly synchronised i. e. prophase, metaphase and anaphase occur more or less at the same time. Individual cells in very young endosperm are rarely found. The substance from embryo sacs with young endosperm suitable for study is colourless and has a delicate whitish suspension of endosperm cells.

- b) The characteristic features of old endosperm are: cells are usually rounded, starch grains are numerous and during mitosis usually surround the spindle poles, amoeba-like protrusions form rarely. In some species among old endosperm young cells are sometimes found (e. g. *Iris*). The endosperm in some species grows old slowly and in others rapidly, and it may remain young some weeks or a few days only. The liquid of embryo sacs with old endosperm is white or whitish.
8. Mitoses are more easily found in endosperm with large or medium than small chromosomes. It seems also probable that in some endosperms there is periodicity of mitosis (day and night).
 9. Small embryo sacs are more difficult to handle than large ones. If the quantity of embryo sac liquid is small, the liquid from several embryo sacs may be mixed together.
 10. During hot days at noon mitoses are often few. In fruits kept in a Petry dish covered with damp blotting paper for 1—2 days mitoses are often more numerous than on the day they are cut off.

The above is generally true, though in the case of some species, there may be exceptions.

NOTES ON MONOCOTYLEDONS

Numerous species are very suitable for studies on endosperm *in vivo*, especially those from the *Liliaceae*, *Amaryllidaceae* and *Iridaceae* families, and also probably several species from the *Araceae* family. The *Gramineae* are less convenient for experiments because the endosperm from this family grows old very quickly and very soon numerous starch grains appear in it, rendering the observations very difficult.

Plants containing etherous oils are not suitable. We have not succeeded in obtaining the continuation of mitosis in the different *Allium* species (*A. schoenoprasum*, *A. odorum*, *A. cepa*) and it seems that the oils prevent mitosis. In any case the course of mitosis in endosperm of these species is not really noteworthy.

Theoretically all species in which free cell wall formation has been reported may be used for experiments. However, in practice it appeared that all species with very small embryo sacs are very difficult to prepare. E. g. *Lilium*, and *Tulipa* embryo sacs contain small quantities of liquid which makes them difficult to handle, though they are very probably otherwise convenient for study. The method used in the present work gives good results in studies on mitosis from metaphase to telophase, but very often is not satisfactory when prophase is to be observed. In the

latter case the hanging drops in oxygen, used with such good results on *Haemanthus* will probably be more convenient.

In the endosperm of monocotyledons studied during this work the course of mitosis is regular and usually very distinctly visible. In all the monocotyledoneous material which we have studied multiploid nuclei have been observed only very exceptionally.

Ornithogalum umbellatum L.

M a t e r i a l. Suitable embryo sacs are 3.5—4 mm long, greenish, slightly transparent with a delicate sculpture on the surface. The embryo sacs are contained in green fruit approximately 20 mm long and 10 mm in diameter. Embryo sacs from the same fruit, or even from the same fruit chamber may be of different age. Old cells are found in a whitish liquid, young ones in a colourless liquid with a whitish suspension. Mitoses are found both in fruits cut off on the day of the experiment, or 1—2 days earlier. 50—70 per cent of cells continue division from prophase to telophase, also numerous cells begin and finish mitosis after preparation. Few cells are injured during preparation.

O b s e r v a t i o n s. In young endosperm amoeba-like protrusions are formed vigorously. Often the process of the formation of a protrusion may be traced in detail; protrusions are not found in old endosperm. The structure of the cytoplasm is „coarse“ and the cell surface seems to be slightly coagulated. Old cells contain numerous starch grains, agglomerating during mitosis mainly at the poles of the spindle. Chondriosomes are visible neither in young nor in old endosperm. The course of mitosis is not visible as clearly as in *Iris*, *Leucojum* or *Haemanthus*.

Resting nuclei are of the *Tradescantia* type, but the structure is less distinct here. 3—10 nucleoli clearly coacervate in nature are to be found. (Fig. 10). The connection between nucleoli formation and chromosome arms is well visible. The course of mitosis is regular and similar to that found in other objects. The time the different stages of mitosis lasts is given in Table II. In numerous normal spindles small particles inside the spindle are seen. The intensity and changes of Brownian movements are similar to those observed in *Haemanthus* (B a j e r 1953a). The course of anaphase has been carefully observed and is illustrated by graphs (Fig. 1) and Fig. 9 (A—G). The action of the pushing body is usually slight. As the outlines and the poles of the spindle are not sharply defined, the acceleration of the chromosome movement, which 5—10 mins after the beginning of anaphase begins, is the best proof of the pushing body action. Inside the pushing body or on its surface fibers are often visible. It is possible that some of these fibers are interzonal connections. Two kinds of primary cell wall formation are to be observed: from the centre or from the outer parts of the phragmoplast. In cells in which the pushing

Fig. 1. *Ornithogalum umbellatum*. Curves of chromosome movement and spindle elongation (spl) in anaphase. Curves of two sister chromosome groups and resultant curves. In anaphase of cell No. 55 length of the spindle does not change. The band in the curve (cell No. 43) is caused by pushing body action. Note differences in shape of curves for these two cells.

body is active the cell wall is formed later than in cells where such action is not noticeable. The formation of the cell wall lasts several minutes. Chromosomes are of medium size and in prophase single chromosomes are difficult to distinguish. In prophase the at least double, and from metakinesis on the at least quadruple, chromosome structure is well visible. The process of longitudinal splitting of chromosomes before anaphase may be distinctly traced. In metaphase kinetochores are in one plain and the mechanism of the beginning and of the course of anaphase may be exactly observed in numerous cases.

TABLE II

Mitosis in *Cnithogalum umbellatum*

Mk — metakinesis, M — metaphase, A — anaphase, A₀ — beginning of anaphase, Phst — phragmoplast, 1 — late. Times measured to the beginning of the next stage.

No of cell	Duration in min. of		Appearing of nucleoli after A ₀ in min.	Formation of primary cell wall in Phst		Spindle length in μ		Max. separation of chromosomes in A in μ	Notes
	Mk ₁ and M	A		beginning of formation, min. after A ₀	formation in min.	M	A ₁		
7	—	15	55	20	4	37	50	47	strong action of pushing body, cell wall is formed from outer parts of Phst
18	—	22	48	27	2	32	48	46	
21	—	22	62	27	—	35	—	43	
33	15	15	64	16	2	31	36	35	
43	15	22	49	—	—	40	55	49	
50	20	22	54	22	2,5	32	40	37	strong action of pushing body
54	—	22	48	23	2	—	43	36	
57	—	19	70	22	1,5	37	46	37	

Veratrum nigrum L.

Material. A suitable fruit is 6—10 mm long, and contains small flying seeds. Embryo sacs are small and the liquid must be gathered together from 3—6 of them. Embryo sacs suitable for endosperm studies are whitish, half — transparent, and contain colourless liquid. In the white liquid old cells are found. 30—80 per cent of cells continue mitosis after preparation. Strong light seems to injure the cells and stop mitosis. It is probable that a sufficient supply of oxygen would increase the percentage of dividing cells. This material is rather difficult to handle.

Observations. Cells are very small, and the refraction coefficient of cytoplasm is higher than in other object. In old cells numerous starch grains are visible in the cytoplasm and prevent the observations of mitosis. In young cells amoeba-like protrusions are formed. The chromosomes are very small and the course of mitosis is difficult to trace distinctly, e. g. it is difficult to distinguish prophase from metakinesis. Chromosomes are smaller than in *Pisum* but slightly larger than in *Lupinus*. In anaphase strong pushing body action is visible in almost all cells. Metaphase lasts about 10 mins, anaphase less than 10 mins. If the cell

is illuminated anaphase is considerably prolonged. In resting nuclei 1—2 nucleoli are found. Owing to the smallness of nuclei it is difficult to describe their structure. Figs 12 (A—F) illustrate this material and the course of anaphase.

Funckia subcordata Spreng.

Material. The suitable fruit is 30—35 mm long and 8—10 mm in diameter. The seeds are small and flying. Suitable embryo sacs are 5—6 mm long, whitish-pink, and contain small quantities of colourless liquid, and so 2—3 of them must be pressed together. It is difficult to define the most appropriate stage, though among the old cells some young ones may be found. 40—70 per cent of cells continue the division from prophase to telophase.

Observations. Mitosis may be observed in young cells only; in old ones it is difficult to observe the chromosomes and the spindle as they are hidden by very numerous starch grains. In many young cells starch grains also appear. Young cells form vigorously amoeba-like protrusions. The cytoplasm structure is similar to that in *Ornithogalum* and chondriosomes are not visible. The intensity of the Brownian movement indicates that the viscosity index of the spindle is only slightly higher than in *Ornithogalum* and *Iris*. The durations of different stages of mitosis are similar to those in *Iris* and the course of mitosis most resembles *Ornithogalum* mitosis. Chromosomes are numerous and of medium size. Figs. 11 (A—C) illustrate the cells of *Funckia* endosperm.

Asparagus pseudoscaberrimus Bl.

Material. A suitable fruit is green, ball-shaped, and about 5 mm in diameter. Preparation of embryo sacs is difficult. Cells are sensitive to mechanical stimuli and very numerous cells are injured during preparation. Those not damaged continue in 70—95 per cent to divide from prophase to telophase and probably enter and finish mitosis after preparation. Mitoses are easily found, though not numerous, in not more than 20 cells from each embryo sac after preparation.

Observations. Cells are small, usually very regular, ball or ellipsoid in shape. The formation of amoeba-like protrusions has not been observed. Cells are not fixed in agar which prevents the observations of numerous cells with an immersion lens, if an ordinary and not an inversion microscope is used. Numerous cells change their position which makes the observations more difficult. The cross section through the spindle is a circle, which has been confirmed by observations of metaphase and anaphase from the pole (Fig. 14). The high refraction coefficient of the chromosomes and the uniform structure of the cytoplasm make the course of mitosis very plainly visible. In young cells the cytoplasm contains no

TABLE III

Mitosis in *Asparagus pseudosaber*

P — prophase, Mk — metakinesis, M — metaphase, A — anaphase, A₀ — beginning of anaphase, Phst — phragmoplastast, l — late. Times measured to the beginning of the next stage.

No of cell	Duration in min. of			Appearing of nucleoli after A ₀ in min.	Formation of primary cell wall in Phst		Spindle length in μ		Max. separation of chromosomes in A in μ	Notes
	P	Mk ₁ and M	A		beginning of formation, min. after A ₀	formation in min.	M	A ₁		
204	40	10	14	—	—	—	29	22	22	cell wall is formed after several hours
208	35	—	8	—	25	—	—	—	20	
209	50	(MonIy) 6	10	60	20	3	18	25	23	
203	50	—	10	50	24	3	18	25	23	

starch grains. Chondriosomes are not to be seen. Varying numbers of nucleoli (6—14) are found in resting nuclei (Fig. 15). Cells are small and chromosomes numerous. Mitosis is quick and from prophase to telophase lasts 1.5—2 hours. The formation of the spindle in late prophase and the contraction stage resembling that of *Haemanthus* (B a j e r 1954) may be traced very distinctly. Spindle formation lasts 10—15 mins, metakinesis and metaphase 15—20 mins anaphase less than 12 mins, formation of cell wall does not last longer than 3 mins, though usually it takes place several mins after the end of anaphase. Table III illustrates the durations of different stages of mitosis, the course of anaphase the graphs on Fig. 2 and Fig. 11 (A—I). The pushing body action in anaphase is considerable. Chromosomes change their shape during some minutes, and the pushing body acts on chromosomes already partly changed. The first stage of resting nuclei formation is marked by the appearance of clear space with no chromosomes around the sister nuclei. The changes and the intensity of the Brownian movement are of the same rate as in most other objects.

Agapanthus umbellatus var. *minor* (*Agapanthus africanus* (W i l d.) W h e n. ssp. *praecox*).

M a t e r i a l. A suitable fruit is 20 mm long with white flying seeds. Grey and black fruit are too old. The suitable embryo sac liquid is colourless with a whitish suspension. 79—90 per cent of cells in prophase die in late prophase, metakinesis or metaphase. More than 90 per cent of those cells which after preparation are in metakinesis or metaphase continue their division to resting nuclei.

Fig. 2. *Asparagus pseudoscaberr*. Curves of chromosome movement and spindle elongation (spl) in anaphase. Curves for two sister chromosome groups, for two half spindles and resultant curves. Anaphase curve for cell No. 209 plotted from microphotos. Pushing body begins to act almost immediately anaphase begins; there are no bands in the curves.

Observations. *Agapanthus* cells are of middle size and their cytoplasm structure is similar to *Ornithogalum*. Young cells form vigorously amoeba-like protrusions, and in old ones numerous starch grains are present. In resting nuclei usually 2 very large nucleoli are found. Usually chondriosomes are not visible. The structure of the resting nuclei is similar to that in *Ornithogalum*. The duration of the different mitotic stages resembles those in *Iris*, however the time of metaphase and anaphase seems to vary greatly in different cells. The action of the pushing body is evident. The formation of primary cell wall has been observed from the centre of the phragmoplast only. Chromosomes are numerous and their dimensions similar to those in *Iris*. Graphs in Fig. 3 and Figs. 16—17 illustrate the course of anaphase in *Agapanthus* endosperm.

Zephyranthes candida H e r b.

Material. Fruit with suitable embryo sacs are about 5 mm long, and the length of embryo sacs is 3—4 mm. The embryo sacs are covered with very

Fig. 3. *Agapanthus umbellatus*. Curves of chromosome movement and spindle elongation (spl) in anaphase. Curves for two sister chromosome groups, for two half spindles and resultant curves. Anaphase curves for cell No. 334 plotted from microphotos. Action of the pushing body begins in early (cell No. 384) or in middle (cells No. 382, 389) anaphase. The pushing body causes the elongation of the spindle in one direction only and the whole anaphase spindle is shoved in one direction (to the lower part of the graph). Bands on the curves are caused by pushing body action.

delicate sculpture. Preparation is easy. Owing to the low fertility of this species artificial pollination is necessary. About 90 per cent of prepared cells continue division from early prophase. It is also probable that numerous cells enter and finish mitosis after preparation. Mitoses are easily found, and the material is undoubtedly valuable.

Observations. Cells are medium in size and in young ones amoeba-like protrusions are formed. The cytoplasm structure resembles greatly that in *Iris*, and mitosis may be observed very exactly from prophase to telophase. Mitosis develops similarly as in *Iris aphylla*, and the course of mitosis is perfectly normal. The times of different stages of mitosis are given in Table IV, and the course of anaphase is illustrated by Figs. 19—20 (A—F). The action of the pushing body is to be seen in all observed cells. Cell walls are formed both from the centre and from the edges of the phragmoplast. The structure of the resting nucleus is similar to that in *Iris*, but the nucleus contains usually 2 nucleoli.

TABLE IV

Mitosis in *Zephyranthes candida*

P — prophase, Mk — metakinesis, M — metaphase, A — anaphase, A₀ — beginning of anaphase, Phst — phragmoplast, 1 — late. Times measured to the beginning of the next stage.

No of cell	Duration in min			Appearing of nucleoli after A ₀ in min	Formation of primary cell wall in Phst		Spindle length in μ		max. separation of chromosomes in A in μ	Notes
	P	Mk ₁ and M	A		beginning of formation, min after A ₀	formation in min	M	A ₁		
400	—	—	26	90	30	5	22	37	30	
401	120	15	18	—	—	—	23	30	28	cell wall did not form
402	120	15	25	—	—	—	22	25	23	Mk lasts 30 min.
405	90	30 40	26	—	35	8	—	—	—	

Narcissus poeticus L.

This material is useful though only the fertile varieties are practical. Chromosomes are of medium size (similar to *Iris aphylla*), and mitosis is distinctly visible. However, it seems that oxygen is necessary for division, therefore experiments should be carried out with the method providing a free supply of this gas (cf. methods).

Haemanthus puniceus

Material. Embryo sacs of *H. puniceus* are prepared in the same way as in *H. katharinae* and as it has been described in a previous paper (Bajer 1953b). Most cells of *H. puniceus* die in prophase and to observe division from prophase the hanging drop in pure oxygen should be used. From metakinesis on 70 per cent of cells — which is less than in *H. katharinae* — continue division to telophase with no oxygen supply. This material is easy to prepare and observe, and very valuable for numerous experiments.

Observations. The course of mitosis, the duration of different stages, and the differentiation of chromosomes of *H. puniceus* resemble greatly those of *H. katharinae* and it is difficult to distinguish them in vivo. Similarly as in *H. katharinae* mitosis may be followed through in detail, and it is possible to observe the same chromosome from prophase to telophase. Also here, as in *Leucojum* and *H. katharinae* or *H. albiflos*, the uncoiling of chromatids is very plainly visible. The stages of mitosis last longer than in *Leucojum*.

Young cells should be used for study because in old ones starch grains impede observations. Fig. 21 illustrates a small and young cell of *H. puniceus* in metaphase and anaphase.

Leucojum aestivum L.

Material. The fruit of suitable age is approximately 30 mm long and 15 mm in diameter. Embryo sacs are dull white (those whitish-yellow are too old) slightly transparent, and about 7 mm in diameter. They contain much liquid. When pressed out the liquid contains at first no cells, they appear finally as a delicate whitish suspension. Mitoses are easily found even 3—5 days after the fruit is cut off and placed in a Petry dish on damp blotting paper. Petry dishes with fruit ought to be placed in running tap water. A small number of cells is injured during preparation, but the material seems to be sensitive to mechanical stimuli. About 90 per cent of cells after preparation continue the division from early prophase to late telophase. Some cells slightly injured during preparation also continue mitosis. After preparation on one slide 100 or more mitoses may be found. This material is very easily and conveniently handled and it has many advantages.

TABLE V
Mitosis in *Leucojum aestivum*

P — prophase, Mk — metakinesis, M — metaphase, A — anaphase, A₀ — beginning of anaphase, Phst — phragmoplast, 1 — late. Times measured to the beginning of the next stage.

No of cell	Duration in min of			Appearing of nucleoli after A ₀ in min.	Formation of primary cell wall in Phst		Spindle length in μ		max separation of chromosomes in A in μ	Notes
	P	Mk ₁ and M	A		beginning of formation, min after A ₀	formation in min	M	A ₁		
175	90	35	35	120	45	5	60	70	65	
179	—	25	30	105	—	—	42	55	50	
180	—	only M 10	43	—	44	4	60	74	70	
182	—	30	39	160	34	8	40	49	46	
—	—	only M 15	35	—	35	4	50	70	57	

Observations. In young cells the course of mitosis may be traced with many details. Cells are very large and amoeba-like protrusions are vigorously formed also in old cells. In old cells numerous starch grains make the observations of mitosis more difficult. Resting nuclei containing 1—2 nuclei are of the *Tradescantia* type and their structure is plainly visible. In numerous cells, mainly the young ones, chondriosomes are found (cf. Fig. 22). Chromosomes are very long and large and do not seem to be differentiated. The course of mitosis is very regular and similar to that found in other objects. In late prophase just before metakinesis the stage of mitotic contraction (Bajer 1954) may be observed very distinctly. Mitosis develops slowly, and prophase lasts more than 2 hours, metaphase at least 20, and anaphase at least 35 minutes. In Table V the time of the different stages of mitosis is given, and the graphs on Fig. 4 and Figs. 23—24 illustrate the course of anaphase

Fig. 4. *Leucojum aestivum*. Curves of chromosome movement and spindle elongation (spl) in anaphase. Curves for two sister chromosome groups, for two half spindles and resultant curves. Action of pushing body begins in middle anaphase. Chromosomes are separated much farther than in any other object (cf. distances in other graphs).

and telophase. In many cells the action of the pushing body is prominent and begins usually in middle anaphase. The formation of phragmoplast and the changes in it during primary cell wall formation resemble those described in *Haemanthus* and may be clearly observed. Primary cell wall formation begins from the edges or from the centre of the phragmoplast. Also the formation of phragmoplasts and primary cell walls may be observed between nuclei which are not daughter nuclei. In daughter nuclei nucleoli are often placed symmetrically and form as mirror reflections. Inside the spindle Brownian movements of small particles may

be observed throughout mitosis and are similar in intensity to those described in *Haemanthus* (Bajer 1953a).

Double structure of chromosome is seen in prophase, it is at least quadruple in metaphase, and double in anaphase. The contraction of chromosomes begins in anaphase, which may be excellently observed if cine-micrography is used. Also with the use of this method the unwinding of the coiled chromatids may be traced in detail. The structure of the kinetochor resembles those in *Haemanthus* and in metaphase kinetochores are arranged exactly in one plane.

Clivia sp.

Most species seem to be excellent material. Mitosis is very distinctly visible, chromosomes are large (cf. Fig. 26), and chondriosomes may be observed in numerous cases. Oxygen seems to be indispensable in pro-phases. This material is most valuable for numerous experimental studies. It is also of importance that these species flower twice a year (winter and summer).

Iris aphylla and others irises

M a t e r i a l. All fertile species are suitable for experimental studies and the best are those not too fertile. Highly fertile and completely pollinated plants develop small embryo sacs with little liquid. Such embryo sacs are more difficult to prepare than large or average ones. Regularly shaped and not deformed embryo sacs are best. Hybrids e. g. *I. interregna*, are usually half sterile and therefore not convenient, though they have large embryo sacs. Although all fertile species are suitable, such species as *I. sibirica* (Figs. 27—28), *I. graminea*, *I. prismatica* have small embryo sacs and are much more difficult to prepare. Embryo sacs are ready for experiments 3—5 weeks after flowering. The liquid containing endosperm cells ought be colourless with a delicate whitish suspension. White or yellow liquid and well developed embryos indicate that the endosperm is too old. In old endosperm, however, young cells may also be found. Usually even if the embryo sac liquid containing endosperm is pressed out very hard, cells will not be injured and will continue mitosis from early prophase to telophase. In carefully prepared preparations more than 95 per cent of dividing cells form resting nuclei, and numerous cells enter and finish mitosis after preparation. It is also remarkable that mitosis may be continued when the structure of the cytoplasm is completely destroyed, which is often observed in slightly injured cells or in hypotomic solution (Fig. 33). Cells seem to be resistant to mechanical and physical factors and in the same species of the *Iris* genus mitosis may continue in different concentrations of agar glucose e. g. *I. aphylla* and *I. xiphioides*. It will be remembered that in most of the other genera mitosis ceases when closely defined narrow limits of agar and glucose concentrations are exceeded. It is remarkable that in genus *Iris* the concentrations of agar and glucose in which the prepared cells best continue their division differ in even closely related species.

The endosperm of *Iris* species is useful in experimental studies of mitosis from prophase to telophase, on chromosome movement, primary cell wall formation, and

in studies on the action of different physical and chemical factors on mitosis in vivo. Chromosomes are of medium size and indistinctly visible in prophase. They are not convenient for investigating the chromosome structure, though such studies are possible on this material. The great advantage of *Iris* species is their different time of flowering, in consequence of which experiments with the endosperm may be continued for more than 2 months.

In most irises mitoses are easily found, both by day and by night, in embryo sacs cut off 2 or even 5 days earlier. Often in one preparation more than 100 mitoses may be found.

TABLE VI

Mitosis in *Iris*

P — prophase, Mk — metakinesis, M — metaphase, A — anaphase, A₀ — beginning of anaphase, Phst — phragmoplast, e — early, m — middle, 1 — late. Times measured to the beginning of the next stage.

No of cell	Duration in min of			Appearing of nucleoli after A ₀ in min	Formation of primary cell wall in Phst		Spindle length in μ		max. separation of chromosomes in A in μ	Notes
	P	Mk ₁ and M	A		begining of formation, min after A ₀	forma- tion in min	M	A		
<i>Iris aphylla</i>										
83	—	only M 15	20	45—55	26	3	18	32	25	A ₀ after 2h 30 min normal resting nuclei
85	—	30 (M 10)	12	50—60	13	2	23	30	24	mitotic spindles joined
91	ve 65	only M 12	19	—	19	—	28	28	22	
94	e 55	—	19	56	27	5	21	26	22	
96	e 55	20	—	60	—	—	24	—	—	
97	e 55	18	15	75	30	—	26	31	25	
100	m 15	20	22	54	33	8	22	24	22	
107	—	23	11	65	24	6	25	33	30	
132	e 45	15	—	50	—	—	—	36	34	2 h 40 min after A ₀ resting nuclei
133	e 45	25 (M 12)	17	40	—	—	32	38	36	
134	e 40	25	21	—	40	—	32	40	37	..
<i>Iris sibirica</i>										
250	e 40	(only M) 10	15	15	—	—	22	30	26	cell wall did not form
250	m 40	30	—	70	—	—	—	—	28	..
253	e 60	25	17	—	—	—	—	20	—	..
254	—	30	14	—	—	—	18	20	17	..

Observations. In all studied irises the course of mitosis is very similar. Cells are much alike and differ mainly in chromosome and nuclei dimensions, and the number of nucleoli. These differences are of no importance.

The course of mitosis here described is from *Iris aphylla*. The dimensions of cells with resting nuclei, the dimensions of nuclei and the number of nucleoli vary (3—17 nucleoli have been observed). Young cells are most suitable for experimenting. They form differently shaped amoeba-like protrusions (cf. Plate VII).

The formation of these protrusions often lasts several seconds and is easily observed especially if cine-micrography is used. In old cells numerous starch grains always appear round the nuclei or the mitotic spindle. Cytoplasmic structure is uniform and subtle. Vacuoles are either very small or there are none. Large vacuoles are a sign of demixing, and when demixing occurs cytoplasmic structure is usually destroyed and Brownian movements become very vigorous. Chondriosomes are very distinctly visible as long thin threads assembled mostly round the nuclei or the spindle, mainly at the poles.

The structure of resting nuclei is indistinct, through some seem to have a structure resembling *Tradescantia* nuclei.

In the early stages of prophase, changes in chromosomes are difficult to observe while inside the spindle all processes beginning with the contraction stage, which is very typical, may be traced in their finest details. Often however, single chromosomes may be distinguished already in prophase and their changes may be followed. The disappearance of nucleoli lasts about 15 minutes. In late prophase it is distinctly visible that the chromosome structure is at least double, and it is quadruple in metaphase. The spindle forms similarly as in *Haemanthus*. The time the different stages of mitosis last is given in Table VI. A free space with no visible structure appears in prophase round the nucleus and this is the first signs of spindle formation. This manner of spindle formations is seen in almost all endosperms as for example in endosperm of *Leucojum* and *Haemanthus*, but has not been reported in other material, e. g. root tips where polar caps are formed. In endosperm after the free uniform space is formed — in some cells longitudinal fibrils usually arranged parallelly to the long axis of the future spindle are visible in it — typical polar caps appear. Changes in the half spindles cause the contraction stage. Immediately after the contraction stage metakinesis begins. An exact analysis of changes in the *Haemantus* mitotic spindle during its formation, of the contraction stage and metakinesis has been described elsewhere (B a j e r 1954). In *Iris* species the development of these stages is very similar to *Haemanthus* though they are shorter. The nuclear membrane disappears before the spindle is formed, but in metakinesis and in metaphase the spindle is often sharply delimited from the cytoplasm. Also the pushing body, and the phragmoplast are often sharply delimited from the cytoplasm. Late metakinesis is difficult to distinguish

from metaphase and in metaphase kinetochores are arranged exactly in one plane. In irises the metaphase spindle is rounded and often almost exactly ball shaped. The course of anaphase is illustrated best by graphs in Fig. 5 and Fig. 30 (B-C). The band on the curve of the chromosome movement is caused by the spindle elongation i. e. pushing body action.

Fig. 5. *Iris aphylla*. Curves of chromosome movement and spindle elongation (spl) in anaphase. Curves for two sister chromosome groups, for two half spindles and resultant curves. Anaphase curves for cell No. 122 plotted from microphotos. Pushing body action begins in early (cell No. 154) or in late (cell No. 122, 130) anaphase.

In the last two cases distinct bands are seen on the resultant curves.

The changes in oscillation of small particles inside the spindle are of the same character and intensity as of those described in *Haemanthus*. Telophase is very short and in late anaphase the chromosomes shorten and the pushing body acts on the already partly changed chromosomes. The primary cell wall is formed from either the outer or the central parts of a well formed phragmoplast. The primary cell wall begins to form much earlier when it forms from the centre of the phragmoplast than when it forms from its borders.

Danghter nuclei are quickly formed and often 45 minutes after anaphase begins typical resting nuclei with well formed nucleoli appear. 2 hours after anaphase begins sister nuclei are usually difficult to distinguish from cells which have not divided. The course of mitosis is very regular, especially if the endosperm is young.

In mitosis spindles may be placed very near each other (Fig. 29) and even touch. Usually in these cases, and also when several mitoses are enveloped by the same lobe of cytoplasm each stage of mitosis very often begins and ends at the same time in all spindles. The two spindles on Fig. 29 look as if they are melting together to form two hexaploid nuclei; this however will not happen and 4 resting nuclei will originate. In these cells the spindle poles do not melt in anaphase and 4 poles are plainly visible (Fig. 29 B). Observations of similar cases in other endosperms indicate that the joining together of anaphase chromosome groups takes place only when spindle poles of two cells are joined together.

Secale cereale

This species is suitable several days after it flowers, the embryo sacs being then less than 3 mm long. The condition suitable for experiments is very short, some days only; soon starch grains form and prevent observations. If the endosperm is not prepared quickly the cells are damaged. The course of mitosis is distinctly visible in young endosperm and cells are of medium size.

This material is difficult to handle and not convenient for experimental study.

NOTES ON DICOTYLEDONS

In the course of the present investigation it has become evident that cotyledons in embryo sacs do not impede the studies on the endosperm. The observations on the endosperm in vivo are best just after the cotyledons appear i embryo sacs and while they are very small. In dicotyledons as in monocotyledons small embryo sacs are the main difficulty. Also in numerous dicotyledons a free supply of pure oxygen is absolutely necessary for the continuation of mitosis after preparation. This renders the dicotyledons more difficult to handle. In numerous species it is very difficult to find mitosis. This is the case for instance, in the *Cruciferae* family. In *Brassica napus* and *B. oleracea* in more than 100 endosperm preparations, i. e. more than 400 embryo sacs, we have not succeeded in finding even one mitosis, though the cells have a very normal appearance. Nevertheless numerous genera are valuable and easy to handle e. g. *Helianthus* and probably *Paeonia* (cf. Fig. 34).

In some species mitosis is far less visible than in monocotyledons, e. g. in many, though not all *Pisum* cells the refraction index of chromosomes is small and metaphase and anaphase are difficult to trace distinctly. However, in only a few of the dicotyledons used in the course of this investigation starch grains, so cumbersome in monocotyledons, have been found. The endosperm from the classical object for mitosis studies *Vicia* is very inconvenient for all detailed observations. It seems also that etherous oils (*Rutaceae*, numerous *Cruciferae*) interfere in the course of mitosis. The multiploid nuclei which are often found indicate that either the course of mitosis is often disturbed or that endomitosis often occurs.

Pisum sativum var. cukrowy karłowcy.

Material. Mitoses may be found in embryo sacs with well formed cotyledons this makes it difficult to determine the size of the most convenient embryo sacs. Preparation is easy, and it is not difficult to find mitoses. Usually more than 80 per cent of cells continue the mitosis from early prophase to telophase.

TABLE VII

Mitosis in *Pisum sativum*

P — prophase, Mk — metakinesis, M — metaphase, A — anaphase, A₀ — beginning of anaphase, Phst — phragmoplast, 1 — late. Times measured to the beginning of the next stage.

No of cell	Duration in min of			Appearing of nucleoli after A ₀ in min.	Formation of primary cell wall in Phst		Spindle length in μ		max. separation of chromosomes in A in μ	Notes
	P	Mk ₁ and M	A		beginning of formation, min after A ₀	formation in min	M	A ₁		
276	—	20	17	60	—	—	26	35	33	cell wall did not form
278	40	20	12	110	22	10	20	23	20	
279	—	—	8	—	10	6	25	33	31	

Observations. Cells are of medium size and amoeba-like protrusions are vigorously formed. The cytoplasm often forms large lobes with many nuclei or mitotic spindles. It is astonishing how mitoses are synchronised in each such lobe of the cytoplasm. Multiploid nuclei resemble those in *Lupinus*. Chondriosomes are often distinctly visible. Cells are very resistant to preparation and few cells are damaged. Chromosomes are smaller than in *Helianthus* and larger than in *Lupinus* and have a very low refraction index, which makes observations difficult. Due to the low refraction index of the chromosomes it is very difficult to recognise middle or late prophase from anaphase, but early prophase is distinct. The different stages of mitosis are short (cf. Table

VII). In anaphase the action of the pushing body is considerable (Fig. 6). The particles oscillating inside the spindle move similarly as in *Hae-manthus*. Bisquid shaped cells, if they are single and not in lobes, are often seen in late anaphase. However the cleavage furrow and abnormal division have not been observed. It has often been noticed that the primary cell wall is not formed in telophase, and this is probably influenced by the age of the endosperm and the degree of its development. Figs 38-40 illustrate the *Pisum* endosperm.

Fig. 6. *Pisum sativum* and *Lupinus polyphyllus*. Curves of chromosome movement and spindle half spindles and resultant curves. Anaphase curves for cells No. 279 and 272 plotted from microphotos. In *Pisum* (cell No. 283) pushing body action causes the shoving of mitotic spindle in anaphase in one direction, the length of the lower half spindle increases while the length of the upper one hardly changes. the pushing body begins to act at the beginning or in the middle of anaphase. In *Lupinus* (cell No. 229) changes of the spindle length have not been noted.

Lupinus polyphyllus Lind.

M a t e r i a l. Suitable siliquae are 30-45 mm long and the embryo sacs are 4-4.5 mm long and approximately 2.5 mm across. In suitable embryo sacs small cotyledons are found. Preparation is very easy, but it is extremely difficult to find mitosis. We have not succeed to establish the percentage of cells which continue mitosis as we have observed only a few cells in mitosis; of these all divided to telophase.

O b s e r v a t i o n s. Cells are very small and form, though not always, amoeba-like protrusions. Mitotic stages after metakinesis are distinctly visible. The very small cells make it difficult to distinguish early from middle or late prophase. Chromosomes have a high refraction coefficient and in anaphase the movement of single chromosomes may be sometimes traced. The chromosomes are undoubtedly the smallest we have observed in the course of the present studies. Anaphase is very short, lasting about 6 minutes. The action of the pushing body is prominent. Graphs in Fig. 6 and Fig. 36 (A-G) illustrate the course of anaphase in *Lupinus*.

Resting nuclei are very small (Fig. 37) and it is difficult to say anything about their structure. The number of the nucleoli in the nucleus is changeable. Numerous high multiploid nuclei (Fig. 35) are found. Such nuclei may be 20 and more times larger than normal resting nuclei.

Vicia faba

The preparation of embryo sacs of this species is similar to that of *Pisum*. Only a few cells continue mitosis. The refraction coefficient of the chromosomes is low and chromosomes are seen very indistinctly (cf. Fig. 41 A-C). Multiploid nuclei are numerous and in some cases a process similar to endomitosis has been observed. It has not been possible to establish whether it really is endomitosis. *Vicia faba* is one of the most inconvenient species studied in the present work. However, preliminary observations of the other species of the *Vicia* genus seem to indicate that they are a better material than *Vicia faba*.

Borago officinalis L.

Fruits of the proper age are pale-green in colour. It is more convenient not to cut out the embryo sacs, but after cutting off the upper part of the fruit, to press out the liquid together with the endosperm. Cells are very small and form amoeba-like protrusions. Resting nuclei contain varying numbers of nucleoli. Chromosomes are very small with a low refraction coefficient. Prophase is difficult to observe but from metaphase, mitosis may be traced without difficulty. Fig. 43 illustrates the course of anaphase. Mitoses are very difficult to find.

Helianthus annuus L.

Material. Embryo sacs with very small cotyledons contain a colourless liquid with endosperm cells. Embryo sacs with large cotyledons or without them are not suitable for experiments. It is most important to choose embryo sacs of a proper age. Not many embryo sacs should be pressed out into one preparation. Mitoses are easily found but there are only a few in each preparation. Most cells continue mitosis from prophase.

Observations. Cells both with and without amoeba-like protrusions are found. Mitosis is distinctly visible and chromosomes are

Fig. 7. *Helianthus annuus*. Curves of chromosome movement and spindle elongation (spl) in anaphase. Curves for two sister chromosome groups, for two half spindles and resultant curves. Anaphase curves for cell No. 348 plotted from microphotographs. Action of the pushing body begins in early anaphase. Similarly as in previous curves the asymmetry of curves for two sister chromosome groups is caused by the shoving in one direction of the mitotic spindle.

smaller than in *Iris* but larger than in *Pisum*. During mitosis cells change their shape considerably (cf. Figs. 44-45). The different stages of mitosis are short and are given in Table VIII. The action of the pushing body is always visible. The primary cell wall begins to form from the centre of the phragmoplast. The course of anaphase is illustrated by Fig. 44-45 and graphs in Fig. 7. A distinctly visible dark band in the middle of the phragmoplast is the first sign of cell wall formation. In some cases after the cell wall forms the pushing body of the phragmoplast shoves the daughter chromosome groups toward the poles.

As many as 9 nucleoli have been observed in one resting nucleus.

TABLE VIII

Mitosis in *Helianthus annuus*

P—prophase, Mk—metakinesis, M—metaphase, A—anaphase, A₀—beginning of anaphase, Phst—phragmoplast, 1—late. Times measured to the beginning of next stage.

No of cell	Duration in min of			Appearing of nucleoli after A ₀ in min.	Formation of primary cell wall in Phst		Spindle length in μ		max. separation of chromosomes in A in μ	Notes
	P	Mk and M	A		beginning of formation, min after A ₀	formation in min.	M	A ₁		
348	—	15	13	45	16	3.5	26	34	27	
349	30-40	15	—	—	23	—	—	—	—	
350	50	20	13	—	14	5	02	28	24	
354	—	18	8	—	13	4	19	26	22	
255	—	16	12	—	22	7	21	24	30	

DISCUSSION

In our opinion the series of experiments presented in this paper are sufficient to qualify the endosperm as a valuable general material for observations of mitosis in vivo in mono- and dicotyledons. We are also convinced that the endosperm of numerous species is very convenient for studies on the influence of the physico-chemical factors on mitosis. The great advantages of endosperm are evident and have already been enumerated (Bajer 1953 b). The clarity with which the different processes in the endosperm are visible is not equalled by any other plant material. Not only for studies on mitosis but also for those on phragmoplast problems, and primary cell wall formation, endosperm is in many important respects the best plant material. Of course not in all species is the endosperm equally convenient. On the whole, monocotyledons are better material than dicotyledons, though it is possible that among dicotyledons numerous species are excellent. Our present results indicate that *Iris*, *Leucojum*, *Haemanthus* and *Clivia* are the most con-

venient material; also rather convenient are the *Ornithogalum*, *Asparagus*, *Zephyranthes*, *Pisum* and *Helianthus* genera, *Zephyranthes* being the easiest of these four. It is noteworthy that *Iris*, the material used by Jungers (1930) in his research, appears now so suitable for studies in vivo and that we have observed in *Iris* almost all the facts described so carefully by him on fixed material.

Objections may be raised that endosperm degenerates in the later stages of its development. This however is not always the case, and even when it does degenerate in the later stages, it is studied in vivo while still young, and then the degenerating factors seem to have no effect. There are no essential differences in the course of mitosis in young and in old endosperm, and only starch grains make the observations of old cells more difficult. Also the mechanical conditions in old endosperm cells are more inconvenient than in young ones. Furthermore it may also be raised that the endosperm tissue is triploidal. However it is not probable that the triploidy of the endosperm can influence, in any important respect, the essential course of mitosis, though the duration and some mechanical conditions of mitosis may be changed. These are not sufficient reason against using the endosperm for studies on cell division.

Young endosperm is a typically meristematic tissue, and the study of the differences in the course of mitosis as it ages, though not important, may throw some light on the mechanism of mitosis.

The course of mitosis is similar in all objects studied. Prophase has been observed in detail in some objects only. In all plants with both large and small chromosomes mitotic contraction is to be observed in late prophase. In this stage the action of kinetochores begins. An exact analysis of this stage and sufficient evidence to support this statement will be given elsewhere.

From Tables II-VIII and graphs of the present paper it becomes evident that a correlation between the duration of the different stages of mitosis and the dimensions of the chromosomes exists. This correlation is most pronounced in anaphase, which is the stage analysed most exactly. It seems even that the time of anaphase is more or less proportional to the dimension of chromosomes. However, to establish the exact nature of this proportionality, it is necessary to work out mathematically the data given in this paper. In anaphase the action of the pushing body has varying intensities. It is noteworthy that the activity of the pushing body changes in the different cells of one species and seems to be more prominent in young than in old ones. The action of the pushing body may begin at the beginning or in the middle of anaphase. In the latter case the curve of the chromosome movement becomes

characteristically shaped due to the acceleration of this movement in middle anaphase.

Several *Iris*, *Asparagus* and *Leucojum* cells have been examined with the use of cine — micrography. Such an examination explains quite satisfactorily the problem of asymmetric curves of two daughter chromosome groups in anaphase. This type of graph has been used in most of our previous papers. From all previous observations it appears that perfectly symmetrical curves are extremely rare. Often curves are characteristically displaced in one direction (cf. graphs in Figs to this paper), which usually does not appear on the resultant curve. Cine — micrographic analysis of dividing cells has shown that this results from the shoving of the whole anaphase spindle in one direction by the action of the pushing body.

In the endosperm of species with large or medium chromosomes, the chromosome structure is often distinctly visible in vivo. In very large chromosomes (*Leucojum*, *Haemanthus*) each of the prophase chromatids seems to be composed of several elements. Prophase chromosomes may have „lamp-brush“ structure which has lately been described by different authors (Huskins 1948, Galinsky 1949, Mota 1952). In metaphase or very late metakinesis it is well visible as chromosomes split longitudinally into two separate elements. Only the kinetochore does not split and its structure in metaphase seems to be very similar to that described by Lima de Faria (1949) in prophase, though more contracted. In species with very large chromosomes quadruple structure of metaphase chromosomes is very distinctly visible. This indicates that the division of chromosomes into four distinct elements takes place between prophase and metaphase.

In all species described in this paper the observation of the Brownian movements of small particles inside the spindle fully support the data obtained on *Heamanthus* (Bajer 1953b).

The method used in the course of the present work is simple, quick and may be further improved. However, it appeared that in some species most of the cells die in prophase. The reason of this is probably — here as in animal tissue — the lack of oxygen necessary for prophase to continue (Bullough 1952). A free supply of oxygen in endosperm of *Haemanthus* makes possible the observations of normal mitosis even 24 hours after preparation. Beginning with metakinesis the supply of oxygen does not seem to be necessary, because usually metakinesis is continued to telophase. The observations obtained show that a vigorous development of bacteria accelerates the death of prepared cells. Sterile conditions however are not necessary in experiments lasting less than 10 hours i. e. in most experimental studies.

The possibilities of experimental treatment of endosperm in vivo are diverse. The vitality of prepared endosperm of some species is astonishing. In *Haemanthus*, for instance, in pure oxygen mitotic spindles may be experimentally flattened to 5-6 μ , while the thickness of the chromosomes is about 4 μ . In these abnormal conditions, where chromosomes form one layer, mitosis may be continued from early prophase to resting nuclei.

In our opinion experimental studies on endosperm may solve numerous most important problems in the mechanism of mitosis, while further improvements in the methods used will prolong the life of prepared cells. It is even possible that permanent tissue cultures similar to those of animal tissues will be obtained from endosperm.

SUMMARY

1. With a simple method described in detail, the advantages of numerous species for observations of mitosis in vivo are examined. The best and the easiest objects are: *Iris*, *Leucojum*, *Clivia*, and *Haemanthus*, also: *Zephyranthes*, *Ornithogalum*, *Asparagus*, *Helianthus*, and *Pisum*, are convenient. *Veratrum*, *Lupinus*, *Vicia* and *Borago* are very inconvenient.
2. On the whole monocotyledons are more suitable than dicotyledons and objects with small chromosomes are more difficult than those with large or medium ones.
3. Mitosis in objects with small chromosomes is shorter than in those with large ones. The time of anaphase, the stage examined most carefully, seems to be proportional to the dimensions of chromosomes.
4. Observations on pushing body action, mitotic contraction, and living chromosome structure are reported.
5. Endosperm is suggested as material for studies on mitosis and related problems.

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PLATES I — X

Ornithogalum umbellatum

Fig. 8. A — prophase, B — early anaphase, C — late telophase with nuclei and without nucleoli. In A nucleoli are visible, outlines of the spindle are not distinct, Note the change of the cell shape and the lack of protrusions. A — 0 min. B — 47 mins after A, C — 1 h 20 min after A.

Fig. 9. A to G the course of anaphase. In metaphase (A) kinetochores are in one plane, anaphase is very regular, E to F — these though similar are not chromosomal bridges, G — normal nuclei. A — 0 min, B — 1 min after A, C — 3 mins after A, D — 7 mins after A, E — 13 mins after A, F — 19 mins after A, G — 32 mins after A.

Fig. 10. Cell with resting nucleus, nucleolus with distinctly coacervate structure.

Fig. 11. *Funckia subcordata*. A — anaphase, B — telophase with well formed phragmoplast, C — resting nuclei with nucleoli. Numerous starch grains make observations difficult. A — 0 min, B — 22 mins after A, C — 55 mins after A.

Fig. 12. *Veratrum nigrum*, metaphase, anaphase and telophase. Low refraction index of chromosomes and coarse cytoplasmic structure prevent direct observations. Compare the course of anaphase with that in *Asparagus* of which the chromosomes are almost of the same dimensions. Note that amoeba-like protrusions begin to form vigorously in telophase (E — F). A — 0 min, B — 9 mins after A, C — 12 mins after A, D — 16 mins after A, E — 23 mins after A, F — 53 mins after A. Figs. 13—15. *Asparagus pseudoscaberrimus*. Fig. 13. Metaphase, anaphase and telophase, in I almost resting nuclei. Note the pushing body action. Clear structureless space around the daughter chromosome groups in telophase is distinct (F), this is the beginning of nuclei formation (cf. text). In G first stage of cell wall formation. A — 0 min, B — 5 mins after A, C — 5,8 mins after A, D — 7 mins after A, E — 8,5 mins after A, F — 9 mins after A, G — 22,5 mins after A, H — 25 mins after A, I — 39,5 mins after A. Fig. 14. Middle anaphase seen from one of poles; stage similar to Figs. 13. D. Note, the dimensions of the chromosomes. Fig. 15.

Almost bare resting nucleus with 13 nucleoli (not all are distinctly visible).

Figs. 16—18. *Agapanthus umbellatus*. Fig. 16. Metaphase, anaphase and telophase. Note the vigorous formation of amoeba-like protrusions in telophase (16 D and 17 E—D).

Fig. 16. A — 0 min, B — 18,5 mins after A, C — 29,5 mins after A, D — 39,5 mins after A, E — 67,5 mins after A, Fig. 17, A — 0 min, B — 10 mins after A, C — 20 mins after A, D — 31 mins after A, E — 38 mins after A, F — 1 h 23 mins after A.

Fig. 19. *Zephyranthes candida*. A — prophase with nucleoli, B — metaphase and C — telophase with cell wall not yet formed A — 0 min, B — 1 h 10 mins after A, C — 1 h 32 mins after A.

20 μ

AGAPANTHUS
ZEPHYRANTHES

Fig. 20. *Zephyranthes candida*. Metaphase, anaphase and telophase with a cell wall.
A — 0 min, B — 3 mins after A, C — 5 mins after A, D — 11 mins after A, E —
15 mins after A, F — 30 mins after A.

Fig. 21. *Haemanthus puniceus*. Almost bare spindle in metaphase and anaphase.
Cell is very small. A — 0 min, B — 1 h 13 mins after A, C — 2 h 4 mins after A.
Numerous microphotos of mitosis in *Haemanthus* are given in other papers (Bajer
1953, a, b, 1954).

Leucojum aestivum

Figs. 22—23. illustrate one cell. Cell is old, but chondriosomes are well visible as long thin threads (Fig. 22). Fig. 23: A — metaphase, anaphase and resting nuclei (F — with nucleoli arranged symmetrically as mirror reflections. A — 0 min, B — 8,5 mins after A, C — 34 mins after A. D — 54 mins after A, E — 1 h 4 mins after A, F — 12 h later.

Fig. 25. *Leucojum aestivum*. A — metakinesis; note the numerous chromatids coiled round each other. B — early telophase. A — 0 min, B — 43 mins after A.

Fig. 26. *Clivia nobilis*. Cell in prophase. Two nucleoli.

Figs. 27—28. *Iris sibirica*. Fig. 27. A — prophase with nucleoli B — metaphase seen from one of the poles. Note the dimensions on the chromosomes. A — 0 min, B — 1 h 2 mins after A. Fig. 28. Metaphase, anaphase and early telophase. A — 0 min, B — 10 mins after A, C — 28 mins after A.

Iris aphylla

Fig. 29. Anaphase in two spindles touching each other, see in text. A — four spindle poles visible (they are also visible in B), C and D it seems that anaphase of these two spindles have joined, however this is not the case, as 4 nuclei are seen in E. A — 0 min, B — 18 mins after A, C — 38 mins after A, D — 41 mins after A, E — 3 h 20 mins after A.

Fig. 30. A — prophase with nuclear membrane, B — metaphase, C—D — early and late anaphase, and E — resting nuclei with nucleoli. A — 0 min, B — 27 mins after A, C — 44 mins after A, D — 1 h 3 mins after A, E — 4 h after A.

Figs. 31—32. *Iris aphylla*. Fig. 31. A — prophase with nucleoli and nuclear membrane, and B — telophase, A — 0 min, B — 1 h 11 mins after A.

Fig. 32. Metaphase plate seen from one of the spindle poles, compare the chromosome dimensions with those of *Iris sibirica* (Plate VI).

Fig. 33. *Iris xiphioides* A — metakinesis, and B — telophase with hypotonic agar with glucose (0,5 and 3 per cent) Structure of cytoplasm is destroyed and large vacuoles have appeared, pushing body action is slight, it is directed to its borders and not to the poles. A — 0 min, B — 14 mins after A.

Fig. 34. *Paeonia officinalis*. Large multiploid cell in prophase.

Figs. 35—37. *Lupinus polyphylus*. Fig. 35. Large multiploid cell with small and large nucleoli; compare the dimensions of this cell with Fig. 37 which shows resting nucleus of normal size. Fig. 36 metaphase, anaphase and early telophase. A — 0 min, B — 1 mins after A, C — 2 mins after A, D — 3 mins after A, E — 4 mins after A, F — 5 mins after A, G — 12 mins after A.

Figs. 38—40. *Pisum sativum*. Fig. 38 A — prophase with nucleoli and nuclear membrane, B — later stage of prophase, C — metakinesis, D and E — anaphase, F — resting nuclei with nucleoli. A — 0 min, B — 13 mins after A, C — 38 mins after A, D — 50 mins after A, E — 1 h 8 mins after A, F — 2 h after A. Note that prophase stages are distinctly visible, while anaphase is exceptionally dim.

Fig. 39. Metaphase and anaphase in cell in which the course of mitosis is very distinctly visible, note, however, that visibility is much worse than in other objects.

Fig. 40. Resting nucleus with nucleoli, cell with amoeba-like protrusions.

Fig. 41. *Vicia faba*. A — metakinesis, B — late anaphase, C — almost resting nuclei without nucleoli, cell wall has not yet formed. A — 0 min, B — 22 mins after A, C — 2 h after A.

Figs. 42—43. *Borago officinalis*. Fig. 42. Multiploid cell. Fig. 43. Metaphase and anaphase in cell of average size. Anaphase is prolonged by lightening the cell.
A — 0 min, B — 38 mins after A, C — 48 mins after A.

Figs. 44—45. *Helianthus annuus*. Fig. 44. A — late prophase just before metakinesis begins, metaphase, anaphase and telophase. A — 0 min, B — 37 mins after A, C — 44,5 mins after A, D — 48,5 mins after A, E — 58 mins after A, F — 1 h 9 mins after A.

Fig. 45. A — metaphase, B — beginning of anaphase, anaphase and telophase. Note that in B chromosomes are arranged in one plane, in E — the dark band is the first sign of cell wall formation. H — resting nuclei. Compare the dimensions of these two cells (Fig. 44 and 45) of *Helianthus*. In *Helianthus* most cells are of medium size (smaller than those in Fig. 45 and larger than in Fig. 44). A — 0 min, B — 15,3 mins after A, C — 17 mins after A, D — 20 mins after A, E — 24,5 mins after A, F — 27,5 mins after A, G — 33 mins after A, H — 4 h 30 mins after A.