Proceedings of the Iowa Academy of Science

Volume 66 | Annual Issue Article 9

1959

A Modification of the Carbowax Embedding Technique for Plant Tissue

Waldo S. Walker

A Modification of the Carbowax Embedding Technique for Plant Tissue

By WALDO S. WALKER

Abstract. The highly hydrate condition of the wall material of the cells of collenchyma causes considerable difficulty in the preparation of permanent sections of the material which are suitable for quantitative studies. When standard procedures of dehydration and embedding are employed, excessive shrinkage of the wall material is common. In an attempt to avoid this shrinkage, polyethylene glycol (carbowax) was used as the killing, fixing, dehydrating and embedding medium. This method met with considerable success. The schedule outlined is a modification of that used by A. R. Spurr.

The cell walls of certain plant tissues contain great amounts of water. In the living state, the cell walls of collenchyma tissue contain as much as 65-70 percent water (Cohn, 1892). This is in contrast with approximately 25 percent found in the walls of xylem cells in several species of trees.

For the past few years the author has been conducting research which has involved precise measurement of the walls of collenchyma cells to show the influence of various treatments on this tissue. These measurements were taken from thin, transverse and longitudinal sections of stems and petioles. In work which involves quantitative study of the walls of the cells in a tissue such as collenchyma, it is imperative that techniques used in producing sections of the tissue have no damaging effect on the cell walls. Several attempts were undertaken in an effort to obtain thin sections of this tissue using the standard methods such as paraffin, dioxane, and celloidin. When the tissue was placed in the harsh killing, fixing, dehydrating and embedding media required in these procedures, damage to the collenchyma cell walls invariably occurred. Removal of the water from the cell walls resulted in excessive hardening, distortion and shrinkage of the cell walls (Figure 1).

In an effort to find a method by which one could make thin sections of this tissue without causing damage to the cell walls, polyethylene glycol or "carbowax" was tried as an embedding medium. This approach met with considerable success. The main advantage of this method lies in the fact that polyethylene glycol is very soluble in water. Use of this method does not distort the cell walls because no harsh dehydration series is necessary. Tissues that are placed in the polyethylene glycol are kept in this water-based medium throughout the killing, fixing, dehydration, and embedding steps of slide preparation. All dilutions of the polyethylene glycol were made with distilled water.

Several schedules involving the use of polyethylene glycol have been proposed by other workers (Carsten, 1949; Firminger, 1950; Van Horne, 1951; and Spurr, 1957). The schedule used by the author was a modification of that used by A. R. Spurr (1957).

PEG (hereafter to be used as the abbreviation of polyethylene glycol) is available in ten polymers that range in molecular weight from 200 to 20,000. Each of the ten polymers is designated by a number which represents its average molecular weight. PEG Nos. 200, 300, 400, and 600 are water white liquids while those of higher molecular weight are waxy solids which are progressively more hard. Only three of the ten available polymers were used in this work. These were PEG Nos. 400, 1540, and 4000. PEG No. 400 is liquid and is completely soluble in water at 20° C. PEG No. 4000 is very hard and waxy and is 62 percent soluble in water at 20° C. Various percentages of PEG No. 400 are used in the schedule and all such dilutions were made with distilled water.

A special killing and fixing agent was not used in preparation of the material for cutting. In the first step of the procedure, 5 mm. pieces of stem tissue containing collenchyma cells were placed in 5 percent PEG No. 400. After the tissue was placed in this solution, the vials were aspirated until each piece of tissue sank to the bottom of the vial. After 12 hours in this solution, the tissue was transferred to a graduated series of increasing concentrations of PEG No. 400 which included 10, 15, 20, 30, 40, 50, and 70 percent. The tissue was placed in the first five of these solutions for 12 hours each. It was placed in the 70 percent solution for 24 hours. At the end of this period of time, small pieces of PEG No. 1540 were placed in the vials along with the 70 percent solution of PEG No. 400, to allow the former to dissolve into the latter gradually. The tissue was kept in this mixture at room temperature for 12 hours. The vials were then placed in an oven at 37° C for 24 hours and were shaken several times during the 24 hour period to insure thorough mixing of the two densities of PEG. This mixture was then replaced by a melted mixture of 1 part PEG No. 4000 to 5 parts PEG No. 1540 and the vial was placed in an oven at 56° C. These concentrations were not always the same because of the variations in the temperature of the room in which the cutting was to be done. Test mixtures were made with varying concentrations of the two polymers, and sections were cut with the microtome to determine which concentration worked best at the specific room temperature. If the room was warm (summer temperature of 80-90° F.), the proportion of PEG No. 4000 was increased slightly. The pieces of tissue were kept in this mixture for 20 hours, and then this mixture was replaced by fresh PEG of the same concentration. The mixture was changed two more times at 24 hour intervals. The vials were then removed

from the oven, and the embedding mixture which contained the pieces of tissue was poured into Syracuse watch glasses which had been coated with petroleum jelly. After the tissue was oriented in the hardening medium the watch glasses were placed in the refrigerator. The embedding medium which was cooled rapidly had a smoother texture than that which was allowed to cool slowly. The solidified medium was easily lifted out of the watch glasses. Five micron sections of the tissue were then cut with a Spencer rotary microtome. The PEG embedding medium seemed to give a much smoother ribbon than paraffin. Excellent ribbons were obtained with the microtome set as low as 2 microns and as high as 25 microns in thickness. Care was taken throughout the cutting process to keep the microtome dry.

It is at this point that the use of PEG presents the only difficulty in the production of permanent slides. Most standard procedures employ an adhesive such as Haupt's to secure the sections to the slide. Usually a 3 or 4 percent solution of formalin is used to float the ribbons out on the slide and set up the adhesive. This procedure cannot be followed successfully with PEG because the ribbon is water soluble. When the ribbons are placed on the formalin the matrix completely dissolves away from the tissue, leaving the sections of tissue floating free. When the formalin evaporates and the sections settle on the adhesive, the PEG which previously held the cell walls of the tissue in rigid support dissolves away and the walls of the collenchyma cells shrink as they dry.

The problem was solved by dissolving the PEG matrix in distilled water and handling the sections as one would treat free-hand sections. After the matrix was dissolved from the sections, the water was drawn off with a pipette and filter paper and the sections were washed again with the distilled water. They were then stained with an aqueous solution of haemalum for several minutes. The stain was drawn off the sections and they were washed three times with distilled water. The water was drawn off, the stained sections were mounted in glycerin and the coverslip was placed on the slide and sealed with clear fingernail polish.

The success of this procedure lies in the fact that the collenchyma tissue never goes through strong dehydration as is necessary in the paraffin method. Also, after the sections are cut they are always kept in a water solution. Although the process is rather tedious, it is very successful in terms of the results obtained. Figures 1 and 2 show a comparison between a cross section of collenchyma embedded by the paraffin technique and one embedded with carbowax. These sections were cut at 5 microns. The permanence of such slides is prolonged by applying more fingernail polish to the coverslip after

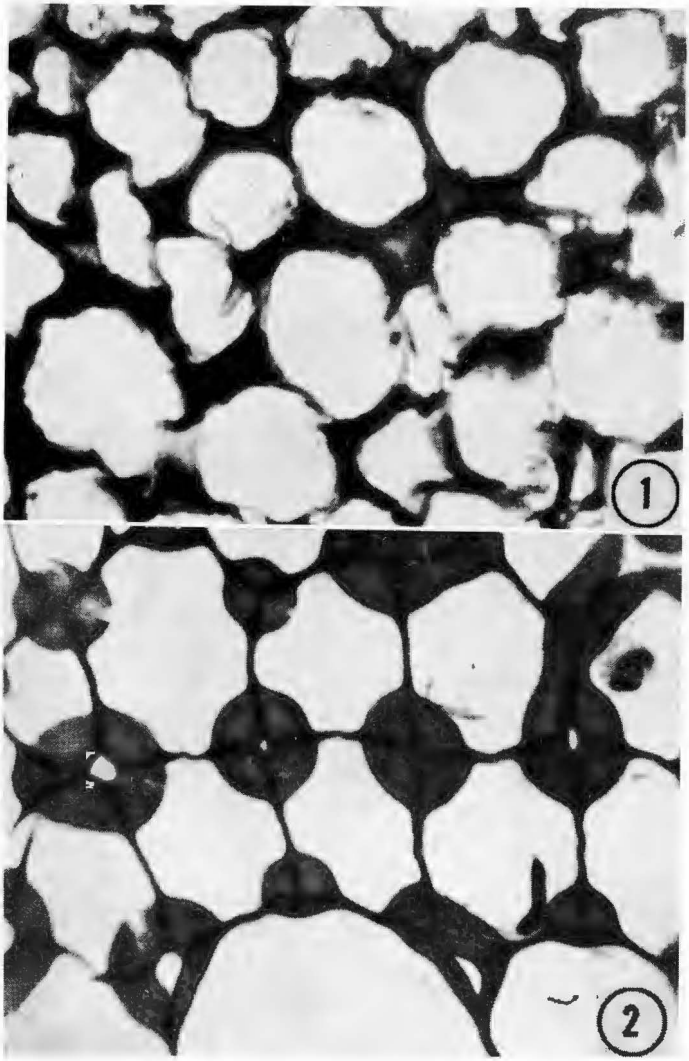

Figure 1. Photomicrograph of cross section of collenchyma of stem of *Datura stramonium* L. This material was killed, fixed, dehydrated, and embedded by the standard paraffin technique.

Figure 2. Photomicrograph of collenchyma tissue taken from a plant of the same age as that shown in Figure 1. This material was killed, fixed, dehydrated and embedded in polyethylene glycol.

8 or 10 months. Slides 15 months old are still in excellent condition, and there has been no fading of the stain.

The above treatment is not recommended for cytological studies of protoplasm due to the plasmolysis which occurs. Perhaps if a more finely graduated series of PEG were used the plasmolysis would be reduced.

When using the technique outlined here, care must be taken that none of the media are acid. Unless the PEG and all other substances applied to the tissue are slightly alkaline, the walls of the collenchyma swell to many times their normal size.

Literature Cited

Carsten, Mary E. 1947. A rapid procedure for the embedding of eyes. Arch. Pathol. 44: 96-100.

Cohn, J. 1892. Beintrage zur Physiologie des Collenchyms. Jahrb. Wiss. Bot. 24: 145-172.

Firminger, H. I. 1950. Carbowax embedding for obtaining thin sections and study of intracellular lipid. Stain Techn., 25: 121-123.

Spurr, A. R. 1957. The effect of boron on cell-wall structure in celery. Am. Jour. of Bot. 44: 637-649.

Van Horne, R. L., and L. C. Zopf. 1951. Water-soluble embedding materials for botanical microtechnique. Jour. Amer. Pharm. Assoc., Sci. Ed. 40: 31-34.

Department of Biology
Grinnell College
Grinnell, Iowa