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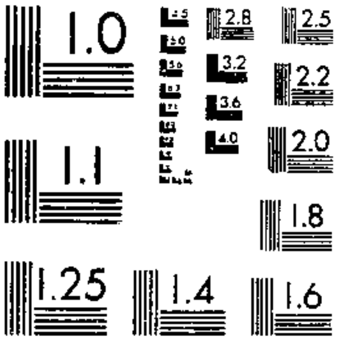

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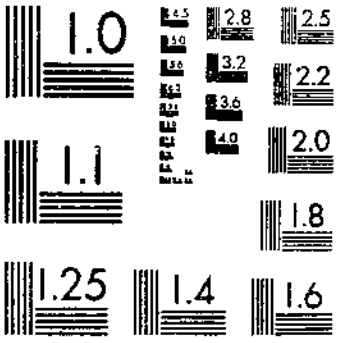

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WASHINGTON, D. C.

VEGETATIVE PROPAGATION FROM THE STANDPOINT OF PLANT ANATOMY

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CONTENTS

INTRODUCTION

The fundamental need for the standardization of horticultural products, which is largely attainable only through the greatly increased employment of vegetative propagation, has been repeatedly emphasized within the last few years (*112*, *162*).[2] The successful control of vegetative propagation involves a thorough understanding of the external factors, together with the internal conditions involved. Although the present study is chiefly a contribution to the knowledge of the internal factors, the importance of the interplay between the external environment and a complex organism is recognized, and where possible the influence of external factors upon the internal anatomical changes, as they proceed, will be discussed.

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[1] So far as this bulletin represents the work of the junior writer, it was chiefly done while he was a collaborator of the U. S. Department of Agriculture, under a National Research Council fellowship in botany. To the National Research Council and to the International Education Board he gratefully acknowledges the receipt of this grant. Many of the anatomical details here presented were obtained by Miss Lettice M. Woffenden and Miss Ursula Tetley, partly at Leeds and partly at Merton, while working under grants from the John Innes Horticultural Institution. The writers are also indebted for many other details to Miss R. M. Tupper-Carey, to various other members and students of the botany department, and to Mr. J. Manby, photographer, of the University of Leeds. Much of the matter contained in this bulletin was presented at the conference on vegetative propagation held at Washington, D. C., April 5, 1927.

[2] Reference is made by italic numbers in parentheses to "Literature cited," p. 89.

No attempt has been made to restate the problems of vegetative propagation in directly practical terms. A number of standard works on horticulture deal with the art of plant propagation, and the reader desirous of specific instruction as to how to propagate a given plant is referred to them (*6*, *189*, *26*, *62*, *72*, *100*, *101*, *8*, *38*). This bulletin is concerned rather with a critical anatomical examination of the internal factors involved in vegetative propagation, though primarily, it is true, for such light as can be thrown upon those forms which now offer more or less difficulty in propagation.

It is certain that the behavior of the plant is closely correlated with its structure and mode of growth. Thus the possibilities of vegetative propagation among monocotyledons, where growth activities are confined to the apical meristem and certain intercalary nodal regions, are more limited in scope and are different from the regenerative possibilities possessed by the dicotyledons and gymnosperms, in which cambial tissues are usually present. This is a clear indication that structural features of the plant are of primary importance in connection with its propagation by vegetative means, and it is from the point of view of plant structure that the problem is considered in the following pages.

THE PROBLEM IN TERMS OF PLANT ANATOMY

By vegetative propagation is meant the process of obtaining asexually an entire plant from a portion. This commonly, but not always, means cutting the plant into pieces and growing complete plants from the bits. With the more simply organized plants the process occurs on a wholesale scale, but in the following pages the writers are concerned with this phenomenon only as it occurs in the flowering plants and, unless otherwise indicated, only in the dicotyledons. These plants consist in the main of shoot and root, the shoot developing from the usually upward-growing shoot apex and the root from the downward-growing root apex. In the seedling plant all this complex organization has emerged from the seed, and ultimately from one single cell in the seed, the fertilized egg cell. The complex organization thus developed is still built up of cells. Though they remain minute they may be exceedingly numerous, and no limit seems to be set to their continued multiplication so long as conditions remain favorable for growth. Hence, if the proper conditions are present, cell multiplication in the fashion characteristic of the individual plant will provide indefinitely a means for plant growth and under certain circumstances therefore for vegetative propagation.

As long as a single cell of the plant remains alive there are reasons for considering that potentially, if it is capable of growth, it is able to give rise to a whole new plant. But in practical experience it has been found that, although individual cells isolated from vegetative parts of the flowering plant may live for months, no one has thus far succeeded in obtaining new plants from them. If, however, instead of single cells, sufficiently large groups of cells are isolated, they may be able to reproduce the whole plant and thus achieve vegetative propagation. The problem of cell multiplication in the higher plant can not, then, be separated from the problem of the organization of cells into the structures characteristic of the higher plant, primarily the shoot and the root. Thus the problem of vegetative propagation becomes that of the development of new shoots and new roots upon isolated pieces of shoots or roots.

Both shoot and root are very complex organizations of living cells, which themselves are but little understood entities, and it is in terms of this organization that the problem must first be visualized. In the higher animals growth occurs as a widely distributed process and takes place simultaneously throughout the greater part of the organism. In the higher plants, on the other hand, the growing regions are largely localized at certain apical growing points. It is here that the characteristic features of the shoot and the root are laid down and that the leaves and reproductive organs are first formed. Ultimately, the differences between shoot and root are to be looked for in the differences in organization between the two types of apical growing points.

In these apical regions the unit of construction is singularly uniform throughout all the flowering plants. It is a relatively small cell compared with the size of cells in other regions of the plant. This "meristematic" cell consists mainly of cytoplasm and nucleus, is of a semifluid consistency, and contains no aqueous drops of sap in any recognizable vacuoles. The nucleus, which is usually central in the protoplast, usually appears rounded like a spherical liquid drop in a liquid medium. There is no doubt that this living cell is a very plastic mass of protoplasm. Behind these meristematic cells other cells are expanding with the intake of water and are vacuolating so that in many cases, as a result, the plastic meristematic cells are stretched over the growing point. The walls of the cells making up the growing point are thin envelopes of cellulose cemented together by a plastic matrix, pectic in nature. The internal hydrostatic pressure does not distend the cells sufficiently to cause them to round off from one another; hence their walls remain in continuous contact and no intercellular spaces appear. Each cell is primarily engaged in the same activity, the construction of living protoplasm out of the simple nutrient materials supplied to it. As a result of such activity the protoplasmic mass increases in both nucleus and cytoplasm until a certain definite limit of size is reached, when cell division occurs and two daughter cells of about the size of the original cell are formed. Through the continuous repetition of this process of growth and division the size of the meristematic protoplasts is maintained within narrow limits. This is a description of the fundamental process of growth as it takes place at the growing point, expressed in terms of the cell units.

Such a continuous construction of new protoplasm requires a continuous supply of nutrient materials. Presumably, this is taken in by the individual protoplast over all its surface, which is in contact with its cellulose envelope. If the whole mass of the protoplast is engaged in the process of protoplasmic synthesis, then with increase in size the mass of protoplasm will increase out of proportion to the surface through which it is nourished; hence, these repeated cell divisions serve to maintain the balance between surface and mass.

The process so far described is characteristic of any meristem at a growing apex, whether of shoot or of root. The differences between individual plants, and still more those between shoot and root of the same plant, in the main must be attributed to differences in the organization of the aggregate of these cells at the growing apices. Certain differences between the arrangement of the cells at the shoot apex and at the root apex are immediately discernible. At the shoot apex these meristematic cells are found at the surface of the growing point, whereas those at the root apex are found beneath certain vacuolated, differentiated cells making up the rootcap. Recently this difference in organization has been correlated with differences in the chemical nature of the walls intervening between the living protoplasts (*113*, *121*) and thus with the nutrition of the protoplasts, because, as already pointed out, the nutrient materials required in the process of protoplasmic synthesis must ultimately reach the individual protoplast through the intervening walls.

Fortunately, the cell walls in these two apical regions show definite differences in microchemical reaction to the iodine cellulose reagents. Although the meristem walls in the region of the shoot apex give the normal blue reaction with iodine in potassium iodide immediately after treatment with 70 per cent sulphuric acid, and with chloriodide of zinc after brief previous treatment with cold aqueous or alcoholic potash, sections of the root apex show neither of these reactions unless they have previously been vigorously boiled in strong alkali. This difference in microchemical reaction has tentatively been correlated with the presence in the root apex of substances, both protein and fatty in nature, which are intimately associated with the cellulose and pectin of the walls in such a way as to mask the characteristic cellulose reactions (*168*). In the shoot apex the only masking substances present in the walls seem to be fatty in nature, and these are much less firmly held by cellulose, so that they are removed by brief treatment with cold alkali. In the case of the shoot apex, most of the fatty substances originally present in the walls when they were first deposited between the dividing protoplasts seem to have "creamed" to the external surface of the shoot. Here they have linked up into a continuous thin fatty layer containing a certain amount of unsaturated chemical compounds such as are always present in vegetable oils. These begin to oxidize and dry and thus form the thin cuticle (*84*, *85*, *110*) which has many of the properties of varnish.

Saying that the carbohydrate mixture of the walls which intervene between the protoplasts of the root apex is impregnated with protein and fat is probably only another way of stating that when the carbohydrates were deposited at the interface between two dividing protoplasts the living protoplasm was incompletely withdrawn from the intervening region, so that the fats and proteins characteristic of the protoplasm are still found in the wall (*115*). These walls separating the meristematic masses are probably of great importance as channels of nutrition for the actively growing protoplasts, and their behavior in this respect is likely to be materially affected by the retention of considerable quantities of the main protoplasmic constituents. If it is assumed that the cellulose wall of the normal plant cell is completely permeable to both water and the solutes contained in it, then the characteristic semipermeability of the living cell is to be ascribed to the protoplasmic envelope within. If, however, sufficient protoplasm is retained within the cellulose wall, this will become much less permeable; when this occurs the behavior of the wall may be expected to be more like that of gelatin than that of filter paper. In aqueous solutions this may well mean that while still permitting diffusion or molecular movement of solutes through the wall, it resists flow or movement of liquid en masse through the wall. At this point the differences between the meristem walls of the shoot and root apices must be left, until with a wider knowledge of the structure of the shoot and of the root the problem of the nutrition of these special meristems can be more fully visualized.

It is difficult to form a comparative estimate of the rate of growth of meristematic cells; but as the processes of growth always involve an increase in size between certain narrow limits, following which cell division occurs, and as the process of division itself requires approximately the same time to take place in each case at a given temperature, in comparing one layer of cells with another a rough estimate of their comparative rates of growth can be made by determining the percentage of cells in similar sections of each layer which are found to be in certain stages of cell division. Comparisons by this method have been made by Schüepp (*139*, *140*) for the shoot and by Lundegårdh (*92*) for the root, with results that will now be considered in some detail.

THE SHOOT APEX

Typical meristematic cells are found at the surface of the shoot apex and for a certain number of layers within. (Pl. 1, A.) Schüepp found that throughout all these layers the proportion of cells in division stages was about the same and that the rate of growth of these cells thus does not appear to be affected by their positions (*139*, *140*). On the other hand, the arrangement of these cells shows that the direction of cell division is not the same throughout all layers. The surface layer, or dermatogen, and the next layer or two, Hanstein's periblem (*53*), invariably divide by the formation of new cross walls at right angles to the surface of the plant. The result is that all new cells thus formed are added to the layer in which they arise and do not contribute to the core of the shoot, over which these surface layers may be regarded as stretched.

In the case of the inner meristematic cells, which divide at the same rate as the outer ones, the divisions are not always in the same plane, so that in this plerome region new cells are being added to the meristematic layer and to the core within. But if new cells are thus being added at the same rate in both regions, in the outer layers only in the superficial plane and in the inner layers in depth as well as in surface, then the only possible result will be a more rapid increase of the surface than of the mass within. Such an increase, in terms of cell division, is represented by the appearance, in the layer beneath the dermatogen, of walls that are no longer at right angles to the surfaces, cells thus being thrust outward from the surface; hence, quite close to the apex are formed folds, the new leaf initials (*140*). Thus it is seen that the superficial leaf initials are the natural result of the method of organization of the meristematic tissue at the shoot apex.

Beneath these characteristic meristematic cells clothing the surface of the shoot apex and continually throwing up folds upon it as it grows are found cells that are changing in various ways. They are vacuolating and thus are growing larger as they become distended with sap. They are forming relatively less protoplasm but more carbohydrate; thus more cellulose is being deposited upon the walls, although at first the rapid extension of the wall prevents any very noticeable increase in thickening. For a time these cells are also forming starch, but this is so rapidly hydrolyzed by the water entering the cells during vacuolation that except when the shoot is grown in the dark (in which case vacuolation is a slower process) this temporary appearance of starch in the meristematic tissue is often missed (*115*).

As the elastic walls of the cells extend under the hydrostatic pressure exerted by the accumulating sap within, they tend to assume a spherical form. The resultant strain upon the amorphous pectin deposit which cements the cells together is too great and intercellular spaces arise. In the early stages at the growing apices these interstices are filled with sap, which only later is displaced by air (*117*). These cells still, although more slowly, continue to divide, and the rate of protoplasmic synthesis likewise slows down; the cells enlarge, but a great part of each cell is now filled with dilute sap, and the protoplasm, which is now also less dense and more watery in nature than previously, is restricted to a narrow envelope between the vacuole and the wall. The nucleus is still dense, and probably in this type of cell, as Gerassimow's experiments have indicated is the case in similar vacuolated Spirogyra cells, growth, so far as it involves the formation of new protoplasm, is now restricted to the nucleus (*39, 40*).

In the shoot apex, vacuolation first appears behind the dome of meristematic cells in the center, which is the region of the future pith. It next appears in the cortical region. Between these two regions of vacuolating cells lies a cylinder of cells which are still meristematic and which, therefore, are compressed between their expanding neighbors on either side. This region is the procambial ring. As the cells are squeezed between the cells within and without, being plastic they extend vertically or longitudinally. Such extension converts them into lathlike cells in which increase in mass is not accompanied by a relative decrease in surface, so that as long as they remain meristematic they can continue to synthesize protoplasm. Thus arise the elongated cells of the procambial strand, from which later the vascular elements differentiate. The protoxylem elements differentiate on the inner side of this ring and the protophloem elements on the outside. In the shoot the new vascular elements thus formed are always isolated from the main vascular strand beneath. They seem rather to be associated with the new leaf initials, appearing first in the procambial ring just where a strand diverges from it and enters a leaf initial. As the isolated xylem elements differentiate, their protoplasts become more permeable, and all cell contents ultimately are lost. Previously, however, owing to the osmotic pressure of the relatively concentrated sap within, such a cell may expand, because it constitutes a system which withdraws water by osmosis from the less concentrated sap in the general vascular system below it in the shoot.

This process of differentiation in the procambial strands appears to be of great importance in the nutrition of the superficial meristem of the shoot. It seems quite probable that water is thus being continually withdrawn from the main supply below and then driven forward into the tissues surrounding the differentiating vascular elements, and with the water would go solutes from these differentiating vascular elements. Thus the protophloem differentiation, of which but few details are yet known, involves a sudden swelling of the developing element, a disappearance of the dense, protoplasmic contents, and a thickening of the wall, along with the assumption of a striking, pearly, highly refractive appearance. These phenomena are followed by the total collapse of the element. The solutes which were thus in evidence must have gone somewhere when they disappeared as the element collapsed; perhaps they added to the solutes present in the sap released by the differentiating xylem, all being then pushed forward into the superficial meristem by the rising tide of sap. And apparently only such a flow of liquid could account for the adequate maintenance of nutrient supplies to the superficial layer of the meristem, so that this layer is able to grow as fast as any layer within. Diffusion does not seem to account for the continuous delivery of solutes to these cells, which are usually more than 10 cell layers, and sometimes 100 or more cell layers, from the end of the nearest differentiating xylem element.

THE ROOT APEX

An attempt by Lundegårdh to determine the proportion of cells in certain division stages in different layers of the root apex (*92*) led to results very different from those obtained by Schüepp for the shoot (*139*, *140*). In the root, the cells to the inside of the meristem, those in the plerome region, were found to be growing by far the most rapidly; the cells farther out showed a rapid falling off in the rate of growth, and the differentiated cells at the outside of the root apex showed no growth at all. (Pl. 1, B.) Other differences in organization are known to be associated with this; the inner cells grow the most rapidly and divide principally by walls laid down at right angles to the main axis of the root, so that most of the new cells contribute to the growth in length of the root. Therefore, there is no tendency for the formation of superficial folds at the root apex, and no exogenous lateral members are formed. Thus it is clear that the main characteristics of root growth are also directly due to the general organization of the meristematic tissues at the apex.

Here, again, vacuolation occurs in the cells behind the meristematic apex, usually first in the cortical cells, in which region also intercellular spaces form first. As in the shoot, these spaces are at first filled with sap, but farther behind the root apex they become displaced with air. The cells within are still meristematic and are compressed by the expanding cortex. They form the stele and are bounded on the outside by the cells which later form the pericycle and the endodermis. As already pointed out, the walls of these cells, unlike those of the corresponding cells in the shoot apex, are as yet heavily impregnated with fatty substances and proteins. Though these substances leave the walls more slowly in the root than in the shoot, they do gradually migrate, and because of their effect on surface tension they tend to accumulate at any surface where the liquid matrix is in contact with air. But as they thus move outward, air is diffusing inward from the intercellular spaces in the cortex, and thus the fatty substances tend to oxidize and condense in a varnishlike strip on the radial and transverse walls of the endodermis (the outermost envelope of cells clothing the stele) inside which air spaces have not yet appeared. Thus the characteristic Casparian strip, an invariable constituent of the endodermal walls in roots of all the flowering plants, is formed.

This Casparian strip appears to be of fundamental importance in the further development of the root. All the vascular differentiation in the root takes place within the endodermis, and the solutes which are released by the differentiating vascular elements are retained within the stele, because outward movement along the walls is precluded by the continuous fatty deposit in the Casparian strip on the radial and transverse walls of every cell of the endodermal cylinder (*119*). Water and solutes may pass outward or inward, moving from cortex to stele and vice versa; but such movement must take place across the protoplasts of the endodermis and is, therefore, under protoplasmic control. Furthermore, this varnishlike layer of the Casparian strip rapidly sets, forming a relatively rigid structure; thus as the cells of the stele within vacuolate, their tendency to expand and round off against one another is materially restricted by the resistance offered by this fine network. As Schwendener (*141*) long ago pointed out, the expansion of the tissues within against this network produces a relatively rigid structure, much as the gas envelope of the balloon may swell against its limiting cord network until it forms a relatively rigid entity. One result is that intercellular spaces are but little developed within the root stele; but still more important, even when formed, in the young developing root they have never been found to contain air, because there are no air spaces in the endodermal cylinder and no air bubble can work past the Casparian strip which firmly cements the cells of the endodermal cylinder together. Within this root stele, vascular differentiation proceeds, but in a very different manner from that taking place behind the shoot apex. Here, in the root, such differentiation takes place much nearer to the deeply sunken meristematic tissue, the differentiating elements being in continuity with the vascular elements already formed. This means that there is little tendency toward the fluctuation of sap pressure such as would be brought about by the appearance and disappearance of new isolated osmotic systems. But the walls intervening between vascular elements and meristem cells are different in nature from the corresponding cells in the shoot. In the root such walls are impregnated with protein and fat which probably offer considerable resistance to flow but not to diffusion. Since there are no fluctuating sap pressures in the root, it must be assumed that the solutes are supplied to the layers beyond the vascular elements largely, if not entirely, by diffusion. Such solutes certainly become available when the protophloem elements swell up and collapse, and the protoxylem elements lose all their organic contents, only a part of which can be retained upon their walls.

But can diffusion supply the necessary solutes with sufficient rapidity to the meristem cells? There can be no doubt of this, provided the distances are sufficiently short. Hill has recently pointed out (*59*) that while the chemist regards diffusion in liquids as a comparatively slow affair, this is because he is dealing with comparatively large space units. Before a steady state is reached, substances are transmitted by diffusion with a speed inversely proportional to the square of the distance, and though this may appear to be a slow method of transmitting substances across a distance measured in centimeters, when the distance is of the order of thousandths of a centimeter, the process will proceed one million times as fast. Between the ends of the differentiating vascular system in the root and the innermost layer of meristem of the root apex, the distances are always measured in microns, as never more than a few small cells intervene. The total distance rarely exceeds a few hundred microns, and for such distances diffusion constitutes an extremely efficient agency for the delivery of nutrient solutes. Moreover, this furnishes a good explanation of why the rate of delivery of the solutes in the root, and with it the rate of growth, falls off with great rapidity in the outer layers of the meristem. Hence it is seen that the difference between the method of nutrition of the shoot meristem and that of the root, determined by differences in the microchemical nature of the walls separating the protoplasmic masses, seems to play the predominant rôle in determining the distribution of growth activity in these two meristems, and thus to determine the general organization of the shoot and the root, new exogenous lateral members appearing only at the shoot apex. If the nutrient supplies reaching the root are adequate, meristematic growth may continue indefinitely, but the new meristem cells must always be found near the vascular supply. New groups of meristem cells do arise in the flanks of the vascular elements within the endodermis, the walls of the protoplasts outside the endodermis being cut off from such supplies (*120*). These new meristems, which give rise to new branch-root apices, form just within the endodermis, in the pericycle, which is a layer made up of living cells relatively slow to vacuolate.

In the light of the foregoing brief analysis of the general structural organization of shoot and root apices, it is possible to take up the consideration of the problems involved in vegetative propagation, which include the formation, in isolated portions of plant tissue, of new shoot apices, new root apices, or both.

Such new shoots and roots, formed as a result of artificial conditions involved in propagation, usually may be regarded as examples of adventive structures. The origin of shoot apices obviously presents different anatomical problems from the origin of root growing points. The origin of adventive shoot and root apices will first be examined in the light of some detailed studies of their formation in isolated pieces of the root of seakale (*Crambe maritima*).[3]

VEGETATIVE PROPAGATION OF CRAMBE MARITIMA

FORMATION OF ADVENTIVE SHOOTS

The organization of the shoot apex and that of the root apex have now been considered. Both types of apices consist in the main of

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[3] The authority for the species is given in this bulletin only for forms not listed in Standardized Plant Names (*3*).

meristematic tissues, apparently similar as regards the structure of the individual cells of which they are composed, but differing between root and shoot in arrangement and organization, both in the meristematic tissues themselves and in the differentiating tissues which arise from the meristems and which probably are very important in their nutrition. The problem of vegetative propagation as generally considered involves the production of such an organized meristematic tissue in some region where normally it does not occur or where if present at all it is dormant at the time a portion of the original plant is isolated.

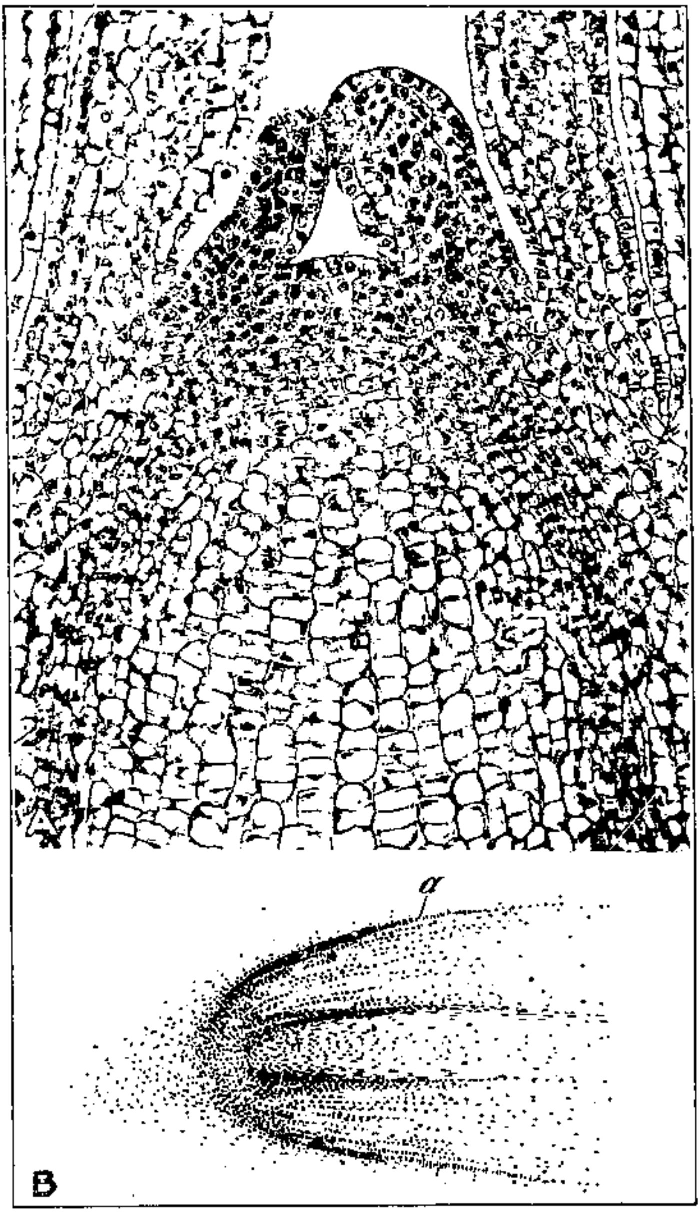
The place of origin of such an adventive growing point differs in different cases of vegetative propagation, but the fundamental problem is always the emergence of an organized meristematic apex from tissues of a different character. Therefore, before attempting a generalized statement of the various ways in which such apical organizations may be induced to appear, it will be well to consider one case in detail and to discuss in turn the problems presented in the different stages of development in such a complex process. Both the processes of shoot and root organization can very well be studied in the regeneration seen at the cut surfaces of pieces of the fleshy root of *Crambe maritima* (Cruciferae).

BUD PRODUCTION

Root cuttings are commonly used in propagating seakale, although it may easily be grown from seed. The root cuttings are sown in the field in the spring, and the roots of the resulting plants are dug in the fall; the lateral roots are trimmed off and stored for sowing the following spring, and the large main roots, which are 2 to 5 centimeters in diameter and about 10 to 20 centimeters in length, are planted in a darkened forcing bed in the greenhouse. Within a few weeks these roots will have expended themselves in throwing up etiolated shoots, each perhaps a meter high and as thick as the root itself. Neither these shoots, which constitute the commercial crop, nor the old roots are commonly used for propagation.

NORMAL ANATOMY OF ROOT

A cross section through a seakale root of the size used for root cuttings (pl. 2, A–D) shows a small core of primary wood, surrounded by a wide ring of very parenchymatous secondary tissue. In the xylem a few vertically extended lignified elements are scattered in radially arranged groups; but in the sectors opposite the protoxylem groups most of the tissue consists of somewhat prosenchymatous parenchyma, for the most part with the long axis of the cells parallel to the axis of the root. Under these circumstances the bulk of this tissue, although filled with starch, seems better described as xylem parenchyma than as ray parenchyma; it is regularly arranged in radial serial order (pl. 3, C), the small amount of expansion of the occasional lignified vessels not leading to any serious distortion, though the parenchyma cells in the immediate neighborhood of these vessels remain small, apparently compressed by their lignified neighbors, and free from starch. These cells, judging from their subsequent behavior in isolated root pieces, seem to retain many meristematic qualities.

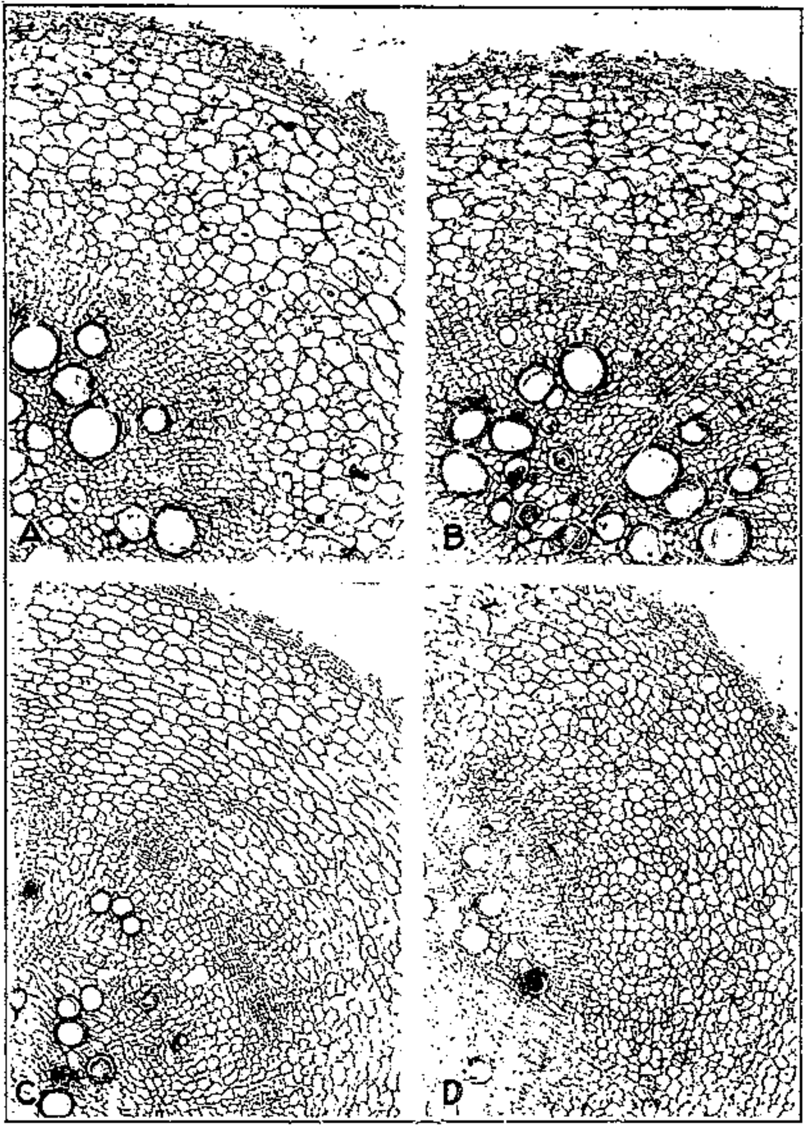


THE APICAL REGIONS OF THE PLANT (LONGITUDINAL SECTIONS)

A.—Shoot apex of *Syringa vulgaris*. The youngest pair of leaf initials and the cells crowning the apex of the shoot are still completely meristematic. Beneath the apex the vacuolating but still dividing cells of the pith are visible, where longitudinal extension is associated with a series of transverse divisions. To either side the longitudinally extended, still meristematic cells of the procumbial strands are evident. × 250.

B.—Root apex of Chlorophytum. The outer cells of the rootcap are vacuolated and have ceased to divide. Behind the dense meristematic apical region vacuolation is visible in the central and the cortical regions; between these two regions lie the meristematic cells of the pericycle and the endodermis. The protoderm (*a*) can be traced well into the apical region. × 40.

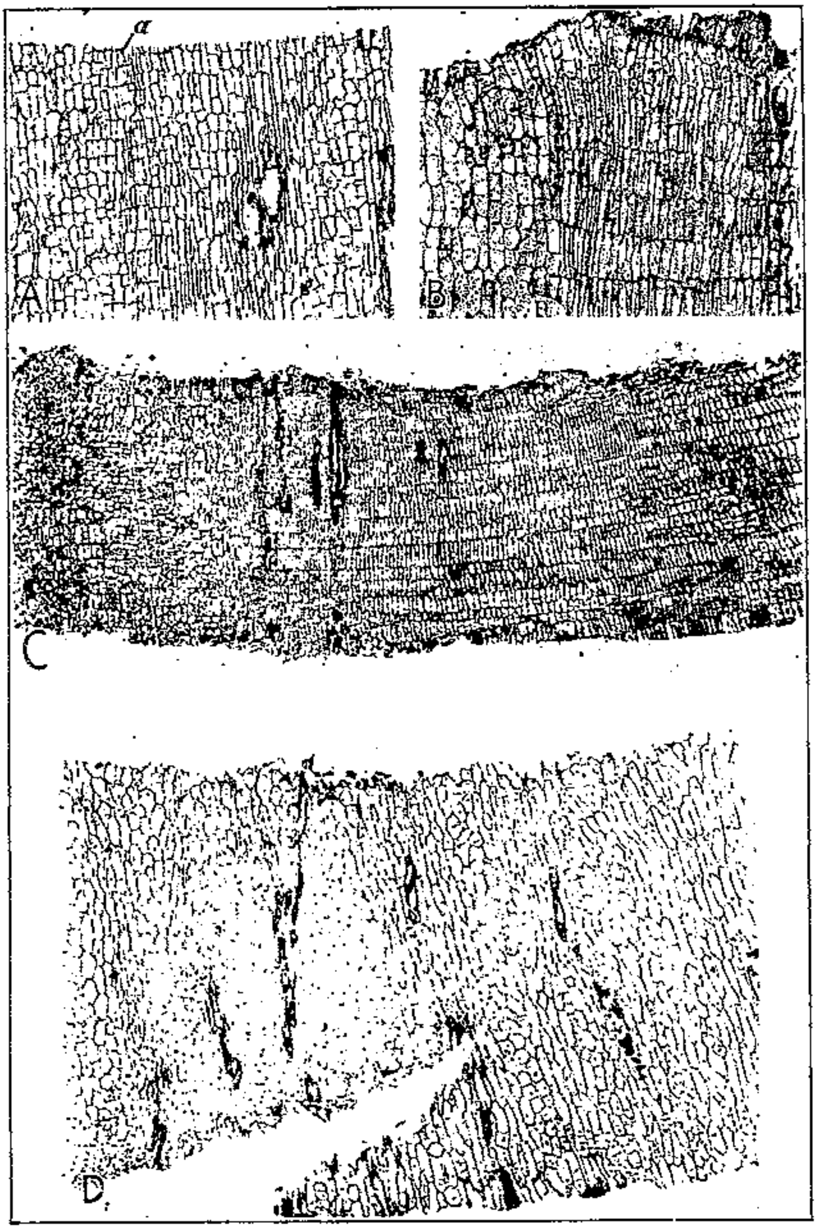
PLATE 2



DIFFERENCES IN CAMBIAL ACTIVITY AT OPPOSITE ENDS OF CRAMBE ROOT CUTTINGS (TRANSVERSE SECTIONS)

A and B.—From 4-day material. × 65. A, Proximal end. Cambium activity is very slight and is restricted to the fascicular cambium. B, Distal end. Meristematic activity is indicated across the rays and around some of the xylem vessels.

C and D.—From 7-day material. C, Proximal end. Meristematic activity is more extensive than in A, but is still chiefly restricted to crescent-shaped regions just within the youngest phloem. Some activity is also indicated around the xylem vessels. × 40. D, Distal end. The activity of the vascular cambium, which has increased in intensity and extent over that shown in C, extends right across the rays. × 45.



MERSITEMATIC ACTIVITY IN ISOLATED ROOT PIECES OF CRAMBE (LONGITUDINAL SECTIONS)

A.—Appearance of tissues beneath freshly cut surface. The phloem lies to the left of the cambium (*a*). × 35.

B.—A similar region three days after cutting. Cells cut through are collapsed and dying, and the walls of those immediately beneath show suberin deposits; starch has disappeared from the living cells below these, but not from the rest of the tissue. This disappearance has proceeded most actively in the neighborhood of the cambium. × 40.

C.—A very thin root slice after three days, showing suberin deposit and beginning of phellogen activity on both surfaces. The section is almost exactly radial and shows the very regular arrangement of the secondary tissues. As in all such short root pieces, there is very little difference in the degree of suberization and extent of phellogen activity between the upper (proximal) and the lower (distal) surfaces. × 20.

D.—After 14 days, suberization and phellogen activity are more marked at the distal surface. Compare extent of suberization and phellogen activity at the two sides of the tear accidentally left on the distal surface when the slice was cut. × 35.

FIRST STAGES IN ADVENTIVE-SHOOT DEVELOPMENT ON CRAMBE ROOTS

A.—Indications of the suberized layers (very heavily stained) at *a*; beneath this are a few wide periderm cells, with typical phellogen showing beneath them on the left. In the center the meristem cells are becoming more nearly isodiametric. × 210.
B.—A slightly later stage. It is now clear that cells beneath the isodiametric cells in the bud initial are themselves becoming meristematic. Typical flattened phellogen cells, with compressed nuclei, are plainly visible on the right. × 235.
C.—A young bud initial appearing in the wound phellogen formed on the side of a piece of xylem cut out from the root with a cork borer. × 210.

On the phloem side of the cambium a similar type of prosenchymatous cell, also arranged in regular serial order near the cambium, is recognizable. As these cells are pressed farther to the outside they become more rounded off from one another and the intervening spaces become very marked and apparently filled with air. At this stage the cells tend to become more nearly isodiametric, because, thus forced to the outside, they have plenty of room to expand, and the regular serial arrangement of the tissues is lost. While the phloem elements near the cambium show sieve plates with callose deposited in the transverse walls, along with lattices on the radial walls, and some evidence of contents characteristic of sieve tubes, much of the secondary phloem is simply storage tissue, most of the cells being filled with starch. Occasional myrosin storage cells are present here as well as in the xylem parenchyma; occasional lignified fibers, either in groups or scattered singly, are also found in the phloem parenchyma. The original elements of the primary phloem are not visible in the periphery. Usually the bounding layer of periderm is somewhat irregular in outline, as ruptures occurring during expansion have been followed by reformation of periderm at deeper levels. The periderm originally arose in the pericycle of the root just within the endodermis.

The young root shows a well-marked endodermis, immediately outside of which are found the curious thickenings of the radial walls of the inner cortical cells. These radial markings which are characteristic of the Cruciferae have been fully described by Van Tieghem (*170*).

Seakale does not seem to offer any exception to the general rule stated by Solereder (*149*), namely, that intraxylary phloem does not occur among any of the Cruciferae.

Active regeneration of both shoots and roots can be seen in pieces of these fleshy roots 2 to 3 centimeters long if they are simply laid horizontally on filter paper in a Petri dish and kept under warm, moist conditions. The observations reported in the following paragraphs are based on such pieces of roots which had been in a germinator at 23° C. for periods of three days and longer.

HEALING OF THE CUT SURFACE

Suberization

A necessary preliminary to all successful vegetative propagation is that the isolated piece of tissue should remain healthy. Necessarily, isolation has involved the exposure of the cut surface, and the first thing that must be considered is the conditions under which the wound surface remains free from serious invasion by the microorganisms which have been offered an ideal medium for growth. Frequently rapid decay does take place at the cut surface, and the cutting disintegrates before the new growing points can be regenerated.

The cut surface in a parenchymatous tissue—other considerations enter when the cut is made across a woody tissue as in the case of hardwood cuttings (*155*)—is always covered immediately by sap and débris from the crushed and broken cells that lay in the path of the knife. This gradually dries, forming a somewhat sticky film, but during the process it constitutes an ideal medium for many microorganisms, and it may be taken for granted that colonies of such organisms are always to be found here. Even the saprophytic organisms, though they may be unable to penetrate the living protoplasts themselves, will be able to spread along the cellulose walls between, in many cases digesting the pectic or cellulose constituents of these walls and disorganizing the tissues generally.

Such disorganization will be followed by death and decay; hence, unless changes in the walls beneath the cut surface, such as prevent the inward migration of microorganisms, take place more quickly than the latter grow and multiply, decay is bound to follow. Such changes in parenchymatous tissues seem usually to be brought about by the deposition, upon the carbohydrates in the wall, of a film made up of fatty substances which rapidly oxidize and dry, in the same way that similar films of unsaturated vegetable fatty substances, when exposed to the air, set to form compounds of a varnishlike consistency. (Pl. 3, A and B.) Such films resist digestion and direct physical penetration by all microorganisms if formed in time.

These deposits, which are obviously of great practical importance, are usually recognized by their reaction to fat stains such as Sudan III. It is necessary in considering the conditions of their formation to examine both the sources of the fatty substances from which they are formed and the conditions under which they set to a suberin deposit. Since the writers' observations upon seakale, so far as suberization is concerned, were by no means so extensive as the investigations that have been carried out with other plants, especially potato tubers, these remarks about suberization are somewhat general and not primarily concerned with seakale. However, so far as experimentation with seakale has been made, all that is said applies equally to it.

The fatty substances themselves undoubtedly arise from the sap which injects the intercellular spaces and the cell walls in the region of the cut. Such a cut can not be made without the cells near the wound (although not actually in the path of the knife) being so strained that an increase of permeability, usually temporary in nature, is brought about in the still-living protoplasts. If this strain is too severe, the increase in permeability is irreversible, and the cell dies. Janse (*67*, *68*) has shown how widespread is this increase in permeability of the living cells as the result of shock. The intercellular spaces in the neighborhood of the cut gradually fill with liquid which is not pure water, but which instead is water that contains solutes, including fatty substances, apparently released from the protoplasts. The result is that these fatty substances, which lower surface tension, accumulate at the water-air surface along the region of the cut; then, provided there is sufficient access of air, they immediately begin to change in chemical nature and are deposited as suberin in the cell walls and on the surfaces bounding the intercellular spaces (*122*).

If sieve tubes are present in the neighborhood of the cut, the contents of these apparently add greatly to the supply of these fatty substances; furthermore, probably because the reaction of the contents of these sieve tubes is usually relatively alkaline, these fatty substances are supplied under conditions favoring rapid condensation to a suberinlike film. In the potato tuber this suberin deposit may, under some conditions, be seen in sections made 12 hours after the cut surface has been exposed, and it is usually visible after 36 hours; it is probably effective before it is detectable by microchemical methods. If formed at this speed it is probably effective in preventing the entry of microorganisms. In the seakale root it forms very rapidly and is a firm layer within three days, but in some fleshy roots it is much slower in formation. It does not form readily on exposed injuries in the parsnip root, a fact undoubtedly closely connected with the observation that such roots seem to be very susceptible to diseases brought about through chance injuries to the root in the soil. Cut slices of red and sugar beets, although visible suberin deposits are slow in forming, usually remain healthy. What prevents the spread of decay in such tissues is an interesting question.

In most shoots or roots, when upper and lower surfaces are exposed by the process of cutting, it is a striking fact that suberization is usually more pronounced and occurs nearer to the cut surface at the lower cut than at the upper cut. This is only one indication, of the many that will be considered, of the polarity at play in the plant.[4]

In this case the formation of the greater amount of suberin at the lower surface suggests a larger supply of fatty substances present, while the fact that the suberin is nearer the exposed surface suggests that there is a larger quantity of sap which does not withdraw into the wall so rapidly as at the upper end; hence, the air-water surface is nearer the exposed surface of the walls. At the upper end, particularly in a piece of shoot, the sap seems to withdraw into the tissues so rapidly that the deposits of suberin occur in a most irregular fashion. Especially does this occur in the center of the pith and in the outer cortex, that is, in the regions which are farther from the vascular system and thus where the sources of sap are less and the air spaces frequently large. Here the deposits in many cases are so irregular, especially in an internodal region, that these tissues are not sealed from the air by a continuous film; in such cases water loss from the carbohydrate walls continues so freely that the tissues do not decay but dry out and wither.

If the sap supply at the cut surface is adequate to give a continuous film of liquid, then as the fatty substances "cream" to the air-water surface the rapidity and effectiveness of suberin formation is dependent upon various factors. In particular, the free access of oxygen is essential. Thus, if the cut surface is kept swimming in water, the fatty substances leach away and oxygen does not reach them while on the walls, and no suberin deposit is formed. This condition is fatal to successful suberization, and no single factor is so likely as an excess of water to produce decay at the cut surface. This is one reason that justifies the procedure, adopted with many difficult cuttings, of exposing the cut surfaces to air for some time before placing them in the soil.

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[4] When questions of polarity are under discussion, there is sometimes the possibility of confusion when the terms "lower" and "upper" alone are employed. The lower surface of an isolated piece of root is, therefore, spoken of as the distal end, and the upper as the proximal. These words are, of course, used in the reverse sense for the shoot, where the upper is the distal end and the lower the proximal.

According to Herklots (*58*), suberization is also aided by a relatively alkaline reaction in the sap, the oxidation of the fatty substances proceeding more rapidly to the alkaline side of pH 6.5. Also, in the case of cut potato tubers, on which most work has been done in this subject, direct exposure to sunlight has often prevented the formation of a continuous suberin deposit at the cut surface, apparently because of a too rapid drying of the sap deposit in the walls and intercellular spaces below the cut (*123*).

CORK FORMATION

Beneath the continuous deposit of suberin the walls and air spaces which are still saturated with sap are to a considerable extent protected from evaporation. Under these conditions there was found in seakale a region below the suberin film in which, with free-hand sections of the tissue transferred directly from the knife to strong glycerin, the whole tissue appeared translucent, because free from air. In this region a whole series of reactions follow which seem to be initiated as the result of the displacement by sap of the air normally present around these living cells. One of the first changes noticed in seakale cuttings is that starch begins to disappear (apparently being hydrolyzed to sugar); this is accompanied by an increase in the respiratory activity of the cells, and in some cases there is evidence of increased oxidase activity in this region (*17*).

Some of the cells thus greatly depleted in starch become very active in the synthesis of protoplasm and at the same time lose their central vacuoles. Starch is not lost from the outermost dead cells, which are more or less cut off from the active cells by the deposit of suberin on the intervening walls. Within the suberin deposit there gradually emerges a characteristic layer of cells which contain but very little starch and are dense in protoplasm with the nuclei prominent; and in these cells divisions parallel to the surface now occur. Accompanied by and even preceding the appearance of this characteristic layer, occurs the enlargement of many of the cells at right angles to the surface. This is evidently closely correlated with an intake of water, following upon the release of pressure incident upon the act of cutting, but other factors are obviously involved.

The cells thus cut off by this layer, meristematic in nature and without vacuoles, show more tendency for division than for enlargement, so that soon they are flattened between the more actively enlarging cells beneath them and the relatively rigid suberized cells above. This layer continues to divide by walls parallel to the surface; the cells thus formed to the outside vacuolate and develop internal suberin lamellae such as are characteristic of cork or periderm cells. These flattened meristematic cells, which within a few days form a continuous layer across the cut surface below the suberin deposit, function as a cork phellogen, and the permanent protection of the cut surface against the entrance of microorganisms or loss of water is mainly effected by the sheet of periderm produced from this phellogen.

This layer of periderm has certain qualities not found in the original suberin film, which, being merely a thin, rigid film deposited on walls that originally were relatively elastic, is readily broken by the strains set up by changes in the water content of the underlying tissues. If the cells beneath the cut are losing water at a fairly rapid rate, the consequent contraction beneath this semirigid surface leads to deep cracks in the suberin layer; these occur under conditions in which the underlying tissues show but slight tendency toward the accumulation of sap in the intercellular spaces. The result is that air and microorganisms obtain access to the deeper lying tissues under conditions that militate against fresh suberization at the newly exposed surface within the crack, and thus is favored the withering of the tissues, or, with a recurrence of moisture, the resumption of decay. On the other hand, if a sheet of periderm is formed beneath the original deposit of suberin, in the periderm tissue the suberin lamellae of the cells are deposited within them and these are cemented together by a general fatty impregnation of the intervening cellulose walls and middle lamellae (*187*). The result is a layer with very much greater resilience than that of the original suberin film, and one which does not so readily crack under the strains resulting from loss or gain of water by the underlying tissues, and which, because of its depth and composition, is a much more effective protection against the entrance of decay or the loss of moisture.

The same polarity is indicated in the formation of the phellogen at the upper and the lower cut surfaces as is displayed in the production of the original suberin deposit. The phellogen appears first at the lower surface, usually in the neighborhood of the original vascular cambium; from here its formation rapidly spreads across the parenchyma on the xylem side of the cambium, but its formation across the phloem parenchyma is very slow in seakale, and toward the periphery both the suberin layer and the cork phellogen are usually sunk farther into the tissue away from the exposed surface. This is probably correlated with the presence of larger air spaces in this region and with the natural tendency for the level of the liquid retained in these spaces to recede farther from the surface.

At the upper surface of the cutting the cork phellogen appears at a later time and spreads even more slowly toward the periphery. This difference in the rate of formation of the suberin deposit and the cork phellogen at the two ends was more marked in the longer cuttings, but it was evident even in the thin transverse disks, as is indicated by Plate 3, C and D.

Considerable discussion has taken place during recent years regarding the causes controlling phellogen production at the cut surfaces of parenchymatous tissues. Haberlandt's school (*48*, *49*), regarding phellogen activity as one of the manifestations of growth that is promoted by hormones, have assumed two sources for these: (1) The dead or dying cells at the cut surface; and (2) the phloem.

Such hormones remain as yet purely hypothetical. There is no doubt that from the dead and dying cells, which become completely permeable, there are released substances which contribute to the supply of fatty substances involved in the original suberin deposit. However, as was pointed out above, such a deposit within a very few hours after the trauma becomes a barrier between these dead and dying cells and the cells which some time later become active as a phellogen. Hence any transfer, across this suberin deposit, of substances that function as hormones and stimulate phellogen activity seems extremely improbable. Furthermore, such substances certainly are not present in normal cases of phellogen activity in a plant that is intact.

The other source of hormones is assumed because of Haberlandt's striking experiments with small disks of parenchyma cut from potato tubers and left under moist conditions (*48*). He found that such disks produced cork phellogen at their exposed surfaces only when they contained a sieve tube. In the potato tuber, as Artschwager demonstrated (*4*), the sieve tubes form a very irregular network throughout the parenchyma of both pith and cortex, so that such isolated disks of parenchyma often contain sieve tubes.

Such experimental evidence, pointing toward the direct influence of sieve tubes upon phellogen production, seems very strong and is in accord with the general position of the phellogen in the normal plant, where it is usually found facing the phloem, although, in the case of epidermal cork, at some little distance from it. The movement of solutes in the phloem is a phenomenon as yet very little understood, but there is considerable evidence to indicate that phloem differentiation, and possibly therefore translocation, usually takes place in a downward direction, both in shoots and in roots. Further evidence of downward differentiation in secondary phloem has been obtained at Leeds in recent years; this seems to suggest that the tendency to downward movement of substances in the phloem is maintained in isolated pieces of tissue, and that polarity, as regards both suberization and meristem formation, is closely associated with this polar organization of the phloem. When, however, very short pieces are cut out, exudation of substances takes place freely from both cut ends, and these are now so close together that the effect of polarity, as shown in the downward transference of the remaining contents of the phloem, is greatly lessened. In experiments with isolated pieces of short internodes of Cucurbita and Coleus considerable evidence has been obtained that the contents of the phloem gradually shift mainly toward the basal end of the isolated segment.

Apart from this suggestion it is difficult to give any explanation of the marked polarity of these isolated pieces of shoot and root save that they are undoubtedly correlated with the polar manner in which these tissues are laid down in the root at the growing apex; the shoot, on the contrary, is organized segment by segment, with vascular differentiation proceeding downward in each internodal segment (p. 6).

It would be unwise at the present state of the knowledge to overemphasize the rôle of the phloem in phellogen activity, particularly in view of some of these experiments with seakale. Disks of secondary xylem parenchyma, which were entirely free from any of the outer ring of secondary phloem, were cut out of the center of the root with a cork borer. The disks of tissue thus obtained produced phellogen freely at all surfaces. Unfortunately, it is impossible to describe this tissue as being absolutely free from phloem, because such disks always contain occasional slender strands which run radially out through the tissue and which are the vascular connections of the original lateral roots that long ago ceased to be active. No evidences of phloem were noticed in these root traces, nor is there any reason to assume that any was present.

In any case, if phloem is necessary, its rôle as a source of "leptohormones" still remains entirely hypothetical; and it is doubtful whether the adoption of a terminology taken from the physiology of animals, the higher ones of which have elaborately developed special organs of internal secretion, without the support of critical experimental evidence derived from plants themselves, does anything except delay the understanding of the problem by employing a phraseology which, with the present limited knowledge of the product and processes concerned, can not have a precise connotation.

The Leeds studies of developmental anatomy have suggested an alternative explanation of the contribution made by the phloem to meristematic activity. The cytological characteristic of the appearance of the phellogen is the emergence of densely protoplasmic cells in a region where previously the cells either had been storing carbohydrates or were swollen with the hydrostatic pressures of large vacuoles, the protoplasm making up but a thin layer around the outside of the cell. The new protoplasts are characterized by a disappearance of the central vacuole along with an increase in protoplasm. Pearsall and Priestley (*104*) have suggested that the behavior of the cell in relation to these processes is closely correlated with the pH of the external sap bathing the protoplast. The maintenance of intense activity in protein synthesis suggests a ready transference of the water thus released by synthetic chemical condensations into neighboring vacuolated protoplasts; it is only over a limited range of pH, in the neighborhood of the isoelectric points of the main constituent proteins, that the protoplast is likely to behave in this manner.

Microchemical reactions show that the suberin deposit, when first forming, is relatively acid in reaction (*58*), as also are the young differentiating cork cells at the time suberin lamellae are being deposited. On the other hand, according to Sachs (*130*) the contents of the sieve tubes usually are relatively alkaline, in many cases actually alkaline to litmus. Thus in a plant which is intact the sap present in the walls and intercellular spaces lying between sieve tubes and the young suberin deposits would show a gradient of hydrogen-ion concentration, across which the phellogen forms. The plastic meristem cells, although compressed by their neighboring vacuolated cells so that they are elongated parallel to the cut surface, never divide at right angles to the surface—that is, by a wall of minimal area, as is usual in a cell at equilibrium with its surroundings (*35*, *165*)—but by walls which lie at right angles to this gradient, as might be expected if the gradient is influencing the synthetic activity of the protoplasm.

This argument can not be carried further in this place, but it has been developed in general relation to the present problem elsewhere (*117*). It at least provides an alternative explanation of the relation of the sieve tube to phellogen activity, and one which permits of an understanding of the circumstances under which the part played by the phloem might sometimes be played by other tissues.

The cut surface of the root is, in the xylem region, interrupted by the presence of tracheids and vessels. These were probably full of sap at the time of cutting, but the sap is rapidly displaced by the air entering the surface with the result that near any xylem vessel present at the cut surface the subsequent air-water interface is found on the flanks of this vessel. Consequently, suberization and cork formation will be found surrounding such a xylem element, the cork cells being cut off toward the empty cavity rather than toward the surface of the cut. In many cases, however, the new cells push through the walls of the vessel as do tyloses, and then rapidly push out through the open ends, so that ultimately the vessels are completely plugged.

APPEARANCE OF THE BUD

Of the process occurring at the wounded surface of seakale cuttings, suberization and cork formation are the ones that have so far been considered because they are associated with the healing of the cut, a necessary factor in successful propagation.

However, these are not the only processes occurring in the early stages following upon the act of cutting. As already indicated, they are associated with an injection of the intercellular spaces near the surface with sap, and this in turn is associated with a loss of starch from the living cells near the cut surface. This disappearance of starch is probably correlated with an increased production of soluble organic solutes, sugars and acids, in the vacuole of these cells, with a resultant increase in osmotic pressure. Certainly many of the cells, in which extension of the elastic walls is not prevented by too rapid deposition of suberin, undergo considerable enlargement, and the free walls markedly round off under the internal osmotic pressure. New cells appear, arising as the result of sporadic cell divisions, or forming serially in a chain from the phellogen cells; these in turn may also swell and round off instead of developing internal suberin lamellae while still regular in outline, in which case they contribute to the formation of callus instead of to the formation of cork. These tissues are certainly to a large extent interchangeable, callus arising under conditions of greater moisture, while the same cells would have contributed to the suberized envelope or to the cork proper if the surrounding air had been drier. These alternative processes have been fully discussed by Küster (*82*) and by Grau (*46*).

In the meristematic cells near the cut surface, at the early stages in development most division occurs in a plane at right angles to the cut surface, the new dividing walls being laid down in a plane parallel to this surface. This is illustrated by the frequency with which mitotic figures are found in longitudinal sections through the material at this stage, while series cut transversely and hence parallel to the cut surface show very few.

The first stages in the appearance of a bud in this material are illustrated in Plate 4. The figures, which are taken from 6-day material, show in line with the cork phellogen a group of meristematic cells which without a doubt originally formed part of this layer. These cells now differ from normal cork-phellogen cells in their shape and in the plane in which successive divisions occur. They are no longer compressed parallel to the surface, and as a result they are larger and their nuclei are completely rounded and lie free in the center of the cells, as occurs in the normal meristematic cells of the apical growing point.

In all probability these changes are the result of a change in the conditions existing in the neighborhood of the original phellogen. Here the meristem cells are compressed against the relatively rigid suberized cells outside by the expanding vacuolated cells beneath. But beneath these uncompressed cells the pressure is released because, as the photographs show, these internal cells are becoming meristematic also. This seems to be the keynote to the emergence of an apical bud, an extension of the tendency to become meristematic from the single cell layer of the phellogen to the living cells within. The result is the development of a small group of meristematic cells, in which the shapes, determined by the mutual pressures, differ from those of the cork-phellogen cells, and in which division no longer takes place exclusively in a plane at right angles to the cut surface. (Pls. 4, C and 5, A.)

On the other hand, the outermost cells of this meristematic group very soon show a definite tendency to divide entirely by walls at right angles, not to the surface of the cut, but to the surface of this group of active cells. This is the method of growth characteristic of the shoot dermatogen, and it soon leads to the development of the typical foliar lobes upon this mass of meristematic tissue. (Pl. 5, E.) At the same time this tendency to become meristematic spreads inward into the tissue beneath this original group. (Pl. 5, B and D.) The cells thus filled with protoplasm and free from vacuoles are surrounded on all sides by the ordinary vacuolated parenchymatous tissue, the result being that these cells become elongated in a direction vertical to the cut surface and so appear to run outward into the lobed mass of meristematic tissue above, forming the first indication of the procambial strands of the new shoot. (Pl. 5, E.) Thus the early differentiation of the new shoot tissues, behind the apical meristem, seems to take place downward, just as it normally does in every subsequent internode of the new shoot; hence the ordinary characteristics of the organization of the shoot apex and of the differentiating shoot beneath it are already in evidence.

Vascular differentiation now proceeds in the procambial strand subtending the newly organized shoot apex; and, as is characteristic of the shoot, this vascular differentiation occurs sporadically here and there along the line of differentiating cells, not in direct connection with the vascular supply of the mother tissue. (Pl. 5, E.) With this, vascular connection is made only later.

The new shoot apex is now characteristically organized and rapidly thrusts itself out from the surface of the mother plant; and the usual active cell divisions, characteristic of internodal growth, proceed along the flanks of the newly differentiating vascular system. It does not seem necessary, therefore, to follow the development of this new shoot system further.

It will be clear from this account that the new shoot organization has developed from the cork phellogen, a point that was reported earlier by Simon (*144*) for similar buds emanating from the callus at the ends of isolated shoots of Populus. Similarly, he described the differentiation of the new vascular elements going on beneath the bud and made clear that such differentiation need not always be preceded by differentiation of clearly outlined procambial strands, for he found that cells of the callus parenchyma lying in the path of the new vascular channel frequently differentiated directly into conducting elements. The general significance of this place of origin of the stem growing point will be considered in a later section, but it seems necessary here to refer to some recent statement of similar cases of bud formation in callus—statements which appear to be completely contradictory to these.

Taylor (*163*) and Graham and Stewart (*45*) described buds arising from the callus at the cut surface of fleshy roots of *Acanthus montanus* T. And. and *Anchusa italica* and referred to them as arising from the vascular cambium. Their statements are supported neither by detailed developmental figures nor by structural descriptions of the bud development. A. *italica* is mentioned again on page 58. Studies upon bud formation in Acanthus were carried out at Leeds some years ago, and it was found that root cuttings of this plant behaved in general like those of seakale. In Acanthus also the meristematic activity of the cells beneath the suberized surface, leading to the production of new cork and callus cells, was found to be most active in the region of the former vascular cambium. But these new cell divisions were found to occur in meristem cells which became flattened parallel to the surface, cut off new cells to the outside, and in all respects behaved as a cork phellogen.

It is true that if one individual cell in this phellogen is considered it may have previously been functioning as a cell of the vascular cambium. But it can not be too strongly emphasized that all experience suggests that there are no fundamental differences between individual meristem cells and that the single cells have the same general meristematic properties whether found in the vascular cambium, the phellogen, the root apex, or the shoot apex. The behavior of the individual meristem cells is determined by the tissue organization of which they form a part, and in the case of Acanthus, beneath the cut surface these meristem cells are functioning as members of a cork phellogen and not as vascular cambium cells; hence it is a definite misuse of terms to describe such tissue as any longer being vascular cambium. On the contrary, these buds seemed just as certainly to arise in the typical cork phellogen as did those of the seakale just described; however, the meristematic activity associated with these buds was not confined to this one layer of cells, for, as was emphasized in connection with seakale, the cells beneath this layer very soon similarly became meristematic and contributed to the further development of the new shoot. Hence, sections of Acanthus as well as of seakale cut through young shoots, still hardly discernible by the unaided eye, show much activity in the tissues behind the phellogen, and it might be easy in many such cases to assume that the shoot had been formed in the inner tissues.

As Schmidt has pointed out (*136*), it is probable that in many cases only the superficial layers of the shoot are actually derived from the cork phellogen, whereas in other cases, as seems to be true with seakale, the entire new shoot except the base is derived from this phellogen layer. This point is obviously of very great importance in the question of the formation and the reversions of periclinal chimeras (*160*).

When disks 1 centimeter each across and 3 to 5 millimeters thick were removed with a cork borer from the xylem region of a large seakale root, it was found that all surfaces exposed became covered with a cork cambium which was capable of forming buds. Capacity to form buds on the sides and root end was also observed on other slices less than 1 centimeter in length, and also in one case upon a piece of a very large root, 5 centimeters thick and 5 centimeters in length, which stood inverted. Centrifuging also aided in the production of buds upon the root end, as was reported by Jones (*69*). The actual formation of the lateral and distal buds seemed to follow the same course as for those forming on the shoot end. Plate 5, C, shows various stages of development of buds on the three exposed surfaces of such a disk.

It must be clearly recognized that different factors are undoubtedly at play, in determining the initiation of these adventive buds, from the factors responsible for their further development. In one disk, 11 millimeters in diameter and 3.5 millimeters thick, 14 days after it had been cut out with a cork borer from the xylem of a main root there were counted 131 separate stem growing points which had arisen in the wound cork cambium. These were distributed as follows: Proximal (shoot) surface, 57; distal, 27; side, 41; and internal (in cork cambium which had formed around vessels), 6. Considering the surfaces exposed in the first three categories, there was roughly 1 bud formed for each 1.8 square millimeters on the shoot end, 1 for each 3.5 square millimeters on the root end; and 1 for each 2 square millimeters of area on the side. This, it will be remembered, was in tissue which contained no true cambium and no phloem at the time it was cut out of the parent root, except such slight traces as might have been present in the remains of the original lateral roots.

Many more buds were initiated on small pieces than on large ones. Except for the one bud that formed on the one large inverted piece, no buds formed on the root end of any cuttings more than 5 millimeters thick unless the piece had been centrifuged. Furthermore, all buds that arose did so from cork cambiums which had formed in either the region of the vascular cambium or the xylem parenchyma; and not a single stem growing point, out of several thousand observed in these seakale studies, came from the phloem parenchyma. This tissue did not seem to possess the power to form buds, notwithstanding the fact that it did, in time, form a cork cambium. It is possible, however, that since this cork cambium was present, had steps been taken to inhibit the formation of buds in the other regions, new stem growing points might have developed in the phloem region. The lack of development of buds in this region seems to be closely connected with the greater development of intercellular air spaces here. In the regeneration experiments of Simon with Populus (*144*), buds were obtained upon the exposed surface of the pith, but their development in these cases was very slow indeed, obviously because the deeper lying meristem differentiation which should lead to the formation of a procambial strand and ultimately end at a functioning vascular supply could not take place. Simon obtained the further development of these buds by cutting a channel through the wood; this permitted vascular differentiation from the apical group of meristem cells to proceed backward to the young differentiating vascular elements associated with the cambium.

Thus it is seen that the formation of an adventive bud seems to require the organization of a superficial group of meristem cells—

the conditions for their functioning approximating closely those that prevail at a phellogen—along with a sap supply which permits the maintenance of this meristematic activity that is subsequently associated with vascular differentiation beneath this superficial group of meristem.

FORMATION OF ADVENTIVE ROOTS

The development of new roots from any isolated root system may take place either laterally or from the wound callus. In Crambe the first roots in evidence after isolation emerge from the normal, lateral surface of the root, when, as is pointed out in the next section (p. 60), they usually appear as branches from the older lateral roots already present in the tissue of the main root. Very rarely in old roots the new roots may emerge laterally from the neighborhood of the cambium of the main axis.

These types of root production are not considered further in this section, but attention is confined to root production from the wound callus which occurs in Crambe as in some other fleshy roots; such adventive roots appear some days after the separation of the root.

If the root piece is sufficiently long, marked polarity is noticeable in the isolated segment. Bud production begins very early and is manifested much more vigorously at the proximal end than at the root end, though under exceptional circumstances (p. 21) buds may appear at the distal end also. In the case of seakale, after about 10 to 12 days, with cuttings kept at about 25° C., vigorous root production from the cut surface was in evidence at the distal end only, and bud production from this end seemed entirely inhibited. The roots usually appeared in a ring over the site of the original vascular cambium. Other roots were occasionally seen to be arising from the xylem region, but no roots emerged from any part of the surface outside the cambium ring. Van der Lek (*86*), considering comparable roots arising upon stems, termed them "wound roots" to distinguish them from the "morphological roots" emerged through a surface that was intact, but this terminology does not seem fortunate, for presumably even wound roots have a morphology.

It will be remembered that when the pieces of root were very short so that distal and proximal surfaces were but a few millimeters apart, both surfaces bore buds freely. On such disks of root no new roots emerged from either surface. Thus from the outset the longer pieces of root differed more in the behavior of the opposite ends, the more activity being displayed at the distal surface; this difference in behavior between the long and the short cuttings evidently increased with time. In the long pieces, during the first few days suberization proceeded more markedly at the distal than at the proximal end, and phellogen activity was likewise more pronounced at the distal end. These differences were also recognizable to a lesser degree in the short slices, but in the further development of the meristematic activities at the two ends by far the greater manifestation of polarity was seen in the long pieces. Although the distal ends of the short pieces formed buds and in the long pieces great meristematic activity was displayed, buds did not form at the distal end, but the new meristems in the longer cuttings were organized instead into root apices. This new development, characteristic of the distal end of a root piece 2 centimeters or more in length, will now be examined in some detail.

The fact that in such material root production appeared to be confined to the distal callus suggests that in such a root a redistribution of meristematic activity continues to take place after isolation, finally resulting in shoot production at one end and root production at the other. Symptoms of such a reorganization of meristematic activity throughout the root piece can be seen in transverse sections through such root pieces, taken after different intervals of time. In the normal root, meristematic activity is mainly, but not entirely, confined to the region of the vascular cambium. Here typical meristematic activity takes place in the region between xylem and phloem. However, growth activity is not confined to the immediate neighborhood of the vascular elements, for interfascicular activity also takes place in the cambial ring, thus the rays keep pace with the increase in the xylem and phloem. In such a fleshy root, moreover, where xylem and phloem are very parenchymatous, cell divisions continue to occur in both the xylem and the phloem parenchyma, and, as has been seen, the regularity of the tissues becomes less and less distinct, particularly in the outer phloem.

After such a root has been isolated, 4-day material shows that near the proximal end the meristematic divisions of the vascular cambium were still strictly confined to the region between xylem and phloem, and in the rays but very slight activity was evident. (Pl. 2, A.) On the other hand, near the distal end (pl. 2, B) it is seen that the meristematic activity of the cambium has extended across the rays as well. Each of these sections was taken about 1 millimeter beneath the cut surface. Plate 2, C, illustrates the state of affairs 2 or 3 millimeters below the proximal surface as seen after seven days. The change in distribution of meristematic activity is here very striking; the activity of the vascular cambium appears to be entirely confined to a region close to the groups of recently formed phloem, which thus comes to be seated outside crescent-shaped rows of actively dividing cells, to the inside of which new groups of short tracheids or vessel elements have already started to differentiate. These are the vascular groups with which are later connected the procambial strands of the buds formed at this end. This section also provides definite evidence of cell division around some of the groups of older vessels—indications that call to mind the cambium that forms in this manner as a normal thing in many fleshy roots like *Oenanthe crocata* L., producing concentric rings of secondary xylem and phloem irregularly throughout the root.

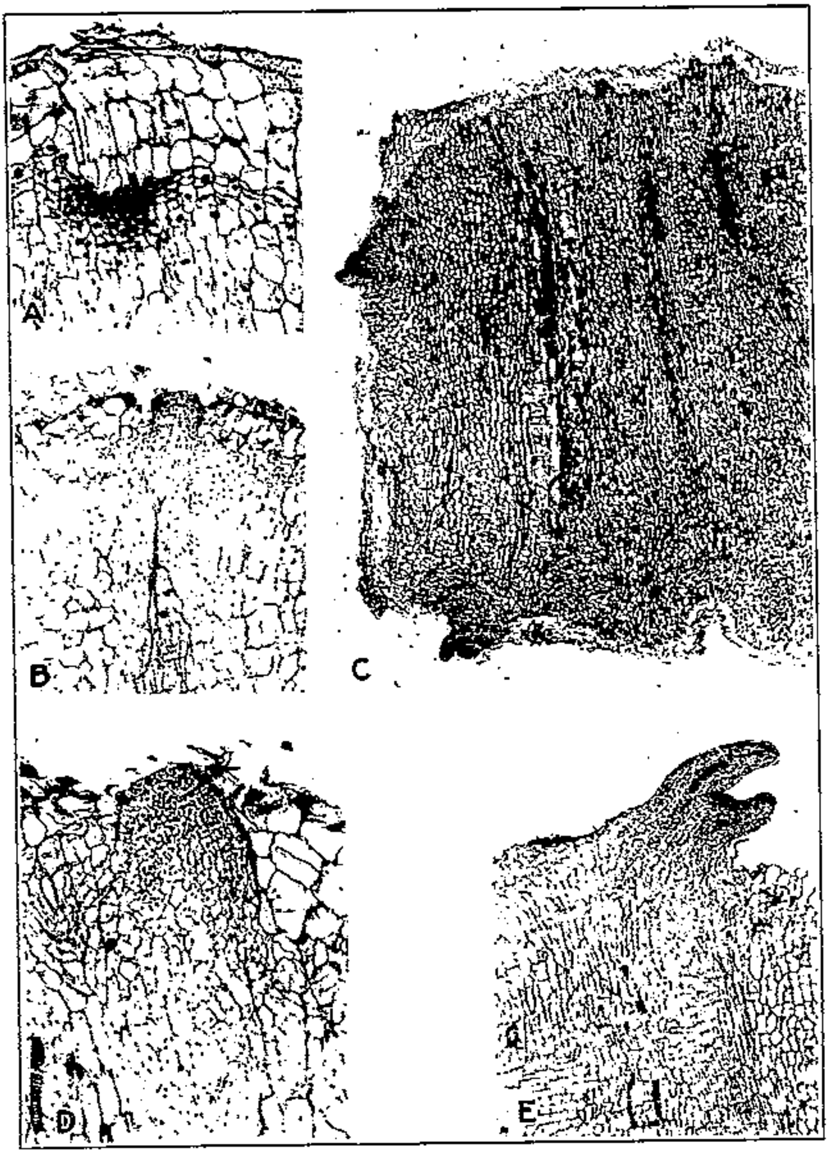
At the lower (distal) end, as Plate 2, D, shows, after seven days similar rings of meristematic activity around groups of vessels are apparent in the interior of the root, but the ring of vascular cambium is here not broken up at all; greater cambial activity has been taking place at this end than before, both between xylem and phloem and across the rays. The products of this activity, to the inside, have differentiated into the short tracheids and vessel segments characteristic of wound wood (p. 33), and to the outside have been cut off cells which presumably must be regarded as phloem parenchyma.

This increased activity of the vascular cambium at the distal end gives, in longitudinal section, a new appearance which is completely missing at the proximal end. In this plane it is seen that the cambial activity has spread around the ends of the differentiated, lignified xylem elements; these old vessels are now covered at the cut surface by the parenchyma cut off during the early stages of the calluslike cell multiplication, and the new cambium differentiates through this parenchyma.

Beneath the suberized cut surface and separated by a parenchymatous semimeristematic tissue two layers of meristem are now stretching; the outer meristem, which forms first, constitutes the cork phellogen, while the inner one is intimately associated with the vascular cambium. The inner line of meristem does not extend into the phloem region at all, but it stretches only across the xylem region. As shown in Plate 6, it seems to merge into the cambium at the side and into the cambiumlike groups which surround the vascular elements scattered within the xylem. This plate also shows that this new cambium has cut off new tracheids to the inside and cells very much like phloem and phloem parenchyma to the outside. Hence, in all respects this new cambium seems to be a regenerated vascular cambium. It is in this mass of tissue, part of it definitely meristematic and part potentially meristematic, extending from cork phellogen to the layer in connection with the vascular cambium, that root initials now originate.

Early stages in root initiation are shown in Plate 7, A–D. In the layer just beneath the phellogen, cells that were already semimeristematic become filled with protoplasm and the nucleus becomes spherical. The phellogen cells themselves, thus released from the pressure exerted from beneath them by vacuolating cells, are no longer so compressed; for a time they remain meristematic in appearance, but they do not undergo rapid division, and therefore there is no increase in the surface of the newly organizing meristem. On the other hand, for several layers within, the cells become meristematic and divide frequently. This happens throughout quite a mass of the subjacent tissue, so that as the root apex becomes more definitely organized (pl. 7, D and pl. 8, B) all the cells throughout a considerable width of tissue appear equally meristematic. Under these conditions there is no indication of a slender ring of meristematic tissue undergoing compression by vacuolated cells within and without, such as is shown by the differentiating bud, so that no procambial strands can be detected, there being only a solid core of meristem of which the inner cells are probably dividing more rapidly than the outer ones (p. 7). Even before the root emerges this core of meristematic tissue has extended down practically as far as the new transverse meristem. However, in every case the roots were seen to arise in the superficial position indicated, opposite either the vascular cambium itself or a strand of cambiumlike tissue surrounding a group of xylem vessels. Hence, as the root organization begins its forward movement, there is a continuity of meristematic tissue between the new cells of the growing point and the newly formed lignified vascular elements of the wound wood, which are connected with the older vessels. In Plate 8, C, the continuity of the vertically extended cells of the vascular cambium with the meristem of the emerging root is very clearly seen, and near the base of the root some of the newly formed xylem elements are visible.

In the case of bud formation at the cut surface the newly organized meristematic apices could not be attributed to any single meristematic

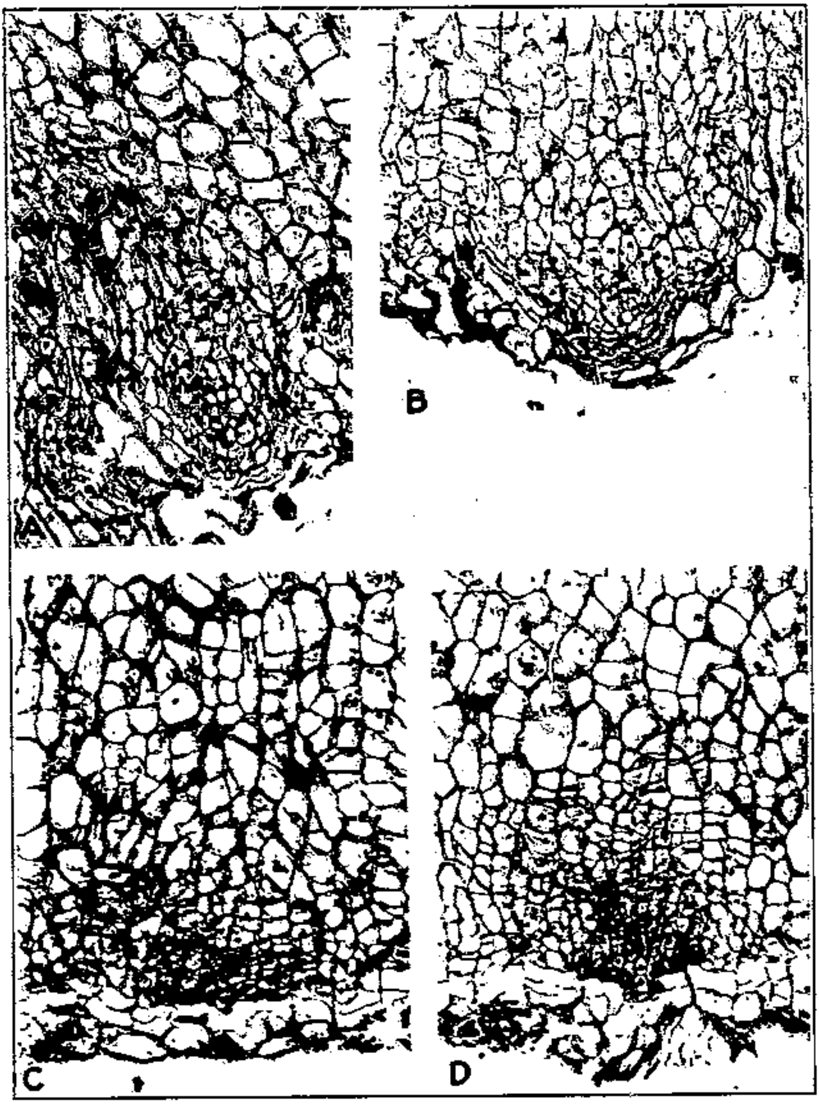


Later Stages in Adventive-Shoot Development on Crambe Roots

A.—A bud initial, showing its position beneath the wound cork. Dead cells still filled with starch are shown at the cut surface above the suberized cells. × 90.
B.—A young bud breaking through the wound cork just above the original cambium. × 50.
C.—Longitudinal section through a disk of xylem cut out from the root with a cork borer. A wound phellogen is active on every surface of this disk, and bud initials are also present on each surface. × 15.
D and E.—Stages of bud formation at the proximal surface, illustrating the gradual extension of meristematic activity into the deeper layers of the tissue. In E, procambial strands are differentiating in the two young leaf initials and thence backward into the meristematic tissues beneath the bud. D, × 110; E, × 30.

LONGITUDINAL SECTION THROUGH DISTAL END OF ROOT PIECE OF CRAMBE AFTER 14 DAYS

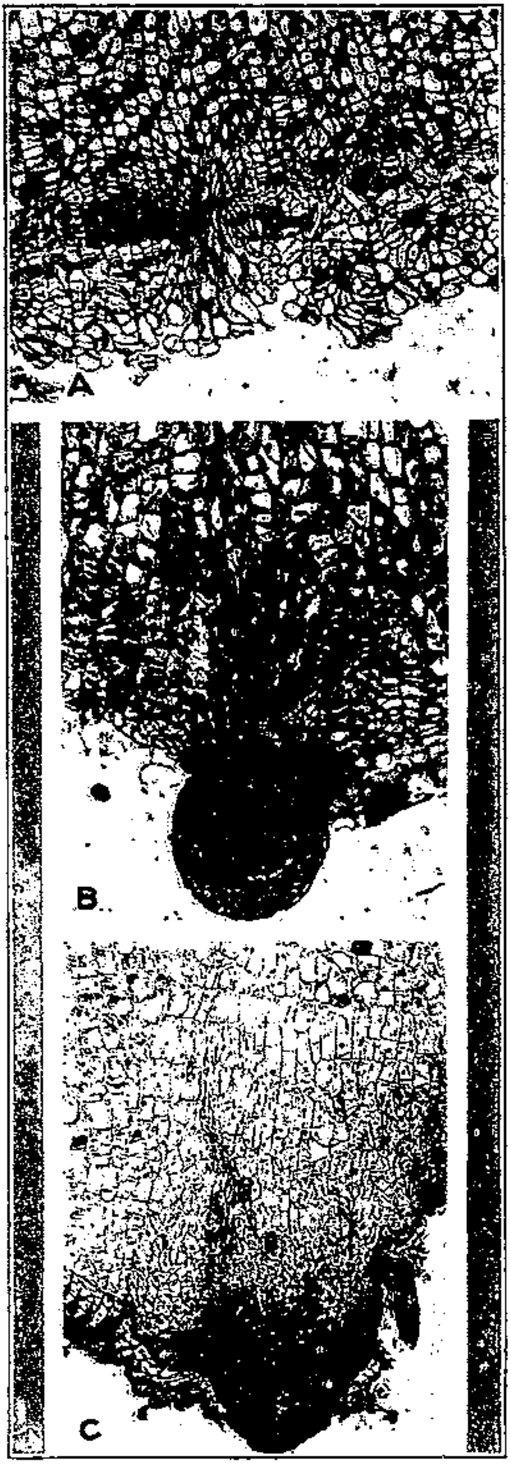
The cork phallogen is visible beneath the suberized layer, and it has formed afresh in the outer layers of the callus, where this tissue has burst through the original suberized layers. Just beneath the position of the original cambium a root has grown out. In the deeper layers of the tissue below the cut surface there can be traced a line of meristematic tissue from which new xylem elements are being cut off toward the inner surface. On the outer side the tissue cut off directly opposite these xylem groups has all the appearance of phloem. This meristematic layer extends only from cambium to cambium. × 50.



FORMATION OF ADVENTIVE ROOTS ON CRAMBE

A–D.—Longitudinal sections through the distal end of root pieces 12 days after isolation. A, On the left note the irregular gash, lined on all sides by phellogen lying beneath deeply stained deposits of suberin. To the right of this gash, just below the cut surface, the meristematic cells of a very young root initial are present. × 110. B–D, Progressively older stages in the organization of a young root initial. × 110.

If these sections are compared with those shown in Plates 4 and 5, it will be seen that at the time and place of initiation the phellogen is less conspicuous beneath the wound cork and seems to take less part in the meristematic transformations associated with the organization of root initials than with shoot initials.



OLDER STAGES IN ROOT FORMATION IN CRAMBE (LONGITUDINAL SECTIONS)

(For explanatory legend see p. 25)

tissue of the original root, though they were clearly associated with the wound phellogen. In this later development of meristematic activity, occurring at the distal end alone, there is unmistakable indication of close correlation existing between root production and the increased meristematic activity associated with the vascular cambium at this end. When the root initial is originally organized the phellogen of the cut surface may form an integral part of its surface layer. But this layer, as is appropriate to a rootcap, ceases to be an active meristematic layer; meristematic characteristics and cell division appear within deeper and deeper layers of the tissue at the wound surface, so that by the time the root is organized and ready to emerge, its inner, actively dividing layers are closely associated with the normal activity of the vascular cambium.

For the internal factors contributing to root formation, therefore, one must look to the factors contributing to this reorganization of meristematic activity which occurs throughout the isolated root. Unfortunately, the anatomical story of polarity, as already indicated, is still largely a matter of speculation. Polarity is bound up with the organization of tissues as a whole, so that to a considerable extent the longer the piece of root employed the more accentuated is this effect.

In the discussion of the relation of phloem to phellogen activity the possible significance of the downward movement in the phloem of solutes essential for meristematic activity has been considered. Such a correlation seems suggested by the increased meristematic activity in evidence at the distal end of the longer pieces, considered in relation to the decreased activity of the vascular cambium at the upper end, and the way in which this diminished activity is confined to crescentlike regions around the groups of phloem. On the other hand, such a correlation between the activity of the phloem and that of the vascular cambium and phellogen is clearly not the only factor concerned, as is shown by the appearance of meristematic activity around many of the internal groups of vessels. As already pointed out, the parenchyma found around these groups is always compressed and relatively free from intercellular air spaces, and to a large extent the distribution of meristematic activity at the proximal end of the root piece could be understood on the assumption that it was necessarily confined to those regions which alone had access to adequate supplies of solutes.

At the distal end, solutes accumulate by the continued polar distributive activity of the phloem, and at this end most of the tissues lying just beneath the cut surface remain charged with sap, so that cambial activity takes place not only near the phloem but also across the rays. Likewise, the entire xylem region, several cell layers back from the cut, becomes more or less meristematic. Between the cork phellogen and the deeper lying meristem which forms subsequently, there is thus left a layer of semimeristematic parenchyma in which

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EXPLANATORY LEGEND FOR PLATE 8

OLDER STAGES IN ROOT FORMATION IN CRAMBE (LONGITUDINAL SECTIONS)

A.—Distal end after 12 days, showing manner in which proliferation of callus forces tissue out through the barrier of suberized cells. Where this happens very little meristematic activity is seen, though a phellogen is reorganizing outside the suberized layers in several places. Above the suberized layer, on the left of the section, a root initial is organizing. × 50.

B.—Distal end after 12 days, showing a root apex emerging from the cut surface. × 100.

C.—Distal end near emergence of a large root (very deeply stained). Behind this a connection can be traced between the meristem cells of the root apex and the vascular cambium of the main root. On the right of this line of meristem cells new xylem elements are differentiating. × 60.

the intercellular spaces are small and injected and in which therefore, the supply of water and solutes are adequate for growth. This tissue, as it multiplies, expands with considerable pressure against the barrier of suberized cells at the surface. Often here and there the inelastic film of suberized and dead cells is broken; with, as an immediate result, the emergence and exposure of the parenchymatous cells within, which "flow out" through the gap as a large-celled, loose, spongy tissue in which the large intercellular spaces fill with air. As Plate 8, A, illustrates, where such rupture occurs, organized meristematic activity ceases. The meristematic cells of the newly organizing root initials as well as those of the shoot initials were always seen to be confined to the region of tightly packed cells lying beneath this still-effective suberized barrier. Neither roots nor shoots were seen to arise opposite breaks in the cork cambium.

The development of shoot apices and root apices taking place at the cut surface of Crambe has been considered. It is clear that internal factors, which are at present far too little understood, determine which, if either, of these types of meristematic activity will be manifested. Shoot meristems appear first, arising in the layer of actively growing cells which lie nearest the cut surface (cork cambium) and under conditions which allow the supplies of food necessary for the continued development of this meristem to reach it through the underlying tissue. This activity may appear at either cut surface, anywhere over the cambium or the xylem parenchyma, and possibly under special conditions even over the phloem parenchyma. At the distal surface only, and later in time, special conditions of meristematic growth prevail, in which activity is so organized that the inner layers of the meristematic tissue are the more active in division, and from an early stage in their development these are closely associated with the meristem which is giving rise to differentiated xylem within—the vascular cambium. Thus, from an early stage, the dense core of the root meristem is organized in contact with the vascular stele. The cells in the outer layers, previously phellogen, whether from lack of food or from some other reason fail to divide actively, and contribute instead to the rootcap of the new structure. Hence, a type of organized meristem emerges from a meristematic matrix that, had it been given certain other internal correlating factors, would have utilized the same energy in the production of a shoot.

That meristematic tissue with such potentialities for shoot production should actually give rise instead to root initials can not be a matter of surprise, in view of the fact that many years ago Beijerinck described the transformation of a typical shoot apex into a root meristem (*11*). Lateral shoot buds appear at intervals along the root of *Rumex acetosella* L. and under normal conditions often grow out into new shoots. But if a piece of this root system is isolated and placed vertically in damp soil, while the upper buds grow out into shoots, in some cases one or more lower bud initials will grow on into roots. Plate 9, C, shows such a root; at its base rudimentary bud scales can be seen. It was obtained in experiments at Leeds, in which Beijerinck's original observations were confirmed by Edmondson.[5]

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[5] EDMONDSON, W. E. THE TRANSITION FROM BUD INITIAL TO ROOT IN RUMEX ACETOSELLA. Leeds. 1926. [Unpublished thesis.]

Furthermore, in a later section (p. 78) a case is illustrated where shoot and root apices were organized very close to one another within the parenchyma near the cut surface of a root of *Cichorium intybus*. Here shoot and root meristems must have originally come from groups of cells that were as close to one another as the phellogen and the deeper lying meristematic layer in the distal callus of the root of Crambe.

Root production seems to be much more closely related than shoot production to the normal activity of the vascular cambium. It is, therefore, perhaps worth emphasizing the striking parallel that exists between the normal vascular cambium, which cuts off more cells to the inside, and the root meristem, which is the more active on the inner surface, facing the stele (*117*). The general conditions governing these two forms of growth activity, the adventive shoot apex and root apex, will be reviewed once more and from a wider angle, in the next section, where a wider range of phenomena is discussed in connection with their organization during vegetative propagation.

THE GENERALIZED PROBLEM

ADVENTIVE SHOOTS

Apparently the apical organization and development of the shoot always proceed along the lines indicated in the preceding sections so that to this extent the problem of the origin of new shoots in vegetative propagation is always the same. But the organization of the tissues in which they may arise varies greatly and the problem of the development of adventive shoots is different, when such structures develop upon isolated leaves or stems, from the problem as outlined above for seakale, and it is still more distinct in the case of buds arising on uninjured roots.

In all cases it is necessary to distinguish clearly between the further development of a meristematic shoot apex, dormant but already organized, and the initiation of such an apex as the result of developmental changes brought about by special conditions such as the isolation of a portion of the plant. Shoot apices may be present in a dormant state upon any portion of the plant, either shoot or root, and the extremely diverse manner in which such primordia commonly occur in different species makes it difficult to use the qualifying term "adventive" (or "adventitious") with a precise connotation.

Koch (*78*) described very fully the process of branching in the flowering plant. He showed that the meristematic "anlagen," constituting the lateral buds, in the case of some water plants as well as occasionally in inflorescences, may appear upon the apex, even before the subtending leaf initials appear. On the other hand, in practically all trees and shrubs the new lateral buds arise some time after the leaf initials have appeared at the growing point, at a time when the internodal development taking place makes these axillary meristematic groups distinct in origin from the meristematic tissue crowning the apex. It would probably be incorrect, therefore, to define, as Hofmeister (*61*) did, an adventive bud as any bud which arises on the axis, without genetic meristematic connection with the original apical meristem, since such a definition would undoubtedly include as adventive a number of axillary buds that have emerged in their regular place, in acropetal order, upon the shoot. On the other hand, it is equally difficult to draw a clear distinction between different buds based upon their order of development, or even upon the relation of injury to their emergence, as Sachs (*131*) did. Thus, every axillary bud is the result of the activity of a little axillary cushion of meristem which is usually located in the axil between stem and leaf, but which may be on the surface of either leaf or stem near the axil. The activity of this meristematic mass is not always exhausted by the formation of a single bud; in many plants, as a regular thing, other buds may be organized in series near the first buds, either above or below them, or on the flanks. Such buds were termed "Beiknospen" by Sandt (*134*), who investigated the phenomenon very fully. This employment of the prefix "bei" needs to be borne in mind because of the possibility of confusion arising from the entirely different way in which the term "Beiwurzeln" is employed (p. 60).

These "Beiknospen" may be originally present in the leaf axil; at other times, as the result of injury or removal of the original single bud, an exactly similar behavior of the axillary meristematic cushion may cause their production; in the latter case, such "Beiknospen" would be termed "adventive" by most workers. Similarly, the dormant bud which emerges long after the usual time of appearance as determined by its acropetal position on the axis may be the further development of a meristematic cushion which had been left on the axis at almost any stage of development between that of an original group of meristematic cells and that of a definitely organized shoot apex.

Again in many plants, including *Cardamine pratensis*, *Atherurus ternatus* [*Pinellia tuberifera* Ten.] (*52*) and *Torenia asiatica* L. (*185*), buds regularly arise upon leaf veins or petioles, and thus, though a feature of the plant's normal development, certainly fall into the category of "adventive," using this term as originally defined by Du Petit-Thouars (*34*), who classified as adventive all buds not terminal or axillary. These leaf-borne buds, however, do not coincide so well with the remainder of his original definition—buds arising later in life than the normally situated structures.

However, if the term "adventive" were employed with shoots as De Candolle (*20*) and many later workers have employed it for roots—only roots arising on other organs than roots being described as adventive—these shoots, emerging normally from leaf tissue, and therefore from a portion of the shoot, could not come into the category of adventive, while all the numerous cases in which shoots appear upon the root as a part of the normal development of the dicotyledon root system (*11*) would be included.

There seems, therefore, only one of two alternatives to be followed in the practical employment of the expression "adventive." In dealing with such diversely organized structures as flowering plants, either the term must be loosely employed or all but a single group must be ruled out. If the term is used in a general sense, there seems little reason to depart far from the original practice, in which any shoot organization appearing in any position other than terminal or axillary, or which appears at some relatively late stage in development, may be termed "adventive." On the other hand, if a precise use of the word is desired, it must be restricted to the numerous cases where, as the result of the abnormal conditions resulting from injury (as in cutting propagation), buds arise in tissues which, whether meristematic or permanent, were not previously organized as meristematic shoot apices.

This strict delimitation of the term would make possible precision in its use, but at the cost of some practical convenience; consequently for the present the writers will continue its use in the general sense, because the problem with which they are concerned is the development of buds in a portion of the plant isolated for vegetative propagation. Such buds may emerge from dormant apical shoot organizations, from meristematic cushions in which this capacity to organize so is latent or only partly indicated at the moment of separation, or, as was seen in the case of seakale, such buds may be an entirely new development resulting from the injury and isolation which is essentially a part of cutting propagation. As has been said, the main differences between these types appear in the organization of the tissue upon which the shoot apex emerges. For that reason it will be convenient to discuss first the development of shoots upon shoots and then of shoots upon roots.

SHOOTS UPON SHOOTS

Monocotyledons

In the great majority of cases when isolated portions of shoots are used for vegetative propagation, the system isolated includes some normal axillary buds and the shoot system of the new plant arises from these. In the case of most monocotyledons this is practically the only possible method of vegetative propagation, as permanent tissues very seldom again become meristematic in such plants, while in the older regions of the plant meristematic tissues are found only in the node and the basal portion of the leaf.

In a number of monocotyledons isolated portions of shoot or root will produce a type of cork at the injured surface, but, as Philipp (*108*) pointed out, this cork is usually different in nature from that characteristic of the dicotyledon. Instead of the original permanent tissue differentiating into a phellogen layer which then remains active for an indefinite period, the cells in one or more layers beneath the cut surface divide into a limited number of segments by walls parallel to the cut. None of the individual cells thus formed function as meristematic phellogen; all become suberized, and in many cases no further cork formation takes place beyond that occurring in this single layer. In other cases, cells adjacent to those which first divided, in turn become active and divide, so that quite a depth of suberized cells may be produced; but the seriation of the blocks of cells in the tissues, as seen in section, is determined by the arrangement of the cells in the original parenchyma in which the divisions occurred, and there is no continuous serial order of the cells such as results when a common phellogen is responsible for their formation. In the origin of the adventive shoots of *Crambe maritima* it was clear that the new shoot organizations arose from the meristematic cells of the phellogen. No layers with such meristematic potentialities appear during the process of cork formation in most monocotyledons, and it is not surprising that no case has so far been described of adventive-shoot formation from the cut stem or root surface in any monocotyledon where cork formation is of this type, which is termed by Philipp "etagen" cork, in contradistinction to the ordinary "initial" cork of the dicotyledon.

In some monocotyledons typical phellogen activities are displayed, as are also certain other characteristic cambial layers. It is from such members of the Liliaceae alone that bud formation has been described, either from the stem callus, as in *Aloe arborescens* var. *frutescens* Link (*69*), or from the root callus as in Dracaena and Dioscorea. In several species of Lilium, in Ornithogalum, and in *Pinellia tuberifera*, bulbils, or shoot organizations, appear upon the leaf-lamina base as a normal thing, very much as similar structures occur within the ovary in Allium. Such buds are, of course, commonly employed in propagation. Furthermore, in the case of many fleshy monocotyledon leaves in which the basal meristem normally persists for some time, when these leaves are isolated, adventive buds appear at or near the cut surface, arising from cells lying just below suberized cells, near the point of injury. Examples are Haemanthus (*43*), Drimia (*44*), and Sansevieria.

Whether the shoot organization is already present in the plant at the time of separation, or forms at the leaf base only after injury, its development follows the same regular course.

The leaf initials arise as folds, almost completely surrounding the meristematic apex; beneath these the internodal tissues subsequently differentiate as cylinder within cylinder, while at the base of each differentiating internode, root initials are developed in connection with the procambial ring. Apparently, in every case in the monocotyledons new roots are formed in connection with such nodal or leaf-base buds, in the new shoot itself; hence at a very early stage the new individual is completely independent of the parent plant. Of course, in many cases where the stem is used as a cutting, roots arise from the main axis as well as from the base of a developed bud. This point will be briefly considered in the next section. Schubert (*137*, *138*), in his account of the propagation of monocotyledons by cuttings, gave a list of species in which propagation has been successfully effected from leaf bases.

DICOTYLEDONS

In the dicotyledon, meristematic tissues are much more widely distributed, their regeneration from permanent tissues occurs very much more frequently, and therefore the modes of organization of adventive buds are much more numerous.

Again, the most familiar example is that in which an axillary bud, left in the isolated portion of the shoot, continues to develop and produces the shoot system of the new plant. The development of such an axillary bud conforms in general very closely to the story of the development of adventive buds from wound callus in Crambe. Such an axillary bud constitutes merely an "anlage" of meristematic tissue in the axil of the leaf. Contemporaneously with its development, the differentiation of the internode in the axis above was proceeding; the procambial strand of a leaf situated higher on the axis ends just above the insertion of this bud, so that just beneath it in the procambial ring occurs a gap which is filled with parenchymatous, nonmeristematic tissue. When the procambial ring differentiates into a vascular system, the xylem and the phloem differentiate downward, this tendency to differentiate passing obliquely around the sides of the branch gap and the leaf gap, which still remain parenchymatous. However, when the organization of this axillary shoot apex continues, the parenchymatous tissue becomes traversed by the procambial strands of the new branch system. In this process of differentiation the vascular elements appear as separate osmotic systems during the early stages, and only gradually link up, by backward differentiation, with the sides of the leaf gaps and with the leaf traces below. Thus the organization of this lateral shoot apex takes place from the surface inward as in other cases, the process sometimes here extending over several months.

ADVENTIVE BUDS UPON THE STEM

If no buds are left upon the detached portion of the axis, in very many cases new buds can be formed. Frequently these arise from small shoot apices which were lying dormant, perhaps in the axil of a scale leaf or as accessory buds ("Beiknospen") at the base of an original bud. The recent extensive observations of Plett (*109*)[6] show that even when such initials are absent new buds may still be formed. Thus, out of 401 species examined, 38 species produced buds upon internodal cuttings, of which 27 also produced roots. On the other hand, although only 11 species formed buds alone, 67 species produced roots alone, indicating a relatively greater tendency for root production than for bud production. In such internodal cuttings, where no remnant of the original bud base and meristematic cushion is left, the new buds usually arise from the wound callus. Plett found that the following species produced buds in this manner: *Rudbeckia laciniata*, *Boltonia latisquama*, *Centaurea calocephala f. aurea* Dry., *C. rupestris* L., *Scabiosa arvensis* L., *Salvia sylvestris* L., *Physostegia virginiana*, *Solanum nigrum*, *Lycopersicum esculentum*, *Nicotiana tabacum*, *Sinningia purpurea* Hort., *Acanthus montanus*, *A. mollis*, *A. spinosus*, *A. longifolius* Tour., *Fittonia argyroneura*, *Passiflora caerulea*, and *Cleome spinosa*. He found that in *Maurandia lophospermum*, Begonia, and several Peperomias the buds arose in the cortex, in Torenia probably from the epidermis, in Apocynum they appeared as lenticellike outgrowths in the cortex, and in Lysimachia from one epidermal cell.

Failure to obtain buds from internodal cuttings obviously can not be accepted as conclusive, as repetition under other conditions might lead to success. In the absence of the anatomical details in each particular case of failure it is difficult to determine in just what stage of the regenerative process the failure occurs.

In a few cases Plett[6] found that buds arising at the distal end of such cuttings were in direct vascular connection through the internode with the roots arising from the basal end; thus in such cases the original cutting remains an integral part of the new vegetatively propagated plant. In the great majority of cases, however,

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[6] PLETT, W. UNTERSUCHUNGEN ÜBER DIE REGENERATIONSERSCHEINUNGEN AN INTERNODIEN. 46 p., illus. Hamburg, 1921. [Unpublished Inaug. Diss. Auszug published 1921 (*109*).]

this does not happen, the establishment of the new shoots as independent plants being conditional upon the development of roots from their bases.

In an experimental study upon callus formation occurring in woody twigs such as Populus, largely dealing with the influence of external factors, Simon (*144*) paid some attention to anatomy. He pointed out in the first place that the somewhat vigorous callus production taking place in these shoots in moist air differed greatly between the two ends of the shoot. At the upper (distal) end he found the callus to be very irregular in form; the vascular cambium seemed to take a comparatively minor rôle in its formation, and the callus was developed through the repeated division of the cells of all the living tissues at the exposed surface, including the pith. However, the cells in the neighborhood of the vascular cambium were the most active in the process. At the lower end the callus was much more regular in appearance, being in the main a mass of tissue cut off by the vascular cambium. This layer continued to form downward into the callus, arching inward toward the wood and cutting off both parenchyma and wood to the inside; to the outside it formed less tissue, all of it parenchymatous in nature, while still farther out a certain amount of calluslike growth and division occurred. At both ends near the outer surface of the callus could usually be traced a meristematic layer which was forming new cells, mainly toward the surface, thus contributing to the formation of the callus or cork, according to the relative humidity.

Simon noted (*144, p. 363–364*) that buds arose from the superficial cells of the callus if they formed in the early stages of its development, but when a well-marked meristem or pheliogen had appeared near the surface, then the buds arose from the neighborhood of this meristem. His results are, therefore, closely in line with the observations upon Crambe.

Simon (*144*) also found that according to the conditions under which the shoots were maintained the position of the buds could be modified. Thus, while usually buds appeared at the distal callus and roots at the proximal, if the formation of the distal callus was repressed by some means and the shoots were supplied with plenty of water, numerous buds arose at the basal callus; inverted cuttings formed a few buds on the proximal callus. If, however, the distal callus, now the lower, stood in water, then 75 per cent of the cuttings produced buds from the proximal callus. Tittman (*166*) had similarly shown for Populus that with repression of the distal callus and with the removal of the axillary buds, adventive buds could be obtained from the basal callus.

Plett[8] noted in his extensive experiments with internodes that, although the roots almost invariably appeared at the proximal end of the cutting, the buds seemed to arise anywhere. Only in *Salvia sylvestris* and *Acanthus mollis* were the buds confined to the distal callus; in several species, including *Boltonia latisquama*, *Physostegia virginiana*, and Nicotiana, most buds were formed upon the basal callus. Gravity and light seemed to produce very little effect upon the position and production of these buds, but moisture exerted a

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[8] See footnote 6 on p. 31.

very marked influence. Plett concluded, as Simon was inclined to do before him, that the marked polarity shown by regenerating roots as compared with the irregularity in position of the regenerating buds might be connected with the relatively different origins of roots and shoots. As has been seen, the buds are almost always exogenous in origin, but the roots, which are generally associated with the vascular cambium or pericycle, are endogenous. Plett, therefore, suggested that the inner tissues of the plant seem to be constructed with a polarized, axial symmetry, whereas the outer layers and the tissues of the callus are not polarized.

From the standpoint of anatomy the differences in the distribution of buds and roots upon these internodal cuttings become somewhat clearer. A considerable amount of unpublished work has been done at Leeds upon internodal cuttings, partly upon herbaceous plants such as Helianthus, Phaseolus, Vicia, and Pelargonium, and partly upon woody plants such as Acer, Ligustrum, and Malus. In all this work the general truth of Simon's conclusion (*144*) has been confirmed, namely, that the callus usually differs in origin as well as in behavior at the opposite ends of isolated pieces of shoots. (Pl. 9, A, B.)

At the proximal end of shoot cuttings, from the very early stages, the vascular cambium is intimately associated with the production of the mass of new tissue. The cushion of tissue thus arising at the base is largely formed by the cambium ring through tangential divisions which cut off cells to the inside of the cambium, and just as also occurs in the normal activity of the cambium many of the cells thus formed differentiate into short tracheids or vessel segments. Frequently at the base of the shoot the wood thus formed differs materially from the normal wood in having fewer vessels and more parenchyma cells; the vessel segments formed are also shorter and have a narrower lumen. This type of wound wood may be limited to the very base of the shoot or it may not be detected at all; it corresponds very closely with the type of wood frequently formed in the neighborhood of ringing wounds (*79*, *156*). In addition to this "dynamic wedge," as Hartig called it (*54*), the cells of the cortex, phloem, wood parenchyma, and pith may undergo a certain amount of extension and division, giving rise to a tissue which is carried outward along with the main mass of tissue arising from the cambium.

The fleshy roots of Crambe, as indeed of many other types of isolated root pieces, show much less indication of such cambial activity at the equivalent, i. e. distal end. As has been already brought out, while callus was formed vigorously over the whole surface, the cambium playing the leading rôle in such activity, it was formed rather by divisions parallel to the surface and not by divisions with the orientation usual in the cambium.

At the surface of the callus formed at the basal (proximal) end of the shoot cutting, even though arising originally from the vascular cambium, another meristematic layer soon emerges just below the surface. In this layer, as has been seen, divisions take place by walls parallel to the surface, giving rise either to cork or to callus. From this phellogen buds frequently appear; in the callus of a fleshy root such as seakale, this layer is visible very early and bud production is both early and widespread. On the other hand, as was originally pointed out by Stoll (*154*) the origin of the roots can usually be found in the neighborhood of the layer of dividing cells which is still genetically connected with the vascular cambium; they are thus in most cases distinctly endogenous.

At the distal end of the shoot cutting in many cases callus production is very slight indeed. If, as commonly happens, the atmosphere around the cut surface is not saturated with moisture, the sap originally injected into the intercellular spaces beneath the cut surface seems to be rapidly absorbed, so that the original fatty deposits which give rise to suberized membranes are very irregular and often well below the surface. Even if the air is saturated and callus does appear, as Swingle (*162*) and several others have pointed out, this is less than that arising at the basal end. More important in this connection, however, is the fact that while the tissues adjacent to the cut may give rise to a callus by cell extension and multiplication, the production of a regular "dynamic wedge," such as that occurring at the basal end by the tangential divisions of the cambial cells, has never been observed at the distal callus, although the individual cells in the region of the cambium may be especially active in the process of callus formation. The new cell divisions are irregular and occur mainly by walls parallel to the cut; hence none of the new tissue can be regarded as the direct result of the normal activity of the vascular cambium. A further important point is the fact that none of this tissue shows any tendency at this stage to differentiate into xylem, as it does at the basal end; in internodal shoots of woody plants practically no xylem formation, either of the nature of wound wood or of the normal type, has been observed in the Leeds experiments to occur at the distal end of woody cuttings.

In the distal callus a phellogen also appears near the surface, and in this buds may arise. Xylem formation in this callus then occurs in connection with the procambial strands of these new buds as they differentiate. Hence, although buds may appear at the surface of the callus formed at this end of the cutting, or occasionally at the other end as well, in view of the fact that the origin of new roots seems to be so closely connected with the activity of the vascular cambium it is not surprising to find roots confined to the basal end.

Likewise, in cuttings of fleshy roots, as has been said, cambial activity at the corresponding proximal end of the cutting is also less than that occurring at the distal end, and root production from this end of the root piece very seldom if ever occurs from such wound tissue. Anatomy thus seems to throw a little light upon the more extensive distribution of buds in these isolated shoot pieces as compared with the localized production of new root initials.

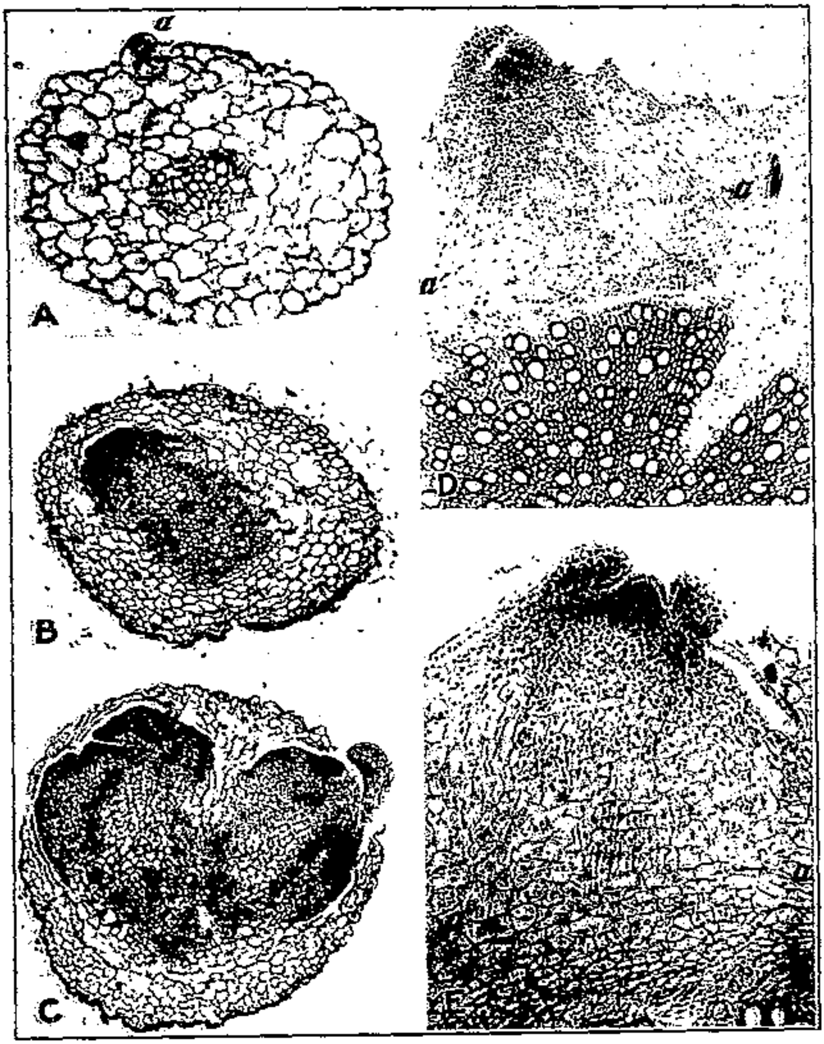
ADVENTIVE BUDS UPON THE HYPOCOTYL

The dicotyledon seedling usually bears its first buds in the axils between cotyledons and epicotyl, but in a number of seedlings buds frequently occur upon the hypocotyl, below the cotyledons; in many other cases, though buds do not normally occur in this position, they may also be formed upon the hypocotyl as the result of special treatment (*10*, *18*, *81*). Extra-axillary buds occur here far more fre-

PLATE 9

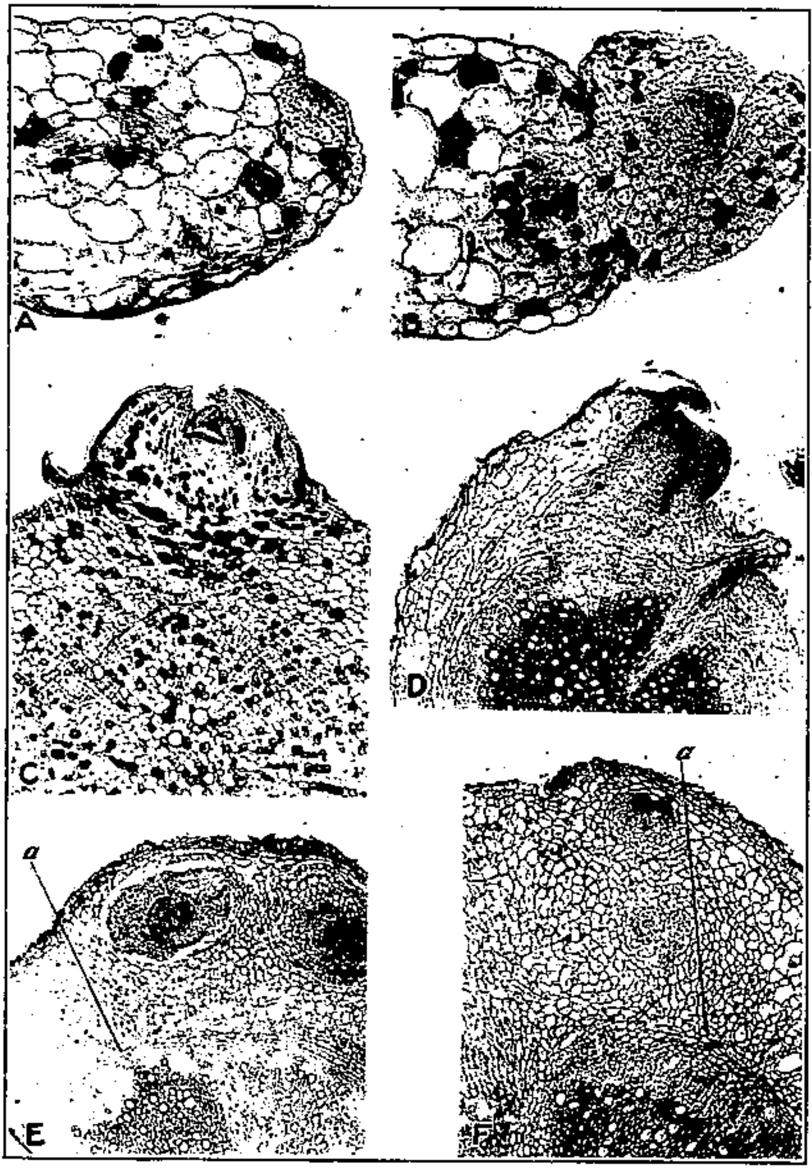
A and B.—Longitudinal sections of the callus from an isolated internode of Forsythia: A, Distal; B, proximal. In A, the callus has formed mainly by transverse divisions and subsequent cell proliferation of the parenchymatous tissues of pericycle, phloem, and cambium. In B, note the active part taken by repeated longitudinal tangential divisions of the cambium, so that new tissues, with differentiating tracheids, have been cut off toward the xylem. The cambium lies around an arc of tissue so produced. This characteristic cambial activity does not occur at the distal end. × 55.

C.—Longitudinal median section of a root of *Rumex acetosella* through a lateral structure which, although originally organized as a shoot apex, owing to experimental treatment has grown forward as a root apex. At the base of this lateral root small scale leaves are visible (*a*). The apex, however, now has a typical root meristem. × 30.



FORMATION OF ADVENTIVE SHOOTS ON HYPOCOTYLS AND ROOTS (TRANSVERSE SECTIONS)

A.—Hypocotyl of *Linaria macedonica*. The beginning of an epidermal bud is visible at *a*. × 100.
B and C.—Seedling roots of *Convolvulus arvensis* just below the hypocotyl showing stages in emergence of endogenous buds. The procambial strands in these buds are differentiating backward from the apex of the buds toward the vascular system of the root. The buds lie opposite protoxylem groups in the root. × 45.
D and E.—Root of *Linaria repens*, showing exogenous buds arising in the neighborhood of branch roots. Especially in E, the centripetal development of the procambial strands is clearly indicated. In each of these sections, in the region just below the bud, a gap is visible in the primary endodermis of the main root. The endodermis is each case is indicated by the arc *a*, *a*. × 100.



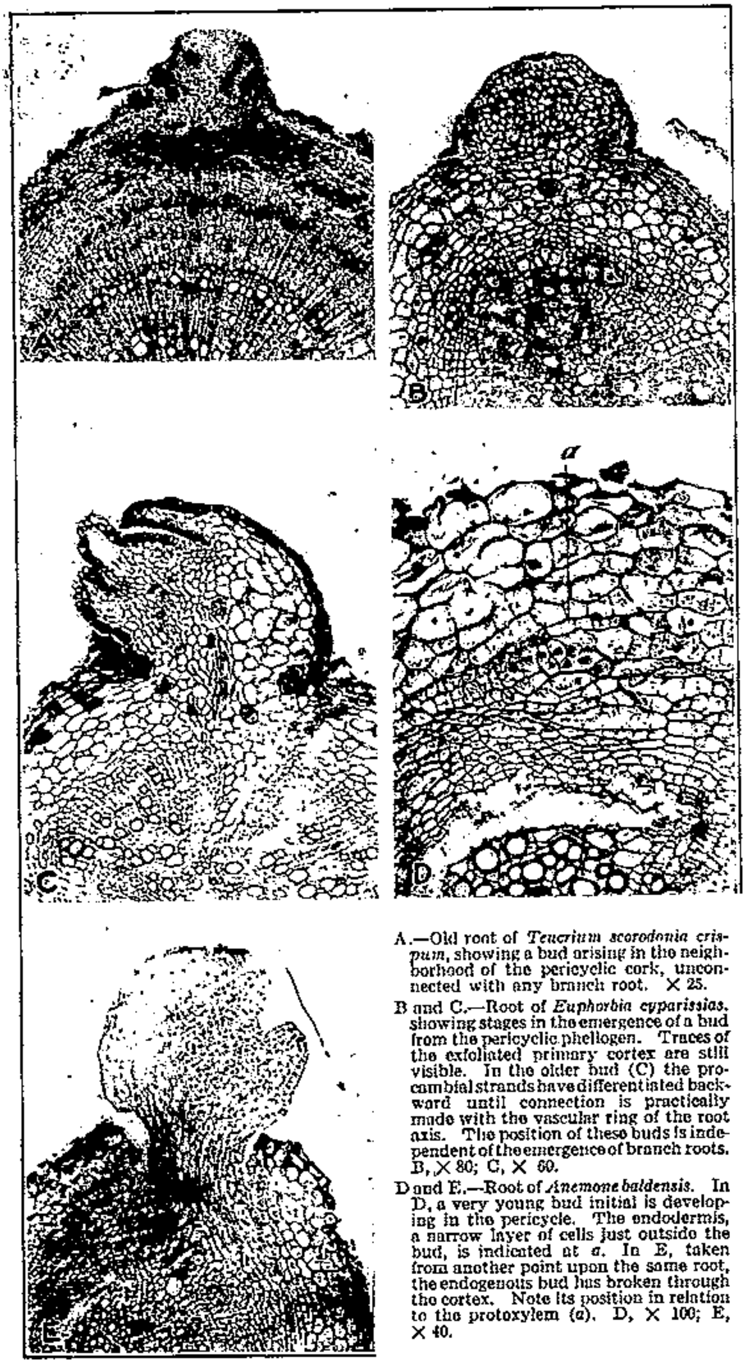
Adventive Shoots on Roots and Leaves

A and B.—Leaf of Bryophyllum, showing adventive buds forming near the margin of the leaf: A, very early stage (× 70); B, with first leaves developed (× 80).

C.—Root of *Epilobium angustifolium*, showing polyderm at the surface of the root (the primary cortex is exfoliated) and a bud arising from just within the polyderm. This bud actually arises near the base of a branch root. × 45.

D.—Root of *Linaria repens*. The procambial strands of the exogenous bud lying to the left of the old branch root have differentiated through the primary cortex back to the region of the vascular cambium. × 50.

E and F.—Root of *Helenium autumnale*, variety Riverton Gem, showing buds differentiating in the primary cortex outside the endodermis (*a*). These buds lie just within the exogenous phellogen. E, × 50; F, × 35.



A.—Old root of *Teucrium scorodonia crispum*, showing a bud arising in the neighborhood of the pericyclic cork, unconnected with any branch root. × 25.

B and C.—Root of *Euphorbia cyparissias*, showing stages in the emergence of a bud from the pericyclic phellogen. Traces of the exfoliated primary cortex are still visible. In the older bud (C) the procambial strands have differentiated backward until connection is practically made with the vascular ring of the root axis. The position of these buds is independent of the emergence of branch roots. B, × 80; C, × 60.

D and E.—Root of *Anemone baldensis*. In D, a very young bud initial is developing in the pericycle. The endodermis, a narrow layer of cells just outside the bud, is indicated at *a*. In E, taken from another point upon the same root, the endogenous bud has broken through the cortex. Note its position in relation to the protoxylem (*a*). D, × 100; E, × 40.

FORMATION OF ADVENTIVE SHOOTS ON ROOTS (TRANSVERSE SECTIONS) quently than on the epicotyl, while, as will be seen later, adventive buds upon the root system just below the hypocotyl are of still more frequent occurrence. It is probable that factors governing the appearance of buds upon the hypocotyl may be very similar in nature to those determining the appearance of buds upon roots, and it may be profitable to bear in mind the facts presented in this brief section in connection with the data dealing with the production of buds upon roots.

A number of plants bearing such hypocotyledonary buds are considered in Beijerinck's monograph (*11*) where references to the earlier literature will be found. A few examples may be discussed to illustrate the points that have already emerged from anatomical study, but many more data are still needed.

Anagallis and Linaria show examples of hypocotyledonary buds which are epidermal in origin. (Pl. 10, A.) But though the first cells to become meristematic are epidermal, as Van Tieghem (*169*) pointed out long ago, the position of the buds seems to be determined by the arrangement of the vascular system. In diarch roots the branch roots appear in four rows, one on each flank of the two protoxylem groups, and when buds are found they appear in the same position. In the hypocotyl, similarly, the buds are formed opposite the primary vascular rays, though with the few scattered buds usually found it is difficult to say whether they form in four rows. Although the first cells to become meristematic are those of the epidermis, the subsequent growth of the bud involves the gradual extension of this meristematic tendency into the inner tissues. First the cells of the cortex lying just inside the superficial meristematic group and then the cells of the endodermis and pericycle become meristematic, and thus a path of meristem is formed all the way between the vascular system and the new superficial meristem. In this meristematic sector the procambial strands differentiate, and from these the vascular elements. Thus the vascular connection with the main stele is made in the way that throughout has proved characteristic of the shoot organization. This centripetal meristematic differentiation below the original superficial meristem of the hypocotyledonary bud was clearly described by Van Tieghem. It is illustrated for the exogenous buds upon the root of *Linaria repens* Mill. (pls. 10, D, E; 11, D), where an exactly comparable form of differentiation is displayed. The exogenous position of these buds, in view of the existence of a functioning secondary endodermis around the stele within, is a matter of considerable interest. Unfortunately, there are not sufficient data to discuss this problem in reference to the hypocotyl; it must suffice to point out that the buds are similarly exogenous in the root of Linaria, whereas in *Convolvulus arvensis* L., where the buds arising upon the hypocotyl are clearly figured by Beijerinck (*11*) as being endogenous, those arising on the root are likewise endogenous. (Pl. 10, B, C.) This difference in bud position on the root is discussed in a later section, and for the present it can only be assumed that the same factors operate in determining the position of hypocotyledonary buds as those determining the position of root-borne buds.

Boodle (*14*) illustrated a similar centripetal differentiation of the vascular supply of the adventive leaves arising upon the hypocotyl, or seedling tuber, of *Cyclamen persicum.*

ADVENTIVE BUDS UPON THE LEAF

The appearance of buds upon isolated leaves is obviously only a special case of their appearance upon shoots. Although the usual position in which buds occur upon the shoot is generally in the axil of the leaf, cases have been described where they appear elsewhere on the axis as a regular feature, notably upon the hypocotyl. Similarly, buds may occur normally upon the leaf. A number of such cases among the monocotyledons have already been referred to. Although there is usually not the same persistent meristematic base in the dicotyledon leaf, buds here also occur upon the leaf, always upon the upper surface. Usually they are to be found at the junction of the leafstalk and lamina, as in *Tolmiea menziesi* Torr. and Gray (*129*), *Cardamine pratensis*, *Radicula nasturtium-aquaticum* (*Nasturtium officinale*) (*52*), and others. In Bryophyllum they are found in the serrations, apparently at the margin of the leaf, but actually still on the upper surface. (Pl. 11, A, B.) The shoots thus found normally upon the leaf show various degrees of development, from that of a well-organized shoot apex to but a small group of meristematic tissue in the case of Bryophyllum. In any case the further development of this meristematic apex usually depends upon the separation from the parent plant of the leaf bearing it, and as a result very extensive series of experiments upon the internal correlating factors controlling growth have been carried out with such material, particularly with the leaves of Bryophyllum (*41*, *90*). Such studies have, however, dealt but slightly with the anatomical aspects of the problem.

When leaves without buds are isolated, in many cases adventive shoots are formed upon the isolated structures. The most extensive series of such experiments recorded in the literature appear to be those of Lindemuth (*88*, *89*) and Stingl (*153*). These have been summarized by Janse (*66*) as follows: Species whose leaves give neither roots nor buds, 47 and 43 per cent; species giving only roots, 52 and 46 per cent; and species giving buds with or without roots, 5 and 15 per cent, according to Lindemuth and Stingl, respectively.

Probably the difference between these percentages as given by the two different workers is mainly due to the fact that Stingl included 20 species of monocotyledons in his trials, and Lindemuth only 1, and monocotyledons never seem to form roots alone, their adventive shoots always being accompanied by roots.

When bud initials are thus organized anew, they practically always appear toward the basal (proximal) end of the isolated leaf system, usually at the base of the leafstalk or at the junction of petiole and blade. Winkler (*185*) described in some detail the widespread production of buds over the upper surface of the leaf in *Torenia asiatica* L., regarding their extensive distribution in this case as a striking phenomenon; but even here the buds do not occur near the margin at the apex. Nevertheless, there seems to be considerable latitude in their place of appearance, and Winkler's attempt to create different categories of buds according to their position does not seem to help in the understanding of this widespread distribution. In cases where veins were cut through in leaves lying on moist sand or other suitable propagating material, buds also frequently arose at the end of the vein, just above the cut.

The details of the development of adventive shoots arising on the leaf are particularly well known for Begonia from the work of Regel (*124*), Vöchting (*175*), Hansen (*52*), Sachs (*133*), and Hartsema (*56*). In this case the bud is often described as arising from a single epidermal cell, but this description may be misleading; it is only true in the same sense that in the development of the bud upon the hypocotyl (p. 35) the bud may first arise in one epidermal cell. This cell divides first by a wall parallel to the surface of the leaf, but Hartsema pointed out that in *Begonia rex*, although this division occurred by the third day, in some cases by the second day some of the mesophyll cells below had already divided.

No noticeable increase in size takes place in the epidermal cell, but changes in cell contents are continuous, and Hartsema observed protoplasmic streaming in sections of living leaves mounted in sugar solution. These movements were associated with a gradual increase in the amount of protoplasm present and with a shifting of the moving stream of protoplasm from a rotation around the wall to a circulation in and out of the center. With these phenomena was observed a transference of the nucleus toward the center of the cell, a movement suggesting an increase in the relative density of the cytoplasm. Cell divisions continued until ultimately the original epidermal cell, with its outline still clearly discernible, was seen to be filled with a mass of small, densely meristematic cells. To this extent the new shoot is the outcome of a single cell.

But now the cells of the mesophyll beneath this active epidermal cell were seen to be changing in the same way and likewise becoming meristematic; it is this differentiation which permits of the subsequent linking up of the new shoot with the vascular system of the original leaf. The possibility of the occasional complete formation of the new shoot from one cell is not excluded, because, if one of these groups of meristematic tissue formed within a single cell should be isolated from the leaf, it is probable that under some conditions it could provide itself with a root; usually, however, in the leaf the cells lying between the epidermis and the vascular bundle cooperate in the production of the new shoot, which may thus depend for a time upon the roots produced upon the vascular system of the leaf in a manner described in a later section (p. 64).

It is essential to distinguish sharply, however, between the mesophyll cells being active in the formation of the vascular connections at the base of the new shoot, and such inner tissues taking part in the organization of the growing point itself. In most cases this distinction seems to be of little value; yet in the case of periclinal chimeras and certain variegated plants, which may also be, in many respects, periclinal chimeras, this genetic constitution of the growing point assumes a great significance. The junior writer has given a review of the subject of graft hybrids (*160*).

Beinling (*12*), in describing the formation of buds upon isolated leaves of Peperomia, where the buds arise from cells of the ground parenchyma close to the cut surface of the leafstalk or the leaf blade, pointed out that these buds when quite young are often cut off by cork from the tissue of the parent leaf and in that case never become linked with the vascular bundles of the leaf. When this occurs, the cells giving rise to the new shoot are always closely associated with the phellogen appearing beneath the cut surface.

Similarly, in *Torenia asiatica* the adventive shoots are described by Winkler (*185*) as commencing their origin with changes in a single epidermal cell. In fact, in all cases referred to in the literature of adventive shoots arising upon leaves there is nothing to modify the general impression that such shoots appear as exogenous structures, their linkage with any existing vascular system always being the result of subsequent differentiation beneath the original superficial meristem. In this respect, as reference to page 64 will show, there is a very clear distinction between the mode of origin of adventive shoots and that of roots upon leaf cuttings.

An interesting point about leaf cuttings is their apparent inability in many cases to form shoots, though they may root and remain alive for many months.

Although a number of isolated cases are known, the majority of species of dicotyledons which readily produce adventive shoots upon leaves are to be found in the families Droseraceae, Crassulaceae, Begoniaceae, and Gesneriaceae (*11*). As Küster pointed out (*81*), almost all these plants possess a very succulent type of leaf, the mesophyll consisting largely of a dense mass of vacuolated parenchyma, with comparatively small intercellular air spaces. The ability to form leaf buds is probably intimately associated with this type of structure, because when such leaves are isolated and held under moist conditions the injury may readily be followed by the injection of these small intercellular spaces with sap, at first directly in the vicinity of the injury and subsequently elsewhere through the injection of air spaces in the neighborhood of veins which become overcharged with sap. Under these conditions, circumstances may readily become favorable (1) for the increased nutrition of epidermal or subepidermal cells, with a tendency for them to become meristematic, and (2) for the subsequent differentiation of the tissues beneath these epidermal groups and hence their linking up with the main vascular supply.

In the majority of leaf petioles, while the intercellular spaces are frequently smaller and injection may more readily occur, Lohr (*91*) found that secondary changes were much more marked. Cambial activity in the vascular bundles was strikingly manifested, in many cases a closed ring of cambium and vascular tissue being formed where normally only a broken circle of bundles would have been present. Phellogen activity was often seen also, sometimes occurring sporadically at the bases of trichomes, but in other cases as a continuous epidermal, subepidermal, or deeper seated layer, in petioles where normally phellogen does not occur. This observation, coupled with that of Beinling (*12*), as to the source of adventive shoots in Peperomia and with the close connection seen to exist between phellogen and adventive shoots, should further emphasize the need for caution in the interpretation of negative results as regards adventive-shoot production in leaf-propagation experiments. To take a specific example, Lohr (*91*) reported negative results as regards bud production with the leaf of *Iresine lindeni*, though the leaf rooted in one month and an abundant phellogen activity was evident after two months. On the other hand, Lindemuth (*89*), who noted particularly the cell enlargement shown in the lamina of this same type of leaves (from 10 by 12 cm. to 12.5 by 15 cm.), obtained buds. Hence as long as tissue can be stimulated to such superficial activity as phellogen production, it seems unwise to give up hope of obtaining buds. *Camellia japonica* is frequently cited as another good example of a leaf that will produce only roots, but Janse (*65*) noted an exceptional case where buds were also obtained.

Furthermore, when roots are obtained, the roots themselves may provide the future buds. Vöchting (*176*) described the following interesting case: In *Thladiantha dubia* Hook. a tuberous swelling appeared at the proximal end of the root which had developed from the leaf, and from this swelling a bud arose. Winkler (*186*) depicted a similar case, two buds being obtained from the base of an isolated tendril of *Passiflora caerulea.* The tendril coiled spirally and became woody, and then a white calluslike swelling developed at the base of the tendril; after about three months a root grew out from this swelling, and many months later the buds also appeared upon it.

In this connection it is perhaps worth while drawing attention once again to some old observations published by Agricola in 1716 (*1*). The leaves of apple, cherry, walnut, and chestnut were taken, their cut ends were covered with wax, and the bases of the leaves were buried to a third of their length in moist earth. The leaves gradually decayed until little but the midribs remained. But at the base of the midrib of some of the leaves a swelling developed from which roots arose, and in a year a small shoot likewise appeared. Attempts made at Leeds to repeat these observations have not been successful, but the soil-moisture and oxygen conditions under which success is possible are evidently quite limited, and, as has been emphasized, the difficulties in the way of propagation tests lead the writers to attach much more importance to recorded positive experimental results than to unsuccessful attempts at repetition.

The suggestion put forward above as to the anatomical factors contributing to success in obtaining the formation of buds throws some light upon a number of somewhat isolated observations upon propagation from cotyledons. Smith (*148*) carried out a very extensive series of observations, confined solely to this type of leaf, and in no case observed an adventive shoot, although the cotyledons frequently increased in size considerably beyond their normal dimensions, and roots developed in many cases. On the other hand, Küster (*81*), with three genera of Cucurbitaceae (Cucumis, Cucurbita, and Luffa), and Hill (*60*), with Cyclamen, obtained adventive shoots from cotyledons, although normal leaves of these genera have furnished no known examples of adventive shoots. In these four genera the cotyledons contain smaller intercellular spaces than do the later formed assimilating leaves, and it is probably in this fact, rather than in their greater food reserves, that the explanation for the more ready production of buds in the cotyledons is to be found. Thus, the production of buds upon hypocotyls is increased rather than decreased by the removal of the cotyledons. Likewise, with isolated green leaves left in the light in the propagating frame, there is no reason to think that supplies of assimilates fail or that development is hindered for lack of food. On the contrary, active growth may be proceeding in the leafstalk (*94*), while the leaf tissue is gorged with starch, and roots may have long since appeared; but still in many leaves buds are not produced. Consideration of these special cases of adventive-shoot production upon leaves seems, therefore, to strengthen the standpoint already arrived at as to the importance in shoot production of the existence of internal conditions favorable for the growth of a superficial meristem and for the subsequent differentiation of the vascular system beneath it.

SHOOTS UPON ROOTS

Apart from the normal axillary buds and those originating in close connection with the axillary meristem, adventive buds occur comparatively rarely upon the shoot. In only a few families are such structures produced upon isolated leaves, and their regeneration upon isolated pieces of shoot is comparatively rare; likewise it has been seen that their normal appearance upon the shoot elsewhere than in the leaf axil is very rare. Buds are more frequently found normally upon the hypocotyl than upon the epicotyl, and they can likewise be more readily induced experimentally upon the hypocotyl. In accordance with this general distribution, buds upon root systems are relatively of much more common occurrence; in many species they occur normally and in many others are more or less readily induced by wounding; hence with dicotyledons, methods of propagation making use of root-borne buds are commonly practiced.

Beijerinck's comprehensive monograph (*11*) contains most of the known facts as to the distribution of root buds, to which very little could be added as the result of more recent work. However, an attempt will be made here to examine from the standpoint of anatomy the facts as to the occurrence and distribution of root-borne buds. From this standpoint it immediately becomes clear that the whole problem of adventive-shoot formation upon the root is governed by internal structural factors which differ from those concerned in adventive-bud production on the shoot.

The Influence of Root Anatomy

In a previous section (p. 7) events occurring behind the growing apex of the root were considered up to the point where a central stele, bounded by an endodermis, has become distinct from the cortex; the latter is bounded to the outside by a few layers of cells, the radial and transverse walls of which are free from intercellular air spaces, and the outermost layer of which usually functions as the piliferous layer. The distinction in structure and function between stele and cortex is thus very sharp in the root. It was pointed out that within the stele the vascular elements, xylem and phloem, are differentiating. The walls of the xylem elements are composed of lignified, permeable cellulose; within them there is no semipermeable membrane, and undoubtedly the aqueous sap present in the xylem moves freely into the walls of all the surrounding tissues up to and including the inner tangential walls of the endodermis. But, as has been brought out, the flow or diffusion of the sap outward along the radial and transverse walls of the endodermis is prevented by the Casparian strip.

Since the endodermis forms a continuous cylinder, one cell thick, extending from the meristematic apex of the root up to the region of the hypocotyl, it may be visualized as a chimney in which the bricks are represented by the living protoplasts and the intervening mortar by the Casparian strips. (Fig. 1.) A striking feature brought out by microchemical work upon the root is the marked consistency with which the endodermal protoplasts adhere to the Casparian strip. They may be pulled away from other portions of the wall by plasmolyzing agents, or in dead material by strong acids, but even under such conditions it is only with great difficulty that the protoplasts can be separated from the Casparian strip, and in life it seems doubtful if they are ever so separated (*119*). The result is that, although the vascular system and parenchymatous tissue of the stele may be charged with water and solutes under pressure (when the absorbing system of the root is at work), this system does not leak, because it is bounded by this endodermal chimney in which both flow and diffusion outward through the walls are completely prevented.

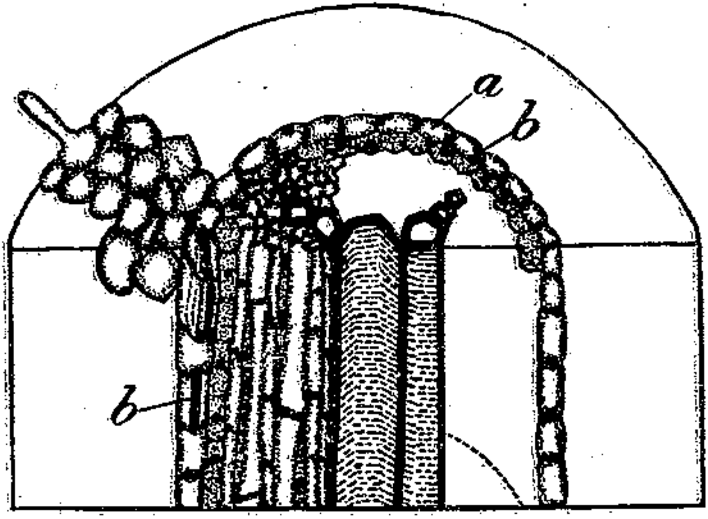


FIGURE 1.—Diagram of a young root as seen in transverse and median longitudinal section. The vascular elements are inclosed within the cylinder of the endodermis (*a*). Sap may move outward from the vascular elements until the endodermis is reached; then further outward movement is stopped by the fat-impregnated Casparian strip (*b*), which is continuous around both radial and transverse walls of all the endodermal cells

Since transference of water and solutes between cortex and stele takes place only across the living protoplasts of the endodermis, movement through these protoplasmic membranes will be practically confined to diffusion. This molecular movement through the walls will be largely determined by the osmotic gradient between the inner and the outer tangential wall. With the inner wall containing the xylem sap and the outer wall presumably containing nothing more concentrated than the soil solution, the movement of water through the protoplast would normally be inward and not outward. Of the actual movement of solutes through the endodermal protoplast, as yet nothing is definitely known. The subject has recently been discussed elsewhere in some detail (*142*). It seems safe to say that there is little likelihood of loss of solutes from stele to cortex occurring through these protoplasts.

Thus it is seen that in the young root a set of conditions is present which must greatly affect the question of propagation. Although the living cells of both cortex and stele may potentially be capable of meristematic growth, the solutes from the vascular supply, which alone can furnish the necessary nourishment, are almost completely confined within the stele, and hence any flow of sap capable of displacing air from the intercellular spaces is not possible outside the stele. The result is, as Tetley (*164*) pointed out, that growth activities in the root are confined within the stele and that the cortex of the root is singularly uniform in character throughout all the flowering plants.

Within the stele, the cells abutting upon differentiating vascular elements themselves in turn tend to undergo the same fate, and it is the cells farthest from the vascular elements—the pericycle—that longest retain their meristematic character (*117*). These pericylic cells seem to play a very important part in propagation.

In the growing root, as the nutrient supply available becomes capable of maintaining more meristematic activity than is represented by the terminal apical group, additional divisions take place in the pericycle by tangential walls, the number of layers of meristematic cells being thus multiplied. These regions practically always lie opposite the protoxylem, except in diarch roots or in those in which mucilage canals are developed just outside and exactly opposite the protoxylem groups. Van Tieghem (*169*), who first made this generalization, pointed out that it suggested a spatial relationship between this special meristematic activity and the xylem and phloem, of such a character that, when the radial sectors occupied by these adjacent tissues in the root were not too wide, the position of this extra meristem (opposite the protoxylem) left it contiguous to both xylem and phloem. On the other hand, in the diarch root, where xylem and phloem lie in radii 90° apart, a comparable position is obtained for the meristem only when it lies between the radii passing through the centers of xylem and phloem. Hence, while in roots containing more than two sets of bundles there are as many extra groups of meristematic activity as there are protoxylem groups, in a diarch root there are four groups, one on either flank of each group of protoxylem.

In the young root these extra groups of meristematic activity are usually soon organized as local root apices, which may grow out almost at once or which may lie dormant for some time.

ENDOGENOUS BUDS ON YOUNG ROOTS

In some cases these extra meristematic groups arising in the pericycle are differently organized from the outset and develop as shoot apices. In these cases the distribution of such buds follows the same laws as Van Tieghem (*169*) found for the branch roots. Beijerinck (*11*) cited *Prunus domestica*, *Convolvulus arvensis*, and *Ajuga genevensis* as having buds of this type in the young root, and he described them as replacing root initials; in certain other cases (*Sisymbrium alliaria* Scop. and *Anemone sylvestris*) buds of this type occur in the same vertical series as do the lateral roots. Buds arising in this manner are definitely endogenous in character, though such position of origin is usually regarded as characteristic of root initials; however, the difference between shoot and root can not be based upon their position of origin, for buds may be either endogenous or exogenous, while in the next section roots that are exogenous will be described. The difference between roots and shoots, whatever their origin, lies in their organization as growing structures.

The endogenous buds of *Convolvulus arvensis* and *Anemone baldensis* L. are shown in Plate 10, B and C, and Plate 12, D and E. It will be seen that the emergent bud with its leaf initials, even after it has burst through the cortex of the root, is still quite unconnected with the stele of the parent root by any vascular strand. Such a connection differentiates independently in the axis of the new shoot structure and then by a further centripetal differentiation becomes attached to the vascular system of the stele. In *A. sylvestris* and *A. hupehensis* the buds apparently always arise in the pericycle, no cork having been seen in these roots; in *A. baldensis* and *A. japonica* pericyclic cork occurs, and the buds sometimes originate in this and sometimes in the pericycle before the cork appears.

ROOT BUD SPORTS

The practical employment in propagation of such endogenous buds provides an opportunity of sometimes obtaining bud sports which, in general, can be understood from the standpoint of anatomy. In the shoot organization, as was pointed out, the dermatogen and one or more inner layers of cells divide continually by walls laid down at right angles to the surface. The result is that throughout the whole shoot a certain number of superficial layers covering stem, leaf, and flower are all the products of the activity of these same superficial meristematic layers. This mode of growth renders possible the existence of graft hybrids, which are periclinal chimeras, a "skin" of one parent incasing a core of the other. Also in many variegated plants, even though the inner and outer tissues may be of the same species, they may still be essentially this same type of periclinal chimera (*9*).

As long as these plants are propagated by stem or leaf cuttings, their character remains essentially the same. But whenever root propagation is adopted, if the new buds arise endogenously, a new situation is displayed. As has been seen, the sequence of events occurring at the root apex does not readily permit of the isolation of a series of superficial layers of meristem that form the whole superficial envelope of the root, because in every case the most external layers soon cease to be meristematic. But usually the activities of a fairly distinct dermatogen (or, better, protoderm) (*50*), through repeated divisions by walls at right angles to the surface, has given rise to the piliferous layer. Within these superficial tissues the pericycle, which is often regarded in histogenesis as the outermost layers of the stele, certainly has no connection with any of these external layers, and it is from this pericycle that the cells arise which form the dermatogen of the root-borne bud. Naturally, therefore, any special characteristics possessed by the "skin" layers of the plant are entirely missing from this endogenous root-borne bud, and the shoot system developing from it shows only the characteristics of the "core." Hence, where such buds can be obtained from roots, the student of graft hybrids or variegation has a method of analysis that may prove helpful in many cases. But it is necessary to ascertain anatomically that the buds are definitely endogenous in origin; as shown later in discussing certain root-borne Compositae buds, more variation is possible in this character than is usually suspected.

Even if the buds that emerge from a root system are exogenous in origin, however, it should presumably be the exception rather than the rule for the superficial layers of the new shoot to have any direct genetic connection with the surface layers of the parent shoot, since, in most cases of root-cutting propagation, the roots used will have had an endogenous origin (p. 60). As a result, none of the root system will have any genetic connection with the superficial region of the old shoot. Furthermore, even if the original root used as a cutting formed part of the true seedling root system, in which case the primary cortex possesses superficial layers that may be genetically similar to the superficial layers of the shoot, when the branch root system arises from this main root axis the cortex of the branch root will be formed by a meristem arising entirely from the inner layers of the main root. Only exogenous buds regenerated from the main axis of such a seedling root would therefore be likely to repeat in the new shoot any special characteristics possessed by the superficial layers of the parent shoot.

Endogenous buds arising in the pericycle at a relatively early stage of root development are comparatively rare; usually in the young root the first indication of additional meristematic activity in the pericycle is the organization of additional root initials. As the root grows older, but still at a comparatively early stage, another form of meristematic activity becomes apparent in the stele of the dicotyledon; in the parenchymatous or procambial tissues still persisting between phloem and xylem repeated divisions occur by tangential longitudinal walls and thus a vascular cambium comes into existence. As this layer is carried outward by the differentiation of the xylem elements recently formed to the inside, it is completed as a continuous ring by a series of tangential divisions occurring in the pericycle cells lying just outside the protoxylem groups. The vascular system of the root is now completely rearranged as regards the internal correlations likely to influence the future meristematic activities of the pericycle. As pointed out previously, the sap found in the xylem is usually at a very different hydrogen-ion concentration from that in the phloem. There is no doubt that in the young root, because of the character of the solutes that they contain, both xylem and phloem influence directly the behavior of the pericycle (*117*).

But after the vascular cambium has formed, a continuous sheet of meristematic tissue cuts off the xylem with its characteristic solutes from the pericycle without. Internally, the latter layer is now bounded partly by phloem and partly by parenchymatous ray tissue and no longer abuts upon any protoxylem. Hence it is not so surprising to find that from this time on the meristematic activities of the pericycle are different in character. After secondary thickening has commenced in the root, no further root initials originate in the pericycle. On the contrary, in this layer a phellogen frequently becomes active and cuts off a cork layer beneath the endodermis. This fact seems quite in keeping with the marked correlation, previously referred to (p. 16), between the proximity of phloem and the maintenance of a phellogen.

ENDOGENOUS BUDS AND PERICYCLIC PHELLOGEN

It has been seen that in very many cases shoot meristems are closely associated with phellogen; hence it is not surprising to find that the pericyclic phellogen occasionally gives rise to buds. Examples are *Teucrium scorodonia crispum* and *Euphorbia cyparissias*, which are illustrated in Plate 12, A–C. Other examples that have been noted at Leeds include *Ailanthus glandulosa* and *Coronilla varia*. In *Statice limonium* L. the buds also arise in the phellogen but near the point of origin of a branch root. In this connection see also the note as to the behavior of the thicker portions of the root of *Thladiantha dubia* (p. 49).

Buds arising in association with a pericyclic phellogen are obviously still endogenous in origin, but they frequently do not appear to be so, a fact that explains many misconceptions in the literature. Frequently, by the time the pericyclic phellogen has formed, the endodermis has undergone changes that make it completely impermeable to all outward movement of water and solutes. In any case, with the formation of a sheet of periderm all transference of materials between stele and cortex is precluded. As this stage is reached the cortex of the root becomes brown and withered. The stele continues to increase in girth as a result of the activity of the vascular cambium, and the withered cortical tissues, unable to adjust themselves to the increasing strain, crack and flake away, exposing the endodermis itself as a cracking layer; thus the cork phellogen from which the buds emerge appears to be the superficial layer of the root. Misled by this fact, Wilson (*183*) recently described the buds arising upon the roots of *Roripa austriaca* Spach. as exogenous in origin, but it is important to correct this misstatement. The phellogen of this root, from which buds arise, is pericyclic in origin, and the buds are endogenous. Below will be described several types of phellogen, which, being exogenously placed in the root, give rise to definitely exogenous buds. If clear cases of pericyclic phellogen are confused with these, the interpretation of the phenomena of bud sports arising on roots will be delayed and much unnecessary confusion created in a problem that is already sufficiently complicated.

The appearance of buds in a pericyclic phellogen is by no means a very common occurrence. Several cases have been referred to and a few others might be cited; to these must be added the similar cases in which buds arise from the meristem layer lying internal to a "polyderm." This tissue (*97, 114*) is in general much like periderm, but easily distinguished from it by the fact that all layers of cells do not give the characteristic suberin reaction with Sudan III. Although the cells of this tissue are arranged in radial series, not all the rings of cells are suberized. They seem to be formed by a succession of jerks of meristematic activity; the cells lying just within the innermost suberized layer start into activity and cut off radial series consisting of one or more cells, of which an outer cell becomes suberized while the innermost one remains alive and later resumes meristematic activity. Such an innermost cell, with its meristematic potentialities, is closely associated with the organization of the bud initial in the root of *Rubus idaeus*. (Pl. 13, D, E.)

When bud initials thus arise in the phellogen or the meristematic layer of polyderm, they are usually found opposite the primary rays of the root. It seems likely that this position is determined less by the original meristematic potentialities of the root pericycle at this point, opposite the protoxylem, than by the increased supply of solutes available opposite a parenchymatous stelar tissue as compared with that available in front of the sheet of prosenchymatous phloem elements, among which fibers are frequently included.

ADVENTIVE BUDS AND BRANCHING OF THE ROOT

By far the most common case of bud formation in the root and the one which is almost the invariable rule with uninjured roots, is the case in which the bud arises in connection with the emergence of a branch root. A brief consideration of the phenomena involved in the emergence of a branch root throws a great deal of light upon this fact.

The new root was described by Van Tieghem and Douliot (*172*) as digesting its way through the endodermis and cortex, but their evidence for the existence of this "secretory pocket" at the apex of the emerging root was very inconclusive; an alternative interpretation is that the root emerges by the crushing and disorganization of the cortical cells and tissues.

Although the statement that the young root secretes digestive enzymes and thus makes its way to the surface is still commonly repeated in the textbooks, almost all critical examination of the question has led to the contrary conclusion. Thus, Vonhöne in 1880 (*178*), reported that while both enzymatic secretions and mechanical pressure played a part in the process, mechanical causes were mainly responsible. Pfeffer (*107*) came to the conclusion that the outward thrust of the root was purely mechanical, as also did Peirce (*105*). Lenz (*87*) more recently examined the same problem very fully and came to the same conclusion. Evidence as to the effectiveness of internal secretions in this case needs very careful examination; in the light of wider knowledge of the effects of pressure upon parenchymatous tissues (*67*, *68*) it is realized that under compression the intercellular spaces in the cortex become filled with sap, and as a result cell division such as noted by Peirce might occur in front of the advancing tip.

One very strong argument against digestion as a method of emergence is the existence of the Casparian strip. Microscopic observation shows the endodermal layer stretched and compressed in front of the advancing tip. The digestion of the endodermis involves the production of an enzyme or hydrolyzing agent capable of breaking up the varnishlike substance impregnating the Casparian strip. This substance resists concentrated sulphuric acid and in the test tube is broken down only by prolonged boiling in strong alkali. No enzymes capable of digesting such a substance are known, and it seems certain that the Casparian strip must be broken through by mechanical means. However, once this resistant, impregnated region of the wall is ruptured, the pressure exerted would be ample to break through the other walls intervening between the root apex and the exterior. Undoubtedly, therefore, mechanical pressure is adequate to force the root apex to the surface if the endodermis is once broken. The assumption of a special secretory apparatus seems unnecessary, and the evidence for its existence is as yet quite inconclusive.

This point has been argued in some detail because the general conclusion is of rather startling significance. In the unbranched root the vascular sap necessary for any prolonged meristematic activity is strictly confined within the stele, but the emergence of a branch root means a forcible and quite possibly a sudden break in the continuity of the endodermis. Such a break may be accompanied by a leak of sap from the endodermis into the cortex in the neighborhood of the gap.

That such leaks actually do occur seems clearly indicated by the fact that the region on the parent root around the base of the branch is frequently characterized by active cell division and proliferation, resulting in calluslike outgrowths. As Devaux (*29*) showed, such a callus is frequently the seat of a certain amount of phellogen activity, and Beijerinck's observations (*11*) showed that when buds occur normally upon the root system they are nearly always found on this callus, and usually very intimately associated with the cork phellogen when such is present.

The figures in Beijerinck's paper (*11*) clearly illustrate the great diversity exhibited by these calluslike growths occurring around the base of branch roots. The subterranean position in which such callus grows and the difficulty in obtaining undamaged specimens have caused the very wide distribution of this morphological character upon the roots of the dicotyledon to be but little suspected. The development of these curious cushions of tissue around branch roots deserves far more extended investigation than it has yet received, and its discussion in relation to the present problem must necessarily be confined to a mere indication of certain of the more salient features in its relation to bud formation.

It is obvious that if this tissue receives a free supply of sap from the root stele, where sap is often contained under pressure, it may here flow out along the walls, displacing the air in the intercellular spaces, so that the supply of water and solutes ultimately reaches the external walls of the cushion and in some cases even leaks from them. Root excretions have been reported by different observers, although the evidence as to their occurrence is contradictory. Unfortunately, in such physiological inquiries there seems to be not a single reference to the morphology of the root system under observation. Clearly the possibility seems very great that such root excretions may synchronize with the rupture which occurs when the branch roots emerge. The supply of solutes thus brought to the surface of a rapidly proliferating calluslike tissue has also great significance from the standpoint of plant pathology. The special vulnerability of this particular point of the root system to the entry of pathogenic organisms seems obvious (*190*).

Sometimes this calluslike cushion develops as a protuberance above and below the base of the branch root; at other times it is more developed on the sides, while frequently it forms a ring around the emerging rootlet. The cushion may be small and barely visible to the naked eye, though usually it is clearly discernible when the root is cleaned free from soil; in some cases, like *Populus alba*, it grows into a corallike mass several centimeters in thickness, from which buds may apparently arise at any point. On the smaller cushions the buds may be quite regularly arranged. Sometimes a single bud is present at each branch, as in *Epilobium angustifolium*, where the bud at its emergence seems to be closely related to the meristem lying just within the polyderm. (Pl. 11, C.) In other cases, such as Linaria, several buds may be formed at the same time upon a single cushion, though only one is shown in Plate 11, D.

It has been seen that some significance attaches to the point of origin of the bud and that it is desirable to be clear as to whether its origin is endogenous or exogenous. With the development of buds upon the callus formed around the base of branch roots, this point may be very difficult to determine. There are here two separate problems to consider; the origin of the cushion itself and the point of origin of the bud upon this cushion. In most cases the first branch roots appear when the parent root is young and the primary cortex still healthy. As the branch root emerges, the tissues of the primary cortex around its base may be stimulated into meristematic activity, sometimes forming phellogen, sometimes bud initials. Thus in Linaria buds arise exogenously from the cortex but apparently only in association with the break in the endodermis produced by the emergence of lateral roots. So far as exogenous buds found upon the hypocotyl in this genus have been examined, the evidence seems to indicate that in the hypocotyl also the development of exogenous buds is closely connected with breaks in the endodermis and in some cases at least here also caused by the emergence of lateral roots.

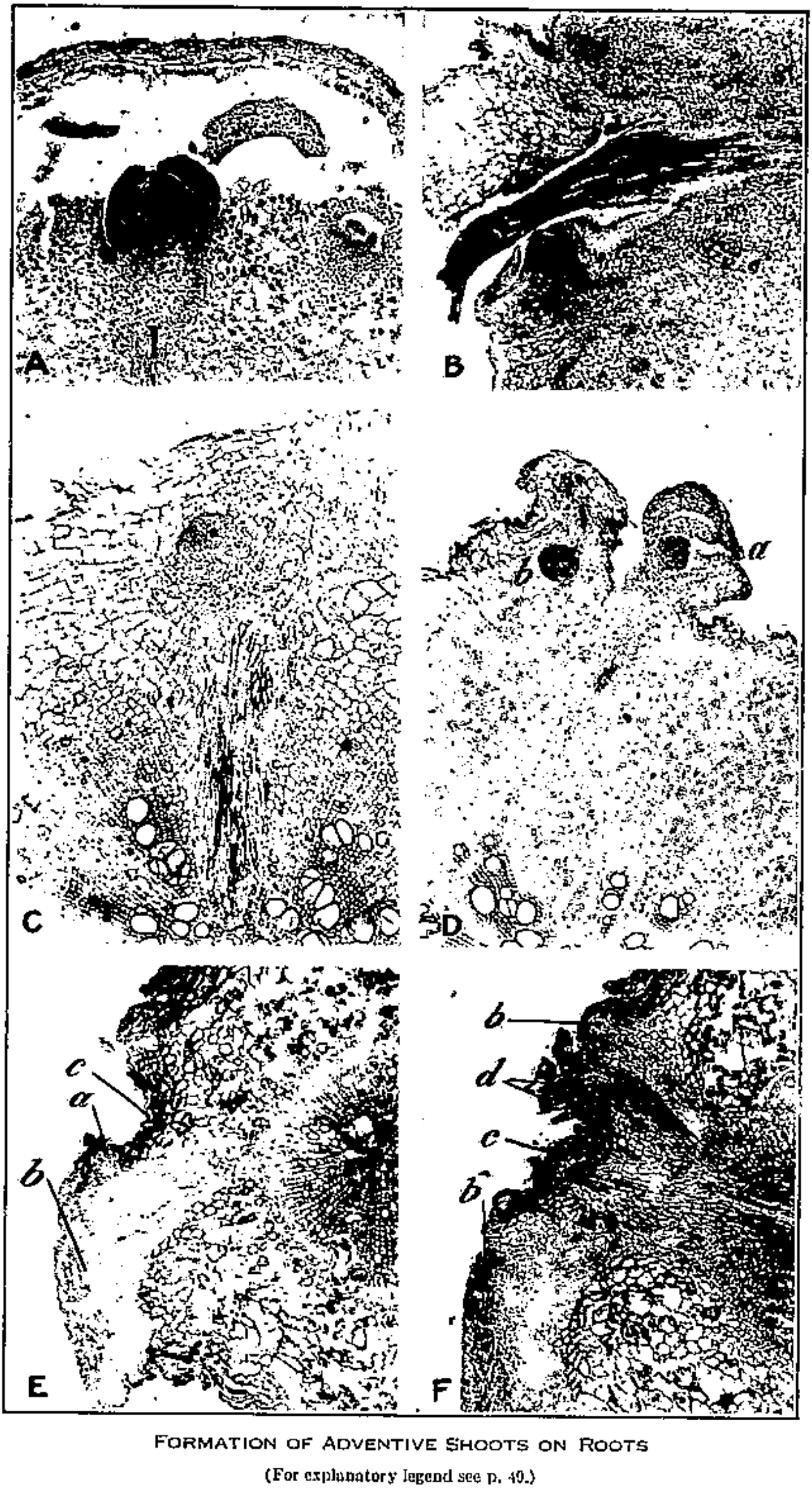
Similarly, Plate 11, E and F (*Helenium autumnale* variety Riverton Gem) shows bud initials developing in the primary cortex of the old root, well outside the endodermis; these are formed only near the base of a branch root. In the larger bud (pl. 11, E) it will be noticed to what an extent the development of the shoot apex and leaf initials has proceeded without there being any indication of the procambial differentiation which will ultimately connect the buds with the stele of the root.

In many cases, however, either the branch roots appear later and traverse a shriveled cortex in which no response to leakage of sap from the stele is possible, or a pericyclic phellogen prevents the movement of materials outward into the primary superficial tissues. In such cases a vigorous cushion of tissue may still be formed around the base of the emerging rootlet, but it arises mainly if not entirely through the activity of the pericyclic phellogen.

The photographs of *Barbarea vulgaris*, *Isatis tinctoria* L., and the Princess variety of Pelargonium (pl. 13, A–C; pl. 14, E, F) illustrate cases of this type where the bud seems traceable to tissues which are closely associated with the pericyclic phellogen. In *Asclepias incarnata* the cork is exogenous (p. 52), but the buds form below the cork phellogen. (Pl. 14, A.) They are clustered around the branch root and appear to be endogenous, in contrast to the definitely exogenous examples furnished by Linaria and Helenium.

FORMATION OF ADVENTIVE SHOOTS ON ROOTS

A and B.—Root of *Barbarea vulgaris*, showing adventive buds arising in the pericyclic phellogen near the emergence of a branch root. A, × 90; B, × 65.
C.—Longitudinal section of root of *Isatis tinctoria*, a bud initial just to the left of the lateral root, closely connected with the pericyclic phellogen. × 45.
D and E.—Transverse sections of root of *Rubus idaeus*, showing stages in the origin of a bud (*a*) from the neighborhood of the meristematic layer within the polyderm (*b*). D, × 90; E, × 50.



FORMATION OF ADVENTIVE SHOOTS ON ROOTS

(For explanatory legend see p. 49.)

These photographs of Pelargonium (pl. 14, E, F) also illustrate another anatomical complication. The bud-bearing callus tissue is here seen to form a somewhat wide and irregular mass around the base of the emerging rootlet. In Pelargonium, as in most dicotyledons, many of the rootlets emerging from a main root are temporary and die away at the close of the growing season, to be succeeded the following spring by a new crop of roots which emerge upon the main "scaffold" roots, usually appearing from the bases of the roots of the previous season; these arise either endogenously, as branches in the tissue of the root where it is still buried in the main axis, as in *Bocconia cordata* (pl. 22, A), or, less frequently, they emerge from the vascular cambium of the main stele in this region, as the new branch root appears to have done in the Pelargonium shown in Plate 14, F. These branch roots penetrate through the callus cushion, throwing the tissue in this region into still greater confusion, so that the exact source of the buds may be very difficult to trace.

Plate 14, B, shows a section of the root of *Thladiantha dubia* (♀ plant). This root exhibits a peculiar habit of growth, being dilated at intervals along its length. From these swollen portions no branch roots arise, though buds occasionally form, arising from the cork phellogen and, therefore, quite independently of branches; but in the narrow portion, the root branches freely, and buds are restricted to the regions around the bases of the branch roots. In the figure an old emergent branch root is seen, around the base of which the increase in girth of the mother root appears to have been retarded so that the branch emerges from a depression rather than from a cushion; but buds appear around the rim, and in section they seem to have a definite relation to the pericyclic cork phellogen.

Another mode of connection of the bud with the base of an emergent root is illustrated by Plate 14, C and D. These photographs of buds in different stages of development in Bocconia make it evident that these buds arise as outgrowths upon the flank of the emergent root and not in the tissues of the parent root. The buds are clearly endogenous in their relation to the parent root, arising well within the endodermis, probably within the pericyclic phellogen. It is impossible to define very clearly their position in relation to the tissues of the branch root, though they are probably endogenous here also.

EXPLANATORY LEGEND FOR PLATE 14

Formation of Adventive Shoots on Roots

A.—Root of *Asclepias incurnata.* Though the primary cortex is much broken up in the preparation, the buds can be seen to lie well below the exogenous phellogen. The larger bud is in close proximity with the vascular supply passing out to a branch root. ×85.

B.—Root of *Thladiantha dubia.* Just below the place of emergence of the branch root a bud initial has arisen in the neighborhood of the phellogen. ×80.

C and D.—Root of *Bocconia cordata.* C, A young bud initial arising upon the flank of a lateral branch root. ×45. D, A bud at the surface (*a*), closely connected with the old branch root, of which some traces are seen to the left of the bud. From the flank of the branch root a new initial can be seen at *b*, arising endogenously. ×85.

E and F.—Root of the Princess variety of Pelargonium: E shows a small bud initial (*a*) in the large meristematic cushion (*b*) near the point of emergence of a branch root (*c*). F shows how new branch roots (*d*) arise in this region after the old branch root has died off (*b*, meristematic cushion; *c*, old branch root). These new roots emerge through the meristematic cushion around the stump of the dead root. Both ×30.

In Phlox the callus is borne upon a region of the branch root which is already clear of the parent root. (Pl. 15, A.) The discussion of the casual factors contributing to the formation of the endodermis (p. 8) brought out that significance appeared to be attached to the diffusion of fatty substances from the wall of the stele and their contact with the air from the intercellular spaces of the cortex. In the case of an emerging rootlet there may well be a material difference in the extent to which air is present in the young cortex so long as this tissue is buried in the parent root; hence in this region and, after subsequent cell extension, for some distance out from the parent root, the endodermis of the branch root may not have developed under normal conditions. If under these changed conditions the diffusing unsaturated fatty materials have not met air as they leave the stelar tissue, they would continue to diffuse outwards. It is therefore very suggestive to find that in Phlox in this region of the branch root where the callus is borne the fatty substances have failed to form the usual continuous Casparian strip in the primary endodermis, but have formed instead irregular deposits through the cortex; these are deeply stained in the photographs. This feature has been noted only in the regions where the bud-bearing callus is present and in the region just behind this, where the branch is emerging from the parent root. Callus formation in this root is sometimes very extensive, and much cell division takes place within the main cortex, so that sometimes this may even be split open. (Pl. 15, B, C.)

Thus, especially in the case of normal root-borne buds, it is seen that in general a close relation exists between the presence of these buds and the existence of gaps or leaks in the endodermal cylinder where the branch root emerges, a gap which is usually characterized by the presence of a callus cushion. In some plants, although no buds appear upon this cushion normally, they may appear when the root system is isolated.

ROOT BRANCHING WITHOUT ENDODERMAL LEAKAGE

There are a number of dicotyledon roots upon which no bud formation has ever been induced; and one reason for this may be the fact that in these roots the emergence of branches is not associated with any leakage in the endodermal system. Of course it must be remembered that negative evidence as to bud formation may always be upset, and the fact, for instance, that in the large family of the Crassulaceae no case of bud production from the root has ever been recorded may very well be due, as Beijerinck (*11*) pointed out, to the fact that vegetative propagation from portions of the shoot can so readily be carried out. Nevertheless, there is certainly good reason to associate the absence of buds in certain cases, and especially normally occurring buds, with the absence of leaks in the endodermis.

The branch root as it emerges from the parent axis and grows on as a normal root system invariably develops a primary endodermis with a normal Casparian strip in the layer of cells lying at the boundary between the stele and the cortex. This endodermis may arise so early in the development of the new root that a Casparian strip may actually develop upon a cell which at the same time or but very little earlier was developing a Casparian strip as a constituent of the endodermis of the parent axis. In this case the endodermis of the parent root makes a perfectly continuous connection with the primary endodermis of the young emerging branch, so that only a very temporary leak of sap from the stele into the cortex is likely to take place. The point of union is then a ring of cells which often appear to bear two bands of Casparian strip around their walls. In section such cells often show the characteristic outline of the Casparian strip in three places in the wall instead of two. Van Tieghem (*171, p. 705*) drew attention to this phenomenon, describing such cells as "triplissée."

Plate 15, D, shows a young branch root of Artemisia. The apex of the new root has not yet emerged from the tissues of the parent root, but already the endodermis has differentiated behind the meristematic apex and is seen to be in strict continuity with the endodermis of the main stele.

In other cases, although there is a lack of continuity between the endodermis of the main axis and that of the branch root, the temporary leakage at the base of the branch root seems to be stopped by the deposition of fatty substances in a continuous layer over the walls of the parenchymatous cells in the region of the gap. The various varieties of Pelargonium, commonly propagated vegetatively, have formed very erratic material as to propagation from root cuttings. The irregularity of the basal callus formed in the Princess variety has already been considered. The Monstrum variety has given uniformly negative results as regards propagation from root cuttings. Examination of the root of this variety in the neighborhood of a branch shows the endodermis of branch and axis to be joined by a group of irregular suberized cells. (Pl. 16, A.) The striking swellings shown on the radial walls of the cortical cells in the main axis in this section are not the Casparian strip but curious swollen rigid bars, impregnated with silica, which seem to be characteristic of the radial and transverse walls of the exodermis in Pelargonium (*173*).

Repeated attempts to obtain buds upon the roots of *Cheiranthus cheiri* proved entirely unsuccessful. In this plant the young root forms pericyclic cork at a very early age, and those of the main root and of the branch are continuous, and no signs of any swelling at the base of the branch root are shown. (Pl. 16, B.)

Callus and bud formation at the base of branch roots have now been traced to the presence of a break in the endodermis at this point, and the absence of the buds and callus in many cases is apparently due to endodermal continuity. There yet remain for consideration various other possibilities of leakage taking place through the endodermis.

LEAKAGE THROUGH THE OLD ENDODERMIS WITH CONSEQUENT FORMATION OF EXOGENOUS CORK AND BUDS

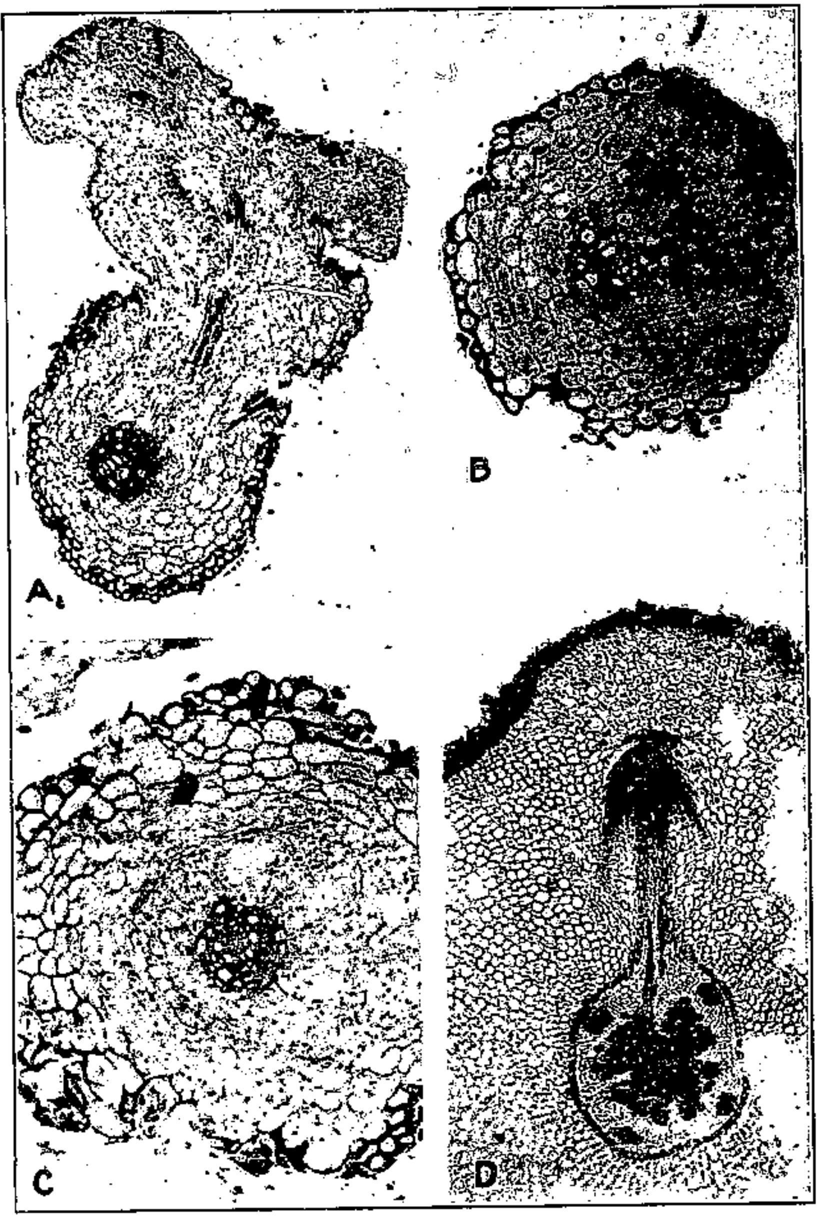
An endodermis does not necessarily remain an effective barrier against the outward movement of water and solutes. As has been seen, the retention of solutes and the osmotic control of water movement depend upon the semipermeability of the living protoplasts of the endodermal cells. This semipermeability is a function of the vitality of the cell, which usually becomes more permeable with age.

Thus, where the endodermis retains the primary structure throughout life, the older regions of the root may be subject to considerable leakage of solutes from the stele, and this is probably the explanation of the exogenous cork formation occurring in *Philodendron erubescens* C. Koch and *Monstera deliciosa* (*122*). In *Cichorium intybus* the cork forms exogenously outside the primary endodermis, but in this case it seems probable that leakage through the old endodermis is facilitated by the actual disruption of this layer which occurs with increase in girth of the stele. In many cases (pl. 16, C, D; pl. 17, B) the buds in this species occur well below the position of the exogenous phellogen, and in some cases at least they appear to initiate just within the endodermis, so that, though the cork is exogenous, the buds may be either endogenous or exogenous.

Usually the endodermal cells signalize their increase in permeability by the "creaming" of their fatty contents to the surface, where they form a continuous suberin lamella upon a base of cellulose. This is known as the secondary endodermal stage and is followed practically always in the angiosperm root by a tertiary stage in which an inner lamella of cellulose is deposited within the suberin lamella (*119*). After all cells of the endodermis have passed into the secondary or the tertiary stage, the endodermal layer becomes practically impermeable to both water and solutes; consequently this stage is usually followed by exfoliation of the primary cortex. But if passage cells are left—that is, cells which remain in the primary stage—the protoplasts of these cells may for a time allow solutes and water to move in either direction through them until, as a result of successive alternations of sap movement and drying of the tissues, the passage cells also become choked with the débris left by the ebb and flow. Until this happens such a tertiary endodermis may be associated with a living primary cortex in which exogenous cork and buds may appear, as in *Aristolochia clematitis* L. (Pl. 17, A.)

A wide range of different types, as regards the nature of the endodermal barrier and the position of the phellogen, is met with in the Solanaceae. In Lycium and Nicotiana, pericyclic cork was found to form very early in young, thin roots. In older roots of Lycium, buds were found emerging from this pericyclic phellogen, in association with the place of emergence of branches. In Nicotiana, buds were obtained only by cutting off the stem and inverting the root system, keeping the older region of the root in moist air. Buds then appeared in association with the phellogen, arising upon the older parts of the root system, at the base of branch roots. With *Cestrum newelli* Nich. and *Datura suaveolens*, in roots up to about 1 centimeter in diameter, the endodermis was found to remain primary, but probably was relatively permeable, as an exogenous phellogen was active outside it. In Capsicum and Physalis many cells of the endodermis had passed into the secondary, impermeable stage, but the presence of a number of passage cells explained the presence of exogenous cork.

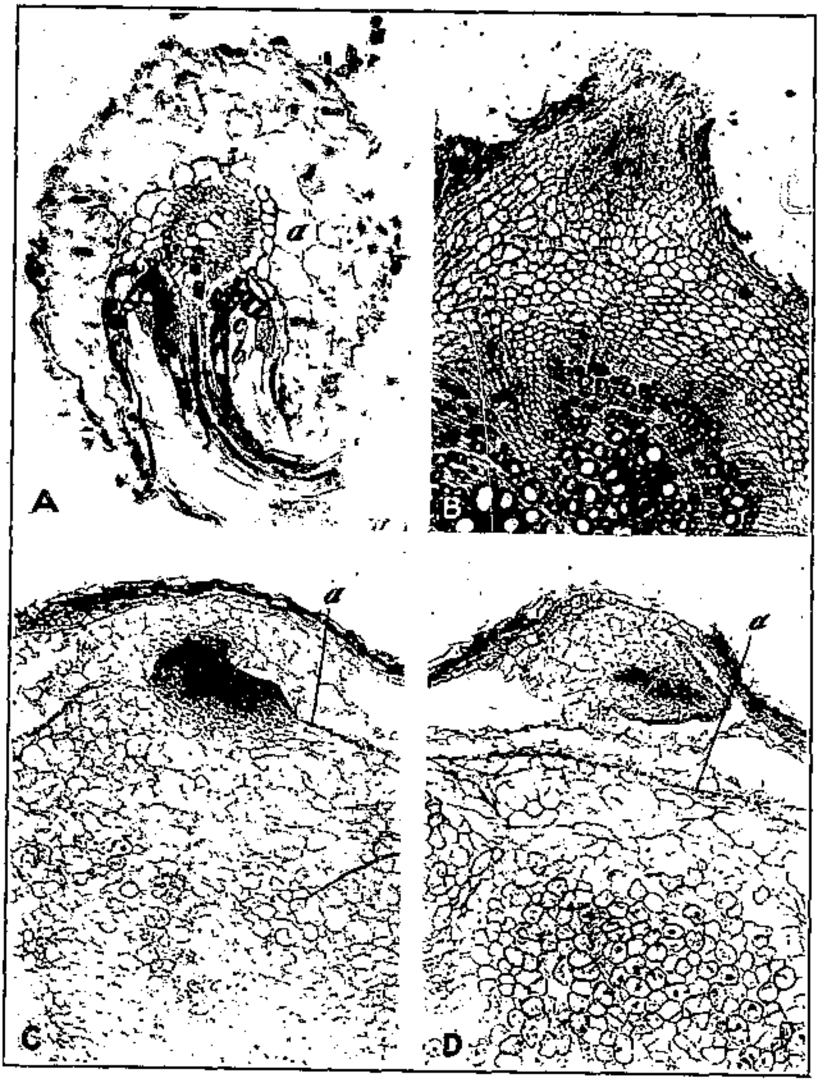
In *Solanum capsicastrum*, *S. crispum* Rz. and P., *S. dulcamara*, and *S. nigrum*, the increase in girth of the main roots, through cambial activity, takes place very rapidly. The endodermis is thus stretched until the cells are greatly extended in a tangential direction. Since they are no longer meristematic, and hence no increase



ROOT BRANCHING WITH AND WITHOUT ENDODERMAL LEAKS

A-C.—Root of Phlox, showing the manner in which the branch root dilates at its base as it emerges from the parent root. A, Branch root in longitudinal section at point of emergence, showing swollen hypertrophied cushion on which adventive buds are borne. × 50. B, Normal appearance of branch root as seen in transverse section. The primary endodermis, not visible in the photograph, is continuous. × 75. C, Same root close to the place of its emergence from the parent root. Cell division and extension have taken place in both stele and cortex, and the primary endodermis is much interrupted in this region. × 85.

D.—Transverse hand section of the root of Artemisia, showing an early stage in the emergence of the branch root. The endodermis of the parent root is clearly continuous with the endodermis of the branch root. × 40.

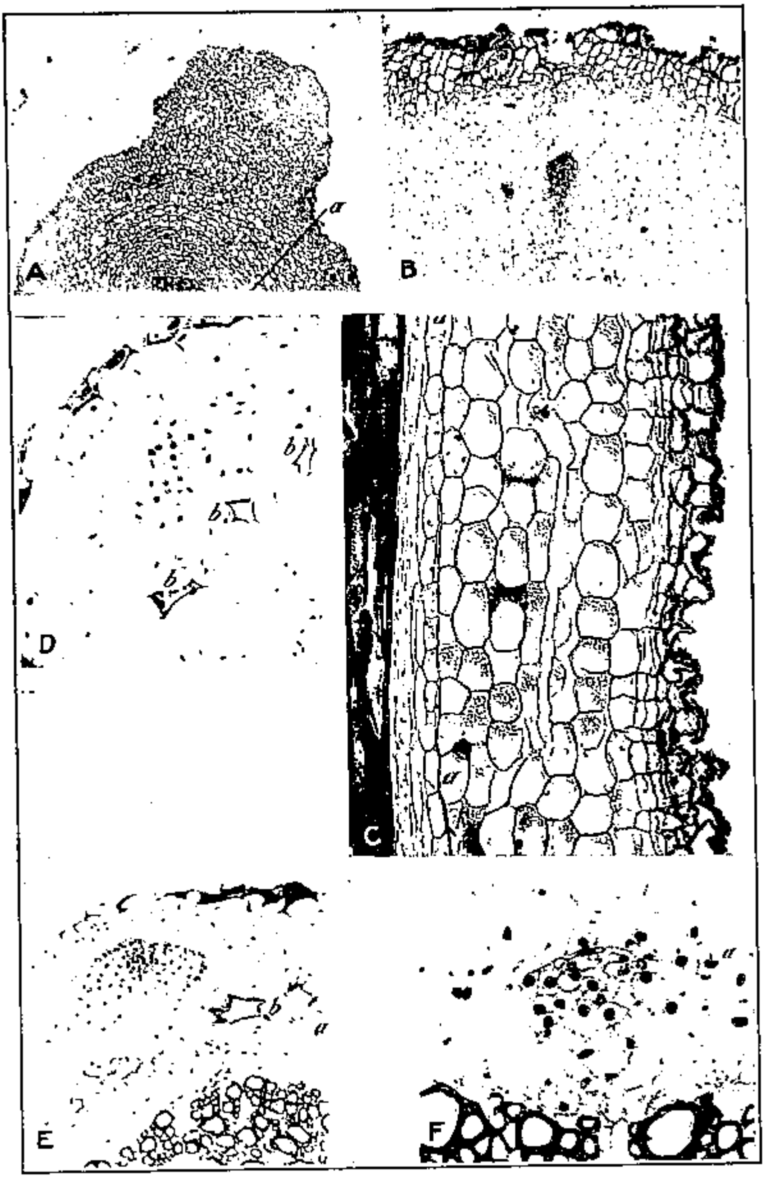


ENDODERMAL STRUCTURE AND ADVENTIVE-SHOOT PRODUCTION

A.—Section of root of the Monstrum variety of Pelargonium, passing radially through the emerging branch root. Between the endodermis of the main root (*a*) and the endodermis of the branch (*b*) lies a group of cells (*c*) with fat-impregnated walls. × 135.

B.—Root of *Cheiranthus cheiri* at the point of emergence of a branch root. The cork of the parent root is continuous with that of the branch root. × 100.

C and D.—Transverse sections of the fleshy root of *Cichorium intybus*. The endodermis (*a*) is primary and evidently permeable, for the exogenous phellogen is active outside it. In C the bud is well below the phellogen and probably arose within the endodermis. In D another bud is shown which lies just beneath the phellogen and undoubtedly is completely outside the endodermis. Both × 65.



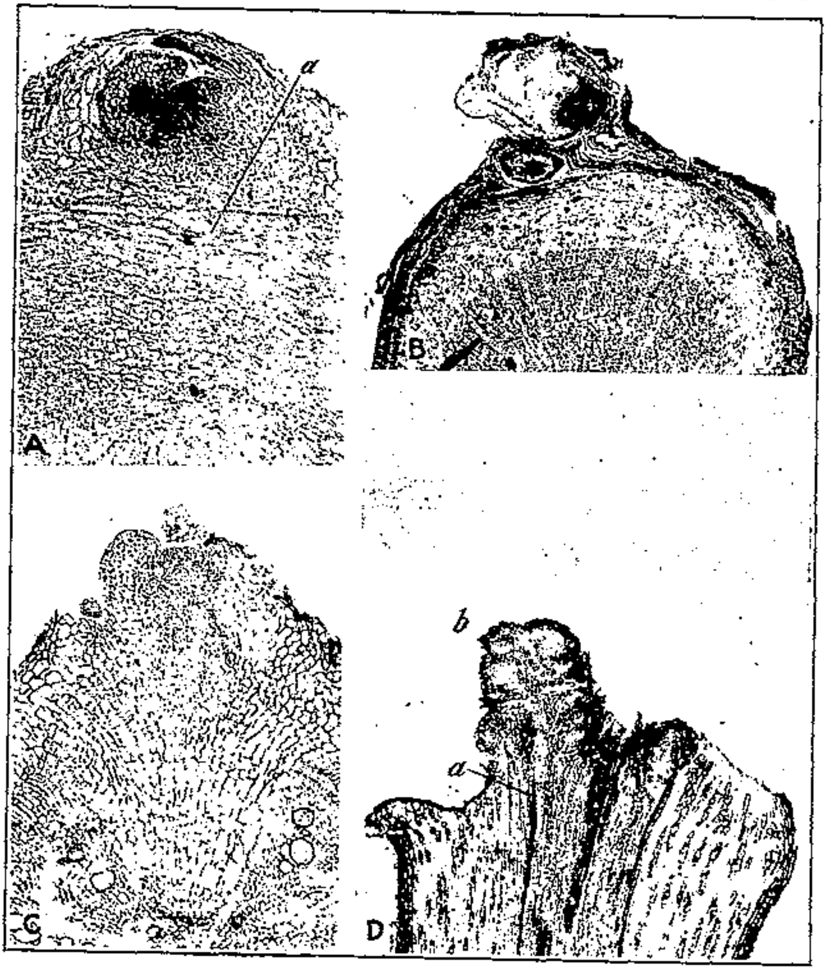
ENDODERMAL STRUCTURE AND ADVENTIVE-SHOOT PRODUCTION

A.—Transverse section of root of *Aristolochia clematitis*, showing a bud arising just beneath the exogenous phellogen. The position of the endodermis is indicated at *a*. × 25.

B.—Longitudinal section from the proximal end of an isolated root piece of *Cichorium intybus*, showing the formation of a new shoot initial some distance below the cork phellogen. × 30.

C.—Longitudinal section of root of *Solanum capsicastrum*. The exogenous cork is visible on the right. Between this and the stele, at *a*, *a*, traces of the collapsed primary endodermis are visible. × 75.

D–F.—Bud initials in transverse sections of Gaillardia roots. D and E show leakage through the primary endodermis (*a*), indicated by the heavily stained secretions (*b*) in the intercellular spaces just outside the endodermis. These leakages are associated with the exogenous buds. D, × 100; E, × 55. In F no development of a secretory system is shown outside the primary endodermis (*a*), and meristematic activity is visible just within the endodermis. × 140.



FORMATION OF ADVENTIVE SHOOTS ON ROOTS

A.—Root of *Cnicus arvensis*, showing an exogenous bud obtained after isolation of the root system; *a*, the endodermis. × 50.

B.—Root of *Fuchsia caledonia*, showing a mass of callus which has formed beneath the polyderm at the base of a branch root and which bears several young shoot initials. × 10.

C.—Root of *Romneya coulteri*, showing a bud obtained after isolation. Although opposite a primary xylem group, it showed no connection with the emergence of a branch root. × 35.

D.—Longitudinal section through an isolated root piece of *Filipendula ulmaria*, showing a callus, bearing a bud initial at *b*, arising from the activity of the tissues of the stele, within the secondary endodermis (*a*). The cells at the cut surface of the primary cortex merely suberized and took no part in callus formation. × 25.

in their numbers is possible, with the increasing tension this layer is ruptured. At the time this happens the primary cortex is still present on the root. After the rupture it is very difficult among the crowded parenchymatous tissue to find traces of the crushed and disorganized endodermis, but it is possible to trace the process and see how this disruption occurs. (Pl. 17, C.) On occasional roots of *S. nigrum*, an endogenous pericylic phellogen is present, formed before the endodermis is disorganized. Usually, immediately after the rupture, vigorous meristematic acivity sets in near the surface of the root and a cork-producing exogenous phellogen arises. In close connection with this phellogen, buds are often found. Beijerinck (*11*) called particular attention to the vigorous capacity for vegetative propagation displayed by the roots of *Solanum dulcamara*, which he described as being in many cases covered with buds.

The same disorganization of the secondary endodermis, taking place before the primary cortex disappears as the result of the increase in girth of the stele, is seen in *Asclepias incarnata.* In this plant, however, the exogenous cork is visible in very thin roots when the secondary endodermis still seems to be practically continuous, while in the thick, older roots all traces of this endodermis are usually lost. Buds occur normally upon the roots; they apparently commence to form several cell layers below the active phellogen and not necessarily in connection with branch roots. (Pl. 14, A.) From the anatomical conditions existing at the time of their formation, it is very difficult to decide whether or not these roots are endogenous in the sense of arising from the original horizon of the pericycle.

In the Compositae, events seem to follow a course slightly different from that just considered. As Tetley pointed out (*164*) in this family the stele seems to release unusual quantities of fats and fat-soluble substances, especially from the phloem. The result is that these substances dissolve in the Casparian strip and pass through it, collecting in the intercellular spaces of the cortex just outside the endodermis. They appear first in the regions opposite the primary phloem in the young root, but as secondary growth proceeds they continue to be released, so that in time the intercellular spaces all around the main part of the cortex may become impregnated with this material, which vigorously reduces osmic acid and readily takes up Sudan III and other fat stains.

The presence of this intercellular secretory system, characteristic of many of the Compositae, seems to stimulate considerable meristematic activity in the cortex. The first evidence of this is the appearance of groups of densely meristematic cells bordering upon the intercellular spaces. These groups of cells are usually described as epithema, but in their course of development they follow the secretion and are probably caused by its presence, instead of being the active agents in secretion, as has usually been assumed. Exactly the same sequence of events has recently been described by Hanes (*51*) for the epithema clothing the resin canals of the conifer.

But, as Plate 17, D and E, shows for Gaillardia, the leakage of these fatty substances through the endodermis may also be associated with exogenous bud initiation. These buds were found to occur only in places where the intercellular secretions were accumulating, and, as is shown in Plate 17, F, where these secretions were not yet present outside the endodermis, evidence was obtained for the existence of endogenous meristematic activity; thus in Gaillardia, it seems that buds might be either endogenous or exogenous in origin. From the material examined at Leeds, they appear to be usually exogenous.

EFFECT OF ISOLATION UPON BUD INITIATION

In the account so far presented of the development of adventive buds upon the root system all buds have been treated in the category of "adventive," whether occurring as a normal thing upon the growing root system or formed only as the result of isolating a part of the root in an attempt at propagation. *Teucrium scorodonia crispum* (Stansfield) Rayner may be cited as an example of the latter case. In spite of repeated attempts, buds had never been successfully obtained upon this variety of the wood sage until the entire root system was isolated, the stump of the plant inverted in the soil, and with the ends of the root buried, the proximal portions of the root system exposed in very moist air. Buds then arose from the bases of the branch roots, and the leaves upon the shoots retained the crisped character of the original strain. Buds around the bases of branch roots were also obtained in *Clerodendron trichotomum* and *Geranium sanguineum* only after pieces of root had been cut off and left partially buried in a moist, porous soil. The same was true for *Cnicus arvensis* Hoffm., except that in this case the buds arose exogenously, upon the main root. (Pl. 18, A.)

With plants of *Fuchsia caledonia* Hort. in which the entire stem had been removed, but in which the root system was left undisturbed in the soil, a few buds were obtained from the cushions occurring at the base of the branch roots. As Plate 18, B, shows, such buds arose from the meristem layer, lying just within the polyderm. Plants of *Romneya coulteri*, similarly decapitated, produced buds from the neighborhood of the pericyclic phellogen which showed no connection with the branch roots. (Pl. 18, C.)

Where lateral buds are thus obtained upon the root surface as the result of isolation, it is of course always possible that more extended observation might reveal the buds occurring upon plants that were intact, growing under certain environmental conditions. There are, however, a very large number of cases where the buds produced after isolation arise from the surface of the callus, which gradually covers the wound. In such cases the root may also bear buds normally in a lateral position, as occurs in *Crambe maritima*, *Cichorium intybus*, *Verbascum nigrum*, and others, or it may be that the only root-borne buds known for such plants are "wound buds," of which *Primula denticulata* and *Morisia hypogaea* Gay. may be mentioned as examples investigated at Leeds.

CALLUS BUDS

Cases of adventive buds arising from the wound callus are not entirely limited to fleshy roots, one example (Crambe) of which has been discussed rather fully, although they arise much more commonly in such roots. This fact is apparently closely correlated with the larger reserves of food and water which such roots contain. Naturally, if functioning roots are present upon the isolated root segment, or can be developed from existing branches, the water supply will be much more certain, and this is probably in part the reason for the success obtained with Teucrium and Fuchsia. Sometimes, again chiefly with fleshy roots, young roots may be regenerated from the wound surface, but, as has been discussed, such new root initials usually appear comparatively slowly and long after the proximal callus is covered with bud initials.

Primula denticulata, *Filipendula ulmaria*, and *Ajuga reptans* (pl. 18, D, and pl. 19, A) furnish examples of callus buds which are formed in relatively slender roots containing a secondary or tertiary endodermis. As was pointed out above, the endodermis restricts the flow of sap to the cortical regions, and, although the primary cortex was still present in the mother roots in these cases, it will be seen that this region has taken no part whatever in the production of the bud-bearing callus. *Plantago lanceolata* L. also shows buds on the callus of a thin root; buds were also found around the base of branch roots in this species. (Pl. 19, B.) Buds were found in the same position on the roots of *P. media* L., which Beijerinck (*11*) described as differing from *P. lanceolata* in its apparent inability to bud. Although in the isolated root of *Acanthus montanus*, illustrated in Plate 19, C, the endodermis was still probably in the primary stage, callus production was confined almost entirely to the cambium region, though a cork phellogen has formed beneath the suberized cells at the cut surface of the pith and cortex.

In Plate 20, B (*Solanum capsicastrum*), a marked phellogen is shown in the cortex. As already described on page 52, the secondary or tertiary endodermis in this root is disrupted and scattered by the increase in girth which the stele within undergoes at a very early stage. In fact here, as in most of the relatively fleshy roots that are to be considered in this connection, any distinction between cortex and stele can be disregarded. If the endodermis is still present in such roots it is usually leaky or disorganized, as in the case of many Compositae and Solanaceae; in other cases the primary cortex has long since disappeared as the result of the formation of pericyclic cork as in *Crambe maritima;* when this occurs, the secondary phloem is either so parenchymatous or such continued division of the parenchymatous pericycle cells originally present has taken place that the outer portion of the root, though within the original pericycle, is as parenchymatous as typical cortex.

FLESHY ROOTS

The typical fleshy root is thus one in which the activity of the vascular cambium has produced a disproportionately large amount of parenchyma, although there may be a core of lignified elements near the center. Both this parenchyma and the residual primary parenchyma lying between xylem and phloem or making up the pericycle display considerable activity in growth. In the fleshy roots of many of the Umbelliferae the secondary tissue is exceedingly irregular, rings of xylem and phloem surrounding isolated groups of primary xylem which have been broken up and scattered by the enlargement and multiplication of the parenchyma cells originally present among the vessels.

In Beta, most of the root is produced by the activity of a succession of concentric rings of cambium which commence their activity afresh farther and farther toward the periphery of the root. The parenchyma between the successive rings of vascular tissue, however, remains active in division and practically keeps pace in its increase in girth with that occurring in the neighboring rings of parenchymatous vascular tissue.

Where tissues behave so anomalously as these, it would be unwise to press any generalization based upon the usual sharp categories of vascular differentiation. In other roots correlations have been made between the places of emergence of adventive structures and the relative position of xylem and phloem, but it is doubtful whether such correlations can be applied to many of these fleshy roots in which the original organization of the primary tissues is sometimes completely masked and in which the behavior of the secondary tissues is so anomalous. The main general characteristic of the fleshy root is the presence of a large amount of parenchyma which shows a considerable tendency to divide. This may very well be correlated with the high water content of these fleshy tissues, and with the fact that the intercellular spaces are small and are often filled with sap rather than air.

In England the propagation of the hop (*Humulus lupulus*) is accomplished by earthing up in summer the bases of the twining stems or even by laying the spirally coiled stems along the ground, partly buried in soil so that half of each spiral turn is in the ground. The portions of the stem thus earthed up remain vigorous and turgid, while the rest of the bine dries and ceases to grow. The earthed-up portions not only remain alive, but they swell up and undergo considerable permanent increase in girth. Anatomical examination shows that vigorous growth and division of the xylem parenchyma have occurred and scattered the dead lignified elements. The cambium also remains alive and vigorous, and the ray cells extend radially, and the cortical tissues also remain vigorous.

These swollen portions of the bine can then be used for the propagation of the plant in the spring, when vigorous endogenous root production takes place from the neighborhood of the cambium and the surface of the swollen shoot becomes covered with adventive buds. In the spiral layers the portions of the stem which have been exposed to the air all winter are dry and dead. The striking difference shown by the alternate portions seems mainly to be due to the water content of the tissues; in the one case the high water content has not only maintained the cambium in a vigorous condition but has also promoted a very widespread cell division throughout the parenchymatous tissues, followed by the vigorous production of exogenous adventive shoots. Anatomical details of this method of propagation of the hop merit further examination. These few facts suffice to call attention to this case as an example of the marked effect upon the cell growth and the production of adventive structures, resulting from the maintenance of turgidity in living parenchymatous tissue. Just these conditions prevail in the fleshy roots under consideration, and they probably have much to do with the vigorous activity in vegetative propagation which such roots commonly show.

On page 31 adventive-bud formation from the callus was described for the shoot, and many of the facts there brought out apply equally to the buds which arise from root callus. Such adventive-bud production is not very common upon the shoot, and, as Beijerinck (*11*) pointed out, in the case of decapitated trees the nearer the cut is to the base of the trunk the greater the bud production. From this standpoint the much greater bud production displayed by the root than by the shoot in such a plant as Crambe is perhaps intelligible, the tissues containing more sap toward the base of the plant.

But by no means can buds be obtained from the callus in all fleshy roots. The necessary healing of the cut surface in such a parenchymatous tissue depends upon suberization, and the meristem forms later. Cocks [9] found that isolated roots of hyacinth, narcissus, and other monocotyledons suberized very slowly and at irregular depths below the cut surface; "etagen" cork later arose beneath the suberin block, but frequently decay set in before this process of healing was completed.

Formation of wound callus and phellogen among the monocotyledons seems almost entirely restricted to the Liliaceae, Dioscoreaceae, and Orchidaceae. Although Beijerinck's (*11*) account of bud formation on these roots suggests that further observations are needed, occasional instances of bud formation upon isolated root systems among these three families seem to have been observed.

Likewise in the dicotyledons the healing of isolated root systems is by no means always accomplished. It must be remembered that at the cut surface the primary cortex, if present, is often isolated from the stele, and as a result it frequently suberizes slowly and inefficiently and provides a surface from which decay may spread. Furthermore, within the endodermis the tissues may be mainly vascular and incapable of the wound response characteristic of parenchymatous tissues. As their liquid contents are displaced by air, the xylem tracheae, especially in woody roots, sometimes become filled with tyloses from the neighboring parenchyma cells (*75*) (pl. 20, A), and a phellogen may thus be enabled to spread across the cut surface; but frequently such roots decay before any signs of adventive buds have appeared.

In the more parenchymatous roots there is great variation in the rate and extent of suberization and phellogen formation displayed. Bebbington [10] examined the healing taking place in slices of red and sugar beets and parsnip; he found that in these plants the wound phellogen was slow in forming. In Beta he found that a very thin suberin film had formed over the cells near the cut surface by the end of two weeks, but even after three months the phellogen, which first appeared above the outermost ring of cambium, had not yet reached the interior of the root. On the other hand, in the root of *Erodium macradenum* L. (pl. 20, D), in which a very vigorous cork formation occurs in the pericycle as a normal thing, both proximal callus and distal callus were cut off by a very vigorous periderm.

This correlation between a vigorous normal phellogen and a vigorous wound phellogen is probably more than a mere coincidence.

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[9] COCKS, A. M. THE GROWTH HABIT OF THE MONOCOTYLEDONOUS ROOT SYSTEM. Leeds. 1925. [Unpublished thesis.]

[10] BEBBINGTON, A. G. WOUND HEALING IN STORAGE ROOTS. Leeds. 1926. [Unpublished thesis.]

Pastinaca sativa and *Daucus carota* are examples of roots in which normal pericyclic periderm found at the surface of the swollen root is often slight or even absent, the surface being in the latter case protected simply by the remains of a suberized endodermis or by an irregular suberization upon the superficial cells. Bebbington[11] investigated the results as regards wound healing obtained with slices of two individual roots of Daucus, one root possessing a normal periderm and the other not. At the cut surfaces of slices subjected to comparable external conditions the root with the periderm formed a wound phellogen and the root without normal phellogen did not.

Whether the periderm is but slightly developed, as in Pastinaca and Daucus, or is present as an extensive and complete layer, as in Erodium, the amount of phellogen activity is always greater at the distal (root) end of long pieces of root, however the cutting is placed in regard to gravity. In roots as in stems frequently a definite phellogen activity can be traced at the lower (in this case the distal) end, but not at the proximal end. This has been seen in *Eryngium alpinum*, *Radicula armoracia*, and *Taraxacum officinale*. (Pl. 20, C.)

Even though there may be no distinct phellogen layer found at the proximal end, patches of callus cells near the surface may become meristematic and organize themselves as shoot meristems. Indications of adventive buds forming at the proximal end of the root are shown in Plate 20, C; similarly they have been seen in *Anchusa italica* (pl. 21, A) and *Symphytum officinale* var. *argenteum* at the proximal end (pl. 21, B) and at both cut surfaces of *Centaurea montana* and *C. babylonica* (pl. 21, C), in all these cases there being no indication of any previous phellogen formation in the callus. When phellogen is present, as a rule the relation of the buds to it seems to be essentially as described for *Crambe maritima*. In *Cichorium intybus*, however, although the adventive shoots occasionally begin closely associated with the phellogen, they usually appear some cells below. (Pl. 17, B.) It was pointed out previously that although normal cork formation is exogenous in this plant, lateral buds sometimes appear to be endogenous. A still further peculiarity exhibited by this species is referred to under "Adventure embryos" (p. 78).

It is thus seen that the connection between phellogen and adventive-bud formation is by no means obligate in either root-borne or shoot-borne callus. In the case of very vigorous periderm formation, as in Erodium, there may be very few buds produced, and conversely vigorous bud formation may occur from a callus in which no phellogen can be recognized. The generalization that still seems to hold is that a tissue organization which is favorable for phellogen activity is also favorable for the formation of adventive shoots. In the case of seakale, a third type of meristematic activity was also seen to occur at the distal end; beneath the phellogen a meristem formed, which is intimately associated with the vascular cambium and which also cuts off toward the inside elements that are quickly lignified. The presence of this layer seems to be associated with root formation from the wound tissue at this end.

Indications of the presence of similar layers, though not necessarily having any connection with root formation, have also been

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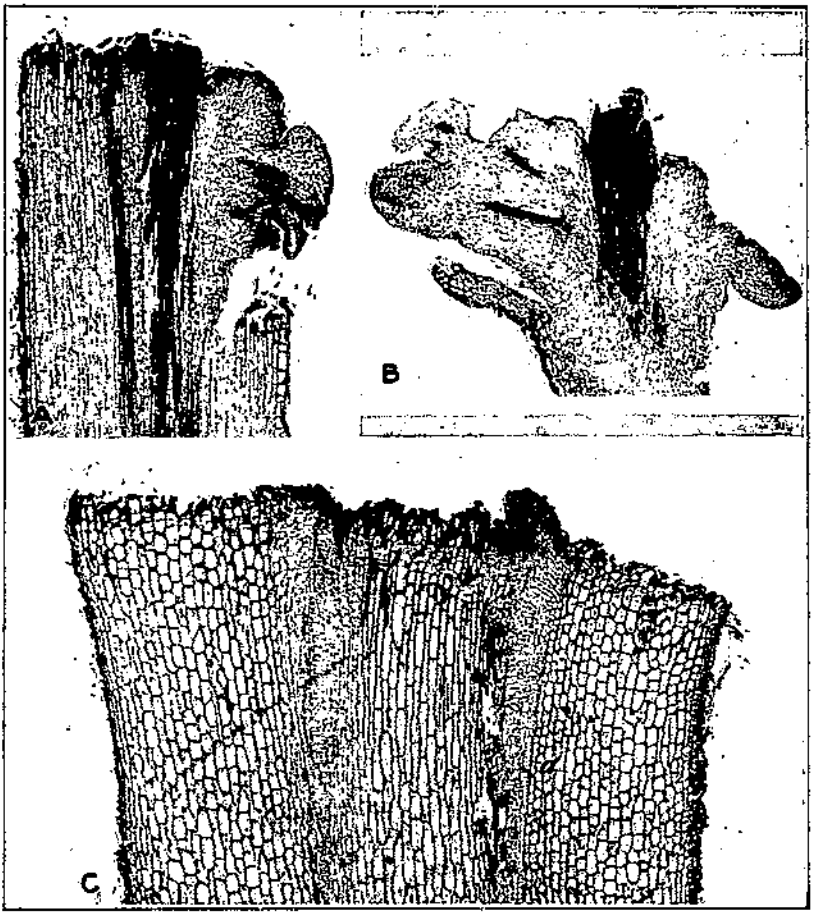
[11] See footnote 10 on p. 67.

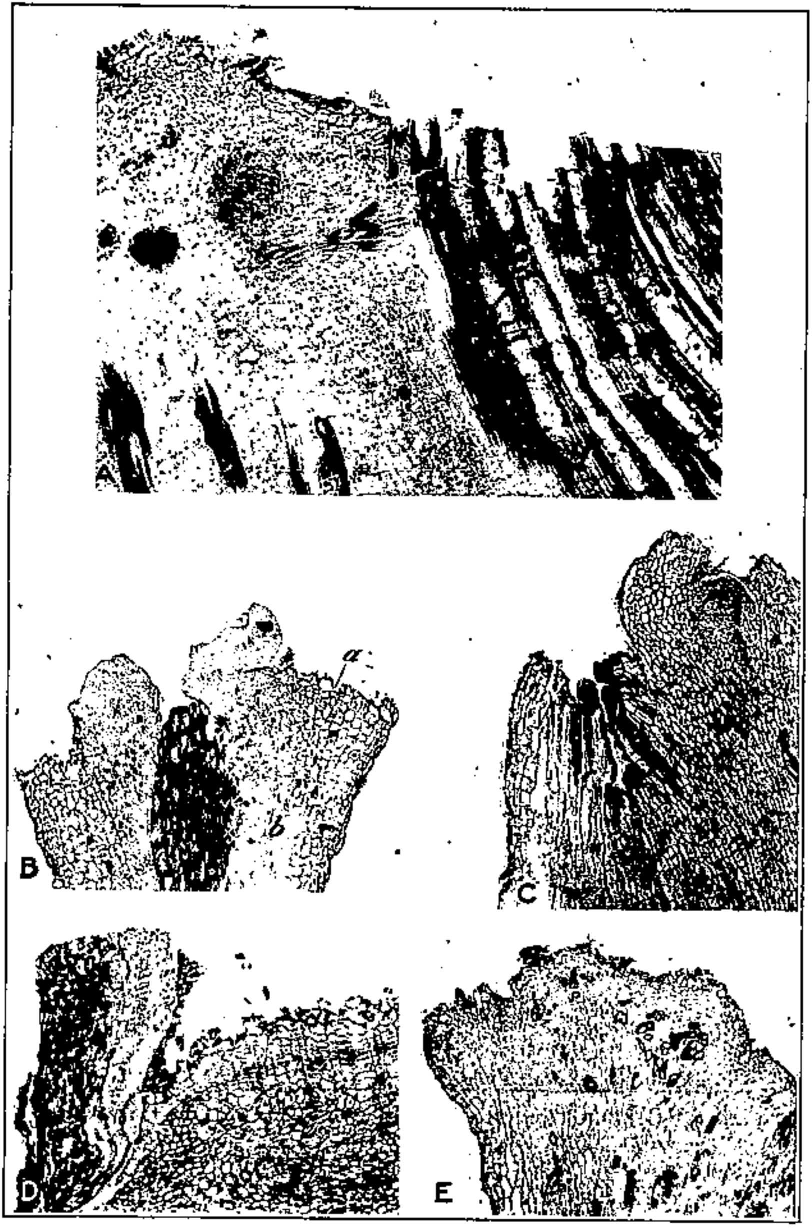


ACTIVITY OF STELAR TISSUES IN FORMATION OF SHOOT-BEARING CALLUS

A.—Longitudinal section of root piece of *Ajuga reptans*. On the left the cells of the primary cortex have merely suberized. All callus growth and meristematic activity have developed from the tissues of the stele, and the callus bearing the bud on the right side of the section has no direct genetic connection with the primary cortex beneath it. × 35.

B.—Buds developing freely upon the callus arising from the stelar tissues at the end of an isolated root piece of *Plantago lanceolata*. The section is obliquely longitudinal and runs through the cortex of the root below and the protruding end of the central core of the xylem above. The xylem near the cut surface is discolored and disorganized. × 25.

C.—Longitudinal section of an isolated fleshy root of *Acanthus montanus*. The endodermis (*a*) is primary, and although callus formation and buds are found mainly over the region of the vascular cambium, a cork phellogen can be traced entirely across the tissues of the primary cortex. × 15.



MERISTEMATIC ACTIVITY IN ISOLATED ROOTS (LONGITUDINAL SECTIONS)

A.—Isolated root of *Sphaeralcea pedata* Torr., showing a bud-bearing callus over the region of the cambium and tyloses in the old xylem vessels. × 60.

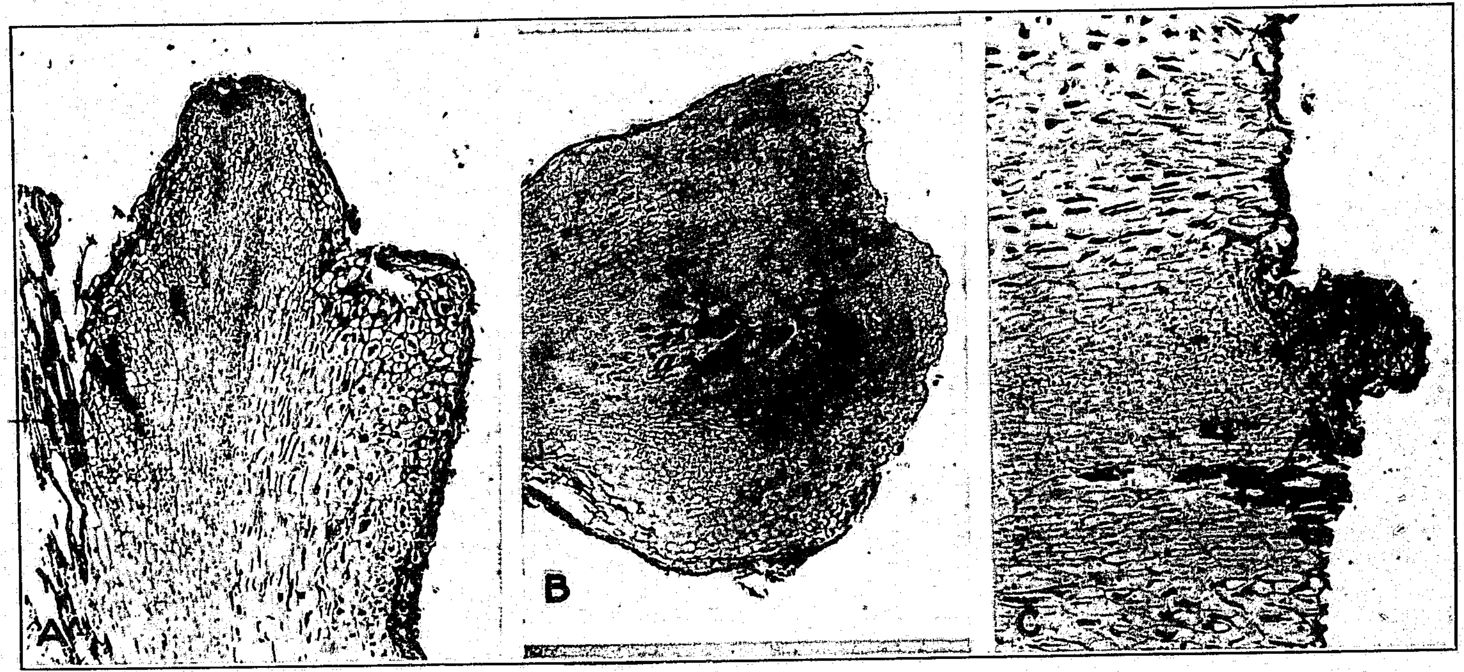
B.—End of a budding root piece of *Solanum capsicastrum*. Although the callus with buds arises from the cut surface of the stele, an active phellogen (*a*) is formed below the suberized cells near the cut surface of the cortex. The endodermis (*b*) is collapsed and broken, and an exogenous cork is present at the cut surface of the root (see pl. 17, C). × 25.

C.—Proximal end of an isolated root piece of *Taraxacum officinale*. No cork phellogen is visible at the cut surface, but over the region of the cambium a shoot initial is developing. × 35.

D.—Distal end of a root piece of *Erodium macradenum*. The cork upon the normal root surface (at the left) has many layers, and the phellogen formed beneath the cut surface has also been very active. × 40.

E.—Characteristic type of callus formed at the distal end of an isolated root of *Silene nutans*. Some distance below the cut surface an active meristem is cutting off short xylem tracheids to the inside. × 25.

PLATE 21



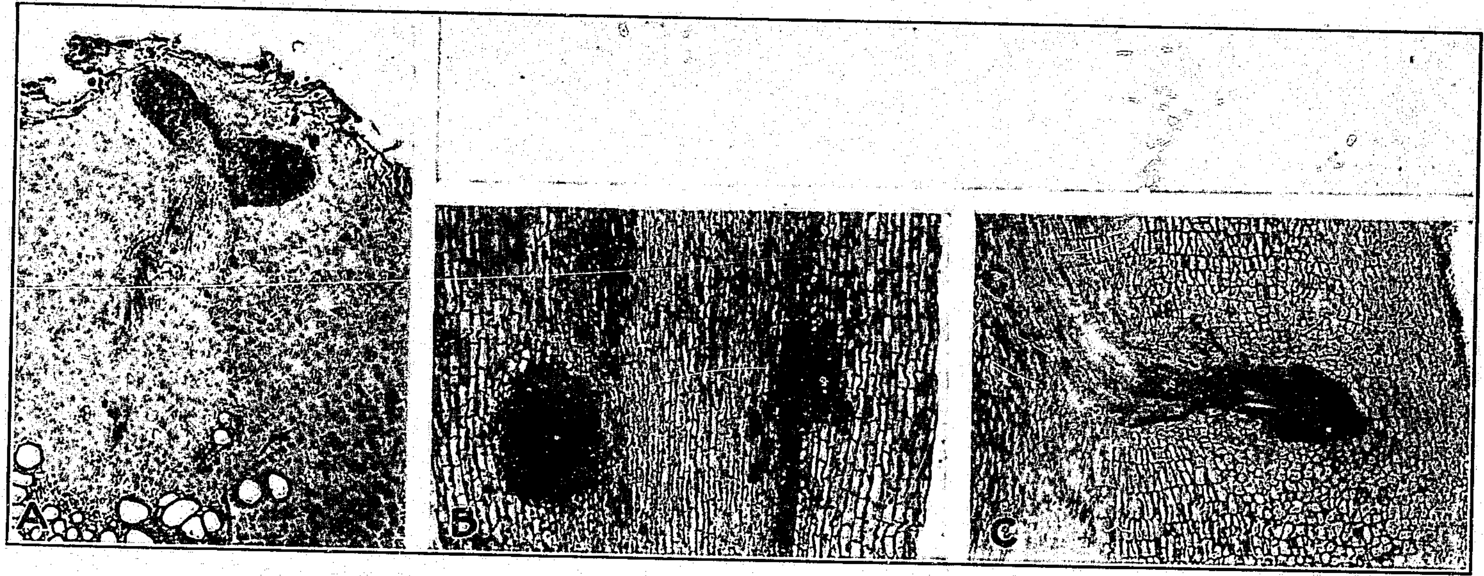
ACTIVITY OF STELAR TISSUES IN FORMATION OF SHOOT-BEARING CALLUS

A.—Longitudinal section through the proximal end of an isolated root piece of *Anchusa italica*, variety Dropmore. The bud initial arising at the cut surface of the callus developed above the cambial region has been preceded by many active cell divisions with periclinal walls, but there is no clearly organized phellogen. The backward differentiation of the procambial strands of the new buds is clearly indicated. × 15.

B.—Oblique longitudinal section of the proximal end of an isolated root piece of *Symphytum officinale* var. *argenteum*. At *a* are the deeply stained ends of the xylem elements originally exposed at the surface of the cut. These elements are nearly buried beneath the enveloping callus which has grown up over them from the cambial region. Buds are now forming on the inner side of the fused mass of callus. × 15.

C.—Bud-bearing callus at the exposed proximal end of a root piece of *Centaurea montana*. Suberin, but no phellogen, is visible at the cut surface. × 15.

PLATE 22



ROOTS ARISING ON ROOTS

A.—Transverse section of a root of *Bocconia cordata*. Part of the emerging vascular strand of an old branch root is visible, and from this two new branch roots are arising endogenously. × 45.

B.—Longitudinal section of a young radicle of Cucurbita. A lateral root initial is present in the pericycle on either side of the stele in which the vascular elements are as yet but slightly differentiated. × 45.

C.—Longitudinal section of an isolated root piece of *Cichorium intybus* in which a new branch root is arising from the neighborhood of the vascular cambium. × 30.

observed in *Eryngium alpinum* and *Taraxacum officinale*, as well as in the parsnip and beet (Bebbington).[12] In *Silene nutans* L., *Scabiosa columbaria* L., the Souvenir de Bonn Abutilon, *Solidago recurvata* Willd., and the Belladonna Delphinium the only meristem found in the callus was of this type, indications of phellogen activity being slight or nonexistent; and it is perhaps significant that no buds were obtained from the cut surface of these roots, notwithstanding the vigorous callus formation. (Pl. 20, E.) In the parsnip, Bebbington[12] observed such an inner meristematic layer forming across the xylem beneath the cut surface, and Kupfer (*80*) found roots produced from the wound callus, but only in pieces of xylem from which cambium and phloem had been removed.

Other fleshy roots with which negative or but slightly positive results were obtained include *Althaea officinalis* (negative), *Hieracium maculatum* Schrank (negative, with well-marked wound cork in callus), *Maclura pomifera* (a bud on one specimen), and *Inula helenium* (negative). In *I. conyza* DC, buds arose at the proximal surface. Kupfer (*80*) also records negative results as regards bud production, for the fleshy roots of *Daucus carota*, *Brassica rapa*, *Raphanus sativus*, and *Tragopogon porrifolius*.

Crambe maritima behaved differently from most fleshy roots in the freedom with which it formed buds over the xylem region; probably the difference is to be correlated with the very parenchymatous nature of the xylem in this plant. In *Cichorium intybus*, callus was more pronounced above the rays than over the xylem; but in most roots the callus forms most markedly above the cambial region. Presumably the regions made up of parenchymatous or meristematic cells are the ones contributing the most to the formation of the callus. In the case of *Radicula armoracia*, although buds arise at the normal surface and from the callus, no buds were obtained from isolated portions containing xylem tissue alone in the Leeds laboratory, though Kupfer (*80*) succeeded in obtaining them from slices of xylem tissue alone. In *Pastinaca sativa* such isolated pieces of xylem tissue on one occasion gave rise to roots from the callus, the only occasion on which roots were obtained from the wound callus in the parsnip.

Evidently, the great diversity exhibited by bud production from roots precludes any absolute classification of types; nevertheless, it seems clear that certain anatomical features of the root are of marked importance in determining its behavior in vegetative propagation.

ADVENTIVE ROOTS

ROOTS UPON ROOTS

The same difficulty in giving precision to the term "adventive" exists in the case of the root apex as in that of the shoot apex. Even though branch roots usually arise in regular acropetal succession behind the growing apex, they do not necessarily begin in tissue which has remained meristematic since its separation from the apex of the parent root. As the result of injury, new root initials commonly arise, out of order, and from nonmeristematic tissue, but

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[12] See footnote 10 on p. 57.

there is no very clearly defined order about the crops of secondary branch roots, which in many species arise regularly on the scaffold roots, replacing the crop of roots which had died down at the end of an earlier growing season.

Many roots arising upon roots, therefore, would fall under most definitions of "adventive," and there would be difficulty in confining the term, as has frequently been proposed, to roots arising on the shoot. Wettstein, therefore, classified all roots arising on the shoot as "Beiwurzeln" (*181*), but, as pointed out on page 28, this may lead to confusion in view of the very different way in which the same prefix is employed with the shoot. In this bulletin the qualification "adventive" will be used in a very general sense, meaning any root not arising in normal acropetal succession upon the young root. Indeed, brief consideration must be given first to normal root production, as the adventive root displays no special distinguishing characteristics whatever as regards its apical organization, and it seems that the same internal factors govern its production as govern that of the normal root.

In the dicotyledon the first root apex, organized in the embryo, must be regarded as essentially exogenous, although its subsequent development is characteristically endogenous, since the greatest activity in the construction of protoplasm and cell multiplication is shown by the layers of meristematic cells which border upon the differentiating stele. Just within the endodermal cylinder as it differentiates lies the pericycle, which is usually a single layer of cells in thickness and which frequently remains meristematic for a considerable time. Within the pericycle, at more or less regular intervals, local centers of greater meristematic activity appear, usually opposite the protoxylem, except in diarch roots, in which case the new centers appear in rows along the flank of each protoxylem group (p. 42). At a very short distance from the growing apex these may be clearly discernible as branch-root initials. Whatever the internal factors producing the greater meristematic activity in this region, it is frequently clear that their influence extends out beyond the endodermis, and in many cases the cells in the cortex opposite the protoxylem contain a greater amount of protoplasm and exhibit toward dyes a behavior which is different from that of the rest of the cortex (*142*). (Pl. 22, B.)

The production of the branch-root initial seems to be the natural response of the root type of apical organization to a plentiful supply of food in the presence of other internal conditions favoring increased meristematic growth. While in the shoot organization the corresponding conditions lead to the vigorous production of new folds of meristematic tissue upon the surface, in the root it is seen that such an increase of meristematic activity shows itself as pockets or groups of meristem which necessarily lie within the confining endodermis. Thus, even though the cells outside the endodermis may show a tendency to become densely protoplasmic along with other cytological changes, they never grow and multiply and hence do not contribute to the new apical meristem which ultimately pushes its way out through them.

The existence of these normal endogenous apices behind the main apex is very closely correlated with the properties of the pericycle.

The cells in this layer, as well as those in the other layers within the stele, ultimately vacuolate, and in some monocotyledons they may do this very early. Thus in Hyacinthus, for example, the cells of the pericycle vacuolate within a few centimeters of the growing apex, the cells opposite the protoxylem being the last to do so. So far there is no record of such cells ever returning to the meristematic condition. Although various experimental methods have been tried, so far no branch roots have ever been induced upon roots of this plant, and a number of other monocotyledons show similar behavior.

In other monocotyledons, according to Brenchley and Jackson (*16*, *64*) roots of two types are found with very different anatomical characteristics, the one kind branching freely and the other practically not at all. The same two types may also be present in some dicotyledons (*182*).[13] The anatomical characters that are associated with branching deserve further investigation. So far as examined, the branching roots seem to have the narrower steles and show the heavier lignification of the xylem; they frequently are earlier in forming a secondary endodermis and as a result shed their primary cortex earlier. They are, therefore, thin and fibrous, while the nonbranching roots in the same plant are relatively thicker, white, and soft in texture.

Cocks[14] found with Camassia that when this bulbous monocotyledon was grown with its roots in water, they developed slowly, being thick and unbranched and packed with starch. Transference to relatively dry fiber produced a thinner, more freely branching type of root. Transference back to water again led to the formation of the thick unbranched type. Branching was induced in the thick unbranched region of the root by cutting off the tip and transferring the root to fiber. Cocks also found that the normal root type has a secondary or tertiary endodermis, with occasional passage cells, the swollen fleshy type a primary endodermis.

In those monocotyledons in which the root normally has the power of branching, probably even after vacuolation the pericycle may be able to return to the meristematic condition and give an adventive root initial as the result of wounding. Additional investigations by Cocks[14] suggest, however, that this happens only rarely in this group; on the other hand, it appears to happen frequently in the dicotyledons, provided that the injury consists only in the removal of a distal portion of the root, leaving the proximal portion, which regenerates the new root meristem, in contact with the shoot.

When a small piece of root system is completely isolated, the regeneration of new root meristems is certainly a very rare occurrence; however, if remains of old branch roots are present on the isolated portion, these will probably contain latent root initials which will now grow out. (Pl. 22, A.) Except for the type of organization of new root initials described for the fleshy root of Crambe, the production of new root initials upon isolated pieces of root appears to be a very rare occurrence, in marked contrast to the production of adventive buds.

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[13] BROWN, F. M. V. THE ANATOMY OF MARSH PLANTS. Leeds, 1928. [Unpublished thesis.]

[14] See footnote 9 on p. 57.

In the older dicotyledon root, as already indicated, with the formation of two cylinders of interclary meristem, the pericycle ceases to play its all-important rôle in the formation of initials upon the main axis. Roots appear in the neighborhood of the vascular cambium, in the region of the primary rays, usually facing a protoxylem group. (Pl. 22, C.) Such a position reminds one of the position of the young root in the pericycle, but it is difficult to know whether at this stage the governing factor is the position of the protoxylem or of the ray; the close association displayed between root initials and vascular rays will be considered just below in relation to shoot-borne roots.

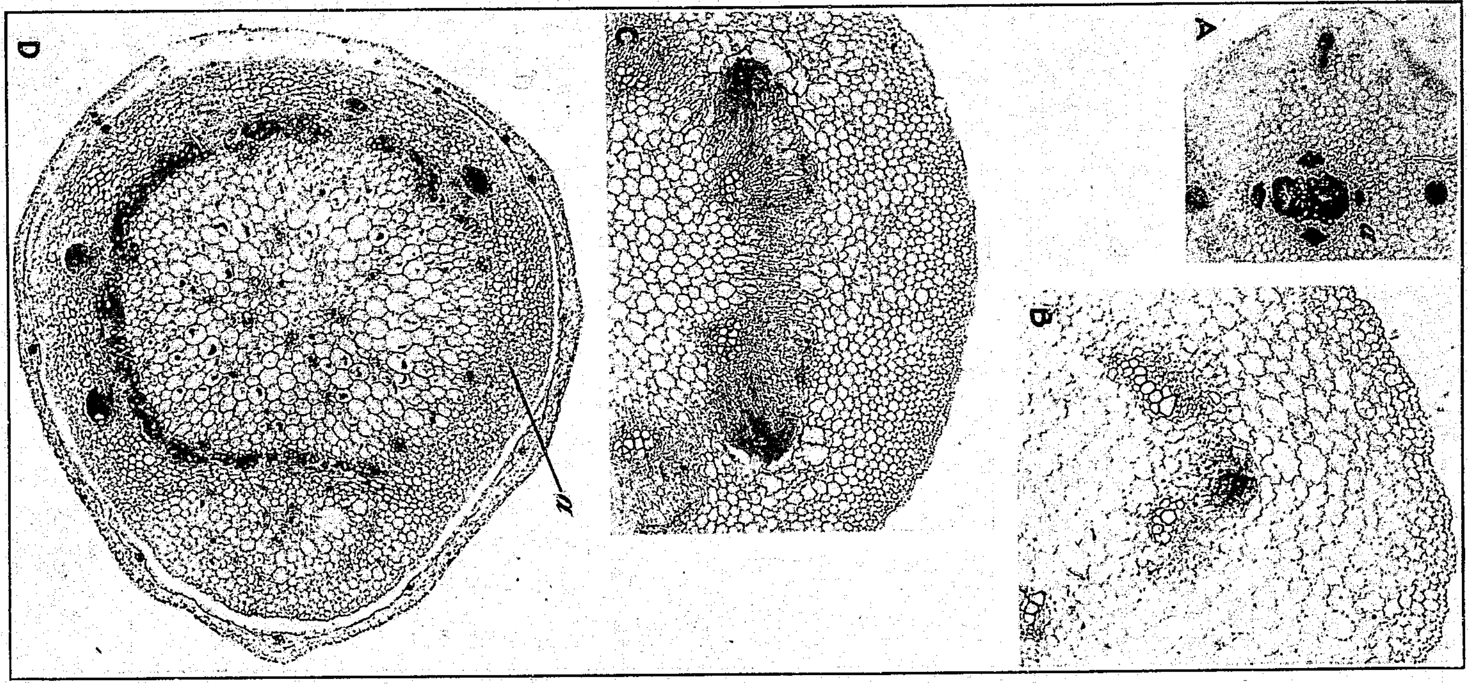
ROOTS UPON SHOOTS

The anatomical facts as to the place of origin of shoot-borne adventive roots are relatively simple and clear, but much confusion has been caused by failure to distinguish between the position of early formed roots and those formed later. Root formation in dicotyledon shoots may begin very early, indeed, even in the still-extending internode, in which case at first sight the position of the roots seems very different from the position of roots arising upon old stems which have ceased to extend longitudinally and in which radial growth alone is proceeding. Undoubtedly, however, there is a definite transition from one type of root origin to the other.

In the shoot, behind the apical meristem, vacuolation begins early in the pith and inner cortex, while between these two tissues is left a cylinder of dense meristematic tissue, the procambial ring. To either side of this ring cells are vacuolating and differentiating, but the outer and inner limits of the ring are bordered by the cells free from air spaces—on the inside the parenchyma, and on the outside the starch sheath; usually in water plants or underground stems and often in etiolated plants (pl. 23, A) the starch sheath becomes a functional endodermis (*141*, *118*).

As the vascular tissue differentiates within the procambial ring, the living cells, thus linked to the stele by a tissue free from air spaces, will receive a more adequate flow of nutrients from the supply within the conducting elements than will the cells farther removed. In the case of the xylem, the lignified elements have no semipermeable containing layer, and the liquid contents of these elements must be common to the walls of all this tissue. It is not surprising, therefore, to find that almost without exception new roots are always formed from tissues which are thus closely connected with the vascular supply. With root initials arising very early, when the procambial layer is young and has as yet not increased in girth, the usual position is in the cells lying just within the starch sheath—the pericycle, as it is often called, though in the stem the pericycle is much less clearly definable than in the root. Vascular differentiation usually, but by no means always, proceeds more quickly in those portions of the procambial ring which are immediately connected with differentiating leaf folds above. In such cases the new root initials practically always arise near, but to the side of, such a vascular group—that is, on a primary ray.

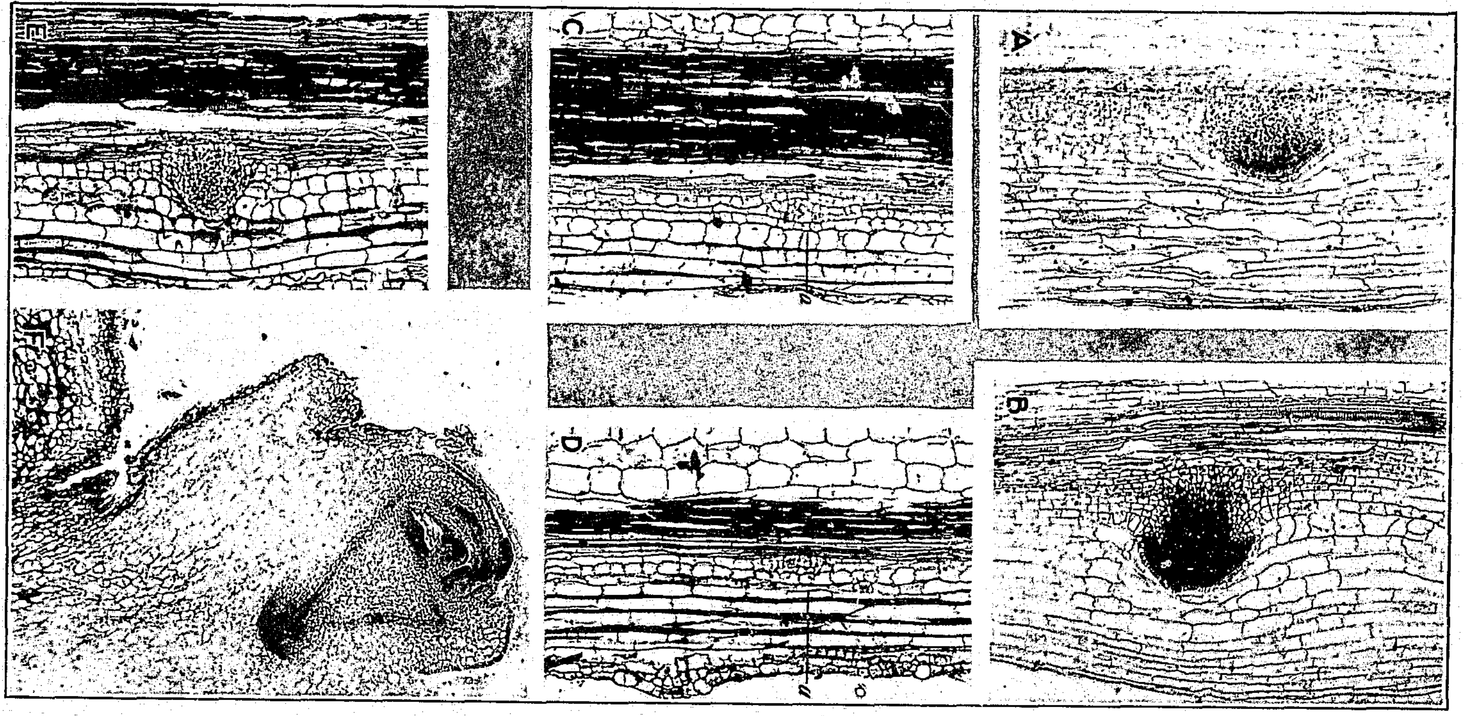
Root initials arising upon the young stem as in Lycopus, *Veronica beccabunga* L., and many others, are best described as pericyclic in position. Even in such a case, with a root appearing very early,



ADVENTIVE ROOTS ON STEMS AND HYPOCOTYLS (TRANSVERSE SECTIONS)

A.—Hand section of an etiolated shoot of *Pisum sativum*, heavily stained with safranin. At *a* the primary endodermis surrounds the narrow stele. This is not found in shoots grown in light. × 20.
B.—An isolated piece of hypocotyl of *Helianthus annuus*, taken near the proximal end. A young root initial is forming on the flank of one of the vascular bundles. × 60.
C.—An isolated piece of hypocotyl of *Ricinus communis*, taken near the proximal end and showing the formation of adventive roots on the flanks of two of the vascular strands. × 30.
D.—Young node of *Tradescantia fluminensis*. Several root initials are forming just outside the vascular ring. At *a* the procambial strands of an axillary bud are visible. × 20.

PLATE 24



A and B.—Longitudinal sections through proximal ends of isolated pieces of epicotyl of *Vicia faba*, showing two stages in the organization of an adventive root. × 30.
C–E.—Longitudinal sections through the epicotyl of *Lycopersicum esculentum*, showing at *a* early stages in root formation in the pericycle. These sections were obtained from internodes left isolated from the plant for two days, but such root initials also develop in the intact shoot on the growing plant. × 20.
F.—Longitudinal section of proximal callus on a fleshy root of *Cichorium intybus*. An adventive bud with procambial strands is well developed at the surface but buried in the tissues of the callus; at the base of the procambial strands a typical root initial has formed, so that in this region the tissue organization is that of a typical embryo. × 35.

its origin is not to be traced to a single cell layer, much less to a single cell, for the cells at this position in the procambial ring remain or again become meristematic; thus a group is now organized as a root apex, its outermost layer in contact with the starch sheath, and its inner layer in close contact with the differentiating vascular elements.

But as the stem grows older, changes in the arrangement of the tissues occur. In particular, the activity of the cambium increases the width of the tissues in the region of the original procambial ring. During all or a part of the first year of growth, in spite of this increase in girth, the starch sheath remains a continuous barrier to the advance of air inward along the intercellular spaces from the cortex. But sooner or later at the outer end of the rays, the cells vacuolate and round off, and air spaces appear between them. According to Klebahn (*73*), such intercellular spaces are formed throughout the radial course of the ray and often provide a channel by which the aeration systems of pith and cortex are in communication, but in the neighborhood of the vascular cambium the cells are more recently formed and are more compressed, so that the intercellular spaces are relatively smaller and more frequently filled with sap. Under these conditions it is not surprising to find the site of formation of the new roots moving inward from the region of the pericycle to the living cells of the ray that lie close to the newly differentiated xylem and phloem. From this stage onward new root initials are thus found in association with the vascular cambium; they still arise in the rays and are still not organized from single cells, resulting rather from the organization of a group of the cells bordering upon the vascular cambium which have remained or again become meristematic, and which may be connected to the newly differentiated vascular elements by a tissue free from air spaces. Plate 23, C and D, and Plate 24, A to E, show various stages of root organization in isolated nodeless segments of the hypocotyls of Helianthus and Ricinus and the epicotyls of Vicia and Solanum. In Ricinus it is clear that meristematic cells have been constructed by every layer of the primary ray from cambium to outer layer of pericycle; in *Vicia faba* the new root apex has clearly emerged endogenously from within the starch sheath. In both cases new tracheids are differentiated at the base of the new root apex, which will link the vascular bundles of the axis with the new root.

In the light of this more recent developmental work (on other plants), the assumption made by the junior writer (*159*) that the stem-borne roots of the apple originate from single cells seems to be erroneous; undoubtedly here also the deciding factor is the organization of a group of cells rather than some definite change taking place in one single cell.

Van Tieghem and Douliot (*172*), while pointing out the common endogenous origin of adventive roots from stems, confined their studies almost entirely to roots arising before the differentiation of the vascular cambium, or at a stage but little later; however, they drew attention to this change in the placing of roots which occurs with increasing age of the stem. In fact, all work regarding root initiation seems to conform with the picture as just drawn, with the exception provided by the Cruciferae, which will be referred to below.

The apparently divergent statements found in the literature regarding the place of origin of stem-borne roots seem to offer no exception to the foregoing, but seem rather understandable on the basis that most observations have been made with very young stems (*172, 181*); hence the statement made by Schüepp (*140*) is in apparent disagreement with the above. Furthermore, the fact that more than one layer of cells take part in the formation of the root has also led to a certain amount of confusion. The points that should again be emphasized are the pericyclic origin of roots on young stems, and the origin in the neighborhood of the cambium on older stems; in either case the roots are generally intimately associated with the rays; furthermore, their origin involves more than one layer of cells. This general viewpoint seems to hold equally well whether one is dealing with roots that grow out immediately after they have formed or with those that lie dormant for a lesser or greater period. In this latter connection see Trécul (*167*), Borthwick (*15*), Van der Lek (*86*), and Swingle (*157*).

Endogenous roots, though frequently more common in the neighborhood of a leaf insertion, may occur anywhere on node or internode. On the other hand exogenous roots, which seem to be restricted to the Cruciferae, occur only in the axils of the leaves, and are thus obviously associated with the axillary bud. This point was clearly brought out in Hansen's observations upon the origin of such roots in *Cardamine pratensis* (*52*). Here, the young root initial is found as a small protuberance upon the side of the base of the adventive, root-borne bud; it is clearly exogenous, the dermatogen in this region contributing the outermost layer of the rootcap of the newly organized root apex. While this group of meristematic tissue on the side of the bud thus organizes as a typical root apex, the part above develops the normal fold of the shoot apex, and the juxtaposition of tissues is such as is characteristic of the embryo, where meristems of both shoot and root are closely in contact and both exogenous. The organization of the root apex here is thus quite characteristic of a root, and all subsequent branches from this root are of the normal endogenous type. Once again it is seen that the distinction between root and shoot does not lie in their relative position of origin, but rather in their characteristic apical organization (p. 4).

The production of roots upon leaf cuttings is only a special case of adventive-root production upon the shoot and requires very little special consideration. In every case examined in detail by Regel (*124*), Hansen (*52*), Beinling (*12*), and Hartsema (*56*), the new root initials have been found to arise from parenchymatous tissue in close connection with the vascular cambium. Usually, therefore, such roots also are organized in the primary rays, though Hansen described them in Begonia as also arising occasionally from parenchyma that had been produced as callus tissue at the cut surface. Here again, the roots do not arise by the repeated division of any single cell from the cambium or any other one layer, but they result from the meristematic activity of a group of cells. Hansen stated that the calyptrogen first becomes recognizable and organization proceeds thence inward. This seems closely in line with the description and figures given of the organization of the root apex in the callus upon Crambe roots.

It will be remembered that root initials are formed on leaves much more readily than are buds, and indeed in many cases buds have never been obtained; if buds do arise, another crop of roots frequently arise from them in the normal endogenous manner, from the procambial ring of the newly organized shoot.

Adventive roots indeed arise very much more readily from shoots than do adventive buds, and the commonest method of vegetative propagation is the isolation of a shoot system with buds upon it, in the expectation that the cutting will root and become an independent plant. However, some plants root so uncertainly that this method of propagation can not be used in all cases. Thus many apples and many other fruit trees as well as a number of other hardwood trees form roots but rarely, while on the other hand the willow, the black currant, many varieties of apple, and many other plants (*42*, *86*, *175*) usually contain, as a normal thing, latent root meristems in the neighborhood of the cambial ring. In fact the difficulty in connection with root production from the shoot is not the statement of such simple anatomical generalizations as to their place of origin as have been given, but rather the correlation of these anatomical facts with the widespread divergence in behavior between different shoots. This problem is not a simple one, as is shown by the apple. Within this species there is a very great difference shown. The varieties that produce burrknots (*57*, *157*) contain as a regular and normal thing latent root initials which push outward into the bark and create the characteristic burrknots, while on the other hand very few varieties of apple have ever been rooted from ordinary hardwood or softwood cuttings. The recent papers by Graevenitz (*42*), Shaw (*143*), Vierheller (*174*), Yerkes (*188*), Auchter (*5*), Knight and Witt (*76*), and Maney (*93*) but emphasize this difficulty.

The papers just cited show that the problem thus indicated—the reasons for success or failure in rooting cuttings—is under vigorous attack from many angles. Therefore, in the hope of opening up some new avenues of approach to this important problem it seems worth while to analyze it from the anatomical standpoint more closely than has yet been done, even though such an attempt must necessarily be somewhat speculative. In making this attempt it has been possible to draw upon some unpublished anatomical investigations which were carried out in the Leeds botanical laboratory by W. A. Sledge and Elsie Briggs, and which, it is hoped, will be made more fully available later.

FACTORS GOVERNING ROOT PRODUCTION FROM THE SHOOT

AIR AND MOISTURE

In view of the characteristic apical organization of the root, root initials arising in the tissues of the shoot require ready, and therefore close, access to the vascular supply. Usually no air spaces exist between the meristem and the source of food, any intercellular spaces being injected with sap, so that the walls between the cambium and the meristem remain saturated and thus permit a steady diffusion of solutes. On the other hand, oxygen is necessary for the respiration of the newly organized meristem, though, as Swingle pointed out (*162*), some species are much more tolerant of oxygen deficiency than others. Probably this oxygen reaches the meristem mainly through the wider intercellular spaces of the cortex, which usually remain uninjected. The practice of clearing the latex from the base of cuttings of plants containing laticiferous systems (*152*) may possibly find its justification in the freedom of the cortical air spaces thus insured.

The balance between injection of the intercellular spaces behind the newly organized root apex and the free movement of air in the spaces in the cortex beyond it, is obviously a delicate one in the case of sensitive cuttings. Horticultural practice often prescribes in such cases that the base of the shoot should be rammed firmly against the side of the container. This should bring about at one and the same time aeration of the wider cortical intercellular spaces, because the cutting has a pocket of air around its base, and injection of the narrower intercellular spaces in the neighborhood of the vascular ring because of the response of these parenchymatous tissues to the pressure employed (*67*). Such a treatment may, therefore, be of very material significance in the case of quick-rooting herbaceous material; it is hardly likely to be effective in the case of slow-rooting hardwood cuttings. The difficulty frequently experienced in rooting cuttings with a large pith may be due to the fact that in such stems the air in the pith within the vascular ring may make it difficult to keep the intercellular spaces in the rays injected in the region of the cambium, and it may in some cases be avoided by using cuttings with a heel (*152*). Similarly, in experiments in which liquids are injected into the vascular system in the effort to assist root production (*27, 76*), where the pull is applied at the distal end (as by the transpiring leaves themselves), it is transmitted downward through the xylem alone, since the cortical air spaces are in ready contact with the outside air. Thus by this method the xylem and the tissues just bordering upon it are alone injected; on the other hand, when the same difference of pressure is used to drive a liquid in from below, the air in the cortex is easily displaced by the entering liquid, and thus any special meristematic activity in the region of the cambium would most certainly tend to be subjected to oxygen deficiency.

Swingle's experiments (*162*) have shown conclusively that the aeration conditions which are optimal for root production are not necessarily, if ever, identical with those which are optimal for callus production, although callus production has often been treated as synonymous with root production. In all cases examined callusing seemed to demand the presence of less oxygen than did rooting, in many cases actual inhibition of callusing being observed while rooting progressed apparently normally.

Corbett (*25*) emphasized the difference between rooting and callusing, and Balfour (*7*) showed that in some cases root production could be brought about by paring down the bulk of the excessive callus originally produced. In the detailed discussion of callus and meristematic activity at the distal end of the roots of Crambe, it was made clear that the cell proliferation and division in callus production and the meristematic activity of the phellogen which produces cork near the surface of such a callus were distinct from the subsequent deeper lying meristematic activity which was more closely associated with root production. Therefore, the removal of the superficial layers of callus may remove another meristematic tissue which is drawing upon a limited food supply, and leave more food available for the meristematic activity, associated with the vascular cambium, in connection with which roots are organized.

LIGHT AND ETIOLATION

Light certainly has little influence upon the development of the endogenous meristems at the base of a cutting, and usually it seems to have little influence upon the subsequent growth of the root apex (*116*), but it has been seen to favor the initiation and growth of any superficial shoot meristems which may be competing for the available food supplies of the isolated shoot (*41*). It seems, therefore, wiser to exclude light from the basal region of the cutting on which root production is desired. Still more important, however, may be the previous etiolation of the region of the shoot from which, at a later date, when isolated, root production is desired. The consideration of this problem requires a brief digression into the subject of the growth organization of the normal shoot.

The shoot growth unit.—As Beijerinck (*11*) pointed out, when root initials are present or can be induced experimentally, they are usually distributed around a leaf insertion, sometimes in the leaf axil, but more frequently to either side of it and a little below it. To this extent their occurrence is in accordance with Chauveaud's conception of a "phyllorhize" (*23*) or Celakovský's, of the "Sproszgliede" (*21*); that is, a section of the axis with its attendant leaf forms a natural growth unit, made up of a leaf and a portion of the axis below down to the insertion of the next leaf vertically beneath. This growth unit, it is argued, should naturally terminate at this point in a root initial. Thus in a simply organized vascular plant, as the sporeling of Ceratopteris, successive leaf-root units are formed; each bears on its ventral surface, in its turn, a growing point which gives rise to a similar new unit. The axis of this fern is thus built up of these merged contributions of the successive "phyllorhizes," while the roots do not form a separate branching system but arise at the base of each successive "leaf-shoot" unit.

In the dicotyledon, Celakovský and Chauveaud, among others, have given reasons for regarding the shoot axis as still built up by the fusion of similar successive growth units, only in this case the root is missing at the base of the unit, where it meets the insertion of the next leaf below. But Beijerinck's observations (*11*) have emphasized the fact that the adventive roots ("Beiwurzeln") appearing on the stem do usually occur in this position. Why, then, are they sometimes missing, and why does every effort to induce their formation in this position meet with failure? One possible answer is that at this point, where the shoot unit meets the shoot unit next below it, a new meristematic center is now invariably present which may draw upon the food supplies that would otherwise be available for the root; this is the axillary bud, a competing meristematic center that is not met with in most of the vascular cryptogams.

Further, in the dicotyledon, instead of the "phyllorhize" habit of growth, with root initials formed near the termination of each unit, the food materials move downward toward the main root system at the base of the shoot axis, which remains continuously active throughout life, new crops of roots being formed upon the scaffold roots from the food supplies brought down from the shoot. This difference is undoubtedly correlated with the change of organization rendered possible by the development of a vascular cambium. The layer of new vascular elements thus added to the axis throughout its length each spring, puts the new shoot units at the periphery of the branches of the axis into effective communication with the new root system similarly developing at the peripheries of the root system. Therefore, even when the supply of food at the base of a potential "phyllorhize" is not used in the organization of an axillary shoot, instead of being diverted to root organization at this point it may move on downward in the continuous chain of vascular connection constructed by the cambium.

In these few words a very complex and controversial morphological and phylogenetic problem is just touched upon, further information in regard to which may be obtained in a monograph by Chauveaud (*23*). It has been discussed primarily because of the suggestion that immediately follows as to the significance of etiolation, but it seems probable that considerable significance may be attached to this growth-unit conception in the interpretation of the physiology of the shoot.

Etiolation prior to cutting.—Darkness certainly inhibits the superficial meristematic growth of the shoot apex. Internodal extension, in connection with existing leaf-shoot units, may take place vigorously, but no new growth units form, and if the meristematic apex is but slightly organized, as in the embryonic epicotyl of an epigeous seedling, in darkness it makes little or no further growth, and the hypocotyl alone extends until the food supplies of the seedling are exhausted, the epicotyl never emerging from between the cotyledons (*115*, *118*).

Similarly, at the East Malling Research Station, Director R. G. Hatton and his associates have had marked success in obtaining roots upon fruit-tree layers which root with difficulty by keeping the new shoots etiolated for several centimeters; however, they found it necessary to compromise between number of shoots and freedom of rooting. Although the shoot which has been continuously etiolated roots by far the most readily if the buds are buried, even though but a thin covering of earth is employed and even though this is not put on until just before visible activity of the buds is to be expected in March, nevertheless decidedly fewer shoots per stool are obtained than when the covering of soil is applied some weeks after bud break has occurred.

Likewise, Knight and Witt (*76*), working with plum and apple, Reid (*126*), and Blackie, Graham, and Stewart (*13*), working with camphor, Smith (*146*, *147*), working with clematis, and Priestley and Ewing (*118*), working with broadbean, have recorded a considerable enhancement of the capacity of the shoot to root through etiolation, a fact which has been on record since very early times (*33*). In all such cases the development of the young axillary shoot meristem is retarded by darkness, and frequently under these conditions food which might have been utilized in its growth appears to be employed in the organization of root initials in the neighorhood of the cambium. However, the presence of a functioning assimilatory region above the etiolated part seems to be desirable in most of these cases.

Various other suggestions have been made as to the significance of etiolation of the shoot prior to its removal for purposes of propagation. Priestley and Ewing (*118*) pointed out that in the epicotyl of *Vicia faba* and other hypogeal seedlings, etiolation led to the production of a primary endodermis in place of a starch sheath, a change which, as has been seen, tends to favor endogenous growth activities as against exogenous. Reid (*126*) and Smith (*146*, *147*) drew attention to various histological changes that certainly would favor the emergence of rootlets though it is not clear how such changes would influence their initiation. Knight and Witt (*75*) found that etiolated cuttings of Prunus produced most of the roots laterally; these were the "morphological" roots of Van der Lek (*86*), while without previous etiolation the few roots obtained almost all emerged through the wound callus.

The general considerations just advanced increase the significance of these recent observations and suggest that etiolation of the shoot prior to its removal for purposes of propagation (i. e. partial layerage) is a line of experimental attack worthy of considerable physiological and anatomical attention.

THE HARDWOOD CUTTING

Other recent extensive series of experiments upon the propagation of cuttings are at first sight apparently in direct disagreement with the generalization just attempted. From the standpoint of the preceding paragraphs, the development of the axillary buds may be a definite contributing factor in the failure of the shoot to root. Van der Lek (*86*) concluded as the result of a very extensive series of experiments that the development of roots upon hardwood cuttings—that is, woody twigs with dormant buds and no leaves—is materially favored by the development of the buds. However, generalizing from Van der Lek's experimental results requires caution. As Swingle has already pointed out (*162*), the further development of preformed roots of burrknot apples was affected but slightly, if at all, by the presence of buds upon the cuttings. The general question of the relation of bud start to the initiation of root apices on the stem beneath deserves further examination.

CAMBIAL ACTIVITY AND ROOT PRODUCTION

Apparently, the cambium furnishes the connecting link between activity of the bud and the initiation of new roots. As has been seen, the new root initials are always formed in tissues in close association with an active cambium. During the winter months the cambium of the branch is usually in a dormant state, and upon the tree in the spring cambial activity begins beneath each bud and works thence downward along the stem (*47*, *156*). This seemed to suggest a ready explanation of the observations of Van der Lek

(*86*) and of the correlation often reported by others between bud activity and root production, especially as a similar renewal of activity beneath the buds on willow cuttings, which then spreads basipetally down the cutting, has already been recorded by Hartig (*55*). But an anatomical examination of the question shows that it is not so simple.

When a piece of shoot is isolated, although but slightly less callus may form at the distal end than at the basal end, cambial activity itself seems to be confined to the basal end of the cutting. Sledge[16] found that this renewed activity spreads slowly up the stem, but usually cambial activity is also working downward from breaking buds higher up on the isolated shoot. Examination of such cuttings at frequent intervals has revealed much material in which there is a neutral, dormant zone lying between the pushing buds and the active basal region. At the base the cambium at first frequently forms wood of the "wound wood" type,[17] but in some cases the wood is indistinguishable from the normal wood. After the cambial activity has become continuous throughout the shoot, the wood formed throughout is of the type usually characteristic of the stem.

These observations render it impossible to ascribe in all cases the advantage of the presence of buds to the start thus given to the cambium. Cambial activity at the base is certainly essential to root initiation in this region, but the activity may be quite independent of the bud, as indeed is shown very clearly by experiments with internodal cuttings carried out in winter with short shoot pieces in which the cambium is still dormant. In practically every case of this kind, Sledge[16] found that cambial activity was initiated at the basal end of the cutting.

It seems necessary to reserve an opinion as to the relation between bud development and root initiation. Van der Lek's cuttings were under unusual conditions, being exposed to the light in a very moist atmosphere. Under these circumstances probably even the basal buds would commence activity, and the cambium activity thus initiated would soon affect the root initials beneath it, or, as in the grape cuttings, the tissues disposed toward root formation. With cuttings which have several basal internodes buried in the sand, the lower buds rarely develop; usually cambial activity, and often rooting as well, will commence at the base before this cambial activity is linked with that working down from the pushing buds. For continued root development a shoot system developing in the light is essential, but the connection between the just-emerging bud and root initiation at the base of the hardwood cutting is far from firmly established.

Interest seems rather to be transferred to the conditions governing meristematic activity in the neighborhood of the proximal wound and that delicate internal balance which converts this activity from the production of ordinary new vascular tissue to the organization of root growing points. Almost certainly one essential for the resumption of cambial activity is the displacement by sap of the air in the

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[16] SLEDGE, W. A. Unpublished research at University of Leeds.

[17] The writers' attention was first drawn to this point by Dr. R. C. Knight, of the East Malling Research Station.

intercellular spaces in the neighborhood of the cambium. Sledge[19] found that if sections cut from woody shoots just before and just after resumption of cambial activity in the spring are transferred directly from the knife to strong glycerin, a marked difference in the appearance of the tissues will be noted in the two cases. Sections cut before the resumption of cambial activity appear dark with the trapped air contained in the vascular rays, even in the cambial region; but as activity begins, the whole of this region becomes translucent throughout, and air is confined to the inner part of the wood, the pith, and the outer regions of the phloem.

When the dormant twig is cut out of the tree, the narrow intercellular spaces near the cambium will become injected with sap; this is one response of the delicate living tissues in this region to the shock. At the distal end this condition does not seem to persist, and everything points to a rapid drying back of the sap from the neighborhood of the cut surface in many twigs until the neighborhood of a node is reached. At the basal end this condition of injection seems to persist whether the cut surface is nodal or internodal, and as a result cambial activity is initiated.

Here, once again the difficult problem of polarity arises, and it is impossible at the present time to evaluate the part played by the phloem and the other tissues in thus maintaining the conditions for cambial activity at the proximal end. This problem will be again considered in connection with the influence of metabolic factors upon the initiation of roots. First of all, however, the specially difficult case of the scion apple must receive brief examination.

The propagation of the apple.—In many unsuccessful trials at Leeds and at Washington with the apple the cuttings either rotted or dried out. Rotting is understandable in these cases in view of the fact that suberization of the cut surfaces is often very irregular in the pith region. Frequently a well-callused base of such a cutting shows a hole in the center of the callus, leading into a disorganized pith. If such a cutting does not root within a reasonable time it is almost certain to decay. In fact, with older cuttings containing burrknots the cut base in many cases rots because of ineffective healing of the cut surface, even though roots may have long since appeared.

On the other hand, the drying out of the twig means that the cambium layer is certainly unable to continue to function, for the tissues in its neighborhood are no longer injected with the necessary moisture. (See the note regarding the propagation of the hop on page 56.) Examination of such cuttings usually shows that the cambium had commenced activity at the base, and frequently this activity has exhausted the starch throughout the entire cutting. In such cases the starch usually disappears from the cutting from the base upward, instead of from the pushing bud downward, as happens when the shoot is left on the tree (*156*). This depletion of the food reserves may be a very important contributing factor in the failure to produce roots. In this connection Winkler's work (*184*) on the relation between starch content and root production in the grape cutting is important.

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[19] See footnote 16 on p. 70.

Cambial activity commences at the base of the shoot, and thus are produced differentiating xylem elements which, if protected beneath by a suitable callus material, will act as an osmotic system and draw water from the soil around the base of the cutting. Examination of the withered cutting clearly shows that the sap movement has not produced its usual effect. Either leakage away from the wood through the disorganized pith on the inside or a certain lack of effectiveness in the system of differentiating xylem and basal callus seems to be responsible. The writers' attempts to remedy this defect by sealing the base of the cutting did not prove effective.

Some of the difficulties of cutting propagation are avoided by the employment of the nurse-root method (*5*, *143*). This method involves the grafting of a piece of stock root upon the base of the scion that roots with difficulty. In some cases the scion is buried very deeply in the ground in the hope of getting roots upon this original piece, while in others short scions are used, and the root system is obtained at the base of the new growth. The root system of the cutting is thus in either case at first furnished by the easily rooting stock, but with many varieties after a year or two roots develop upon the scion; the nurse root can then be removed from many of the cuttings, and the rooted plant can be replanted as a complete tree. The chief difficulty offered by such a method, however, is the greater relative development of stem than of roots upon the scion, so that the scion when removed has insufficient roots to maintain it. In this system of treatment the difficulty of maintaining a sap supply in the scion is met by the vigorous growth and root production of the nurse root which absorbs ample water from the soil and delivers it to the scion. At the same time disorganization and decay at an imperfectly blocked pith surface in the scion are avoided by the presence of the graft union.

Auchter (*5*) emphasized the need for deep planting. Such deep planting will obviously materially aid the nurse root in the task of keeping the scion tissues well supplied with sap and at the same time will favor meristematic activity in the cambium rather than in the bud.

However, the problem is by no means solved. Root production on the scion depends not only upon a vigorous cambial activity but upon an abundant supply of elaborated materials in the neighborhood of the cambium. Much of this material will move down out of the scion tissues into the stock, where vigorous root production takes place. To some extent this movement may be checked at the base of the new growth, where a wire ring is sometimes applied for this purpose, or at the point of the graft union; nevertheless the drain of the stock-root system upon the supply of elaborated food will materially reduce root production in the scion.

In this connection Chandler's (*22*) modification of the nurse-root method is of great interest. He grafted the stock-root piece into the side of the scion so that a short piece of the scion projected below the union. The result was that materials to some extent moved past the point of union down into the stub of the scion; here root production took place, sometimes laterally, in some varieties mainly through the callus formed over the base of the stub; in a very few cases roots also formed above the union. The success of this method suggests that, provided the scion is kept supplied with abundant sap, suberization and callus formation at its exposed base will be satisfactory, and root formation from the wound callus is thus added as a possible method of root formation.

However, this method seems to have one very serious if not fundamental drawback. These conditions are extremely favorable for the production of what have long been called crown galls. Even though the recent work of Riker and Keitt (*128*) seems to indicate that such overgrowths are not so serious as has long been thought, they are at least undesirable.

If by the devices indicated the cambial region in the isolated shoot system can be kept full of sap, the result seems likely to be continued meristematic activity and thus in time the production of roots. This may also be the explanation of the successful rooting of softwood cuttings of apple, reported by Stewart (*152*). In such a shoot, as has been seen (p. 62), air is prevented from approaching nearer the cambium than the outer wall of the starch sheath, and when such material is severed for propagation it at least starts with the tissues well provided with sap. In fact, one of the most commonly used criteria for ascertaining that the shoot is in proper condition for use as a softwood cutting is a state of high turgidity; the desirable shoot usually breaks off clean when bent, and the unsuitable shoot collapses and crushes.

Unfortunately, none of these methods or that of root-cutting propagation described by Yerkes (*188*) has the simplicity or certainty requisite for extensive employment in the commercial nursery, although a variety of methods are available which may enable the investigator to obtain scion varieties of apples upon their own roots. For the nursery practice at present the only method seems to be grafting either upon stocks grown from seed or upon vegetatively obtained layers or root cuttings.

THE SOFTWOOD CUTTING

In the softwood cutting the shoot is taken at a time when its leaves are still functioning. There is some disagreement among practical horticulturists as to the rôle played by the leaves in the propagating frame, since the most obvious effect of the leaves is their tendency to dry out the cutting. Hence, the more leaves remaining, the more saturated must be the atmosphere within the propagating frame in order to avoid draining the sap from the neighborhood of the vascular ring, where its presence is all important for root production. Provided this contingency can be avoided, the presence of the leaves seems highly desirable, since the older leaves, in the light, continue to supply a considerable amount of food to the axis. The quantitative studies of Loeb (*90*) on Bryophyllum give some indication of how important the leaf may be in this connection, and in such herbaceous cuttings the relation between rooting and the downward movement of materials from the leaf through the vascular channels is much more evident than in the case of hardwood cuttings.

From the time the procambial ring first shows its close connection with the leaf initial, cambial activity is obviously closely allied to the growth of the leaf. In the shoot the first tangential divisions initiating cambial activity in the procambial strand follow close

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upon, if they do not actually precede, the earliest signs of vascular differentiation. Jost (*70, 71*) has shown that this process of cambial division is greatly dependent upon the growth of the lateral organs, whether leaves or flowers, above it on the axis. His experiments with the epicotyl of the seedling make it clear that the controlling factor is not the food supplied by the cotyledons lying below, but rather the actual growth processes occurring in the young lateral initials above.

In the twig, the subsequent dependence of cambial activity upon growth processes in the bud seem to be only a continuation of the same phenomenon. In experiments with decussate-leaved Coleus, using isolated pairs of internodes with one single node between them, it is easy to see that great influence was exerted by the growing leaves, both upon ordinary cambial activity and upon root production in the axis beneath. Here again the factor affecting growth was not simply food supplies; although the entire basal region of such cuttings is usually gorged with starch, nevertheless cambial activity and root production lag behind on the flanks of the proximal internodes which are not subtending a leaf. Plett (*109*) [20] in his experiments with internodal herbaceous cuttings noted an accumulation of the starch toward the proximal end of these cuttings in many cases, especially in Begonia, *Sanchezia nobilis* Hook., and *Sinningia purpurea* Hort. In these cuttings and in others in which some leaves were left, starch accumulation often coincided with greater rooting, but Plett also found an influence of the bud upon root formation in the internode below, which seemed quite independent of the localization of food supplies.

The basal movement of the carbohydrate contents, as indicated by starch distribution, in such internodal cuttings may be very striking. In some of Briggs's observations upon isolated nodes of Coleus the congested appearance of the phloem in this basal region certainly suggested that it had taken a part in the movement. Greenwood [21] also found that if isolated shoots of Pelargonium and other herbaceous cuttings were ringed, cambial activity began at the proximal surface above the ring, as well as at the basal end of the cutting. In some cases there was also distinct evidence of a greater depletion of carbohydrates from the upper end of internodal cuttings and some indication of starch accumulation at the basal (proximal) end.

If the direct course of the vascular channels beneath a leaf is broken by a cut in which a mica slit is inserted, then within a few days root production and cambial activity can be diverted to another flank in these plastic tissues. Anatomical investigations made by Simon (*145*) and confirmed by Miss Elsie Briggs, show, however, that this diversion is preceded by the differentiation of new vascular strands on the other side of the stem.

The evidence is, therefore, again very strong for associating root initiation with cambial activity and this once more with the activity of the leaf and probably with the transmission through the phloem of some substances which accumulate in the region where roots are ultimately produced.

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[20] See footnote 6 on p. 31.

[21] GREENWOOD, L. THE PROBLEM OF POLARITY IN ISOLATED PIECES OF STEM AND IN INVERTED GRAFTS. Leeds. 1927. [Unpublished thesis.]

Sledge[22] has observed in internodal cuttings of privet, apple, and other plants that after a time definite evidences of disorganization, which apparently begins in the phloem, are visible at the distal end. Early signs include a swelling and vacuolation of the phloem parenchyma cells, accompanied by a gradual disorganization of their nuclei, following which the cells of the cortical parenchyma frequently swell and discolor. This change is always associated with a very marked alkalinity of the tissue. The reaction is very definite and sap diffusing from the phloem is as alkaline as pH 8. These observations suggest a continued basal movement of substances in the phloem which may ultimately result in a disorganization of this tissue at the exhausted distal end, in which, when carbohydrates fail, respiratory metabolic activity leads to the destruction of protein with consequent nuclear disorganization and the development of an alkaline reaction. The continued downward movement of substances through the phloem may at least be a contributory factor to the polarity of the isolated shoot segment and the partial cause of the accumulation of carbohydrates at the proximal end. The relation of such an accumulation to subsequent root initiation is indeed difficult to determine. Such carbohydrate accumulation has to take a particular form; possibly the development of a relatively acid reaction, which is often associated with a high carbohydrate-nitrogen ratio and a diminished degree of succulence (*103*, *63*), is a necessary condition.

Reid's (*125*) observations on the effect of the carbohydrate ratio on the rooting of the tomato are direct experimental evidence of a nitrogen correlation which is generally recognized, and which is the partial explanation for the restricting of initiation and development of roots above a ring extending into the cambium and above ligatures constricting the bark. Such a relative increase in carbohydrates is probably associated with rooting in the pendulous tips of certain varieties of Rubus in the later part of the growing season, in the drooping stolons of *Cornus californica*, and is probably the factor linking flower-bud and burrknot formation in the apple (*157*).

Temperature may exercise a very definite influence upon root initiation by its influence upon the balance of metabolism. Bushnell (*19*), analyzing the potato plant as affected by temperature, shows that carbohydrate accumulation in the plant increases rapidly with a fall in temperature, supposedly because, although photosynthesis may have a comparatively slight temperature coefficient, the utilization of sugar in respiration diminishes very rapidly with a fall in temperature. This brings to mind an interesting comment upon the early observation of Vöchting (*177*), confirmed by Bushnell, as to the geographical distribution of the potato; stolon and tuber formation is favored by low temperature and leafy-shoot production by higher temperatures. From such an effect upon the metabolic balances of the plant, lower temperatures might similarly be expected to favor root production, while on the other hand with a rising temperature, exudation pressures, which depend upon growth and differentiation and which are intimately connected with the maintenance of shoot meristems, are likely to be greater. This is

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[22] See footnote 16 on p. 70.

an almost unexplored field. Beijerinck (*11*) recorded the observation that the apple and the pear produce root-borne buds (suckers) more readily in a warm climate. Likewise in the fruit trees of Europe many observations suggest two maximum periods of root growth, one in the spring at the expense of stored food reserves and the other in late summer or fall after foliage production has reached its maximum (*106*).

However, accumulation of carbohydrates does not necessarily mean the production of conditions favorable to root initiation. Plett (*109*) [23] found it necessary to distinguish between the accumulation of starch at the base of an internode and the production of roots from this region. There is indeed a good deal of evidence which suggests that in particular the presence of starch should not be regarded as a necessary indication of a disposition to produce roots. Ahrns (*2*) showed that in an isolated leaf system starch is only retained or produced under certain conditions, even when the carbohydrate content is very high. Thus, if the leaves are allowed to lose water and wilt, the starch rapidly disappears and the sugar content in the mesophyll greatly increases. On the other hand, if the leaves are kept saturated with water, the starch, even in the dark, disappears very slowly and the sugar concentration remains low (*111*). Starch appearance in this condition, therefore, seems to be an indication of a condition of high hydration of the tissues, and it may be recalled that, when dealing with root production from roots, the swollen starch-filled root was associated with an indisposition to branch. In the case of the shoot similarly the consideration of one striking case in which high carbohydrate content does not necessarily involve a ready disposition to root, may conclude this section.

A TYPICAL CONUNDRUM (THE POTATO TUBER)

Probably the reader requires no further reminder that these pages represent only the effort to state a problem and not the attempt to justify a solution. The production of roots upon shoots bristles with difficulties in the way of any generalization, and the tentative efforts made above seem likely to be shipwrecked by such a homely object as the potato. The tubers of the potato are in part the products of cambial activity, and they contain a cambium capable of further meristematic growth. Very abundant food reserves, especially carbohydrates, are present; and they also have both internally and externally a very widely distributed phloem, very parenchymatous it is true, but with sieve tubes ramifying through it. Yet from the tissues of the tuber itself, notwithstanding numerous experimental treatments, apparently there has not been obtained one authentic case of root production. The same seems to be true for tubers of certain species of Dioscorea (*176*).

Although cambium, carbohydrates, and phloem are all present, conditions are still definitely unsuitable for root production. On the other hand, in the stolons an endodermis is present, and endogenous roots are produced with exceeding freedom. Cases where roots seemed to emerge from the tubers have, on examination, proved to be cases of roots arising from the bases of stolons not completely

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[23] See footnote 6 on p. 31.

freed from the tuber, or (*135*) from the point of insertion of the tuber upon the parent stolon.

As the end of the stolon swells and the new tuber forms, the endodermis disappears, and a cork phellogen begins activity, at first in the epidermis and later in the subepidermal layer. Thus the formation of the tuber is associated with a superficial meristematic activity, which, however, is not followed by free bud production, for the regeneration of buds from the tuber, though occurring from wound tissue occasionally, seems to be usually limited to the neighborhood of the eyes. In the resting tuber probably the intercellular spaces in the cambial region, as elsewhere, are filled with air; with the outgrowth of the buds the cambium may temporarily become active again, but root production does not follow. A certain vague correlation is here suggested between the failure of root formation and the swollen, starch-packed, unlignified, unbranched root. Evidently food supplies are abundant, but at no stage of depletion do conditions become propitious to the initiation of new roots.

This case seems worth its position at the end of this account of root production on the shoot, if only to show how far still is the interpretation of the phenomena from being complete. It is obvious that the problem of root initiation is far from settled.

ADVENTIVE EMBRYOS

Although both adventive roots and adventive shoots have been considered in some detail, a few words must be said about the appearance of these two structures when they arise together, even though at the present time the employment of adventive embryos as a means of growing uniform plants seems more an alluring vision than a definite promise.

As is too well known to stress here, with very many plants the desirable features of vegetative propagation as compared with seed propagation are more than offset by the great difficulty of handling. Hence the prospect of combining the desirable features of vegetative propagation with the ease of handling characteristic of seed propagation, is indeed inviting. It has been known for many years that one type of polyembryony is characterized by the formation of embryos, by tissue of the nucellus, which genetically represents only the seed parent. These embryos push into the embryo sac and compete for space and food with the true embryo. Apparently in most cases the true embryo wins the struggle; hence the rarity of development of the asexual embryos makes it almost impossible to recognize them when they actually do appear.

Recently, however, Frost (*37*) has called attention to the fact that in certain citrus hybrids the true embryo is apparently always crowded out by one or more complete adventive embryos, so that in these cases "vegetative reproduction by seeds" is achieved. Such facts regarding the occurrence of this phenomenon as are known are chiefly genetical in nature, and apparently nothing at all is known regarding it from the standpoint of causal anatomy.

Attention has been called (*77*, *161*) to another type of asexual seed production which is apparently not dependent upon the stimulus of fertilization as were the cases referred to by Frost (*37*). Kobel (*77*) found that unfertilized flowers of apple set seed under conditions not at all understood and pointed out the importance which such asexually produced seeds would have for use as grafting stocks.

If such a use of terms is permissible, it may be said that Plate 24, F, illustrates a still more asexual type of adventive-embryo formation, but one which does not seem to have the importance possessed by the two types just referred to. In this case of *Cichorium intybus* apparently a bud has arisen on the callus tissue around a branch root (p. 47). However, instead of the procambial strands differentiating backward through the callus, and ultimately connecting with the vascular supply of the main root, a root growing point has arisen just behind the bud and in such a position as to suggest that the two structures differentiated in the callus simultaneously. Here again nothing can be said about the causes underlying such a remarkable deviation from the course of events usually shown, but shoot and root initials also develop in equally close juxtaposition in the axillary buds of many of the Cruciferae (p. 64), where, just as in the true embryo, the root initial associated with the bud is organized in an exogenous position, though its subsequent growth is of the usual endogenous type.

DISCUSSION

THEORY AND PRACTICE

No attempt has been made in the previous pages to describe exhaustively the wide range of phenomena that are covered by the term "vegetative propagation." Not only do these phenomena differ as to position and mode of growth of the adventive structures concerned, but there are endless differences of details as to the time these growths take to form, and their response to season. Any effort to enunciate a general law governing these phenomena, therefore, meets with the almost inevitable sequel that, from among the multitude of slight individual variations, some example is forthcoming which appears, in its behavior, to refute the generalization.

So impressed are some of the most experienced workers in this field with this aspect of the problem that they incline toward the idea that the only adequate generalization is that each plant, or at least each species, is a law unto itself and requires individual study before its behavior as a self-propagating unit can be determined. There is obviously a substratum of truth in this standpoint; the test of practical experience alone will determine the method of propagating a new species, but if this view is exaggerated it becomes the negation of science and discourages all effort to ascertain the biological framework common to a group of plants which are universally recognized as forming a natural group. Throughout the previous pages, therefore, an effort has been made to present the varied phenomena from a consistent standpoint, but with as little consequent distortion as possible.

Both botany as a descriptive science and horticulture as, to a large extent, systematized and recorded practice, tend to be smothered under a mass of detailed observations, which convey no message because they have not been brought into contact with any generalized statement of the problem of plant growth and behavior. Therefore, in the writers' opinion, there would be no justification for this restatement of the problem of vegetative propagation from the standpoint of anatomy, if it were not associated with an effort to combine the facts into some working hypothesis regarding the factors controlling growth and development. Such a working hypothesis has been found in the suggestion as to the internal factors controlling the normal growth and development of shoot and root. All the varied phenomena of adventive shoot and root production that have been considered, have been briefly interpreted as they came under review, from the standpoint of the internal conditions assumed to be necessary for the production and maintenance of the "normal" activity of a meristematic apex. Undoubtedly this interpretation will need modification as the infinitely varied details of these complex processes are rendered available by further investigation, but at least it provides a standpoint which should promote rather than hinder fuller inquiry, and it is certain that in such further examination more light will be obtained upon the little understood phenomena governing normal growth.

Since any working hypothesis that is to justify its existence should open new avenues of inquiry, it is desirable not merely to examine the recorded facts, but also to consider whether the standpoint adopted adds anything to the interpretation of the complex interrelations existing between the internal phenomena of propagation and the contributing external factors. This brings the observer into the very diversified record of practical experience, where, owing to the almost inevitable horticultural method of varying several external factors at once, generalization must be cautious and tentative.

It may be emphasized that the experienced horticulturalist who claims that every species is a law unto itself implicitly displays in his procedure as determined by his experience, a system of wide generalization as to plant behavior. His attack upon a new plant is governed by this generalized experience; he assumes a common biological response from his material, and his decision as to the procedure to follow in such a case is not determined haphazard, but by his knowledge and observation of that particular plant's normal habit of growth. As botany and horticulture become more closely allied it may be expected that the experienced propagator, recognizing the aim common to workers in both fields, with his wide and sympathetic understanding of the plant's behavior, will more and more help in the task of interpreting this behavior in terms of the still-rudimentary science which aims at expressing the facts as to the structure and physiology of the plant.

THEORIES OF POLARITY

All attempts to generalize as to the factors governing the production of new shoots and roots upon the plant seem to have been stimulated by a recognition of the innate polarity exhibited by the plant. The isolated shoot tends to produce adventive roots more readily than adventive shoots, and to produce them at the lower (proximal) end. The isolated root produces adventive buds more readily than adventive roots, and these buds appear most frequently at the upper (proximal) end. Thus, each portion of the plant acts as though it were trying to restore the old balance by the regeneration of the missing structure.

THEORY OF FORMATIVE SUBSTANCES

From the impression of this innate polarity exhibited by any isolated part of the plant, the conception developed that such an isolated system still contains two moving currents of formative substances, shoot-forming materials moving toward the shoot end, and root-forming materials moving toward the root pole. Largely through the writings of Sachs (*132*) this " formative-stuffs " hypothesis has had a wide popularity. A summary of this theory was given by Darwin (*28*) and another more recently by Hartsema (*56*). It is not proposed to give an account of its early development, as, apart from one special field of investigation, this standpoint is now only of historical interest.

In reference to this one problem, however—the effect of the condition of the plant at the time it is used for propagation upon the nature of the structures formed in regeneration—the phraseology of the " formative-stuffs " hypothesis is still usually employed, and in this field is found the only positive experimental evidence which has been brought forward in support of such hypothesis.

EFFECT OF MATURITY OF THE PLANT UPON ITS REGENERATION

In his experiments with Begonia, Sachs (*133*) noted that adventive shoots grown from leaf cuttings taken from plants in flower themselves seemed to reach the flowering stage precociously early. These results were immediately expressed in the phraseology of the " formative-stuffs " hypothesis and were interpreted as due to the large amount of " flower-forming substances " which were naturally present in leaves isolated from plants at the flowering stage. Figdor (*36*) made similar observations with leaf cuttings of *Monophyllaea horsfieldii* R. Br., Wakker (*179*) with *Begonia rex*, Stewart (*151*) with stem cuttings of Gardenia; and indeed this particular characteristic of propagation has been repeatedly recorded in horticultural literature. Doposcheg-Uhlár (*30*) and Winkler (*185*) have pointed out that when leaf cuttings are taken from plants in which the leaf form changes with the maturity of the plant, the shoots from the juvenile forms take longer to reach the adult form of leaf than do adventive shoots arising on the adult-leaf form.

The phenomenon is not restricted to leaf cuttings. Stewart (*150*) recorded a striking experiment in which shoots were grown from a series of pieces taken from a single long root of Acanthus. The buds arising in the older pieces of the root produced the adult type of leaf very much earlier than did the buds from the youngest piece of root; this developed in succession a series of leaves passing from the juvenile to the adult form. Similarly in some species of Begonia and some Gesneriaceae, when the leaf cuttings are taken in the fall, swollen tuberous structures, closely covered with small modified leaves are produced instead of the usual vegetative buds.

Very few systematic attempts have been made to understand these propagation phenomena, though in at least one case an interesting experimental confirmation of the " formative-stuffs " hypothesis has apparently been supplied. Doposcheg-Uhlár (*30*) studied the development of tubers from leaf cuttings of "*Gesnera graciosa*"; a number of these tubers, which are freely formed in the fall, were collected, ground in sand in 50 per cent glycerin and left in the extracting medium for eight days. The extract was then filtered off, left in a desiccator for some months, during which a brownish yellow precipitate settled out. The following summer the glycerin extract was precipitated with alcohol, and the precipitate was washed with alcohol and finally dissolved in water and made a clear solution. Leaves of the Gesneria plants were then in some cases separated from the plant, the leafstalk being at the time of cutting immersed in this solution, while other leaves were injected with it. On the first occasion, at the end of July, when this was done, neither experimental leaves nor normally treated leaf cuttings developed anything but roots. In a later experiment, September 1, while the few normally treated leaves developed only leafy buds, the majority of the experimental leaves developed both buds and tubers, and in some cases tubers only. By November all the leaves of both checks and experimental plants had developed tubers.

These experiments are put forward by Doposcheg-Uhlár (*30*) as evidence that tuber formation is here to be regarded as caused by a "growth enzyme" of the general type suggested by Beijerinck (*11*) which accumulates in the leaves of these plants in the fall. The experimental method is assumed to extract this enzyme in the fall and to retain it so that it can be inoculated into the leaves the next summer and thus bring them to the point of tuber formation before they otherwise would reach it. However, the experimental evidence is not strong in support of this remarkable suggestion.

If such an enzyme were obtained by this method (which remains a pure hypothesis), then it should be most vigorous in the extract utilized in July; but no tubers were obtained at this time. By September, as is clear from the subsequent behavior of the normal plants, all leaves were approaching the period when tuber formation takes place, and it seems probable that the treatments adopted, i. e., immersing the leafstalk in the solution as it was severed, or injecting it subsequently with the solution, would have been equally effective in producing tubers if the solution had previously been boiled or indeed had been only water. The experimental treatment adopted would have left many tissues which take part in regeneration injected with liquid, and such treatment, quite apart from any question of enzyme activity, would have been sufficient to produce profound modifications in the behavior of the regenerating tissues. The check plants in this experiment are useless because they did not receive identical treatment with either boiled solution of the enzyme or pure water.

Doposcheg-Uhlár (*30*) himself showed in previous experiments that cuttings of *Achimenes longiflora* grown in water culture, might form either leafy aerial shoots or tubers below the level of the liquid, according to whether a culture solution, pure water, or tap water was employed. He also concluded that the different behavior in propagation shown by the juvenile, simple leaves of *Begonia carolineaefolia* Regel, as compared with that of the later formed compound leaves, was to be attributed mainly to the larger store of water and organic and mineral constituents present in the compound leaf.

FORMATIVE INFLUENCE OF QUALITATIVE OR QUANTITATIVE METABOLIC DIFFERENCES

The experimental basis for the hypothesis of "formative stuffs" in this type of experiment is, therefore, still quite inadequate. There is a growing tendency at the present time to associate flower formation, together with the frequently associated change in leaf form and in the general habit of the plant, with an altering balance of metabolism such as is expressed in the conception of the carbohydrate-nitrogen ratio. The experiments of Klebs (*74*) have thus led to the development of a new standpoint, especially in dealing with the flower formation, in which the process is related rather to quantitative seasonal differences in nutrition, than to qualitative differences in the nature of the substances reaching the growing point. Modern work in fruit culture especially is giving this new standpoint a very firm physiological foundation; however, the causal anatomy of the changes in the growing shoot apex which are involved constitutes a still-untouched field.

There are not wanting suggestions, moreover, that the new standpoint will prove equally valuable in relation to this similar problem in vegetative propagation. In fact, the junior writer (*158*) has already called attention to the striking similarity shown in the apple between the initiation of roots (burrknots) and the initiation of fruit buds. Goebel (*41*) in his general outlook on the problem of regeneration, emphasized the constitution and organization of the parent plant at the time of regeneration, rather than its content in particular formative substances.

Winkler (*185*) called attention to the fact that the practical gardener often prefers the method of propagation by seed to that by leaf, because the shoots from the leaf cuttings are apt to flower while still small and ineffective plants, whether the cuttings were taken from leaves at the time of flowering or earlier. He pointed out that the adventive shoots in the leaf naturally develop with a different metabolic balance at the outset, and that this difference may be greater in buds arising from leaves later in the summer. This changed metabolic balance may well be responsible for lessened growth and precocious flower production.

In the experiments of Dostal (*31, 32*) and of Němec (*98*), different formative powers in regeneration were shown by different parts of the shoot at the same time. Němec (*98*) found that while the basal region of the leaf of *Streptocarpus wendlandii* Spreng. produced adventive shoots giving only flower primordia, at the base of veins situated higher in the leaf adventive vegetative shoots appeared, while in between these two regions adventive structures often intermediate in character were obtained. To judge from its vigor in producing roots, the carbohydrate food supplies available were most plentiful at the base of the leaf, but this portion showed only a weak development of fertile adventive shoots. This is another indication that shoot production is not directly determined by the concentration of organic nutrients. The formative effect of the leaf upon the shoot organization produced in this plant could be obviated by removing the new shoot initials from the leaf at an early stage.

Dostal (*32*) similarly showed with *Circaea intermedia* Ehrh. that if pairs of leaves were isolated from the same plant, runners, flowering shoots, or transitional forms between these two structures were obtained according to their position on the plant. These experiments, however, clearly are better interpreted as the result of the effect of quantitative differences in the distribution of the same substances about the plant when it is severed for propagation, rather than as the result of the localized distribution of different formative substances. In particular, the latter hypothesis would need to be strained very far to justify a qualitative distribution of formative substances sufficient to explain the striking differences in regenerative power shown by a number of pieces of the same leaf, observed in some of Němec's experiments with Streptocarpus.

DIFFERENT "ANLAGEN" RATHER THAN DIFFERENT FOOD SUPPLIES

In the days when knowledge of the vascular channels and of food transport were so vague, special systems transporting different nutrient materials to shoot and root could easily be assumed. To take the origin of new apices within the pericycle of a young root as a concrete example, however, it is clear that these structures, appearing in the same position, draw upon the same vascular system and the same sources of supplies. This is true for all cases of shoots and roots. In the same plant all apices are dependent upon the same vascular system for the sources of materials used in their growth. The method of nutrition may be different, but the source of supplies is the same, and the differences between shoot and root can be no longer traced to an assumed difference in the formative qualities of the nutrient materials.

Another method of escaping the dilemma is evidenced by the readiness of many investigators of the present day to find refuge in the magic word "hormone." Growth in the plant quite probably is in part controlled by the diffusion, from one tissue to another, of chemical substances which affect permeability, protoplasmic powers of synthesis, and cell division, but it must be emphasized that at present the experimental basis for the assumption of any definite substances with specific chemical and biological properties is very meager and inadequate. Unless a hypothesis is both the basis and the result of an experimental examination which helps the elucidation of the problem of shoot and root formation, it becomes a stumbling block rather than an aid to understanding. The many facile generalizations as to growth-promoting and growth-inhibiting substances found in the literature to-day, too frequently constitute a refuge in words from any further attempt at understanding a difficult problem.

Loeb (*90*) began his interpretation of the phenomena of regeneration in Bryophyllum with the working hypothesis that the failure of the adventive or axillary shoot to grow out under certain conditions, might be due to the movement of growth-inhibiting substances into the neighborhood of these shoot organizations from the region of the growing dominant apical bud. But as he continued the quantitative analysis of the phenomena of regeneration, he became more and more impressed with the competitive character of the demands of the different growing organizations, whether shoot or root, upon the quantity of material available for growth. Implicitly, though nowhere very explicitly, Loeb abandoned the assumption of growth-inhibiting materials. On the new basis, the inhibition produced by one actively growing center upon the others is due to the fact that the materials available for growth are all moving to this already growing center and are there being utilized.

Loeb (*90*) found that the drain upon the available food supply was of the same effectiveness for equal amounts of new growth, whether the growth centers were shoot or root. This clearly indicates that such new structures utilize the same food reserves and not specifically different substances. But the phenomena of polarity remain to be explained, and Loeb concluded that in different regions of the plant this common supply of nutrients finds "anlagen" of different nature and therefore gives rise to different structures in the two regions. The explanation is thus shifted from the hypothesis of chemical differences in nutrient materials to an anatomical basis. With Loeb, however, this explanation remained completely hypothetical, for he made no attempt to identify these different "anlagen."

This anatomical basis for polarity can be critically examined in the light of the facts given in the preceding sections. It has been seen that there is no constant difference in the position of shoot and root "anlagen"; either may be exogenous, and either may be endogenous. Furthermore, in the detailed studies regarding the regeneration of shoot and roots in the neighborhood of the cut surface in Crambe, it becomes clear that given different conditions, a given group of cells might behave as cork phellogen, as the dermatogen of a new shoot apex, or as the outer layers of a root initial. Similarly, a given cell of the pericycle lying opposite a protoxylem group, clearly has the potentiality of forming part of a vascular cambium, a pericyclic phellogen, a root initial, or a shoot initial. As a rule, when once the meristem cells are organized into a characteristic "anlage" the organization does not change its character whatever the food supplied to it, though the exception to this rule is provided by the bud upon the root of *Rumex acetosella* which has been experimentally induced to grow on into a root apex (p. 26). Usually, if the food supplies permit of growth taking place, the organization of the meristematic apex determines the nature of the structure produced. The problem of polarity in regeneration then lies rather with the internal factors controlling the initiation of these "anlagen." Jones (*69*) has tried to utilize Child's theory of "axial gradients" (*24*) in interpreting the phenomena of polarity, but this theory has never grappled with a double axial gradient extending out from shoot pole and root pole at opposite ends of the growing organism, and, as Jones admits, such an assumption "adds little to an understanding of the causes underlying the facts."

ORGANIZATION AS THE BASIS OF DIRECTED MERISTEMATIC ACTIVITY

The anatomical and developmental study of regeneration has shown that any single meristematic cell is capable of forming part of an organized group which may function as any of the meristematic organizations characteristic of the plant, whether phellogen, cambium, or root or shoot apex. Possibly, like the germ cell, any single cell might be capable of giving rise to all such structures by continued cell division; but forming part, as it does from the beginning, of a cell organization, what the individual cell does is determined by its relative position in the organism. This is the main point that appears to emerge from this reexamination of the subject. The behavior of the living meristematic cell is determined by its position in a group and the behavior of the group by its relative position in a complex organization. Potentially any cell is capable of meristematic growth and may produce any cell organization characteristic of the species; practically, however, its possibilities are definitely limited by its position in a complex organization.

With the possible exception of some of the monocotyledons where the essential internal factors seem never to function, any living cell may return to the meristematic condition. Miehe (*96*) has recently suggested that the meristematic state is due to the presence in the cell of a hypothetical type of plasma, the archiplasm. No evidence is supplied for the existence of this special plasma and no effort made to seek it, so that this seems only another example of the too-common tendency to substitute words for working hypotheses (*83*). Miehe's suggestion seems completely incompatible with the striking observations made many years ago by Noll (*99*) upon *Bryopsis muscosa* Lamour.

In this coenocytic alga, Noll (*99*) found that the protoplasm and nuclei could be seen in the living filament, streaming from the side of the siphon where the protoplasm had been lying between central vacuole and wall, into the mass of protoplasm which filled the apex of the filament. As it slowly moved to this position, it always appeared to change in consistency, becoming more dense and less watery. Such a change in density was also suggested by the position of the chloroplasts, which he saw embedded in the protoplasm along the sides of the siphon, but which accumulated at the vacuole surface in the apex. At the apex, growth was proceeding, including presumably the manufacture of more protoplasm and the multiplication of nuclei. The protoplasm then streamed out of the apex again and down the siphon, so that the actual protoplasmic and nuclear content of the apex was always changing, but nevertheless growth activities remained centered there.

It seems impossible to think that in this case the behavior of the protoplasm is determined by the taking up of some hypothetical archiplasm as it enters the apex from the side of the siphon, and which is left behind again as the moving protoplasm leaves the apex. The phenomenon points rather to the behavior of the protoplasm being determined by its position in the siphon, and likewise the behavior of the meristem cell in the flowering plant is determined by its position in that more highly organized structure.

Relative position in a complex organism thus seems to determine whether each individual cell shall be meristematic and densely filled with protoplasm, semimeristematic, vacuolated and still dividing, or greatly extended with sap and showing no signs of growth activity. Hence the result of this anatomical study is to stress the significance of the position, not of preformed "anlagen" but of the living cells which by their coordinated activity give rise to shoot or root primordia. In the first section the different behavior of shoot and root apices was traced to differences in their organization. In the case of the adventive primordia so significant in propagation, their emergence is now seen to depend upon the response of the living cells of the plant to their new environment produced in the process of propagation when a portion of the completely organized plant is isolated and thus the relation of its living tissue systems to one another and to the external world is profoundly modified.

INFLUENCE OF ORGANIZATION UPON THE EMERGENCE OF THE ADVENTIVE MERISTEM

The problem of the initiation and maintenance of the meristematic condition in the cell, as a result of its position in a complex organization, already has been briefly considered. Two hypotheses have been advanced of recent years to interpret such phenomena. One, which invokes the operation of "wound hormones" as well as similar substances issuing from the phloem, does not seem at all applicable, in its present form, in accounting for the appearance of the meristematic condition except as the result of injury. The other hypothesis, that the hydrogen-ion concentration at the surface of the meristematic protoplast is an important condition of its characteristic activity, has recently been applied by the senior writer (*117*) to interpret the distribution of meristematic tissues in the plant that is intact as well as in the injured one.

There are sound a priori reasons for anticipating a significant effect of external pH upon a meristematic protoplast. The conditions found to exist in meristem cells in the higher plants suggest that the ratio of the total mass of the cell to its surface is a very important limiting factor determining size. This suggests that nutrients entering through the surface are utilized by all the mass of protoplasm and not merely by the nucleus. Under such conditions the external pH is likely to have a very direct influence upon the activity of the cell, because it profoundly influences the behavior of protein substances to water. This view is still a working hypothesis both requiring and permitting experimental examination; preliminary observations seem to point towards its general truth (*58*, *180*), but at the most it can be but one contributing factor.

The pH prevailing at the surface of the meristematic protoplast will also have a very great influence upon the permeability of the cell. It has been assumed that the protoplasmic surface is bathed in a medium which is near its isoelectric point; Michaelis (*95*) has shown that this would tend to make the protoplasm permeable both to anions and cations. Such an effect may have the greatest influence on the rapid entry of the solutes required for protoplasmic synthesis.

While a beginning has thus been made upon the problems of the initiation and maintenance of meristematic condition in general, the problem of the initiation of the one type rather than another seems to evade even formulation. At present it is difficult to indicate a possible experimental line of attack.

When the original apical meristems were under consideration, their differences in organization were connected with the difference in mode of nutrition, which seems to be determined by the chemical nature of their walls, and this in turn by the extent to which proteins and similar substances are withdrawn from the carbohydrate framework. It is very suggestive, therefore, to realize that the ease with which proteins and similar amphoteric substances separate from carbohydrate walls is a function of their relative electric charges and thus again very sensitive to the pH (*102*).

If, therefore, the meristematic condition were possible over but a limited range of pH around the isoelectric point of the protoplasmic proteins, at the relatively acid end of that range the proteins would only sluggishly clear from the cleavage plane on which carbohydrate was deposited, while at the relatively alkaline end of the range the proteins would leave the walls very promptly. Under these conditions the acid end of the range would favor the apical organization of the root, and alkaline conditions the shoot meristem. This is a very tentative suggestion indeed, but its consideration has been encouraged by the gradual development of the hypothesis of hydrogen-ion reaction in relation to the intercalary meristems.

The key to the difference between adventive shoot and root may quite probably be found in the oft-repeated ringing experiment, in which when the phloem is completely severed, buds subsequently appear below the ring and roots above. The buds are here found on the side of the root system and thus on the side which has by far the greater tendency toward positive sap pressures. Roots, on the other hand, are formed on the side where the downward movement (in the phloem?) brings materials to the ring.

Beijerinck's observations (*11*), referred to previously (p. 57), that bud production is more marked the nearer the cut surface is to the uninjured root system, together with the relatively ready production of buds upon the succulent type of leaf and upon fleshy roots, all accord with the apparent rôle of positive exudation pressures in the formation of shoot initials. Here, also, may lie the reason for the correlation, so clearly brought out by Beijerinck, between the position of the xylem and the adventive bud; in the radially organized root as well as in the dorsiventral leaf the adventive bud invariably appears in a superficial position opposite the xylem.

The opposed characteristics of phellogen and vascular cambium are very striking. The vascular cambium cuts off most cells to the inside, and these in their process of differentiation, lignification, and death, tend to maintain the acid reaction of the xylem sap. The phellogen cuts off most cells to the outside, which similarly tend to develop an acid reaction as they differentiate, suberize, and die. The cells formed towards the phloem by both meristems remain alive; they usually retain carbohydrate walls essentially free from other impregnating substances. It has been shown that there are reasons for regarding each of these meristems as lying across a gradient of hydrogen-ion concentration, but with the gradient running in opposite directions, the phloem which is outside one and inside the other, always supplies the alkaline end of the gradient. Now it is at least suggestive, since the pH can be indicated as possibly determining meristem organization as well as meristem activity, that in an axis bearing both these intercalary meristems, adventive shoot organizations are always formed in close association with the phellogen. The new root initial, on the other hand, is associated with the vascular cambium, the effective cells usually lying in a primary ray; these cells become more densely filled with protoplasm and more definitely meristematic than they were, but their walls are still impregnated with protein, and the most actively dividing layer is to the inside, abutting upon the cambium and the relatively acid supplies of the xylem.

Whether a group of meristem cells in the callus of seakale organize as a stem apex or a root apex, therefore, may depend in part upon the pH of the liquid bathing them. In this connection Riehm (*127*) found that when fragments of leaves of *Cardamine pratensis* were floated in a solution of KH_2PO_4 (relatively acid) they became meristematic and gave buds in three days and roots two days later, but when they were floated on K_2HPO_4 (relatively alkaline) roots were formed in three days and buds only after two weeks.

These suggestions can only be regarded as an indication of the type of problem that has emerged and requires solution. The main result of the present examination of the problems of vegetative propagation is the conclusion that success in this field depends upon the initiation and maintenance of meristematic tissues which are organized as adventive shoot and root apices. At least the clear recognition of the nature of the problem is the first step towards its solution, and the suggestion that the hydrogen-ion concentration in the neighborhood of the new meristem, a result of the organization of the living tissues around it, may play an effective part in the process, must at present simply be regarded as an illustration of the manner in which tissue organization in the isolated plant system may modify meristem growth and organization.

CONCLUSION

The detailed analysis of the anatomical basis of vegetative propagation presented in this bulletin does not readily admit of a summarized presentation, but the general conclusion that emerges can be concisely stated.

The problems of vegetative propagation among the flowering plants, viewed from the standpoint of causal anatomy, are the problems of the initiation and development of an adventive shoot or root, or both, upon an isolated portion of a living plant. The essential processes may or may not have started before severance from the parent plant.

Such adventive structures are not determined in their nature and position by the movement of special formative food substances in the plant, or by the existence of preformed "anlagen." Their production invariably involves the formation and maintenance of a meristematic tissue which is organized as either shoot or root apex. The internal and external conditions concerned with the initiation and maintenance of these meristematic groups of cells are examined. The conclusion is that "the behavior of the living meristematic cell is determined by its position" (p. 84) in the living tissue, and the manner in which position may influence subsequent growth and activity of the cell is explored in a preliminary manner, but in the main this must remain a problem for future investigation.

The subsequent development of such adventive apical meristems requires internal and external conditions essentially equivalent to those required for the development of the corresponding shoot or root apices upon the parent plants—conditions which are defined as closely as possible in the light of the writers' very meager information regarding the physiology of plant growth and differentiation.

LITERATURE CITED

(1) Agricola, G. A.
1716. NEU- UND NIEERHÖRTER, DOCH IN DER NATUR UND VERNUNFT WOHLBEGRÜNDETER VERSUCH DER UNIVERSAL VERMEHRUNG ALLER BÄUME, STAUDEN, UND BLUMENGEWÄCHSE. 184 p., illus. Regensberg.

(2) Ahrns, W.
1924. WEITERE UNTERSUCHUNGEN ÜBER DIE ABHÄNGIGKEIT DES GEGENSEITIGEN MENGENVERHÄLTNISSES DER KOHLENHYDRATE IM LAUBBLATT VON WASSERGEHALT. Bot. Arch. 5: 234–259.

(3) American Joint Committee on Horticultural Nomenclature.
1923. STANDARDIZED PLANT NAMES. Prepared by F. L. Olmsted, F. V. Coville, and H. P. Kelsey. 546 p. Salem, Mass.

(4) Artschwager, E. F.
1918. ANATOMY OF THE POTATO PLANT, WITH SPECIAL REFERENCE TO THE ONTOGENY OF THE VASCULAR SYSTEM. Jour. Agr. Research 14: 221–252, illus.

(5) Auchter, E. C.
[1926]. AN EXPERIMENT IN PROPAGATING APPLE TREES ON THEIR OWN ROOTS. Amer. Soc. Hort. Sci. Proc. (1925) 22: 205–211, illus.

(6) Bailey, L. H.
1920. THE NURSERY-MANUAL; A COMPLETE GUIDE TO THE MULTIPLICATION OF PLANTS. 456 p., illus. New York.

(7) Balfour, I. B.
1913. SOME PROBLEMS OF PROPAGATION. Jour. Roy. Hort. Soc. 38: 447–460, illus.

(8) Baltet, C.
1910. THE ART OF GRAFTING AND BUDDING. Transl. from the French. Ed. 6, 230 p., illus. London.

(9) Bateson, W.
1921. ROOT-CUTTINGS AND CHIMAERAS. II. Jour. Genetics 11: 91–97, illus.

(10) Beals, C. M.
1923. AN HISTOLOGICAL STUDY OF REGENERATIVE PHENOMENA IN PLANTS. Ann. Missouri Bot. Gard. 10: 369–384, illus.

(11) Beijerinck, M. W.
1887. BEOBACHTUNGEN UND BETRACHTUNGEN ÜBER WURZELKNOSPEN UND NEBENWURZELN. Verhandel. K. Akad. Wetensch. Amsterdam 25, 146 p., illus.

(12) Beinling, E.
1879. UNTERSUCHUNGEN ÜBER DIE ENTSTEHUNG DER ADVENTIVEN WURZELN UND LAUBKNOSPEN AN BLATTSTECKLINGEN VON PEPEROMIA. Cohn Beitr. Biol. Pflanz. 3: 25–50, illus.

(13) Blackie, J. J., Graham, R. T. D., and Stewart, L. B.
1926. THE PROPAGATION OF CAMPHOR. Roy. Bot. Gard. Kew, Bul. Misc. Inform. 1926: 380–381.

(14) Boodle, L. A.
1920. THE MODE OF ORIGIN AND THE VASCULAR SUPPLY OF THE ADVENTITIOUS LEAVES OF CYCLAMEN. Ann. Bot. [London] 34: [431]–437, illus.

(15) Borthwick, A. W.
1905. THE PRODUCTION OF ADVENTITIOUS ROOTS AND THEIR RELATION TO BIRD'S-EYE FORMATION (MASER-HOLZ) IN THE WOOD OF VARIOUS TREES. Notes Roy. Bot. Gard. Edinb. 4: 15–36, illus.

(16) Brenchley, W. E., and Jackson, V. G.
1921. ROOT DEVELOPMENT IN BARLEY AND WHEAT UNDER DIFFERENT CONDITIONS OF GROWTH. Ann. Bot. [London] 35: [533]–556, illus.

(17) Brieger, F.
1924. UNTERSUCHUNGEN ÜBER DEN WUNDREIZ. Ber. Deut. Bot. Gesell. 42: (79)–(90), illus.

(18) Burns, G. P., and Hedden, M. E.
1906. CONDITIONS INFLUENCING REGENERATION OF THE HYPOCOTYL. Bot. Centbl., Beihefte (I) 19: [383]–392, illus.

(19) Bushnell, J.
1925. THE RELATION OF TEMPERATURE TO GROWTH AND RESPIRATION IN THE POTATO PLANT. Minn. Agr. Expt. Sta. Tech. Bul. 34. 29 p., illus.

(20) Candolle, A. P. de
1827. ORGANOGRAPHIE VEGETALE. 2 v., illus. Paris.

(21) Celakovský, L. J.
1901. DIE GLIEDERUNG DER KAULOME. Bot. Ztg. 59: [79]–114, illus.

(22) Chandler, W. H.
[1926]. POLARITY IN THE FORMATION OF SCION ROOTS. Amer. Soc. Hort. Sci. Proc. (1925) 22: 218–222, illus.

(23) Chauveaud, G.
1921. LA CONSTITUTION DES PLANTES VASCULAIRES RÉVÉLÉE PAR LEUR ONTOGENIE. 155 p., illus. Paris.

(24) Child, C. M.
1915. INDIVIDUALITY IN ORGANISMS. 213 p., illus. Chicago.

(25) Corbett, L. C.
1897. THE DEVELOPMENT OF ROOTS FROM CUTTINGS. W. Va. Agr. Expt. Sta. Ann. Rpt. (1895/96) 9: 196–199.

(26) ———
1902. THE PROPAGATION OF PLANTS. U. S. Dept. Agr. Farmers' Bul. 157, 24 p., illus.

(27) Curtis, O. F.
1918. STIMULATION OF ROOT GROWTH IN CUTTINGS BY TREATMENT WITH CHEMICAL COMPOUNDS. N. Y. Cornell Agr. Expt. Sta. Mem. 14, p. [69]–138, illus.

(28) Darwin, F.
1881. THE THEORY OF THE GROWTH OF CUTTINGS; ILLUSTRATED BY OBSERVATION ON THE BRAMBLE, RUBUS FRUTICOSUS. Jour. Linn. Soc. [London] Bot. 18: 406–419.

(29) Devaux, H.
1900. RECHERCHES SUR LES LENTICELLES. ÉTUDE SUR LES CONDITIONS PHYSIOLOGIQUES DE L'ACCROISSEMENT ET DE LA DIFFÉRENCIATION DE LA CELLULE ET DES TISSUES. Ann. Sci. Nat., Bot. (8) 12: 1–240, illus.

(30) Doposcheg-Uhlár, J.
1911. STUDIEN ZUR REGENERATION UND POLARITÄT DER PFLANZEN. Flora [Jena] 102: [24]–86, illus.

(31) Dostal, R.
1911. ZUR EXPERIMENTELLEN MORPHOGENESIS BEI CIRCAEA UND EINIGEN ANDEREN PFLANZEN. Flora [Jena] 103: 1–53, illus.

(32) ———
1926. ÜBER DIE WACHSTUMSREGULIERENDE WIRKUNG DES LAUBBLATTES. Acta Soc. Sci. Nat. Moravicae 3: 83–209, illus.

(33) Duhamel du Monceau, H. L.
1768. TRAITÉ DES ARBRES FRUITIERS. 2 v., illus. Paris.

(34) Du Petit-Thouars, A. A.
1809. ESSAI SUR LA VÉGÉTATION. 304 p., illus. Paris.

(35) Errera, L.
1886. SUR UNE CONDITION FONDAMENTALE D'ÉQUILIBRE DE CELLULES VIVANTS. Compt. Rend. Acad. Sci [Paris] 103: 822–824.

(36) Figdor, W.
1907. ÜBER RESTITUTIONSERSCHEINUNGEN AN BLÄTTERN VON GESNERIACEEN. Jahrb. Wiss. Bot. 44: [41]–56, illus.

(37) Frost, H. B.
1926. POLYEMBRYONY, HETEROZYGOSIS AND CHIMERAS IN CITRUS. Hilgardia 1: 365–402, illus.

(38) Fuller, A. S.
1887. THE PROPAGATION OF PLANTS. 349 p., illus. New York.

(39) Gerassimow, J. J. (Gerasimov, I. I.)
1901. ÜBER DEN EINFLUSS DES KERNS AUF DAS WACHSTUM DER ZELLE. Bul. Soc. Imp. Nat. Moscow (n. s.) 15: 185–220, illus.

(40) ———
1904. ZUR PHYSIOLOGIE DER ZELLE. Bul. Soc. Imp. Nat. Moscow (n. s.) 18: 1–134, illus.

(41) Goebel, K.
1902. UEBER REGENERATION IM PFLANZENREICH. Biol. Zentbl. 22: 385–397, 417–438, 481–505, illus.

(42) GRAEVENITZ, L. VON.
1913. ÜBER WURZELBILDUNG AN STECKHOLZ. 50 p. Weida i. Th. (Inaug. Diss. Jena.)
(43) GRAHAM, R. J. D., and STEWART, L. B.
1922–23. VEGETATIVE PROPAGATION OF HAEMANTHUS HIRSUTUS, BAKER. Bot. Soc. Edinb. Trans. and Proc. 28: 183–184.
(44) ——— and STEWART, L. B.
1924. VEGETATIVE PROPAGATION OF ORNITHOGALUM AND DRIMIA. Bot. Soc. Edinb. Trans. and Proc. 29: 69–71.
(45) ——— and STEWART, L. B.
1927. REGENERATION FROM ROOTS OF ANCHUSA: ANCHUSA ITALICA. Bot. Soc. Edinb. Trans. and Proc. 29: 333–334.
(46) GRAU, E.
1917. UNTERSUCHUNGEN ÜBER DIE REGENERATION DER VEGETATIONSPUNKTE AN ABGESCHNITTENEN SPROSSEN IM HINBLICK AUF DIE BILDUNG DER PFROPFBASTARDE. 74 p., illus. Königsberg. (Inaug. Diss.)
(47) GROSSENBACHER, J. G.
1915. THE PERIODICITY AND DISTRIBUTION OF RADIAL GROWTH IN TREES AND THEIR RELATION TO THE DEVELOPMENT OF "ANNUAL" RINGS. Wis. Acad. Sci., Arts, and Letters, Trans. 18 (1): 1–77.
(48) HABERLANDT, G.
1913–14. ZUR PHYSIOLOGIE DER ZELLTEILUNG. Sitzber. Preuss. Akad. Wiss. 1913: 318–345, illus.; 1914: 1096–1111, illus.
(49) ———
1921. WUNDHORMONE ALS ERREGER VON ZELLTEILUNGEN. Beitr. Allg. Bot. 2: 1–54, illus.
(50) ———
1914. PHYSIOLOGICAL PLANT ANATOMY. Transl. from 4th German ed. by M. Drummond. 777 p., illus. London.
(51) HANES, C. S.
1927. RESIN CANALS IN SEEDLING CONIFERS. Jour. Linn. Soc. [London] Bot. 47: 613–636, illus.
(52) HANSEN, A.
1881. VERGLEICHENDE UNTERSUCHUNGEN ÜBER ADVENTIVBILDUNGEN BEI DEN PFLANZEN. Abhandl. Senckenberg. Naturf. Gesell. 12: [147]–198, illus.
(53) HANSTEIN, J.
1868. DIE SCHEITELZELLGRUPPE IM VEGETATIONSPUNKT DER PHANEROGAMEN. Abhandl. Geb. Naturw. Math. u. Med. 1868: 109–134, illus.
(54) HARTIG, G. L.
1851. LEHRBUCH FÜR FÖRSTER UND FÜR DIE, WELCHEN ES WERDEN WOLLEN. Aufl. 9, Bd. 1, 334 p., illus. Stuttgart and Tübingen.
(55) HARTIG, T.
1862. UEBER DIE BEWEGUNG DES SAFTES IN DEN HOLZPFLANZEN. Bot. Ztg. 20: [73]–76, [81]–87, [97]–100, [105]–109, illus.
(56) HARTSEMA, A. M.
1926. ANATOMISCHE UND EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DAS AUFTRETEN VON NEUBILDUNGEN AN BLÄTTERN VON BEGONIA REX. Rec. Trav. Bot. Néerland 25: 305–361, illus.
(57) HATTON, R. G., WORMALD, H., and WITT, A. W.
1926. ON "BURR-KNOTS" OF FRUIT TREES. Jour. Pomol. and Hort. Sci. 5: 195–204, illus.
(58) HERKLOTS, G. A. C.
1924. THE EFFECTS OF AN ARTIFICIALLY CONTROLLED HYDRION CONCENTRATION UPON WOUND HEALING IN THE POTATO. New Phytol. 23: [240]–255, illus.
(59) HILL, A. V.
1928. THE DIFFUSION OF OXYGEN AND LACTIC ACID THROUGH TISSUES. Roy. Soc. [London] Proc., Ser. B. 140: 39–96, illus.
(60) HILL, A. W.
1920. STUDIES IN SEED GERMINATION. EXPERIMENTS WITH CYCLAMEN. Ann. Bot. [London] 34: [417]–429, illus.
(61) HOFMEISTER, W.
1868. ALLGEMEINE MORPHOLOGIE DER GEWÄCHSE. *His* Handb. Physiol. Bot. 1: [405]–664, illus.

(62) HOTTES, A. C.
1922. PRACTICAL PLANT PROPAGATION. Ed. 2, 224 p., illus. New York.
(63) HURD, A. M.
1923. ACIDITY OF CORN AND ITS RELATION TO VEGETATIVE VIGOR. Jour. Agr. Research 25: 457–469, illus.
(64) JACKSON, V. G.
1922. ANATOMICAL STRUCTURE OF THE ROOTS OF BARLEY. Ann. Bot. [London] 36: [21]–39, illus.
(65) JANSE, J. M.
1921. EIN BLATTSTECKLING VON CAMELLIA JAPONICA MIT ADVENTIVKNOSPE. Flora [Jena] 114: 401–404, illus.
(66) ———
1925. ERNÄHRUNG, ADVENTIVBILDUNG UND POLARITÄT. Flora [Jena] 118–119: [257]–288.
(67) ———
1926. ON THE NEW PHENOMENA CAUSED BY IRRITATION OF ROOTS. K. Akad. Wetensch. Amsterdam Proc. 29: [834]–842. (Transl. from Verslag Wis. en Natuurk. Afd. 35: 418–427.)
(68) ———
1927. EINE NEUE ENTEILUNG DER PFLANZENBEWEGUNGEN. Flora [Jena] 122: 1–32, illus.
(69) JONES, W. N.
1925. POLARITY PHENOMENA IN SEAKALE ROOTS. Ann. Bot. [London] 39: [359]–372, illus.
(70) JOST, L.
1891. UEBER DICKENWACHSTHUM UND JAHRESRINGBILDUNG. Bot. Ztg. 49: [485]–495, [501]–510, 525–531, [541]–547, [557]–563, [573]–579, [589]–596, [605]–611, [625]–630, illus.
(71) ———
1893. UEBER BEZIEHUNGEN ZWISCHEN DER BLATTENTWICKELUNG UND DER GEFÄFASSBILDUNG IN DER PFLANZE. Bot. Ztg. 51: [89]–138, illus.
(72) KAINS, M. G.
1916. PLANT PROPAGATION. 322 p., illus. New York.
(73) KLEBAHN, H.
1883. UEBER DIE STRUCTUR UND DIE FUNCTION DER LENTICELLEN, SOWIE ÜBER DEN ERSATZ DERSELBEN BEI EINIGEN LENTICELLENFREIEN HOLZGEWÄCHSEN. Ber. Deut. Bot. Gesell. 1: 113–121, illus.
(74) KLEBS, G.
1914. ÜBER DAS TREIBEN DER EINHEIMISCHEN BÄUME SPEZIELL DER BUCHE. Heidelberger Akad. Wiss. Math-Naturw. Kl. Abhandl. 3, 116 p., illus.
(75) KLEIN, G.
1923. ZUR ÄTIOLOGIE DER THYLLEN. Ztschr. Bot. 15: [417]–439.
(76) KNIGHT, R. C.
1926–27. THE PROPAGATION OF FRUIT TREE STOCKS BY STEM CUTTINGS I–II. Jour. Pomol. and Hort. Sci. 5: 248–266; 6: 47–60. (A. W. Witt, joint author of II.)
(77) KOBEL, F.
1927. ZYTOLOGISCHE UNTERSUCHUNGEN AN PRUNOIDEEN U. POMOIDEEN. Arch. Julius Klaus-Stiftung Vererbungsforsch. Sozialanthropol. u. Rassenhyg. 3: 1–84, illus.
(78) KOCH, L.
1893. DIE VEGETATIVE VERZWEIGUNG DER HÖHEREN GEWÄCHSE. Jahrb. Wiss. Bot. 25: [380]–488, illus.
(79) KRIEG, A.
1908. BEITRÄGE ZUR KENNTNIS DER KALLUS- U. WUNDHOLZBILDUNG GERINGELTER ZWEIGE UND DEREN HISTOLOGISCHEN VERÄNDERUNGEN. 68 p., illus. Würzburg.
(80) KUPFER, E.
1907. STUDIES IN PLANT REGENERATION. Mem. Torrey Bot. Club 12: 195–241, illus.
(81) KÜSTER, E.
1903. BEOBACHTUNGEN ÜBER REGENERATIONSERSCHEINUNGEN AN PFLANZEN. Bot. Centbl. Beihefte 14: [316]–326, illus.
(82) ———
1925. PATHOLOGISCHE PFLANZENANATOMIE. Aufl. 3, 558 p., illus. Jena.

(83) KÜSTER, E.
1928. DAS VERHALTEN PFLANZLICHER ZELLEN IN VITRO UND IN VIVO. Arch. Expt. Zellforsch. Besonders Gewebezüchtung 6: 28–42.
(84) LEE, B.
1925. THE PLANT CUTICLE. II. A MACROCHEMICAL STUDY. Ann. Bot. [London] 39: [755]–768.
(85) ——— and PRIESTLEY, J. H.
1924. THE PLANT CUTICLE. I. ITS STRUCTURE, DISTRIBUTION, AND FUNCTION. Ann. Bot. [London] 38: [525]–545.
(86) LEK, H. A. A. VAN DER.
1924. ROOT DEVELOPMENT IN WOODY CUTTINGS. Meded. Landbouwhoogesch. [Wageningen], deel 28, verhandel. 1, 230 p., illus. (In Dutch. English summary, p. 211–230.)
(87) LENZ, F.
1911. ÜBER DEN DURCHBRUCH DER SEITENWURZELN. Cohn. Beitr. Biol. Pflanz. 10: 235–264, illus.
(88) LINDEMUTH, H.
1903. WEITERE MITTEILUNGEN ÜBER REGENERATIVE WURZEL- U. SPROSSBILDUNG AUF LAUBBLÄTTERN (BLATTSTECKLINGEN). Gartenflora 52: 619–625.
(89) ———.
1904. ÜBER GRÖSSERWERDEN ISOLIERTER AUSGEWACHSENER BLÄTTER NACH IHRER BEWURZELUNG. Ber. Deut. Bot. Gesell. 22: [171]–174, illus.
(90) LOEB, J.
1924. REGENERATION. 143 p., illus. New York and London.
(91) LOHR, T.
1908. BEOBACHTUNGEN UND UNTERSUCHUNGEN AN SPROSSLOSEN BLATTSTECKLINGEN. 35 p., illus. Bonn. (Inaug. Diss. Rhein. Friedrichs-Wilhelm's Univ.)
(92) LUNDEGÅRDH, H.
1914. DAS WACHSTUM DES VEGETATIONSPUNKTES. Ber. Deut. Bot. Gesell. 32: 77–83, illus.
(93) MANEY, T. J.
[1926]. THE PROPAGATION OF OWN ROOTED APPLE STOCKS. Amer. Soc. Hort. Sci. Proc. (1925) 22: 211–217.
(94) MATHUSE, O.
1906. ÜBER ABNORMALES SEKUNDÄRES WACHSTUM VON LAUBBLÄTTERN, INSBESONDERE VON BLATTSTECKLINGEN DICOTYLER PFLANZEN. Bot. Centbl., Beihefte (I) 20: 174^1–174^{48}.
(95) MICHAELIS, L.
1926. DIE PERMEABILITÄT VON MEMBRANEN. Naturwissenschaften 14: 33–42.
(96) MIEHE, H.
1926. DAS ARCHIPLASMA. BETRACHTUNGEN ÜBER DIE ORGANISATION DES PFLANZENKÖRPERS. 92 p. Jena.
(97) MYLIUS, G.
1913. DAS POLYDERM. EINE VERGLEICHENDE UNTERSUCHUNG ÜBER DIE PHYSIOLOGISCHEN SCHEIDEN POLYDERM, PERIDERM UND ENDODERMIS. Biblioth. Bot. Heft 79, 119 p., illus.
(98) NĚMEC, B.
1911. WEITERE UNTERSUCHUNGEN ÜBER DIE REGENERATION. IV. Česka Akad. Císaře Františka Josefa Bul. Internatl. Cl. Sci., Math. et Med. 16: 238–256, illus.
(99) NOLL, F.
1903. BEOBACHTUNGEN UND BETRACHTUNGEN ÜBER EMBRYONALE SUBSTANZ. Biol. Zentbl. 23: 281–297, 321–337, 401–427.
(100) OLIVER, G. W.
1903. THE PROPAGATION OF TROPICAL FRUIT TREES AND OTHER PLANTS. U. S. Dept. Agr., Bur. Plant Indus. Bul. 46, 28 p., illus.
(101) ——— and HOTTES, A. C.
1926. PLANT CULTURE. A WORKING HANDBOOK OF EVERY DAY PRACTICE FOR ALL WHO GROW FLOWERING AND ORNAMENTAL PLANTS IN THE GARDEN AND GREENHOUSE. Ed. 5, 442 p., illus. New York.
(102) PEARSALL, W. H.
1925. HYDROGEN ION CONCENTRATION AND GROWTH. Sci. Prog. [London] 20: 58–67.

(103) Pearsall, W. H. and Ewing, J.
1929. THE RELATION OF NITROGEN METABOLISM TO PLANT SUCCULENCE. Ann. Bot. [London] 43: 27–34.

(104) ——— and Priestley, J. H.
1923. MERISTEMATIC TISSUES AND PROTEIN ISO-ELECTRIC POINTS. New Phytol. 22: 185–191.

(105) Peirce, G. J.
1894. DAS EINDRIGEN VON WURZELN IN LEBENDE GEWEBE. Bot. Ztg. 52: [169]–176, illus. 1894.

(106) Petersen, O. G.
1898. ÉTUDES SUR LES PHÉNOMÈNES VITAUX DES RACINES DES ARBRES. Overs. K. Danske Vidensk. Selsk. Forhandl. 1898: 1–68, illus. (In Danish. French résumé, p. 58–68.)

(107) Pfeffer, W.
1893. DRUCK- UND ARBEITSLEISTUNG DURCH WACHSENDE PFLANZEN. K. Sächs. Gesell. Wiss., Abhandl. Math. Phys. Cl. 20: [235]–474, illus.

(108) Philipp, M.
1923 ÜBER DIE VERKORKTEN ABSCHLUSSGEWEBE DER MONOKOTYLEN. Biblioth. Bot., Heft 92, 27 p., illus.

(109) Plett, W.
1921. UNTERSUCHUNGEN ÜBER DIE REGENERATIONSERSCHEINUNGEN AN INTERNODIEN. [8] p. Hamburg. (Auszug, Inaug. Diss. Hamburg Univ., unpublished). (See also footnote 6, p. 31.)

(110) Priestley, J. H.
1921. SUBERIN AND CUTIN. New Phytol. 20: 17–29.

(111) ———
1924. THE FIRST SUGAR OF PHOTOSYNTHESIS AND THE RÔLE OF CANE SUGAR IN THE PLANT. New Phytol. 23: 255–265.

(112) ———
1925. VEGETATIVE PROPAGATION. (Abstract) Imp. Bot. Conf. London Rpt. Proc. 1924, p. 338–339.

(113) ———
1926. PROBLEMS OF VEGETATIVE PROPAGATION. (BEING THE MASTERS LECTURES FOR 1925). Jour. Roy. Hort. Soc. 51: 1–16, illus.

(114) ———
1926. THE RELATION OF CORK FORMATION TO THE ENDODERMIS IN THE SHOOT OF THE DICOTYLEDON. Leeds Phil. Soc., Sci. Sect., Proc. 1: 123–135, illus.

(115) ———
1926. LIGHT AND GROWTH II. ON THE ANATOMY OF ETIOLATED PLANTS. New Phytol. 25: 145–170, illus.

(116) ———
1926. LIGHT AND GROWTH. III. AN INTERPRETATION OF PHOTOTROPIC GROWTH CURVATURES. New Phytol. 25: 213–226.

(117) ———
1928. THE MERISTEMATIC TISSUES OF THE PLANT. Cambridge Phil. Soc. Biol. Rev. and Biol. Proc. 3: 1–20, illus.

(118) ——— and Ewing, J.
1923. PHYSIOLOGICAL STUDIES IN PLANT ANATOMY. VI. ETIOLATION. New Phytol. 22: 30–44.

(119) ——— and North, E. E.
1922. PHYSIOLOGICAL STUDIES IN PLANT ANATOMY. III. THE STRUCTURE OF THE ENDODERMIS IN RELATION TO ITS FUNCTION. New Phytol. 21: 113–139, illus.

(120) ——— and Pearsall, W. H.
1922. GROWTH STUDIES. II. AN INTERPRETATION OF SOME GROWTH-CURVES. Ann. Bot. [London] 36: [239]–249, illus.

(121) ——— and Tupper-Carey, R. M.
1922. PHYSIOLOGICAL STUDIES IN PLANT ANATOMY. IV. THE WATER RELATIONS OF THE PLANT GROWING POINT. New Phytol. 21: 210–229, illus.

(122) ——— and Woffenden, L. M.
1922. PHYSIOLOGICAL STUDIES IN PLANT ANATOMY. V. CAUSAL FACTORS IN CORK FORMATION. New Phytol. 21: 252–268.

(123) Priestley, J. H. and Woffenden, L. M.
1923. THE HEALING OF WOUNDS IN POTATO TUBERS AND THEIR PROPAGATION BY CUT SETS. Ann. Appl. Biol. 10: 96–115, illus.

(124) Regel, F.
1876. DIE VERMEHRUNG DER BEGONIACEEN AUS IHREN BLÄTTERN. Jenaische Ztschr. Med. u. Naturw. (N. F. 3) 10: [447]–492, illus.

(125) Reid, M. E.
1926. GROWTH OF TOMATO CUTTINGS IN RELATION TO STORED CARBOHYDRATE AND NITROGENOUS COMPOUNDS. Amer. Jour. Bot. 13: 548–574, illus.

(126) Reid, O.
1923. THE PROPAGATION OF CAMPHOR BY STEM CUTTINGS. Bot. Soc. Edinb. Trans. and Proc. 28: 184–188, illus.

(127) Riehm, E.
1905. BEOBACHTUNGEN AN ISOLIERTEN BLÄTTERN. Ztschr. Naturw. (1904) 77: 281–314 [311–344].

(128) Riker, A. J., and Keitt, G. W.
1926. STUDIES OF CROWNGALL AND WOUND OVERGROWTH ON APPLE NURSERY STOCK. Phytopathology 16: 765–808, illus.

(129) Rzimann, G.
1926. ÜBER ORGANBILDUNG AN ADVENTIVKNOSPEN VON TOLMIEA MENZEISII (TOR. ET A. GRAY). Sitzber. Akad. Wiss. Wien, Math. Naturw. Kl. Abt. 1, 135: 291–315.

(130) Sachs, J.
1862. UEBER SAURE, ALKALISCHE UND NEUTRALE REAKTION DER SÄFTE LEBENDER PFLANZENZELLEN. Bot. Ztg. 20: [257]–265.

(131) ———
1878. UEBER DIE ANORDNUNG DER ZELLEN IN JÜNGSTEN PFLANZENTHEILEN. Arb. Bot. Inst. Würzburg 2: 46–104, illus.

(132) ———
1880–82. STOFF UND FORM DER PFLANZENORGANE. Art. Bot. Inst. Würzburg 2: 452–488, 689–718, illus.

(133) ———
1892. PHYSIOLOGISCHE NOTIZEN. I. Flora [Jena] 75: 1–3.

(134) Sandt, W.
1925. ZUR KENNTNIS DER BEIKNOSPEN. Bot. Abhandl., Heft 7, 160 p., illus.

(135) Schlumberger, O.
1913. ÜBER EINEN EIGENARTIGEN FALL ABNORMER WURZELBILDUNG AN KARTOFFELKNOLLEN. Ber. Deut. Bot. Gesell. 31: 60–63, illus.

(136) Schmidt, A.
1924. HISTOLOGISCHE STUDIEN AN PHANEROGAMEN VEGETATIONSPUNKTEN. Bot. Arch. 8: 345–404, illus.

(137) Schubert, O.
1913. BEDINGUNGEN ZUR STECKLINGSBILDUNG UND PFROPFUNG VON MONOKOTYLEN. Centbl. Bakt. [etc.] (II) 38: 309–443, illus.

(138) ———
1914. UBER DIE VERMEHRUNG MONOKOTYLER PFLANZEN DURCH STECKLINGE. Ber. K. Lehranst. Wein, Obst u. Gartenbau Geisenheim 1913: 114–119.

(139) Schüepp, O.
1914. WACHSTUM UND FORMWECHSEL DES SPROSSVEGETATIONSPUNKTES DER ANGIOSPERMEN. Ber. Deut. Bot. Gesell. 32: 329–339, illus.

(140) ———
1926. MERISTEME. Handb. Pflanz. Anat., Abt. 1, Teil 2, Histologie, Bd. 4, 114 p., illus.

(141) Schwendener, H.
1882. DIE SCHUTZSCHEIDEN UND IHRE VERSTÄRKUNGEN. Abhandl. K. Akad. Wiss. Berlin, Phys. Math. Cl. 1882, Abt. 3, 75 p., illus.

(142) Scott, L. I., and Priestley, J. H.
1928. THE ROOT AS AN ABSORBING ORGAN. I. A RECONSIDERATION OF THE ENTRY OF WATER AND SALTS IN THE ABSORBING REGION. New Phytol. 27: [125]–140, illus.

(143) Shaw, J. K.
1919. THE PROPAGATION OF APPLE TREES ON THEIR OWN ROOTS. Mass. Agr. Expt. Sta. Bul. 190, p. 73–96, illus.

(144) Simon, S.
1908. EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE DIFFERENZIERUNGSVORGÄNGE IM CALLUSGEWEBE VON HOLZGEWACHSEN. Jahrb. Wiss. Bot. 45: 351–478, illus.

(145) ———
1908. EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE ENTSTEHUNG VON GEFÄSSVERBINDUNGEN. Ber. Deut. Bot. Gesell. 26: [364]–396, illus.

(146) Smith, E. P.
1924. THE ANATOMY AND PROPAGATION OF CLEMATIS. Bot. Soc. Edinb. Trans. and Proc. 29: 17–26, illus.

(147) ———
1928. A COMPARATIVE STUDY OF THE STEM STRUCTURE OF THE GENUS CLEMATIS, WITH SPECIAL REFERENCE TO ANATOMICAL CHANGES INDUCED BY VEGETATIVE PROPAGATION. Roy. Soc. Edinb. Trans. 55: 643–664, illus.

(148) Smith, L. H.
1907. BEOBACHTUNGEN ÜBER REGENERATION UND WACHSTUM AN ISOLIERTEN TEILEN VON PFLANZENEMBRYONEN. 85 p., illus. Halle. (Inaug. Diss.)

(149) Solereder, H.
1908. SYSTEMATIC ANATOMY OF THE DICOTYLEDONS. . . . Transl. by L. A. Boodle and F. E. Fritsch, rev. by D. H. Scott. 2 v., illus. Oxford.

(150) Stewart, L. B.
1922. NOTE ON JUVENILE CHARACTERS IN ROOT AND STEM CUTTINGS OF ACANTHUS MONTANUS. Bot. Soc. Edinb. Trans. and Proc. 28: 117–118.

(151) ———
1924. REMARKS ON THE MORPHOLOGY AND PROPAGATION OF GARDENIA SP. Bot. Soc. Edinb. Trans. and Proc. 29: 41–42, illus.

(152) ———
1927. METHODS OF PROPAGATION. Jour. Roy. Hort. Soc. 52: 33–39, illus.

(153) Stingl, G.
1908. ÜBER REGENERATIVE NEUBILDUNGEN AN ISOLIERTEN BLÄTTERN PHANEROGAMER PFLANZEN. Flora [Jena] 99: 178–192, illus.

(154) Stoll, R.
1874. UEBER DIE BILDUNG DES KALLUS BEI STECKLINGEN. Bot. Ztg. 32: 737–742, 753–768, 785–798, illus.

(155) Swarbrick, T.
1926. THE HEALING OF WOUNDS IN WOODY STEMS. Jour. Pomol. and Hort. Sci. 5: 98–114, illus.

(156) ———
1926. THE HEALING OF WOUNDS IN WOODY STEMS. II. CONTRIBUTIONS TO THE PHYSIOLOGICAL ANATOMY OF RINGED APPLE SHOOTS. Jour. Pomol. and Hort. Sci. 6: 29–46, illus.

(157) Swingle, C. F.
1925. BURR-KNOT OF APPLE TREES. ITS RELATION TO CROWNGALL AND TO VEGETATIVE PROPAGATION. Jour. Heredity 16: 313–320, illus.

(158) ———
[1926]. THE USE OF BURRKNOTS IN THE VEGETATIVE PROPAGATION OF APPLE VARIETIES. Amer. Soc. Hort. Sci. Proc. (1925) 22: 228–230.

(159) ———
1927. BURRKNOT FORMATION IN RELATION TO THE VASCULAR SYSTEM OF THE APPLE STEM. Jour. Agr. Research 34: 533–544, illus.

(160) ———
1927. GRAFT HYBRIDS IN PLANTS. Jour. Heredity 18: 73–94, illus.

(161) ———
1928. VEGETATIVE PROPAGATION OF THE APPLE BY SEED. Science (n. s.) 67: 296.

(162) ———
1929. A PHYSIOLOGICAL STUDY OF ROOTING AND CALLUSING IN APPLE AND WILLOW. Jour. Agr. Research 39: 81–128, illus.

(163) Taylor, G.
1926. THE ORIGIN OF ADVENTITIOUS GROWTHS IN ACANTHUS MONTANUS. Bot. Soc. Edinb. Trans. and Proc. 29: [291]–296, illus.

(164) Tetley, U.
1925. THE SECRETORY SYSTEM OF THE ROOTS OF THE COMPOSITAE. New Phytol. 24: 138–162, illus.
(165) Thompson, D'A. W.
1917. ON GROWTH AND FORM. 793 p., illus. Cambridge.
(166) Tittman, H.
1895. PHYSIOLOGISCHE UNTERSUCHUNGEN ÜBER CALLUSBILDUNG AN STECKLINGEN HOLZIGER GEWÄCHSE. Jahrb. Wiss. Bot. 27: [164]–195, illus.
(167). Trécul, A.
1846. RECHERCHES SUR L'ORIGINE DES RACINES. Ann. Sci. Nat., Bot. (3) 6: 303–345, illus.
(168) Tupper-Carey, R. M., and Priestley, J. H.
1923. THE COMPOSITION OF THE CELL-WALL AT THE APICAL MERISTEM OF STEM AND ROOT. Roy. Soc. [London] Proc., Ser. B 95: 109–131.
(169) Van Tieghem, P.
1887. RÉCHERCHES SUR LA DEPOSITION DES RADICELLES ET DES BOURGEONS. Ann. Sci. Nat., Bot. (7) 5: 130–151.
(170) ———
1887. SUR LE RÉSEAU SOUS-ENDODERMIQUE DE LA RACINE DES CRUCIFÈRES. Bul. Soc. Bot. France 34: 125–130.
(171) ———
1891. TRAITÉ DE BOTANIQUE. Ed. 2, t. 1, 1034 p., illus. Paris.
(172) ——— and Douliot, H.
1888. RÉCHERCHES COMPARATIVES SUR L'ORIGINE DES MEMBRES ENDOGÈNES DANS LES PLANTS VASCULAIRES. Ann. Sci. Nat., Bot. (7) 8: 1–660, illus.
(173) ——— and Monal.
1888. SUR LE RÉSEAU SOUS-ÉPIDERMIQUE DE LA RACINE DES GÉRANIACÉES. Bul. Soc. Bot. France 35: 274.
(174) Vierheller, A. F.
[1924]. INVESTIGATIONS IN THE ROOTING OF APPLE CUTTINGS. Amer. Soc. Hort. Sci. Proc. (1923) 20: 250–255, illus.
(175) Vöchting, H.
1878. ÜBER ORGANBILDUNG IM PFLANZENREICH. Theil 1, 258 p., illus. Bonn.
(176) ———
1900. ZUR PHYSIOLOGIE DER KNOLLENGEWÄCHSE. Jahrb. Wiss. Bot. 34: 1–148, illus.
(177) ———
1902. UEBER DIE KEIMUNG DER KARTOFFELKNOLLEN. EXPERIMENTELLE UNTERSUCHUNGEN. Bot. Ztg. 60: [87] 114, illus.
(178) Vonhöne, H.
1880. UEBER DAS HERVORBRECHEN ENDOGENER ORGANE AUS DEM MUTTERORGANE. Flora [Jena] 63: [227]–234, [243]–257, 268–274, illus.
(179) Wakker, J. H.
1885. ONDERZOEKINGEN OVER ADVENTIEVE KNOPPEN. 106 p. Haarlem.
(180) Weber, F.
1924. THEORIE DER MERISTEMBILDUNG. Naturwissenschaften 16: [289] 296.
(181) Wettstein, F.
1906. ENTWICKLUNG DER BEIWURZELN EINIGER DIKOTYLEN SUMPF- UND WASSERPFLANZEN. Bot. Centbl., Beihefte (II) 20: 1–66, illus.
(182) White, P. R.
1927. STUDIES OF THE PHYSIOLOGICAL ANATOMY OF THE STRAWBERRY. Jour. Agr. Research 35: 481–492, illus.
(183) Wilson, C. L.
1927. ADVENTITIOUS ROOTS AND SHOOTS IN AN INTRODUCED WEED. Bul. Torrey Bot. Club 54: 35–38, illus.
(184) Winkler, A. J.
1927. SOME FACTORS INFLUENCING THE ROOTING OF VINE CUTTINGS. Hilgardia 2: 329–349, illus.
(185) Winkler, H.
1903. ÜBER REGENERATIVE SPROSSBILDUNG AUF DEN BLÄTTERN VON TORENIA ASIATICA L. Ber. Deut. Bot. Gesell. 21: 96–107, illus.

(186) WINKLER, H.
1905. ÜBER REGENERATIVE SPROSSBILDUNG AN DEN RANKEN, BLÄTTERN UND INTERNODEN VON PASSIFLORA COERULEA L. Ber. Deut. Bot. Gesell. 23: 45–48, illus.

(187) WOFFENDEN, L. M., and PRIESTLEY, J. H.
1924. THE TOXIC ACTION OF COAL GAS UPON PLANTS. II. THE EFFECT OF COAL GAS UPON CORK AND LENTICEL FORMATION. Ann. Appl. Biol. 11: 42–53, illus.

(188) YERKES, G. E.
[1924.] EXPERIMENTS IN THE PROPAGATION OF FRUIT-TREE STOCKS. Amer. Soc. Hort. Sci. Proc. (1923) 20: 241–244.

(189) ———
1929. PROPAGATION OF TREES AND SHRUBS. U. S. Dept. Agr. Farmers' Bul. 1567, 52 p., illus.

(190) ZELLER, S. M.
1926. OBSERVATIONS ON INFECTIONS OF APPLE AND PRUNE ROOTS BY ARMILLARIA MELLEA VAHL. Phytopathology 16: 479–484, illus.

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