2. Collecting and Subdividing Plant Materials

The preservation of structural details of cells and tissues is influenced by the condition of the plant at the time of collecting and by the subsequent preparation for killing (fixation). For the study of normal structure, select healthy, representative plants. Remove the plant or the desired part with the least possible injury to the sample. If the material is to be killed at once, follow the procedure outlined in Chap. 3. If the material cannot be killed promptly, it should be stored and transported in such manner that bruising, desiccation, molding, and other injuries are minimized. Do not use material that has been obviously damaged in storage or shipment. The unsatisfactory slides obtained from such material are likely to be interpreted by uncritical observers as the result of poor technique. Dried herbarium specimens can be softened and sectioned to make slides in which it is possible to determine the gross features of vascular arrangement or carpellary organization (Hyland, 1941). However, such material is not suitable for detailed microscopic study.

The following general directions are introduced at this point for the use of readers who have selected subjects on which to work. The reader who seeks suggestions concerning suitable and tested subjects should turn to Part II and use the recommendations made therein in conjunction with the present chapter.

LEAVES

Remove a leaf or leaflet by cutting the petiole, without squeezing or pulling the petiole. The vascular bundles in the petioles of some plants become dislodged easily. For transportation or brief storage, place the leaves between sheets of wet toweling paper and keep in a closed container such as a tin can or a Mason jar. If the leaves appear to be wilted on arrival in the laboratory, freshen them in a moist chamber or in water before processing.

STEMS

Leafy stems can be kept fresh for several days by standing them in a container of water, preferably in a refrigerator. If such storage is not practicable, cut the stems into the longest pieces that will fit into the available closed container without folding or crushing. Wrap the pieces promptly in wet paper and store in a cool place. Dormant woody twigs, large limbs, and disks cut from logs can be kept for weeks in a refrigerator without appreciable injury.

ROOTS

Do not collect roots or other underground organs by pulling up the plant. The delicate cortex is easily damaged, in fact, the woody stele may be pulled out of the cortex, leaving the cortex in the ground. To collect roots without damaging them, dig up the plant, soak the mass of soil in water until thoroughly softened. Wash the soil away carefully, cut off the desired roots and brush them gently with a camel's hair brush to remove as much soil as possible. Wrap the pieces and store as in the case of stems.

FLORAL ORGANS

Remove entire flowers or flower clusters and wrap in wet paper. Store in a closed container in a cool place. Large buds like those of lily can be kept in a Mason jar of water until you are ready to dissect and preserve them. Fruits may be collected and stored in a similar manner.

LIVERWORTS AND MOSSES

Remove groups or mats of the material with a generous quantity of the substratum. Store in a moist chamber until the plants are turgid. Saturate the substratum in order to permit the removal of complete plants without excessive damage. Dissect out the desired parts under a binocular and transfer to the preserving fluid promptly.

ALGAE

Collect in a quantity of the water in which the plants are growing, and keep in a cool place in subdued light. Many filamentous forms disintegrate rapidly in the laboratory, and even in the greenhouse unless the temperature and light can be carefully controlled. It is best to kill algae promptly after collecting.

FLESHY FUNGI

The larger fleshy fungi can be transported and stored, wrapped

loosely in waxed paper. Sporulation continues and may indeed be promoted in this manner. However, since molding and disintegration take place during prolonged storage, material should be processed promptly. Small fungi should be wrapped in moist paper, enclosed in waxed paper, and processed as soon as possible.

PATHOLOGICAL MATERIAL

Particular care should be exercised to insure that the condition of the host tissues is not altered by handling, in order that abnormal structure may be properly interpreted as an histological symptom of the disease. Prevent wilting of the material, or revive it in a moist chamber, but avoid the development of bacteria, molds, or other secondary organisms. For a pathological investigation, always collect normal, disease-free tissues of age comparable with the diseased samples. It is imperative to work out the best technique for preserving the "normal" condition of the host before attempting an authoritative interpretation of slides of pathological material.

The foregoing general remarks will serve as a basis from which the worker can develop effective methods and habits of collecting and handling material in accordance with facilities and circumstances. Hold rigidly to the view that the finished slide should represent the original structure of the plant, whether that structure is presumably normal or pathological or is the result of experimental treatment.

The handling of materials that are to be used for bulk specimens or whole mounts is described in Chap. 10. The preparation of permanent slides from microtome sections consists essentially of the following processes:

1. Selecting desired plants or parts of plants and, if necessary, subdividing into suitable pieces.
2. The killing and preservation of the contents of cells and the preservation of cellular structures in a condition approximating that in the living plant.
3. Embedding in a matrix if necessary, in order to support the tissues for sectioning. See page 91 for the sectioning of unembedded tissues.
4. Sectioning of the tissues into very thin slices.
5. Staining the slices and covering with a cemented cover glass to make a permanent slide.

Subdividing Material for Processing

Some preliminary remarks concerning the action of reagents in the preservation of cells and tissues will aid in understanding the following description of this process. The reagents used for killing

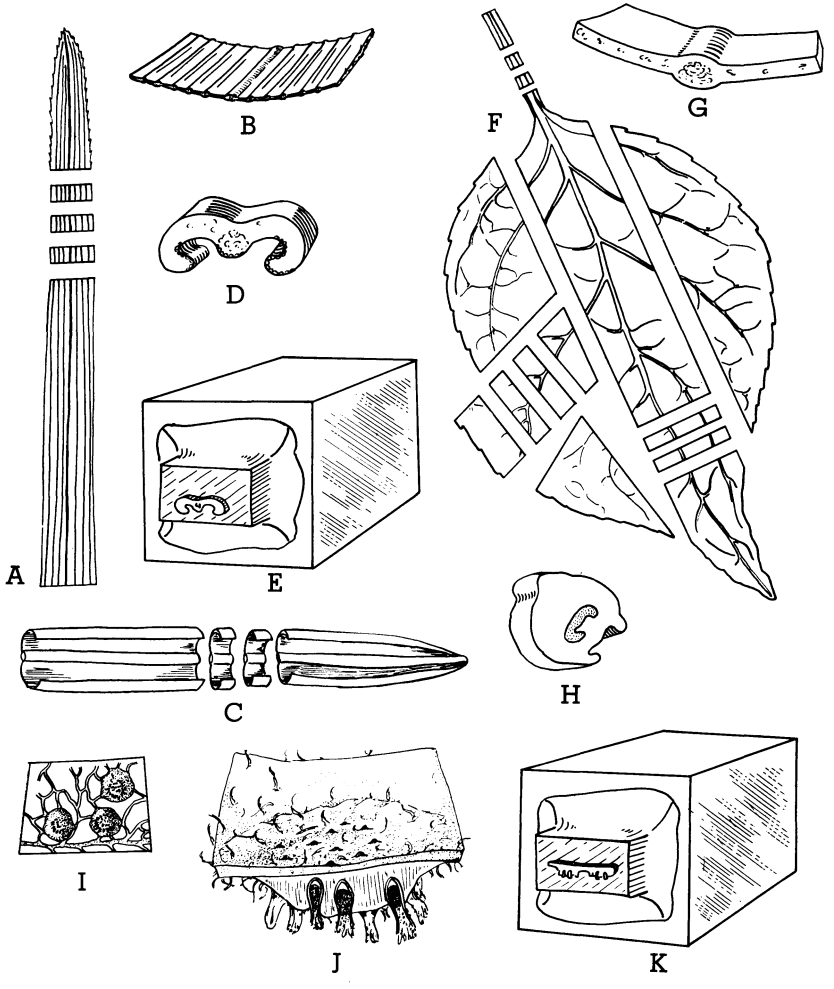

FIG. 2.1—Methods of subdividing leaves for embedding: *A–D,* long narrow leaves and transverse pieces removed from such leaves; *E,* embedded piece of leaf fastened to mounting block; *F–H,* large broad leaf and excised pieces of blade and petiole; *I,* portion of leaf with fungus pustules; *J,* enlarged view of excised aecia; *K,* embedded piece of leaf bearing aecia, fastened to mounting block.

cells contain ingredients that are toxic to protoplasm. In order to stop life processes quickly and without distortion of structure, the killing fluid must reach the innermost cells of a piece of tissue before disintegration takes place. Most reagents penetrate very slowly through the cuticle or cork on the surfaces of plant organs, but

penetration is much more rapid through cut surfaces. Therefore, it is desirable to cut the organ being studied into the smallest pieces that will show the necessary relationship of parts.

The subdividing of soft fresh material is best done with a razor blade, with the material placed on a sheet of wet blotting paper or held carefully against a finger. Excessive pressure against the support is likely to rupture delicate tissues as in the mesophyll of leaves (Fig. 11.1) or the chlorenchyma of a stem (Fig. 11.2). Such damage does not become visible until the sections in the ribbon are examined or possibly not until the finished slide is examined. The usual results are peeling of the epidermis and distortion of the crushed tissues.

Leaves are almost invariably cut into small pieces for processing. Narrow leaves that are not much over 5 mm. wide, may be cut into complete transverse pieces measuring 2 to 4 mm. along the rib (Fig. 2.1 *A–D*). Examples of this type are bluegrass, garden pinks, hedge mustard, and some narrow-leaved milkweeds. Broad leaves should be cut into small pieces, selected to include midrib, lateral veins, fungus pustules, fern sori, or other desired structures (Fig. 2.1 *F, G, I, J*). The enlarged views of the pieces of leaf (Fig. 2.1 *B, D, G, I, J*) and the pieces of embedded tissue mounted on blocks ready for sectioning (*E* and *K*) will aid in visualizing the orientation of pieces. Particular care should be used in subdividing pathological material (Fig. 2.1 *I, J*). If it is necessary to know which is the long axis of the leaf, cut all pieces so that the shorter dimension is along the long axis of the leaf, or vice versa, and record the method in your notes.

Herbaceous stems, roots, petioles, and other more or less cylindrical organs are usually cut into short sections or disks. When cutting out sections or subdividing pieces, do not roll or press the pieces. Keep the material moist, and work rapidly. After the final subdivision, drop the pieces into the killing fluid promptly. By means of descriptions and sketches, like those in Figs. 2.1 and 2.2, keep an accurate record of the part of the plant from which the pieces of tissue were obtained.

Figure 2.2 gives additional suggestions for subdividing organs. A stem that does not exceed 2 mm. in diameter should be cut into sections 2 mm. long if highly cutinized, but may be as long as 10 mm. if the surface is permeable. An organ 5 mm. in diameter should be cut into 5-mm. lengths. An organ 1 cm. in diameter should be cut into disks 2 to 5 mm. thick. Stems of larger diameter are usually cut into 5-mm. disks that are halved or quartered longitudinally or divided into wedge-shaped pieces.

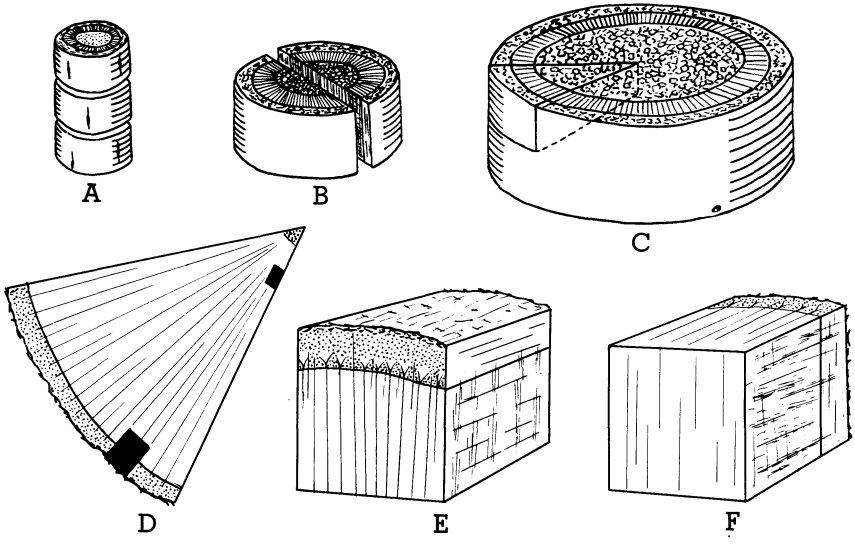

FIG. 2.2—Methods of subdividing massive cylindrical organs: *A–C*, sample includes portions of all tissues in the axis; *D* shows the position of pieces removed from a large log; *E* and *F*, enlarged views of trimmed pieces removed from large log.

Woody twigs having a diameter up to 5 mm. should be cut into 15-mm. lengths. Larger twigs should be cut into shorter pieces because the impermeable cork makes penetration by reagents difficult, except through the cut ends. Do not cut the twigs into pieces with pruning shears or a knife. Rough handling will bruise the cambium, phloem, the fragile primary cortex and cork cambium, resulting in the separation of the outer layers during sectioning or during staining. Use a razor blade and cut through the twig by chipping a groove deeper and deeper around the twig until it is cut through. An excellent tool for cutting twigs into sections is a fine-toothed high-speed saw, such as a rotary dental saw or a jig saw, especially the vibrating diaphragm type.

To make slides of transverse, radial, and tangential sections in the region of the cambium of old trees, use tissues removed from newly felled logs or limbs having a diameter of at least 10 cm. Cut disks 2 to 3 cm. thick from portions of the log that were not bruised in felling. Wrap the disks in wet burlap and take into the laboratory

at once for further trimming. Split a disk radially into pieces having uninjured blocks of bark firmly attached to the wood. Trim off enough of the inner part of the wedge of wood to leave a block of sapwood with several annual rings and with cambium and all outer tissues intact (Fig. 2.2 *D-F*). With a razor blade split a thin layer from the two radial faces, from the inner tangential surface and from the transverse faces of the block, thereby removing tissues that were compressed during the preliminary trimming. Keep the material wet during these operations. Drop the pieces into the killing fluid at once after final trimming.

Wood from dead logs, dry lumber, or furniture wood requires proper trimming to establish the future cutting planes. It is usually easy to establish the radial plane by splitting the wood longitudinally, parallel to a ray. At right angles to this plane, split the block longitudinally along the tangential plane, and then trim in the third or transverse plane. Rough splitting can be done best with a plane bit, and rough crosscutting with a fine-toothed high-speed mechanical saw. Finally, trim all faces with a razor blade to remove surface tissues that were damaged by the rough trimming. Subsequent processing of the wood is described in the section on the preparation of hard tissues.

The handling of more specialized and difficult materials such as buds, floral organs, and fruits is described to better advantage in Part II in conjunction with detailed directions for processing such materials. The handling of plant bodies and organs of the lower phyla is also described in Part II.

The foregoing brief outline of methods of collecting and preparing material for preservation can be modified and adapted to meet most problems. The principal preliminary operations and precautions necessary for successful processing may be summarized as follows:

1. Use fresh, normal material.
2. Remove pieces having the desired features and oriented so as to establish planes in which microtome sections are to be cut.
3. Cut into suitable pieces, with minimum bruising, compression, or desiccation.
4. Immerse the pieces promptly into the killing (fixing) fluid (Chap. 3), and promote quick penetration of the fluid by removing air with an aspirator (Fig. 3.1).
5. Record the necessary data concerning species, location, date, parts selected, and killing fluid used.