

A DISSECTION OF THE CHROMOSOMES IN THE POLLEN  
MOTHER CELLS OF *TRADESCANTIA VIRGINICA* L.

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PLATE I.

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The micro dissection method is proving of great value for studying the physical nature of the fresh, unstained, and living cell.

Barber's pipette holder,<sup>1</sup> a mechanical device which was primarily intended by its designer for the isolation of bacteria and for which it is admirably fitted, has hitherto been quite successfully used for the dissection of cells. With it Kite and one of us<sup>2</sup> have been able to make preliminary studies on the nuclear network and even to drag individual chromosomes out of the cell. The procedure, however, was exceedingly difficult owing to the fact that Barber's instrument lacks the necessary control over the movements of the dissecting needles.

With a recently devised micro manipulator<sup>3</sup> which works on a principle entirely different from that of Barber's instrument, the movements of micro needles under the highest magnifications of the microscope can be controlled to such an extent as to render possible an actual dissection of the chromosomes of a cell. A description of such operative work on the germ cells of certain insects is shortly to be published.

For the dissections recorded in this paper a type of micro needle was used which has not yet been described. The needle is made of glass in the usual way except that during the moment when its tip is being

<sup>1</sup>Barber, M. A., *Philippine J. Sc.*, 1914, ix, 307.

<sup>2</sup>Kite, G. L., and Chambers, R., *Science*, 1912, xxxvi, 639. Chambers, R. *Science*, 1914, xl, 824; 1915, xli, 290.

<sup>3</sup>Chambers, R., *Anat. Rec.*, 1922-23, xxiv, 1.

drawn in the micro burner, a peculiar twist is given which causes the finely tapering tip of the needle to bend sharply at about 20 to 30 microns from its apex. This gives one a remarkably fine hooked needle with a very slender tapering tip set on a relatively stout shank to ensure adequate rigidity.

The pollen mother cells of *Tradescantia* were selected, owing to the ease with which the desired stages can be obtained and because of the conspicuous chromosomes which are present during the mitotic stages. The fully grown cell is more or less spherical and about 65 microns in diameter. An anther of young flower buds was crushed and the pollen mother cells set free on a cover-slip in a drop of equal parts of 10 per cent saccharose and of sap expressed from a freshly cut end of the *Tradescantia* stalk. The drop was spread out in a thin layer on a cover-slip which was then inverted over a Barber moist chamber on the stage of the microscope. The micro needles which were used for the dissection projected into the chamber.

All the observations and experiments recorded were made with a Zeiss 2 mm. apochromatic objective, N. A. 1.40, and a No. 8 compensating ocular. It is worthy of note that the photographs illustrating the experiment were taken with the microscope set up for micro dissection purposes which necessitated a substage condenser with a free working distance of  $\frac{3}{8}$  inch. This distance was secured by using a Leitz substage aplanatic condenser with the top lens removed. A layer of immersion oil was placed between the condenser and the floor of the chamber, so that the breaking of the light rays occurred only as they passed into the space of the chamber to reach the hanging drop containing the cells. The combination of lenses used with this arrangement and the illuminant passing through a B filter resolves the structure of the *Surirella gemma* shell but not that of the *Amphipleura pellucida*.

Fig. 1 is a photograph of living, unstained pollen mother cells as the chromosomes are collecting in the equator prior to the first maturation division. Note the ring shape of two of the chromosomes in one of the cells. The chromosomes lie in an optically structureless or hyaline area, which occupies a large part of the cell. About this hyaline area is a relatively thin zone of cytoplasm in which occur highly refractive globules apparently of a fatty nature. In killed and stained cells this hyaline area exhibits fibrous streaks, the so called

spindle fibers of the metaphase. The cell is enclosed within a thin cellulose wall which is somewhat out of focus in this figure.

In the attempt to dissect the cells, it was found necessary to remove the investing cellulose wall as it is too stiff to allow the ready penetration of the micro needle.

Fig. 2 shows the way in which this is done. The cellulose wall is first punctured and torn with a needle. The needle is then thrust through the gap and inserted into the viscous cytoplasm of the cell. With a second needle the opposite side of the cellulose wall is caught, and, on moving this needle while the first is kept stationary, the cellulose wall is pulled cleanly off the cell. In the early prophase stages of the cell this was not possible as the wall was found to be much less developed and was closely adherent to the underlying cytoplasm. When the cell reaches the metaphase stage, the investing wall has become much stiffer and is separable from the cytoplasm.

Fig. 3 shows the cellulose wall after it has been removed and the naked cell lying beside it. The chromosomes are somewhat out of focus but they can still be seen in the hyaline area.

That this hyaline area is jelly-like in consistency is shown in Fig. 4, in which it has been stretched by inserting two needles and then moving them apart. Chromosomes which lie in the stretched part of the area tend also to be stretched. On releasing one needle the stretched area gradually tends to revert to its original shape.

Fig. 5 shows that the cytoplasm can be torn away from the hyaline nuclear jelly in the form of glutinous, granular strips.

Fig. 6 follows Fig. 3 in demonstrating the removal of chromosomes out of the jellied area into which a vertical needle has been thrust. The tip of this needle is out of focus and its hollow shaft shows in the photograph in cross-section as a ring. The other needle with its tip also out of focus lies diagonally to the plane of the figure and is in position preparatory to removing one of the chromosomes.

In fresh preparations of pollen mother cells it is difficult to pull the chromosomes out of the jelly. The chromosomes are considerably more solid than the material in which they lie, but in the attempt to extricate one, they all tend to clump and to stick together. After some time, however, the hyaline area tends to liquify and it is then easier to isolate the chromosomes.

Fig. 7 shows two isolated chromosomes. Since these chromosomes are from a cell which has not yet undergone the first maturation division, they are morphologically tetravalent. One of them is a definite ring with transverse constrictions. The other is almost a closed V with one part on end viewed in optical section. It is distinctly a cylinder exhibiting a medulla, which possesses a refractive index quite different from that of its cortex. This structure is in accordance with that of the prophase filaments already reported and substantiates the findings recorded in a discussion on the structure of the chromosome, by one of us.<sup>4</sup>

Fig. 8 shows one half of the tetravalent ring-shaped chromosome the other half having been dissected away. Notice the tip of the dissecting needle at the lower left corner of the figure.

Fig. 9 exhibits the elasticity of the chromosome material. The chromosome of the previous figure was caught at its two ends by the needles and stretched. It is to be noted that the constricted area stretches more than the rest of the rod and is thus drawn out into a thin filament. Stretching the chromosome slightly more than is shown in the figure causes a break at the constriction, whereupon the slender filamentous portion gradually draws back into the more massive body of the chromosome.

Regarding the structure and consistency of the metaphase spindle and of the chromosomes of these cells, we can draw the following conclusions.

The spindle area is a hyaline, jelly-like mass, less solid than the surrounding cytoplasm and distinctly separated from it. In the living and fresh state there is no evidence during metaphase of spindle fibers which form so prominent a feature in coagulated and stained material.

In this jelly lie the much denser but homogeneous chromosomes. The chromosome of the *Tradescantia* pollen mother cell is an elastic, jelly-like, nodulated, cylinder and possesses a cortex which differs markedly in refractive index from its central core.

<sup>4</sup>Sands, H. C., *Am. J. Bot.*, 1923, x (in press).

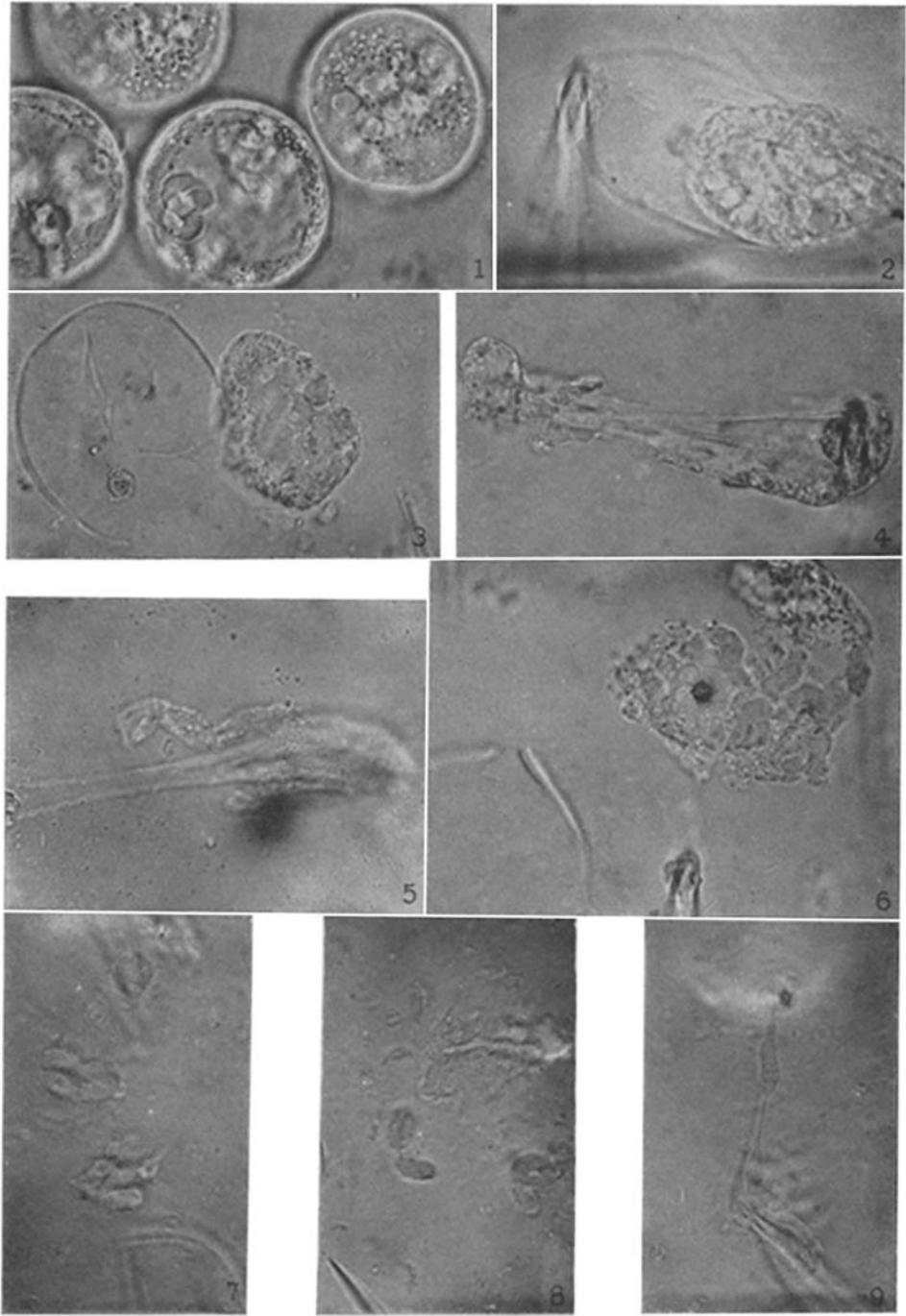
## EXPLANATION OF PLATE 1.

FIGS. 1 to 9. Photomicrographs of living unstained pollen mother cell of *Tradescantia virginica* L. isolated in plant sap and saccharose solution and dissected to ascertain the consistency of the nuclear area and of the chromosomes.

Photographs taken with a No. 8 compensating ocular and a Zeiss 2 mm. apochromatic objective. Substage equipped with a Leitz aplanatic condensor with the top lens removed. Source of illumination a Leitz Liliput arc lamp with the light rays passing through a B filter. This combination resolves the beaded lines on the shell of *Surirella gemma* but not those of *Amphipectera pellucida*.

The photographs were reduced to about  $\frac{1}{8}$  of the original size.

For a description of the figures see the text.



(Chambers and Sands: *Tradescantia virginica* L.)