

13. Vegetative Organs of Vascular Plants

The vegetative organs of the vascular plants are the leaf, the stem, and the root. These organs can be studied either from the standpoint of developmental morphology and histogenesis, or they may be studied from the comparative viewpoint by a comparison of the mature organ in its diverse forms. A combination of the two viewpoints has much merit, and the following presentation of materials and methods affords suitable material for such studies.

Meristems

This section is limited to the apical meristems or growing points of stems and roots and the associated organ primordia. Lateral meristems are more properly discussed in connection with secondary growth of older stems and roots. The study of the activities of meristems is in part a study of mitosis. Some prepared slides of meristems are intended to show critical details of mitosis; however, some slides also are prepared to show tissue systems and organ primordia. For either type of slide, meristematic tissues are processed by the most critical methods that time and facilities permit.

THE ROOT TIP

Growing points of roots are obtainable from seedlings sprouted on blotting paper, from sprouted bulbs, from older plants in pots, or from plants dug up in the field. Regardless of the source or length of a root, the meristematic region is confined to the terminal 1 to 2 mm. (Fig. 13.1). Penetration of reagents occurs over the entire surface. For elementary work, adequate cellular detail is obtained with the entire root tip. The pieces are thus large enough to be handled easily. Longitudinal sections show the relationship of the root cap, the meristem, and the older tissues, and slides can be made by quantity-production methods. For more critical studies the root tip should be cut into the smallest possible pieces or prepared by one of the modern smear methods. The best of these methods have been admirably assembled by Johansen (1940) and Smith (1947). There

is a daily periodicity in the rate of mitotic activity, a periodicity that is characteristic for each species. In order to have many mitotic figures on the slides, root tips should be collected at periods when many cells are dividing.

Bulbs of *Allium cepa*, *Hyacinthus*, *Crocus*, *Tulipa*, and to a lesser extent of *Lilium* are good sources of root-tip material. The following suggestions, based on onion, will furnish the basis for other related subjects. The simplest method of sprouting the bulbs is in individual containers of water, using a tall narrow bottle, jar, or drinking glass of such diameter that the lower third of the bulb is submerged. Change the water twice a day. Bulbs sprout well in moist, steam-sterilized sphagnum. The peaks of mitotic activity for onion are from 1:00 to 2:00 P.M. and 11:00 P.M. to midnight.

Onion root tips are preserved satisfactorily in fluids of the Nawaschin type; one of the most consistently satisfactory is III. The comparatively expensive butyl alcohol and dioxan methods give good results, but a closely graded acetone-xylene series and careful infiltration yield excellent preparations for general class use. Bouin's solution yields excellent mitotic figures, especially prophases and telophases. Occasional lots killed with Bouin are extremely poor. Fluids containing osmic acid are favored by some workers for critical cytological work, but the use of this expensive reagent is not justified for routine work. The formula must be adapted to the plant being studied, and the reader who wishes to use such fluids must consult the research literature for details.

Safranin-fast green and the triple stain give the most complete picture of the entire cell, differentiating the cell wall, the texture of the cytoplasm, the achromatic figure, and nuclear structure. Iron hematoxylin stains chromatin an opaque, contrasty blue-black against a gray cytoplasm. Gentian violet-iodine gives a brilliant blue-black translucent chromatin on a perfectly colorless, almost invisible background. Select the stain combination that gives the desired effect.

Hyacinthus and *Narcissus* also are suitable sources of root-tip preparations. The methods are essentially as given for the onion. *Gladiolus* has very small chromosomes, and preparations are of value mainly to illustrate comparative chromosome sizes. The possibilities of the numerous kinds of bulbs, corms, and rhizomes available in field and garden have been by no means fully explored.

Root tips of corn are obtainable by sprouting kernels in a moist chamber. When seminal roots and some lateral roots have developed, cut off the meristematic tips. Root tips also may be obtained from

pot-bound plants and the plants can be repotted without apparent damage. Mitotic activity is usually rapid during the early forenoon. Maize cytologists favor a formula that is practically identical with Craff III. Choose a stain by the criteria discussed in connection with the onion.

Vicia faba, the horse bean, has 12 large chromosomes, 2 of them about twice as large as the others. Obtain root tips by sprouting seeds in a moist chamber or from plants grown in pots of sphagnum. Kill in Nawaschin or in Craff II, and stain as with onion. The radicles of many other legumes also are easy to obtain and to process. Sections may be stained for either histological or cytological details (Fig. 13.2), or a good compromise may be obtained with safranin-fast green.

The common trailing *Zebrina* grown in greenhouses has large chromosomes. Obtain root tips from cuttings rooted in sand. The periodicity is an uncertain factor, and the worker must chance obtaining abundant mitotic figures. Bouin's solution and Craff II usually give acceptable results.

APICAL MERISTEMS OF THE STEM

The origin of the tissues of the stem and of lateral organs on the stem is revealed by a study of the meristematic tip or apex of a stem. This growing apex may be found at the tip of a growing axis, or in a dormant terminal or axillary bud. One of the easiest subjects to handle is the shoot from the sprouting kernel of corn. Sorghum, oats, and other small grains also may be used, but the coleoptile is small and not so easy to handle as that of corn. The growing apex of these Gramineae is a broad dome, from which the leaf primordia arise as lateral protuberances. Successive leaf primordia are laid down during this period of rapid growth and may be seen in graded order in a good median longitudinal section. Transverse sections show the lateral extension of the meristematic margins of the leaf primordia. The oat sprout develops axillary buds earlier than do the other suggested grasses.

To obtain growing apices of stems of *Zea*, germinate the corn in sphagnum or sand. When the coleoptile is approximately 5 cm. long, cut out a section 5 mm. long at the coleoptile node (Fig. 13.1A). This region, which contains the growing apex, can be located easily by holding the shoot before a bright light — the region of the coleoptile node and compact growing apex area is dark. Older seedlings show more advanced axillary buds than the young sprouts. One

week to 10 days after emergence, the seedling has leafy axillary buds (Fig. 13.3 *a*). The tassel primordia become evident 25 to 30 days after emergence (Fig. 13.3 *b*). Instructive vegetative and floral apices can be obtained from the sprouts that arise from sods or clumps of the larger perennial grasses, such as brome grass or orchard grass. Inflorescences are initiated during April (Fig. 13.3 *c*), and vegetative tips are obtainable from the new sprouts that emerge in midsummer or later. Excellent preservation of these gramineous growing apices can be obtained with Craff I. The air must be completely evacuated from the tightly overlapping leaves encircling the stem tip. Improper infiltration results in the collapse of the meristematic tissues and breaking of the tip and leaf primordia during sectioning. Cut sections of maize 10 to 12 μ thick and *Avena* 6 to 8 μ . Stain in tannic acid-ferric chloride, in hemalum-safranin, or in safranin-fast green.

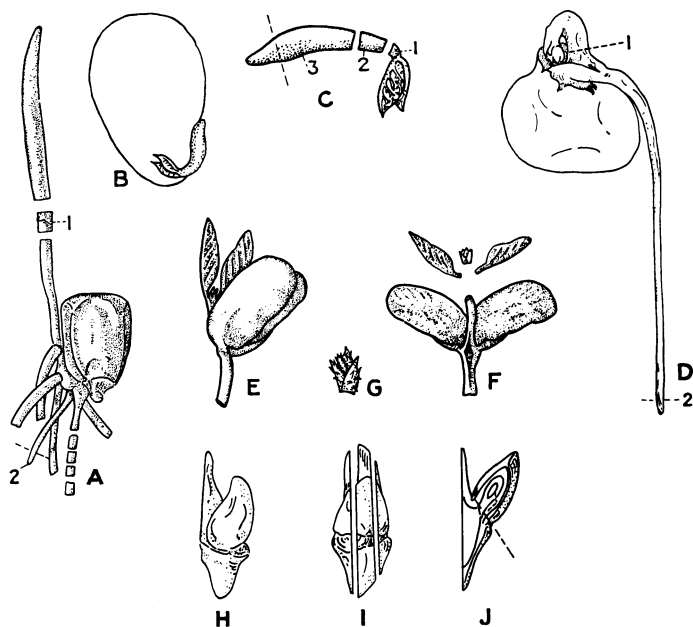


FIG. 13.1—Methods of obtaining apical meristems. *A*, seedling of corn, growing apex of stem is at coleoptile node 1, root tip removed from seminal root 2; *B*, half of kidney bean seed with embryo in place; *C*, parts of embryo, 1 contains stem tip, 2 is discarded, 3 is used for root tip; *D*, sprouting pea, epicotyl cut off at 1 is used for stem tip, root tip cut off at 2; *E* and *F*, sprouting soybean dissected to obtain terminal bud *G*; *H*–*J*, bud of basswood removed from twig and divided for killing.

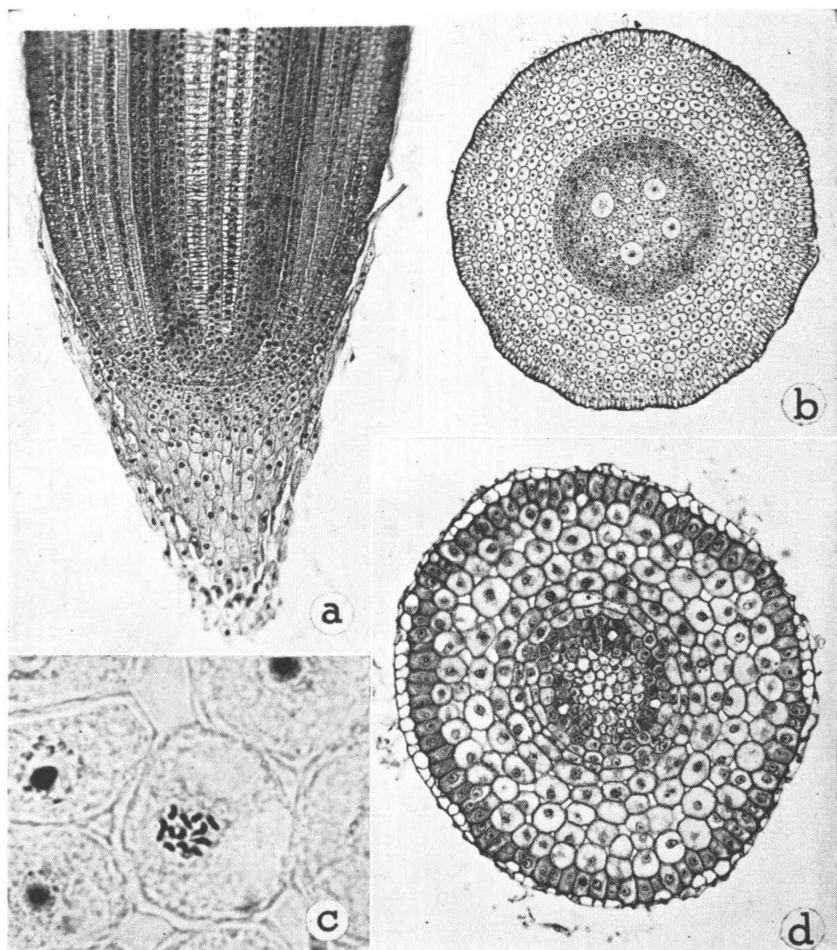


FIG. 13.2—Root tip meristems and histogens. *a, b, Zea mays*; *c, Melilotus alba*; *d, Lotus tenuis*, chromosome complement, iron-hematoxylin.

A good dicotyledonous stem growing apex can be obtained from young seedlings of Lima beans, kidney beans, soybeans, flax and peas, sprouted in sphagnum or sand. Lima and kidney beans have a large epicotyl of simple structure at the postdormant stage, after the seed coat has been ruptured but the plumule has not yet emerged. Peel off the seed coat, separate the cotyledons, and remove the epicotyl and radicle (Fig. 13.1 *B, C*). Good fixation can be obtained with Craff II followed by the acetone-xylene series. Cut the paraffin sections at right angles to the flat, overlapping plumule leaves. Stain as

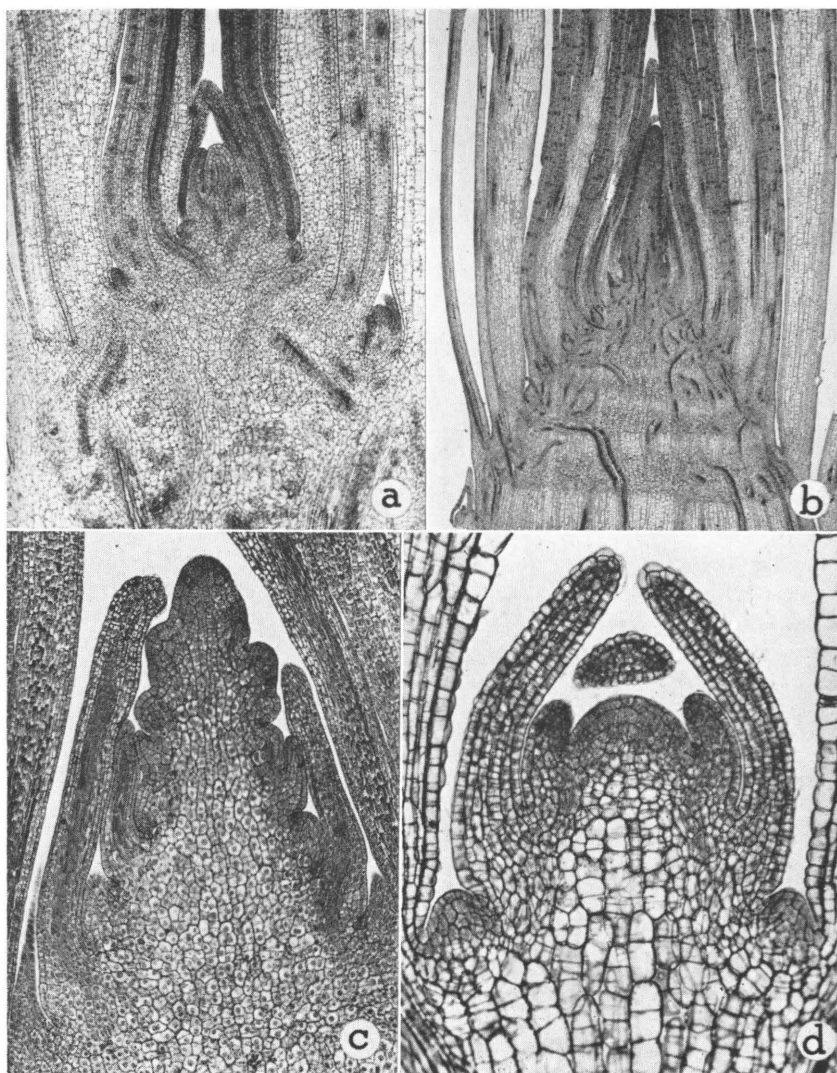


FIG. 13.3—Vegetative and floral apices of stems: *a*, *Zea*, vegetative, 1 week after emergence; *b*, *Zea*, tassel primordia, 7 weeks after emergence; *c*, *Bromus inermis*, inflorescence primordia in April; *d*, *Linum*, plumular bud of seedling.

recommended for corn seedling. The median section will show a broad apical meristem, two small leaf primordia, and fragments of the folded plumule leaves. The radicle can be used for histological or cytological preparations of the growing point.

Peas show a more advanced condition at a corresponding stage of germination. The pea bud is perfectly glabrous. Sprouts showing axillary bud primordia are obtained when the sprout has emerged from the seed in the form of a loop (Fig. 13.1 *D*). Increasing complexity develops rapidly as the sprout becomes straight.

The epicotyl in sprouting soybean is more advanced in organization than in beans or peas. Extract the soybean bud from the burst seed. For an older stage, permit the epicotyl to elongate until the tips of the plumule leaves just protrude beyond the cotyledons. Remove the cotyledons, pull the plumule leaves apart, and remove the entire bud (Fig. 13.1 *E-G*). Soybean buds are pubescent and must be pumped with an aspirator until they sink in the killing fluid. Large multicellular hairs in the axils of the leaf primordia are easily mistaken for axillary buds by elementary students. The bud is a desirable item for advanced teaching (Fig. 13.4 *b*). The growing apex of the flax seedling is glabrous and very simple in organization (Fig. 13.3 *d*). When the appressed cotyledons of the seedling have begun to diverge, make a transverse cut 1 mm. below the cotyledonary node and another cut 2 mm. above the node. The cotyledons serve as a guide to orientation in the microtome. Section at right angles to the flat sides of the cotyledons.

Axillary buds of *Coleus*, tomato, and other herbaceous plants or the buds from potato eyes also are desirable subjects. Before dropping buds of this type into the killing fluid, it is best to dissect away some of the outer bud scales.

Uniformly good fixation of buds of the above legumes and other recommended herbaceous plants has been obtained with Craff II and the dioxan method, the ethyl-normal butyl series or the dioxan-normal butyl series. Some good results, with occasional unexplained failures, have been obtained with dioxan alone.

Buds of trees and shrubs collected at different seasons show the initiation of leaves and flowers, or the dormant condition. Expanding spring buds of the maples, basswood (*Tilia glabra*), and tulip poplar (*Liriodendron tulipifera*) are recommended. In the shrubs, lilac (*Syringa*), honeysuckle (*Lonicera*), and elderberry (*Sambucus*) are excellent subjects. Remove the buds from the twig as shown in Fig. 13.1 *H-J*. Slice off a longitudinal slice from each side, peel off some

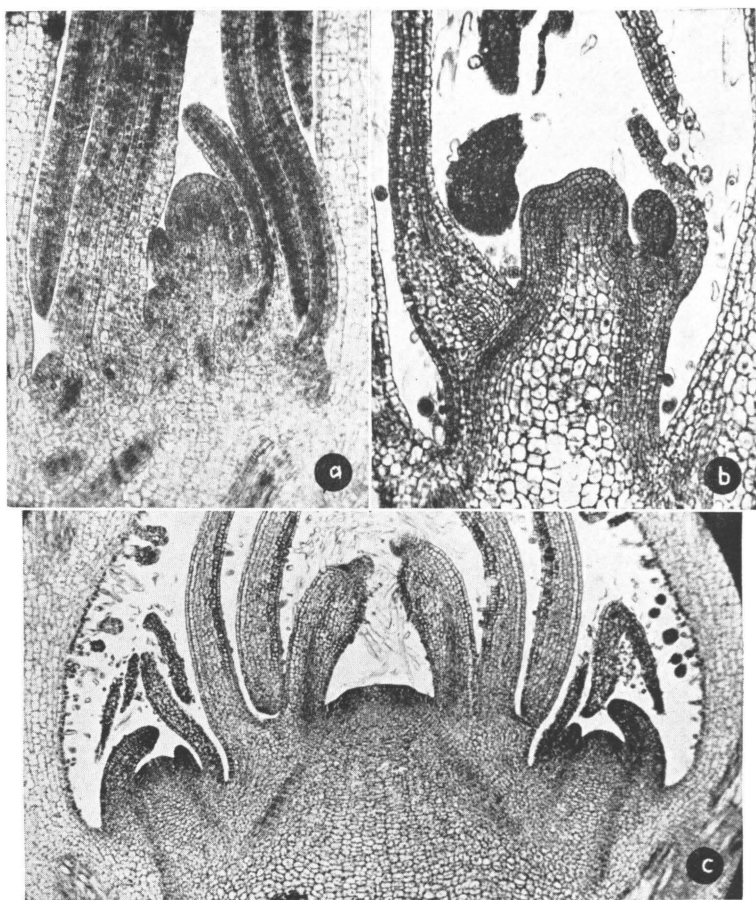
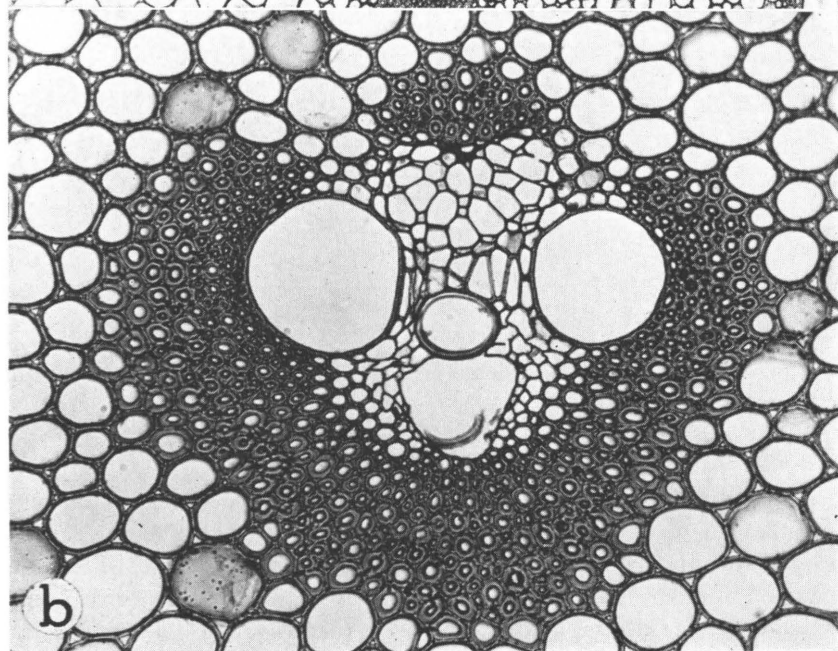
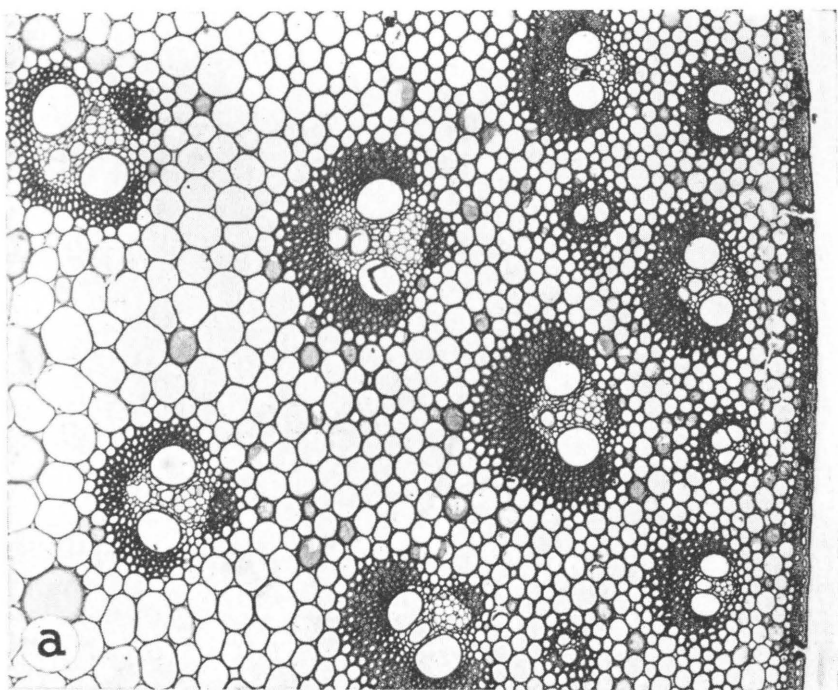


FIG. 13.4—Apical meristems of stems: *a*, *Zea mays*, seedling 1 week old, Craff I, acetone-xylene; *b*, *Glycine max*, seedling 1 week old, Craff II, three-grade dioxan; *c*, *Acer saccharinum*, dormant bud from large tree, FAA, alcohol-xylene.

of the tougher outer scales under a magnifier, drop into the killing fluid promptly, and pump vigorously. FAA penetrates well and gives acceptable fixation (Fig. 13.4 *c*). If more perfect preservation of the protoplast is desired, dissect away most of the larger leaves, and kill the remaining growing apex and small leaf primordia in Craff II. Dehydrate the more brittle buds in butyl alcohol. Use any of the stains recommended for previous growing points.

FIG. 13.5—Stem of *Zea*: *a*, sector; *b*, single bundle near periphery of stem.



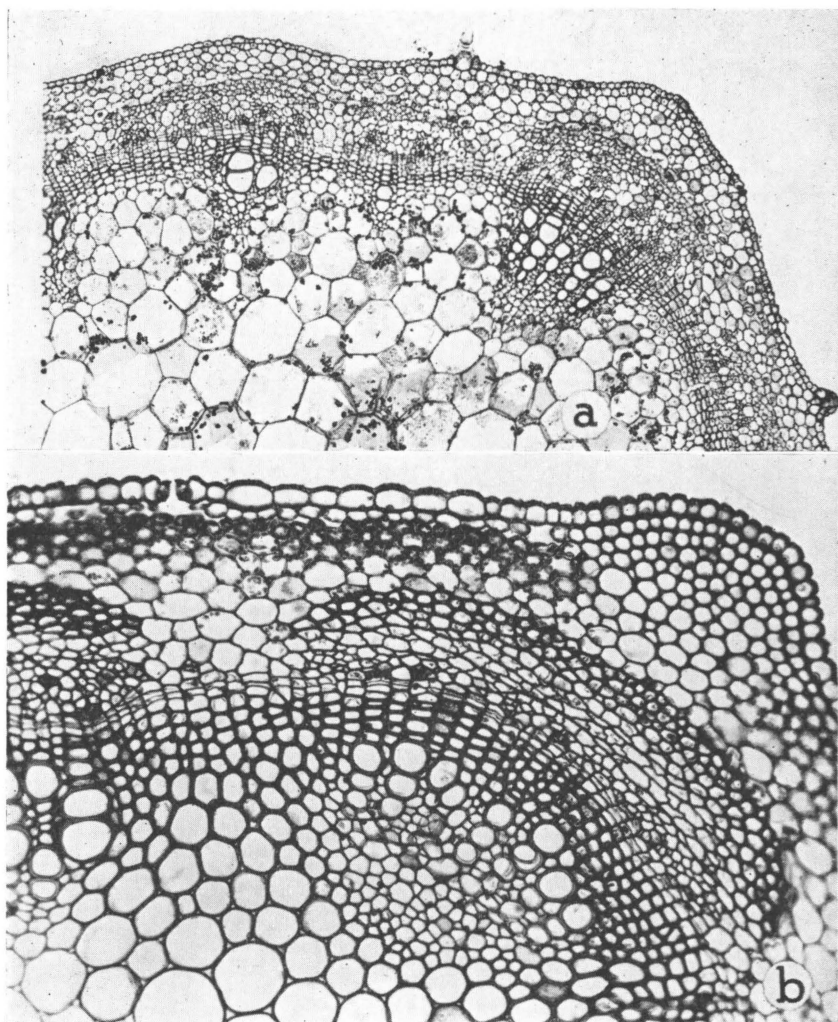


FIG. 13.6—*a*, Stem of soybean, *Glycine max*; *b*, stem of alfalfa, *Medicago sativa*.

The Stem

The techniques used for the processing of stems range from the foregoing methods used for the delicate meristematic tip to the rather drastic and apparently crude methods necessary to make slides of seasoned lumber. The portion of stem to be selected for sectioning depends on the degree of differentiation that is to be demonstrated.

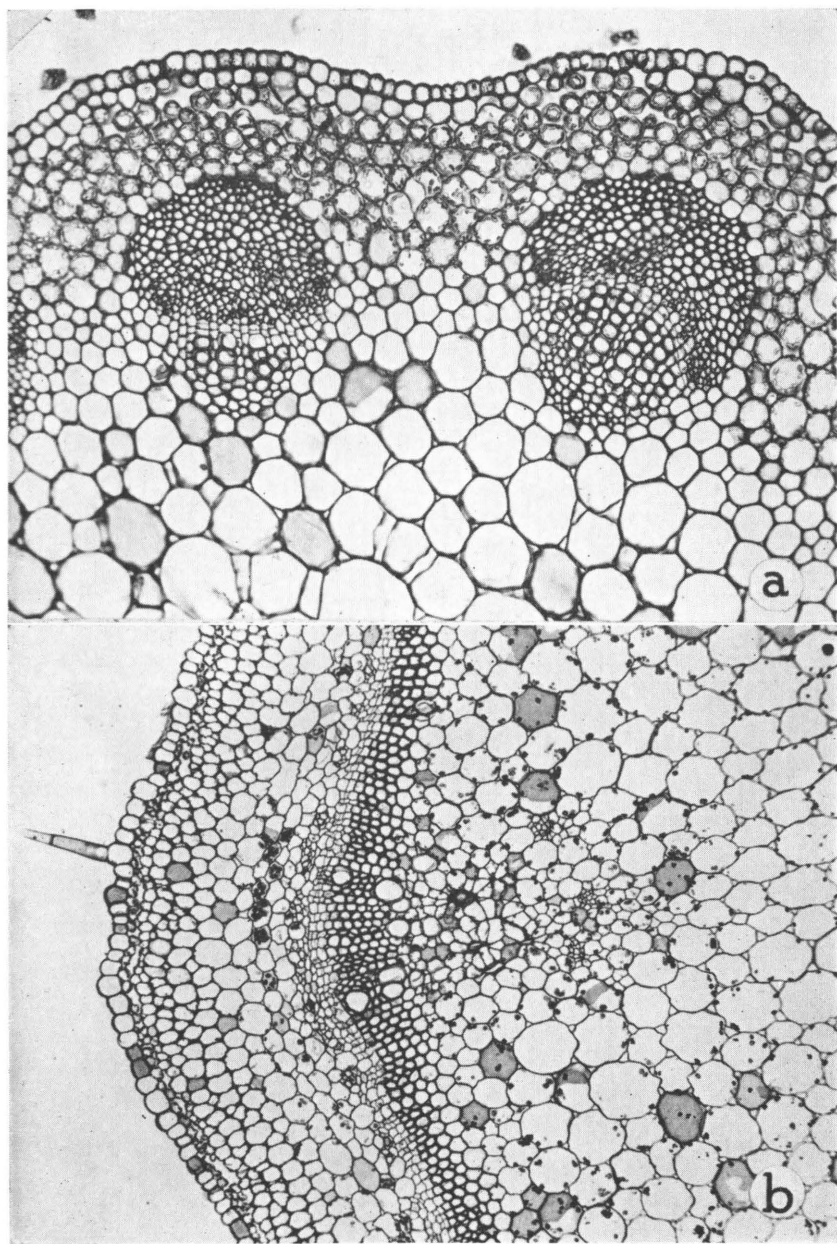
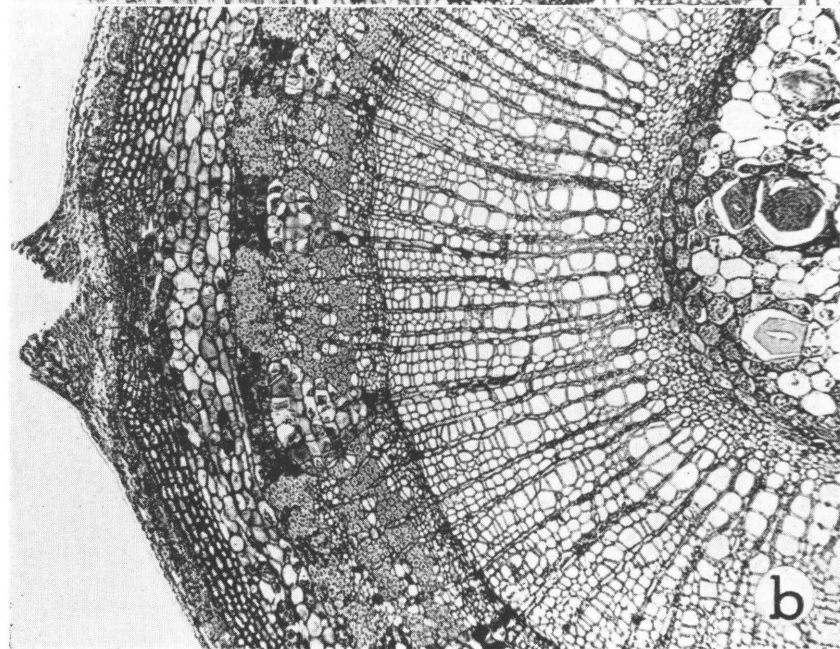
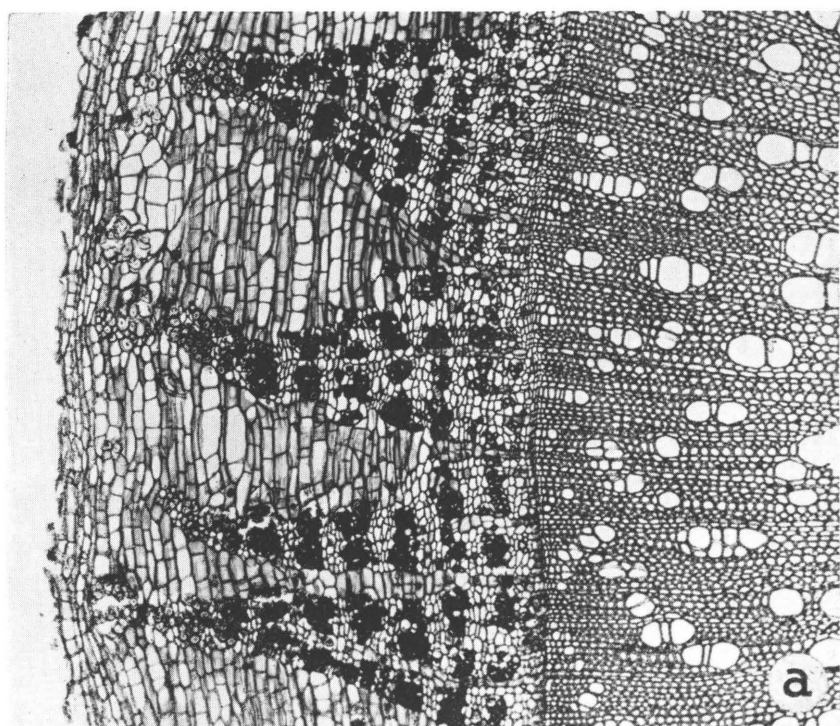


FIG. 13.7—*a*, Stem of alsike clover, *Trifolium hybridum*; *b*, stem of tomato, *Lycopersicum esculentum*.



MONOCOTYLEDONOUS STEMS

It is convenient to discuss first the monocotyledonous stem because these stems reach a climax of differentiation in one growing season and do not present the problems raised by the secondary growth of dicotyledonous and gymnosperm stems. Maize may well be used as the standard subject for the grass stem. Complete transverse pieces of seedlings will show the overlapping whorls of leaves encircling the stem. Nodal pieces show the axillary buds, the potential ears. From the older plants use only the internodal pieces of stem, stripping away the leaves. A pot-grown plant will become fairly well lignified and yet be so small that a complete cross section, or at least a quarter sector, can be placed on a slide. However, such plants give an inaccurate picture of the number and structure of the bundles. To show the well-developed and lignified bundle sheath and cortex or rind, use large, field-grown plants at about the time of pollination. Cut the stem into short disks, and divide each disk longitudinally.

Young stems collected before the internodes have become exposed should be killed in a mild fluid like Craff II. Mature stems must be killed in *FAA* and pumped until they sink. The dry, air-filled pith is difficult to infiltrate; it is therefore desirable to exhaust again in the anhydrous stage of dehydration. The use of normal or tertiary butyl alcohol permits paraffin embedding of all but the toughest stems, which must be cut in celloidin (Fig. 13.5). Transverse and longitudinal sections of corn stem take a brilliant safranin-fast green stain. The hemalum-safranin combination is the second choice. Iron hematoxylin-safranin is used only if the middle lamella is to be emphasized.

Other important plants that illustrate the large grass type of stem are sugar cane and sorghum. Wheat, oats, and other small grains and field grasses illustrate the small hollow culm. The most easily available grass rhizome is that of quack grass, *Agropyron repens*. The hydrophytic monocots have interesting culms and rhizomes. Species of *Carex* having triangular stems, as well as round-stemmed species, and the cat tail, *Typha*, should not be overlooked. These subjects can be prepared by the methods outlined for maize.

Monocot stems of the nongramineous type may be obtained from several easily available plants. The trailing *Zebrina* grown in green-houses has a soft stem that can be sectioned in paraffin. *Asparagus*

FIG. 13.8—*a*, Stem of hemp, *Cannabis sativa*; *b*, stem of basswood, *Tilia*.

sprengeri, an important plant in the florist trade, has a thin woody stem. The younger portions near the tip can be cut in paraffin, the old woody stems must be cut in celloidin. Wild species of *Smilax* have a woody stem. Kill the woody stems of *Asparagus* and *Smilax* in *FAA*, and cut in celloidin only if a sample embedded in paraffin cannot be cut.

DICOTYLEDONOUS AND CONIFEROUS STEMS

The apical meristematic regions of dicot stems have been discussed in the section on apical meristems. The tissue systems of these stems differentiate very rapidly close to the apex, and the first few internodes below the terminal bud show the fully developed primary tissues and the initiation of secondary activity. A convenient though artificial and arbitrary classification of stem types is in common use. *Herbaceous* stems develop comparatively little secondary wood, and, if a complete cylinder of wood is produced, it is laid down late in the growth period. *Woody* stems begin the formation of a complete cylinder of secondary wood early in the season and produce an extensive cylinder of highly lignified xylem. Every possible gradation of woodiness between these two types may be found in the plants about us. The following examples are recommended either because they are of economic importance or because they present some structural feature of fundamental importance.

Plants that can be grown quickly in pots are convenient subjects for the herbaceous stem. Plants of kidney beans, peas, and soybeans attain usable size in a short time. Actively growing field materials are the best source for sweet clover, alsike clover, and alfalfa (Fig. 13.7). Any of these legume stems can be killed in Craf III. The softer internodes can be carried through an acetone-xylene or alcohol-xylene series. The harder stems, especially soybean, cut better after dioxan or the butyl alcohols (Fig. 13.6).

The cultivated sunflower, *Helianthus*, and *Chrysanthemum* are good representatives of the Compositae. The common fleabane, *Erigeron*, is a suitable native subject in this family. The above stems seem to withstand the dehydrating action of *FAA* without marked plasmolysis, and a strong Nawaschin modification like Craf IV or V is satisfactory. The butyl alcohol process is recommended for these rather tough stems.

Bicollateral bundles are characteristic of the Cucurbitaceae and Solanaceae (Fig. 13.7). Important members of these families can be obtained easily. Seedlings of squash, pumpkin, or melon grow rapidly

and furnish long hypocotyls as well as epicotyl materials. Do not use *FAA*; kill in Craff II, and use alcohol-xylene for tender stems and butyl alcohol for tough ones. Tomato and tobacco seedlings grow slowly, but they are almost indispensable subjects. Potato plants are easily grown from tubers. Stems of these plants are not killed properly by *FAA* but are preserved with excellent cellular detail in Craff II. Old, tough stems of tomato and tobacco must be processed in *TBA* or sectioned in celloidin. Small potato tubers, 3 to 6 mm. in diameter are easy to section. Kill in Craff I, and embed in paraffin by a slow, closely graded process. Longitudinal sections show that the tuber is a stem with an apical meristem which produces leaf primordia.

Medullary bundles and anomalous cambial activity occur in the *Chenopodiaceae*. The common weed *Chenopodium album* is probably the most readily available representative. Several related weeds are equally interesting. Kill in *FAA* or Craff III, and process in butyl alcohol or dioxan.

The foregoing methods recommended for specific herbaceous stems can be used with an extensive range of plants in many species of economic importance or academic interest. For instance, commercial fibers of primary and secondary derivation can be illustrated with the stem of *Cannabis sativa* (Fig. 13.8). As a broad general recommendation, use a mild chrome-acetic-formalin on tender materials, and process in alcohol-xylene or acetone-xylene. For moderately hard stems use Craff III, and for very hard stems use *FAA*, followed by dehydration and infiltration in dioxan or butyl alcohol.

The bush fruits like raspberry, blackberry, currant, gooseberry, and other plants having similar semiwoody stems may be handled like herbaceous stems while in the tender growing stages, but they eventually become too hard to process by the foregoing methods. Such hard materials usually must be handled like woody stems, as described in the following pages.

For the study of twigs of woody plants, material collected during the winter has some advantages. The previous season's xylem is fully lignified, secondary phloem is fully matured and firm, the cambium is clearly distinguishable as a layer immediately adjacent to the wood, and the cambium does not slip readily. However, if the development of cambial derivatives is to be studied, stems must be collected at intervals during the growing season. Such materials must be processed with greater care than dormant stems. Twigs should be taken to the laboratory promptly and cut into short pieces for killing as described in Chap. 2.

Many species of forest, orchard, and shade trees make excellent preparations for the study of young woody stems. The basswood, *Tilia* (Fig. 13.8 *b*), has become a great favorite, but there is no advantage in studying basswood in a region where it is not native. Species of *Populus*, *Fraxinus*, and *Acer* are easily sectioned. The apple and other fruit trees have been neglected as class materials, although they are easy to section. Tougher woods like oak, hickory, or locust are much more difficult to cut, and complete perfect sections are not obtained with such certainty. The standard coniferous subjects are the white pines, *Pinus strobus* in the east, and *P. lambertiana* or *P. flexilis* and several other five-needle pines in the west. These are representative of the five-needle or soft pines. For the hard pine type many more species are available, such as several species of yellow pine, the scrub pines, and jack pines. There is not much choice among the numerous two- and three-needle hard pines.

The principal American genera of conifers should be represented in a comprehensive stock of slides. Some of these trees are used as ornamentals, the commonest ones being *Abies*, *Larix*, *Tsuga*, *Thuja*, *Picea*, *Pseudotsuga*, and *Juniperus*. Shrubby conifers are among the commonest ornamentals, and specimens of shrubby species in the genera *Juniperus*, *Thuja*, and *Taxus* are readily available.

The methods of handling the woody dicots and coniferous stems are decidedly stereotyped. The subdividing of such materials is illustrated in Fig. 2.2. The impermeability of the cork on woody twigs necessitates the use of a fluid of good penetrating powers, and *FAA* has long been the standard fluid. Stems may be left in this fluid for years. Preserved stems can be rinsed in several changes of 70% alcohol at 1-day intervals and sectioned without embedding. The celloidin method is recommended because of the ease and certainty of attaining high productivity by quantity production methods.

Woody stems having bark tissues are usually stained with the combinations recommended for herbaceous stems. Hemalum-safranin, safranin-fast green, and safranin-aniline blue have become standard stains. The method of handling sections and the staining processes are described in Chap. 8.

Transverse, radial, and tangential sections of the cambial region of woody plants make instructive preparations that are indispensable for a critical study of the three-dimensional aspects of cambium, the mechanism of abscission, and the structure of developing and mature elements of the xylem and phloem. The excessive use of transverse sections and the neglect of longitudinal sections build up an in-

complete or even incorrect picture of the woody stem in the mind of the student.

Twigs are not satisfactory for making longitudinal sections in quantities. Unembedded twigs cannot be held in the microtome horizontally for longitudinal sections. If an embedded and blocked twig is sectioned longitudinally, only the outermost sections are strictly tangential, and only a few slices from the center are true radial sections, cut parallel to a ray. For first-class preparations cut accurately on the three desired planes, use blocks of wood and attached bark removed from living trees as illustrated in Fig. 2.2. Sectioning of such blocks is quite impossible without embedding in celloidin; whereas, with the celloidin method, perfect sections can be produced in quantities (Fig. 13.9 *b, c*). Collect the material in the winter when the cambium is firm. Soft wood like basswood, white pine, apple, or silver maple can be cut without special softening. Kill in *FAA*, and embed in celloidin. Hard woods like oak or locust must be treated with hydrofluoric acid after killing and hardening in *FAA*. The protoplasts cannot be expected to be in perfect condition after treatment in *HF*. The process is described in Chap. 8.

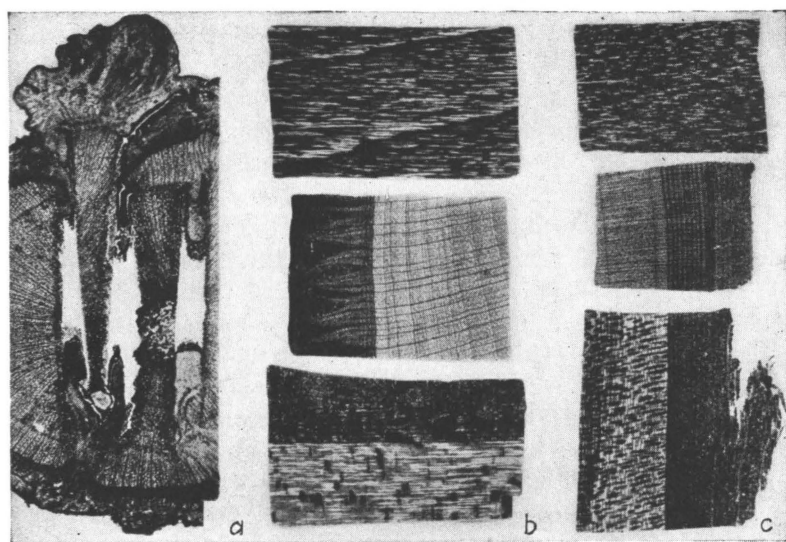


FIG. 13.9—Illustrations of material cut by the celloidin method: *a*, cross section of apple graft union; *b*, sapwood region of sector from 20-year-old trunk of *Tilia*, sections in three planes; *c*, sections from approximately 40-year-old trunk of apple tree. All subjects killed in *FAA*. The *Tilia* stem was infiltrated in Cellosolve solution of celloidin.

The desirability of using choice sections showing the bark in three planes cannot be overemphasized. In addition to serving as supplementary class material for studying the structure and development of the stem, such preparations serve as reference material for research, especially for pathological studies. Even in wood technology, in which the work is largely confined to the microscopic structure of the wood, preparations showing the cambium, phloem, cortex, and periderm are a valuable supplement.

Seasoned lumber is frequently used as a source of material for slides, and excellent preparations can be made from such material. However, parenchymatous elements such as xylem parenchyma and the epithelial cells of resin canals are collapsed and distorted. The preparations are adequate for diagnostic purposes and for the study of nonliving elements of the xylem. For best results, use properly seasoned wood and prepare the blocks so that sections can be cut accurately along the three conventional planes as described in Chap. 2. The specialized sectioning methods necessary for dry or hard woods are described on pages 84–85.

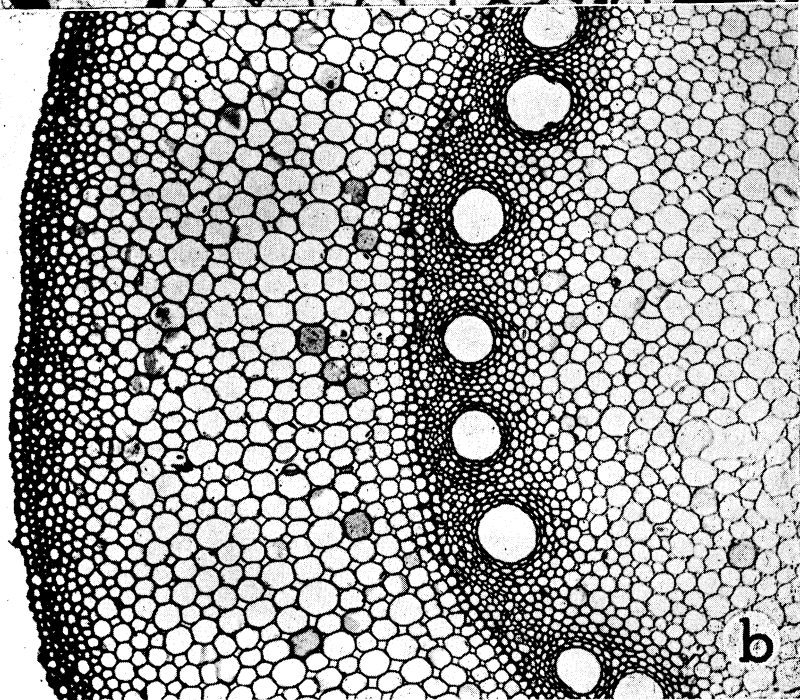
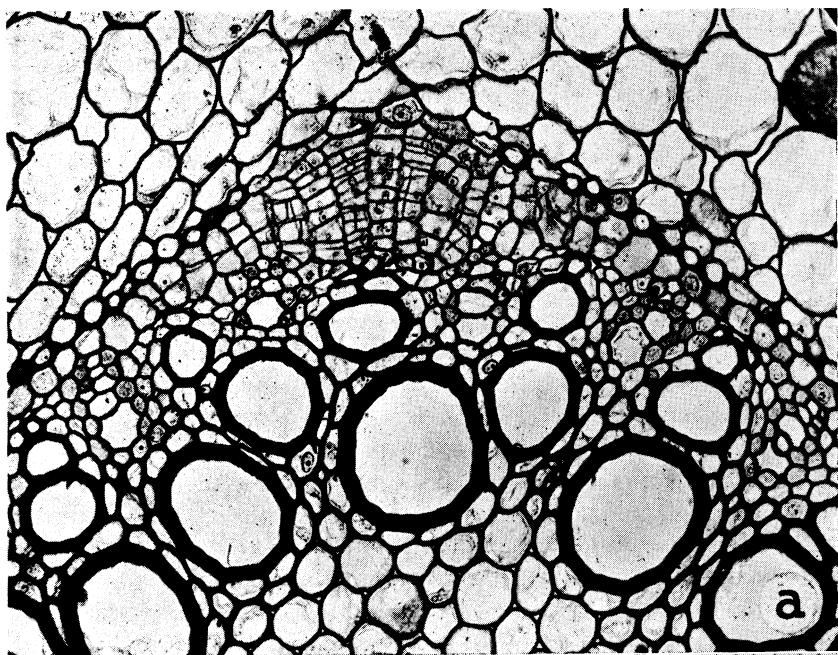
The Root

The processing of roots of seed plants for anatomical study is similar to the methods used for stems. The meristematic root tip is usually prepared by careful cytological methods; sections may then be stained either with a cytological stain for nuclear structures or stained with some histological combination. Batches of root tips that do not have abundant mitotic figures are usually set aside for histological preparations. The methods of obtaining root tips are described in Chap. 9.

The histogens of roots are evident at the root tip, especially if the preparation is stained to show cell walls as well as nuclei. The primary tissues are evident at the beginning of the root-hair zone, where the emerging root hairs can be detected with a hand lens. Initiation of lateral root primordia can be demonstrated at the upper limits of the root-hair zone, where the old root hairs are beginning to collapse. At this level the primary tissues are usually clearly differentiated, without being excessively woody.

Favorable subjects for illustrating the monocot root are maize and *Asparagus officinalis*, the garden asparagus. Germinate corn in

FIG. 13.10—*a*, Transverse section of root of *Asparagus officinalis* showing initiation of lateral root; *b*, brace root of *Zea*.



sphagnum (not in sand!), and remove pieces of root from the desired region. Roots grown in a moist chamber or water culture have excessively spongy, fragile cortical parenchyma, unlike the structure found in plants in a more normal environment. Tissues of the tip, the hair zone, and for some distance above, are fixed well in Craff II; the older tough roots must be killed in *FAA*. The brace roots from field-grown plants may also be used (Fig. 13.10). Roots of sugar cane, sorghum, and the small grains are processed by the above methods.

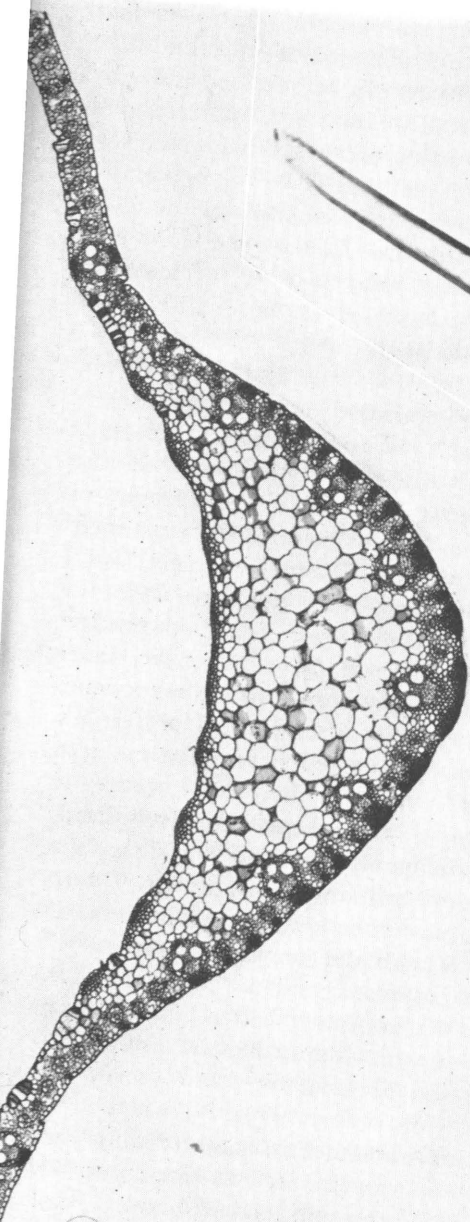
Asparagus roots are obtainable readily from volunteer seedlings that occur in the vicinity of asparagus beds. The softer portions of the root, within 3 cm. of the tip, are killed in Craff II, but older roots that have an impermeable hypodermis and endodermis must be killed in *FAA*. The butyl alcohol method is suggested for the harder pieces (Fig. 13.10).

Acorus calamus is almost a classical subject for the monocot root. This water plant is abundant in suitable locations, but material is often inaccessible, and the root has no advantages over *Asparagus*. The processing methods are identical for these two plants.

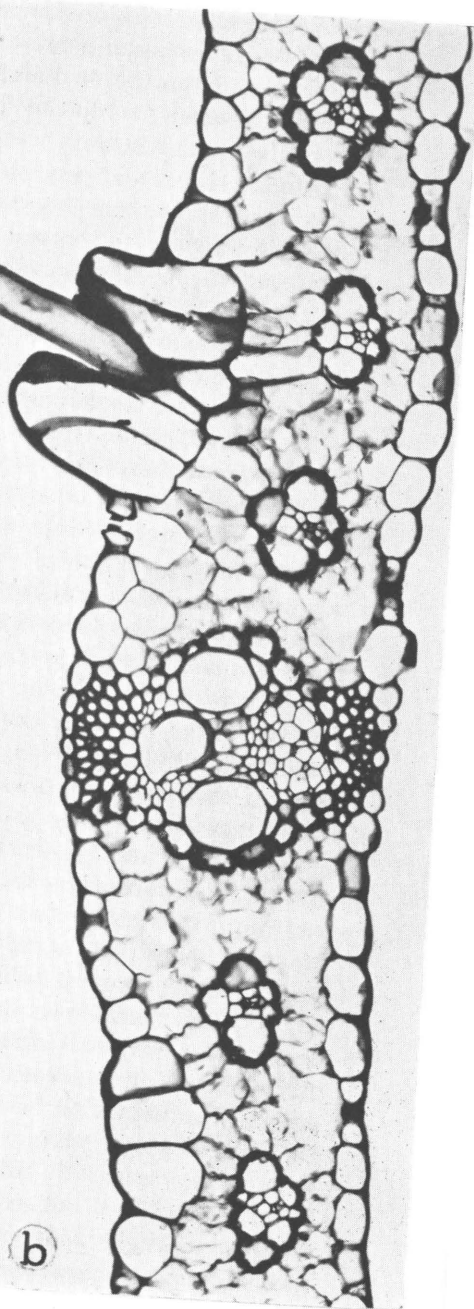
Smilax hispida root has a remarkably thickened endodermis, with laminated cell walls impregnated with brown coloring matter. Kill the roots in *FAA*, and try a batch with the butyl alcohol method, using celloidin if sections cannot be cut in paraffin. Safranin-fast green gives a brilliant contrast in which the prominent endodermis is reddish brown.

Young dicotyledonous roots are obtained readily from the large-seeded legumes, beans, peas, soybeans, and especially the horse bean, *Vicia faba*. The early stages, including the emergence of lateral roots, can be obtained from roots grown in a moist chamber of sphagnum. These roots do not become excessively spongy when grown in this manner. The older roots showing extensive secondary growth must be taken from plants grown in soil. Soybean and horse bean should be killed in Craff III. Young roots of the apple are particularly interesting because of the prominent Casparian strips in the endodermis. Material is not so easy to obtain as with plants that can be grown quickly from seed. Volunteer seedlings of apple can be dug up carefully and abundant roots of various ages obtained. Kill the youngest roots in Craff III and woody roots in *FAA*. Trial batches of older roots may

FIG. 13.11—Transverse sections of leaf of *Zea*: *a*, photographed at 14X with 48mm. Micro Tessar, reproduced at 28X; *b*, detail of trichome and blade.



a



b

well be processed with tertiary butyl alcohol, and any age classes that cannot be cut in paraffin must be cut unembedded or in celloidin.

Flax root has a simple diarch stele. Roots can be obtained easily by germinating seeds in blotting paper. Radish, mustard, cabbage, and many other roots also may be grown in this manner to adequate size for primary tissues and processed by the methods given for apple.

Ranunculus root has long been popular as an example of the dicot root. The large fleshy roots of the buttercups, *R. septentrionalis* and *R. fascicularis*, are easy to obtain and to process, using the methods given for apple. The buttercup root is less likely to be of interest to the student than the roots of economic plants.

Large taproots like those of alfalfa, *Medicago*, and sweet clover, *Melilotus*, are handled like the older semiwoody roots of apple.

The tough, woody stems, rhizomes, and roots of ferns, horsetails and club mosses are most conveniently discussed at this point because they are handled like other woody materials. Collect the rhizomes of ferns in the spring, just after the fronds have fully expanded. Acceptable preservation of rhizomes can be obtained with *FAA*, and it is not improbable that for investigational work this formula could be adjusted to give good fixation with given species. For routine preparation of many species, uniformly good results have been obtained with Craf II. The subjects become very brittle after xylene, but very large rhizomes can be cut readily after *n*-butyl or tertiary butyl alcohols. It has been customary to embed hard rhizomes like those of *Pteris aquilina* in celloidin. However, the *TBA* process is satisfactory for portions of the rhizome that are not excessively hard, but have the woody structures adequately lignified to show the mature condition of tissues. The most brilliant and satisfactory stain is safranin with fast green. The presence of yellow deposits in the cells produces undesirable staining effects with the hematoxylins.

The fleshy root of *Botrychium* is recommended. Tests with *B. virginianum* have shown that Craf I gives much better fixation than does *FAA*. A very striking color contrast is obtained with safranin-fast green. Roots of the Boston fern and of available native ferns are processed as above.

Species of *Lycopodium* occur in abundance in some regions, and some species, especially tropical ones, are cultivated in conservatories. Stems may be fixed in *FAA* and carried through tertiary butyl alcohol to paraffin. It is usually necessary to soak the mounted specimen in warm water before sectioning. Roots are easy to process successfully by the same methods.

Selaginella has highly localized distribution, but excellent preserved material is obtainable from dealers, and several species are extensively cultivated in greenhouses. The processing is the same as for *Lycopodium*, the infiltration must be slow and thorough, because the stele is literally suspended in a highly parenchymatous cylinder and is easily torn in cutting.

The vegetative organs of *Isoetes* are studied only in advanced work in anatomy, and there is comparatively little demand for slides. Roots should be severed and divided into short pieces. The compact stem and rhizophore may be processed entire or quartered. The methods used for *Lycopodium* are satisfactory. The highly silicified stem of *Equisetum* has long been a problem for technicians. Penetration is difficult with an aqueous killing fluid, but *FAA* is satisfactory. The older stems must be desilicified by treating with hydrofluoric acid. Transfer directly from *FAA* to the acid diluted with twice its volume of 95% alcohol. After 2 days in acid, wash in 50% alcohol, making at least five changes at 4-hr. intervals. Observe the precautions concerning the use of HF given in Chap. 8. Dehydrate in *TBA*, and infiltrate slowly and thoroughly. Rhizomes and roots do not need to be desilicified, otherwise the processing is the same as for aerial stems.

The Leaf

The mesophytic dicotyledonous broad leaf is the type most commonly used for the study of the so-called typical leaf. Some general directions apply for the handling of most types of leaves. Leaves are easily damaged during processing by apparently minor mishaps. It is therefore desirable to kill duplicate batches in each of the formulas used, keeping one batch in the preserving fluid while the other one is embedded and tested. Consult Fig. 2.1 concerning the usual methods of subdividing leaves. Good results can be obtained with many leaves by killing in *FAA*. Soft leaves having small veins can be dehydrated in acetone or ethyl alcohol, whereas leathery leaves, or leaves with thick or wiry veins should be processed in butyl alcohol or dioxan. One batch of each subject may well be killed in *FAA* and another batch in one of the fluids given in the following specific recommendations.

The firm leaves of the trees and shrubs are represented by apple, cherry, rose, lilac, and privet. Leaves of apple and related plants may be killed in *FAA*, but occasional batches exhibit considerable plasmolysis (Fig. 11.1). Consistently good results can be obtained

with Craf II and *TBA* dehydration. The latter reagent minimizes the brittleness of these subjects. A disadvantage of the rosaceous leaf is the presence of excessive brown pigmentation in the cell walls and masses of yellow gummy materials in the cells. The embedded pieces of leaf in the paraffin block are decidedly dark, and the staining effects tend to be muddy, especially with the hematoxylin. The use of a safranin-fast green or safranin-aniline blue combination makes slides with fairly clean color contrasts. Lilac and privet leaves can be processed by the above methods. Many other trees and shrubs have leaves in this firm-textured category. Geranium leaf is firm and easy to process. Craf V gives excellent results. Do not use pieces with large veins unless *TBA* is used for dehydration.

Leaves of softer character than the foregoing are illustrated by various easily obtainable legumes. Kidney bean, soybean, clovers, and alfalfa have more or less pubescent leaves; peas and horse bean have practically glabrous leaves. All of these leaves have been killed successfully in *FAA*, but failure occurs often enough to justify more critical methods. Excellent preservation of alfalfa and soybean leaf has been obtained with Craf III followed by an acetone-*TBA* series (Fig. 11.1). The thinness of the cell walls requires a stain of good contrast, such as hemalum, followed by safranin, the xylem stain. The coal-tar dye counterstains are likely to yield weakly stained parenchyma and barely visible plastids.

The leaves of the Solanaceae and Cucurbitaceae represent the very tender type of broad leaf. Subdividing of fresh leaves must be done with the greatest care because of the open and fragile construction of the parenchyma. The glandular hairs should also be preserved intact. The best killing is obtained with a mild fluid, such as Craf I. Practically perfect preservation of tobacco leaf has been obtained consistently with this fluid. Although the blade is soft in leaves of this type, the veins are large and firm, justifying the use of *TBA*.

Begonia leaf is an interesting tender leaf. The epidermal cells on both sides are enormous, the two layers occupying more than two-thirds of the thickness of the leaf. The narrow interior layer consists of poorly defined palisade and extremely loose spongy parenchyma. All these interior cells contain chlorophyll; each cell has relatively few, but very large chloroplasts. A leaf of this type is obviously difficult to preserve. Good killing has been obtained with chrome-acetic 0.5-0.5, washed by diffusion in a large volume of water, followed by the ethyl alcohol-xylene series. Cut 15 μ thick in order to keep the large epidermal cells intact. Coleus is another common

greenhouse plant with soft leaves. They are preserved well by *FAA* and particularly well by Craff II.

The stereotyped construction of the mesophytic leaf permits the use of innumerable species to illustrate the type, making it possible to utilize plants that are readily accessible and characteristic of the region rather than use some classical species as if it had special virtues.

Most broad leaves are distinctly dorsiventral, the columnar palisade cells being on the upper or ventral side. Leaves that normally assume a vertical position do not have such distinctive palisade cells, the upper and lower tissue zones are nearly alike, and the dorsiventrality is obscured. The garden beet and sugar beet are good examples. These leaves can be fixed successfully in *FAA* or Craff III.

The study of the dicotyledonous leaf would be far from complete without a study of deviations from the typical mesophyte. Perhaps the most striking variations are the xerophytic adaptations. The leaves of species of *Dianthus* show a range from the relatively large, flat leaves of the greenhouse carnation to the waxy, narrow, rolled leaves of the rock garden species. These easily obtainable leaves are well preserved by Craff II. The tough cuticle becomes brittle after xylene but cuts well after *TBA* or dioxan. A brilliant stain is obtained with safranin-fast green.

Nerium oleander has leaves of unique xerophytic structure. The lower surface is indented with globose cavities or infoldings of the epidermis. Each pocket is lined with numerous hairs and contains many stomates. The upper epidermis is firm and highly cutinized. There are two to three layers of tough thick-walled hypodermal cells below the epidermis, and the deep-seated palisade cells are long and narrow. Killing fluids penetrate with difficulty. *FAA* is the most rapid of the satisfactory formulas but may cause slight plasmolysis. If the pieces cut transversely out of the fresh leaf are very narrow, not over 1 mm. wide along the linear dimension of the midrib, good penetration and fixation are obtained with Craff 0.30–1.0–5.0. Brittleness in paraffin is minimized by the use of butyl alcohol or dioxan. Safranin-fast green gives a brilliant and highly differential stain.

Leaves of citrus fruits are also of the leathery type and have an added interesting feature, the pear-shaped oil glands in the epidermis. The spongy parenchyma is compact and firm, and the palisade cells are small and closely spaced. The impervious character of the surface and compactness of the interior necessitate the use of *FAA*, which produces acceptable results. If the pieces of leaf are cut very narrow, Craff III produces excellent fixation.

Hedera helix is a remarkably efficient xerophyte that can withstand severe drought. However, the leaf has no striking structural adaptations; its tissue organization is that of a stereotyped mesophyte. This very fact makes the leaf an interesting subject for comparative studies. Kill in Craf II.

The leaf of either of the common rubber plants, *Ficus elastica* or *F. pandurata*, is an interesting leathery, latex-bearing leaf. The small, compact epidermal cells are overlaid by a very thick cuticle. Under the upper epidermis there are two layers of large water-storage cells, under which there are two layers of small, short palisade cells. Two layers of compact hypodermal cells occur adjacent to the lower epidermis. The spongy parenchyma is very open and is transversed by the prominent latex vessels. The latex does not seem to be preserved in stainable form by *FAA*, but very well by the chromic acid fluids. Excellent results are obtainable with chrome-acetic 0.5–0.5 or Craf I.

Other illustrations of lactiferous leaves are easily obtainable. The leaf of the ubiquitous dandelion can be preserved in perfect condition by Craf I. Leaves of the common cultivated poinsettia and of the cultivated and native *Euphorbias* are well preserved by Craf I. These leaves are not brittle and may therefore be put through an alcohol-xylene or acetone-xylene series.

The *succulents* have very fleshy leaves that can be preserved well in Craf I and dehydrated carefully in normal butyl alcohol. The tissues are highly susceptible to damage, and a procedure that produces severe distortion should not be condemned without a repetition of the process.

✓ The gramineous leaf is represented by maize, sugar cane, sorghum, foxtail, and bluegrass. Corn illustrates well the border parenchyma of the vascular bundles (Fig. 13.11), but sorghum, and especially sugar cane, have more striking motor cells. Bluegrass is a good representative of the narrow type with prominent *bulliform* motor cells along the midrib. Foxtail is intermediate between the very broad and very narrow types. Acceptable preservation can be obtained with *FAA*, but for nearly perfect fixation use Craf III. This procedure has been repeated many times with corn, with uniformly good results. Prior to the introduction of the butyl alcohols and dioxan the older midribs of corn and other grass leaves were difficult to section without considerable breakage, but the use of these reagents has minimized the difficulty. //

Monocotyledonous leaves other than the gramineous type should be included in a comprehensive collection. The leaves of lily repre-

sent the broad flat type. The extremely large stomates are the standard subject for studying sectional views of the stomate. Kill in Craff II and process by the alcohol-xylene method. The thinness of the cell walls necessitates a contrasting wall stain like hemalum. The stomates are shown with almost diagrammatic clarity. Leaves of *Zebrina*, *Rhoeo*, *Tradescantia*, *Polygonatum biflorum*, and *Smilacina racemosa* can be prepared by the above method.

The tender, cylindrical, hollow leaf of onion is preserved in excellent condition in Craff I and processed like those of lily.

The tough leaf of *Iris* is difficult to section. Fair cellular fixation can be obtained with *FAA* and excellent preservation with Craff I if the pieces are very narrow. The *TBA* process minimizes brittleness in paraffin.

The favorite subjects for the study of coniferous leaves are *Pinus strobus*, a soft pine, and *P. laricio austriaca*, *P. sylvestris*, or other hard pine. Needles collected in the late fall and in winter are very hard and become extremely brittle in the paraffin. The cells contain much granular resinous material which remains in the finished preparation. In July the needles are full grown, with all structural features fully developed, but they are still sufficiently soft to cut readily, and do not have excessive deposits in the cells. Kill in Craff III, and process in *TBA*; safranin-fast green yields a beautiful preparation. Longitudinal as well as cross sections should be made. Needles of the spruces (*Picea*) are also in the tough, wiry category and should be processed like those of pine.

The flat type of needle is represented by Douglas fir (*Pseudotsuga taxifolia*), the hemlock (*Tsuga*), or the fir (*Abies*). These may be killed in the fluids recommended above. Although these relatively soft needles can be processed through xylene, dioxan and butyl alcohol improve the cutting properties.

The broad leaf of *Ginkgo biloba* should not be omitted from a study of the gymnosperm leaf. Collect leaves in July, and kill in Craff III.

The leaves of cycads are difficult subjects because of their tough, xerophytic features. Select pinnules that are not fully matured and toughened. Cut transversely into narrow pieces and kill in *FAA*. The nonalcoholic fluids penetrate poorly, but excellent fixation in small pieces is obtained with Craff II. Xylene renders the tissues very brittle, but tertiary butyl alcohol permits satisfactory sectioning.

Fern leaves are readily obtainable from the common Boston fern. Use pinnules that have expanded to maximum size but are still bright,

shiny green. Old leaves contain discoloring deposits in the cells. Kill in *FAA* or Craf II. Other conservatory or native ferns may be prepared by the same methods.

The foregoing recommendations dealt with mature leaves. Advanced students are invariably interested in the development of the leaf. The place and mode of origin of leaf primordia and the early stages of leaf development are evident at the growing points of stems, and the processing of suitable materials is discussed in the section dealing with the stem.