HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 10 DECEMBER, 1936 NUMBER 11

CONTENTS

UNIVERSITY OF CALIFORNIA • BERKELEY, CALIFORNIA

HILGARDIA · VOLUME 10, NUMBER 11
DECEMBER, 1936

DEVELOPMENT OF THE FLOWER AND MACRO-GAMETOPHYTE OF ALLIUM CEPA

H. A. JONES AND S. L. EMSWELLER

ONTOGENY AND STRUCTURE OF COLLENCHYMA AND OF VASCULAR TISSUES IN CELERY PETIOLES

KATHERINE ESAU

VESSEL DEVELOPMENT IN CELERY

KATHERINE ESAU

ONTOGENY AND STRUCTURE OF COLLENCHYMA AND OF VASCULAR TISSUES IN CELERY PETIOLES

KATHERINE ESAU

ONTOGENY AND STRUCTURE OF COLLENCHYMA AND OF VASCULAR TISSUES IN CELERY PETIOLES[1]

KATHERINE ESAU[2]

INTRODUCTION

EFFORTS TO OBTAIN STRINGLESS varieties of celery (*Apium graveolens* L.) drew attention to the nature of the so-called celery strings. The two structures that constitute these strings, the vascular bundles and the collenchyma strands, differ greatly from each other in their development, their histology, and their physical properties. This paper deals with the ontogeny and structure of the tissues that make up the strings and gives some information regarding their relative strength.

The present work treats of the histological part of the problem in considerable detail and adds to our knowledge of histogenesis and tissue differentiation. It compares the mode of origin of collenchyma with that of the vascular tissue and describes in detail the phloem and its transformation, in the final stages, into the collenchymatous bundle cap.

MATERIAL AND METHODS

In preparing the material for histological study, the procedure was as follows. Pieces of petioles were killed and fixed for 48 hours in Karpechenko's chrom-acetic-formalin solution (Rawlins, 1933,[3] p. 13). The fixed material, washed in three changes of pure dehydrated dioxan (Graupner and Weissberger, 1931), was placed in a paraffin oven in a mixture of dioxan and paraffin. The latter was changed four times to expel the dioxan. Three to four days after placement in the oven the material was embedded in paraffin.

Without being soaked in water, the embedded material was cut 10 microns thick on a rotary microtome. Instead of a microtome knife, Gillette razor blades, clamped into a Spencer razor-blade holder, were used. Very little difficulty was experienced in cutting the material, even the collenchyma and xylem of the oldest petioles. The protoplasts, however, commonly shrank throughout the material, though no attempt was made to determine at what stage of the process this shrinkage occurred.

For staining, Heidenhain's haematoxylin was used in most cases,

[1] Received for publication September 1, 1936.

[2] Junior Botanist in the Experiment Station.

[3] See "Literature Cited" at the end of the paper for complete data on citations, which are referred to in the text by author and date of publication.

although the combination safranin-anilin blue was also resorted to. The haematoxylin stain was prepared according to Hance (1933). The anilin blue, used in a weak solution in 90 per cent alcohol, was applied to sections previously stained in safranin. It rapidly stains the cytoplasm and walls of cellulose and gives a good contrast with safranin, which is retained by the lignified walls, the nucleoli, and the slime of sieve tubes.

Preparation of the material for comparing the relative strength of collenchyma and of the vascular bundles is mentioned in connection with the method of measuring this strength.

The material used for the illustrations was taken from three celery plants, each of a different variety. Figure 1, *A* to *C* was drawn from sections through the growing point and the youngest leaves of the Utah variety. Figures 1, *D* to *I*, figures 2–5, and plates 1–8 were taken from the Golden Plume plant. Table 3 gives the origin of the material for figures 7 and 8.

GROSS ANATOMY OF PETIOLES

Celery petioles have crescent shapes in transverse sections, with prominent ribs on the abaxial (dorsal) side (plate 1, *A*). A large collenchyma strand is present in each rib under the epidermis (plate 2). No collenchyma strands occur on the adaxial (ventral) side, but here the two to three subepidermal layers of cells are collenchymatously thickened (plate 2). The two lateral points of the crescent are also prominently reinforced by collenchyma.

The main vascular system of the petiole is distributed on its abaxial side and is composed of vascular strands of various sizes (plates 1 and 2). Each collenchyma strand lies on the same radius with one of the larger bundles; the smaller bundles have no collenchyma strands opposing them (plate 2). A row of very small vascular bundles occurs on the adaxial side (plates 1 and 2). The vascular strands are embedded in large-cell parenchyma with intercellular spaces. In pithy plants this parenchyma breaks down in certain regions, leaving prominent internal cavities (plate 2).

Oil ducts are a conspicuous feature of the celery petioles. There is an oil duct subjacent to each collenchyma strand (plates 2; 3; 4, *A*; and 6); numerous others occur in the parenchyma surrounding the vascular bundles (plates 1, *B;* and 3). Oil ducts also differentiate in the metaphloem (plate 5).

The collenchyma strands are more uniform in size and are fewer than the vascular bundles. The largest bundles exceed the collenchyma strands in diameter (plate 2).

The large vascular bundles are collateral; and the xylem occurs on the adaxial, the phloem on the abaxial side of the bundles (plate 2). The xylem, roughly triangular in cross-sectional outline, is covered dorsally and laterally by a crescent-shaped structure that includes the phloem and the bundle cap (plates 2 and 5). In mature bundles the xylem is

TABLE 1

CERTAIN GROSS MORPHOLOGICAL CHARACTERISTICS OF CELERY PETIOLES

Variety	Leaf No.	Length of petiole, cm	Area of median cross section, cm^2	Collenchyma		Vascular bundle		
				Number of strands	Av. size of strand, mm^2	Number of bundles	Av. size of bundle, mm^2	Av. size of bundle cap, mm^2
Golden Plume	11	7	3.1	..	.....	..	.....	.....
	12	9	3.1	22	0.273	33	0.648	0.396
	14	10	3.0	..	.....	..	.....	.....
	15	10	3.2	..	.....	..	.....	.....
	16	11	...	16	0.514	23	0.905	0.525
	17	17	3.3	..	.....	..	.....	.....
	18	12	3.1	..	.....	..	.....	.....
	21	14	3.2	..	.....	..	.....	.....
	24	15	...	13	0.663	17	0.862	0.500
	26	15	...	13	0.503	13	0.873	0.506
Tall Golden Self-blanching	10	14	0.9	..	.....	..	.....	.....
	12	15	...	15	0.135	17	0.364	0.225
	13	20	0.9	..	.....	..	.....	.....
	15	20	1.0	..	.....	..	.....	.....
	16	24	...	15	0.163	16	0.460	0.236
	17	22	0.9	..	.....	..	.....	.....
	23	15	...	12	0.193	13	0.474	0.276
	24	18	...	12	0.258	14	0.636	0.332
	25	18	1.2	..	.....	..	.....	.....
	26	16	...	12	0.242	13	0.605	0.336
	27	15	1.2	..	.....	..	.....	.....
Utah	9	9	1.9	15	0.176	16	0.556	0.352
	14	20	1.9	14	0.248	17	0.718	0.438
	15	24	1.9	..	.....	..	.....	.....
	16	21	2.4	16	0.258	17	0.744	0.479
	17	24	1.9	..	.....	..	.....	.....
	18	23	1.9	..	.....	..	.....	.....
	22	21	2.2	..	.....	..	.....	.....
	23	19	1.8	15	0.257	15	0.809	0.500

separated by cambium from the functioning phloem (plate 5). The latter is a small-celled tissue, which, on the outside, merges with the tissue of the bundle cap.

Some of the small bundles, especially those on the adaxial side, consist of phloem only. Solitary xylem vessels without the accompanying phloem also occur. If xylem and phloem are both present in the adaxial bundles, their relative position is reversed as compared with that in the

abaxial bundles; that is, the xylem lies on the abaxial, the phloem on the adaxial side of the bundle.

The collenchyma and the large vascular bundles occur as continuous parallel strands extending from the base of the petiole to the place where the lamina is inserted. The vascular strands may have a few anastomoses connecting them laterally with each other. Both the collenchyma and the vascular strands have a larger diameter at the base than at the apex. From the base up they decrease rather rapidly in thickness and then remain fairly uniform to the apex of the petiole.

Table 1 shows certain size relations of parts of celery petioles in three different plants. The area of the petiole was measured in its median cross section. The areas of the strands of collenchyma and of the vascular tissues represent averages of basal and apical areas of all strands of a given cross section of the petiole, except of the smallest vascular strands, which had no corresponding collenchyma strand and were not measured. The number of vascular bundles represents the sum of abaxial bundles, including the smallest. The number of collenchyma strands is the sum of abaxial strands, except the collenchyma reinforcing the two marginal ribs.

The areas of the vascular bundles and of the collenchyma strands were drawn from their transverse sections by means of a camera lucida at a magnification of 44 diameters and were then measured with a planimeter.

The number of each leaf indicates its relative distance from the growing point. In counting, a leaf 0.5 cm in length, located near the growing point, was given the number 1, so that the highest number indicates the oldest leaf in each plant.

As table 1 shows, the first leaves on the plant tend to have fewer vascular bundles and fewer collenchyma strands than those formed later. The vascular strands average considerably larger in diameter than the corresponding collenchyma strands.

Plates 1 and 2 show size relations of parts of petioles in three stages of development. These photographs, all of the same magnification, were taken at the base of petioles of leaves 1, 7, and 13 of the Golden Plume plant.

HISTOGENESIS OF PETIOLES

A leaf primordium has a very broad base and a narrow apex, which soon becomes lobed and gives rise to the pinnate leaf blade. The base develops into a petiole through elongation, tissue differentiation, and maturation.

Using Schüepp's (1926, p. 4) general classification of meristems, we may distinguish three kinds of meristematic tissue in the primordial

petiole: the protoderm, the procambium, and the ground meristem. In this terminology, procambium is the meristem that gives rise to the primary xylem and phloem and the vascular cambium; the protoderm differentiates into the epidermis; the ground meristem into the pith and cortex.

The three meristems may be distinguished on the basis of size and degree of vacuolation of their cells. The ground-meristem cells are comparatively large, particularly on the adaxial side of the petiole; and they are the first cells to develop conspicuous vacuoles and intercellular spaces. The protoderm has small cells, somewhat more dense than those of the ground meristem. The least vacuolate and the smallest in cross section are the procambium cells, which are also distinguished by being longer than broad. Cell-division figures are more abundant in the protoderm and in the procambium than in the ground meristem.

As seen in transverse section, a petiole in the primordial state is crescent-shaped, like the more mature one. Considering a cross section through the thickest part of the petiole of a leaf approximately 250 microns high, we find, beginning with the adaxial side (fig. 1, *A*, beginning below), the following layers: (1) the adaxial protoderm, consisting of one layer of cells; (2) several layers of comparatively large cells of the ground meristem (the adaxial meristem of Foster, 1935*a*); (3) the procambial region with small-cell procambium strands and slightly larger embryonic cells intervening between these strands; (4) two to three layers of abaxial cortical ground meristem; and (5) one layer of abaxial protoderm.

The cells of the protoderm divide only anticlinally. In the ground meristem two types of divisions occur—periclinal and anticlinal. In the procambium, periclinal divisions generally predominate; but anticlinal walls are also formed.

The procambial tissue of a leaf is initiated in the ground meristem (see also Foster, 1935*a*, p. 116) of the primordium through a rapid succession of periclinal longitudinal divisions. The procambium strands of the larger vascular bundles arise earlier than those of the smaller bundles. The small abaxial bundles and all the adaxial bundles are initiated in those regions of the ground meristem where vacuolation is conspicuous. Foster describes a similar late initiation of procambium in the lateral strands of the cataphylls of *Carya* (1935*a*, p. 105) and in the cortical bundles of transitional leaf forms of the same plant (1935*b*, p. 172).

In the Umbelliferae, according to Ambronn (1881), collenchyma differentiates in close connection with the vascular bundles; in fact,

Ambronn states that they both arise from the same procambium strand; but through differentiation of a layer of parenchyma within the procambium strand the latter becomes separated into two parts—the outer, giving rise to collenchyma, and the inner, forming the vascular tissue.

The histogenesis of celery petioles deviates from the scheme given by Ambronn for the Umbelliferae. As was mentioned earlier, approximately three layers of cells separate the procambium strand from the protoderm during the first stages of tissue specialization (fig. 1, *A*). Later very active periclinal divisions occur on the abaxial side of the procambium strand, the resulting cells becoming arranged in radial rows. Such cells are shown in figure 1, *B* between the procambium (*pr*) and the oil duct (*od*). In view of the absence of a clear demarcation between the procambial and the ground-meristem cells on the periphery of the procambium strand, it is difficult to determine accurately whether these cells are derived from the outer procambium cells or from the ground meristem. Through the appearance of anticlinal walls in later stages of development the original radial arrangement of the cells is lost (fig. 1, *E*).

Immediately outside this active region an oil duct differentiates before any xylem or phloem elements are in evidence in the petiole (fig. 1, *B*). The oil ducts arise as schizogenous intercellular spaces, but may be distinguished from ordinary intercellular spaces by their rather large size and the characteristic divisions of cells lining the space.

During the developmental processes just described, important changes are initiated in the layers located between the oil duct and the protoderm. Although the ground-meristem cells of this region early manifest vacuolation and development of intercellular spaces, they undergo sporadic divisions (fig. 1, *A* and *B*). After the appearance of the oil duct, divisions become increasingly numerous (figs. 1, *C* and 5, *A*); and, in consequence, a strand of elongated dense cells, similar to procambial cells, is formed. This strand increases in size and subsequently differentiates into collenchyma.

Through the rapid division of cells on the abaxial side of the procambium strand, and the divisions leading to the formation of collenchyma, the abaxial portion of the petiole lying on the same radius with the procambium strand becomes elevated in the form of a rib. The ground-meristem cells between the ribs adjust themselves to the active abaxial elevation by increase in size and by a few divisions.

Developmental processes occurring on the abaxial side of large bundles do not take place near those small bundles which are not associated with collenchyma strands (fig. 1, *C*, right).

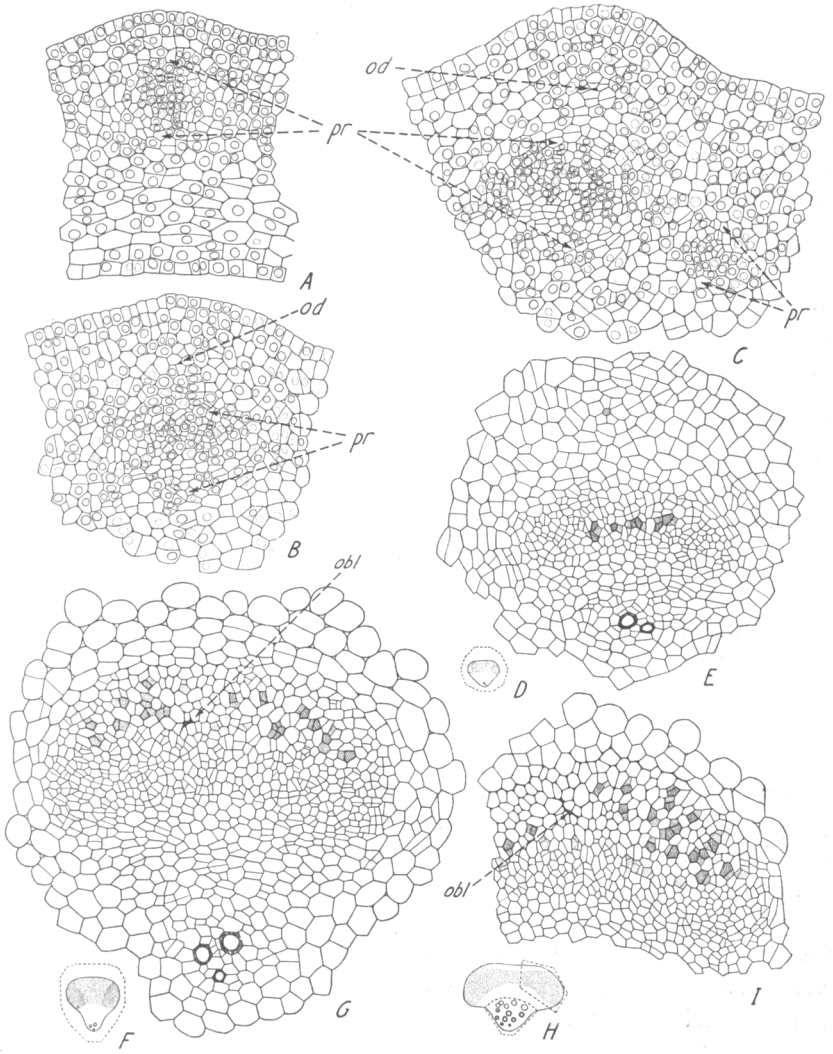

Fig. 1.—*A–C*, Transverse sections through parts of petioles showing differentiation of procambium strand (× 184); *E, G, I*, early differentiation of vascular tissues in transverse sections (× 184); *D, F, H*, diagrams of entire bundles from which drawings *E, G, I* were made (× 38); *obl*, obliteration; *od*, oil duct; *pr*, procambium.

The description given above shows that, in contrast to the Umbelliferae studied by Ambronn (1881), celery is characterized by the independent origin of collenchyma and vascular bundles within the petioles. The early differentiation of the oil duct facilitates recognition of regions and clearly limits the primordial collenchyma to the subepidermal layers of the ground meristem.

Although Ambronn (1881) considers that in the Umbelliferae the collenchyma and the vascular tissue arise from the same procambium strand, in most other dicotyledons he found these two kinds of tissues to arise independently of each other. None of the plants studied by Wisselingh (1882) offered an example of common origin of collenchyma and vascular tissue. The Umbelliferae, however, were not included in Wisselingh's material.

ONTOGENY AND STRUCTURE OF THE VASCULAR BUNDLES

Procambium and Cambium.—In the formation of the procambium in petioles, longitudinal divisions in certain regions of the ground meristem occur in rapid succession, forming strands of elongated cells with small transverse diameters. These divisions are periclinal and anticlinal; but as the anticlinal walls tend to be formed perpendicularly to the periclinal ones, the procambial cells show rather regular radial arrangement (fig. 1, *A*). In the phloem region of the procambium strand this arrangement is soon destroyed by the appearance of the less regularly placed anticlinal walls (fig. 1, *C*, *E*, and *G*). In the xylem region the radial arrangement is lost after the vessels, enlarging, induce divisions and spatial adjustments in the xylem parenchyma (fig. 1, *E* and *G*; plates 3 and 4). In the region of the strand where cambium is to differentiate, the radial rows of cells remain in evidence because periclinal divisions predominate here.

In longitudinal sections the procambium cells are longer than broad because the periclinal divisions are not immediately followed by transverse divisions. Cells, moreover, do not enlarge much between successive longitudinal divisions.

At first the procambium cells are no longer than the adjacent ground-meristem cells, but are much narrower. In a Golden Plume petiole 2.5 cm long, with the first xylem and phloem elements mature, parenchyma cells were approximately 35 microns long and 25 microns wide; procambium cells 40 $\times$ 6 microns large. When the procambium is changing into cambium, ground-parenchyma cells are broader than high. They measured, in the Golden Plume plant, 40 $\times$ 50 microns; the corresponding procambium cells 60 $\times$ 8 microns.

The division of the procambium cells between the xylem and phloem causes a steady enlargement of the procambium strand, particularly in radial extent. At the same time, new cells are added to the strand on its periphery. In this process the ground-meristem cells surrounding the procambium stretch somewhat, perpendicularly to the periphery of the strand, then divide by a wall that is periclinal with respect to the strand. Thus shell-like layers are formed around the young procambium strand (fig. 1, *C*, left). Cells of the layers nearest the strand then divide by anticlinal walls and become part of the procambium itself.

Differentiation of primary vascular tissues begins long before the procambium strand reaches its full size. The addition of cells on the periphery of the procambium ceases, however, in regions opposite (in radial direction) the mature elements, but continues right and left from these (fig. 1, *E*). This lateral growth is very active, so that the bundle increases in width tangentially, particularly in the phloem region (fig. 1, *G*). As more phloem elements mature, left and right from the first ones, the addition of new procambium cells becomes limited more and more to the extreme margins of the two lobes of phloem. The phloem, in consequence, assumes its characteristic crescent shape (fig. 1, *G* and *H*). Plate 3 shows a bundle in which addition of new procambium cells has not yet ceased.

The first phloem and xylem elements differentiate rather close to each other (fig. 1, *E*) ; but continued division of procambial cells in the center of the bundle increases the distance between them (fig. 1, *G* and plates 3 and 4). The procambium cells formed centrifugally from the xylem differentiate into new elements of this tissue, while new phloem cells arise on the opposite side of the bundle. Since, however, the differentiation of elements progresses faster than the division of procambium cells, the xylem and phloem gradually approach each other (figs. 1, *D, F, H,* and 2, *B, C.*)

As the layer of dividing procambium cells narrows down to a few rows of cells (plates 3 and 4), the meristem shows an increasing similarity to the cambium of herbaceous dicotyledons. Eventually cells appear with short radial diameters and become arranged, in longitudinal sections, in horizontal tiers. The longitudinal divisions are predominantly periclinal, and the resulting cells retain a radial arrangement in the mature state.

These characteristics, however, appear in the meristem even before the petiole ceases to elongate, so that the vascular tissues produced by this meristem are morphologically primary tissues. In bundles like that shown in plate 4, for example, mature sieve tubes and vessels are longer

than their mother cells; and the vessels have spiral secondary thickenings.

Because of the terminology generally adopted in modern botanical texts, the meristem lying between the xylem and phloem is here called procambium as long as it forms primary vascular tissues. Thus bundles in plates 3 and 4, which were taken from elongating petioles, have no cambium yet.

When petioles cease to elongate, continued tangential divisions in the procambium give rise to the metaxylem and metaphloem. In their mature state the cells of these tissues show a definite radial arrangement.

Vascular bundles with primary tissues complete are shown in plates 5 and 7. Although the meristem between the xylem and phloem in these bundles has differentiated into the cambium the latter had not produced any tissues at this stage. In general no secondary tissues were present in petioles used in this study.

The early ontogeny of the vascular bundles in celery does not fully agree with the generalized description of the differentiation of primary vascular tissues given by Eames and MacDaniels (1925, p. 87). These authors state that at the beginning of differentiation of vascular tissues "the portions of procambium which form the first xylem and those which form the first phloem are situated well apart," and their figures 42 (p. 84) and 44 (p. 87) indicate that the procambium reaches its full radial extent before the first xylem and phloem differentiate. Eames and MacDaniels give no details about the course of events in the median region of the procambium strand; they merely remark on page 130 that "just before the formation of such a sheet of tissue (cambium) there is a transitional period during which cell division is taking place in various planes in the central procambial zone, but tending in later stages of development to occur more and more in the tangential plane only."

In celery the mode of development of the primary vascular bundles resembles that given by de Bary (1884, pp. 389–390) for "thick, and especially collateral bundles." In such bundles the longitudinal divisions that initiate them in primordial organs "often still go on for a long time on the boundary between phloem and xylem, when at the edges of the bundle the differentiation of the tissues is already completed."

Phloem as a Whole.—The development of this tissue is shown in transverse sections in figures 1, *D* to 2, *H*. The small diagrams of entire bundles accompanying the phloem sections show the relative size of the bundles from which the phloem was drawn. Quadrangles drawn in interrupted lines in the diagrams indicate the position of sections that

were drawn in detail. Some drawings and photomicrographs were made of the same bundles. Thus figure 2, *C* and *D* corresponds to plate 3; 2, *E* and *F* to plate 4; 2, *G* and *H* to plate 5.

The first protophloem sieve tubes differentiate in the upper median region of the procambium strand (fig. 1, *E*). Each sieve tube has one companion cell. The subsequent sieve tubes arise to the right and left of the first sieve tubes and also in the centrifugal direction from these. Figure 1, *G* shows this stage of development. At *obl* are some of the first sieve tubes in a crushed condition. Functioning sieve tubes are indicated by hatching and are also distinguished by their comparatively thick walls. The companion cells are brought out by dense stippling. Light stippling in certain other cells marks immature sieve tubes and companion cells.

Differentiation right and left from the obliterated elements becomes increasingly prominent (fig. 1, *H*). Then sieve tubes begin to arise centripetally from those formed earlier, while the older ones are continually crushed and obliterated (figs. 1, *I* and 2, *A* and *D*).

Phloem tissue transitional between the protophloem and the metaphloem is depicted in figure 2, *E*. The origin of this tissue from a regularly dividing meristem has become rather conspicuous. Oil ducts, which already have appeared at the stage shown in figure 2, *A*, are a regular feature of the phloem in 2, *E*.

Figure 2, *G* shows part of a bundle in which the functioning phloem is composed of metaphloem only. The sieve tubes of the protophloem have been almost completely obliterated. In the protophloem, parenchyma constitutes a prominent part of the tissue, whereas in the metaphloem, sieve tubes and companion cells predominate.

In the small bundles that have no xylem associated with the phloem (see p. 433) the first protophloem elements differentiate in the center of the procambium strand, while new elements appear uniformly in all directions from the intial ones. If any cambium-like layer is developed in these bundles, it completely surrounds the phloem and cuts off cells only toward the phloem, not toward the outside.

The sieve tubes of proto- and metaphloem, though alike in structure, differ in size. As shown by a glance at the phloem in figures 1 and 2, sieve tubes become progressively larger in their transverse diameters, so that the last metaphloem sieve tubes are the largest. Their elements are also longer than those of the protophloem sieve tubes.

The sieve tubes usually have only one companion cell when viewed in cross section, but in longitudinal sections they may have one or two companion cells (fig. 3, *C*, *D*, *F*, and *G*). In forming protophloem sieve

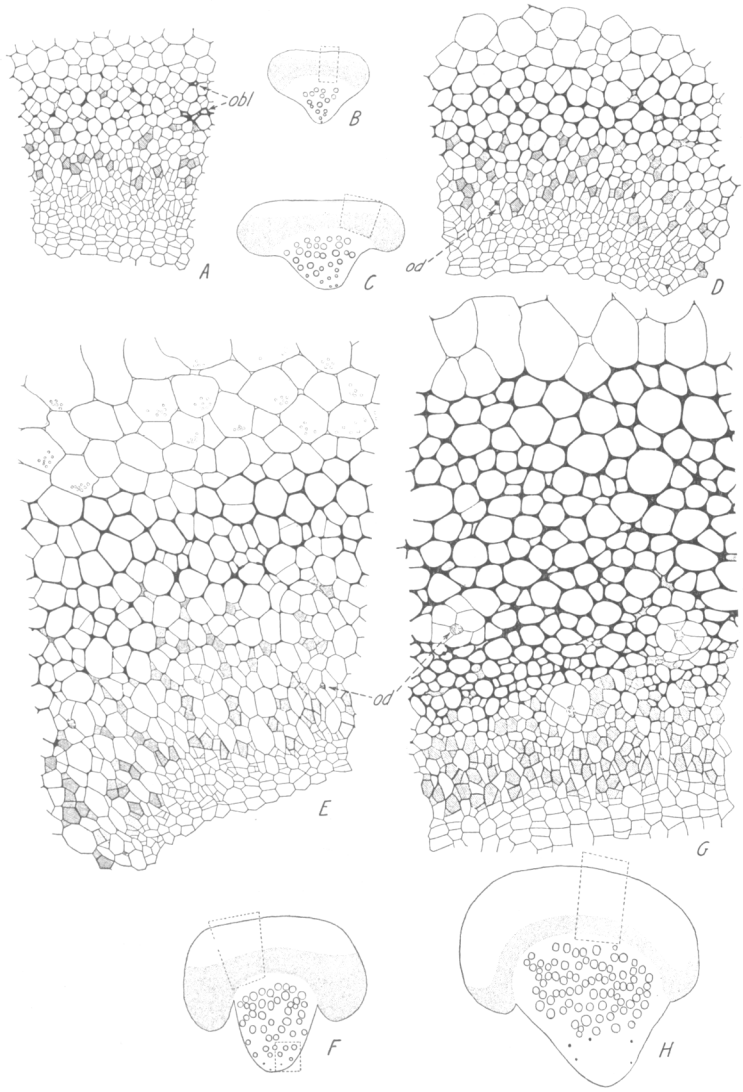

Fig. 2.—*A, D, E, G,* Transverse sections through parts of vascular bundles showing development of phloem (×158); *B, C, F, H,* diagrams of entire vascular bundles from which drawings *A, B, E,* and *G* were made (×34); *obl,* obliteration; *od,* oil duct.

tubes a procambium cell divides several times, the second-before-last division giving rise to a phloem parenchyma cell and a sieve-tube-companion mother cell. The latter then divides longitudinally to form a sieve-tube element and a sister cell, which may give rise to one companion cell or to more than one by dividing transversely.

The relation among sieve tubes, parenchyma, and companion cells is clearly shown in figure 3, *H* and *I*, where the groups of cells derived from one procambium cell are still clearly outlined. The sieve tubes are indicated by *st*, and the companions are the smallest cells of each group. To the left below in 3, *H*, are two phloem parenchyma cells derived from one procambium cell. Similar groupings may be found in figure 3, *M*.

In the metaphloem (fig. 3, *L*) some parenchyma cells develop from a procambium cell without division. More frequently the phloem mother cell divides by a longitudinal wall, one of the resulting cells becoming a parenchyma, the other a sieve-tube-companion mother cell; or both derivatives of the phloem mother cell give rise to sieve tubes with their companions.

In the division of the sieve-tube-companion mother cell, one of the daughter cells is larger than the other. This is the young sieve tube (fig. 3, *C*, *H*, *I*, and *M*). Besides being smaller, the companion cells are also distinguished, in a mature state, by their dense protoplasts and prominent nuclei (fig. 3, *I*, left, and *M*, above).

Sieve Tubes.—The ontogeny of the sieve tube follows a course similar to that of a sieve tube in the sugar beet (Esau, 1934). Figure 3, *C–F* illustrates four stages in sieve-tube development. The element in *D* shows increase in size and vacuolation in comparison with that in *C*. There is also a slime body above the nucleus and disk-like plastids in the cytoplasm.

The sieve-tube plastids of celery, though less prominent structures than those of the beet, are similar in shape. Plastids of celery sieve tubes, like those of many other plants, stain red with iodine.

In figure 3, *E* the walls of the sieve-tube element are thickened, vacuolation is very prominent, and the nucleus and the slime body (below the nucleus) are disintegrating. Figure 3, *G* depicts a similar stage.

A mature element is shown in figure 3, *F*. It contains a thin parietal layer of cytoplasm enclosing a large central vacuole. The plastids are included in the cytoplasm. The end walls have the characteristic sieve plates with very fine perforations. Slime accumulations are not prominent, being present only on the lower sides of the plates.

The end walls of sieve-tube elements are transverse or slightly oblique. Only one sieve plate is present on the end wall, but the longitudinal

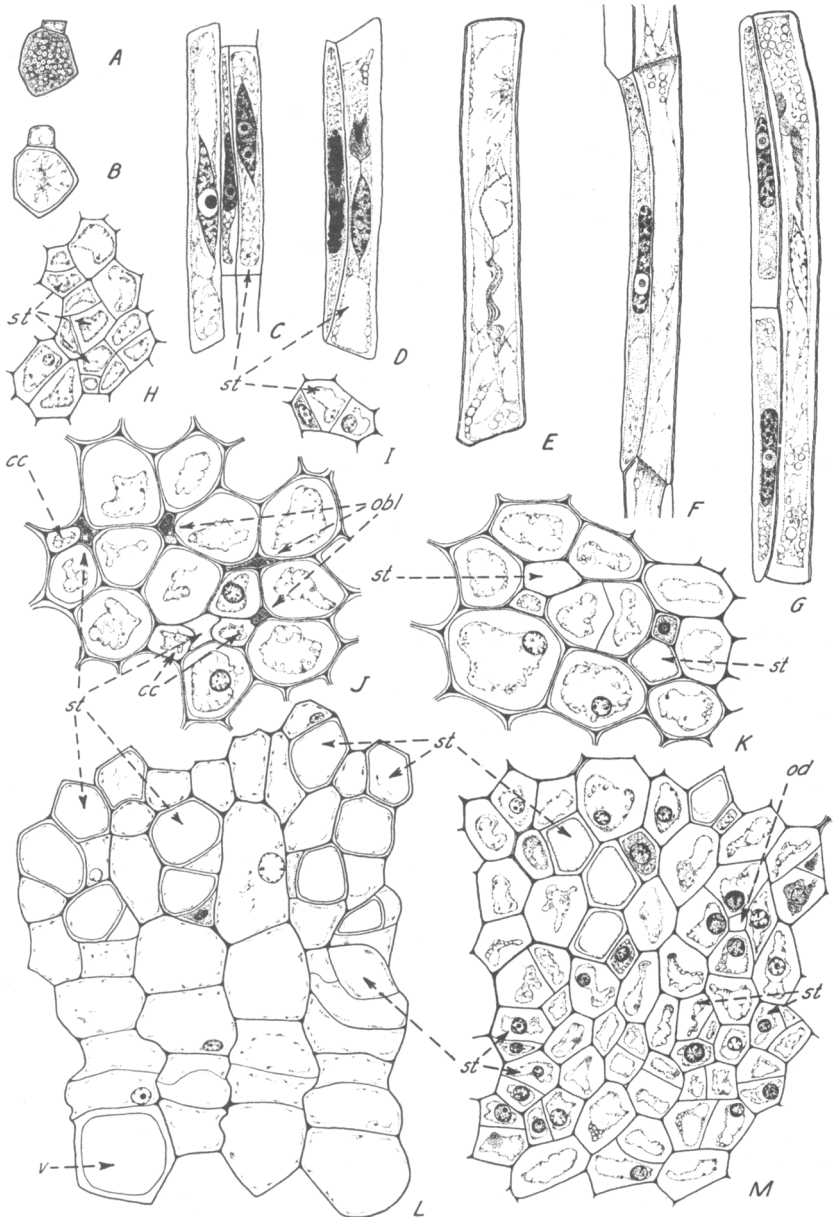

Fig. 3.—Details of phloem structure: *A, B,* two transverse sections of one sieve-tube element with a sieve plate at *A*; *C–G,* different stages of sieve-tube differentiation shown in longitudinal sections; *H–M,* different stages of phloem development shown in transverse sections; *cc,* companion cell; *obl,* obliteration; *od,* oil duct; *st,* sieve tube; *v,* vessel. (× 700).

walls have sieve fields. Figure 3, *A* shows a sieve plate with light-colored callus cylinders and black-stained cytoplasmic strands passing through the center of these cylinders. Figure 3, *B* depicts another transverse section through the same sieve-tube element as in 3, *A*, but at a slightly higher level. Here the thickening of the longitudinal wall is visible, and also the slime. The companion cell appears above the sieve tube in *A* and *B*.

Although the celery sieve tubes are rather small and do not represent favorable material for the study of such elements, some information was gathered on the behavior of walls in mature versus old sieve tubes in prepared sections. In early stages of differentiation the sieve tube develops thickenings on longitudinal walls (fig. 3, *E*). By the time the nucleus disappears and the end walls show sieve-plate perforations, this deposition of wall material is very marked; usually it appears to be of uniform thickness on all longitudinal walls (fig. 3, *L* and *M*). The wall next to the companion cell may or may not be thickened. When the sieve tube begins to approach senility the wall thickening disappears, so that a functionless element has no thicker walls than one just emerging from the mother-cell stage.

Figure 3, *K* shows, at the right, a functioning sieve tube with a thick wall; at the left, a functionless element with a thin wall. Figure 2 also brings out the difference in thickness of young and old sieve tubes.

Crushing of the sieve tube ensues after the loss of the thick wall (fig. 3, *J*, below). This crushing, accomplished by growth and expansion of adjacent parenchyma cells, continues until the lumen of the sieve tube is entirely closed. Definitive callus may be recognized on sieve plates of elements being obliterated. Companion cells also are crushed, although sometimes considerably later than the sieve tubes. Figure 3, *J* shows several stages in obliteration of sieve tubes and companion cells. Figure 2, *G* illustrates the same phenomenon at a lower magnification.

Besides the peculiarity of wall structure, the sieve tubes are distinguished by the behavior of their protoplasts in prepared sections. In young sieve-tube elements still containing nuclei, the cytoplasm shrinks as much as that of any other cell that was living at the time of sampling (fig. 3, *M*). In mature functioning elements, however, the cytoplasm fails to shrink and adheres closely to the walls (fig. 3, *L* and *M*, above).

A similar differential shrinkage of protoplasts in young and old sieve tubes was observed by Strasburger (1891, p. 194) in *Robinia pseudoacacia* upon treatment of the material with alcohol. Crafts (1933) found that the protoplasts of mature sieve tubes of potato phloem, in contrast to those of the young elements, cannot be plasmolyzed with

hypertonic sucrose solutions. The lack of shrinkage of sieve-tube protoplasts in prepared sections and the loss of susceptibility to plasmolytic agents in fresh sections is, perhaps, traceable to the same property of mature sieve-tube cytoplasm—its complete permeability.

Oil Ducts.—Among the elements of metaphloem, oil ducts are a prominent part, but they begin to develop in the protophloem (fig. 2, *A*). Gradually these structures gain in prominence (fig. 2, *D–G*). They are very conspicuous in the phloem in plate 5.

Oil ducts of the phloem, as well as those of the cortex, develop as schizogenous intercellular spaces. Figure 4, *E* and *F* shows two stages in phloem oil-duct development. In *E* the intercellular space has just made its appearance, whereas in *F* it has grown to a large cavity.

In the formation of an oil duct in the cortex, cells undergo several divisions. As a result, the mature duct is lined with many cells, whereas there are usually only three cells in contact with the cavity when the duct is initiated. (Compare oil ducts in figures 1, *C* and *E;* and 5, *A–D*). In the phloem, a similar process may take place; but frequently the intercellular space increases in size so rapidly that contact of the duct with new cells is brought about by separation of pairs of cells to their common line of union with a third cell (fig. 4, *E* and *F*).

Lining the oil duct are active cells elaborating an ethereal oil that later accumulates in the duct. These cells have prominent nuclei (figs. 3, *M* and 4, *I*) and thin walls (fig. 4, *F;* plate 5). Contrary to Sayre's statement (1929), no lignification was observed in them.

The product of the oil ducts often appears as coarsely coagulated material within the cavity. Frequently the contents of the duct stain densely (plates 4, *A* and 5).

In the sections examined the oil ducts, as far as visible, were long, continuous structures. In longitudinal view the cells lining the cavity are arranged end to end, as shown for a cortical duct in figure 4, *I*. Here the two rows of active cells to the right and left of the duct are conspicuous by virtue of their dense cytoplasm.

Bundle Cap.—The caps of the vascular bundles in celery arise in a manner similar to that of the bundle cap in sugar-beet leaves (Esau, 1933 and 1934). This structure is composed of phloem-parenchyma cells that have enlarged and have thickened their walls subsequent to the obliteration of sieve tubes and companion cells.

The cells of the phloem parenchyma are living. Their walls are thin in the functioning phloem (figs. 2, *A, D, E, G;* 3, *L* and *M*) but thick in the nonfunctioning tissue (figs. 2, *E* and *G,* above; 3, *J* and *K*). They are of nonlignified cellulose. The wall thickening is somewhat more

prominent in the corners than elsewhere, and its resemblance to the thickenings of collenchyma walls leads to the use of the term "collenchymatous bundle cap." The ontogeny of the bundle cap, however, and its mechanical properties, which are discussed later in this paper, clearly differentiate it from true collenchyma.

When first formed, the phloem parenchyma cells have transverse or slightly oblique end walls (fig. 3, *C*, left). Later, through readjustments, perhaps in the nature of symplastic growth (Priestley, 1930), the cells become more tapering and assume the appearance shown in plate 7, right. The horizontal walls, visible in these cells, are thin partitions formed in late stages of ontogeny when the cells have undergone some elongation. The walls of the mature cap cells are rather thick and have simple pits.

Parenchyma cells in actively growing phloem undergo marked spatial rearrangements. Certain of these cells force others apart and make new contacts. Sometimes a cell surrounded by other parenchyma cells later comes in contact with a sieve tube or a companion cell. This phenomenon, very common in celery phloem, is much like that observed in the xylem of rapidly expanding vessels (page 448).

Figures 1, *G, I,* and 2, *A, D, E,* and *G* show details of bundle-cap development. The phenomenon of sieve-tube obliteration is most marked in 2, *G*. The diagrams of entire bundles in 1, *F, H,* and 2, *B, C, F,* and *H* show the relative growth in size of the bundle cap, which is represented by the white area above the stippled portion. The latter includes the differentiating and the functioning phloem.

Xylem.—The xylem tissue in celery shows a rather simple structure, containing only vessels and xylem parenchyma. The early ontogeny of the xylem is shown in figure 1, *E* and *G,* whereas figures 1, *D, F, H,* and 2, *B, C, F, H,* together with plates 3, 4, and 5, illustrate the relative increase in the amount of xylem in progressively older bundles.

The xylem is endarch, differentiating centrifugally. The first protoxylem elements have small diameters (plate 3, below); the succeeding ones become progressively larger (plates 3 and 4). When growth of the bundle slows down, vessels smaller in diameter are again produced; but their proportion with respect to the xylem parenchyma increases.

Plates 5 and 7 show on the adaxial side of the bundle the first protoxylem with obliterated vessels. Then follows the intact part of the protoxylem. Next to the cambium appears the metaxylem, containing scalariform and reticulate elements and a small proportion of xylem parenchyma.

The majority of vessels in celery petioles have spiral secondary thick-

enings. The first vessels formed may show annular bands, but usually only in localized regions of the vessels.

The secondary walls of the xylem vessels show lignin reaction with phloroglucin and HCl.

Spirals while being formed are laid down rather closely over the primary walls, but the coils become separated first through continued longitudinal growth of elements and later, after maturation, by passive stretching caused by growth in length of the petiole. Although the scalariform and reticulate elements of the metaxylem undergo no stretching after maturation, their mother cells are longer in comparison with the procambium cells from which they were derived.

The xylem mother cell develops into a vessel element without any divisions; but it rapidly increases in width and length. The meristem cells giving rise to xylem parenchyma cells, however, undergo longitudinal and transverse divisions, so that the resulting cells are narrower and shorter than the vessel elements.

Vessel mother cells grow and expand so rapidly that the spatial relation of cells in the xylem is considerably affected. There is not only a flattening and distortion of adjacent cells, but actual tearing apart of cells, so that new contacts are made by the expanding vessel. Such phenomena have already been noted by Priestley and his co-workers (1935) in woody angiosperms.

Figures 4, *C* and *D* show two sections from the same portion of xylem, one being 10 microns below the other. Whereas in 4, *C* vessels *a* and *b* are separated by a parenchyma cell, in 4, *D* they are in contact with each other. The expanding vessel *a* has torn apart parenchyma cells that intervened between it and vessel *b*.

The separation of parenchyma cells from each other does not occur uniformly along the entire wall, and the two cells being forced apart remain in contact in several places. This situation is made possible by the growth of walls in localized regions, so that the cells appear to have arms or protuberances connecting one cell to the other.

Figure 4, *G* shows an early stage in the separation of parenchyma cells; 4, *H* a later stage in the process. Both drawings represent longitudinal sections. The lack of contact between some of the protuberances is an artifact obtained in cutting thin tangential sections off a cylindrical body, the vessel. Where protuberances are not in contact, a portion of them was sliced off with the preceding section of the paraffin ribbon.

Priestley and his co-workers (1935) suggest that the places where cells remain in contact by means of arms are the pitted places.

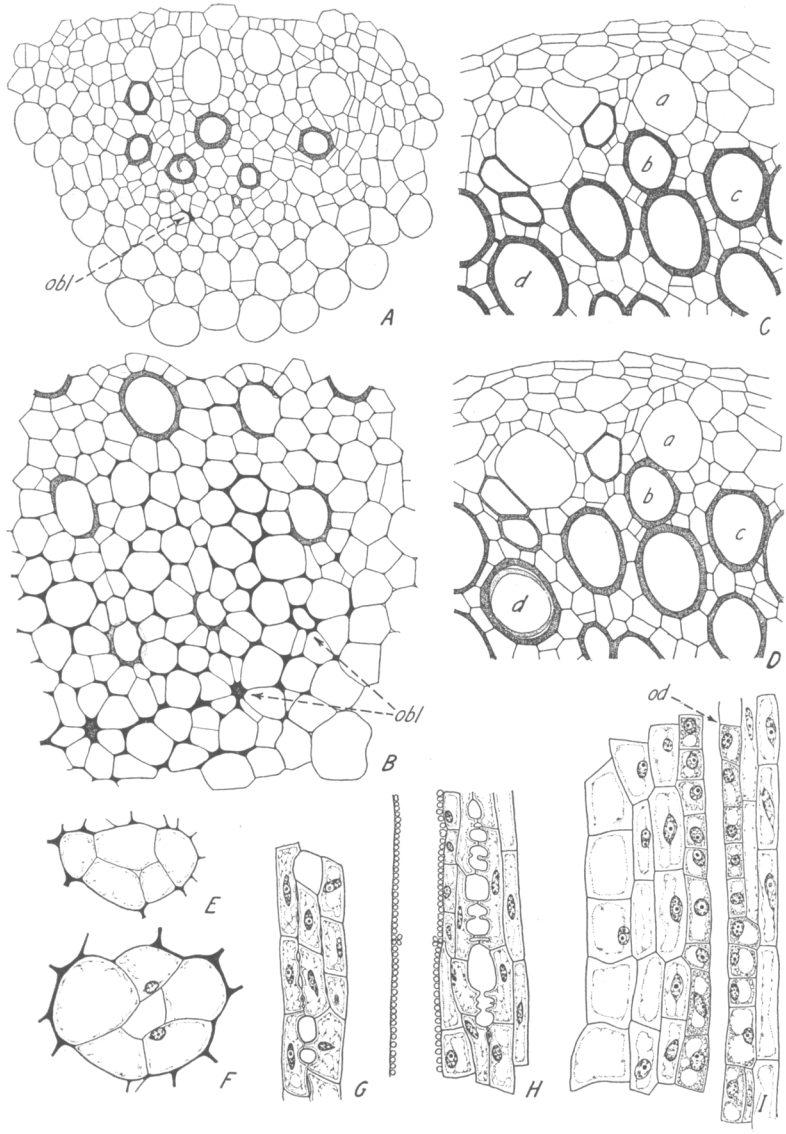

Fig. 4.—*A, B,* Two stages of protoxylem development; *C, D,* two successive sections of the same xylem region showing relation of vessels to surrounding cells; *E, F,* oil ducts from metaphloem; *G, H,* tearing apart of xylem parenchyma cells; *I,* oil duct from cortex; *A–F,* transverse sections; *G–J,* longitudinal sections; *obl,* obliteration; *od,* oil duct. (*A–D, G* and *H,* × 211; *E, F,* × 467; *I,* × 301.)

Xylem-parenchyma cells assist in obliterating the nonfunctioning vessels in the protoxylem. When a vessel becomes stretched so that its spiral is unable to keep the vessel open, adjacent parenchyma cells elongate anticlinally with respect to the vessel, thereby closing its lumen. This change in shape of parenchyma cells may be accompanied or preceded by periclinal divisions.

Figure 4, *A* and *B* shows stages in obliteration of vessels. In *A* only one of the protoxylem vessels has been obliterated at *obl;* the others are still intact, although in the smallest ones the lumina have been narrowed down. The upper part of the drawing shows some xylem mother cells without secondary thickenings. This section was taken from a bundle represented in figure 1, *H*.

In figure 4, *B*, which was drawn from the bundle represented in outline in figure 2, *F*, and which also occurs in plate 4, further progress in protoxylem obliteration may be noted. The lowest elements have been closed by the much enlarged xylem-parenchyma cells, whereas the one above and to the right has been partly crushed. Two more elements above have also been subjected to much stretching and therefore show only portions of their loose spirals. Xylem-parenchyma cells begin to encroach upon these vessels. Three larger vessels above have wide-open lumina, but their spirals are also somewhat loose and appear only partially in sections. Above, to the left, is a functioning vessel with a close spiral appearing as a ring in cross section.

Upon obliteration of protoxylem vessels, xylem-parenchyma cells thicken their walls in a manner similar to that of the bundle-cap cells. Plate 5 shows to the left the characteristic appearance of the nonfunctioning protoxylem, with parenchyma cells radiating from the points where vessels were crushed. Darkly stained material has accumulated in place of the destroyed cells. Plate 7 shows, at the left, the same region in longitudinal section. Pits in the thick walls of xylem parenchyma appear as light dots.

Primary and Secondary Walls in the Vascular Tissues.—In the analysis of the wall structure of vascular as well as other tissues, the recent revision of cell-wall terminology made by Kerr and Bailey has been adopted in this paper. For clarity of descriptions of walls, the following quotation is taken from the work of those authors (Kerr and Bailey, 1934, p. 342) :

> There are two distinct and fundamentally different categories of cell walls. Meristematic elements and such of their derivatives as retain a potentiality for growth and enlargement have walls which are characterized by their capacity for growth and extension and for undergoing reversible changes, e.g. in thickness. On the contrary,

tissue cells which undergo irreversible changes and thus lose their potentiality for growth and enlargement may form supplementary or *secondary wall* which tends to be more or less conspicuously laminated.

The first category of walls mentioned includes the primary walls.

Applying this classification to walls in celery petioles, we find that the only elements showing secondary walls are the vessels. The walls of the phloem parenchyma, even after the latter is transformed into the bundle cap, must be classed as primary, because its cells are living and can undergo reversible changes. In connection with sieve-tube obliteration the parenchyma cells continue to grow and divide after their walls have been thickened. In the sugar beet (Esau, 1933) bundle-cap cells lose their wall thickenings when anomalous cambium is formed in the bundle cap.

The sieve tubes develop rather thick walls when they emerge from the mother-cell stage, but the thickening disappears again when the elements become senile. Because of their transitory nature, sieve-tube wall thickenings are interpreted as primary, pending more detailed studies.

The xylem parenchyma develops rather thick walls in the old protoxylem regions, but these walls are similar to those of the bundle-cap cells. Also like the latter, xylem parenchyma cells are living in their mature state.

Vessels, however, have secondary walls. After these appear, the cells lose their protoplasts and become incapable of reversible changes.

It is beyond the scope of this investigation to attempt to differentiate between the primary wall and the intercellular substance (Kerr and Bailey, 1934).

ONTOGENY AND STRUCTURE OF THE COLLENCHYMA

Ontogeny of Collenchyma.—The collenchyma in the ribs of celery petioles is of a type in which the major portion of the wall material is deposited in the corners of cells (plate 6).

As was mentioned earlier in this paper (p. 436), collenchyma arises within the ground meristem, between the oil duct and the protoderm. This part of the ground meristem develops intercellular spaces before the divisions initiating collenchyma become evident.

Figure 5, *A*, depicting the initiation of collenchyma, shows that periclinal divisions predominate at first but are soon followed by anticlinal longitudinal divisions. The subepidermal layer does not at once take part in these active divisions. Eventually, however, it contributes some cells to the collenchyma by cutting off tangentially a row of cells toward the collenchyma strand (fig. 5, *B*, *C*, and *D*). The resulting cells of the outer layer then grow as large as the subepidermal cells of regions where

no collenchyma is present, and remain as a transitional layer between the collenchyma and the epidermis. This differential growth of the subepidermal cells is shown successively in plates 3; 4, *A*, and 6.

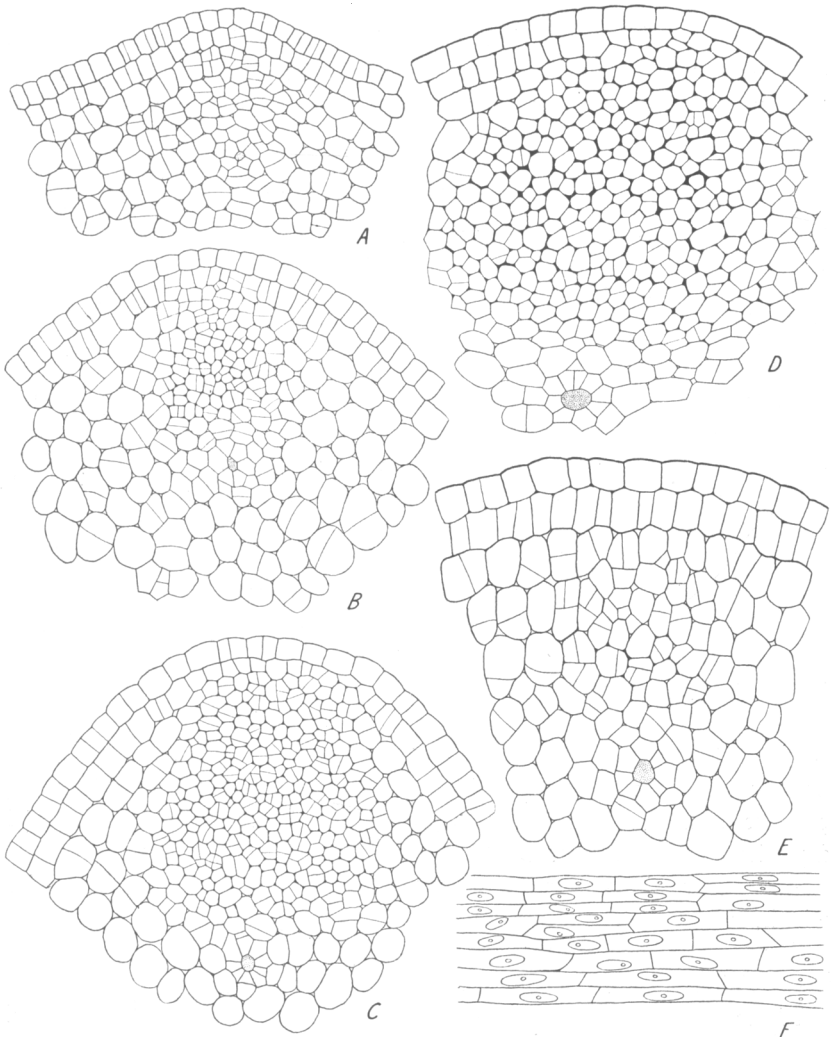

Fig. 5.—*A–E*, Transverse sections of collenchyma (× 215); *A–D*, four successive stages of development; *E*, late initiation of collenchyma; stippled cells representing oil ducts; *F*, young collenchyma in longitudinal view. (× 307.)

Growth of the young collenchyma strand somewhat resembles the growth of a procambium strand. The early divisions lead to the formation of a strand of elongated cells of small diameters. This strand continues to grow through repeated divisions within the strand itself and

through the addition, on the periphery, of new cells derived from the ground meristem (fig. 5, *A*, *B*, and *C*).

Divisions follow one another so rapidly that cells enlarge very little from division to division and soon appear much smaller than the ground-parenchyma cells about them (fig. 5, *A*, *B*, and *C*). Only when divisions slow down do collenchyma cells begin to enlarge (fig. 5, *D*).

The rapid succession of divisions in the young collenchyma strand causes a close packing of cells and loss of those intercellular spaces that were present among the initiating ground-meristem cells. Intercellular spaces occur among recently divided cells in figure 5, *A*, but are limited to the peripheral layers of collenchyma in 5, *B*. Perhaps the deposition of wall material in the corners aids in closing intercellular spaces along with the packing of dividing cells. The collenchyma strand in plate 7 has no intercellular spaces. The small lumina visible in the photograph are cross-sectional views of the tapering ends of collenchyma cells.

Although the deposition of the characteristic wall thickenings begins in very early ontogeny of collenchyma (fig. 5, *B*), it does not make much progress until cell division slows down (fig. 5, *D*).

Meristematic collenchyma cells are longer than wide and have transverse or somewhat oblique end walls (fig. 5, *F*). While increasing in length, collenchyma cells develop tapering end walls, probably through processes akin to that termed by Priestley (1930) symplastic growth. Some thin transverse walls develop in the later stages.

Plate 8 shows the long tapering ends of mature collenchyma cells and the thin transverse partitions. No pitting is evident. It seems to be limited to the thin portions of walls.

Some collenchyma strands are initiated later than others, in a more mature region of the petiole. Figure 5, *E* shows origin of such a collenchyma strand. It was taken from the same transverse section of the petiole as 5, *D*. As the intercellular spaces are large when a bundle of this kind is initiated and as divisions are less rapid than in younger regions, intercellular spaces are frequently retained in the mature strand.

Using Kerr and Bailey's terminology (1934), the wall thickenings of collenchyma should be considered as primary. Collenchyma cells are living, retain a potentiality for growth and enlargement; their walls have a capacity for undergoing reversible changes, as evidenced in the case of phellogen formation in collenchyma of woody species.

Chemical Nature of Collenchyma Walls.—The walls of collenchyma are chiefly of cellulose and contain a high percentage of water. Anderson (1927) was able to show the presence of pectic materials as well as of cellulose, and the increase of pectic materials in amount toward the

"middle lamella." Using agents that dissolve out the pectic substances, he has found that cellulose and the pectic substances alternate in layers. The collenchyma walls are doubly refractive in polarized light, before and after removal of pectic substances.

Cohn (1892) has attempted to determine the amount of water in collenchyma walls, using the following procedure. He carefully peeled out the strands of collenchyma, soaked them in water for 24 hours, then weighed them after having removed excessive moisture with filter paper. After drying the strands for some time at 95°–100° C, he weighed them again; and from drawings of their cross sections he obtained the ratio between walls and lumina. He also determined the specific weight of cellulose needed for calculating the volume of wall substance. Assuming that the lumina of cells are filled with water, he arrived at values of 60 to 75 per cent of water in fresh weight of collenchyma walls. In contrast, he found that walls of wood from several different species of trees contained approximately 25 per cent water, those of the lignified "bast" of *Vitis vinifera* 34 per cent, of maple 39 per cent. He also ascertained that, after heating, collenchyma walls lost their ability to absorb moisture and became in this respect like fibers.

By examining under a microscope the behavior of collenchyma walls upon addition of dehydrating agents, Cohn learned that the transverse contraction of walls was much more pronounced than the longitudinal. The values obtained for collenchyma of *Eupatorium cannabicum* were as follows: contraction in radial direction 27 per cent, in tangential 10 per cent, and in longitudinal ½ to ¾ per cent.

Anderson (1927) suggests that alternation of layers of cellulose and pectin explains the strong transverse contraction, the pectic material being capable of holding large amounts of water. Heating destroys the colloidal nature of pectic substances and makes them unable to absorb large amounts of water again.

MECHANICAL STRENGTH OF COLLENCHYMA AND OF THE VASCULAR TISSUES

In view of Sayre's (1929) statement that collenchyma is apparently the only tissue having a definite relation to stringiness in celery, an attempt was made to compare the strength of collenchyma with that of the vascular tissues.

Sayre reports that collenchyma in tough, stringy specimens is especially hard, judging by its tendency to break under the microtome knife. To support his contention, he gives several photomicrographs with broken and unbroken collenchyma. His figure 3, however, shows con-

siderable tearing in the xylem also; in figure 6 the bundle cap and phloem are badly torn; and in figure 10 even the soft ground parenchyma is seriously damaged. Moreover, the breaking of collenchyma often occurred in streaks, leaving other parts of the same collenchyma strand intact (Sayre, figs. 10 and 13). Such irregular tearing must have been caused by a defect in the microtome knife.

By proper technique, tearing may be reduced to a minimum. In this study, collenchyma strands showed very little tearing (plates 2, 3, 4, 6, and 8), even in old petioles of very stringy varieties, and offered less difficulty in cutting than the xylem. Evidently, therefore, the degree of breaking of collenchyma in paraffin material is not a reliable measure of its toughness. As will be shown, however, Sayre is correct in assuming that collenchyma is tougher than the vascular strands.

Experiments to measure toughness of celery were made in 1881 by Ambronn, who used a method similar to Schwendener's (1874) for determining elasticity of fibers. A strand of tissue was broken by fastening one end of it with a clamp and suspending weights to the other end. By means of a scale with a pointer, brought in motion by the added weight, the elongation of the strand could be determined. Having made drawings of cross sections of strands, Ambronn determined the area occupied by walls and calculated the tensile strength of the tissue.

Ambronn found that collenchyma could support 10 to 12 kilograms per mm^2, values that compare very favorably with the 15 to 22 kilograms per mm^2 obtained by other workers for fibers. The latter structures are, however, more elastic than the collenchyma, because they regain their original length even after having been subjected to a tension of 15 to 20 kilograms per mm^2, whereas collenchyma cells pass their elastic limit at 1½ to 2 kilograms per mm^2. Fibers elongate 1.5 per cent of their original length before they break; collenchyma strands 2 to 2½ per cent. From his data Ambronn concludes that as a supporting tissue collenchyma is particularly adapted to growing parts of plants. Its toughness offers firm support; but its low elasticity, combined with an ability to respond to tension by stretching, prevents it from hindering the elongation of growing organs.

In the present study the strength of collenchyma and vascular bundles was compared by determining the breaking load of individual strands of these tissues.

Strands of collenchyma or of vascular tissue were peeled out of fresh turgid petioles. With care, one can obtain strands several centimeters long, almost free of adhering parenchyma. The vascular bundles were used entire or were separated along the cambium into bundle cap and

xylem. The bundle cap, of course, included the functioning phloem; but to simplify description it is called simply the bundle cap. Cells of the bundle cap undoubtedly determine the strength of the abaxial part of the vascular bundle, the phloem being a soft thin-wall tissue.

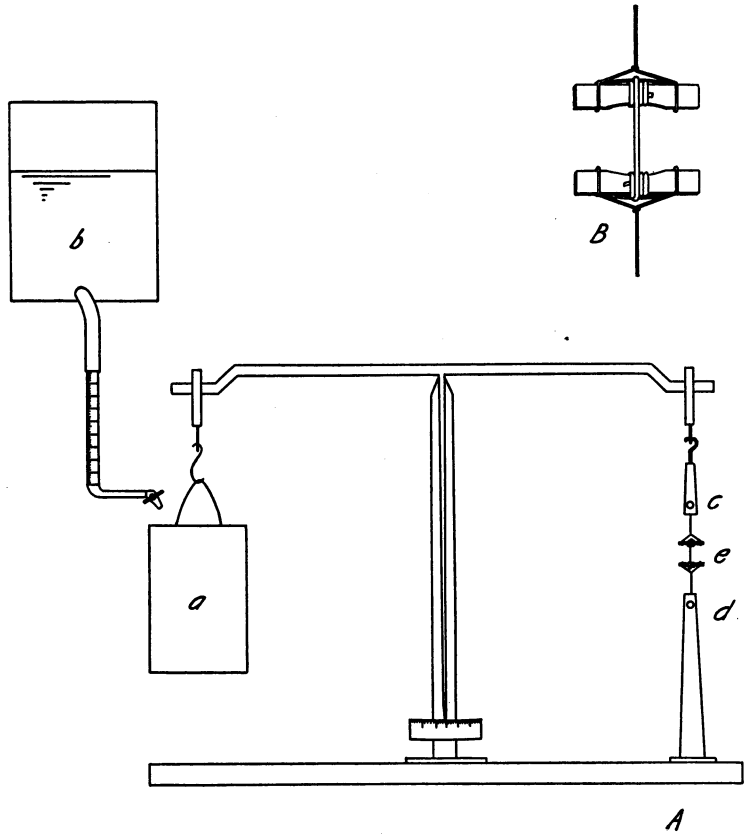

Fig. 6.—*A*, Apparatus used in determining breaking load of collenchyma and vascular bundles; *B*, detailed diagram showing method by which strands of tissue are attached.

To keep the strands fresh, they were placed in petri dishes on moist filter paper and were taken out one by one.

The apparatus to measure the breaking load consisted of a specially adapted balance shown in figure 6, *A*. On the left arm, container *a* for water was suspended directly under a burette leading from a stationary container *b* with the stock supply of water. On the right arm, clamp *c* was suspended above the stationary clamp *d*.

The celery string at *e* was not directly clamped into *c* and *d*, because

TABLE 2

RELATIVE STRENGTH OF COLLENCHYMA AND OF THE VASCULAR BUNDLES

Variety	Leaf No.	Breaking load in grams				Average area in mm²			
		Collenchyma	Vascular bundle	Bundle cap	Xylem	Collenchyma	Vascular bundle	Bundle cap	Xylem
Golden Plume	13	730	185	...	...	0.471	0.522	.....	.....
		1,000	215	...	...	0.976	0.805	.....	.....
		1,020	250	...	...	1.330	0.747	.....	.....
	20	1,210	...	355	260	0.532	.....	0.804	0.491
		1,300	...	240	95	0.905	.....	0.616	0.322
		1,500	...	230	140	0.583	.....	0.613	0.413
		1,500	...	265	150	0.951	.....	0.638	0.414
	22	1,205	275	...	...	0.960	0.584	.....	.....
		1,300	...	275	155	0.785	.....	0.904	0.400
		1,315	525	...	...	0.976	0.983	.....	.....
		1,340	390	...	...	1.046	0.910	.....	.....
		1,525	365	...	...	0.995	1.111	.....	.....
	24	1,170	...	380	170	0.475	.....	0.581	0.328
		1,275	...	290	225	0.497	.....	0.558	0.348
		1,500	...	340	150	0.491	.....	0.469	0.316
Tall Golden Self-blanching	20	720	305	...	...	0.413	0.558	.....	.....
		720	390	...	...	0.442	0.721	.....	.....
		940	500	...	...	0.305	0.796	.....	.....
	21	800	...	190	95	0.266	.....	0.380	0.266
		1,100	...	265	130	0.396	.....	0.417	0.324
		1,160	...	255	130	0.326	.....	0.408	0.336
		1,200	...	210	130	0.427	.....	0.350	0.314
	22	800	...	210	80	0.291	.....	0.320	0.251
		850	320	...	...	0.372	0.542	.....	.....
		860	405	...	...	0.301	0.632	.....	.....
		880	440	...	...	0.368	0.695	.....	.....
		890	240	...	...	0.367	0.504	.....	.....
		940	260	...	...	0.304	0.729	.....	.....
Utah	11	420	164	...	...	0.238	0.628	.....	.....
		425	300	...	...	0.202	0.775	.....	.....
		505	216	...	...	0.246	0.541	.....	.....
	12	305	122	...	...	0.253	0.316	.....	.....
		430	222	...	...	0.217	0.728	.....	.....
		500	204	...	...	0.227	0.677	.....	.....
	13	540	300	...	...	0.362	0.852	.....	.....
	24	1,325	375	...	...	0.411	1.116	.....	.....
		1,350	390	...	...	0.308	1.134	.....	.....
		1,700	475	...	...	0.359	1.253	.....	.....

then it would either slip out or break at one or the other points of attachment; but it was fastened as shown at *B* in figure 6. Each end of the strand was wrapped around a small bar of hardwood, made very smooth where it would come in contact with the strand. Care was also exercised in laying the last winding of the strand over the underlying coils of the same kind of tissue. The bars were then placed in wire loops, and these loops were clamped by their free ends into *c* and *d* of *A* in figure 6. The exposed length of the strand was approximately 1.5 cm, but the entire strand was 12 to 15 cm long. It is difficult to fasten shorter strands securely.

This method of fastening the strands proved fairly satisfactory. Although they broke sometimes in the middle, sometimes near one or the other end, this behavior did not seem to increase the variability of values obtained. The determinations were rejected, however, when the string broke at points of contact with the wood.

After the strand had been secured in position, water was poured into *a* at an approximate rate of 200 cc per minute. The flow was discontinued as soon as the strand snapped. The volume of water in *a* was measured, and the value obtained was later expressed in grams.

Table 2 shows the results of determining the breaking load for collenchyma and vascular tissue in different petioles of different varieties. In every case the value for collenchyma occurs in the same line with the value or values for the vascular bundle that was lying on the same radius with the collenchyma strand.

The average area of the strand represents the average between the areas at its base and at its apex. Although this area does not exactly correspond to the area in the median region of the bundle or in the region where the strand broke, it indicates the comparative size of strands.

In view of the exploratory nature of the experiment, the tensile strength was not determined in this study. The wall area at the breaking point of the strand would have had to be measured in tensile-strength calculations. This task would have presented difficulties as regards the heterogeneous tissues in the vascular bundles.

As table 2 shows, collenchyma strands are much tougher than the xylem, or the bundle cap, or the two tissues taken together. A comparison of the average areas indicates that the difference in the breaking-load values for collenchyma and vascular tissues is not determined by a difference in size of the strands of these tissues. The collenchyma strands were often thinner than the entire vascular bundles and even thinner than bundle caps taken alone. Where the collenchyma strands

were thicker than the strands of vascular tissues the difference in size was not proportionate to the difference in toughness.

The strength difference of collenchyma of two of the plants did, however, depend on the size of strings, those of the Tall Golden Self-blanching being thinner and weaker than those of the Golden Plume.

TABLE 3

CHARACTERISTICS OF COLLENCHYMA AND BUNDLE CAP SHOWN IN FIGURES 7 AND 8

Variety	Leaf No.	Collenchyma			Bundle cap		
		Figure No.	Per cent of area occupied by wall	Size of strand, mm²	Figure No.	Per cent of area occupied wall	Size of strand, mm²
Golden Plume	26	7, *A*	23.4	0.754	7, *D*	21.1	0.506
	26	7, *B*	35.1	0.671	7, *E*	26.1	0.516
	26	7, *C*	52.3	0.232	7, *F*	26.6	0.351
	24	7, *G*	21.3	1.142	7, *J*	10.4	0.780
	24	7, *H*	35.9	0.589	....	....	.....
	24	7, *I*	43.7	0.403	....	....	.....
	23	7, *M*	21.1	0.713	7, *K*	19.4	0.496
	23	7, *N*	20.4	0.770	7, *L*	14.6	0.336
	12	7, *O*	25.4	0.491	7, *Q*	21.7	0.494
	12	7, *P*	40.6	0.325	7, *R*	22.2	0.925
Tall Golden Self-blanching	24	8, *A*	38.4	0.207	8, *C*	32.0	0.325
	24	8, *B*	44.0	0.356	8, *D*	25.6	0.351
	16	8, *E*	45.4	0.191	8, *G*	29.8	0.227
	16	8, *F*	57.0	0.088	8, *H*	25.7	0.072
	12	8, *I*	36.1	0.114	....	....	.....
	12	8, *J*	34.3	0.186	....	....	.....
	12	8, *K*	35.2	0.181	....	....	.....
	12	8, *L*	42.1	0.041	....	....	.....
Utah	23	8, *M*	56.4	0.227	8, *P*	21.8	0.594
	24	8, *N*	46.1	0.330	8, *Q*	21.3	0.589
	14	8, *O*	30.6	0.310	....	....	.....
	10	8, *R*	34.9	0.165	....	....	.....

The collenchyma strands of the old leaf (No. 24) of Utah, though small, were very tenacious.

Collenchyma of the younger leaves, compared with that of the older ones, showed a greater reduction in breaking-load values than the corresponding vascular bundles. The increase in toughness with age of tissue probably results from an increase in thickness of cell walls.

Though xylem strands were weaker than the corresponding bundle caps, this difference was largely determined by their relatively small size.

All the data given above are, of course, far too limited to permit any conclusions regarding the relative stringiness of different celery varieties.

The difference in the physical properties of collenchyma and the

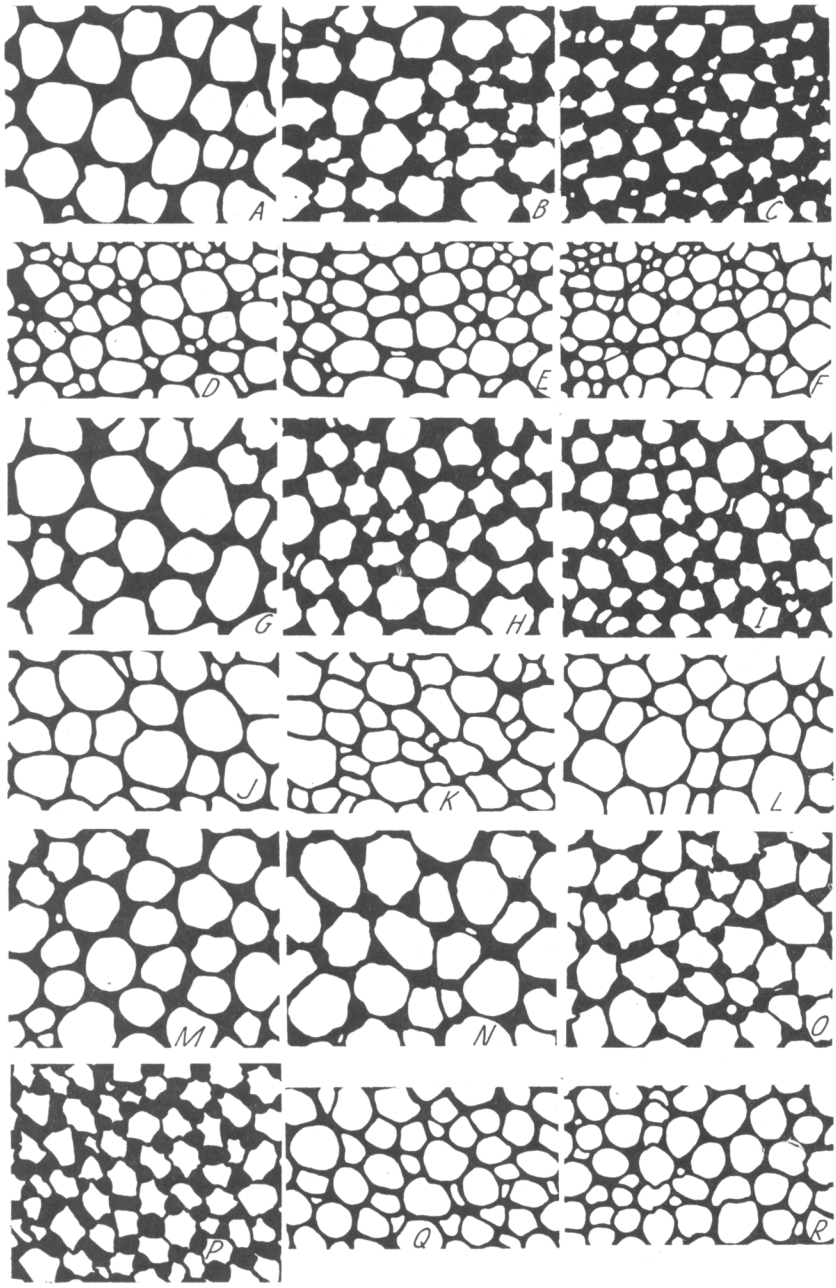

Fig. 7.—Transverse sections of collenchyma (*A–C, G–I, M–P*); and bundle cap (*D–F, J–L, Q, R*) from fresh petioles. Further explanations in table 3. (×205.)

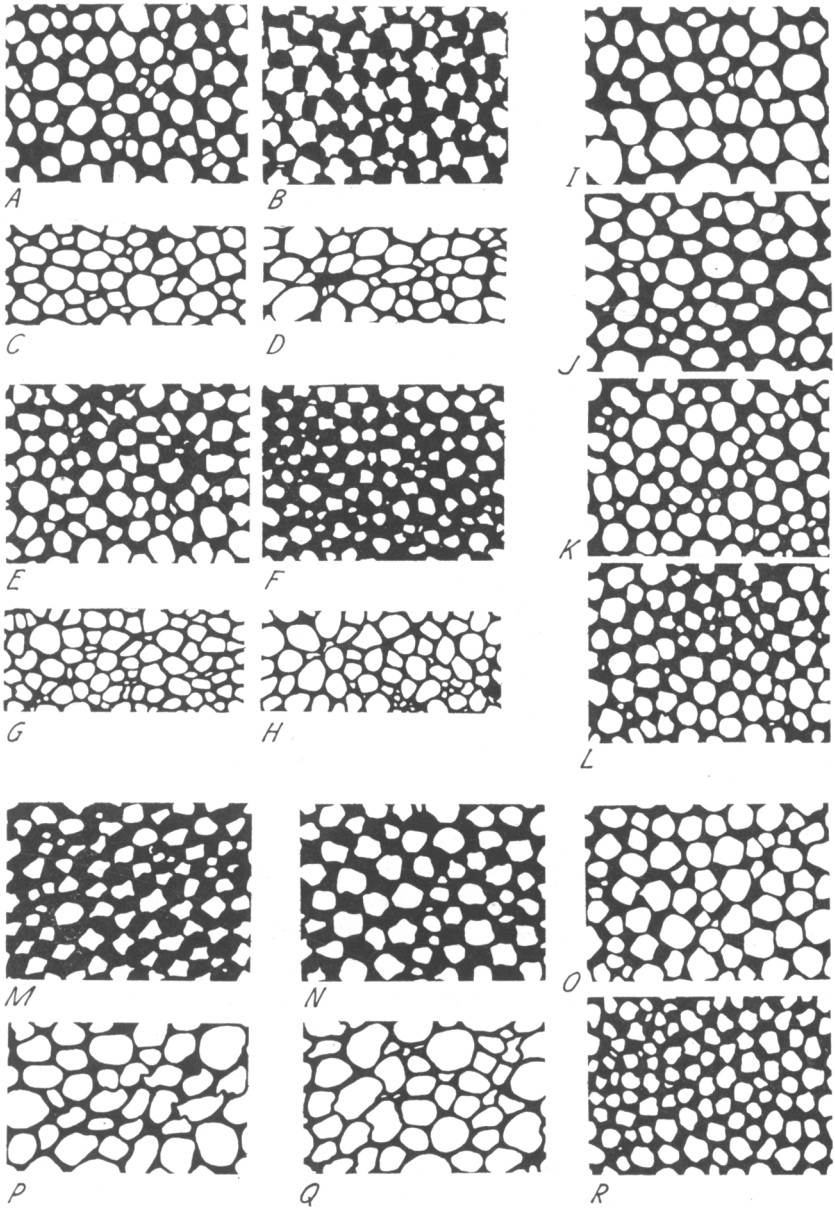

Fig. 8.—Transverse sections of collenchyma (*A*, *B*, *E*, *F*, *I–O*, *R*); and bundle cap (*C*, *D*, *G*, *H*, *P*, *Q*) from fresh petioles. Further explanations in table 3. (× 205.)

bundle cap, tissues that have certain morphological characteristics in common, is, however, worthy of note. The difference in toughness between these two tissues might partly be explained by the difference in wall thickness..

Figures 7 and 8 compare collenchyma and bundle-cap structure in camera-lucida drawings from fresh sections of petioles. Table 3 gives the origin of each section, the size of the strand from which it was drawn, and the percentage of area occupied by walls in each case. This last value was obtained by measuring the reflection of light from each drawing by means of a photoelectric cell.

Figures 7 and 8 and table 3 clearly show that collenchyma has considerably thicker walls than the bundle cap and in most cases has more wall material per unit area. They also show that while the bundle cap is fairly uniform in structure in different bundles of the same petiole, the collenchyma varies markedly in cell size and wall thickness. The smaller collenchyma strands tend to have smaller cells and thicker walls. This relation is particularly obvious in the material from the Golden Plume plant.

DISCUSSION

The two kinds of structures commonly called "celery strings" differ greatly in their ontogeny, structure, and mechanical properties. One of these structures, the collenchyma strand, is made up of a very tough tissue which, to be broken by pulling, requires two to four times as heavy a load as the other structure, the vascular bundle.

Considering the known facts about the structure of tissues tested in this study for their toughness, we find some histological characteristics that might account for the observed differences in strength.

The collenchyma has the appearance of a strong tissue having long overlapping cells with thick walls. The xylem, for the most part, is composed of spiral vessels and thin-wall parenchyma with transverse end walls. The coils of the spirals in the vessels are separated from each other by a thin primary wall. No fibers occur in the xylem. The phloem, a living parenchymatous thin-wall tissue with no fibers, is probably, next to the cambium, the weakest tissue in the bundle. The cap cells, although prosenchymatous in shape like the collenchyma cells, are phloem-parenchyma cells that acquire thick walls rather late in their ontogeny. These walls, moreover, are not so thick as those of the collenchyma.

From the histological point of view the strength difference between collenchyma and the bundle cap is particularly interesting. Bundle

caps of the type found in celery are often called "collenchymatous structures" or even simply "collenchyma." As ontogenetic studies reported in this paper clearly show, these two types of tissues have a different mode of development. They differ in their mature structure as well as in their mechanical properties.

A consideration of the relative toughness of strings in different varieties of celery is beyond the scope of this study. A few remarks, however, should be made here regarding the possible value of breaking-load determinations in comparing varieties for stringiness. Resistance to a pulling force may not be the only measure of stringiness of a variety. Conceivably, other characteristics might play a more prominent rôle than toughness, or at least an equal rôle, in determining the brittleness of strings still imbedded in the other tissues of the petiole. One characteristic of this sort appears to be the turgidity of the tissues. Celery petioles with strong strings will appear stringless when broken after they have been cooled. Possibly, under natural conditions, celery varieties differ with regard to tissue tensions. In the Utah plant used in this experiment, the strings removed from the plant coiled like springs; but in other varieties they remained straight. This behavior, apparently, indicates that in the sample of Utah variety the strings were under greater tension within the plant than in the other varieties.

Ambronn (1881) has found that collenchyma is subjected within plants to tensions equal to 9 to 12 atmospheres, this tension being brought about by the turgor pressure of the surrounding growing parenchyma cells. Whether the vascular bundles are under similar tension has not been determined.

In studying comparative stringiness of celery varieties, one should consider the problem of tissue tensions along with the relative toughness of tissues. One should also seriously consider the fact that environment greatly influences the structure of plants, particularly with regard to the amount of wall material deposited by the cells.

SUMMARY

Celery petioles contain a semicircle of large collateral vascular bundles on the abaxial side and a row of very small bundles on the adaxial side. A subepidermal collenchyma strand occurs in the rib opposite each major vascular bundle on the abaxial side. Both the collenchyma and the large vascular bundles constitute the "celery strings" of growers and consumers.

The petiole of a primordial leaf contains the following meristems: the protoderm, the procambium, and the ground meristem. The protoderm

develops into the epidermis. The ground parenchyma and the collenchyma come from the ground meristem, but the collenchyma is initiated within the ground meristem through longitudinal divisions resulting in the formation of a strand similar to the procambium.

The procambium differentiates into primary phloem and xylem and the cambium. It arises in the ground meristem of the primordial petioles and increases in size partly through continued division of the first-formed cells, partly through addition of new cells from the ground meristem.

The first protoxylem and protophloem elements lie rather close together in the primordial bundles; but as the procambium cells between them continue to divide, they are moved farther apart. Through the division of the procambium cells the bundle grows in radial extent while differentiation is in progress. The division of the procambium cells increases in regularity; and as the periclinal walls predominate, the last-formed vascular tissues (metaphloem and metaxylem) show regular radial arrangement of cells.

The vascular bundles usually contain only primary tissues, although cambium differentiates between the xylem and phloem in the old leaves.

The protoxylem is endarch and is composed of spiral vessels and parenchyma. The vessels of the metaxylem have scalariform and reticulate secondary thickenings. No fibers occur in the xylem.

Differentiating vessels expand so rapidly that they tear the adjacent parenchyma cells apart and come in contact with new cells. Protoxylem vessels, which mature in rapidly elongating petioles, undergo much stretching; eventually their lumina are closed by the adjacent parenchyma cells.

The phloem contains sieve tubes, companion cells, and phloem-parenchyma cells. Slime bodies occur in differentiating sieve tubes, one in each element. Mature sieve tubes have no nuclei, but contain cytoplasm and plastids. A single sieve plate occurs on the transverse or the somewhat oblique end wall. The sieve tubes and their companion cells develop in rapid succession, the old elements being obliterated.

Phloem parenchyma cells of the old phloem continue to enlarge for some time and develop thick primary walls. When fully developed they constitute the structure known as the bundle cap.

When collenchyma is initiated in the ground meristem, the divisions occur in rapid succession so that the new cells remain small and become closely packed. The collenchymatous wall thickenings appear long before the cells cease to enlongate. Mature collenchyma cells are prosenchymatous in nature, being long, with tapering ends.

Mechanically the collenchyma is much stronger than the vascular tissue. The breaking load of collenchyma may be two to four times that of the entire vascular bundles or the bundle cap.

ACKNOWLEDGMENTS

The writer is grateful to Professor J. F. Wilson for lending the apparatus to measure the breaking load of tissues and to Dr. F. A. Brooks and Mr. C. E. Barbee for making possible the use of the photoelectric cell.

LITERATURE CITED

AMBRONN, H.
1881. Ueber die Entwickelungsgeschichte und die mechanischen Eigenschaften des Collenchyms. Ein Beitrag zur Kenntniss des mechanischen Gewebesystems. Jahrb. Wiss. Bot. 12:473–541.

ANDERSON, D.
1927. Ueber die Struktur der Kollenchymzellwand auf Grund mikrochemischer Untersuchungen. Sitzber. Akad. Wiss. Wien. Math.–Naturw. Kl. 136:429–40.

BARY, A. DE.
1884. Comparative anatomy of the vegetative organs of the phanerogams and ferns. [English translation by F. O. Bower and D. H. Scott.] 659 p. Clarendon Press, Oxford.

COHN, J.
1892. Beiträge zur Physiologie des Collenchyms. Jahrb. Wiss. Bot. 24:144–72.

CRAFTS, A. S.
1933. Sieve-tube structure and translocation in the potato. Plant Physiology 8:81–104.

EAMES, A. J. and L. H. MACDANIELS.
1925. An introduction to plant anatomy. 364 p. McGraw-Hill, New York.

ESAU, K.
1933. Pathologic changes in the anatomy of leaves of the sugar beet, *Beta vulgaris* L., affected by curly top. Phytopathology 23:679–712.
1934. Ontogeny of phloem in the sugar beet (*Beta vulgaris* L.). Amer. Jour. Bot. 21:632–44.

FOSTER, A. S.
1935*a*. A histogenetic study of foliar determination in *Carya buckleyi* var. *arkansana*. Amer. Jour. Bot. 22:88–147.
1935*b*. Comparative histogenesis of foliar transition forms in Carya. Univ. California Pubs., Bot. 19:149–86.

GRAUPNER, H., and A. WEISSBERGER.
1931. Ueber die Verwendung des Dioxans beim Einbetten mikroskopischer Objekte. Mitteilungen zur mikroskopischen Technik I. Zool. Anz. 96:204–6.

HANCE, R. T.
1933. Improving the staining action of iron haematoxylin. Science 77:287.

KERR, TH., and I. W. BAILEY.
1934. The cambium and its derivative tissues. No. X. Structure, optical properties, and chemical composition of the so-called middle lamella. Jour. Arnold Arboretum 15:327–49.

PRIESTLEY, J. H.
1930. Studies in the physiology of cambial activity. II. The concept of sliding growth. New Phytol. 29:96–140.

PRIESTLEY, J. H., L. I. SCOTT, and M. E. MALINS.
1935. Vessel development in the angiosperm. Leeds Phil. Soc. Proc. 3:42–54.

RAWLINS, T. E.
1933. Phytopathological and botanical research methods. 156 p. J. Wiley & Sons, New York.

SAYRE, C. B.
1929. Quality in celery as related to structure. Illinois Univ. Agr. Exp. Sta. Bul. 336:559–88.

SCHÜEPP, OTTO.
1926. Meristeme. 114 p. In: K. Linsbauer's Handbuch der Pflanzenanatomie 1. Abt. 2. Teil: Histologie Bd. IV, Lief. 16. Gebrüder Borntraeger, Berlin.

SCHWENDENER, S.
1874. Das mechanische Princip im anatomischen Bau der Monocotylen. 179 p. Wilhelm Engelman, Leipzig.

STRASBURGER, E.
1891. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. Histologische Beiträge, Bd. 3. 1,000 p. Gustav Fischer, Jena.

WISSELINGH, C. VAN.
1882. Contribution à la connaissance du collenchyme. Arch. Néerland. Sci. Exact. et Nat. 17:23–58.

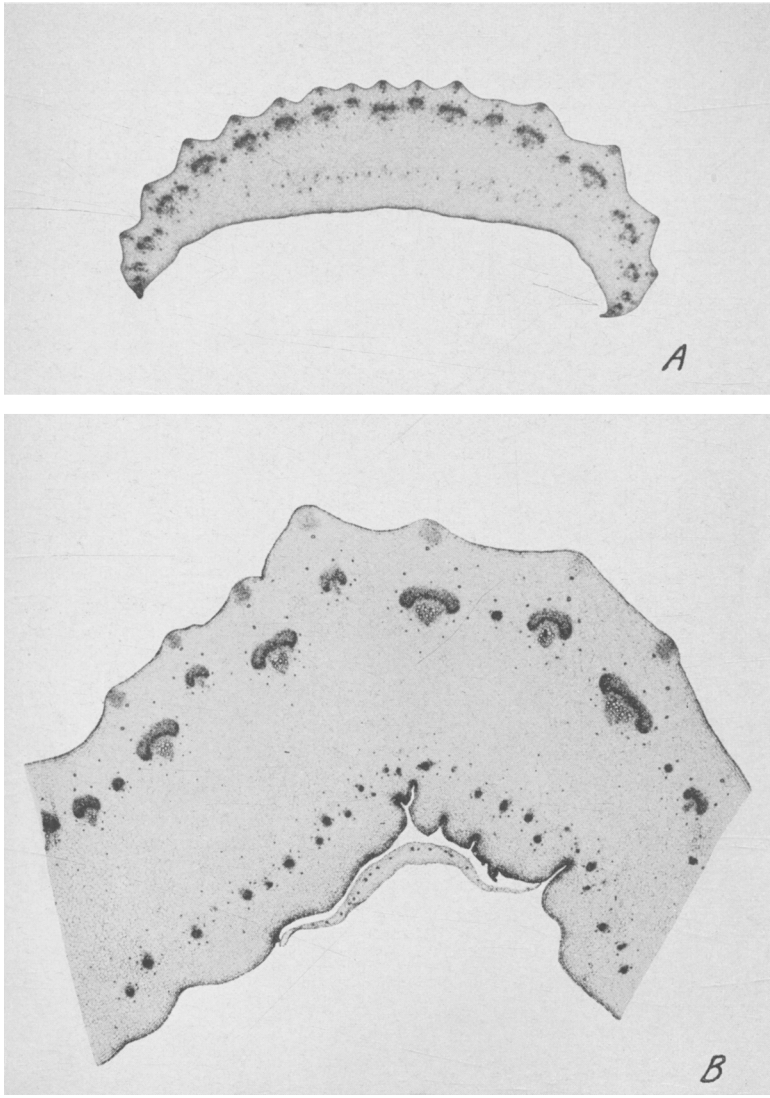

Plate 1.—*A*, Transverse section through a young petiole near its base; *B*, similar section through a somewhat older petiole. (× 11.)

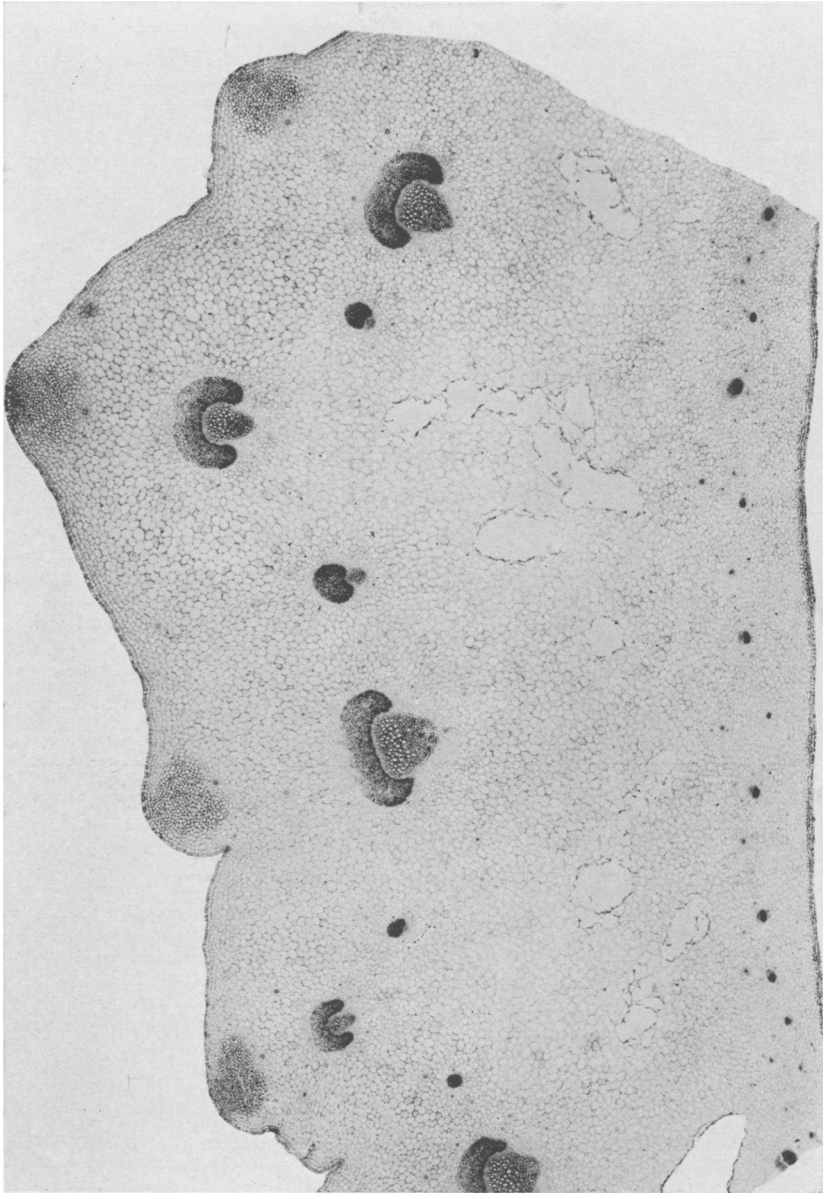

Plate 2.—Transverse section through a petiole, more mature than those in plate 1. (× 11.)

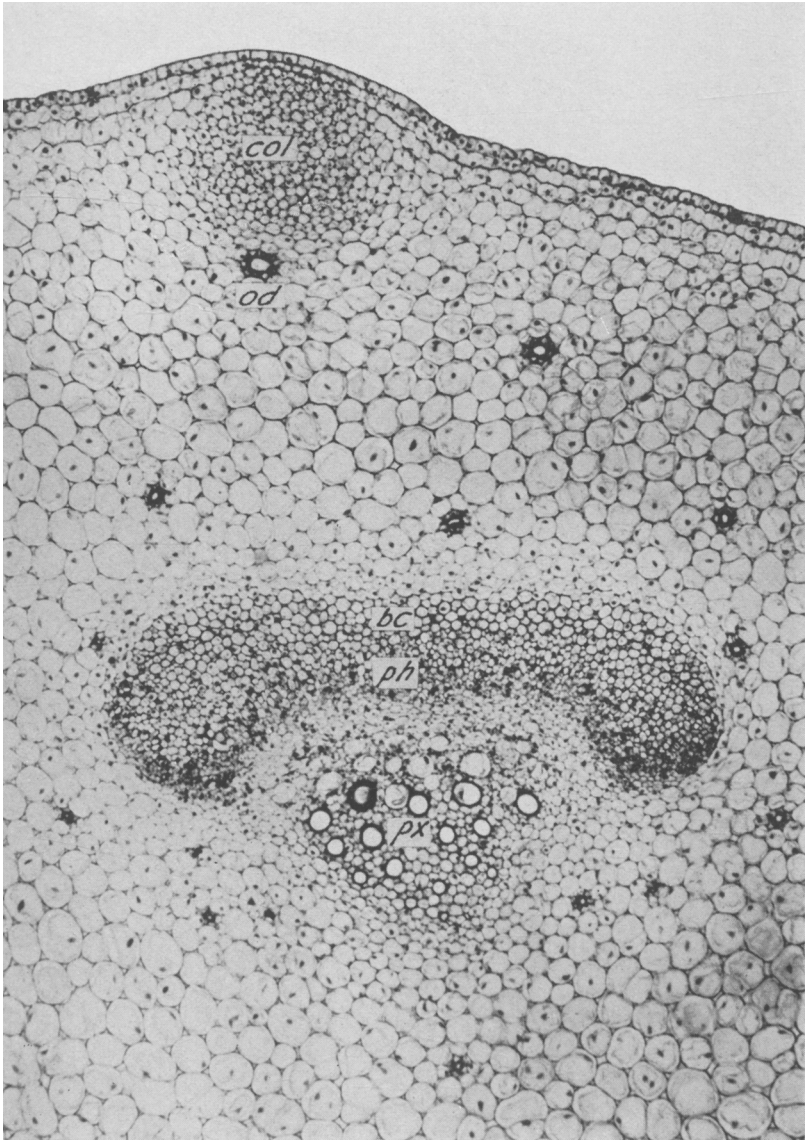

Plate 3.—Transverse section through a portion of a petiole showing a comparatively young vascular bundle and, above it, near the epidermis, a collenchyma strand. *bc,* bundle cap; ***col,*** collenchyma; *ph,* phloem; *px,* protoxylem. (× 90.)

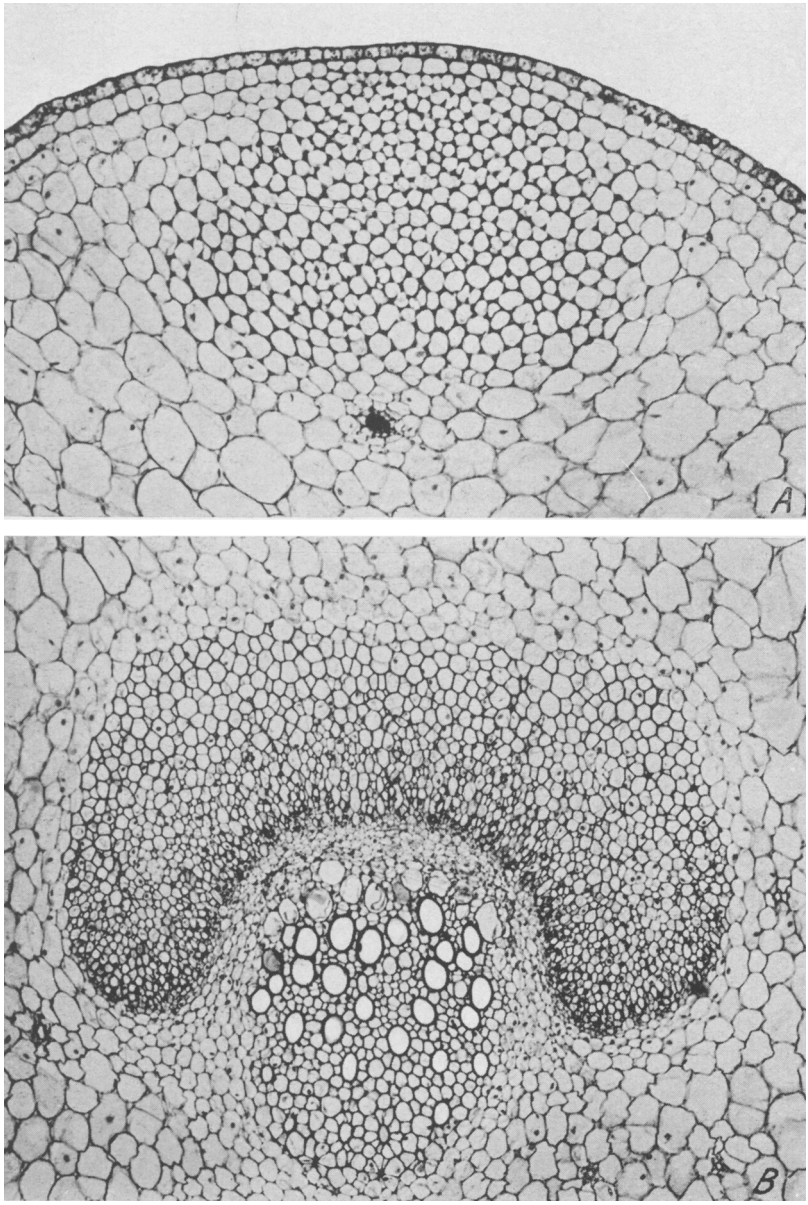

Plate 4.—Transverse sections of collenchyma (*A*), and of a vascular bundle (*B*), from a more mature petiole than in plate 3. (× 90.)

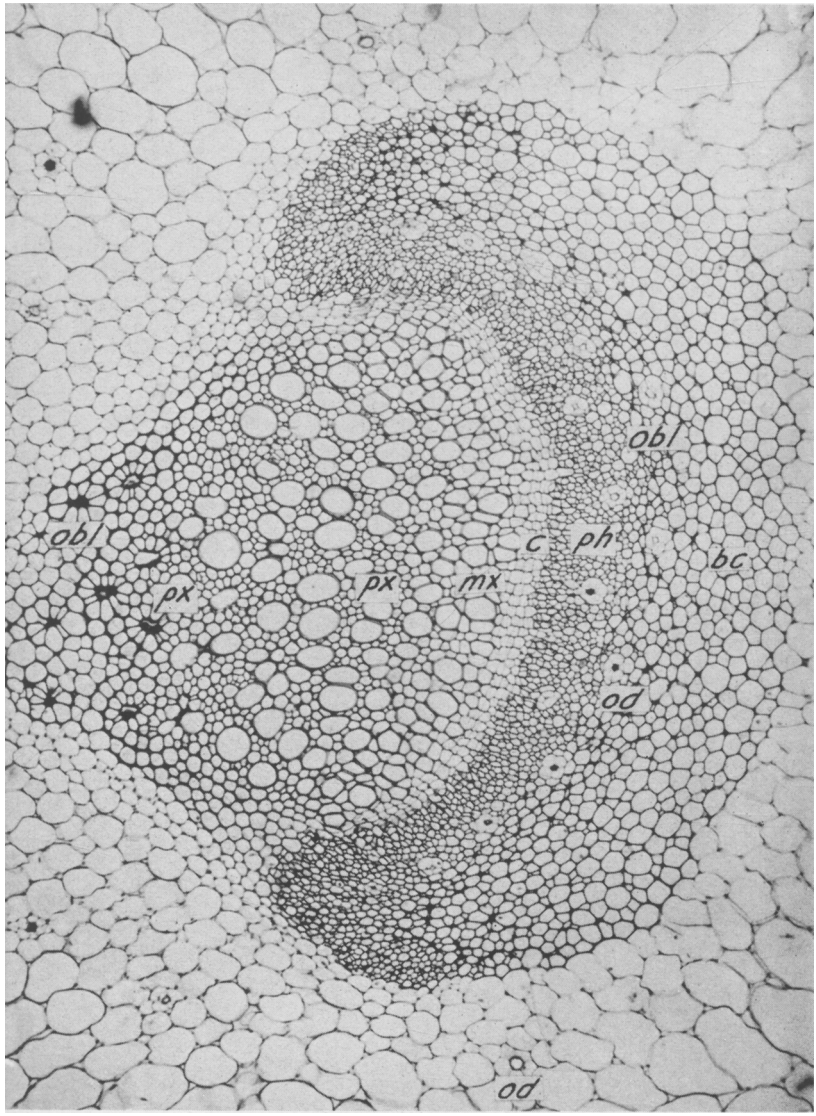

Plate 5.—Transverse section of a vascular bundle from a petiole that had ceased to elongate. The xylem is at the left; the phloem and bundle cap are at the right. *bc*, bundle cap; *c*, cambium; *mx*, metaxylem; *obl*, obliterated elements; *od*, oil duct; *ph*, phloem; *px*, protoxylem. (× 90.)

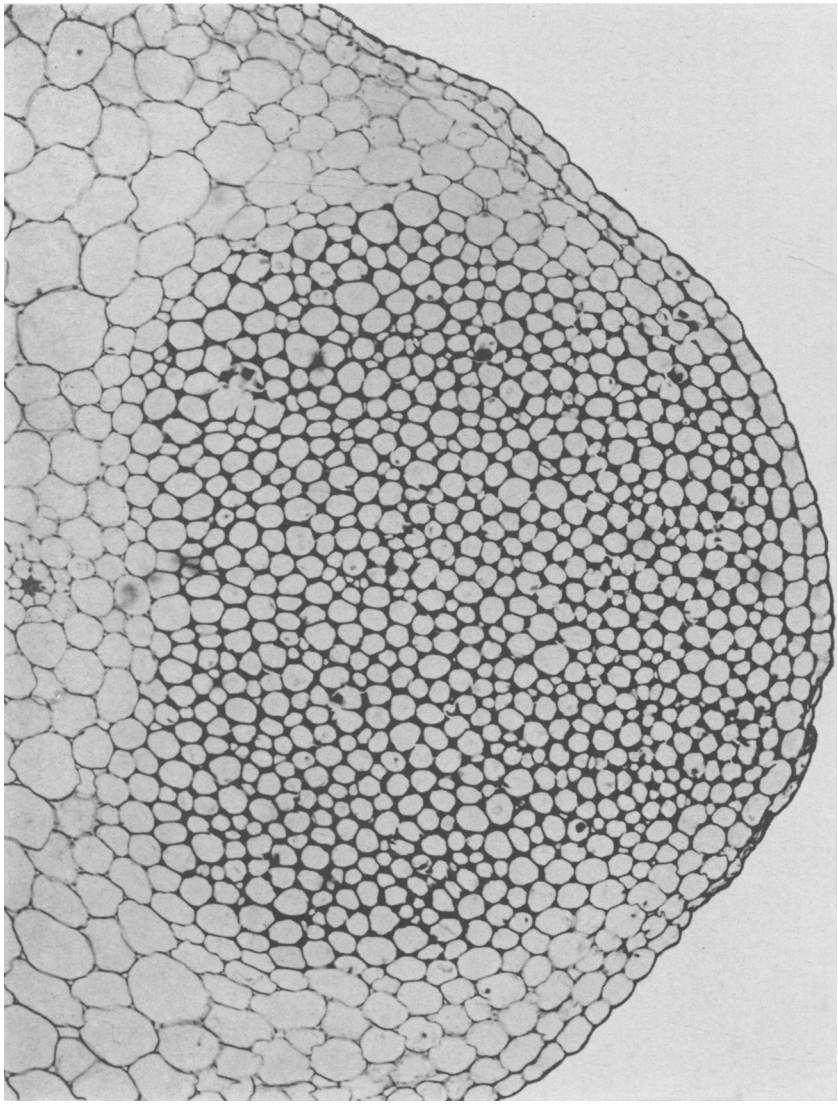

Plate 6.—Transverse section of a collenchyma strand from a petiole that had ceased to elongate. (× 90.)

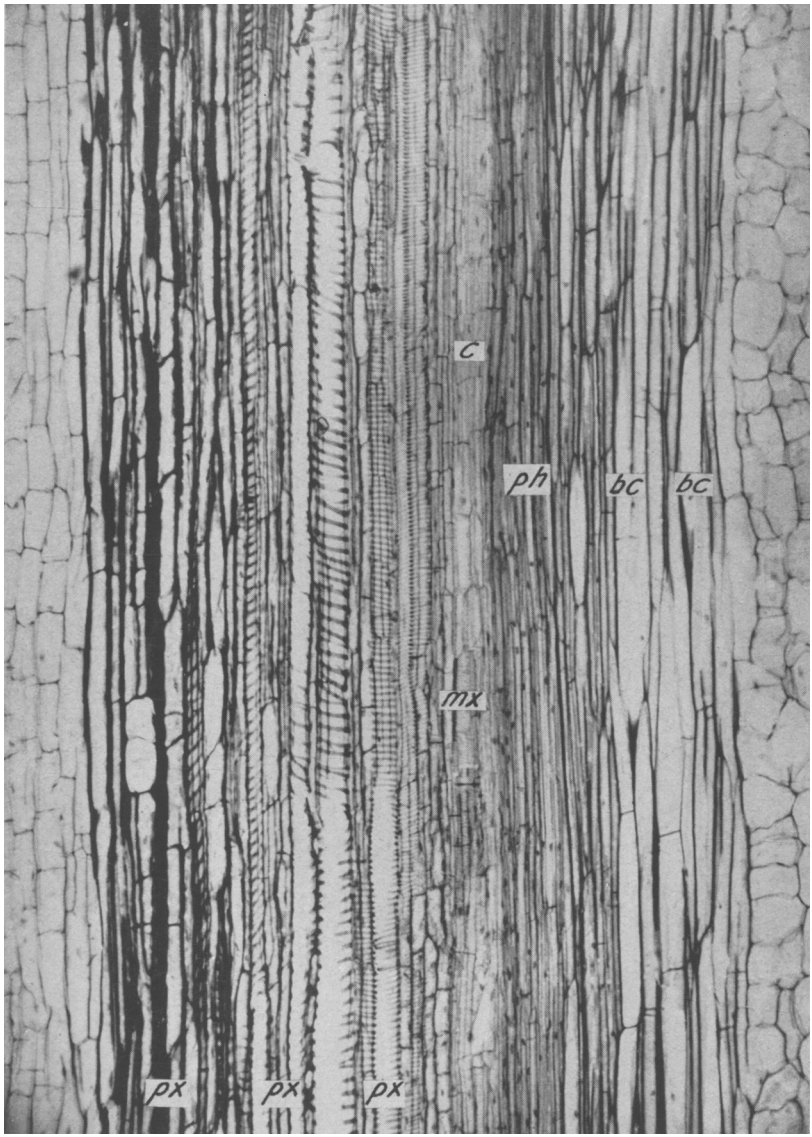

Plate 7.—Longitudinal section of a vascular bundle at a stage of development similar to that in plate 5. Tissues from left to right: parenchyma, old protoxylem (*px*), functioning protoxylem (*px*), differentiating metaxylem (*mx*), cambium (*c*), phloem (*ph*), bundle cap (*bc*), and parenchyma. (× 90.)

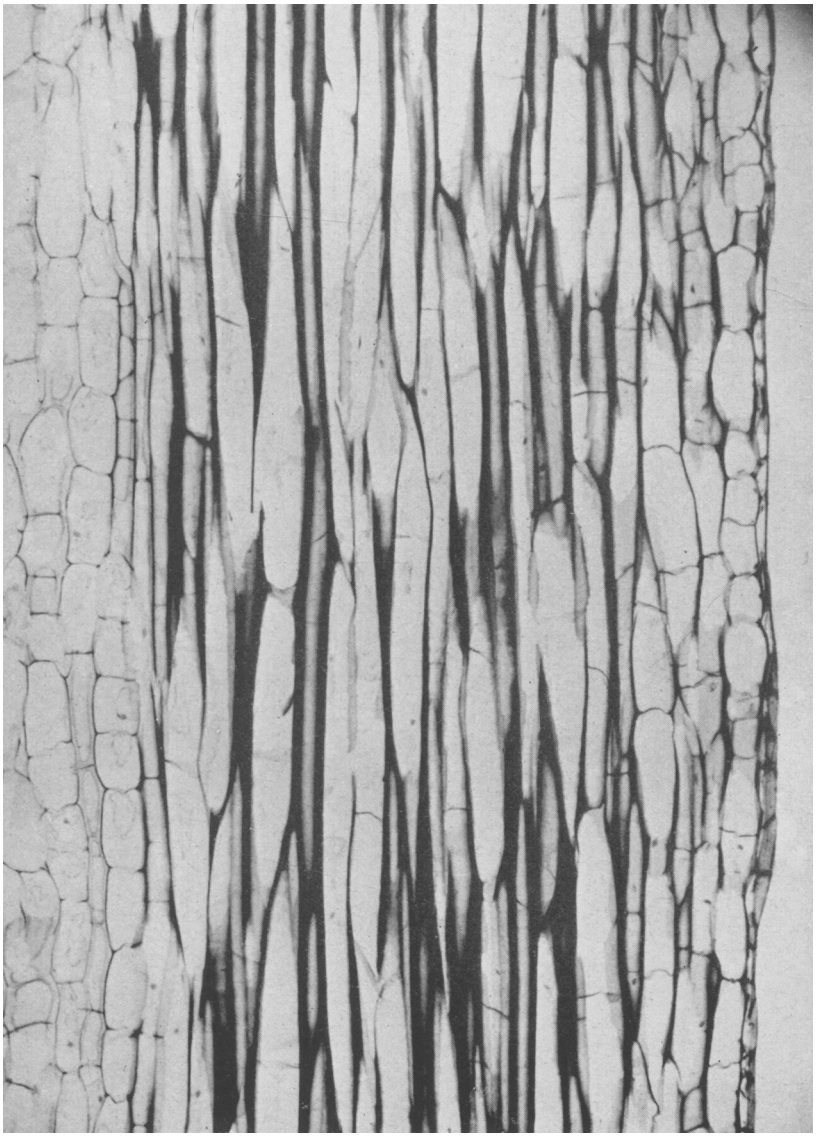

Plate 8.—Longitudinal section of a collenchyma strand at a stage of development similar to that in plate 6. (× 90.)