DEVELOPMENT AND ANATOMY OF PRIMARY STRUCTURES IN THE SEEDLING OF CUCURBITA MAXIMA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 488

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(WITH SIX FIGURES)

Introduction

Since HARTIG (14) discovered the sieve tube and internal phloem in *Cucurbita pepo* in 1854, numerous studies have been made of the anatomy of the Cucurbitaceae. The investigations have usually been incidental to larger comparative surveys with emphasis on ontogeny and phylogeny. They show a haphazard distribution among the different genera, and at the same time fail to indicate the interrelationships of the various phases studied. The present paper gives a more complete description of the development and the histological anatomy of the seedling of a single species.

After the work of HARTIG, VON MOHL (29) made similar observations on the phloem in several plants, including *Cucurbita pepo*. To both investigators the significance of this tissue was admittedly unknown. Later, when the physiological and cellular nature of phloem was better understood, FISCHER (10) made an extensive study of this tissue in the Cucurbitaceae, noting its character and its unusual distribution. BRAEMER (3), studying the drug plants *Bryonia dioica*, *Citrullus colocynthis*, and *Ecballium elaterium*, differed with FISCHER in regard to the interpretation of certain tissues which the latter called phloem and which BRAEMER considered a segmented lactiferous system quite distinct from phloem.

Meanwhile DE BARY (8) originated the term bicollateral for this type of bundle with internal and external phloem. He cited the Cucurbitaceae as the type. This formulation of a term precipitated a controversy as to whether the internal phloem is actually part of the bundle or whether it is independent and the bundle therefore not bicollateral. Various investigators (15, 21, 25, 12, 1, 28, 5, 30, 19)

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have affirmed or denied the direct relationship of this inner phloem to the vascular bundle.

Other anatomical aspects of the Cucurbitaceae also have been studied. The root tip was described by JANCZEWSKI (17). The root itself was studied by VAN TIEGHEM (26) and by RUTLEDGE (24). JANCZEWSKI (18) and VAN TIEGHEM with DOULIOT (27) traced the development of lateral roots but disagreed completely in their interpretations. The transition has been reported by various workers. GÉRARD (13) briefly described it in *Cucurbita maxima*. Later DANGEARD (7) gave a short account, agreeing with GÉRARD, but the following year LAMOUNETTE (21) presented a somewhat different interpretation for the same species. RUTLEDGE (24) has given a more detailed description of the transition in this species. Others (10, 30, 19) have mentioned the transition but only as incidental to some other study. The peg specifically has been studied by FLAHAULT (11), NOLL (23), CROCKER, KNIGHT, and ROBERTS (6), and by others.

In the hypocotyl the primary structure of the bundle in general has been described by the investigators involved in the bicollaterality controversy. Although most of their work is based on the upper axis or stem derived from the epicotyl, much of it may be applied to the bundles in the hypocotyl. In addition to those already mentioned, HOLROYD (16) and ZIMMERMANN (31) have referred to primary structures for a number of different genera, including *Cucurbita*. The course of the bundle in the hypocotyl, through the cotyledonary node and into the cotyledons, has been vaguely mentioned by DANGEARD (7) for several genera, not including *Cucurbita*, and by JEAN (19) for *C. pepo*. The relationship of the epicotyl to this lower axis has been overlooked, except for an incorrect interpretation by DANGEARD (7) and a description of the lower two or three internodes of the stem by MANTEUFFEL (22).

Material and methods

In the present study observations were limited to *Cucurbita maxima* Duchesne. This species is probably subtropical and American in origin (9). As listed by CASTETTER and ERWIN (4), many horticultural varieties have been developed; that used for the present work was the winter squash, Blue Hubbard. For a study of the

seedling, several plantings were made for daily collection up to seven or eight days. Older material was collected approximately each week during the first half of the growing season. All the material was grown under ordinary garden conditions except that the bases of the older plants were protected by wire cages against the destructive activity of the squash vine borer, *Meletittia satyriniformis* Hübner. In fixing the material, a modification of Navashin's solution and a chromacetic solution were used. The material was cut at various thicknesses: at 6 μ for the study of the origin of lateral roots, at 10 μ for transverse sections of young material, at 15 μ for older material. Longitudinal sections were more satisfactory at 20 μ for xylem but at about 10 μ for phloem. Flemming's triple stain was used, also light green and fast green with safranin. Gourley's method for staining and clearing the vascular system, and a slight modification, produced excellent material for tracing the course of the vascular bundles in the various organs of the plant.

Investigation

Seed and seedling

The seed of *Cucurbita maxima* is characteristically dull white, flat, and elongated or broadly oval with a strong marginal rim which is interrupted at the micropylar end. The anatomy of the seed has been described by Barber (2) and Kondo (20). In planting, the seed lies flat. Under favorable conditions, at the end of two days the root has protruded between the halves of the seed coat and turned at right angles downward in the soil (fig. 1*A*). The growth of the root is rapid; by the next day it may be 3 cm. in length. This same day, the third day (all dates have reference to the time of planting), the peg begins to develop as a small lateral ridge flattened against the hypocotyl and placed in the angle formed by the horizontal hypocotyl and the perpendicular root (fig. 1*B*). In one or two specimens this outgrowth extended farther around the axis. On the fourth day many lateral roots show just below the peg, and the peg itself has enlarged considerably. At the same time the hypocotyl begins to elongate upward but the cotyledons remain horizontally within the seed coat. The latter soon begins to split, because the lower half is firmly held in place by attachment to the lower surface of the hori-

zontal peg and the upper half is being forced away by the upward growth of the arched hypocotyl (fig. 1*C*). Finally the seed coat splits sufficiently and the cotyledons are drawn out. The recurved hypocotyl with the cotyledons breaks above the soil level about the sixth day, following which the seedling soon becomes fully erect.

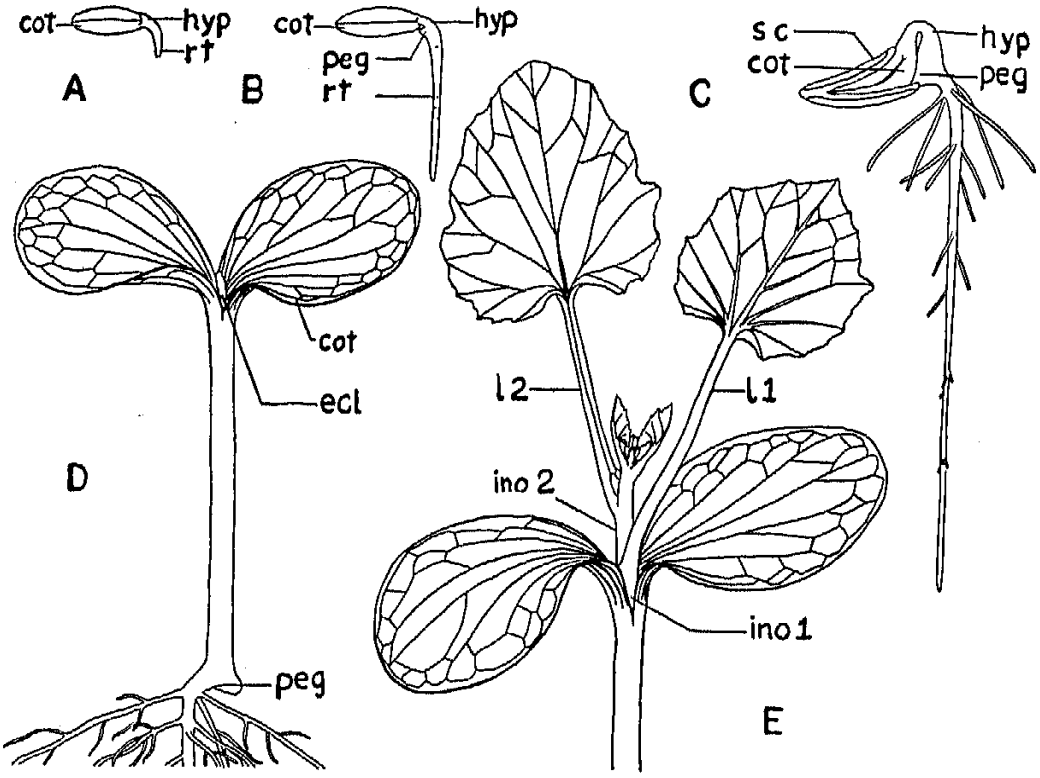

FIG. 1.—Development of seedling: *A*, two days, axis before formation of peg, seed coat removed (*cot*, cotyledon; *hyp*, hypocotyl; *rt*, root); *B*, three days, development of peg; *C*, five days, function of peg in splitting seed coat (*sc*) and withdrawal of cotyledons; *D*, eight days, seedling in which primary growth is completed (*ecl*, epicotyl); *E*, older plant, position of first foliage leaves (*l1* and *l2*) and relative lengths of first (*ino1*) and second (*ino2*) internodes.

The determination of the seedling phase in the life cycle is necessarily somewhat arbitrary. In this case the limit has been placed at about eight days, when the primary structures seem to be completed and most of the stored nutritive material has been utilized so that the young plant is independent and commencing the growth which results in secondary structures (fig. 1*D*). At the end of eight days, then, the plant shows a well branched root system. The primary root is slender, gradually increasing in diameter up to the peg, at

which level the axis enlarges abruptly. This is caused in part by the lateral extension of the peg, and in part by a general enlargement which is maintained through the length of the hypocotyl except for a slight taper below the cotyledons. The hypocotyl is slender and averages about 7.5 cm. in length after elongation is completed. In this variety the surface of the hypocotyl is smooth. The expanded cotyledons are oval, with broad bases, rather thick and green, as is the rest of the plant above the soil. The upper surface of the cotyledon is somewhat hairy but the lower surface is glabrous with prominent veins. The slowly developing epicotyl scarcely shows as yet.

Primary structures

Root tip.—Janczewski (17) classified the root tip of *Cucurbita maxima* as the fourth type. He designated this form as characteristic of only the Cucurbitaceae and the Leguminosae. In this type the identification of several separate histogens is impossible; instead there is one common generative zone from which all tissues arise. In a median longitudinal section of the primary root tip in *C. maxima* this generative zone forms a shallow curve across the tip. At the center the meristem may show about seven layers of cambiform cells, the number diminishing toward the margins. Outwardly this generative zone renews the root cap. At the same time the marginal portions give rise to the dermatogen, the dermatogen and root cap layers dividing from each other so as to effect the "stair-step" appearance in their differentiation, such as Janczewski described for the third type of root. The remainder of the root is derived from cells differentiating inwardly from the central portion of the generative zone. These cells undergo several further divisions which are often irregularly periclinal; then that tissue directly in the center forms the plerome, or stele, and the remaining adjacent cells form the periblem, or cortex.

Root axis.—From this type of apical meristem differentiation of the various tissues is difficult to follow. In the seedlings studied it is further complicated by the rapid elongation of the primary root and the consequent delayed maturation of the tissues. Approximate delimitation of cortex and stele is soon possible, however, because the cells composing the innermost layer of the cortex continue to

undergo tangential divisions for a longer period of time. Within the stele the first tissue to mature is the protophloem. It consists of four arcs of tissue only two or three cells wide, but tangentially extending ten or more cells. In the center of each band, usually wedged between two larger pericyclic cells, a single cell differentiates through changes in its wall and its contents. This cell with its lightly staining contents is conspicuous among the others in which the cytoplasm is much more dense. This protophloem element, although small in diameter, may be considerably elongated. Soon other similar cells differentiate in the band of protophloem; they also are adjacent to the pericycle or may be one or two cells deeper. Among them are other larger parenchymatous cells of the protophloem.

In contrast with the first formed phloem, consisting only of parenchyma, the metaphloem possesses sieve tubes and companion cells. The former are elongated but are small in diameter with relatively small sieve plates. The companion cells are short and have the nucleus and dense cytoplasm usually characteristic of this type of cell. The greater part of the metaphloem is composed of parenchymatous cells, usually larger in diameter than the sieve tubes, variable in length, but generally several times their width. In addition there are some scattered cells with darkly staining and coarsely reticulate cytoplasm.

Paralleling the maturation of the protophloem, the protoxylem has also begun differentiation. The first elements observable are annular with relatively heavy ring thickenings. There are four of these, one at the apex of each protoxylem point. Usually these elements are soon torn, thus initiating the protoxylem lacunae which may later become conspicuous. At the time that this first protoxylem is maturing, the tetrarch exarch pattern of further differentiation is marked out by the successively larger cells in the four primary xylem arms. The two or three cells next within the oldest protoxylem differentiate as delicate annular or spiral elements. The transition from annular to spiral is irregular. Similarly the next two or three elements show a transition in thickening from a delicate spiral to a heavy scalariform pattern. Because of these intermediate types it is not always possible to distinguish protoxylem from metaxylem, but certainly the next vessels form part of the metaxylem. They are

considerably larger than the outer preceding xylem elements and show closely reticulated thickenings. In addition to the elements just described, a variable number of adjacent cells may undergo similar differentiation.

After maturation of these four strands of primary xylem, a considerable area in which further differentiation is retarded remains in the center. It at first has the appearance of pith. This fact may account for VAN TIEGHEM'S (26) report of pith in the root, since he studied a young section only 1 cm. from the root tip. GÉRARD (13) also noted pith in the root; as he was primarily studying the region of transition, he may have observed a section of the transition at a level in which pith was differentiated, since this tissue descends rather deeply. As RUTLEDGE (24) has noted for *Cucurbita maxima* and as seen in the variety used for this study, ultimately two or more cells at the center mature into conspicuously large, pitted vessels (*cf.* fig. 4*A*). The parenchymatous cells surrounding these vessels are isodiametric or horizontally elongated, their walls reticulately thickened and often sinuous. These cells may abut directly against the reticulate vessels in the four earlier formed xylem strands, or two or three layers of vertically elongated, thin walled xylem parenchyma cells may intervene.

Between the primary xylem and phloem a band of tissue remains undifferentiated; this tissue constitutes the cambium. Exterior to these primary vascular tissues is the pericycle. Over the phloem it is composed of a single layer of cells, large in diameter and somewhat elongated. Over the protoxylem points it consists of about four layers of cells, smaller in diameter and only slightly elongated.

During maturation of the stele, the cortex has also matured. At first the innermost layer of cortical cells continues to show tangential divisions, adding to the cortex. At the same time the cells in the outermost layers undergo radial divisions. Finally these different divisions cease, and, with maturation of the scalariform elements the innermost layer becomes the endodermis with narrow Casparian strips. The cells are more elongated and somewhat larger in diameter than those of the pericycle. The parenchymatous cells of the cortex are still larger; they are elongated and show conspicuous intercellular

spaces. Completing the axis is the epidermis, composed of elongated tabular cells many of which form root hairs.

ORIGIN OF SECONDARY ROOTS.—Concerning the origin of secondary roots in the Cucurbitaceae, two radically divergent opinions have been expressed. JANCZEWSKI (18) stated that the origin of lateral roots in the Leguminosae and Cucurbitaceae differed from other Phanerogams in that the cortex was involved. In describing the development he declared that the pericycle of the mother root gave rise to the stele of the lateral root, and the endodermis and adjacent cortical layers gave rise to the primary cortex at the surface of which the generative zone developed only later. VAN TIEGHEM and DOULIOT (27), in a reinvestigation of this work, rejected JANCZEWSKI'S interpretation. In both *Cucurbita maxima* and *C. pepo* they noted a precise origin of the lateral root from the two pericyclic layers of the mother root, with the endodermis and five or six inner cortical layers forming only a digestive pocket aiding in the outward growth of the young root. The observations made in the present study support the view expressed by JANCZEWSKI.

The primordium of the secondary root originates early in the ontogeny of the primary root, after the differentiation of the protophloem but before that of the protoxylem. In several root tips this showed an initiation of the primordium within 1 mm. of the apical meristem, a longitudinal section in particular showing a primordium 0.8 mm. from the generative zone. The first definite indications of development, as traced in transverse sections, are radial divisions in the two or three inner cortical parenchyma layers opposite the protoxylem point (fig. 2*A*). These divisions are conspicuous in the flatly oval, regularly arranged cells of the cortex. Meanwhile the innermost layer of the cortex continues to exhibit tangential divisions, although in the remainder of the root these are infrequent. At the same time the cells of the pericycle commence dividing rather irregularly, except that the outermost layer, just beneath the tangentially dividing layer of the cortex, also shows tangential divisions. In this way the exact identity of these two layers is soon lost. Longitudinal sections show that the tangential and radial divisions are accompanied by frequent transverse divisions.

Gradually more layers of the cortex become involved in the area

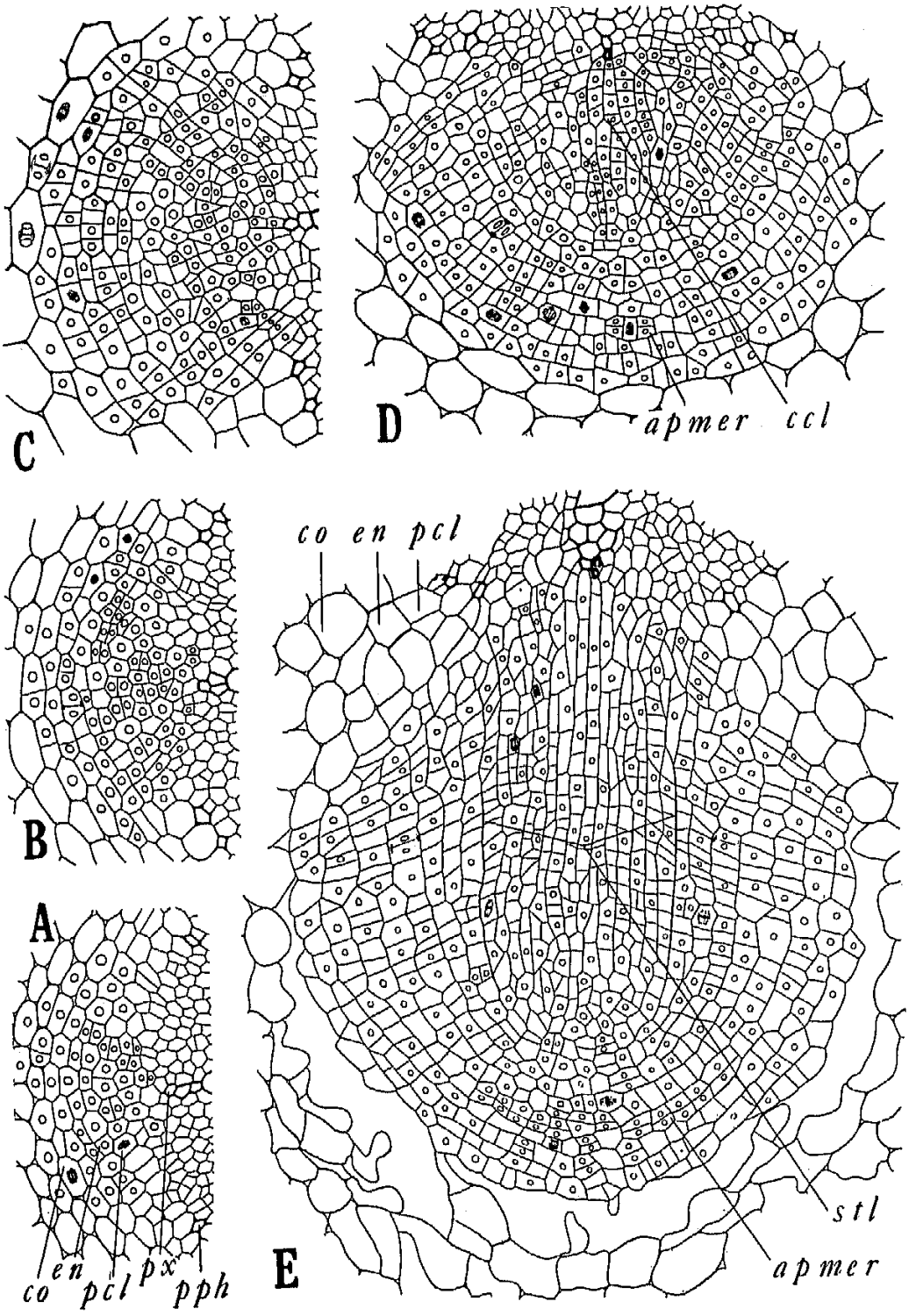

FIG. 2.—Transverse sections from primary root showing origin of secondary root: *A*, first radial divisions in cortex (*co*), and tangential divisions in endodermal (*en*) and outermost pericyclic (*pcl*) layers, nucleated cells in region of primordium (*pph*, protophloem; *px*, protoxylem); *B* and *C*, further divisions in cortex and pericycle; *D*, continued divisions in cortex and pericycle with formation of central cylinder (*ccl*) and origin of apical meristem (*apmer*); *E*, secondary root nearly through cortex of primary root; stele (*stl*) and apical meristem established.

showing radial divisions (fig. 2*B*, *C*). The lateral extent of this zone also increases, the base extending toward the phloem on either side. The cortical cells composing it remain meristematic. They show only a single radial division at first but later several divisions occur, those cells earlier involved showing the greater number. The outline of the mother cell remains traceable, also the layered arrangement of the cortex, although the latter is somewhat distorted by the greater activity and growth of the tissue of the pericycle. In this last region successive divisions produce numerous small, radially elongated cells. With this the lateral root is beginning to take form (fig. 2*D*), the central cylinder of narrow elongated cells forming a stele derived from the pericycle, and curved over this a cap of cortical tissue (and possibly of some pericyclic tissue).

Growth of this primordium continues by further divisions and elongation in the central cylinder. The cortical cap maintains its form and position over the stele by means of successive divisions of those cells nearest the phloem of the parent root. Finally, when all but about three layers of the cortex form part of the cap, a generative zone similar to that already described for the primary root tip develops (fig. 2*D*, *E*). It arises by tangential divisions in approximately the second, third, or fourth outermost layers of the cortically derived part of the root primordium. With growth commencing in this apical area and continuing at the base for a short time, the cortical tissue of the primary root soon tears away around the tip of the primordium, thus freeing the young root to continue growth.

TRANSITION AND THE PEG

TRANSITION.—The early descriptions by GÉRARD (13) and DANGEARD (7) gave the essential idea of the transition; that is, doubling of the number of xylem strands, similar doubling of the phloem, superimposition of these two tissues with reorientation of the xylem from centripetal, through tangential, to centrifugal maturation, resulting in eight bundles separated by narrow medullary rays. LAMOUNETTE (21) presented a somewhat different account for *Cucurbita maxima*. He traced the division of the four xylem bundles of the root and the formation of four transition bundles alternate in posi-

tion with the former. Of the four bundles, two divided into two each, forming a total of six which completed the transition and con-

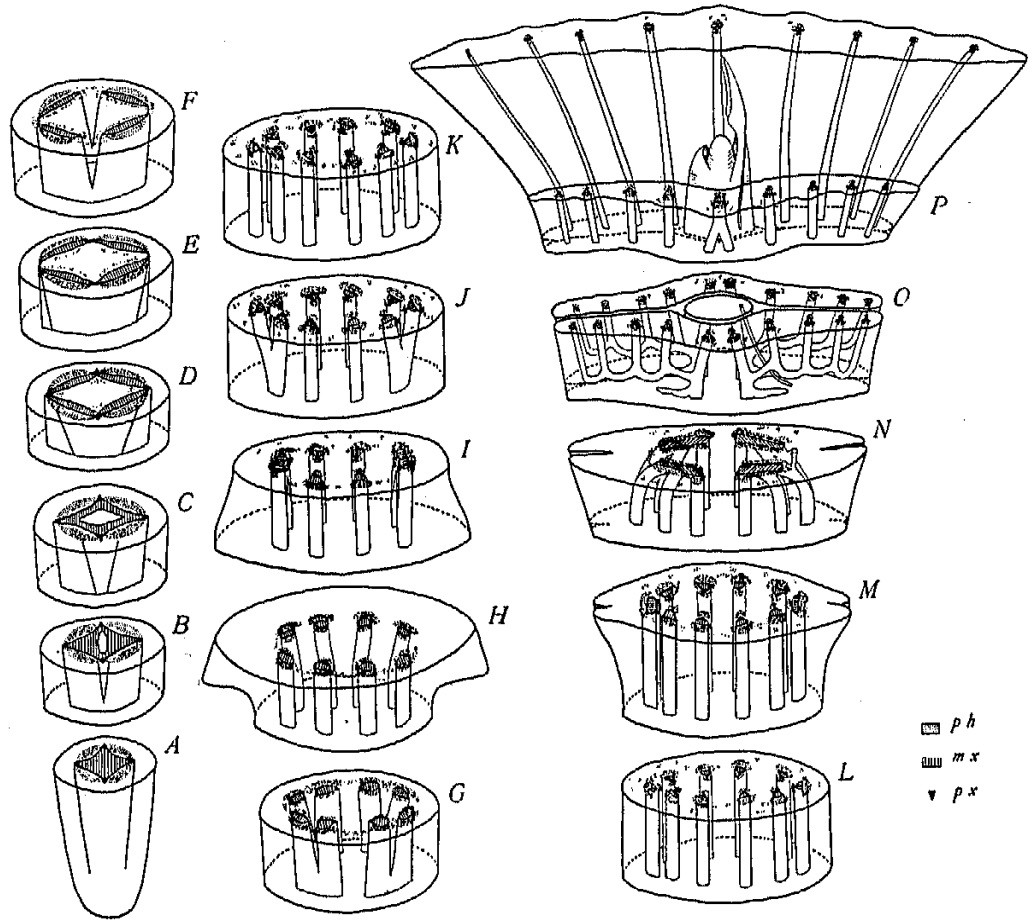

FIG. 3.—Diagram of a seedling, series of transverse sections: *A*, root; *B*, differentiation of pith beginning the transition; *C*, enlargement of pith area with metaxylem in peripheral position; *D*, differentiation of internal phloem; *E*, divergence of each primary xylem strand into two tangential arms; *F*, establishment of dissected siphonostele of four tangential transition bundles; *G*, formation of eight bundles, each with protoxylem still in tangential position; *H*, abrupt enlargement of axis at peg, with protoxylem differentiation becoming endarch; *I*, endarch bundles completing transition, anastomosing of two end pairs of bundles; *J*, end bundles dividing into three to form total of ten bundles which continue through the hypocotyl (*K*, *L*, and *M*); *N*, cotyledonary node, formation of cotyledonary plate by tangential anastomoses, insertion of first foliage leaf trace on dividing end bundle at right; *O*, two median traces to each cotyledon continuing from cotyledonary plate, branching of each trace laterally to establish principal veins in blade of cotyledon; *P*, base of cotyledons, the epicotyl.

tinued through the hypcotyl. Recently RUTLEDGE (24) has supported the two earlier writers. The following agrees with RUTLEDGE except for some details in description and interpretation.

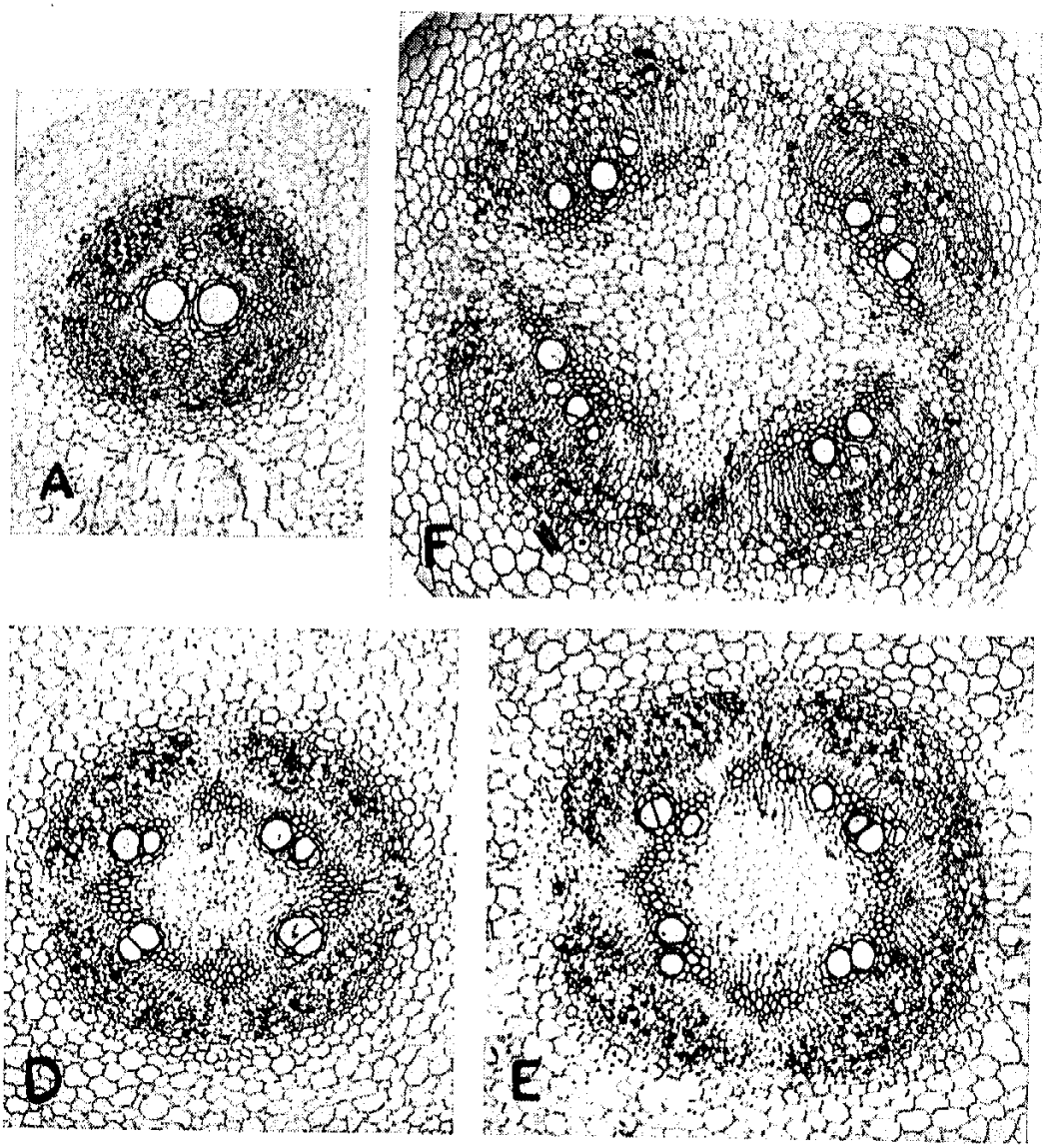

FIG. 4.—Vascular tissues in transition region, series of transverse sections (lettered to correspond with fig. 3): *A*, root just below transition showing differentiation of additional reticulate xylem parenchyma between large metaxylem vessels (*B* and *C* omitted); *D*, central pith and differentiation of internal phloem, identifiable by darkly staining cells within triangular protoxylem strands, metaxylem in peripheral position within external phloem; *E*, divergence of each primary xylem strand into two tangentially extending arms joining the metaxylem; *F*, dissected siphonostele of four transition bundles with tangential band of metaxylem tipped at either end by protoxylem. External phloem masses joined across rays by connective phloem, also phloem along inner face of bundles connected to outer phloem along the rays.

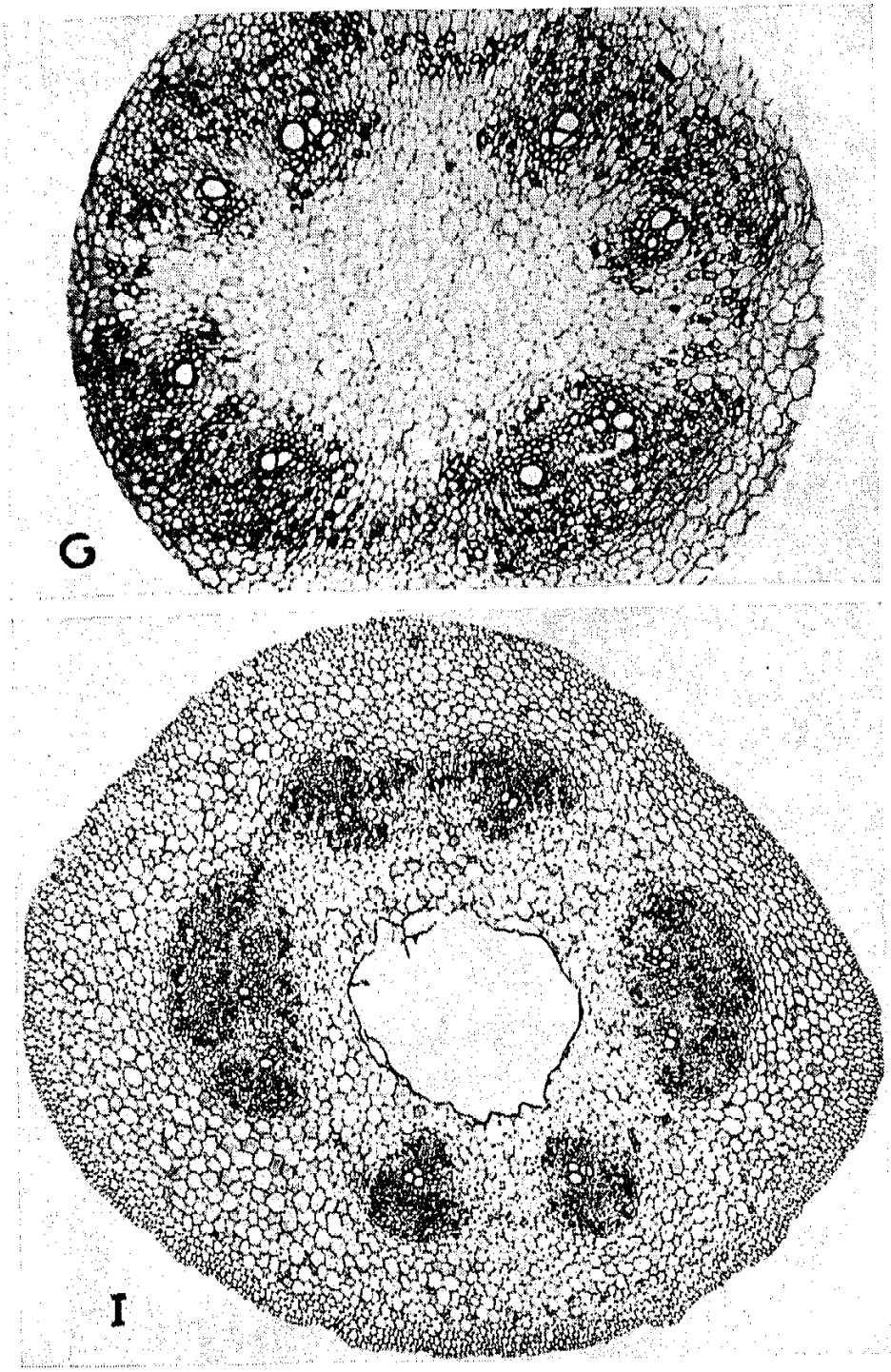

FIG. 5.—Vascular tissues in transition region, transverse sections (lettered to correspond with fig. 3): *G*, eight bundles (one transition bundle just dividing) with phloem differentiating along the new rays, maturation of protoxylem still tangential (*H* omitted); *I*, complete endarch bundles just above peg, also branching of anastomosed end bundle to form the ten vascular bundles of hypocotyl. Differentiation of connective phloem in cortex pericycle, and (less frequently) along the rays.

In *C. maxima* the transition region extends from the exarch protostele in the root somewhat below the peg to the completely endarch dissected siphonostele at a level just above the peg in the stemlike hypocotyl. This involves a portion of the axis over 1 cm. in length in a seedling of about seven days, the exact distance varying with the different specimens. The following description is based on material of that approximate age.

The protostele in proximity to the transition region shows some departure from the structure described as typical of the root (fig. 4*A*). The reticulately thickened xylem parenchyma cells between the large central pitted vessels have increased in number, especially along a median line extending between two opposite protoxylem points. This has separated the pitted vessels into two groups. At the same time these large vessels gradually increase to four or more in number.

The transition, in tracing the course from root to stem, begins where certain of these median cells differentiate as thin walled, vertically elongated pith parenchyma instead of reticulate xylem parenchyma. A narrow band extending from one primary xylem arm to the opposite and in a line at right angles to the plane of the cotyledons results (fig. 3*B*). At higher levels this pith increases in width, continuing to the other two lateral arms of the primary xylem (fig. 3*C*). Concurrent with these changes the pitted vessels by progressively tangential differentiation separate into four groups, which attain positions nearer the periphery of the stele, just within the cambial initials internal to the four phloem groups and alternate with the protoxylem points.

These changes take place gradually, in a spatial relation. Thus about 8 mm. above the wholly rootlike pattern of tissues, the protoxylem and first formed metaxylem still retain the same linear exarch relationship. The inner reticulate tracheae are more numerous, and so broaden the base as to form triangular masses of these primary xylem strands (fig. 4*D*). Connecting these triangles tangentially, and in this way producing a hollow diamond inclosing the pith, is the remaining and later formed metaxylem with the large pitted vessels. By differentiation the number of these vessels again in-

creases from the approximate four to an approximate eight, two in each tangential mass of metaxylem. These vessels are surrounded by reticulate xylem parenchyma which may abut directly against the reticulate tracheae in the triangular masses, or more usually is separated from them by several layers of thin walled xylem parenchyma, as described for the root. External to the metaxylem are the cambial initials, already active at this stage but with no secondary differentiation. Outside this the primary phloem has maintained its original position and relative arrangement. Similarly the pericycle, cortex, and epidermis show continuity with the lower root.

At successive levels above this, reorientation of tissues occurs more rapidly. Because of slight variations in the rate of this reorientation, all four parts of the stele, even though they undergo the same changes, may not show exactly similar structure at any given level. This is especially true of the upper levels. Continuing up the axis 1 or 2 mm., the pith increases in extent and there is a differentiation of internal phloem (figs. 3*D*, 4*D*). Certain cells, frequently those internal to the xylem triangles but several cells removed, undergo divisions producing groups of smaller cells. Among these are some of the conspicuously darkly staining cells already described in the outer phloem. Others are simple phloem parenchyma. A few cells exhibit end walls which are perforated with numerous and somewhat irregularly arranged simple pits. In this way they are similar to the sieve tubes of the outer primary phloem, but the cells are very much larger, and companion cells have not been noted except as the darkly staining cells take this place.

At this level the xylem is not characteristically exarch as in the root. By the maturation of parenchyma in the inner face of the metaxylem immediately subtending the protoxylem, these first formed reticulate tracheae gradually assume a more tangential position in relation to the annular and spiral elements. At successively higher levels each of the original triangular masses becomes divided into two arms spreading toward the tangential bands of the later formed metaxylem. The latter shows the formation of additional small reticulate tracheae which are arranged at first adjacent to the two larger pitted vessels but which progressively differentiate to a position between the two vessels.

Continuing upward, this process of splitting extends to the protoxylem, resulting in irregularly double rows of annular and spiral elements, so that about 1 mm. higher a hollow square of xylem is formed with protoxylem touching at the corners and the sides formed of metaxylem composed mostly of reticulate tracheae, but with about eight separate pitted vessels (figs. 3*E*, 4*E*). Within this square the internal phloem is distributed along all four faces but remains as scattered groups of cells separated from the lignified elements by several layers of parenchymatous cells, some of which may show divisions initiating cambial activity. The parenchymatous pith is composed of large, somewhat elongated cells, circular in cross section, with conspicuous intercellular spaces. The tissues external to the xylem are arranged much as before, except that the lateral extent of the phloem is greater and the rays opposite the protoxylem points are consequently narrower.

In the next 1–2 mm. higher, the pattern of the primary structures is usually complicated by the development of lateral roots at the protoxylem points. Above these, however, each protoxylem point is completely divided into two parts by the differentiation of an intervening parenchymatous ray. As a result, a dissected siphonostele of four tangential bundles is established at this level (figs. 3*F*, 4*F*). Each of these transition bundles consists of phloem and a tangential band of metaxylem tipped at either end by annular and spiral protoxylem elements. The metaxylem forms a continuous layer of small reticulate tracheae with the two or more larger and later matured pitted vessels occupying a position on the outer face of the tangential band. The internal phloem is continuous across the inner face of these bundles but is interrupted at the four primary rays. It is more closely associated with the xylem than at the lower levels. The outer phloem is still directly continuous with that of the root, having maintained both its original arrangement and position. In addition there is frequently differentiation of phloem across the rays connecting the adjacent areas of external phloem, and differentiation in the parenchyma along the side of the ray connecting the inner and outer phloem masses around each bundle.

The diameter of the axis increases more rapidly toward the level of the peg, the pith is larger, and the bundles are separated by

wider rays. At the same time, and about 1 mm. above the establishment of four bundles, the number of bundles increases to eight (figs. 3*G*, 5*G*). This results from the maturation of parenchyma through the center of each tangential bundle. This parenchyma becomes established first in the band of metaxylem interrupting it in the approximate center and so dividing it that each part shows one of the large pitted vessels and other associated tracheae.

Differentiation of the ray through the phloem masses takes place at higher levels, occurring first through the internal phloem and, still higher, through the outer phloem. These eight bundles show characteristics of transition in that the differentiation of the xylem, in passing from the protoxylem to the first formed metaxylem, is still tangential; but the later formation of the large pitted vessels on the outer face of the xylem mass is centrifugal or endarch. The outer phloem retains its relative arrangement except that it now consists of eight masses instead of four. Similarly the internal phloem is present along the inner face of each bundle.

Progressive differentiation from the internal phloem and from the outer phloem in the newly developed parenchymatous ray establishes phloem interconnections along this lateral face just as occurred along the opposite face at a lower level. The internal phloem is separated from the lignified elements by one or two layers of parenchymatous cells which may or may not show cambial activity. Likewise the parenchyma between the lateral phloem and the vascular tissues may show cambial activity. The delimitation of distinct phloem areas is difficult because of this differentiation of phloem across the rays and along the sides of the bundles. Outside the phloem, the parenchymatous pericycle has become irregularly several layered. Next this is the endodermis still traceable as a continuous single layer around the stele, then the parenchymatous cortical tissue, and finally the epidermis which forms root hairs up to the approximate level of the peg.

With the development of the peg the diameter of the axis suddenly increases (fig. 3*H*). In the lower levels this results from increase in cortical tissue on the one side to form the peg. At a level about midway in the peg, however, the stele may also participate in the general widening, the two median bundles on the side toward

the peg curving abruptly outward and the two adjacent bundles also partly sharing in this outward differentiation. Meanwhile, in a distance of about 1.5 mm., at the upper levels of the peg, reorientation of the primary xylem from tangential to centrifugal or endarch differentiation is completed (figs. 3*I*, 5*I*).

Not only does the xylem change in its relative arrangement, but also in the character of the elements composing it. The emphasis on scalariform and reticulate elements characteristic of the root passes to emphasis on spiral or loosely scalariform elements. At the same time there is a gradual decrease in the diameter of the vessels from root to hypocotyl. The phloem shows greater continuity in its character. The outer phloem forms a broad mass capping each bundle, and scattered phloem groups occur across the rays connecting these various outer masses. The internal phloem maintains its position on the inner face of each bundle, but the interconnections with the outer phloem are less frequent. Apparently phloem may also differentiate in the pericycle over the vascular bundles, as the darkly staining cells of the phloem may be found directly adjacent to the endodermis. The endodermis may be traced over the bundles by its small Casparian thickenings, but it is indistinguishable across the rays. At still higher levels it can be identified above the bundles chiefly by its starch containing cells. The remainder of the cortex is composed of large parenchymatous cells with conspicuous intercellular spaces. A cutinized epidermis completes the axis. With the establishment of this dissected siphonostele of endarch bundles the transition is concluded.

The transition in *Cucurbita maxima* is of a fairly simple type. Two features, however, the internal phloem and the peg, add particular interest to it. Differing opinions have been expressed concerning the internal phloem. GÉRARD (13) first described it in the transition region, indicating that this tissue was derived from the outer phloem by inward migration along the rays. LAMOUNETTE (21) denied this, stating that there were no interconnections between the outer and inner phloem, the internal phloem ending blindly below. Further investigation has shown that there is continuity or interconnection of inner and outer phloem, and it is not to be considered that the one developed from the other. Confusion may have arisen

from the fact that the downward extent of the internal phloem seems to vary with the age of the seedling. In a young plant of three or four days it is possible to identify tissue which is internal to the xylem mass of each endarch bundle and which will mature directly into internal phloem. This tissue may be traced to a lower level (approximately fig. 4*G*) where these eight bundles show a tangential arrangement of the xylem, but lower than this it gradually becomes indistinguishable. Progressive downward differentiation takes place so that at about seven days phloem is readily identified at a level where the pith is entirely inclosed by xylem, as described in the transition (fig. 4*D*). Differentiation in the rays seems to correlate with differentiation along the inner face of the bundle. In older stages, therefore, the inner and outer phloem do show interconnections along the rays, but also the internal phloem ends blindly at a still lower level.

PEG.—The peg has presented controversial material in regard to the factors influencing its development, but morphologically the structure is simple. Although the pattern of the transition is already formulated in the embryo, there is no trace of the enlargement which forms the peg. As noted, only on the third day after planting does the peg begin to form as a lateral ridge. Cross sections show that it results from numerous cambial-like divisions in the tissues along a plane extending tangentially across the axis. At lower levels the line of this activity usually involves only the cortex, but at higher levels this plane of divisions may also involve the vascular tissue, which accounts for the abruptly outward course of these bundles.

The broad lower face of the peg is at right angles to the axis, or even at an acute angle; it bears root hairs and often shows traces of reticulately thickened and branched parenchymatous cells which were part of the seed coat to which this surface of the peg was firmly attached during germination. The upper part of the peg gradually merges with the hypocotyl, and shows the smooth epidermis of the latter. CROCKER, KNIGHT, and ROBERTS (6) have pointed out that the position of the peg is determined by external influences. Certainly it does not show a constant morphological relationship to the transition. In some cases the greatest dimension of the peg occurs at the level of the four tangential transition bundles, and in other

specimens this level of maximum extent occurs where there are eight or more completely endarch bundles.

HYPOCOTYL

COURSE OF THE BUNDLES.—In the transition from root to hypocotyl a change in the form of the axis occurs, from the circular root to the oval hypocotyl. The peg at the base is on one side of this oval. This form continues up to the divergence of the cotyledons, the longer diameter of the oval parallel to the plane of the cotyledons. Of the eight original bundles in the upper levels of the transition, two are at each end of the oval outline and two along each side (fig. 3*H*). Usually just above the peg and often before the vascular bundles becomes endarch, the number of these bundles increases. This is brought about through anastomosing and branching of the eight bundles, most of these changes taking place within a few millimeters above the peg. Ten was the smallest number of bundles found in the hypocotyls observed; twelve is a common number; and as many as sixteen have been counted. This necessarily involves a wide variety of patterns, the simplest and most frequently recurring of which is described. At the upper levels of the transition the two bundles at each end of the oval anastomose to form a single large bundle which continues up the axis for a short distance before it divides into three (figs. 3*I*, 5*I*). These six with the two along each broad face of the oval make ten vascular strands, the basic number. Frequently an additional bundle is formed on each side of the oval by simple branching of one or the other of the original bundles. This pattern of ten or twelve bundles may continue up the axis to the cotyledonary node (fig. 3*I–M*), or any one of these bundles, especially those at the end of the oval, may give rise to one or more additional bundles. Similarly the anastomosing and divergence at the lower levels may vary considerably; the anastomosing may be omitted and branching may be more frequent. These bundles are arranged in a single ring, in contrast to the two rings characteristic of the upper stem of the Cucurbitaceae.

RUTLEDGE (24) described a similar pattern as an exceptional case. He accounts for the increase in number by the formation of additional bundles between the original bundles, frequently between

those on either side of the oval. He does not make it clear as to whether these bundles are independent in origin, or are anastomosed with the adjacent ones. That the latter is the case is shown in material stained and cleared by Gourley's method. The continuity of all parts of the vascular system is evident. It is true that the additional bundles are usually smaller than the others and branching at first involves only phloem, but xylem is later differentiated. Also in certain cases in very young seedlings these additional bundles, after several lateral anastomoses just below the cotyledonary plate, continue for a short time as small strands of phloem which then end blindly.

Contrary to these observations is a statement by DANGEARD (7) that the hypocotyledonary axis in the Cucurbitaceae is later modified by the descent of foliar traces. Since the entire vascular pattern of twelve bundles as described was traced in the procambial strands of an embryo before germination, there is hardly evidence in *Cucurbita maxima* to support this view of DANGEARD.

DIFFERENTIATION IN THE AXIS.—The pattern of the hypocotyl formulated in the embryo consists of tissue showing little differentiation. The epidermis is composed of small closely arranged cells. The parenchyma of the cortex and a similar parenchyma of the stele consist of short, thin walled cells filled with stored nutritive matter and showing characteristic intercellular spaces. Surrounded by this fundamental parenchyma, about halfway between the center and the epidermis, are the provascular strands varying in form and size according to their position in the pattern. They are composed of small elongated cells without the dense contents of the parenchyma cells and without intercellular spaces (fig. 6*A*).

In further differentiation, the first changes occur in the provascular strands. Several elements of the protoxylem mature by the third day. These elements do not differentiate on the inner margin of the procambial tissue, but several cells within it (fig. 6*B*). They are annular and coarsely spiral elements. Unlike the root they are not necessarily adjacent to one another, but may be separated by xylem parenchyma. These first elements are small in diameter, but the later matured elements are larger and the thickening of their walls

is more closely spiral. In the hypocotyl a spiral-scalariform vessel of elongated segments of relatively large diameter seems to be the

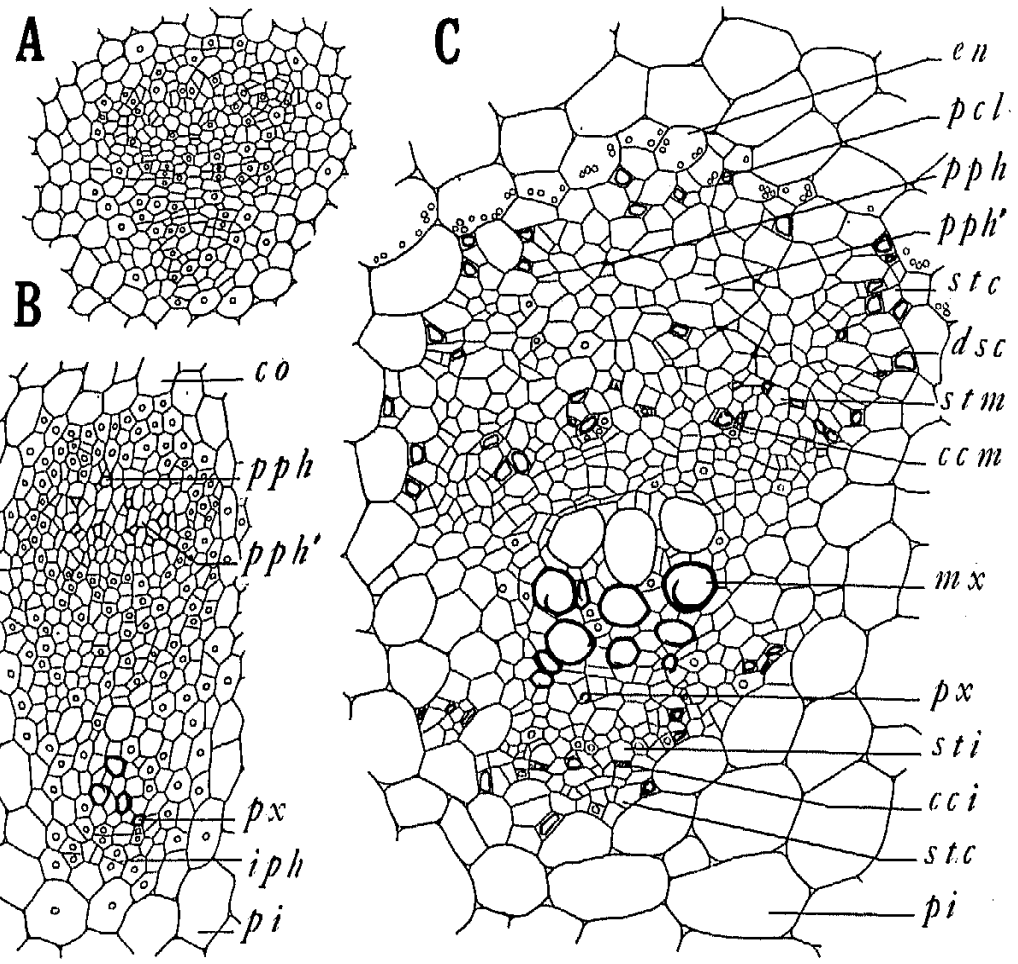

FIG. 6.—Differentiation of vascular bundle in hypocotyl: *A*, provascular strand in embryo just before germination; *B*, vascular bundle of three-day seedling showing outer protophloem with elongated elements (*pph*) and other parenchymatous cells (*pph'*), protoxylem (*px*), and several recently divided cells in undifferentiated internal phloem (*iph*); *C*, vascular bundle of seven-day seedling with primary differentiation nearly completed, showing external phloem with crushed elongated protophloem elements (*pph*) and other protophloem cells (*pph'*) enlarging to become fibers, differentiation of metaphloem with sieve tubes (*stm*) and companion cells (*ccm*), of internal phloem with sieve tubes (*sti*) and companion cells (*cci*), of connective phloem in pericycle (*pcl*) adjacent to endodermis (*en*) and in association with external and internal phloem, chiefly along periphery. Connective phloem conspicuous because of its darkly staining cells (*dsc*); it also shows sieve tubes (*stc*) (*pi*, pith).

end point of primary differentiation, instead of the large, pitted, short segmented trachea such as was found in the root. At the end of primary growth the first protoxylem elements have been torn but

a lacuna is not formed; instead the vessels are closed in by the adjacent enlarging parenchymatous cells.

On the third day the external protophloem is also identifiable (fig. 6*B*). It is similar to that found in the root, with the small, elongated, lightly staining cells interspersed among the larger undifferentiated parenchymatous cells. This protophloem forms a small oval mass of tissue toward the outer face of the bundle. The external metaphloem is much later in differentiation, the sieve tubes and companion cells being first identified in material five days old (fig. 6*C*). In addition the metaphloem shows the darkly staining cells and undifferentiated parenchyma as described for the root. During this later development the small elongated cells of the protophloem are crushed and resorbed. The accompanying parenchymatous cells become enlarged and very elongated. By the eighth day there is scarcely a trace of the crushed cells, and the remaining parenchymatous cells are identifiable as those which later become the lignified phloem fibers characteristic of the hypocotyl (fig. 6*C*).

In the meantime the internal phloem has matured. Since the protoxylem apparently does not form on the inner margin of the provascular strand, one or more layers of procambial tissue remain undifferentiated. There are exceptions to this in some of the smaller additional bundles where the xylem is seemingly formed along the inner margin. The determination of the exact extent of this procambial tissue is impossible. This inner tissue does not show differentiation of protophloem as found in the outer phloem. Instead, during the third and fourth days, cell divisions in these inner layers and adjacent cells increase the extent of this meristematic area (fig. 6*B*). About the fifth day sieve tubes and companion cells are differentiated (fig. 6*C*). Thus the internal phloem corresponds in composition and maturation with the outer metaphloem. Differentiation takes place in those cells toward the pith, and an undifferentiated parenchyma intervenes between the internal phloem and the xylem. It is this zone which later gives rise to the internal cambium but remains inactive during the primary growth.

While the external and internal phloem is maturing, there is also differentiation of phloem strands in non-vascular regions. This unusual distribution was first described by FISCHER (10), who classi-

fied the phloem into several types according to its position: ectocyclic if external to the pericyclic ring of fibers (of the upper stem); entocyclic if within this sclerenchymatous ring; and commissural if connecting these two types and the vascular phloem of the bundles. This classification seems hardly necessary for a study of the hypocotyl. In the first place there is no separation of the ectocyclic and entocyclic systems, but all of these phloem strands form a much branched and interconnected system. So also it is hardly possible to distinguish the commissural phloem. Moreover all this phloem appears structurally similar. On this basis it is simpler to refer to this as connective phloem.

In the primary growth of *Cucurbita maxima* these connective phloem strands appear in the cortical region, in the pericycle, and in the rays around the bundles, although not directly adjacent to the vascular tissue except where the strands occasionally anastomose with either the internal or external phloem. This phloem is abundantly formed in the pericycle. This is especially true over the bundles as noted in the transition region. GÉRARD (13) first described this as interruption of the pericycle by the fiber-phloem bundles. Later RUTLEDGE (24), finding phloem directly adjacent to the endodermis, concluded that the pericycle was lacking here. But since this phloem is definitely external to the protophloem (fig. 6*C*), it seems more logical to interpret it as differentiation of connective phloem in the pericycle over the bundle.

The connective phloem does not show in the earliest stages of the seedling, but about the fourth or fifth day it begins to develop. This probably accounts for the retarded development of the internal phloem in the transition and similarly along the rays. Certain cells undergo several longitudinal divisions each, resulting in the differentiation of one sieve tube, one or more darkly staining cells, which occupy the position of companion cells, and several parenchymatous cells, all from the one mother cell. In transverse sections of the axis this phloem appears in isolated patches, except where occasional horizontal anastomoses occur. Longitudinally the branched but connected nature of this system is more evident, showing where adjacent mother cells have so differentiated that the sieve tubes are continuous with others vertically or laterally.

Completing the primary maturation of the axis, the other tissues exhibit fewer changes. The epidermis consists of short tabular cells. Stomata are present in the epidermis. Immediately within are several layers of collenchyma. The cortical parenchyma shows large elongated, chlorophyll-containing cells, and the characteristic intercellular spaces have persisted. The endodermis can be traced over the vascular bundles by its conspicuously short cells, which are without Casparian thickenings but contain starch. Across the rays its identity is lost. The pericyclic and pith parenchyma are similar to that of the cortex, except that the inner pith in this variety does not show differentiation of phloem in the primary condition. At the center of the axis a pith lacuna is formed during the later phases of primary growth. This lacuna extends from the upper levels of the transition to just below the cotyledonary node.

Up to this point the description of phloem has followed in general the interpretation given by FISCHER (10); but from certain observations, the question has arisen as to whether there are two types of tissue involved in the phloem. This idea is supported by the work of BRAEMER (3), and a number of his observations parallel those made in *Cucurbita maxima*. The one type of phloem is found only in the vascular bundles, hence can be referred to as fascicular phloem. It is differentiated in both the outer and the inner phloem (fig. 6*C*). In it the sieve plates show a callus formation; the perforations of the plate seem small and regular in arrangement. The cell contents of the sieve tube are homogeneous and rather translucent. The companion cells, two or more to the length of one sieve tube, are filled with dense cytoplasm and clearly show a nucleus. The fascicular phloem is derived from procambial tissue and at the most the sieve tube and the accompanying companion cells originate from the one mother cell.

The second type will be referred to as connective phloem since it is this type only which is found in the non-vascular regions and already has been designated by that name. In addition it is associated with both vascular areas of phloem, but chiefly along the periphery of these (fig. 6*C*). The sieve plates of the connective phloem do not show a callus formation but react to stains as do the adjacent cellulose walls. The perforations in the sieve plates are seemingly larger

and less regular. The sieve tubes may be much shorter than those of the fascicular phloem, and the cell contents show darkly stained, plastid-like bodies scattered through the clear cytoplasm. In this second type of phloem the so-called darkly staining cells are often associated with the sieve tubes as companion cells, but apparently not regularly so. They may be of relatively large size with densely staining, coarsely reticulate cytoplasm and obliterated nuclear outline. In origin this connective phloem appears to be derived from partially differentiated parenchymatous cells. As previously noted, the one mother cell may give rise to a sieve tube, two or more darkly staining cells, and several parenchymatous cells. It is in this connective phloem that BRAEMER found the active constituents of the drugs in the several plants which he studied. Because of this fact, and because it differs so much from the fascicular phloem, he considered it as a segmented lactiferous system. This reinterpretation would eliminate the more unusual aspects of phloem distribution in *C. maxima*. The observations here presented are admittedly limited, however, and suggest only that further work is needed on this subject.

Before concluding the discussion of phloem, reference should be made to the subject of the bicollateral bundle. HÉRAIL (15), an early worker on the problem, formulated two criteria for bicollaterality: (a) the derivation of the internal phloem from the procambial strand, and (b) simultaneous development of the inner and outer phloem. He considered that the Cucurbitaceae had definitely bicollateral bundles. The present description of primary differentiation in the bundle of *C. maxima* is in agreement with this; the procambial tissue gives rise to phloem on the inner face of the bundle and the differentiation of this inner phloem is concurrent with that of the outer metaphloem. Later LAMOUNETTE (21) described the derivation of the internal phloem from the pith only. His work seems to be on an arbitrary basis inasmuch as it is so often impossible to distinguish between procambial tissue of the vascular strand and the pith. Also the consideration of two types of phloem gives further reason for discarding this latter interpretation. On this basis phloem derived from the pith would be only connective phloem. Actually the vascular type is also found in the inner phloem.

BARANETSKY (1), COL (5), and WORSDELL (30) have rejected these two criteria of HÉRAIL and reinterpreted the bundle from a phylogenetic point of view. They have considered it as not bicollateral but composed of two independent bundles, of which the inner may differentiate only phloem or it may later develop the complementary cambium and xylem. VON FABER (28) found that these potentialities need not in the least destroy the unity of the bicollateral bundle. He reaffirmed the concept of its bicollateral nature on the basis of the ontogeny as he found it for the bundle of *Cucurbita pepo* traced from the apical meristem. The present observations on the bundle in the hypocotyl of *C. maxima* as traced from the embryo agree essentially with the work of VON FABER.

Accepting these criteria with emphasis on the ontogeny of the bundle, that of *C. maxima* is bicollateral. On the other hand, the phylogenetic interpretation is equally important, but the limited scope of the present study provides no answer for this aspect of the question.

COTYLEDONARY NODE

NODE.—At the apex of the hypocotyl the divergence of the cotyledons forms a complicated first node. Like the lower hypocotyl it may show many variations in its vascular pattern, but again can be reduced to a basic form. Tracing upward from the ten vascular bundles previously described in the hypocotyl, the first change in course occurs in those bundles at either end of the oval outline. About 3–4 mm. below the divergence of the cotyledons (fig. 3*N*) the middle bundle at each end divides into two parts, which separate and pass tangentially upward away from each other and toward the adjacent bundles with which they anastomose. These latter continue the tangential course until each end bundle anastomoses with the respectively adjacent central bundle. This reduces the total number of bundles to four, two in the center of each broad side of the hypocotyl. The upward continuations of these four bundles form the two traces to each of the cotyledons. In the course of this general convergence any intervening additional bundles take part. Additional vascular strands between the central bundles on either side of the oval may add considerable variation to the pattern, but ulti-

mately they also anastomose with the adjacent original bundles. The tangential anastomosing produces the effect of four sloping transverse bundles which might be considered the four parts of a cotyledonary plate (fig. 3*N*). Each of these transverse bundles represents essentially the rejoined parts of a single tangential bundle of the transition.

COTYLEDONS.—The four traces just described continue upward and outward into the broad bases of the cotyledons. Before the divergence of the cotyledons is completed, each trace branches laterally, a single vascular bundle differentiating toward the margin of the cotyledon (fig. 3*O*). The course of this bundle is undulating but in general downward. Occasionally there are direct connections between it and that part of the cotyledonary plate immediately below. Four or more large veins diverge upward from this basal lateral vein. The two median traces also continue into the cotyledon; they may anastomose to form one midvein or they may persist independently but with one subordinate to the other. Thus there is established at the base of the cotyledon a complement of at least nine vascular bundles (fig. 3*O*, *P*) which extend into the blade as the principal veins, diverged slightly and interconnected by a network of smaller veins.

From the hypocotyl into the base of the cotyledons the tissues show a simple continuity. The vascular bundles have a structure similar to those described for the axis. They occupy a position at the center of a rather thick mesophyll which is some fifteen or more cells in depth, is composed of parenchyma like that of the cortex in the axis, and shows the same intercellular spaces and connective phloem. On the adaxial surfaces over the vascular bundles there are areas of collenchyma.

The mesophyll in the blade of the cotyledon has a different appearance. In the areas intervening between the large veins the three adaxial layers are closely arranged palisade tissue, and the remaining twelve layers, more or less, form a spongy parenchyma. All this tissue is photosynthetic, although in the unexpanded cotyledon it functioned as a storage tissue. The compact parenchyma is limited to areas adjacent to the more important veins and scattered connective phloem is still found in this. These principal veins are midway

between the two surfaces, but the smaller veins are found interrupting the third palisade layer nearer the adaxial surface. Internal phloem is present in the veins even when only one xylem element is differentiated. The upper epidermis is composed of somewhat larger cells than the lower and it may bear multicellular hairs, but the lower surface is smooth. Stomata appear in both surfaces.

EPICOTYL.—At germination the epicotyl consists of a small growing point overarched by the primordium of the first leaf. In eight days the primordia of six leaves may have differentiated, but the whole structure remains inconspicuous and hidden at the base of and between the two cotyledons (fig. 1*D*). In about two weeks the first leaf has expanded. It is alternate with the cotyledons and is usually found immediately above these, resulting in a short first internode while the internodes next following are much longer (fig. 1*E*). Observation of the embryo before germination reveals that the median trace to the first leaf is already identifiable as a procambial strand although the remainder of the tissue in the epicotyl is undifferentiated. This trace shows correspondingly early differentiation of the xylem elements. Possibly the formation of the short first internode is correlated with this precocious maturation.

Material collected at the end of five weeks, stained, and cleared by Gourley's method showed the vascular pattern of the lower nodes and internodes and the relationship of the epicotyl to the lower part of the plant. Contrary to DANGEARD (7), the trace to the first leaf may have the lowest insertion, which is on one or (with branching) on both divergences of the first dividing end bundle of the cotyledonary node (fig. 3*N*). The other bundles of the stem are later differentiated against the lower and more lateral portions of the transverse bundles which form the cotyledonary plate. This produces a leaf gap opposite each cotyledon. The number of bundles and the pattern formed in the first internode are irregular in the several specimens examined. It is only in the second and third internodes that the characteristic two rings of bundles are established. MANTEUFFEL (22) has noted a similar variation in the lower nodes of *Cucurbita pepo*. In general his description of the course of bundles in the upper nodes and the divergence of the leaf traces is similar to that found in *C. maxima*.

Summary

1. The root tip of *Cucurbita maxima* possesses a single histogen from which all the primary root tissues arise.

2. The primary root is exarch, tetrarch. Differentiation of the large central metaxylem vessels is retarded; pith is not present.

3. The primordium of a secondary root is formed from the cortex, including the endodermis, as well as the pericycle of the primary root.

4. The transition extends from approximately 1 cm. below the peg to just above it. At the lowest level pith differentiates in the center and the metaxylem takes a peripheral position just within the phloem. Each primary xylem strand diverges into two arms extending laterally and joining the metaxylem. These arms separate, resulting in a siphonostele of four tangential transition bundles. These divide into two parts each, forming a total of eight bundles which become endarch.

5. Of these eight bundles usually two pairs anastomose, then divide into three, producing a total of ten bundles which continue through the hypocotyl. Additional bundles may arise.

6. The bundle is considered bicollateral on the basis of ontogeny; it shows a differentiation of internal phloem from the procambial tissue at the same time that the external metaphloem differentiates. (The study of a single species allows no interpretation on the basis of phylogeny.)

7. A suggestion is made concerning the differentiation of two types of phloem, the one called fascicular phloem and the other called connective phloem. Differences in origin, structure, and distribution of the two types are described.

8. In the cotyledonary node tangential anastomoses produce a cotyledonary plate of four parts. Continuations from these form two traces to each cotyledon. Before the cotyledon diverges completely, each trace branches laterally to form a basal vein from which arise four or more bundles which are the principal veins in the blade of the cotyledon.

9. The bundles of the epicotyl differentiate against the parts of the cotyledonary plate. The epicotyl is retarded in its development

except for the median trace to the first foliage leaf. The early differentiation of this trace may account for the characteristic short first internode.

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