ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XXIV — Nr 3
1955

A simple method for examining the action of chemicals on mitosis in living endosperm

by

J. MOLÈ-BAJER

Endosperm showed itself to be excellent material for detailed observations of the course of mitosis in different plants (B a j e r 1953, a, b, 1954, and B a j e r and M o l è - B a j e r 1954). It was also suggested that it is suitable for studies on the influence of chemical substances on mitosis. The purpose of this work was to elaborate a simple and useful technique for such studies. Very detailed experiments were made on the influence of respiratory inhibitors on different stages of mitosis. The results of these studies will be published later and here only a detailed description of the method employed will be given.

Qualitative methods of the work with very small amounts are found in G l i c k's (1949) book.

The method for the observation of endosperm *in vivo* consists in pressing the endosperm on to agar with a suitable sugar concentration. The contents of the embryosac in a suitable stage of development, which varies between 3 and 6 weeks after pollination (B a j e r and M o l è - B a j e r 1954), is pressed on to a large cover slip smeared with agar. On the cover slip a vaseline or *constant* paraffine ring is made and the area inside the ring is smeared with a very thin layer of hot agar. 0.4—0.5% agar and 3—3.5% of glucose is suitable for different species. The concentration of glucose must be experimentally determined. The large cover slip on which the endosperm is smeared is covered with a smaller one also smeared with agar and the two glasses are sealed with hot vaseline. In the case of large embryosacs (*Clivia, Haemanthus, Leucojum*) containing over 500 µl of liquid, the pressing out should be done very carefully. Then the first drops of liquid contain no cells and may be cast away. This is necessary as on the area of 2 square cm of agar, no more than 20—25 µl of the liquid containing endosperm should be pressed. If the quantity of liquid is small the cells or endosperm lobes — found in young embryosacs — flatten slowly. This process is of great importance for the studies of the course of mitosis *in vivo*, but during the studies on the influence of chemicals on mitosis should rather be avoided. Too small a quantity of liquid leads to the death of cells and too large gives rise to a drop of very nonuniform thickness, which should also be avoided. The drop should be thin and uniform.

It appeared that the atmosphere of oxygen, applied previously, is not necessary; however for some plants light seems to cause death or to retard the course of mitosis, especially till the beginning of anaphase, i. e. the first stages of mitosis are the most susceptible, and therefore for observations the weakest light, with heat rays eliminated, should be applied.

*

In the above described method an artificial medium is applied (agar with glucose), but the cells are however surrounded by the natural medium: liquid from the embryosac. As the chemical composition of the liquid is not known this makes more difficult the proper dosing of the chemical agents, whose influence is to be studied. If the normal state of the cells is dependent on these two mediums (agar and liquid), which is assumed to be certain, the chemical substances after preparation may be added either to 1) agar, or to 2) liquid. Also the third method — injection to the embryosacs before preparation — may be applied. However as this last method presents serious difficulties in quantitative studies, it will not be discussed further.

Before the detailed description some general remarks concerning the two (1, 2) will be given. The osmotic pressure of the liquid containing endosperm is not known. This makes it practically impossible not to influence it during the action of chemicals, though the changes may be very slight. Most probably slight changes of the osmotic pressure have no influence on the course of mitosis, which is proved by the following facts: in some plants the division is continued in different concentrations of the glucose in the agar, and also the mixing of the endosperm liquid from some embryosacs of different age does not change the percentage of dividing cells, though most probably their osmotic pressure is not the same.

Only substances on which glucose does not act can be used. E. g. if the colchicine influence is examined, instead of glucose, mannit should be applied, as the glucose opposes the colchicine action (C o r n m a n 1950).

1. ADDITION OF THE CHEMICALS TO THE ENDOSPERM LIQUID

In order to change the osmotic pressure of the endosperm liquid a slight degree only, which does not influence the course of mitosis, when a certain amount of any substance is added, e. g. solution of the chemical agent, it would be necessary to subtract previously from the endosperm liquid the same amount of osmotically active substances. It is evident that this is impossible, and a different method must be used. This way is to add the smallest possible volume of the solvents of very high concentration, then the osmotic pressure is only slightly changed, while

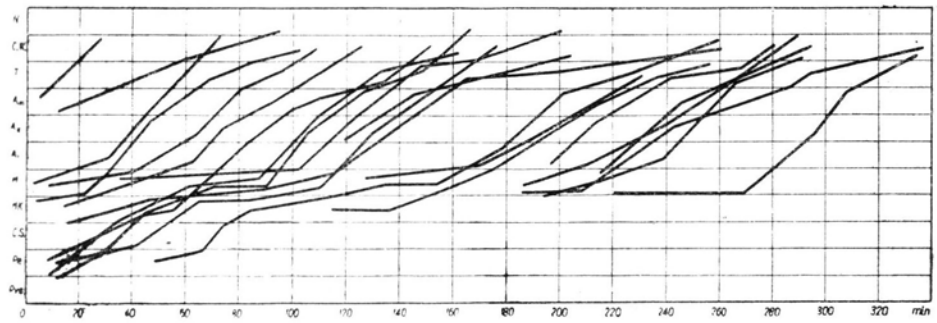

Fig. 1. Control. Properly made preparation; all the cells continue the division. Pve — early prophase before spindle formation, Pe — early prophase, C. S. — contraction stage, Mk — metakinesis, M — metaphase, $A_{I, II, III}$ — anaphase early, middle and late, T — telophase, C. W. — cell wall formation, N — appearing of nucleoli. *Haemanthus Katharinae.*

the endosperm equals O. Intervals on the ordinate are either equal for each stage, as is seen on Figs. 1—2, or they may be proportional to the average duration of different stages of mitosis. However it must be stressed that the durations of different stages in normal mitoses differ considerably and average durations must be drawn from several observations. If the microscope is supplied with a cross table with nonius, which makes it possible to point exactly the position of each cell in preparation and to find it in a moment, after some practice simultaneously 20—30 cells may be observed. The stage of each cell is confirmed every few minutes and the time is exactly marked on the graph. For each preparation, in and not in the control, such a graph is made. The courses of the curves on the

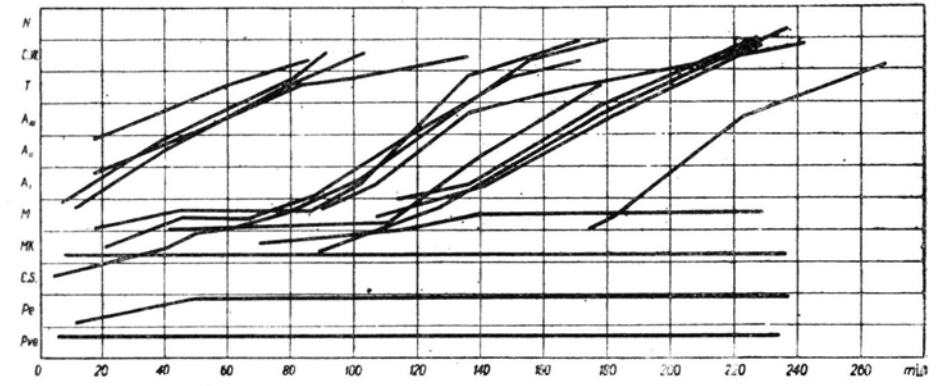

Fig. 2. The drying preparation. The hanging drop too thin — the drying of the drop caused death of most cells in early stages (prophase). — Partly explanation in Fig. 1. *Haemanthus Katharinae.*

In general this method is worse than the following one, but it makes possible however the use of thermo-labile substances, which is not possible in the second one.

2. CHEMICAL SUBSTANCES ADDED TO AGAR

In this method the chemical agent is added to the agar with the glucose. The same quantity (calibrated micropipette) of agar with sugar and the substance whose influence is to be studied is smeared on the same area of the cover slip. This is easily achieved, as the edges of the area are determined by the vaseline ring, whose dimensions may be always the same. A short weight amount of endosperm is pressed out and a sufficient quantity of liquid is added on a torsion balance. It seems however that the weighing is not necessary to get exact results, as it appeared that after some practice the volume of the pressed endosperm liquid changes no more than 10%, which was confirmed during control weighing.

The area of agar should be large — about 4 square cm — and liquid with endosperm should be evenly placed, since this is of importance for equal diffusion.

This method appeared much better than the first one. In practice on a properly made preparation only one layer of cells is present and as a result all cells touch the agar. The main defect of this method is that the substances which are destroyed by high temperature (above 80°C) cannot be applied as agar in a hot state must be used for the smearing of the cover slips.

3. ACTION OF GASES

The simplest methods are employed if the action of gases on endosperm is examined. A large cover slip with the endosperm prepared in the usual manner is fixed to a specially adopted chamber (B a j e r 1954) which permits the observations on a given gas. Also on the bottom of the chamber the reaction which results in a gas production may be started. The qualitative work in this last case also presents no difficulties. Such a method was applied for the studies of the influence of HCN (to KCN solution HCl was added) and gave quite satisfactory results.

4. RECORDING THE RESULTS

A simple method for recording the results was applied. During the observations the following graph is made: ordinate — different stages of mitosis, abscissa — durations of observations. The time of preparing

large volume may cause either diminishing or increasing of the osmotic pressure. An additional difficulty arises as very often it is not possible to make a high concentration of the substance, as it may be only slightly soluble in water. In practice this method may be used for substances which act in low concentrations only. If this method is employed the concentrated solution of the substance is added with the aid of a micropipette. The making of such quantitative pipettes is described by Glick (1949) and they showed themselves to be really very handy. The endosperm with liquid may also be taken with a calibrated pipette or, better still, the cover slip with vaseline is weighed and only agar is added with a calibrated pipette. A torsion balance is quite sufficient and very quick and convenient. Then the endosperm is added in excess, the excess of liquid is removed on to the pan with a pipette, and the solution of the agent is given. The making of the preparation should be quick, and after some practice it takes only a few minutes.

There are different types of torsion balances; some of them are described by Brauner (1929), Portsmouth (1948) and Glick (1949). Of very high sensitivity are those based on the turn of a quartz string (torsion balance of Nernst). The most convenient is described by Portsmouth, and the simplest described by Brauner. This last type was used: it is of sufficient accuracy and its construction offers no difficulty. A long glass rod (length about 40 cm) of about 2 mm diameter is fixed on one end and about 10 cm from the other end the pan is suspended. The pan is made from a cover slip fixed to a 5—8 cm long glass rod, which is hoop bent on the opposite end and is suspended on a slight U-bend made for this purpose on a long glass rod. The long glass rod from the fixed end may be bent into a spiral. The part of the rod on the opposite end of the rod fixing moves on the ground of the mirror scale. All weight should be manteled as it is sensitive even to air currents. The accuracy of the balance depends on the length and the thickness of the long glass rod and the point of the pan suspension. Calibration is necessary, and may be easily done with small analytic weights. Calibrations indicate also the limits in which the balance can be used i. e. the region of the proportionality of the bending forward in relation to the weight.

If the volume of the endosperm liquid is measured with a pipette many cells are injured or killed and this may be a source of serious error (cf. Fig. 2).

Also the mixing of the endosperm liquid and the added solution may result in injuring many cells. The most harmless method is to give the solution of chemical substance not to the endosperm liquid but immediately on to the agar after smearing; this makes it possible to spread the solution much more equally and uniformly.

In the calculation of the final concentration the volume of agar — which should be the slightest possible — should also be counted.

control graphs are slightly different, this being caused by different durations of mitosis in different cells, however if the control is sufficiently large the error is easily eliminated.

The graph of such types gives:

1. the number of cells (from which the percentage may be calculated) in which mitosis is continued depending on the stage of mitosis (Figs. 1—2),

2. the stage in which the course of mitosis is stopped or delayed (Fig. 2),

   a. in the case of immediate delay action of the factor causing complete stoppage of the course of mitosis independent of the stage, at the moment when the continuation of mitosis ceases the curve changes to a straight line parallel to the abscissa, c. f. Fig. 2,

   b. in the event of weak influence of the factor particular stages of mitosis may be prolonged and the angle of the incline of curves for single cells may be changed or particular stages may be completely stopped,

3. the moment in which the substance begins to affect the mitosis — from comparison with the control,

4. average velocity of division in and not in the control.

It is very easy to eliminate the divisions which were in late stage, that e. g. the course of mitosis ended before the action began — in cases in which the substance acts slowly, and so on. It is also evident that disturbances of different kinds may be easily noticed.

*

These methods were employed for the studies of substances with small molecules and gases (nitrogen, oxygen, KCN and NaN_3). In all cases the process of diffusion is immediate and if the substance has any influence it affects mitosis a few minutes after preparation. Most probably the concentration of active substance in the agar and in the endosperm liquid were quickly equalled, as in not one case was the correlation of the substance action and the time found i. e. the results caused by the active substance do not increase in time. For substances with larger molecules the diffusion would be slower, but experimental determining of the time of the substance action, and the stability of such action however cause no difficulty.

As was stressed above, these methods are useful for substances which act in low concentrations i. e. do not change notably the osmotic pressure, but most substances, however, whose influence was studied on the course of mitosis in the last years have this property.

CONCLUSIONS AND SUMMARY

A simple method for studies of the influence of chemical substances on mitosis in endosperm *in vivo* is described. The method makes possible exact qualitative experiments.

Chemical substances may be:

1. added to agar with glucose on which the endosperm is smeared,
2. added to the liquid containing endosperm cells,
3. injected to the embryosac before preparation.

The two first methods are described and the advantages and difficulties which arise are stressed.

Also a simple method of recording the results is recommended. This method consists in making a special graph from which the following data may be obtained:

1. the number of cells (%) in which mitosis is continued depending on the stage of mitosis,
2. the stage in which the course of mitosis is stopped or delayed,
3. the moment in which the substance begins to affect the mitosis,
4. average velocity of division.

Laboratory of Plant Physiology
Jagiellonian University
Kraków, Grodzka 53

(Entered 3.II.1955)

LITERATURE

B a j e r A., 1953 a, Absolute viscosity and living mitotic spindle structure. Acta Soc. Bot. Poloniae. 22 : 331—348.

B a j e r A., 1953 b, Endosperm valuable material for experimental studies of mitosis in vivo. Ibid. 22 : 475—482.

B a j e r A., 1954, Cine-micrographic studies on mitosis in endosperm. I. Ibid. 23 : 383—412.

B a j e r A. and J. M o l è - B a j e r, 1954, Endosperm material for study on the physiology of cell division. Ibid. 23 : 69—98.

B r a u n e r L., 1929, Pflanzenphysiologisches Praktikum, I Teil. G. Fischer. Jena. p. 112.

C o r n m a n I., 1950, Alleviation of mitotic poisoning by glucose. Journ. Cell. and Comp. Physiol. 35 : 301—302.

G l i c k D., 1949. Techniques of histo- and cytochemistry. Interscience Publ. New York. p. 531.

P o r t s m o u t h C. B., 1948, A simple torsion balance. Ann. Bot. 12 : 331—333.