9. Sectioning Unembedded Tissues

Materials that have sufficient rigidity to withstand the impact of the sectioning knife can be sectioned without embedding. The most rapid and simple method consists of grasping a piece of fresh or preserved plant material in the fingers and slicing with a razor or a razor blade. Extremely thin sections can be cut in this way by a skilled and experienced worker. These methods receive entirely too little attention in teaching and research. These seemingly crude methods can yield excellent preparations for teaching. The student who collects his own materials and makes his own preparations, even though crude ones, gains an understanding of plant structure that cannot be imparted solely by thrusting a neatly labeled finished slide before him. Much wasted effort could be spared in research by adequate preliminary survey work conducted by freehand sectioning.

Written directions are of little instructional value for this work. Patience, experience, and perhaps inherent skill are the chief requirements. Sectioning can be aided by enclosing the material between pieces of pith or cork. Split a cylinder of pith lengthwise and cut a longitudinal groove or a recess in the pith of appropriate size and shape to receive the specimen. Wrap the two pieces of pith together with thread, and soak in water. The pith expands and encloses the material firmly enough to be sliced with a sharp blade. The tissues and the knife should be kept wet with water and the sections floated in water. The subsequent handling of the sections is described at the end of the discussion of sectioning. A drip siphon is a useful device when doing extensive freehand sectioning. Place a 2- to 5-liter bottle of distilled water above the work table, and install a siphon that terminates in a glass tube drawn to a fine aperture. Adjust the siphon with a screw clamp so that a drop of water is released every 2 or 3 sec. and drops into a waste container. The worker can have both hands occupied with the sectioning, and a drop of water for wetting the material or floating a section is instantly available at any time.

Sectioning Unembedded Tissues With the Microtome

Rigid materials or large objects from which it is difficult to obtain complete sections freehand should be cut with a microtome. Any sliding microtome, from the inexpensive table microtome to the most elaborate precision microtome, may be used. A fresh twig of white pine, basswood, or cottonwood makes an excellent subject. Clamp a 3-cm. length of twig into the microtome with 1 cm. projecting above the clamp. Set the knife so that it makes a long slanting cut, and make sure that there will be ample clearance between the tissue clamp and the knife carriage. Keep the twig and the knife flooded with water while cutting. Remove the slices from the knife, and float them in a watch glass of water. Examine the floating sections with a binocular or a hand lens, discard imperfect sections, and continue sectioning and selecting until enough satisfactory sections have been cut. The ratio of perfect sections obtainable by this method is much less than is possible by the celloidin method. Nevertheless, the quality and output by sectioning fresh material are an agreeable surprise to workers who give it a fair trial.

The above method can be used with unembedded tissues that have been killed in 70% alcohol and stored in that fluid, or with materials that have been killed in any fixing fluid and dehydrated to 70% alcohol. This degree of dehydration is usually necessary to make the tissues sufficiently firm. However, woody tissues may be killed in *FAA,* rinsed in several changes of 50% alcohol to eliminate much of the acid, and sectioned as above. When cutting tissues from a wet preservative or when cutting partly dehydrated tissues, keep the tissues and the knife flooded with water or with dilute alcohol of approximately the same water concentration as the preservative. Float the sections in a dish of the fluid in which they were cut.

Sections of living tissues frequently do not have satisfactory staining reactions, especially with the stains used for permanent slides. Furthermore, these staining processes usually induce severe plasmolysis and alteration of the protoplasm. If good preservation of the protoplasm is desired, transfer sections cut from living material into a killing fluid, in which the protoplasm is fixed and hardened. For sections of woody twigs or firm herbaceous stems, *FAA* is recommended. For critical studies try Craf II or III (Chap. 3) or an experimentally determined modification. The quality of preservation produced by a formula can be ascertained by examining sections in a drop of the fluid. Thin sections are killed and hardened almost

instantly. After 10 min. in the fluid, rinse the sections in water, and proceed with the staining.

The preceding methods permit the study of living cells, or cells in which protoplasmic details were fixed by reagents. If these methods of sectioning unembedded material fail to give satisfactory results, the materials must be treated by methods which may distort or destroy fine protoplasmic details. Nevertheless, the following methods are useful, within the stated limitations.

Dry lumber of soft woods such as white pine, basswood, or willow can be sectioned successfully without embeddding. Trim the wood carefully into blocks measuring approximately 1 by 1 by 2 cm., and prepare the blocks by alternately boiling in water and pumping in cold water, until the pieces are thoroughly saturated and sink. Hard woods that cannot be cut after this treatment may be sectioned by one of the following methods.

The live-steam method of sectioning wood is based on the principle used in the manufacture of veneer. If a jet of superheated steam is directed upon the surface of a block of wood, the surface becomes soft enough to permit the cutting of a thin section. Steam can be generated in a flask, but a safer device is a small steam generator, provided with a pressure gauge, water level gauge, safety valve, and a water inlet. Such generators are obtainable from dealers in chemical apparatus. The steam from the generator passes through a copper tube, in which there is a superheating coil heated by a Bunsen burner, and the superheated steam emerges through the small orifice of a nozzle which can be adjusted over the specimen. Successive sections are cut at experimentally determined intervals of steaming. For additional details and variations of the apparatus refer to Crowell (1930) and Davis and Stover (1936).

Jeffrey's vulcanization method makes possible the sectioning of extremely hard materials, such as walnut shells. Materials are sealed in a chamber made from a section of pipe, and heated in a dental vulcanizer at approximately 160°C. for 1 to 5 hr. This is followed by treatment in hydrofluoric acid. Materials may be cut unembedded or in celloidin. Because of the special equipment necessary, the procedure is not described here, and interested workers are referred to Jeffrey (1928).

Sectioning With the Freezing Microtome

Unembedded tissues that are too soft or fragile to stand up under the impact of the knife can in some cases be frozen and then

sectioned. The device used on the sliding microtome for this purpose is known as a freezing attachment. The various freezing attachments are described in the catalogues of the manufacturers of microtomes. The device replaces the usual tissue clamp or object carrier of the microtome. Freezing is accomplished by the evaporation or expansion of a freezing agent – ether, CO_2 gas, or solid CO_2 (dry ice). The piece of tissue to be frozen is usually enveloped in a fluid or semifluid medium, which on freezing affords additional support.

Gum arabic is probably the best-known supporting medium. Make an aqueous solution of gum arabic of thick, sirupy consistency. Add a few crystals of carbolic acid. Dip the specimen to be cut into the gum. Freeze a 2- to 3-mm. layer of gum on the supporting disk of the freezer. Place the specimen on the disk, wrapping a generous quantity of the gum around the specimen. Turn on the freezer, and, as the gum begins to congeal, wrap more gum on the specimen until the material is well supported. Proceed with the sectioning.

Gelatin is another satisfactory supporting medium. Make a gelatin solution that is semifluid at room temperatures. Add 0.1 per cent carbolic acid as a preservative. Warm on a water bath for use, and use in the manner described above for gum arabic. A semifluid solution of agar is another excellent medium, used like gelatin.

Freehand or microtome sections can be mounted in a drop of water or 50% glycerin and studied with the microscope. If glycerin is used, the water can be evaporated and the cover glass sealed with lacquer or paraffin, making a semipermanent mount. The mounting media described on page 102 also can be used. Sections of dark-colored woods, or other materials having adequate coloration, can thus be made into semipermanent or permanent slides without staining.

Staining

The staining of sections of unembedded tissues is essentially the same as the staining of celloidin sections. The worker who has had previous experience with paraffin sections can follow the staining charts in Chap. 7, making the necessary modifications. For the benefit of workers who wish to use the present chapter without having had previous experience, an outline of some simple, practical processes is offered. Sections cut in alcohol should be progressively transferred or run down to water before staining. Add an equal volume of water to the alcohol containing the sections. Mix gently, pour off half of the liquid, and add an equal volume of water. Pour off all the liquid,

and rinse the sections with two changes of water. Proceed with the staining process.

The most easily controlled stain combination consists of a self-mordanting hematoxylin (Chap. 7) followed by an aniline dye. Assume that sections of a firm woody subject are to be stained. Drain off the water in which the sections are floating in a watch glass, and flood the sections with hemalum or similar stain. After 5 min. remove a section with a brush, rinse in distilled water and then in tap water and examine with a microscope. Lignified cells, such as tracheids and phloem sclerenchyma, should be practically colorless. Soft tissues like pith, cortex, and cambium should have blue-stained walls. Nuclei should be blue-black. If the sections are overstained the entire protoplasts become blackened, obscuring cellular detail, and the walls of lignified cells become stained. Sections can be destained with ½% HCl and thoroughly washed in tap water. When the correct intensity of blue is attained, cover the sections with safranin. Try an interval of 15 min. in safranin. Locate this point in the staining schedule given in Staining Chart III. Rinse the sections in water, and cover with 50% alcohol. The safranin will dissolve out of the nonlignified tissues faster than out of lignified cell walls. Slower destaining can be obtained with 50% acetone. When the lignified cells are still deep red, rinse the sections quickly in anhydrous alcohol or acetone. Acetone stops destaining action better than does alcohol. Complete the process as shown in Staining Chart III. Mount the sections on slides as described in Chap. 7.

Having gained some familiarity with the above stain and with the use of staining schedules, study the discussion of staining in the paraffin method (Chap. 7) and the celloidin method described earlier in this chapter, and try some of other stain combinations described in those chapters.

Sectioning by Encasing in Water-Soluble Waxes

These little-known methods are intermediate between embedding methods and sectioning without embedding. As a matrix, use one of the water-soluble wax-like synthetics, such as glycerol monostearate, which melts at 55°C. The living or fixed material is transferred directly from water to the melted matrix, which is then hardened, fastened to a wood mounting block, and sectioned. Very little infiltration occurs, but the material is encased and held with sufficient rigidity to make fairly good sections. This method has been used successfully with nearly mature clover seeds and shows much promise

for similar subjects. Other synthetic stearates should be tried (Johansen 1940, McLane 1951).

Microchemical Tests

One of the important advantages of sectioning fresh untreated material is the avoidance of the chemical and physical alterations that are undoubtedly produced by the processing necessary for embedding. Although the protoplasm is probably changed by the handling incident to sectioning and mounting fresh material, the nonliving constituents of cells and tissues probably are not markedly changed chemically. This makes possible the use of microchemical tests that reveal with more or less accuracy the chemical nature of important structures. Although the science of chemical microscopy is highly developed, it occupies a minor place or is virtually ignored in many botanical curricula. However, certain chemical tests are generally regarded as indispensable in even an elementary study and are therefore included here.

Starch; Iodine-Potassium Iodide Test (IKI)

Water	100 cc.
Potassium iodide	1 g.
Iodine	1 g.

Place a drop of the reagent directly upon the specimen. Most starches give a blue-black color. Waxy starch, found in some genetic stocks of maize, turns yellow or brown. By using a very dilute solution of the reagent and imparting only a trace of color to the starch, the laminations in the granules may be observed with the microscope. When testing entire living cells such as those of *Spirogyra* or leaves like those of *Elodea,* the aqueous reagent reacts very slowly, and a reagent made with 70% alcohol should be used.

Sugars; Osazone Test

Solution A

Glycerin (warm)	10 cc.
Phenylhydrazine-hydrochloride	1.0 g.

Solution B

Glycerin	10 cc.
Sodium acetate	1.0 g.

Mix a drop of each solution on a slide, float the sections in the mixture, place the slide over the mouth of a wide-mouthed flask containing boiling water, and heat for 10 to 15 min. Glucose and fructose produce fascicles of yellowish needles; maltose produces fan-shaped clusters of flattened needles. After 30 to 60 min. of heating, sucrose becomes hydrolyzed and reacts to form needles like those produced by glucose.

Reducing Sugars; Fehling's Solution Test

Although this is not a microtest, it is included because it is essential for a systematic examination of the prominent chemical constituents of cells and tissues.

Solution A

Water	1 l.
Copper sulphate	79.28 g.

Solution B

Water	1 l.
Sodium potassium tartrate	346 g.
Sodium hydroxide	100 g.

Mix equal volumes of *A* and *B* in a test tube, add a quantity of the finely pulverized materials to be tested, heat to boiling. A brick-red precipitate indicates reducing sugars.

If a negative or slight test is obtained for reducing sugars, a test for sucrose can be made by first hydrolyzing the sucrose. Add 1 cc. concentrated HCl to 10 cc. of the extract to be tested. Heat in a water bath at 70°C. for 5 min. Cool and neutralize with sodium carbonate, and test for the resulting reducing sugar with Fehling solution.

Lignin; Phloroglucin Test

Solution A

Phloroglucin, 1% to 2% in 95% alcohol

Solution B

Hydrochloric acid (try concentrated acid, as well as acid diluted with 1 to 3 volumes of water.)

Float the sections in a drop of phloroglucin on a slide, and cover with a cover glass. Place a small drop of the acid at one edge of the cover glass. Examine with a microscope. Lignified walls become violet-red.

Cellulose; Iodine-Sulphuric Acid Test

Mount sections or crushed fragments in IKI. Observe with the microscope, and locate blue-stained starch. Place a drop of 75 per cent H_2SO_4 at one side of the cover glass. As the acid diffuses in, note that cellulose walls swell and become blue.

Cellulose; Chloriodide of Zinc Test

Water	14 cc.
Zinc chloride	30 g.
Potassium iodide	5 g.
Iodine	0.9 g.

Mount thin sections in a drop of the reagent. Cellulose becomes blue.

Proteins; Millon's Reagent Test

Concentrated nitric acid	9 cc.
Mercury	1 g.

When dissolved, dilute with an equal volume of water. Place the specimen on a slide, drain or blot off excess water, put on just enough reagent to cover the material, and heat with a small flame. Proteins give a brick-red color. This is not a highly satisfactory reagent. Futhermore, it is highly corrosive and must be used with care. Do not permit inexperienced students to use this reagent on a microscope. The instructor should set up a demonstration microscope, after draining excess reagent from under the cover glass.

Fats and Oils; Sudan III

Alcohol (80%)	100 cc.
Sudan III	0.5 g.

Cut very thin sections or smear a fragment of the material on a slide, flood with the dye, and cover with a cover glass. After 10 to 20 min. the microscopic globules of fat should assume the bright, clear color of the dye. Cotyledons of the soybean and peanut are good subjects.