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ONTOGENY AND STRUCTURE OF THE PHLOEM OF TOBACCO[1]

KATHERINE ESAU[2]

INTRODUCTION

THIS STUDY ON PHLOEM ANATOMY of tobacco was originally intended to serve as a basis for certain projected investigations on the host-virus relations in the curly-top disease. During the work, however, the discordant statements discovered in the literature concerning some problems of histogenesis and nomenclature prompted a more detailed developmental study than at first contemplated.

The principal problems considered were as follows: the origin and development of external and internal phloem; the distinction between the primary and secondary phloem; the comparative structure of the mature and the degenerating phloem; and the origin of the so-called "pericyclic fibers" that occur on the periphery of the phloem in mature stems. The study of development and structure of the sieve tubes, though secondary, furnished information about the morphologic characteristics of these elements in phloem of different stages of development.

The material for study was limited to petioles and stems of two species of tobacco: *Nicotiana tabacum* L. and *Nicotiana glauca* Graham. The first species was selected as an experimental plant because it is used in many virus studies; the second as a plant used, together with *N. tabacum,* in certain important curly-top investigations. Moreover, for studying secondary phloem, *N. glauca,* being a woody shrub, was more suitable than the herbaceous *N. tabacum.*

The healthy phloem of Solanaceae, and of *Nicotiana* in particular, has been studied by several investigators. Crafts (1934) described the ontogeny of the sieve tubes and the structure of mature phloem of *N. tabacum*

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and *N. glauca,* but without giving any information on the ontogeny of the tissue as a whole. De Toni and Paoletti (1891) and Avery (1933*a*, 1933*b*) include some incidental information on the phloem of *N. tabacum.* Two articles by Artschwager (1918, 1924) describe the development and structure of the phloem of potato, but lack detail regarding the early ontogeny of this tissue. Information on the phloem of Solanaceae is also available in the comparative works on the internal phloem (Vesque, 1875; Petersen, 1882; Hérail, 1885; Weiss, 1883; Lamounette, 1890; Baranetzky, 1900), the pericycle (Morot, 1885; Léger, 1897*b*), and the sieve tube (Russow, 1882*a*, 1882*b*; Fischer, 1885*b*, 1886; Lecomte, 1889).

The specific contributions to the knowledge of the phloem of Solanaceae by the investigators mentioned above have been considered in the main part of this paper.

In view of its wide scope, the present investigation has been divided into several distinct topics, each accompanied by a pertinent review of literature.

The discussion following the main part of the paper brings out general histogenetic problems particularly worthy of future study.

MATERIAL AND METHODS

The younger specimens of *Nicotiana tabacum* and *N. glauca* were grown from seed in a greenhouse and outside; but the old *N. glauca* plants were sampled in their natural habitat.

Most of the material was killed in chrom-acetic-formalin solution prepared as recommended by Rawlins (1933). This killing agent caused considerable shrinkage of collenchyma and phloem walls. To obtain sections with walls of more nearly normal thickness, a certain amount of material was killed in a formalin-acetic-alcohol solution with a high percentage of acetic acid (Rawlins, 1933, p. 14, solution No. 2).

In preparation for imbedding in paraffin the material was washed in 70 per cent ethyl alcohol and was then passed through an ethyl-butyl-alcohol series. Certain samples from old stems were washed and dehydrated in dioxan (Esau, 1936).

To facilitate the sectioning of old stems on the microtome, pieces of imbedded material were soaked in water for at least 24 hours before cutting. This treatment was necessary after the dioxan as well as the ethyl-butyl-alcohol dehydration.

Heidenhain's iron-alum-hematoxylin method of staining was used almost entirely. After fixation with chrom-acetic-formalin, this stain clearly brought out both the walls and protoplasts, if the destaining was not carried too far. After fixation with formalin-acetic-alcohol the phloem walls tended to stain lightly.

The dehydrating agents also affected the results of staining. The outlines of cytoplasmic structures and of the walls were considerably sharper after the ethyl-butyl-alcohol than after the dioxan treatment.

The principal merit of the dioxan dehydration in this study was in reducing the distortion of tissues with large intercellular spaces.

The paraffin material was sectioned with Gillette blue safety-razor blades, 10 microns thick, on a Spencer rotary microtome.

For a rather detailed study of sieve tubes, particularly of the callus deposits on the sieve plate, the classic sieve-tube-callus stain, anilin blue (Wilhelm, 1880; Russow, 1881), was employed. Fresh sections were first treated with iodine-potassium-iodide solution, then stained with anilin blue, and finally washed and mounted in a glycerin mounting medium. Anilin blue used alone was satisfactory for the location of the callus; but iodine was necessary for studying cytoplasmic structures.

Drawings were made from anilin blue as well as from paraffin material; the photomicrographs only from permanent slides. Most illustrations were made from material fixed in chrom-acetic-formalin solution and stained with iron-alum-hematoxylin. When other combinations were used in preparing material for illustrations, the reagents employed are indicated in the legends.

The equipment for photomicrography consisted of a Zeiss research microscope with the 9 × Winkler-Zeiss photo-ocular, the Zeiss low-voltage microscope lamp, and the Zeiss Miflex camera attachment with a 6½ × 9 cm camera. Eastman commercial panchromatic films were used as successfully as the Wratten M plates.

GROSS ANATOMY OF THE VASCULAR SYSTEM

Since *Nicotiana tabacum* and *N. glauca* show marked similarity in the structure of their phloem, the two are described jointly, except when an observation is available with one species but not with the other.

In a transverse section of an internode, the vascular tissues of a one-year-old tobacco stem are composed of the following layers: a continuous ring of xylem; the external phloem, separated by cambium from the xylem; and the internal phloem, lying around the periphery of the pith next to the primary xylem (fig. 1, *A* and *B*). The single vascular bundle of the leaf trace is crescent-shaped, with the phloem tissue practically surrounding the xylem (fig. 1, *C*).

In the primary condition the external phloem consists of groups of small cells separated from each other by large parenchyma cells (plate 3, *A*). These groups consist of sieve tubes, companion cells, and phloem-parenchyma cells. The large cells usually lie along the same radii as the parenchyma cells of the xylem and in their regular radial arrangement

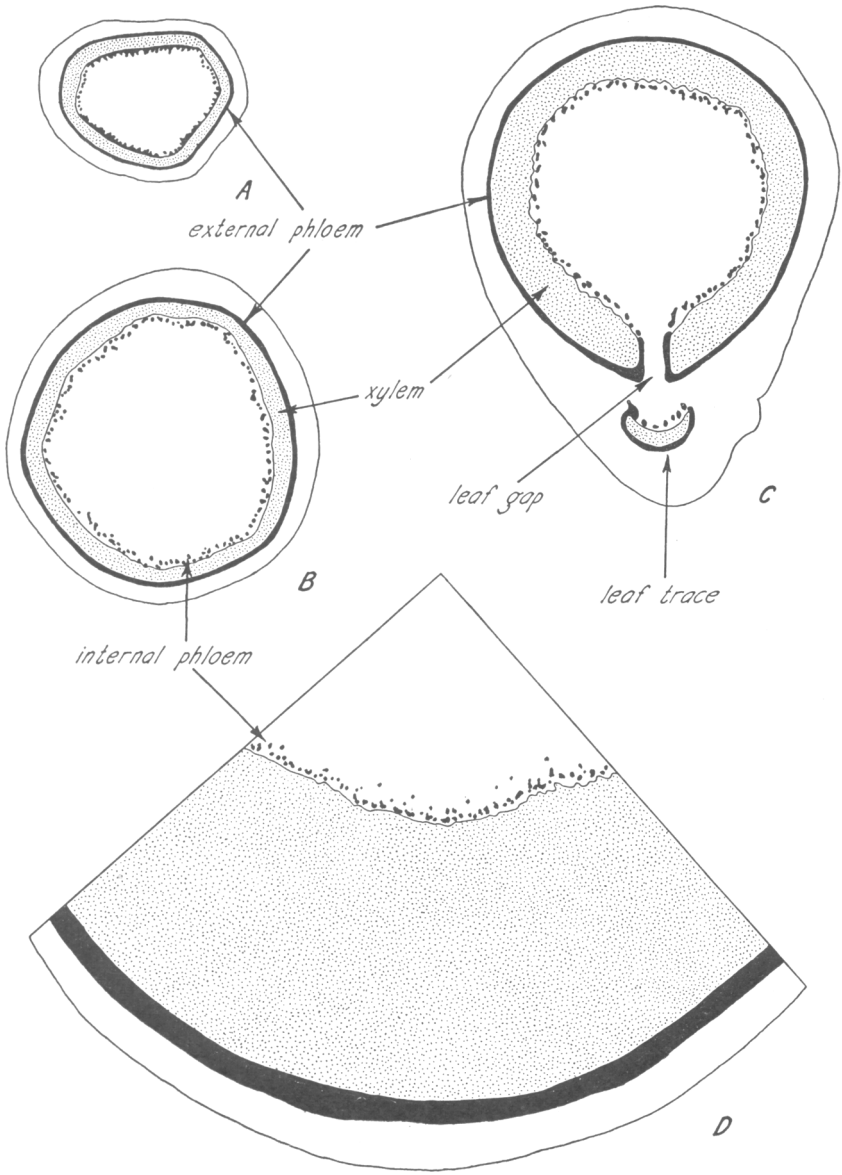

Fig. 1.—*A–C*, Transverse sections of *Nicotiana tabacum* stem sampled at the end of the first year of growth; *A*, internode nearest the inflorescence; *B*, ninth internode below *A*; *C*, eighteenth node below *A*, showing continuity of external and internal phloem in the leaf-gap region. *D*, Portion of a transverse section of a two-year-old stem taken 1 foot above the ground level. All fresh unstained sections. (All × 3.5.)

resemble rays (plate 3, *A*). These cells are called "primary rays" in the present paper.

In longitudinal sections the groups of smaller cells appear like strands passing through large-celled parenchyma and anastomosing with each other.

The external phloem is increased in amount through the formation of secondary phloem by the vascular cambium. In this phloem, sieve tubes, companion cells, and phloem-parenchyma cells are arranged in radial rows instead of groups, several rows together forming wedge-shaped masses with bases near the cambium. The triangular masses are separated from each other by secondary rays lying on the same radii as the primary rays (plate 6, *A*). New secondary rays are formed within the wedge-shaped masses as these become wider. When the cells of the new rays enlarge, the phloem appears broken up into radial strands (plate 13).

In the internal phloem the sieve tubes, companion cells, and phloem parenchyma form groups farther apart from each other than similar groups of the primary external phloem (plates 3, *A* and *B*, and 4, *A*). The internal phloem forms anastomosing strands when viewed in longitudinal sections (plate 5, *A*).

Whereas the external phloem develops, through the activity of the vascular cambium, into a rather continuous layer of tissue, with rays as an integral part of the system (plates 6, *A* and 7, *A*), the internal phloem always remains divided into discrete strands (plates 6, *B* and 7, *B*).

The internal and the external phloem are delimited by fibers on their free margins—on the outer in the external phloem, on the inner in the internal (plates 4, *A*; 6, and 7). In petioles these fibers usually do not reach their full development and remain rather thin-walled (plate 3).

The external and the internal phloem of the stem are connected with each other through the leaf-gap region (fig. 1, *C*). In two-year-old branches of *Nicotiana glauca* this gap is closed, and the continuity of the two kinds of phloem is completely broken.

During growth in thickness the xylem develops much more rapidly than the phloem (fig. 1). In young stems of tobacco the inner phloem appears comparable in amount to the outer (fig. 1, *A*), but during secondary growth the external phloem becomes much more massive than the internal (fig. 1, *D*).

According to certain workers, the internal phloem is usually better developed quantitatively than the external (Vesque, 1875; Petersen, 1882; Scott and Brebner, 1889, 1891; Perrot 1899, p. 167; Artschwager, 1918); these observations referred, however, to stems in primary state of development.

The extent of the external phloem of the stem is increased by the vas-

cular cambium throughout the life of the tobacco plant. No continuous cambium is associated with the internal phloem, but this tissue increases in amount through cell division on the abaxial side of each phloem group and through formation of new groups.

The longevity of the internal phloem was not determined. In samples examined it was still growing at the end of the second year.

Petersen (1882) was able to follow actively growing internal phloem into a six-year-old twig of *Lycium*.

ORDER OF APPEARANCE OF THE FIRST PHLOEM AND XYLEM ELEMENTS IN THE APEX OF THE SHOOT

As is well known, the differentiation of the vascular tissue of the stem is intimately connected with the development of leaves. The first differentiated vascular elements of the stem appear in localized regions that constitute the leaf traces.

The order of development of the xylem of a leaf trace became known before that of the phloem, because the xylem elements were usually recognized more easily than sieve tubes. With dicotyledons, apparently, differentiation of leaf-trace vessels often commences at the entrance of the trace into the leaf, or in the leaf itself, and then gradually extends downward into the stem and upward into the apex of the primordium (Trécul, 1881, 1891; Weiss, 1883; De Bary, 1884, p. 391; Priestley and Swingle, 1929).

Judging from literature examined thus far, the differentiation of the phloem of a trace has rarely been considered. Baranetzky (1900) wrote that in contrast to vessels, sieve tubes differentiate almost simultaneously over the entire extent of the trace. Chang (1935) found that the first sieve tube of a given leaf develops in acropetal direction, from the stem toward the leaves.

Considerable information is, however, available on the relative time of appearance of the first phloem and first xylem elements in a given region. As early as 1863, Sanio reported that in stems certain phloem elements differentiate before the xylem. Somewhat later (1865) he identified these elements as sieve tubes and cambiform.

Russow (1872) and Léger (1897*b*) found the appearance of phloem before xylem a general characteristic of stems of cryptogams as well as phanerogams. Chang (1935) stated that the development of the vascular tissue in a leaf primordium begins with the differentiation of a sieve tube. In roots also, sieve tubes mature first among vascular elements (Russow, 1872; Lesage, 1891; Chauveaud, 1897, 1900).

The approximate distance from the growing point of the first differentiated sieve tube has been determined in certain roots. In the rye,

Chauveaud (1897) found the first mature sieve tube 1 mm from the growing point. Crooks (1933) observed such an element 0.4 mm from the promeristem in a flax-seedling root. The distance between the growing point and the first mature sieve tube in lateral root tips of sugar beet was about 0.4 mm in a healthy plant and about 0.2 mm in a curly-top-diseased plant (Esau, 1935).

As far as the writer is aware, the spatial relation of the first sieve tube to the growing point of the shoot has not been considered in literature.

Students on internal phloem agree that this tissue differentiates more or less late as compared with the external phloem (Weiss, 1883; Hérail, 1885; Lamounette, 1890; Léger, 1897*b*; Baranetzky, 1900; Avery, 1933*b*). Some of these papers also indicate that protoxylem frequently differentiates before the internal phloem (Weiss, 1883; Léger, 1897*b*; Baranetzky, 1900). According to Avery (1933*b*), the first xylem and external phloem are conspicuous in a tobacco-leaf primordium that is a little over 1 mm in length, whereas the internal phloem appears in a leaf approximately twice this length. Even in the Cucurbitaceae, where the internal phloem is closely associated with the rest of the vascular tissues, the first internal sieve tube is preceded by a protoxylem element and an external sieve tube, in the order named (Léger, 1897*b*).

In contrast to other workers, Artschwager (1918) reported that in the potato stem internal sieve tubes differentiate before the external and that protoxylem matures before the protophloem. The accuracy of this observation may be questioned, however, because the first stages of vascular differentiation are incompletely described.

In the present study on the order of differentiation of the first phloem and xylem elements in tobacco, serial transverse sections of shoot tips of the main axes and of the lateral branches served as material for observation.

Figures 2 and 3 show the differentiation of the vascular elements in the main stem tip and young leaves of a three-months-old plant of *Nicotiana tabacum* grown in a greenhouse.

The section in figure 2, *A*, was cut through the promeristem of the growing point. The latter appears in the center of the figure and the successively older leaves are spirally arranged around the stem tip.

Besides the numbered leaves, a primordium 30 microns in height was present above leaf 1. The trace of this primordium is evident in the stem, between leaves 3 and 5, in figure 2, *C*. The lengths of the leaves numbered 1 to 5, beginning with the smallest, were as follows: 90, 160, 290, 410, and 560 microns. The larger leaves were not measured.

The dots and circles correspond in number to the sieve tubes and xylem elements that have been identified with certainty. The only sieve tubes

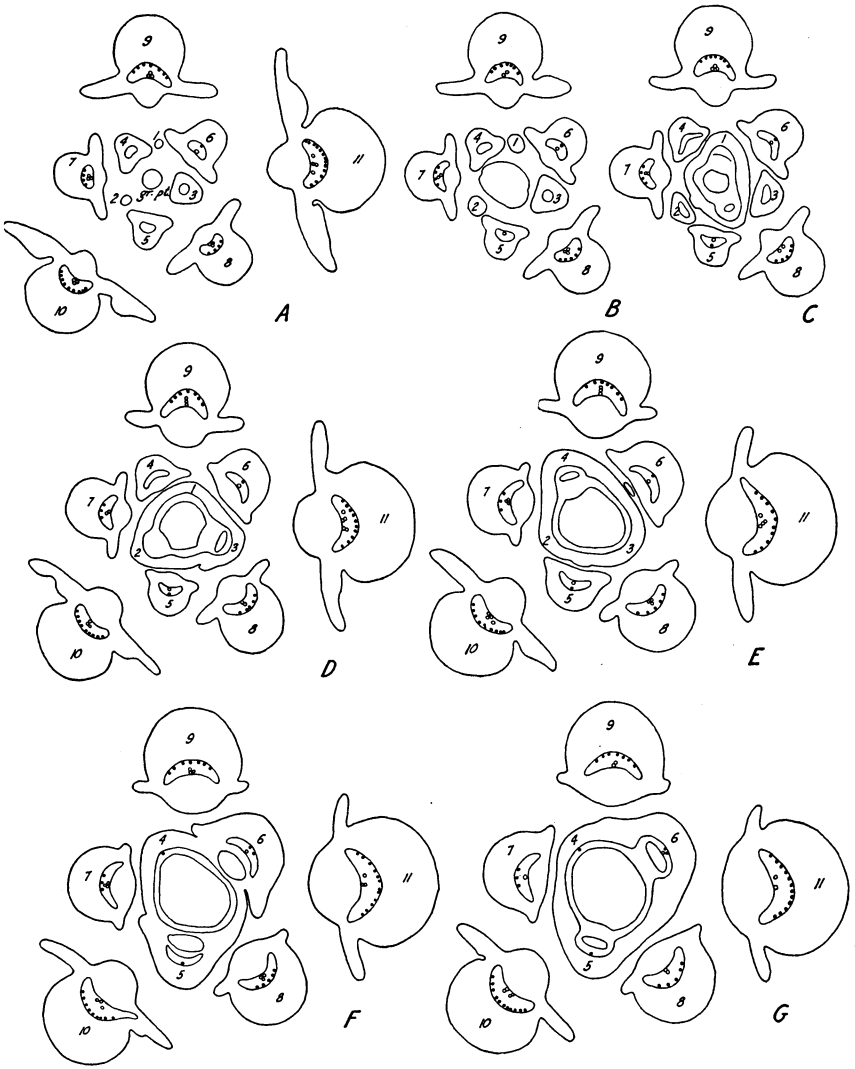

Fig. 2.—Successive transverse sections through apex of a three-months-old *Nicotiana tabacum* plant, showing the stem and young leaves. *A*, Section through the growing point (*gr. pt.*). The succeeding sections were taken the following number of microns below the growing point: *B*, 40; *C*, 110; *D*, 190; *E*, 260; *F*, 300; *G*, 340. The areas outlined within the leaves and stem indicate the procambium regions. The sieve tubes are represented by dots; the xylem elements by circles. (All × 23.5.)

counted were those that showed the characteristic scarcity of contents and thickened walls of differentiated sieve tubes. The recorded xylem elements had secondary walls and were free of protoplasts.

Henceforth, for the sake of brevity, the xylem elements will frequently be called vessels, although the dissolution of end walls has not been verified. The first xylem elements in tobacco leaves usually have spiral secondary thickenings.

In the plant shown in figures 2 and 3, leaves 1 to 5 contained no vascular elements at the level of the growing point (fig. 2, *A*). Leaf 6 had one sieve tube and one xylem element. Mature vascular elements became successively more numerous in progressively older leaves, the number of sieve tubes increasing much more rapidly than that of vessels.

At 40 microns below the growing point (fig. 2, *B*) leaf 5 had one mature vessel and one immature sieve tube which is not indicated in this figure. At 110 microns below the growing point, leaf 5 showed two fully differentiated vascular elements: one vessel and one sieve tube (fig. 2, *C*).

Leaf 1 had disappeared as a discrete structure in the section shown in figure 2, *C*, but the buttress and trace of this leaf were still evident. At successively lower levels leaves 2, 3, and 4 became fused with the stem. The traces of leaves 2 and 3 appear in figure 2, *D;* that of leaf 4 in figure 2, *E*. None of these traces contained mature vascular elements in these sections.

At 300 microns below the growing point, a sieve tube became discernible in the trace of leaf 4 (fig. 2, *F*). From here this sieve tube was traced down to the 670-micron level (fig. 3, *D*), where it became part of the group of sieve tubes of the trace of leaf 7.

Figure 2, *F*, shows fusion of leaves 5 and 6 with the stem. The mature xylem element has disappeared from the trace of leaf 5. An immature vessel containing a protoplast, though still discernible in this trace, also disappeared at a lower level. The entire length of the mature xylem element of leaf 5 was about 250 microns.

Leaf 6 shows two mature sieve tubes in figure 2, *F* and *G*. These elements further increase in number in sections of figure 3 and appear to the right and left from the first mature element until they form a continuous row with the sieve tubes of the traces of leaves 9 and 11 (fig. 3, *F*). The trace of leaf 5 shows one sieve tube in 3, *B*, three in 3, *D*. The number of sieve tubes of trace 4 increases to two in figure 3, *B*.

In contrast to the sieve tubes, the number of vessels remains about the same in sections of figure 3 except for the appearance of the numerous vessels of leaf 12 in figure 3, *F*. The sieve tubes are first arranged in groups (fig. 3, *C* and *D*), but in older sections they are rather uniformly distributed around the periphery of the procambium ring (fig. 3, *F*).

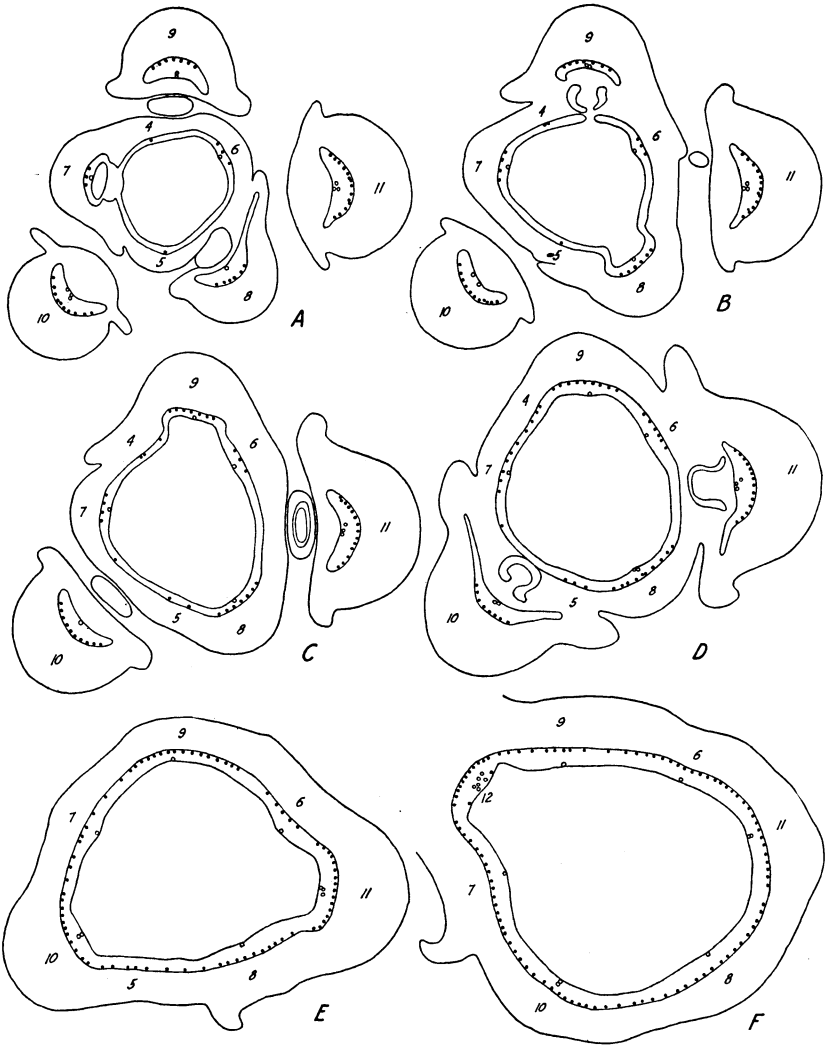

Fig. 3.—Successive transverse sections through the same plant as in figure 2. The sections were taken the following number of microns below the growing point: *A*, 430; *B*, 510; *C*, 580; *D*, 670; *E*, 830; *F*, 950. Regions and elements are designated as in figure 2. (All × 23.5.)

The vessels remain localized in the first points of origin (fig. 3, *F*) until the stem is considerably older.

The set of sections of *Nicotiana tabacum* shoot represented in figures 2 and 3 clearly show the contrast between the xylem and phloem in the order of differentiation of the first elements. As illustrated by leaf 5, the first vessel of a foliar trace appears in the primordium independently of

the xylem of the stem, whereas the first sieve tube (leaf 4) differentiates acropetally, from the stem into the leaf. The gradual decrease in number of sieve tubes of a trace at successively higher levels (leaf 6) also indicates upward differentiation of the phloem of a leaf trace. At lower levels, traces of leaves 4 and 5 show that the first sieve tubes differentiate in advance of the first vessels; but near the apex of leaf 5 the vessel is somewhat ahead of the sieve tube. In other words, in the stem proper the first sieve tubes differentiate before the vessels.

The internal phloem conspicuously lags behind the external in time of appearance. Leaf 9 was the first to show meristematic groups of the internal phloem. Immature internal sieve tubes were evident in leaf 11; the first mature elements in leaf 12. A comparison of figures 2, *F* and 3, *F*, shows that within the stem proper the distance from the growing point to the first external sieve tube equalled about one-third of the distance to the first internal sieve tube.

The results obtained with *Nicotiana tabacum* on the order of development of the first vascular elements were checked by the use of three shoot tips of *N. glauca,* taken from well-developed young lateral branches of a plant that was growing in natural habitat.

The observations on the shoot tips of *Nicotiana glauca* confirmed the general results obtained with *N. tabacum*. The numerical values obtained with one of these shoots were used in constructing the diagram in figure 4. Here the relative height of leaves and length of the vascular elements were drawn to scale. The term *vessel* designated spiral elements that had fully developed secondary walls, but were not necessarily free of protoplasts. Elements marked as *sieve tubes* had attained maximum diameter and maximum wall thickness and showed scarcity of cytoplasm. *Immature vessels* had no secondary walls but showed the characteristic vacuolation of the protoplast. *Immature sieve tubes,* although with comparatively thick walls, had dense protoplasts.

Only one sieve tube and one xylem element were drawn in each leaf of figure 4, because the addition of others would have greatly complicated the diagram. Information on the increase in number of elements in successively older leaves of a similar shoot was previously given in figures 2 and 3.

Figure 4 shows a mature sieve tube in the stem, 324 microns below the growing point. This sieve tube belongs to the trace of leaf 4. An immature portion of the sieve tube occurring above the mature indicates upward differentiation of this element.

In leaf 5 the sieve tube has differentiated to the place of insertion of this leaf on the stem. Immature sieve-tube elements extend for some 275 microns into the leaf. At the insertion of the leaf at the highest point

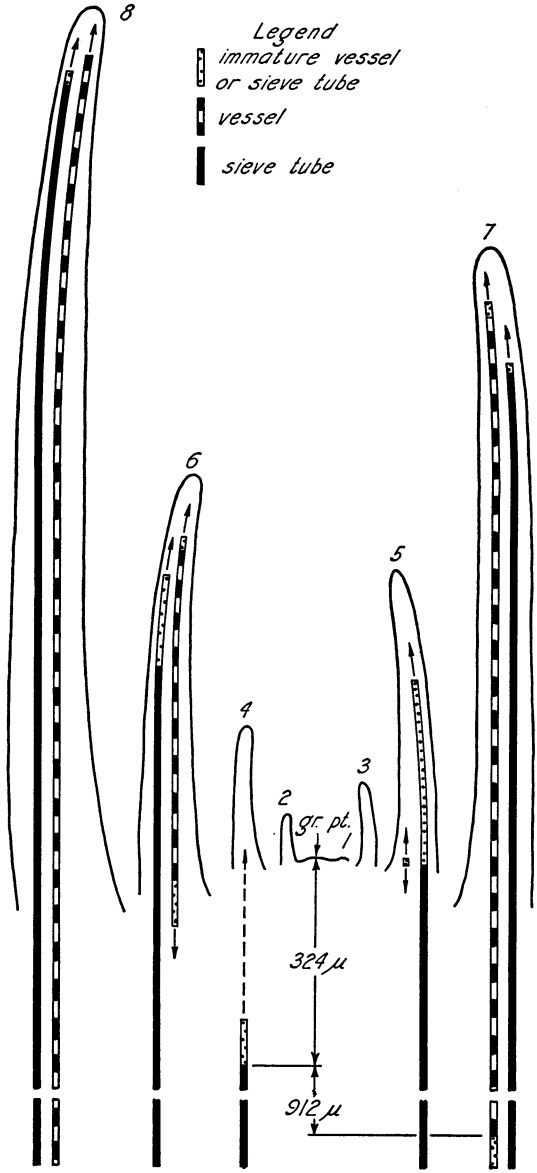

Fig. 4.—Diagram of the apical region of a *Nicotiana glauca* plant, showing the relative height of the leaves and the position of the first sieve tube and of the first vessel in each leaf. The phyllotaxy has been disregarded, but the leaves were placed at their proper levels below the growing point (*gr.pt.*). The leaves, beginning with the youngest, measured the following number of microns in length: 5, 82, 132, 220, 476, 660, 1032, 1424. (×103.)

reached by the mature sieve tube, the first protoxylem element commenced to vacuolate.

Leaf 6 shows considerable progress in the development of the vessel. This element has differentiated mainly upward and, in so doing, has left the sieve tube slightly behind. The vessel of leaf 6 was, however, not yet fully developed. Though it had secondary walls it apparently contained protoplasts throughout its length. The upward differentiation of the

TABLE 1

LEAF SIZE AND DIFFERENTIATION OF FIRST VASCULAR ELEMENTS

Species	Length of leaf that contains:		Distance of the first sieve tube from the growing point
	First sieve tube in its trace	First xylem element	
	microns	*microns*	*microns*
Nicotiana tabacum	410	560	300
Nicotiana glauca	220	476	324
	340	552	240
	284	462	352

sieve tube of leaf 6 made progress in comparison with leaf 5, but the immature portion decreased in length. This decrease apparently indicates a retardation in the rate of differentiation.

The spiral element of leaf 7 had secondary walls down to 1,236 microns below the growing point and was free of protoplasts for most of this length. Below this level, mother cells with primary walls replaced the vessel. These immature but highly vacuolate cells were discernible to the last section in the series, some 2,000 microns below the growing point.

As far as could be ascertained, the vessel in leaf 8 had established a connection with the xylem of the stem. At 600 microns below the growing point it was connected laterally with another element; then the second element alone was perceptible to the last section in the series.

The two other shoot tips of *Nicotiana glauca* used in this study gave results similar to those depicted in figure 4. They showed upward development of the sieve tubes of leaf traces; independent origin of leaf-trace vessels; the slightly more rapid acropetal differentiation of vessels as compared with sieve tubes. Table 1 shows that all three stem tips of *N. glauca* and the one shoot of *N. tabacum* yielded comparable values with regard to distance between promeristem and nearest sieve tube within the stem, and to size of leaves with first vessel elements.

As in *Nicotiana tabacum,* the internal phloem of *N. glauca* develops later than the external phloem and later than the first xylem of a given trace or leaf.

ORIGIN OF THE PHLOEM

External Phloem.—The primary phloem is usually considered to arise from procambium; the secondary from the vascular cambium. According to the common concept (Eames and MacDaniels, 1925, p. 87 and 130), the procambium is a rather massive tissue characterized by a more or less disorderly arrangement of cells; the cambium, on the contrary, forms a narrow zone between the xylem and phloem, shows a predominance of tangential longitudinal divisions, and gives rise to radially seriated derivatives.

Yet many works on ontogeny of vascular tissues show that, as a rule, the meristem forming the first vascular tissues in stems and leaves is characterized by a predominance of periclinal divisions, so that the resulting "primary tissues" may be arranged in an orderly manner. This method of development occurs in gymnosperms (Koch, 1891; Strasburger, 1891, p. 90) and monocotyledons (Strasburger, 1891, p. 90; Dauphiné, 1917), as well as dicotyledons (Sanio, 1863; Strasburger, 1887; 1891, p. 226; Gidon, 1900; Decrock, 1901; Bouygues, 1902; Kostytschew, 1922, 1924; Thoday, 1922; Carstens, 1931; Priestley and Scott, 1936; Esau, 1936).

A particularly detailed analysis of the origin of primary tissues is given by Chauveaud (1911), who considers that the alternate (or radial) and the superposed (or collateral) arrangements of vascular tissues belong to different evolutionary stages. The alternate arrangement, more primitive from the evolutionary point of view, occurs in the root but is omitted in stem and leaf. In the root, superposed elements appear after the alternate; in the stem, vascular bundles are collateral from the beginning of differentiation. In all organs, stems, leaves, and roots, the superposed elements are produced by a meristem whose cells are arranged like those of a typical cambium; the alternate, on the other hand, is derived from procambium. Chauveaud therefore considers that the superposed elements are of secondary origin and that, from the ontogenetic point of view, stems and leaves have no primary vascular tissues.

Dauphiné (1917), accepting Chauveaud's interpretation, finds it applicable to monocotyledons as well as dicotyledons and gymnosperms.

The origin of the so-called "primary tissues" from a cambiumlike meristem has been noted in the xylem more frequently than in the phloem. Certain workers have even emphasized that orderly divisions were initiated after the appearance of the phloem (Sanio, 1863; Bouygues, 1902; Thoday, 1922). Not all primary phloem, however, arises before tangential divisions begin to predominate in the meristem: the metaphloem may resemble secondary phloem in radial arrangement of cells (Strasburger. 1891, p. 90, 261–62).

Although meristems forming the so-called primary and secondary vascular tissues may show similar radial arrangement of cells, they differ from each other in certain respects.

As is well known, the cambium of a species that has much secondary growth displays a certain complexity of structure, caused by the presence of ray and fusiform initials. The meristem giving rise to primary tissues, on the other hand, is more homogeneous in structure than the cambium of old stems.

Gidon (1900, p. 32–34) points out that the cells of procambium are polygonal, those of the cambium rectangular, in transverse sections. He also mentions that the derivatives of the procambium are, in general, individualized for some time before they differentiate into mature elements, whereas in cambium the differentiation takes place close to the initiating layer.

Sanio (1873–74) and Strasburger (1882, p. 39–40) report in the cambium a marked difference between the thick radial and the thin tangential walls, a contrast not characteristic of the procambium.

Strasburger (1891, p. 285, 318) infers that growth termed secondary begins after elongation of an organ is completed. Elements differentiating during elongation differ structurally from those formed without longitudinal stretching. This is particularly evident in the xylem.

The review above might suffice to indicate that, along with similarities, the vascular meristems in the younger and older parts of the plant display certain differences in structure and method of tissue formation that cause marked contrasts between the resulting mature tissues. At the same time, it shows that further comparative studies on genesis of vascular tissues must precede any reëvaluation of the terms *procambium, cambium, primary tissues,* and *secondary tissues.*

Lacking a sure basis for such a reëvaluation, this paper will retain the old terminology, with the understanding that procambium may resemble cambium in producing radial series of cells, but that certain differences in structure and development justify the separate treatment of the two forms of vascular meristem and of the tissues they produce. At the same time procambium and cambium are regarded not as two distinct meristems, but as two developmental stages of the vascular meristem.

The tobacco exemplifies a genus in which tangential divisions are evidenced in the early ontogeny of the vascular tissues, particularly of the xylem. When the first sieve tube and the first xylem element are perceptible in a petiole (fig. 5, *A*) or in a portion of a stem (fig. 6, *C*) the procambium cells are characterized, in transverse sections, by comparatively small size and by dense protoplasts. These cells are still no more orderly arranged than the cells of the adjacent ground meristem. But in further

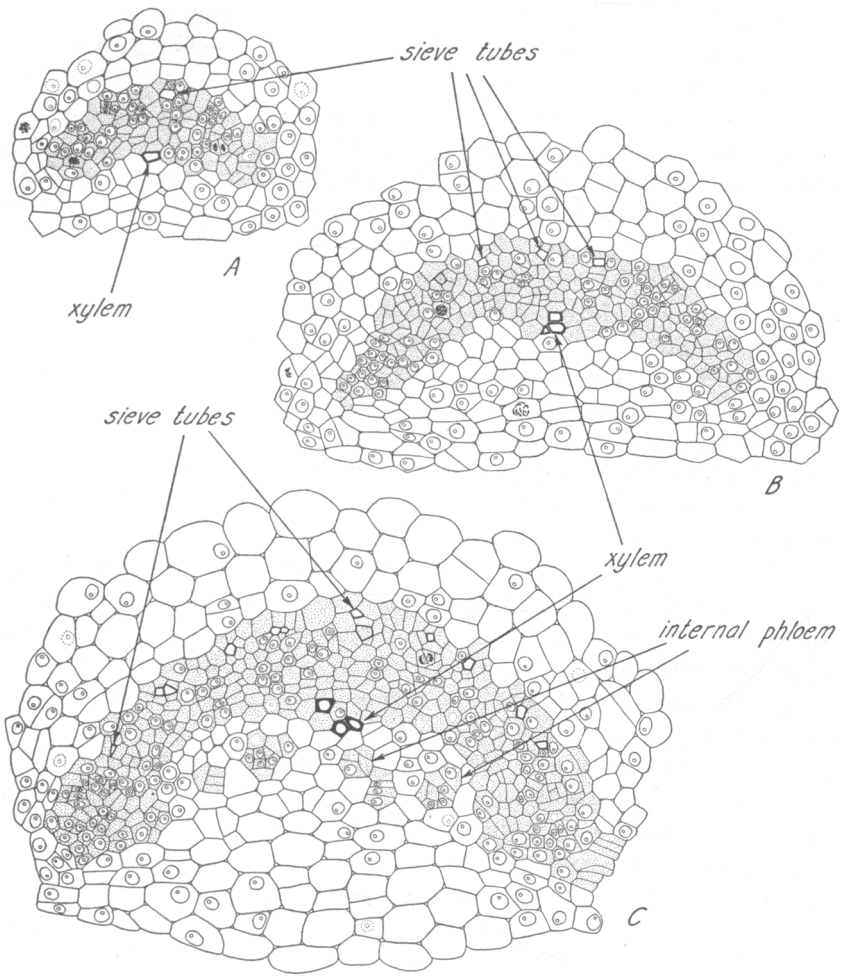

Fig. 5.—Transverse sections through median portions of *Nicotiana tabacum* petioles, showing three stages of differentiation of vascular tissues (stippled areas). *A*, Section from leaf 5 of figure 2, *D* with first sieve tube and first vessel in the procambium strand; *B* and *C*, sections from leaves 7 and 10 respectively, taken between sections *D* and *E* of figure 2; *C*, depicts initiation of the internal phloem. Immature sieve tubes are stippled. (All × 239.)

differentiation of protoxylem the new vessel mother cells appear in the same radial rows with the preceding (figs. 5, *B* and *C*, and 6, *D*). This tendency toward radial seriation is first displayed in the portion of the procambium that lies between the first sieve tube and the first xylem elements (fig. 5, *C*). Then it appears right and left from the initial position, closely following the differentiation of sieve tubes on the abaxial side of the procambium region.

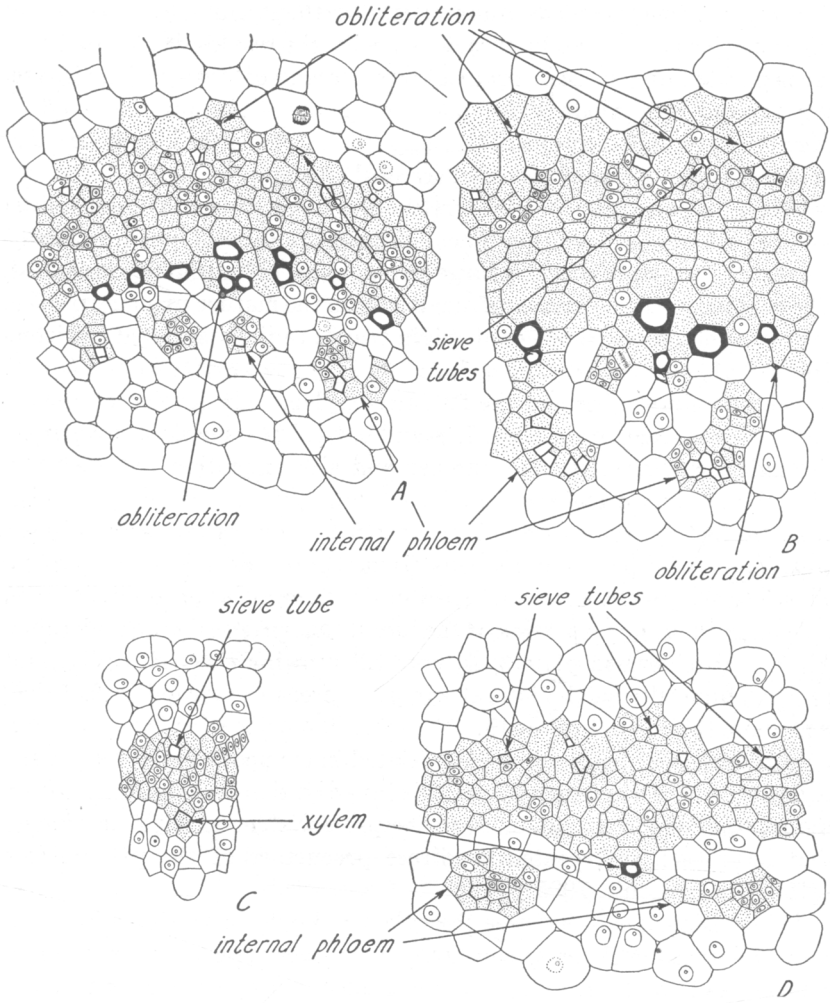

Fig. 6.—Transverse sections through portions of petioles (*A* and *B*) and stem (*C* and *D*) of *Nicotiana tabacum*, showing differentiation of vascular tissues (stippled areas). *A*, Section from leaf 12 taken between sections *F* and *G* of figure 2; the trace of this leaf occurs in figure 3, *F*. *B*, Section from the same petiole as was used in plate 2, *A*; *A* and *B* show obliteration of first sieve tubes and vessels. *C*, Trace of leaf 5 from section in figure 3, *A*; it shows the first mature sieve tube and immature vessel. *D*, Trace of leaf 10 taken below the section in figure 3, *F*; initials of the internal phloem are in evidence. Immature sieve tubes and vessels are stippled. (All × 239.)

With advancing development, the predominance of periclinal walls becomes more pronounced (fig. 6, *A* and *B*). Through these tangential divisions mother cells are added to the phloem as well as to the xylem, but the two sorts of mother cells differ in the kind of subsequent divisions that take place in them.

The xylem mother cells differentiate, without further longitudinal divisions, into vessels and parenchyma cells, the two kinds of cells forming separate but continuous radial rows. During differentiation the vessels expand only moderately, not perceptibly distorting the original radial seriation. As a result of this method of differentiation, the mature primary xylem is a very orderly arranged tissue (plate 3, *A*).

Certain phloem mother cells, on the other hand, divide by longitudinal walls of different orientation. These divisions form small groups of cells, separated from each other by large parenchyma cells, radially arranged. The presence of these groups of small cells in the mature primary phloem somewhat obscures evidences of origin from a radially seriated meristem (plate 3, *A*).

The first sieve tubes and the first protoxylem cells develop at a short distance from each other (figs. 5, *A* and 6, *C*), but later they move apart through the increase in the number and size of mother cells between them (fig. 6, *B* and *D*).

In the petiole the procambium strand also grows laterally through addition of new procambium cells from the ground meristem (fig. 5). This lateral growth does not cease until differentiation of xylem and phloem in the median portion of the bundle has progressed considerably. The bundle shown in plate 2, *A*, was still growing through addition of procambium cells on the lateral wings, whereas the strand in 2, *B* had terminated this type of growth.

In the stem the procambium is initiated in connection with leaf traces and early forms a complete ring. This investigation cannot well consider the relation of the procambium to the promeristem or to the meristems that have been interpreted as direct precursors of the procambium (Helm, 1931; Louis, 1935).

The division of cells between the xylem and phloem continues after the stem has ceased to elongate, but the vascular meristem acquires the characteristics of cambium and gives rise to secondary vascular tissues.

In tobacco the procambium and cambium, as defined in this paper, may be differentiated on the basis of structure and methods of cell division.

In tangential longitudinal sections the procambium appears rather homogeneous (fig. 10, *A*), whereas, the cambium shows a striking contrast between short and long initials (fig. 10, *B*). The long initials of the procambium have one or both end walls transverse, whereas those of the cambium are true fusiform initials with two tapering ends (fig. 10, *B*). Radial longitudinal divisions are common in the procambial zone (fig. 10, *A* at *b*); the fusiform initials of the cambium divide by oblique walls (fig. 10, *B* at *a*). The sieve tubes and vessels derived from procambium

are longer than the initial cells; the conducting cells derived from the cambium are not longer than the fusiform initials, the sieve tubes even being much shorter because of transverse divisions in sieve-tube mother cells.

In transverse sections the active cambium is characterized by comparatively thick radial walls and very thin tangential walls (plate 18, *B*). In the procambium the two kinds of walls are less different in thickness (plate 18, *A*). Procambium cells tend to be polygonal (plate 18, *A*); those of the cambium are more nearly rectangular (plate 18, *B*).

The homogeneity of the cambium in transverse sections is disturbed by the appearance of rows of very small cells—the sections of the tapering ends of fusiform initials (plate 18, *B*). This feature is absent in the procambium.

The change from procambium to cambium is gradual, as is the transition between the primary and secondary vascular tissues.

The petioles of not-too-vigorous plants complete their development without cambium and secondary vascular tissues. Such a petiole was used in plate 3, *A*.

In vigorously growing leaves the vascular meristem may develop certain characteristics of the cambium, at the same time continuing to produce, as long as the leaf elongates, vascular tissues with certain primary characteristics. Such a mixture of primary and secondary characteristics was observed in the bundle illustrated in plate 3, *B*. The vascular meristem showed a distinct segregation into ray and fusiform initials, but the vessels continued to develop spiral thickenings.

Internal Phloem.—The early papers on phloem recorded merely the presence of internal phloem in different families (Hartig, 1854; Mohl, 1855; Hanstein, 1864; Schreiber, 1865). Vesque (1875), besides describing new families with internal phloem, also studied the advanced developmental stages of this tissue. De Bary (1884, p. 319) introduced the term *bicollateral bundle* and suggested that the internal phloem constitutes part of the vascular bundles, although in some plant groups, as in Solanaceae, some internal strands appear to be independent.

After De Bary, workers paid considerable attention to the origin of the internal phloem. Petersen (1882), like De Bary, regarded the internal phloem as part of a vascular bundle. Hérail (1885), however, said that this was true only in Cucurbitaceae, whereas in other families, among them Solanaceae, this phloem arose in the pith. Lamounette (1890), on the other hand, decided that even in Cucurbitaceae the internal phloem arose independently from the rest of the vascular tissues—namely, in the parenchyma which is adjacent to but sharply delimited from the procambium.

Several other papers deal rather incidentally with the origin of internal phloem. De Toni and Paoletti (1891) thought that in *Nicotiana tabacum* this phloem arises in the pith. Artschwager (1918) holds it to be of procambial origin in the potato. Avery (1933*b*) considers that the internal phloem of *N. tabacum* arises in the provascular meristem.

Baranetzky's (1900) paper is the most illuminating study on the origin of internal phloem. This worker observed that the procambium is sharply delimited on the outer margin but remains, for some time, rather indefinite on the inner margin. Here new cells are added to the vascular meristem through divisions in the adjacent ground meristem. Thus the procambium becomes thicker even after differentiation of first protoxylem, and the new cells develop into phloem bundles. In other words, the internal phloem is not sharply distinguished from the rest of the vascular tissues with regard to its origin.

The procambium of tobacco also is not clearly delimited from the adjacent ground meristem, and it does not reach its ultimate extent before the differentiation of xylem and phloem is initiated. As is well known, procambium develops from certain other meristematic cells through a rapid succession of longitudinal divisions. Such divisions continue on the adaxial side of the procambium after the external phloem and the protoxylem make their appearance. At this time, the cells adjacent to the procambium on its adaxial side show characteristics similar to those of the ground meristem somewhat farther away and are here interpreted as ground-meristem cells. They are converted into procambium cells through longitudinal divisions. A similar metamorphosis of ground meristem occurs on the lateral wings of a young procambium bundle of a petiole (fig. 5).

Not all ground-meristem cells on the adaxial side of the xylem divide, however. Many remain undivided, enlarge, and vacuolate so that the young internal-phloem strands soon appear like islands of small cells among larger cells (figs. 5, *C* and 6, *D*).

When the primary ray cells within the external phloem enlarge and vacuolate, a considerable similarity is established between the primary external phloem and the internal phloem (plates 2, *C* and 3, *A*).

The ground meristem is not the only source of internal phloem. The cells on the inner margin of the vascular bundles retain their potentiality to produce new phloem strands, apparently as long as the plant lives. These cells do not remain meristematic, however, but enlarge and vacuolate like their neighbors until, at an earlier or later stage of differentiation, they divide and develop into phloem bundles. Some of these cells lie outside the xylem and resemble pith cells; others are parenchyma cells of the primary xylem. Thus in contrast to the external phloem, the

inner phloem is initiated not only in meristematic cells, but also in partially and fully differentiated parenchyma cells.

The internal phloem is commonly regarded as primary in origin (Eames and MacDaniels, 1925, p. 251). The strands arising in mature parenchyma cells are not, however, primary in the usual sense of the term.

ONTOGENY OF THE PHLOEM

Protophloem.—The earliest phloem, the protophloem, consists of small cells. The sieve plates of the sieve tubes are comparatively indistinct. These characteristics explain the failure of certain workers to recognize sieve tubes in the protophloem (Eames and MacDaniels, 1925, p. 90).

The presence of sieve tubes in protophloem is, however, frequently recorded in literature. Russow (1872, p. 4; 1881), originator of the term *protophloem,* concluded that this tissue contained sieve tubes or related elements in all vascular plants. De Bary (1884, p. 325) agreed with Russow. Strasburger (1891, p. 261–62), who used the term *cribral primanen* instead of *protophloem,* recorded sieve tubes with calloused sieve plates in this tissue.

Several French workers (Lesage, 1891; Léger, 1895, 1897*a,* 1897*b;* Chauveaud, 1897, 1900) have studied in much detail certain thick-walled glistening elements that appeared in the phloem as soon as differentiation started. Léger (1897*a,* 1897*b*) and Chauveaud (1897, 1900) found sieve plates in these elements and thus identified them as sieve tubes. Léger (1897*a*) also pointed out that these elements were characteristic of the first phloem of any plant organ.

Among the recent workers, Chang (1935) especially carefully obtained proof of the presence of sieve tubes in the protophloem of stems and leaves of certain dicotyledons. Sieve tubes in the first phloem were also described by Esau (1934, 1935, 1936).

Companion cells are not constant members of protophloem in many plants, particularly in roots (Chauveaud, 1900; Chang, 1935; Esau, 1935). Even if the procambium cell divides shortly before sieve-tube differentiation, the sister cell or cells of the sieve tube may remain indistinguishable from adjacent parenchyma or may differentiate into sieve tubes.

The protophloem sieve tubes of tobacco are readily discernible in both petioles and stems (figs. 5, and 6, *C* and *D*). In prepared sections these cells are characterized by comparatively thick walls. Their lumen appears clear because the chromatic cytoplasmic portion of the protoplast becomes reduced to a thin parietal layer. Immediately after the acquisition of its special characteristics, the sieve tube tends to round off and thus seems highly turgid. The clear, wide lumen contrasts strikingly

with the rather densely stained protoplasts of the adjacent cells. Sieve plates are, of course, not always evident in transverse sections, but are readily located in longitudinal views.

In prepared sections, particularly those treated with the chrom-acetic-formalin killing agent, the walls of the first sieve tubes are considerably thinner than in fresh sections. These walls, highly refractive in untreated material, resemble collenchyma walls. Although they absorb the same stains as other cellulose walls, they apparently have a very high affinity for stains during the height of differentiation of the sieve tube.

In a given vascular bundle in a petiole, or a leaf trace in the stem, vascular differentiation begins with one sieve tube and one protoxylem element (figs. 5, *A* and 6, *C*). The subsequent sieve tubes appear to the right and left of the first (figs. 5, *B* and 6, *D*) until they reach the margins of the petiolar bundle (fig. 5, *C*) or form a complete ring in a stem (fig. 3, *F*).

These sieve tubes are usually accompanied by sister cells that later become crushed together with the associated sieve tubes. These companion cells are not easily recognized in very young regions where many of the procambium cells are similar in size to the sieve tubes and their companions (figs. 5 and 6, *C*) and have as dense protoplasts as the companion cells. But in somewhat older regions the cells around the sieve tubes enlarge, while the sieve tubes and companion cells remain small, or even become smaller as they approach obliteration. The companion cell may then be recognized without difficulty (fig. 6, *A*, *B*, and *D*).

After the sieve tubes have appeared on the abaxial margin of the procambium, a second layer of these elements, all with companion cells, begins to differentiate in a centripetal direction from the initial layer. The first sieve tube of the second row develops near the first sieve tube of the first layer (figs. 5, *C* and 6, *D*).

While the second layer of sieve tubes is differentiating, the first row begins to undergo obliteration. The collapse of sieve tubes occurs in the same order as differentiation; that is, the first mature sieve tube is also the first to undergo obliteration.

The longevity of the first sieve tube in tobacco appears somewhat greater than that recorded in certain other plants. In the sugar beet the first sieve tube was observed in the third leaf primordium and began collapsing in the fifth (Esau, 1934). In *Tropeolum,* according to Chang (1935), such an element was evident in the third or fourth primordium and was partly degenerated in the eighth. In tobacco the first protophloem sieve tube was perceptible in the fifth (fig. 4) or sixth (fig. 2) leaf and had collapsed in the twelfth or thirteenth.

After the outermost row of sieve tubes has been crushed, the next

deeper row undergoes obliteration, while new sieve tubes differentiate in still deeper layers of the procambium. Meanwhile, xylem elements appear right and left from the first protoxylem cells (fig. 6, *A* and *B*).

About two rows of sieve tubes arise in the procambium before radial seriation of cells becomes clearly evident in this meristem. In the section shown in figure 6, *B*, the sieve tubes of the first row are obliterated, the second row contains mature elements, and the procambium between the xylem and phloem consists of radially arranged cells.

Figure 6, *B*, was drawn from a portion of the bundle shown in plate 2, *A*. In this bundle nine sieve tubes of the first row were crushed, and seventeen were open and mature. Some of these mature elements were on the margins of the first row, others in the median portion of the second row.

The sieve tubes of the first two or three rows usually appear singly or in pairs, rather uniformly distributed among parenchyma cells (fig. 5, *C* and 6, *A*). These sieve tubes have a very short existence; they mature during rapid elongation of the stem or leaf and, within a short time, become stretched and crushed. The portion of the phloem containing these short-lived sieve tubes is here called protophloem.

Metaphloem.—The original subdivision of the primary phloem into protophloem and metaphloem was rather arbitrary. It was suggested not because of possible differences between these tissue layers, but because the primary xylem was subdivided into protoxylem (Russow, 1872, p. 3–4) and metaxylem (van Tieghem, 1887) and because the proto- and metaphloem layers synchronized in their appearance with the proto- and metaxylem, respectively.

As Chauveaud (1911) has pointed out, the classification into protophloem, metaphloem, and secondary phloem may be inappropriate from the standpoint of origin of these layers; but these terms conveniently designate the successive stages in the development of the phloem and are used in this sense in the present work.

The metaphloem of tobacco is illustrated in plates 2, *C*; 3, *A*; 8, *A*; 9, *A*. In contrast to the protophloem it contains groups of sieve tubes, companions, and phloem parenchyma cells. Since it arises in plant parts that show a decreasing rate of elongation, it is more persistent than the protophloem, as well as more extensive.

The transition from protophloem to metaphloem is not sudden. The size of mature cells increases gradually; and although the metaphloem sieve tubes are grouped, these groups are very small at first (plate 8, *A*).

Whereas the first protophloem sieve tubes appear rather uniformly spaced around the periphery of procambium of the stem (fig. 3, *E*), the metaphloem is somewhat more prominent in the regions of leaf traces.

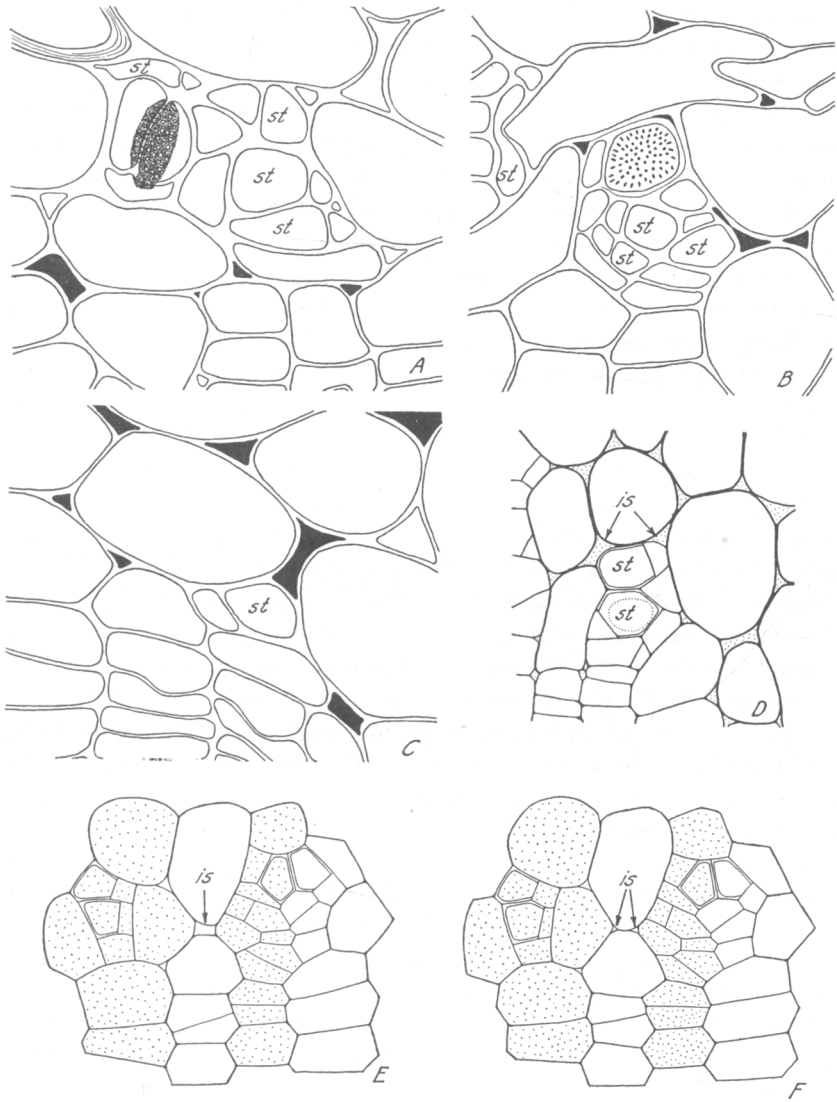

Fig. 7.—Transverse sections through the external primary phloem of *Nicotiana tabacum*. *A–C*, Drawings from fresh unstained sections mounted in lactose solution; the intercellular spaces shown in black contained air after sectioning. *D*, Section taken from the same petiole as in plates 3, *B* and 10, *B;* intercellular spaces are stippled. *E, F*, Two successive sections, one micron apart from each other, taken from the same petiole as plates 2, *C* and 18, *A;* the stippling sets apart radial rows of cells, each row being derived from a different initial. *D–F*, Drawing from paraffin material. Details are: *is*, intercellular space; *st*, sieve tube. (*A–C*, × 661; *D–F*, × 437.)

With the appearance of the metaphloem the radial seriation in procambium becomes increasingly prominent. The primary rays, differentiating from phloem mother cells without longitudinal divisions, clearly indicate the origin of the metaphloem from an orderly dividing meristem. In the formation of the small-celled groups, however, two or more initials lying side by side divide by longitudinal walls and jointly produce a group of small cells (fig. 7, *E* and *F*, right; plate 3, *A*). The divisions are followed by some growth of the new cells, so that they encroach upon the undivided ray cells. This spatial readjustment is accompanied by partial separation of ray cells from each other and by formation of interrupted intercellular spaces between them (fig. 7, *E* and *F*).

In the small-celled groups the companion cells are usually somewhat smaller than the other members of the group (plate 9, *A*). The phloem parenchyma cells vary in size, some being similar to the sieve tubes, others larger. Their relative size apparently depends on the number of divisions in the phloem mother cell that gives rise to them. Such a cell may divide, for example, into three daughter cells, two of which develop into relatively large phloem parenchyma cells, while the third divides again. If this dividing daughter cell produces not only sieve tubes and companions but also phloem-parenchyma cells, the latter remain comparatively small (fig. 7, *E* and *F*, left).

The phloem-parenchyma cells are narrower, but not longer, than the primary ray cells.

The sieve tubes of the metaphloem are in contact with a variety of cells. They occur next to other sieve tubes and next to phloem-parenchyma or primary ray cells (fig. 8; plate 11, *A*).

Fibers of the Primary Phloem.—After the obliteration of sieve tubes, the protophloem undergoes such radical changes that it can no longer be recognized as phloem tissue. The cells surrounding the functionless sieve tubes rapidly enlarge (fig. 6, *B*). When the sieve tubes and their companions are completely crushed between the enlarging cells, thickened places temporarily indicate the former position of obliterated elements (plates 8, *A*; 9, *A*; 10, *A*).

The enlarging cells may divide longitudinally (fig. 6, *B*, near median arrow, above); but transverse divisions do not occur in them. They rapidly elongate until, in the stem, they reach about 1 mm in length. Their nuclei divide, however, so that four or eight nuclei frequently occur in the same cell.

Plate 11, *B*, shows two nuclei in one of these cells. Both large cells with nuclei in plate 9, *A*, had other nuclei in adjacent sections.

In the petioles these cells usually develop no true secondary walls (plates 3; 9, *A*; 10, *A*); but in the stem they gradually acquire secondary

thickenings (plate 11) and develop into fibers (fig. 8; plates 4, *A;* 6, *A;* 7, *A;* 16, *A*). Because of their origin in the primary phloem, principally in the protophloem, these fibers are here interpreted as protophloem or primary phloem fibers.

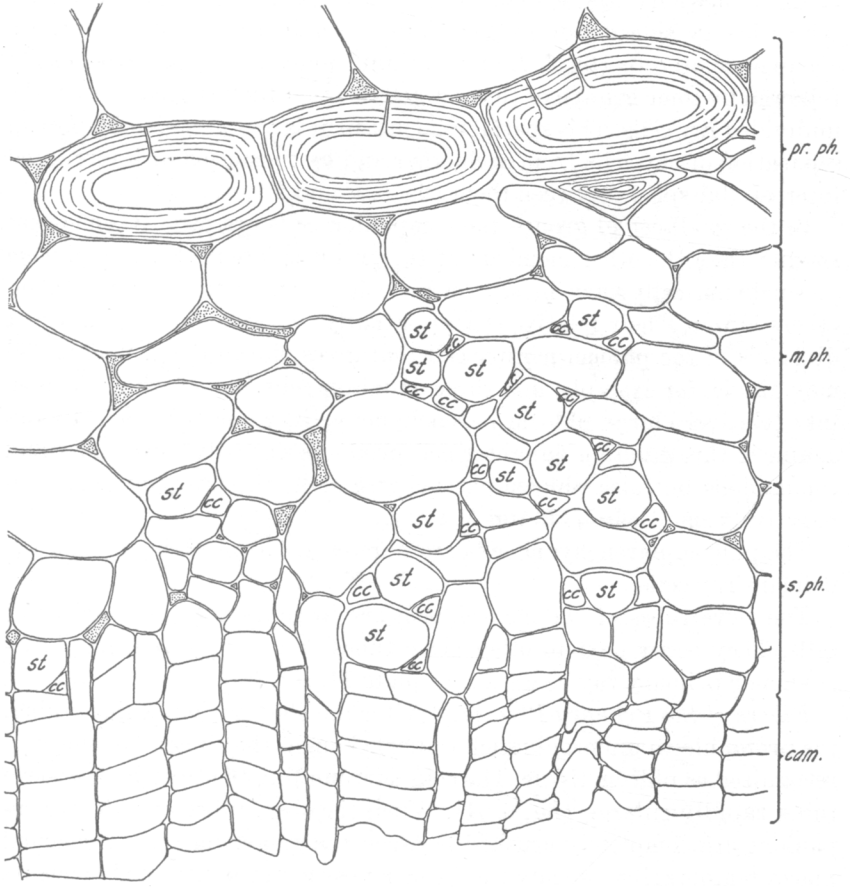

Fig. 8.—Transverse section through the external phloem of *Nicotiana tabacum* from a somewhat older stem than in plate 4, *A*. The intercellular spaces are stippled. Details are: *cam*, cambium; *cc*, companion cell; *m.ph.*, metaphloem; *pr.ph.*, protophloem; *s.ph.*, secondary phloem; *st*, sieve tubes. (× 492.)

Certain cells of the protophloem retain thin walls, so that the sclerenchyma cells appear separated by parenchyma cells into irregular groups (plate 4, *A*).

The starch sheath occurs immediately outside the fibers.

Avery (1933*b*) defines the fibers on the inner and outer periphery of the vascular system in *Nicotiana tabacum* as pericyclic fibers. This defi-

nition agrees with the usual interpretation of similar cells in other plants (Eames and MacDaniels, 1925, p. 100, 256).

Before van Tieghem (1882), such fibers were termed bast fibers, together with the fibers of secondary phloem (Hartig, 1853; De Bary, 1884, p. 526–27). Vesque (1875) named them external or primary phloem fibers in contrast to the fibers of the secondary phloem.

The term *pericycle* was first used by van Tieghem (1882) in reference to the ring of sclerenchyma and subjacent parenchyma outside the vascular bundles of *Cucurbita,* but he also applied this term to the similar region in other plants, even if this region was composed of discrete strands of fibers lying on the outer periphery of the phloem. On the basis of extensive comparative studies, Morot (1885) concluded that the pericycle occurred in roots and stems of practically all plants and that it was derived from the same fundamental meristem as the rays and the pith.

D'Arbaumont (1886) contested Morot's viewpoint because ontogenetic studies on many woody and herbaceous species indicated to him that the pericycle arose from procambium.

Léger (1895, 1897*a,* 1897*b*), clearly understanding the development and old-age metamorphosis of primary phloem, furnished conclusive proof that, in most plants, the so-called "pericyclic fibers" are part of the phloem. He also interpreted as phloem tissue the collenchymatous or sclerified bundle caps characteristic of the vascular bundles of certain families. Léger worked on a very large number of plants in thirty-two different families, including cryptogams as well as phanerogams.

Among subsequent workers Gidon (1900) recorded absence of pericycle in Nyctaginaceae. Esau (1933, 1934, 1936) showed that collenchymatous bundle caps in sugar beet and celery originate from phloem.

Artschwager (1918) refers to fibers on periphery of phloem in potato as phloem fibers, but does not show their ontogenetic relation to the phloem. He interprets the parenchyma cells between the groups of fibers and even those between the groups of external primary phloem as pericycle.

Secondary Phloem.—During the first year of growth the tobacco stem completes the development of the primary phloem and produces considerable secondary phloem (plate 7, *A*). The latter appears to differentiate very slowly at first. Through several internodes that have ceased to elongate, the external phloem shows hardly any change in amount, while the xylem grows much in thickness (fig. 1, *B* and *C;* plate 4, *A*). A marked addition of phloem from the cambium occurs in the second year of growth (figs. 1, *D* and 6, *A*).

The protophloem, as previously described, loses its original appear-

ance in the elongating portion of the stem, so that it is represented by fibers and parenchyma in more mature stems (plate 4, *A*). The metaphloem remains relatively unchanged until secondary growth becomes well established. Then the crushing of sieve tubes in this tissue becomes evident—first near the protophloem fibers (plate 7, *A*), later throughout the entire metaphloem (plate 6, *A*). By the end of the second year the metaphloem appears functionless, although its conducting cells are not yet completely crushed in a several-year-old stem (plates 13; 14, *A;* and 16, *A*).

The transition from the metaphloem to the secondary phloem is gradual because both these tissues arise from a radially seriated meristem. Figure 8 shows the external phloem at the beginning of secondary growth. Some of the metaphloem sieve tubes lie in the same radial rows as the secondary sieve tubes. Plates 3, *B* and 10, *B,* also show phloem during transition from primary to secondary growth.

In the metaphloem, as previously shown, the radial seriation of cells is largely lost during development of the tissue (plate 3, *A*). Mature secondary phloem, on the contrary, clearly reflects the origin from a radially seriated meristem (plates 6, *A;* 7, *A;* 15, and 16, *B*).

The secondary phloem is derived from a nonstratified cambium with long fusiform initials (figs. 10, *B;* 11), which divide by tangential longitudinal walls and by very oblique anticlinal walls (figs. 10, *B,* at *a;* 11 at *e, d,* and *g*). Frequently the oblique walls have the shape of sigmoid curves in tangential views (figs. 10, *B* at *a,* and 11 at *e*).

As shown by figures 11 and 12, mature cells of the longitudinal system are shorter than the fusiform initials from which they were derived. This condition is caused by the appearance of the somewhat oblique to transverse divisions in the phloem mother cells (fig. 11, at *a, b*). These divisions occur in the formation of sieve tubes, as well as phloem parenchyma (fig. 12). A similar method of development of sieve tubes was previously recorded for *Vitis* (Janczewski, 1881) and *Tilia* (Strasburger, 1891, p. 222). Sometimes two rather oblique walls cut off a short fusiform cell (fig. 11 at *e* and *f*) that matures into a sieve tube or a phloem-parenchyma cell.

Sieve plates develop in different kinds of walls: in the nearly transverse and slightly oblique (fig. 12 at *d*); in the very oblique (fig. 12 at *e*); and in the longitudinal walls (fig. 12 at *f*). Shorter walls usually bear only one sieve plate (fig. 12 at *d*); longer walls have several (fig. 12 at *e* and *f*); occasionally a long wall bears one sieve plate of somewhat limited size (fig. 12 at *g*), or a short wall has several plates (fig. 12 at *h*).

All the walls bearing sieve plates are, however, anticlinal, so that the sieve plates of longitudinal and oblique walls are visible in face view

only in radial sections (plate 12). The transverse sieve plates appear in face view only in transverse sections. The other sieve plates are seen in sectional views in cross sections (plate 15).

The wall that separates the sieve tube from the companion cell is the last to be laid down in the developing phloem. This wall has an orientation intermediate between anticlinal and periclinal (plates 15 and 16, *B*).

The companion cell may be as long as the sieve-tube element (fig. 12, at *b*) or shorter (fig. 12, to the right from *f*). In the latter case the sieve-tube element shows the characteristic flared ends (fig. 12, at *f*). Sometimes the companion cell is formed near the oblique end wall of the sieve-tube element, the new partition being laid down parallel to the oblique wall (fig. 12, at *i*).

The cell that has been cut off from the sieve-tube mother cell directly differentiates into a companion cell without any further divisions. Usually only one companion cell is associated with each sieve-tube element.

Scattered fibers occur in the secondary phloem (plate 16, *B*); these are much shorter and narrower than the protophloem fibers. A fusiform phloem mother cell directly differentiates into a fiber without division. The fibers of the secondary phloem show no multinucleate condition.

The rays of the secondary phloem are one to two cells wide and one to many cells high, as viewed in tangential sections (figs. 10, *B*, 11, and 12).

In the formation of new ray initials, those a few cells in height may be cut out of a fusiform initial, somewhat as a companion cell is cut out of a sieve-tube mother cell; but the resulting small cell divides by one or more transverse walls (fig. 10, *B* at *b*). Initials of high rays reach the extent of one or more fusiform initials. Frequently portions of one or two fusiform initials contribute jointly to the formation of one ray. The wall between the remaining portion of the fusiform initial and the nearest ray cell is then either oblique (figs. 10, *B* at *c*; 11 at *h*) or transverse (fig. 10, *B* at *d*).

In young phloem the ray cells are usually radially elongated (plates 6, *A*; 13; 15); sometimes their longitudinal diameter is longer than the transverse (plate 12). In old phloem the ray cells become stretched tangentially because of the increasing circumference of the stem (plates 5, *B*; 13, above). In response to the tangential stretching, the ray cells not only elongate tangentially but also divide by radial walls (plate 5, *B*). The cells are also pulled apart so that large intercellular spaces develop among them (plate 13).

The parts of secondary phloem that contain sieve tubes, companion cells, and phloem parenchyma cells do not become wider under the influence of the tangential stretching. Rather, they become narrower because

certain cells collapse. Thus the rays of the old phloem become wider than the blocks of cells of the longitudinal system and more conspicuous than in the young phloem. In the oldest secondary phloem the longitudinal system appears like a network of anastomosing strands sharply separated from the ray parenchyma (plate 5, *B*). In contrast to the old, the young phloem forms a rather homogeneous tissue system (plate 4, *B*).

The sieve tubes of the secondary phloem occur in contact with all types of cells, including rays (plate 15) and fibers (plate 16, *B*).

Internal Phloem.—When internal phloem arises near the inner limit of the xylem, the cell that is to become a phloem strand divides several times, usually first by periclinal and then by anticlinal walls. Since the resulting cells do not enlarge very much, the entire group retains for a while the shape of the cell from which it was derived (fig. 5, *C*). The groups may subsequently enlarge, through appearance of similar divisions in adjacent cells. One or two cells or a part of a cell may combine with the previously divided cell in forming a phloem group (fig. 6, *A, B, D*). This addition of new cells occurs on the abaxial side of the groups. New independent strands also arise in centrifugal direction from those formed earlier; that is, they are formed nearer the protoxylem (fig. 6, *B*).

In each group, phloem elements are differentiated in centrifugal order. The first sieve tubes appear farthest from the protoxylem, on the adaxial side of a strand (fig. 6, *A* and *B*); then they differentiate progressively nearer the xylem. Companion cells and large and small parenchyma cells differentiate along with the sieve tubes. The latter may lie in contact with any cell of the group and also on the periphery of a strand (plate 9, *B*). Usually, however, companion cells intervene between the sieve tubes and the parenchyma outside the bundle.

The groups of internal phloem of a given petiolar bundle or of a leaf trace within the stem appear first right and left from the first protoxylem; then they differentiate nearer the median portion of the bundle or trace and also farther toward the margins of the expanding procambium strand. Maturation of bundles occurs in the same order as their differentiation.

The size of the individual phloem strands depends not only upon the initial number of cells that undergo divisions to make up one strand, but also on the duration of meristematic activity within each group. While maturation, followed by obliteration, is taking place on the adaxial end of a phloem bundle, cell division continues for a time on the abaxial side. The divisions occur in a rather orderly manner, tangential walls predominating, so that the meristematic cells resemble cambium (plate 8, *B*). This meristem, however, gives cells only toward the bundle, not toward the outside.

Cell division in the first phloem groups is of short duration, and the cambiumlike layer disappears when all its cells mature into the various phloem elements (plate 9, *B*). Because of this limited duration of meristematic activity, the first phloem groups remain small; but those formed later may attain much larger size if the rest of the plant shows vigorous growth (plate 3, *B*).

As differentiation progresses toward the abaxial side in each bundle, sieve tubes and their companions are obliterated on the adaxial side. As in the external phloem, the cells near the sieve tubes enlarge, divide longitudinally (sometimes), and finally crush the sieve tubes and companion cells between them (plates 8, *B*; 9, *B*).

The earliest phloem groups usually lose all their sieve tubes and companion cells. The remaining cells undergo modifications similar to those of the fibers of the external protophloem. They become very long, their nuclei divide several times, and their walls thicken (plates 3, *B*; 4, *A*; 6, *B*; 7, *B*). In contrast to the petioles, stems show more or less prominent secondary thickenings in mature internal fibers (plates 6, *B*; 7, *B*).

Thus the first internal phloem strands have much in common with the external protophloem. They mature in elongating organs, lose their sieve tubes rapidly, and develop long multinucleate fibers. But in contrast to the external phloem, the internal tissue is, from its beginning, divided into discrete strands.

Although the first internal phloem groups arise in rather close proximity to the protoxylem (figs. 5, *C*; 6, *D*; plate 2, *A* and *B*), they later appear at a considerable distance from the inner margin of the xylem (plates 3, *B*; 4, *A*). This spatial rearrangement is brought about by the division and enlargement of parenchyma cells surrounding the phloem groups and by the interpolation of new phloem strands between the functioning xylem and the first phloem strands. The internal margin of the xylem appears, moreover, to recede toward the abaxial side, because of the crushing of protoxylem (plate 2, *C*).

The addition of new phloem strands seems to continue as long as the rest of the vascular tissues grow, although disintegration of the pith may destroy the internal phloem without stopping the activity of the vascular cambium.

Already in young organs the inner phloem groups differentiate from parenchyma cells that lie among the first protoxylem vessels (fig. 6, *B*; plate 2, *A*); but in old stems these phloem strands actually enclose obliterated protoxylem elements (plate 6, *B*).

As the presence of crushed xylem among internal phloem shows, the latter arises in the parenchyma of the primary xylem. These parenchyma cells have comparatively thick walls and large vacuoles, with intercellu-

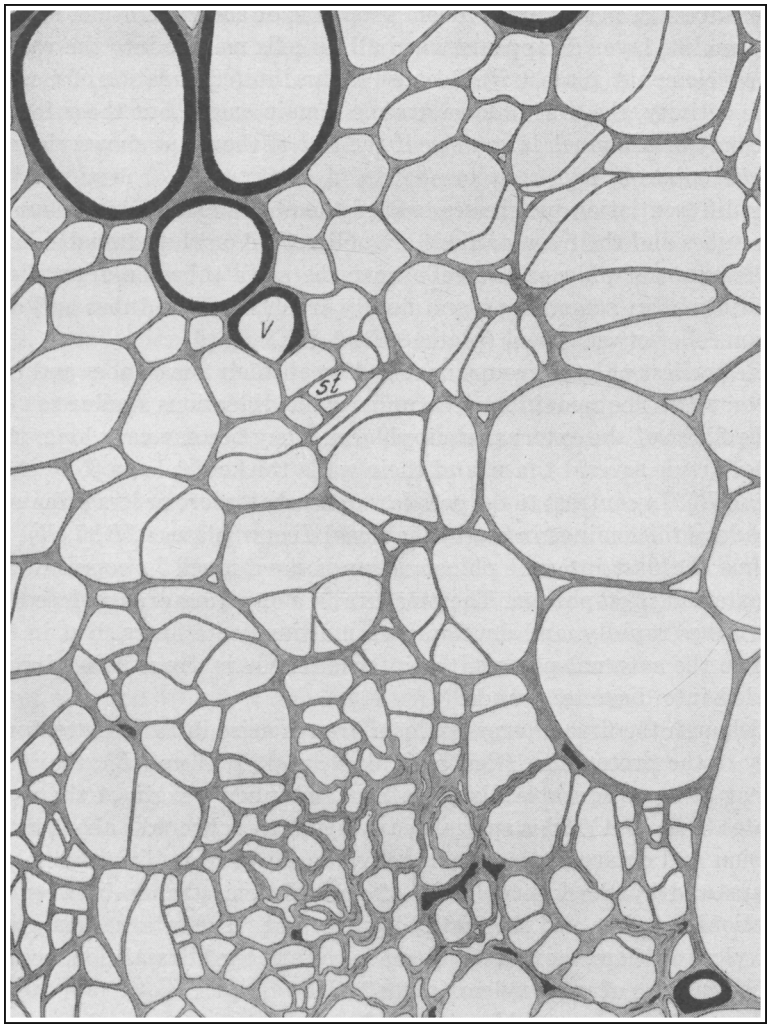

Fig. 9.—Transverse section through the internal phloem of a two-year-old stem of *Nicotiana tabacum*, which was also used in plate 6, *B*. Some primary xylem occurs in the upper part of the figure; mature phloem is below. Parenchyma cells between the xylem and phloem have recently divided to produce more phloem. Formalin-acetic-alcohol fixation. Details are: *st*, sieve tube; *v*, vessel. (× 448.)

lar spaces among them. But they divide readily (fig. 9). When sieve tubes differentiate among the products of division, they may occur very close to intact vessels, sometimes with only one parenchyma cell separating the two (fig. 9).

The increase in size of the internal-phloem strands through division

of cells within the strand has been described by most students of internal phloem. Many workers have regarded the dividing region as a kind of cambium, because the divisions are frequently localized in few layers of cells and occur by formation of periclinal walls. Vesque (1875) applied the name of *false cambium* to this meristematic region; Scott and Brebner (1889), *local cambium;* Petersen (1882), *inner cambium.* Baranetzky (1900) chose *unilateral cambium* because, he said, this meristem gave cells in one direction only, no xylem being formed. Lamounette (1890) used Vesque's term *false cambium* in reference to the internal phloem of *Nicotiana tabacum.*

SIEVE TUBES

Types of Sieve Tubes.—Plants with pronounced secondary growth show morphological differences between primary and secondary sieve tubes (Lecomte, 1889)—differences largely determined by the shape of meristematic cells and by methods of division of mother cells.

Tobacco exemplifies such a relation. The procambial cells have mostly transverse end walls (fig. 10, *A*), and the primary sieve tubes are characterized by transverse or nearly transverse terminal walls (plate 1, *F* and *G*). The secondary sieve tubes, derived from fusiform initials that divide several times by nearly transverse walls, show very oblique to nearly transverse terminal walls (fig. 12).

The primary sieve tubes elongate during their ontogeny, becoming longer than the initials from which they were derived. The secondary sieve tubes do not elongate, and, because of transverse divisions in their mother cells, are much shorter than the initials that gave rise to them. (Compare figs. 11 and 12.)

The primary sieve tubes are characterized by simple sieve plates—that is, plates consisting of one unit covering the entire terminal wall. The occasional sieve plates on longitudinal walls are also simple, occupying only a small part of the wall. In the secondary sieve tubes, sieve plates are not only simple but are also combined into groups, sometimes called compound sieve plates (figs. 12 at *e* and *f;* 13, *D;* 14; plate 12).

The primary sieve tubes, like all members of the longitudinal system of the primary phloem, are narrower than the corresponding elements of the secondary phloem. (Compare plates 4, *A* and 6, *A;* 11 and 16, *B.*) The primary phloem, including the sieve tubes, is characterized by somewhat thinner walls than the secondary phloem. (Compare plates 11 and 16, *B.*)

The sieve tubes of the internal phloem are mostly of the type found in the external primary phloem, but the secondary type occurs here also in older parts of the stem.

Development of the Sieve-Tube Protoplast.—In the present description of the development of the specific internal organization of tobacco sieve tubes, three problems are considered: the occurrence and morphol-

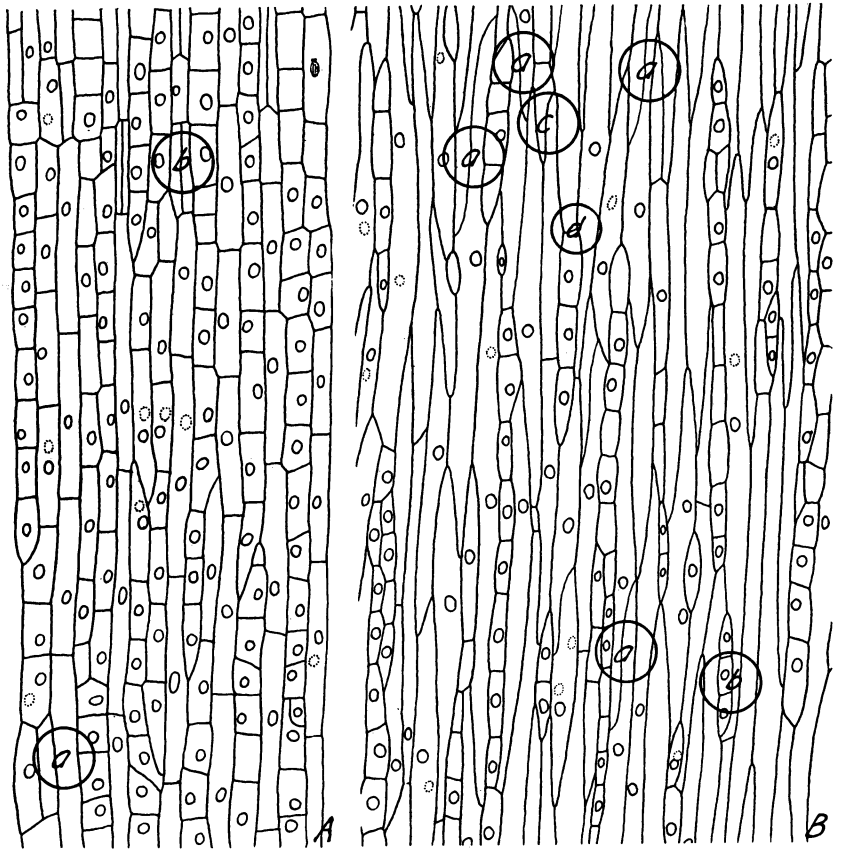

Fig. 10.—*A*, Tangential longitudinal section of procambium from the same petiole of *Nicotiana tabacum* as in plates 2, *C*, and 18, *A*. *B*, Tangential longitudinal section of cambium from the same portion of *N. tabacum* as was used in plate 18, *B*. Further explanations in the text. (Both × 142.)

ogy of the slime bodies; the disintegration of the nucleus; and the occurrence and structure of the sieve-tube plastids.

Although the appearance and disintegration of slime bodies of *Nicotiana tabacum* and *N. glauca* were described in detail by Crafts (1934), these structures are considered again here, because the writer was unable to confirm Craft's statement that slime bodies of tobacco occur only in primary sieve tubes.

The tobacco slime body, as described by Crafts is homogeneous in

structure in fresh and anilin-blue material and, at the same time, is peculiarly twisted or coiled. It is usually single in a given cell but may be accompanied by several others.

The particular type of slime body found in tobacco is characteristic of

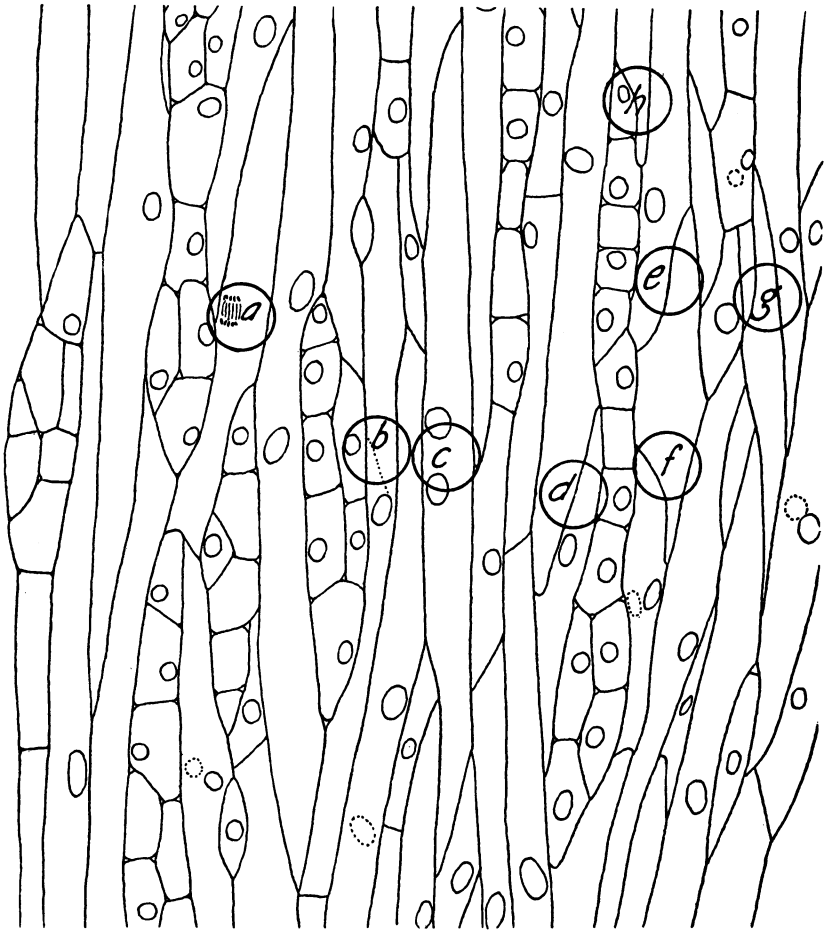

Fig. 11.—Tangential longitudinal section of cambium from a one-year-old stem of *Nicotiana glauca*. Further explanations in the text. (× 200.)

Solanaceae in general (Nelson, 1922; Kotila and Coons, 1923; Doolittle and McKinney, 1923; Kofoid *et al.*, 1923; Artschwager, 1924; Crafts, 1933, 1934). Morphologically it is distinct from the slime drops of Cucurbitaceae (Wilhelm, 1880, p. 41 and 55; Fischer, 1886; Lecomte, 1889; Crafts, 1932) or from the spindle-shaped slime bodies of Leguminosae (Strasburger, 1891, p. 193–94, 199; Baccarini, 1892; Staritz, 1893;

Mrazek, 1910; Nelson, 1922; Doolittle and McKinney, 1923; Bailey, 1923). In some plants slime bodies show no tendency toward constancy of shape, as, for example, in *Vitis* (Wilhelm, 1880, p. 16–17).

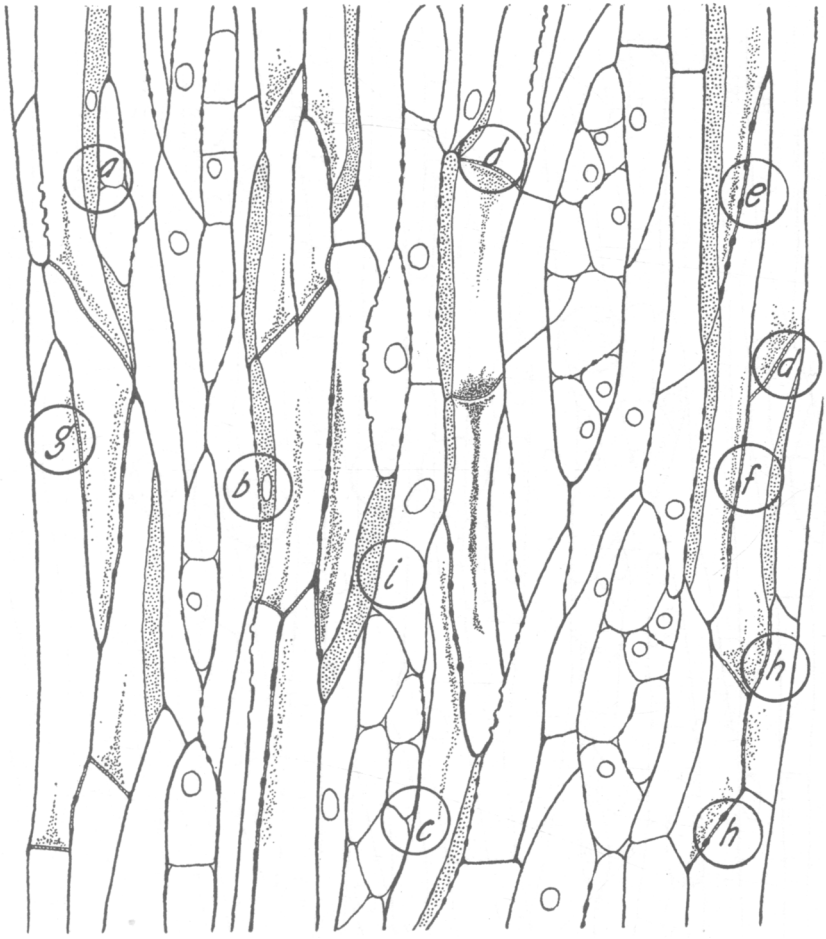

Fig. 12.—Tangential longitudinal section of secondary phloem from the same stem of *Nicotiana glauca* as in figure 11. The sieve tubes are brought out by stippling near the sieve plates; this stippling represents slime. The companion cells are stippled throughout. Further explanations in the text. (×200.)

Workers generally agree, however, that all types of slime bodies are proteinaceous in nature and are products of the sieve-tube protoplast.

Wilhelm (1880, p. 17) already recognized that the slime bodies and the proteinaceous coagulable material of mature sieve tubes are identical. Fischer (1885*a*, 1886) reported that slime bodies became dissolved in

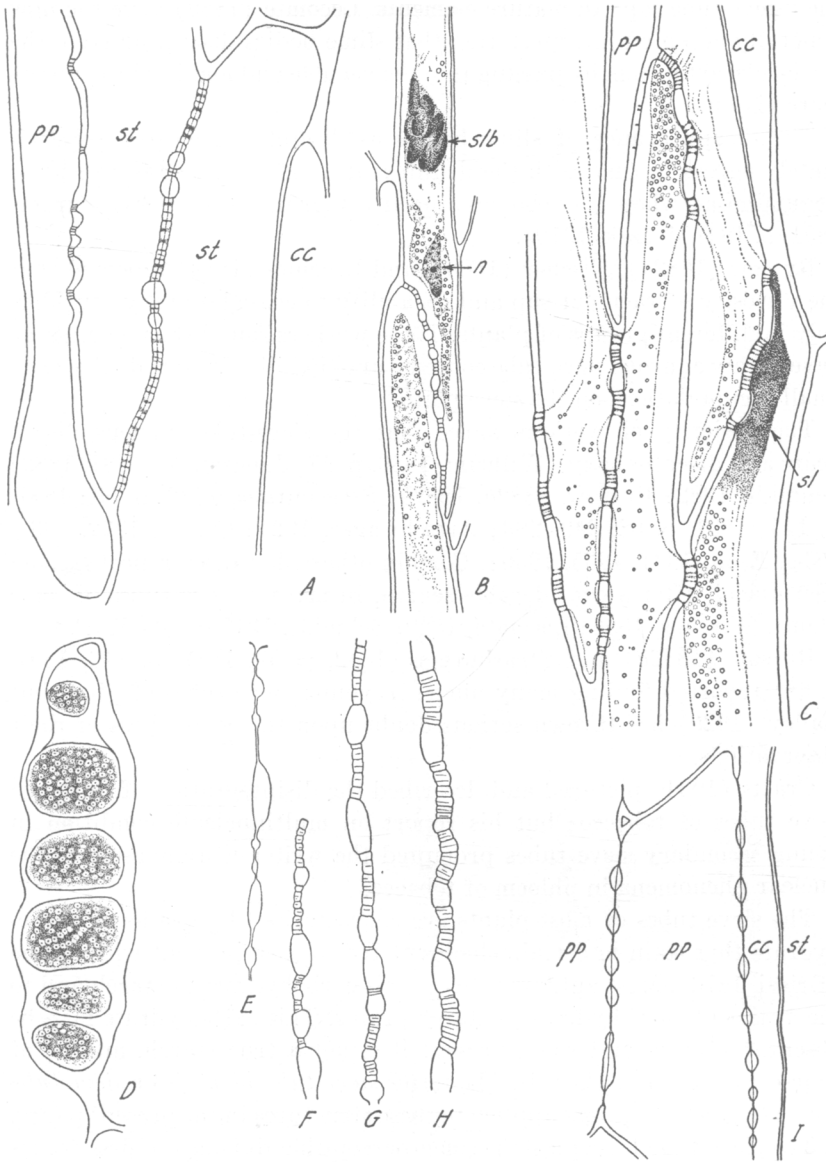

Fig. 13.—Details of secondary phloem elements: *A*, *D*, and *I*, from paraffin preparations of *Nicotiana glauca*; *B*, *C*, *E–H*, from *N. tabacum* treated with I_2KI and anilin blue. *A*, Wall structure of different phloem elements in tangential section; sieve plates between *st* and *st*. *B*, Portions of two young sieve-tube elements, the upper one containing a slime body. *C*, Illustration of cytoplasmic attachment to sieve plates. *D*, Sieve plates of an inclined wall as viewed in radial section. *E–H*, Sieve plates in four successive stages of development. *I*, Wall structure of different phloem elements in tangential section. Details are: *cc*, companion cell; *n*, nucleus; *pp*, phloem parenchyma; *sl*, slime; *slb*, slime body; *st*, sieve tube. (*A*, *D–I*, ×700; *B* and *C*, ×450.)

the "sieve-tube sap" in mature elements. Lecomte (1889) gave the now generally accepted interpretation that slime bodies disappear from the sieve-tube cytoplasm by passing into the vacuole and making its contents more viscous.

The disappearance of slime bodies as discrete structures approximately agrees in time with the disintegration of the nucleus, except in Leguminosae, where these bodies appear rather persistent (Strasburger, 1891, p. 194; Mrazek, 1910).

Russow (1882*a*), Fischer (1886), and Lecomte (1889) reported that the viscosity of vacuolar sap and its ability to coagulate vary considerably in different groups of plants. These workers found that Solanaceae have highly aqueous sieve-tube contents and, in killed material, give very small accumulations on the sieve plate.

The majority of workers agree that the sieve-tube nucleus disappears as a discrete body (Wilhelm, 1880, p. 17; Janczewski, 1881, 1882; Schmidt, 1882; Russow, 1882*a*, 1882*b*; Strasburger, 1882, p. 59; 1887, p. 145; 1891, p. 68, 249, 289; Artschwager, 1924; Crafts, 1932, 1933, 1934; Esau, 1934, 1935, 1936). Certain others, however, report having seen nuclei, after a considerable search, in mature sieve tubes of some plants (Fischer, 1886; Lecomte, 1889; Schmidt, 1917, p. 48–57).

Russow (1882*a*) and Strasburger (1882, p. 59, 1887, p. 146, 1891, p. 68) reported finding many nuclei in young sieve tubes of pine; but Bailey (1920) has thrown serious doubt upon the correctness of their observations.

Crafts (1934) pictured and described the disintegration of nuclei in sieve tubes of tobacco; but his report on multinucleate condition in young secondary sieve tubes prompted the writer to reinvestigate the nuclear phenomena in phloem of tobacco.

The sieve tubes of most plants are characterized by grains of carbohydrate that stain in certain shades of red upon treatment with iodine (Briosi, 1873). Most authors refer to this carbohydrate as starch and to the grains of carbohydrate as starch grains. This carbohydrate may be absent, however; and when present it is not a true starch, and is, of course, produced in plastids. The terms *sieve-tube plastids* or *sieve-tube leucoplasts* are therefore applied to these structures in the present paper.

The sieve-tube leucoplasts become perceptible during the first stages of specialization of a sieve tube (Briosi, 1873; Wilhelm, 1880, p. 17; Fischer, 1886; Crafts, 1934; Esau, 1934, 1935, 1936). If carbohydrate accumulates in the plastids, it appears long before the sieve tube is mature.

The characteristic staining reaction of the sieve-tube carbohydrate upon treatment with iodine has been observed by numerous workers

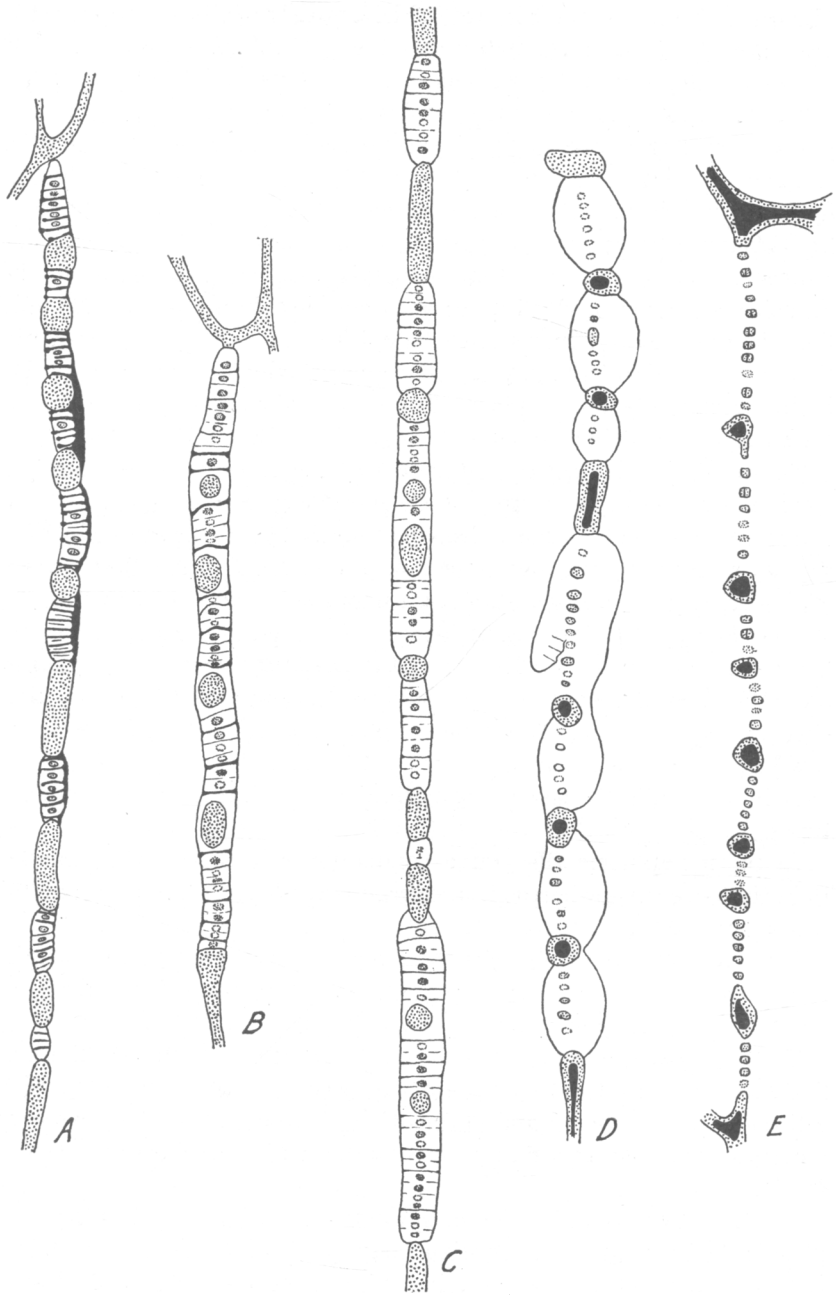

Fig. 14.—Longitudinal sections of sieve plates of *Nicotiana glauca* in different stages of metamorphosis: *A*, the earliest stage; *E*, the latest. The sieve plate walls are stippled; the callus is shown in white. Further explanations in the text. (×1190.)

(Briosi, 1873; Russow, 1882*a*, 1882*b*; Lecomte, 1889; Strasburger, 1891, and others). If this carbohydrate is absent, then the leucoplasts, if at all discernible, stain yellow with iodine (Strasburger, 1891; Esau, 1934).

The leucoplasts arise in the cytoplasm of young sieve tubes, but apparently may enter the vacuole in mature elements. This is the opinion of Fischer (1886) and Crafts (1933, 1934). The latter regards this release of plastids into the vacuole as one indication of a breakdown of limits between cytoplasm and vacuole in mature sieve tubes.

Lecomte (1889) thought that sieve-tube plastids never occur in the vacuole; Strasburger (1891, p. 68–69), however, found that in the pine flocculent masses and grains of carbohydrate occur in the watery liquid of the sieve tubes, whereas the leucoplasts, which stain yellow with iodine, occur only in cytoplasm. The carbohydrate-free leucoplasts of the sugar beet remain confined to the cytoplasm (Esau, 1934, 1935).

The plastids, whether free of carbohydrate or containing some, are retained by the sieve tube until this element begins to collapse.

Strasburger (1891, p. 249) observed that carbohydrate grains in *Vitis* first appear in groups, but later fall apart. He decided that several grains arise in one leucoplast, but are liberated upon its maturation.

The characteristic specialization of the sieve-tube protoplast in tobacco begins after the last division of the sieve-tube mother cell—that is, after the formation of the companion cell. In the protophloem this division practically halves the sieve-tube mother cell (plate 1, *D*), and the dividing sieve-tube mother cells (plate 1, *A*) may be recognized as such only if they lie in the same longitudinal row with differentiating sieve tubes.

In the metaphloem the young companion cell is somewhat smaller than the sieve tube, whereas in the secondary phloem it appears only as a fragment of the sieve-tube element.

During maturation the sieve tube expands somewhat more than the companion cell, so that even in the primary phloem the sieve tube is, as a rule, the evidently larger of the two cells (plate 1, *D–F*).

The protoplasts of the young sieve tube and its companion cell are at first similar (plate 1, *D*); but gradually the cytoplasm of the companion cell increases in density and chromaticity, while that of the sieve tube undergoes rapid and prominent vacuolation and stains lightly (plate 1, *D–F*).

The first striking indication of specialization of the sieve-tube protoplast is the appearance of the slime body (plate 1, *E*). In paraffin material the slime bodies have a distinct fibrous structure (plate 1, *E–G*), showing considerable similarity to the kinoplasmosomes that are concerned with the formation of cell plate in dividing cells (plate 1, *A–C*).

The kinoplasmosomes, however, occur in pairs and stand in relation to the cell plate when viewed in sections perpendicular to the plane of division (plate 1, *A*, lower cell; *B* and *C*). In sections parallel to the new wall the kinoplasmosomes are not fibrous in structure and show no resemblance to slime bodies (plate 1, *A*, upper cell). In transverse sections, kinoplasmosomes and slime bodies may be confused with greatest ease.

The slime bodies of tobacco usually appear singly in each sieve-tube element, but sometimes one large body is accompanied by several small ones (plate 1, *G*). The slime bodies increase in size during their development (plate 1, *E* and *F*); then they break down. The phenomena associated with disintegration have been described by Crafts (1934). Suffice it to mention that the slime body disintegrates at the same time as the nucleus.

Slime bodies are characteristic of primary and secondary sieve tubes. In vigorously growing stems of *Nicotiana tabacum* and *N. glauca* they are as common in the secondary as in the primary phloem. A secondary sieve tube containing a slime body is depicted in figure 13, *B*.

The disappearance of the nucleus as a discrete body also indicates specialization of the sieve-tube protoplast. The nucleus breaks down before the connecting strands through the sieve plates have developed their maximum size and chromaticity.

As long as the nucleus is intact, the sieve-tube cytoplasm can be pulled away from the young sieve plate as readily as from other walls (plate 1, *E* and *F*), even when the sieve plate is already well defined (fig. 13, *B*). During disintegration of nucleus and after the completion of this process the connection between cytoplasm and sieve plate becomes firm (plate 1, *G*; fig. 13, *C*).

Before disintegration the nucleus swells up and loses its chromaticity.

There is only one nucleus in each young sieve-tube element of the secondary as well as the primary phloem. Frequently two or more nuclei belonging to different fusiform phloem mother cells or cambium initials of the same radial row, appear in the same focal plane in tangential sections (fig. 11 at *c*). Such views might be mistaken for pictures of multinucleate cells. These cells are not yet young sieve tubes, however, because fusiform cells divide several times before they give rise to sieve tubes.

The leucoplasts of tobacco sieve tubes can be recognized during the first stages of development of the sieve tube. Though readily discernible in iodine—anilin-blue preparations, in paraffin material they stain poorly and are relatively inconspicuous even in mature sieve tubes.

The plastids accumulate carbohydrate before the sieve plate reaches maximum differentiation. The carbohydrate was present in the cells shown in figure 13, *B*. As in other plants, it stains red with iodine.

In mature sieve tubes the plastids abound and tend to accumulate near the sieve plates in sectioned material (fig. 13, *C*).

It is difficult to decide whether the individual bodies visible in mature sieve tubes are grains of carbohydrate or are the entire plastids. They frequently occur in small clumps, as though several originated together in the manner described by Strasburger in *Vitis* (1891, p. 249). On the other hand, each granule resembles in appearance the entire plastid in a sugar-beet sieve tube (Esau, 1934). It has the shape of a disk with the margins more densely stained than the center.

The pink-stained grains of tobacco were observed, agitated by Brownian movement, in the vacuole of mature elements treated with iodine and anilin blue. They remain discrete until definitive callus completes its development.

Companion cells show no carbohydrate grains, but phloem parenchyma contains large leucoplasts that accumulate starch.

Sieve Plates.—As the existing terminology regarding the sieve plate and related structures is rather loose, the terms used in this paper must be circumscribed.

The sieve plate, a term first used by Hanstein (1864, p. 25), has come to mean in angiosperms a characteristically differentiated wall or wall region of a sieve tube with relatively large connecting strands passing through the wall, each strand being surrounded by a cylinder of callus.

Similar structures, but less highly differentiated, with barely perceptible connecting strands and rather small callus deposits, frequently occur on longitudinal walls. These formations were first described by Nägeli (1861, p. 222–23), who termed them sieve fields. Wilhelm (1880, p. 14) pointed out that in *Vitis* the sieve fields and sieve plates imperceptibly merged one into another.

Russow (1882*a*, p. 269) and Strasburger (1887, p. 145–46; 1891, p. 65; 1901, p. 527–30) applied the term *sieve field* to a unit of a gymnosperm sieve plate. This unit consists of one callus cylinder and several connecting strands passing through it.

In comparing the pine sieve plate with that of *Wistaria,* Strasburger (1901, p. 527–30) concluded that in *Wistaria* the connecting strands of each sieve field have been replaced by a single large strand, the closing membrane between the small strands having been dissolved. The development of sieve pores in an angiosperm has evidently been interpreted thus in Eames and MacDaniels' (1925, p. 70) comparative description of sieve tubes of lower and higher groups of plants.

Strasburger apparently had no intention of giving the term *sieve field* a strictly circumscribed meaning, for he also used it in the sense of Nägeli (Strasburger, 1887, p. 163, legend to fig. 73, *C*); then he employed

it to designate a unit in the so-called "compound sieve plate" (Strasburger, 1891, p. 236). In this latter sense the term *sieve field* was used in some more recent works also (Eames and MacDaniels, 1925, p. 195; Crafts, 1934).

Strasburger frequently speaks of "sieve pits" in reference to Nägeli's sieve fields (1891, p. 236, 260; 1901, p. 531) and in reference to a unit of a compound sieve plate (1901, p. 530). Schmidt (1917, p. 32–33) and Crafts (1932, 1933) also employ the term *pit* to designate those structures on lateral walls that resemble sieve plates.

Jeffrey (1917, p. 125), Hemenway (1913), MacDaniels (1918), and Eames and MacDaniels (1925, p. 72) use *lattice* to define structures similar to a sieve field in Nägeli's terminology.

Hill (1908) has shown that a sieve field, in the sense of Nägeli, is characterized, like a sieve plate, by deposition of callose and by a considerable chromaticity of connecting strands. If a sieve field occurs between a sieve tube and a parenchyma cell, it shows the callus and chromatic connecting strands only on the sieve-tube side. In the parenchyma half of the wall an ordinary simple-pit structure is present, so that each chromatic strand appears to be continuous with an ordinary plasmodesmon. A similar connection apparently exists between sieve tubes and albuminous cells in gymnosperms (Strasburger, 1891, p. 60–61; Hill, 1901). Strasburger (1891, p. 61–62) and Perrot (1899, p. 70–71) called these structures one-sided sieve pits.

In the present paper the term *sieve field* is used in the sense of Nägeli to denote the weakly developed structures resembling sieve plates, found on longitudinal walls. Such structures, if they occur between a sieve tube and a parenchymatous cell, are called one-sided sieve fields in Strasburger's sense. A unit in the so-called compound sieve plate is designated as a sieve plate.

The sieve plates of tobacco, whether single on an end wall (fig. 7, *B*) or in groups on inclined (fig. 13, *A* and *D*) and longitudinal walls, are characterized by rather small connecting strands. Plate 17 illustrates the striking contrast in size of connecting strands between sieve plates of *Cucurbita* and those of *Nicotiana*. As in all angiosperms, each connecting strand is surrounded by a callus cylinder which appears as a white ring around the densely stained connecting strand (fig. 13, *D;* plate 17, *B* and *C*).

The origin of the connecting strands and of the callus cylinders was not studied in this investigation; nor were any attempts made to determine the nature of the connecting strands and to prove their actual continuity through the sieve plate. As far as could be ascertained, the strands appeared continuous (fig. 13, *C*); and their thickness and chro-

maticity was greatest immediately after the disintegration of the nucleus and slime bodies. By using anilin blue one may detect the presence of the callus cylinders before the disintegration of nucleus and slime body—that is, before the strands attain their maximum size. Callus was visible at the stage depicted in figure 13, *B*.

Before the callus appears, the sieve plates resemble simple pits. The contrast between the pit area and the bars separating the pits from each other is very marked at this time (fig. 13, *E*). Later the bars, which in section appear like beads, show little increase in thickness, while the pit or the sieve-plate areas thicken rapidly (fig. 13, *E–H*).

This increase in thickness largely results from the growth of callus cylinders. Having increased in height, they spread laterally, covering thereby the original wall of the sieve plate. When this wall is completely imbedded in the callus, the former has, in sections, the appearance of small dots surrounded by the translucent callus (fig. 14, *A*). When the connecting strands attain their maximum thickness, the calloused plate becomes about as thick as the cellulose bars separating the sieve plates (figs. 13, *H;* 14, *A*).

When a sieve plate already covered with callus is seen in face view, in prepared sections, the callus cylinders surrounding the connecting strands are still discernible; the original sieve-plate wall, being less translucent than the callus, is visible through the latter. When the light passes through a practically solid callose layer, as around the connecting strands, it appears brighter than elsewhere and, therefore, the callus cylinders stand out as white rings. With anilin blue the darkest shade of the sky-blue color is obtained in the region of the callus cylinders.

The growth of the callus is uninterrupted until it reaches the final, the definitive stage (Lecomte, 1889). It gradually increases in height, thereby surpassing the cellulose bars in thickness and even burying some of them (fig. 14, *B* and *C*). It also progressively reduces the size of the pores of the sieve plate, so that the connecting strands become thinner (fig. 14, *C*). Finally the callus reaches the definitive stage, forming large bulging masses on both sides of each sieve plate (fig. 14, *D*). At this time the connecting strands are so thin that they may be shown only with particularly exacting staining technique.

The attachment of the cytoplasm to the sieve plate is firm when the connecting strands are most evident (fig. 13, *C*) ; weak before the strands reach their maximum size (fig. 13, *B*) and after definitive callus develops.

Sieve fields in Nägeli's sense seem not to occur between sieve tubes of tobacco. The sieve plates of all walls, as far as determined, have the same structure with regard to size of connecting strands and of callus cylinders. This observation agrees with that of Russow (1882*a*) on *Nicotiana*.

There are, however, one-sided sieve fields between sieve tubes and phloem-parenchyma cells. In these the connecting strands and callus cylinders are very small, but may be detected in anilin-blue material. The heavy and chromatic part of the strand, as well as the callus cylinder, extends only through the sieve-tube wall. This situation is indicated in figure 13, *C*, by lines reaching only about half way in one of the walls between a sieve tube and a phloem-parenchyma cell. The protoplast has been torn away from the sieve field because of its weak connection with this structure.

The wall bearing one-sided sieve fields is usually wavy, the sieve field bulging into the parenchyma cell, and the portions of the walls between the sieve fields bending toward the sieve tube (fig. 13, *A*).

Sieve plates also may bulge slightly, one way or the other (figs. 13, *C* and *H*; 14, *A*).

Longitudinal Walls of the Sieve Tubes.—Sieve-tube walls not covered with sieve plates or sieve fields show a peculiar transitory thickening that reaches its maximum prominence during the height of the development of the element.

Many workers have studied the development and disappearance of these thick walls. The French investigators who observed them in the protophloem (Lesage, 1891; Léger, 1895, 1897*a*, 1897*b*; Chauveaud, 1897, 1900) called them *nacré* walls because of their pearly luster.

The *nacré* development appears at the same time or slightly before the maturation of the sieve plate (Léger, 1897*b*; Chauveaud, 1897, 1900; Chang, 1935). The thickening is not permanent; it disappears in aging sieve tubes (Léger, 1895, 1897*a*, 1897*b*; Chauveaud, 1897, 1900; Russow, 1882*a*, 1882*b*; Hill, 1901; Chang, 1935; Esau, 1936).

Thick walls have been observed in secondary as well as primary sieve tubes (Léger, 1897*a*; Russow, 1882*a*, 1882*b*).

Investigators agree that the thick glistening walls of differentiated sieve tubes are of cellulose (Schmidt, 1917, p. 8–9). Certain chemicals cause this wall to swell (Schmidt, 1917, p. 10–13; Zimmerman, 1922, p. 42–46), and certain stains are readily adsorbed by it (Chauveaud, 1900; Chang, 1935).

The inner margin of the special thickening may be crenulated (Léger, 1897*a*; Schmidt, 1917, p. 12; Zimmerman, 1922, p. 42). Since the thickening is well set off from the original wall of the meristematic cell, some workers consider this layer distinct from the primary wall (Léger, 1897*a*; 1897*b*; Schmidt, 1917, p. 10; Zimmerman, 1922, p. 42–46).

The sieve tubes of tobacco develop thick walls while slime bodies and nuclei are still present. They appear to be highly hydrated walls because they shrink perceptibly when treated for imbedding in paraffin. Never-

theless, in actively differentiating primary phloem, the characteristic thick walls of the sieve tubes are evident in paraffin material (fig. 7, *D–F;* plates 8, *A;* 10, *B,* and 11, *A*). When the sieve tube is approaching obliteration, the thick wall disappears (plate 11, *B*).

In secondary phloem, which in general has thicker walls than the primary, the special wall of sieve tubes is usually inconspicuous in paraffin material (plate 16, *B*).

Degeneration and Obliteration of Sieve Tubes.—Hartig (1837, p. 160; pl. I, fig. 46), the discoverer of the sieve tube, already recognized that sieve tubes, and certain small cells associated with them, are crushed in the old phloem. He also saw formation of the cork cambium in this phloem.

Wigand (1863, p. 119), not knowing, apparently, of Hartig's interpretation, failed to understand the structure of old phloem and referred to it as horn prosenchyma or horn bast. Oudemans (1862), on the other hand, saw that this tissue consisted of flattened and folded cells, whereas Rauwenhoff (1869), who was familiar with Hartig's paper, stated that the crushed cells were sieve tubes.

Wilhelm (1880, p. 46) first described the process of obliteration of sieve tubes. He saw that sieve tubes were crushed after the formation of definitive callus and that their lumina became completely closed.

Bliesenick (1891) especially studied the problem of obliteration. He found that large masses of callus may or may not be formed before obliteration; that only sieve tubes and companion cells are crushed in angiosperms; and that, as previously shown by Strasburger (1891, p. 55), sieve tubes and albuminous cells are together destroyed in the gymnosperms.

Léger (1895, 1897*a,* 1897*b*), who studied obliteration in primary phloem in much detail, reported that sieve tubes lose their thick walls before being crushed but that sometimes, when crushing is only slight or absent, the sieve tubes remain open and resemble parenchyma cells.

Details on obliteration of primary sieve tubes also occur in papers by Esau (1933, 1934, 1935, 1936), and Chang (1935).

The primary sieve tubes are crushed and obliterated directly after definitive callus develops. In the secondary phloem, crushing usually progresses very slowly, and the definitive callus disappears before the obliteration of the lumen is attained (Janczewski, 1878, 1881, 1882; Russow, 1881, 1882*a,* 1882*b;* Lecomte, 1889; Strasburger, 1891, p. 194, 224, 245, 260; Hill, 1901). Callus-free sieve tubes apparently lack any protoplasmic structures (Russow, 1881; Janczewski, 1881; Strasburger, 1891, p. 245).

The observations on obliteration of sieve tubes in tobacco agree in

general with those reported for other plants. The primary sieve tubes lose their thick walls, develop definitive callus on sieve plates, and then collapse together with their companion cells. Partly crushed sieve tubes and companion cells are discernible in the lower median position of plate 10, *A*, and in the center of plate 11, *A*.

The protophloem sieve tubes are so thoroughly crushed that all traces of them gradually disappear (plates 8 and 9). They are first crushed along the lumen, the sieve plate persisting for a while.

As previously mentioned, the sieve tubes of the metaphloem last longer than those of the protophloem, and after they are crushed their lumina are not completely closed for a year or more (plate 14, *A*; 16, *A*).

The obliteration of the secondary sieve tubes is also slow and rather imperfect (plate 14, *B*). The relatively large masses of secondary phloem that accumulate during years of growth continue to show only partly closed sieve tubes (plate 13). The crushing of secondary sieve tubes apparently does not depend on the addition of new cells by the cambium, for it occurs in tangential direction (plate 13).

The behavior of definitive callus in functionless sieve tubes was studied in *Nicotiana glauca,* because the other species of tobacco did not furnish sufficiently old stems. In *N. glauca* the definitive callus is removed approximately during the second season of its existence.

Fresh definitive callus shows a characteristic affinity for anilin blue. Before callus disappears, its chromaticity is reduced and the connection with the sieve plate is weakened, so that in sections the callus frequently separates, more or less completely, from the sieve plate (fig. 14, *D*). The unattached lumps of callus show projections on the side facing the plate. These are portions of callus cylinders that formerly occurred within the sieve-plate pores.

The definitive callus seems to be removed from the sieve tube by dissolution. It appears soft on the external margin, for its outline becomes less sharp. Frequently the outer margin develops deep indentions, connected by fine lines with the pores of the sieve plate. Whether these lines are the much-reduced connecting strands has not been ascertained.

After the definitive callus is lost, the pores of the sieve plate appear completely open (fig. 14, *E*).

During the removal of the callus a peculiar change apparently takes place in the structure of the walls of the sieve-tube element. The so-called "middle lamella" appears to increase in thickness and shows marked chromaticity (fig. 14, *D* and *E*).

The protoplasts of the sieve tubes that have lost their definitive callus are completely degenerated, as evidenced by deep staining and lack of structure (plates 5, *B* and 12).

The Series of Transformations in the Structure of the Sieve Tube.—Phloem workers early formulated their ideas as to the stage of development that may be interpreted as the functioning stage. After extensively studying sieve tubes of all groups of vascular plants, Janczewski (1881, 1882) divided sieve-tube existence into the following stages: (1) evolutive stage, lasting from the cambial state to the time of "perforation of the sieve plate"; (2) active stage, covering the period when the connecting strands of the sieve plate are clearly evident; (3) transitional stage, when definitive callus develops on the plate; and (4) passive stage, characterized by absence of callus and cytoplasm.

Russow (1881, p. 77) suggested, "The specific function of the sieve tube begins with callus formation and lasts only as long as callus structures are present."

When, through the researches of Russow (1883), Kuhla (1900), Strasburger (1901), and Hill (1901, 1908), the connecting strands came to be regarded as modified plasmodesmata, the active stage was identified with the time of maximum differentiation of the connecting strands.

Whatever the nature of the connecting strands—whether they consist of slime and cytoplasm (Hill, 1901, 1908; Mühldorf, 1937) or of cytoplasm only (Schmidt, 1917, p. 31; Crafts, 1932), the sieve tube is generally regarded as mature when the strands have attained their maximum size and chromaticity. This development is closely preceded by disintegration of the nucleus and slime bodies and by the development of thick walls. Crafts (1932, 1933, 1934) reports also that at maturity the sieve-tube cytoplasm is completely permeable.

Mature sieve tubes occur very close to the undifferentiated procambium or cambium cells, because the developmental stage of a sieve tube is of very short duration.

Russow (1882*a*, p. 279) applied the term *boundary cells* to the first differentiated sieve tubes because, during the active season, these cells sharply delimit the phloem from the cambium. During dormancy this boundary is much less definite (Russow, 1882*a*, 1882*b*; Strasburger 1891, p. 66; Hill, 1901).

The French workers paid particular attention to the development of thick walls during maturation of the sieve tube. Léger (1895) used the term *differenciation nacré* to designate mature sieve tubes with thick glistening walls; but Chauveaud (1897, 1900) chose *maximum differenciation,* because, he said (Chauveaud, 1911), *nacré* walls occur in other cells beside the sieve tubes and should be regarded as one of the many characteristics of differentiated sieve tubes.

Primary sieve tubes, being obliterated more or less early during the first season, are characterized by a very short duration of the mature

stage. The secondary sieve tubes usually remain in mature stage one to two years (Wilhelm, 1880, p. 37; Janczewski, 1881; Strasburger, 1891, p. 70, 195, 244; Hill, 1908). They retain the definitive callus—that is, remain in transitional stage—for about a year (Russow, 1881; Strasburger, 1891, p. 70).

To avoid implications that certain structures and certain functions are definitely related, the stages in the period of existence of the tobacco sieve tube were designated as follows: (1) developmental stage, lasting from sieve-tube mother cell to a specialized cell; (2) mature stage, during maximum differentiation of connecting strands and maximum wall thickness; (3) transitional stage, during maximum development of definitive callus to its loss; (4) degenerative stage, beginning with the removal of definitive callus and ending with the more or less complete crushing of the element.

In the primary sieve tubes of tobacco, particularly in the protophloem, all stages are of short duration; the obliteration takes place almost as soon as the definitive callus develops. The callus is not removed before crushing, but is probably dissolved later. In the secondary sieve tubes all four stages may be clearly distinguished.

As far as could be ascertained, the period of maturity and the transitional stage each last not more than one year.

In an approximately four-year-old vigorous stem of *Nicotiana glauca,* still blooming when collected on the last of October, none of the phloem was yet cut off by cork cambium, but most of the sieve tubes were in the stage of degeneration. Plate 13 shows this phloem and depicts the relative proportions of the tissue in different stages of transformation. The area marked *Pr. Ph.* includes functionless primary phloem, a portion of which is also shown in plate 14, *A*. The zone marked *Sec. Ph.* I is secondary phloem containing degenerating sieve tubes free of definitive callus. A portion of this tissue is shown, at higher magnification, in plate 14, *B*. *Sec. Ph.* II is secondary phloem with sieve tubes in transitional stage; *Sec. Ph.* III, the same phloem, with mature sieve tubes. Enlarged views of mature phloem are illustrated in plate 15. *Sec. Ph.* IV contained developing sieve tubes with nuclei and slime bodies. The cambium was directly subjacent to the fourth layer of phloem.

If the mature stage is also the functioning stage, the amount of active tissue is remarkably small in comparison with the functionless phloem.

CERTAIN ASPECTS OF ARRANGEMENT AND INTERCONNECTION OF PHLOEM CELLS

Intercellular Spaces.—Lecomte (1889) found that, except in a few species, plants are characterized by absence of intercellular spaces in the phloem. The tissue has rather thick corners and bears certain resemblance to collenchyma. Crafts (1931) also remarks that intercellular spaces are usually lacking in phloem: if present, he thinks, they are filled with solution and have no connection with the air-filled intercellular spaces of the cortex. He suggests that this arrangement permits the development of high pressures characteristic of the phloem contents.

The tobacco species used in the present study contain intercellular spaces in the phloem, although they are largely limited to the rays. In the primary phloem the small groups containing sieve tubes, companion cells, and phloem parenchyma show compact arrangement of cells; but intercellular spaces are present immediately outside these groups (fig. 7, *A* and *B;* plate 8, *A*) and are very prominent in the primary-ray region (fig. 8, plates 10, *B* and 11). The internal phloem groups are also free of intercellular spaces (plate 8, *B* and 9, *B*). The spaces may be next to sieve tubes (fig. 7, *B* and *D;* plate 10, *A*) or to companion cells (fig. 7, *B* and *D;* plate 11, *A*). In freehand sections of fresh material, many intercellular spaces contain air (indicated in black in fig. 7, *A–C*). Possibly, nevertheless, in the intact plant these spaces are filled with solution.

Intercellular spaces appear during the early ontogeny of primary phloem (fig. 7, *D–F;* 8; plate 11). In figure 7, *D*, the lower sieve tube still had a nucleus.

In the secondary phloem, prominent intercellular spaces pass in radial direction through the rays (fig. 12). Spaces are also present among parenchyma cells of the longitudinal system (plate 16, *B*). Occasionally spaces seem to occur next to sieve tubes (fig. 12, at *c*) or to companion cells (fig. 12, at *a* and *i*).

In the old phloem, intercellular spaces become very prominent, particularly in the rays. With the increase in stem circumference the ray cells, as well as the cortical cells, are stretched tangentially until they partly separate from the adjacent cells of the same tangential layer. They remain connected, however, along parts of radial walls, where pits occur. Connecting strands are visible in these places in anilin-blue material. Upon further stretching, these connections resemble protuberances.

The presence of intercellular spaces in the phloem of tobacco, in contrast to many other plants, shows the need for thorough consideration of this problem; but the question of the contents of intercellular spaces in intact plants must be remembered in further studies on this subject.

Pits in Phloem Walls.—Information on pitting of phloem walls is incomplete and rather conflicting in the literature on phloem structure.

The sieve tubes and companion cells are generally held to be intmately connected. Several writers have reported presence of simple pits between these two types of cells (Wilhelm, 1880, p. 29, 42; Russow, 1883; Koch, 1884; Fischer, 1885*b*, 1886; Lecomte, 1889; Strasburger, 1891, p. 286; Hill, 1908). Beginning with Russow (1883), workers frequently searched for connecting strands in walls between sieve tubes and companion cells. Russow himself could not demonstrate such connections; but Kuhla (1900), Hill (1908), and Livingston (1935) reported cytoplasmic strands in these walls. Hill stated that on the sieve-tube side the strands were converted into slime and were accompanied by callus. Kuhla and Livingston, however, did not consider the special characteristics of the sieve-tube walls and studied the distribution of plasmodesmata without regard to the callus development. Livingston used *Nicotiana tabacum* in his studies and gave figure 12, plate 1, to support his statement that plasmodesmata interconnect sieve tubes and companion cells. The identity of sieve tubes and companion cells is, however, uncertain in this figure.

Sometimes workers observed no pitting in the wall between the sieve tube and the companion or albuminous cell. Strasburger (1891, p. 73, 286) pointed out that pits are evident only when the walls are thick. Kuhla (1900) reported that in *Cucurbita* the connecting strands between sieve tubes and companion cells are restricted to pits, but that in *Viscum* the wall between these two kinds of elements is so thin that no pits occur and plasmodesmata are scattered.

Many workers saw no connections between sieve tubes and phloem parenchyma nor between sieve tubes and rays (Wilhelm, 1880, p. 29; Russow, 1883; Fischer, 1885*b*, 1886; Strasburger, 1891, p. 60, 195, 225, 274). Kuhla (1900), on the other hand, reported pits and plasmodesmata between sieve tubes and phloem parenchyma cells. Hill (1908) described these pits as one-sided sieve fields.

Pits are absent between phloem parenchyma and companion cells, according to Fischer (1886); between albuminous and other parenchyma cells in pine, according to Strasburger (1891, p. 60). The latter worker, however, saw pits between companion and ray cells in certain species of Angiosperms (Strasburger, 1891, p. 195, 212, 225, 237, 274). Kuhla (1900) found few pits with fine plasmodesmata between companion and phloem-parenchyma cells in *Cucurbita* and *Viscum*.

The recognition of pitting among phloem-parenchyma and ray cells and also between these two kinds of cells did not present much difficulty to workers (Wilhelm, 1880, p. 30; Russow, 1882*c*, 1883; Fischer, 1886;

Strasburger, 1891, p. 60, 237, 247; Hill, 1908). Russow (1883) found, in many plants, that pits in phloem parenchyma are localized in radial walls, whereas in terminal walls plasmodesmata are scattered.

Pits occur between parenchymatous members of the phloem and the fibers (Strasburger, 1891, p. 60, 237).

The present study of connections between phloem cells of tobacco was preliminary in nature; no special plasmodesmata technique was employed. The object was to gain some information on the occurrence of pits in phloem walls. In conformity with Crafts' (1934) observation, *Nicotiana glauca* proved more satisfactory than *N. tabacum* for the study of pitting.

Figure 12 shows the distribution of pits in a tangential section of *Nicotiana glauca* phloem. Sieve plates are represented by double lines interconnected by numerous cross lines with each other; the one-sided sieve fields between sieve tubes and parenchyma cells are shown as indentions appearing on the sieve-tube side of the wall; parenchyma walls with simple pits are shown as lines with alternating thick and thin places.

The distribution of sieve plates has been adequately discussed in the part on ontogeny of secondary phloem.

Sieve tubes are rather frequently connected by one-sided sieve fields with phloem-parenchyma cells. These structures are evident in radial and in tangential longitudinal sections. Several sieve fields are shown in figure 12 at low magnification, and in figure 13, *A*, at high magnification.

The wall between the sieve tube and companion cell is very thin and, as far as could be ascertained, shows no pitting (fig. 13, *A* and *I*). This appears to be true of both the primary and the secondary phloem. In anilin-blue material, cytoplasmic connections were recognized between parenchyma cells, but not between sieve tubes and companion cells. This technique is, however, not adequate to demonstrate plasmodesmata when the latter are extremely fine.

Occasional simple pits occur between companion and phloem-parenchyma cells (fig. 12, at *b* and elsewhere; fig. 13, *I*). The wall between a ray and a companion cell is also pitted sometimes.

Simple pits are common among and between phloem-parenchyma and ray cells (fig. 12). Connecting strands may be demonstrated in these pits by the anilin-blue technique.

As the review of literature and the data on tobacco show, the problem of connections between cells of the mature phloem is far from clearly understood. A comprehensive study of phloem walls by the use of techniques showing plasmodesmata, combined with methods used in demonstrating callus, should greatly elucidate the problem of cytoplasmic interrelation among phloem cells.

DISCUSSION OF THE GENERAL HISTOGENETIC ASPECTS INVOLVED IN THE PRESENT INVESTIGATION

The present investigation, though primarily a study of ontogeny of the phloem tissue, touches upon several general problems of developmental anatomy that deserve attention from morphologists.

Among these problems the origin of the vascular tissues is of particular interest in relation not only to histogenesis but also to nomenclature. Sufficient data have been presented here to show that no creditable ontogenetic study of xylem or phloem can omit the problem of distinguishing between cambium and procambium.

The common method of separating these two forms of vascular meristem is based mainly on studies of cell arrangement as demonstrated in transverse sections. Orderly radial arrangement of cells is used as the principal criterion for recognizing the cambium.

If radial seriation of cells continues to be considered as the major character distinguishing between cambium and procambium, and between secondary and primary vascular tissues (Eames and MacDaniels, 1925, p. 51), then we must agree with Chauveaud (1911) that stems and leaves possess no primary vascular tissues.

Yet a young elongating aerial organ and an old stem show important differences as to method of tissue formation and structure of the vascular meristem. The present study illustrates how these differences may be utilized in characterizing the so-called "primary" and "secondary phloem." It also indicates that a reëvaluation of the present classifications of vascular meristems into procambium and cambium, and of vascular tissues into primary and secondary, requires for a background comparative developmental studies of a wide scope. The interpretation of a meristem as cambium and of xylem and phloem as secondary only because of radial seriation of cells (Arber, 1917–1919, 1922; Priestley and Scott, 1936) does not solve the problem of classifying vascular tissues.

The problem of origin of internal phloem also involves the question of classifying tissues into primary and secondary. The strands of internal phloem originate in successively more mature cells; and if the first strands are interpreted as primary, because they arise in meristematic cells, the subsequent strands should be considered secondary because they are initiated in more or less mature cells. Yet the developmental and structural characteristics of the phloem strands are apparently independent of the degree of differentiation of initiating cells. Any striking differences seem to be determined by the position in the stem. Long multinucleate fibers occur, for example, only in the strands that are

formed on the inner periphery of the phloem in rapidly elongating parts of organs.

The present study draws attention to the problem of leaf-trace differentiation. Workers generally agree that the procambium of a leaf trace originates at the base of a leaf primordium and differentiates then acropetally into the primordium, basipetally into the foliar buttress and the stem proper (Foster, 1936). The downward differentiation gives rise to the leaf trace. It is also agreed that the xylem commonly differentiates in the same manner as the procambium. The present study, as well as Chang's (1935) paper, indicates that the phloem of a leaf trace follows a different course of development from that of xylem. It differentiates, at least in the species considered in these studies, acropetally from the stem into the leaf.

Judging from this information, sieve tubes appear first in the younger, lower portion of the procambium of the trace before they differentiate in the older, higher portion of this meristem. No data seem to be available to test the correctness of this implication.

To elucidate the relation between the first sieve tubes and the procambium of a leaf trace, further studies like Louis' (1935) would be required; but they should be combined with studies on older portions of stem where vascular differentiation takes place.

Developmental studies on phloem reveal the weaknesses of the present concept of the pericycle. As Léger (1897*b*) has adequately proved from the ontogenetic point of view, the so-called "pericycle" of most stems is not a distinct tissue region but is part of the phloem. Léger's work, however, did not find sufficient recognition; instead Morot's (1885) interpretation largely came to dominate our concept of pericycle as a layer originally independent of vascular tissues.

In this connection, van Tieghem (1886) considered only mature structures when he defined the pericycle, and placed the definition of this region outside any ontogenetic inferences. Morot (1885), however, introduced the idea that pericycle arises outside the procambium.

Léger's (1897*b*) work alone would seem a good basis for reconsidering the present concept of pericycle; but in view of Baranetzky's (1900) conclusion, that even in Cucurbitaceae at least part of the sclerenchyma ring arises in the procambium, further comparative studies on the origin of pericycle would be desirable.

SUMMARY

The differentiation of the first phloem elements in the apex of the shoot of *Nicotiana* is determined by the development of leaf primordia. The first sieve tube within the stem appears about 300 microns below the

growing point in the lower portion of the trace of the fourth or fifth leaf primordium.

Within the trace the sieve tubes differentiate in acropetal order. When the first mature sieve tube appears at the base of the leaf, a xylem element is initiated opposite in the procambium. While the sieve tube continues its upward differentiation, the xylem element develops downward, as well as upward.

The first sieve tube, as seen in transverse sections, appears in a median position within the procambium region of a petiole or a leaf trace. New sieve tubes arise right and left from the first one until they become evenly distributed on the periphery of the procambium and form in the stem a continuous ring. The subsequent sieve tubes differentiate in centrifugal direction from the first layer.

The first xylem develops more slowly than the first phloem and remains restricted to a few elements in the median position of the petiolar bundle or trace until the phloem has formed a continuous layer of sieve tubes about the periphery of procambium.

In the apex of the leaf the xylem differentiates somewhat in advance of the phloem; but at the base of the leaf and in the stem, sieve tubes of the external phloem appear first among the vascular elements. They are followed by protoxylem elements. The sieve tubes of the internal phloem develop, in stem and leaf, after the first protoxylem cells.

After the differentiation of some external sieve tubes, divisions by tangential walls become established in the procambium so that primary xylem arises in definite radial rows.

Beginning with approximately the third centripetal layer of sieve tubes, the phloem also arises from the radially seriated meristem; but the appearance of longitudinal walls of various orientation in certain phloem mother cells disturbs the orderly arrangement of the differentiating tissue. These divisions occur in the formation of strands consisting of sieve tubes, companion cells, and phloem-parenchyma cells. Primary rays alternate with these strands and by the orderly arrangement of their cells indicate their origin from a radially seriated meristem.

The vascular meristem, including the stage of orderly divisions, is here called "procambium" as long as it shows the following characteristics: more or less polygonal cells in transverse sections; scarcity of oblique end walls; lack of sharp distinction between initials of transverse and longitudinal systems; absence of oblique anticlinal divisions. The sieve tubes derived from this meristem elongate after the beginning of specialization. The procambium thus characterized is active mainly in elongating organs. The tissues derived from it are here called "primary xylem" and "primary phloem."

In the part of the stem that has definitely ceased to elongate, the vascular meristem shows, in contrast to the procambium, the following characteristics: more or less rectangular cells in transverse sections; presence of distinct ray and fusiform initials; occurrence of oblique anticlinal divisions. The sieve-tube elements are considerably shorter than the fusiform initials from which they arose because of transverse divisions in phloem mother cells. This meristem is the cambium and gives rise to secondary phloem and xylem.

The primary phloem may conveniently be divided into protophloem and metaphloem. The former arises during the most rapid elongation of the stem, and its sieve tubes and companion cells have a very brief existence. The metaphloem, more persistent, is formed—in contrast to the protophloem—by an obviously radially seriated meristem.

Whereas the radial arrangement of cells is largely disturbed in the differentiating primary phloem, it is very evident in the mature secondary phloem.

The internal phloem differentiates from strands of meristem that arise through cell division on the adaxial side of the xylem. These cells appear as ground-meristem cells when internal phloem just begins to differentiate, but they cannot be sharply delimited from the procambium. Later the strands of internal phloem arise in progressively older cells and progressively closer to the protoxylem. Eventually, parenchyma of the protoxylem develops strands of phloem.

The strands of internal phloem are larger and better defined than the strands of the primary external phloem. They further enlarge for a limited time through division of cells on the abaxial side of each strand. Rapidly expanding parenchyma cells, resembling pith cells, surround the internal-phloem groups.

The strands of the internal and of the primary external phloem anastomose among themselves. The external and the internal phloem layers are connected with each other through the leaf gaps.

The earliest phloem on the adaxial as well as the abaxial side of the stele is short-lived and undergoes a striking transformation after the obliteration of sieve tubes and companion cells. Elongated parenchyma cells lying near the crushed elements develop into fibers. These, in the present paper, are called "primary phloem" fibers instead of pericyclic fibers because they arise in the same procambium as the nearest sieve tubes.

The sieve tubes of the primary phloem have transverse end walls, each usually bearing one sieve plate. The sieve tubes of the secondary phloem have oblique and transverse terminal walls. Rows of small sieve plates occur on a longitudinal or an oblique wall; one sieve plate occurs on a

transverse wall. The sieve tubes of the internal phloem show mostly transverse sieve plates, but oblique walls with numerous sieve plates occur in strands formed in older stem regions.

The protoplasts of mature sieve tubes in all phloem regions are characterized by absence of discrete nuclei and presence of plastids carrying a carbohydrate that stains red with iodine. One nucleus and one large slime body, with sometimes a few small ones, occur in young sieve tubes of the secondary as well as the primary phloem. The slime bodies and the nucleus disintegrate at approximately the same time.

The very fine connecting strands through the sieve plates are each surrounded by a callus cylinder, as in other angiosperms. After the disintegration of slime body and nucleus, the connecting strands show maximum diameter and chromaticity. Later the callus cylinders enlarge and constrict the strands. The callose spreads also over the cellulose network in older sieve tubes and eventually becomes deposited on both sides of the plate in great masses, called definitive callus.

The primary sieve tubes are crushed after the development of the definitive callus, the secondary lose their callus in approximately the second year after its formation. The secondary sieve tubes are only partially crushed.

The entire life span of a sieve tube may be divided into four stages: (1) developmental stage, during which the protoplast specializes and the sieve plate begins its characteristic development; (2) mature stage, beginning with the maximum differentiation of the connecting strands and ending with the development of definitive callus; (3) transitional stage, from maximum development of callus to its loss; (4) degenerative stage, from loss of callus to more or less complete crushing, accompanied by death of protoplast. In primary sieve tubes the transitional stage is omitted.

Each sieve tube usually has only one companion cell, which may be as long as the sieve tube or shorter. The wall between the sieve tube and the companion cell is very thin and apparently has no pits.

One-sided sieve fields occur between sieve tubes and phloem-parenchyma cells; simple pits among and between phloem-parenchyma and ray cells. Companion cells sometimes show simple pits in walls adjacent to other parenchymatous members of the phloem.

The phloem of tobacco shows rather loose cell arrangement. Although groups containing sieve tubes are free of intercellular spaces, the latter are very prominent among ray cells.

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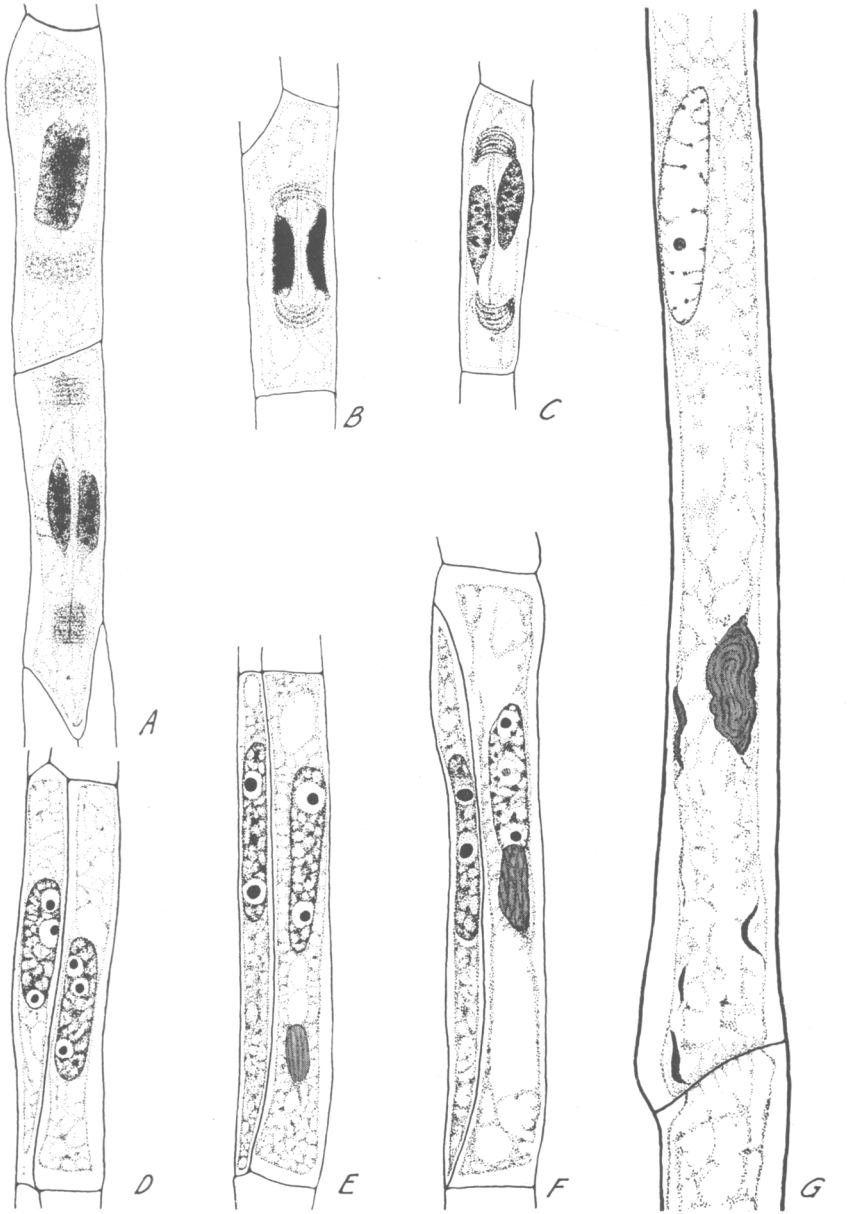

Plate 1.—Ontogeny of primary sieve tubes of *Nicotiana tabacum*. *A–C*, Dividing phloem mother cells. *D*, Young sieve tube, to the right, and companion cell, to the left. *E*, *F*, Two further stages in the development of sieve tubes and companion cells; each sieve tube contains one slime body. *G*, Portion of a sieve tube with one large and several small slime bodies and a degenerating nucleus. (All × 992.)

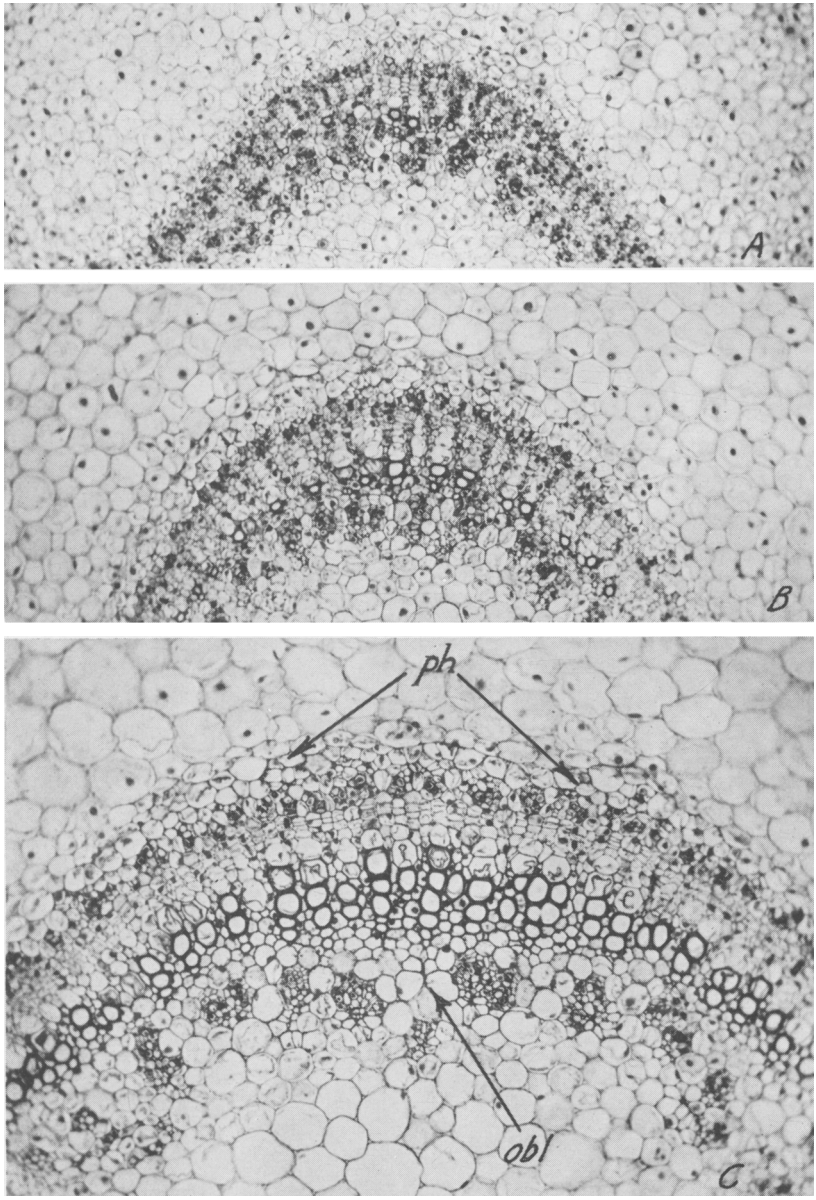

Plate 2.—Vascular bundles of petioles from *Nicotiana tabacum* in three stages of development. The leaves used for these sections came from the same plant as that in plate 3, *A*, and measured the following number of millimeters in length: *A*, 19; *B*, 36; *C*, 79. The petiole in *A* was also used for figure 6, *B*; the one in *C*, for plate 8. Details are: *ph*, external limit of phloem; *obl*, obliterated xylem elements. (All × 90.)

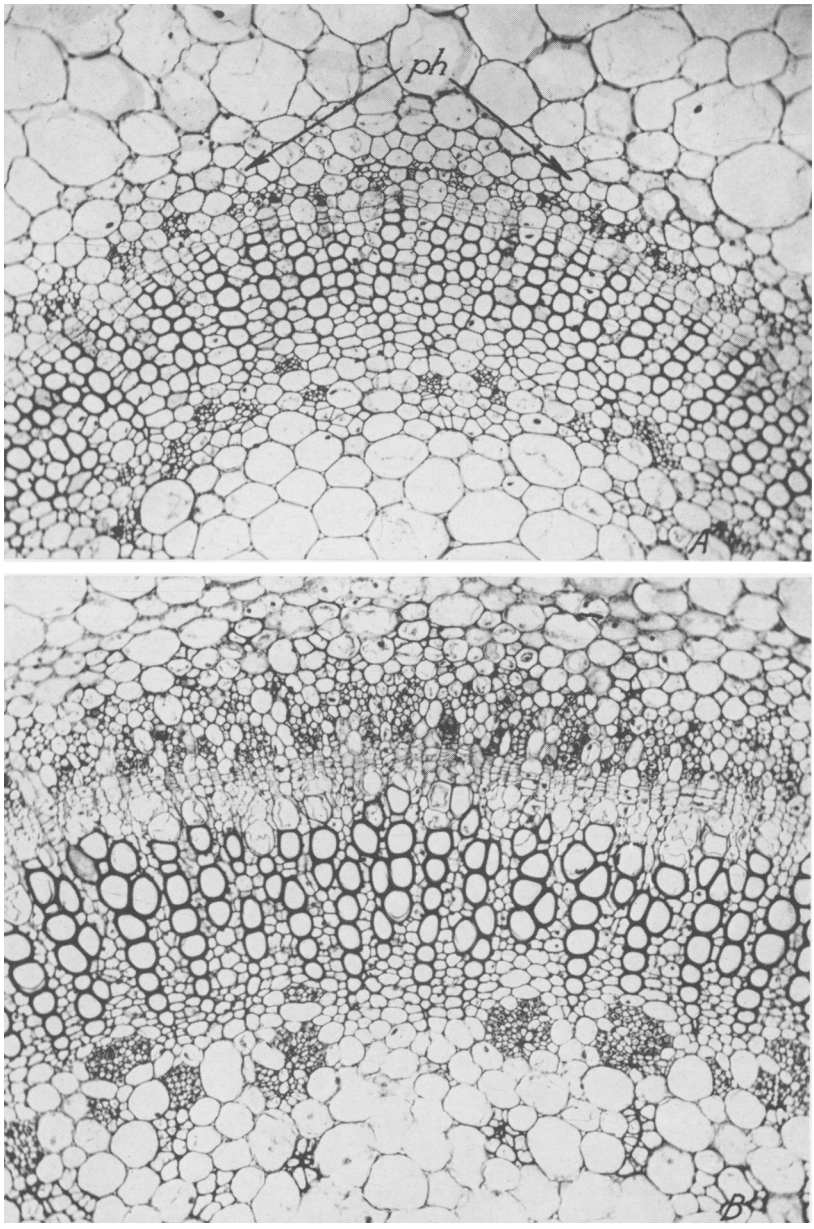

Plate 3.—Vascular tissues of *Nicotiana tabacum* petioles: *A*, from a mature leaf, 181 millimeters long, of a three-months-old plant grown in a greenhouse; *B*, from an immature leaf, 360 millimeters long, of a vigorous two-year-old plant of the same species grown outdoors. The petiole of *A* was used also in plate 9; that of *B*, in plate 10. The external limit of phloem is indicated at *ph*. (Both × 72.)

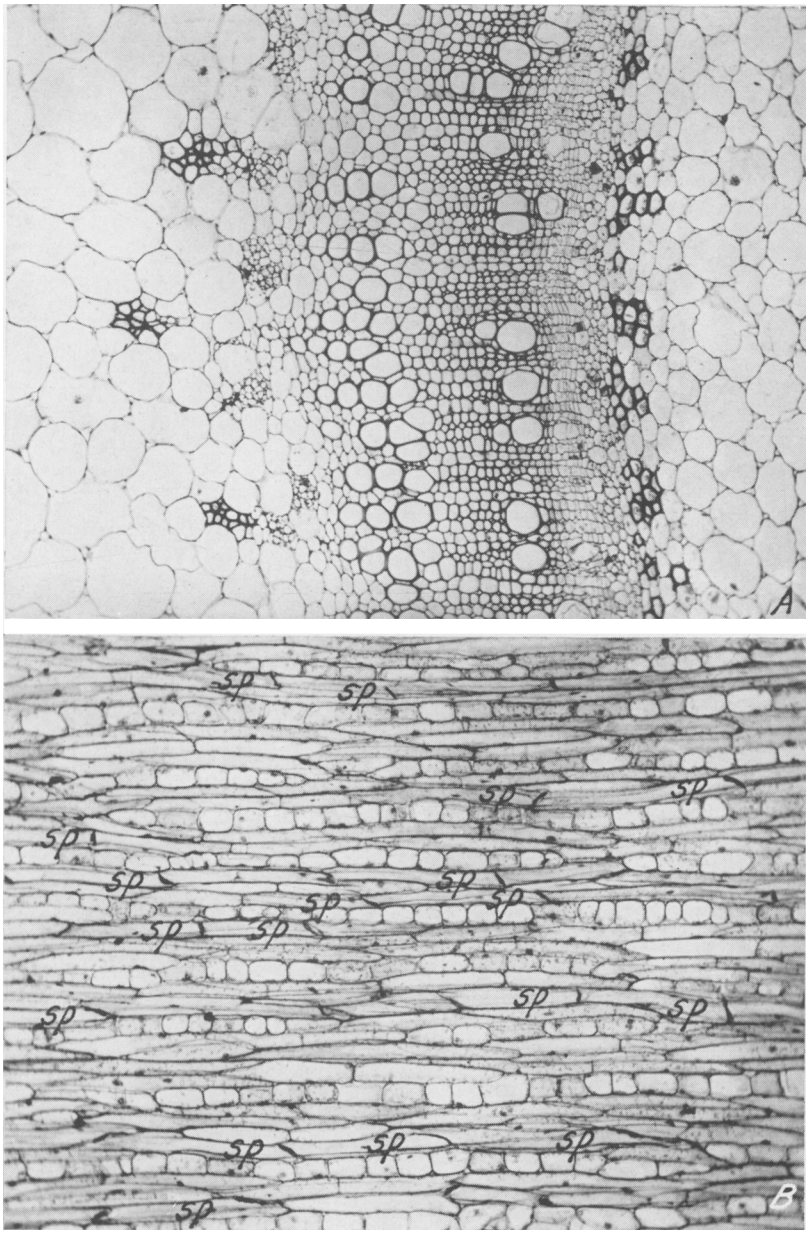

Plate 4.—*A*, Transverse section through the vascular tissues of a stem of a three-months-old plant of *Nicotiana tabacum*. *B*, Tangential longitudinal section of young secondary phloem from an approximately one-year-old stem, 3 centimeters in diameter. Sieve plates are indicated at *sp*. (Both × 90.)

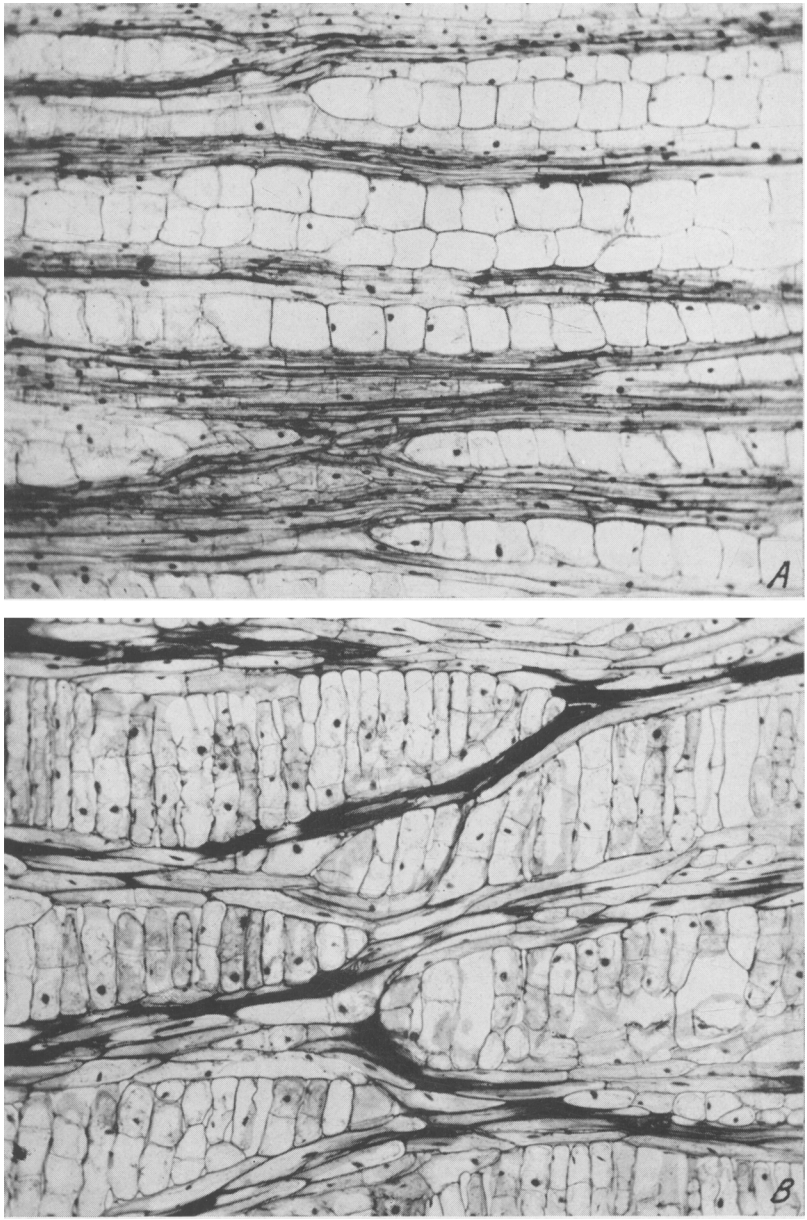

Plate 5.—*A*, Tangential longitudinal section through the internal phloem of a three-months-old stem of *Nicotiana tabacum*. *B*, Tangential section through old secondary phloem of *N. glauca* stem, 3 centimeters in diameter, sampled at the end of the first season's growth. (Both × 90.)

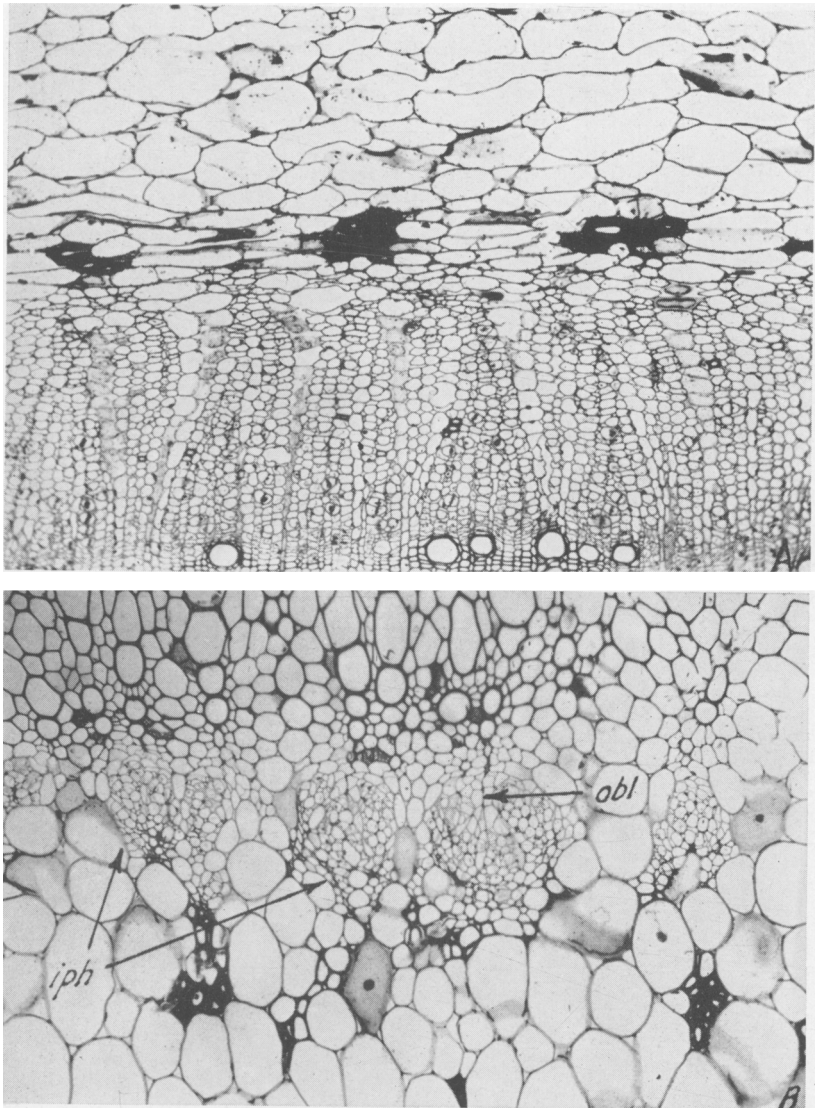

Plate 6.—Transverse sections from a two-year-old stem of *Nicotiana tabacum*, 3 centimeters in diameter, sampled 3 feet above the ground level. Formalin-acetic-alcohol fixation. *A*, External secondary phloem, with fibers and cortex above and some secondary xylem below. *B*, Internal phloem, with primary xylem above and pith parenchyma below; figure 9 shows this phloem at higher magnification. Details are: *iph*, internal phloem; *obl*, obliterated xylem elements. (Both × 90.)

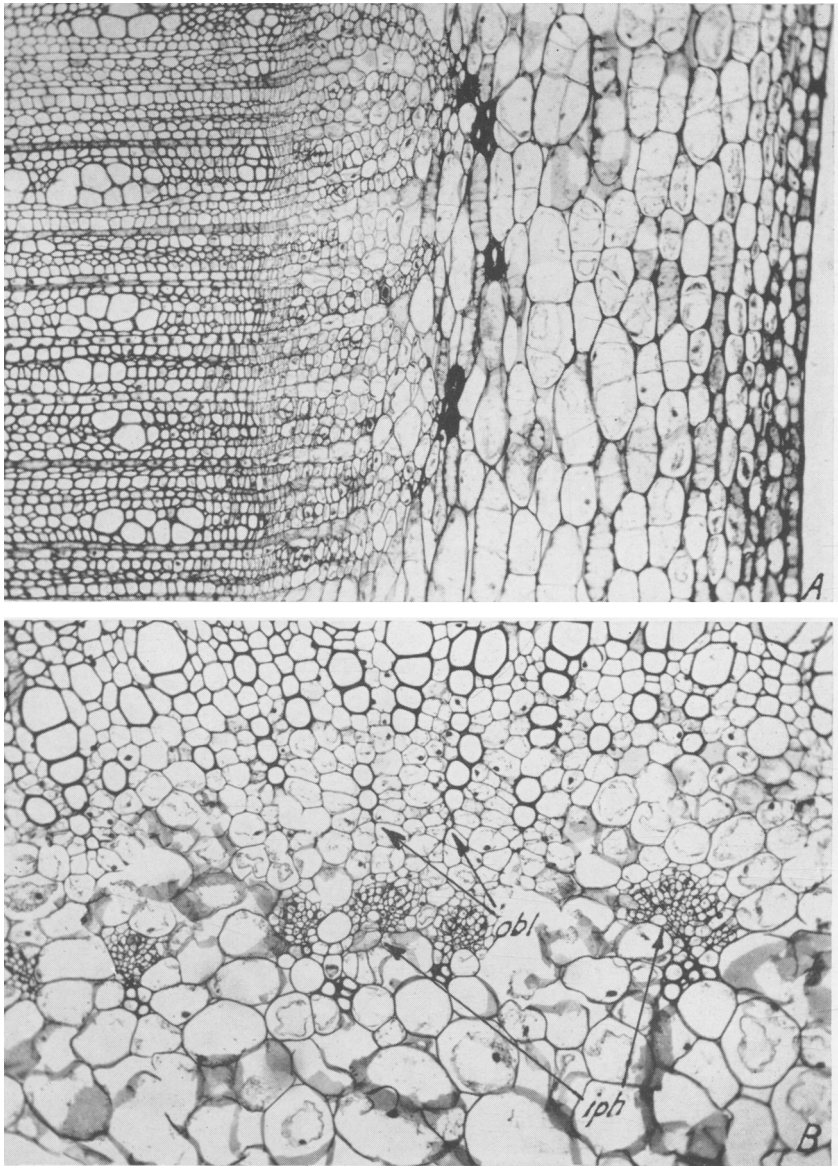

Plate 7.—Transverse sections from a stem of *Nicotiana glauca* sampled at the end of the first season. *A*, Tissues from left to right are as follows: secondary xylem; cambium; secondary, then primary external phloem; primary phloem fibers; cortex; and epidermis. *B*, Internal phloem with primary xylem above and pith parenchyma below. Details are: *iph*, internal phloem; *obl*, obliterated xylem elements. (Both × 90.)

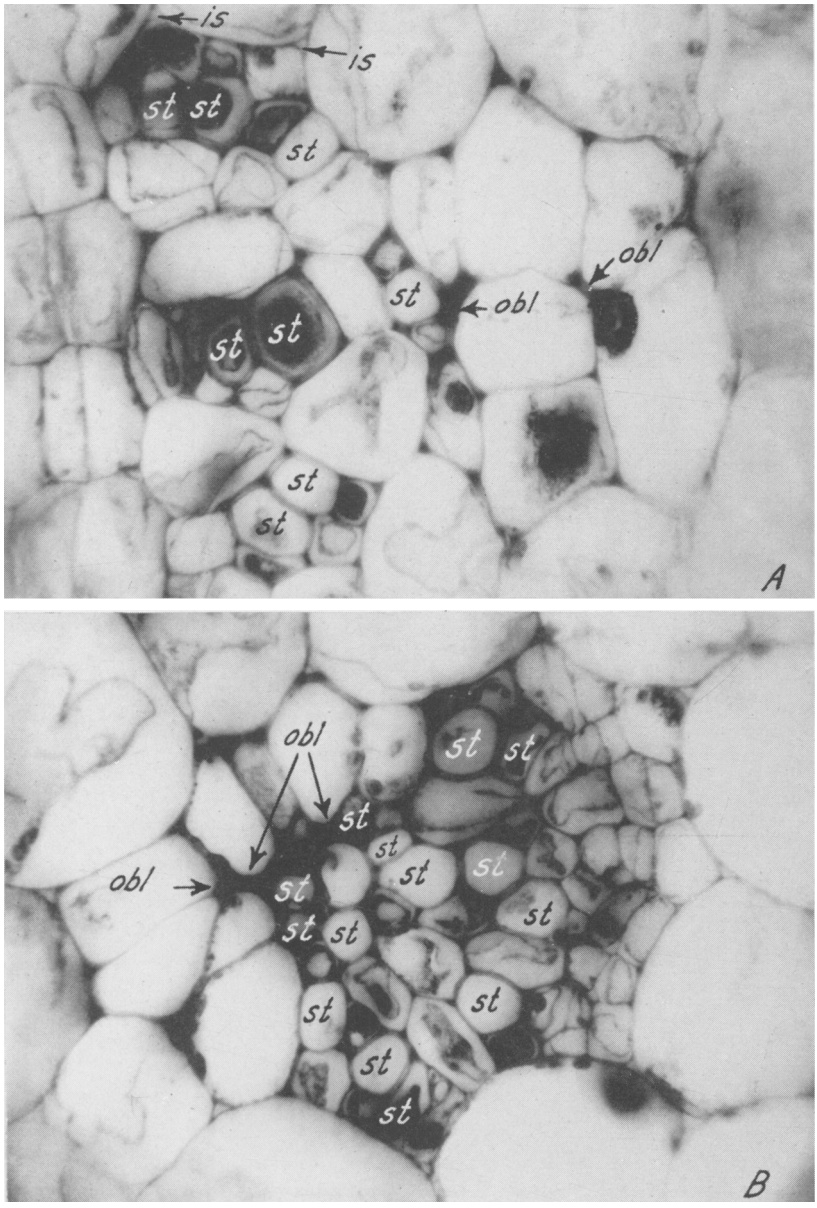

Plate 8.—Transverse sections through phloem of *Nicotiana tabacum* from the same petiole as in plate 2, *C*. *A*, External phloem; *B*, internal phloem. Details are: *is*, intercellular space; *obl*, obliterated elements; *st*, sieve tube. (Both × 810.)

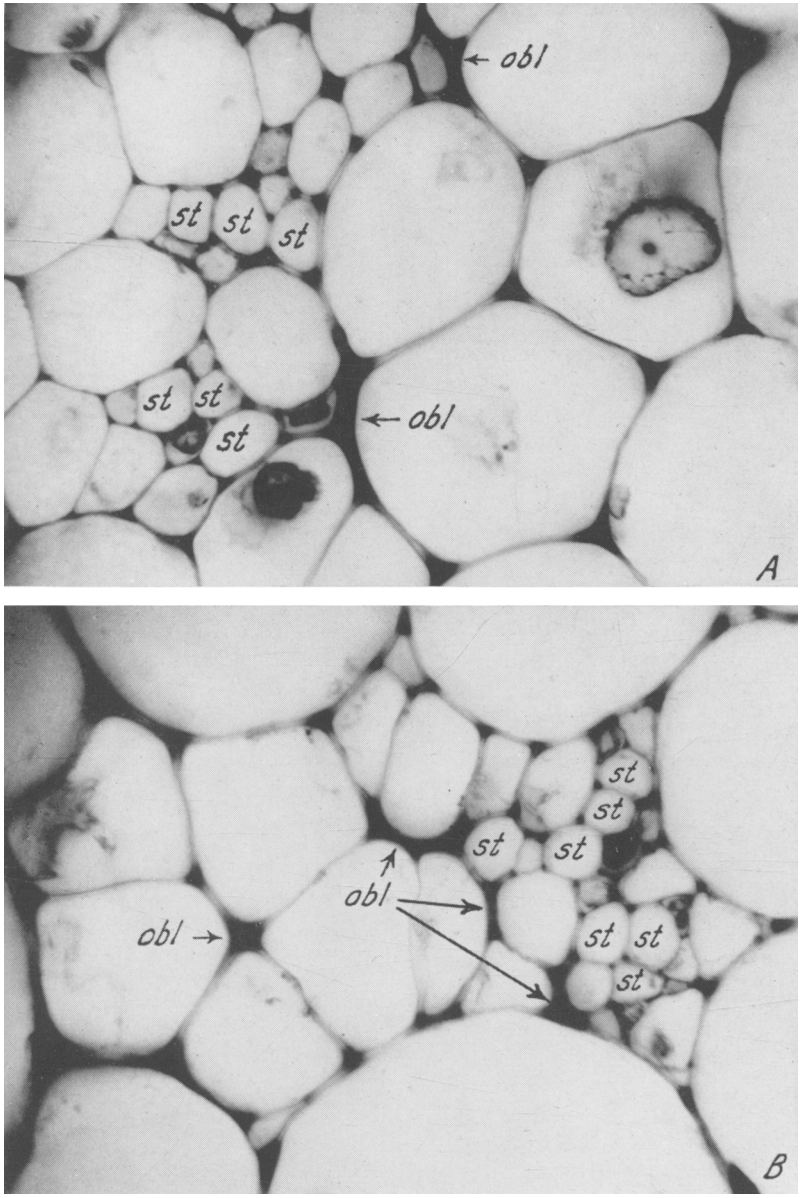

Plate 9.—Transverse sections through phloem of *Nicotiana tabacum* from the same petiole as in plate 3, *A*. *A*, External phloem; *B*, internal phloem. Details are: *obl*, obliterated elements; *st*, sieve tube. (Both × 810.)

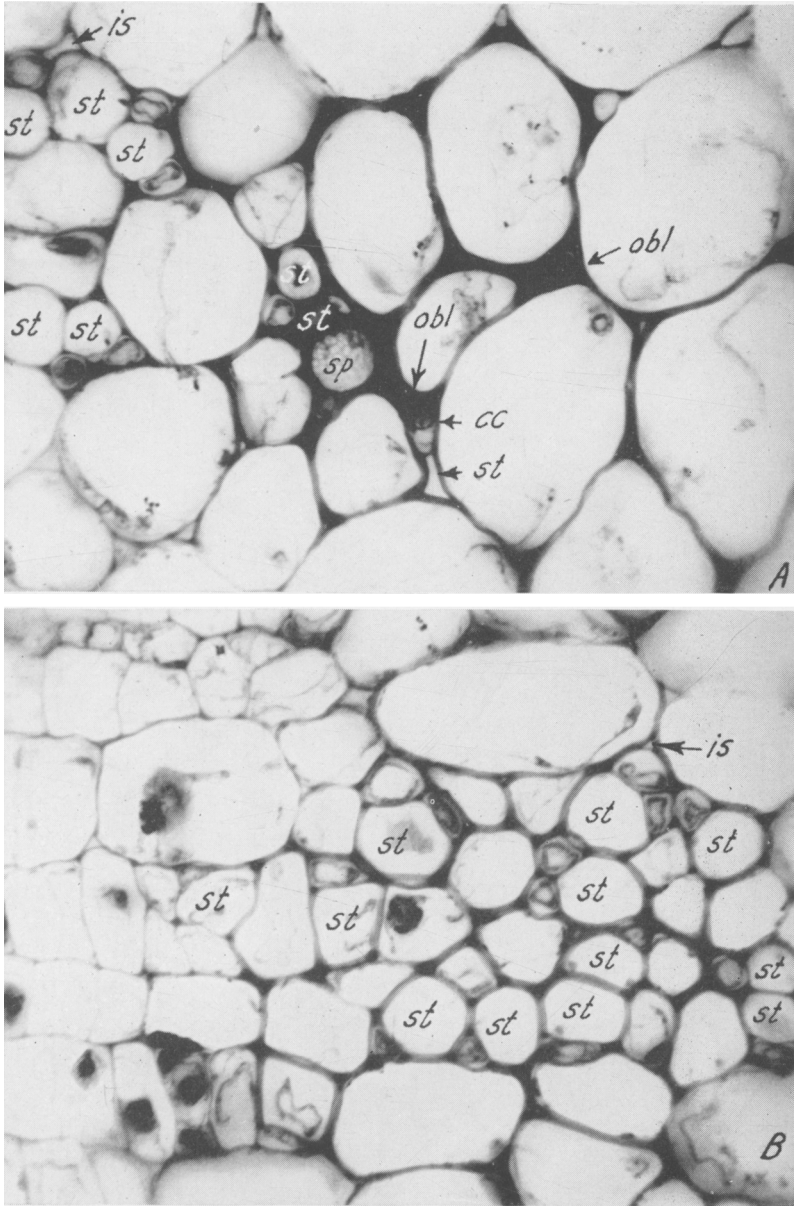

Plate 10.—Transverse sections through external phloem of *Nicotiana tabacum* from the same petiole as in plate 3, *B*. *A*, Outer part of phloem with partly and completely crushed sieve tubes and companion cells. *B*, Inner part of phloem with mature sieve tubes, right, and undifferentiated elements, left. Details are: *is*, intercellular space; *obl*, obliterated elements; *sp*, sieve plate; *st*, sieve tube. (Both × 810.)

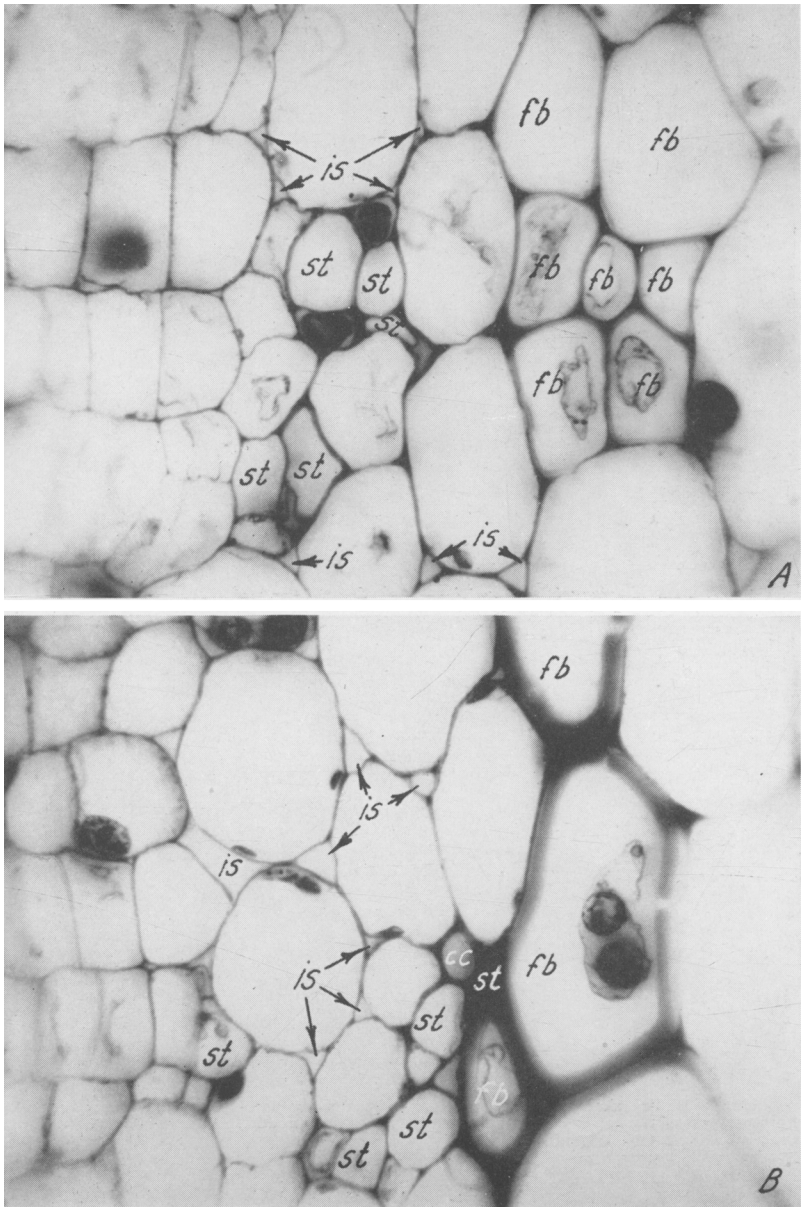

Plate 11.—Transverse sections through primary external phloem of a three-months-old stem of *Nicotiana tabacum*. *A*, Section from a higher internode; *B*, from a lower. Details are: *cc*, companion cell; *fb*, fiber; *is*, intercellular space; *st*, sieve tube. (Both × 810.)

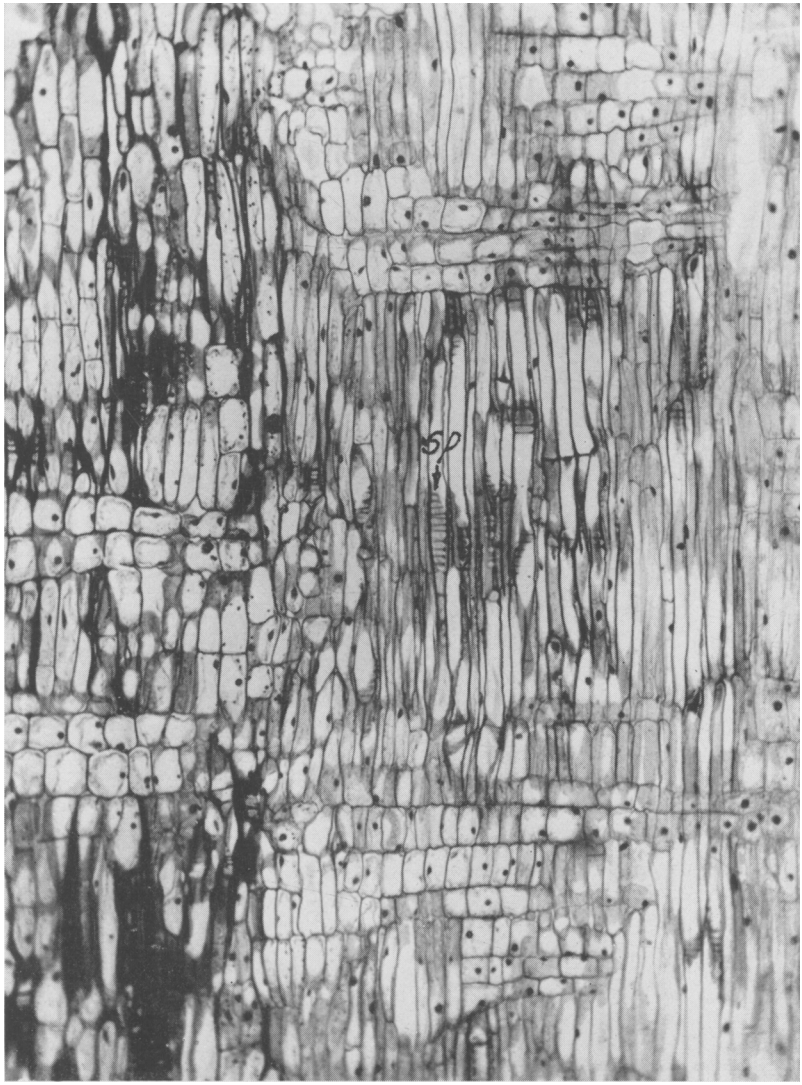

Plate 12.—Radial view of secondary phloem from a two-year-old stem of *Nicotiana glauca*, with functionless phloem to the left, mature phloem in the middle, and cambium to the right. A sieve plate is indicated at *sp*. (× 117.)

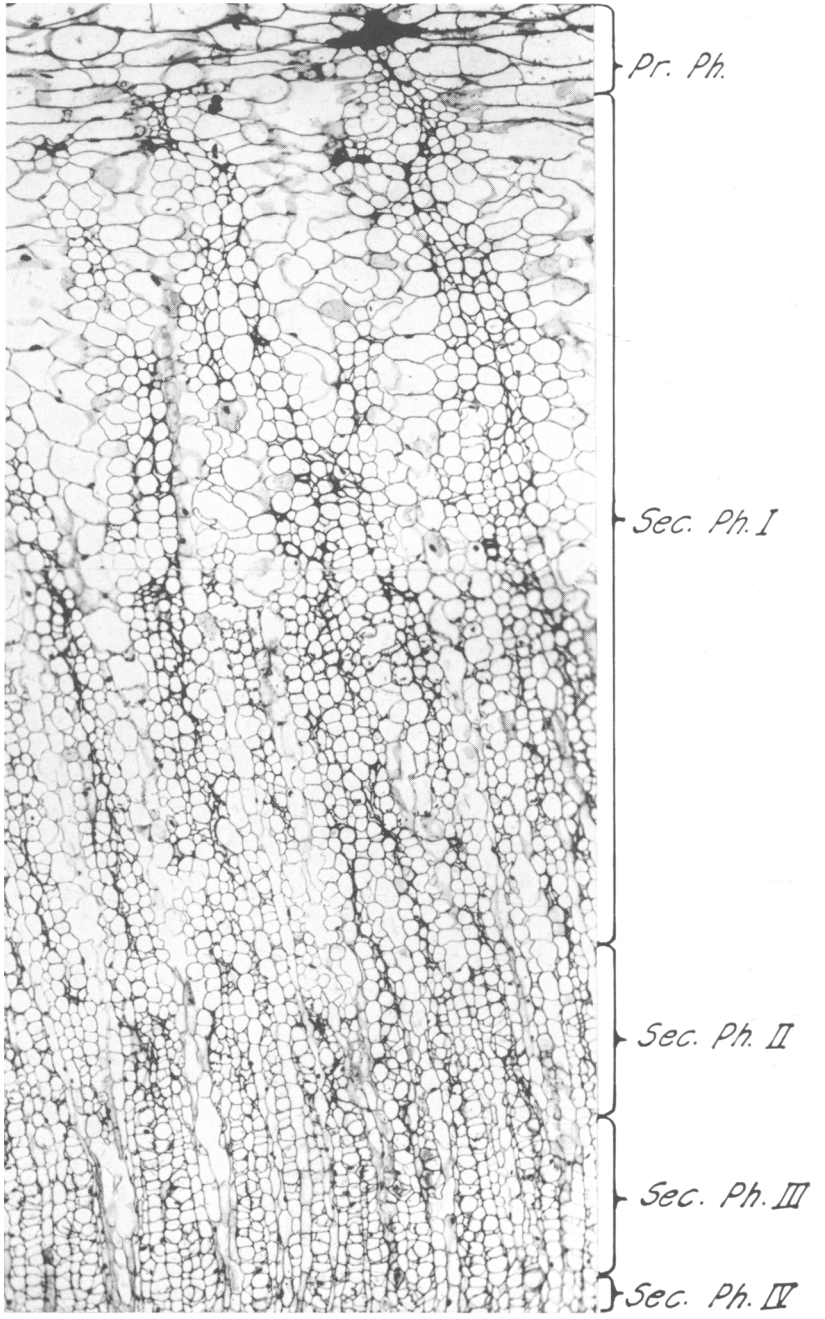

Plate 13.—Transverse section of external phloem from an approximately four-year-old stem of *Nicotiana glauca*. *Pr. Ph.*, primary phloem; *Sec. Ph.*, secondary phloem. Further explanations in the text. (× 90.)

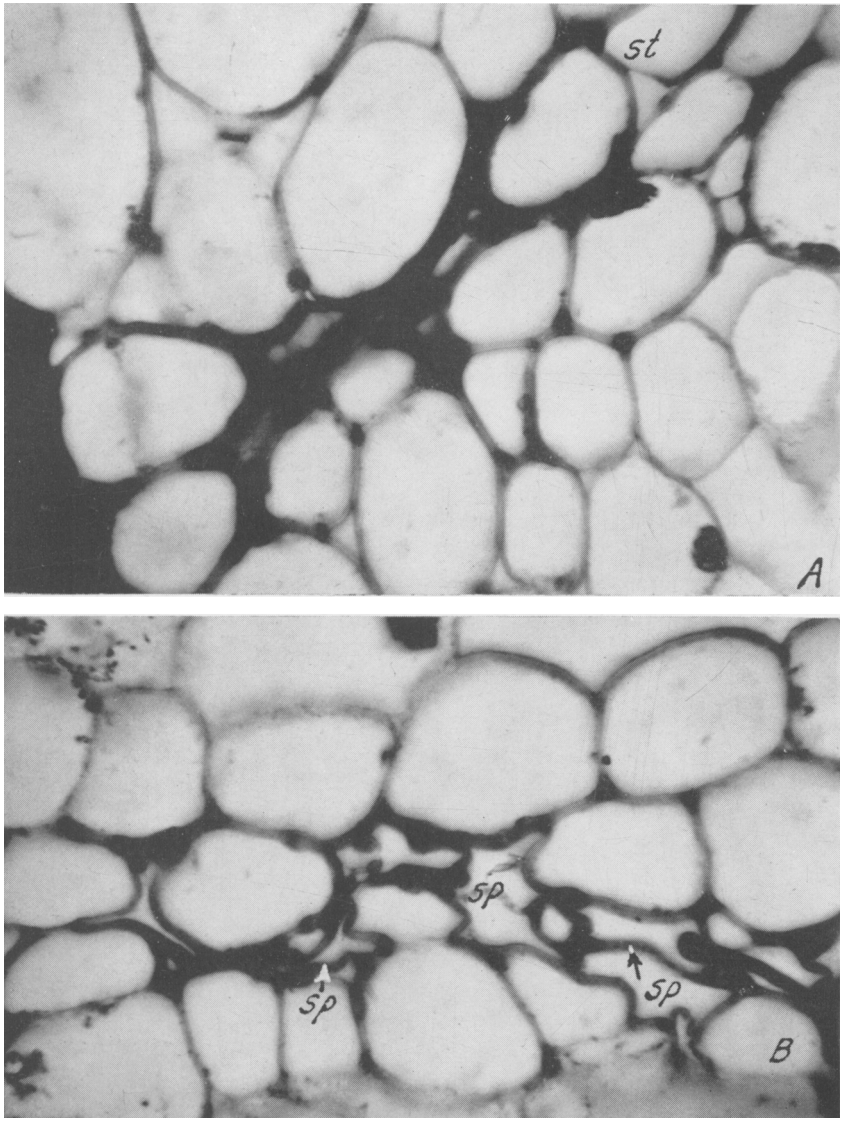

Plate 14.—Transverse sections through the same phloem as in plate 13. *A*, Crushed metaphloem from the region marked *Pr. Ph.* in plate 13. *B*, Crushed secondary phloem from the region marked *Sec. Ph. I* in plate 13. Details are: *sp*, sieve plate; *st*, sieve tube. (Both × 810.)

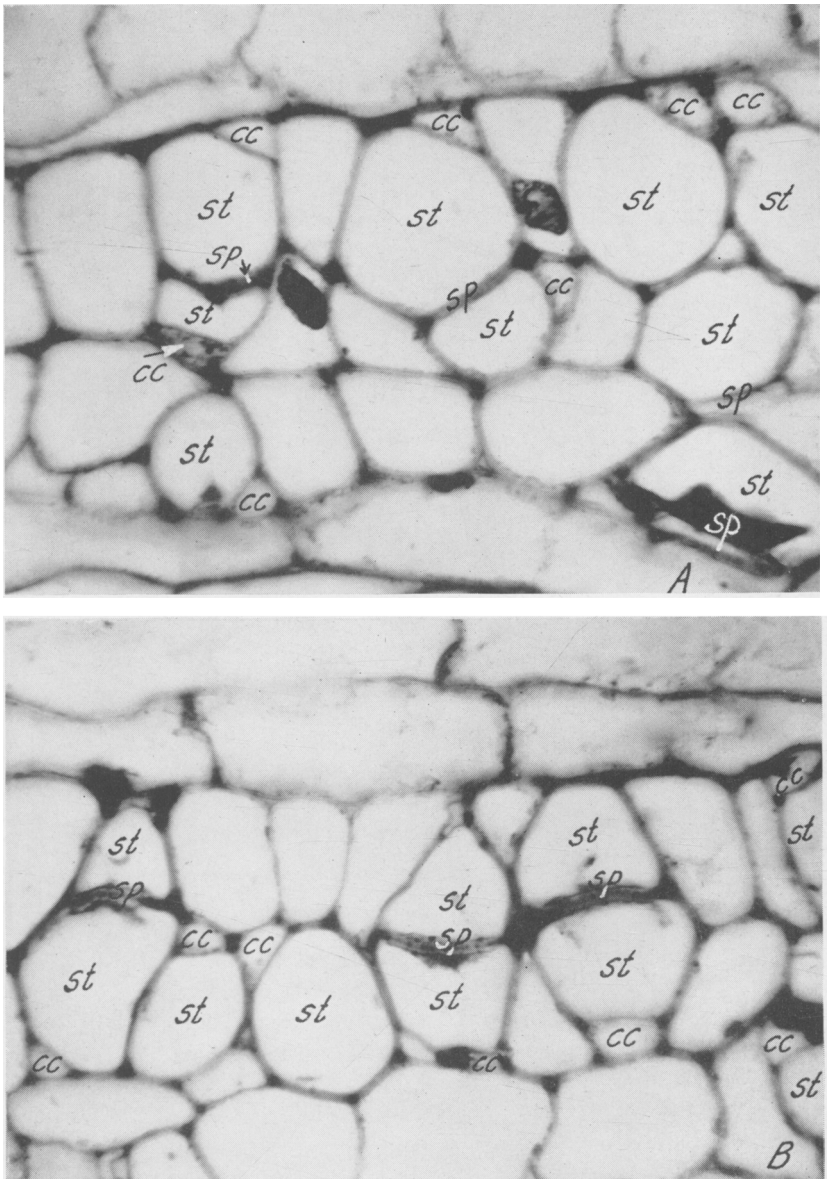

Plate 15.—Transverse sections through the same phloem as in plate 13; *A*, from the inner, *B*, from the outer part of the secondary phloem marked *Sec. Ph. III* in plate 13. Details are: *cc*, companion cell; *sp*, sieve plate; *st*, sieve tube. (Both × 810.)

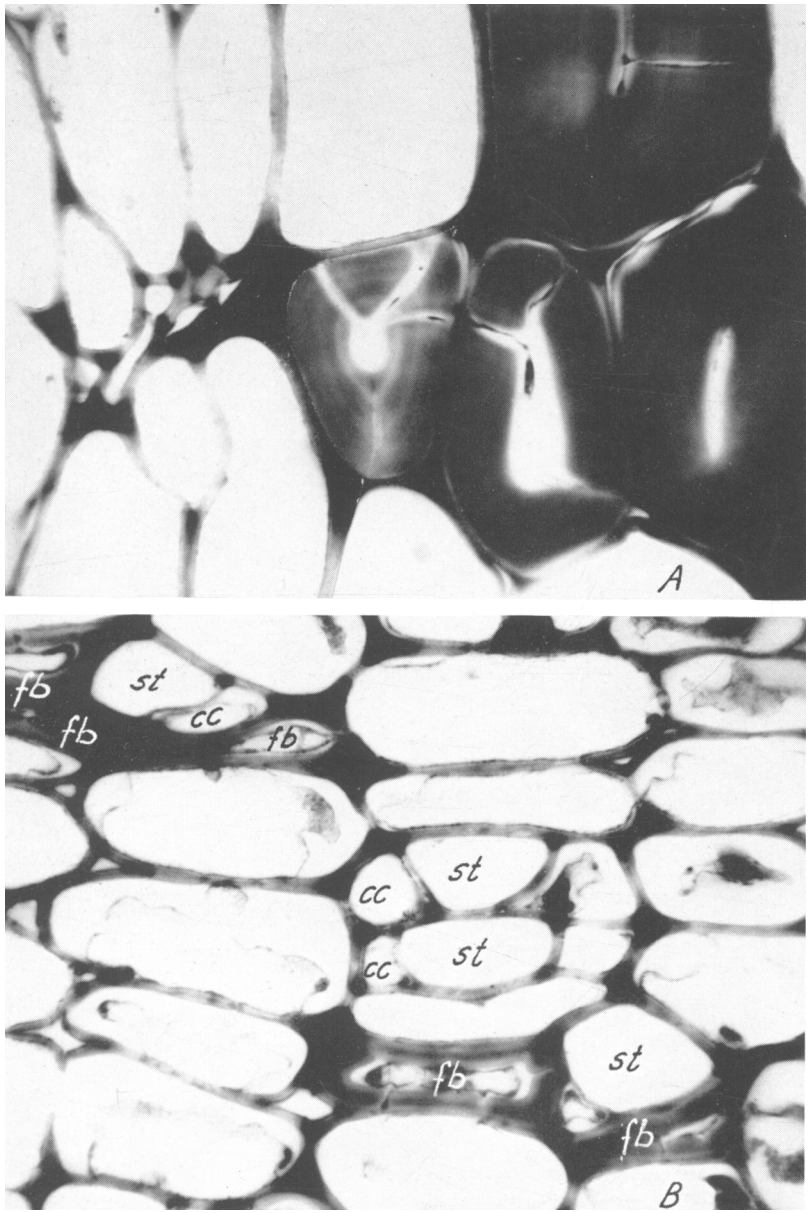

Plate 16.—Transverse sections through the external phloem of *Nicotiana tabacum* from approximately one-year-old stem, 3 centimeters in diameter. *A*, Primary phloem fibers and crushed metaphloem. *B*, Secondary phloem; *cc*, companion cell; *fb*, fiber; *st*, sieve tube. (Both × 810.)

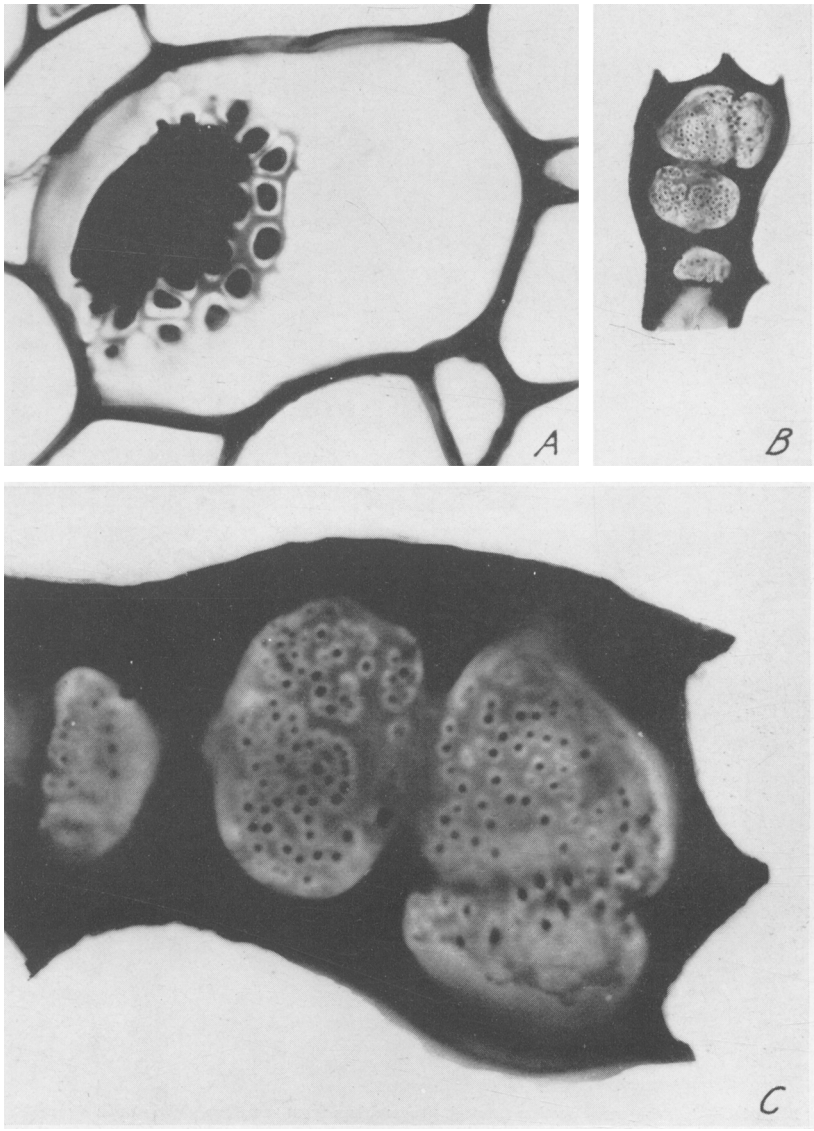

Plate 17.—Surface views of sieve plates. *A*, Portion of a *Cucurbita pepo* sieve plate partly covered with slime. *B*, Sieve plates from secondary phloem of *Nicotiana glauca*. *C*, Same as *B*, but at higher magnification. In each sieve plate the connecting strands are stained black; the callus cylinders enclosing the connecting strands are unstained. (*A* and *B*, × 810; *C*, × 2430.)

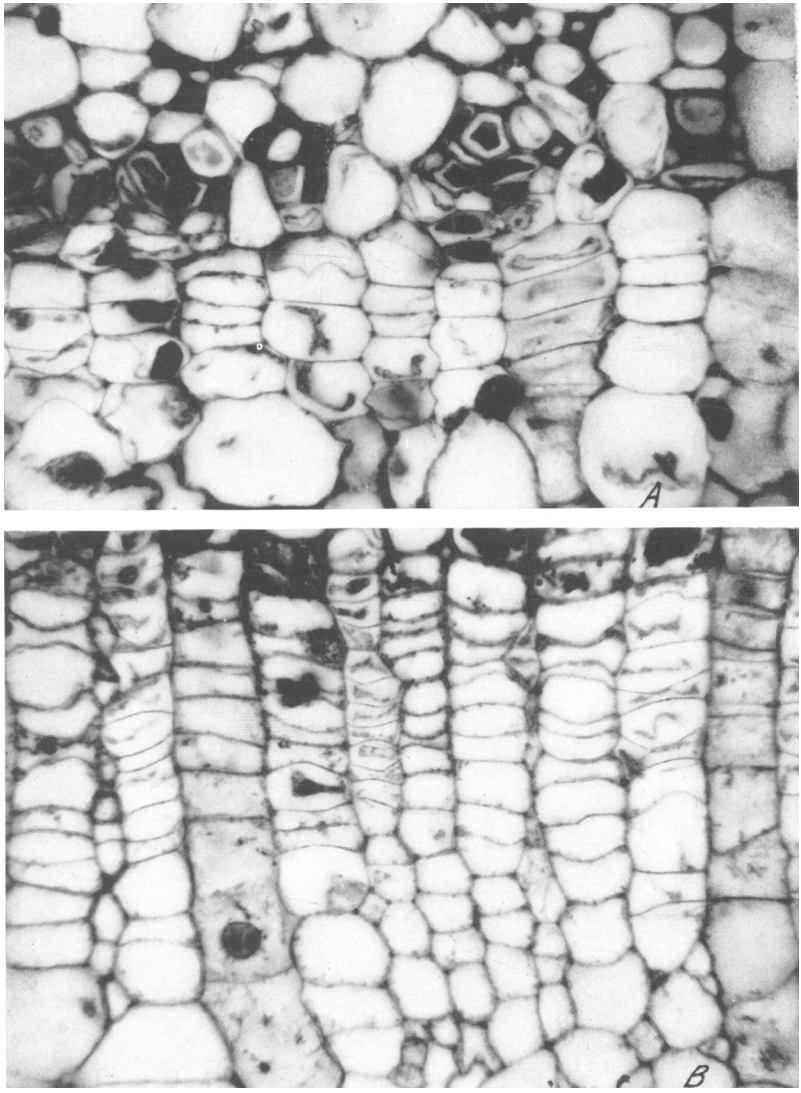

Plate 18.—*A*, Transverse section through procambium from the same petiole as in figure 10, *A* and plate 2, *C*. *B*, Transverse section through cambium from the same stem as in figure 10, *B*; this stem was of a degree of development similar to that used in plate 6. (Both × 540.)