PROTOPLASMIC VISCOSITY IN PLANTS

I. PROTOPLASMIC VISCOSITY OF DIVIDING CELLS IN FLORAL BUDS OF TOBACCO

By **DONTCHO KOSTOFF**

Sofia University, Bulgaria

With 12 Text-figures

Received for publication, April 12, 1930

Methods for investigating protoplasmic viscosity date from the last century but more recently the old have been perfected and new offered chiefly by WEBER, HEILBRONN, HEILBRUNN, and CHAMBERS [Literature in WEBER (1924), HEILBRONN (1922), HEILBRUNN (1928) and CHAMBERS (1925)] so that now one can attack problems in this field with a greater degree of confidence. My aim is not to discuss here methods, since these have been presented and discussed in a summarical work by WEBER (1924), but to present the results obtained by applying the method of centrifuging in particular and, considering the results so obtained and those reported by other investigators, to give acceptable interpretations of certain biophysical phenomena occurring in the living cell and tissue in various conditions.

Attention was given first to the relative values of the protoplasmic viscosity in the dividing cells of plants of *Nicotiana Tabacum*. Floral buds, at a stage when meiotic divisions usually occur, were collected with floral stems as long as possible and wrapped in slightly moistened cotton. Thus prepared the buds were placed in the tubes of the centrifuging machine on a cotton layer of about 15 mm. filling the bottom of the tubes. The buds were so wrapped in the cotton that they could be inserted into the tubes easily without forcing and a horizontal position maintained in order that during the centrifuging the centrifugal force would act in a direction perpendicular to the style of the bud. Care was taken to avoid all possible pressures or mechanical injuries

and to prepare the buds for centrifuging as quickly as possible, usually within from 15 to 20 seconds. Series of buds were centrifuged at the rate of 2200 revolutions per minute, r = 90 mm., 18—22° C., for periods of 1 minute, 5, 10, 15, 20, and 25 minutes; then promptly, within 15 to 20 seconds, removed and fixed in the following derivative of BOUIN's fixative: 75 cc. saturated solution of picric acid, 25 cc. formaldehyde, 8 cc. acetic acid, 1·5 g. chromic acid, and 1 g. urea. The apical end of the bud and anthers was opened by a sharp blade to facilitate immediate fixation. After dehydration and clearing, fixed material was imbedded in paraffin and sections made of 8 and 10 microns were stained with iron alum haematoxylin.

In the investigation of this material so prepared especial attention was given to the relative values of the protoplasmic viscosity in the pollen mother cells (PMC). In the buds centrifuged 1 and 5 minutes no striking change could be observed in the PMC. The material centrifuged 10 minutes showed slight changes in the cellular content of the PMC when compared with conditions of untreated material. Such changes were more striking in material centrifuged 15, 20, and 25 minutes. Since the changes effected after centrifuging for 25 minutes, in most instances, and for 20 minutes, in some instances, were found to be irreversible I shall describe here mainly those observed in the material centrifuged for 15 minutes. In the latter case the changes were found to be reversible and usually returned to normal in from 1 to $1^1/_2$ hours after centrifuging if the buds were kept in moist cotton at the same temperature (18—22° C.).

WEBER (and his students) and HEILBRUNN (for Literature see HEILBRUNN, 1928) used the arrangement of the cell content subsequent to centrifuging for estimating the viscosity of the protoplasm. Using similar criteria in estimating the protoplasmic viscosity in centrifuged floral buds of *Nicotiana Tabacum*, relative values can be obtained for changes in various stages during the meiotic and mitotic cell divisions based on the arrangement of the cellular content produced by the centrifuging.

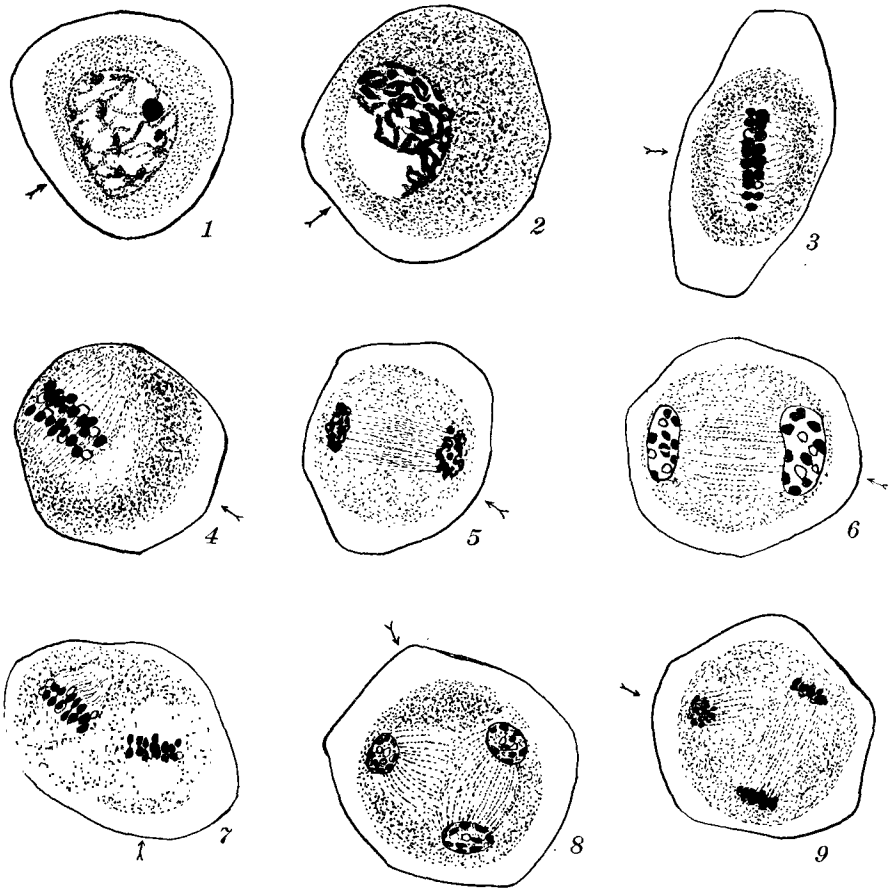
The conditions in the PMC will be described first. In the prophase (fig. 1) the volume of the nucleus is increased when compared with that of the resting nucleus. The nuclei of the PMC in prophases have a tendency to appear closer to the centripetal wall after centrifuging. The arrow in fig. 1, and in all the other figures also, shows the direction of the activity of the centrifugal force. Its direction is very

easy to determine in the sections of the buds from the position of the starch grains and the nuclei of old, highly vacuolated cells; since the buds were centrifuged with the style at right angles to the force and the sections prepared transversely to the style. The starch and most of the nuclei of the old, vacuolated cells were thrown to the centrifugal wall even after centrifuging for only 1 minute.

The nuclei during diakinesis have a position similar to that of nuclei in the prophase (fig. 2). There are two ways to explain such a condition, both being equally true and complementary. One is that the density of the nucleus decreases during the prophase and diakinesis as compared with that of the nucleus during the resting stage; the other that the density of the cytoplasm increases at this time, the density of the cytoplasm apparently increasing on account of the increase in the volume of the nucleus. During the prophase the nucleoli tend to approach the centrifugal side of the nucleus, a condition not to be observed in the case of the resting stages. The spireme, however, during the prophase remains unaffected in distribution after 15 minutes centrifuging. During the diakinesis the pairing chromosomes tend to occupy the centrifugal side of the nucleus after 15 minutes centrifuging (fig. 2). These conditions indicate that the nuclear content in which are floating the nucleoli and pairing chromosomes during the prophase and diakinesis respectively is less viscous during these two stages than during the resting stage. On account of this the cytoplasmic viscosity (CV) seems to be noticeably increased, evidenced morphologically by the absence of any deformations of the cells during these stages after centrifuging. Therefore, while the density of the nucleus during the resting stage is greater than that of the cytoplasm, during the prophase and diakinesis the density of the cytoplasm is greater than that of the nucleus.

With the metaphase the nuclear membrane disappears, the spindle forms, and the chromosomes arrange equatorially. During this phase the cytoplasm seems to be less viscous than during any other division phase. Cells in heterotypic metaphase on which centrifugal force has acted 15 minutes at right angles to the equatorial plane are often flattened, the spindle fibers bent, and the spindle as a whole slightly compressed (fig. 3). The same is true of conditions following the same treatment of the homeotypic metaphase (fig. 7). When the centrifugal force acts at right angles to the spindle during the metaphase or very early anaphase the whole spindle is moved toward the centrifugal wall (fig. 4). Centrifuging for periods of 15 minutes has no marked effect

on the late anaphase and telophase in either of the meiotic divisions (figs. 5, 6, 8, and 9), i. e. the CV is higher during the anaphase and



Drawings of pollen mother cells (figs. 1—9) and somatic cells (10—12) from centrifuged floral buds of *Nicotiana Tabacum* made with the aid of a Zeiß microscope with 15 × ocular, 1.25 mm. oil immersion objective, and a camera lucida (reduced).

Fig. 1. Prophase.
Fig. 2. Diakinesis.
Fig. 3. Late heterotypic metaphase.
Fig. 4. Early heterotypic anaphase.
Fig. 5. Late heterotypic anaphase.
Fig. 6. Late heterotypic telophase (interkinesis).
Fig. 7. Late homeotypic metaphase.
Fig. 8. Homeotypic telophase.
Fig. 9. Homeotypic anaphase.

telophase than during the metaphase and very early anaphase. With the dissolution of the nuclear membrane preceding the metaphase in both meiotic divisions, the nuclear content apparently plays a part in the decrease of CV noted at this period. Highly chromatolyzed chromosomes were equally distributed inside the nuclei of the telophases in both meiotic divisions after centrifuging 15 minutes (figs. 6 and 8) and not tending to occupy the centrifugal area of the nuclei as in the case of those in the diakinesis.

Generalizing the observations just given on the conditions in the pollen mother cells one can conclude that there is an increase of CV occurring during the prophase and diakinesis followed by a decrease during the heterotypic metaphase and early anaphase with another increase during the late anaphase and telophase, the same cycle being

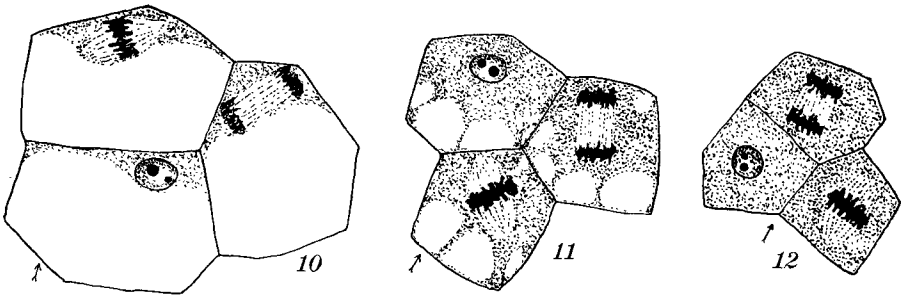


Fig. 10. Old, highly vacuolated cells with resting and dividing nuclei.
Fig. 11. Slightly vacuolated cells with resting and dividing nuclei.
Fig. 12. Young, meristematic cells with resting and dividing nuclei.

repeated in the homeotypic division. Generally speaking, this is in agreement with the observations made by previous investigators on other objects.

The investigation of PV in the dividing somatic cells by the centrifuging method presents a more complicated problem, since the embryonic meristematic cells are not sufficiently expanded and their cell walls offer greater resistance to deformations (fig. 12) than in the case of others, and because the expanded cells are more or less vacuolated (figs. 10 and 11). Under the latter conditions the vacuoles usually mask the effect so that one cannot estimate with very great accuracy how much is due to the CV and how much to the vacuolation. Therefore, in these investigations one can speak only of tendencies.

The protoplasm of old, highly vacuolated cells like those illustrated in fig. 10 occupies a small area in the centrifugal portion of the cell after centrifuging. Such cells cannot be taken into consideration in estimating the PV for the degree of injury is relatively great and the effect of the centrifuging usually irreversible in the case of these cells.

The effect of centrifuging slightly vacuolated cells, like those shown in fig. 11, can be considered in estimating the PV. In these instances the protoplasm also tends to occupy the centrifugal region of the cells but cytoplasmic threads still remain in contact with the centripetal walls and the effect of the centrifuging is here reversible.

The resting nuclei are less dense than the starch grains, the latter reach the centrifugal walls after 45 seconds of centrifuging in the case of cells like those shown in fig. 11, while the nuclei in the same cells approach the centrifugal walls only after 20 minutes of centrifuging, not even then to the same extent, although the nuclei should meet *relatively* less resistance on their way through the cytoplasm since they have greater volume than the starch grains. These observations indicate that the nucleus is very slighly denser than the cytoplasm during the resting stage.

The nucleoli in the somatic cells so treated with centrifuging had the same position in the nuclei of these cells as in those of the controls.

From studying the centrifuged buds it appears that the CV in the dividing somatic cells, estimated from the movement of the cell content toward the centrifugal walls and from the fineness of the cytoplasmic threads by which the cell content is attached to the centripetal walls, is higher in the prophase and anaphase and lower in the resting stage and metaphase. It is well known that one can pull finer threads from a more viscous medium than a less viscous one. Thus, the phases in which finer and occasionally more numerous cytoplasmic threads occur between the cell content removed to the centrifugal walls and the centripetal walls should have a higher CV. Observations on numerous sections indicate that resting stages have coarser cytoplasmic threads of the kind just mentioned after centrifuging than in the case of dividing phases.

The latter can be arranged as follows in regard to the fineness of these connecting cytoplasmic threads: resting stage, metaphase and early anaphase, late anaphase and telophase, and finally the prophase where the finest and sometimes the most numerous threads appear. There are, of course, occasional instances where, for example, the threads in the anaphase appear less fine than those in the resting stage but

the majority of instancies show the tendency to be as stated above. On such a basis the CV would appear to increase in the various phases in the following order: resting stage, metaphase and early anaphase, late anaphase and telophase, and finally the prophase with the highest CV. Generally speaking, these observations on *Nicotiana Tabacum* are in agreement with the observations on *Sphacelaria fusca* reported by ZIMMERMANN (1923).

The condition of the cytoplasmic viscosity as described here and in a few other instances, correlated with the data presented by previous investigators has been used as a basis for interpreting the irregularities in the cell divisions in various instances and for interpreting the hybrid vigor as given elsewhere [KOSTOFF (1929/30, 1930)].

Literature cited

CHAMBERS, R., 1925. The physical structure of the protoplasm as determined by microdissection and injection. Cowdry's General Cytology, University of Chicago Press, Chicago 1925.

HEILBRONN, A. L., 1922. Eine neue Methode zur Bestimmung der Viskosität lebender Protoplasten. Jahrb. f. wiss. Bot. **61**, 284—338.

HEILBRUNN, L. V., 1928. The colloid chemistry of protoplasm. Gebr. Borntraeger, Berlin.

KOSTOFF, D., 1929/30. Biologia na callusa (Biology of the callus). Ann. Univ. of Sofia **8**, 297—316. Bulgarian with english summary.

—, 1930. Ontogeny, genetics, and cytology of *Nicotiana* hybrids. Genetica **12**, 33—139.

WEBER, FR., 1924. Methoden der Viskositätsbestimmung des lebenden Protoplasmas. Abderhaldens Handb. d. biol. Arbeitsmeth., Abt. XI, Teil 2, S. 656—718.

ZIMMMERANN, W., 1923. Zytologische Untersuchungen an *Sphacelaria fusca* AG. Ein Beitrag zur Entwicklungsphysiologie der Zelle. Zeitschr. f. Bot. **15**, 113—175.