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COMPARATIVE ANATOMY OF ENDOGENOUS BUD AND LATERAL ROOT FORMATION IN CONVOLVULUS ARVENSIS ROOTS CULTURED IN VITRO[1]

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ABSTRACT

The anatomy of endogenous bud and lateral root formation was studied in *Convolvulus arvensis* roots cultured in vitro. Although early stages in the initiation of buds and roots were found to be identical, continued development of primordia into roots or buds was accompanied by differences in participating primary root tissues, in rates of development, and in orientation of cell divisions. Evidence for the existence of a primordium capable of developing into either a bud or root is discussed with reference to *Convolvulus* roots and other plant parts shown to have similar morphogenetic potentials.

THE FORMATION of buds from roots has been discussed extensively by Beijerinck (1887), van Tieghem and Duliot (1888), and Priestley and Swingle (1929). Prior to the monograph of Beijerinck, numerous investigators had contributed to a recognition of the variety of plants which form buds from their roots. Irmisch (1857) compiled a list of these plants comprising 42 species, 38 of them dicots; this list later grew to 132 species, 124 of them dicots (Wittrock, 1884). Among this large number of plants which develop buds from their roots, *Convolvulus arvensis*, the field bindweed, was recognized as one of a much smaller group of plants, representing several families, in which buds develop from the pericycle in a location identical to that of lateral root formation.

The anatomy of bud formation from *Convolvulus* roots was first studied by Irmisch (1857) and Vogl (1863). They recognized that bud formation was endogenous and occurred on the protoxylem radii, but because of the occurrence of extensive secondary growth in roots they were studying, they were unable to determine the exact location of origin.

Beijerinck studied bud formation on the hypocotyl and root of *Convolvulus* seedlings. He found that buds arose from the pericycle at sites opposite the protoxylem poles and showed no evidence of endodermal participation during their development. His attempts to obtain a series of stages in the formation of lateral roots were unsuccessful because of the small number which were present. Beijerinck suggested that endogenous buds develop in place of lateral roots.

Van Tieghem and Duliot described the formation of bud primordia by growth and division in all directions of an arc of pericycle cells, which produced a hemispherical protuberance. They believed that as a bud primordium developed it sequentially digested the endodermis and then all the cortical layers in its path to the periphery of the root. Lateral root formation in *Convolvulus siculus* and *Convolvulus tricolor* also proceeded by tangential divisions of six to eight pericycle cells surrounding a protoxylem pole. As the pericycle cells continued to divide, the endodermis divided radially and then tangentially; consequently the endodermis remained single-layered at the flanks of the lateral root, whereas at the tip it became several layers thick. Developmental stages of lateral root formation in *Convolvulus arvensis* were not illustrated, although one drawing of a lateral root before emergence showed a single-layered endodermis which had undergone only radial divisions.

These investigators outlined the major steps in the anatomy of bud formation from *Convolvulus* roots. As part of a study of the physiology of organ formation in cultured *Convolvulus* roots, a reinvestigation of the anatomy of endogenous bud and root formation was undertaken. Culturing these roots has enabled such a study to be made in the absence of secondary growth and has allowed stages of organ formation to be placed in rather precise sequence by their time of development in organ culture.

MATERIALS AND METHODS—*Convolvulus arvensis* root segments were cultured in 10-cm petri plates on a modified Bonner pea root medium (Torrey, 1958) solidified with agar and adjusted to a post-autoclave *p*H of 4.5.

Prior to a study of the anatomy of bud and root formation the distribution of buds and roots in cleared root segments was determined. Intact roots or isolated segments in culture characteristically developed protuberances which could not be classified reliably as buds or roots. However,

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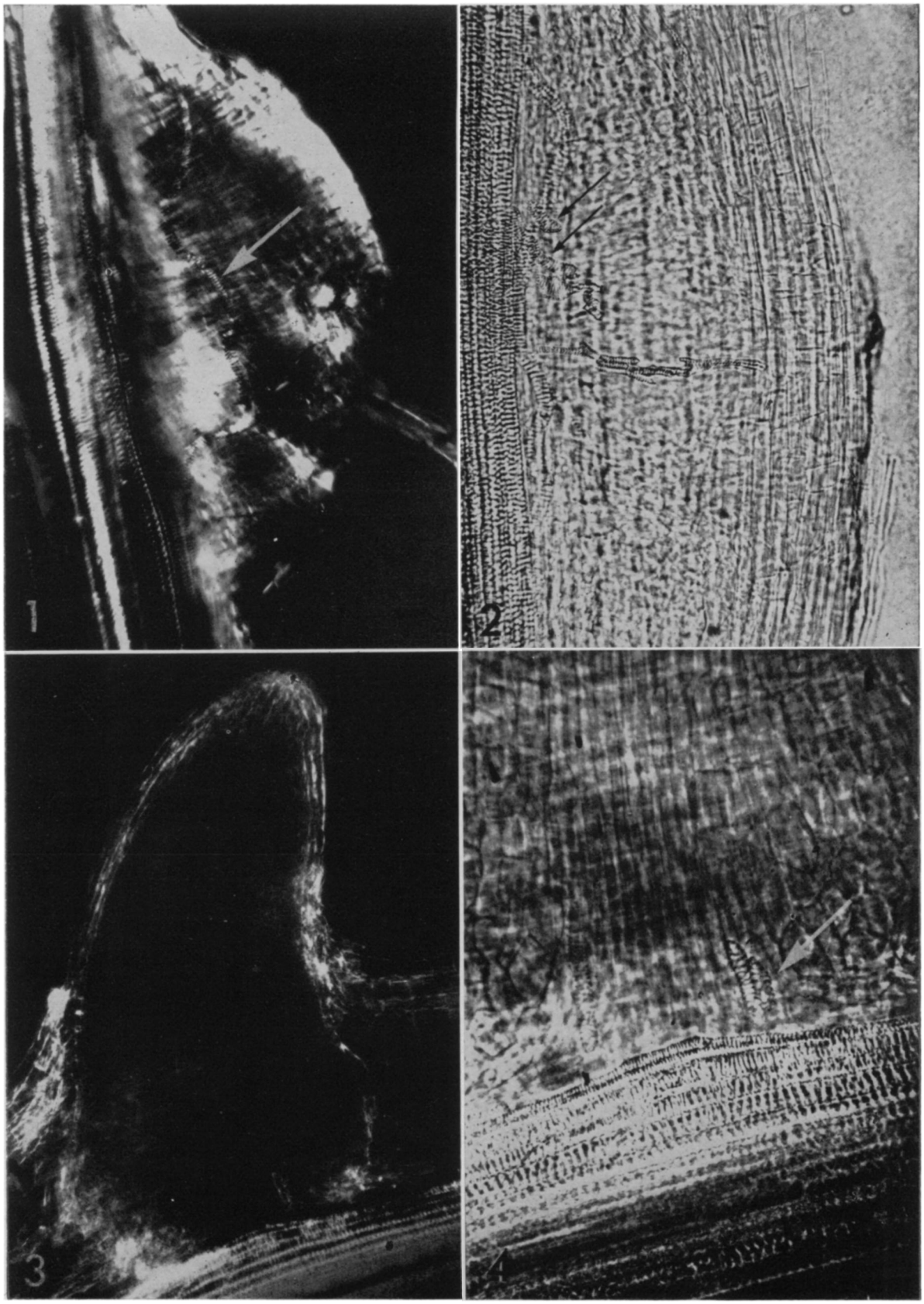


See page 498 for caption.

in segments cleared by the method of Jacobs (1952) organs could be distinguished before they protruded from the root epidermis. It was found that buds could be distinguished from roots by the following features: (1) buds are broader organs than roots; (2) the terminal protoxylem elements of one primary root xylem pole become separated from the rest of the xylem elements in that pole during bud formation, and in a longitudinal view these protoxylem elements appear to form arcs which run through portions of the bud primordium; (3) the first xylem of the lateral root differentiates at right angles to the xylem of the parent root segment, whereas the first xylem of the bud develops obliquely with respect to the parent xylem. These characteristics are illustrated in Fig. 1–4. Figure 1 shows a longitudinal view of a root segment with a cleared bud illustrating the raised terminal protoxylem elements running through the middle of the bud. Figure 2 shows a bud in which some new xylem has differentiated. Figure 3 shows a cleared lateral root; Fig. 4 shows a more highly magnified view of the first mature xylem elements of the lateral root. The pattern of xylem differentiation was adopted as the most important of these criteria, and unless otherwise noted, primordia were not recorded unless they could be positively identified on this basis.

Figure 5 shows the composite distribution of organs formed in 30 segments 15 mm long, excised from the region of the root axis 15–165 mm from the root apex and cleared after 6 weeks in culture. The distribution of organs indicates that proximal[3] regions of the segment, which do not form roots, but which do have a high probability of forming buds can be excised. Distal regions, on the other hand, have a high probability of forming roots and also form a few buds.

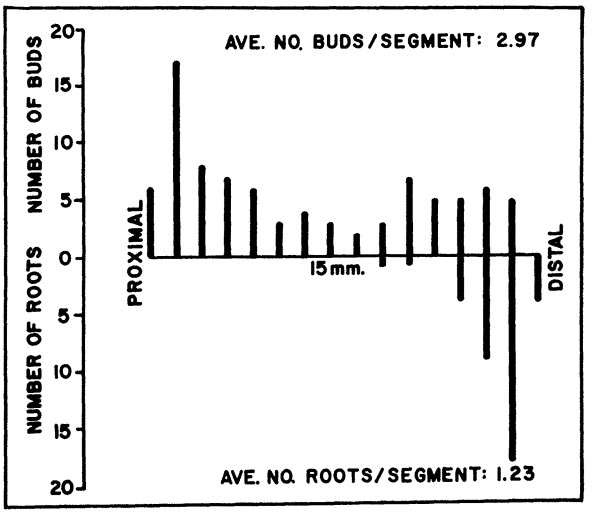


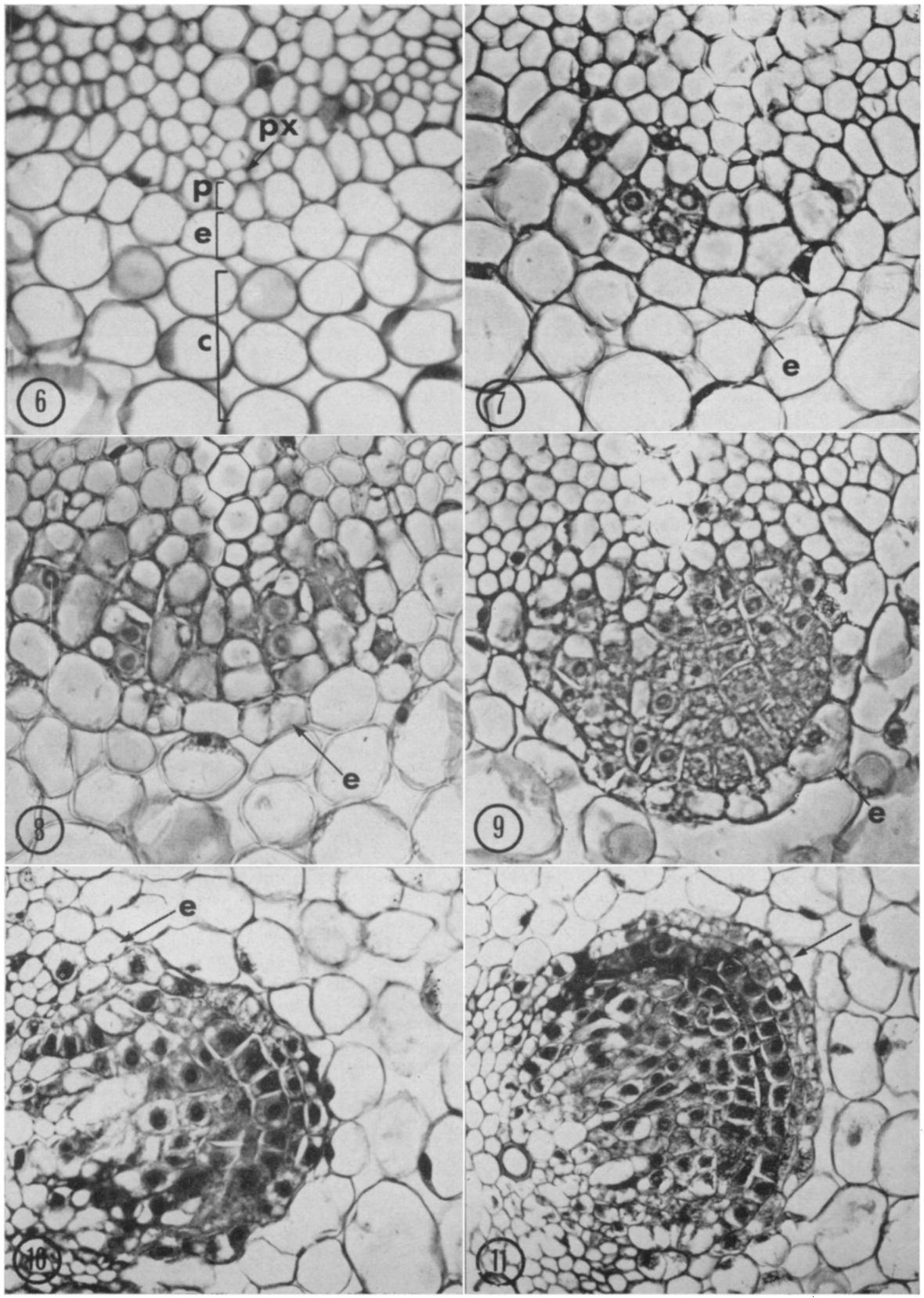
Fig. 5. The distribution of buds and roots in 30 segments, 15 mm long, cultured for six weeks, cleared, and counted. Each bar represents the total number of buds or roots recorded in each 1-mm interval.

This polarity of organ formation, observed in cleared root segments, aided in the anatomical investigation of bud and root formation. Six root segments, 15 mm long, were excised from isolated roots grown in culture for about 6 weeks. The portion of the root axis used was the region 15–105 mm from the root apex. The segments were placed in a petri plate, each with its distal end to the periphery of the plate. At varying time intervals after excision, 3-mm segments were excised from the proximal and the distal end of a segment and examined for stages in the formation of buds and

[3] The terms "proximal" and "distal" refer to the segment orientation with respect to the root from which it was excised. The distal end of a segment is the end which was closest to the root tip, and the proximal end is the end which was farthest from the root tip.

Fig. 1–4.—Fig. 1. Lateral view of a cleared bud not yet protruding from the root segment. The overlying epidermis and cortex of the root have been removed. Terminal protoxylem elements of one xylem arm have been separated and raised from the rest of the xylem elements, traversing the middle of the bud (unlabeled arrow). Photographed in polarized light, ×95.—Fig. 2. Lateral view of a cleared bud not yet protruding from the root segment. The overlying epidermis of the root has been removed. The first xylem to differentiate in association with the bud is marked by arrows. The raised protoxylem elements are in another focal plane, ×125.—Fig. 3. Cleared lateral root protruding from the cortex of the parent root showing the first mature xylem of the lateral root. The overlying epidermis of the parent root has been removed. Photographed in dark field illumination, ×90.—Fig. 4. Higher magnification of the lateral root–parent root junction showing the first mature xylem elements of two poles of the lateral root (unlabeled arrow). Terminal protoxylem elements of the xylem arm of the parent root remain adjacent to the rest of the xylem elements in that pole. Photographed in phase contrast, ×240.

Fig. 6–11.—Fig. 6. Transection of a root showing the primary tissues in the region of a xylem pole. px, protoxylem; p, pericycle; e, endodermis; c, cortex, ×520.—Fig. 7–11. Transections near the distal end of root segments. e, endodermis. —Fig. 7. The same region shown in Fig. 6—24 hours after excision of the segment. Pericycle cells have undergone tangential division. Photographed in phase contrast, ×505.—Fig. 8. A primordium 36 hours after excision. Outer daughter cells resulting from the first pericycle division have divided tangentially a second time. Photographed in phase contrast, ×360.—Fig. 9. Root primordium 44 hours after excision of the segment. Radial files of four cells derived from the pericycle have formed. The outermost cells have undergone tangential enlargement and divided radially. Some endodermal cells have divided radially. Photographed in phase contrast, ×385.—Fig. 10. Root primordium 60 hours after excision of the segment. Divisions of the endodermal cells have continued, resulting in two layers of cells. Cortical cells contiguous with the primordium have collapsed, ×290.—Fig. 11. Root primordium 60 hours after excision of the segment. Cells derived from the endodermis have formed a triple layer at one point (unlabeled arrow). Cells contiguous with the primordium have collapsed, ×260.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roots, respectively. The 3-mm segments were frozen in 10% methyl cyclohexane in isopentane (v/v) as described by Branton and Jacobson (1962), fixed and dehydrated in 0.1% $HgCl_2$ in methyl alcohol (Bell, 1959) at −70 C, transferred to n-propyl alcohol, gradually infiltrated with polyethylene glycol 400 distearate (Sidman, Mottla, and Feder, 1961), and embedded in Tissuemat. Sections were stained with safranin, hematoxylin, and fast green.

RESULTS—The following series of figures for bud and for root formation are each assigned a time in hours or days. This time refers to the length of time from excision of the 15-mm segment to fixation of the terminal 3-mm portion of the segment. The stage depicted in most cases corresponds to the largest primordia which have formed within the given time period. The terminology adopted for planes of division is described by Esau (1965).

Figure 6 is of a section of the mature primary root structure, showing a region where primordia have not been initiated. Proceeding from the protoxylem pole (labeled "px" in Fig. 6) towards the outside of the root, the first cell layer is the pericycle (p), followed by the endodermis (e) with Casparian strips in cross section, and then three cell layers of the cortex (c).

Lateral root formation—Figures 7–13 illustrate stages of root formation, obtained from transections of distal ends of cultured root segments fixed at successive time intervals. Figure 7, at 24 hours, shows a young primordium. Five or six pericycle cells, forming an arc which surrounds the protoxylem pole, have each undergone radial enlargement and one tangential division.

By 36 hours (Fig. 8) further radial enlargement has occurred and outer daughter cells have also divided tangentially. Some of the endodermal cells show an increase in protoplasmic content.

Tangential divisions continue in both the outer and inner rows of daughter cells of the pericycle until radial files of four cells are formed. At 44 hours (Fig. 9) the outermost cell in this radial file has undergone tangential enlargement followed by radial division. Endodermal cells at this stage also show radial divisions.

The activation of divisions in the endodermis continues and by 60 hours (Fig. 10) tangential divisions have produced two cell layers derived from the endodermis. The effect on the cortical cells of radial growth by the root primordium is shown in this figure by the collapse of cells contiguous with the primordium.

Figure 11 shows at 60 hours a larger primordium in which cells derived from the endodermis form a triple layer at one point. Several collapsed cells of the inner cortex are still evident.

By 72 hours (3 days) the young lateral root (Fig. 12) is more than halfway through the cortex. Wall material remaining from collapsed cortical cells is visible around the tip of the root. Flattening of cells not contiguous with the root tip, but positioned between it and the epidermis, is still absent.

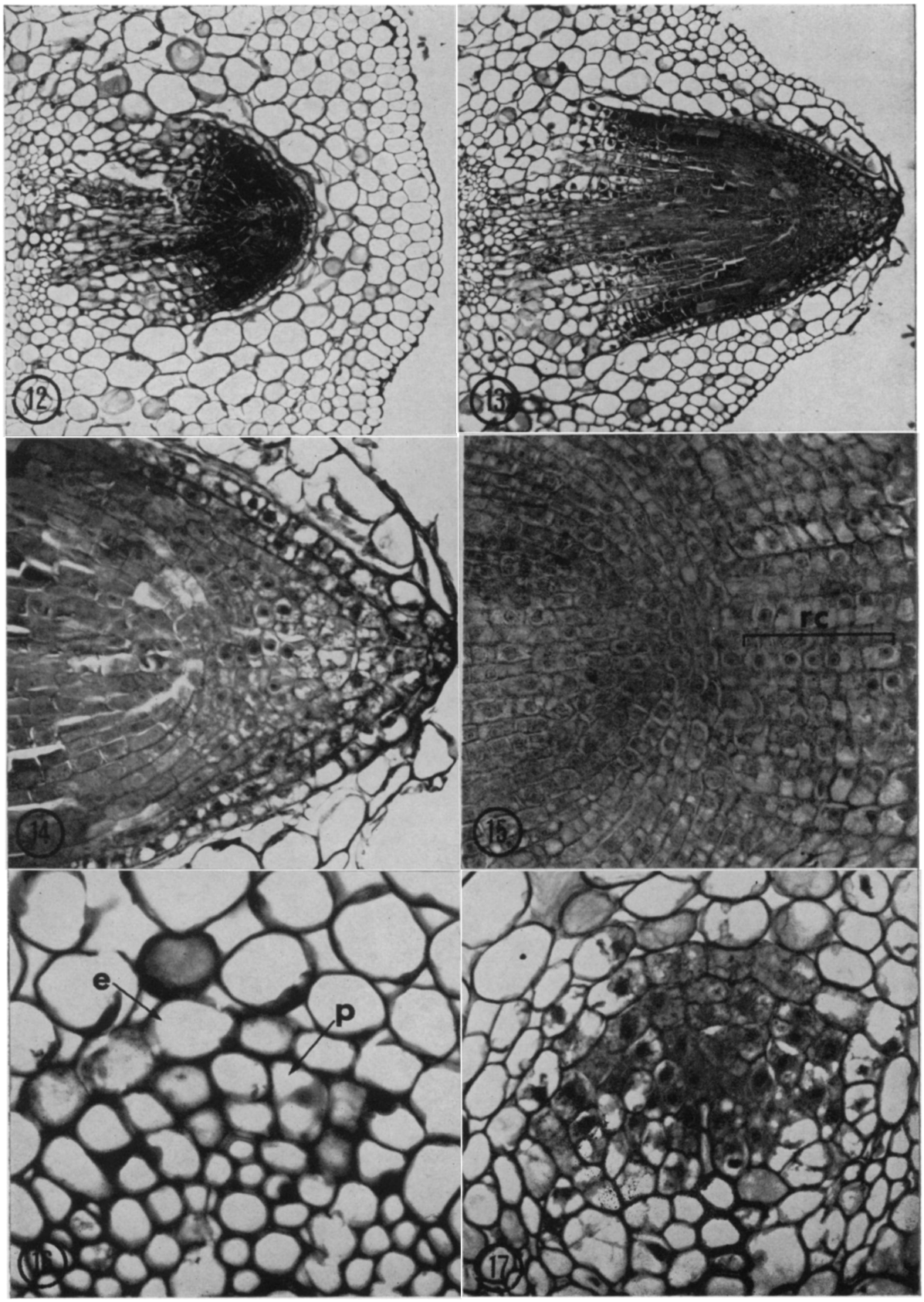
Figure 13 shows a lateral root just completing penetration of the cortex and epidermis after 96 hours (4 days).

A thorough study of the fate of the layers of cells derived from the endodermis has not been made. Figure 14 is a higher magnification of Fig. 13, showing the "root cap" and apical initials. Figure 15 shows the same region of such a lateral root after it has reached full diameter and growth rate. This root cap shows a columnar structure resulting from transverse divisions in root cap initial cells. There is a discontinuity in the files of cells of the columella and the central cylinder. Though files of cells are also evident in the root tip of the young lateral root (Fig. 14), the root cap area shows only the beginnings of a columnar structure. It is probable that the "root cap" of the lateral root at the stage shown in Fig. 13 is largely derived from the endodermis and later sloughed as root cap initials begin transverse divisions to form files characteristic of the larger lateral root.

Endogenous bud formation—Figures 16–21 illustrate stages in bud development seen in transections of the proximal ends of cultured root segments. Figure 16 shows a young primordium after 44 hours. As in the root primordium, six or seven pericycle cells around the protoxylem pole have undergone radial enlargement and one tangential division (cf. Fig. 7).

By 72 hours (Fig. 17), or 3 days, radial files of three to four cells have formed. Although only tangential divisions have occurred, alignment of the files of daughter cells is less precise than was observed in the young root primordium. Enlargement and division of the cells lateral to the protoxylem also occur, resulting in the compression

Fig. 12–17.—Fig. 12. Transection near the distal end of a root segment showing a lateral root primordium 72 hours after excision of the segment. Wall material remaining from collapsed cortical cells is visible around the tip of the primordium, ×170.—Fig. 13. Transection near the distal end of a root segment showing a lateral root 96 hours after excision of the segment. Root apex is just penetrating the outer cortex and epidermis, ×110.—Fig. 14. A more highly magnified view of the apex of the lateral root shown in Fig. 13. Root cap area shows only a limited columnar structure, ×280.—Fig. 15. Longitudinal section of a portion of the root apex of a lateral root after it has reached full diameter and growth rate. The root cap (rc) shows a columnar structure, ×305.—Fig. 16. Transection near the proximal end of a root segment 44 hours after excision of the segment. Pericycle cells (p) have undergone tangential division. e, endodermis, ×590.—Fig. 17. Transection near the proximal end of a root segment 72 hours after excision of the segment. Divisions of the pericycle cells have resulted in radial files of three cells. Enlargement and division of cells lateral to the protoxylem have crushed the terminal protoxylem elements, ×340.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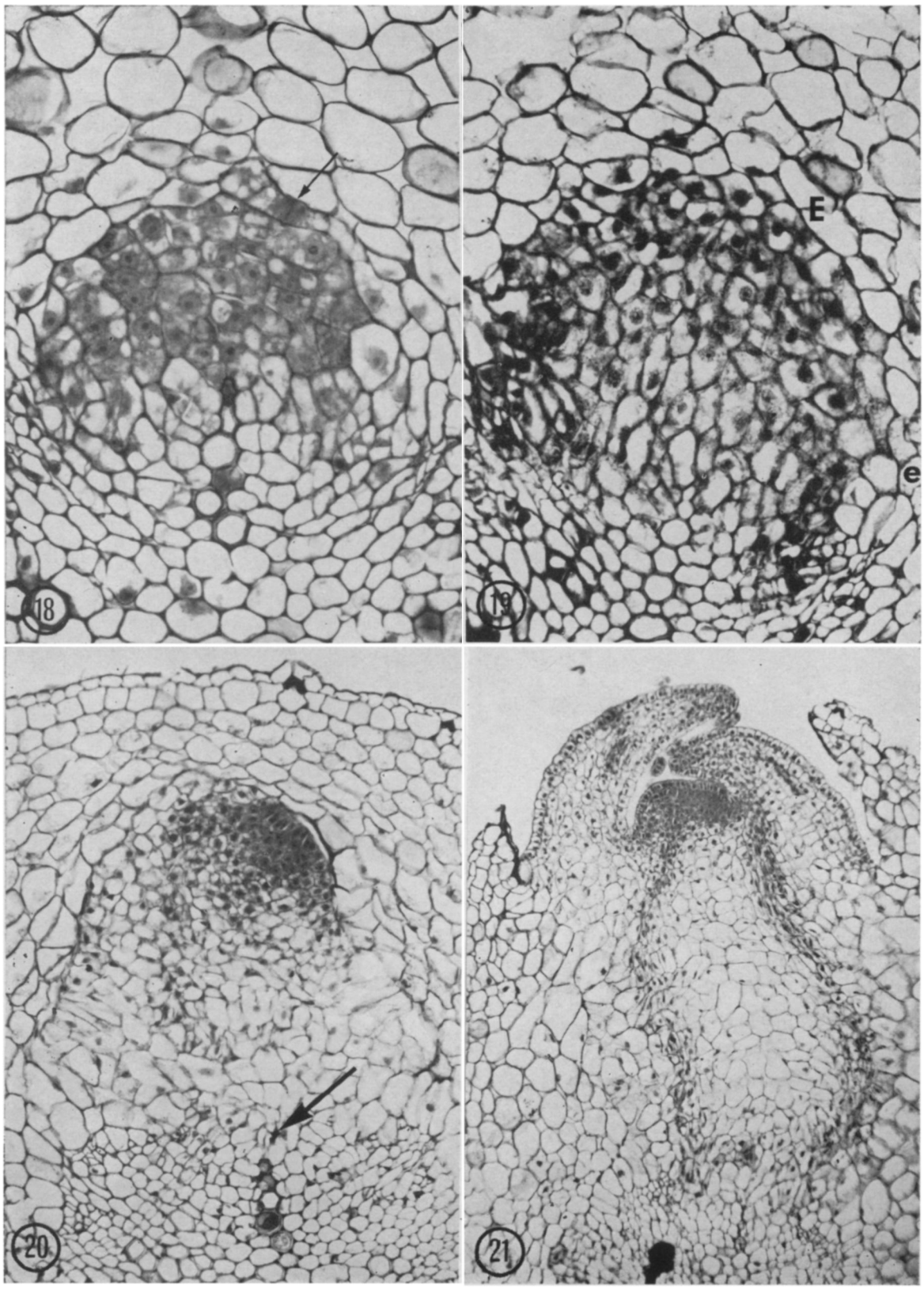
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of terminal protoxylem elements. The rate of development of buds can be compared to that of roots by consulting Fig. 12, also at 72 hours, in which the root is more than halfway through the cortex.

By 5 days (Fig. 18) cells of the endodermis of the bud primordium show only enlargement and the beginning of radial divisions, illustrated by the metaphase plate in one of the endodermal cells. The root primordium at a comparable size already has a two-layered endodermis (Fig. 10).

Figure 19 shows a bud primordium at about 8 days. Endodermal cells have undergone extensive enlargement and have divided radially By the time bud primordia have reached this size, a distinction among cells of the bud primordium, endodermal cells, and adjacent cortical cells cannot always be made. As yet, there is no histogen organization typical of the bud apex.

At 11 days (Fig. 20) a meristematic dome of cells has developed at the outer periphery of the primordium. Cortical cells around the flanks and at the tip of the primordium show flattening in the directions which would be predicted as a result of pressure exerted by the developing bud. Cells between the epidermis and the bud apex are flattened so that their longest axis, seen in cross section, is parallel to the epidermis. Cells along the flank of the bud primordium are obliquely flattened from the radial and tangential growth of the bud primordium. Continued divisions and enlargement of the cells lateral to the terminal xylem elements have completely crushed the terminal protoxylem element and separated it from the rest of the xylem arm (see arrow in Fig. 20). This process confirms one of the diagnostic features of cleared buds illustrated in Fig. 1. Such an effect has been observed in only one root but in nearly all cleared or sectioned buds.

By about 2 weeks (Fig. 21) a bud apex has developed which has penetrated the outer cortex and epidermis of the root and which shows a clear single-layered tunica and two leaf primordia.

Although the series of stages from a single-layered pericycle to a clearly recognizable organ are figured separately for the root (Fig. 7–13) and the bud (Fig. 16–21), there are no entirely consistent anatomical features which allow the earliest stages of organ formation at the distal end (Fig. 7, 8) to be distinguished from the earliest stages at the proximal end (Fig. 16–17). The inclusion of separate figures for these stages is based only on the procedure of describing root and bud formation at opposite ends of the 15-mm segments. In fact, from a consideration of the data in the next section, one may not exclude the possibility that the primordia at these earliest stages were present prior to excision of the segments.

Organ formation in intact cultured roots—Although there were no externally visible primordia for at least several hundred millimeters behind the tips of *Convolvulus* roots grown in culture, histological sections of the region of the root from which the 15-mm segments were routinely excised revealed that small primordia were present at the time of segment excision. Therefore, it was necessary to determine the nature and stage of development of these primordia which, because of their small size, could be done only by a histological study. Rather than section the entire lengths of roots, representative 5-mm lengths along each root were fixed and sectioned. Seven root cultures, grown in the dark for about 6 weeks, were observed for the 24-hour period before fixation to confirm that they were growing at a normal rate (greater than 20 mm/day). From these roots 5-mm segments were excised and immediately prepared for histological study. The first column in Table 1 shows the distance from the root tip to the 5-mm segments studied. Data from the seven 5-mm segments, each the same distance from the root tip, were pooled so that the number of primordia reported in the second column of Table 1 represents the total number of primordia observed for that interval in all seven roots.

Primordia were rated by class. Primordia which showed one or a few tangential divisions in the pericycle, but not a complete arc of divided cells, were assigned to Class 1. Class 2 corresponds to primordia such as those illustrated in Fig. 7, 16; Class 3 to those shown in Fig. 8, 17; Class 4 to that shown in Fig. 18; Class 5 to that shown in Fig. 19; and Class 6 to that shown in Fig. 20. No primordia were found which could be identified as root primordia (this would require that they be at least as large as the primordium illustrated in Fig. 9), so all primordia have been assigned to classes corresponding to the developmental stages of bud formation.

From Table 1 it is clear that primordia are initiated at a distance of only 5–10 mm from the actively growing root apex and that the first

Fig. 18–21. Transections near the proximal end of root segments.—Fig. 18. Bud primordium five days after excision of the segment. Division of endodermal cells has not occurred with the exception of one cell in metaphase (unlabeled arrow), ×320.—Fig. 19. Bud primordium about eight days after excision of the segment. Endodermal cells have undergone enlargement and divided radially. e, endodermal cell at the flank of the primordium, which has not enlarged; E, endodermal cell which has enlarged and divided radially, ×270.—Fig. 20. Bud primordium about 11 days after excision of the segment. A meristematic dome of cells has developed at the periphery of the primordium. The terminal protoxylem element (unlabeled arrow) has been crushed and separated from the rest of the xylem arm, ×185.—Fig. 21. Bud about 14 days after excision of the segment. The bud apex, which has penetrated the outer cortex and epidermis of the root, has a single-layered tunica and two leaf primordia, ×135.

TABLE 1. *Primordia present in the terminal 135 mm of Convolvulus roots based on a histological analysis of segments from seven roots cultured for about six weeks*

Distance of 5-mm segment from root tip (mm)	Total number of primordia/ 7 segments	Class:	1	2	3	4	5	6
		Corresponding bud primordium stage shown by Fig.:	—	16	17	18	19	20
		Bud primordium age:	—	44 hr	72 hr	5 da	8 da	11 da
		Corresponding root primordium stage shown by Fig.:	—	7	8	—	—	—
		Root primordium age:	—	24 hr	36 hr	—	—	—
5–10	2		—	2	—	—	—	—
15–20	11		—	11	—	—	—	—
30–35	14		6	7	1	—	—	—
50–55	12		2	9	1	—	—	—
70–75	14		1	11	2	—	—	—
90–95	16		4	12	—	—	—	—
110–115	13		1	11	1	—	—	—
130–135	10		—	9	1	—	—	—
150–155	17		2	12	2	1	—	—
170–175	8		1	4	3	—	—	—
190–195	11		1	6	2	1	1	—
210–215	14		2	7	—	3	2	—
230–235	8		—	8	—	—	—	—
250–255	8		—	5	—	2	1	—

stages in bud and root formation (Fig. 7, 8, 16, 17) could be accounted for by primordia found to be present at the time of excision in the region of the root axis from which the 15-mm segments were excised (15–105 mm from the root apex). However, later stages in the formation of buds (Fig. 18–21) or roots (Fig. 9–13) cannot be explained by pre-existing primordia at the same stage and must represent organ development after segment excision. Although primordia representing more advanced stages in bud development were observed at increasing distances from the root apex, they never occurred in greater numbers than could be accounted for by initiation of all primordia within the first 20 mm of the root. Since few, if any, new primordia appeared to be initiated after the first 20 mm, and since the largest proportion of the primordia was always in the first two classes, most primordia must have been inhibited from further development. Those primordia which did develop became recognizable bud primordia.

The age of a 15-mm region of the root axis may be figured either by the number of days the region has been cultured as an excised segment or, in the intact root, by the increase in distance of the region from the root tip. Since the mean growth rate of *Convolvulus* roots is about 23 mm/day, each 23-mm interval between the 15-mm region and the root tip is equivalent to one day in culture for the excised segment. This relationship forms the basis for the following comparisons of the rate of bud development in excised segments to the rate of bud development in the intact root.

A bud at the 5-day stage (Class 4; Fig. 18) of development is no larger than a bud which might be seen in the same region of the root axis after the root had grown an additional 23 mm/day for 5 days. The region of the root axis from which the segments were excised (15–105 mm from the root apex) would be 130–220 mm from the apex after 5 days of growth (Table 1). Similarly, a bud at the 8-day stage (Class 5; Fig. 19) is no larger than a bud that might be found 199–289 mm from the root apex. Consequently, some bud primordia may develop in the intact cultured roots at a rate comparable to that observed in excised segments. No evidence was obtained for lateral root development in this region of the intact root except for stages too small to distinguish as bud or root primordia.

To extend the analysis of organ production by root cultures, entire roots were cleared and the distribution of organs along their length was determined. Roots used in this study were checked for a 24-hour period to confirm that they were growing at a rate of at least 20 mm/day. Primordia were assigned to one of the following four groups: (A) primordia which could not be identified as root or bud; (B) larger primordia in which the terminal xylem element was separated, but no new xylem had differentiated in association with the primordium; (C) buds; and (D) roots. The identification of primordia in cleared whole

TABLE 2. *Organ production in whole root cultures*

Group[b]	Root 1[a]				Root 2				Root 3			
	A	B	C	D	A	B	C	D	A	B	C	D
Distance from tip (mm)												
0–100	—	—	—	—	—	—	—	—	1	—	—	—
100–200	—	—	—	—	4	—	—	—	1	—	—	—
200–300	1	—	—	—	2	—	—	—	4	—	—	—
300–400	2	—	1	—	2	2	—	—	—	—	—	—
400–500	7	—	—	—	5	—	—	—	4	—	—	—
500–600	8	1	1	—	8	7	2	—	5	1	1	
600–700	8	—	1	—	6	6	1	—	7	—	—	—
700–800	7	—	1	—	11	1	3	—	8	2	1	—
800–900	12	1	—	—	23	11	1	—	9	1	1	—
900–1000	9	2	—	—	14	5	1	—	15	1	5	—
1000–1100	18	1	6	1	6	3	7	—	4	—	2	—
1100–1200	2	—	1	6	14	2	7	—	—	—	—	2
1200–1300	3	—	4	3	5	2	5	1	3	—	2	1
1300–1400					2	—	—	5	1	—	3	—
1400–1500					2	—	2	1				

[a] Root 1 was 1270 mm long; root 2 was 1450 mm long; root 3 was 1330 mm long.
[b] A, indeterminate primordium; B, primordium with separated protoxylem; C, bud primordium; D, root primordium.

roots is more difficult and less certain at the early stages than in sectioned roots. Even so, root primoridia can be classified definitely between the 72- and 96-hour stage (Fig. 12, 13). Bud primordia can be recognized by the 11-day stage (Fig. 20). Results from the three roots studied in this way are presented in Table 2. All organs within each 100-mm interval of the root axis have been grouped.

Although a few buds may develop in the presence of the root tip at the same rate as they develop in excised segments, the data presented in Table 2 show that excision of segments from the root axis greatly promotes the number of buds which develop. Ten 15-mm segments were found to produce 30 buds after 4 weeks in culture. No 150-mm region of these roots of a comparable age or older produced nearly as many buds.

Excision of segments from the root axis also greatly affects lateral root formation. Segments 15 mm in length develop lateral roots large enough to recognize in cleared root segments in only 3–4 days (equivalent to less than 100 mm growth of the main axis). In intact roots (Table 2) no lateral roots were recorded closer to the tip than about 1,000 mm (equivalent to about 6 weeks' growth).

DISCUSSION—Although the methods used in this anatomical investigation of *Convolvulus* root and bud formation involved excision of segments, it is clear that excision is not required to set off primordium initiation. As a result of cutting no callus was formed, and although occasional cell divisions may have occurred at the cut surface, most meristematic activity was associated with the formation of roots and buds. Similarly, under the conditions of root culture used in this investigation, there was no evidence of vascular cambium or phellogen activity accompanying or preceding organ formation.

In the earliest stages there were no differences observed in cellular patterns of bud and root formation. Both organs originate from an arc of pericycle cells by tangential divisions of these cells. Later stages differ not only in their rates of development, but also in the behavior of the endodermis, in cellular patterns of organization, and in participation of central cylinder cells around the protoxylem pole. Van Tieghem and Duliot believed that the bud digests first the endodermis and, subsequently, all the cortical layers until it reaches the outside of the root. In this study there was no indication of digestion of any cells during bud development, but there was some indication of mechanical distortion. Rather than being digested, the endodermis underwent radial divisions and great cell enlargement. Because of this enlargement in bud primordia, it became difficult to distinguish the endodermis from other inner cortical layers or from the apical primordial cells prior to organization of the bud apex. A study of longitudinal sections might have clarified the fate of the endodermis, but no study of this type was made. Therefore, the possible participation of the endodermis and other cortical cells in the organization of the bud apex cannot be ruled out.

In root development the activation of the endo-

dermis to repeated divisions culminated in a series of layers of cells. Cortical collapse at the advancing margins of these endodermal derivatives suggested at least partial enzymatic penetration. It is possible that the activation of the endodermis by the developing root may aid in its penetration of the cortex, but such an interpretation is based only on the appearance of the cortex during the radial growth of bud and root primordia.

Though the earliest stages of root and bud initiation appeared identical, conclusive evidence for an undetermined primordium, or an undetermined primordium site, has not been obtained. There is ample evidence in favor of the existence of an undetermined primordium which may develop into a leaf or a bud. Wardlaw (1949) showed by surgical isolation that an incipient leaf site on the apex of *Dryopteris aristata* could instead develop into a bud. In an accompanying histological study, he concluded that small leaf and bud primordia were indistinguishable. Cutter (1956), using apices of the same fern, showed that the three youngest leaf primordia could be induced to develop as buds by surgical isolation. By excising and culturing young fern leaf primordia of *Osmunda*, Steeves (1961) was able to obtain development of the first nine leaf primordia into buds.

The case regarding the existence of primordia which may develop into either a bud or a root is much less secure. Beijerinck described the conversion of a bud developing on the roots of *Rumex acetosella* into a root. The change from bud-like vascular organization to root-like vascular organization also corresponded to the change in morphology of the organ. Such an organ thereby possessed one or two leaf primordia at its base, but terminated in a root apex. This observation was experimentally confirmed by Edmonson in 1925 (cited by Priestley and Swingle, 1929). In *Rumex acetosella* buds are formed at intervals along the length of the root. If a segment of the root is excised and placed upright in damp soil, buds near the lower end sometimes develop into roots.

Dore (1955) studied the formation of roots and buds from thickened root cuttings of horseradish (*Armoracia rusticana*). These organs arose only after the cortex had been sloughed; they were restricted to sites in association with old lateral root traces. Usually only one, or occasionally two, primordia developed at each site: either two roots, two buds, or one of each.

Evidence that an undetermined primordium existed in these cuttings was based on the early histological identity of all primordia and on the fact that submergence of the cuttings in water caused the proportion of buds to roots to decrease while the total number of organs remained constant. Such evidence may be obtained only in a system with a limited number and capacity of sites for organ formation. In a later paper, Dore and Williams (1956) found that five organs/lateral root trace were formed in 1-mm cuttings. The average value of one or two organs/site was observed in longer segments. It is attractive to suggest that similarity in young stages of primordium formation indicates an undetermined primordium, but such similarity cannot be accepted as proof.

In root and bud formation in *Convolvulus*, although only pericycle cells around protoxylem radii participate in primordial initiation, restrictions on the number of primordial sites in the longitudinal direction are little understood. Hormonal treatment of *Convolvulus* roots can greatly increase the number of organs (Bonnett and Torrey, 1965), making any statistical proof of different developmental potentialities of early primordia based on a limited number of available sites untenable

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STRUCTURE OF THE SHOOT APEX IN LACTUCA SATIVA[1]

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ABSTRACT

Histological observations of the leaf lettuce 'Black Seeded Simpson' showed the dormant embryo to possess two visible leaves and a flat to slightly depressed plumular apex. Observations conducted over a 12-day period of germination and growth showed the development of L_1 and L_2, emergence of L_3 and L_4, and periodic changes in size of the apex which were associated with leaf emergence. Thus the dormant embryo of *Lactuca* appears to be considerably more advanced in development than was previously believed. The shoot apex appeared flat to slightly depressed at all developmental stages studied.

THE LACTUCA embryo is used frequently to exemplify embryonic simplicity among dicotyledonous plants. The mature *Lactuca* embryo was shown by Esau (1953) to possess a radicle and a hypocotyl with two cotyledons subtending an epicotyl having a convex apex without differentiated leaf primordia. Jones (1927) did not describe the *Lactuca* epicotyl but sketched transverse sections of developing achenes showing the embryo with cotyledons and a plumule that consisted of two unidentified structures. Hayward (1938) provided the only information about the shoot apex in developing seedlings. The shoot apex of a 6-day-old seedling of 'New York Regular' was shown to be convex and subtended by a single, well-developed leaf. He also mentioned that after emergence the cotyledons become oriented almost vertically; then by expansion they expose a minute epicotyl, and within a few days the first leaf appears. Thus it may be inferred that the shoot apex of the dormant embryo is convex and possesses at best only a single, poorly developed leaf primordium.

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Additional information pertinent to *Lactuca:* (a) the young seedling stems do not undergo internodal elongation and form either a head or a basal rosette of leaves, and (b) the leaves are crowded on the axis in a 2/5 spiral phyllotaxy (Hayward, 1938).

Generally, apical meristems consist of meristematic initials and their immediate derivations of a shoot and occur as dome shaped structures that extend above the base of the last formed leaf or leaf pair. However, shoot apices of seed plants vary anatomically from a shape which is convex to one that is flat and even partially depressed, as for example in *Drimys* (Gifford, 1950), *Helianthus* (Esau, 1945), and *Rauwolfia* (Mia, 1960). There is evidence that apices change both in size and shape during plant growth and development; therefore, the plumular or embryonic apex may be considerably different from the seedling shoot apex. Seasonal changes also appear to play an important role in determining apical size and shape. There are many excellent reviews of plant apices and related subjects; therefore, no effort will be made to provide a complete literature review. Instead, readers are asked to consult Foster (1939, 1949), Philipson (1949, 1954), Popham (1951), Buvat (1952), Esau (1953), Gifford (1954), Sinnott (1960), or Clowes (1961) among many others. A more recent collection of pertinent data appears in the Brookhaven Symposium in Biology (1964) concerning meristems and differentiation.

The present study characterizes the plumular or embryonic shoot apex and also apical changes