

CONTRIBUTIONS TO OUR KNOWLEDGE OF *BALANOPHORA.*

I. The Morphology and Parasitism of the Tuber.

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1. *Introduction.*

THE origin and development of the curious root-parasites belonging to the family of the *Balanophoraceæ* have been the problems of investigation since, if not before, the time of Micheli, not only on account of the extreme reduction of their floral organs, but also on account of their parasitism and habit. Some of the earlier investigators of these plants were constrained to read in the developmental course of the tuber and of the inflorescence a strange similarity to the *Clavarieæ* or the *Agaricaceæ*, while others regarded them as abnormal outgrowths of the roots of forest-trees; nevertheless, the phanerogamic basis of their life-history was at no time doubted.

Of the numerous treatises on the morphology of the tuber and its anatomical relationship with the host, those of Unger, Goeppert (of both of which the original papers were not available to the writers), Hooker, Solms-Laubach and Heinricher seem to be the most valuable contributions. Hooker³ in his monographic work, "Structure and affinities of the *Balanophoraceæ*" has furnished us with a fund of information pertaining to the anatomy and histology of the tuber, as well as the taxonomic relationships within the family. Some of his assertions, however, particularly those in which he maintains that the woody vascular system which traverses the ground tissue of the tuber of *Balanophora* belongs to the parasite are contradictory to Goeppert's findings, and incorrect. But Goeppert's observations have since been confirmed by Solms-Laubach,⁴ who has established the identity of the vascular system of the host-root and those traversing the parasite-tuber. Solms-Laubach has also given a short resumé of the important literature upon the subject, selected out of the numerous philosophic dissertations and nature-phantasies on these problematic plants. Heinricher's work (of which unfortunately only a single

paper² was available to the writers) in the main confirms the later observations of Solms-Laubach on the existence of an "absorption tissue" within the vascular steles of the host inside the tuber. A curious situation arises here in the fact that van Tieghem⁵ in his extensive work on the *Balanophorales*, published in the same year as an earlier paper of Heinricher, surprisingly enough records observations radically opposite to Heinricher's in that he revives Hooker's view refuted by Solms-Laubach nearly 40 years earlier.

On the origin and the early development of the floriferous branch, the previous literature is inadequate. Solms-Laubach has attempted to locate the growing region of the tuber; in the absence (as he believed) of growing apices, he infers that the early establishments of the endogenous floral branches must be laid by the formation of secondary meristems.

Finally, on the morphological nature of the tuber, one need only mention Goebel's interpretation, which is the last word on the subject at the present day. Goebel¹ considers the tuberous vegetative body of the parasite as corresponding to the hypocotylous segment of the seedling, the only segment which develops while all the other embryo-organs are suppressed, and which later gives rise to the floral branch adventitiously.

2. Material and Methods.

The present study is based on investigations made on two species of *Balanophora*, *B. indica* and another which has been provisionally identified as *B. dioica*, R. Br. Tubers at various stages of development were collected in January 1933, *B. indica* at Coonoor, Nilgiris, and *B. dioica* at Makut on the Coorg-Malabar boundary, S. India, and fixed on the spot in formacetic alcohol. Paraffin sections 8-10 μ thick of selected parts of the tuber were stained, some in iron-alum Hæmatoxylin, but most of them in Saffranin-Light-green combination. Most of the figures were drawn with zeichenapparat, the rest being semi-diagrammatic.

3. Observations.

(a) *External Morphology*.—A fully grown tuber of *B. dioica* is about twice the size of a man's fist, and runs out into several lobes. Each of the projecting lobes is almost certain to contain within it the beginnings of an inflorescence. While young plants almost as tiny as a pea often bear a single, small-sized floral branch, the older ones produce several bigger ones, year after year during their long flowering season, till the tubers attain their maximum size and then die down. The rough outer surface of the tuber is spread over with the scattered mycelia of a fungus, which attacks the inflorescences when they are shed. There are sometimes no pustules. The

lobes of the tuber are often hollowed out by insects, which lay their eggs within, and the entire association comprising of the tuberous parasite, the host-root system growing with it, the fungus on its surface and the insects which lodge within it and probably fertilise the flowers provide an interesting system of inter-related life-histories.

In *B. indica* the tubers are bigger and may be lodged on roots nearly half-an-inch thick. Young tubers, however, resemble those of *B. dioica*, but do not put forth an inflorescence till they have attained a considerable size. Pustules are present, but fungal mycelia have not been seen. In both species, the tubers are partly or wholly subterranean.

The youngest tubers obtained are shown in Pl. XLVIII, Fig. 1. The smallest of them is not more than 1.5 mm. in diameter, but already shows an internal structure, qualitatively not very different from that observed in the older tubers. One tiny tubercle (*a*) appears as though it had burst out from the interior of the host-root whose outer bark forms a short collar at the base of the tubercle. Such tubers have, before this, given rise to the belief that the parasite tissues remain within the host-root, and burst out into tubers at certain stages, somewhat after the manner of *Rafflesia* or *Pilostyles* (see Heinricher).² This extremely interesting question, however, is outside the scope of the present study.

Pl. XLIX, Fig. 1 shows a tuber of *B. indica* in diagrammatic longitudinal section, showing the vascular bundles (slightly exaggerated) permeating the body of the parasite-tuber as a profusely branching system. It was reconstructed from preparations obtained from dissected and macerated material and serves to give a preliminary idea of the body of the tuber in relation to the disposition of the strands which (as has frequently been proved ere this) belong to the host-root system.

(b) *Anatomy*.—The ground-tissue of the tuber consists for the most part of undifferentiated parenchymatous cells, most of which are filled with a sticky resinous substance, termed 'Balanophorin' by Goeppert. The outermost cells of this tissue bounding on the exterior are large and have thickened walls towards the exterior, and no epidermis is differentiated. With the growth of the tuber, these cells are broken down and wear off, rendering the outer surface rough. The ground-tissue of the tuber is more or less irregularly traversed by a branching system of woody strands, conspicuous by their brown colour and their relative hardness in the white and fleshy parenchyma of the tuber. Each of them is equivalent to a root of the host-plant, though extremely modified in appearance, structure and behaviour, and may be termed an "intra-matrical host-root" for want of a better

descriptive term. The entire system of these intra-matrical roots is directly traceable to and is continuous with the woody root of the host on which the tuber is perched, and from which it derives its nourishment. These well-known facts have to be re-asserted in view of the opinions to the contrary expressed by van Tieghem⁵ in his work on the "Inovuleæ", to which one will have to refer again in a later paragraph.

(1) *The Intra-matrical Root.*—The cross-section of one of these intra-matrical roots taken near one of the distal ends of the system (*i.e.*, near the periphery of the tuber) discloses an extremely simple structure. In the specimens of *B. indica*, collected for this investigation, the intra-matrical host tissue being conspicuously smaller celled than the parasite tissues, the distinction between the two is extremely easy and well-marked. The entire region of the host tissues is surrounded by a sheath of three or four layers of parasite parenchyma; the cells of this sheath do not differ from the general parenchyma of the tuber, save that they appear often slightly elongated along the length of the root (flattened as it were by the increase in girth of the root), and are characterised by the absence of balanophorin. The sheath is delimited by the slightly thickened cell-walls of the outermost cell-layer of the sheath. The innermost sheath-cells contiguous to the host tissues possess slightly bigger and more prominent nuclei and slightly denser plasma than the rest of the cells of the parasite-parenchyma. The host-elements thus delimited by this sheath consist in this region mainly of an undifferentiated small-celled tissue. This tissue is interspersed with large cells in such a manner that the small-celled tissue appears as though it fills the interstices between these big cells. The latter possess thin walls and large nuclei. In *B. indica*, they generally showed plasmolysed contents, but in *B. dioica* they were observed to possess a dense plasma sometimes uniformly granular, sometimes filled with minute droplets, and their nuclei were oval or slightly distorted; appearances, in short, which led van Tieghem to regard them as secretory cells. In their size and the appearance of their nuclei, these cells resemble the cells of the parasite-tuber, and (as will be seen later) their long and somewhat irregular rows can be traced along the length of the intra-matrical root up to the growing region of the parasite, where they are formed and differentiated. They are collectively called the "Secondary Thallus" by Strigl according to Heinricher,² and are believed by him to have an absorptive (haustorial) function. Here and there adjoining these absorptive-cells, but dispersed in the small-celled host tissue apparently without a definite order or regularity are a few tracheids with pitted or scalariform thickening. These are the protoxylem elements of the intra-matrical root. The small-celled tissue extends into the interstices of the

innermost cells of the sheath so that in a cross-section, the young intra-matrical root has the outline of an irregular cog-wheel with sharply-pointed cogs. Neither the strands of the phloem nor the layers of cambium are to be seen in this portion of the root, nor is there the evidence of a differentiated endodermis or pericycle enclosing the poorly-developed primary stele. The entire small-celled tissue retains its cell-contents and the nuclei also remain as prominent as in a meristematic tissue. But it has not been seen to undergo cell-multiplication in this region such as has been observed in the meristematic tissue situated at the distal end of the intra-matrical root.

In a slightly older portion of the same root, we find the scattered tracheids of the protoxylem supplemented by metaxylem elements, whose formation is not regularly centripetal as in a normal root. Like the development of the protoxylem, the development of the metaxylem also seems to be rendered irregular by the presence of the absorptive cells. No spiral or annular vessels have been noticed either in the protoxylem or in the metaxylem, and one also notes that except in the formation of tracheids, no elongation either of the individual cells or of the root as a whole takes place, almost all the host tissues remaining as they were first formed.

In the small-celled tissue lying outside this central region of mixed protoxylem, metaxylem and parasitic absorptive cells layers of cambium are now gradually differentiated. These may be made out in Pl. XI, Fig. 3 but are seen better at a later stage such as the one shown in Fig. 4. This shows a broad and fully-formed band of cambial cells; in this particular instance, the metaxylem-arrangement is more root-like. There is still no pericycle but from the location of the cambial layers, one may assume that the narrow band of host tissue lying exteriorly to this cambium to correspond to the cortex of the intra-matrical root.

The still older roots within the tuber possess a more or less wide ring of secondary wood, without any medullary rays. The formation of secondary wood proceeds from the cambial layers just described. The secondary tissues are formed in the close proximity of the outermost groups of the primary xylem; and a continuous ring of wood and wood-parenchyma is formed, in which a few radial rows of narrow pitted or scalariform vessels are seen. The cells of the host tissue lying outside this woody ring lose their dense cell-contents, and the outermost of them undergo some of the changes usually seen in bark-formation; although no phellogen as such seems to be developed, some of the cells in this region take a deep stain, not unlike suberised cells. Little further change takes place in this tissue and it remains sandwiched between the parasitic parenchyma and the growing woody cylinder of the intra-matrical root (Pl. L, Fig. 1). The maximum

development of secondary tissues in any tuber is seen in the main intra-matrical root at the basal region of the tuber, close to the point of attachment on the extra-matrical host-root. From this region, the intra-matrical host-root is directly traceable to its extra-matrical portion, where it shows the normal dicotyledonous root-structure (Pl. I, Fig. 2). The absorptive cells of the parasite are absent in the extra-matrical portion, and the primary stele has the centripetal metaxylem. The woody cylinders of the two are continuous with the normal bark-forming cortex of the extra-matrical host-root.

(2) *The growing points.**—Situated at the point of termination of the intra-matrical host-root, surmounting, as it were, the distal extremity of it, are a few layers of cells, which establish a continuity between the absorptive-cells within the intra-matrical root on the one hand and the parenchyma of the tuber outside it, on the other. It is perfectly continuous with the latter and as the region is quite localised, it is readily recognised. The cells are smaller than the average parenchymatous cells of the parasite, prismatic or rectangular, thin-walled and provided with dense protoplasm and large nuclei, in short, undoubtedly meristematic, and so obvious that one is at a loss to know how it could have been overlooked by Solms-Laubach, van Tieghem and others (Pl. XLIX, Fig. 2).

One has little doubt that this constitutes the primary meristem of the parasite. As every branch of the host-root system within the tuber is surmounted by a meristematic region of the parasite, it follows that the parasite has as many growing points as the intra-matrical system has ultimate branches in the tuber. As the growth of the tuber and that of the host-root system within it take place side by side and are entirely correlated with one another, it is necessary to describe the growth of the latter before proceeding to examine the growth of the tuber.

(3) *The Growth of the Intra-matrical Roots.*—That region of the small-celled host-tissue immediately adjoining the parasitic meristem differs from the rest of the same tissue in that cell-divisions are observed to take place in it, whereas, elsewhere, as said before, the tissue seems to be inactive. Though the division-spindles observed in this apical region of the intra-matrical root are few and far between, yet it seems safe to infer that it constitutes a slow-growing (or perhaps periodically active) apical growing

* Since the above was written, a summary by Dr. A. Sperlich, of Heinricher's work on the growth and parasitism of the *Balanophoraceæ* in "Handbuch der Pflanzenanatomie, Band IX/2 Die Absorptionsorgane der parasitischen Samenpflanzen" (Berlin, 1925, p. 20-23) was obtained. The results show a large measure of correspondence, almost entirely in the part dealing with the growing points of the tuber.

point of this root. The two meristems are thus contiguous. In *B. indica* the intra-matrical meristem of the particular host-plant is very easily distinguished from the parasite-meristem by the smaller size of its cells; an extremely fortunate circumstance, for though the host-tissues within the tuber are generally smaller-celled than those outside the tuber, yet there may not always be a difference between the host and the parasite-meristems in the size of their cells. This may very naturally depend upon the species of the host. That such is the case is shown by *B. dioica* in our collection; here the difference in the size of the two contiguous meristems is not so great as in the specimens of *B. indica*, so that the cells could be referred to either of the two meristems only with some difficulty. There is, however, a marked difference between the host-parenchyma and the parasite-parenchyma, for while the parasite cells increase rapidly in size and alter in general appearance both within and without the intra-matrical root, the host-cells remain more or less as they were first formed, and offer as sharp a contrast with the host tissues in the advanced stages as in the case of *B. indica* described earlier (Pl. XLIX, Fig. 3).

The presence of the dividing nuclei in the two meristems shows that cell-multiplication takes place in both the meristems side by side. The line of demarcation between these two adjacent meristems is by no means straight, but is similar to what is observed in the case of the sheath-cells and the small-celled host tissue adjoining it. In other words, the host-meristem runs into the interstices between the nearest cells of the parasite-meristem. Thus the parasite-cells which are formed and differentiated towards the host-meristem, are already separated from their neighbours by the wedging in of the host-meristem cells and are thus isolated in long, irregular rows along the length of the intra-matrical root. Sometimes at an early stage they divide, but always grow considerably in size soon after their division and undergo changes which characterise them as absorptive cells already described (Pl. XLIX, Fig. 2). They take no part in the development or the secondary growth of the intra-matrical root, but are present in such large numbers from the earliest stages of development of the primary stele of the host, that the normal development of the proto- and meta-xylem of the latter is interfered with and effectively disturbed (Pl. XLIX, Fig. 3).

The growth of the two meristems seems to be simultaneous. In their growth against one another, it is difficult to say which of them is the aggressor, though from the nature of the relationship, one would naturally imagine that the parasite cells push themselves into the host-meristem. The manner in which the cells of the latter wedge their way in between the meristematic cells of the parasite and often isolate them, indicates, on the

other hand; that they have at least some share in the interlocking growth of the two tissues.

(4) *The Growth of the Tuber.*—The parasitic meristem, though well marked out from the parenchyma of the tuber yet, is uninterruptedly continuous with it. From the meristematic cells in the centre of the growing region to the differentiated cells of the parenchyma is a gradual transition in the size and appearance of the cells, in which the progressive differentiation of the meristematic cells into the ground-tissue of the parasite can be observed. It is easily seen that while the cells formed by the meristematic region towards the intra-matrical root go to form the absorptive tissue mentioned above, the rest add to the parasite parenchyma. They increase in size gradually, become somewhat rounded, and quickly develop a large, central vacuole, soon filled with *Balanophorin*. To a certain extent, their growth in size, coupled with a mutual adjustment between the cells, renders possible the continued activity of the parasite-meristem. Towards the periphery, however, the parenchymatous cells appear to become secondarily meristematic and to divide further. This is particularly well seen in *B. indica*, where the tubers attain considerable sizes. In these tubers, at the periphery, a few layers of cells are observed to possess slightly denser contents and more prominent nuclei than the cells of the ground-tissue do. The cell-walls are often straight, unlike those of the more interiorly-situated cells, which serve mainly for the storage of reserve material. Though here and there a cell-division has been observed in this region, the growth here is admittedly more general in the sense that it is not localised, and consequently, this region shows no indication of rapid growth. The contrast between the central inactive parenchyma and the peripheral growing region is quite distinct, particularly where the tuber is about to branch into two, or where a lobe enclosing a young floriferous shoot is rapidly growing in size. At the extreme periphery, the cells are slightly flattened and at the outermost layers, the cells burst their thickly-suberised walls or split away from one another and thereby make the surface of the tuber roughly granular.

(5) *The Conducting System of the Parasite.*—Whether the parasite tuber possesses a vascular system of its own during its vegetative growth and whether it is continuous with the intra-matrical strands of the host have been controversial questions since the time of Goeppert and Hooker, but as yet (so far as the available literature shows) no satisfactory solution to these questions has been put forward. Running in various directions here and there across the parenchyma of the tuber are strands of three or four rows of parasite-cells. These are narrower, if not longer, than the ordinary cells

of the parasite-parenchyma, and possess slightly denser plasma and darker-staining nuclei. In these strands very often spiral tracheids are developed. The strands, here and there, appear to establish connections with the intra-matrical host-root. They reach the innermost cells of the sheath, which resemble to some extent, the absorptive cells within the host-tissues and which are, not infrequently, contiguous to the outermost of the absorptive-cells. The spiral tracheids of the parasite-system, however, stop short at the sheath of the intra-matrical root, and thus create the impression that the two systems, *viz.*, the intra-matrical roots and the parasite strands, are quite discontinuous, a statement which is only partly true.

The conducting system does not show any regularity of distribution and arrangement. It is easily overlooked in a section owing to its more or less tortuous course, and its indistinct connection with the host tissues is not observed at first sight. During the early formation of a floriferous branch, a considerable number of these strands is formed in connection with the neighbouring intra-matrical roots and these strands organise themselves into a loose anastomosing system in the vicinity of the primordium of the floriferous shoot. The strands here are generally better developed than elsewhere, the tracheids being formed in larger numbers; but their best development takes place within the stalk of the inflorescence during its later growth and elongation.

Thus it will be seen that in *B. indica*, the parasite-tuber possesses during its vegetative growth a rudimentary system of feebly-developed strands with spiral tracheids. This, however, may not be true of all species and at all stages of the development of the tuber. It may depend upon the size attained by the tuber or may be associated with the origin of the floriferous branch. The large difference of opinion seen in the previous literature on this point makes it difficult to generalise the facts seen in one or two species. Two facts seem to be definite: that the rudimentary vascular system is developed without any definite arrangement or orientation and according to the needs arising in the course of the growth of the tuber; and that its best and most orderly development takes place in and in connection with the floriferous branch.

(c) *The Origin of the Inflorescence.*

(1) *The formation of the inflorescence-cavity.*—For this part of the investigation, *B. dioica* provided an extremely suitable material, for in it, the first indications of the formation of a floriferous branch are to be seen in a lobe at a very early stage of development, when it is no bigger than a hanging drop of water. Such a lobe shows in its interior a minute cavity

which seems to arise in the following manner: Where a cavity is about to develop, the paranchymatous cells of the parasite are depleted of their storage material, and the common walls of three or four contiguous cells swell up and gradually disintegrate, often after splitting from one another. The swelling of the cell-wall extends to the adjoining cells, but sometimes a single cell is observed to disintegrate, causing a cavity to be formed (Pl. LI, Figs. 5 and 6). This cavity is lined by the swollen, deeply-staining walls of the surrounding cells, some of which may disintegrate too. The cavity grows in size, first by the splitting and stretching apart of the surrounding cells, which takes place at certain points and extends radially outwards (Pl. LI, Fig. 7); and later by the enlargement of the containing lobe, the parenchyma of which now becomes secondarily meristematic, particularly above and around the cavity. Thus the cavity arises lysigenously and enlarges to a large extent schizogenously.

Though as a rule, a lobe gives rise to a single floriferous shoot, in many cases, in the interior of the lobe, swollen or partly split cell-walls are seen side by side with a half-developed floriferous branch, which suggests that the formation of more than one cavity is initiated, but of these only one reaches the full development (Pl. LI, Fig. 5).

So far as the present observations go, the cavity does not seem to have a predefined position in the interior of the lobe in which it arises; nor does it arise in direct connection with any of the host-roots in the lobe. But often the region where a cavity is to be formed is connected with the source of nourishment (the intra-matrical root) by a conducting strand of the parasite, in which, as said before, spiral tracheids are developed (Pl. LI, Fig. 6). This strand approaches close to the region of the swollen cell-walls. As the vascular strands are found fairly developed at the time when the cavity is yet in its early stages of formation, it seems probable that the cavity arises in relation to an already existing or developing vascular strand; though the cavities do not abut directly on a vascular strand, but are situated only in their immediate neighbourhood, it would be reasonable to presume that a cavity will naturally arise in the vicinity of a conducting strand. There is no indication, however, to show that particular regions in the tuber-parenchyma are set apart during their formation from the primary meristem to give rise later to cavities and inflorescences; at any rate, such predefined regions are not visibly differentiated in the parenchyma in which they later arise and the probability is that the formation of a cavity is determined by relatively external conditions such as the size attained by the lobe, its rate of growth or the season of flowering, while its exact position seems to be largely dependent upon the vascular strands of the parasite.

(2) *The formation of the primordium.*—The inflorescence takes its origin on the side of the cavity turned towards the vascular strand; as this is not always the side nearest to the centre of the lobe, the long-axis of the inflorescence-primordium—when it is formed,—is not always parallel to the long-axis of the lobe containing it. The primordium, however, never arises on the side of the cavity towards the periphery of the lobe. At first, when the schizogenous extension of the cavity is still incomplete, the cavity is uneven in shape, but during its later growth, the angularities are smoothed out. The primordium then shows itself as a short conical outgrowth projecting into the cavity. The cells of this outgrowth and those situated between this and the vascular strand appear slightly smaller in size than elsewhere, and possess thin and straight walls, but it is only at a later stage that they develop somewhat denser contents and prominent nuclei (Pl. LI, Figs. 9 and 10). The outgrowth may arise immediately or some time after the formation of the cavity. In the former case, the primordium keeps pace with the enlargement of the cavity and no vacant space is to be seen in the cavity, save in the earliest stages. On the other hand, when the primordium is delayed in taking origin, it is some time before it fills the slowly-growing cavity.

At a very early stage, when the floriferous branch is little more than an ovoid mass of undifferentiated cells, scales grow up from or near its narrow base on all sides one by one. Each scale is at first 2 or 3 cells thick, and extends from the base, over the top and sometimes on to the other side of the slightly enlarged head of the primordium (Pl. LI, Figs. 11 and 12). They arise in acropetal succession, rapidly increasing in number. Meanwhile, spiral tracheids make their appearance along the axis of the ovoid primordium. The first strands are centrally situated; gradually more tracheidal strands are differentiated in the vicinity of these at the base of the primordium. These strands extend proximally along the short stalk and establish themselves as a system continuous with the already-differentiated strands in the neighbourhood of the cavity, which again are connected proximally with the intra-matrical roots and terminate in the sheaths of the latter. Their course in the parenchyma of the lobe below the base of the primordium does not follow a definite plan being largely determined by the disposition of the intra-matrical host-roots in the neighbourhood. In the stalk of the floriferous branch, when fully developed, they have a more or less definite orientation, and in cross-section, appear to be arranged in concentric circles. On their disposition and later development in this region, we have the accounts of Hooker and van Tieghem, to which one can add but little.

In the later stages, a comparatively rapid growth of the lobe brought about by the secondary meristematic activity of the parenchyma of the entire lobe, results in a corresponding increase in the size of the cavity. The growth of the inflorescence with the development of scale-leaves covering the head, however, proceeds at a quicker pace, so that finally the parenchymatous crust formed by the lobe-tissue is burst open at the apex. The floriferous branch thereafter grows out by the elongation of the stalk, leaving the crust as an irregularly lobed collar round its base.

4. Discussion.

(a) *The Intra-matrical System.*—Ph. van Tieghem's paper on the "Inovuleæ" makes it necessary to demonstrate here once again some facts discovered well-nigh a century ago. As pointed out earlier in the account, van Tieghem believes, as Hooker did, that the system of "cordons vasculaires" in the tuber of *Balanophora* belongs to the parasite and is constituted entirely of parasitic tissues. The most important reason for this belief is that he finds in the primary stele of this vascular cylinder a centrifugal arrangement of the proto- and meta-xylem groups, which is typical of the epicotyl or the stem. That being the case (he proceeds) the root of the host cannot be conceived of as giving rise to a stem-stele, and therefore this stele must belong to the parasite. This having been further confirmed by the absence of the root-cap, root-hairs, etc., he takes the absorptive cells to be medullary secretory tissue, and the entire tuber is described as possessing a polyarch stele.

A striking feature of the cross-section of the intra-matrical host-root in *B. indica* is the obvious difference between the tissues of the parasite and those of the host, in the size of the cells. In spite of the intimate association of these contiguous tissues, this persistent difference makes the distinction between the two easy and unmistakable, even in the meristematic region. In a longitudinal section, this small-celled tissue can be traced downwards to the host-root, and although, outside the tuber, in the normally developing root, the cells are bigger in size, almost everyone of the host tissues within the tuber is in uninterrupted organic continuity with those outside it. This is particularly well seen in young tubercles. Again a few tubers with the attached portions of the host-roots were macerated in boiling solution of caustic soda and these on washing away the parasitic tissues, gave beautiful "skeletons" of the parasite, consisting of the entire system of the intra-matrical roots, perfectly continuous with the main host-root (Pl. XLVIII, Fig. 2). In short, the continuity of the intra- and extra-matrical portions is so obvious even at a glance that it seems superfluous to enter into details. The structure of the intra-matrical roots, it must be admitted, does not

possess all the characteristics of a root-stele, but assuredly enough, it does not possess those of the stem-type either. From the region of the primary meristem onwards, one observes the host tissues sundered apart by the absorptive-cells, their normal course of development entirely altered. The formation of the proto- and meta-xylem is irregular and phloem-groups are indistinguishable; the rest of the tissues remain undifferentiated till the development of secondary wood. These abnormalities are only to be expected in a system which is under the direct influence of and in immediate contact with the parasite, which establishes with its host an extremely intimate relationship.

Van Tieghem's error, however, is not quite unaccountable. The relation and association of the two tissues, host and parasite, are so intimate everywhere, even in the region of growth, that no distinction can possibly be made between the two, save where the difference in the size of the cells is sharply marked. The instance of *B. dioica* has already been cited, where the two meristems are not so easily distinguished from one another as in *B. indica*. Such was probably the case in the material investigated by van Tieghem. Nevertheless, it is astonishing how he could have failed to note the perfect continuity of the intra- and extra-matrical host-roots.

Though it has long been known that the intra-matrical vascular system belongs to the host, the fact that each of those strands corresponds to an entire root seems to have been noticed only by Heinricher or probably Solms-Laubach, before him. (Unfortunately neither of these papers was available to the writers.) It has already been pointed out that almost every one of the tissues of the normal host-root has a counterpart in a well-grown intra-matrical root, whose structure, however, is considerably modified under the influence of its parasitic environments. The most important of these modifications are the absence of a root-cap, a differentiated epidermis and root-hairs, besides the malformations of the primary stele, the imperfect development of the phloem and bark and the dichotomous mode of branching. That all these modifications induced in the root structure are attributable solely and exclusively to the influence of the parasite upon it was strikingly confirmed in a certain tuber of *B. indica*, in which the intra-matrical system had grown out beyond the tuber (Pl. XLVIII, Fig. 3). This tuber was found growing upside down, probably having been turned over by gardeners working in the neighbourhood or by rodents. It was partly buried in the moist soil with the host-root above it, and must have been lying thus for a considerable time, for the tender, pale-white roots which grew out of tiny pits in the tuber were already about a couple of inches long. They had no root-hairs, but in all other respects (including

the possession of a root-cap) they showed the normal structure of young dicotyledonous roots, and their respective tissues were continuous with those of intra-matrical system (Pl. XLVIII, Fig. 4). The undifferentiated parenchyma of the latter was re-elaborated in them into the usual tissue layers of the roots, namely, the endodermis, the pericycle, the cortex and the epidermis, and the normal size of the cells was restored (Pl. XLVIII, Fig. 5). Absorptive cells of the parasite were totally absent and lateral roots were produced endogenously (Pl. XLVIII, Fig. 6). Altogether this little find entirely laid open the facts of the case, and was so interesting that it had to be laid aside for a separate study.

The above facts undeniably go to show that despite the continued influence of the parasite upon it, and the more or less fundamental changes brought about in its structure and behaviour, the host-root system within the tuber is yet capable of re-assuming the normal form of growth when this influence is removed.

How these roots originally arise from the parent host-root and become modified is yet an unsolved question. At least two alternatives have been suggested. One that the radicle of the parasite seedling penetrating into the young host-root splits apart its vascular cylinder and these split portions are drawn in to the parasite-tuber. If this were the case, it should be assumed that each of these strands develops itself, by the activity of its cambium, into a (potentially) complete root. The other alternative is that the influence of the contact or the early penetration by the parasite radicle on the host-root, stimulates the latter to produce a lateral root into the parasite tubercle, this induced rootlet later giving rise to the intra-matrical root-system. The growth of one of the intra-matrical root branches into a normal root outside the tuber is strong evidence in favour of the second alternative. It is only by a study of germination and of the early stages of tuber-formation that this problem can be finally solved. At present, the question is best left open.

(b) *The Mode of Growth.*—The location of the growing regions deeply imbedded within the tissues of the parasite and the "centrifugal" growth of the tuber appear at first sight to be contrary to the normal modes of growth generally obtaining in plants. Indeed, the entire structure and developmental course of the parasite-body is profoundly modified by the absence of apical or intercalary growing-regions in the ordinary sense of these terms. That the growth, however, is really apical and essentially similar to that of the other flowering plants is evident on a little reflection. But before interpreting this "centrifugal" growth of *Balanophora* in terms of apical intercalary growth, it is necessary to conceive of the tuber as an enlargement of

the hypocotylous segment of the seedling, which alone develops, while the other embryo organs are, for the time being, suppressed. This is Goebel's interpretation. Goebel¹ considers that *Balanophora* in this respect, stands a step ahead of *Orobanche*, in which the radicle penetrates into the host-root and gives rise to a tiny tubercle outside the host-root, and from this the floriferous shoot arises adventitiously. This is exactly what obtains in *Balanophora*, only here the development of the tubercle proceeds to a far greater extent with resultant modifications. Now one of these modifications is the growth of the host-root and its penetration into the tuber. In this case, one has to consider that the radicular growing point is itself carried inward into the tuber by the growth of the host-root and comes to be centrally situated. Later, by inducing a repeated forking of the host-root, it divides itself into numerous centres of cell-multiplication, and results in the formation of a tuberous, lobed body. Under these conditions, the growth of the host-root is inseparably bound up with its penetration by the parasite-cells, and the parasite is thereby enabled to possess a meristematic tissue outside the root as distinct from an absorptive tissue inside it. These relationships of the host and the parasite are diagrammatically shown in Plate XLIX, Fig. 13.

The endogenous origin of the inflorescence-shoot has a significance bearing upon the morphological interpretation of the tuber. Goebel's hypocotylar interpretation is based primarily on two grounds; namely, the endogenous origin of the floriferous shoot and the evidence from allied plant-parasites, such as *Cynomorium coccineum* and the *Orobanchaceæ*, in which the stages of germination and the formation of hypocotylar tubercle have been investigated. In the absence of corresponding investigations in *Balanophora*, the adventitious formation of the inflorescence may be considered a little further.

Cases of origin of shoot-buds from radicular organs are well known; *Linaria vulgaris*, *Solanum dulcamara* and many of the *Rosaceæ* (*Phyrus*, *Prunus*, *Rubus*, etc.) are among the outstanding examples. But the interesting feature of the adventitious bud of *Balanophora* is the development of the cavity and the formation of a free surface prior to the appearance of the inflorescence primordium. A digestion of the tissues in contact with the growing point as in the formation of a lateral root does not take place here and though one or two cells do appear to undergo disintegration, they do so long before the formation of a meristematic region abutting on the cavity. The cavity is mainly schizogenous in origin. In the light of the mode of growth of *Balanophora*-tuber, where the growing point is covered over all round by the tuber-tissue, the inflorescence arising

on a free surface of a cavity may be given an altogether different interpretation. It may be suggested that the inflorescence is truly exogenous, for with this mode of growth, the bud which would normally be formed on the surface of the vegetative body, would find its growth arrested at the very start by the overlying tuber-tissues. The formation of a free surface for the development of the bud is thereby hindered and postponed and takes place at a later stage. In other words, the formation of the cavity here is the re-formation of the free surface, previously closed over at the meristematic region, which would mean that the bud is really exogenous in origin.

The above interpretation is somewhat hypothetical, for at present there is little evidence to support it. So far as the present observations show, the cavity is entirely a secondary formation, and not an enlargement of a pre-existing cavity or a "free surface" grown over. The inflorescence should for the present be regarded as endogenous and with the assumption of Goebel's interpretation, the schizogenous nature of the cavity may be looked upon as a minor adaptation.

5. *Summary,*

The paper deals with the morphological relationships of the host and parasite, as well as the manner of origin of the inflorescence. The growth of the intra-matrical host-roots and the development of their tissues which proceeds side by side with the growth and development of the tuber, is described. The intimate relationships of the host and parasite are shown by their contiguous meristems which grow more or less against one another while also adding to their respective tissue systems. While the host-meristem penetrates into the cell-interstices of the parasite-meristem, the latter develops cells towards the host-meristem which are pushed into the host-root during its growth, and form the absorptive tissue of the parasite.

The growth of the tuber proceeds primarily from the parasite meristems. The cells of the relatively peripheral layers of the tuber too multiply, though not rapidly, and render possible the continued activity of the interiorly-situated primary meristems.

The rudimentary conducting system of the tuber establishes connections with the intra-matrical root by means of cells with dense contents. These cells can be traced upto the cells which abut directly on the host tissues.

That the intra-matrical system traversing the tuber consists of the entire roots of the host was definitely proved in a certain tuber, in which

these roots had grown out beyond the tuber, and though these were continuous, tissue for tissue, with their intra-matrical portions, they showed the typical dicotyledonous root structure, normal in every respect; thus indicating that the abnormal appearance and behaviour of the intra-matrical roots is due solely to the influence of the parasite.

Prior to the formation of the floriferous shoot in a lobe of the tuber, a minute cavity arises in the parenchyma in the vicinity of a parasitic vascular strand, often due to the disintegration of one or two cells. It enlarges schizogenously and also by the growth of the lobe as a whole. The inflorescence primordium grows into this cavity as an outgrowth from the side of the vascular strand. By a relatively quick growth and the rapid development of scale leaves from the base, it soon outgrows the cavity and finally bursts through the lobe covering it. Vascular connections between the tissues of the primordium and the conducting strand below are formed in very early stages and attain their full development in later stages.

Though the origin of the inflorescence within a newly-formed cavity presents certain peculiarities, the evidence now available is insufficient to test the validity of Goebel's hypocotylar interpretation of the tuber.

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EXPLANATION TO PLATES.

The magnifications given in the explanatory paragraphs below refer to the original drawing. Where the drawings have been photographed, the reduction in size of the entire plate has been mentioned separately in the case of each plate.

PLATE XLVIII.—(1) Young tubercles of *B. indica* with host-roots (Photograph) $\times 4$. The Intra-matrical System: (2) *B. indica*. The woody portions of the intra-matrical root system obtained by macerating tubers. In each case, extra-matrical main-root may be seen. The intra-matrical portion is

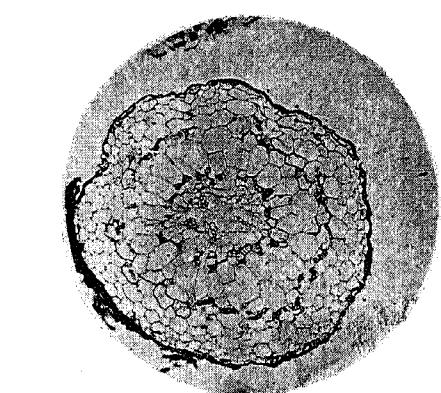
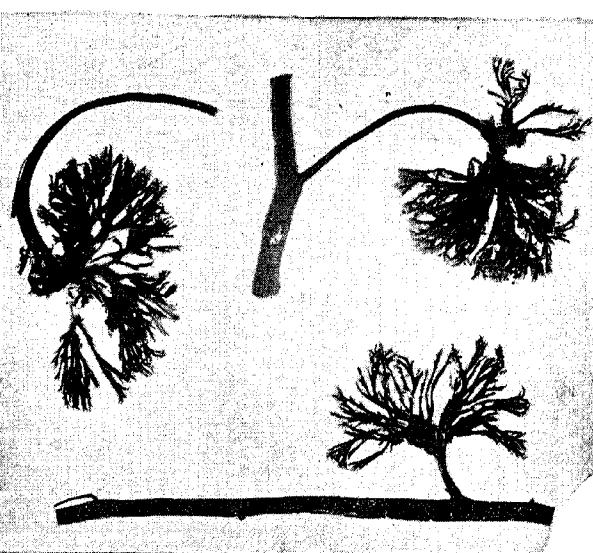
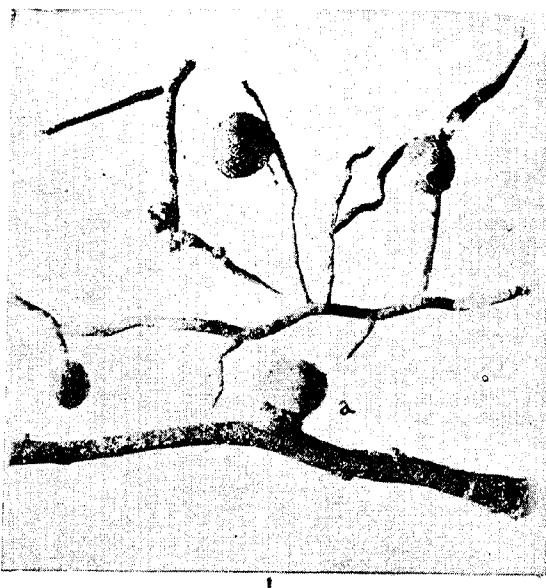
distinguished by its profuse branching (actual size photograph). (3) Parts of the tuber showing the host-roots growing out of the tuber. Description in the text. $\times \frac{1}{2}$ (Photograph). The Structure of out-grown root. (4) Longitudinal section of the tuber, showing the growth of the intra-matrical roots out of the tuber. The continuity of the intra- and extra-matrical portions may be observed at *a* and *b*. In the centre *c*, is a cross-section of one of these extra-matrical rootlets. $\times 20$ (Photomicrograph). (5) Transverse section through one of the rootlets grown out of the tuber. Note the normal development of the tissue systems, particularly the pentarch primary stele. $\times 80$ (Photomicrograph). (6) Longitudinal section through a root grown out of the tuber, showing the origin of a lateral root. $\times 100$ (Photomicrograph).

PLATE XLIX.—The Anatomy of the Tuber (*B. indica*, except Fig. 4). (1) Diagrammatic longitudinal section of a tuber showing the distribution (slightly exaggerated) of the intra-matrical host-root system within the tuber (actual size). (2) Longitudinal section through a growing point at the distal termination of an intra-matrical root, showing the small-celled host tissue of the parasite. Capping the host tissues is the meristem of the parasite and the host tissue immediately below it is the meristem of the intra-matrical host-root. \times about 150. (3) Part of a section of a tuber showing a young intra-matrical root in transverse section. The irregular development of proto- and meta-xylem and the indications of a cambial ring may be made out in the host tissues. The outer boundary of the sheath of parasite-cells is shown on the left. (The cell-contents of the host-cells have not been shown.) (4) Part of a section of a tuber of *B. dioica* showing a slightly older intra-matrical root, with developed proto- and meta-xylem and a wide and fully-formed cambial ring. \times about 75. (13) Diagrammatic longitudinal section of a *Balanophora*-tuber illustrating the truly apical growing regions of the parasite pushed inwards by the growth of the host-root. The host tissues (shown dark) are interspersed with parasite absorptive-cells. The host and parasite systems are separated, as it were, from one another for the sake of clear demarcation. In the parasite-tuber, the meristem (closely dotted) may be seen wedged in at several points by the penetrating meristem of the host-root. The outer limit of the sheath of parasite cells is represented by a fine dotted line on either side of the intra-matrical root. (\times about 17.)

PLATE L.—The Anatomy of the Host-Root. (1) Part of a longitudinal section of the tuber of *B. indica* showing the intra-matrical host-root in cross-section. Description in text. $\times 64$. (Photomicrograph). (2) Transverse section of the extra-matrical portion of the host-root. $\times 64$ (Photomicrograph).

PLATE LI.—The Origin of inflorescence in *B. dioica*. (5) Semi-diagrammatic longitudinal section of one of the lobes showing an inflorescence primordium and a tiny "accessory" cavity with a parasitic conducting strand, just below the primordium. $\times 10$. (6) The inflorescence cavity arising in the parasite-parenchyma in the neighbourhood of a parasitic vascular strand; one or two tracheids may be seen in the latter. The contents of the cavity indicate the degeneration of a cell at the place. $\times 90$. (7) The inflorescence-cavity growing schizogenously. $\times 38$. (8) The same at a later stage. $\times 38$. (9) Longitudinal section through the cavity showing the early indications of the formation of the inflorescence-primordium. The development of thin-walled cells in the region between

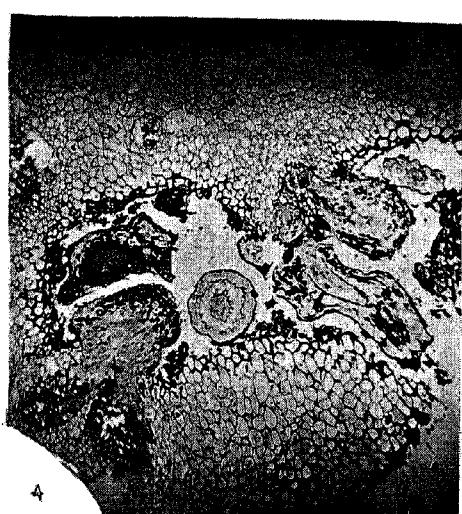
the vascular strand and the cavity is noteworthy. $\times 90$.
(10) Longitudinal section showing the inflorescence-primordium; early stages. $\times 90$. (11) Semi-diagrammatic longitudinal section of a lobe containing an inflorescence primordium. $\times 24$. (12) Semi-diagrammatic longitudinal section of the inflorescence-primordium at a later stage, showing the development of scale leaves. $\times 33$.



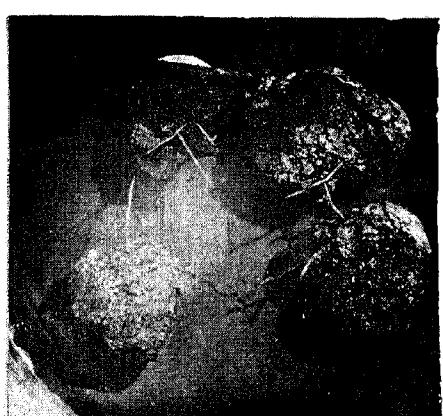
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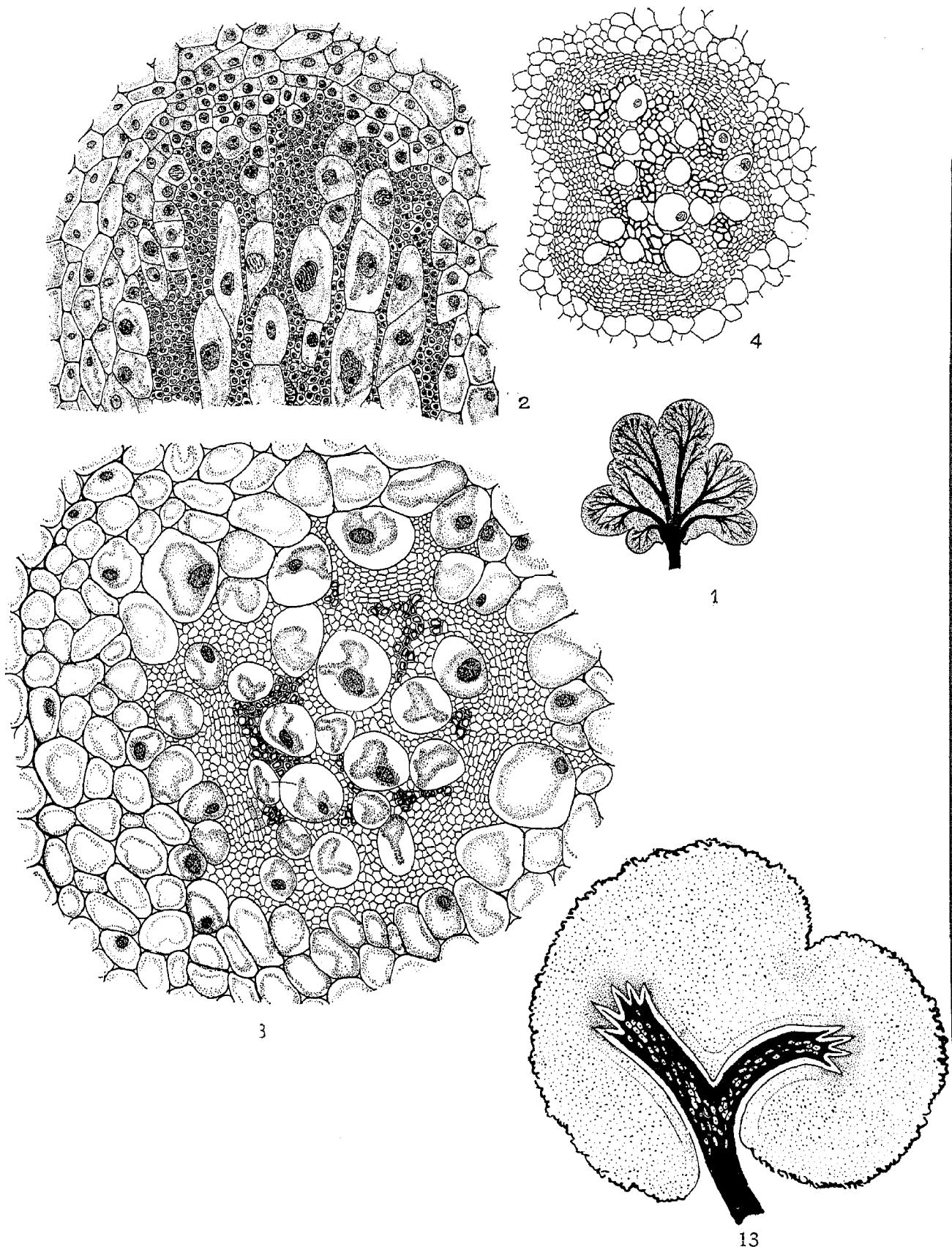
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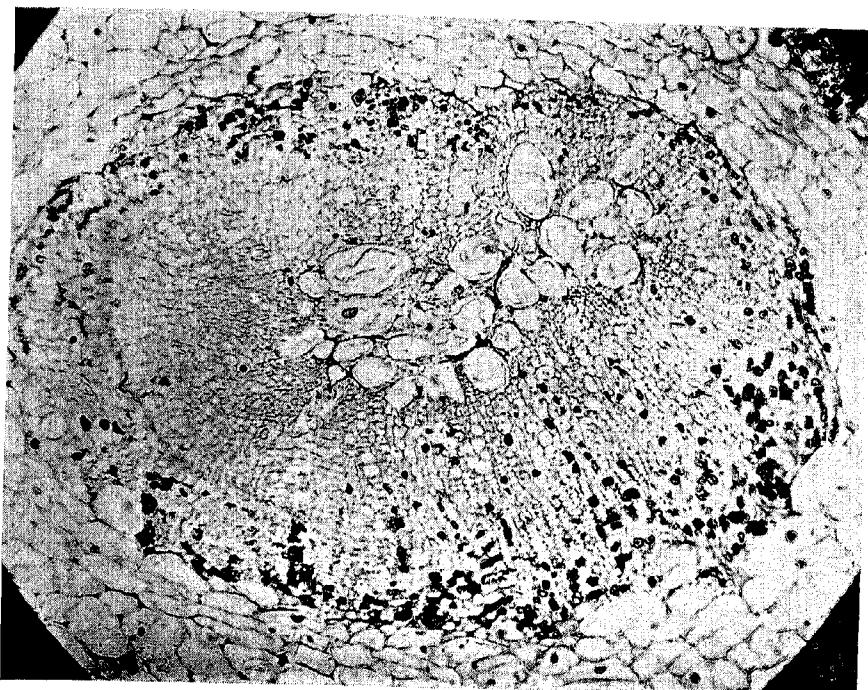


FIG. 1.

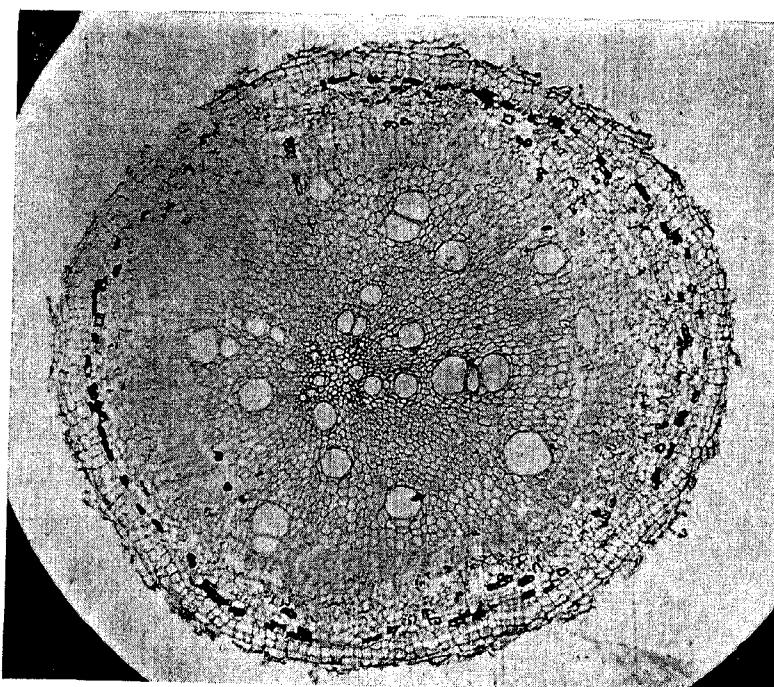


FIG. 2.

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