THE
VEGETATIVE PROPAGATION
OF ACANTHUS.

PHYSIOLOGICAL-ANATOMICAL STUDIES
IN REGENERATION.

By

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Thesis presented for the Degree of

DOCTOR OF PHILOSOPHY
UNIVERSITY OF EDINBURGH,
JUNE, 1933.

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INTRODUCTION.

INTRODUCTION.

The vegetative propagation of Acanthus has already provided material for investigation from different points of view, viz., that of external morphology, which STEWART (18) has shown varies in the leaf with the age of the cutting used, and that of the origin of the adventitious growths, which has been described by TAYLOR (19); and in view of the many interesting problems arising in connection with the regenerative phenomena of this plant, the following work was undertaken.

The material used was grown at the ROYAL BOTANIC GARDEN, EDINBURGH; and the writer wishes to thank the Regius Keeper, Professor Sir Wm.Wright Smith, for the facilities provided enabling this investigation to be carried out. He also desires to thank Dr.R.J.D.Graham, at whose suggestion the work was started, and whose guidance throughout has been of the greatest value, and also the staff of the Propagating Department under Mr.L.B.Stewart and Mr.R.E.Cooper, whose attention to the cultivation of the plants under observation and helpful suggestions during the course of the investigation are greatly appreciated.

SCOPE OF THE PRESENT INVESTIGATION

The present work, while dealing with the origin of the regenerated organs, pursues the subject further and following the developmental anatomical features of these members subsequent to their initiation, shows how their internal construction is influenced in the first place by the internal organisation of the parent axis from which they arise, and secondly (in the case of roots) by the external environmental conditions in which they are produced.

That the normal ontogenetic processes of an organ have an influence on its regenerative phenomena is shown, especially in the case of leaf propagation, and in view of such a fact it has been found necessary to describe in some detail the differentiation of tissues occurring in the root and shoot as they pass from their embryonic condition to the attainment of maturity.

These previously mentioned papers have shown how remarkably plastic this plant is when considered from the viewpoint of vegetative propagation; the present paper shows an equally remarkable plasticity in internal tissue organisation, and as the facts emerge it becomes very suggestive that the two sets of phenomena cannot be considered apart.

Lastly, several points encountered during the investigation of leaf propagation suggested that an interesting/

interesting comparison might be made between the anatomical changes occurring therein and the anatomy of the seedling. Accordingly, seedlings were raised, and as these exhibit some points of interest other than those for which they were originally intended, a fuller account of their development is given than was originally considered necessary.

METHODS.

The cuttings used were grown in peat fibre in a propagating case maintained by bottom heat at a temperature of from 75° to 80° F. (except in special cases to be mentioned as they arise), and were examined periodically (usually daily). The seedlings were raised in a similar manner.

The various stages chosen for examination were, when microtome sections were desired, killed and fixed for twenty four hours in Blé's fluid, after which they were immersed in boiling water for thirty minutes, as recommended by TAYLOR (19), to remove the mucilage from the tissues. After this the material was brought up through gradually increasing strengths of alcohol into absolute alcohol, then into mixtures of alcohol and xylol, and after pure xylol, was embedded in paraffin. The sections were stained with saffranin and haematoxylon.

During some stages of the investigation, recourse to hand sections was made. At first the material was treated with boiling water as described above, but this was seen to have a rather drastic effect on some of the finer organs and tissues examined. It was found that this difficulty of the presence of mucilage could be sufficiently overcome, however, to allow of reasonable ease of cutting simply by leaving the material in methylated spirit for twenty four hours, after/

after which time enough of the mucilage was removed to prevent the sections from sticking to the razor.

Where cell and tissue measurements were made of delicate tissue, hand sections cut from fresh material in water had to be used, to obviate shrinkage which in some cases was caused by the spirit.

Iodine was used for testing starch, Sudan III for fats and suberin, and acetic acid for calcium oxalate.

GENERAL MORPHOLOGICAL AND ANATOMICAL FEATURES OF THE PLANT.

Acanthus montanus, a native of tropical Africa, is an evergreen shrub with unbranched, rather weak stems, and prickly leaves. A notable feature of the plant is the rather copious production of aerial roots, which are produced on the stem from below upwards to within the top few internodes. These have previously been described as stilt roots or prop roots. SCHIMPER (15) says that "the stilt roots which give Rhizophora mucronata such a peculiar appearance, occur relatively strongly developed elsewhere only in the herbaceous Acanthus." The term 'stilt root' conveys the impression that the roots are produced by the plant to give support to a stem unable to support itself.

Examination of any plant, under cultivation at least, is sufficient to cast doubt over this assumption: these roots are obviously much too slender and lacking in strength to act as supporting organs, in fact, if the plant has to be maintained in an upright position, it has to be staked, notwithstanding the fact that the stem may be well furnished with these appendages.

While it is not denied that these organs when produced in sufficient number would not assist in keeping a somewhat lax stem off the ground, the primary/

primary reason for their production must be assigned to some other cause. A significant fact in this connection is the lack of secondary growth in breadth of the stem. Examination of any stem reveals the fact that from the top few internodes downwards no increase in diameter has taken place. This, as will be referred to again, is due to lack of cambial activity and hence production of secondary conducting tissue; a want which must be compensated for as the plant increases in size, and it is suggested that the method by which this want is overcome is by the production of these aerial roots, in the same way as Palms and other species lacking in cambium have overcome a similar difficulty.

This view is supported if one compares the habit of the plant with its chief anatomical features. These are mainly a lack of secondary tissue production in stem and root, the retention in the latter of a relatively large pith for a considerable period, and the persistence of the cortex, periderm production, where occurring, taking place in the outermost cortical layers. These features are all those of a typical herbaceous plant, but nevertheless Acanthus montanus, as cultivated at least, does not show the typical herbaceous habit in that the aerial parts do not die down each year. (It is to be noticed that SCHIMPER (15) refers to Acanthus as being herbaceous but, unfortunately, does not say which species is referred/

referred to, as other species very closely related to the one under consideration behave as true herbaceous perennials.)

Assuming then that the acquisition of a herbaceous anatomy has provided the plant with a conducting system insufficient for its growing needs, the production of aerial roots is understandable as a response to supply this deficiency; and the purely mechanical concept of these roots being produced as struts must take a secondary place. That they have undoubtedly also a mechanical function is seen by their anatomy, which differs from the soil root, or soil portion of an aerial root after it has penetrated the substratum, in the more peripheral position of the conducting system and the relatively greater production of strengthening tissue. This difference is shown more clearly if old roots be compared, when it is seen that in the soil root the conducting system is a central core, while in the aerial root a large pith is present. In fact, a transverse section of an old aerial root is more like that of a stem than of a root, a fact which is recognised in other aerial roots, e.g. Pandanus and Rhizophora. While this difference has without doubt a mechanical value, it will be shown later on that in Acanthus in the first place the difference can be traced to the root tip, where it is produced as a response to environmental factors/

factors which are probably nutritive in nature, and from which it follows that any value the difference in construction has to the plant from the mechanical point of view is secondary rather than primary. In view of these facts the term prop root or stilt root has been discarded in this paper, and these organs will simply be referred to as aerial roots.

THE SEEDLING

Germination

Unfortunately, no seeds of Acanthus montanus have so far been procurable. Seedlings were raised, however, of A. mollis, A. niger, and A. spinosus; and as the general anatomical features of A. mollis in the adult plant were seen to closely correspond to those of A. montanus, it has been assumed that the anatomical changes occurring in the transition from root to shoot in this species (for the details of which the seedlings were raised) will be the same as in the former, and that the application of the facts as obtained to the case under consideration will in no way be invalidated.

The seed is large with two very fleshy cotyledons, and a very small embryo. On germination, the cotyledons remain below ground, but open apart, rupturing the seed coat. Neither hypocotyl nor epicotyl elongate, the first leaves emerging from within the cotyledons, at the top of the radicle. These being followed by other two or three pairs of leaves without elongation of the internodes, a rosette is formed at the base of the cotyledons. (Fig. 1). At the end of the growing season these leaves fall off, leaving a crown consisting of a large terminal bud, and a number of smaller axillary ones, each bud being covered with thick bud scales.

The/

The root system at first consists of radicle only, but later is supplemented by the development of adventitious roots from the hypocotyl. This region is demarcated from the radicle by its stouter diameter, and by the whiter colour and smoother texture of its skin. The adventitious roots, of which two are usually produced, originate below the insertion of the first pair of foliage leaves, i.e. they are in the intercotyledonary plane.

In this condition the seedling remains till the next growing season, when the terminal bud breaks through the bud scales and develops into the shoot.

<u>Seedling Anatomy</u>.

The tap root of the young plant is characterised in the primary condition by a diarch stele, the two root poles being situated on the periphery of a pith of from twelve to fifteen cells in area. The stele is delimited externally by a pericycle, which in turn is circumscribed by a well marked endodermis, and a fairly large cortex. Secondary Changes beginning soon, the symmetry of the primary arrangement is quickly lost.

The initiation of the vascular system is by the formation of the two xylem strands; these consist of protoxylem only. As the root tissues mature the metaxylem is deposited laterally on either side of the protoxylem, so that in passing from the distal to the/

the proximal part of the root increase of the xylem tissue takes place mainly in the tangential direction. The order of development of the metaxylem is centrifugal, and due to this fact and the poor development of protoxylem, the pith does not become encroached upon by lignified tissue. (This point will be dealt with again in describing the ontogeny of the adventitious root.)

Towards the basal end of the root a transverse section shows two plates of xylem each separated by a fairly extensive pith, a tissue which has itself increased in diameter. Each plate consists of a small central portion of protoxylem flanked on either side by a much larger triangular-shaped group of metaxylem vessels.

In this condition the conducting system enters the hypocotyl, in which region the protoxylem diminishes and finally disappears, and the four groups of metaxylem move apart and finally arrange themselves in four equidistant tracts.

The anatomical transition occurring in the hypocotyl is illustrated in Fig. 2.

The cotyledons are sessile. The single cotyledonary strand after entering the main axis bifurcates, and each half remaining in the cotyledonary plane pursues a downward course, and becoming the metaxylem of the root unites laterally with the protoxylem, as described. The foliar traces from the first leaves enter/

enter the axis at a slightly higher level, at right angles to the cotyledonary traces, and dividing, become superimposed on the latter.

The essential features of the transition, then, are the lateral union of the cotyledonary traces with the protoxylem of the root, and the disappearance of the latter tissue above the level of union.

It will be observed that no rotation of the root poles has taken place, a phenomenon rendered unnecessary by the position of these tissues in the cotyledonary plane, and by the deposition of the metaxylem on their tangential flanks.

This type of transition though in one sense really a modification of VAN TIEGHEM'S Type three, to which type it has already been referred by LEE (10), differs in the fact that no root pole rotation occurs.

VAN TIEGHEM'S transitional types have been criticised by DANGEARD (4) who found it unnecessary in some cases to account for a transition of root strands into stem strands by means of a rotation of the protoxylem through 180°; this author found that continuity between root and shoot was effected by means of the metaxylem, which was continued directly with the cotyledonary traces. He evolved a system of classification of types different to VAN TIEGHEM'S, one of his types being that just described. The presence of the remains of the protoxylem between the cotyledonary traces he attributes to poor intercalary growth, and due/

due to the same fact, the region of transition is short.

Branch roots soon arise on the tap root, and having their origin opposite the two xylem tracts, appear in two diametrically opposite rows. They, like the radicle, are characterised by a diarch stele.

The adventitious roots produced later in development, it was noted, were hypocotylar in origin, and appeared below the point of insertion of the first pair of foliage leaves. A transverse section of the axis at the level of emergence of these roots shows that they each arise between, and in connection with, two of the vascular strands in the intercotyledonary plane. As this is the plane in which the foliar traces enter the axis, it seems evident that the vascular supply with which these roots are connected is that from the leaf.

The vascular system of these members differs from that of both tap and branch root in the possession of a larger number of root poles, of which from four to five have been observed at the point of insertion on the parent axis. Towards the growing apex of the root this number decreases to three, which figure is thereafter constantly maintained as the foundation number of the stelar system of such roots.

The first foliage leaves borne on the seedling differ from those on an older plant by the possession of/

of a much thinner lamina and by the absence of spines. All the leaves of the rosette produced during the first year of growth are characterised thus, and only when the plant breaks into growth in the second year are the leaves characteristic of the mature plant formed. In short, Acanthus passes through a definite and easily recognisable juvenile form, of which the external morphological features are expressed in the nature of the leaf.

ANATOMY OF THE PLANT

THE STEM

General

In outline the stem is either round or square, and is of fairly uniform breadth throughout its length. Branching does not occur, unless as a response to injury, as the axillary buds never develop. In arrangement the leaves are opposite and decussate. An interesting feature is that the bud on the adult plant does not possess bud scales, thus differing from the dormant bud of the seedling.

As the age, and hence the internal construction, of the portion of stem used for propagative purposes exerts to some extent an influence on the internal construction, (at least at its point of insertion), of a root arising from it, the process of tissue differentiation undergone as the stem matures requires some attention. The ontogeny of the stem, therefore, will be described, as is seen in a series of transverse sections passing from the growing apex backwards.

Ontogeny

The growing apex is relatively broad and blunt, and is covered by a dermatogen whose cells are heavily impregnated with a substance of fatty consistency. The histogenetic regions characteristic of the normal shoot/

shoot are well defined, and give rise to an embryonic pith of 330μ in diameter, which is circumscribed by a procambial ring 130μ broad, and an embryonic cortex 860μ broad. In the procambium a few protoxylem elements are matured in two diametrically opposite strands, these having differentiated from the two leaf papillae terminating the shoot. The young axis dilates towards its proximal end where the petiole strands from the first pair of expanded leaves intrude into the central cylinder. The leaves being opposite and decussate, these petiole strands enter the axis in a plane at right angles to that of the first formed strands.

In outline they are arc-shaped, and their union with the procambial ring affects the configuration of the latter in that it becomes somewhat more square. The petiole has, in addition to these main strands, two small accessory ones, which enter the main axis one on either side of the main one, and unite with the procambium a little lower down, their places of union being the corners of that tissue. The lignified tissue in the petiolar arc consists of a number of more or less radiating rows of elements, separated from each other by a row or two of parenchymatous cells. In this condition the arc unites with the central cylinder, but below its point of insertion the rows of parenchyma increase in breadth, with resulting separation of the rows of xylem. All these parenchymatous/

parenchymatous rows of cells do not increase in equal proportions, but do so in such a manner as to separate the xylem into groups. Usually three, or at least three main groups, of the latter are isolated, which on passing down the internode become more widely separated, one finally migrating to each corner of the central cylinder, and one remaining in a more or less central position between these other two. Frequently besides these main groups, are other smaller ones consisting of only a few rows of xylem elements, which occupy a position anywhere between the main strands. The two traces from the leaf papillae are at this stage now only composed of one or two very small vessels.

Fig. 3 shows a transverse section through the first internode at this stage having a central cylinder somewhat squarish in outline, and with six main vascular strands, one at each corner and one in the centre of the two sides in the plane of the first foliage leaves. The centres of the other two sides may or may not be occupied by the leaf papillae strands, depending upon the degree of downward differentiation of the latter. Towards the lower end of the internode the vascular system becomes gradually less well developed, and may at the extreme proximal end only consist of a few small vessels in each strand. The scanty development of the vascular system at the base of the internode can be attributed to the fact that differentiation/

differentiation of this tissue proceeds downwards from the node, and also to the fact that intercalary growing is still in progress. This is reflected in the activity of the procambium, which here has attained a breadth of 265μ. The greater dilation of the procambium here relative to xylem maturation has its effect on the configuration of the stele, which in this region tends again to become circular, the influence of the shape of the petiole strand having been removed, as it were. It follows then, that in any young internode the outline of the central cylinder will be to some extent dependent upon the intensity of intercalary growth and primary dilation relative to xylem differentiation. Hence the stem may be either square or circular in outline in any given internode, or may even vary somewhat in the internode itself. The configuration usually alters again after secondary growth has commenced, when very often a completely circular vascular system is formed.

The vessels differentiated downwards from the petioles constitute the protoxylem of the stem. They run through two internodes before fusing with the strands from the corresponding pair of leaves below. They vary from 8.3μx 16.5μ to 23μx 25μ in diameter, and lie on the medullary side of the procambium. In outline they are hexagonal or circular.

From this stage onwards lignification proceeds in/

in the procambium, which matures elements more or less continuously round the pith, but in a particular manner. On the primary strands, and centrifugally to the protoxylem, elements of two types are found - vessels similar in outline to these first formed ones, but of larger size, and smaller ones between the latter. Between the primary strands, however, only one type of element is formed, that being the small one: these elements here are dispersed in fairly regular rows. All these elements are metaxylem. With the lateral union of the primary strands by lignification across the interfascicular procambial portions, the vascular cylinder becomes complete. A well marked cambium is formed, and the stem has completed its primary growth. This condition is shown usually up to the fourth internode.

<u>Transverse section of the fourth internode.</u>

As most of the cuttings grown in this investigation were made by cutting the stem through the fourth node or internode, the internal morphology of this region is given in greater detail. An average diameter of the stem is 4 mm., and in outline may be round or square. In the centre is a pith of from 1.8-2mm. in diameter. The cells in the centre are large and thin-walled, varying from 92μx 92μ to 166μx 166μ or 166μx 200μ; round the periphery the cells are smaller, from 8.5μx 8.5μ to 33μx 33μ.

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In the region of the primary vascular strands these peripheral cells are extremely thick-walled, the thickening being cellulosic, and the cell therefore being collenchymatous. Intercellular spaces throughout the pith are small, and all the cells are packed with starch grains, except the collenchyma, which contain acicular crystals of calcium oxalate. Mucilage is abundant in all the tissues.

The vascular system (Fig. 4) consists of a number, usually of from eight to ten primary vascular strands of from 300 μ to 433 μ broad, and intruding into the pith.

Of these primary strands, the larger are situated diametrically opposite, and when four large strands occupy the corners of the pith, the central cylinder becomes sQuarish in outline. Varying amount of lignified tissue is present between the primary rays, but usually at this stage the interfascicular procambium has not given rise to more than four rows in depth of elements at the most. In any section degrees of differentiation can be seen from the formation of the first row of elements to the formation of a strip of tissue 100 μ broad. The primary strands are composed of large elements 25μ x 25μ to 50μ x 83μ in diameter, and small ones 8.3μ x 8.3 μ to 16.5μ x 16.5μ in diameter. Between the rays the lignified tissue consists of the latter type of small elements only. Seen in longitudinal section the xylem elements, both large/

large and small, have simple pits on their walls. A large amount of wood parenchyma is present, and in the interfascicular portions of the ring rays of parenchyma, from one to three cells wide, extend outwards from the pith. SOLEREDER (17), referring probably to these, speaks of the narrow medullary rays in the Acanthaceae. No true medullary rays, however, are present, if by that term is meant the projection of strips of pith outwards between the xylem masses, as is common in other herbaceous types of stem. While in function they may be similar, in Acanthus they are procambial in origin; a more appropriate term is therefore 'wood ray' or 'xylem ray', as used by EAMES and MacDANIELS (6).

The cambium is two to three layered and continuous. The phloem consists of little groups of small large nucleolated cells, and is in turn circumscribed by a two to three layered pericycle. The cells of the latter tissue are from 20μx 20μ to 33μx 50μ in diameter. Pericyclic fibres, 13μx 16.5μ to 16.5μx 16.5μ in diameter, with very much thickened and lignified walls occur, either solitary or in small groups. The pericyclic cells have raphides of calcium oxalate.

A well defined starch sheath is present, whose cells are slightly elongated tangentially and measure from 33μx 50μ to 41μx 66.5μ.

The cortex is sharply differentiated into inner

and/

and outer regions. The former is of from 11-12 cells in breadth, its cells being thin-walled and large; nearest the starch sheath they are from 33μx 50μ to 58μx 66.5μ in diameter, but passing outwards they increase from 130μx 130μ to 166.5μx 200μ in diameter. Intercellular spaces are large and the cells contain starch.

The outer cortex is small-celled and collenchymatous. It is from six to seven cells broad, these measuring 25μx 25μ to 66.5μx 83μ in diameter, and being devoid of starch grains. A cork cambium has been differentiated in the outermost cortical cells, and is beginning to cut off the cork cells replacing the epidermis.

Secondary thickening in the stem.

A well defined continuous cambium is present from the stage when the primary body is complete onwards, but its activity is weak. It commences to differentiate secondary tissue as soon as the primary increase has ceased, so that the two processes are continuous, and no demarcation can be seen between the two tissues. Histologically the xylem matured from the cambium is similar to that from the procambium, large and small elements being formed opposite the primary vascular strands, small elements only in the interfascicular regions.

So feeble is secondary xylem production that the stem/

stem never shows any marked increase in thickness from about the fourth internode downwards. The cortex is persistent, and only becomes encroached upon to a slight extent by distension of the stelar tissues.

The anatomy of the stem of Acanthus montanus is clearly that of the herbaceous type, especially in the reduced intensity of cambial activity.

Anomalous production of conducting tissue has been described by HÉRAIL (8) in Acanthus spinosus, A. mollis, and A. longifolius. In these species medullary bundles are formed round the periphery of the pith by some of the cells of that tissue becoming activated, producing both xylem and phloem. No similar phenomenon has been observed in A. montanus during the present investigation, but in several older parts of stems it is seen that the primary vascular strands have continued their protrusion into the pith. These projections have the appearance of having had their origin in the peripheral medullary cells in these regions. No phloem has been recognised, but lignified elements similar to those already existing in the primary rays have been differentiated.

The nodal plexus.

As the anatomical features of this region often have an influence on the internal morphology of a root arising from it when a nodal cutting is made, the construction of the plexus if of some importance.

The/

The petiole before its point of insertion on the main axis has a vascular system consisting of a main strand, completely circular in outline, and two small accessory cortical strands situated laterally and on the adaxial side of the main strand. At the point of insertion the main strand becomes an arc, the open side of the arc facing the adaxial side. Here the conducting system from the axillary bud fuses with the petiole arc. The former is dome-shaped, with its base facing the abaxial side, and hence the open side of the arc. As the central cylinder of the stem is approached, the two unite, and fuse as one complex of elements with the former.

The accessory strands at the point of insertion migrate downwards across the cortex of the petiole towards the abaxial side, and on entry into the stem they traverse the cortex of the latter at an angle more inclined to the vertical than the main strand; compared with which strand, therefore, they unite with the central cylinder of the stem at a lower level.

Fig. 5 shows a transverse section through the node above the level of union of the accessory petiolar strands, and Fig. 6 is a construction of the node from longitudinal and transverse sections.

THE ROOT/

THE ROOT

General

The plants available for this investigation having been all raised from cuttings, a full description of root anatomy and development has been possible with adventitious roots only, the seedlings raised not having reached a sufficient age to study the developmental features of the tap root.

As the object of the investigation, however, is to show the relationship between anatomy and the production of adventitious growths, the developmental anatomy of the tap root is of no immediate value, (unless perhaps for comparative purposes), and the lack of information on this subject in no way affects the results obtained.

The adventitous root system of the plant includes the aerially produced roots as well as those produced in the soil.

The aerial root is green, except for a region behind the root tip, and is covered with a dense mass of hairs giving it a felty appearance. Branching never occurs except as a response to injury. Lenticels are present. As has been pointed out before, the general anatomical configuration of its tissues more nearly approaches that of a stem than a root. Starch grains are rare, but mucilage is abundant, especially at the tip.

The/

The soil root is a branched, fleshy organ with a large reserve of starch and also contains mucilage. The conducting tissue is more centralised than in the aerial root. Externally this root is provided with a persistent covering of root hairs, which are dense in the older parts, and as in the aerial root, present a felty appearance.

Ontogeny of the soil root.

The following account of the ontogeny of the adventitious root of Acanthus produced in the soil is based on a series of transverse sections taken at intervals from the apex backwards in roots of various lengths. The length of these intervals and the lengths of the roots examined will be given when the case arises. Before emergence from the parent organ the root shows internal differentiation into plerome, periblem and dermatogen with root cap; these layers are distinct except at the apex, where, as will be shown later on, intense and uniform cell division in all three has resulted in their non-stratification. When the root is from 5-8 mm. long, the plerome gives rise to an embryonic pith and a procambium in which differentiation of stelar tissue commences immediately (Fig. 7). The pith has a diameter of 83μ-100μ; its cells, numbering from fifteen to eighteen, measure 16.5μ in diameter, and have no intercellular spaces. The procambial ring, 116.5μ to 127μ broad, consists of cells varying from 8.3μ to 16.5μ in diameter, the smallest being/

being those on the cortical side.

The lignified elements are arranged in three xylem arches, each of which consists of one or two small vessels 8.3μ in diameter, and one large one, 16.5μ in diameter, developed centripetally to the former. These vessels lie on the cortical side of the procambium, lignification not yet having proceeded internally towards the pith. The three alternating phloem masses consist each of three or four cells 5μ-6.5μ in diameter. These have large nuclei, and even at this early stage an occasional fibre may be seen lying towards their external face.

The surrounding cortex is from ten to twelve cells broad, and measures 330μ: some of its cells are isodiametric, varying in size from 46.5μ towards the periphery to 58μ towards the centre of the tissue: others are elongated tangentially, varying from 25μ x 50μ to 33μ x 50μ. Intercellular spaces are present.

The stelar tissues are delimited externally by a single layered pericycle, and outside this is an endodermis with Casparian dots on the radial walls.

The piliferous layer does not produce root hairs till after the stelar system has become established: in several actively growing roots no hairs were produced for a distance of 4 cm. behind the apex, whereas xylem differentiation can be seen after a distance of just over 5mm. The distance behind the apex at which/

which root hair production commences varies with environmental conditions, but in each case examined, root hair production was preceeded by stelar elaboration. In the region where root hair production is active, a sudden increase in the diameter of the root takes place: for example, an increase from 1,000μ to 1,240μ has been noted. This increase is due to both cell division and to dilation of the individual cells. Thus the pith now measures 115μ x 130μ, its cells, numbering about thirty vary from 16.5μ to 23μ x 26.5μ in diameter.

No intercellular spaces have yet been formed. The breadth of the procambial ring remains the same, but the central cylinder has increased, an increase due to the pith. To maintain its continuity round that tissue, the procambium must have increased radially. The cortex has now a breadth of 465μ, is ten to fourteen cells broad, these measuring from 33μ to 66.5μ in diameter. Increase in diameter of the root must be attributed mainly to activity in the pith. If the ratio central cylinder/total diameter be compared between this region of the root and the hairless region between it and the apex, it is found to be 1/5.4 and 1/4.7 respectively; if, however, the ratio pith/central cylinder be compared for the same regions, it is seen to be 1/1.9 and 1/2.33, i.e. an increase in the diameter of the root is accompanied by a decrease in the relative diametric proportions of

central/

central cylinder to pith, or, the pith is expanding radially more quickly than the central cylinder. That this property of the pith has a marked effect upon the internal organisation of the root will be shown later on: while the value to the plant of the rapid increase in total diameter, may be, as JOST (9) pointed out that a certain tenseness is given to the root (thus rendering it more stable in the soil) when increase in thickness occurs after a period of rapid elongation. JOST also correlated this phenomenon with a concurrent decrease in length. No attempt was made to discover if a decrease in length took place in the Acanthus root in this region, but the tendency for the cortical cells, especially the peripheral ones, to become elongated radially, in contradistinction to the tangential elongation as noticed in the region between this and the apex, suggests that a slight contraction in longitudinal dimensions may have taken place.

Maturation of stelar tissue continues by the addition of phloem cells on the tangential sides of the first formed ones and by addition of more lignified elements to each xylem ray. The elements added at this stage are intermediate in size between the two sizes of existing vessels, and are deposited centripetally and tangentially to the last formed large vessels, the latter becoming enclosed in a circle of elements of narrower lumina.

Towards the proximal end of this region of root hair production, a cambium arises in the outermost cortical layers, beneath the piliferous layer. This latter, by the dilation of the root, becomes sloughed off, but is replaced by this secondary cambial activity, providing the root with a persistent piliferous layer, and hence persistent root hairs.

Persistent root hairs have been described in members of the Compositae, Primulaceae and Leguminosae, and WHITAKER (24) has shown that their presence is correlated with the lack of secondary thickening characteristic of the herbaceous type of root.

The case for Acanthus agrees with this statement. The cortex remains throughout the life of the root, becoming a storage tissue: its preservation is ensured by the lack of increase of the vascular cylinder, as will be shown, and by the non-development of a pericyclic phellogen. WHITAKER found in the genera he studied that the development of these hairs occurred either at the proximal end of the root, or at the distal. This is not the case in Acanthus. Here the root hairs are developed from the distal to the proximal end; on any particular root or part of a root they may be seen either as a continuous covering, or an interrupted one. On thick old roots, or on the proximal thicker parts of young roots they are usually continuous, while on the younger more actively growing part of the root they tend to be discontinuous.

Their/

Their disposition is given below on two roots, one 28 cm., the other 3 cm. long.

TABLE I

Distribution of Root Hairs.

Root 28 cm. long.

From tip backwards to 3.8 cm.	.... no hairs
From 3.8 cm. " " 15.3 cm.	.... hairs
From 15.3 cm. " " 16.8 cm.	.... no hairs
From 16.8 cm. " " 28 cm.	.... hairs & branch roots

Root 3 cm. long.

From tip backwards to 7 mm.	.... no hairs
From 7 mm. " " 17 mm.	.... hairs
From 17 mm. " " 30 mm.	.... no hairs

It is an accepted fact that root hair production occurs behind the rapidly elongating region of the root apex, and SNOW (16) has shown that root hair production is correlated, not with the rate of growth of the organ, but with differential elongation of outer and inner tissues, being dependent on the ratio between "the capacity of the epidermal cells to elongate and their ability to do so." Thus, an epidermis tending to elongate more quickly than it is able to do because of the slower elongation of the internal tissues throws its outer free walls into bulges, which extend into root hairs. In other words, when internal and external tissues are elongating equally, no hairs are produced: when the internal tissues are elongating/

elongating more slowly than the external, root hair production commences. In the young Acanthus root, then, the phenomenon of alternating hairless with hairy regions suggests alternating periods of rapid elongation of all tissues followed by periods of slower and differential growth, the slowest growth being in the central tissues.

The root hair production started in the first region of differential growth behind the apex is continued backwards by means of the cortical cambium: hence it is natural to find that the hairless regions will show a decreasing length, passing from the apex backwards, till on the older parts of the roots, the hairy covering is continuous.

Further, if the hairless regions are those of rapid, and the hairy regions those of slower elongation, it might be expected that the latter would show an increased radial growth when compared with the former. This is usually found to be the case if the regions immediately behind the apex are considered. It has been seen that in passing from the region behind the apex to the region where root hair production commenced, that an increase in diameter in one particular root took place from 1,000μ to 1,240μ: passing further backwards now into the next hairless region, a decrease to 1,150μ was observed.

This difference is exaggerated in roots plunged straight into alcohol, in which medium the tissues in the/

the hairless region assume a shrunken appearance, especially in the cortex where the cells, mostly of the periphery, become elongated radially: The tissues in the root hair region remain comparatively turgid. On account of this fact, measurements were made from hand sections of fresh material cut in water.

Despite the fact that the root has shown a decrease in diameter, cellular division in the pith has resulted in an increase, though slight, in that tissue. Cell increase has taken place from thirty to fifty, but no increase in the size of the cells has occurred: the presence of nuclei in some shows that this tissue is still in a state of activity.

The relative proportions of the tissues in the three regions just considered can be tabulated as under. (It will be seen that the tissue measurements which have been given are those of the diameters. From the physiological point of view, however, measurements giving the surface of contact between the various tissues would have been of more value, i.e. where actual size of tissue is considered, the area rather than the diameter is of more importance. When, however, the figures are used for comparative purposes, such as in the table below, where they are stated as ratios, the final result is independent of the actual measurements.)

TABLE II/

TABLE II

Comparative Dimensions of Tissues in Young Part of Root.

	$\frac{\text{Central Cylinder}}{\text{Total Diameter}}$	$\frac{\text{Pith}}{\text{Central Cylinder}}$
Region before root hair production	1/4.7	1/2.33
Region of root hair production	1/5.4	1/1.9
Region behind root hair production	1/4.2	1/1.9

Thus, between the last two regions, a decrease in the proportion of central cylinder to total diameter has been effected concurrently with the maintenance of the relative proportions of pith to central cylinder, indicating a lag in radial expansion of cortical and procambial tissues proportional to pith. The effect of this will be discussed later on.

From this region backwards secondary roots are produced; and in the procambial ring a fourth xylem arch has made its appearance (Fig.10, ii). This arch is first seen by the appearance of a vessel of larger size - 25 μ in diameter, round which smaller ones, 13 μ in diameter, are deposited. A regular feature of the development of the Acanthus root is the increase in the number of xylem archs in transverse sections taken from the apex backwards, a feature which will be discussed later. In the formation of these archs , the first formed small elements characteristic/

characteristic of the first three tracts may be either absent or present.

Further increase in the lignified tissue of each ray takes place in a characteristic manner. Maturation of narrow elements, which as was seen were deposited round the large ones, continues in a centripetal direction towards the pith. At the same time, however, deposition of large vessels, measuring from 25μ x 33μ to 30μ x 33μ occurs tangentially to the first formed centripetally developed group, and their order of deposition is centrifugal. These latter are the second xylem elements of the primary wood, or the metaxylem of VAN TIEGHEM (20), who described them as arising in precisely the same manner in many species. In Acanthus, no centripetal deposition of metaxylem relative to protoxylem occurs. The first group of elements are all protoxylem, and the metaxylem is tangentially disposed and centrifugally developed relative to them, This phenomenon, contrary to the usual centripetal deposition of metaxylem relative to protoxylem in many roots, results in the preservation of the pith as a parenchymatous tissue, which eventually becomes a storage organ. The xylem archs at this stage present the appearance of an inverted 'T', and consist of large vessels embedded in a mesh of narrow ones. As lignification continues, the deposition of metaxylem, while losing some of its regularity, extends towards the pericycle as two arms on/

on either side of the protoxylem, and always is composed of large and small elements, while towards the pith, small elements only are matured. The net result of this process is a greater increase of each ray tangentially than radially.

Passing backwards into the next region of root hairs, diametric increase to 1,665μ has taken place, an increase again which must be attributed mainly to the central cylinder. This has now a diameter of 616.5μ x 665μ (compared with 265μ x 280μ in the previously described region); and again the seat of dilation has been the pith, which has increased from 130μ x 150μ to 465μ x 475μ. This tissue has now a diameter of fourteen cells, these measuring from 25μ to 66.5μ (compared with 16.5μ to 28μ previously). The cortex shows an increase in breadth from 460μ to 560μ, due mainly to dilation of the cells.

Increase in the number of xylem rays continues by the addition of one arch at a time, so that towards the distal end of a root a number varying from ten to thirteen has been observed (Fig. 10). Tangential growth of each arch continues as described so that eventually, when they become united, the pith is circumscribed by a complete lignified cylinder.

A transverse section through the root in the region having seven xylem archs showed the following features (Fig. 8). The pith has increased to a diameter of 600μ x 665μ, its largest cells, in the centre,/

centre, measure 83μ, while those round the periphery increase 25μ. It is not easy to determine whether these small peripheral cells are really pith cells, or procambial cells which may now be maturing into pith cells. As has been seen, the root up to this stage has shown continued radial growth by cellular activity of its own tissue. The indications now are, however, that increase in diameter is due to the innermost procambial cells, which only giving rise to narrow lignified elements on the xylem side, are maturing into pith cells on the interior.

The xylem rays have not yet united, and have radial and tangential dimensions varying from 58μ-66.5μ and 130μ-200μ respectively.

Between these xylem rays and the alternating phloem a well defined, and almost continuous cambium is differentiated. A noteworthy point is that between the xylem rays the procambium, due to stretching over a dilating pith, is much narrower, so that here the cambium almost abuts on to the pith. Union of the rays is effected a little further back by the lignification, on the periphery of the pith, of a narrow strip of small elements only, across these remaining portions of the procambium. The xylem archs are not usually disposed at equal intervals round the pith, so that some of the last portions to lignify are longer than others. A transverse section through the region in which the xylem ring was almost complete/

complete showed one lignified portion 130μ long. The xylem varied in breadth from 16.3μ (on the sides of the rays where it consisted of one element only) to 130μ (in the primary ray).

The stele now assumes an aspect differing from previously described regions. The xylem cylinder when complete, is cylindrical on the pith side, but on its outer face consists of a series of ridges and furrows, due to continual deposition of both wide and narrow elements opposite the primary rays, but narrow elements mainly in the interfascicular regions. The irregularity of the outer face causes the outline of the cambium, which follows the outline of the xylem, to be sinuous. With the completion of the xylem ring, and the ensuing commencement of cambial activity, the root has completed its primary growth (Fig. 9.); the most outstanding features of this have been the continual dilation of the pith, and the increase in the number of root poles. Secondary increase due to the cambium follows immediately on completion of primary growth, and as secondary and primary tissues are histologically similar (with the exception of the protoxylem), no difference can be detected between the two.

From the time of completion of the vascular ring, and just before the event, onwards, differences in development occur, these differences being correlated with the behaviour of the pith relative to the xylem/

xylem cylinder.

Two sets of conditions arise -

(1) In which dilation of the pith ceases before, or when, the cylinder is complete.

(2) In which the cylinder is complete before dilation of the pith has ceased.

(1) If in a root in which the lignified cylinder has become complete the pith is mature and has ceased to dilate (a condition usually attained when about 14 cells in diameter and measuring 665μ), further development consists simply in the maturation of vascular tissue, from procambium at first, and then from cambium, in the normal way. (Figs. 9; 10, i-vii) The pith when mature has intercellular spaces and its cells are packed with starch grains. The cortex is likewise, but here the intercellular spaces are larger. The outermost cortical layer continues to be meristematic, producing a piliferous layer externally and cortical tissue internally. The peripheral cortical tissue is thus secondary, but no sharp distinction is seen between primary and secondary regions; the only distinguishing features of the latter tissue being the smaller size of the cells, their tendency to tangential elongation, and the less frequent occurrence of intercellular spaces.

Secondary xylem formation of the normal type is very scanty, and as no internal phellogen arises to cut off a cork layer, the cortex never becomes exfoliated/

exfoliated, but remains as a storage organ for starch. In old woody roots, the demand for an increase in conducting tissue is satisfied by development of lignified tissue in the pith. It has not been observed whether this is due to activity of an intrastelar cambium arising later in development in that tissue (except in the special case to be considered shortly), or whether it is due simply to maturation of the pith cells themselves into vessels. Development of this tissue takes place from the periphery of the pith inwards, which tissue may become either partially or completely transformed. Histologically this lignified tissue is similar to that of the vascular ring, but no phloem has ever been observed.

(2) The case is quite otherwise, however, if the lignified cylinder is complete, or almost complete, before the pith has stopped increasing in diameter. The latter exerts a distending force on the former, which being rapid, and incapable of stretching, ruptures. The rupture occurs, first at any rate, in one of the least lignified interfascicular regions, but as dilation of the pith continues, complete fragmentation of the stele ensures; the isolated fragments corresponding to the primary rays.

Thus a root which before the xylem ring became continuous had a pith measuring 300μ x 500μ in diameter, and one unlignified interfascicular strip of procambium/

~~to~~ 130μ long had, when the pith had increased to 330μ x 580μ, an unlignified strip 250μ long (Fig. 11) In the region behind this, the pith had increased to 465μ x 665μ, the unlignified ray had become 330μ long, while other gaps of 50μ to 83μ had occurred between the primary rays. Reunion of the xylem rays across these gaps takes place by cambial activity, when dilation of the pith ceases. Before this, however, other changes have occurred. Ensuing on its tendency to follow the outline of the xylem, the cambium has penetrated into the bottom of the furrows on the external face of the cylinder, and when rupture of the lignified ring has taken place in these furrows, the cambium likewise becomes broken. (Fig. 12) The fractured ends of the cambium now continue their way round the sides of the xylem fragments and enter the pith, round the periphery of which tissue they penetrate to various degrees. In some cases they join up to form an almost continuous ring. Reunion of the extra xylary cambium is effected on cessation of pith dilation by certain cells in the path of the original cambium becoming meristematic, and restoring the continuity of that tissue, which then proceeds to mature the xylem closing the gaps. The intraxylary cambium differentiates lignified tissue round the periphery of the pith. (Fig. 14) Phloem only in a special case (to be dealt with later) has been observed, but when such is produced, it is towards the/

the centre of the pith. (Occasionally, when no phloem is produced, an occasional fibre may be seen).

It is thus seen that dilation of the pith after the establishment of the continuous vascular ring leads to rupture of the latter, and following on this, the production of anomalous secondary thickening of the nature of inverted stelar tissue in the pith,

Anomalies in the vascular system of the Acanthacene have been described by HÉRAIL(8), who confined his attention, however, to the stem. In Hexacentris and Mendonica, irregular deposition of large and small lignified elements on the outside of the stele gives the latter a furrowed outline, as described here in the root of Acanthus. In these genera, however, secondary xylem formation is more active, and the rapidly increasing ridges finally rupture the cambium across the furrows; union is effected later by the the fractured ends of the cambium growing together as observed in Acanthus. From HÉRAIL's work it is obvious then that the irregularity in the outline of the xylem is in itself, provided it becomes sufficiently pronounced, sufficient to cause discontinuity of the cambial ring.

In the Acanthus root under consideration, however, there is the extra contributory cause, namely, the dilation of the pith , probably a much more disturbing factor than xylem irregularity.

The disintegrating effect on the stele of a distending/

distending pith has been shown in some of the lower plants. WARDLAW(23) has shown that in the stems of Psilotum, Tmesipteris and Lycopodium, passage from a young stem to an old one is accompanied by "progressive decentralisation and disintegration of the xylem". These phenomena he interprets on BOWER's Size Factor theory, the structural changes being effected to maintain a high proportion of surface to bulk in the xylem tract.

Such an interpretation may be used to explain the development of the primary state common to all Acanthus roots - the concurrent dilation of the pith, decentralisation of the stele and increase in the number of xylem tracts being expressions of the same underlying principle. The fact still remains to be accounted for however, that fragmentation of the xylem occurs only in some roots and not in others. It is a significant fact that the anomaly, where it occurs, is due to dilation of the pith after the establishment of the xylem ring, whereas in the other case, continuity of the xylem is preceeded by maturity of the pith. The controlling factors, then, must be there either delaying pith development relative to xylem formation, or causing it to develop after it normally would have stopped.

Now, if the origins of these roots be compared, it is seen that the roots which exhibit normal structure are those produced from an old piece of stem/

stem, or at least a stem in which primary increase in tissues has stopped and the xylem ray is complete, whereas roots showing the anomaly are those borne on a stem still developing, or on a petiole. (This latter case will be considered in greater detail later). Further, the anomaly has only been observed at the proximal end of such roots, just outside the point of emergence from the parent organ. In the case of a root arising from a stem where primary tissue is mature, the base of the root is in continuity with tissue which has ceased to dilate, and therefore can have no distending influence on the basal root regions. (Fig.15) In the case of a root however, arising from a stem still undergoing primary tissue dilation, the tissue of the stem abutting on the root base, if increase takes place, will have a distending effect on the basal root tissues. This effect is all the more pronounced if the root arises from the stem while still in the discreet vascular strand stage. (Fig.16): the root, arising in the proximity of one of the strands or between two strands, has a base broad enough to abut on the interfascicular meristematic tissue. This tissue, actively dividing, considerably disintegrates the stelar system of the young root inside the stem, and this activity progressing a little way down the root, gives rise to the phenomena just described. In fact in roots, produced from the first or second nodes or internodes, the stelar system of the/

the root at its extreme proximal end often consists of a number of quite isolated xylem strands, each completely surrounded by a cambium.

Figs 14, ii to v show the proximal end of a root produced from a cutting made through the first node. . Here the arc-shaped portion of the xylem is derived from the primary ray of the stem, while the isolated strand (acc.str.) is derived from one of the accessory leaf traces, the cutting having been made just above the point of union of this strand with the central cylinder of the stem.

It is thus seen that the degree of stelar complexity of the parent organ exerts an influence on the internal construction of the adventitious root produced from it, at least at its point of insertion, this influence depending upon whether or not the tissue of the young root is in connection with dilating tissue of the stem.

The Branch Root.

The branch root is somewhat slender, and is characterised in the majority of cases by a diarch stele. The pith is small, and becomes completely lignified in a much earlier stage of development than in the parent root. No fragmentation of the xylem cylinder has ever been noticed in the branch root, and pith dilation only occurs to a slight extent,

Ontogeny of the aerial root. /

Ontogeny of the aerial root.

A point of considerable interest in the ontogeny of the soil root of Acanthus is the increasing number of xylem archs passing from the apex backwards. Before this question is discussed, however, it is necessary to follow the development of the aerial root in a similar manner.

The aerial root is initiated in the pericycle as is the soil root, and emerges with an internal organisation similar to the latter. The growing point is broad, but soon differs from the soil root in that as it enters the atmosphere it becomes broader, and bulkier, in contradistinction to the thinning of the root after entering the soil. The reason for this becomes obvious if a series of transverse sections behind the tip be examined. Immediately behind the apex the three histogenetic regions are well defined, but their relative proportions differ from those of the soil root, the outstanding difference being the narrowness of the **cortex** and the breadth of the central cylinder compared to that of the soil root. The following figures show a comparison between the two types of roots.

TABLE III

	Soil Root.	Aerial Root.
Total diameter	1500 μ	1500 μ
Cortex	400 μ	250 μ
Central cylinder	580 μ	1000 μ
Pith	470 μ	500 μ

The most distinctive feature as shown by these figures then, is the rapid increase in breadth of the procambium, at the expense of the **cortex**. This region

is/

is immediately behind the root cap: the next stage in development , following closely on the above, is the rapid increase in breadth of the cortex. Thus in the above root the cortex attained a breadth of 415·5μ while the central cylinder was still 1000μ in diameter. To this rapid increase in breadth, of first the central cylinder and then the cortex, is attributed the bluntness of the growing point of the aerial root.

A very important difference between the two types of roots is the large diameter attained by the central cylinder of the aerial root immediately behind the apex, largely due to procambial dilation, compared with that of the soil root.

In fact, in the former, the procambium attains almost its mature dimensions before the stelar elaboration commences, whereas as has been shown, the soil root shows progressive increase of procambium from the apex backwards. (See Figs.7,10,18) Correlated with this type of growth, the aerial root elongates very slowly, a fact which suggests that in air the histogenetic tissues in the apex are increasing radially much more actively than longitudinally, whereas in the soil, the reverse is the case.

Resulting from this difference between the two types of root apex are differences in the anatomical features of the mature tissues. In the aerial root the pith and the procambial ring attain their full size/

size, or almost so, before differentiation of phloem and xylem commences. Before lignification begins the procambium is ridged and furrowed, the number of ridges being usually about ten. In the ridges the primary xylem groups are found, by the differentiation in each of from one to three rows of narrow elements, matured centripetally.

The development of the xylem cylinder proceeds as in the soil root, elements being matured tangentially and centripetally, the latter, however only to a limited extent; the greater tangential deposition of elements soon leads to the lateral union of the primary rays, forming a continuous ring round the pith. No increase in the number of primary rays takes place in the aerial root, as in the soil root, as here, all the rays are matured at the same time behind the apex.

The xylem ring differs histologically from that of the soil root in the small number of large vessels formed compared to the large amount of narrow elements.

The large metaxylem vessels are disposed much more regularly on the sides of the protoxylem than in the soil root. On a completion of the ring, the cambium becomes continuous and proceeds immediately to differentiate secondary tissue. Large vessels are deposited mainly in lines continuous with the metaxylem, the rest of the tissue consisting of narrow elements. Primary rays of parenchyma are usually formed/

formed between the primary xylem rays; and after secondary growth, secondary rays commence opposite the protoxylem.

All secondary formation of conducting tissue in the aerial root is normal, on the outside of the primary body, no conducting tissue being formed in the pith. Secondary increase is much more pronounced than in the soil root, producing a wide xylem cylinder. The cortex is never exfoliated. Figs. 19 and 20 show an aerial root before and after secondary growth has taken place.

Consideration must now be given to the nature of the covering of the root. Behind the apex, while primary tissue growth is proceeding, a cambium on the outside of the cortex cuts off a layer of suberised tissue. This provides the root with a protective layer for some distance backwards, but later, notably after the completion of the xylem ring, this cambium produces a dense mass of hairs. These persist from this stage onwards back to the proximal part of the root, and may be replaced again by a cork layer only in old and very woody roots, after considerable secondary increase has taken place, and the cortex itself has become impregnated with fatty substances.

These hairs are unicellular and resemble the root hairs of the soil root, and are produced as thickly as to provide the root with a thick felt like covering.

The/

The presence of starch grains is only noticed in old woody roots, when it is present in both pith and cortex.

The main features distinguishing the aerial root from the soil root, then are (1) its much broader growing point, (2) differentiation of the primary xylem body round a mature pith, (3)secondary increase of conducting tissue on the outside only of the primary, (4) retention of the pith, (which is larger relative to cortex than in the soil root).

The aerial root of Acanthus on entering the soil shows a change in internal construction corresponding to the change in environment. This consists in the reduction of the breadth of the growing point and the ensuing assumption of the characters of the soil root - decrease in size of pith and contraction of the stele into a more central position and lack of secondary tissue formation external to the primary. The change takes place gradually, a series of transverse sections above and below soil level showing just a gradual decrease in the diameter of the pith, concurrent with an increase in the amount of large vessels and decrease in small ones.

Comparative Anatomy of soil root and aerial root, and its bearing on ontogeny.

It has been shown that the soil root differs ontogenetically from the aerial root in the gradual increase in the number of root poles passing from the growing/

growing apex backwards, or in other words, the reduction in the number of these in the younger parts compared to the older. Reduction phenomena in the number of root poles in the radicle have been pointed out by DODEL (5) who showed that in Phaseolus that organ possessed more xylem tracts towards its proximal than at its distal end. These extra strands he showed were accessory cotyledonary traces, alternating with the usual root poles; they differ from the strands in Acanthus, however, in that they only pursue a short downward course into the root before terminating, either singly, or after uniting in pairs. BOND (3) describes similar phenomena in Vicia as well as in Phaseolus. The results of both of these authors show that in these species examined a correlation exists between the presence of a pith and the number of xylem strands. Thus in the radicles of these species a pith exists in the proximal region of the root where it merges into hypocotyl, but disappears towards the distal region. Here a central xylem core is formed, consisting of fewer archs than in the conducting system differentiated round the pith at the proximal end. BOND furthermore found that decotylation led to slower growth of the radicle, with the ensuing retention of the pith and accessory strands for a longer period.

In the root system of the Corsican Pine ALDRICH-BLAKE (1) has shown that not only root pole reduction/

reduction, but the reverse process of augmentation may take place, the latter process accompanying an increase in diameter of the root. In view of the above considerations, then, it is not unnatural to expect that in the root of Acanthus the number of archs should show a correlation with the dimensions of the pith. The following table shows how reduction has occurred in a root 28 cm. long.

TABLE IV.

Xylem arch reduction in the soil root.

Distance behind tip.	No.of xylem archs.
8 mm.	3 - the 4th just forming
4 cm.	4
8 cm.	4
12 cm.	5
16 cm.	6
20 cm.	6
24 cm.	8
26 cm.	9
28 cm.	10

Likewise, as has been shown previously, the pith shows a gradual diminution from the proximal to the distal end, and that there is a correlation between the two sets of phenomena can be seen if the relative dimensions of the tissues be compared. This is illustrated by the following figures.

TABLE V./

TABLE V.

Comparison between stelar structure and tissue dimensions in the soil root.

Degree of Stelar differentiation.	Diameter of pith. / Diameter of central cylinder.	Diameter of central cylinder. / Total diameter of root.
3 xylem archs	1:2.33	1:4.7
4 " "	1:1.9	1:4.2
5 " "	1:1.4	1:2.6
7 " "	1:1.4	1:2.4

Thus increase in the number of archs is accompanied by a decrease of central cylinder relative to pith, but an increase in central cylinder relative to total diameter. The actual increase in diameter of the central cylinder is clearly to be attributed mainly to the pith: hence the determining factor accompanying root pole increase is the increase of pith relative to total diameter; in other words, when the pith has attained a given relative diamater, stelar complexity will have attained a correspondingly high degree.

Comparing new soil root and aerial root, it has been seen that in the former the complete primary stelar system is only established gradually over a considerable length of root, whereas in the aerial root the whole primary system is established at the same time, shortly behind the apex.

The following figures obtained in an aerial root may be contrasted with those in Table V.

TABLE VI/

TABLE VI.

Comparison between stelar structure and tissue dimensions in the aerial root.

Degree of stelar differentiation.	Diameter of pith. Diameter of central cylinder.	Diameter of central cylinder. Total diameter of root.
Procambium just noticeable	1:2	1:1.5
Histogenetic regions well defined	1:1.5	1:1.8
Before xylem formation	1:1.2	1:1.8
Xylem differentiation begun (19 archs)	1:1:2	1:1:09
Tangential elongation of archs	1:1.2	1:1.09
Secondary thickening commenced	1:1.4	1:1.06

(The last figures show that after commencement of secondary thickening the proportions are upset, and are therefore not valid for comparative purposes. It must therefore be emphasised that the phenomena under consideration are those of the primary root body only).

The increase of the ratio central cylinder total diameter 1:1.5 to 1:1.8 and its subsequent decrease to 1:1.09 is due to the rapid dilation of the cortex behind the tip, as previously described. Apart from this however, the figures show that in the aerial root the final relative proportions of all tissues are attained much sooner behind the growing point than they are in the soil root; and consequent upon this precocious attainment of the mature size of the central cylinder is the formation ab initio of the total number of primary xylem archs.

The question now arises, to what is this difference/

difference in organisation behind the growing point due?

On its initiation in the parent axis the aerial root and soil root have a precisely similar internal morphology; the subsequent difference in organisation must therefore occur after emergence into either soil or air.

Examination of a young soil root throws some light on this point, as is shown in the following Table of figures obtained in a root 15 mm. long.

TABLE VII.

Comparison between stelar structure and tissue dimensions in a young soil root.

Distance behind Top.	Degree of Stelar differentiation.	Diameter of pith. / Diameter of central cylinder.	Diameter of central cylinder. / Total Diameter of root.
5 mm.	No lignified elements.	1:1.23	1:2.5
10 mm.	6 xylem archs.	1:1.3	1:2.5
15 mm.	10 xylem archs.	1:1.27	1:2.85

These figures show that roughly the same proportion between pith and central cylinder have been attained (cf. previous tables) as in the older root and the aerial root when the vascular ring has the larger number of xylem archs.

Further, this ratio has been attained much sooner behind the apex in the young soil root than in the old, and hence approximates more nearly to the aerial root./

root. From the latter it differs, however, in that root pole reduction has begun. In other words, this young soil root resembles the aerial root in that the degree of stelar complexity is greater behind the growing point than in the older root, but as elongation has proceeded this degree has diminished, and its diminution is correlated with the relatively less proportional radial dilation of central tissues to peripheral tissues.

Reference to Table IV again shows this point. In the proximal part of that root, i.e., the part of the root formed when young, a new xylem ray appears each 2 cm., while in the distal region the root had elongated 11.2 cm. between the deposition of the fourth and fifth archs, i.e. stelar elaboration has been a quicker process behind the apex of the root when it was young than when it has become older. Further, it is seen that between the appearance of the xylem archs in the younger part of the root there has been much more rapid elongation than has occurred in the older parts when their tissue differentiation was proceeding. In this phenomena lies the clue to the problem. The young root after its emergence is dilating radially more rapidly proportional to longtitudinal growth, but after it has penetrated the soil for some distance, it elongates more rapidly with a consequent reduction of radial increase.

In the aerial root, on the other hand, the tissues/

tissues dilate more quickly behind the growing point, with which is correlated the slow longtitudinal growth of such an organ.

The facts, then as understood so far for the development of the root, may be summarised as follows:-

(1) The root initial is the same in soil and aerial root; it is broad, and has a high degree of stelar elaboration behind the apex.

(2) On entry into the soil, rapid longtitudinal increase takes place with a concurrent diminution in radial increase and in stelar complexity.

(3) On entry into the atmosphere, slow longtitudinal increase is accompanied by greater proportional dilation of the tissues and the maintenance of a high degree of stelar complexity behind the apex.

(4) In all cases the degree of stelar elaboration is correlated with the relative proportion of central cylinder especially pith, to total root diameter. The cortex remaining practically the same breadth after its embryonic state has been passed, differential growth between central tissues and cortical tissues is suggested. When the central tissues dilate more rapidly relative to cortex, a stele with a correspondingly large number of xylem archs is formed; when dilation of the central tissues is relatively slower than the cortex, only a few xylem archs are formed.

The fact has been referred to previously that in

the/

the root of Acanthus the tissues at the extreme tip, under the root cap, do not show definite stratification into the three histogenetic regions until further back; this confusion of tissues, according to FLAHAULT (7), is due to the intensity and equality of cellular increase of all regions in this area i.e. central cylinder and cortex are differentiating with equal regularity. It follows, then, that the width of central cylinder relative to cortex as initially laid down will be maintained as long as growth in this manner proceeds, and any condition altering the relative growth of the two tissue regions will be followed by the differences in stelar complexity already described,

Consider now the root entering the soil. A period of rapid elongation ensues, followed, as has been shown when discussing root hair production,by a period of slower elongation. The first phase, being devoid of root hairs, is indicative of equal longtitudinal extension of all tissues; the second phase, where root hair production commences, was shown to be due to differential growth between the peripheral and central tissues.

Fundamentally the same process, therefore, appears to central both degree of stelar complexity and root hair production. It was seen that the stele has its foundations laid down before root hair production commences, i.e. while uniform elongation of all/

all tissues is taking place behind the apex. With the slowing down of longtitudinal increase in the region behind this differential growth between central and peripheral tissues ensues, resulting in greater dilation of the former tissues proportional to the latter, or in other words the slowing down of longtitudinal increase is more marked centrally than peripherally. The result for the central tissues is increase in stelar elaboration, and for the peripheral tissues the production of root hairs.

Considering now the aerial root, slow growth in length maintains the equal longtitudinal increase of all tissues. Hence for some distance backwards from the tip no root hairs are produced. Further back, however, root hair production becomes intense, suggesting a greater tendency to elongate of the epidermal than the central tissues. This may possibly be due to the fact that in this case the epidermis becomes green, and hence photosynthetic; the occasional starch grains seen in the green parts of the root have been suggested by TAYLOR (19) as being the transitory products of photosynthesis.

On the other hand, in this region xylem differentiation is proceeding much more rapidly than in the more distal region, and the effect of maturation of lignified tissue in itself might be a retarding one on the peripheral tissue.

Again, differential growth here might simply

ensue/

ensue on the slowing down of longtitudinal increase, the central tissues slowing down more rapidly than the peripheral ones.

To conclude this discussion, then, it is seen that determining factor on the ultimate construction of the primary body of the root is to be found in the nature of histogen differentiation behind the growing point. In the soil the plerome elongating more rapidly is contracted in breadth, and leads to centralisation of the stelar tissues; in the air it elongates slowly and becomes dilated, leading to stelar decentralisation. That is difference is due to the effect of environmental conditions on the growing apex is strongly suggested by the fact that the aerial root in penetrating the soil assumes corresponding internal features, but what the factors are which exert the determining influence it has not been possible at present to investigate.

THE LEAF.

General.

The leaf exhibits varying external morphological features according to whether it is of juvenile or adult form. The juvenile form has a thin lamina with an outline varying from entire, or almost so, to serrate, or slightly notched, depending upon the degree of juvenility showing. The less juvenile the leaf, the more deeply notched is the margin. All juvenile forms show an absence of spines, which outgrowths characterise the adult leaf only. The latter form has a thick leathery lamina, is much darker green in colour than the juvenile leaf, and has a relatively deeply cut margin. The venation is recticulate.

Anatomy of the petiole.

The petiole in transverse section (Fig. 21) is cylindrical, with an average diameter of about 3 mm. The vascular system consists of one main central strand about 700 μ in diameter, and two small accessory ones, from 58 μ to 83 μ in diameter, situated in the cortex towards the adaxial side of the main strand.

The cortex is clearly demarcated into an inner region with large cells, ranging from 33 μ x 33 μ to 130 μ x 130 μ in diameter and containing starch grains, and an outer, consisting of three to four rows of small collenchymatous cells, from 25 μ x 25 μ to 33 μ x 33 μ.

The main strand of the vascular system leaves the stem as an arc, but after the departure of the bud trace the arc closes in so that the strand becomes cylindrical. The pith is small, 250μ x 330μ; in the centre the cells vary from 41.5μ x 41.5μ to 66.5μ x 66.5μ in diameter, and are thin walled; round the periphery, they are smaller, from 25μ x 25μ to 33μ x 33μ, and collenchymatous. Starch is stored in the pith.

The xylem is in a continuous ring, consisting of vessels and parenchyma cells. No cambium is normally present. Phloem is present to a considerable extent, and is surrounded by a pericycle one or two cells broad, and a well marked starch sheath. Pericyclic and phloem fibres are few, or absent altogether.

The accessory strands each consist of a central core of from four to six xylem elements, surrounded by phloem and a single layered pericycle, in which a few fibres are sometimes present. No starch sheath has been observed.

VEGETATIVE PROPAGATION OF THE PLANT.

REGENERATION FROM THE STEM.

General.

Propagation of this plant by means of stem cuttings is very easily effected. For the purpose of investigation various types of cuttings were made, as follows:-

Isolated apical portions of the stem, the cut being made through the nodes or internodes from the first downwards. Some of these were defoliated, others were inserted with their leaves on.

Isolated internodes, of various lengths down to ¼ inch; some of these were inverted.

Isolated nodes; these in some cases were defoliated.

A noticeable feature was the quickness with which foliated cuttings rooted compared to their defoliated neighbours.

Where any buds existed on the cutting, either terminal or axillary, shoot production was due simply to growth of one of these organs, and the only regenerated members were the roots. In the case of internodal cuttings, both buds and roots are regenerated. The buds always appear at the organic apical end of the cutting, no matter whether it is inserted normally or inverted, and precede the roots. The buds, of which a number are produced, appear in a ring above/

above the region of the vascular cambium; as a rule only one, or two, develop at the expense of the others. An attempt to see how small a piece of stem would regenerate new organs shewed that isolated pieces of stem ¼ inch long inserted in sand at a temperature of 75°F. could regenerate a number of buds. So far however, no roots have been induced on such pieces.

Whether or not still smaller pieces have the ability to produce adventitious growths cannot at present be stated, no cutting smaller than ¼ inch having been made.

The roots are usually produced in two opposite rows, and emerge either through the cut end or through the sides of the cutting at its basal (proximal) end.

Rooting of this plant can be induced on the stem simply by causing the latter to assume a horizontal position. A growth curvature ensues, bringing the distal regions of the stem into a vertical position, and in the region of curvature one or two roots are produced.

The shoot produced from the cutting sooner or later usually forms an adventitious root towards its basal end, above its point of union with the parent stem, suggesting that the vascular supply from the latter to the new growth is insufficient.

Healing of the wound.

This is brought about in the first place by the deposition of **suberin** on the cell walls of the parenchymatous tissue underlying the cut surface, a process which can be observed three days after insertion of the cutting, but becomes more obvious later. All experience suggests that this formation of suberin is due to the oxidation of the unsaturated fatty substances present in the cell sap, which rise to the surface of the wound, and condensing, give rise to a hard varnish like layer. This implies access to a free oxygen supply, a necessity which is suggested by the fact that cuttings inserted in moss litter kept saturated with water rotted, possibly due to the leaching away into the water of the fatty substances as they rise to the surface.

Soon after the formation of this protective layer meristematic activity commences in the underlying cells. The first observable change is the disappearance of the starch grains; after this a layer of cells arises, which are flattened in the plane parallel to the cut surface. These are densely protoplasmic and have large nuclei. Cell division takes place in this layer parallel to the cut sruface, cutting of cells to the outside which round off and develop suberised walls.

This layer is interrupted by the elements of the xylem; here, however, the cut ends of the vessels become/

become closed by the formation of closely packed cellular tissue, having the appearance of tyloses. Towards the ends of the vessels these cells have suberised walls, but towards the interior their cellulosic nature is unchanged. Thus a complete layer of protective tissue is formed across the wounded surface, so that after a week the cuts end may be completely blocked.

In the case of cuttings with two cut surfaces to heal, marked polarity is shown in that the production of the blocking material, in that activity begins earlier, and is more pronounced, at the proximal than at the distal end.

This difference is shown even in the suberisation of the cut surface prior to meristematic activity. Towards the proximal surface this process takes place so quickly that in some parts cells become suberised before the removal of their starch contents, so that little areas of tissue appear in which the starch grains appear completely enclosed in a suberin sheath. Towards the distal end, suberisation is much less marked, and the lack of starch grains in the cell layers beneath the surface suggests that the process has taken place at a much slower rate.

A more marked difference still is shown in the production of the blocking material at either end. At the distal end meristematic activity is immediately beneath the cut surface, and only one or two cell layers/

layers are cut off, whereas at the proximal end the activity is more deeply seated, and four or five layers of cells are produced. The deposits of suberin are likewise heavier at the proximal than at the distal end.

This blocking material produced at the cut surface is usually referred to as callus. In this case, however, where its cells become suberised, it is really of the nature of a cork layer, and the meristematic tissue giving rise to it is a cork cambium.

Initiation of the shoot.

A stem cutting after a fortnight in the propagating pit shows at its distal end a number of meristems, which appear in a ring above the vascular cambium, and finally emerge as adventitious buds. According to TAYLOR (19), these meristems have their origin in the vascular cambium. PRIESTLEY and SWINGLE (14), however, refer the initiation of these organs to the cork cambium underlying the cut surface, a few cells of which ceasing to cut off suberised cells become organised to constitute the bud primordum, this being the case no matter whether the parent organ is stem or root. They criticise TAYLOR, attributing to him the statement that the buds in the fleshy roots of Acanthus montanus arise in the cambium. Reference to TAYLOR's paper, however, will show that he referred the origin of the buds in the roots/

roots to the pericycle, and in the stem only to the cambium, the latter statement seemingly having been mistaken by PRIESTLEY and SWINGLE for the former.

The process of bud initiation as observed during the present investigation however, goes to show that it is erroneous to attribute the tissue constituting the young bud to any distinct tissue of the parent organ. In the stem, it is true, the cells which give rise to bud primordia may have originally been cut off from a cambium; this layer, however, simply gives rise to a mass of uniform undifferentiated tissue, towards the outside of which the bud initials are laid down. Again, the peripheral cell layers of this tissue may be functioning as a cork cambium, and some of these cells may contribute to the tissue of the young bud; but they only contribute to it, they do not give rise to it. The tissue comprising the shoot primordium, even before it has its external surface thrown out into leaf papillae is derived from a mass of tissue lying between, and including, the cork cambium and the stelar tissues of the parent organ, the appearance of having come from the latter being simply due to the affinity of the new growths for the conducting system, to which they ultimately become united.

Initiation of the root.

According to TAYLOR the root has its origin in the/

the cambium, but as this statement is not borne out by the present investigation, the subject will be considered in some detail.

VAN TIEGHEM and DOULIOT (21) came to the general conclusion that a definite correlation existed between the precocity of an endogenous member and the seat of its origin, and found that in a Dicotyledenous stem adventitious growths were pericyclic in origin till, by ageing of the stem and the consequent alteration of the pericycle (by becoming thick walled, or by producing a periderm), it lost its rhizogenous faculty. When this occurred, the seat of root production changed to more deeply lying tissues, occurring first in the external primary phloem parenchyma, then in the internal phloem parenchyma, or in the secondary phloem parenchyma, derived from the vascular cambium depending upon the degree of lateness of initiation of the root primordium compared to the degree of maturity of the stem. In general, then, in young stems the roots are pericyclic; in older stems, after the formation of a vascular cambium the latter gives rise to the adventitious growths. This point has later been emphasised by PRIESTLY and SWINGLE, who offer a physiological explanation based on the necessity of the proximity of the new organ to tissue continuously bathed in sap from the conducting system of the parent. In a young stem the tissues surrounding the xylem lack intercellular spaces and are well supplied with/

with sap; as the stem ages and cambial activity increases the width of the stelar tissues air spaces appear between the cells in the outer ends of the rays, but the more newly formed cells in the neighbourhood of the cambium are more compressed, and have smaller intercellular spaces which are more easily injected with sap. Hence the roots arise in this region.

During the course of the present investigation, however, no roots having their origin in the cambium have been observed, no matter the age of the cutting used. All roots in the stem of Acanthus are pericyclic in origin, with the exception of those arising on a stem in which elaboration of stelar tissues is still in an embryonic state, such as in the first node or internode, in which case they must be attributed to the procambium. The fact that in this plant the old as well as the young stem produces these adventitious growths from the pericycle may possibly be attributed to the fact that no matter the age of the stem, the starch sheath is a continuous, flattened layer, with no intercellular spaces, and hence may act to a certain extent as a hindrance to the radial diffusion of sap outwards from the stele. This condition would ensure an easy and constant supply of liquid for the pericycle beneath it, and the latter retaining its primary characteristics by neither becoming thick walled nor the seat of periderm formation, has in no way its rhizogenous faculties impaired./

impaired.

That this persistent property of the pericycle is correlated with lack of secondary thickening is clear, an interesting fact if it is allowed that the production of aerial roots on the normal plant is due to the same cause.

The first sign of activity in the pericycle is seen when some of the cells of that tissue become densely protoplasmic and their nuclei become large. These cells then elongate radially and divide in the tangential plane. (Fig.22) All of the two or three layers of the pericycle divide, but according to VAN TIEGHAM and DOULIOT (21) it is the outermost layer only which, by two tangential divisions, gives rise to the three meristematic regions of the young root. The innermost pericyclic layer or layers gives rise to the base of the central cylinder only. The differentiation of the young root into dermatogen, periblem and plerome is due to differential growth of the cells composing it. It is seen that when activity in the pericycle commences, the activity spreads from a small group of cells both in radial directions to the cells on either side. There is thus from the beginning in the pericyclic arc a medium group of cells which is a few cell divisions ahead of those on either side; this more pronounced activity of the central cell is maintained, so that the adventitious growth becomes recognisable as a dome, with/

with its base on the internal pericyclic layers and its apex pushing out the endodermis. (Fig. 23). Segmentation of the central core of the dome which at first took place entirely in the tangential plane soon takes place obliquely, and finally in the radial plane. This latter type of division takes place mainly towards the exterior of the central core, the more deeply situated cells not being so active. In this way the central cylinder becomes demarcated from the cortex, and itself becomes differentiated into procambium and pith.

The cortex is derived from the central layer of cells formed by the division of the outermost pericyclic layer, and is soon distinct from the central cylinder, except at the apex, where the cell layers merge and no definite stratification can be seen. According to LEMAIRE (11) this occurs in root apices where the central cylinder and cortex are differentiating at the same time and when cell divisions are equally intense in both regions, a phenomenon attributed by FLAHAULT (4) to the bulkiness of the root tip. This author, working with radicles, came to the conclusion that in bulky roots confusion of tissues at the apex followed the intensity of cellular division occurring in these types, whereas in smaller roots the primary meristems are quite distinct.

The piliferous layer and root cap have their common origin in the outermost of the three layers cut off by the outermost pericyclic layer.

The endodermis covering the root initial divides radially to form a sheath over the apex of the latter. This finally becomes detached. According to VAN TIEGHAM and DOU~~B~~LIOT (21) it is digestive in Acanthus, but this statement is not supported by observation. No evidence of cells in process of digestion or of starch grains in process of erosion can be seen. The appearance of the cortical cells, on the other hand, strongly suggest that the root forces its way out mechanically through this tissue by pressure alone.

REGENERATION FROM THE ROOT.

This has been described by TAYLOR (19), who refers both buds and roots to a pericyclic origin. The subject was not investigated in any detail by the present writer; attention is drawn to the fact, however, that the root produced on a root cutting of Acanthus is simply a branch root, and shows the typical construction of such, thus differing from the adventitious root of the stem cutting. As to the pericyclic origin of the buds, the same argument holds in this case also as in that of the latter type of cutting.

An attempt to discover from how small cuttings adventitious growths could be produced showed that isolated pieces of root measuring about $\frac{1}{4}$ inch long and $\frac{1}{8}$ inch broad could produce buds. No roots so far have been produced. In pieces smaller than this no healing was effected, and the cuttings rotted.

REGENERATION FROM THE LEAF.

General.

Isolated leaves grown under the same conditions as already described for the stem after a week regenerate a root system from the cut end of the petiole. The roots are initiated in the vascular system, of which the main strand may produce one large one, or two small ones, and each accessory strand may produce one. Thus from one to four roots may be formed. After it has produced a branch root system a swelling appears on one side at the top of each root, just below its point of emergence from the petiole. This swelling increases in size, and soon gives rise to one or more small buds. Usually only one develops into a shoot, or if more than one develops, one is much stronger than the other, or others. The shoot, after it has a few leaves expanded, generally produces an adventitious root of its own. (Fig.24). After this, an abcission layer forms across the base of the petiole, and the leaf withers away. Fig. 25 shows a leaf cutting one year old, in which the remains of the petiole can still be seen attached to the individual to which it gave rise. Even after the shoot has been produced the swelling from which it arose may continue growth, attaining in some cases the size of a pea. The other buds may be induced into growth by removal of this shoot, and an interesting feature is the/

the haphazard manner in which the buds are formed over the surface. No polarity such as is characteristic of root or shoot appears in this organ. Fig.26 shows one such intumescence one year old, and isolated from its aerial parts. It will be seen that other buds are starting growth, and that they occur indiscriminately practically all over the surface, showing that the factors determining the position of adventitious growths in root and shoot cuttings are absent here. A very strong resemblance between this organ and the tuberous swelling produced by the leaf cutting of Zamioculcas is seen. In the latter, no polarity occurs, so that no matter in which position the tuber is planted, a shoot is produced from the top.

For such an organ the term protocorm has been suggested, but in view of the discussion to follow later, the matter will not be pursued further here.

The first leaves produced on the shoot show juvenile characters. If propagation be now effected from one of these juvenile forms, the leaves produced in turn from the new shoot show still further development of juvenile characters, notably in a great reduction in size and in simplicity in outline. In this way, after two generations of cuttings a leaf was produced which was barely one inch long when full grown and had an entirely simple outline except for a notch at the apex.

Figs.27-29 show an adult leaf, a juvenile produced/

produced from an adult leaf cutting, and a juvenile leaf from a juvenile leaf cutting.

The leaf of Acanthus shows remarkable plasticity in its regenerative powers. Thus a leaf severed in two by a cut at right angles to the midrib through the centre of the leaf will produce adventitious roots from the base of the midrib in each half; or if the leaf be split longtitudinally down through the centre of the midrib and petiole, and each half planted horizontally, adventitious roots are produced in two or three places along the midrib, as well as from the end of the petiole. (Fig.30).

Attempts to regenerate new growths from the isolated petiole have met with repeated failure. The same fate attended attempts to produce buds on the petiole after the root system was established. This was done by planting the leaf, and then, after the roots were produced, removing the blade, leaving only the petiole with roots attached. In most cases, however, all growth stopped; or if growth did proceed long enough, in which case it was very slow, for bud production, these were found in the normal manner at the top of the root. In no case did they make any further growth, however, after their appearance.

Healing of the cut surface.

This is essentially the same process as that described in stem regeneration, and therefore will not/

not be further enlarged upon. Healing takes place in from three to five days.

Initiation of the root.

The root arises a little way up from the cut end of the petiole, and grows downward and out through the base. In origin it is pericyclic, and its initiation is the same as that described in the stem-borne root. The young root is usually of such a size that its base covers half of the vascular ring of the petiole strand. (Fig.35) Pericyclic activity is not confined, however, to the arc alone which gives rise to root but extends considerably much further round the cylinder on either side. In the case where two roots arise from the main strand, the pericycle becomes, of course, meristematic the whole way round.

Moreover, activity is not confined to the pericycle. From this region it spreads inwards into the phloem and panenchyma of the xylem ring. This tissue, which is simply the unlignified part of the procambium divides rapidly and in many cases displaces some of the xylem elements laterally and radially. A cambium now makes its appearance, being more pronounced in the region of the petiole immediately above that in which the root is produced. This cambium proceeds to form new xylem elements which considerably increase the breadth of the xylem ring, and, differentiating downwards, enter into the root. In one case an increase in breadth from 185μ to 450μ - 500μ was noted.

A/

A longtitudinal section through a petiole from which a root has arisen shows that the latter has emerged obliquely to the petiole strand. Over the proximal front of the root the blocking material from the cut surface has formed a dome, and activity at the base of the pith has caused a slight dilation of that tissue radially, resulting in a distension of the vascular ring. This tissue, due to the above fact, and also to the activity of its parenchyma, has lost its compactness at the base of the petiole.

The root from the accessory strand is likewise pericyclic in origin.

Formation of the swelling.

A root borne on a petiole has, when about one inch long, the same external appearance as a stem-borne root in that so sign of any swelling can be seen. A series of transverse sections cut from the tip upwards shows the same sequence of events as in the normal root, until the region just below the petiolar base is reached. The three xylem strands at the distal end, (Fig.31) become augmented as the root increases in diameter towards the proximal end, and the construction of the stele up to a certain point corresponds in every way with a stem borne root, with the exception that the alteration in outline from cylindrical to arc-shaped, by the greater increase in breadth of one of the interfascicular portions of the ring/

ring, and the indentation of the cambium, becomes accentuated, This is shown in Figs. 31, 32.

Concurrently with this, the stele becomes compressed on two sides, resulting in the reduction of the pith to a strip of tissue only one or two cells broad. (Fig. 31), with on one side a row of three or four xylem strands and on the other a delimiting tissue consisting only of endodermis pericycle and cambium, with a xylem strand only at each end.

Above this region signs of activity are seen in the stelar tissues, especially at one of the narrower ends; at this point (p., Fig. 31) slight dilation of the pith has occurred, a condition which has been accompanied by activity in the cambium, which has become more pronounced round that end. Higher still, changes have occurred which have no parallel in the development of the stem-borne root. The activated cambium at the dilated end of the stele has ruptured between the two xylem strands, and the broken ends, growing out almost at right angles to their previous path, have entered the cortex. This development is shown in Figs. 31 – 33

The penetration of the cortex by the cambium extends almost to the periphery, and the cortical tissue between the two strands becomes densely protoplasmic and shows signs of cell division. This is more pronounced in a group of cells towards the periphery (b., Fig. 32).

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The region above this shows a still more advanced stage of central activity in that a sheet of meristematic tissue, roughly triangular, (c.Fig.31) extends from the stele outwards. In this tissue the identity of the cambium as such has practically disappeared, the whole region having a more or less uniform appearance. Close examination, however, reveals the existence of a few tracheids in paths corresponding to those in which the cambium would lie. (tr.Figs.31,32). Further, the densely protoplasmic area (b.Fig.32) noted previously is more pronounced, both in density and extent, and activity in the pith with its distending effect on the stelar system has caused a rupture of the cambium at one or two points between some of the xylem strands, (r.Fig.31), the cambium in the indented point of the ring, however, remaining complete. The cambium itself is in an active state of division, a condition more marked in the fragments on the outside of the arc than in the unbroken part on the opposite side. Passing higher, cambial activity has increased especially at the narrow end of the stele opposite to that in which it was noted lower down the root. At this end a rupture has been effected, but no intrusion of the cortex has taken place; cell division of the cambium here takes place in situ, and merely increases the width of the tissue. Also, activity of the pith has resulted in the displacement of the primary xylem rays, which have become noticeable merely/

merely as scattered lignified elements. (pr. x. Figs.31-32)

The final disintegration of the primary structure occurs at the base of the root, just above the previously described region. Here the vascular cambium has united along the outside of the arc, and has curved round that narrow end of the pith where activity was last noted to be most intense. (Figs.31,32) Activity at the opposite end, i.e. where lower down the cambium intruded into the cortex, has not had the same result here, the whole of the cambium functioning in the same manner, which is the formation of secondary stelar tissue. The remaining tissues, cortex and pith, are also in a state of activity, especially the latter.

The outline of the stele, at this stage is characterised by its incompleteness, due to its formation from the cambium from the outside of the arc, and round one end only, it is hook shaped, (Figs.31,32), and it is worthy of note that the missing part of the system is on that side from which lower down, the cambium invaded the cortex. Also, the stelar tissue is entirely secondary, the primary body being in a state of complete disintegration, and consisting only of a few scattered vessels (pr.x. Figs.33,34) which lie across the pith obliquely, facing across, or towards, the gap in the secondary body.

Leaving now the root, transverse sections through the base of the petiole show that the vascular system here/

here is dual in origin, in that the xylem being matured from the petiolar cambium is differentiating downwards round the true root system. Moreover, the secondary tissue of the root is in continuity with this secondary petiolar tissue, and a gap (Fig.36) which has been formed at the base of the petiole strand due to dilation of the pith is in continuity with the the gap in the secondary root tissue.

It will be clear then that the arc shaped outline of the root stele towards its proximal end is due to its mode of union with the petiolar conducting system, especially if it is borne in mind that originally only a part of the continuous ring of the main strand, i.e., an arc, took part in the elaboration of new tissue.

These series of changes have been described as taking place from the distal to the proximal end of the root, but obviously, the order in which they would take place in the plant would be in the reverse diraction, secondary changes proceeding from the older to the younger parts. By considering them in the manner just adopted, however, a reconstructed account is made possible of the sequence of events in each particular region, as within limits, the stage at present reached by the more distal parts will reflect to some extent the order of development of the more proximal.

Considering now the changes that have occurred

as/

as a whole, the most salient features are (1) the beginning of secondary changes in the conducting system at a time when in normal root development the primary body is not complete, a series of changes having as their necessary precursor the activation of the vascular cambium, and (2) the destruction of the primary body by dilation of the pith.

The fact must be taken into account here that the region under consideration is at the junction of petiole and root, a region in which the materials drifting downwards from the leaf will meet the upwardly moving solutes from the root. An accumulation of material will follow, which having no outlet as when a piece of stem, however small, and with a bud, or the potentiality of forming a bud, is inserted between root and leaf, will be productive of peculiar physiological conditions with accompanying abnormal anatomical requirements, principal among which will be a gradually increasing pressure on the surrounding tissues and the demand for an augmented conducting system. Now, it was seen that the earlier changes which occurred were increased activity in the pith and the commencement of activity of the vascular cambium; the former, ultimately resulting in the complete disintegration of the primary conducting system, makes increase in that body impossible, and the premature activation of the cambium, which if not already resulting as a response to the physiological conditions/

conditions prevailing, hence would become a necessity. The ensuing development of secondary vascular tissue will follow the same course down to the petiolar base as in the normal root, with the exception perhaps that the dilation of the pith accentuates the gap in the stele, but it is evident from the material examined that pith dilation is unable to do any more than this, due to the resistance offered by the vascular system of the petiole. Once outside the petiole, however, conditions are altered. The slender system of the young root will be unable to resist the pressure exerted by the increasing pith, especially when it is taken into account that this latter tissue commences excessive dilation when the state of the root still consists of isolated xylem strands. Under these circumstances rupture of the cambium is easily effected, with its concomitant effect upon the outline of the stele. Now the root emerged from the petiole with an arc shaped conducting system, into the interior of which arc the cambium on that side had penetrated; as obviously this part of the cambium ring can stand more outward displacement than the other parts before rupturing, the break occurs elsewhere, and in each case examined has been at once of the two narrow ends of the now compressed stele. Hence the secondary tissue develops with a gap which is roughly at right angles to the indentation on the primary body lower down the root.

Given/

Given now a vascular system, incomplete, but with the potentiality of forming a complete cylinder, differentiating downwards round an expanding pith, the tendency will be for new cells formed from both of these tissues to be laid down, at the gap, in a direction resultant from two opposing forces - that of the cambium to form a cylinder and enclose the pith, and the tendency of the latter to keep the cambial cylinder incomplete. The new pith tissue continues to distend the stele, but mainly is proliferated across the gap and towards the cortex; in doing so, it displaces and carries out with it the primary xylem and accompanying cambium which were situated at that corner. The result for the remainder of the cambium is that the end on the other side of the gap continues its course into the cortex (Fig.31,cam$_1$.): while the other end (Fig.31,cam$_2$) continues its normal path round the side of the stele, but in doing so encloses the cambium (Fig.31,cam$_3$) situated originally across the mouth of the arc. Due to these phenomena the stele, therefore becomes hook shaped. That the development of newly formed tissue in one direction more than another has also a physiological basis is seen when it is taken into account that round the outside of the young secondary stelar system, though no endodermis may be found, there occurs a fatty deposit. This will hinder the passage of material into the cortex from the conducting system on that side of the root/

root, but on the other wise, where no such barrier exists, diffusion will rapidly take place in that direction, and will be conducive to the intence meristematic activity which is seen there.

Considering now the region where the first signs of abnormal changes were observed in passing upwards from the tip, it was noticed that here the cambium had penetrated the cortex in which tissue or tracheidal system was being formed. This condition occurs at the bottom of the gap in the cambium, a level down to which the formation of secondary tissue proceeding higher up has not descended. In all probability the rupture in the cambium would occur over the whole length of the gap at the same time, and occurred in both the longtitudinal and transverse planes; hence the cambium would enter the cortex from the proximal and distal ends more or less simultaneously. The cambium above the gap forms secondary tissue, while the portion below, active, but not in a sufficiently advanced state to form secondary tissue, forms a tracheidal system.

The net result of all this reorganisation of tissue, then, is the escape of material from the material from the tissues of the stele across a gap formed by a rupture in that tissue, with the resulting increase in cellular activity around and outside that gap, the whole acting as a safety valve to relieve the pressure at that region. The continuance of this activity/

activity results in the alteration of the outline of the root, the newly forming tissue finally growing out as the swelling which, it was seen, gives rise to the adventitious buds.

That this swelling is produced from internal root tissue is obvious from the foregoing account, and is not a growth of callus, as stated by TAYLOR (19).

Initiation of the bud.

It was noticed that in the region where the cambium had penetrated the cortex with the resulting differentiation of tracheids, that the cortical tissue surrounding these had become meristematic (Fig.32), a condition more pronounced in a small group of cells between the tracheidal system and the periphery (Fig.32, b.). Closer examination of this group reveals the fact that the cell divisions are occurring according to a definite plan.

Fig. 38 shows that the plane of division in the peripheral cells is anticlinal, while in the deeper seated tissue cell divisions are occurring in all planes, a condition clearly comparable to that of the growing apex of a shoot, where the outermost tissue, increasing in surface only, and overlying a tissue which increases in bulk, finally becomes thrown into folds.

Precisely the same development takes place in the cellular group under consideration, so that it soon becomes/

becomes organised into a young bud, with two leaf papillae surrounding an apical growing point and a differentiating procambial strand.

This bud is found on the side of the intumescence, which still is increasing in size, and with the vascular system of which the procambium of the bud is in continuity. A longtitudinal section (Fig. 39) of a young stage of bud development shows that the procambium is united with the vascular system of the root on the proximal and distal sides of the gap; later on, however, when the swelling has attained a more considerable size, it is seen that the procambium has differentiated backwards into the pericycle of the petiole, so that at this stage the axes of this tissue and of the petiolar strand are in one straight line. At this period of development the bud grows downwards into the soil, and only later does it bend round and grow upwards. This is usually only the case with the first shoot produced, the later ones as a rule growing straight outwards or upwards. This is possibly due to the fact that the first is being differentiated at the same time as the swelling is being formed, and is pushed downwards by the enlarging mass of tissue behind it, the later buds being initiated when the growth of the intumescence has slowed down.

The anatomy of theswollen region itself at this stage shows some interesting features, and deserves consideration in further detail.

It/

It was seen that in the region above that in which the bud initial was formed that rapid enlargement of the pith was concurrent with the formation of a hook shaped sheet of secondary stelar tissue. The rapidly enlarging pith gives rise to the parenchyma of the swelling and is eventually traversed by the cambium, giving a continuous ring of conducting tissue.

This tissue in the lateral outgrowth differs from that in the root, however, in that in the latter the xylem is in a continuous mass of radiating rows, whereas in the former it consists of discreet strands. (Fig. 42vi). All this tissue being produced from a cambium, the maturation of lignified elements is in the centrifugal direction; the phloem, moreover is collateral with the xylem. In other words, these discreet strands are typical stem strands, and it is to them, in fact, that the conducting system of the shoot is united, In function, therefore, they must be considered as primary tissue, although they are derived from a cambium which at the same time is differentiating secondary tissue in the same transverse plane.

That this secondary tissue belongs to the root proper is seen by a series of transverse sections cut from this region downwards. (Fig~~s~~.42) (The construction of the root stele beneath the swelling shows the peculiar feature of having a transverse section (Fig. 43) in which all the xylem is maturing in/

in the centrifugal direction, and is collateral with the phloem instead of alternating with it. The gap in the system has not yet closed in, and a piece of cambium enclosed as previously described is forming inverted vascular tissue round the periphery of the pith; in this case phloem as well as xylem has been seen).

It is obvious now that the vascular system of the swollen outgrowth is interpolated between true root conducting system and that of the young shoot. With the latter it is connected directly, the conducting tissue simply contracting and running out into the stem (Fig.42). The union between the conducting elements of the swelling and those of the root is not continuous throughout its length, complete continuity only occurring where the elements in the outgrowth run out into the shoot. Above and below this a gap occurs. Where the conducting system of root, swelling and stem are continuous the latter two are oblique, or at right angles to the former, and in this region a considerable number of tracheids run longtitudinal across the interior of the conducting system (Fig.42) These are probably only temporary in function, playing no part in the construction of the permanent tissues formed.

The union between other buds is precisely the same, their own proper vascular system uniting with the system of discreet strands in the intumescence.

An/

An unusual feature is seen in a root produced from a petiole which has been split longtitudinally, as previously described. The base of the root abuts on the two cut ends of the half ring of the petiolar strand, from each of which a separate vascular system grows down into the root, thus providing the latter with two quite distinct steles. (Figs.45,46).

After the complete union between shoot and root has been effected, and the shoot has expanded its leaves, an abciss layer is formed across the base of the petiole, proceeding from the cortex inwards (Fig. 41). When this layer is complete the leaf withers away.

Recapitulating now the outstanding anatomical features of this proximal region of the root, it is seen that here the primary system of the root disappears, and is replaced by tissue which matures centrifugally and is in continuation with the stelar tissue differentiating downwards from the base of the petiole. On one side of the swelling the tissue is continuous: on the other it consists of discreet collateral strands, i.e. here all root anatomy is entirely lost, despite the fact that originally the structure was undoubtedly that of a normal root. In other words, this region is one in which true root structure becomes transformed into true stem structure.

Compare/

Compare this now with the transitional features between stem and root in the normal plant, i.e., in the hypocotyl of the seedling.

This was seen to consist of -

1. The reduction and final disappearance of the protoxylem of the root passing upwards to the base.
2. The continuation downwards of the cotyledonary traces as the metaxylem of the root; in the hypocotyl this tissue completely replacing the root poles.
3. The centrifugal maturation of these elements in the root.

The resemblance between these structural changes and the essential stages of bud production as described is obvious. Objection may be taken to the fact that in the one case, that of the seedling, all the tissues under consideration are primary, whereas in the other, secondary tissues enter into the structure of the body. That this is of no significance however, is seen by the fact that at one stage the primary body and the secondary are both derived from the same tissue, namely, the cambium, and hence the distinction between the two types as far as their origin is concerned cannot be of any value.

Looked upon from the structural point of view, these new tissues form the foundation of the stelar system of the new organs regenerated, and therefore in function must be regarded as primary.

It/

It is not unreasonable, therefore, to look upon this swollen portion of the root as a region whose internal construction is analogous to that of the hypocotyl, both functioning as regions in which the alternating xylem and phloem tracts of the root become the collateral strands of the stem, and in which maturation of the first lignified elements of the primary body alters in direction from centripetal to centrifugal.

As far as its regenerative properties are concerned, this intumescence also suggests a region intermediate between stem and root, notably in the lack of polarity shown in the production of adventitious buds. Such lack of polarity is the characteristic feature of the tuberous swelling regenerated from the leaf of Zamioculcas, one of the Aroideae, an organ for which as previously mentioned, the term 'protocorm' has been suggested.

Now in the seedlings of this family the tubers have their origin in the hypocotyl, a fact which appears significant if considered along with the case of Acanthus.

The question of polarity in plants in general may be considered thus:- On the shoot the adventitious buds are usually produced on the distal end and the roots on the proximal, while on the root the buds are usually on the proximal and the roots on the distal. In the case of the swollen structure on the Acanthus/

Acanthus root, however, buds are produced freely all over the surface, indicating that in this region the factors causing the difference in polarity between root and shoot counteract one another.

If this region really shows an anology, then, with the hypocotyl, it would be expected that in this latter part of the axis a similar lack of polarity should be seen in the regeneration of new growths.

Attempts to regenerate new growths from the hypocotyl in Acanthus have so far not been made, and therefore it is not possible to discuss the problem further in the case of this plant, but PRIESTLEY and SWINGLE (14) have shown that in plants in general adventitious buds occur more freely on the root than on the hypocotyl, and more freely on the hypocotyl than on the epicotyl, suggesting again that from the point of view of regeneration the hypocotyl is intermediate between shoot and root.

Owing to lack of more evidence on this subject this discussion must be terminated with the suggestion that in propagation from the leaf of Acanthus not only root and shoot, but a region resembling hypocotyl, are regenerated - in other words, a return to a seedling-like condition has been effected.

INFLUENCE OF THE INTERNAL ORGANISATION OF THE PARENT ON THAT OF THE REGENERATED ORGAN.

The development of the bud on the root as seen in leaf propagation shows how the initiation of the latter resulted from a series of changes of organisation of the parent axis, while the study of the ontogeny of the root shows how the organisation of the latter is influenced by external environmental factors.

It now remains to show how the organisation of the parent axis exerts an influence on the organisation of tissues in the regenerated organ. This is seen most clearly in a general survey of the phenomenon of root production.

The anatomical features of the radicle, branch root, and adventitious root have been already described. These types will now be compared, as in doing so the differences in their construction throw some light upon the internal environmental factors influencing their stelar morphology.

It has been seen that in the seedling the adventitious hypocotylar roots possessed a larger number of root poles than both radicle and branch roots. The difference between the number of root poles in the branch root and in the hypocotylar root has been shown by DODEL (5) in Phaseolus, which is attributed by this author to the fact that the branch root, arising from one single strand of the parent organ, derives its conducting system from that strand only,/

only, whereas the hypocotylar root, arising from a double cotyledonary trace, derives its conducting system from two strands; hence the possession of a larger number of root poles than the branch root. The same interpretation may be placed on the phenomena observed in the present case, except that here the hypocotylar roots, though situated certainly on cotyledonary traces, are in the planes of the first pair of foliage leaves, and therefore in the quadrants into which the foliar traces differentiate downwards. It will be remembered that neither hypocotyl nor epicotyl elongate, and so the foliar traces are inserted on the axis immediately above the cotyledonary traces. In view of this fact then, it is difficult to say whether the traces to which the roots are connected are really foliar or cotyledonary, but their orientation rather suggests that they are connected with the leaf strands.

Whichever be the case, the fact that they are connected with a larger vascular supply than the branch root is reflected in their correspondingly more elaborate vascular structure than the latter.

BOIRIVANT (2), examining several genera belonging to various families, and comparing adventitious roots with radicles and branch roots, found that adventitious roots anatomically more nearly resembled radicles than branch roots. The same is the case here, if size of root and dimension of tissues/

tissues be taken into consideration, notwithstanding the fact that the radicle and the branch root both possess only two root poles, while the adventitious root has three or more. It can be shown, however, that the possession of either two or more root poles behind the tip does not necessarily depend entirely upon the size of the root but upon the nature of its origin, e.g. the first adventitious roots on the seedling have three or more xylem strands, and these roots are frequently of smaller diameter than the radicle with its two strands.

Apart from this fact, if the behaviour of the tissues be considered from the point of view of their subsequent development, the adventitious root shows more similarity to the radicle than to the branch root, especially in the dilation and non-lignification of the pith.

Similarity in size is seen, however, between adventitious root and radicle, in these former organs when regenerated from stem cuttings.

The relationship between the anatomy of the root and the conducting system of the parent axis has been referred to. This is brought out more clearly by a study of the effect of the age of the stem on the adventitious root arising from it.

Roots arising from a young piece of stem before the formation of secondary xylem has commenced, i.e., when the conducting system still consists of discreet strands/

strands arise in the majority of cases opposite one of these strands.

That this is not always the case is shown by Fig. 16, which shows a root arising between two strands. In general, it may be said that the root in origin may be fascicular or interfascicular, but with a distinct preference for the former position, - i.e. opposite the primary strand. According to LEMAIRE (11) the ability of a root to arise in either the fascicular or interfascicular regions of the vascular ring is due to the continuity of the pericycle, while the association between adventitious roots and primary rays has been emphasised by PRIESTLEY and SWINGLE (14).

In such cases, when the root arises from a tissue which has not completed full primary growth, the dilating effect of the latter exerts an influence on the basal regions of the former, as previously shown. Moreover, that the configuration of the stelar system of the root at its point of insertion on the stem is influenced by configuration of the stele of the latter, is shown by roots arising from a nodal cutting. Fig. 17 shows such a cutting, in which it is seen that the root has arisen from the leaf trace, and one of the necessary strands has entered the root and formed an isolated strand. On an older stem, however, as previously shown, where the tissues are mature, the configuration of the root tissues at the point of insertion are perfectly normal, while the/

the case of the root with the double vascular system produced from a petiole cut longtitudinally provides a further striking case of the influence of the anatomy of the parent on that of the regenerated organ.

Influence of the leaf on root production.

It was pointed out previously that foliated cuttings rooted much more quickly than defoliated ones; this, taken together with the fact that in leaf propagation removal of the blade from the petiole even after establishment of the root causes cessation of further growth suggests that the possession of a photosynthetic area is a valuable, if not necessary, asset. The facts as they appear from anatomical considerations also support this view - notably in the affinity of the root for the primary ray of the stem, which, as was seen, is the downward prolongation of the foliar trace. Attention to such a phenomenon has been previously drawn by VAN der LEK (22), who, working with hardwood cuttings, showed that the root initials occurred opposite the vascular strands on the stem, while LOEB (12) in Bryophyllum showed the correlation between the leaf and the production of adventitious roots.

PARKER and SAMSON (13) showed in some grasses that leafage removal resulted in a poorly developed root structure, while BOND (3) has shown in Vicia and Phaseolus seedlings that decotylation resulted in a more poorly developed root than in normal seedlings.

It would appear therefore, that the presence of the leaf is a factor governing root production and in view of such a fact it is doubtful if the common horticultural practice of removing some of the leaves prior/

prior to inserting the cuttings is a sound one. The current idea appears to be that leafage removal will reduce transpiration and hence help to maintain the turgidity necessary for growth; but provided that this difficulty can be overcome by providing the cuttings with an atmosphere sufficiently saturated to check transpiration, such as is the practice in the ROYAL BOTANIC GARDEN, EDINBURGH, under the aegis of Mr.L.B.STEWART, whose success as a propagator is known, there is no doubt that the propagation of a plant vegetatively has a better chance of success if the cutting be made with its leaf area entire.

CONCLUSIONS.

The results of these investigations show that in the first place, to understand fully the phenomena associated with the production of adventitious growths the normal ontogenetic processes of the plant must be taken into consideration.

It is also shown that the structure of the regenerated organ is influenced by two sets of factors - (1) The internal construction of the parent at its moment of initiation, and (2) the external environmental factors into which it emerges. Further, comparison between the different types of roots shows that the adventitious root produced from a stem cutting shows more resemblance to the radicle than it does to the branch root.

It has also been suggested that in leaf propagation a return to a seedling-like condition has been effected, in that not only root and shoot, but also a transitional region between the two analogous to the hypocotyl, have been regenerated.

Finally the experience of other workers taken together with the facts as elucidated for Acanthus show that there is an intimate association between the leaf and the production of roots, and that the presence of a leaf area on the cutting is desirable as promoting the latter phenomenon.

SUMMARY.

1. The normal anatomy of Acanthus montanus is that of a herbaceous plant. This implies a lack of secondary tissue production, and as the plant does not show the herbaceous habit, but is an evergreen perennial, the necessity for an increase in conducting tissue is supplied by the production of aerial adventitious roots.

2. The poor development of secondary tissue is due to non activity of the cambium, and correlated with this is the production of the adventitious roots from the pericycle, no matter the age of the stem bearing them.

3. The adventitious soil and aerial roots are initiated in the same manner, and constructed in the same way, but changes in organisation follow their emergence into the different environments of soil and air, changes which can be traced to the effect of environment in the growing apex. The soil root has a centralised conducting system and the aerial root a more peripheral one; the latter changing into the former on entry into the soil.

4. Regeneration can be effected from root, stem, or leaf. Adventitious roots are pericyclic in origin; adventitious shoots arise from a mass of meristematic tissue between the conducting system of the parent and the peripheral tissues.

5./

5. This is most clearly seen in regeneration from the leaf, in which bud production occurs on a swelling produced at the top of the adventitious root.

6. In this region the primary conducting system of the root is completely disintegrated and is replaced by prematurely former secondary tissue. This region is shown to have an anology to the transition from root to stem in the hypocotyl of the seedling.

7. The leaves on regenerated shoots show varying degrees of juvenility.

8. The anatomy of the regenerated organ is shown to be influenced by the anatomy of the parent at the point of union between the two.

9. A correlation exists between the presence or absence of leaves and the facility with which adventitious roots are produced. The latter arise mostly in proximity to the primary rays of the stem, which are the downward prolongation of the leaf traces.

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ILLUSTRATIONS.

Fig.1. Seedling of Acanthus mollis (actual size): one cotyledon has been removed.

cot. - cotyledon.
hyp. - hypocotyl.
ad.rt. - adventitious root.

Fig.2. Transition from root to stem anatomy in seedling.

p.x. - protoxylem.
m.x. - metaxylem.
c.t. - cotyledonary trace.
l.t.i. - trace of first foliage leaf.
l.t.2. - trace of second foliage leaf.
ad.rt. - adventitious root.

i. Behind root tip.
ii-v. Decrease in protoxylem and increase in metaxylem, passing upwards.
vi. In hypocotyl.
Vii. Intrusion of cotyledonary traces.
viii.and ix. Above hypocotyl.

Fig.3. T.S. first internode (x 26).

f.t.p. - foliar trace from leaf papilla.
f.t.l. - foliar trace from youngest expanded leaves.

Fig. 4. - T.S. fourth internode (x 16).

Fig. 5. - T.S. node (x 16).

pt.st. - main strand of petiole.
acc.st.- accessory strand.

Fig. 6. Nodal plexus.

pt.st. - main strand of petiole.
acc.st.- accessory strand.

Fig. 7. T.S. soil root behind tip (x 26).

Fig. 8. T.S. root with seven archs (x 26).

Fig. 9. T.S. older soil root. (x 16).

Fig.10. Development of adventitious soil root.

i.- iv. Stages common to all roots.
v.-vii. Development after stage iv.of normal root.
viii.-xiii. Anomalous development, after stage iv., of a root whose stelar system becomes disintegrated.

The endodermis is represented by a continuous line, the cambium by a dotted line; lignified tissue black, remainder of stele cross-hatched.
The isolated strand which separates off in xi, xii, and xiii is probably derived from an accessory strand of the petiole. (cf. Fig.17)

Fig.11. T.S. root before rupture of cambium (x 320)

Fig.12. Rupture of cambium in root (x 320)

Fig.13. T.S. root with disintegrated stele (x 26)

Fig.14. Re-union of the cambium and completion of the xylem ring (x 320)

Fig.15. T.S. young stem with adventitious root (x 26.)
Fig. 16. T.S. older stem with ditto (x 26)
Fig.17. Development of root from a nodal cutting.

i. L.S.'s through half of the node, showing the base of the root which has arisen from the main petiolar strand.
ii.-v. T.S.'s through stem and root.
ii. Stem above region of root junction.
iii. Root at junction with stem.
iv.& v. Root below junction.

pet.st. - main strand of petiole.
pet.st.(r) - root strand connected with above.
acc.st. - accessory strand.
acc.st.(r) - root strand connected with above.
ad.rt. - junction of adventitious root and stem.

Fig.18. T.S. young aerial root before formation of xylem (x 26).

Fig.19. T.S. aerial root before commencement of secondary growth (x 16).

Fig.20. T.S. aerial root after secondary growth (x 16).

Fig.21. T.S. petiole (x 26).

Fig.22. T.S. stem, showing commencement of pericyclic activity (x 320).

Fig.23. T.S. stem, showing root initial (x 71).

Fig.24/

<u>Fig.24.</u> Young petiole cutting (half actual size)

pet. - petiole from which regeneration has taken place.
int. - swelling on base of adventitious petiolar root.
b. - buds.
pet.rt.- root from petiole.

<u>Fig.25.</u> As in Fig.24, after 1 year's growth. (half actual size).

<u>Fig.26.</u> Swelling on petiole-borne root, after 1 year's growth, isolated from its shoot. (actual size).

sh. - base of shoot which has been cut off.
s.p. - scar left by base of petiole of leaf cutting.
b. - buds now starting into growth.

<u>Figs.27-29.</u> Leaf types in Acanthus (actual size).

Fig.27 Adult form.
Figs.28 & 29 Juvenile forms.

The type illustrated in Fig.28 was produced when the adult leaf was used as a cutting, while that in Fig.29 arose from a leaf cutting of the type in Fig.28.

<u>Fig.30.</u> Regeneration from a leaf halfed longtitudinally.

<u>Fig.31.</u> Changes leading to formation of swelling on top of petiole-borne root.

p. - dilating region of pith.
c. - meristematic region in cortex.
r. - ruptures in cambium.
pr.x.- primary xylem.

i. Behind root tip.
ii.& iii. Compression of stele to form an arc, and dilation of pith.
iv.& v. Rupture of cambium and penetration of latter into cortex.
vi, vii & viii. Reduction of primary vascular system and formation of secondary.

Fig.32/

Fig.32. As in Fig.31.

b. - Region of greatest activity in cortex.
pr.x. - Primary xylem.

i. As in Fig.31, iii.
ii. As in Fig.31, v.
iii. As in Fig.31, vii.
iv. As in Fig.31, viii.

Fig.33. Rupture of cambium and intrusion into cortex (x 320).

Fig.34. Formation of secondary stelar tissue in root swelling.

tr. - tracheids.
pr.x. - remains of primary xylem.

Fig.35. T.S. petiole with root initial (x 26).

Fig.36. T.S. petiole at junction with root at the stage when the swelling is being formed, showing the gap in the conducting system (x 26).

Fig.37. Formation of secondary tissue and beginning of swelling in root (x 26).

Fig.38. T.S. edge of swelling, showing first stage in bud formation (x 320)

tr. - tracheids.

Fig.39. L.S. petiole and root showing origin of bud.

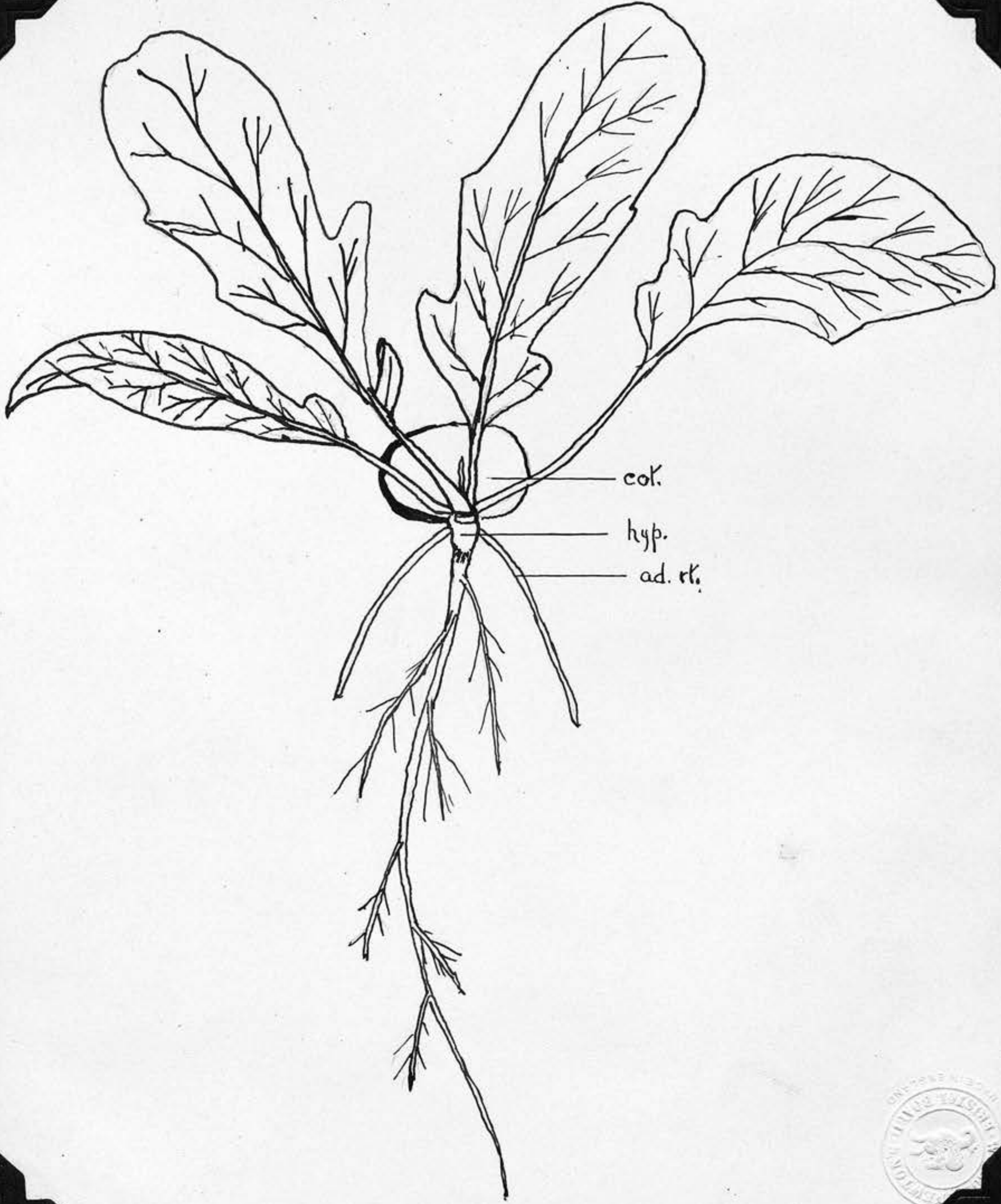
pet.st. - petiole strands.
tr. - tracheids.
pr.x. - remains of primary xylem at the gap in the primary system.
pro.c. - procambium of bud.
cal. - callus.

Fig.40. Further stage of development of Fig.39.

Fig.41. The same as in Figs.39, and 40, continued.

tr. - tracheids.
ab. - abciss layer.
b_2 - young bud.
b_1 - first formed bud, now developed into a shoot, with two leaves, whose petiole bases are shown (pet.b.l.)

Fig.42./



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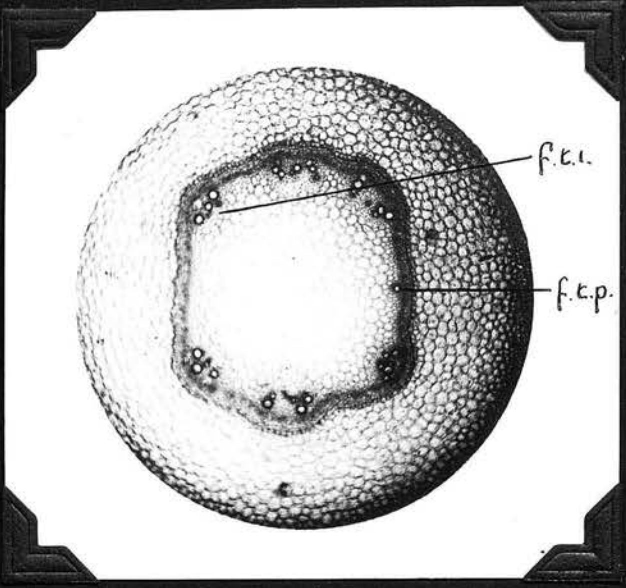

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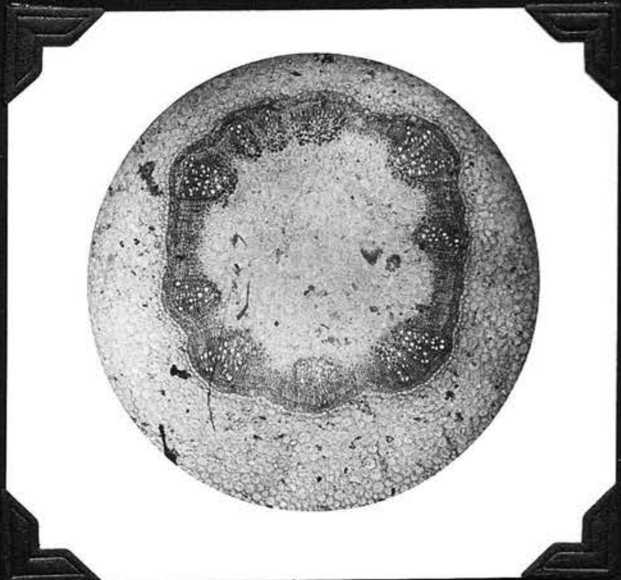

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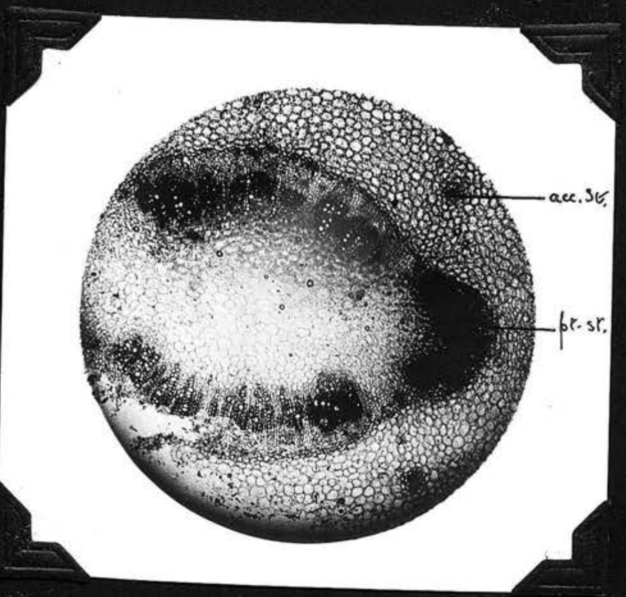

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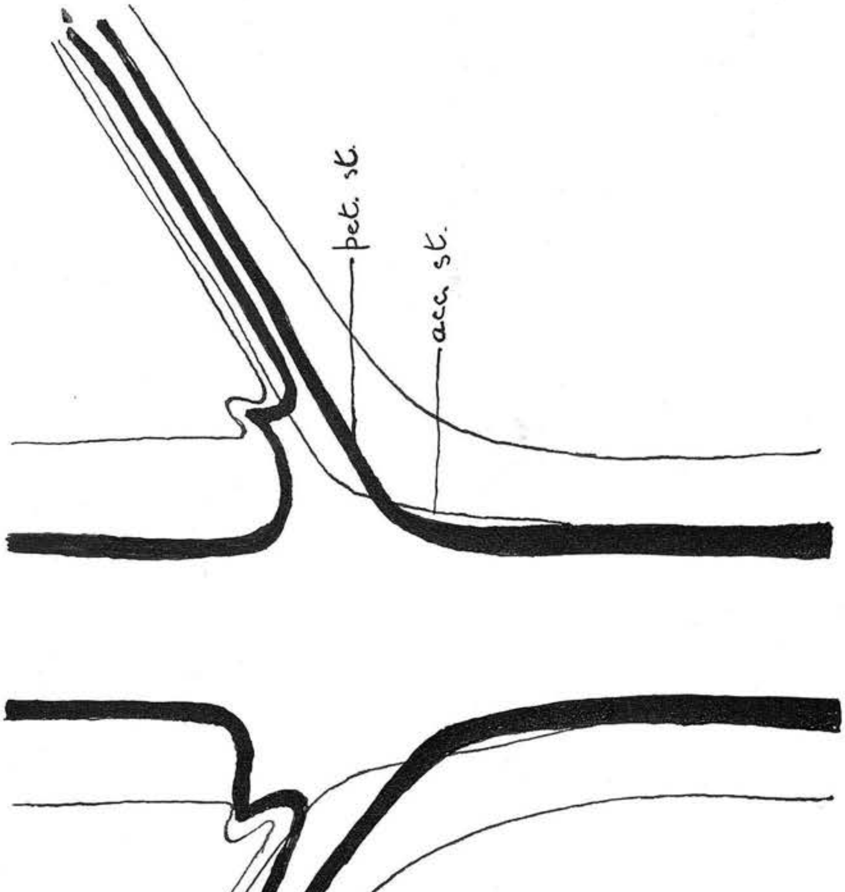

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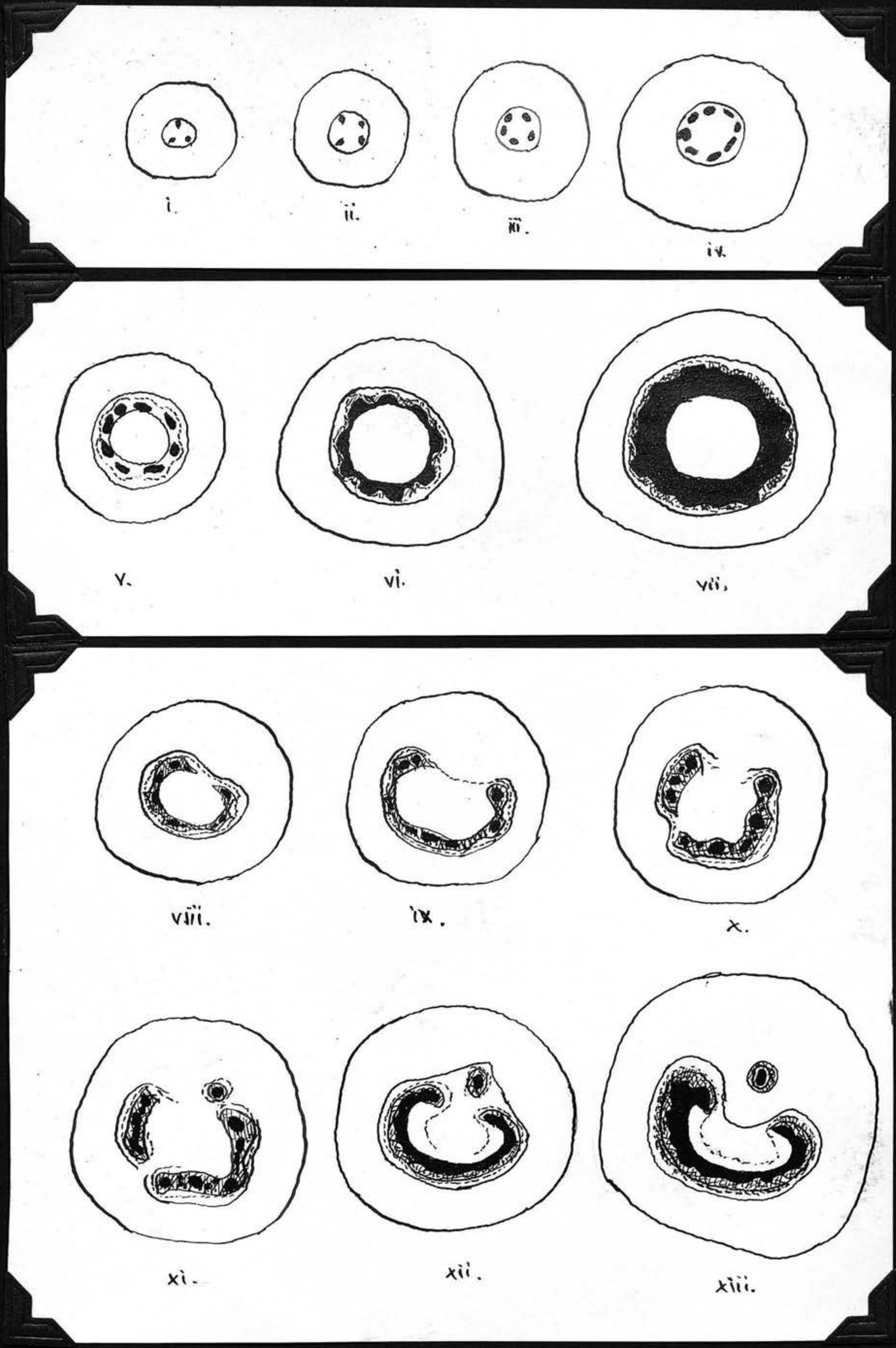

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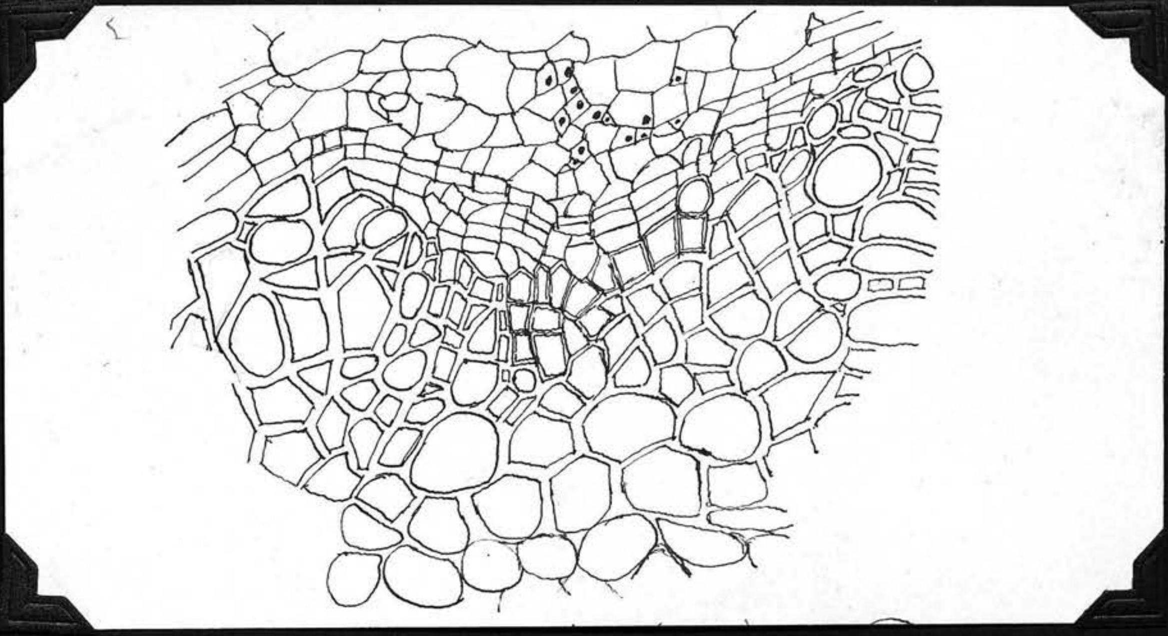

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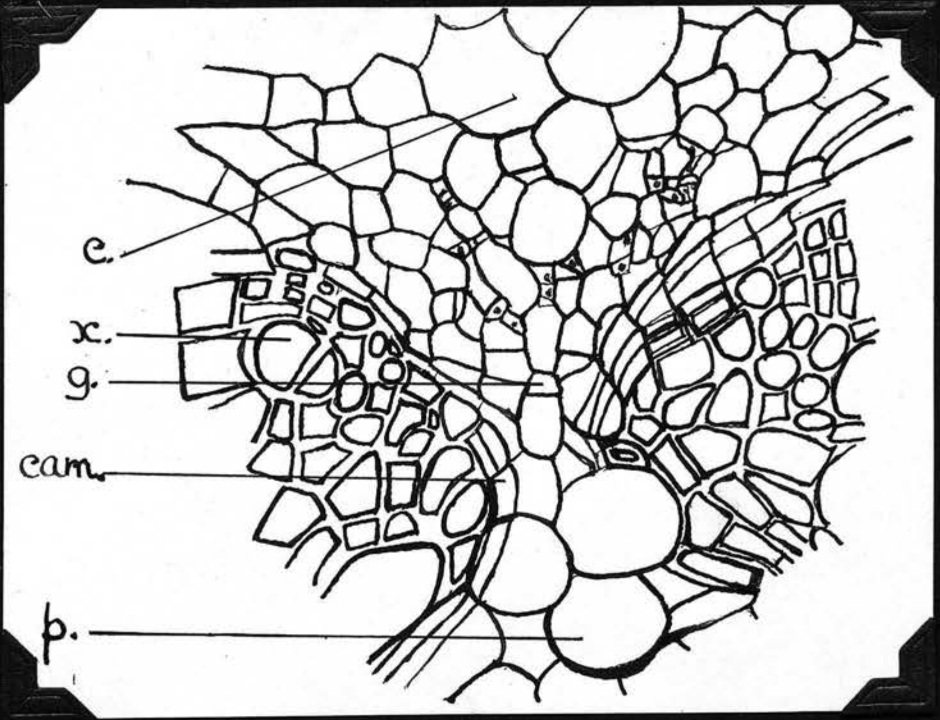
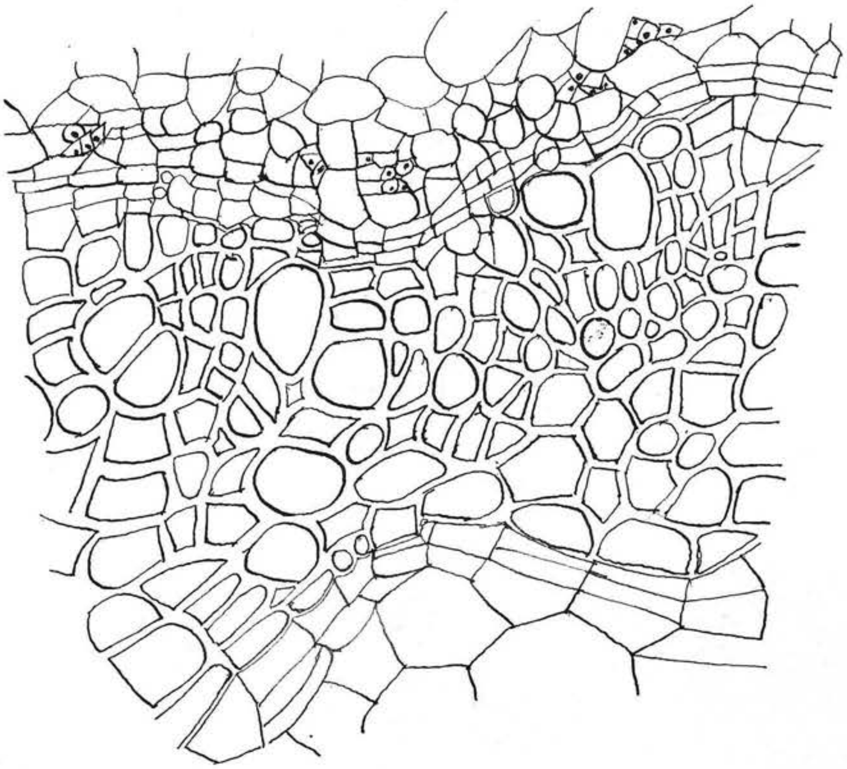


Fig. 12

Fig. 13



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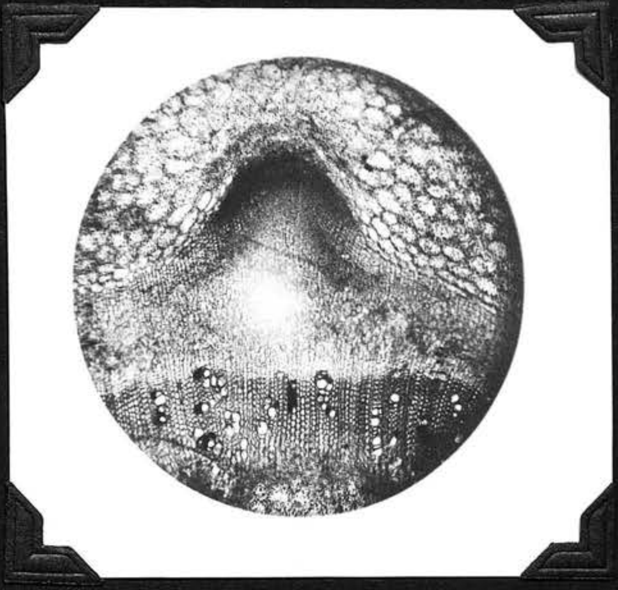

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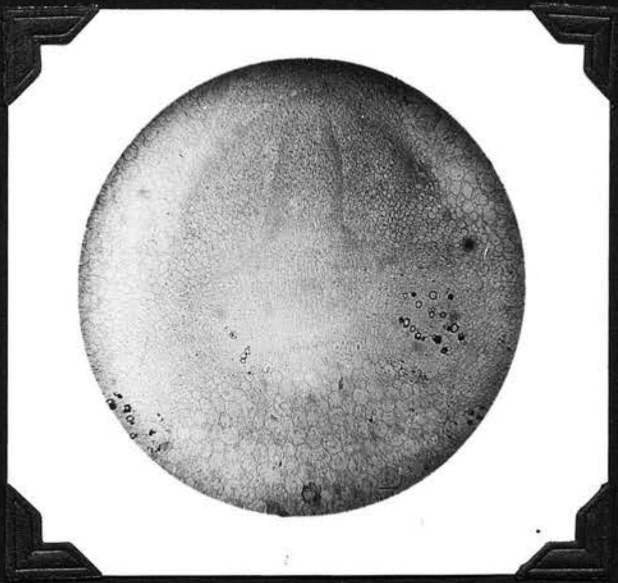

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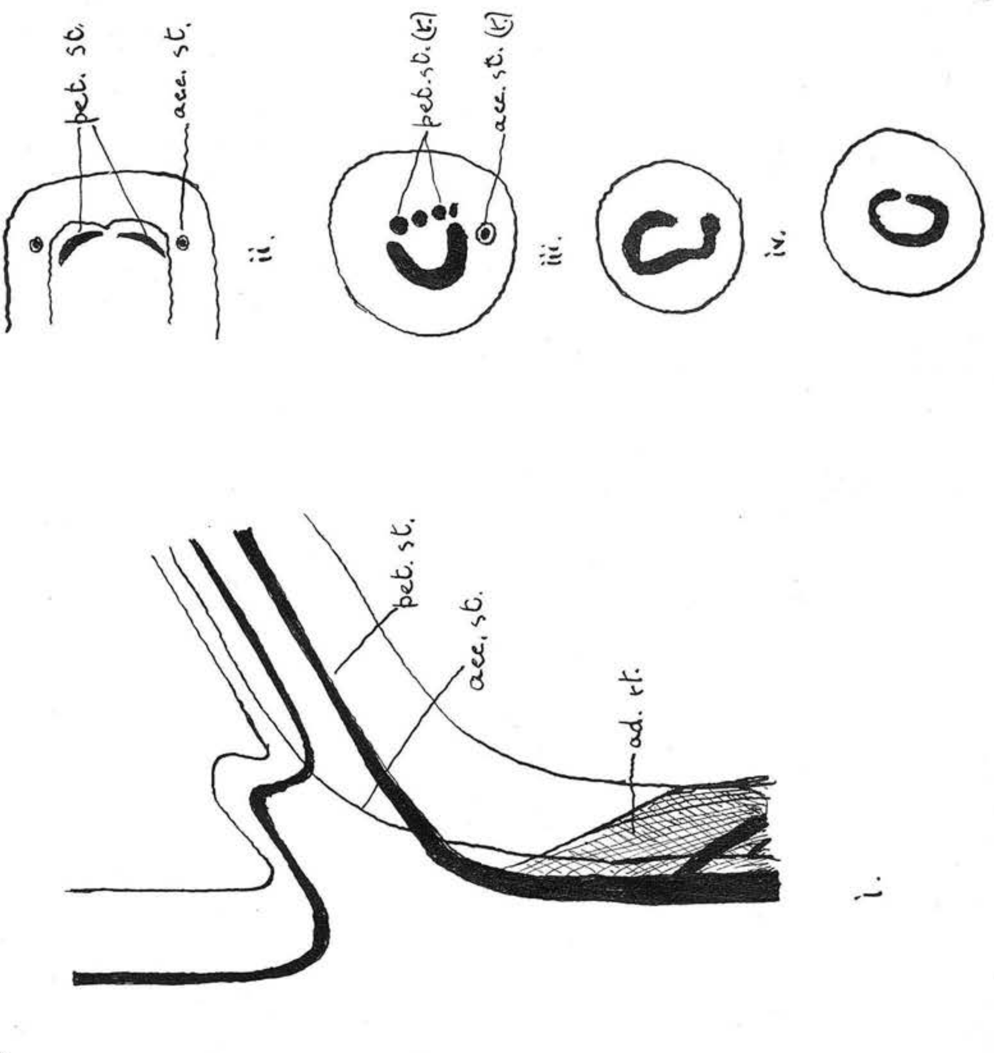

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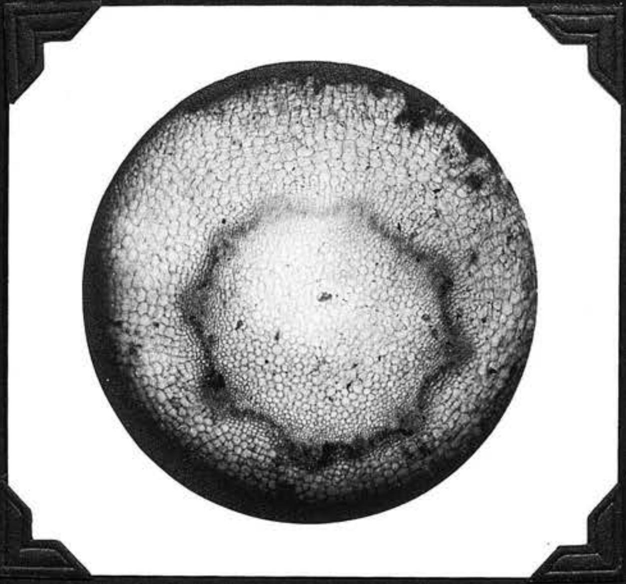

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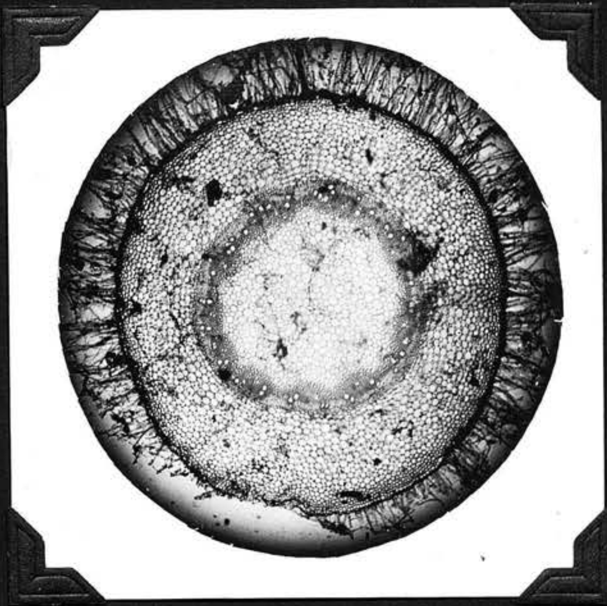
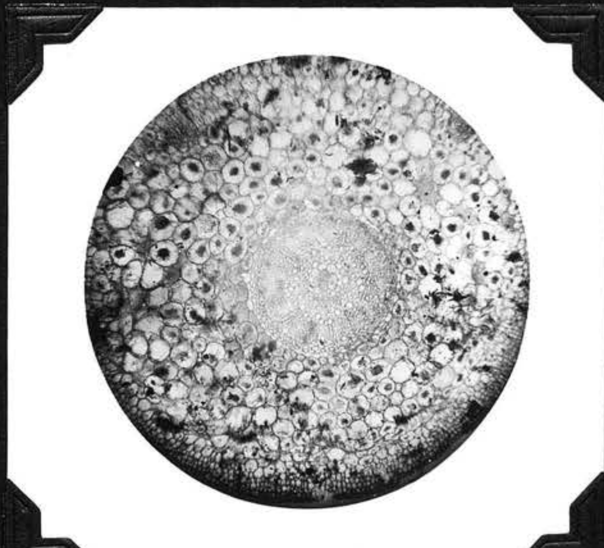


Fig. 19

Fig. 20



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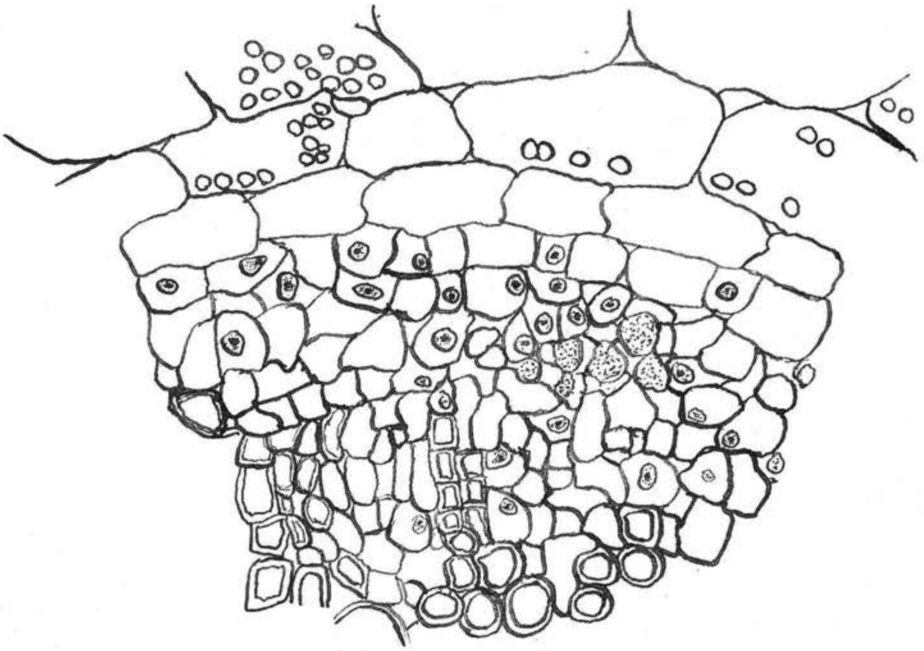

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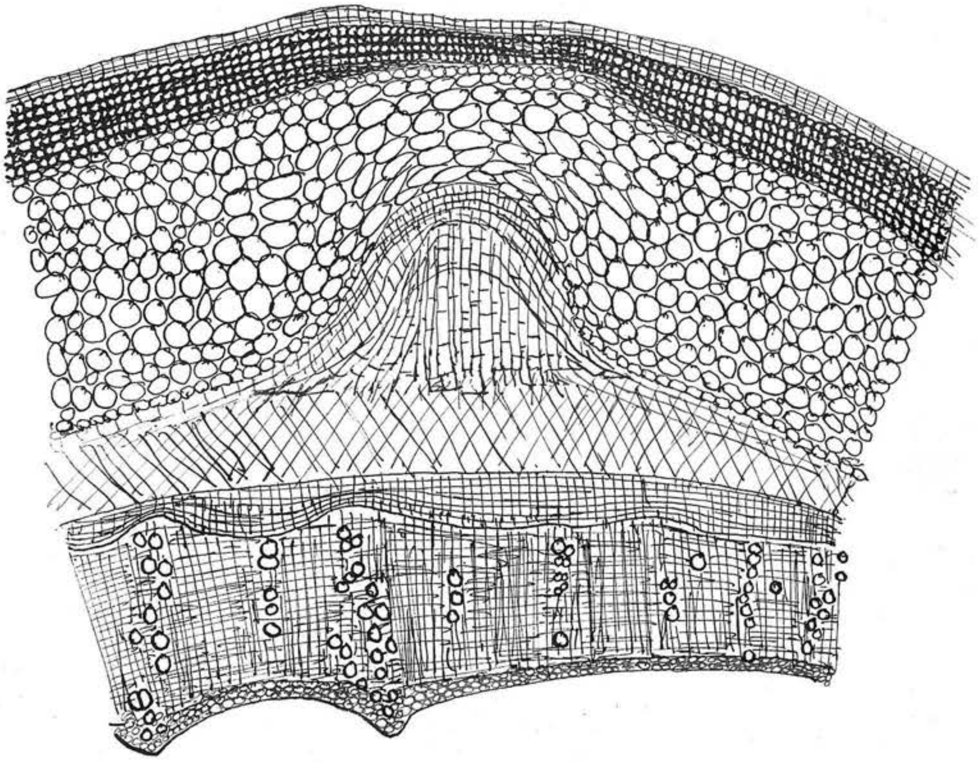

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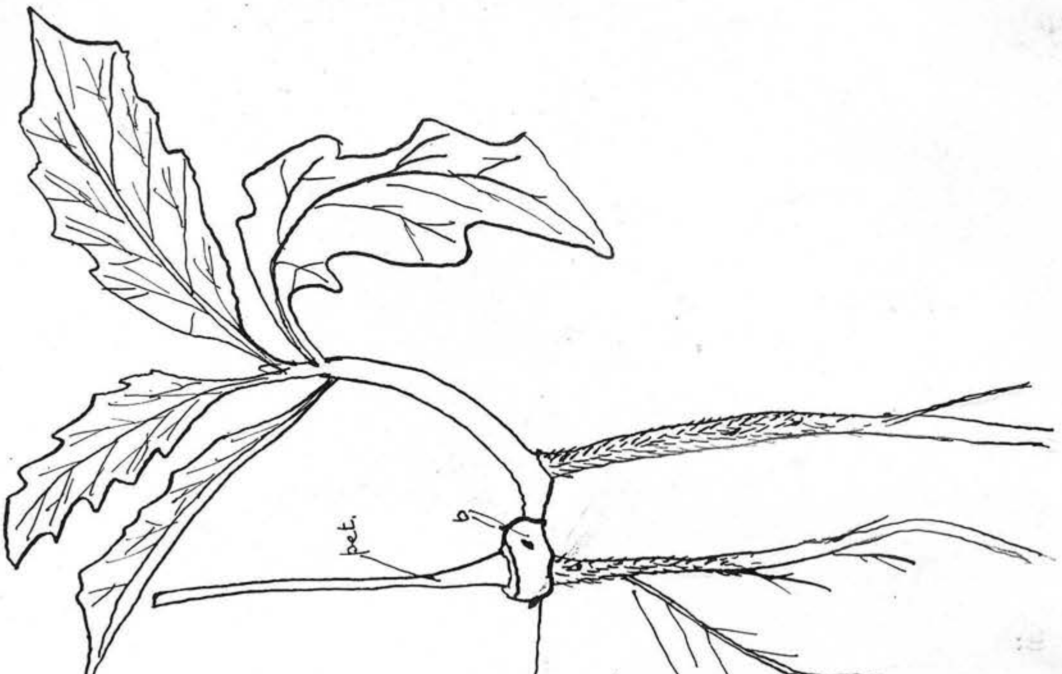


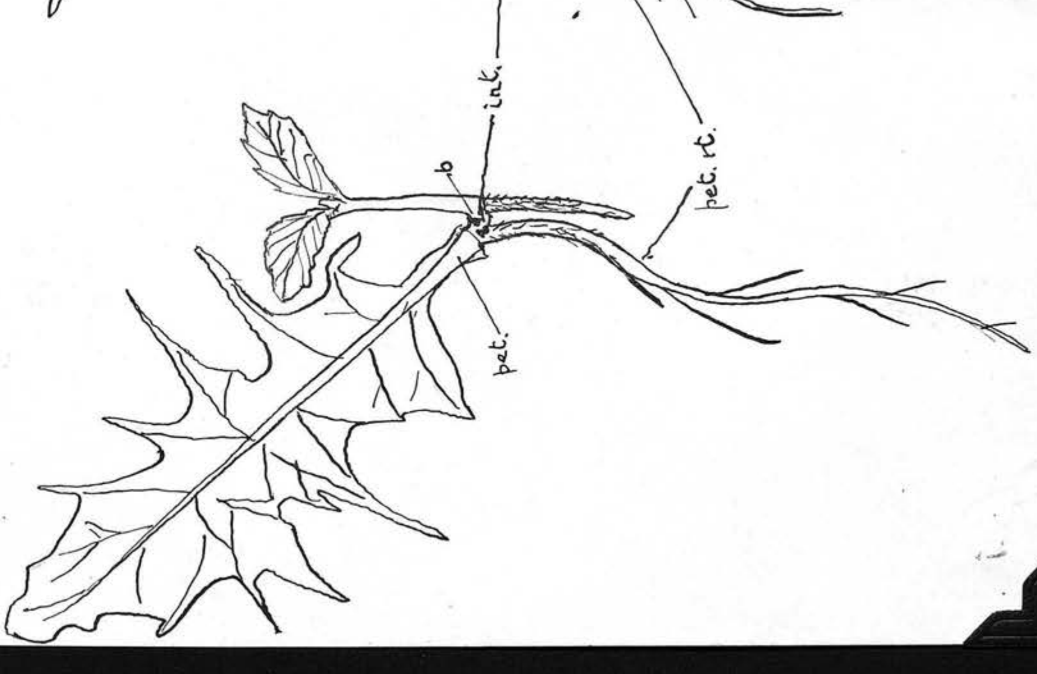
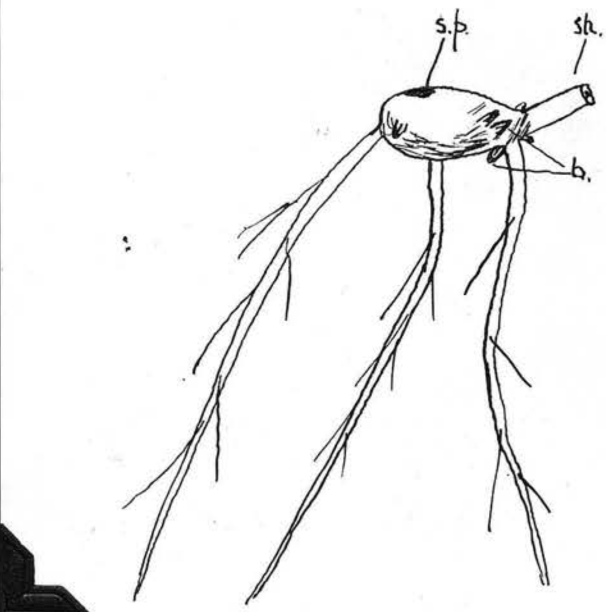


Fig. 24

Fig. 25



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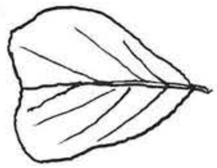


Fig. 29.

Fig 28

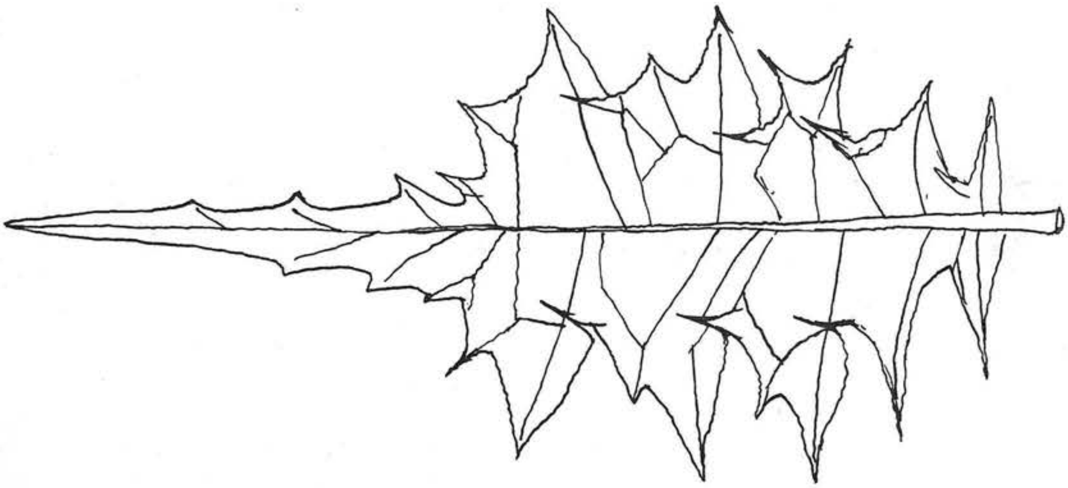
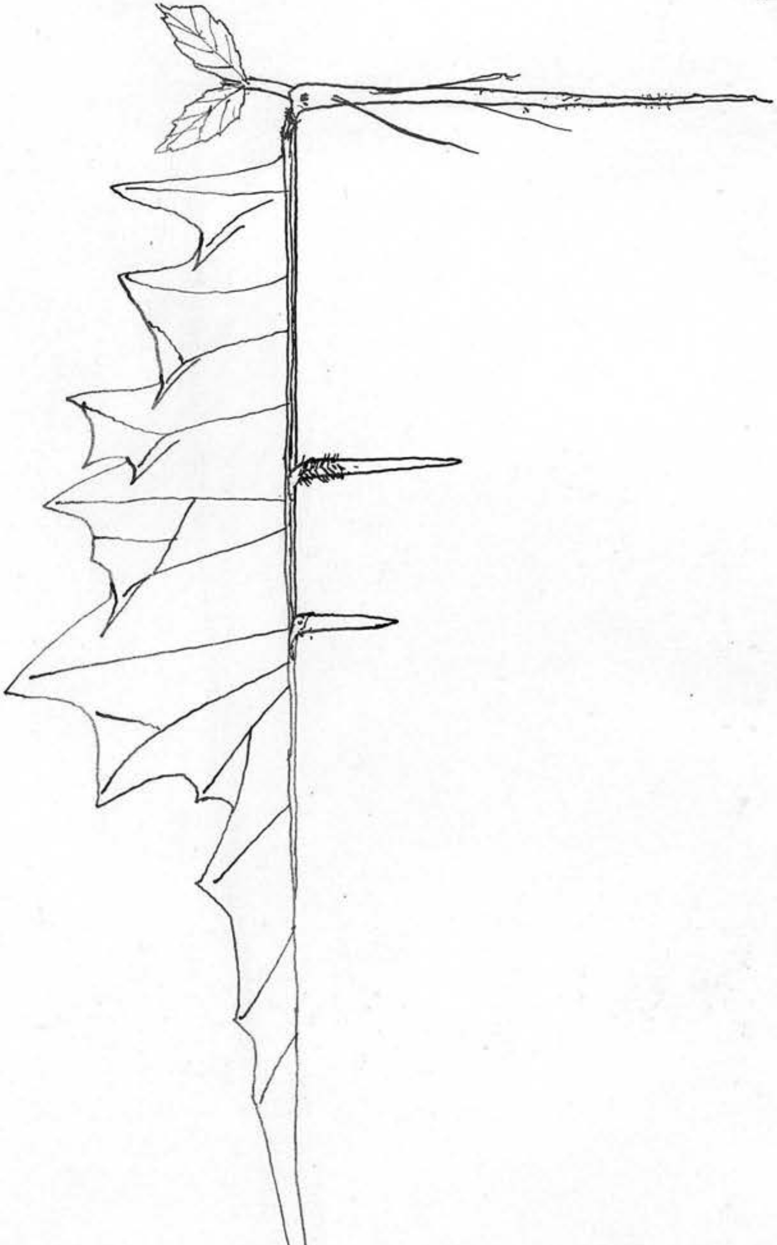


Fig. 27.

Fig. 27

Fig. 28

Fig. 29



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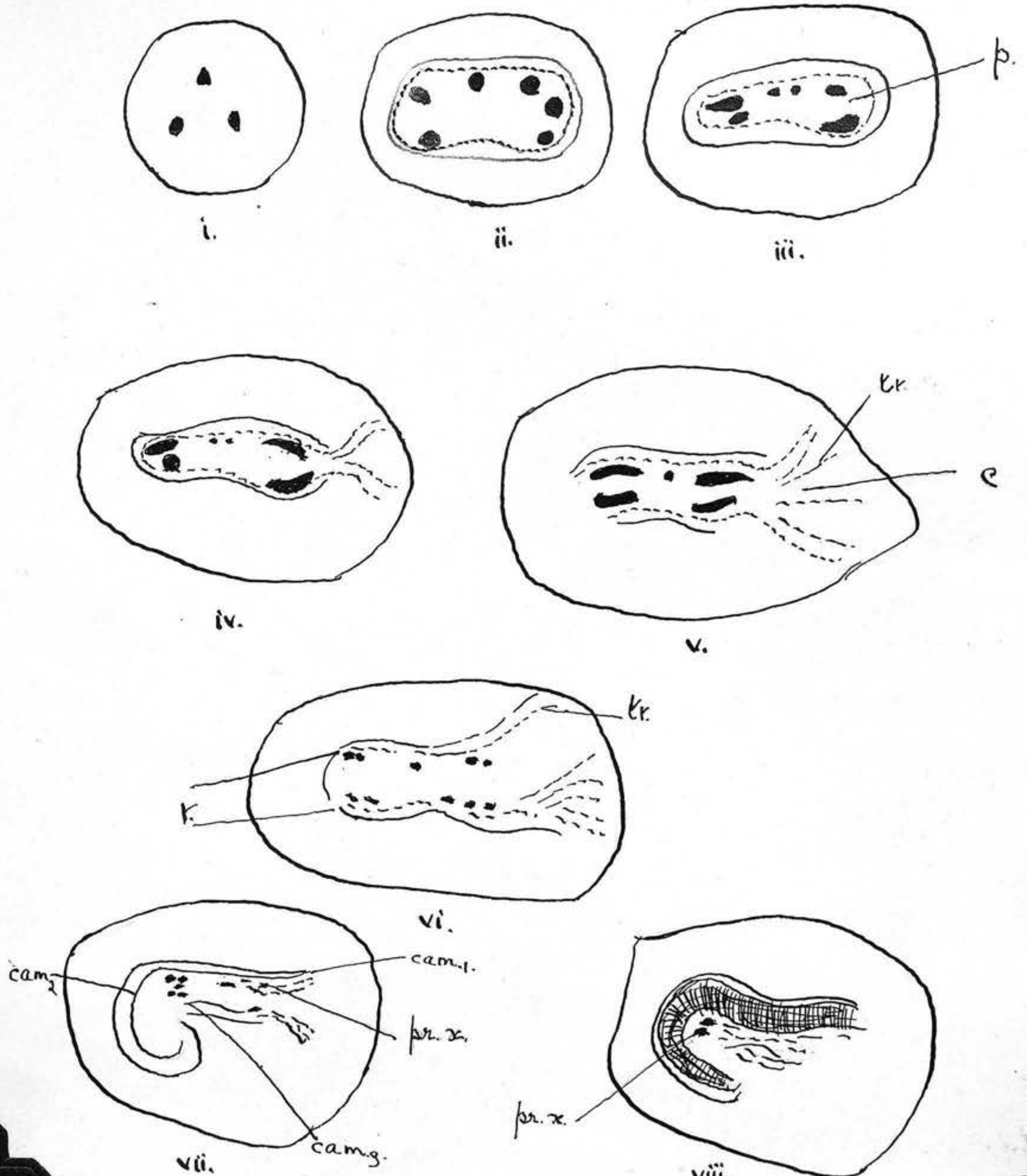

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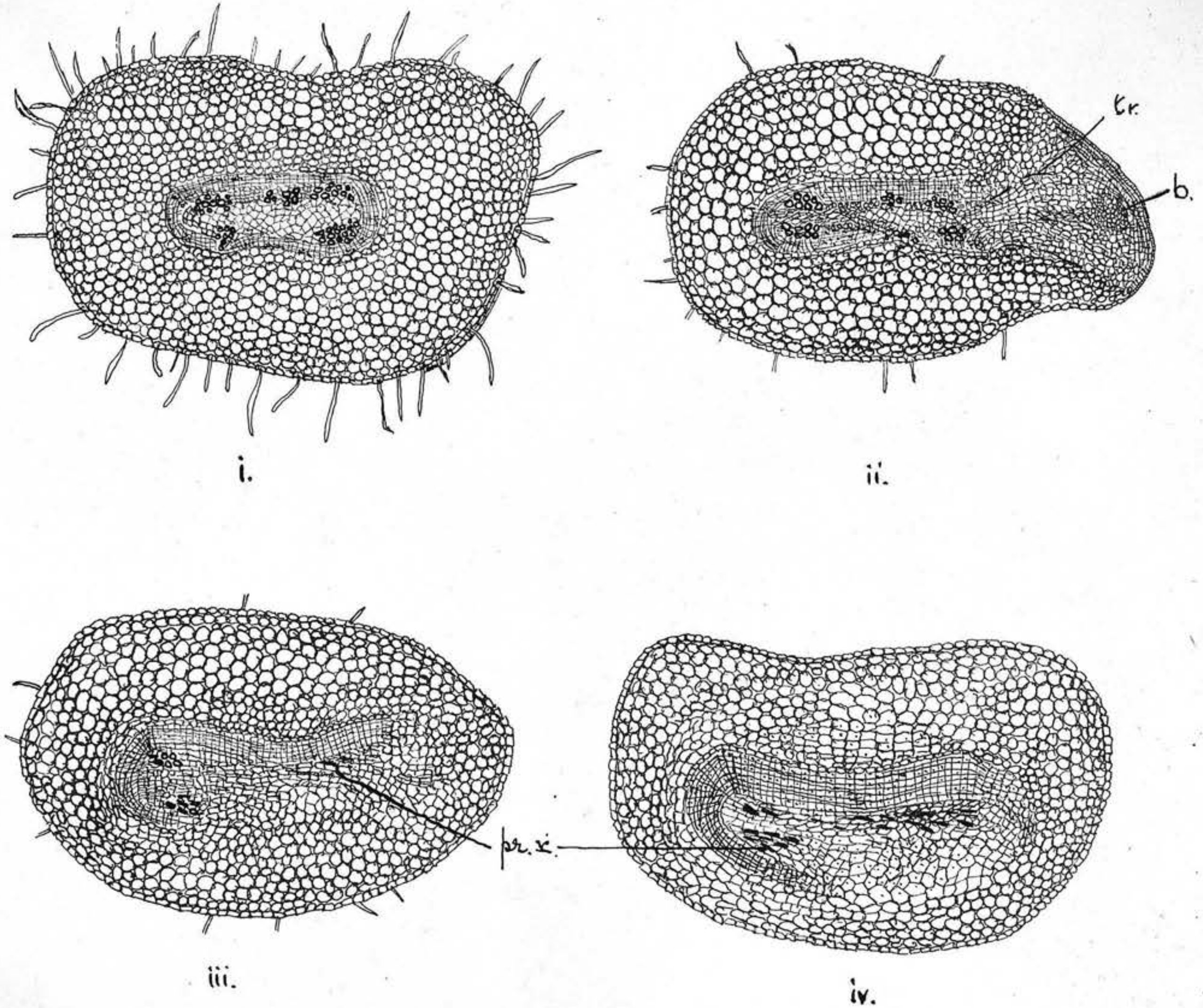

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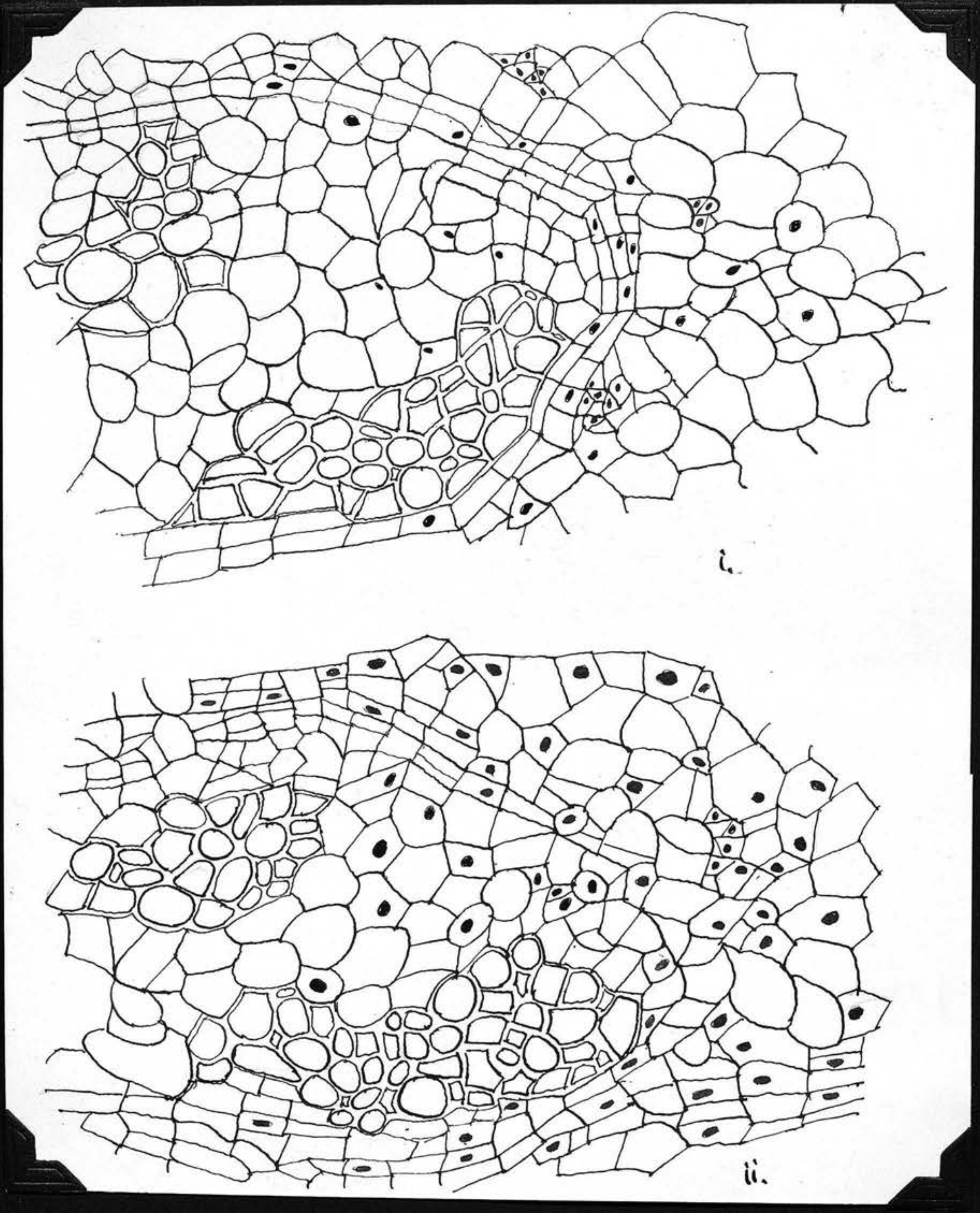

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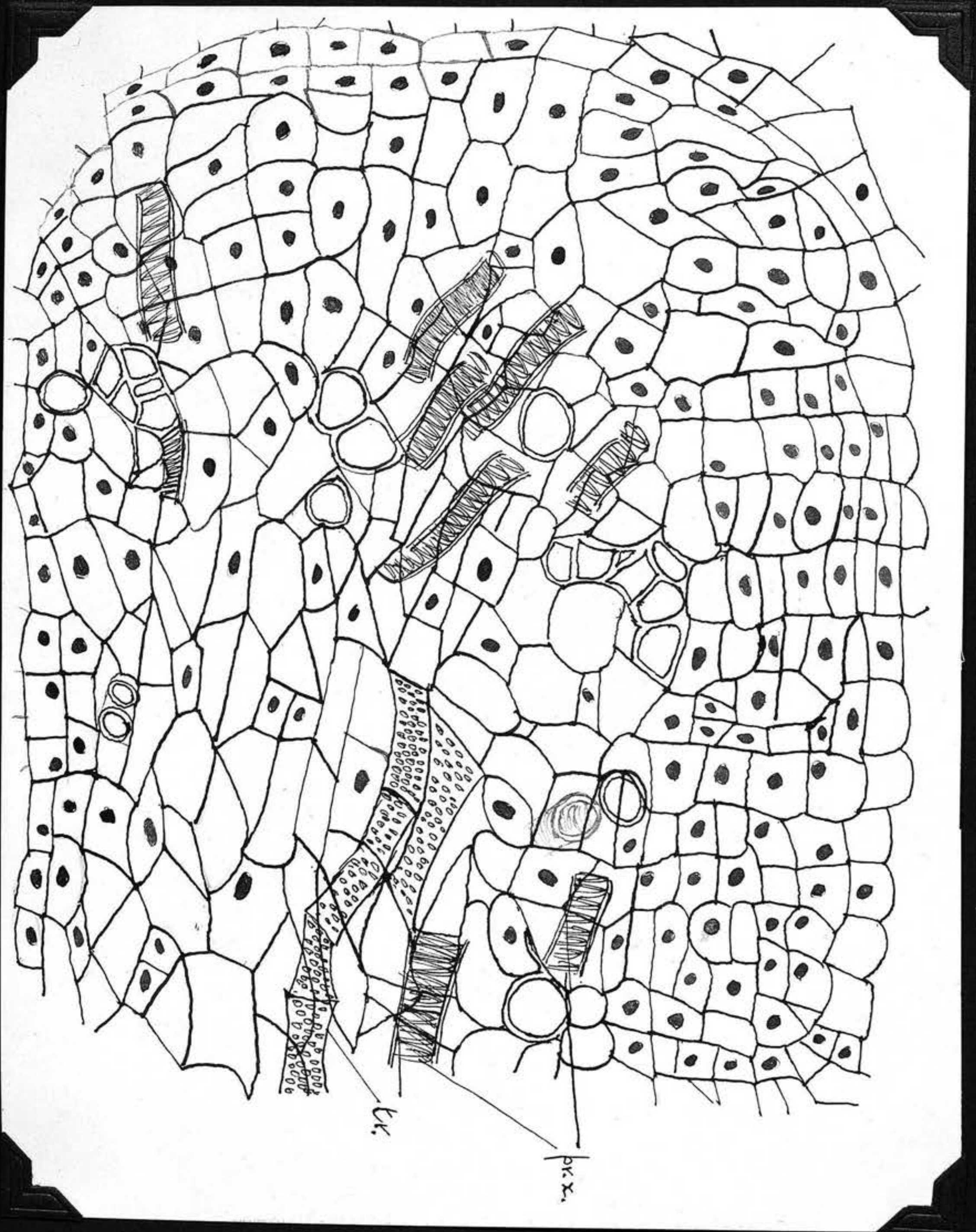

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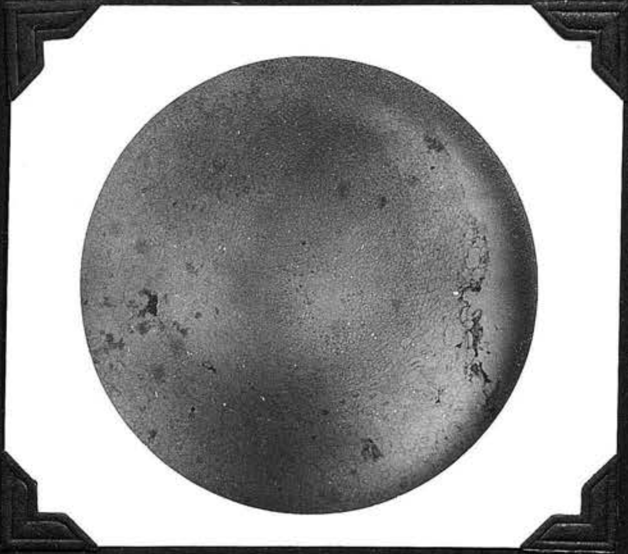
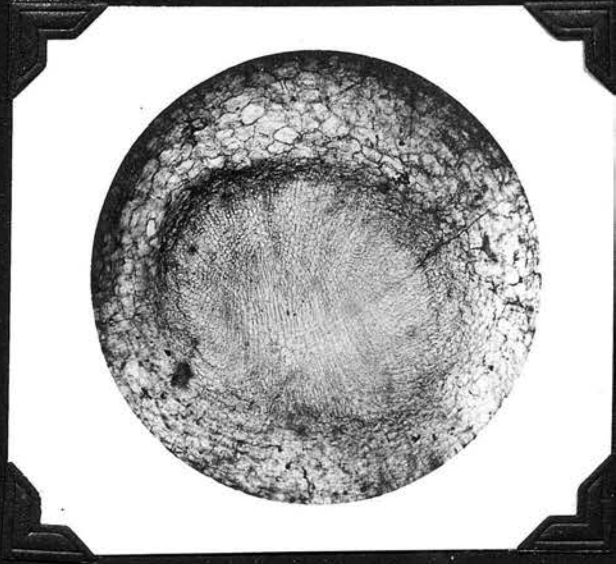
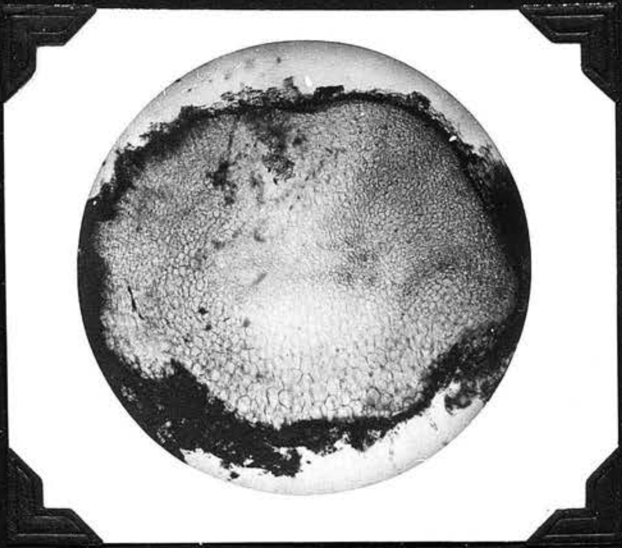


Fig. 35

Fig. 36





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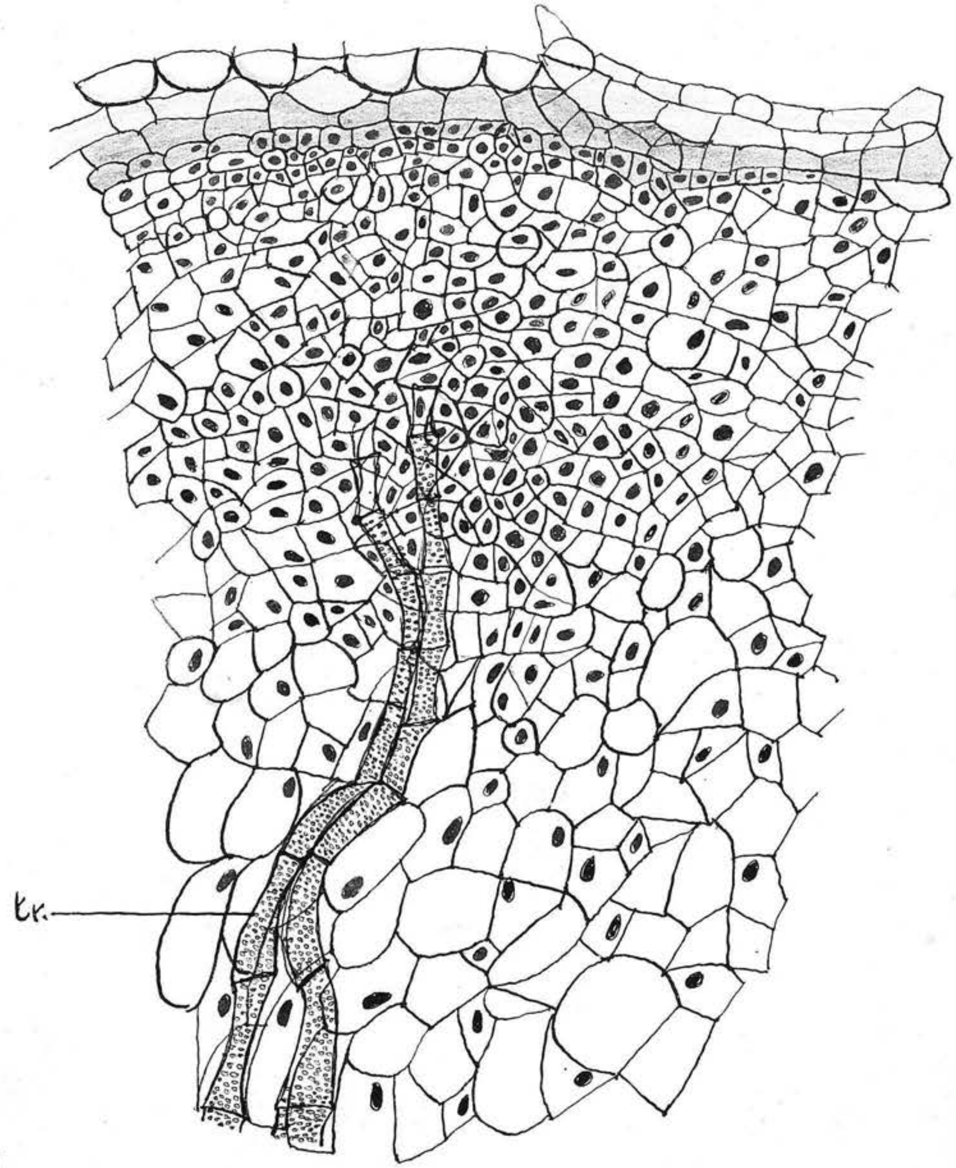

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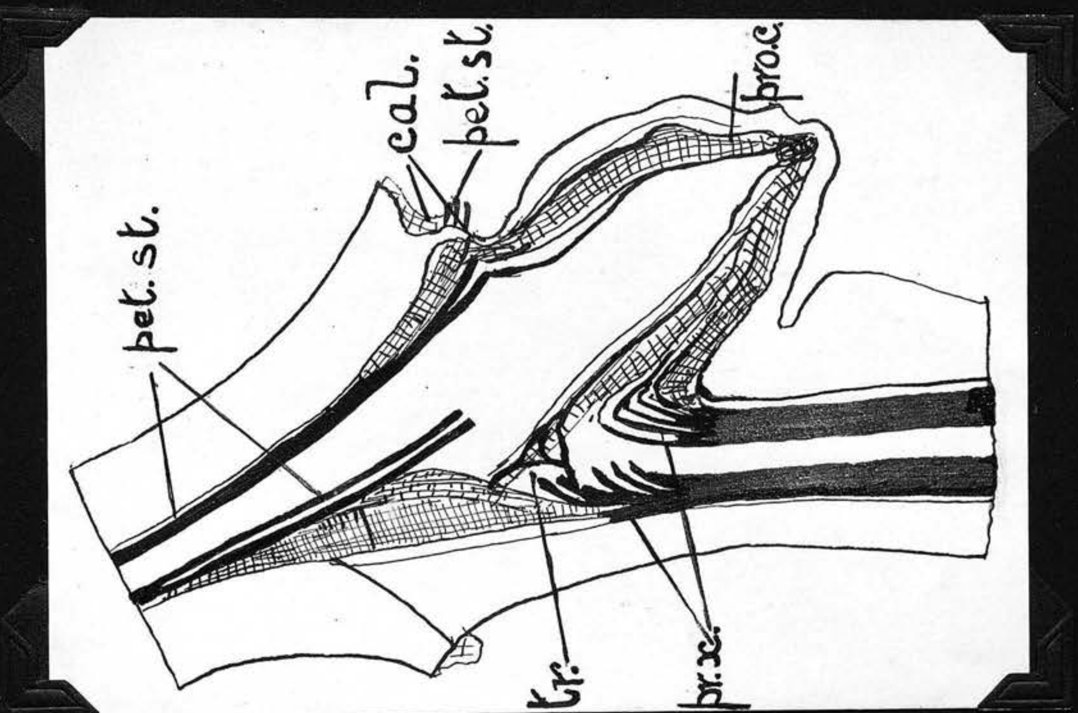


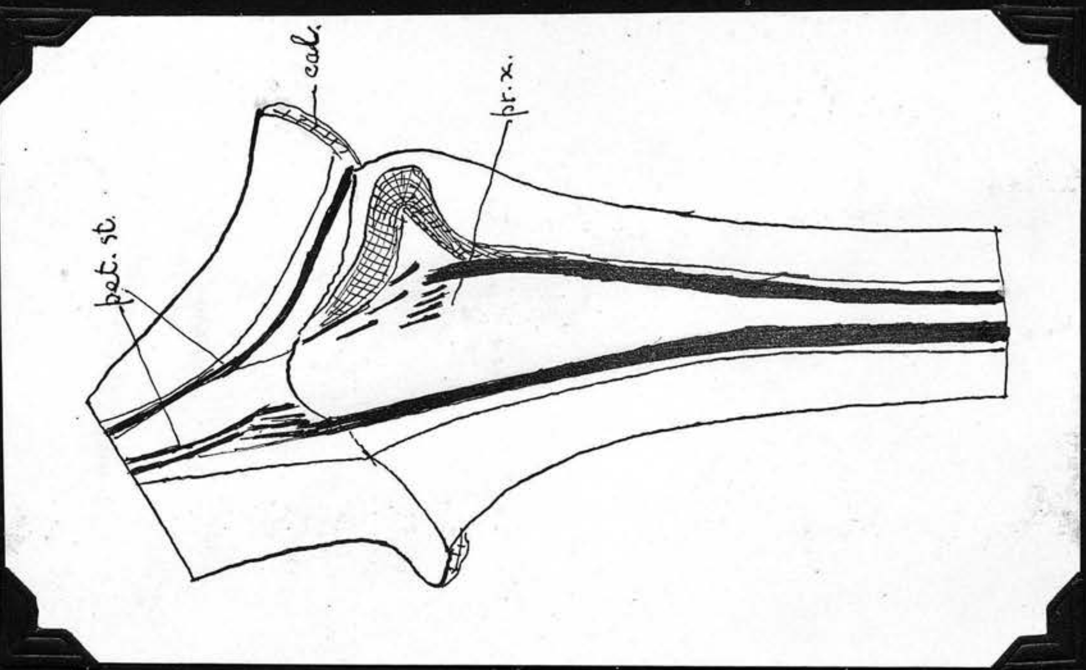
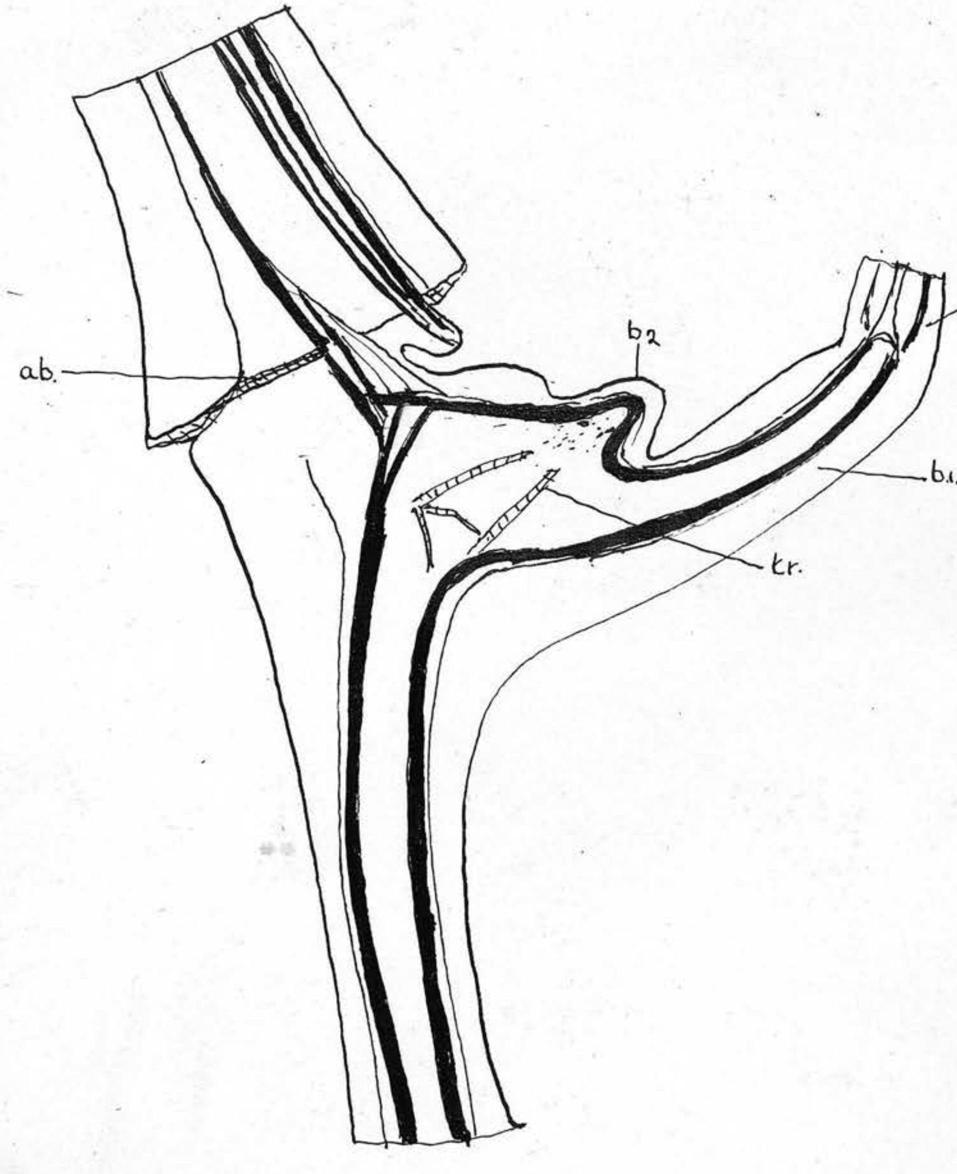


Fig. 39

Fig. 40



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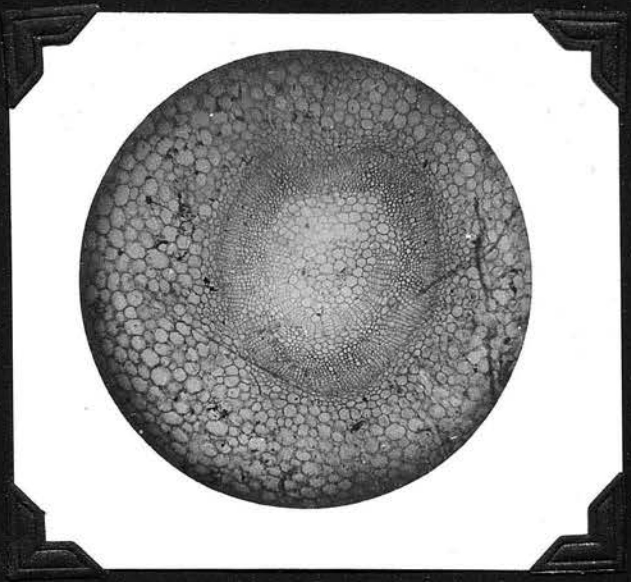

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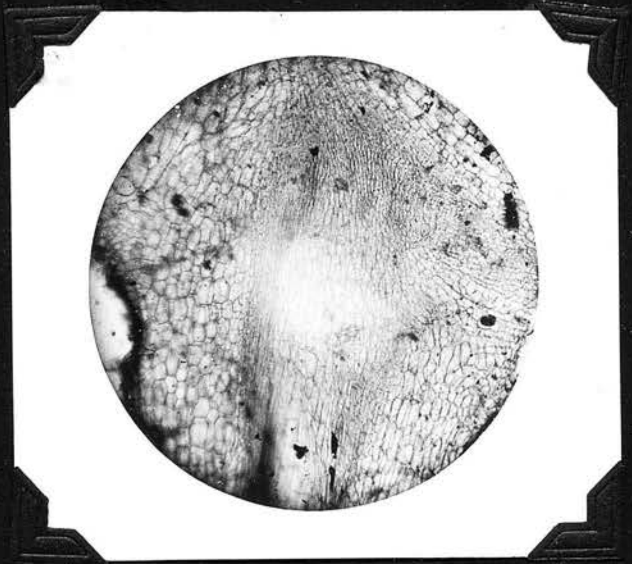

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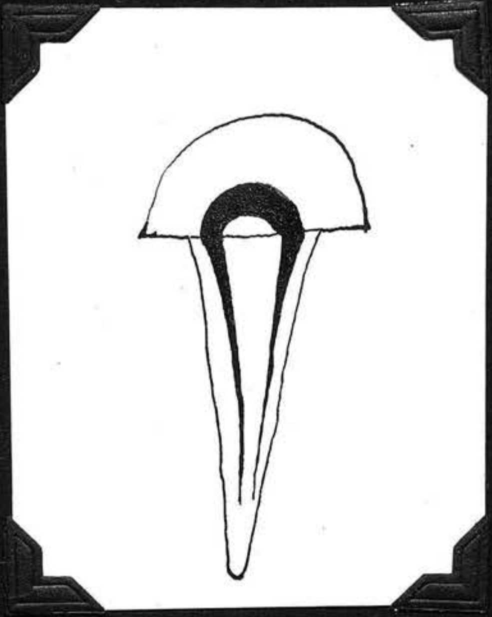

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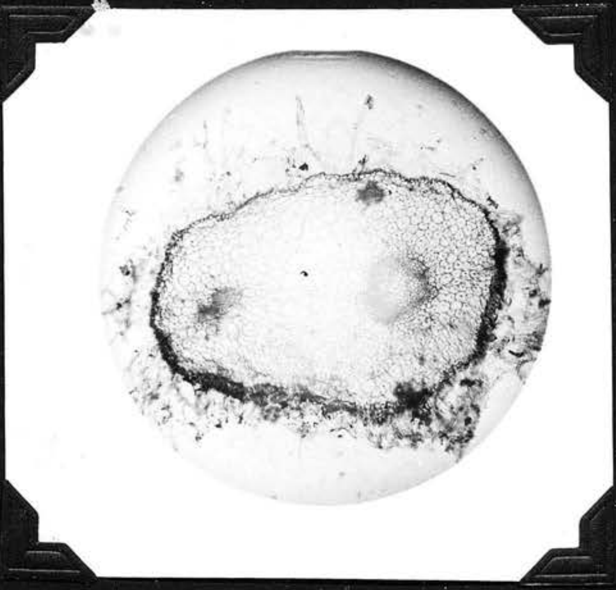

Fig. 46