Studies in the Morphology and Anatomy of the Ophioglossaceae.

I. On the branching of *Botrychium Lunaria*, with notes on the anatomy of young and old rhizomes.

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With Plates XX and XXI and fourteen Figures in the Text.

IN these studies some results obtained by the re-investigation of examples of all three genera of the Ophioglossaceae will be described. The affinities of this group of plants have long been a matter of doubt and dispute, but of recent years the evidence for the early view that the Ophioglossaceae are a primitive group of Ferns has been greatly strengthened. This has resulted less from their further study than from the increase of our knowledge regarding other primitive Ferns, both extinct and recent. While my study of the Ophioglossaceae has led me to hold this view as a highly probable working hypothesis, the object of these papers is not to expound it, but to examine in detail some facts which must be taken into consideration, whatever conclusion as to the phylogeny of the group is ultimately arrived at. It is hoped that this will be of assistance to investigators who are engaged in the study of extinct Ferns. The full discussion of results will be deferred until the last of these studies, but the bearing of particular points of interest will be briefly considered in each paper.

EXTERNAL FEATURES.

The general morphology, anatomy, and life-history of *Botrychium Lunaria* are well known from the earlier researches of Braun, Roeper, Hofmeister, Russow, Holle, and others, supplemented by the study of various points of detail by later investigators. Familiar as our native species of *Botrychium* is in laboratory work, it is difficult to collect a complete account of the anatomy of its rhizome from the literature. More

attention has been paid to such larger species as *B. ternatum* and *B. virginianum*. Russow¹ studied *B. rutaefolium* (A. B. and Koch.), a form of *B. ternatum*, while the structure of *B. virginianum* is well known from the work of Jeffrey² and Campbell.³ A knowledge of these species of the section *Phyllotrichium* does not, however, explain the structure of *B. (Eubotrychium) Lunaria*; this appears to be of special importance for the interpretation of the stelar anatomy in the genus.

The starting-point of my re-examination of *B. Lunaria* was the need of fuller information regarding the internal endodermis of this plant for comparison with *Helminthostachys*. The internal endodermis was described for young plants of *B. Lunaria* by Poirault⁴ and Van Tieghem,⁵ and while my work has been in hand Bower⁶ has given a more detailed description than we previously possessed of this feature in the anatomy. Part of the present paper traverses some of the ground covered by Bower. The study of the progression in structure from the embryonic to the adult regions of the rhizome has led to the recognition of some new points in the structure of the stele and leaf-trace and to the investigation of the branching of the rhizome.

The description given by Hofmeister,⁷ supplemented by the investigations of Bruchmann,⁸ shows that the young sporophyte is attached to the subterranean prothallus by a fairly large foot. The first leaves have the form of small scales, and the first roots, corresponding to these, develop on a region of the stem, which remains very short. According to Bruchmann, elongation of the axis does not become marked until five or six roots have developed, and it is not until the eighth or tenth leaf that the plant appears above the surface of the soil. The detailed study of the anatomy and growth relations of very young sporophytes of successive ages would be of great interest, but lack of sufficient suitable material prevented me from attacking the problem in this way. Uninjured plants, however, even when of large size, retain at the base the first-formed region of the rhizome. The structure of this region can thus be easily studied and the transition to the adult structure followed.

When a number of plants are compared they are found to present considerable variety in external form. A number of roots are always attached close together at the lower end of the rhizome, and this *basal region* evidently bears the crowded roots that are such a conspicuous feature of the young sporophytes figured by Hofmeister and Bruchmann. Sometimes the transition to the thicker region of the rhizome with adult structure is a direct one; the diameter of the rhizome increases until the full size

¹ Vergleichende Untersuchungen, p. 119.

² Trans. Canad. Inst., vol. v, p. 265.

³ Most recently in The Eusporangiatae, to which work reference may also be made for the general bibliography of the subject.

⁴ Ann. Sci. Nat., Sér. 7, t. 18, 1893, pp. 169, 170.

⁵ Journ. de Botanique, 1890, p. 405.

⁶ Annals of Botany, 1911, p. 537.

⁷ Higher Cryptogamia. Ray Soc., 1862, p. 307.

⁸ Flora, 1906, p. 203.

is attained, but the leaf-scars and roots are closely crowded, and there is no appearance of specially elongated internodes. Often, however, an *intermediate region* is present between the basal and the adult regions; in this intermediate region the rhizome is slender and the leaf-scars and roots are distant. It is of variable length, being sometimes represented by one or a few internodes, while in other cases it attains a length of several inches and bears a considerable number of leaves. Possibly the occurrence of this slender elongated region, and its greater or less development, may be related to the depth of the parent prothallus beneath the surface of the ground. Above the intermediate region, when present, the rhizome increases in diameter rather suddenly and then maintains the greater diameter. In this *adult region* the leaves and roots are again crowded, and there is no appearance of internodes.

The distinction here made on the ground of external form between basal, intermediate, and adult regions of the rhizome has to be borne in mind in considering the anatomy. When the stelar structure is followed from below upwards the basal region is found to exhibit the transition from a solid protosteles to a medullated stele. In the region above this a medullated stele with a distinct ring of primary xylem, but little or no secondary thickening, is met with. When the intermediate region is marked the leaf-gaps are frequently very long, and it is in this region that the internal endodermis discovered by Poirault, and recently re-described by Bower, may occur. A transitional region with increasing development of secondary wood around the primary xylem leads to the adult region; in this the much larger stele has a marked development of secondary xylem, the leaf-gaps are short and often overlap, and an internal endodermis is always wanting.

The nature of the secondary thickening in *Botrychium Lunaria* will be discussed with some other points of detailed anatomy below. It will be convenient first to consider the general progression in stelar structure from the basal to the adult regions of complete plants, the relations of the external endodermis to the internal endodermis when the latter is present, and the bearing of these facts on the nature of the pith.

PROGRESSION IN STELAR ANATOMY FROM THE BASAL TO THE ADULT REGIONS OF THE RHIZOME.

For the study of the vascular skeleton of *B. Lunaria* six small plants were cut into complete series of transverse sections. The results obtained by detailed examination of these plants are recorded in the accompanying reconstructions of the vascular system (Text-figs. 3-8). The results have been checked by less complete study of larger plants and by comparison with longitudinal sections. In this way it has been possible to analyse the facts in greater detail than was done by Bower in the paper in which he

directed attention to the problem of the medullation of the Ophioglossaceae. The facts to be described are in substantial agreement with his results, but they go beyond them and have led to somewhat different conclusions.

Before describing the six plants in order the relations of the endodermis to a departing leaf-trace as seen in transverse sections may be referred to. The ordinary type in the adult region is represented in Text-fig. 1, *a-d*. The earlier preparations for the departure of this trace are seen in Text-fig. 9, 20, 21; this shows how the xylem separates first on one side, and that

TEXT-FIG. 1. Endodermal relations in the departure of a leaf-trace from the adult region of the stele. Description in text.

a bulge in the endodermis indicates the commencing departure of the trace. This arc of endodermis accompanies the trace on its abaxial side (Text-fig. 1, *a b, c*). On the separation of the trace an open gap is left in the endodermis of the stele (Text-fig. 1, *c*), but the endodermis continues inwards on either side for a short distance round the margins of the gap in

the xylem. The interest of this particular node is that it shows clearly that the inward extension of the endodermis is evident before the external endodermis is interrupted (Text-fig. 1, *a, b*). In other cases this may not be obvious. The most natural way of regarding the extension of the endodermis seems to be as an assumption of endodermal characters by certain intrastelar cells and not as indicating an intrusion of cortex. This is more clearly shown in Text-fig. 2, which represents the departure of one of the earlier traces of plant A (cf. Text-fig. 3). In this case the new formation of endodermis appears as a reparatory band, completely bridging across the gap before the trace has actually separated. There is thus never an open gap in the endodermis. The adult type

TEXT-FIG. 2. Endodermal relations in the departure of a leaf-trace with no gap in the external endodermis. Description in text.

may be placed in relation to this by regarding the new formation of endodermis to either side as corresponding to an incomplete reparatory band. These two types of leaf-trace departure do not exhaust the variety of endodermal relations found at the leaf-gap in *B. Lunaria*. Other examples where the process is complicated by the presence of an internal

endodermis are seen in Text-fig. 9. 5 and 12-14, and will be referred to later. This account will, however, suffice to make clear the longitudinal reconstructions, to the consideration of which we may now turn.

The conventions upon which the diagrams (Text-figs. 3-8) have been constructed must be borne in mind in making deductions from them. The diagrams represent the stele of the stem and the leaf-traces departing from it. Only the xylem and the endodermis are indicated, but it will be understood that conjunctive parenchyma, phloem, and pericycle come in order between the xylem and the external endodermis; phloem is wanting opposite the leaf-gaps and there is no internal phloem. The leaf-traces, which are really spirally arranged, are represented as if they formed two orthostichies and arose alternately. The level of origin of root-traces is indicated, but it must not be inferred that they arise vertically below the leaves as shown; for this reason the endodermis is not represented as disturbed by the root-traces. The position of the apex of the stem is indicated by a star, and the crosses in the leaf axils mark the position of vestigial lateral buds. The xylem of the stele and leaf-traces is represented as if seen in longitudinal section. So also is the external endodermis, which is represented by a line. The internal endodermis is supposed to be seen in surface view also, as if the stele were split in half; the extent of an incomplete internal endodermis can thus be represented. The surface view of the internal endodermis is dotted, and the slight inward extension of the endodermis round the margin of the leaf-gap is similarly shaded. When the internal endodermis was complete its section is represented by a line. The convention by which the endodermis is represented will be readily understood by comparing the reconstruction in Text-fig. 8 with the transverse sections of the same stele in Text-fig. 9. The diagrams were all constructed to the same scales, but the longitudinal and transverse scales are different, so that the stele is represented as broader in proportion to its length than is actually the case. Owing to its length, Text-fig. 7 has had to be reduced as compared with the other reconstructions.

All the six plants showed at the base a small stele with solid xylem. This basal region was complete in all except plant E, and with this exception all the plants showed the crowded first-formed roots. A number of roots were attached before any leaf-trace or leaf-gap was evident. From Bruchmann's account of the development of the sporophyte we may perhaps infer the existence of as many earlier scale-leaves as there are basal roots. If this is so, the lowest evident leaf-trace in these plants would not mark the first leaf; presumably the vascular tissue in the first developed scale-leaves was either absent or so small in amount that it did not affect the stele. In some cases it may have been displaced by the cortical growth and become unrecognizable; an example of this is seen in plant F, where, however, a corresponding leaf-gap is present in the stele.

In all six plants the solid xylem of the basal region becomes medullated a little below the first evident leaf-trace, but the plants present considerable variety in the transition from the basal region to the adult structure, and the differences are very marked in relation to the degree of development of an internal endodermis. While doubtless not exhausting the possible range of variation, these plants appear to afford sufficient data for a critical study of the nature of the internal endodermis in *B. Lunaria*. Plants A and B have no internal endodermis in their gradual passage to the adult

structure. In plant C there is a deeper inward extension than usual of the endodermis at one long leaf-gap, but the internal endodermis is never complete. Plant D is similar to the preceding one, but the internal endodermis is completed for a short distance. In plants E and F there is a still more marked intermediate region in the rhizome, and the internal endodermis is complete for a considerable distance, not only at the leaf-gap, but above and below this.

TEXT-FIG. 3. Reconstruction of the stele of plant A. Description in text.

In plant A (Text-fig. 3) the stele begins below with a slender solid strand of tracheides. In this, as it widens out, a small parenchymatous pith appears, and the medullated condition persists throughout the further growth of the plant. Before the preparation for the departure of the first recognizable leaf-trace is evident, five roots have been given off close together from the solid stele and lower portion of the medullated stele. No trace of any vascular supply to the scale-leaves, which were presumably related to these roots, was found. The first evident leaf-trace is very small, and dies out in the cortex a short distance from the stele; the second is only a small strand, but reaches the base of what was evidently a large scale-leaf. The third passes out from the stele as a considerable

trace, while the fourth and succeeding traces are larger and probably supplied the first green leaves. The departure of the first three leaf-traces involves no breach in the external endodermis, a complete reparatory band of endodermis being formed internal to the departing trace (cf. Text-fig. 2). The fourth and following leaf-traces depart from the stele, which shows a marked increase in diameter, according to the adult type (cf. Text-fig. 1).

This is the plant described by Bower on p. 540 of his paper, and a section at the level of departure of the fourth leaf-trace is figured by

him to show the presence of tracheides mixed with the pith-cells.¹ It need only be added regarding this feature that tracheides occurred in the middle of the pith of the adult region of this plant close to the apex. Their position and that of the tracheides figured by Bower are indicated in the reconstruction. This plant is of interest as showing a direct and gradual transition to the adult type of stele without the intervention of any marked intermediate region. The leaf-gaps are all short, and below the adult region they are at once closed by endodermis. There is nothing in the structure of the plant or in the endodermal relations to suggest that its pith is in any way due to intrusion of cortex, while the position of the tracheides in the pith seems direct evidence against this.

Plant B, the stele of which is reconstructed in Text-fig. 4, shows a direct and even more rapid transition to the adult type of stelar construction; in it also there is no internal endodermis and nothing suggestive of cortical intrusion throughout the whole plant. The slender solid strand of xylem at the base of the plant increases in diameter on passing upwards; scattered parenchyma cells appear in it and then a definite pith is established. Four roots are attached close together in the region below the first leaf-gap. The small leaf-trace in relation to this dies out in the cortex, and the leaf-gap closes at once; unfortunately the endodermal markings were not very clear, but the relations appeared to be similar to those at the lower leaf-gaps of the preceding plant. On the closure of the leaf-gap a root-

TEXT-FIG. 4. Reconstruction of the stele of plant B. Description in text.

¹ Bower, loc. cit., Pl. XLV, Fig. 10.

trace arises from the stele to one side of the position of the gap, followed by a second from the other side of the stele. The next leaf-gap opens in the xylem ring and a small trace departs. The endodermal relations at this and the succeeding leaf-gaps are of the adult type, in that a gap remains for some

distance in the endodermis, the edges of the latter being continued round for a short distance. After the trace has separated from the stele a root arises from the latter to one side of the gap. It is unnecessary to follow this plant node by node, as the relations are evident from the reconstruction. The stele is relatively bulky and the primary xylem thick, the same type of leaf-trace departure is maintained, and shortly after the separation of each trace one or two root-traces arise from the stele. There is thus evident a certain segmental regularity in the relations of roots and leaves, which is also shown more or less distinctly in the other plants.

In plant C (Text-fig. 5) the solid xylem strand increases in diameter and bears several root-traces. About the level of the fourth root parenchyma appears in the centre of the stele and is continuous with the outer conjunctive parenchyma of the stele at what must probably be regarded as the first leaf-gap, although no leaf-trace passes off. Above this the xylem of the medullated stele is again completed, and two root-traces pass off. The leaf-trace which passes off from the second leaf-gap dies out in the cortex, while the third trace reaches a leaf. The endodermis is completed preparatory to the departure of both these traces. The third gap in the xylem remains open for some distance, and when it closes preparations for the fourth leaf-trace are evident. The departure of this trace is very gradual and the leaf-gap is correspondingly elongated.

Before any interruption of the external endodermis an incomplete internal endodermis appears. This forms two strips extending from either side of the incipient trace; on the separation of the latter the internal endodermis becomes continuous on either side with the margins of the gap in the

TEXT-FIG. 5. Reconstruction of the stele of plant C. Description in text.

external endodermis left by the departure of the leaf-trace. The condition is thus much as in the adult region, except that the endodermis is continued much more deeply into the pith. At no level, however, is the internal endodermis complete. When the external endodermis forms a complete ring, and before the gap in the xylem closes, the endodermal markings within the stele cease. The next leaf-trace is much larger, its departure follows the adult type, and the leaf-gap is short. The stele has now become wider and the zone of xylem thicker, with indications of secondary growth. This plant thus shows an imperfect internal endodermis associated with one extended leaf-trace departure.

Plant D may be described very briefly, since, as Text-fig. 6 will show, it only differs in one essential point from the preceding plant. Here also the first leaf-traces are small and the endodermal relations at the leaf-gap conform to the adult type. The interest of this plant lies in the slow departure and the long leaf-gap left by the third leaf-trace. This constitutes the specialized intermediate region. After the xylem of the third leaf-trace has separated from the rest of the xylem, but before any interruption of the external endodermis, endodermal markings appear on the radial walls of cells within the arms of the horseshoe-shaped xylem of the stem. This internal endodermis is at first quite unconnected with the external endodermis. As the leaf-trace separates and the external endodermis is interrupted, these bands of internal endodermis become continuous with the free ends of the gap in the outer endodermis. This then appears to extend deeply into the pith, lining the tube of xylem, but not forming a complete internal endodermis. The condition is thus the same as in the long leaf-gap of the preceding plant. On passing further up the gap in plant D, however, the internal endodermis does become complete for some distance.

TEXT-FIG. 6. Reconstruction of the stele of plant D. Description in text.

TEXT-FIG. 7. Reconstruction of the stele of plant E. Description in text.

Still further up, the endodermal markings disappear from the cells lining the inner portion of the concavity, and only the slight inward curves of endodermis usual in the adult type of leaf-gap are found. The endodermal gap then closes, and shortly afterwards the xylem ring. Thus the plant resembles plant C, except in that the internal endodermis becomes complete for a short distance in the elongated leaf-gap.

Plant E is the longest plant that was studied in complete series, and a glance at the reconstruction of the stele (Text-fig. 7) will show that a fairly extended intermediate region is formed by the very gradual departure of one leaf-trace, while above this came a rapid transition to the adult region. Owing to its length, this reconstruction has had to be reproduced on a somewhat lower scale than the others of the series. The base of this plant was imperfect, and, although the transition from a stele with solid xylem to one with a pith could just be followed, it is clear that at least one and probably a number of roots are missing from the base. The first recognizable leaf-trace dies out as soon as it leaves the stele. The next two are larger and the leaf-gap in the xylem more extended, but in all these three leaf-trace departures the endodermis is completed before the departure of the trace, and there is no gap in the endodermis. Owing to the place of development of the reparatory band of endodermis, there is an appearance of dipping-in opposite the leaf-gap; this dip is soon lost, the endodermis becoming convex outwards, and its explanation does not involve the need of 'pocketing'. In the third leaf-trace the endodermis is also completed around the trace itself. Shortly after the departure of this trace and before the gap in the xylem has closed the usual root is attached, and a little higher up the origin of the fourth leaf-trace, which is the interesting feature of this plant, commences. The departure of this trace is extremely gradual, and as soon as its position is evident in the

xylem ring the internal endodermis appears. This soon forms a complete ring, as seen in transverse sections, but the first appearance of the endodermal markings is irregular and the internal endodermis does not form a closed pocket at the lower end. As the distance widens between the leaf-trace and the xylem of the stele, both being still enclosed by the external endodermis, endodermal markings appear on the walls of cells connecting the outer and inner endodermal layers. Thus on the separation of the leaf-trace, first on one side and then on the other, both the trace and the vascular tissue of the stem have complete endodermal sheaths. That of the stem consists of the external endodermis connected round the margins of the leaf-gap with the internal endodermis. Above the departure of the leaf-trace first one and then another root-trace arises from the stele. At the upper end of the leaf-gap the incurved ends of the external endodermis approximate, and this again becomes a complete and independent tube. The internal endodermis persists for a short distance as an imperfect tube, but all endodermal markings have disappeared from the pith before the gap in the xylem closes. Above this remarkable leaf-insertion the stele increases in diameter, and the xylem ring is thicker and shows secondary thickening. A number of leaf-traces are given off with comparatively short intervals between them, the endodermal relations at their departure, like the general features of the stele, being of the adult type.

The last of the six plants (Plant F) shows much in common with the preceding specimen. Its structure is illustrated by the reconstruction of the stele (Text-fig. 8) and by the series of selected transverse sections in Text-fig. 9. The numbers 1-21 refer to the slides of this series, on each of which an equal number of sections was mounted; where the changes were rapid and several sections from one slide are figured they are distinguished by letters, e.g. 2 *a*, 2 *b*, 2 *c*, 2 *d*. Comparison of this series of sections with the reconstruction will make the relations of this rather complicated plant clearer, and will also show the general nature of all the reconstructions.

The first root was in line with the base of the stem, and the stele of the root when followed up passed into the solid core of xylem of the stem stele (Text-fig. 9. 1). This increased in diameter and showed a zone of tracheides radiating from a central group; a root is attached in this region (2 *a*). Parenchyma appears mingled with the central tracheides (2 *b*) and soon forms a distinct pith (2 *c*, 2 *d*). After two roots have been attached a small leaf-gap with no evident departing trace forms in the xylem ring, but a trace is recognizable close to the periphery of the cortex, having apparently been separated by the cortical growth (Text-fig. 9. 3. Compare the reconstruction, Text-fig. 8). When this gap has closed, two roots, one of which is shown in Text-fig. 9. 3, arise from the stele. Then another root-trace arises (4 *b*), and immediately above this the second leaf-trace separates (5, 6).

While the first leaf-gap is closing and the second is forming, an internal endodermis appears in the pith. It is at first incomplete (4*a*), but soon becomes a complete tube (4*b*). The external and internal endodermis become

TEXT-FIG. 8. Reconstruction of the stele of plant F. Description in text. The divisions on the left correspond to the numbers in Fig. 9.

continuous as the second leaf-trace separates (5, 6). As the trace departs a root is given off from the stele to one side in the usual fashion (5). The second leaf-gap is a long one. The gap in the external endodermis remains open for some distance (6, 7) and then closes, the external endodermis again becoming a complete tube (8, 9). The internal endodermis is for some distance a complete tube (9), but then becomes incomplete (10-18). Before the second leaf-gap in the xylem closes the third gap opens (11, 12), the internal endodermis being present as an incomplete arc opposite to the new gap. As the preparations for the departure of this leaf-trace advance, endodermal markings appear on the cells bridging across from the internal to the external endodermis. Then the trace separates (13*a*, 13*b*) with a complete sheath of endodermis, the adaxial portion of which is continuous below with the internal endodermis; the external endodermis at the leaf-gap is continued inwards for a short distance by the side portions of the arc of internal endodermis (13*b*). The outer endodermis almost at once becomes continuous across the gap (13*c*), and at this level a root-trace is attached, followed by another on the other side of the gap (15). The third gap then closes in the xylem ring, and the fourth leaf-trace prepares to separate (14-16). The next root-trace is attached

to one side of the incipient fourth leaf-gap (16, 17). The internal endodermis, which has persisted as a more or less incomplete layer (13c-16), now becomes localized as an arc internal to the departing fourth leaf-trace (17, 18). It then dies out and plays no part in the actual departure of

TEXT-FIG. 9. Selected transverse sections of plant F, showing the progressive changes in the stele and the endodermal relations from the basal to the adult regions. Description in text.

this trace (19, 20), which follows the adult type, as do all the succeeding leaf-traces. Three traces depart in rapid succession, and then two roots are borne just below the meristematic region of the plant.

This plant has thus a marked intermediate region between the basal

and adult regions. In the intermediate region a more or less complete internal endodermis is present, extending over the origin of three leaf-traces. The distribution of the internal endodermis, which can be followed from the series of cross-sections and the reconstruction (Text-fig. 8), does not lend support to the idea of pocketing at the leaf-gaps. It is limited to the slender intermediate region, while in other respects the medullation of the stele does not differ from what is found in the regions below and above. Both the transverse sections and the reconstruction show how, after the stele has widened from the base of the plant (1-4), its diameter decreases greatly in the intermediate region (6-12) and then increases again till the uniform diameter of the adult region is attained (19-21).

Comparison of the vascular systems of these six small plants of *B. Lunaria* allows of a number of conclusions being drawn with tolerable certainty. On the whole, the distribution of the leaves and roots suggests a repetition of corresponding segments of the plant body whatever the ultimate explanation of the segmentation may be. Usually one or two roots are inserted on the stele close to the sides of the leaf-gap just after a leaf-trace has separated. There is not, however, a rigid relation of roots to leaves, for a number of leaf-traces may be given off without any corresponding roots. It may further be noted that it is difficult to distinguish nodes and internodes in *B. Lunaria*. When a long internode appears to be present this is found to be due to the extension both of the region in which the trace is gradually separating from the stele and of the leaf-gap left on the separation of the trace. Both these regions may be regarded as belonging to the leaf-base. The result attained is, however, the same as if definite nodes and internodes were present; the longitudinal extension of the shoot is increased, and the individual leaves are more widely separated. In this way the distance between a departing leaf-trace and the nearest roots below may be greatly increased, as is seen most clearly in the fourth leaf-trace of plant E.

All the plants showed a similar transition from a stele with a solid xylem to one with a pith. The appearance of a pith in the stele was never associated with the presence of an internal endodermis, and the transition from the basal region to the typical adult structure may be a direct and gradual one without the development of an internal endodermis in any region. The transition to the adult type of relation of leaf-trace to stele may take place close to the base of the plant (Plant B), or a number of the lower leaf-traces may depart without leaving endodermal gaps (Plant A). In both these cases, while there is a gradual increase in the size and complexity of the stem and its stele, there is no specialized intermediate region to be distinguished.

The development of an internal endodermis appears to be associated with the presence of a definite intermediate region, in which there has been

greater growth in length with extension of the leaf-trace departures and leaf-gaps. There may be an absolute decrease in diameter of the stem and stele as compared with the region below (Plant F), and in any case there is delay in assuming the full diameter of the adult region. In the plants selected for detailed examination the intermediate region only involved one or a few leaf-bases, but in other specimens it had a length of some inches. It appears as if intercalated between the basal and adult regions, possibly to bring the apex of the plant more rapidly to the proper depth below the surface of the soil.

The degree of development of an internal endodermis in the intermediate region varies, as comparison of plants C, D, E, and F shows. The endodermal markings appear before the leaf-trace has separated and (like the reparatory band of endodermis formed in the departure of the early leaf-traces) the internal endodermis seems to be a new formation. While transverse sections in which the external and internal endodermis are continuous show the appearance of an endodermal pocket described by Poirault and Bower, the reconstructions make clear that this appearance does not establish the existence of intrusive pocketing. In no case was a completely closed pocket found. Comparison of the mature structure of these plants thus leads to the conclusion that the internal endodermis, when present, is a new differentiation in the pith, and is of no morphological significance as indicating a limit between stele and cortex.

The development of the stele from the apical meristem will not be dealt with in this paper. It may be said, however, that this has been carefully examined and no direct evidence in favour of an actual intrusion of tissue into the stele at the leaf-gap has been obtained. Since the distribution of the endodermis when critically considered does not support the view of pocketing of the cortex into the stele, and direct evidence from the developing region is against any such readjustment of the tissues, it seems justifiable to conclude that the internal endodermis of *Botrychium Lunaria* is a new differentiation within an intrastelar pith. The association of the internal endodermis with the long leaf-insertions of the intermediate region suggests a physiological advantage in its presence, for it is found in regions in which there are long intervals between the roots and leaves.

SOME DETAILS OF THE VASCULAR ANATOMY OF THE RHIZOME.

The general construction of the vascular skeleton of the rhizome of *Botrychium Lunaria* has been considered in the preceding section. In this portion of the paper the structure of the stele in different regions of the stem will be described, especially as regards the xylem, and an account given of the structure of the leaf-trace until it enters the petiole. Further, the occasional development of xylem as a result of cambial activity

occurring in the pericycle will be recorded, and the resulting pericyclic wood described for both the stem and leaf-trace.

The stelar structure in the various regions of the rhizome is most readily appreciated from transverse sections. The chief features of interest concern the xylem, and it will be sufficient to state at the outset that conjunctive parenchyma, sieve-tubes, pericycle, and endodermis are found in the order named outside this. The staining and photographic processes have been directed to demonstrating the xylem as clearly as possible, but the other tissues of the stele are well seen in Pl. XX, Photos. 16, 17, as they occur in a stele of fair size without marked secondary thickening. It will be convenient to commence with the structure of the xylem in the definitely medullated stele, deferring the consideration of the transition from a solid to a medullated xylem till afterwards.

The stele represented in cross-section in Pl. XX, Phot. 1, is typical of a more or less extensive region of the rhizome after medullation has taken place. The wide pith is surrounded by a continuous tube of xylem two or three tracheides in depth. This I regard as primary xylem. No protoxylem is present, except when a leaf-trace begins to be recognizable, and then the endarch xylem of the trace corresponds to an arc of the primary xylem. Judging by the relative position of the protoxylem of the leaf-trace the primary xylem of the stem can be described as centrifugal; the question whether centripetal primary xylem can be recognized will be considered below. There is typically no cambium or secondary xylem, and the occasional radial arrangement of the tracheides can be accounted for by the direction of the divisions in the procambium. The stele shown in Phot. 1 has no internal endodermis, but it is quite characteristic of the intermediate region of the rhizome, where an internal endodermis may occur. The more bulky steles in Photos. 16 and 17 are similar in that the xylem is practically all primary, but the zone of primary xylem is thicker.

The transition to the thicker adult region of the rhizome may be gradual, but often takes place rather suddenly, especially when a marked intermediate region has been present. It is then associated with the beginning of secondary thickening of the xylem, though not due to this. Phot. 2 shows the structure in the transition region. The ring of primary xylem, which was alone present in the stele represented in Phot. 1, is seen around the pith, but active divisions have evidently gone on in the region immediately outside the primary xylem and an irregular zone of secondary wood has been developed. The incompleteness of the secondary xylem brings out the distinction between it and the primary wood.

In the light of these sections the structure of the stele in the fully adult region will readily be understood. This is represented in Phot. 3, and small portions of the vascular ring from two distinct plants are shown in Photos. 4 and 5; the latter figures are to the same scale of magnification

as Photos. 1, 2, 16, 17, while Phot. 3 is less highly magnified. It will be evident that the diameter of the stele has greatly increased, and also that the zone of xylem is much thicker. Next the pith comes a continuous zone of primary xylem, which is best seen in Phot. 4, and outside this the secondary xylem. The two facts that the secondary tracheides are in regular radial rows, and that the medullary rays are confined to the secondary wood, and therefore do not extend to the pith, enable the distinction between primary and secondary xylem to be readily made in *B. Lunaria*. The secondary tracheides are wider and rather shorter than those of the primary xylem. This is seen in Pl. XXI, Phot. 22, which is, however, selected to show another feature of the stele.

While primary and secondary xylem can thus be distinguished in *B. Lunaria* some qualification is necessary as to the sense in which the term secondary xylem is used. The succession indicated by the steles in Photos. 1, 2, and 3 is that found in the extended ontogeny of the plant. At the level of each section the stele was complete, and under normal conditions of growth no additions would be made to the xylem. Even in the case of the large stele of the adult region, with its well-marked zone of secondary xylem, the secondary thickening is not progressive. The tracheides of the primary xylem are the first to be lignified behind the apex, but the tangential divisions in the procambial cells outside the primary xylem are in main part completed close to the apex. What happens in the region somewhat further back is the maturing and lignification of the elements thus arranged in radial rows and forming the secondary xylem. The secondary thickening in this case thus appears to be an arrangement to increase the amount of xylem in the primary construction of the stem, and does not proceed indefinitely. The justification of the comparison of the secondary thickening in *B. Lunaria* with that of the higher plants is, however, clearly shown by the power of the meristem to resume the active addition of new elements to the xylem under special circumstances. A good example of this will be described below in one of the branching plants (Text-fig. 14).

The fact that in a considerable region of the rhizome all the xylem actually formed may be primary explains the divergent statements found in the literature regarding *Botrychium Lunaria*, since, according to the region of the rhizome examined, there may appear to be marked secondary thickening or none. On account of the importance of the memoir in which it appears, it seems advisable to specifically correct the recent statement by Campbell¹, that 'in *Eubotrychium* the cambium is absent, and apparently there is no secondary increase in diameter'.

The further question as to whether, in addition to the centrifugal primary xylem distinguished above, any representative of the well-marked centrifugal xylem found in *Helminthostachys* can be recognized in *B. Lunaria*,

¹ The Eusporangiateae, p. 102.

will be best dealt with by returning to the structure of the stele in the basal region. The strand of xylem at the extreme base of the plant is very slender, but soon becomes a thicker though still solid strand as it is traced upwards (Phots. 6, 7). It is composed of a small central group of irregularly arranged tracheides surrounded by a zone of tracheides that show a tendency to radial arrangement. I was at first inclined to interpret this as a centrarch structure, regarding the central tracheides as representing the protoxylem. Further study has not supported this interpretation, nor does it seem necessary to regard the radial arrangement of the peripheral tracheides as evidence of their being secondary xylem. It is sufficient and more useful for comparative purposes to distinguish the central and peripheral xylem in this region of the stele. At a slightly higher level (Phot. 8) parenchymatous cells appear among the tracheides, and, as described and figured by Bower, soon form a small pith in a central position. The pith forms in the central xylem (Phot. 9), and elements of this can usually be detected at the inner edge of the tube of xylem just after the pith has been initiated. The appearance of the pith is related to the first leaf-gap in the sense that, when this forms, the pith and the parenchyma outside the xylem become continuous, but there seems no evidence for the pith being derived by intrusion either of the outer conjunctive parenchyma or of the cortex.

The difficulty of arriving at a definite conclusion as to whether the central as well as the peripheral primary xylem is present in the expanded medullated stele higher up the rhizome is owing to the fact that protoxylem is only present in the leaf-traces entering the stele. Thus in the rest of the stele the protoxylem is wanting as a guide to the possible distinction of centripetal primary xylem from centrifugal primary xylem. It has been shown above that practically all the xylem of such a medullated stele as that represented in Phot. 1 is to be regarded as primary centrifugal xylem. But indirect evidence from several sources points to the existence of central or centripetal primary xylem also; as the question is of some importance the evidence will be stated fully.

While the inner surface of the tube of primary xylem abutting on the pith is often quite regular and only occasional tracheides could be suspected of belonging to the central xylem, this is not always the case. Thus in the stele represented in Phots. 16 and 17 the inner surface of the xylem is very irregular, and the groups of tracheides projecting into the pith are open to the suspicion of representing the central xylem. This is even more the case when, as often happens, a tracheide is met with separated from the xylem tube by one or several parenchymatous cells. A case of this kind is figured by Bower from one of the small plants reconstructed above, and, as stated previously, isolated tracheides developed in the centre of the pith of the adult region of this plant. The reconstruction in Text-

fig. 3 will show the position of these, and should be compared with the figure in Bower's paper.¹ The distinction of such irregular tracheides near the periphery of the pith from the outer primary xylem is strengthened when the departure of the leaf-trace is taken into account. The trace, at its departure, is always endarch, and the central tracheides disappear internal to it. But the position of the protoxylem of the nascent trace agrees with the distinction here drawn between outer and inner primary xylem in the rest of the xylem ring (cf. Phot. 16).

The distinction of the outer and inner xylem is also brought out in the mode of departure of some of the earlier leaf-traces from the small medullated stele. The endarch leaf-trace departs from the outer xylem only, so that where the inner xylem is fairly represented it bridges across the gap left by the departing trace. This is shown in Phot. 10, where a tracheide separates the pith of the stele (on the left) from the parenchyma adaxial to the small trace, the xylem of which is just about to separate. In Phot. 11 the same stele is shown a few sections higher up. The xylem of the trace has separated, but the pith is completely shut off from the leaf-gap in the outer xylem by a bridge of tracheides of the central xylem.² A similar case to Phot. 10 is represented in Fig. 12, Pl. XLV of Professor Bower's paper, though not interpreted in the same way. Such a case as shown in Photos. 10, 11 at once suggests comparison with those Osmundaceae in which the leaf-trace 'pocket' is shut off from the pith by the inner tracheides of the xylem, and it is possible to interpret the appearances described and discussed by Gwynne-Vaughan³ in the seedlings of *Osmunda* on these lines. How close the comparison is will be clear from the four sections of another plant of *B. Lunaria* at successively higher levels in Photos. 12-15. In Phot. 12 the xylem of a small leaf-trace is about to depart from the stem stele. The prominent group of parenchyma immediately within the trace is not the pith of the stele. This is represented by the small group of parenchyma more to the right. A little higher up the pith of the stem stele has disappeared, and the solid xylem now consists of a group of central xylem surrounded by peripheral xylem. The xylem of the leaf-trace, now separated from that of the stele, has departed in a protostelic fashion at the expense of the peripheral xylem only. That this interpretation is correct is shown by the higher section (Phot. 14), in which the pith of the stele has

¹ Annals of Botany, xxv, Pl. XLV, Fig. 10.

² While there was nothing abnormal about this plant, which was studied in a complete series, the lower region of the stele, as shown in Photos. 10 and 11, presented one remarkable feature. An external endodermis was present in the usual position, separated by one layer of cells (the pericycle) from the phloem. In addition to this another cylinder of cells, with the usual endodermal markings on the radial walls, was present some three layers of cells further out in the cortex. Such an anomalous development of endodermis has a bearing on the possibility of new formation of endodermis in *Botrychium*.

³ Loc. cit., Pl. XLIV, Figs. 3, 4.

commenced to reappear and from now on persists and enlarges. Not till the level reached in Phot. 15, where the small leaf-trace is dying out in the cortex, does the pith communicate with the leaf-gap parenchyma. The relations in this case are essentially similar to those described by Gwynne-Vaughan in *Osmunda*. The plant from which Photos. 12-15 were taken was further of interest in that after the stele had expanded to a medullated stele like that shown in Phot. 16, it again contracted and had scattered tracheides in the small pith.

It was pointed out above that occasionally tracheides may be developed in the pith of normal plants. A more extreme case of the same kind was met with in a small injured stem, which bore a branch. This will be described later on, and it is sufficient to refer to the reconstruction of this specimen (Text-fig. 11), to the series of transverse sections (Text-fig. 10), and to Phot. 31. These show that the development of tracheides internal to the position of the protoxylem had led to this region of the stem possessing a veritable mixed pith of tracheides and parenchyma.

The analysis of the xylem of *Botrychium Lunaria* in different regions of the rhizome thus leads to the recognition of secondary xylem, centrifugal (peripheral or outer) primary xylem, which is constantly present, and centripetal (central or inner) primary xylem, which is more or less clearly indicated. This brings the construction of the primary xylem of *Botrychium* into line with that of *Helminthostachys*, where centrifugal and centripetal primary xylem are well marked. That the centripetal xylem of *B. Lunaria* when present usually disappears opposite a departing leaf-trace and takes no part in its formation only strengthens the comparison with the centripetal primary xylem of *Helminthostachys*.

The leaf-trace of *Botrychium Lunaria* is usually and correctly described as departing from the stele of the stem as a single endarch strand of xylem, accompanied on its abaxial side by conjunctive parenchyma, phloem, pericycle, and endodermis in the same succession as these tissues are found in the stele of the stem (Photos. 3, 16, 17). On passing from the stem into the leaf-base the single trace divides into two strands (Phot. 21), which become inclined so as to face one another. The leaf-trace, however, exhibits considerable variety in its construction, and some of the main features observed will now be referred to.

When the stele of the stem has marked secondary thickening, the leaf-trace on its separation shows, outside the arc of primary xylem, secondary tracheides arranged in radial rows and separated by medullary rays. This secondary xylem diminishes in amount and disappears as the leaf-trace nears its departure from the stem (cf. Photos. 3, 20). From this point onwards the xylem of the leaf-trace is altogether primary, and is continuous, when traced downwards, with the primary xylem of the stem stele. When the trace departs from a stele without secondary

thickening its xylem is of course wholly primary from the beginning (Phots. 16, 17).

No trace of centripetal xylem has been seen to the inside of the leaf-trace when it separates from the stele of the stem,¹ even when the latter shows remains of the central xylem (Phots. 16, 17). The protoxylem of the departing trace abuts directly on the medullary parenchyma. The layer of cells to the inside of the xylem-ring can often be distinguished as of different appearance from the other cells of the pith, and this layer of cells is always clearly recognizable on the adaxial face of the xylem of the departing leaf-trace. As the trace separates the next layer of cells may assume endodermal characters, and so complete the endodermis of the leaf-trace adaxially (Phot. 18). Whether a complete endodermis is present or not the adaxial parenchyma can be recognized as a part of the leaf-trace; it usually increases by cell-division as the trace departs, so that the outline of the latter becomes oval. As the trace widens out on its passage through the cortex it assumes a rather flattened C-shape, the concavity being filled up or lined by the adaxial parenchyma. No phloem has been observed on the adaxial face of the leaf-trace.

The most interesting variant of the usual structure of the leaf-trace as described above is the development of tracheides on the adaxial side of the protoxylem; indications of these are found in most cases, and sometimes the adaxial xylem is well marked. The metaxylem of the trace then appears to curve round at the two ends, while more scattered tracheides are developed across the adaxial face of the trace joining the two ends (Phots. 18, 19). The adaxial tracheides are seen in longitudinal section in Phot. 20. The adaxial xylem is most marked when the trace is passing through the cortex of the stem, but occasional tracheides have been met with after the trace has entered the petiole and divided. A common state of affairs is for only a slight adaxial extension of xylem to take place at the two ends of the trace. This may probably be related to the tendency of the xylem of the trace to form a hook, as is shown in Phots. 21 and 27.

Such leaf-traces might be described as mesarch, but it is questionable whether this is a sufficient or right interpretation. It has been pointed out that the trace is never truly mesarch at its departure, the central xylem, if present, disappearing before the trace departs. It is, of course, possible to regard the adaxial xylem in the trace as a reappearance of the central or centripetal xylem, in which case the trace would be truly mesarch. But it seems to be better regarded as due to a more or less complete adaxial extension or completion of the centrifugal xylem of the trace; this produces a trace the xylem of which has the form of a ring enclosing more or less parenchyma. It should be mentioned that, even when the endodermis and xylem are thus completed, no corresponding completion of the phloem has

¹ Except in the case of one branching plant described below (Text-fig. 14).

been observed. The interpretation of these leaf-traces suggested here is supported by the fact that when truly mesarch leaf-traces are met with in *Helminthostachys*, the adaxial completion of the centrifugal xylem may also take place and the two processes be clearly distinguished.¹

The interpretation of the leaf-traces of *Botrychium Lunaria*, as derived from the outer xylem of the stele only, is supported by the distribution of the xylem in small steles, which are giving off leaf-traces (cf. Photos. 10–11, 12–15). It need only be added to what was said above regarding these figures that the xylem of such small leaf-traces may show a solid and apparently mesarch structure due to the completion of the metaxylem adaxially.

It is not uncommon to find that the adaxial xylem becomes separated from the rest of the leaf-trace on nearing the periphery of the stem, as is shown in Text-fig. 9. 21. The main portion of the leaf-trace then passes out into the base of the petiole, while the adaxial xylem appears to die out in the cortex. The separation appears to be due to the active divisions at the periphery of the rhizome, associated with the development of periderm, affecting the old leaf-trace as it traverses this region. I was at first inclined to regard this disposition of the adaxial xylem as indicating a vestigial vascular supply to the vestigial axillary buds to be described below. While, however, the adaxial xylem of the trace would naturally be concerned in the supply of these buds, and may in a sense be related to their presence, the separation of the adaxial xylem from the main body of the trace is brought about secondarily, and it seems impossible to attach significance to it.

There remains for description the occasional development of xylem elements as a result of cambial activity starting in the pericycle. This was found both in specimens that had branched and in a normal unbranched rhizome. In Pl. XXI, Phot. 23 a portion of the stem stele of the rhizome bearing the branch represented in Photos. 32–34 is more highly magnified. The xylem is primary and only about four tracheides deep; outside it and separated by conjunctive parenchyma comes the phloem, the small sieve-tubes of which are clearly seen. Outside the sieve-tubes radial divisions, which had resulted in the development of a number of short lignified tracheides, are seen to have taken place in the pericyclic cells. The same thing had happened in the adult rhizome, a portion of which in radial section is represented in Phot. 22. From right to left this shows the pith, the primary xylem, secondary xylem, and cambium; outside this lies a crushed and darkly stained sieve-tube, and just outside this a zone of tissue derived from active periclinal divisions in the pericycle. From the inner segments thus cut off groups of short tracheides have developed. In this case there appears to be no disturbing reason for the development of pericyclic xylem, as there

¹ Cf. Lang, Proc. Manch. Lit. and Phil. Soc., vol. lvi, Pt. II, p. 5, Figs. 3, 4.

may be in the case of a developing branch making special demands on the water-supply from the main axis. In Fig. 24 the lower region of the leaf-trace, which subtended the branch in the branching specimen, is shown in transverse section. In it a complete arc of pericyclic xylem has been formed on the abaxial side. This, as will be shown, was not continuous with the pericyclic xylem of the stem, and did not directly contribute to the supply of the branch. This description, along with the figures, will serve to put on record an anomalous development for which I know no parallel in the Filicales.

VESTIGIAL AXILLARY BUDS AND BRANCHING OF THE RHIZOME.

The plants of *Botrychium Lunaria* that come under observation are usually unbranched. In 1859, however, Roeper¹ described and figured rhizomes bearing numerous lateral branches, and in 1875 Holle² gave a brief account with a diagrammatic figure of the only branch he had met with. Bruchmann³ states that he had found a dozen examples of branching, and that young plants appeared to be more liable to branch than older ones. He does not enter into the anatomy of the branched plants, but describes and figures the origin of lateral growing points. While regarding these as the initial stages of the development of branches, he makes no remarks on their frequency or their constant relations to the leaves. Bower⁴ placed Bruchmann's observations on *B. Lunaria* in relation to the axillary structures previously described in *Helminthostachys* by Gwynne-Vaughan,⁵ and interpreted by him as vestigial lateral buds. It may be added that Velenovsky figures a branched specimen of *B. matricariaefolium*, and that Campbell records a case of branching in *B. lanuginosum* due to the apex having been destroyed and two lateral shoots developed as adventitious buds. I have communicated a note on the branching of the Ophioglossaceae to the Proceedings of the Manchester Literary and Philosophical Society,⁶ and examples in all three genera will be described in these studies.

The study of complete series of sections through small plants, and of transverse and longitudinal sections of older and larger rhizomes, has shown that a structure comparable to the vestigial bud of *Helminthostachys* is of practically constant occurrence in every leaf-axil of *Botrychium Lunaria*. The position of these vestigial buds, wherever recognized, is indicated by the crosses in the stelar reconstructions (Text-figs. 3-8). In *Botrychium*, as in *Helminthostachys*, the structure is not obviously bud-like, but in both plants its nature is established by the occasional development of actual branches in the corresponding position.

¹ Bot. Zeit., xvii, 1859, p. 257, Taf. XII.

² Loc. cit., p. 226, Taf. II, Figs. 60-62.

³ Ann. of Bot., xvi, p. 170.

⁴ Bot. Zeit., xxxiii, 1874, p. 301, Taf. III, Fig. 4.

⁵ Land Flora, p. 443, footnote.

⁶ Feb. 20, 1912.

In the examples most clearly comparable to *Helminthostachys*, a canal or slit extends downwards and inwards from the axil of the leaf. Usually the outer portion of the slit is completely occluded, and no trace of it is evident passing through the periderm. The inner portion of the slit is always recognizable, and though it does not extend so far towards the stele as in *Helminthostachys*, it exhibits a similar slight curvature forwards at the inner end. The cells bounding the side of the slit away from the apex of the stem have the same glandular appearance as was noted by Gwynne-Vaughan in *Helminthostachys*; these cells, which often break down in preparations, extend round to the inner end of the canal. There is a more or less marked projection of the tissue of the inner wall of the slit, i. e. of the surface belonging to the stem, into the concavity of the canal. The cells forming this projection are small and have a healthy appearance. The general relations of the whole structure to the leaf-trace and the regions as described above are shown in the two longitudinal sections (Photos. 25, 26). Phot. 28 shows the position of the vestigial bud in relation to the leaf-gap and the subtending leaf-trace, the section passing through the base of the vestigial bud below the level of the canal. Phot. 27 is a similar section of a larger stem less highly magnified; it passes through the slit leading down from the leaf-axil.

While this description and these figures serve to show the appearance and structure of these vestigial buds, the position of the actual apex is still a matter of doubt, and the detailed consideration of this question must be postponed. I incline to regard the apex as being situated either at the inner end of the slit or towards the leaf-base, and not to be represented by the more marked projection of tissue from the stem side of the slit. Comparison with other species or the study of buds in a more active state will be necessary to clear up this point. It is clear, however, that the slit represents a shut-in portion of the external surface of the shoot, and the whole structure may be appropriately compared, as was done by Bower, to the sunken, shut-in, and often dormant buds of *Equisetum*.

Four plants of *Botrychium Lunaria*, which bore developed branches, have been studied by means of successive transverse sections, and in another example the base of a lateral branch was seen in longitudinal section. In every case the apex of the main axis had been destroyed, and at a greater or less distance behind it a vestigial bud had developed to produce a branch, by means of which the growth of the plant was carried on; in one specimen two vestigial buds had thus been developed. In the example of a lateral branch described and figured by Holle, the vascular supply was derived from the adaxial side of the leaf-trace and not from the stele of the main stem. The general position of all the branches I have studied agrees with this, but their vascular connexions exhibit differences of detail which make it necessary to describe all the examples. The variations found are of

interest for comparison with other Ferns, and especially with *Helminthostachys*.

The structure and vascular relations of the first branched specimen will be readily understood from the transverse sections at different levels (Text-fig. 10), together with the reconstruction of the stele (Text-fig. 11). Three transverse sections at different levels are shown in Photos. 29-31. The reconstruction of the vascular skeleton is to the same scale as those of the unbranched plants above. The outline cross-sections are, however, drawn to a slightly smaller scale, and show the cortex as well as the stele. The branch was evidently borne on a small plant the apex of which had been destroyed. The stele of the main axis is small, and at the level where the series begins shows the leaf-gap left by the departing trace, to which the branch is related. This leaf-gap has a deeply extending, but incomplete, internal endodermis (Text-fig. 10, *a*); this diminishes in extent before the gap in the endodermis closes (*b, c*). The endodermis of the departing leaf-trace becomes complete on the side towards the stele (*b*). As this takes place, tracheides destined to supply the branch begin to appear at first to either side of the xylem of the trace; they ultimately form an adaxial curved band joining the two ends of the leaf-trace xylem (*c*). This stage is represented in Phot. 29. The adaxial xylem increases in amount and becomes separate from the xylem of the trace, first on one side (Text-fig. 10, *d, e*; Phot. 30), and ultimately on the other also. Even before it is completely detached, the xylem of the branch arranges itself in the form of a medullated stele with scattered tracheides in the pith (Text-fig. 10, *e*; Phot. 31). Until this stage the stele of the branch has been enclosed by an endodermis common to it and the subtending leaf-trace, but the endodermis outside the latter now becomes indistinct and disappears. The endodermis becomes complete around the branch stele as this separates from the leaf-trace, but previously the endodermis on the adaxial side of the branch has become continuous with that of the main axis (Text-fig. 10, *e, f*; Phot. 31). Not only is this the case, but tracheides are developed between the xylem of the main stele and that of the branch, so that the xylem of the two is continuous for a short distance (Text-fig. 10, *e*; Phot. 31). Above this the two steles again separate, and each has its own complete endodermis (Text-fig. 10, *g*). A little higher the stele of the main axis disappears and the branch alone remains (*h*).

Before tracing the stele of the branch further the structure of the stele of the main axis must be described. It was mentioned above that this had an imperfect but extensive internal endodermis continuous with the external endodermis at the leaf-gap (Text-fig. 10, *a*). The outer endodermis then becomes complete, the inner endodermis disappearing (*b, c, d*), and later the xylem-ring is practically completed (*e, f*). Just at this level the connexion with the xylem of the branch takes place. From a little below the closure

of the xylem-gap scattered tracheides are present throughout the pith of the axis (Text-fig. 10, *d, e, f, g*; Phot. 31). Their distribution is shown diagrammatically in the reconstruction (Text-fig. 11). Still higher, on approaching the injured end of the axis, the xylem ceases to be lignified.

The development of the tracheides throughout the pith of this stele is

TEXT-FIG. 10. Series of transverse sections of the first branching specimen. Description in text.

a matter of some interest. Comparison with uninjured plants shows that normally no tracheides would have been developed in this position. Their development may be related to the influence on the young main axis of the wounding or of the development of the branch; probably the former is the important factor. Reasons have been given earlier in the paper for recognizing a poorly developed central or centripetal xylem in normal stems of *Botrychium Lunaria*. The scattered tracheides mixed with parenchyma in the pith of this wounded stele are most naturally regarded as a fuller development of this centripetal primary xylem. A point of interest is that

they appear in a pith which lower down was delimited by an internal endodermis, and might therefore be supposed to have been derived by cortical intrusion. The development of these tracheides throughout the pith thus strengthens the evidence against the cortical nature of the pith in *Botrychium*.

The origin of the stele of the branch and its relations to the subtending leaf-trace have been considered. The branch stele soon assumes the characters of a complete and independent stele with a large pith (Text-fig. 10, *g*), from which the scattered tracheides present lower down (*e*, *f*) have disappeared. Surrounding the pith is a wide zone of xylem with tracheides in radial rows, conjunctive tissue and phloem; the external endodermis is complete, and there is no trace of an internal endodermis. The branch stele is much larger than that of the main axis, and resembles in structure an adult rhizome with primary and secondary xylem. From this bulky stele two root-traces are first given off (Text-fig. 10, *g*, *h*), and then a first and second leaf-trace above these two roots respectively. There is nothing to indicate that the bulky pith in the lower part of the stele is of cortical origin. The gap left by the departure of the first leaf-trace closes almost at once in the endodermis, and a little later in the xylem-ring. This trace evidently supplied a reduced leaf, and, though of good size at its origin, rapidly diminishes in the cortex when it divides into two. A vestigial bud is present in the axil of this leaf (Text-fig. 10, *i*), and this is also the case for the second trace, which, however, dies out before reaching the periphery of the cortex.

TEXT-FIG. 11. Reconstruction of the relations of the steles of the main rhizome and the branch in the first branching specimen.

An indication of internal endodermis is found before this trace separates, and the gap in the external endodermis is repaired almost as soon as made.

Above the origin of these two first leaf-traces the diameter of the stele of the branch diminishes until it is about that of the original main axis. The complete stele below the origin of the third leaf-trace has no indication of internal endodermis (Text-fig. 10, *k*). As the xylem of the trace separates, but while the external endodermis is still continuous, two strips of internal endodermis develop (*l*), and, on the separation of the trace, these join the edges of the gap in the external endodermis, and the internal endodermis becomes a complete lining to the stele (*m, n*). The branch thus on the whole repeats the development often found in plants developed from an embryo, and it is probable that the departure of the third leaf-trace would be a gradual one with a long leaf-gap.

The structure of the other branching specimens may be more briefly described, and the vascular relations in each case will be clear from the diagrams of transverse sections without the need of reconstructions.

The main axis, which bore the second branch, had had its apex cut off; the single branch had originated a considerable distance behind the wounded end on what must have been a mature region of the rhizome. The portion of the rhizome which bore the branch had the general structure characteristic of a marked intermediate region. The leaf-trace subtending the branch departed very gradually, and the long leaf-gap had an internal endodermis. Text-fig. 12, *a*, shows the previous leaf-gap still open and the subtending leaf-trace ready to depart; the gradual separation of the trace can be followed in Text-fig. 12, *a-g*. In this plant pericyclic xylem was developed in the stele of the main axis (Text-fig. 12, *g*; Photos. 23, 34). At a lower level a band of pericyclic xylem had developed on the abaxial side of the departing leaf-trace (Text-fig. 12, *b, c*; Photos. 32, 24). This soon dies out and plays no part in the vascular supply to the branch. The xylem of the subtending leaf-trace then shows an adaxial extension leading to the formation of a complete ring of xylem (Text-fig. 12, *d, e*; Phot. 33). This is clearly comparable to the adaxial xylem in the subtending leaf-trace of the first branching specimen, where there was no development of pericyclic xylem. As in that case, the adaxial xylem becomes separated from the departing leaf-trace, and ultimately gives rise to the vascular supply for the branch. The organization of the stele of the branch as followed upwards is, however, more irregular in this specimen. The adaxial xylem, after becoming detached from the leaf-trace (Text-fig. 12, *f*), forms an irregular group of tracheidal strands instead of becoming at once arranged to form the stele of the branch (Text-fig. 12, *g*; Phot. 34). On passing a little higher up these become rearranged to form the small medullated stele of the branch, which at once departs from the main

rhizome (Text-fig. 12, *h*). Meanwhile several irregular groups of tracheides have developed in the tissue between the stele of the branch and that of the main axis. These tracheides, like the adaxial group left behind by the branch, soon die out. Before this happens, however, the latter group passes across and fuses with the inner group of tracheides.

It is unnecessary to follow the stele of the branch in detail. It need only be said that the stele is small at its commencement, and does not exhibit the contraction or reduction shown by the former branch. The vascular relations of this branch to its subtending leaf-trace show a general correspondence with the branch previously described, but are less regular. In both these specimens the chief vascular attachment of the branch is to the adaxial side of the subtending leaf-trace, some distance above its

TEXT-FIG. 12. Series of transverse sections of the second branching specimen. Description in text.

separation from the main stele. In the two specimens now to be described the attachment of the branch is deeper in the axil of the leaf-trace, and more closely related to the stele of the stem.

The vascular relations were especially regular in the third specimen (Text-fig. 13; Photos. 35, 36). Here also the upper end of the main axis had been destroyed, and one axillary bud, a short distance behind the cut end, had produced a branch. As the subtending trace begins to leave the stele additional tracheides are evident to either side of the xylem, and practically connect the departing trace to the sides of the leaf-gap in the xylem (*a*). The additional xylem soon extends across the adaxial face of the leaf-trace (*b*), and the stele of the branch is organized from the first in this adaxial xylem (*b-e*). While this is going on, and even after the leaf-trace has departed (*e*), the xylem of the branch is connected by a tract of tracheides with the margins of the leaf-gap. The xylem of this branch,

derived mainly from the adaxial xylem of the subtending leaf-trace, has thus been throughout in connexion with the xylem of the stem stele. As the branch stele becomes quite independent a certain amount of the xylem between it and the stele of the axis dies out in the cortex. As in the case of the first branch described, the stele of the branch at its base has a greater diameter than the stele of the main axis at the same level; the branch stele soon diminishes in diameter. A slight development of pericyclic xylem occurs in the subtending leaf-trace (Text-fig. 13, *b*), but takes no part in the supply to the branch.

The fourth case of branching in *B. Lunaria* that I have studied is of great interest, but unfortunately only a number of hand sections were available. I owe the specimen to the kindness of my friend Professor

TEXT-FIG. 13. Series of transverse sections of the third branching specimen. Description in text.

Gwynne-Vaughan, who noticed the branching in some sections cut by a student in his laboratory, and obtained and arranged all the sections that had been cut of the piece of rhizome in question. The stout rhizome with typical adult structure of the stele had evidently been injured at the upper end, and the buds in the axils of the two uppermost leaves of the remaining portion of the xylem had developed as branches. The leaf below showed the usual vestigial axillary bud. The steles of the branches in this specimen were relatively small compared with that of the stele of the axis.

The main rhizome had a large stele with a well-marked external endodermis. The broad band of xylem around the large pith showed the distinction of primary and secondary wood described above in other large stems. The arrangement of the elements of the primary xylem was very suggestive of a distinction of centripetal and centrifugal xylem. Outside

the band of secondary xylem continuous with the centrifugal primary xylem, the cambial activity had been resumed, and a further zone of secondary xylem had been formed. This was in great part parenchymatous, but numerous tracheides had been developed in the radial rows of cells produced from the meristem. This resumption of secondary growth must be placed in relation to the development of the branches.

The general vascular relations of the branches to the main axis will be understood from Text-fig. 14. At the level where the series begins (A) one leaf-trace is nearing the periphery of the cortex, and the position of the vestigial bud in relation to this leaf is indicated by the slit in the next diagram. A second leaf-trace has departed leaving a wide gap in

TEXT-FIG. 14. Series of transverse sections of the fourth branching specimen. Description in text.

the stele, and opposite the gap is the vascular supply for the first branch. The xylem for the branch is in part connected with the additional secondary xylem, and in part appears to come from within the leaf-gap. In B the attachment of a root-stele by one side of this gap is seen, and a root was attached on the other side between the levels of A and B. The stele is imperfect in the neighbourhood of the first branch, and the latter cannot be followed further. This section (B), however, shows the first indication of the vascular supply to the second branch. The leaf-trace subtending this branch still forms part of the complete vascular ring of the main stele, but is distinguishable by the outward slope of its tracheides. Internal to the xylem of this trace a group of tracheides has arisen at the periphery of the pith. This group of tracheides increases in size as the trace prepares

to depart (C). As the departure of the trace rapidly takes place the internal group of tracheides passes out through the leaf-gap and, along with an arc of tracheides continuous with the additional secondary xylem of the stele, constitutes the xylem of the base of the branch (D). This becomes arranged to form a definite stele, at first with a gap opposite to the subtending trace (E). This gap closes, and in F the small branch stele is seen in the cortex beside the main stele; the gap in the latter is now closing. Comparison of A and D will make it clear that the origin of the vascular supply to the first branch was similar.

The special interest of this specimen lies in the need of vascular connexions for the branch affecting the stele of the stem before the departure of the subtending leaf-trace, and in the resumption of active secondary thickening producing xylem to which another portion of the vascular supply to the branch is connected.

The fifth branching specimen (Phot. 37) was incomplete, the branch itself having been broken away. It was met with accidentally when cutting the basal region of a plant longitudinally. The specimen shows, however, the relation to the leaf-trace of the commencing tracheidal supply to the branch. The photograph shows the arrested end of the main axis, the departing leaf-trace, and the leaf-gap above it. The endodermis was completed at once on the departure of the trace. The latter itself is small, but from just above the level of its departure from the stele adaxial xylem to supply the branch has been developed. The tracheides of this adaxial xylem are comparatively short and wide. Just below the broken surface indications were evident of the arrangement of this xylem to form the stele of the branch.

If the several branched specimens of *B. Lunaria* described above are compared, it is evident that, while they present considerable differences in detail, the vascular connexions of the branch follow the same general plan. In considering them it is essential to bear in mind that the branch had its origin in a vestigial axillary bud called into activity by the arrest of the normal apical growth of the main axis. While the tissues laid down in the further growth of the bud itself will be strictly primary, any vascular connexions established in the region below the vestigial bud, either with the subtending leaf-trace or the stem stele, must be due to subsequent and, in a sense, secondary changes. This will be more marked when the vestigial bud called into activity is situated on a quite mature region of the rhizome some distance from the apex, and least so when the lateral growing point affected is close to the apical meristem of the main axis where the tissues are still immature.

Direct evidence in favour of the view just expressed could only be obtained from the study of buds in various stages of their growth into branches. The facts regarding the relations of the mature branches are,

however, consistent with and support it, especially if the structure be considered from above down, and not, as in the descriptions given, from below up. Down to a level which probably marks the position of the original bud, the structure of the branch is normal. Below this we come to the vascular connexions to the pre-existing tissues of the main shoot. These must be regarded as developed, under the influence of the developing bud, on the tissues occupying the axil of the leaf-trace. Further study of the anatomy of such structures may throw much light on the immediate causes of the organization of the vascular system. The remarkable fact comes out from the study of these few branches that these secondarily established vascular connexions are such as to allow of comparison with other lateral branches, the vascular supply to which is part of the primary organization of the plant.

While the relative position of the branch remains the same, its vascular supply may appear to come almost wholly from the subtending leaf-trace, or may in considerable part be derived from, or connected with, the tissues of the stem stele. The former case is best shown in the first branch, less clearly in the second, while connexion with the stem stele is marked in the third and fourth specimens. This variation in *Botrychium Lunaria* is of considerable interest when it is borne in mind that the branch in *Helminthostachys* is connected with the stele of the stem at the anterior end of the leaf-gap and not with the subtending leaf-trace.

On considering all the facts, I am inclined to regard the vascular relations of the branch in the first specimen as most nearly representing what would be found if the development of the branch were immediate and primary. The endodermal relations show that this bud must have sprung into activity in a region so close to the apex of the main shoot that the endodermis was not yet differentiated. The preparations for the vascular supply of this branch at once suggest comparison with the more or less complete development of adaxial xylem in normal leaf-traces. It seems possible that the structure in the second and third examples can be brought into line with this; at any rate, whatever be the significance of the xylem connecting the branch and stem steles in these three cases, the main relation of the branch is to the adaxial xylem of the subtending leaf-trace. The fourth example, however, seems to show that the vascular connexions may be more irregular and not always reducible to one plan.

While all the details cannot be reconciled it seems to me that we are justified in looking on *Botrychium Lunaria* as having ancestrally or potentially a branched shoot, the branches springing from the leaf-axils. It seems justifiable further to compare the origin of the vascular supply to the branch with what is found in the branching of the Hymenophyllaceae and of some of the Zygoterideae. That variants should occur in *Botrychium* is not surprising; indeed, it is remarkable that the subsequent establishment of

vascular connexions for its branches should show so much in common with the primary structure in the branches of the groups mentioned.

SUMMARY.

1. Complete rhizomes of *Botrychium Lunaria* exhibit some variety in external form. Above the basal region with its crowded roots an ill-defined segmentation of the shoot can be traced; this is marked by the origin of one or two roots just above the leaf-gap, i. e. near the base of the segment of the shoot corresponding to the next leaf. The transition from the basal to the adult regions of the rhizome may be a gradual and direct one, none of the segments being especially elongated. In other cases a marked intermediate region, due to one or a number of segments being greatly extended and slender, may intervene between the basal and adult regions.

2. The medullation of the stele may take place without interruption of the external endodermis at the lower leaf-gaps, and without the development of an internal endodermis in any part of the plant. The band of endodermis closing the gap caused by the departure of a leaf-trace is a new formation, and may be developed before the internal endodermis is interrupted.

When an internal endodermis is present it is confined to the slender intermediate region of the rhizome, and may be more or less complete. It does not form a complete pocket extending down from the leaf-gap, and its distribution suggests a new formation to meet physiological needs, rather than the intrusion of a phloeotermal layer marking the inner limit of an intrusive cortex. The pith of *Botrychium Lunaria* is therefore regarded as wholly intrastelar and not as intrusive, whether an internal endodermis is present or not. This view is supported by the relations of the tissues in the apical region.

3. Centrifugal (or outer) primary xylem, centripetal (or inner) primary xylem, and secondary xylem can be distinguished in the stele of *B. Lunaria*.

In the non-medullated stele of the basal region the group of central tracheides is surrounded by a zone of tracheides showing indications of radial arrangement. On medullation taking place elements of the central xylem can still be recognized on the inside of the cylinder of outer xylem, and *may* occur scattered through the pith. Only primary xylem is present, and this also holds for the more or less specialized intermediate region of the rhizome. The transition to the fully adult region is marked by the appearance of tracheides arranged in regular radial rows immediately outside the centrifugal primary xylem. The medullary rays present in the zone of secondary xylem are cut off from the pith by the continuous zone of primary xylem.

The xylem is completely developed and lignified a short distance behind the apical meristem. No sharp limit can be drawn between the

early tangential divisions in the procambium giving rise to the elements of primary xylem, and the slightly later ones that give rise to the tracheides of the secondary xylem. The secondary growth is normally not progressive; under exceptional circumstances it may, however, be actively resumed.

The central (or centripetal) primary xylem is usually only represented by a few tracheides at the periphery of the pith. In one wounded stem numerous tracheides, the position of which was clearly centripetal, developed throughout the pith, and tracheides in a similar position are sometimes met with in normal stems.

4. The leaf-trace commonly departs as a segment of the vascular ring of the stele. The centripetal xylem, if present, disappears internal to the leaf-trace, so that the latter at its departure is an endarch collateral strand. In some small rhizomes the trace may depart from the ring of outer or centrifugal xylem, leaving the central xylem complete; or the central xylem may close across the gap before the outer xylem does. In both cases the xylem of the rhizome appears analysed into its outer and inner components.

5. The endodermis may be completed round the adaxial side of the departing leaf-trace, enclosing parenchyma between it and the protoxylem of the trace. Divisions take place in this adaxial parenchyma, and tracheides may develop in it; these extend round from the curved ends of the xylem of the leaf-trace, and form a more or less complete ring of xylem. Indications at least of this adaxial xylem, which is not directly continuous with the inner xylem of the stem, are commonly found. The adaxial xylem is frequently separated from the rest of the trace by continued growth of the parenchyma in the region of the periderm. It may, however, still be represented in the leaf-trace after this has entered the petiole.

A leaf-trace departing from a stele with secondary thickening usually exhibits secondary growth in the lower portion of its course through the cortex.

6. Under exceptional circumstances meristematic activity may commence in the pericycle, and lead to the development of a more or less extensive tract of pericyclic xylem outside the phloem. This has been found in the stem and in the departing leaf-trace. The pericyclic xylem, which suggests comparison with some cases of anomalous secondary thickening, may or may not be associated with the presence of a branch.

7. A single vestigial bud, similar to that described by Gwynne-Vaughan in *Helminthostachys*, is regularly present in the axil of every leaf of *B. Lunaria*. It is connected to the outside by an occluded slit, and remains embedded in the cortical tissue of the rhizome after the leaf has withered.

8. Under special conditions, especially when the growth of the main axis is arrested, branches may develop in the position corresponding to the vestigial buds, and evidently from one or more of the latter having been

stimulated to active growth. While the buds are part of the primary construction of the plant, the vascular connexions of the branch with the main shoot have to be established in more or less mature tissues; this accounts for the variety in detail found in this respect.

The chief vascular supply of the branch is derived from a development of xylem adaxially to the subtending leaf-trace. This xylem is strictly comparable to the adaxial xylem referred to under (5). The vascular supply to the branch may arise from the subtending leaf-trace when the latter has already separated from the stele, or it may arise lower down in the axil of the leaf-trace. A more or less extensive vascular connexion may also be present between the branch and the stele of the stem, in the neighbourhood of the leaf-gap.

The vascular supply to the branch becomes organized into a medullated stele. This is sometimes small, but in other cases is much larger than the stele of the parent rhizome; in the latter cases the stele of the branch diminishes in diameter above its base. The stele of the branch at first shows a gap opposite to the subtending leaf-trace, so that the latter *appears* as if it were derived from the branch stele. The stele of the branch, which shows a general resemblance to that of a plant developed from an mbryo, may acquire an internal endodermis; the origin of the pith, however, is below and independent of this.

CONCLUDING REMARKS.

As stated at the beginning of this paper, any full discussion of conclusions will be deferred until the corresponding facts for other Ophioglossaceae have been described. A brief reference to the conclusions towards which this study of *Botrychium Lunaria* appears to point is, however, necessary.

The re-examination of *B. Lunaria* has revealed a number of features in the anatomy which considerably modify our general conception of the construction of the plant. The most striking of these is the regular presence of a vestigial axillary bud in relation to every leaf, and the reference of the occasional branching of the rhizome to the active growth of one or more of these. This justifies us in regarding *B. Lunaria* as a potentially branched plant, the buds being part of the primary construction of the plant and not adventitious. This feature at once suggests comparison with *Helminthostachys*, and with the Hymenophyllaceae and Zygopterideae,¹ and it is with these latter groups and the Osmundaceae that comparisons would most naturally be made on the ground of general anatomy.

A distinction between inner (or centripetal) and outer (or centrifugal) primary xylem can be traced from the basal region throughout the plant. I am inclined to attach importance to this, especially in the light of the

¹ Cf. Scott, *Annals of Botany*, vol. xxvi, p. 59.

adult stelar structure in *Helminthostachys*, where inner and outer primary xylem are well developed. The two xylems appear to be strictly comparable to the outer and inner xylem in *Zygopteris*, and possibly to the outer and inner xylem in Osmundaceae. In *B. Lunaria* the outer primary xylem is well developed throughout the plant, while the inner xylem is, as a rule, only represented by a few tracheides round the periphery of the pith, which occupies its place in the expanded stele above the basal region. The secondary xylem comes immediately outside the primary, and there is no absolutely sharp distinction between the two. The relation between primary and secondary xylem is comparable to what is described for *Botrychioxylon*¹ and for *Zygopteris corrugata*.

The medullation of the stele has been examined in detail, since it appeared to be important to ascertain all facts which would throw light on the significance to be attached to the presence of an internal endodermis in the lower region of the rhizome. The view reached, that the pith is throughout intrastelar, and that the internal endodermis is a new formation and does not mark a morphological boundary, makes the pith in the Ophioglossaceae strictly comparable to the parenchyma enclosed by the xylem in the Zygopterideae. It would also be comparable to the pith of the Osmundaceae, as the origin of this is explained by Kidston and Gwynne-Vaughan, and to the pith of the Schizaeaceous stele as interpreted by Boodle. The conclusion to which my observations point, that the pith in the Ophioglossaceae is not due to 'pocketing' or 'intrusion of cortex', but is wholly intrastelar, goes beyond that of Bower. He accepted the endodermis provisionally as marking the limit between pith and cortex, and thus arrived at the view that the pith in *Botrychium* was in the basal region intrastelar, and in the upper portion of the plant largely intrusive and cortical. The further facts given in this paper appear inconsistent with any actual intrusion of cortex. The Ophioglossaceae would thus come into line in this respect with all the relatively primitive groups of Ferns, and the excellent statement recently made by P. Bertrand² on the nature of the differentiation of the procambium to form either a solid column of xylem, a medullated stele, or one with internal endodermis and phloem would apply to the Ophioglossaceae as well as to the Osmundaceae.³

The leaf-trace has been shown to exhibit a tendency to the completion of its ring of xylem by the development of adaxial tracheides extending from the ends of the arc of xylem which leaves the stele of the stem. Since any indication of centripetal xylem disappears before the leaf-trace departs from the stele of the stem, it does not appear that the adaxial xylem of the leaf-trace is

¹ Scott, Trans. Linn. Soc., vol. vii, pp. 373 ff.

² Progressus Rei Botanicae, iv, p. 208.

³ The facts ascertained for *Botrychium Lunaria* appear to be irreconcilable with the statement of Jeffrey 'that the pith must in all cases be regarded as a derivation of the cortex' (Bot. Gaz., Dec. 1910, p. 412). But the general question has been sufficiently discussed by Bower (Ann. Bot., xxv, p. 555).

a continuation outwards of the centripetal xylem of the stem. The facts seem more satisfactorily explained by regarding the leaf-trace not as mesarch, but as completed by an adaxial extension of the centrifugal xylem. This view is supported by those cases in *Helminthostachys* in which a truly mesarch trace is completed adaxially; in such cases the two xylems can be clearly distinguished. The Ophioglossaceous leaf-trace on this view would present points of resemblance to the early departing trace in Zygopterideae and to the trace in the Hymenophyllaceae. It would also resemble that of *Osmunda*. Such a view agrees in general with Kidston and Gwynne-Vaughan's interpretation of the C-shaped leaf-trace in the Ferns, but since it regards the xylem of the trace (whether solid or tubular) as composed of centrifugal xylem extended round adaxially, and not as mesarch, it differs in some respects. On this view the hooks of xylem at the outer margin of the leaf-trace would be the last indication of the adaxial extension of the xylem.

The derivation of the vascular supply to axillary branches from the adaxial xylem of the subtending leaf-trace supports this comparison of the leaf-trace with those of the Hymenophyllaceae and Zygopterideae. Further facts are necessary before the variations in the vascular supply to the branches in *Botrychium* can be fully elucidated. In essentials, however, the comparison of the axillary branching in Ophioglossaceae, Hymenophyllaceae, and Zygopterideae is strengthened by the vascular relations in *B. Lunaria*. The Ophioglossaceae may throw light on the variable position of the branches, whether on the stem or the subtending leaf-trace found in the Zygopterideae.

These remarks will indicate, without entering into details, the general bearing of some of the additional features in the anatomy of *B. Lunaria* which have been described in this paper. A deeper analysis of the construction of the rhizome is necessary before such questions as the distinction of stem and leaf in its composition can be discussed. The stelar structure, the medullation, the construction of the leaf-trace, and the nature of the branching are all consistent with a relationship of the Ophioglossaceae to the ancient Fern stock, the general features of which are indicated in the relatively primitive groups of Ferns, such as Zygopterideae, Botryopterideae, Osmundaceae, Hymenophyllaceae, &c. The facts do not appear to point to a direct relationship to any particular known group. In any case it seems at present more important to elucidate the comparative morphology of these more ancient Ferns in all its details than to speculate on the problem of actual relationship.

DESCRIPTION OF FIGURES IN PLATES XX AND XXI.

Illustrating Prof. Lang's paper on *Botrychium Lunaria*.

(All these figures are from untouched photographs.)

PLATE XX.

Phot. 1. Transverse section of medullated stele in the intermediate region of the rhizome. Practically all the xylem is centrifugal and primary. $\times 62$.

Phot. 2. Transverse section of a stele showing the transition to the region with definite secondary thickening. Radial rows of secondary tracheides are developed irregularly outside the narrow zone of centrifugal primary xylem. $\times 62$.

Phot. 3. Transverse section of the stele of the adult region of the rhizome. Around the zone of primary wood (in which centripetal as well as centrifugal xylem may be represented) comes a broad zone of secondary xylem consisting of radial rows of tracheides and medullary rays which only extend inwards as far as the primary xylem. $\times 35$.

Phot. 4. Portion of the vascular ring of a similar stele more highly magnified. The distinction of primary and secondary xylem is well shown. $\times 62$.

Phot. 5. Portion of the vascular ring of the stele in Phot. 3, showing the primary and secondary xylem. Some of the primary xylem next the pith may be centripetal. $\times 62$.

Phot. 6. Transverse section of a stele at the base of a plant. The solid xylem consists mainly of central tracheides around which elements of the outer xylem are commencing to appear. $\times 62$.

Phot. 7. Transverse section of the stele of the same plant at a slightly higher level. The solid xylem consists of the central group of tracheides, and around it the tracheides of the outer xylem arranged more or less radially. A root-trace is departing from the stele. $\times 62$.

Phot. 8. Transverse section of the same stele at a still higher level. Parenchyma has appeared in the central xylem forming the beginning of the pith. The xylem is mostly composed of the outer xylem with elements of the inner xylem around and between the pith-cells. $\times 62$.

Phot. 9. Similar section of the stele of another plant. The two pith-cells in the centre are surrounded by the inner xylem, outside which comes an incomplete zone of tracheides of the outer xylem. $\times 62$.

Phot. 10. Transverse section of a stele near the base of a plant, showing on the right a leaf-trace about to depart. A tracheide of the inner xylem lies opposite the leaf-gap and marks the boundary between the pith and the 'pocket' of parenchyma internal to the departing trace. $\times 62$.

Phot. 11. Transverse section a little higher up than Phot. 10. The leaf-trace has separated from the stele but is still enclosed by the endodermis. Tracheides of the inner xylem have completely shut off the pith from the leaf-trace 'pocket'. The stele in this and the preceding photograph has a second endodermis some three cells further out in the cortex. $\times 62$.

Phot. 12. Transverse section of a stele showing a small pith, and to the left a large parenchymatous 'pocket' internal to a leaf-trace which is about to depart. $\times 62$.

Phot. 13. Transverse section slightly higher up than Phot. 12. The xylem of the trace has separated and the parenchymatous pocket forms a gap in the outer xylem only. The central xylem has become solid again; there is no true pith. $\times 62$.

Phot. 14. Section a little higher up than Phot. 13. The xylem of the trace is further out but still enclosed by the stelar endodermis. The gap in the outer xylem persists. The pith of the stele is reappearing. $\times 62$.

Phot. 15. Section still higher up than the preceding photograph. The pith has increased, almost replacing the central xylem, and communicates with the parenchyma of the gap in the outer xylem, which is almost closed. The leaf-trace is seen dying out in the cortex. A root-trace is attached to the xylem of the stele below. $\times 62$.

Phot. 16. Transverse section of the stele of the same plant as Photos. 12-15, but much higher up. The zone of xylem is wholly primary. The tracheides projecting into the pith correspond to elements of the central xylem. They disappear internal to the leaf-trace which is preparing to depart on the left. $\times 62$.

Phot. 17. Section a little higher up than the preceding photograph. The endarch leaf-trace has separated from the stele. A root-trace is attached to one side of the leaf-gap. $\times 62$.

Phot. 18. Transverse section of a small leaf-trace on its way through the cortex. The endodermis is complete adaxially and a number of adaxial tracheides are present, continuous on the right side with the curved extension of the xylem of the original leaf-trace. $\times 62$.

Phot. 19. Transverse section of a large leaf-trace on its way through the cortex; scattered adaxial tracheides are present on the concave inner face of the trace. $\times 62$.

Phot. 20. Longitudinal section of a leaf-trace passing through the cortex from an adult stele with marked secondary thickening. At the lower end of the photograph the leaf-trace shows shorter and wider secondary tracheides to the outside of the primary xylem; these disappear higher up. Two adaxial tracheides are seen to the inner side of the primary xylem of the trace. $\times 62$.

Phot. 21. Transverse section of a leaf-trace in the base of the petiole. The xylem has just divided into two and shows the hooks, interpreted as the last indication of the adaxial xylem; the hook on the right is most clearly seen. $\times 62$.

PLATE XXI.

Phot. 22. Longitudinal section of an adult stem with secondary thickening. From right to left the photograph shows: pith, primary xylem, secondary xylem, conjunctive parenchyma, phloem (represented by a crushed sieve-tube), and pericyclic xylem. The tracheides of the latter are short and arranged in groups which indicate their origin by irregular tangential divisions in the pericycle. $\times 62$.

Phot. 23. Transverse section of part of the stele of the rhizome of the second branching specimen. Pericyclic xylem is developed and lies outside the phloem. $\times 62$.

Phot. 24. Transverse section of the leaf-trace subtending the branch of the second branching specimen. An almost complete band of pericyclic xylem is present outside the phloem on the abaxial side of the trace. $\times 62$.

Phot. 25, 26. Longitudinal radial sections of the rhizome, showing the position and appearance of the enclosed vestigial axillary buds. $\times 35$.

Phot. 27. Transverse section of the stele of the adult region of the rhizome, showing a departing leaf-trace and the axillary slit leading down to the vestigial axillary bud. $\times 40$.

Phot. 28. Transverse section of another rhizome passing just below the level of the axillary slit, i.e. through the region in which the apex of the vestigial bud is probably situated. $\times 62$.

Phot. 29–31. Three transverse sections of the first branching specimen. In Phot. 29 the adaxial xylem of the subtending leaf-trace is completed. In Phot. 30 this has separated on one side from the leaf-trace and is becoming arranged to form the stele of the branch. In Phot. 31 the branch stele is still connected with the xylem of the subtending leaf-trace, but has also become continuous with the xylem of the main axis. Tracheides have developed throughout the pith of the main axis. $\times 40$.

Phot. 32–34. Three transverse sections of the second branching specimen. In Phot. 32 the subtending leaf-trace shows the pericyclic xylem. In Phot. 33 this has almost disappeared, but the band of adaxial xylem to supply the branch is complete. In Phot. 34 the adaxial xylem has separated from the subtending leaf-trace as an irregular group of tracheides from which the branch stele will be organized. $\times 40$.

Phot. 35, 36. Two transverse sections of the third branching specimen. In Phot. 35 the adaxial xylem forming the stele of the branch is still connected with the subtending leaf-trace and also with the xylem of the main stele in the neighbourhood of the leaf-gap. In Phot. 36 the subtending leaf-trace is departing, but the stele of the branch is still in connexion with the xylem of the main stele. $\times 40$.

Phot. 37. Longitudinal section of the fifth branching specimen, showing the departure of the subtending leaf-trace from the injured main stele, and the development of short tracheides adaxially to the leaf-trace. These were the first indications of the vascular supply to an axillary branch which had been broken off. $\times 40$.

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W.H.L. photo.

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LANG — BOTRYCHUM LUNARIA

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