CEYLON J. SCI. (Bio. Sci.) Vol. 5, No. 1, July 1964

# The Development of Buds on the Rhizome of *Polypodium vulgare L.*

by

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(With one plate and three text figures)

## INTRODUCTION

According to Mettenius (1860), lateral buds in ferns, like those of phanerogams, have a fixed and constant relationship to the bases of leaves. In some species of *Trichomanes* each leaf has a bud in its axil. Starting with this condition, Mettenius derived the positional relationships of buds in other ferns through transitional forms: in *Polypodium vulgare* the bud has moved the length of an internode, and appears opposite the next leaf. Mettenius also stated that when a bud developed early it became as strong as the main axis, so that a dichotomy might appear to have taken place. Hofmeister (1862), however, denied the presence of axillary buds in the ferns, and considered that ferns branched in two ways —by bifurcation of the end of a shoot, and by the formation of adventitious buds. Branching in *P. vulgare* would then be due to bifurcation. Klein (1884) reported that in *P. vulgare* leaves and lateral shoots arose quite independently of each other, so that buds were not axillary. He made detailed investigations of the morphology and of cell arrangement at the shoot apex, and attributed each lateral shoot to the development of a single superficial cell of the apical meristem, situated very close to the apical cell. Velenovsky (1905) and later Bower (1923) considered that lateral branches in *P. vulgare*, and in most other ferns, originated in a dichotomy of the shoot apex. Goebel (1928), however, distinguished between branching and true dichotomy. He noted that in dorsiventral ferns the apical cell of the rhizome did not divide into two equal halves, in the manner of *Dictyota*, but, as Klein had shown, the lateral buds were laid down very near the apex. Goebel showed how a 'monopodial' condition and also 'axillary' branching might be derived from a development of this nature.

Since these interpretations of branching in *P. vulgare* have been based largely on investigations of mature parts of the plant, it was felt that investigation of the shoot apex might throw light on the mode of inception of the buds and the nature of branching. Similar methods have been used successfully by Wardlaw (1943 et seq.) in his investigations of the mode of branching in *Matteuccia struthiopteris*, *Onoclea sensibilis* and other radially constructed ferns, where he found that the buds develop from bud rudiments or detached meristems which are described as "small areas of the original apical meristem which become separated off, or detached, and persist in characteristic positions along the shoot", and that truly adventitious buds probably never occur in the ferns.

## MATERIALS AND METHODS

Collections of *P. vulgare* were made at Freshfield in Lancashire, England, and at Anglesey in Wales, and some additional material was obtained from Lochinvar, Sutherland, Scotland.

Direct observation of the shoot apex was made under a dissecting microscope magnifying 125 times. The scales were removed from the apex by means of a fine pair of forceps and sharp steel needles. After observation or treatment, the distal region of the rhizome was wrapped in moistened cotton wool and the specimens were placed on moist peat in pans covered with glass plates, by which method apices could successfully be kept growing in the laboratory for periods of several months.

## THE SHOOT APEX

When the covering of scales is removed, the distal end of the rhizome is seen to be somewhat flattened, with the apical meristem appearing as a blunt cone seated on the subadjacent tissue known as the subapical region. Still further out is the mature region of the shoot. Leaf primordia may be seen on the dorsal surface of the shoot apex, and emerging young roots on the ventral surface, while buds are seen in a median position (Plate, I,1).

## THE APICAL MERISTEM

The apical meristem, as defined by Wardlaw (1943*a*), consists of 'a superficial layer of prism-shaped cells of distinctive appearance and reaction'. It consists of an apical cell and prism-shaped cells surrounding it, which are formed by the division of the apical cell and its segments. In mature rhizomes of *P. vulgare*, the apical meristem is often seated in a shallow depression ('Scheitelgrube'). The youngest leaf primordium also lies in this depression, the shape of which varies as this primordium is left behind during the growth of the apical meristem. The apical cell of *P. vulgare* is three-sided in surface view, and the apical meristem has, in general, a similar construction to that of other leptosporangiate ferns. The apical cell is eccentrically placed on the meristem, being closer to the ventral edge. Thus there are more prismatic cells dorsal and lateral to the apical cell than ventral to it.

## THE INCEPTION AND DEVELOPMENT OF BUDS

The development of buds can usually be traced back from mature regions of the rhizome into the subapical region of the shoot apex, where they appear as shiny rounded humps. Closer to the apical meristem, however, buds become more difficult to distinguish from the surrounding tissues by visual observation, and direct observation alone cannot decide with certainty whether buds have their origin at the apical meristem, and, if so, their exact point of origin. It was possible, however, to obtain relevant information by marking particular positions on the shoot apex with Indian ink and observing the development of organs and tissues in relation to the marks. It was found that bud rudiments originate at, and later separate from, the lateral margins of the apical meristem during growth: in short, they originate as detached meristems. The position of buds in relation to the leaves indicates that bud rudiments are formed alternately with the leaf primordia. The apical meristem undergoes rhythmic alterations in size and shape during the inception and development of leaf primordia in two alternating positions on its dorsal side: the inception of the bud rudiments can be related to this rhythmic activity and to the special organization and mode of growth of the apical meristem. The apical meristem broadens out alternately in its horizontal plane during the inception of leaf primordia, and it is at the edge of this widened part, just below the leaf sites, that the areas which will later be recognized as bud rudiments are defined. On the continued growth of the apical meristem the bud rudiments, i.e., the detached areas of the apical meristem, are left behind. It is only after a detached meristem has reached a position some distance from the apical meristem that its further development with organization into a bud can be fully recognized.

The formation and development of the detached meristems may be illustrated by longitudinal sections of shoot apices in various planes. Plate I, 2, a horizontal longitudinal section of a shoot apex, shows the lateral extension of the apical meristem in the plane of origin of the detached meristems. The detached meristem in Plate I, 3, has separated from the apical meristem; both are made up of similar prismatic cells, the intervening cells undergoing periclinal divisions and differentiating into parenchyma. It will be seen that the prismatic cells constituting the detached meristem retain their activity to some extent, and have underlying prevascular tissue, which is continuous with the prevascular tissue proximal to the apical meristem.

At earlier stages the detached meristem is made up of a circular group of prismatic cells which are all alike, with no apical cell distinguishable among them. As the detached meristem develops, however, a prismatic cell in its centre enlarges and gives rise by oblique divisions to a tetrahedral apical cell. This is contrary to Klein's (1884) view that the inception of a bud starts with the formation of a 'three-sided' apical cell very close to the apical cell of the shoot, but in general agreement with Wardlaw's (1943*a*, 1943*b*) findings in *Matteuccia struthiopteris* and *Onoclea sensibilis*.

The prevascular tissue proximal to the detached meristem is initially a solid strand. With the further development of the detached meristem this strand widens, with the development of a central pith, and the vascular system of the fully differentiated bud is dictyostelic, joining the axial vascular system at one of its lateral gaps in the form of discrete strands.

The detached meristem is at first situated in the same terminal depression as the apical meristem and the youngest leaf primordium on its side of the apex. Later, as the detached meristem becomes further separated from the leaf primordium and the apical meristem, it comes to lie in a shallow depression of its own, of similar origin to the apical depression, and at this stage it is recognizable as a bud by visual observation. The bud soon becomes surrounded and covered over by a ring of scales, and as it grows it gradually becomes raised above the level of the parent shoot. It forms one or two leaves, and then remains inactive in the form of a lateral hump on the shoot until, under appropriate conditions, it is subsequently able to resume growth.

## EXPERIMENTAL OBSERVATIONS

Since the formation of detached meristems is linked with apical activity, it was considered that experimental damage to the apical meristem, thus terminating or modifying its activity, might throw light on those aspects of apical organization and regulation which are connected with bud formation. In particular, precise information as to the location of bud sites in the meristem might be obtained by inducing precocious bud growth.

Accordingly, the apical meristems of a number of shoot apices were punctured in various ways. This operation was carried out on batches of shoot apices which had been collected at different times of the year, and was done under the dissecting microscope, using in most instances a fine-pointed steel needle held in the hand. As a result of such punctures, a central region of the apical meristem, comprising about a quarter of its area, and including the apical cell, became necrosed.

One of more than 50 apices so treated is illustrated in Fig. 1. At the time of puncturing, $P_1$ was on the apical meristem, but, 11 days later, was observed to have separated from it. Two buds appeared on either side of the apical meristem as shiny, rounded humps one at its edge just ventral to $P_1$, the other at the opposite edge just ventral to the $I_1$ position. $I_1$ itself was not yet visible. Plate I, 4, shows a horizontal longitudinal section of this shoot apex, 18 days after puncturing. The damaged apical meristem and the two

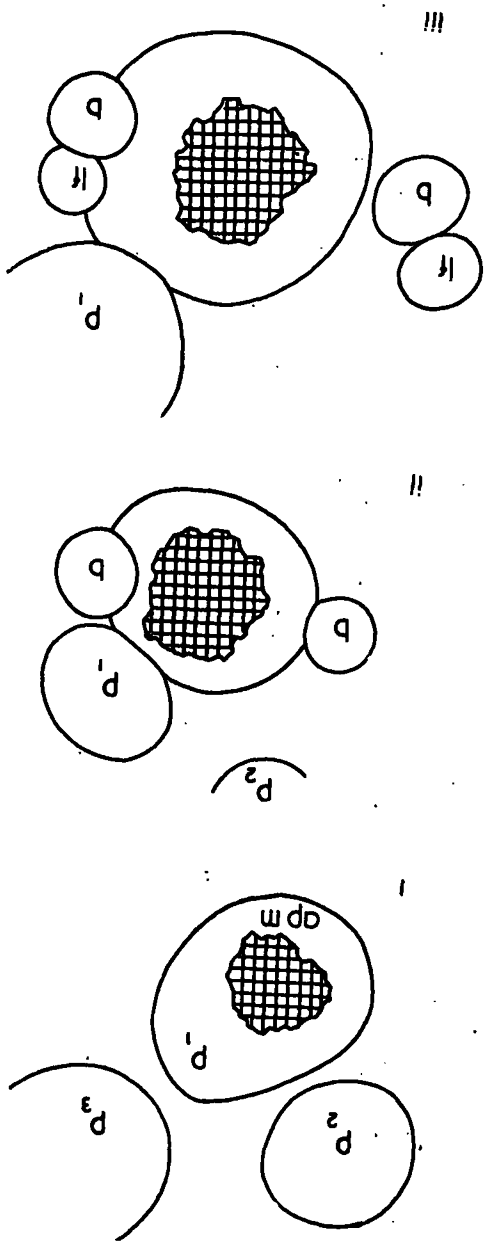



Fig. 1. Shoot apex, with the apical meristem experimentally punctured. (i)—at the time of puncture; (ii)—11 days later; (iii)—18 days later. buds *b*, *b*, have formed at the edge of the apical meristem. *lf*, leaf formed by bud (×90).

new buds can be seen. The apical meristem has become parenchymatous, and some wound tissue has formed. The prevascular tissue beneath the two new buds is in connection with the axial vascular system. In about half of the shoot apices treated in this way two buds were formed; in the other half only one bud was formed, in one of the bud positions. It is probable that a bud appears only if the site on the apical meristem has already attained the necessary stage of development at the time of puncturing the apical meristem, this depending on the plastochrone stage. All the shoots in which the apical meristem was punctured developed either one or two buds at the apex.

It was further observed that the period of time between the experimental treatment and the first appearance of a bud at the shoot apex depended on the extent of injury to the apical meristem. When relatively little of the apical meristem was left undamaged, a bud appeared in less than a week, whereas when most of the apical meristem was undamaged, it was a longer time before a bud could be observed. Working with *Dryopteris aristata*, Wardlaw and Cutter (1955) observed a similar relation. In both forms this may be due to both (*a*) a greater residual inhibitory effect where more of the apical meristem was undamaged, and (*b*) lower availability of nutrients in the same conditions owing to competition with the larger undamaged portion of the apical meristem.

It is of interest that after injury to the apical meristem it is the youngest buds that develop most vigorously and not the older ones at the side of the shoot: situated closest to the apical meristem, they are probably in a more favourable position than the others to tap the nutrients that passed previously to the active apical meristem. The experimentally induced buds always appeared in the same position in relation to the apical meristem – in the flattened region on its sides, just ventral to the leaf sites – thus confirming the earlier observation that detached meristems separate from the apical meristem at these locations. When the apical meristem is punctured, the shoot ceases to grow; the detached meristems remain at the edge of the apical meristem and develop into buds after the lapse of some time. The inception and growth of leaf primordia at the damaged apical meristem – while it continues – seems to have no inhibitory influence on this development.

Light puncturing of the apical meristem produced somewhat different results. The position of the apical cell can generally be estimated, and the apical meristems of some shoots were punctured lightly, using a micromanipulator and a fine-pointed tungsten needle, so as to restrict necrosis to the apical cell group. One of these shoot apices is illustrated in Fig. 2, 17 days after the puncture. Two leaves, $I_1$ and $I_2$, have formed in their

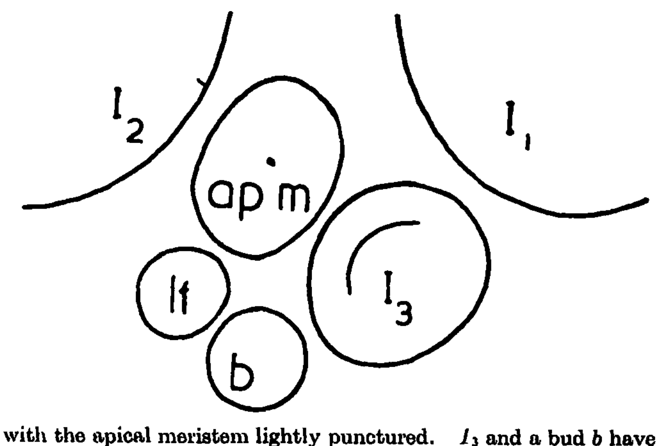



Fig. 2. Shoot apex, with the apical meristem lightly punctured. $I_3$ and a bud *b* have formed ventrally. *lf*, leaf formed by bud. (× 125).

normal positions: their growth centres may already have been determined when the apical meristem was punctured. A leaf primordium has formed on the ventral region of the apical meristem, and opposite $I_2$. It is now a dorsiventral structure, orientated in the direction of the punctured apical meristem. A younger leaf primordium, nearly opposite $I_1$, and a bud meristem *b* have also formed ventral to the damaged apical meristem. The leaf primordium *lf* and the bud meristem appeared almost simultaneously, but $I_3$ appeared earlier. The relationship of *lf* to the bud meristem is not certain: they may have had their origin independently from the prismatic cells of the damaged apical meristem, or the leaf primordium may have been formed by the bud meristem. The site of formation of $I_3$ is significant, since its presence suggests that the inhibition of leaf primordia on the ventral side of the apical meristem must have been overcome as a result of damage to the apical cell group. In the shoot apex in Fig. 3, also lightly punctured, no new leaves have formed on the dorsal side, but a bud has arisen ventrally, opposite $P_2$. Concurrently with the formation of this bud, a leaf primordium was formed dorsal to it, from prismatic cells at the ventral edge of the apical meristem. In this specimen the inhibitory effect on the formation of leaves ventrally seems to have been reduced to the extent that a leaf primordium could arise there in relation to a new bud. This leaf later became dorsiventral, orientated between the damaged apical meristem and the new bud. In most instances, light puncturing was followed by the formation of a single bud in the ventral region of the apical meristem, usually ventro-laterally, opposite one of the leaves. The formation of buds in this region under these conditions may be due to the presence of a large area of undamaged meristematic tissue where, although buds do not form normally, they can form in the absence of the regulatory effect of the apical cell group. In such specimens the formation of buds on the normal bud sites did not take place, probably owing to the inhibitory effect of the new ventral bud.

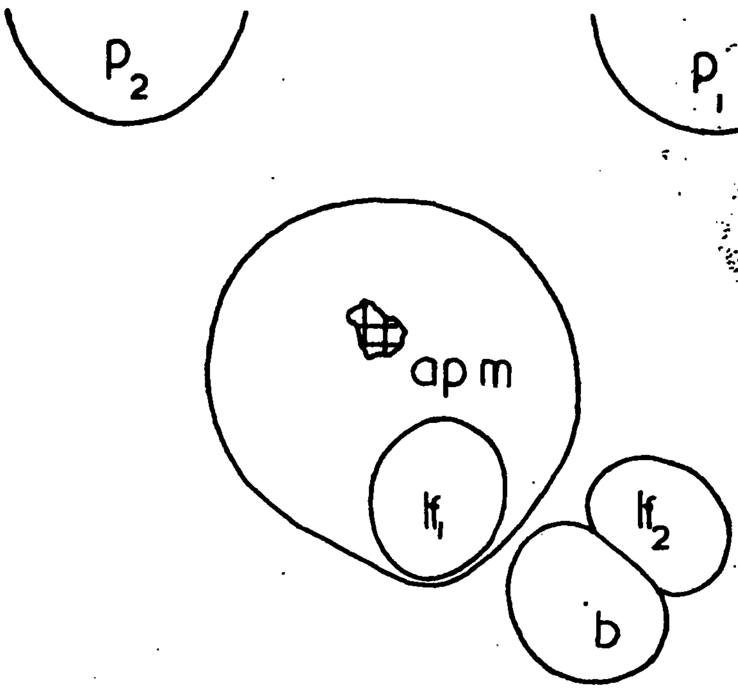


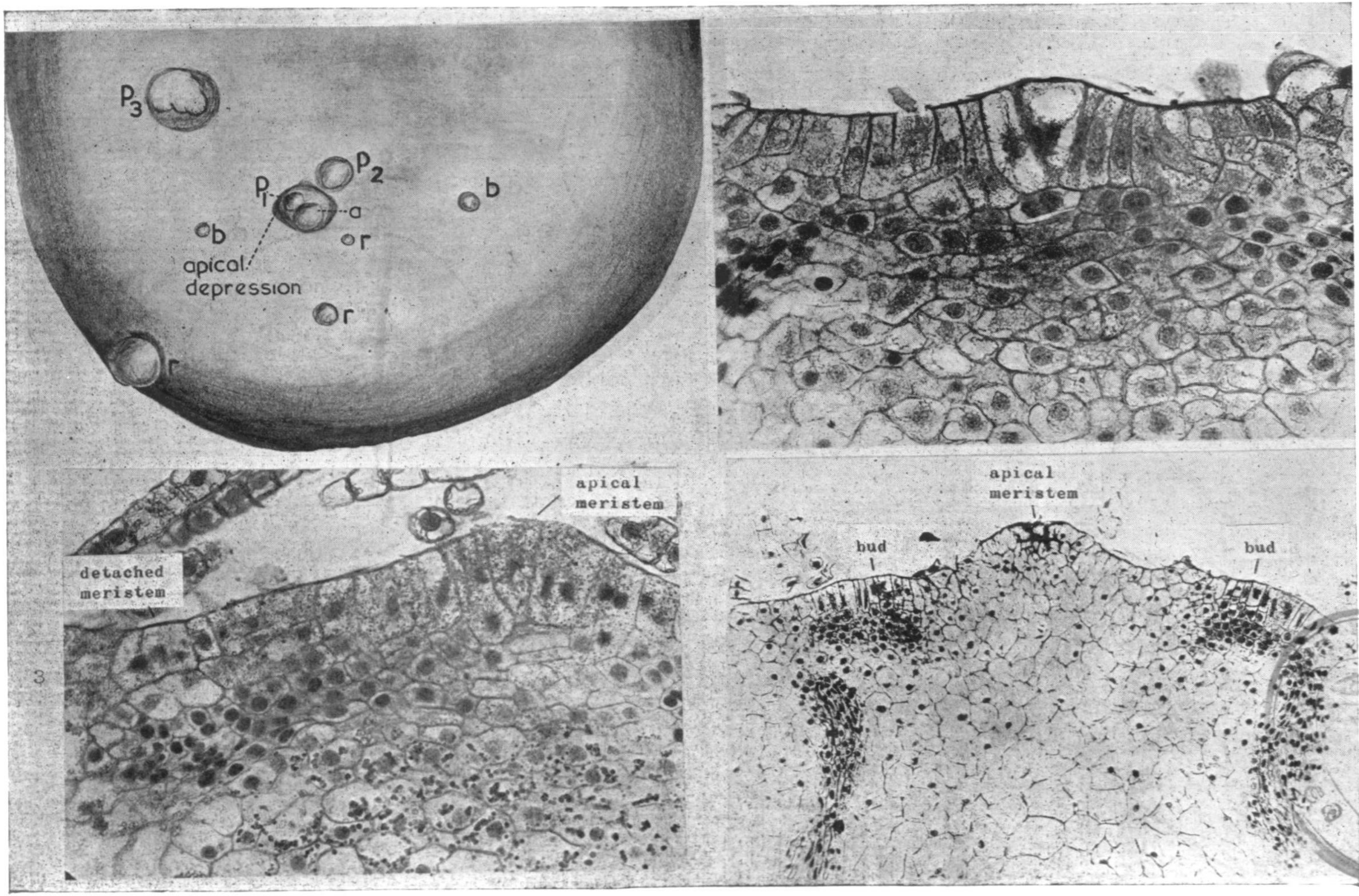

Fig. 3. Shoot apex, with the apical meristem lightly punctured. A bud *b* and a leaf $lf_1$ have formed ventrally. $lf_2$, leaf formed by bud, ( × 125).

## DISCUSSION

During the formation of vegetative buds in *P. vulgare*, groups of prismatic cells whose meristematic nature is preserved, i.e. detached meristems, separate from the lateral edges of the apical meristem, this process being linked with the rhythmic changes in size and shape of the apical meristem in these regions, and taking place at intervals of one plastochrone. It is important to determine why specific sites on the apical meristem are able to retain their potentialities for meristematic growth. A solution to this problem can be reached only with the aid of more precise knowledge than is available at present of factors that regulate the characteristic organization of the apical meristem and the distribution of growth in it. It is significant in this connection that bud sites occupy a median position at the apical meristem, between the leaf-bearing dorsal region and the root-bearing ventral region, these positional relationships being probably determined by the interaction of metabolic and other factors. Bud rudiments are formed at the flattened edge of the apical meristem, whereas leaves originate relatively high up on the apical cone, closer to the apical cell.

The extent of vascular connection between the bud and the axis varies with the time at which the bud develops from the detached meristem. Wardlaw (1943*a*, 1943*b*) found that whereas in *Onoclea sensibilis* buds induced experimentally in the immediate vicinity of the apical meristem were in vascular connection with the axis, those induced in older regions formed no such connection; and the quiescent detached meristems of *Matteuccia struthiopteris* and *O. sensibilis* had no vascular trace. In *P. vulgare*, however, the detached meristem is in vascular connection with the axis from the first. These differences seem to be related to the activity of the meristematic cells of the detached meristem, the active bud rudiment of *P. vulgare* promoting the formation of prevascular tissue beneath. These differences can also be observed in angiosperms (Philipson, 1949; Esau, 1954). Bell (1956) has claimed that the buds of *Elaphoglossum* which, whether dormant or not, have a vascular connection with the stele, are of a different nature from the buds with no vascular connection which Wardlaw has investigated in *Matteuccia* and elsewhere. According to Bell, lateral buds in *Elaphoglossum* "represent a potential branching system which has been inherited from the ancestors of the genus". Since the buds of *P. vulgare* resemble those of *Matteuccia* and *Onoclea* in their formation, and also seem to resemble those of *Elaphoglossum* in their vascularization, further investigation of bud formation in a wider range of fern species may well show that the differences referred to by Bell are only apparent.

Buds have been induced to form in the ventral half of the apical meristem of *P. vulgare* by experimental means, but not in the dorsal half. The explanation for this is probably that leaf growth centres already present in the dorsal region inhibit the appearance of buds in the vicinity, whereas in the absence of the regulatory effect of the apical cell group a bud can arise in the extensive prismatic tissue of the ventral region. A further interesting result obtained by light puncturing was the inception of leaves on the ventral side of the apical meristem, independent of buds, as well as in association with them. In these instances, the regulatory effect of the apical meristem that restricts leaf formation to the dorsal half appears to have been overcome.


P3
P2
P1
a
b
b
r
r
r
apical depression
apical meristem
detached meristem
apical meristem
bud
bud

1

2

3

4

Plate

## SUMMARY

1. The origin of buds in *Polypodium vulgare* L. is traced to detached meristems separating laterally from the apical meristem.

2. These meristems normally develop into recognizable buds when some distance away from the apical meristem, but on the destruction of the apical meristem, buds were formed at the points of origin of the detached meristems.

3. When the apical cell group was lightly punctured, buds, and in some instances leaves, had their inception at the ventral half of the apical meristem.

4. The buds are in vascular connection with the axis from their inception.

## ACKNOWLEDGEMENTS

The writer wishes to thank Professor C. W. Wardlaw of the Department of Botany, University of Manchester, where the work was done, for his helpful guidance and encouragement, and Dr. E. G. Cutter for many valuable suggestions. His thanks are also due to Mr. E. Ashby and Mr. G. Barker for the photographic illustrations.

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### EXPLANATION OF PLATE

1. Shoot apex of adult rhizome, showing apical meristem and positions of leaves $P_1$—$P_8$, buds and roots: b, b, buds; r, r, roots. (× 35).
2. Horizontal longitudinal section of shoot apex, showing lateral extension of apical meristem (× 330).
3. Horizontal longitudinal section of shoot apex, showing a detached meristem (left) close to the apical meristem, and separated from it by parenchyma (× 330).
4. Horizontal longitudinal section of shoot apex illustrated in Fig. 1 (iii). Punctured apical meristem in middle, flanked by buds (× 110).