Angiosperm Development—Fruits, Seeds, Meristems, and Secondary Growth

LABORATORY 29

OVERVIEW

Angiosperms protect their seeds inside fruits that develop from the ovary tissues of flowers. Seeds, liberated from their fruits, germinate and form the **epicotyl,** which develops into the shoot system, and the **hypocotyl,** which develops into the root system. **Apical meristems,** located at the tips of growing roots and stems, give rise to the **primary tissues** and increase the length of both stems and roots. In dicots, **lateral (secondary) meristems** give rise to **secondary tissues,** which increase the width of the stem and root, making them woody.

During this laboratory you will examine fruits, their seeds, how the seeds germinate, and how a plant develops through the activities of both apical and lateral meristems.

STUDENT PREPARATION

Prepare for this laboratory by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency.

✓ EXERCISE A Fruits

A **fruit** is a mature seed-containing ovary, a cluster of mature ovaries, or an ovary and closely associated tissues. Recall that the ovary is the enlarged portion of a flower carpel and contains one or more ovules. (Review flower structure, Laboratory 24, Exercise C.) Seeds develop from fertilized ovules, thus seeds are inside fruits. As the ovary develops into a fruit, the ovary wall thickens and becomes the **pericarp.** The evolution of such covered seeds marked a great evolutionary advance for angiosperms.

Fertilization most often initiates development of the fruit as well as the seed but, in some plants, pollination alone serves as the stimulus for fruit development.

Structural adaptations of various fruits have facilitated the worldwide dispersal of many plants. The structural organization of fruits reflects that of the flowers from which they develop. Fruits can be classified into three major types:

1. **Aggregate fruits** Consist of a number of enlarged multiple ovaries of a single flower, massed on or scattered over the surface of a single receptacle (the part of the flower stalk that bears the floral organs). The separate ovaries are called fruitlets. Examples include the raspberry, blackberry, and strawberry.
2. **Multiple fruits** Consist of the enlarged ovaries of several flowers more or less coalesced into one mass. Examples include the mulberry, fig, and pineapple.

3. **Simple fruits** Arise from the ovary (composed of a carpel or several united carpels) of a single flower. Simple fruits are divided into several categories based on the consistency of the pericarp and on structure and dehiscence (manner of opening). The two major groups include fleshy fruits and dry fruits.

   a. **Fleshy fruits** The thickened pericarp sometimes becomes differentiated into three distinct layers: proceeding from the outside to the inside of the fruit, the **exocarp,** the **mesocarp,** and the **endocarp.** The development and consistency of these layers differ among types of fruit. There are several types of fleshy fruits (Figure 29A-1), including berries (examples: grapes and tomatoes), drupes (examples: cherries and peaches), pomes (examples: pears and apples), hesperidia (citrus), and pepos (squash, melons, and cucumbers).

   b. **Dry fruits** Dry fruits (Figure 29A-2) are simple fruits usually classified according to whether they are **dehiscent** (split open when ripe) or **indehiscent** (do not split open when ripe). Further distinctions are made according to their mechanisms of dehiscence and other features of structure. Peas and beans are examples of dehiscent fruits. Sunflower seeds and wheat are indehiscent fruits.

Objectives

- ☐ Define the term "fruit."
- ☐ Relate fruit structure to the ovary and ovules of a flower.
- ☐ Distinguish among aggregate, multiple, and simple fruits.

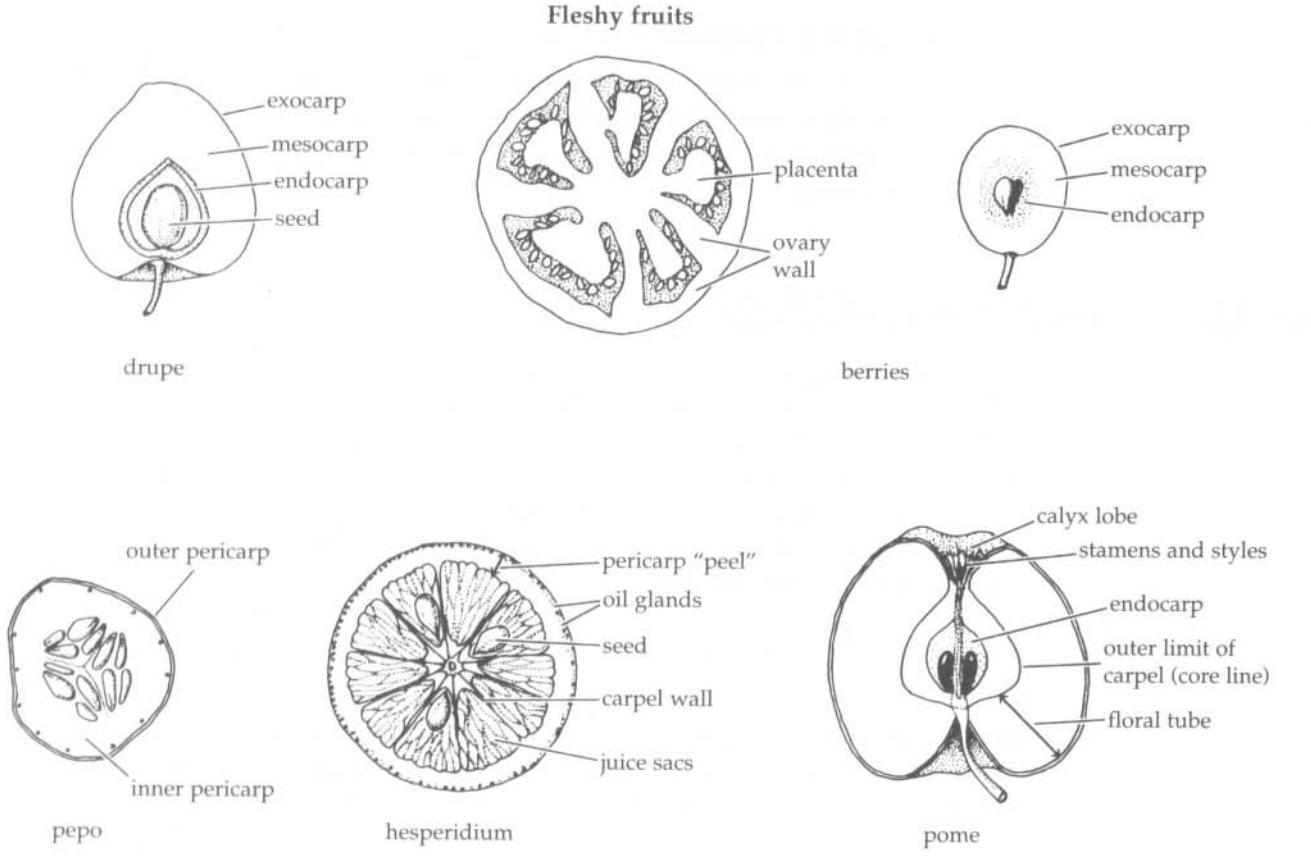


Figure 29A-1 *Examples of fleshy fruits.*

- ☐ Relate the structure of angiosperm flowers to their fruits.
- ☐ Recognize common types of fleshy fruits.
- ☐ Recognize specific examples of dry (dehiscent and indehiscent) fruits.

Procedure

1. Observe the cut peach and coconut on demonstration. The peach and the coconut are classified as **drupes:** in both fruits, the single seed is enclosed by a hard, stony endocarp (Figure 29A-1). In the peach the mesocarp is fleshy and edible, whereas in the coconut the mesocarp is fibrous. The exocarp of the peach forms the skin. The exocarp of the coconut is a hard covering that is usually removed, along with most of the mesocarp, before it reaches the produce department. The coconut you buy is the seed enclosed in the endocarp with remnants of the fibrous mesocarp still attached.
2. Cut a grape in half lengthwise. The grape is a **berry** (Figure 29A-1). The endocarp, mesocarp, and exocarp (pericarp) are fleshy. Mesocarp and endocarp are not well differentiated. The exocarp forms the skin.
3. Examine the cut apple on the demonstration table. The apple is a **pome** (Figure 29A-1). The endocarp surrounding the seeds is papery or leathery. The core constitutes the extent of the ovary, and the exocarp and mesocarp are indistinguishable. The flesh of the fruit derives from the enlarged bases of the petals and stamens that surround the ovary, not from the ovary itself.
4. Using the key to simple fruits (Table 29A-1) and the diagrams in Figures 29A-1 and 29A-2, try to identify each of the fruits on demonstration. For those fruits commonly eaten as food, identify the part of the fruit usually eaten (exocarp, endocarp, or the entire fruit). Since seed dispersal is closely associated with fruit structure, suggest the probable means of seed dispersal for each fruit. Record your observations in Table 29A-2.

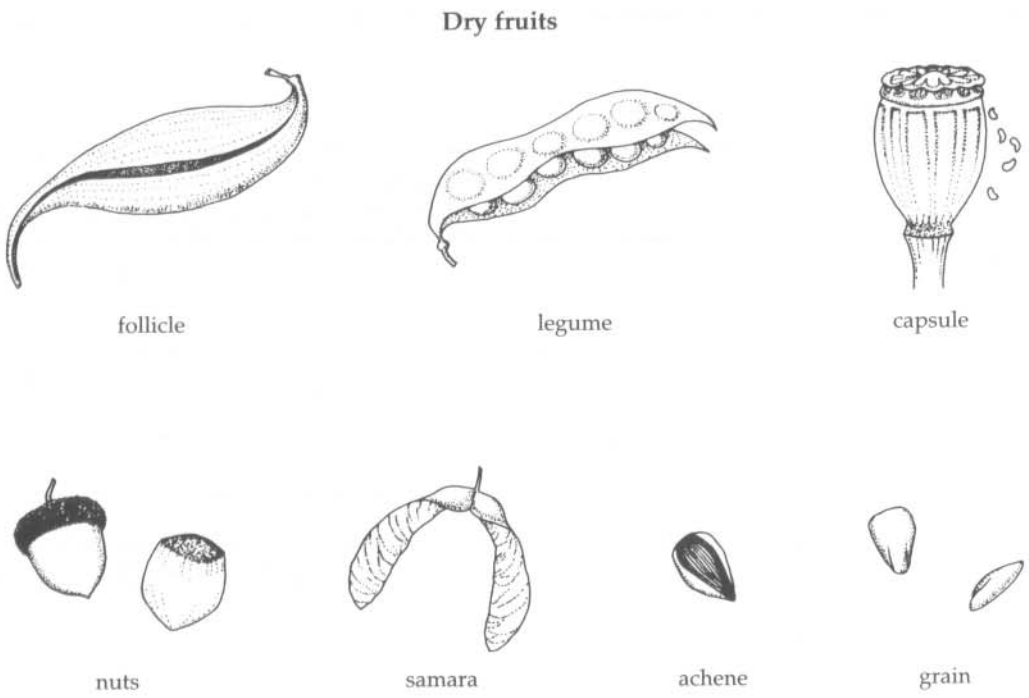


Figure 29A-2 *Examples of dry fruits.*

Table 29A-1 A Dichotomous Key to Identify Simple Fruits

1. Fruit dry at maturity 2
   2. Fruit splits open (is dehiscent) 3
      3. Fruit contains one chamber or compartment 4
         4. Fruit splits open along one side . . . . . Follicle—example: milkweed
         4. Fruit splits open along two sides . . . . . Legume—examples: peas, beans
      3. Fruit contains two or more chambers and splits along more than two sides . . . . . Capsule—example: lily
   2. Fruit does not split open (is indehiscent) 5
      5. Ovary wall very hard . . . . . Nut—examples: oak, hickory
      5. Ovary wall easily cracked or opened 6
         6. Ovary wall with winglike outgrowth . . . . . Samara—examples: ash, maple, elm
         6. Ovary wall without winglike outgrowth 7
            7. Seed attached at only one place to the inside of the ovary wall . . . . . Achene—example: sunflower
            7. Seed completely attached to ovary wall so that the two cannot be separated . . . . . Grain—example: wheat, rice, corn
1. Fruit fleshy at maturity 8
   8. Fruit containing only one seed (located inside the pit) . . . . . Drupe: the exocarp is skinlike; the mesocarp, fleshy; and the endocarp, stony—examples: cherry, peach, almond, olive
   8. Fruit containing several seeds 9
      9. Fruit has a firm leathery or hard rind 10
         10. Fruit has a hard rind and no sections; rind inseparable . . . . . Pepo—examples: cucumber and squash
         10. Fruit has leathery rind and many sections; rind separable . . . . . Hesperidium: citrus fruits
      9. Fruit has a peel that is not leathery and may be eaten 11
         11. Fruit has a core . . . . . Pome—examples: pear, apple
         11. Fruit more or less fleshy throughout; seeds may be eaten . . . . . Berry—examples: grape, pepper, tomato

Table 29A-2 Identifications of Common Fruits

Plant (common name)	Fruit Type	Portion Eaten (if applicable)	Means of Seed Dispersal
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

✓ EXERCISE B Seed Structure

Angiosperm and gymnosperm seeds are integumented ovules. The integuments form the seed coat. The ovule itself contains the embryo sac within which double fertilization occurs. One sperm nucleus from the pollen grain unites with the egg to form a zygote. The other sperm nucleus unites with two polar nuclei to form a triploid primary endosperm nucleus. The zygote divides mitotically to form the embryo. The primary endosperm nucleus divides mitotically to form the endosperm, food for the embryo.

Objectives

- ☐ Identify the parts of a monocot and a dicot seed and describe the function of each.
- ☐ Describe the function of endosperm and the cotyledons.
- ☐ Compare the mechanisms of endosperm storage in monocots and dicots.

✓ PART 1 Examining the Dicot Bean Seed

Procedure

1. Obtain a water-soaked bean (*Phaseolus vulgaris*) seed. Examine the **seed coat** and note the **hilum,** the former point of attachment of the ovule to the ovary.
2. Gently remove the seed coat with your fingernails. Integuments around the ovule thicken and harden into this protective covering.
3. Pull the two cotyledons apart. The embryo consists of three parts:

   Cotyledons Leaflike food storage organs (seed leaves). In most dicots, the endosperm is absorbed during embryonic development and the seeds develop two fleshy, food-storing cotyledons.

   Hypocotyl Located below the attachment of the cotyledons, this portion of the embryo will develop into the embryonic root, or **radicle**.

   Epicotyl Located above the attachment of the cotyledons, this portion of the embryo will develop into the embryonic shoot. The first true leaves or **plumule** develop from the epicotyl.

4. In Figure 29B-1a, label the seed coat **a**, cotyledon **b**, hilum **c**, hypocotyl **d**, epicotyl **e,** first leaves or plumule **f**.

PART 2 Examining the Monocot Corn Seed

1. Examine the water-soaked kernels of corn (*Zea mays*). The kernel is a one-seeded fruit with the **pericarp** fused with the seed coat.
2. Cut a kernel lengthwise, bisecting the embryo. The **single cotyledon** or seed leaf is the broad surface of the embryo pressed against the starch-rich **endosperm.** In monocots, the cotyledon performs an absorbing rather than a food-storing function. The root tip (at the end of the embryonic root or radicle) is enclosed by a protective sheath called the **coleorhiza.** The shoot tip is enclosed in a conical sheath called the **coleoptile.**
3. In Figure 29B-1b, label the pericarp **a**, endosperm **b**, cotyledon **c**, coleoptile **d**, epicotyl **e**, hypocotyl **f**, coleorhiza **g**.

   a. What is the difference between the functions of the cotyledon in corn and in the bean?

   _____

   b. Which part of the corn seed contains the food source for the developing embryo?

   _____

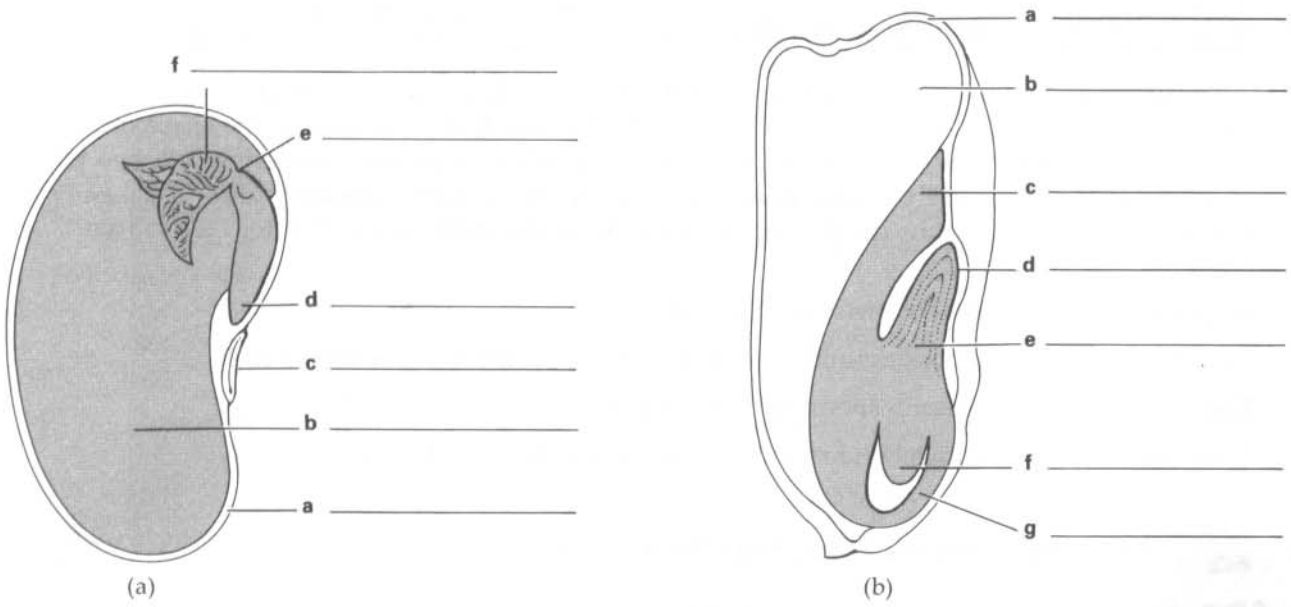


Figure 29B-1 *(a) The bean seed, a dicot. (b) A seed of corn, a monocot.*

EXERCISE C Found a Peanut

The peanut, a dicot, provides excellent material with which to check your understanding of fruits, seeds, and embryos.

Objectives

☐ Diagram and discuss the functions of the parts of a peanut.

Procedure

1. Obtain a peanut. *a. What type of fruit is it? (Should we be calling the peanut a fruit?)* _____

   b. Is the peanut really a nut? _____ *Why or why not?* _____

   _____

   c. What part of a fruit does the "shell" of a peanut represent? _____

2. Open the peanut. *d. On how many sides does it split open? (This should give you a clue for answering question a.)* _____

3. Remove the edible part. *e. What part of the fruit is the brown skin on the surface of the parts you eat?* _____ *f. What is the part of the fruit that you are used to calling the "nut?"* _____

4. Open the seed carefully. *g. Is the peanut a monocot or a dicot?* _____

   h. What does each half of the seed represent? _____

5. Look carefully for the embryo at the end of the seed. You should be able to see the hypocotyl, epicotyl, and some little leafy-looking structures, the first true leaves of the stem.

   i. Why are these little leafy structures not the cotyledons? _____

   _____

EXERCISE D Seedling Development

As seed germination occurs, the first structure to emerge from most seeds is the **radicle** (embryonic root), formed from the hypocotyl. The continuation of this root is called the **primary root. Branch roots** (lateral roots) develop on the primary root. In monocots, the primary root is short-lived and the root system of the adult plant develops from **adventitious roots** that arise from the first **node** (place on the stem where leaves are attached). Lateral roots are then produced from the adventitious roots.

The way in which the shoot emerges from the seed differs among various plants. In some plants, such as beans, the cotyledons are carried above the soil by the hypocotyl. This first forms a hook and then straightens out to lift up the cotyledons, which become photosynthetic. The food stored in the cotyledons is digested and transported to areas of the growing seedling. The cotyledons shrink and eventually fall off the stem.

In other dicot plants, such as the garden pea, the epicotyl forms a hook that carries the plumule (epicotyl plus first foliage leaves) above the ground while the cotyledons remain below ground. In corn the root and shoot emerge from the protective coleoptile (the first seedling leaf), and the single cotyledon remains below ground.

Objectives

- ☐ Distinguish between the germination processes in beans, peas, and corn.
- ☐ Describe the process of seedling development by stating the fates of the hypocotyl, epicotyl, cotyledons, shoot apex, and root apex.

PART I Comparing Germination in Beans, Peas, and Corn

Procedure

1. Examine the different stages of germinating bean seedlings on demonstration. In the bean, a dicot, seedling emergence is due to expansion of the hypocotyl. Note in particular the **hypocotyl arch** (hook).

   a. What is the advantage of having the hypocotyl arch pull the cotyledons up through the soil, rather than push them up? _____

   _____

   Once the arch breaks through the soil surface and is exposed to light, it straightens out to hold the cotyledons and epicotyl in an upright position. Identify the following parts on the live germinated bean seedling: seed coat, cotyledon, first leaves, hypocotyl, epicotyl, and primary root. Refer to Figure 29D-1a.

   b. What does the hypocotyl become? _____

2. Examine the germinated pea seedlings on demonstration. In the pea (a dicot) seedling, the cotyledons remain underground. The epicotyl forms a hook which then straightens out and pulls the plumule above ground. Identify the following parts on the live germinated pea seedling: seed coat, cotyledon, first leaves, hypocotyl, epicotyl, and primary root. Refer to Figure 29D-1b.

3. Study the different stages of germinated corn on demonstration. Identify the coleoptile. Note that it is initially a closed tubular structure that grows to the soil surface. Once the coleoptile emerges from the soil, it ceases to grow in length, splits, and exposes the rolled leaves within. The shoot apex is at the base of the underground coleoptile. The "stalk" of the seedling is formed of rolled leaf bases. Only after the corn plant is several inches in height does the stem start to grow. The single cotyledon remains below ground. The radicle, originally enclosed in the coleorhiza, forms the primary root. Adventitious roots will later develop from the stem (Figure 29D-1c).

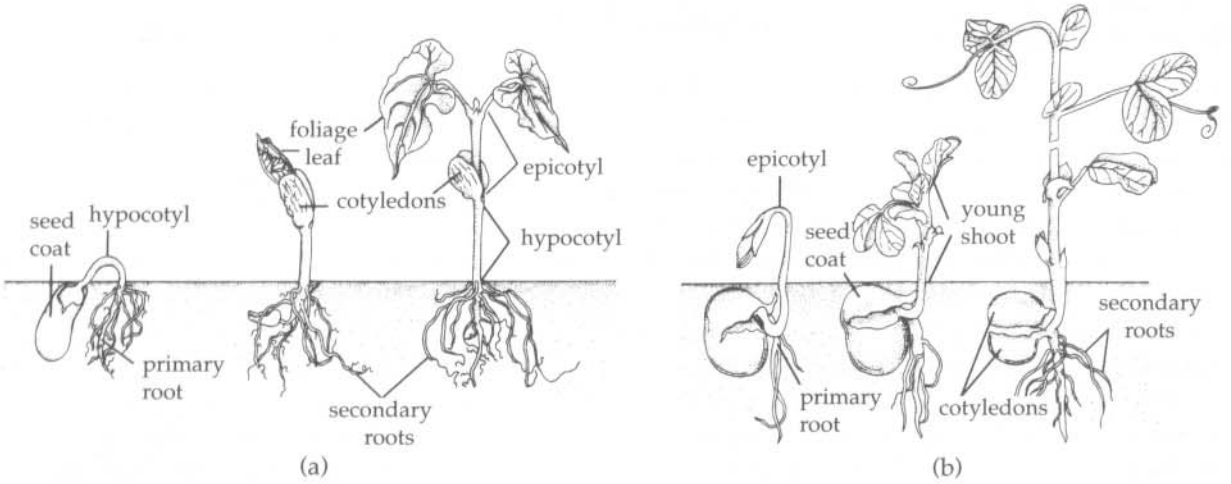
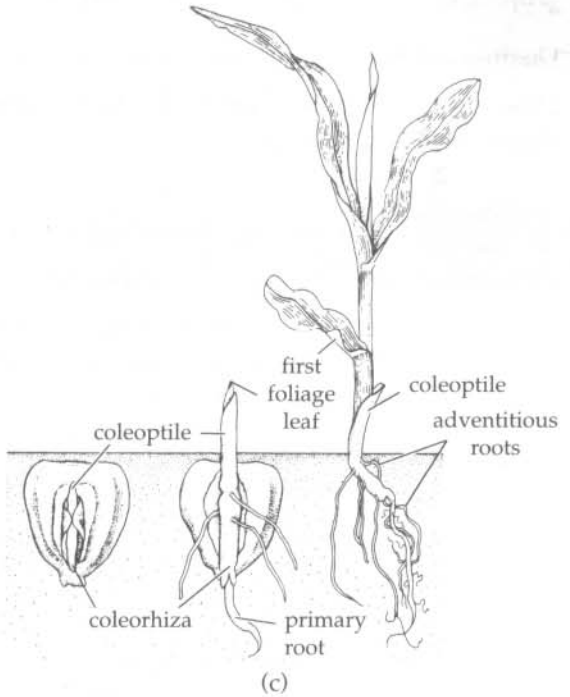


Figure 29D-1 *(a) During seedling emergence in the dicot bean, the hypocotyl expands and forms an arch that lifts the cotyledons up and out of the ground. (b) During germination of the dicot pea seedling, cotyledons remain below ground and the epicotyl forms a hook to pull the plumule above ground. (c) During germination of the monocot corn seed, the single cotyledon remains below ground and the shoot emerges from the coleoptile.*



4. Identify the leaf, coleoptile, primary root, and adventitious roots of the germinated corn seedling.

5. Cut a germinating corn seedling in half and add Lugol's solution (I_2KI) to it. Add I_2KI to the ungerminated seed examined earlier (Exercise B) and compare the color reaction.

   c. Which test is positive? _____

   d. What has happened to the food reserves of the endosperm in the germinated seedling? _____

   _____

6. In the following table (Table 29D-1), list the above-ground and below-ground structures you observed in the bean, pea, and corn seedlings, including the function of each. What are the major differences in the germination processes for each seed type?

Table 29D-1 Above and Below Ground Structures

	Bean	Pea	Corn
Above ground			
Below ground			

PART 2 Observing the Germination and Development of Seeds

Observe the development of bean, pea, corn, and other seeds in a germination chamber.

Procedure

1. Fill a clear plastic cup with wet soil or wrap a moist piece of blotter paper around the inside surface of the sides of the cup and pour an inch of water into the cup.
2. Place seeds against the plastic about an inch below the soil or between the blotter and the cup. You should be able to see the seeds. Cover with a Petri dish lid.
3. Observe the seeds for two weeks (or longer). Remember to keep the soil moist. Keep the blotter paper moist by keeping a one-fourth inch of water in the bottom of the cup. The blotter paper will act as a wick and soak up the water.
4. In Table 29D-2 record your observations over 10 days.

Table 29D-2 Seed Germination

Type of Seed	Time until Emergence of Epicotyl	Time until Emergence of Hypocotyl	Fate of Cotyledons	Fate of Hypocotyl and Epicotyl	Day 5	Day 10
					Length of Hypocotyl and Epicotyl General Appearance	Length of Root and Shoot General Appearance

EXERCISE E Studying the Stem Tip and Root Tip

The developing shoot (epicotyl) and root (hypocotyl) are dependent upon the presence of **apical meristems**—the growing points or areas of mitotic activity at the tips of the shoot and root. These are responsible for primary growth and the laying down of primary tissues.

Three primary meristematic tissues are present in the root and shoot tip. (1) The **protoderm** is the outermost layer of cells along the surface of the apical tissue and is responsible for production of the

epidermis. (2) The **procambium** forms lengthwise columns of tissue that will differentiate into vascular bundles composed of xylem and phloem. (3) The **ground meristem** adds cells to the remaining space, giving rise to the cortex and the pith. Development of the stem and root from these meristematic tissues occurs through cell division, cell enlargement, and cell differentiation.

Objectives

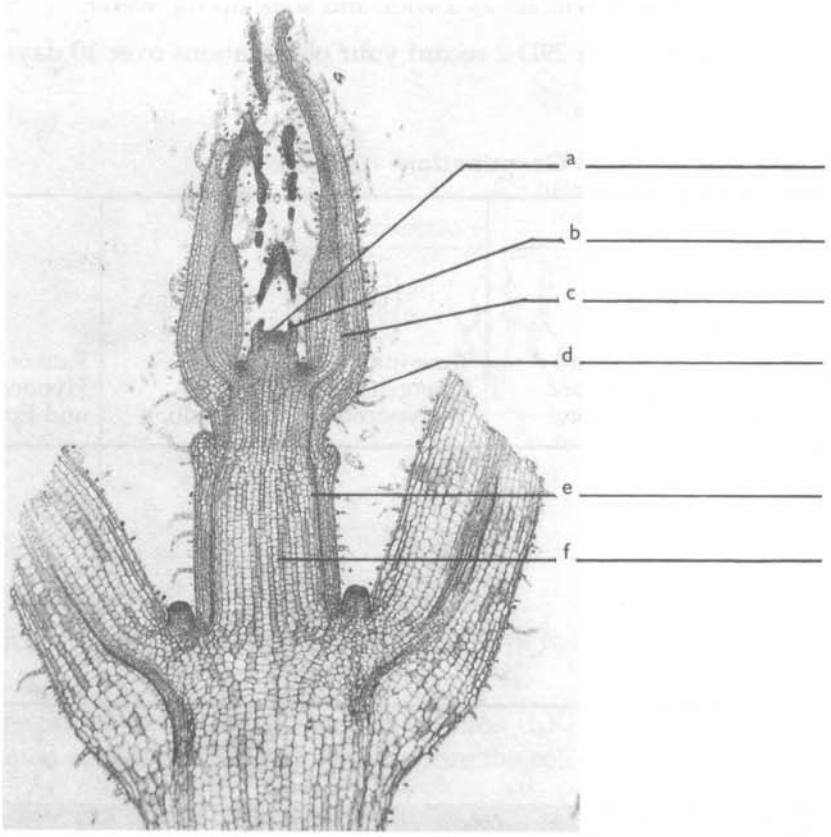
- ☐ Distinguish between apical and lateral meristems in the root and shoot.
- ☐ Identify the types of tissues formed by apical meristems and describe their location in the young root and shoot.

✓ PART I Examining the Stem Tip

Procedure

1. Locate the apical buds at the tips of all branches on a living *Coleus* plant.
2. Look at the **axil** of each leaf between the leaf base and the stem axis and identify the **axillary bud.** When branches develop, axillary buds become apical buds.
3. Use low power (10×) to examine a prepared slide of the apical bud of *Coleus* (longitudinal section), then switch to high power (40×). Locate each of the following structures and label them in Figure 29E-1.

Figure 29E-1 *Stem tip of the* Coleus *plant.*



- **a Meristematic cells** Meristematic cells are small with relatively large nuclei and dense cytoplasm, and are stained more intensely than surrounding nonmeristematic cells. Note the mound of meristematic cells at the stem tip and in the regions along the sides of the stem in the axils of the young leaves.

b Leaf primordia The size of these rudimentary leaves increases from the tip downward. Notice that leaf primordia are visible only on alternate nodes, and intervening nodes appear as mounds of tissue.

a. Is the arrangement of Coleus *leaves alternate or opposite?* _____

c Vascular tissue Strands of vascular tissue are seen in lengthwise view along the sides of the stem and in older leaves.

d Protoderm Outermost layer developing into the epidermis.

e Procambium Located inside the protoderm; gives rise to the primary vascular tissue.

f Ground meristem Inner cells giving rise to ground tissue, including the cortex.

4. Examine the head of a cabbage or a brussels sprout that has been cut lengthwise through the center. Compare it with the *Coleus* stem tip.

b. What may the entire head be called? _____

✓ PART 2 Examining the Root Tip

Procedure

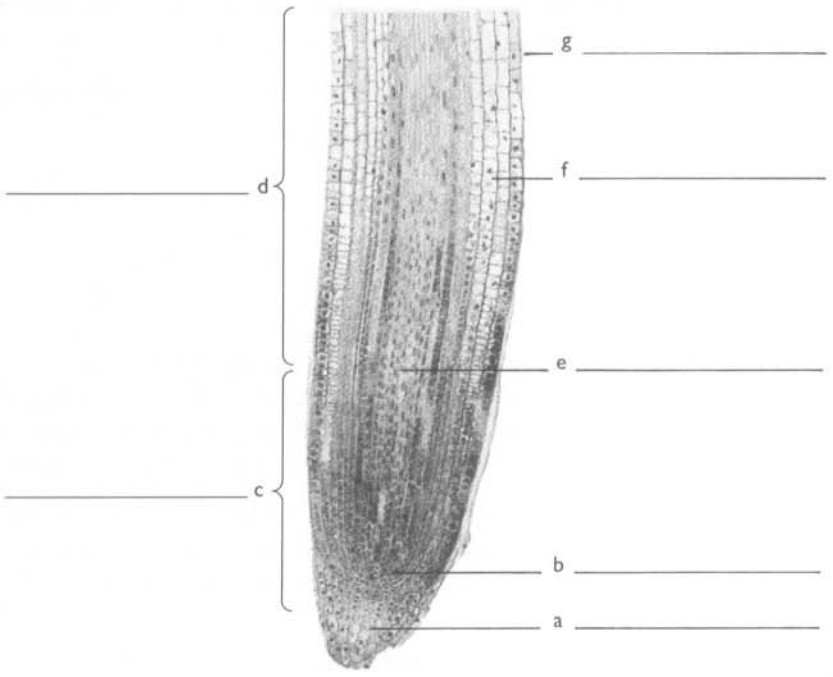
1. Place a young radish or rye seedling in a large drop of water on a slide.
2. Use a razor blade to cut off the grain and shoot of the seedling and carefully lower the cover glass over the root. Study the following structures under low power (10×) and label them in Figure 29E-2.

a Root cap Located at the tip of the root, covering the apical meristem. Cells of the root cap are usually loosely arranged and often have broken away from the root.

b Apical meristem Located behind the root cap; a **region of cell division, c** Cells are tightly packed and appear dark.

d Region of elongation Region behind the apical meristem where cells elongate. Region ends at level of first root hairs.

Figure 29E-2 *Root tip.*



e Procambium Innermost area of the root; matures to form the central vascular cylinder.

f Ground meristem Middle tissue layer; forms the cortex.

g Protoderm Outer layer of cells; differentiates into epidermis.

EXTENDING YOUR INVESTIGATION: GROWING LONGER

The elongation of cells in the region of elongation produces most of the increase in length of the root. Do you think that there is any increase in length beyond this region? To test your answer, formulate a hypothesis that predicts what will happen to the length of the region of elongation relative to the region of maturation in the growing root.

HYPOTHESIS:

NULL HYPOTHESIS:

Identify the **independent variable** in this experiment.

Identify the **dependent variable** in this experiment.

If you observe the root tip of a germinating pea seed over a period of 24 hours, what do you **predict** you will observe?

Use the following procedure to test your hypothesis.

PROCEDURE:

1. Obtain three pea (*Pisum*) seedlings of equal size with straight roots about 2 to 3 cm long. One at a time, lay each seedling flat on a moist paper towel and then, with a fine indelible ink pen (e.g., Sharpie™), make marks exactly 2 mm apart along the entire length of the root. Record the number of marks and length of the root in Table 29E-1. In the space below, diagram one of the seedlings showing the distribution of the marks.

Pea Seedling *Pea Seedling after 24 Hours*

2. Obtain 3 pieces of glass tubing each 4 cm long with an inner diameter of 5 mm. Insert the root of each seedling into a tube so the cotyledons of each seedling are resting on one end of the tube. Place the tubes upright in a plastic cup containing 25 ml of water. Cover the cup with a Petri dish or plastic wrap and set it in a dark, warm place.
3. Examine the seedlings after 24 hours. Measure the distances between the marks and the total extent of root growth. Record your data in Table 29E-1. Draw the same seedling (see above) as before, showing the new distribution marks.

RESULTS: What was the result of your experiment? Which portion of the root elongated?

Do your data and results support your hypothesis?

Your null hypothesis?

Was your prediction correct?

What do you **conclude** about the way in which root growth occurs?

Table 29E-1 Root Length of Germinating Pea Seedlings

Pea Seedling	Number of Marks 2 mm Apart	Total Length of Root at Start (mm)	Root Length after 24 Hours	
			Distances Between Marks	Total Growth (mm)
1				
2				
3				

✓ EXERCISE F Secondary Growth of Angiosperms—The Woody Stem (*Tilia*)

We have observed that all vascular plants have primary meristems in two locations: stem tips (buds) and root tips. These are responsible for primary growth and production of primary tissues. Leaves are limited to primary growth, but the stems and roots of woody plants (not herbaceous plants) can thicken as a result of **secondary growth.**

There are two secondary meristems in woody plants: the **vascular cambium** and the **cork cambium.** The vascular cambium consists of a row of cells that lies between the xylem and phloem of the vascular bundles and continues between individual vascular bundles, thus forming a continuous cylindrical sheath of cells within the stem. The meristematic cells divide and produce new cells along both interior and exterior faces of the vascular cambium. Cells produced along the interior face differentiate into secondary xylem, whereas those produced along the exterior face differentiate into secondary phloem (Figure 29F-1).

During each growing season a new layer of secondary xylem is added to the inside, and a new layer of secondary phloem to the outside of the vascular cambium. Xylem layers build up and form the woody core of the plant. The xylem cells are dead and contain **lignin** to strengthen their cell walls. In contrast, secondary phloem does not build up: older layers of phloem, as well as the cortex and epidermis formed during primary growth, are continuously sloughed off the stem and new protective tissues are formed by the **cork cambium.** Because the **bark** layers external to the vascular cambium are continuously sloughed, these outer regions do not add significantly to the cumulative thickness of the woody stem and root: wood is primarily secondary xylem.

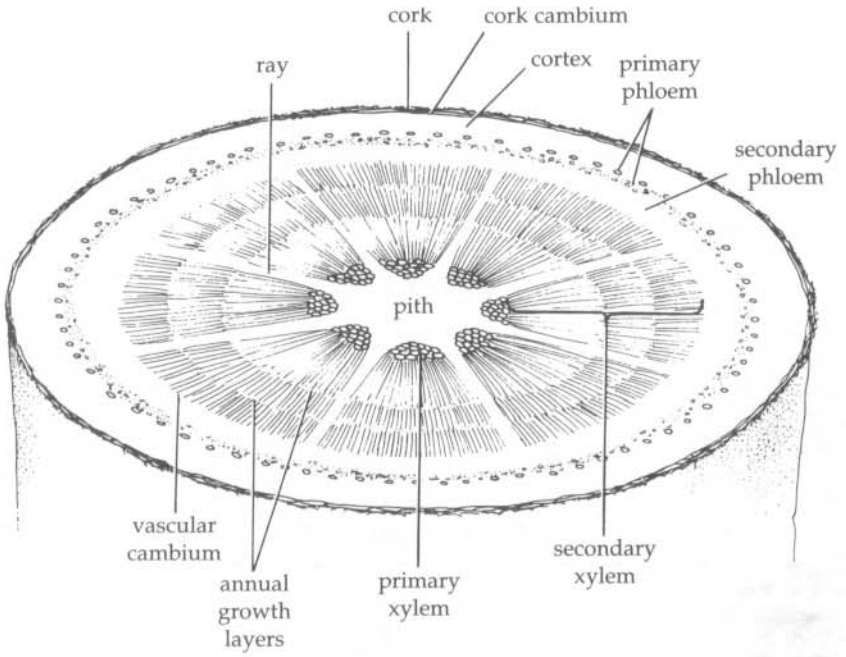


Figure 29F-1 *Cross section of a 3-year-old stem, showing annual growth layers. On the perimeter of the outermost growth layer of xylem is the vascular cambium, encircled by a band of secondary phloem. The primary phloem and also the cortex will eventually be sloughed off. The tissues outside the vascular cambium, including the phloem, constitute the bark.*

Objectives

- ☐ Describe how wood is formed.
- ☐ Distinguish between summer wood, spring wood, heartwood, and sapwood.
- ☐ Describe how to determine the age of a tree.
- ☐ Describe the importance of secondary meristems to the woody plant.

Procedure

1. Examine a prepared slide of a woody stem (*Tilia*, basswood). Locate the following structures and label them in Figure 29F-2.

   a Pith Located in the center of the stem, just as in the herbaceous dicot stem of alfalfa (Laboratory 28).

   Vascular tissue Xylem. The closely spaced bundles of **secondary xylem b** form a wide, continuous ring around the pith. Clusters of **primary xylem** cells **c** are located toward the interior around the pith.

   d Vascular cambium Situated at the outer edge of the xylem, these thin-walled cells give rise to the secondary xylem and phloem and the **vascular rays e**, ribbonlike aggregates of two to three cells extending radially through the xylem and widening to form triangular areas toward the outside.

   f Annual rings In temperate zone woody plants, the secondary xylem cells formed by vascular cambium early in the growing season when more moisture is present, called **spring wood g**, have a larger diameter than those formed later, called **summer wood h**. The contrasting cell types in spring wood and summer wood produce the annual ring, an indication of the xylem produced during one growing season. You can determine the age of a tree by counting its annual rings.

   i Vascular tissue Phloem. This appears as wedge-shaped areas between widened vascular rays. The banded appearance is the result of layers of thick-walled **phloem fibers j** interspersed with layers of **sieve tubes** and **companion cells k**.

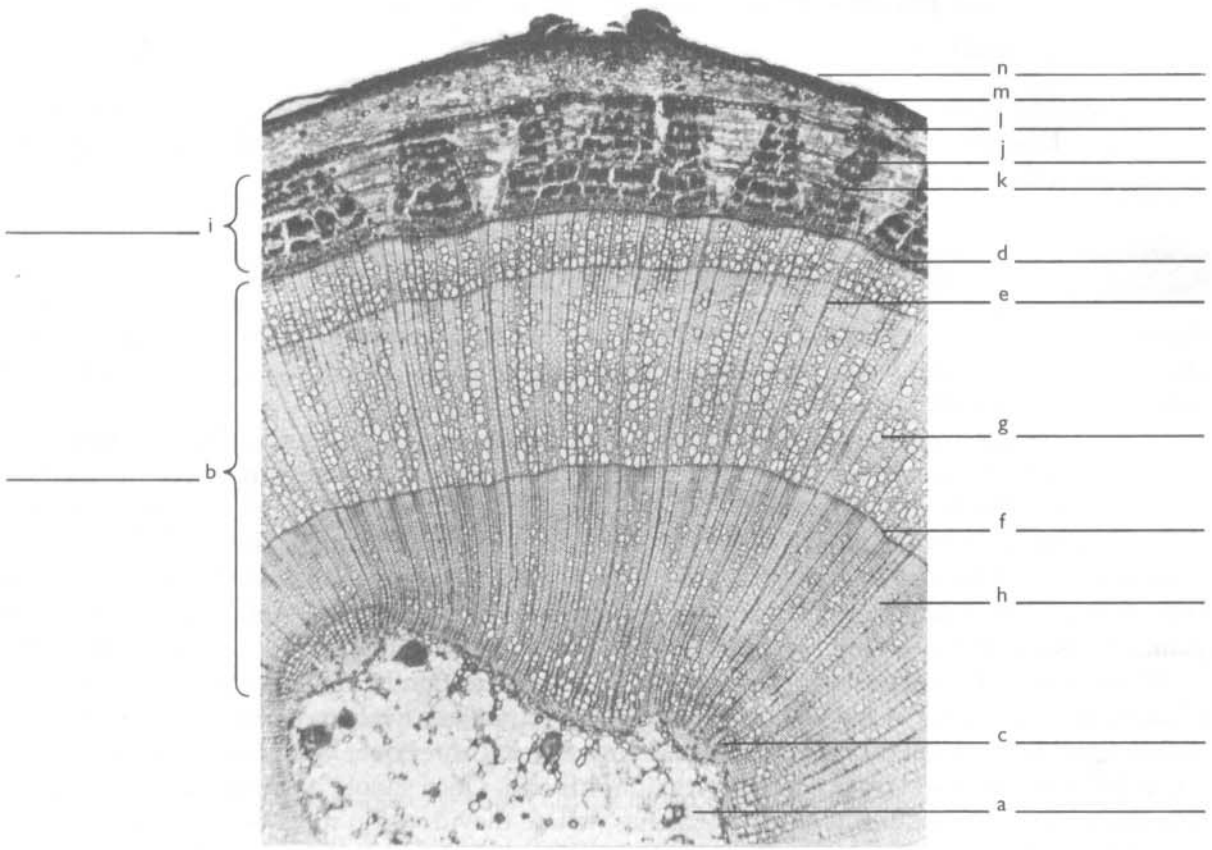


Figure 29F-2 *Cross section of* Tilia *stem.*

a. How old is the basswood section you are examining? _____

b. In the tropics, rainfall and temperature are the same year round. Do you think that trees grown in the tropics would have annual rings? _____

c. Which kind of wood within an annual ring is always *located to the inside?* _____

Why? _____

_____

l **Cortex** Located outside the phloem, the cortex varies in thickness and is somewhat distorted by the pressure exerted by increasing amounts of xylem and phloem tissue. Parenchyma, collenchyma, and fibers are present.

m **Cork** Located just beneath the **epidermis n**. Cork is formed by the cork cambium, which develops in the outer cortex about the time that second-year growth occurs.

Wood All tissues outside the vascular cambium are known as *bark.* Tissues lying inside the cambium are referred to as *wood.* As trees grow older, the older xylem cells lose their ability to conduct water due to the accumulation of resin substances and other metabolic waste products within the cells. This wood is called **heartwood.** The younger conductive wood surrounding the heartwood is called **sapwood.** Heartwood is often darker than sapwood.

2. Examine the tree cross sections on demonstration. Identify bark, cambial region, annual rings, spring wood, heartwood, sapwood, summer wood, rays, and pith.

d. Which structures of woody stems are included in the bark? _____

In wood? _____

3. The wood samples on demonstration have been cut transversely, radially, and tangentially. Determine how each has been cut in relation to growth rings and rays. Note the characteristic wood pattern of various cuts.

EXERCISE G Plant Tissue Culture*

Small pieces of tissue, **explants,** removed from a plant and grown in a specialized medium can give rise to **clones,** new individuals having the same genetic makeup as the parent plant. In this experiment, you will culture explants in vitro to produce clones.

When explants are removed from the parent plant, the excised tissues respond to being "wounded" by stimulating cell division to form a protective layer of wound tissue, sometimes accompanied by the formation of **callus** tissue. Callus tissue is often described as undifferentiated; the cells of explants and those of callus formations undergo "dedifferentiation" during which they become less specialized and more generalized (however, not *all* cells reach the same state of generalization). The "dedifferentiated" cells, with proper stimulation by hormones, particularly auxins and cytokinins, can regenerate entire plants. Cells capable of giving rise to all the structures of a mature plant are said to be **totipotent.**

Cauliflowers, *Brassica oleracea,* a member of the mustard family, can be used to demonstrate plant tissue culture or cloning techniques. The "head" of the cauliflower is composed of a mass of apical meristems within floral buds. The buds can be induced to produce leafy shoots by treatment with a mixture of the auxin **indoleacetic acid** (IAA) and the cytokinin **kinetin.** Once shoots have formed, they are transferred to a second growth medium without hormones, where they develop roots. Rooted shoots can then be placed in soil to propagate the multiple copies or clones of the original parent plant. Development from explant to plant requires 6 weeks.

Procedure

Ideally, this procedure should be carried out under a laminar-flow hood. However, plant tissue culture can be done almost anywhere if simple precautions are taken to maintain aseptic conditions. Choose an area where there is little or no air movement. Clean your work area with a 1% bleach solution. Plan to have all necessary tools nearby and accessible with a minimal amount of movement. *Do not* lean over the work surface (hair is a good source of bacteria). Carry out all manipulations *in front of you.*

1. Break apart or cut cauliflower into 0.5-inch to 1-inch sections.
2. Place three or four pieces in a small jar or Erlenmeyer flask containing 100 ml of tap water and 3 drops of dishwashing detergent (Joy™). Shake periodically for 5 minutes. Drain off the liquid and rinse the flask and the cauliflower well in tap water.
3. Add 50 ml of 70% ethanol and shake for 1 minute.
4. Drain off the ethanol (into a waste beaker) and add 100 ml of 10% bleach. Shake gently for 15 minutes.
5. Drain off the bleach solution. Using sterile forceps that have been dipped in 95% ethanol, flamed, and cooled, transfer the cauliflower pieces to a jar containing 100 ml of sterile distilled water. Swirl for 2 minutes.
6. Carefully drain off the water and, using sterile forceps, transfer the pieces of tissue to a sterile Petri dish.

**Adapted from Janice H. Haldeman and Jane P. Ellis, "Using cauliflower to demonstrate plant tissue culture," The American Biology Teacher, vol. 50, no. 3, March 1988.*

7. Use a sterile scalpel to make slices of the explant tissue (approximately 1 cm by 1 cm and 3 mm in thickness). Make sure that each slice contains bud tissue.
8. Use sterile forceps to transfer four slices of tissue to a Petri dish containing Medium A (Murashige and Skoog Minimal Organic Medium) supplemented with sucrose but without hormones. Seal the edges of the dish with a strip of paraffin film.
9. Place the dish of explants 12 to 15 inches below a fluorescent light source connected to a timer set to provide 16 hours of light and 8 hours of dark. Culturing should take place at room temperature (26 ± 2°C).

Next Laboratory Period

1. Check for dead or contaminated explants: those that appear fuzzy (covered by fungus) or slick (covered by bacteria) should be discarded. Healthy explants will show bud development and some evidence of "greening" (tips may redden due to the production of anthocyanins).
2. Using aseptic technique, transfer healthy explants to a sterile jar containing Medium B (same as Medium A, but supplemented with 2.5 mg/l kinetin and 8.0 mg/l IAA).
3. Return explants to the light source and allow approximately 2 weeks for shoots to develop.

Rooting New Shoots

As shoots form, you will notice some root development. These roots have not formed from bud apical meristem tissue but from the vascular cambium of the non-bud tissue of the explant. These first roots do not serve the newly formed shoots and must be removed so that the shoots themselves can form roots.

1. Use sterile forceps to transfer a shoot cluster to an empty sterile Petri dish.
2. Use a sterile scalpel to cut shoots at the base, separating them so that each shoot includes at least two leaves and a bud. Prepare at least six shoots.
3. Place three shoots in a sterile jar of Medium A, gently pushing the end of the shoot into the medium so that the stem stands up vertically.
4. Return the explants to the same light source. Root development will take place in approximately 1 week.

Transplanting

When roots (three to four) ranging from 0.5 to 1.5 cm in length have developed, the "clones" can be transferred to soil. Aseptic technique is no longer critical.

1. Carefully remove rooted shoots from the Medium A jars and rinse with tap water to remove all traces of Medium A.
2. Obtain a pot of soil. Make a hole in the soil with your pencil and place the plant into the hole (the roots and approximately one-third of the stem should be buried). Press the soil firmly around the base of the stem.
3. Water thoroughly and place a plastic bag over the pot to retain moisture. Return the plants to the light source or place them on a windowsill in indirect light.

Alternative Procedure

Alternatively, your instructor may ask you to culture tobacco leaf disks. In this case, swab a tobacco leaf with 95% ethanol and place it in a sterile Petri dish containing a 20% solution of bleach. After 10 minutes rinse the leaf three times with sterile distilled water and use a sterile cork borer (#4) to cut leaf disks from the tissue between veins. Transfer the leaf disks aseptically to flasks of sterile nutrient medium and gently tap them down onto the surface of the medium using a sterile, round-end glass rod. Place the covered flasks under the same light source used for the cauliflower explants. A callus will form within 3 to 4 weeks, followed by development of roots and shoots (4 to 8 weeks) plus buds and flowers (8 to 12 weeks).

Laboratory Review Questions and Problems

1. Strictly speaking, what is a fruit?

2. Why is a peanut actually a fruit rather than a nut?

3. Distinguish between simple, multiple, and aggregate fruits.

4. Distinguish between fleshy and dry fruits.

5. Identify the following fruits as simple, multiple, or aggregate. For simple fruits, use the dichotomous key in Table 29A-1 to further classify the fruit type.

Fruit	Simple, Multiple, or Aggregate	Type of Simple Fruit
Plum		
Strawberry		
Peanut		
Pineapple		
Banana		
Fig		
Tomato		
Coconut		
Orange		

6. When parents tell children to "eat their vegetables," what do they actually mean? What is a vegetable?

7. For the following "vegetables," identify the parts we consume: roots, stems, leaves, flowers, or fruits. Review Laboratory 28 and refer to your textbook for assistance.

"Vegetable"	Plant Part Eaten	"Vegetable"	Plant Part Eaten
Carrot		Spinach	
Irish potato		Squash	
Sweet potato		Lettuce	
Celery		Onion	
Broccoli		Corn	

8. Fill in the following table to summarize some characteristics of monocots and dicots.

		Monocot	Dicot
SEED	Number of cotyledons		
	Presence of plumule		
	Presence of coleoptile		
	Presence of coleorhiza		
STEM	Presence of vascular cambium (lateral meristem)		
	Presence of primary meristem		

9. Distinguish between each of the following:
Ovary, carpel

Fruit, ovary

Seed, ovule

Indehiscent, dehiscent

Hypocotyl, epicotyl

Monocot, dicot

Branch roots, adventitious roots

Primary meristem, secondary meristem

Wood, secondary xylem

Spring wood, summer wood

Heartwood, sapwood

Bark, cork

10. Which tissues compose the bark of a tree? Why does "girdling," cutting completely through the bark around the circumference of the trunk, kill a tree?