PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Experiments on Phyllotaxis. I. The Effect of Isolating a Primordium

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PHILOSOPHICAL TRANSACTIONS.

I. *Experiments on Phyllotaxis.* I.—*The Effect of Isolating a Primordium.*

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Contents.

Part I.—Introductory.

(1) *Historical.*

Theories concerning the causes of phyllotaxis may be divided conveniently into two groups. According to those of the first group, the arrangement of the leaves depends upon some unknown properties of the stem or the stem-apex. According to those of the second group, the positions in which the leaf-primordia arise are determined by the positions of the older primordia with which they make contact, or of any other members below them with which they may be in contact, such as cotyledons. Among the theories of the first group is that of SCHIMPER and BRAUN, who suggested that spiral sequences were due to a spiral growth impulse which travelled up the stem, the leaves arising at regular intervals along the course of this spiral.

A more recent theory due to CHURCH (1909 and 1920) was that the young leaves were initiated at the points of intersection of two sets of impulses radiating in opposite directions along logarithmic spirals, with the growing point as their pole, and intersecting at right angles. These curves later became visible as the contact parastichies. The leaves were thus laid down independently of each other, their positions being due to unknown properties of the apex through which the impulses radiated.

In a series of papers published since 1923, SCHUEPP investigated different kinds of phyllotaxis systems with special reference to the symmetry or asymmetry of the constructions. He concluded (1928) that the symmetrical or asymmetrical disposition of leaves cannot be explained by mechanical causes, nor by nutrition, but is "the consequence and visible sign of symmetrical or asymmetrical internal structure" (p. 876). The theory of HIRMER (1922) also belongs to the first group.

The second group of theories originated with HOFMEISTER'S observation that each leaf arises in the largest gap between the previous leaves. To explain this fact he suggested (1868) that the outgrowth of primordia produced a tension in the outer cell walls, which tended to resist the outgrowth of further primordia. Each primordium, therefore, arose in a position where the tension was at a minimum and thus came to occupy the space that was farthest removed from the previous primordia. This theory has received no further support, but the observation that leaves arise in the largest gap has been accepted as a fundamental fact by several later writers.

SCHWENDENER realised the truth of HOFMEISTER'S law, but considered that the latter had exaggerated its importance as a factor determining leaf arrangement (1878). In addition, he considered the following factors to be of fundamental importance: (1) The establishment of contact between a young primordium and at least two neighbouring leaves at the end of its first growth phase. (2) The ratio of the sizes of the young leaves to that of the axis on which they arise. He supposed that, when the leaves had been laid down in a manner determined by these factors, they were displaced by mutual pressures, which were the result of the unequal resistances encountered by the extending leaves in the transverse and longitudinal directions. This theory of displacement by contact pressures has received much criticism and very little support, and it is now generally agreed that an actual displacement of leaf insertions rarely, if ever, takes place and cannot be considered as a factor determining phyllotaxis systems.

SCHUMANN accepted SCHWENDENER'S hypothesis concerning the factors that determine the positions in which the leaves arise, but was unable to agree with the theory of displacement by contact pressures (1892, *a* and *b*). His studies of *Costus*, *Pandanus* and other Monocotyledons in which the leaves are arranged in twisted spires led him to the important conclusion that the centre of each primordium is located at the lowest point above the previous primordia. He wrote: "It is, moreover, a general experience that the points that lie farthest from the growing-point are the first to be occupied by new primordia."

Some of the conceptions that originated with HOFMEISTER and SCHWENDENER were worked out more thoroughly by VAN ITERSON in 1907. He investigated the properties of the geometrical patterns in which leaves are arranged. Assuming that the outlines of the insertions of the youngest leaves are approximately circular, and that the narrow zone of the paraboloid apex on which they are situated approximates to part of the surface of a cone, VAN ITERSON made the following assumption: "The outlines of the insertions of young lateral members, with constant phyllotaxis, approximate to a similar system of circles in contact on a circular cone surface" (pp. 202 and 146). A thorough mathematical investigation of the properties of such a system of touching circles on the surface of a cone, a plane and a cylinder, enabled him to state the exact relationships between the following three factors: firstly (*a*) the divergence angle, secondly (*b*) the ratio of the sizes of primordium and axis, or the "relative radius," and thirdly, the numbers of the contact parastichies.

The great value of this investigation is that it revealed the extent to which the phyllotaxis system depends on the value of the factor *b*, the relative radius. For certain values of *b*, only one phyllotaxis system is possible, but for other values of *b* several systems are possible, with different parastichy numbers, as is shown in his book in graph II, fig. II. In the second part of the book, in which these results are applied to plants, VAN ITERSON investigated the positions in which the primordia arise when the phyllotaxis is constant and when it is changing. He concluded that constant phyllotaxis is maintained as a consequence of the following "facts of observation," as he calls them:—

"(1) The relative radius of the insertions of the new primordia (the factor *b*) is the same as that of the young primordia that are already present.
(2) These primordia are in contact with at least two older members.
(3) They are laid down in the larger gaps between the previous leaves.
(4) Small irregularities in the size or position of new members are eliminated during the continued growth of the system or in the course of development."

By his second observation, he does not mean that the primordia arise in contact with their neighbours, but only that contact is usually achieved by the end of the "first growth phase." For plants in which contact is not then achieved, he falls back on SCHWENDENER'S idea of a "field of development" which must be substituted for the areas occupied by the young leaves. He considers that the main difference between constant and changing phyllotaxis is that in the former the relative radius instead of remaining constant decreases or increases. It shortly attains a new constant value and a new phyllotaxis system is established, depending on the new value of *b*, the relative radius, and on the disposition of the leaves laid down before the change (p. 258). Van Iterson's investigations are of great importance for the interpretation of the experiments to be here reported.

In 1913, Schoute suggested that the main problem of phyllotaxis was the determination of leaf centres (p. 174). He supposed that the central point of the leaf is determined first and that the leaf then radiates out from this point. His theory of the determination of leaf centres need not be quoted, since the experiments to be reported here provide evidence that the central point of the leaf is not determined independently of the rest of it. He made the further hypothesis that the growing-point produces a hormone which retards the formation of leaf-centres in the parts below it which it reaches. He considered that, as a result of this retardation, each leaf occupies the farthest possible position from the growing-point—an opinion similar to Schumann's.

Though the theories of phyllotaxis are numerous, they have never been tested by direct experiments, probably on account of the technical difficulties. Yet clearly it should be possible to decide by experiment between the theories of the first and second groups. For if the truth lies with those of the second group, it should be possible, by operating upon the primordia that are arising or just about to rise, to alter the positions of the subsequent primordia. Since a method for performing minute operations had already been practised by one of us, in an investigation of the regeneration of the stem apex (Pilkington, 1929), it occurred to us that similar methods might be used for experiments of this kind upon phyllotaxis. The plant selected for the experiments was *Lupinus albus*, because this plant had been found convenient in the previous investigation, and because it has comparatively large leaf primordia, which can be distinguished easily under a dissecting microscope.

(2) *The Normal Lupin Apex.*

Before the experiments are reported, a preliminary description of the apex and phyllotaxis of the Lupin is necessary. The phyllotaxis of *Lupinus albus* belongs to the series called the "Hauptreihe" by the Germans, and the "Fibonacci" series by Church. The contact parastichies of *L. albus* form a $2 + 3$ system.* The leaves have stipules which extend round the axis, so that the stipules of successive leaves are in contact with each other. If the stipular contacts are included, the contact parastichy system becomes $1 + 2 + 3$.

It was postulated by Van Iterson that the insertions of the young leaf primordia may be approximately represented by circles described on the paraboloid apex, but probably this can only be correct for leaves that have no stipules. No method could be devised for determining exactly the contours of the Lupin primordia, but it was concluded from observations of the apex, made through the dissecting microscope, that the primordia arise as circular humps, with the stipules forming tapering horizontal

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* In a previous paper by one of us (Pilkington, 1929) it was wrongly described as $3 + 5$.

projections on either side. The whole primordium is thus roughly oval in shape with its longer diameter lying transversely to the axis of the shoot.

The divergence angle was measured from sections of terminal buds of six different plants, 8 to 10 successive divergences being measured in each bud. The method of measuring was the following: The buds were embedded in collodion and sections were cut free-hand. The centres of the leaves were located by the positions of the central vascular strands. The three youngest leaves were excluded from the measurements since their vascular strands were not yet differentiated and the centres could not be determined with precision. The centre of the axis was far harder to determine, especially as the vascular cylinder seldom formed a true circle, but the centre was judged by the eye as accurately as possible. The sections were drawn with a drawing eyepiece, and, for greater accuracy, the measurements were made on the drawings viewed through the eyepiece, so that the protractor was seen against the image of the section. The divergences recorded in this determination of the normal angle were all averages of measurements made on not less than three sections at different levels, as were also all the other divergences recorded in this paper. Measurements of any one divergence differing from one another by more than 5° were discarded as unreliable. The mean value of 52 divergence angles measured in this way was found to be 136°·3. The probable error was 1°·88, and the probable error of the mean was 0°·26. The extremes were 143°·16 and 130°·87.

(3) *Methods and Terminology.*

The operations were made with a " cataract knife " under a Leitz binocular dissecting microscope giving a magnification of 30 diameters. The apical parts of the shoots were fixed after from three to five weeks, embedded in collodion and examined by free-hand sections. The convention will be adopted of calling the primordia that were visible at the time of operation P_1, P_2, P_3, etc., P_1 being the youngest. Those arising after the operation will be called I_1, I_2, I_3, etc., I_1 being the first to arise. (The letter I was selected because these primordia were still invisible at the time of operation.) Operations in which the cut was made so as partially to isolate the piece of tissue which was about to give rise to the next primordium, or the next but one, will be called isolations of I_1 or I_2, although these primordia were invisible at the time and sometimes failed to develop. The term " growing-point " will be used to denote the extreme tip of the apex, which is usually considered to represent the centre of growth, and the term " apex " to denote all the part of the stem that lies above the youngest primordia.

(4) *The Nature of the Operations and the Shift of the Growing-point.*

It was found impracticable to remove the youngest visible primordium (P_1) completely by a clean cut, but it was possible to isolate it from the stem apex and the other primordia by a tangential longitudinal cut parallel to the axis of the shoot, which left it still attached below. In the same way it was possible to isolate the piece of tissue about to give rise to I_1 or I_2. After these operations the isolated primordia or isolated pieces of tissue usually developed into normal leaves retaining their connexion with the stem at a lower level, though in one isolation of P_1 and several isolations of I_1 and I_2, they failed to develop.

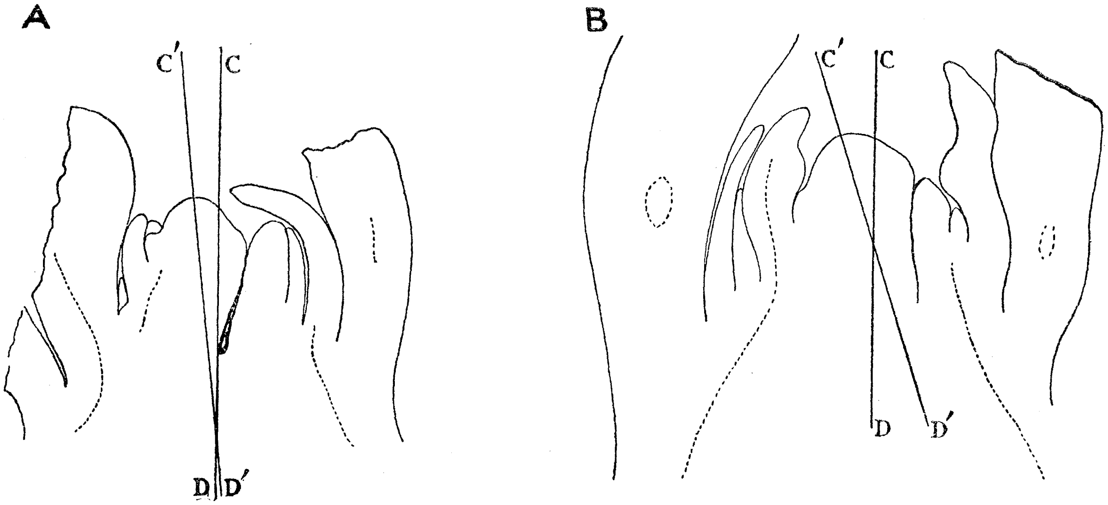

FIG. 1, A.—No. 396. Longitudinal section through stem apex and I_1, 10 days after isolation of the latter. The wound is indicated by a thick line. The line C′D′ marks the direction of growth of the new growing-point and CD that of the old growing point, as indicated by the orientation of the cell walls in the pith below it. × 52.

B.—No. 404. Longitudinal section through stem apex and isolated piece of tissue due to give rise to I_2, 9 days after the operation. The lines CD and C′D′ as in A. × 56.

Figs. 1, A, and 1, B, Nos. 404 and 396, are drawings of longitudinal sections through stem apices and isolated primordia, made at only 10 days and 9 days after the operations. They show the positions of the cuts, and the manner in which they isolated the pieces of tissue that were due to give rise to I_1 or I_2 from the rest of the apex. To ensure that the sections should be at right angles to the planes of the cuts, the points at which the cuts had passed through the outermost leaves of the bud were noted, but, in spite of this precaution, in many apices the sections passed through the wound obliquely and were useless. It is evident from the figures that the stem apex has tilted away from the wound, and this tilt is considered to be the result of a displacement of the centre of growth away from the wound, similar to that which occurred in previous experiments (PILKINGTON, 1929), in which the whole apex was split in half. In spite of many attempts, it was not found possible to devise a satisfactory method for measuring the shift of the growing-point. Its importance will be made clear below. In the experiments to be reported, the apices were left for a much longer time after the operations before they were examined—usually for from 3 to 5 weeks according to temperature.

PART 2.—THE ISOLATIONS OF I_1 AND P_1.

(5) *The Effects on the Subsequent Phyllotaxis.*

The arrangements of the primordia that arose after the isolation of P_1 or I_1 will now be described and compared. For their positions were altered in certain ways, although no primordium ever arose from the actual surface of the wound. The details of the results of 20 isolations of P_1 and 21 of I_1 are recorded in the appended Tables I and II.

When P_1 was isolated the subsequent phyllotaxis system was not much disturbed, but the angles subtended by the next two leaves, I_1 and I_2, at the growing-point exceeded the normal angle of 136°·3 in nearly every experiment. A list of these angles is given in Table I, column 2. They vary from 136° to 165°, and only in two experiments were they less than 145°. Since the growing-point of the stem had been displaced away from the wound in all these experiments, the angle I_1–I_2 was the angle subtended at the new growing-point and not at the old. Some increase in the angle I_1–I_2 would therefore be expected, even if these primordia arose in their normal positions relatively to the older primordia, since the growing-point had been displaced towards the line joining their centres. It will be discussed below whether the shift of the growing-point suffices to account completely for the increase in the angle I_1–I_2 or whether other factors also come into play.

Drawings of two typical experiments, Nos. 222 and 240, are shown in figs. 2 and 3. In each of these drawings, as in all subsequent drawings of experiments, a series of sections at different levels is shown, the highest section being shown at the top of the page. Also a drawing of a section of a normal apex, on a smaller scale, is inset, on which the exact position of the cut in the experiment is indicated, relatively to the isolated primordium, by a straight line. The way in which the position of the cut was determined will be explained in Section 10. It can be seen that the phyllotaxis has not been seriously disturbed. Measurements of the divergence angles of successive leaves after the isolated leaf are given in Table IV. They become gradually closer again to the normal angle of 136°·3 ± 1·88.

When I_1 was isolated, I_2 and I_3 were the next two primordia, and their positions may be compared with those of I_1 and I_2 when P_1 was isolated. The angle I_2–I_3, after isolation of I_1, may therefore be compared with the angle I_1–I_2 after isolation of P_1. This angle, I_2–I_3, increased enormously after isolation of I_1; measurements of such angles are given in Table II, column 2, arranged in three groups. In the 21 isolations of I_1 these angles ranged from 158°·3 to 203°·25. It is evident that their values form a continuous series following on approximately from those of the divergence angles I_1–I_2 obtained after isolation of P_1, of which the largest was 165°. On account of the displacement of the growing-point caused by the cut isolating I_1 the divergence angles of subsequent leaves were again subtended at a new centre of growth, and not at the old one.

Whatever may have been the cause of the increase in the angle between I_2 and I_3, it appeared that the subsequent leaf arrangement depended on the position and form of these two leaves. For in six experiments in which the angle I_2–I_3 was less than 180° (Table II, group 1) the subsequent arrangement was essentially similar to that after the isolation of P_1 (see fig. 4, No. 137). Measurements of the divergences of the subsequent leaves were made in two experiments and are given in Table V, group 1.

But in twelve experiments in which the angle I_1–I_2 exceeded or equalled 180° (Table II, group 3), the direction of the genetic spiral was reversed. Two examples are shown in figs. 5 and 6, Nos. 235 and 377. It can be seen that I_4 lies on the opposite side of the apex from that on which it would normally have arisen (compare the drawings of a section of a normal apex inserted in figs. 5 and 6, on which are indicated the positions of the cuts in these experiments). From this point onwards the direction of the genetic spiral was reversed. Measurements of the subsequent divergence angles are given in Table V, group 3. After a certain number of leaves had been laid down, the divergence angles approached again the normal value of 136°·3. The first few angles after the angle between I_2 and I_3 generally oscillated above and below the normal, as can be seen from the Table, but the oscillations were not always regular.

The fact that I_4 arose on the opposite side of the apex from the normal when the angle I_2–I_3 exceeded 180° supports the theory, mentioned in the introduction, that each primordium arises in the largest gap between the previous primordia. For I_4 occupied the larger gap between I_2 and I_3, on whichever side of the apex it lay. Moreover, it is in accordance with this theory, that when I_4 arose on the opposite side of the apex from that on which it would normally have arisen, the genetic spiral continued in the reverse direction from then onwards. For in normal Fibonacci phyllotaxis, such as that of the Lupin, since three primordia make up a cycle, the gap in which each subsequent primordium falls is the gap between the next older primordium but one, and the next older but two, as can readily be seen from fig. 5, A. And within this gap, the new primordium falls rather closer to the older of the two primordia on each side—a fact of which the explanation will become clear below. Normally, therefore, I_5 would have fallen in the gap between I_2 and I_3, and on the opposite side of the apex from I_4 (see fig. 5, A). But after the isolation of I_1, when I_4 arose between I_2 and I_3 on the opposite side of the apex from its normal position, it did not allow room for I_5 to arise on that side of the apex (see fig. 5). Consequently I_5 also arose on the opposite side of the apex from its normal position, and rather closer to I_2 than to I_3, in the other angle between I_2 and I_3. As a result, I_3, I_4 and I_5 constituted a cycle of primordia, of which the genetic spiral ran in the reverse direction. The subsequent primordia arose, as in an intact plant, in the gaps between the next older primordium but one, and the next older but two, and thus the genetic spiral was continued indefinitely in the reverse direction.

The causes which led, after several oscillations, to the re-establishment of the normal divergence angle were probably comparable with those which lead to the gradual establishment of the normal angle in Lupin seedlings, in which the two first primordia after the cotyledons are nearly at 180° from one another, as also they are in seedlings of other plants with Fibonacci phyllotaxis (see ITERSON, 1907, p. 273 *seq.*). Indeed, we have found that in Lupin seedlings, the divergence angles of the first six primordia, or more, often oscillate similarly, though less regularly, above and below the normal angle, before it is established. It might be expected that when the angle I_2–I_3 was very close to 180°, two new primordia would arise simultaneously, one on each side of the apex. In several experiments I_4 and I_5 did indeed appear to have arisen almost simultaneously, though never absolutely so.

There remain three experiments, those of group 2 of Table II, in which after isolation of I_1 the angle I_2–I_3 was less than 180°, and yet the genetic spiral was reversed. In two of these (Nos. 195 and 155) on account of an unequal development of the stipules of I_3, its stipule above the wound approached closer to I_2 than did its other smaller stipule on the opposite side, so that the larger gap fell within the smaller angle, on the opposite side of the apex from the normal. In the third (No. 132, *b*) owing to the unequal development of the stipules of I_3, the stipules of I_2 and I_3 met without overlapping on either side, so that the gaps available for the next two primordia were probably equal on both sides (see fig. 7).

It therefore appears that the position of I_4 depended not on the position of the centres of I_2 and I_3 but on the region occupied by these primordia as wholes. There seems to be no other conclusion indicated but that I_4 occupied the largest available gap between the edges of I_2 and I_3. This is the most striking piece of evidence obtained from the experiments in support of the theory that the position in which a primordium arises is determined by the positions of the older primordia.

The results of this section may be summarised as follows: When P_1 was isolated from the apex by a longitudinal cut, the divergence angles between the next two primordia, I_1 and I_2, ranged from 136° to 165°. The direction of the genetic spiral remained unaltered and the divergence angles of the succeeding leaves gradually approximated to the normal angle of 136°·3. When the region of the apex that was about to give rise to I_1 was isolated by a longitudinal cut, the divergence angle between the next two primordia, I_2 and I_3, varied from 158°·3 to 203°·25. In those experiments in which the angle exceeded 180° the direction of the genetic spiral was reversed, the leaf I_4 arising on the opposite side of the apex from that on which it would normally have arisen. When the divergence angle I_2–I_3 was less than 180°, the genetic spiral was not reversed, except in three experiments, in which, owing to the unequal development of the stipules of I_3, the larger gap between the stipules of I_2 and I_3 fell nevertheless on the opposite side of the apex from the normal. It depended therefore on the position of the larger gap between the next two primordia after the isolated primordium whether the genetic spiral was reversed or not.

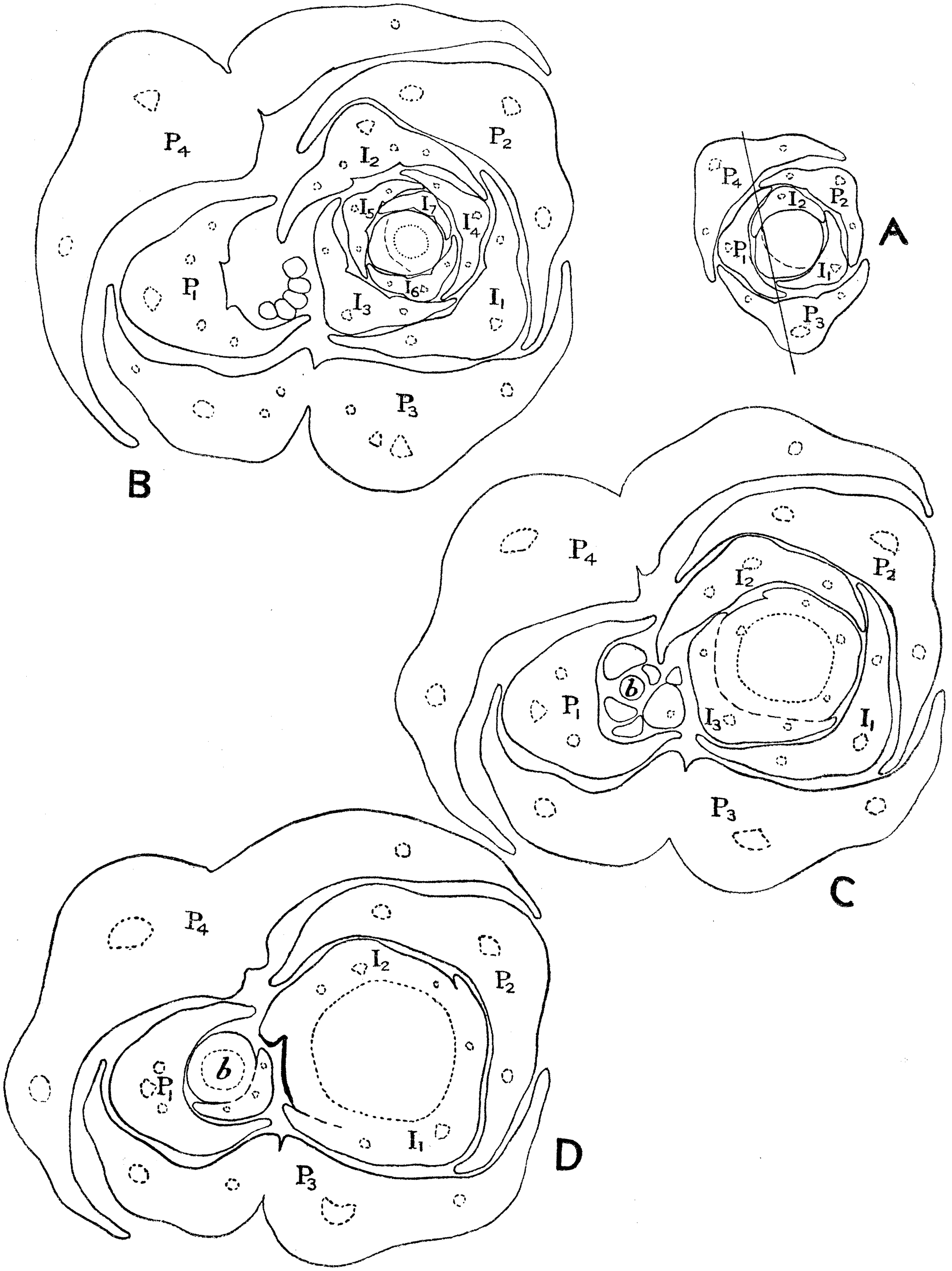

FIG. 2.—No. 222. An Isolation of P_1. Group II. A. Transverse section of normal bud showing position of cut. × 35. B, C, D. Transverse sections of No. 222 at different levels. × 33. A bud " *b* " has arisen in the axil of P_1. The wound scar in D is indicated by a thick black line.

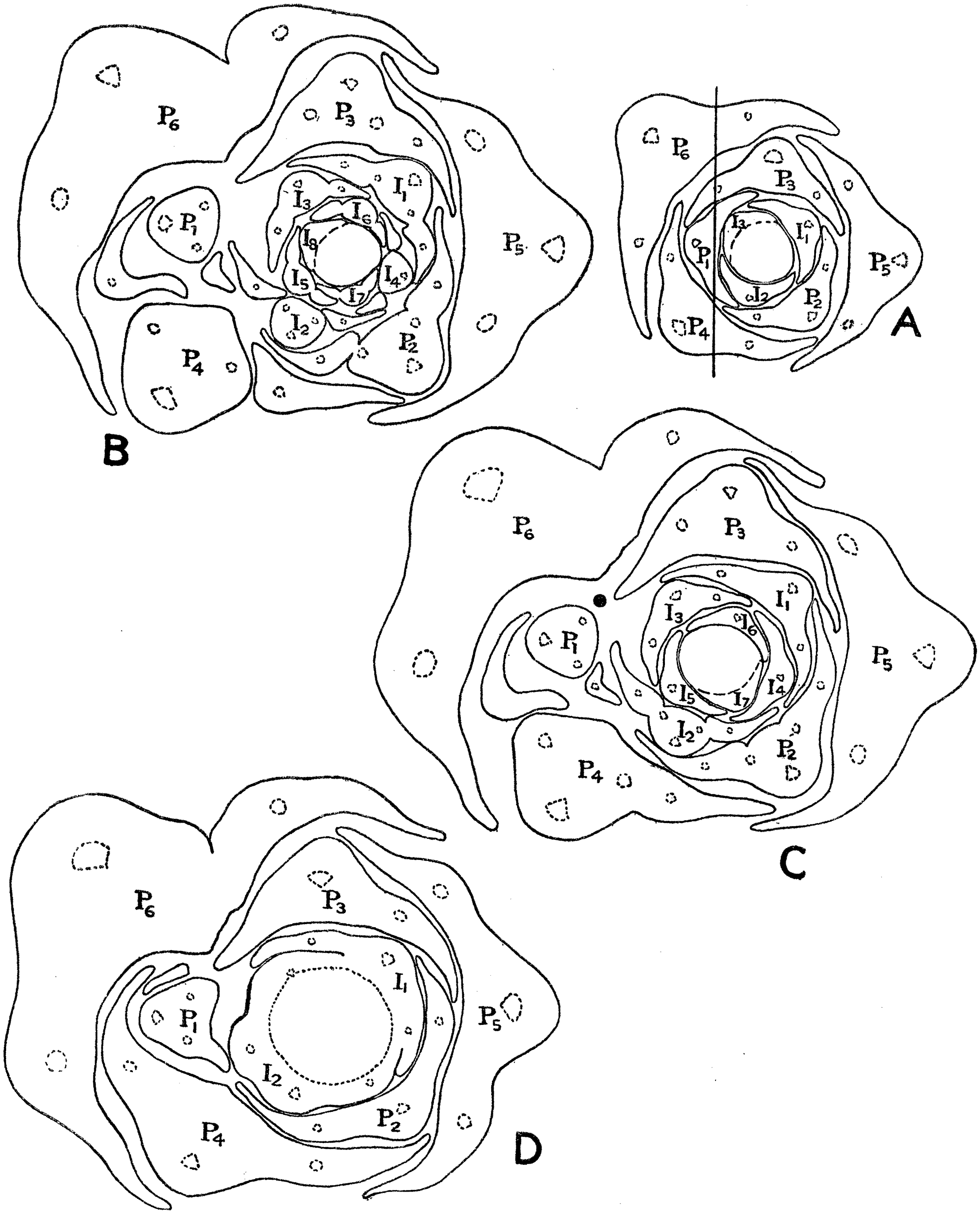

FIG. 3.—No. 240. An Isolation of P_1. Group I. A. Transverse section through normal bud showing position of cut. × 35. B, C, D. Transverse sections through bud of No. 240 at different levels. × 30. The wound scar in D is indicated by a thick black line.

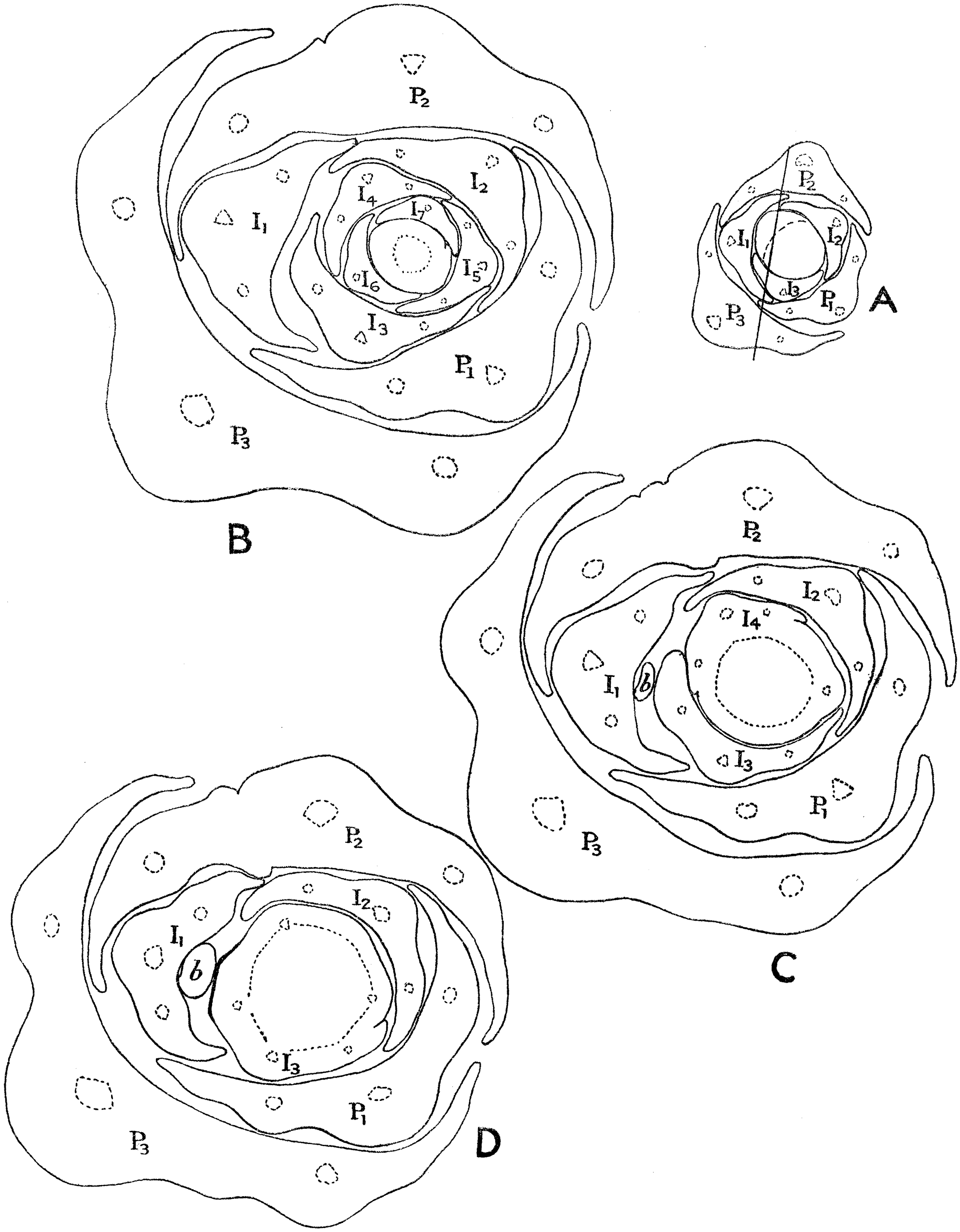

FIG. 4.—No. 137. An Isolation of I_1. Group I. A. Transverse section of normal bud showing position of cut. × 35. B, C, D. Transverse sections of bud of No. 137 at different levels. × 30. A bud "*b*" has arisen in the axil of I_1. The wound scar in D is indicated by a thick black line.

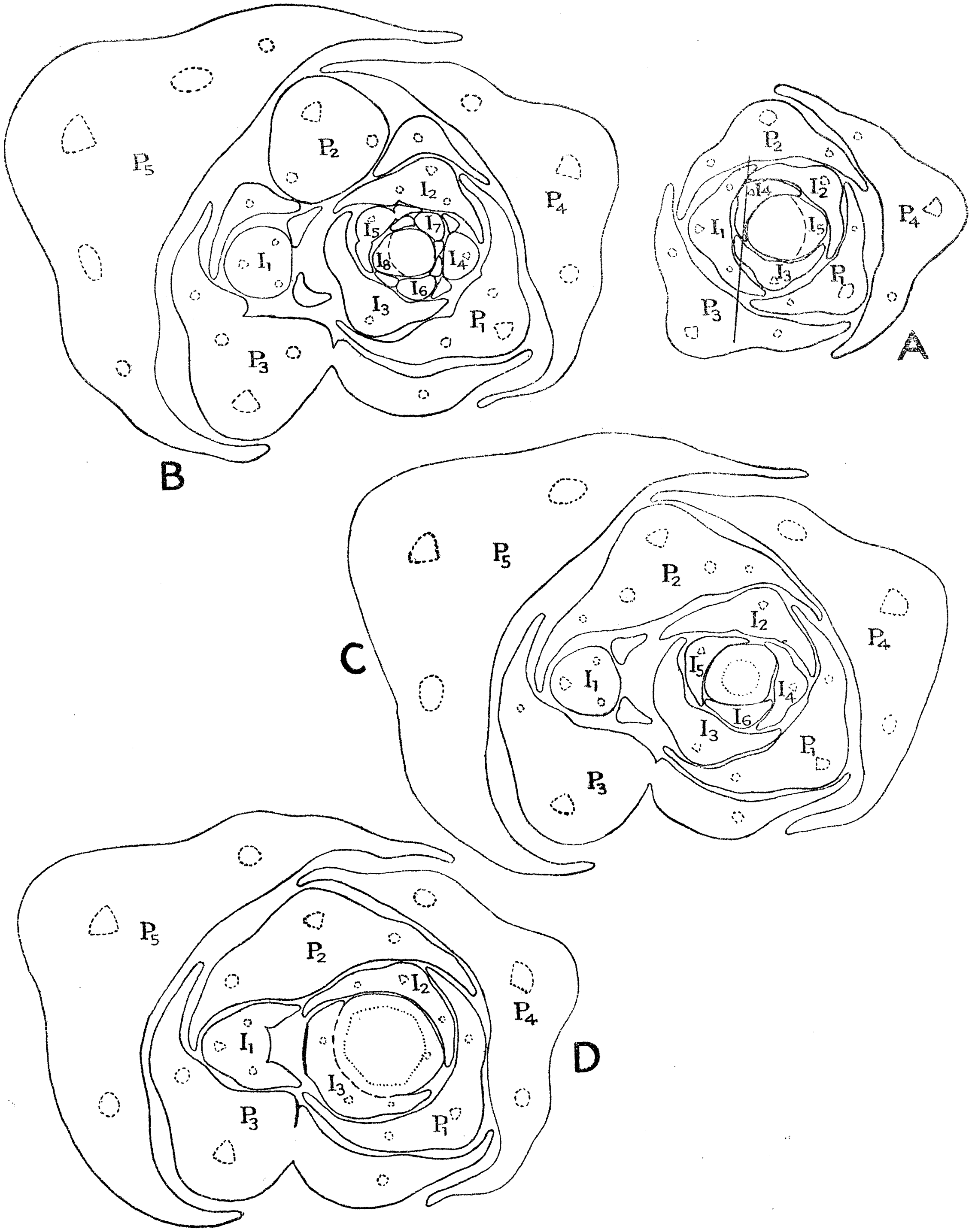

Fig. 5.—No. 235. An Isolation of I_1. Group III. A. Transverse section through normal bud showing position of cut. × 35. B, C, D. Transverse sections through bud of No. 235 at different levels. × 35. The wound scar in D is indicated by a thick black line.

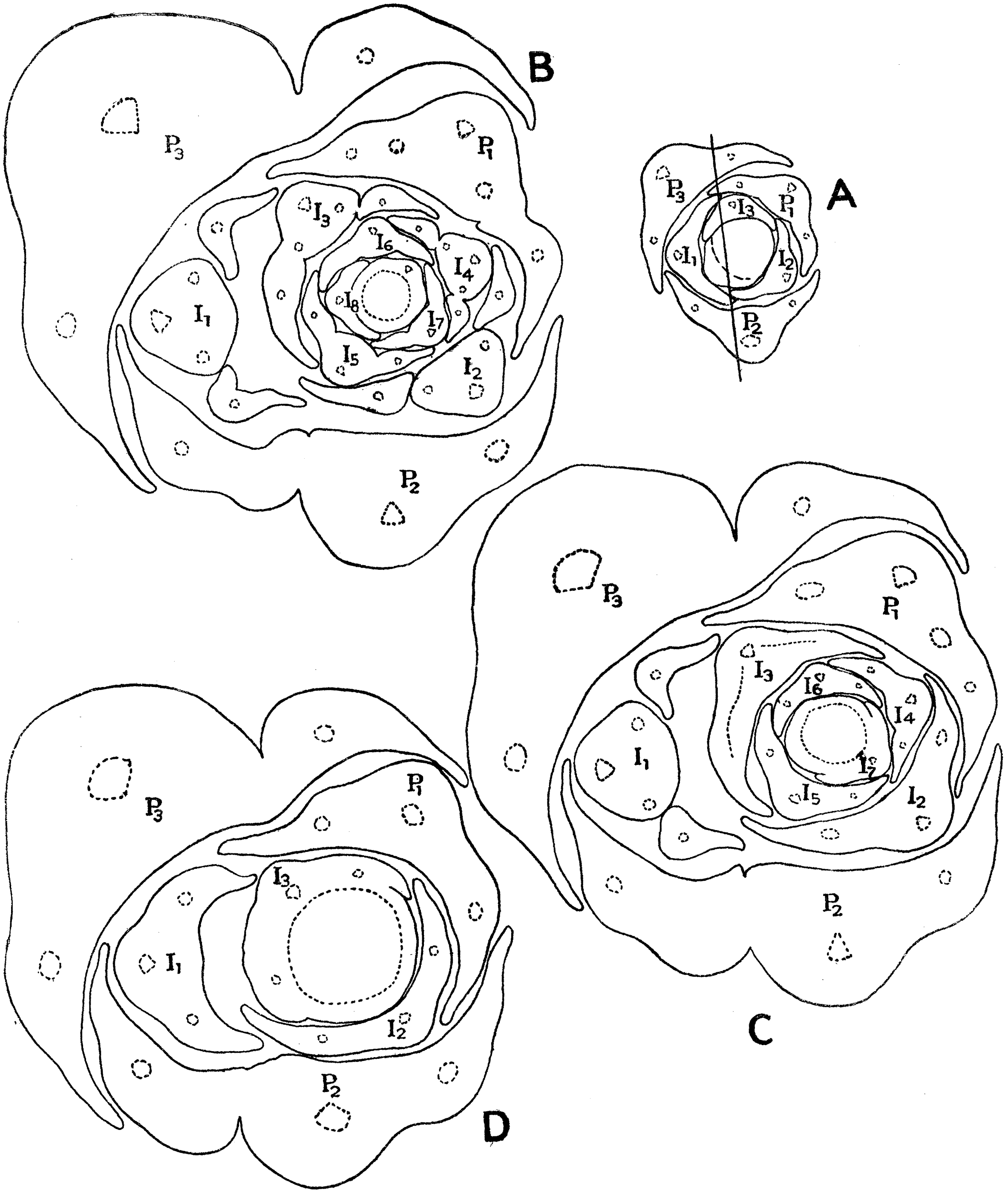

FIG. 6.—No. 377. An Isolation of I_1. Group III. A. Transverse section of normal bud showing position of cut. × 35. B, C, D. Transverse sections of bud of No. 377 at different levels. × 34.

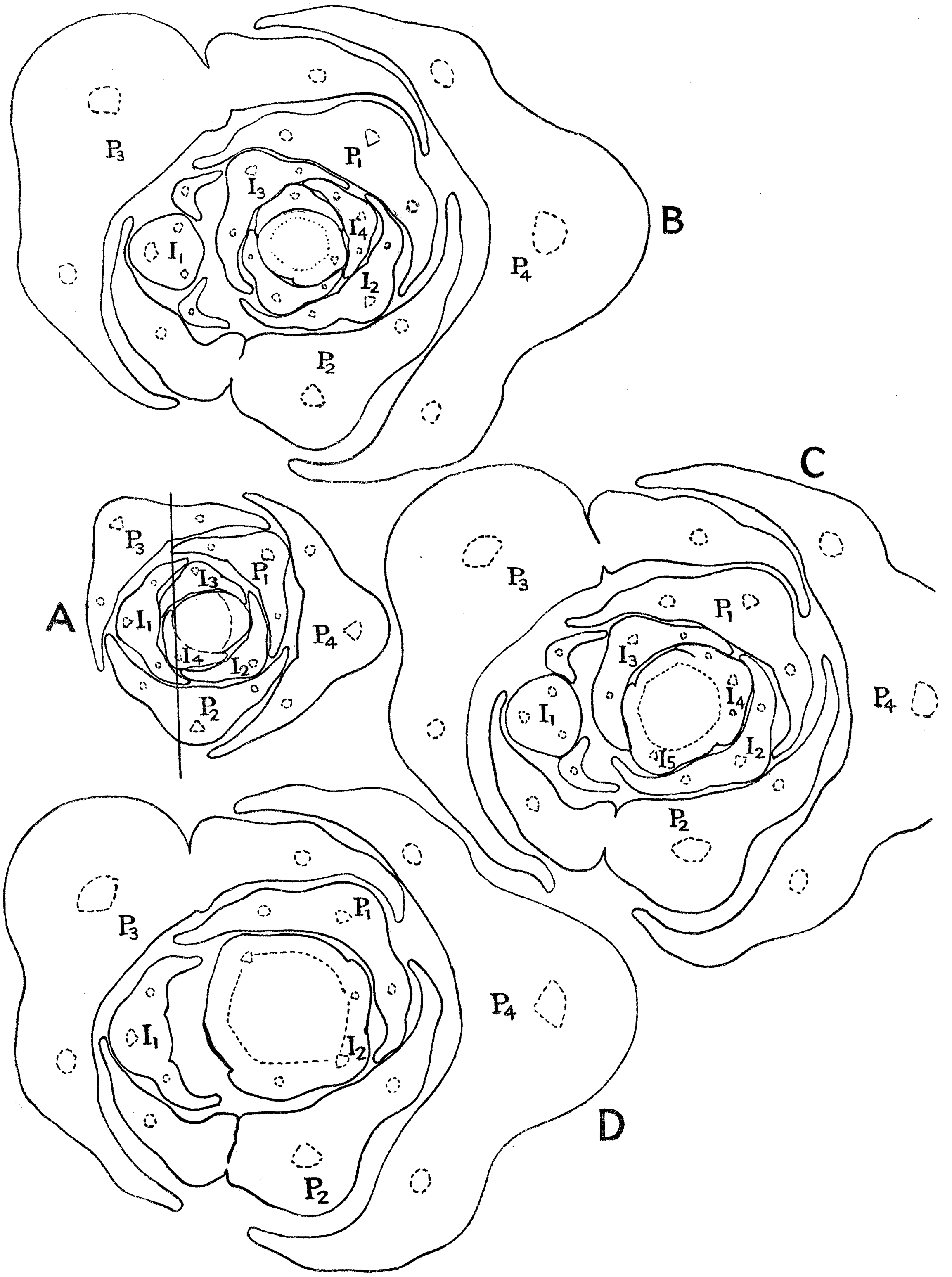

Fig. 7.—No. 132B. An Isolation of I_1. Group II. A. Transverse section of normal bud showing position of cut. × 35. B, C, D. Transverse sections of bud of No. 132B at different levels. × 28. The wound scar in D is indicated by a thick black line.

(6) *The Working Hypothesis.*

So far, in interpreting the results, we have considered each primordium as arising in the largest—that is to say, widest—gap between the older ones. But a more exact conception would be that, as the gaps between the youngest primordia gradually increase in size, new primordia are formed in each gap as soon as it attains a certain minimum width. The application of this conception to the experiments already described is the same as that of the conception of the widest gap previously employed. For of any two gaps that differ in size, the wider gap must also be the one that will first obtain the necessary minimum width, since the whole apical region is growing regularly.

It is further clear that a gap must be at some minimum distance below the growing-point before a primordium can arise in it, since primordia do not arise over the summit of the apex. It would, therefore, be more accurate to say that a primordium forms in each gap as soon as the gap attains both a certain minimum width and a certain minimum "lowness," the term "lowness" being understood to mean distance below the growing-point. This hypothesis may be more briefly stated by saying that each primordium arises in the "first available space," the word "available" being understood to mean both wide enough and "low" enough. Actually matters are simplified by the fact that in the Lupin, both normally and also, probably, in all the experiments here reported, the gap that first becomes low enough is also the gap that first becomes both low enough and wide enough. But it is nevertheless possible that in some other plants, or even in the Lupin in other circumstances, this may not be so.

(7) *The Causes of the Increase in Angle between the next two Primordia.*

It must next be considered what were the causes of the increase in the angle between the next two primordia after the isolated primordium, and further, why the increase was much greater after isolation of I_1 than of P_1. The growing-point, as has been mentioned already, is displaced away from the wound, and it therefore moves towards the gap between the next two primordia. This shift of the growing-point must by itself increase the divergence angle between the next two primordia, since this angle is the angle subtended at the growing-point by the line joining their centres. Unfortunately, it was not found possible to measure the shift of the growing-point, but if on the average it was equal in absolute amount after the isolations of I_1 and P_1 (and it seems unlikely that it was less after isolation of I_1), then it can be understood that it must cause a greater increase in the angle between the next two primordia when I_1 is isolated than when P_1 is isolated. For when I_1 is isolated, the distances of the next two primordia from the old growing-point are less, since they are younger, and consequently an equal absolute shift of the growing-point towards the gap between them must cause a greater increase in their divergence angle.

It was found, however, that the increase in the divergence angle of the next two primordia was not entirely accounted for as the direct result of the shift of the growing-point towards them. It was partly due to the fact that the centre of the second primordium after the isolated primordium arose not in its normal position, but closer to the wound, whereas the centre of the first primordium retained its normal position. This change in the position of the second primordium could not indeed be detected by measurements of divergence angles, since the original position of the growing-point was not known and attempts to locate it by reference to the older leaves proved unsuccessful. But finally a method was adopted of detecting—though not of measuring—changes in the position of the next two primordia after the isolated primordium, by observing their relation to the older leaves with which they made contact. For in transverse sections of normal buds the central vascular strand of each leaf is seen to lie very close to one of the main lateral strands of the older of the two leaves with which it makes contact (see fig. 8), so that changes of position can be detected by observing the relative positions of these strands.

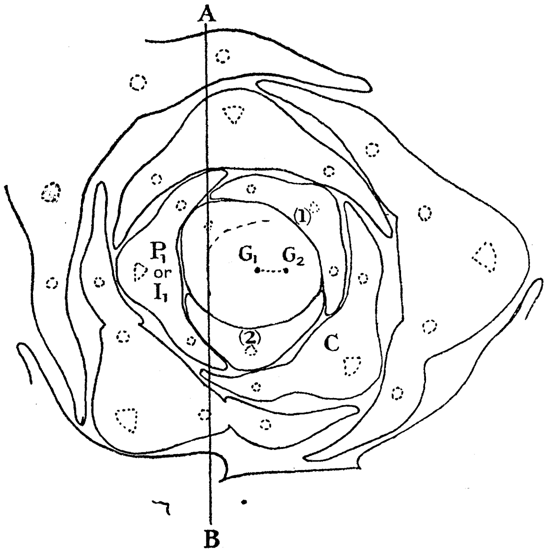

FIG. 8.—Transverse section through a normal bud passing just below the apex. The line AB represents the cut isolating P_1 or I_1. C is the contact leaf. (1) and (2) are the first and second primordia after the isolated primordium. G_1 and G_2 are the positions of the old and the new growing-points, joined by a broken line. × 45.

By this method no significant displacement of the centre of the first primordium after the isolated primordium could be detected, but in many experiments it was obvious that the second primordium had been displaced towards the wound, although no part of that primordium, nor of any other, arose from the actual surface of the wound. In Nos. 222 and 240, for instance (figs. 2 and 3), in which P_1 was isolated, it can be seen that the central strand of I_2 is slightly displaced relatively to the lateral strand of P_2. Also in Nos. 235 and 132, *b* (figs. 5 and 7), in which I_1 was isolated, the central strand of I_3 is clearly displaced relatively to the lateral strand of P_1. The displacement can be detected most accurately in the lower sections of each series. Similar displacements were detected in many other experiments also, of which the figures cannot all be reproduced. Their approximate amounts are recorded in Tables I and II, columns 4. In many of the experiments, however, the displacements could not be estimated at all, because the lateral bundle of the leaf with which the second primordium made contact had been disturbed by the cut. The displacement of the second primordium was always towards the wound, and was nearly always greater after isolation of I_1 than of P_1. It must have contributed towards the increase in the divergence angle between the first and second primordia, and the fact that it was greater after isolation of I_1 must have contributed towards making the increase in this angle greater after isolation of I_1. (It may here be noted that in speaking of "displacements" it is never intended that a primordium is actually moved from one place to another, but merely that it arises in some position other than its normal position.)

(8) *A First Factor causing Displacement of the Second Younger Primordium.*

It must next be considered whether this displacement of the second primordium can be interpreted in terms of the hypothesis of the "first available space." On this hypothesis, the position in which a primordium arises must depend upon the shape of the contour-line that is formed by the upper margins of the older primordia round the apex, and upon the relative distances from the growing-point of the regions immediately above different points in that contour-line. For of any two such regions, the one which at any moment is the farther from the growing-point must also be the one which has first reached, or will first reach, the necessary minimum distance at which a primordium can arise. In the experiments, the contour-line upon which the position of the next two primordia after the isolated primordium depends, is formed on one side of the apex by the outline of the wound, which touches one edge of the second primordium (see fig. 8). The other edge of the second primordium makes contact with an older leaf, which forms the contour line on the opposite side of the apex. This older leaf, which is P_2 when P_1 is isolated, and P_1 when I_1 is isolated, will be referred to throughout as the "contact leaf."

Now the shift of the growing-point which is caused by the wound, alters the ratio of the distances from the growing-point of the wound and the "contact leaf," increasing the former and diminishing the latter, as can be seen from fig. 8. Moreover, if the shift of the growing-point is equal in absolute amount after the isolations of I_1 and P_1, it must cause a greater alteration in the ratio of the distances of wound and contact leaf after isolation of I_1 than of P_1. For the distances of wound and contact leaf are both absolutely smaller when I_1 is isolated, since the wound isolates a younger primordium and the contact leaf is also younger. It was not indeed found possible to measure the shift of the growing-point, but it seems unlikely that it was less after isolation of I_1 than of P_1, and if it was greater the above conclusion is strengthened.

Now it seems probable that one factor that is involved in causing the second primordium to be displaced towards the wound, is the change in the ratio of the distances from the growing-point of wound and contact leaf, which increases the relative amount of space available for leaf formation in the region above the wound and decreases it above the contact leaf. If this is so, it can be understood how it is that the displacement of the second primordium is greater after isolation of I_1 than of P_1: for the change in the ratio of the distances of wound and contact leaf must also be expected to be greater after isolation of I_1, as has just been pointed out.

This interpretation is indeed supported by direct observations. For from sections of the apices cut at different levels, it could be seen that after isolation of I_1, the edge of the second primordium (I_3) always extended laterally into the region above the wound, and often reached right across this region, so as to make contact with I_2 on the other side, as in Nos. 377 and 235, figs. 6 and 5. On the other hand, after isolation of P_1, the edge of the second primordium (I_2) was always found to be situated at a lower level in relation to the wound, so that it made contact with the sloping side of the wound, which seemed to have prevented it from extending farther, as in No. 240, fig. 3. None the less, it is probable that even after isolation of P_1, the edge of the second primordium extended laterally a little beyond its normal position.

After isolation of I_1 in 14 out of 19 apices that were examined, the stipule of the second primordium which extended across the region above the wound, was seen to be larger than the other, so that the primordium as a whole was asymmetrical, as can be seen from Table II. This fact indicates that the stipules of a primordium can to some extent spread out into a space that is available for them, independently of the central part of the primordium, from which the rest of the leaf originates. After isolation of P_1 the stipules of the second primordium did not extend nearly so far into the region above the wound, and only in one experiment out of 20 was this primordium slightly asymmetrical.

(9) *The Shapes of the Primordia and their Representation.*

So far, only the displacement of the *edge* of the second primordium has been considered. But it is necessary to consider also the displacement of its central part; for, as has been already stated, the central vascular strand of the second primordium was displaced from its normal position, and this strand may be considered to mark its morphological centre. For this purpose it is necessary to construct a diagram showing the vertical components of the phyllotaxis system, as well as the horizontal components. But before this can be done, it must be decided what is the probable shape of the young primordia, as seen in surface view.

Van Iterson, from consideration of transverse sections of primordia which show the shapes of what he calls "folioid curves," has concluded that in many plants the primordia at their insertions are circular in surface aspect. In the Lupin, sections of young primordia have a shape which may be considered as approximately composed of a folioid, representing the central part, together with two marginal portions outside the folioid, which represent the stipular rudiments on each side. The transverse diameter of the central part, from which all the leaf except the stipules originates, is approximately equal to 0·45 of the diameter of the whole primordium from edge to edge.

Accordingly in the diagram (fig. 9) each primordium is represented by a central circle, of diameter equal to 0·45 of the total diameter, with marginal portions representing the stipular rudiments attached to it on each side. At the time, indeed, when the position of a primordium is being determined, it is still invisible, but the most probable assumption is that its shape at that time is approximately the same as when it does become visible. Moreover, primordia do not arise in positions which do not allow room for their stipules, and this fact indicates that the areas from which the stipules are due to arise must already form part of the physiological field of the primordium before it is visible.

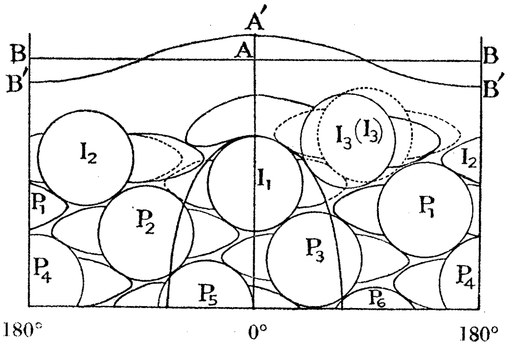

Fig. 9.

Direct observations were also made on the shapes of the youngest primordia on the apex, as seen in the solid, and these confirmed the conclusions drawn from their shapes as seen in section. For it was seen that in the youngest visible primordium, during the greater part of its first plastochron, only the central part was visible, and this was circular in outline, or at least approximately so. But a little later, towards the end of the first plastochron, the stipules of this primordium began to arise from the surface of the apex, on each side of the circular central part, and they appeared as transversely elongated members tapering towards their ends. The manner in which the primordia make contact with one another and the extent to which they overlap, have been determined as accurately as possible from a transverse section of a normal apex, and the diagram has been drawn accordingly. The phyllotaxis system has been represented as if upon the surface of a cylinder which has been unrolled and spread out flat, with primordia all of the same size. Actually the primordia are situated upon a paraboloid apex, and the younger ones are smaller, but in spite of this discrepancy, the diagram will serve the present purpose. For it is intended to show only the way in which the primordia make contact with one another and with the wound, and these contacts can be shown quite correctly even when the primordia are drawn of equal sizes, as if on the surface of a cylinder.

The diagram represents an isolation of I_1, but if the numbering of all the primordia is changed by one step, it will represent equally well an isolation of P_1. The vertical line through the centre of I_1 is taken as the zero line from which the divergence angles are measured. The two lines on each side marked 180° represent the line along which the cylinder is supposed to have been cut, in order to spread it out flat. On account of the shift of the growing-point, the distances of points in the diagram from the old and the new growing-points are represented by their distances from the lines B A B and $B^1A^1B^1$ respectively. The amount of shift of the growing-point is represented by the distance A A^1 or B B^1, which is the distance between the above two lines at 0° and 180°. Since the shift could not be measured, its amount could only be guessed. Though the diagram has been made as accurately as possible, and in accordance with the facts that could be ascertained, it is introduced only to illustrate the argument, and, in view of the many difficulties, it is naturally not claimed that it is a completely exact representation of the phyllotaxis system. The positions which would normally have been occupied by I_3 and by the stipule of I_2 are shown by dotted lines, and their actual positions by unbroken lines.

(10) *A Second Factor displacing the Second Younger Primordium.*

It was found possible to determine the position of the outline of the wound by examining drawings of sections of the apices at various levels. For the older leaves had frequently been cleft by the cut, and it could be seen at what points they had been cleft. Reference was then made to a drawing of a section of a normal apex, and the youngest visible primordium in this drawing, or the youngest but one, was considered as representing the isolated primordium as it would normally have been when it had developed to that stage. Then, on the older leaves of this normal section, the points were marked which corresponded with the points at which in the experiment the corresponding older leaves had been cut into. These points sufficed to determine the plane of the cut, and it could therefore be seen at what points, if any, this plane passed through the primordium of the normal section which represented the isolated primordium.

By this method it was found that when I_1 was isolated, usually the cut did not really isolate the whole of the area which that primordium would normally have occupied by the time that it had become the youngest, or youngest but one, visible primordium. To use a convenient term, the cut usually did not isolate the whole of the " presumptive area " of I_1.*

Thus it was found, as is recorded in Table II, that out of eighteen isolations of I_1, of which the necessary drawings were available, in four (Nos. 144, 194, 235, 319) more than half the presumptive stipular area of I_1, on the side towards P_1, was left on the apex, in four (Nos. 322, 379, 155, 100) about half of that area was left on the apex, and in five (Nos. 132, 195, 377, 321 and 137) less than half or the tip only of the area was left. In four others (Nos. 245, 182, 236 and 133) scarcely any of the area was left on the apex, and in one (No. 104) the amount was rather doubtful.

Now the part of the presumptive stipular area of I_1 that was left on the apex did not give rise to stipules by itself, and it seems that on the side towards I_3 the part of that area left on the apex must have become occupied by the central part of I_3. For when drawings of sections of the apices were examined at a level low enough to pass through the wound, it was found that out of 18 isolations of I_1, of which the necessary drawings were available, in eight the central part of I_3 was right in the gap above the point at which the wound and the contact leaf (P_1) met, and in contact with the wound. In another six of these isolations, I_3 appeared probably to occupy this gap, and only in

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* The term " presumptive area of a primordium " is not quite precise unless it is stated *for what stage of development* of the primordium the area referred to is presumptive. For in the Lupin the insertions of the stipules gradually extend round the stem as the primordium develops, until, at about the stage of P_9, they reach nearly half-way round. But at the early stages of development here considered, this extension has not been more than slight. Here, indeed, since it was the factors displacing I_3 that were being considered, it was relevant to consider the area of the isolated primordium (I_1) that was presumptive for the stage of development of P_1 or P_2, as was actually done. For the position of I_3 was being determined at about the time at which I_1 would normally have reached the stage of P_1 or P_2.

four (Nos. 245, 182, 235 and 322) did I_3 appear to lie not in this gap, but closer to its normal position. In three others (Nos. 379, 358 and 205) the position could not be determined.

Now from fig. 9 it can be seen that when the central part of I_3 lay in this gap, it must have occupied the part of the presumptive stipular area of I_1 that was left on the apex. And this would account, in part at least, for the observed displacements of the central part of I_3 towards the wound. Moreover, it is quite in accordance with the hypothesis of the " first available space " that the central part of I_3 should occupy the gap between wound and contact leaf, if nothing prevented it from doing so: for the point where wound and contact leaf meet is the lowest point in the contour line—that is to say, the farthest from the new growing-point—as can be seen from fig. 9. (It must be remembered that distance from the new growing-point is represented by distance from the line $B^1A^1B^1$, not from B A B).

If this explanation of the displacement of I_3 is correct, the displacement must be expected to have been less when only a small part of the presumptive stipular area of I_1 was left on the apex than when a larger part was left. On the whole, this was found to be so, as far as could be judged, as can be seen from Table II, though unfortunately it was not found possible to measure the displacements exactly, and often their amounts could not even be roughly estimated, for the reason explained in section (4). Moreover, the correlation is obscured by the fact that the I_1 primordia were at slightly different stages of development: for since the displacements of the second primordium were less when P_1 was isolated, it must be expected that in the isolations of I_1 the displacements would be rather less when I_1 was rather older and therefore nearer to the stage of P_1. It was indeed found that in the isolations of P_1 the displacements were actually less when P_1 was older.

In the isolations of P_1, the cuts were intentionally made sometimes rather close to the central part of that primordium, and sometimes rather far from it and closer to the growing-point, so that the isolations fell into two groups. By observations similar to those made after the isolations of I_1, it was found that in the isolations of the first group the amounts of the stipular areas of P_1 left on the apex were actually, for the most part, considerably greater than in those of the second group, as can be seen from Table I, column 3. The parts of the stipular areas of P_1 also that were left on the apex did not develop by themselves in the experiments here reported, and it is probable that they also were invaded to some extent by the central part of the second younger primordium (I_2). For it can be seen from the diagram (fig. 9) that they must have been invaded whenever the second primordium was displaced towards the wound, as it was usually found to have been, though less far than after isolation of I_1. Moreover, in the first group of isolations of P_1, in which the amounts of stipular area left on the apex were greater, the displacements of I_2 were also usually distinctly greater (see Table I), and so also on the average were the angles between I_2 and I_1. This difference may, however, not have been due to the fact that in the first group more of the stipular area of P_1 was left on the apex. The explanation may be that the cuts, being closer to the central part of P_1, presumably did not extend so far up the apex above P_1, and so allowed the stipule of I_2 to extend farther into the region above the wound. Or again, both factors may have contributed.

(11) *Combined Working of the above two Factors displacing the Second Primordium.*

Even in the first group of isolations of P_1, the second primordium (I_2) was usually not displaced so far as was the corresponding primordium (I_3), when I_1 was isolated (see Tables I and II). Moreover, examination of drawings of sections made at rather a low level showed how it was that this was so. For it was seen that when P_1 was isolated, the central part of I_2 was never displaced so far towards the wound as to occupy fully the depression above the point at which wound and contact leaf met, whereas when I_1 was isolated, the central part of I_3 usually arose in this depression, as has been mentioned already. Since, therefore, the point at which wound and contact leaf met was the lowest point in the contour line, it needs to be considered how it was that when P_1 was isolated, the central part of the second younger primordium (I_2) did not arise right in the depression above this point, but in a higher position closer to its normal position.

The explanation is probably as follows :—After isolation of P_1, the second primordium was situated lower down in relation to the wound than after isolation of I_1, as has been pointed out already, and the lateral extension of its stipule was therefore limited by the side of the wound. Consequently, it was not possible for the central part of the second younger primordium to arise in the deepest part of the depression between wound and contact leaf : for if it had done so there would not have been room for its stipule. And the centres of primordia are not found to arise in positions that do not leave sufficient room for the stipules.

This explanation is supported by the fact, already mentioned, that after isolation of P_1, the second younger primordium was nearly always symmetric, like the primordia of an intact plant, its morphological centre being midway between the edges of its stipules. For this indicates that the position of the morphological centre depended on the positions of the edges. On the other hand, after isolation of I_1, the second primordium (I_3) was situated higher up in relation to the wound, so that its stipule was able to extend for a long way through the region above the wound, and usually became abnormally large, making the whole primordium asymmetric (see Table II). Consequently, the displacement of the centre of I_3 towards the wound was usually not limited by the necessity for allowing room for its stipule, and the central part of that primordium was therefore free to arise in contact with the wound, in the deepest part of the depression between wound and contact leaf.

The asymmetry of I_3 is of interest in that it indicates that a primordium may arise in two slightly different ways. For after isolation of P_1 the second primordium (I_2) arose as soon as there was available for it, at a sufficiently low level, a space wide enough to allow room for the edges of its stipules: and the central part of the primordium arose midway between its edges, although this was not the lowest position, within the space. But after isolation of I_1, through the relative increase in available space above the wound, a space became available that was more than wide enough for the stipules of the second primordium (I_3). The central part of that primordium was therefore free to arise in the lowest position within the space, even though in so doing it was not arising in the middle of the space. The stipules extended unequally on each side, and the whole primordium became asymmetric.

The displacement therefore of the second primordium after the isolated primordium seems to have been due mainly to two factors. In the first place, part of the presumptive stipular area of the isolated primordium was usually left on the apex and became available for leaf-formation, with the result that a new depression was available for the central part of the second primordium, lower than its normal position and closer to the wound. Secondly, the shift of the growing-point and the consequent relative lowering of the outline of the wound enabled the stipule of the second primordium to extend to a greater or less extent into the region above the wound, and thereby to allow room for the central part of that primordium to be displaced, either (after isolation of I_1) right into the new depression available for it, or (after isolation of P_1) for a less distance in the same direction.

(12) *A Third Factor displacing the Second Primordium.*

Still a third factor appears to have contributed towards the displacement of the second primordium, and this may be explained as follows:—A few additional isolations of I_1 and P_1 were made, and the apices were examined by longitudinal sections at right angles to the wound after a week or ten days only. It was seen that in the stem apex, just internally to the surface of the wound, several layers of slightly elongated cells had been formed by tangential divisions, with their longer sides parallel to the wound surface. These layers can be seen in the drawing shown in fig. 10, A. They are probably of the same nature as the cambial layers that frequently form internally to wound surfaces, and it can be seen that they extend slightly above the wound and run out to the original surface of the apex. It therefore appears that the cambial layers formed an additional zone of tissue internal to the cut surface of the wound, and reaching to the surface of the apex around the wound.

Now since, after isolation of I_1, the central part of I_3 appeared usually to make contact with the sloping side of the wound, it must have invaded the new cambial zone. This zone must therefore have provided an additional amount of space for leaf-formation, and in invading it the central part of I_3 must have been displaced in the direction of the wound further than it would otherwise have been.

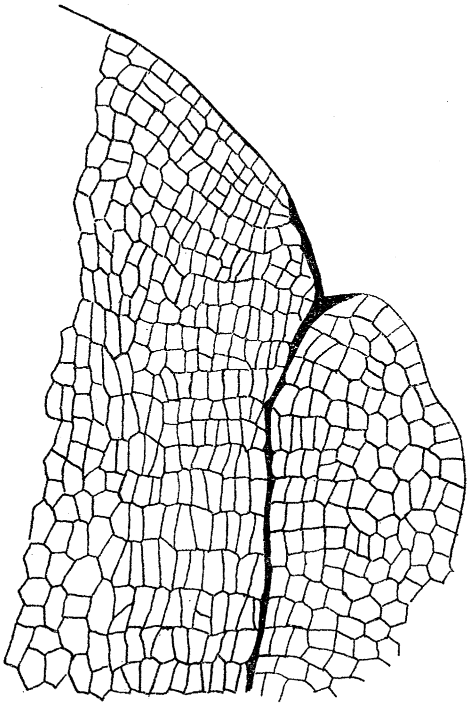

FIG. 10, A.—Part of a longitudinal section of an apex, made nine days after an isolation of I_2, in a plane at right angles to the wound, showing cambial divisions round the wound. The heavy black line marks the wound scar: the isolated piece has not developed into a leaf. Magnification approx. 300 times.

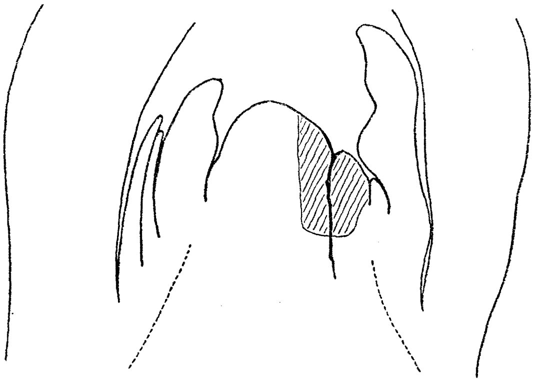

FIG. 10, B.—A small scale drawing of the same apex, in which the portion of tissue drawn in fig. 10, A is shown as a shaded area. × 54.

(13) *The Behaviour of the First Younger Primordium.*

The fact that the centre of the first primordium after the isolated primordium was never displaced, can readily be understood. For the central part of that primordium was situated in a depression between two older leaves (see fig. 9), and the parts of these leaves with which it was in contact were not touched by the cut. Consequently, the centre of the first primordium would not be expected to be displaced. On the other hand, the stipule of the first primordium might be expected to extend beyond its normal position towards the region above the wound, in the same way as did the stipule of the second primordium after isolation of I_1, as a result of the shift of the growing-point and the relative lowering of the wound. And actually it was found that after isolation of I_1 the next primordium (I_2) was asymmetric in 13 out of 17 apices that were examined, its stipule towards the wound being the larger (see Table II). After isolation of P_1, the next primordium (I_1) was asymmetric in one only out of 20 apices examined, probably because, as already explained, the relative lowering of the wound was not so great.

(14) *The Development of the Isolated Primordia.*

An incidental point of interest concerns the manner in which the isolated primordia themselves developed in those experiments in which about half or more of their stipular areas were left on the apex. For out of eleven such isolations of P_1 (those of the first group), in six P_1 developed with only one stipule as in No. **240**, fig. **3** (probably because the cut was slightly oblique), and in four it developed without stipules. In one it did not develop at all. From this it appears that a primordium at the stage of P_1 cannot re-organise itself so as to form a perfect leaf, if deprived of about half or more of one of its stipular areas.

On the other hand, out of nine isolations of I_1 in which half or more of one stipular area was left on the apex, though in four I_1 did not develop at all, yet in the five in which it did develop it was always symmetrical, and never developed with one stipule only. For in three of these experiments it developed into a complete leaf, with well-formed stipules, though two of these leaves were of less than normal size, and in a fourth experiment also it developed into a leaf with stipules, though the stipules were very small, even relatively to the central part of the leaf, which was itself small. In the fifth experiment it developed without any stipules. Since it was evident that in four of these five isolations, just as in those of P_1, the cuts were accidentally slightly oblique, these facts indicate that I_1, when deprived of half or more of its stipular areas, can re-organise itself so as to form complete stipules at the expense of tissue that would otherwise have gone to form the central part of the leaf. Thus, to use a convenient term, a secondary determination of the material of the primordium into central part and stipules has taken place at the stage of P_1, but not, or not irrevocably, at that of I_1.

In the isolations, whether of I_1 or P_1, in which less than half of the stipular areas was left on the apex, the isolated primordium always developed into a complete leaf possessing both stipules. But it could not be seen from the sections whether the stipules were quite of normal size or not, since the isolated leaves became irregularly folded.

Another point that may here be noted is that in the second group of isolations of P_1, in all except one (No. **346**) a bud developed on the isolated piece in the axil of the primordium. Also in three of the isolations of I_1 (Nos. **236**, **195** and **358**) in which the cut passed rather far from the central part of the primordium, a bud developed in this position, but not in the other isolations of I_1 nor in those of P_1 of the first group. These buds must have been formed by re-organisation from part of the apical tissue that was included in the isolated piece above the primordium, and it is doubtful whether they can be considered as axillary buds of a normal kind. It may be noted that, at this stage of development of the Lupin, buds are not usually found in the axils of leaves other than the isolated leaf.

PART 3.—THE ISOLATION OF I_2.

(15) *The Subsequent Phyllotaxis.*

In a further series of 21 experiments, of which the detailed results are given in Table III, the region from which I_2 was due to arise was isolated from the rest of the apex by the method already described. In many of these experiments, the cuts really isolated an area smaller than the whole presumptive area of I_2, and so left on the apex parts of the presumptive stipular areas of the isolated primordium, just as they did when I_1 was isolated. In 12 of the 21 experiments, the isolated primordia failed to develop: this point will be considered below.

The first primordium to arise after the operation was naturally I_1, which was older than the isolated primordium: it arose in its normal position. But the next primordium that arose on the apex was younger than the isolated primordium, and its position varied in different experiments; so that the results fall into three groups according to the position of this primordium. The results of the first group were similar to those of isolating I_1. The next two primordia after the isolated primordium were I_3 and I_4, corresponding to I_2 and I_3 when I_1 was isolated. The contact leaf was I_1, corresponding to P_1 when I_1 was isolated. The central part of I_3 occupied its normal position, as was shown by its contacts, and I_4 was displaced towards the wound, as was shown by its contact with I_1. The angle I_3–I_4 increased greatly, and exceeded 180°, and the genetic spiral was reversed.

In all the experiments of this group, and also in those of the other groups reported below, I_1 arose in its normal position between P_2 and P_3, as could be seen from the contacts that it made with these leaves and with P_1. This is what would be expected on the working hypothesis, since the gap between P_2 and P_3, in which the central part of I_1 arose, was not affected by the wound.

The results of this first group were thus essentially similar to those of isolating I_1, and the explanations proposed for the increase in the angle I_2–I_3 after isolation of I_1, and for the displacement of I_3 towards the wound, apply equally to the increase in the angle I_3–I_4 and the displacement of I_4 after isolation of I_2.

In nine other experiments, those of the second group, another primordium arose before I_3 obliquely above the wound. This primordium will be called $I_{2'}$, since its divergence angle from I_1 was not very different from that of I_2 in a normal plant. An example is shown in fig. 11, No. 167. In all the experiments of this group, part of $I_{2'}$ extended above the wound, but its centre lay between the region directly above the wound and the adjacent primordium P_1, as can be seen in fig. 11. In the experiments of this group, I_3 was regularly displaced a little way away from the wound. This was clearly because the stipule of $I_{2'}$ had encroached upon the position normally occupied by I_3.

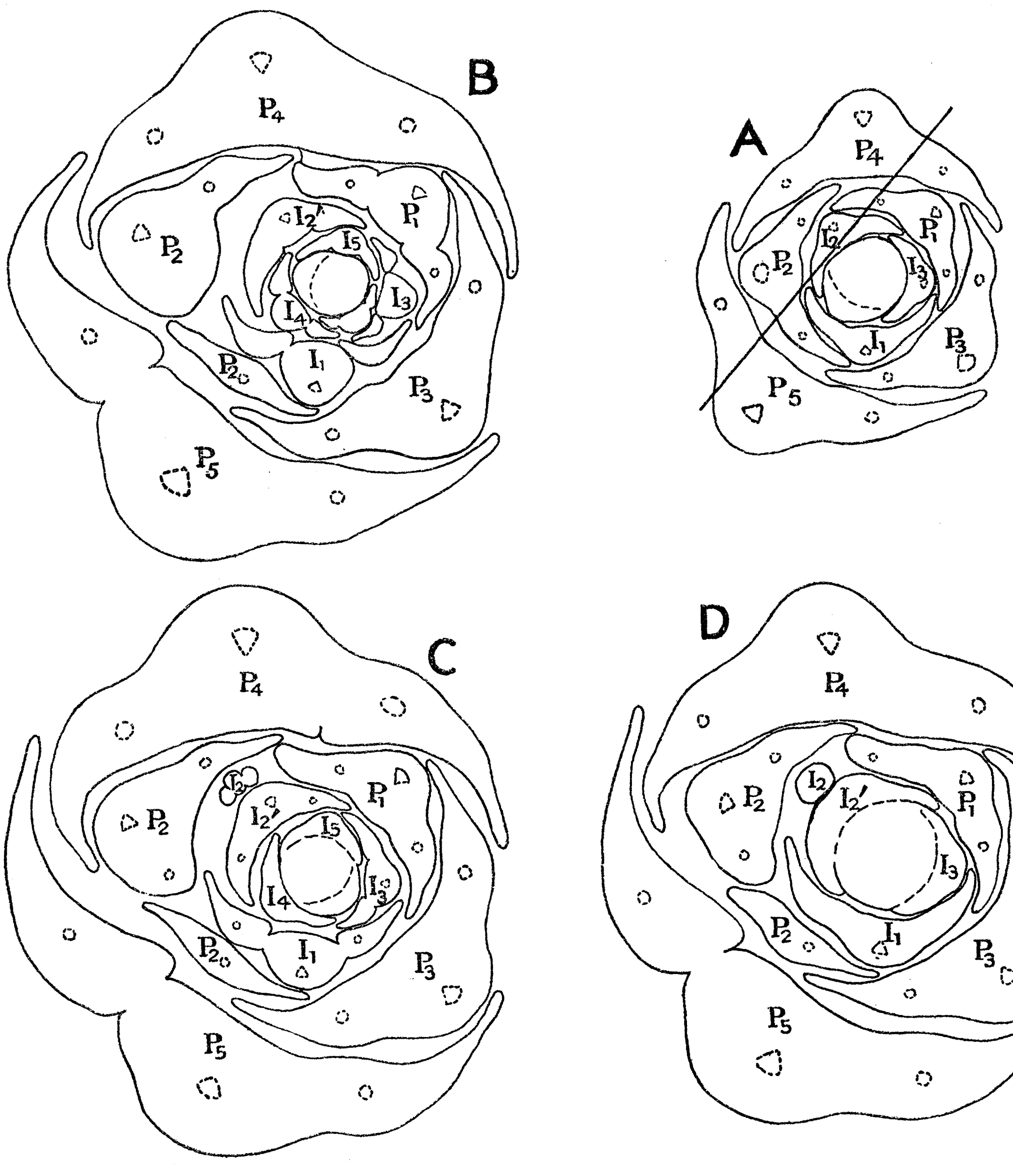

FIG. 11.—No. 167. An Isolation of I$_2$. Group II. A. Transverse section of normal bud showing position of cut. × 35. B, C, D. Transverse sections through bud of No. 167 at different levels. × 33. The wound scar in D is indicated by a thick black line.

In fig. 12 an attempt is made to show the position of I$_2'$ in relation to the wound and the older primordia. The primordia are represented, as before, as if on the surface of an unrolled cylinder, and, on account of the shift of the growing-point, the distances of points in the diagram from the new growing-point are represented, as before, by their distances from the curved line $B^1A^1B^1$. The diagram should be compared with the drawing of No. 167, fig. 11. In this experiment the cut passed obliquely through I$_2$, leaving on the apex the whole of its stipular area next to P$_1$, and probably even some of its central part also. In the diagram therefore the vertical dotted line, which passes through the centre of the wound, is slightly to one side of the vertical line through I_2. I_2' is asymmetrical, one of its stipules extending right across the region above the wound, and making contact with I_1.

It can be seen that in one point the diagram does not quite correspond with the drawing of No. 167. For in the diagram the stipule of I_2' next to P_1 over-reaches the centre of P_1, whereas in the drawing it does not. This error is considerably reduced if allowance is made for the activity of the zone of cambial divisions which, in the manner explained in section (12), increases the area of the surface of the apex all round the wound, and therefore increases the horizontal distance from the wound of the centre of P_1. This new zone of tissue is represented in a second diagram, fig. 13, by the area between the two parallel curved lines, of which the inner one represents the outline of the wound, and the outer one represents the limit of the new zone of tissue. If it is supposed that the new zone had approximately the width indicated at the time when I_2' was arising, the diagram now corresponds more nearly with the drawing of No. 167. For the distance of the centre of P_1 from the wound is increased by the new zone in the manner indicated, and consequently the stipule of I_2' does not reach so far through the region above P_1 as it did in the previous diagram. (For simplicity no attempt has been made to show a cambial zone in the isolated piece, though undoubtedly one was formed there also.)

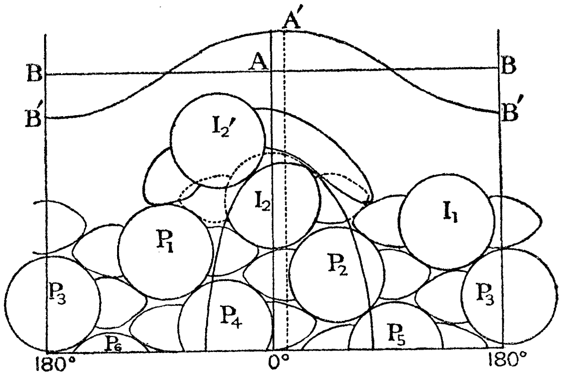

FIG. 12.

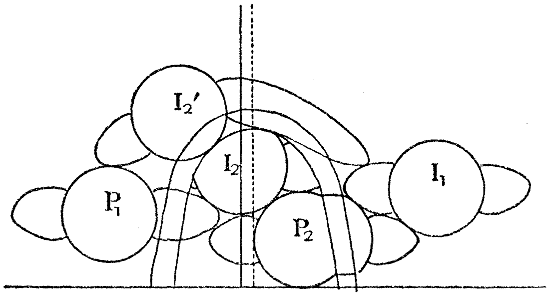

FIG. 13.

The results of this second group were obtained when a large part of the presumptive stipular area of I_2, on the side next to P_1, was left on the apex, and could therefore be occupied by $I_2{}'$. Thus in six of these experiments considerably more than half of this stipular area was left on the apex, in one just about half was left, and in one slightly less than half. In the remaining one the amount was not determined (see Table III). On the other hand, out of the eight experiments of the first group, in two only was about half of this presumptive stipular area left on the apex, and in five less than half was left. In the remaining one (No. 350) the plane of the cut could not be determined with certainty, as the older leaves had been displaced, probably through unsatisfactory embedding. It may be noted that $I_2{}'$ never occupied the gap between I_1 and the wound. This may be explained by the fact that I_1 is situated at a higher level than P_1, being younger, so that the gap between P_1 and the wound became available the sooner.

There remain four experiments, forming a third group, in which $I_2{}'$ occupied a rather different position, its centre lying directly above the wound. Three examples, Nos. 210, 367 and 379, are shown in figs 14–16. In the four experiments of this group, as in most of those of the first group, only a small part, less than half, of the presumptive stipular area of I_2 next to P_1 was left on the apex, so that there was no available gap between P_1 and the wound in which the central part of $I_2{}'$ could arise. The centre of this primordium arose above the centre of the wound and can hardly be said to occupy a gap at all.

The fact that results like those of the second and third groups, in which the next primordium after the isolated primordium arose obliquely or directly above the wound, were obtained only after the isolations of I_2, and never after those of I_1, is probably to be explained as follows. Reasons were given in section (8) for considering that the increase in the space available above the wound, relative to the amount of space available on the other side of the apex, which was due to the shift of the growing-point, was greater after isolation of I_1 than of P_1. For the same reasons it was probably greater still after isolation of I_2, so that, in the isolations of I_2 of the second and third groups, a position obliquely or directly above the wound was the lowest available position for the next primordium. The activity of the zone of cambial divisions round the wound during the plastochron in which I_1 was arising must also have increased the space available around the wound to a greater extent than it was increased after isolation of I_1.

It may be noted incidentally that in No. 367, fig. 15, $I_2{}'$ was abnormal in shape. It consisted of two stipules joined towards the base by an irregularly shaped central region, and the normal central part, from which the rest of the leaf should have arisen, was absent. In No. 379 also (fig. 16), in sections of $I_2{}'$ at low levels, no normal central part was found and no central vascular bundle, but in higher sections a central part with four bundles appeared, and the leaf finally bore five pinnæ. It is possible that when these leaves arose there was not enough room above the wound for them to form normal central parts, but it is uncertain how their irregular shapes should be explained.

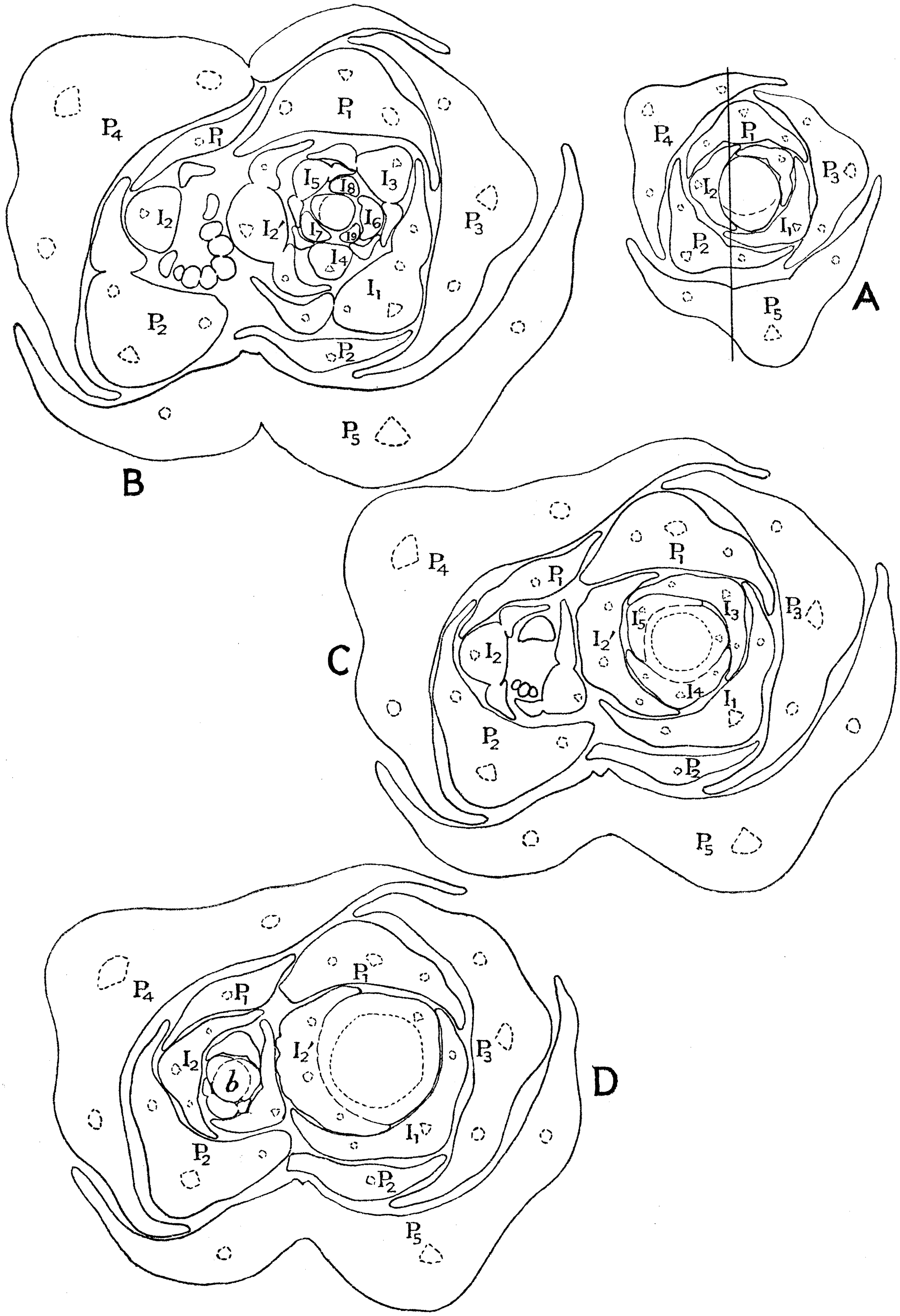

FIG. 14.—No. 210. An Isolation of I$_2$. Group III. A. Transverse section through normal bud showing position of cut. × 35. B, C, D. Transverse sections through bud of No. 210 at different levels. × 30. A bud " *b* " is present in the axil of I$_2$.

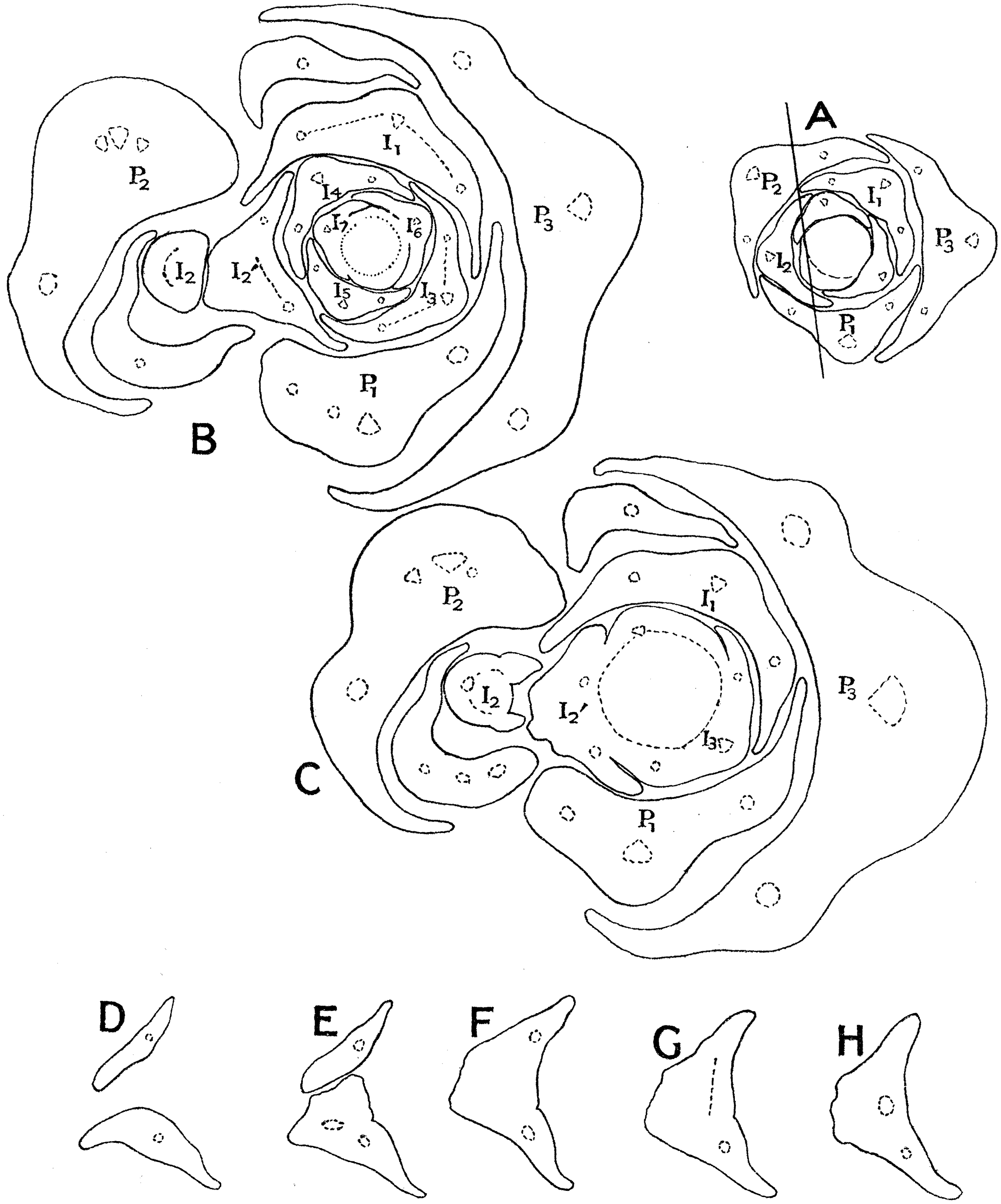

Fig. 15.—No. 367. An Isolation of I_2. Group III. A. Transverse section through normal bud showing position of cut. × 35. B, C. Transverse sections through bud of No. 367 at different levels. × 34. D, E, F, G, H. Transverse sections through I_2', D being the uppermost and H being just above the level shown in C. × 34.

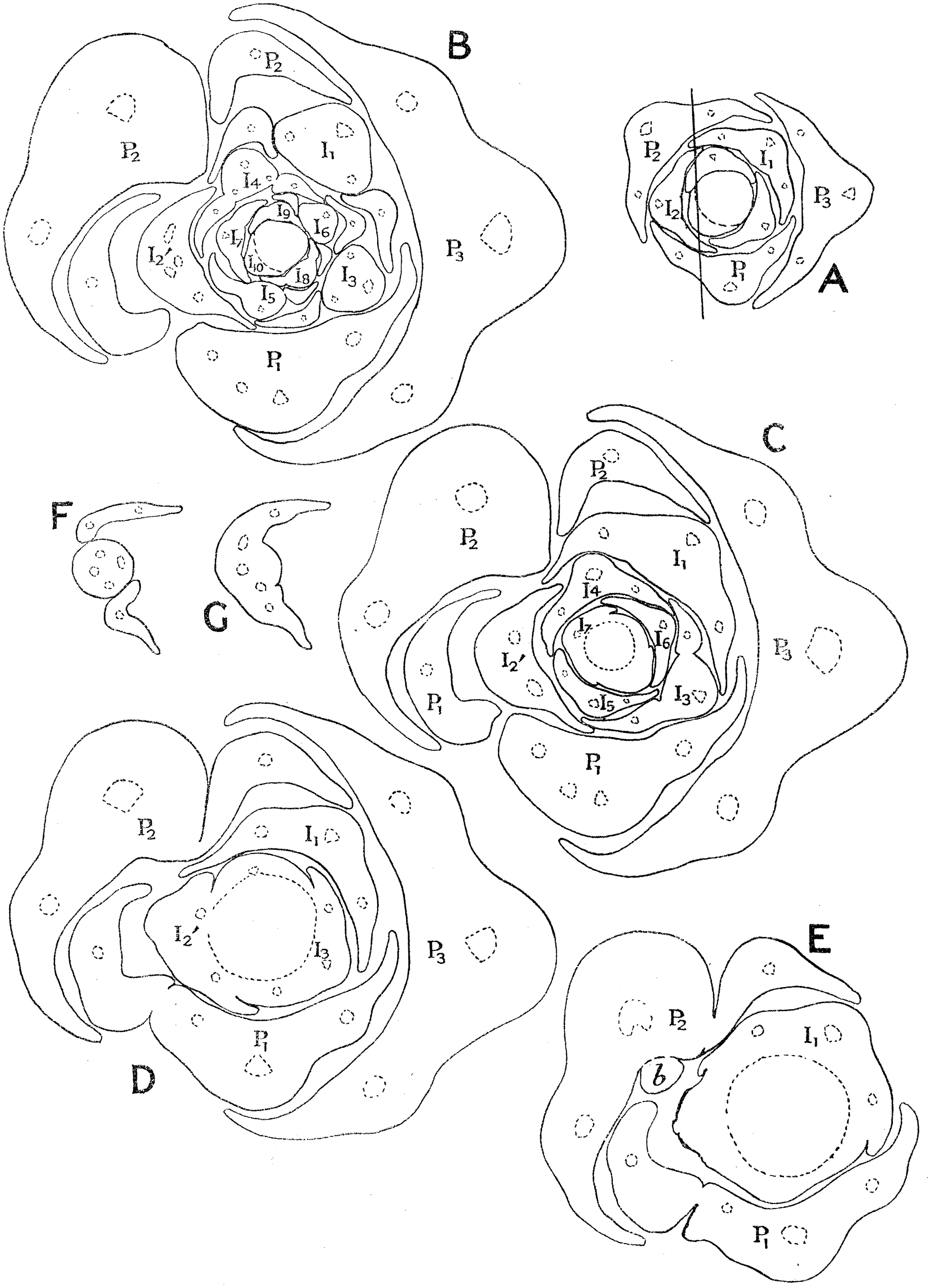

FIG. 16.—No. 379. An Isolation of I$_2$. Group III. A. Transverse section through normal bud showing position of cut. × 35. B, C, D, E. Transverse sections through bud of No. 379 at different levels. × 28. F, G. Further transverse sections through I$_2'$ at higher levels, F being the uppermost. × 28. A bud "*b*" is present in the axil of P$_2$. The wound scar in E is indicated by a thick black line. I$_2$ did not arise.

(16) *The Factors Determining the Different Results.*

After isolation of I_2, the primordia, as has been pointed out already, arrange themselves in one of the three following ways:—

(1) I_3 may arise in its normal position, as the next primordium after I_2, and the genetic spiral is then reversed, just as after isolation of I_1.

(2) Another primordium, $I_2{}'$, may arise before I_3, with its centre between the wound and P_1. The direction of the genetic spiral then remains unchanged.

(3) $I_2{}'$ may arise with its centre directly above the wound. The direction of the genetic spiral is again unchanged.

The decision between these three possible results must depend on which part of the apex first becomes available for the next primordium after I_2, and this may very probably depend in part upon the position of the cut. It has already been pointed out that when the results were of the second kind, it was nearly always found that a large part, half or more, of the presumptive stipular area of I_2 next to P_1 was left on the apex. This part of the stipular area, therefore, which contributed towards forming an available space between the wound and P_1, probably became available before the gap in which I_3 was due to arise. In Nos. 363 and 361, indeed, fig. 17, $I_2{}'$ and I_3 arose almost simultaneously, though $I_2{}'$ arose slightly the sooner, and their adjacent stipules were united. In these two experiments, therefore, the two gaps probably became available almost simultaneously.

When only a small part, less than half, of the stipular areas of I_2 was left on the apex, the results were regularly either of the first or of the third kind. This is understandable, since there was clearly no room for a primordium to arise between the wound and P_1, as can be seen from fig. 14, and consequently results of the second kind were not to be expected. In eight of these experiments, the results were of the first kind, and in four they were of the third kind. Since in all of them (except possibly in No. 350) only a small part of the stipular area of I_2 was left on the apex, the factor deciding between the results of the first and third kinds can hardly have been a difference in the position of the cut. It is therefore suggested that the deciding factor was a difference in the amount of shift of the growing-point, and that when this shift was, for some reason, especially large, the region above the wound became available before the gap in which I_3 was due to arise. A primordium, $I_2{}'$, then arose above the wound, and a result of the third kind was obtained. On the other hand, when the shift of the growing-point was not quite so great, I_3 arose before the region above the wound became available, and then I_3 did not allow sufficient room for $I_2{}'$ to arise at all. This suggestion could not, however, be checked, since it was unfortunately not found possible to measure the shift of the growing-point.

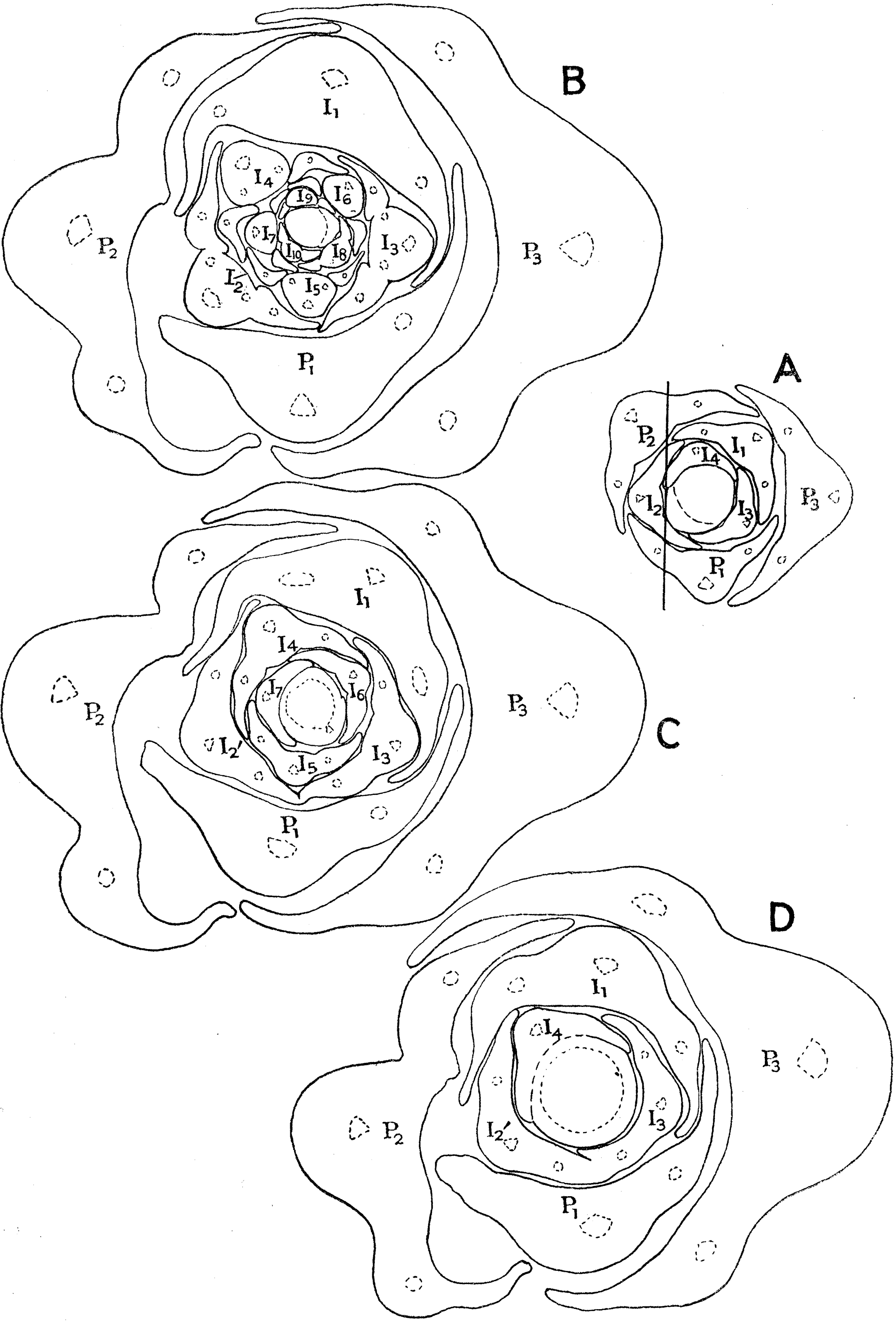

Fig. 17.—No. 363. An Isolation of I_2. Group II. A. Transverse section through normal bud showing position of cut. × 35. B, C, D. Transverse sections through bud of No. 363 at different levels. × 28. P_2 has been displaced towards P_1 during the process of embedding. I_2 did not arise.

(17) *The Development of the Isolated Primordia.*

In eight of the 21 isolations of I_2, the isolated piece of tissue gave rise to a leaf. The isolated leaves varied greatly in size, as they did also when I_1 was isolated. When I_2 developed in isolation, it was always symmetrical, as was also I_1 when isolated. For in seven of the experiments, I_2 developed with both stipules, and in one experiment with no stipules. It never developed with one stipule only (see Table III). In the other thirteen experiments, I_2 failed to develop, although in six of them, at least half of its presumptive stipular areas was included in the isolated piece. The fact that I_2 failed to arise in these six experiments is remarkable and suggests that it may not have been determined at the time when it was isolated, and that determination may only take place in tissues that are in direct organic continuity with the growing-point. But clearly much more evidence is needed on the point.

PART 4.—CONCLUSIONS.

The results of the experiments show definitely that the positions in which primordia arise do depend on the positions of the previous primordia, and in this respect they support the second of the two groups of theories referred to in the introduction. They show further that, as a general rule at least, the new primordia arise in the largest of the gaps between the previous primordia, as was pointed out in section (5) of Part 2: for when the positions of the largest gaps were altered in the experiments, the positions of the new primordia were correspondingly altered.

In Section (6) of Part 1, however, it was pointed out that an addition must be made to the conclusion that the new primordia arise in the largest (that is, widest) gaps. For reasons were given for considering that a more adequate and exact statement would be that each new primordium arises in the first gap that attains both a minimum width and also a minimum distance below the growing-point. Accordingly this statement was adopted as a working hypothesis. It combines one point from the theories of HOFMEISTER, SCHWENDENER and VAN ITERSON with another point from SCHUMANN. For the former three authors considered that the primordia arose in the larger (that is, wider) gaps, whereas SCHUMANN considered that they arose in the positions that were farthest from the growing-point. From SCHOUTE's theory also it would follow that the primordia arise in the gaps that are largest and farthest from the growing-point, but his theory seems to us untenable for the two following reasons. Firstly, the results reported in Section (5) of Part 2, especially the results of the second group of isolations of I_1, show that the positions in which primordia arise depend on the positions of the outlines of the previous primordia, and not on any hypothetical circles of inhibiting influence spreading out from the centres of those primordia, such as SCHOUTE assumes. Secondly, SCHOUTE's assumption that the central point of a primordium is always determined first, independently of the rest of the primordium, is inconsistent with the fact that, in the Lupin at least, the centres of primordia do not arise in positions which would not allow room for their stipules on each side. Moreover, SCHOUTE's assumptions, apart from being inconsistent with the facts, are in any case unnecessary.

With regard to the above working hypothesis, it must be pointed out that the necessary minimum width and minimum distance from the growing-point must both be relative to the size of the stem apex. For in Lupin buds in which the stem apex is small, the primordia are proportionately small, and also less far below the growing-point. Furthermore, in the experiments the isolated pieces of tissue sometimes gave rise to primordia that were considerably smaller than those that arose on the apex. These facts indicate that there is in the plant a considerable regulation of the ratio of the size of a new primordium to the size of the apex on which it arises—a ratio which plays an important part in the writings of SCHWENDENER, CHURCH, and especially VAN ITERSON.

The conclusion reached in Section (5) of Part 2, that the primordia arise in the larger gaps, was based on the behaviour of the third primordium after the isolated primordium and on that of all the subsequent primordia. Their behaviour is explained in just the same way on the working hypothesis (since the larger gaps here, as usually, were also the farther from the growing-point), and the next task was therefore to consider how the behaviour of the first and second primordia after the isolated primordium can be explained. Although the explanation is not so simple and obvious as that of the behaviour of the later-formed primordia, yet it is shown in Sections (7) to (13) of Part 2 that very probable explanations can be offered on the basis of the working hypothesis, and these explanations are supported by various observations. This part of the investigation was inevitably rather lengthy and complicated, but from it there emerged points of interest concerning the shapes of the primordia, the manner in which they occupy the available spaces, and the circumstances in which they become asymmetric. In Part 3 it was shown that the results of isolating I_2 can also very well be explained on the working hypothesis. It is therefore concluded that the working hypothesis, since it covers adequately all the main facts, is probably in the main correct, though future experiments may easily show that it needs to be added to or qualified in various ways.

VAN ITERSON, as was stated in the introduction, has shown in a masterly manner that most of the main facts of phyllotaxis can be explained as necessary consequences, if four so-called "facts of observation" are taken as granted. One of these was that the new primordia arise in the larger gaps between the previous ones, but, until the point was experimentally tested, it was really only an assumption that the positions of the new primordia are actually determined by the positions of these gaps. The present experiments show that they are so determined, and consequently the probability of his theory is greatly increased. His theory is, however, worked out only for plants in which the primordia may be considered to be circular in outline, and consequently it needs to be further considered to what extent it will hold good for plants such as the Lupin, in which, on accouut of the stipules, the outline of the primordia is certainly not circular. Moreover, even with regard to plants with circular primordia, there remains at least one group of facts of which VAN ITERSON has not been able to offer any convincing explanation (1907, p. 247 *seq*). But since it is intended presently to report further experiments on phyllotaxis, discussion of these questions will be postponed.

Here, however, it will just be pointed out that VAN ITERSON'S theory suffices to render intelligible the origin of Fibonacci phyllotaxis systems in seedlings. For he has found that in seedlings of numerous species of plants possessing Fibonacci phyllotaxis, the first two primordia after the cotyledons arise at nearly 180° from one another, and the divergence angles of the subsequent primordia do not approximate to the normal Fibonacci angle of 137°·5 until several primordia have arisen. We have found that this is true for Lupin seedlings also, and the process can be interpreted as a gradual approach to the angle which is to be expected on VAN ITERSON'S theory, when the ratio of the size of a primordium to the size of the apex has sufficiently diminished. In the experiments here reported, the manner in which the divergence angles gradually returned to the normal, after the great increase in angle, is probably comparable with the manner in which the normal angle is gradually established in seedlings.

In conclusion, we wish to express our very real gratitude to Dr. A. H. CHURCH, F.R.S., who by his writings and conversation first aroused our interest in phyllotaxis. Although our experiments have led us to conclusions very different from his, we desire to express our recognition of the great value of the numerous observations with which he has enriched the subject.

Our investigation was carried out in the Department of Botany at Oxford, and part of it was carried out while one of us was holding a research fellowship at Somerville College.

PART 5.—SUMMARY.

1. The phyllotaxis of *Lupinus albus* is of a "Fibonacci" type, with 2 + 3 contact parastichies and successive leaves along the genetic spiral have a mean divergence angle of 136°·3. If the contacts along the genetic spiral are included, the parastichy system is 1 + 2 + 3.

2. Certain primordia were partially isolated from the rest of the apex by vertical tangential cuts: they remained attached below the cuts. As a result, the extreme tip of the stem-apex, or "growing-point," shifted away from the cut.

3. The primordia that were visible at the time of operation were called P_1, P_2, etc., P_1 being the youngest. Those still invisible at that time were called I_1, I_2, etc., I_1 being the oldest.

4. When P_1 was isolated, the divergence angle between the next two primordia increased considerably. When I_1 was isolated the angle between these primordia (I_2 and I_3) increased much more and often exceeded 180°. I_4 then arose on the opposite side of the apex from the normal, and the genetic spiral was reversed in direction and continued

indefinitely in the reverse direction. The normal divergence angle was again approached after a few oscillations above and below.

5. These results support the theory that each primordium arises in the largest gap between those already present. But a more precise hypothesis is that it arises in the first space that becomes both wide enough and distant enough from the growing-point. This is adopted as the working hypothesis.

6. The increase in angle between the next two primordia after the isolated primordium, is partly the direct result of the shift of the growing-point towards the gap between them, and is partly due to the fact that the second primordium is displaced, relatively to the primordia below it, in the direction of the wound.

7. It is shown to be in accordance with expectation that the increase in this angle is greater after isolation of I_1 than of P_1. The displacement of the second primordium is explained, on the working hypothesis, as due to three different factors working together, and the explanation is supported by various observations. The shape of the primordia is discussed and a diagram is constructed.

8. After isolation of I_2, the results were sometimes essentially the same as after isolation of I_1, the genetic spiral being reversed. But sometimes another primordium, I_2', arose before I_3, obliquely above the wound or directly above it, and the spiral was then not reversed. These results are interpreted on the working hypothesis.

9. The isolated primordia developed into leaves in 19 out of 20 isolations of P_1, in 17 out of 21 of I_1, and in 8 out of 21 of I_2. Certain points concerning their development are commented upon.

10. It is concluded that the results show definitely that the positions in which primordia arise depend on the positions and shapes of those already present, and further, that they support strongly the particular form of hypothesis stated above.

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APPENDIX.

TABLE I.—20 Isolations of P_1.

Numbers of experiments (11 in all).	Angle I_1–I_2 in degrees.	Amount of stipular area of P_1 next I_2 left on apex.	Displacement of I_2 relative to P_2.
Group I, with cuts close to P_1. The genetic spiral did not reverse.			
345	145·0	(?)	(?).
239	151·5	About half	Slight.
243	152·5	Nearly all	,,
277	153·0	All	(?).
151	153·0	,,	Medium.
229	153·2	,,	,,
244	156·5	About half	Slight.
247	158·3	Nearly all	Medium.
154	161·0	,,	Slight.
373	162·7	,,	,,
240	163·5	,,	,,
	Mean = 155·5 ± 1·074.		
Group II, with cuts rather far from centre of P_1. The genetic spiral did not reverse.			
(9 in all)			
356, *b*	136·0	Less than half	(?).
226	139·2	,, ,,	None.
346	145·0	More than half (probably)	(?).
354	150·5	Scarcely any	(?).
224	152·7	Very little	None.
222	153·0	Less than half	Slight.
347	153·0	Scarcely any	None.
355	154·7	Less than half	(?).
348	165·0	About half	Slight.
	Mean = 149·9 ± 1·862.		

NOTES.—I_2 was always symmetrical except (slightly) in 373.

I_1 was always symmetrical except possibly in 154.

The central part of I_2 was never in the gap above the point at which wound and P_2 met.

TABLE II.—21 Isolations of I_1.

Numbers of experiments.	Angle I_2–I_3 in degrees.	Amount of presumptive stipular area of I_1 next I_3 left on apex.	Displacement of I_3 relative to P_1.	Symmetry of I_3.	Symmetry of I_2.	Whether centre of I_3 was in depression above point where wound and P_1 met.

Group (1) in which the angle $I_2 - I_3$ was less than 180°, and the direction of the genetic spiral remained unchanged.

182	158·3	Scarcely any	Slight	Sym.	Sym.	No.
245	164	,,	,,	,,	Asym.	,,
236	164	,,	,,	,,	,,	,,
137	165	Less than half	Medium	,,	Sym.	Yes (probably).
322	166·3	About half	,,	Asym. (?)	Asym.	No.
379	171·7	,,	,,	(?)	(?)	(?).

Group (2) in which the angle $I_2 - I_3$ was less than 180°, but the genetic spiral was reversed.

132, *b*	165	Less than half	Medium	Asym.	Asym.	Yes (probably).
155	172	About half	(?)	,,	,,	Yes.
195	173	Very little	Medium	,,	,, (slightly)	,,

Group (3) in which the angle $I_2 - I_3$ equalled or exceeded 180° and the genetic spiral was reversed.

100	180	About half	Medium	Asym.	Asym.	Yes.
242	180	Very little	,,	,,	,,	Yes (probably).
377	180·3	,,	(?)	,,	,,	Yes.
321	181·7	,,	(?)	,,	,,	,,
358	182	(?)	(?)	(?)	(?)	(?).
144	186	Nearly all	Medium	Asym.	Asym.	Yes (probably).
194	187·7	More than half	Large	,,	,,	,, ,,
104	187·8	Nearly all (?)	(?)	,,	Sym.	Yes.
133	190	Scarcely any	Medium	,,	Asym.	,,
205	196	(?)	(?)	,,	,,	(?).
235	198	More than half	Large	,,	,,	Yes (probably).
319	203	,, ,,	,,	,,	Sym.	Yes.

TABLE III.—21 Isolations of I_2.

Group (1) in which the results were similar to those of isolating I_1.

Numbers of experiments.	Angle I_3–I_4 in degrees.	Amount of presumptive stipular area of I_2 next to I_4 left on apex.	Amount of presumptive stipular area of I_2 next to P_1 left on apex.	Development of I_2.	Symmetry of I_4.	Symmetry of I_3.
132	169·7	None	None	Slightly smaller than normal with bud in axil	Asym.	Sym.
327	181	Less than half	Less than half	Did not arise	,,	,,
369	181·3	About half	About half	,, ,,	,,	,,
350	192·7	,,	(?)	,, ,,	,,	Asym.
220	193·3	Very little	About half	Slightly smaller than normal	,,	Sym.
324	193·3	,,	Very little	Did not arise	,,	Asym.
362	197	Less than half	Less than half	Of normal size	(?)	(?).
39	197·7	None	None	About of normal size, with bud in axil	,,	Sym.

Group (2) in which a primordium I_2 arose obliquely above the wound on the side towards P_1.

Numbers of experiments.	Angle I_3–I_4 in degrees.	Amount of presumptive stipular area of I_2 next to I_4 left on apex.	Amount of presumptive stipular area of I_2 next to P_1 left on apex.	Development of I_2.	Angle I_1–I_2'.	Symmetry of I_3.
167	—	More than half	All	Very small with no stipules	138	—
326	—	Less than half	,,	Did not arise	143·7	—
361	—	Nearly all	Nearly all	,, ,,	144·7	—
356	—	None	All	,, ,,	147	—
328	—	,,	About half	,, ,,	150	—
378	—	More than half	Nearly all	,, ,,	150	—
368	—	(?)	(?)	,, ,,	152	—
93, *b*	—	Scarcely any	Less than half	Slightly smaller than normal	155	—
363	—	About half	Nearly all	Did not arise	163·2	—

Group (3) in which a primordium I_2' arose directly above the wound.

Numbers of experiments.	Angle I_3–I_4 in degrees.	Amount of presumptive stipular area of I_2 next to I_4 left on apex.	Amount of presumptive stipular area of I_2 next to P_1 left on apex.	Development of I_2.	Angle I_1–I_2'.	Symmetry of I_3.
320	—	About half	Very little	Did not arise	138	—
210	—	Less than half	Less than half	Smaller than normal, with bud in axil	110	—
367	—	,, ,,	,, ,,	Very small with tiny stipules	(?)	—
379	—	,, ,,	,, ,,	Did not arise	(?)	—

TABLE IV.—Angles subtended at new growing-point after isolation of P_1 by subsequent leaves, in degrees.

	No. 151.	No. 229.	No. 347.	No. 240.	No. 247.
I_1–I_2	153	153·2	153	163·5	158·3
I_2–I_3	122	115·8	132	120·3	119
I_3–I_4	122	142·8	134·2	137·3	140·2
I_4–I_5	141·5	138·7	137·7	131·5	140
I_5–I_6	139	—	141·3	145·5	135·7

	No. 222.	No. 244.	No. 239.	No. 345.	No. 226.
I_1–I_2	153	156·5	151·5	145	139·2
I_2–I_3	121–7	125·5	128–2	118·2	144·5
I_3–I_4	148·2	144	138·2	151·7	124·7
I_4–I_5	131·3	133	138	129·3	140
I_5–I_6	139·3	136·7	133·5	136·7	142
I_6–I_7	134	—	—	139·3	127·3

TABLE V.—Angles subtended at new growing-point after isolation of I_1, in degrees.

	Experiments of group (1).		Experiments of group (2).	
	No. 137.	No. 379.	No. 195.	No. 132*b*.
I_2–I_3	164·6	171·75	173	165
I_3–I_4	124	116	101	106
I_4–I_5	130	144·3	148	133
I_5–I_6	142	145·3	137	151
I_6–I_7	—	135·75	136	123

	Experiments of group (3).				
	No. 377.	No. 144.	No. 133.	No. 205.	No. 235.
I_2–I_3	180·3	186	190	196	198·7
I_3–I_4	110	116	117	125	115
I_4–I_5	144·75	146·5	142	146	149
I_5–I_6	138·3	133	145	127·5	132·3
I_6–I_7	138	141·5	123	143	—
I_7–I_8	—	—	145	132	—