Experiments on Phyllotaxis. II. The Effect of Displacing a Primordium

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Phil. Trans. R. Soc. Lond. B 1932 **222**, 353-400
doi: 10.1098/rstb.1932.0019

IX. *Experiments on Phyllotaxis. II.—The Effect of Displacing a Primordium.*

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(*Communicated by Sir* Frederick Keeble, *F.R.S.*)

(Received January 12, 1933.)

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Part 1.—Introduction.

(1) *Nature and Purpose of the Operations, and Methods.*

Experiments on *Lupinus albus* were reported previously in which the arrangement of the subsequent leaves was changed as a result of the isolation from the stem apex of the region from which the next leaf or the next but one was due to arise (Snow and Snow, 1931). The results led to the conclusion that each new leaf-primordium arises in the first space that attains both a certain minimum width and a certain minimum distance below the apex (p. 36), a conclusion which strongly supports van Iterson's theory of phyllotaxis (1907). The purpose of the present experiments was to test this conclusion further by means of a different operation performed on the same plant. The conclusion of the previous paper will therefore be taken again as a working hypothesis, and an attempt will be made to explain the present results on the basis of it. In the present experiments a slight vertical cut was made in a radial plane through the area from which the next primordium was due to arise, or in other words through the presumptive area of I_1 (for terminology see section 4). The cut sometimes extended downwards a little way below this presumptive area, but probably never reached more than a very little above it. This operation was considered to be a suitable method for testing the conclusions reached previously for the following reasons. Firstly, if the centres of primordia arise only in positions that allow room for their stipules, as the previous results indicated (p. 23), any primordia arising in contact with the sides of the wound should arise with their centres at some distance from it, and consequently at some distance from the normal position of the centre of I_1. Secondly, as a result of these displacements, the positions of the subsequent primordia should also be changed, if they arise in accordance with the working hypothesis.

The methods adopted were the same as those described previously. When the seedlings had expanded four leaves, these leaves were allowed to remain, and the younger leaves which covered over the apex were removed until the apex and the youngest primordia were visible. The apices were then operated upon, and later, usually after three weeks or sometimes four, the buds were pickled, embedded in collodion and examined by means of hand sections. A race of *Lupinus albus* was used which did not produce flower buds too early.

(2) *The Angles Subtended by Normal Leaf-primordia.*

A brief description of the normal apex and phyllotaxis of *Lupinus albus* and of the shapes of the primordia was given previously (1931, pp. 4, 5, 19). The mean divergence angle was found to be 136°·3 ± 0°·26, the probable error of a single angle being 1°·88. Since the ratios of the sizes of the primordia to that of the axis on which they arise were found to be an important factor in determining the phyllotaxis it was thought desirable to determine these ratios more exactly. The most convenient way of doing this is to measure the angles subtended at the centre of the axis by the insertions of the primordia. These angles give a measure of the "arcs" of the primordia—that is, of the fraction of the circumference which they occupy. In transverse sections it was found very difficult to determine the exact limits of the insertions of the youngest primordia, but this could be done more accurately by the following method. The apex together with the four youngest primordia was dissected out and cut off by a horizontal cut. It was then placed on a slide and viewed under the microscope by transmitted light. The axis then appeared a deep green and the primordia inserted on it a pale green, so that their limits could be distinguished more easily. Nine such preparations were made and drawn under a drawing eye piece: an example is shown in fig. 1. The arc of P_1 (the youngest visible primordium) could only be measured in four of the preparations, since in the others it was too young to be clearly distinguished from the apex. Also the measurements of this arc can only indicate an upper limit, since when P_1 first arises it is too small to be measured.

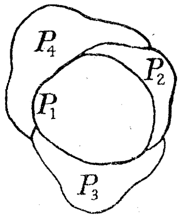

FIG. 1.—Normal apex of *Lupinus albus* with the four youngest leaves, prepared as described in text and drawn as seen in the solid. P_1 is so small that its exact boundary cannot be distinguished. × about 60.

The arcs of the primordia of different ages, measured in degrees, were found to be as follows:—

P_1, in 4 plants, 102, 112, 124 and 129.
P_2, mean in 9 plants, $147 \cdot 3 \pm 1 \cdot 57$ (extremes 138 and 159).
P_3, mean in 9 plants, $156 \cdot 3 \pm 1 \cdot 45$ (extremes 147 and 161).
P_4, mean in 5 plants, $161 \cdot 4 \pm 1 \cdot 27$ (extremes 157 and 167).

These measurements show that the arcs of the primordia increase for several plastochrons at least, though at a steadily diminishing rate.

(3) *The Reaction of the Apex to the Operation.*

In the previous experiments in which P_1 or I_1 or I_2 was isolated from the apex by a vertical tangential cut, it was found that the growing-point shifted away from the wound (1931, p. 6). On account of this shift the displacements of the subsequent leaves could not be directly measured by their divergence angles from the older leaves. It was hoped that in the present experiments the operation, which was a slight radial cut, would not cause the growing-point to shift; but unfortunately it did so. The shift of the growing-point caused the whole stem apex to tilt away from the wound as in the previous experiments. This tilt is clearly shown in fig. 2, A and B, which show longitudinal sections, stained in ruthenium red, through two apices which were pickled only 6 and 10 days respectively after the operations. In order that the tilt of the apex might be seen it was necessary that the sections should pass through the plane of the wound;

and accordingly the position of the wound in relation to the outermost leaves of the bud was noted. But even so the sections frequently passed obliquely through the wounds: indeed the section shown in fig. 2, A, was slightly oblique. Successful longitudinal sections were obtained through six apices, pickled from 6 to 10 days after the operations.

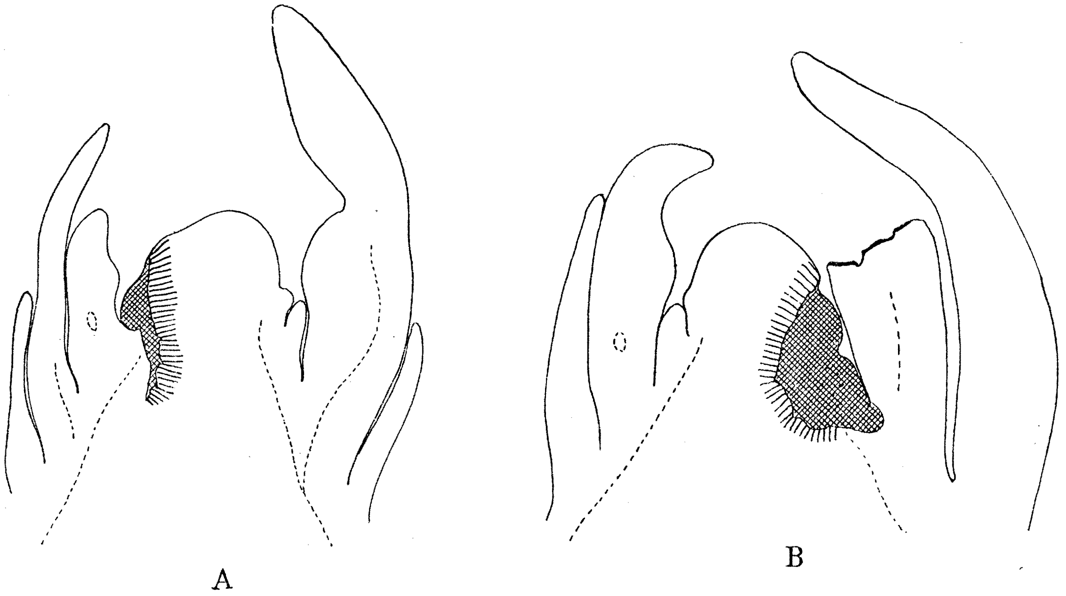

FIG. 2.—A. Longitudinal section through stem apex 10 days after operation. The wound is shaded with cross lines. The wound meristem is shown by lines radiating inwards from the edge of the wound. The stem apex has tilted away from the wound. × 45. B. Longitudinal section through stem apex 7 days after operation. The wound and wound meristem are indicated as in A. The top of the leaf on the right of the apex had been removed previously. × 50.

The wounds as seen in longitudinal sections were patches of dead brown cells clearly delimited from the living cells which surrounded them. They generally extended downwards for some distance below the presumptive area of I_1. The radial depth of the wound varied in different experiments, but it probably always passed through the procambial strands, as in the two experiments shown in fig. 2, in which the procambial strands are drawn with broken lines. It is evident that new procambial strands must have been formed internally to the wound; for transverse sections made through the wound about 3 weeks after the operation showed an unbroken vascular cylinder. A wound meristem was formed beneath the surface of the wound, as in the previous experiments. The tiers of cells formed by this meristem are shown in fig. 2 by lines radiating from the wound.

In order to study further the contour of the wound and the extent of the wound meristem several plants were examined by transverse sections at only 6 or 7 days after the operations. It was found that the two sides of the wound had gaped apart in the upper part as shown in fig. 3, B. The wound meristem was seen internally to the wound, and it was most active internally to the centre of the wound. These observations explain the appearance of the wound as seen in sections of apices pickled 3 or 4 weeks after the operation; for in the latter sections the upper part of the wound appeared as a small V-shaped scar (see fig. 11, No. 24 D), and this can be readily understood, since the two sides must have gaped apart some time before. The very small relative size of the wound scar in these sections was obviously due to the fact that its absolute size remained the same while the surrounding tissues went on growing. In two or three experiments, for instance No. 5, fig. 5, D, the wound was seen to project on a protuberance, which may have been formed by the activity of the wound meristem.

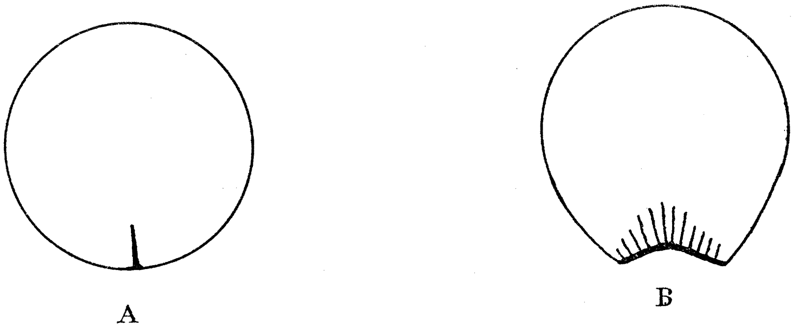

Fig. 3.—A. Diagram of transverse section of apex immediately after operation. The wound is shown by a radial line. B. Diagram of transverse section of apex about 7 days after operation. The sides of the wound have gaped apart; the wound meristem is shown by lines radiating inwards from the wound.

(4) *Terminology and Brief Classification of Results.*

The primordia will be named in the same way as before. Those visible at the time of operation are P_1, P_2, etc., P_1 being the youngest: those arising after the operation are I_1, I_2, etc., I_1 being the first to arise. The "genetic spiral" is a line drawn through successive primordia in the order in which they arise. The direction along this spiral towards the successively younger leaves will be called the "anodic" direction, and the opposite direction the "kathodic." The operation divided the presumptive area of I_1 into two parts, an anodic part and a kathodic. Sometimes both of these parts contributed towards forming new primordia, which arose either before or after I_2, but before I_3. Of these the anodic primordium will be called I_1a, and the kathodic one I_1k. But sometimes only I_1a arose, and sometimes only I_1k, so that the results fell into three main groups which will be described separately. The terms I_1a and I_1k were chosen for convenience only, and not as implying any "homology" with a normal I_1. Similar experiments were performed on P_1 and I_2 also. These did not greatly disturb the phyllotaxis, but the experiments on P_1 were of interest with regard to the regeneration of P_1 itself, and will be reported in another paper.

PART 2.—EXPERIMENTS IN WHICH ONLY I_1A AROSE.

(5) *General.*

In the experiments of this group, the cut made through the presumptive area of I_1 did not pass exactly through the centre of that area, but a little to the kathodic side of it, as is shown by the angles between the wound and P_1, given in Table I, column 2, which were always less than the normal angle of 136°·3, by amounts ranging from 13° to 31°. The measurements of these angles P_1-wound were indeed a little too small, since the growing-point and the young stele beneath it had shifted away from the wound. But the error was probably not greater than 4° at most.* A primordium, which will be called I_1a, arose on the anodic side of the wound soon after the operation, but the region on the kathodic side did not give rise to a primordium until later, when it was partly occupied by I_3.

The results fall into two sub-groups: in the first sub-group, containing seven experiments, I_1a was a complete leaf and was frequently united with I_2 by its anodic stipule; in the second sub-group, containing three experiments, I_1a was a bract, consisting of a thin lamina with an approximately median vascular bundle but without pinnæ. Details are given in Tables I and II. Two experiments of the first sub-group, in which I_1a was a complete leaf, are shown in figs. 5 and 7, Nos. 5 and 7: in both I_1a is united to I_2 by its anodic stipule. An experiment of the second sub-group is shown in fig. 6, No. 9. The position, size and form of I_1a in these experiments will be described and discussed first, and the changes in the subsequent phyllotaxis afterwards.

(6) *Position and Size of* I_1a *when a Leaf.*

(*a*) *Experiments in which* I_1a *did not extend above the Wound.*—In previous experiments it was found that when a leaf arose by the side of a wound the position of its centre was such as to allow room for a stipule between it and the wound (1931, p. 23); and it was concluded that the centre of a leaf is not determined independently of its edges. Similarly

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* This error in the angle P_1-wound was estimated as follows. The growing-point shifts towards the gap between P_1 and I_2 and therefore tends to increase the angle P_1–I_2 (see, for instance, fig. 11, A). Now in the experiments to be described in section 11, the angles P_1–I_2 exceeded by an average amount of 6°·6 the normal value of 87°·4 (see Table III). This increase, therefore, must have been the increase due to the shift of the growing-point, provided that I_2 arose in its normal position. Now I_2 in the experiments of this sub-group did arise in approximately its normal position, so far as could be judged from its contacts with P_1 and P_2. There was indeed no reason to expect that I_2 would be displaced towards P_1, though it might possibly have been expected that it would be slightly displaced *away* from P_1 and towards the wound (see section 11). But if I_2 was at all displaced away from P_1, then the mean increase of 6°·6 in the angle P_1–I_2 was only partly due to the shift of the growing-point towards those primordia. Consequently a mean increase of 6°·6 in this angle is the *most* that can have been directly due to the shift of the growing-point. But this being so, it will be clear from simple consideration of the geometry of a transverse section that the decrease in the angle P_1-wound due to the same cause can only have been about half this amount, or not more than about 3°·5.

TABLE I.—Group 1. Experiments in which only I_1a arose.

Numbers of experiments.	Angle P_1-wound, in degrees.	Angle P_1–I_1a.	Angle I_1a–I_2.	Angle I_2–I_3.	Angle I_1a–I_3.	Difference of angles I_2–I_3 and I_1a–I_3.	Final direction of genetic spiral.
Sub-group 1, in which I_1a was a leaf.							
1	115	163	113 (I_4)	133	114	20	Unchanged
2	100	165	110 (I_4)	136	114	26	,,
3	111	171	107 I($_4$)	143	110	36	,,
4	122	166	106 (I_4)	154	100	48	,,
5	132	175	97 (I_4)	147	116	50	,,
6	107 (approx.)	167·5	101	155 (I_4)	104	54	Reversed
7	105	166	95	155 (I_4)	110	60	,,
Sub-group 2, in which I_1a was a bract.							
8	121	161	—	—	—	—	Unchanged
9	123·5	165	—	—	—	—	,,
10	?	?	—	—	—	—	,,

TABLE II.—Group 1. Further Data.

Numbers of experiments.	Arc of kathodic half of I_1a in degrees.	Arc of anodic half of I_1a in degrees.	Size of P_1 at time of operation.	Whether I_1a extended over the wound.
Sub-group 1, in which I_1a was a leaf.				
1	57	57	Small	Yes
2	65	48	,,	No
3	60	41	Large	,,
4	75	50	Small	Yes
5	80	50	,,	,,
6	60	48	?	No
7	61	44	Large	,,
Sub-group 2, in which I_1a was bract.				
	Total arc of I_1a.			
8	82	—	Large	No
9	77	—	Medium	,,
10	70	—	?	,,

in the present experiments it was found that the centres of the I_1a leaves never arose so close to the wound as to leave no room for their kathodic stipules. Consequently the central part of I_1a did not occupy the lowest unoccupied space, but occupied instead a space at a higher level and further from the wound; and the time of its appearance was delayed until this space became available. The present results, therefore, confirm the conclusion that the central part of a leaf cannot arise in a position which does not allow room for its stipules.

On the hypothesis adopted here, a leaf-primordium is determined as a whole, as soon as a space is available both wide enough and low enough for it. There is therefore a certain minimum area of the apex in which a primordium can be determined, and when a primordium is determined in it, this area constitutes the primary area of the primordium. Consequently it is to be expected that the morphological centre of I_1a will arise at a distance from the wound equal to half the arc covered by this minimum area, provided that its kathodic stipule abuts on the side of the wound and does not reach over the top of it. Actually there were four experiments in which the kathodic stipule of I_1a did not extend over the top of the wound (an example is shown in fig. 7, No. 7), and in these the arcs of the kathodic halves of I_1a, as measured by the angles which they subtended, ranged from 60° to 65°, the mean being 61°·5, or approximately 62° (see Table II). These measurements therefore indicate that the minimum arc over which a leaf-primordium can be determined is approximately twice 62° or 124°, though the number of experiments is clearly too small for estimating it very exactly.

As to the anodic halves of I_1a, they were joined to I_2 in these four experiments, and indeed in all but one of the seven experiments in which I_1a was a leaf. They subtended angles ranging from 41° to 50°. Since these angles were much less than half the angle subtended by the minimum area of a leaf-primordium, as estimated from the measurements already given, it must be concluded that the primary areas of I_1a and I_2 had partially coincided or "overlapped," and that these leaves were joined in consequence. It is probable that two primary areas can only overlap in this way when their centres are at the same level, or nearly so. The degree of union I_1a and I_2 varied considerably. For their united stipules were usually free at the tips, though joined at the base. But in fig. 7, No. 7, the stipules were united even in the highest section examined. Also in fig. 5, No. 5, described in the next section, it appeared that I_2 was inserted slightly higher than I_1a, and had therefore arisen slightly later. But the stipule of I_2 was turned backwards, so that its morphologically upper surface faced outwards and was united with the upper surface of I_1a.

The position of I_1a in the four experiments in which its stipule did not reach over the wound is shown in the diagram, fig. 4, in which primordia are represented as if on the surface of an unrolled cylinder. Such diagrams are imperfect, as was pointed out previously (1931, p. 20), since in the plant the primordia arise from a paraboloid apex, and there are also other difficulties. Nevertheless, they serve as a useful guide to the relative positions and contacts of the primordia. This diagram, like all similar diagrams in this paper, is constructed to such a scale that 1 mm. corresponds to an arc of 3°. The divergence of the leaves before I_1 is taken as 136°; and these leaves are represented as if covering arcs equal to the mean between the arcs of P_2 and P_3, given in section 2. This value was chosen because there is evidence that a primordium is determined at the I_1 stage (or perhaps at a late I_2 stage), and I_1 normally makes contact with P_2 and P_3.

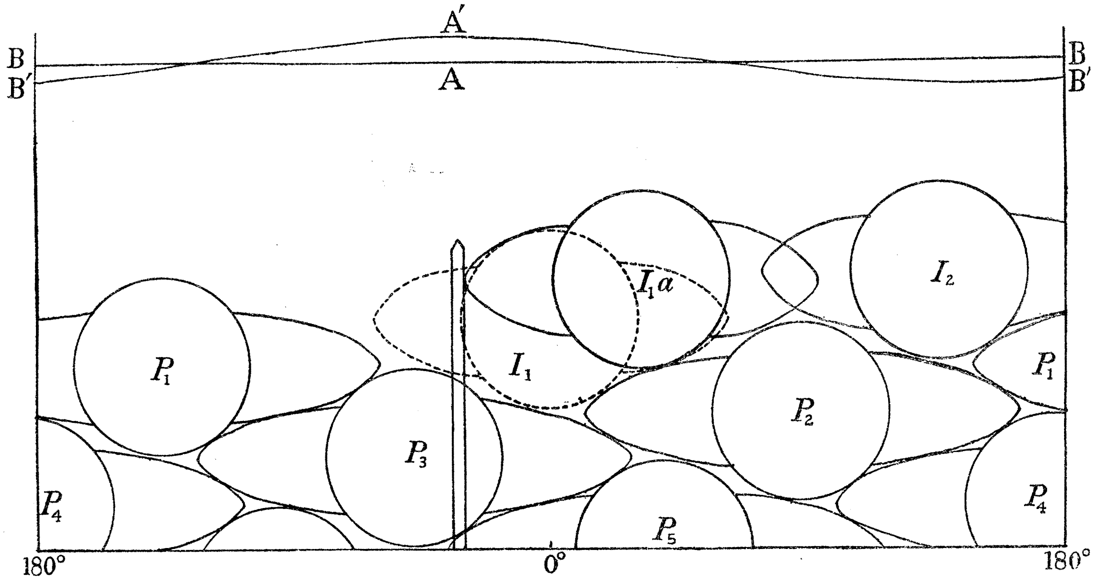

FIG. 4.—Diagram representing the experiments in which I_1a arose alone and did not extend above the wound. Explanation in text.

The correct diameter for the circles representing the central parts of the leaves was difficult to determine as no definite boundary could be seen between the centres and stipules of P_2 and P_3, even when viewed in the solid under the microscope: but, so far as could be judged, the arcs of the central parts of P_2 and P_3 covered rather more than 60°. The diameters of the circles were therefore made to correspond to an arc of 62°. As it was not possible to determine the exact shapes of the stipules, they were drawn so as to make the necessary contacts. The primordia arising after the operation are represented as if covering arcs of 124° this being the value already estimated for the minimum arc of a leaf-primordium. For in considering how these primordia fitted into the available spaces we are concerned with the areas which they occupied when first determined. The presumptive area of I_1 is indicated by a dotted line; its arc also is represented as equal to 124°. The wound is represented by a double line passing through the presumptive area of I_1. The line BAB represents the position of the growing-point before the operation, and the line B′A′B′ its position after it had shifted away from the wound. Since the amount of this shift could not be measured, it was guessed.

On the basis of the working hypothesis it might have been expected that I_1a would have arisen before I_2; for it can be seen from the diagram that the growing-point has shifted towards I_2. And since in the diagram I_1a and I_2 appear to be almost equidistant from the original position of the growing-point, it seems that I_2 must have been nearer to the new position of the growing-point, after it had shifted. It is indeed possible that I_1a may have arisen slightly earlier than I_2, but the interval between I_1a and I_2 must have been very short, to allow their stipules to unite. It is therefore suggested that the shift of the growing-point took some time, and that in these four experiments it had not been completed when I_1a and I_2 were determined. Accordingly in the diagram I_1a and I_2 have been represented with their horizontal axes parallel to the line BAB representing the original position of the growing-point.

(*b*) *Experiments in which* I_1a *did extend above the Wound.*—In three experiments in which I_1a formed a complete leaf (Nos. 1, 4 and 5) its kathodic stipule extended through the region above the wound. These three experiments will now be briefly described. In No. 4 the arc of the kathodic half of I_1a was 75°, or 10° greater than the greatest arc of this half of I_1a in the previous four experiments. The portion of this arc between the centre of I_1a and the highest point of the wound was 40°, or 20° less than the smallest arc of the kathodic half of I_1a in the previous experiments. It follows, therefore, that the primary area of I_1a reached over the top of the wound, and that the kathodic stipule having thus overreached the wound, was able to extend through the vacant area on the other side for a further 10°.

In No. 1, although I_1a extended over the top of the wound, yet it did not appear to have extended beyond its primary area, for though it had reached its 11th plastochron it covered an arc of only 114°, its anodic and kathodic halves both subtending 57°. This leaf therefore occupied slightly less than the minimum area indicated by the previous experiments, but we have not been able to explain its small size, nor how it was that it did not extend into the vacant area on the kathodic side of the wound. But this experiment may be considered exceptional, since the failure of I_1a to extend beyond its primary area was clearly abnormal; it does not provide trustworthy evidence of the size of the minimum arc.

In No. 5, fig. 5, the arc of the kathodic half of I_1a was 80° and the portion of this arc between the centre of I_1a and the top of the wound was only 43°. It is therefore probable that the primary area had reached over the top of the wound and that a further portion of stipule had originated beyond the wound, as in No. 4. But No. 5 is peculiar in that the kathodic stipule of I_1a is divided into two a short way above its insertion level, as is shown in fig. 5. The free part of this stipule is marked "S" in fig. 5, B, C, and D. A similar phenomenon was seen in No. 17, in which I_1 arose on the kathodic side of the wound and its anodic stipule extended across the top of the wound and divided into two above its insertion level.

Since in these three experiments I_1a arose with the tip of its kathodic stipule above the wound, instead of abutting on the side of the wound as in the four previous experiments, its position needs to be specially considered. It might at first seem that in these three experiments, I_1a had arisen later and at a higher level. But if its position is judged by comparison with I_2, it appears that this is not so. For whereas in the previous four experiments I_1a arose at nearly the same time as I_2 and was joined to it, in No. 1 I_1a arose before I_2 and was not joined to it, and in No. 5 I_1a, though joined to I_2, was at a slightly lower level (see fig. 5) and had therefore arisen before I_2. In No. 4 I_1a arose at nearly the same time as I_2, as in the previous experiments. Now in the present three experiments it was recorded that P_1 was young at the time of operation,

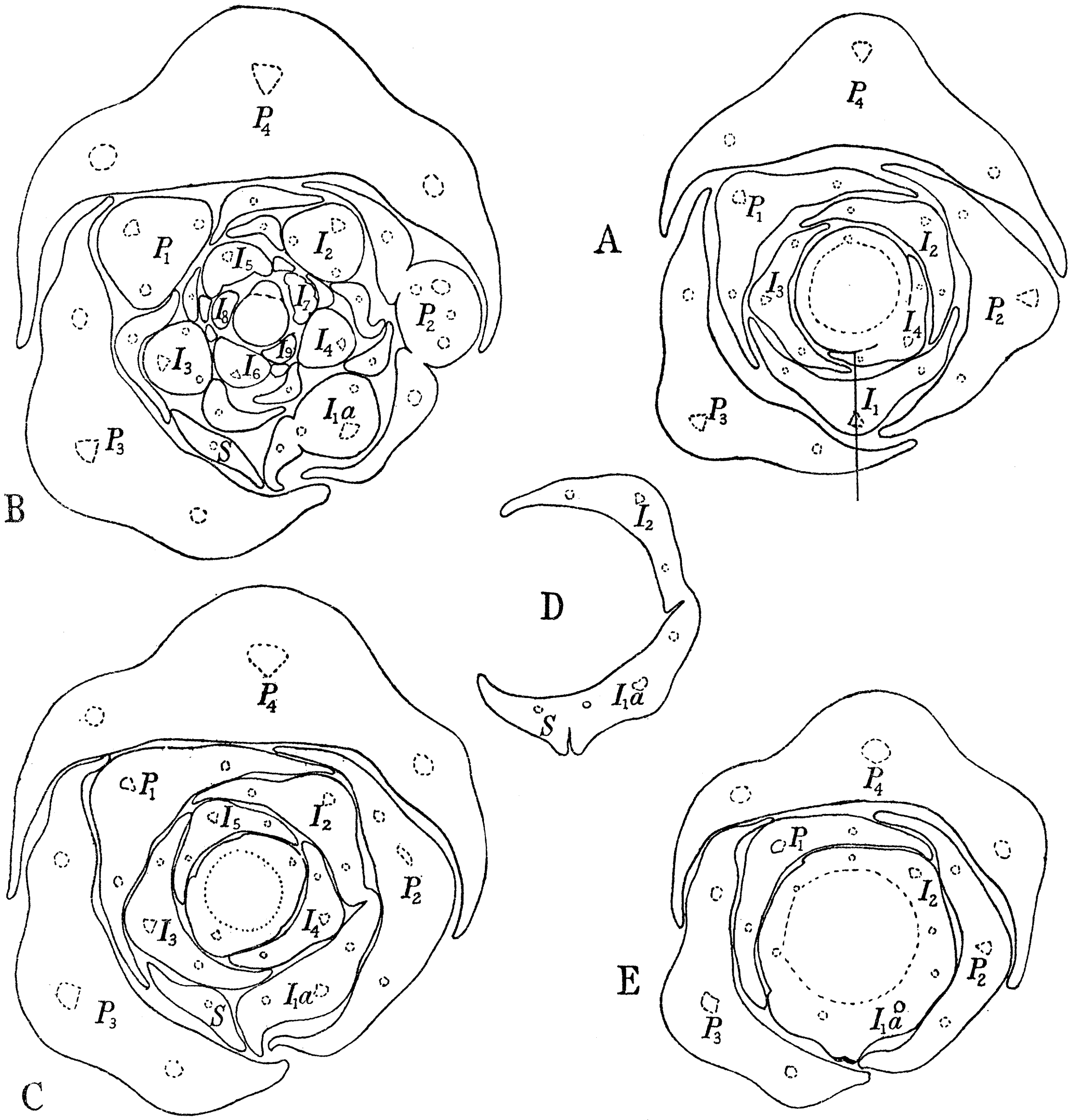

Fig. 5.—No. 5. Only I_1a arose. Sub-group 1. A. Transverse section of normal bud, showing position of wound. B, C, E. Transverse sections of No. 5 at different levels. D. Transverse section of I_1a and I_2 between the levels of C and E. The wound scar is indicated by a thick black line in E. B, C, D × 33. E × 25.

so that I_1 also must have been young. This fact is significant, for the results resemble those of another series of eight experiments in which similar operations were performed on the presumptive area of I_2. These experiments will not be described in detail, but it may be mentioned that in all of them a complete leaf developed on the anodic side of the wound, and that in all but one the kathodic stipule of this leaf arose above the wound, while in all of them its other stipule lay *beneath* the stipule of I_3.

The explanation of these results is almost certainly that the shift of the growing-point away from the wound caused the region above the wound to become available for leaf formation sooner than it would otherwise have been; and further that, for the reason given in the previous paper (1931, p. 18), the affect of the shift was the more pronounced the younger the primordium or presumptive area operated upon. For from this it can be understood how it was that in the experiments in which the primordium operated upon was either I_2 or a young I_1, the primordium I_2a (or I_1a) arose with a stipule above the wound, and also that in all but one of these experiments it arose earlier than the next primordium I_3 (or I_2), instead of simultaneously with it. In the previous four experiments indeed, in which I_1a was almost simultaneous with I_2, it was considered probable that the shift of the growing point was not completed until after I_1a and I_2 were determined. But in the experiments here considered, since the primordium operated upon was either I_2 or a very young I_1, the time which elapsed before a space became available for I_2a or I_1a must have been greater. It is therefore reasonable to suppose that in these experiments the shift of the growing-point was completed before these primordia were determined.

It is clear that in all the seven experiments in which I_1a was a complete leaf, the explanation of the displacement of its centre away from the wound is that its centre could not arise any closer to the wound than would allow room for its kathodic stipule. This can readily be seen in those experiments in which its kathodic stipule abutted on the side of the wound, and even in the three experiments in which the tip of this stipule arose above the wound it can be understood that the thicker part of the stipule, closer to the centre of the leaf, was not able to do so.

(7) *The Conditions of Origin of Leaves and Bracts.*

In three experiments I_1a was a bract, as already stated. An example is shown in fig. 6, No. 9. It must therefore be considered what causes led to this result. In the first place it may be noted that the I_1a bracts differed from the I_1a leaves in two respects. Firstly, they differed in the arcs which they covered at their insertion levels. For the arcs of the I_1a bracts were always considerably smaller than those of the I_1a leaves. The largest arc of an I_1a bract was 82°, while the smallest arc of an I_1a leaf was 88°. (It may be noted that when I_1a was joined to I_2 the exact boundary could not be determined; but for purposes of measurement it was considered to lie mid way between the two vascular strands present in the joined stipules at or near the insertion level. When, as in No. 7, only one strand was present at the insertion level, the boundary was assumed to coincide with this strand.)

Secondly, the bracts arose sooner after the operation than the leaves: for all the bracts arose before I_2, whereas 6 out of the 7 leaves were almost simultaneous with I_2; the 7th arose slightly earlier. Probably both bracts and leaves arose later than I_1 would normally have arisen, but the leaves were delayed for a longer time than the bracts.

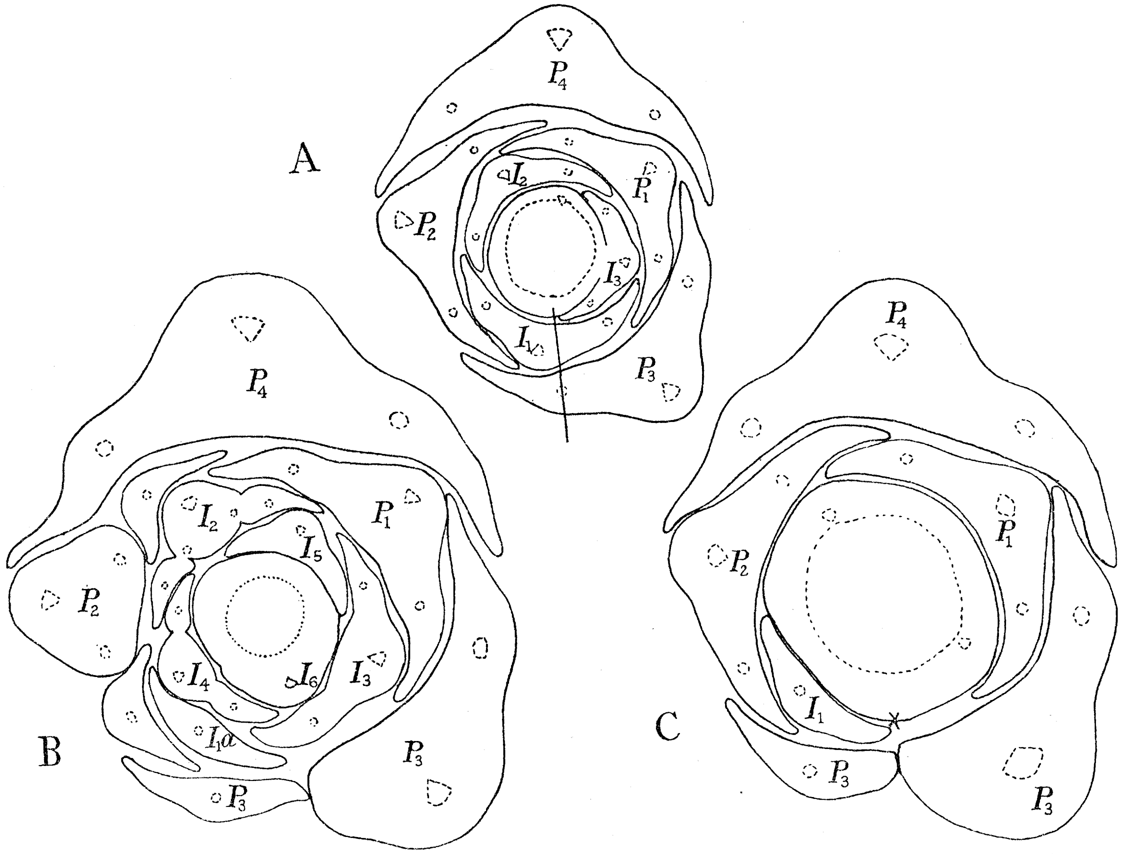

Fig. 6.—No. 9. Only I_1a arose. Sub-group 2. A. Transverse section of a normal bud showing position of wound. B and C. Transverse sections of No. 9 at different horizontal levels. The position of the wound as estimated from its position in lower sections is marked × in C.

There are only two variable factors recorded in these experiments which could account for the difference between the bracts and leaves: one is the stage of the plastochron at the time of the operation and the other is the position of the wound, as indicated by the angles P_1-wound in Table I. These angles in the second sub-group fall well within their range of variation in the first sub-group, and it is therefore unlikely that they were the deciding factor. The stage of the plastochron at the time of the operation could only be roughly estimated by noting the size of P_1 when the operation was made. Its size was judged by the eye and noted as "large," "medium" or "small"; it is recorded in Table II, column 6. Unfortunately in several experiments the size of P_1 was not noted. It can be seen from the table that P_1 was usually smaller in the first sub-group, so that the operations must have been made earlier in the plastochron. Admittedly the data recorded are not nearly enough to make it certain that the difference is significant but they suggest that the bracts resulted from operations made late in the plastochron, and this suggestion is supported by the results of another series of similar operations made on the next older primordium P_1, which will be reported in another paper. In these experiments bracts often developed from the smaller half of P_1 or sometimes from both halves. An example is shown in fig. 15, No. 23, in which P_1 was halved as well as I_1, and both halves of P_1 gave rise to bracts.

These experiments on P_1 therefore increase the probability that the I_1 bracts resulted from operations made late in the plastochrons. Actually the bracts never appeared when the operations were made early in the plastochron but only when P_1 was large or medium at the time of the operation. On the other hand a bract did not always arise when the operation was made late in the plastochron; for in two of the experiments of sub-group 1, P_1 was large at the time of operation, and yet I_1a was a leaf. This fact is at present difficult to explain, but it must be remembered that the estimations of the sizes of P_1 are liable to considerable error.

In spite of this difficulty, therefore, it seems probable that an I_1a bract arises when the operation is made late in the plastochron and when the wound is fairly close to the centre of the presumptive area of I_1, as it actually was in two at least of the three experiments in which bracts arose. As an explanation it is suggested that the presumptive area of I_1 is then so divided that even the larger part of it is too small to give rise to a leaf. And yet the presumptive area is already so far determined in the direction of leaf-formation that the larger part of it cannot be de-differentiated but is bound to go on and form a structure of some kind. On the other hand, when I_1a arose as a complete and symmetrical leaf the original presumptive area of I_1, if it was to any extent "determined" at the time of the operation, must have been completely de-differentiated again as a result of the wound. Consequently the I_1a leaves must have arisen not by reorganization but as completely new leaves, as soon as spaces were available for them both wide enough and far enough below the growing-point.

This explanation is supported by the facts already mentioned, that the I_1a bracts arose sooner than the I_1a leaves, and that they covered much smaller arcs. Indeed the bracts originated almost entirely from part of the normal primary presumptive area of I_1, as will be shown in section 11 and Table V, whereas the I_1a leaves extended far beyond this area. Incidentally, it may be pointed out that the smallest of the bracts covered an arc of 60°, so that it appears that parts of the presumptive area of I_1 that are smaller than this cannot give rise by themselves to any structure at all. But they may be occupied later by parts of other leaves. On the other hand it might be expected that if, as a result of a wound further from the median position, the larger part of the presumptive area of I_1 were larger than in the experiments in which bracts arose, and if the operations were similarly made late in the plastochron, an imperfect leaf would arise, as it did in some of the experiments on P_1. Imperfect leaves were indeed obtained on the *kathodic* side of the wound, as will be described in section 11, after operations made late in the plastochron, and possibly they would have been obtained on the anodic side also, if more experiments had been done.

(8) *Subsequent Phyllotaxis when* I_1a *was a Leaf.*

In all the seven experiments in which I_1a was a complete leaf, except No. 1, it arose almost simultaneously with I_2 and was joined to I_2 by its kathodic stipule in the manner described in the previous section. The causes of the displacement of the centre of I_1a away from the wound and towards I_2 have already been discussed. I_2 appeared to occupy approximately its normal position in the six experiments in which it was joined to I_1a so far as could be judged from its contacts with the older leaves P_1 and P_2, though it may sometimes have been very slightly displaced in the anodic direction by I_1a. The angle I_1a–I_2 was therefore considerably less than the normal on account of the displacement of I_1a towards I_2, its highest value being 113° (see Table I). In No. 1 indeed, I_2 was noticeably displaced in the anodic direction, and this was no doubt due to the fact that in this experiment I_1a arose before I_2 and occupied part of the area which I_2 would normally have occupied. The angle I_1a–I_2 in this experiment is thus slightly larger than in the other six, but still considerably less than the normal.

I_3 appeared to be slightly displaced towards the wound in all seven experiments. Its displacement is consistent with the working hypothesis, for there was a vacant space on the kathodic side of the wound which would normally have given rise to the kathodic half of I_1, and this space was lower than the space which would normally have been occupied by I_3. I_3 therefore occupied this space. The angle I_2–I_3, recorded in Table I, was therefore greater than the normal, except in No. 1, in which the displacement of I_2 towards I_3 more than compensated for the displacement of I_3 towards the wound, and in No. 2 where this angle was normal. The position of I_4 was critical as it determined whether the genetic spiral was reversed or not. In five experiments I_4 arose in approximately the normal position—that is, between I_1a and I_2, and the spiral continued unchanged (see fig. 5, No. 5), while in two experiments I_4 arose on the opposite side of the apex between I_2 and I_3 and the spiral was reversed (see fig. 7, No. 7). It will now be considered whether the position of I_4 can be explained in terms of the working hypothesis. According to the hypothesis I_4 should arise in the gap which first becomes both wide enough and low enough. There were therefore two factors determining which of the gaps became soonest available, firstly, their width, as measured by the subtended angles, and secondly, the ages of the leaves on each side of them. For the younger the leaves on each side of a gap, the nearer to the growing-point it must lie and the longer will be the time before it will become available for a leaf. Now when I_4 was arising the apex was surrounded by I_1a, I_2 and I_3. The three angles between these leaves are given in Table I. The angle I_2–I_3 is regularly the largest. The other two angles, I_1a–I_3 and I_1a–I_2, are more nearly equal, but the former is slightly the larger in all the experiments except one. But I_3 is the youngest of the three primordia and hence the gap between I_1a and I_3 lies nearer the growing-point than the gap between I_1a and I_2. It is probably for this reason that I_4 never occupied the gap between I_1a and I_3. The two gaps available for I_4 were thus the ones between I_1a and I_2 and between I_2 and I_3. Of these, the gap between I_2 and I_3 regularly subtended the larger angle but it was also the nearer to the growing-point since I_3 was younger than I_1a. It is probably for this reason that in five out of the seven experiments I_4 arose in the gap between I_1a and I_2. With regard to the two experiments in which I_4 arose between I_2 and I_3, it must be borne in mind that as a gap increases in width it must also increase in depth, and conversely: for the stipules of the older primordia below the gap taper towards their tips, so that if these primordia are further apart and their stipules do not overreach one another so

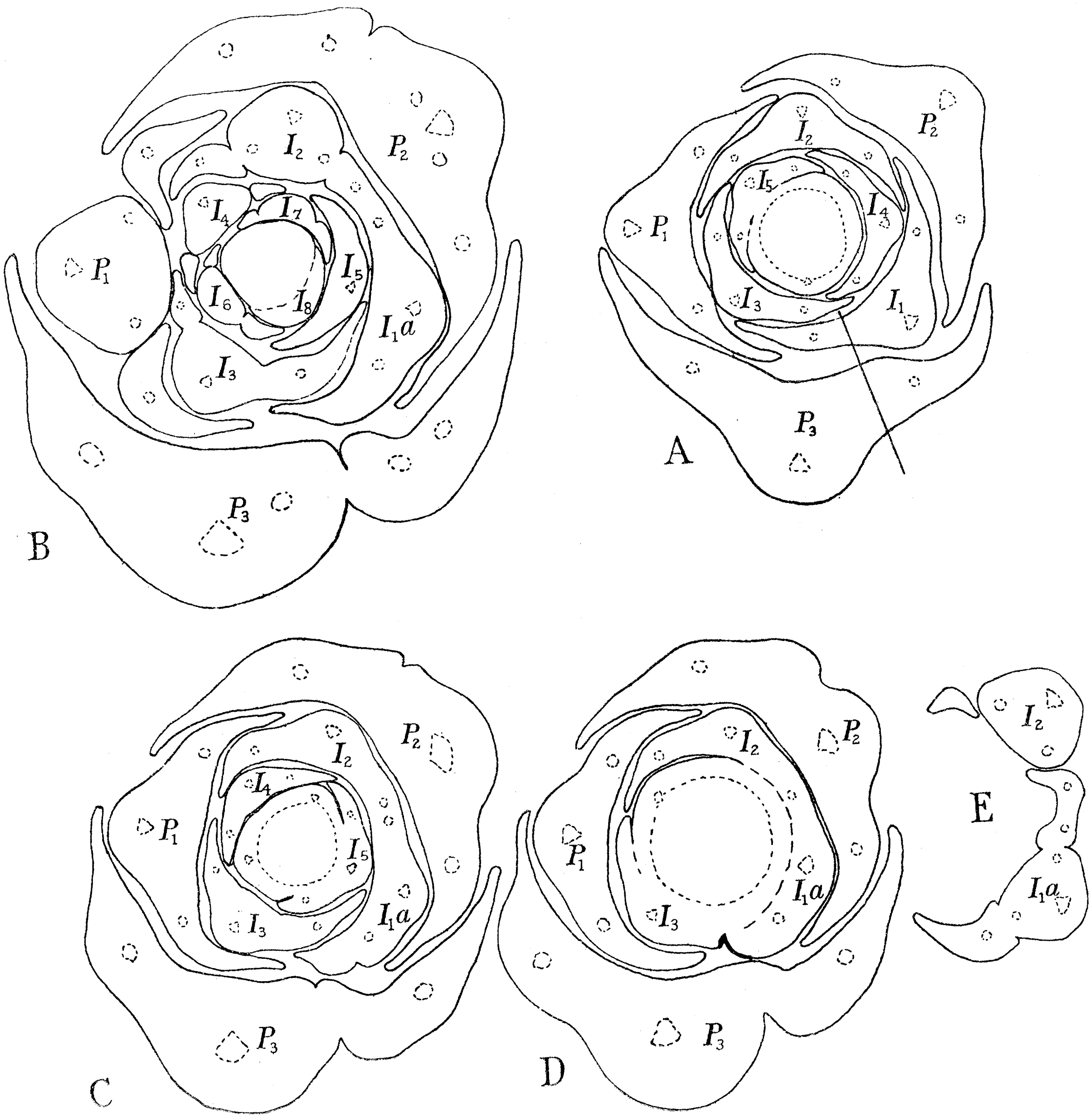

Fig. 7.—No. 7. Only I_1a arose. Sub-group 1. A. Transverse section of a normal bud showing position of wound. B, C, D. Transverse sections of bud of No. 7 at different levels. E. Transverse section of I_1a and I_2 above level of B. The wound scar in D is indicated by a thick black line. B × 45. C, D, E × 35.

much, the depression or gap above the stipules will be deeper. It follows that if the gap I_2–I_3 increased and the gap I_2–I_1a decreased beyond a certain limit the former would become not only wider but deeper than the latter, and would therefore be occupied by I_4. It should therefore be possible to correlate the position of I_4 in these experiments with the difference between the angles I_2–I_3 and I_1a–I_2. This difference is given in column 7 of Table I, and it can be seen that actually the two experiments in which I_4 arose in the angle between I_2 and I_3—Nos. 6 and 7—were those in which the difference in favour of that angle was greatest.

In tracing the direction of the genetic spiral, the usual convention will be followed of always passing from one leaf to the next younger by the shorter way round the stem. When this is done, it can be seen that the genetic spiral in these last two experiments was reversed between I_3 and I_4, as a result of the change in the position of I_1. For the angle I_3–I_4, if measured in the original direction of the spiral, exceeds 180°, so that the shorter way from I_3 to I_4 is the opposite way round the stem. I_5 in turn occupied the gap between I_1a and I_2, since I_4 had occupied the gap in which I_5 would normally have arisen. Also the centre of I_5 lay nearer to I_1a than to I_2, since the stipule of I_4 prevented it from arising in the centre of that gap, as can be seen from fig. 7, No. 7. I_6 and all subsequent primordia made contact with the next older primordium but one and the next older but two as in a normal plant, and thus the genetic spiral continued indefinitely in the reverse direction. The positions of I_5 and the subsequent leaves in the five experiments in which the genetic spiral was not reversed need not be specially described. For their positions did not differ greatly from those in a normal plant as can be seen from fig. 5, No. 5.

To sum up, it has been shown that the final direction of the genetic spiral depended on the position of I_4, which in turn depended on the difference between the angles I_2–I_3 and I_1a–I_2. For when the angle I_2–I_3 exceeded the angle I_1a–I_2 by more than a certain amount, I_4 occupied the former angle and the spiral was reversed. The angle I_1a–I_2 was decreased by the displacement of I_1a away from the wound and towards I_2, while the angle I_2–I_3 was increased by the displacement of I_3 towards the wound and away from I_2. It was not found possible to correlate the varying degrees of displacement of these primordia with the varying positions of the wound on account of complications, such as the fact that the kathodic stipule of I_1a extended over the wound in some experiments and not in others.

(9) *Subsequent Phyllotaxis when* I_1a *was a Bract.*

In three experiments I_1a was a bract, as already stated. Measurements of two of these experiments are given in Table I. It was not possible to measure the third, No. 10, as the sections were too oblique. As was pointed out in section 7, the I_1a bracts subtended angles much smaller than a normal leaf, and so small that the bracts can only have extended slightly, if at all, beyond the presumptive primary area of I_1. Consequently the size of the gap between I_1a and I_2 cannot have been greatly decreased in these experiments, as it was in those in which I_1a was a leaf. The angles between the centres of I_1a and I_2 in these experiments were not recorded, since they were not comparable with the angles that corresponded when I_1a was a complete leaf. I_2 appeared to occupy its normal position and I_3 was displaced towards the wound, as in the previous experiments. I_4 occupied its normal position between I_1a and I_2 as would be expected if, for the reason already given, the gap between I_1a and I_2 was scarcely decreased by the displacement of I_1a. Since I_4 occupied its normal position the genetic spiral was not reversed.

PART 3.—EXPERIMENTS IN WHICH ONLY I_1K AROSE.

(10) *General.*

In fourteen experiments I_1 arose on the kathodic side of the wound only, and in another (No. 16), which will be included in this group, an I_1 leaf arose on the kathodic side of the wound and a bract on the anodic side. Details are given in Tables III and IV.

Table III.—Group 2. Experiments in which only I_1k arose. All angles in degrees.

Numbers of experi- ments.	Angle P_1-wound.	Angle P_1–I_1k.	Angle P_1–I_2.	Angle I_1k–I_2.	Position of I_3.	Final direction of genetic spiral.
Sub-group 1, in which I_1k did not extend above the wound, and arose before I_2.						
11	167	102	95	163	Between P_1 and I_1k	Unchanged
12	160	110	90	160	,,	,,
13	160	120	?	?	,,	,,
14	150	108	95	159	,,	,,
15	155	109	98	153	,,	,,
16	137	100	92	168	,,	,,
Sub-group 2, in which I_1k did extend above the wound, and arose after I_2.						
17	155	97	101	162	Between P_1 and I_2	Reversed
18	157 (approx.)	94	104	162	,,	,,
19	148	86	99	175	,,	,,
20	147	82	96	182	,,	,,
21	140	80	94	186	Between I_2 and the wound	Unchanged
22	132	71	102	187	Between P_1 and I_2	Reversed
23	?	?	?	188	,,	,,
24	132	75	90	195	,,	,,
25	129	63	90	203	Between I_2 and the wound	Unchanged

Note.—In No. 16 an anodic bract also arose.

TABLE IV.—Group 2. Further Data.

Numbers of experiments.	Arc of kathodic half of I_1k, in degrees.	Arc of anodic half of I_1k.	Divergence of centre of I_1k from wound.	Size of P_1 at time of operation.
Sub-group 1.				
11	71	65	65	Small
12	65	50	50	?
13	61	40	40	Very large
14	64	50	50	Medium
15	63	48	48	,,
16	52	37	37	Large
Sub-group 2.				
17	97	98	58	Small
18	94	99	65	Medium
19	77	97	62	Small
20	89	95	65	,,
21	85	95	60	Medium
22	80	112	61	Large
23	82	124	60	Medium
24	85	99	57	Small
25	74	74	66	Medium

In ten of these fifteen experiments the wound lay considerably to the anodic side of the centre of the presumptive area of I_1, as is shown by the angles P_1-wound given in Table III. In four others the wound was median or slightly kathodic, and in one the angle P_1-wound was not measured. In making these statements, allowance has been made for the fact that the angles P_1-wound, as measured, were probably about 3°·5 too small, on account of the shift of the growing-point (see section 5).

In the previous experiments, in which I_1a arose it was found that its centre was displaced far enough to allow room for its kathodic stipule. Similarly in the present experiments the centre of I_1k was usually displaced far enough from the wound to allow room for its anodic stipule. In five experiments, however, its anodic stipule was abnormally small, a fact of which the explanation will be considered in the next section. In nine of the experiments of this group the sequence of the leaves I_1k and I_2 was reversed, I_2 arising before I_1k. None the less it will be convenient to call this leaf I_1k even when it arose after I_2. The experiments may conveniently be divided into two sub-groups. In the first sub-group I_1k arose before I_2 and did not extend over the top of the wound; in the second sub-group I_1k arose after I_2 and did extend over the top of the wound.

(11) *Position, Size and Form of* I_1k *in Sub-group* 1, *and Position of* I_2.

In the six experiments of sub-group 1, in which I_1k arose before I_2, the anodic stipule of I_1k made contact with the side of the wound and did not extend over the top of it.

Since its extension was prevented by the wound, the arc of the anodic half of I_1k should be equal to half the minimum arc of a primordium, if I_1k was determined as a completely new leaf after the operation. It may be recalled that the corresponding measurements made on I_1a indicated values of from 60° to 65° for half the minimum arc.

Measurements of the arc of the anodic half of I_1k are given in Table IV, column 4. In No. 11 the arc of this half was 65° and the total arc of I_1k was 136°. This measurement of the anodic half therefore agrees well with the values of half the minimum arc obtained previously. In the other five experiments, however, the arc of the anodic half of I_1k varied from 52° to 37° and was always much less than that of the kathodic half. The total arcs of I_1k in these five experiments varied from 115° to 88°. These measurements of the anodic halves are certainly less than would have been expected if I_1k in these experiments had been a completely normal leaf determined afresh after the operation. One of these five experiments, No. 12, is shown in fig. 8.

The size of P_1 at the time of operation, is recorded in Table IV. In four of the five

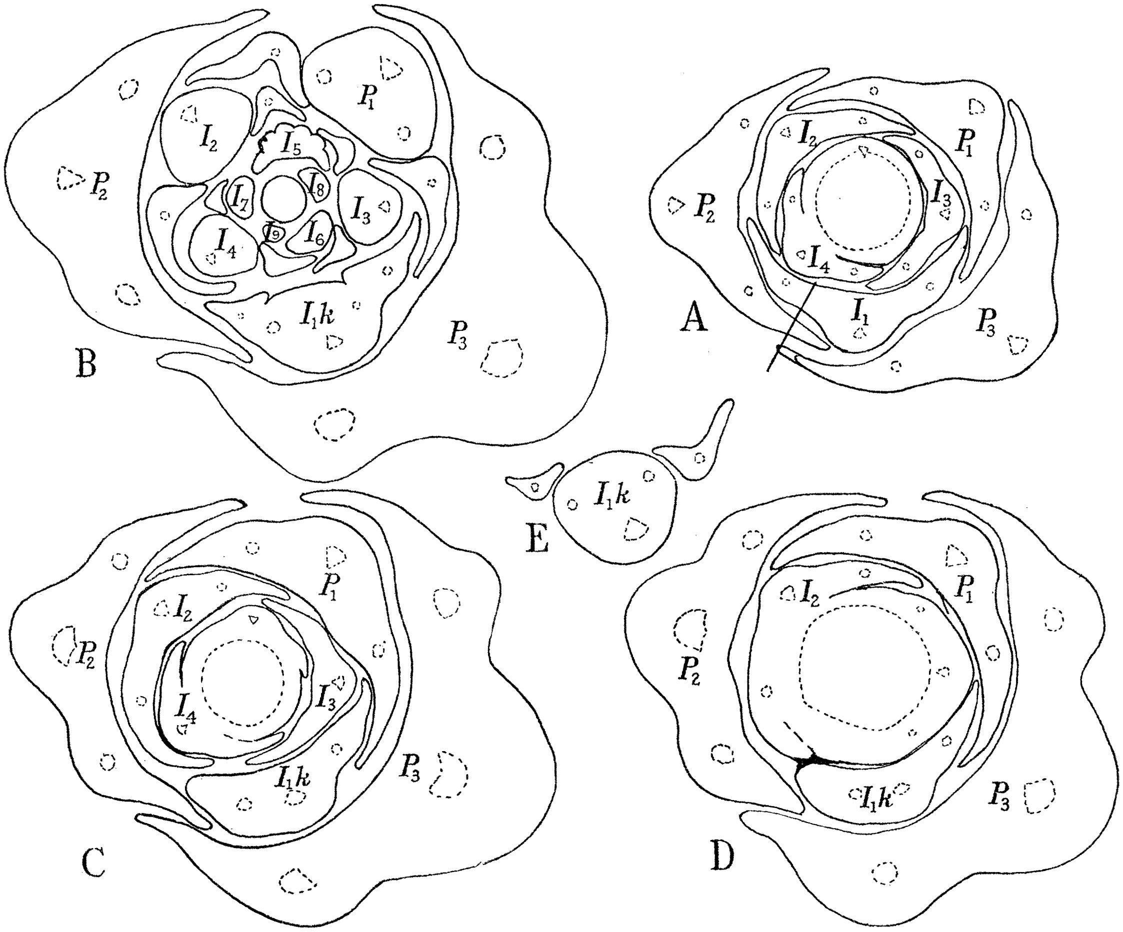

FIG. 8.—No. 12. Only I_1k arose. Sub-group 1. A. Transverse section of bud of a normal plant showing position of cut. B, C, D. Transverse sections through No. 12 at different levels. E. Transverse section of I_1k above level of B. B, C, D, E × 30·5. The wound is shown with a thick black line in D.

experiments in which I_1k was imperfect, P_1 was " medium," " large " or " very large," and in the fifth its size was not recorded, so that it is probable that in all five experiments the operation was made in the latter half of the plastochron. Now in section 7 it was suggested that the I_1a bracts arose when the operations were made towards the end of the plastochron, and that they were formed by the incomplete reorganization of part of the presumptive area of I_1, the operation having been made at a time when the leaf-forming processes had proceeded so far that they could not be completely arrested. Similarly it is now suggested that in the present experiments the five imperfect I_1k leaves were also formed partly by reorganization and not as completely new leaves, since there is evidence that the operations were made towards the end of the plastochron. This suggestion is supported by the fact that in another series of experiments, already mentioned, in which P_1 was split in half, similar imperfect leaves were often formed if the cut was non-median. Usually the larger half of P_1 gave rise to an imperfect leaf, and the smaller half to a bract.

But with regard to the experiments on I_1, if the imperfect leaves and the bracts both resulted from operations made late in the plastochron, the question remains how it was that the five imperfect leaves were all on the kathodic side of the wound, whereas five bracts out of a total of six were on the anodic side. This may indeed have been only a coincidence, but it is probably significant, and an explanation can be suggested. But first it is necessary to consider certain relevant observations collected in Table V. This table includes all the experiments so far reported in which imperfect leaves or bracts arose, except one in which the bract was not measured: it also includes one

TABLE V.—Comparison of Bracts and Leaves. All angles in degrees.

Numbers of experiments.	Divergence of wound from centre of presumptive area of I_1.	Arc of imperfect leaf.	Arc of bract or bracts.	Extension of imperfect leaf beyond presumptive area of I_1.	Extension of bract or bracts beyond presumptive area of I_1.
12	27 (anodic)	115 (I_1k)	—	26	—
13	27 (anodic)	101 (I_1k)	—	12	—
14	17 (anodic)	114 (I_1k)	—	35	—
15	22 (anodic)	111 (I_1k)	—	27	—
16	4 (anodic)	88 (I_1k)	60 (I_1a)	22	2
8	12 (kathodic)	—	82 (I_1a)	—	8
9	9·5 (kathodic)	—	77 (I_1a)	—	5·5
26	1 (kathodic)	—	75 (I_1a)	—	12
			64 (I_1k)	—	3

Notes.—(1) The angles in column 2 are the difference between the normal divergence angle (taken as 136°·5) and the angles P_1-wound, to which 3°·5 has first been added to allow for the fact that these angles, as measured, were about 3°·5 too small (see footnote to section 5).

(2) Columns 5 and 6 are calculated from columns 2, 3 and 4, the arc of the presumptive area of I_1 being taken as 124°.

experiment not yet mentioned, No. 26, in which bracts arose on both sides of the wound. It can be seen from the table that the imperfect leaves all covered arcs that were larger than those of the bracts, though less large than those of normal leaves. For their arcs ranged from 88° to 115°, whereas those of the bracts ranged from 60° to 82°. It can also be seen that the imperfect leaves extended to distances of from 12° to 35° beyond the presumptive primary area of I_1 (the mean being 24°·4), whereas the bracts only extended to distances from 2° to 12° beyond that area, the mean being 6°·1.

In the light of these observations, the following explanation is suggested for the fact that the imperfect leaves were all kathodic, and the bracts all but one anodic. It is suggested that in the experiments in which I_1a bracts arose, I_2, which lies nearer to the anodic side of the wound than to the kathodic, may have prevented I_1a from extending more than a few degrees beyond the presumptive area of I_1. Thus I_1a may have been restricted to so small a space that it was unable to form even an imperfect leaf and developed into a bract, which, as the measurements show, needs less room for its formation. It may indeed be objected that since I_2 arose at a higher level than the I_1a bracts, it could not have set a limit to their extension. But on the other hand it is possible that even when I_2 arose at a higher level than I_1a, it may have been *determined* before the time at which the presumptive area of I_1 (already partially determined) had been completely reorganized so as to form I_1a: for the formation of a new leaf may take a longer time than the formation, by reorganization, of a bract.

In all the experiments of sub-group I, I_2 appeared to have retained its normal position as judged by its contacts with P_1 and P_2. It might have been expected that the kathodic stipule of I_2 would have extended to fill the vacant area on the anodic side of the wound, which was adjacent to it. In one experiment, indeed (No. 12, fig. 8) the kathodic stipule of I_2 did extend into this area and made contact with the wound, as is shown in fig. 8, D. In No. 11 the wound was very far from median and rather wide so that the stipule of I_2 touched the wound without extending abnormally. Nos. 13 and 15 were examined in the solid after 10 and 8 days instead of the usual time, and the overlying primordia made it impossible to see whether I_2 touched the wound. In No. 14, however, it could clearly be seen that I_2 did not touch the wound: but at the level of its insertion a small protuberance was seen on the anodic side of the wound which probably prevented I_2 from occupying that region. Finally in No. 16, as already stated, an anodic bract arose between the wound and I_2.

(12) *Reversed Sequence of* I_1k *and* I_2 *in Sub-group* 2.

The distance of the centre of I_1k from the centre of the presumptive area of I_1, as calculated from the angle P_1–I_1k (Table III, column 3), was regularly greater in sub-group 2 than in sub-group 1. This was due to the following two causes. Firstly, the wound was usually more nearly median in sub-group 2, as is shown in Table III, column 2; secondly, the distance of the centre of I_1k from the wound was generally greater in sub-group 2, as is shown in Table IV, column 4. The reversal of the sequence of I_1k and I_2 in the second sub-group can be explained as due to this greater displacement of I_1k. For it is evident that as I_1k is displaced towards P_1, it must gradually rise to a higher level; and if it is displaced far enough, it will occupy a higher level than I_2, which lies on the other side of P_1, and will therefore arise after I_2. This explanation is supported by the fact that in sub-group 2, in which I_1k arose at a higher level than I_2, the angular divergence of I_1k from the centre of P_1 was less than that of I_2, whereas in sub-group 1 it was greater than that of I_2, as can be seen from Table III, columns 3 and 4.

(13) *The Position, Size and Form of* I_1k *in Sub-group* 2, *and Position of* I_2.

There can be little doubt that in all the experiments of sub-group 2, I_1k was determined as a completely new leaf after the operation. For the arc of its anodic stipule was never abnormally small as in five of the six experiments of sub-group 1; indeed, it was abnormally large for reasons which will be considered later. It can be understood that I_1k was determined as a new leaf, since in only one of these experiments was the operation made late in the plastochron, so far as can be judged from the sizes of P_1 recorded in Table IV, column 6. Consequently it is probable that the leaf-forming processes in the original presumptive area of I_1 had not yet gone so far as to be irreversible.

In all nine experiments of sub-group 2 the anodic stipule of I_1k extended over the top of the wound. I_1k was frequently asymmetric, its anodic stipule being abnormally large, as can be seen in figs. 13, 14 and 15, Nos. 21, 20, and 23. The arcs of the anodic and kathodic halves of I_1k are given in Table IV. The mean of the arcs of the anodic halves is 99°·2, and they are considerably larger than the kathodic halves in all experiments except Nos. 25 and 17, in which they are about equal. Both halves are so large that they must both have extended considerably beyond the primary areas within which they were first determined.

The arcs of the part of I_1k which lay between its centre and the wound ranged from 60° to 66° in seven of the experiments, while in two others they were 57° and 58°. The mean value was 61°·5. Since, therefore, the measurements given in section 7 indicated a mean value of approximately 62° for half the minimum arc of a primordium, it seems that in all the present experiments, except the two mentioned last, practically all the primary area of the anodic half of I_1k must have been situated between the centre of that primordium and the wound, and that the thin tip of the anodic stipule must subsequently have extended considerably beyond its primary area into the vacant space on the other side of the wound. These measurements support the previous estimate of 62° for half the minimum arc, though they really indicate only a lower limit for that value, since part of the anodic half of I_1k reached beyond the wound.

It seemed desirable to know at what stage the extension of the tip of the anodic stipule of I_1k took place, especially as the size of this stipule was found to be an important factor in determining the position of I_3. Since, however, these experiments were examined when I_1k was 6, 7 or 8 plastochrons old they provide no evidence on this point, and consequently it was necessary to examine I_1k at a much earlier stage. As previously stated, the most accurate method of measuring the sizes of the youngest primordia was found to be that of examining the apex together with 3 or 4 of the youngest leaves in the solid under the microscope. A number of additional experiments were therefore carried out in which the wound was made on the anodic side of the centre of the presumptive area of I_1. After only 8, 9 or 10 days, instead of 3 weeks as in most of the other experiments, the apex and the youngest primordia were dissected out and examined in the solid. In most of the experiments the wound was so far from the median that the displacement of I_1k was relatively slight and its anodic stipule made contact with the wound as in the experiments of sub-group 1. In one experiment, however, No. 62 (not included in the tables), I_1k had arisen after I_2 and its anodic stipule extended right over the top of the wound, as is shown in fig. 9. I_3 had also arisen but not I_4, so that I_1k was in its second plastochron. The preparation was drawn under a drawing eyepiece, the centre of I_1k being judged approximately by eye. The arc of the anodic half of I_1k was already 101°, and that of the kathodic half 76°. The arc between the centre of I_1k and the wound was 61°. The anodic stipule must therefore have extended very early in the development of I_1k, probably during its first plastochron and certainly not later than the second plastochron. It was difficult to determine whether I_1k made contact with the top of the wound. For the wound which had opened out laterally was situated on a projecting piece of tissue, and it is possible that the projecting tissues surrounding the wound had been so affected by the operation as to be unavailable for leaf formation.

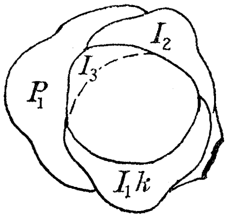

FIG. 9.—No. 62. Only I_1k arose. Apex with four youngest leaves prepared as described in the text and drawn as seen in the solid. The wound scar is drawn with a thick black line. × about 60.

On the basis of these observations, a typical experiment of sub-group 2 is illustrated diagrammatically in fig. 10, which is constructed in the same manner as the previous vertical diagram, fig. 4. The positions of the centres of I_1k and of the wound are the averages of their positions in all the experiments of sub-group 2. The amount of the shift of the growing-point and the vertical height of the wound have been guessed since they could not be measured.

In the previous diagram, fig. 4, representing experiments in which I_1a arose and did not extend above the wound, I_1a and I_2 were orientated with respect to the original position of the growing-point, since reasons were given for considering that in those experiments these primordia were determined before the growing-point had completed its shift. In the present experiments the orientations of I_1k and of I_2 must be considered separately since they arose at different times. I_2 has been orientated in the diagram with respect to the new position of the growing-point (represented by the line B′A′B′), there being no available evidence to show whether in this sub-group it was determined before the growing-point had completed its shift or not. I_2 has also been represented as displaced 4° from its normal position towards the wound, for it can be seen from the diagram that if I_2 is orientated with respect to the new growing-point, it is to be expected that it will be displaced in this way, since it will thus arise in a slightly lower position.* Actually when the position of I_2 in this sub-group was judged by its contacts with P_1 and P_2, it did not appear to have been displaced, except possibly in three of the nine experiments, Nos. 17, 18 and 19; but it is not possible by this method to detect with certainty a displacement of less than about 8°. Strictly speaking I_2 should have been drawn with its horizontal axis a curve parallel to the curve B′A′B′, but owing to the

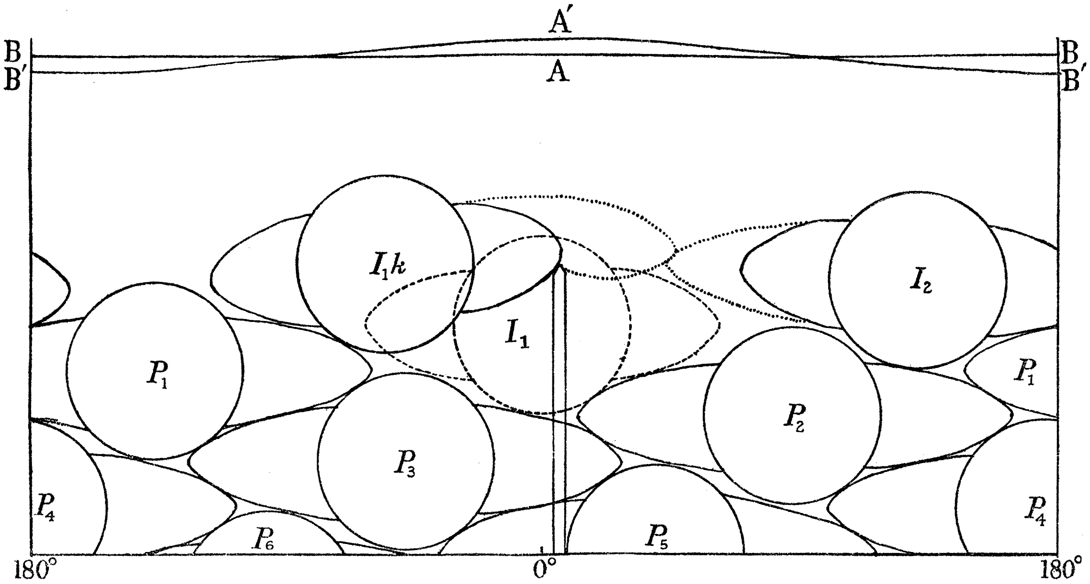

FIG. 10.—Diagram representing the experiments in which I_1k arose alone and extended above the wound. Explanation in text.

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* In the previous paper (1931, p. 25) we said that on the hypothesis adopted I_2 would not be expected to shift after an operation on I_1, since the parts of P_1 and P_2 with which I_2 is in contact are not affected by the operation. But the present vertical diagram shows that nevertheless I_2 must be expected to shift slightly, if it is to be orientated with respect to the new growing-point. In the vertical diagram of the previous paper (1931, fig. 9) we did not notice this point, since there we orientated I_2 (perhaps incorrectly) with respect to the old position of the growing-point. Actually in the previous investigation it looked as if I_2 did not shift after the operations on I_1, nor I_3 after those operations on I_2 which gave comparable results (p. 27). This may have been due to the difficulty of detecting small displacements, or again it is possible that the present diagram is misleading in that it represents the depressions between primordia as less deep than they really are in the plant. For if in the plant these depressions are deeper, then the shift of the growing-point will (to speak figuratively) be less likely to slide the central part of the next primordium out of the depression which it occupies and so to displace it towards the wound. The question of the exact position of the next primordium after the one operated upon, though troublesome, is not of much importance in either investigation, since its displacement is at most so very small.

difficulty of doing this, it has been drawn with a straight horizontal axis, the two ends of which are equidistant from those points of the curve B′A′B′ which are vertically above them.

With regard to I_1k, it is probable that in all the nine experiments of this sub-group, it was not determined until the shift of the growing-point was completed. For otherwise it is unlikely that the region above the top of the wound would have been available for its anodic stipule. Also it can be seen from the diagram that since the anodic stipule of I_1k extended over the top of the wound and since its kathodic stipule appeared to make contact with P_1, I_1k must have been orientated with respect to the new position of the growing-point. It can also be seen from the diagram that, although the tip of the anodic stipule of I_1k extended over the top of the wound, the centre of that primordium could not have arisen closer to the wound without rising to a higher level on account of the shape of the stipule, which increases in thickness towards the centre of the leaf. Incidentally it may be noted that in the two experiments, Nos. 24 and 17, in which the divergences of the centre of I_1k from the wound were least, being only 57° and 58°, the operations were made early in the plastochron, so that, for the reasons given previously (1931, p. 18), the increase in available space above the wound, due to the shift of the growing-point, was probably especially great, and more of the anodic stipule of I_1k was able to arise above the wound.

In none of the experiments of sub-group 2 did the kathodic stipule of I_2 extend to fill the whole of the vacant space on the anodic side of the wound as it did in No. 12 of sub-group 1. This stipule was indeed a little larger than the other stipule of I_2 in all experiments of sub-group 2 except Nos. 22 and 23, but it did not extend so far as to the wound. This can be seen clearly in fig. 11, No. 24, which shows sections through the level of insertion of I_2. It therefore needs to be explained how it was that I_2 did not extend so far as the wound, and the following explanation is suggested. In all these experiments, I_1k must have arisen only slightly after I_2, since it was inserted only very slightly above I_2 on the stem. Moreover, the anodic stipule of I_1k overreached the kathodic stipule of I_2 only very slightly. It is therefore probable that the extension of these stipules was limited by the contact that they made with each other. For since they were inserted at almost the same level, but did not unite, I_1k could only overreach I_2 very slightly, as is clear from the diagram, fig. 10. It is therefore suggested that I_1k and I_2 when first determined occupied only the minimum area of a primordium (or very little more), and then gradually extended until they met. For if this was so it can be understood how it was that the kathodic stipule of I_2 never extended so far as to the wound: for before it could do so, the anodic stipule of I_1k had extended over the top of the wound and blocked the way, although I_1k was a slightly younger leaf than I_2. From this it should not be inferred that a younger leaf can block or limit the *primary area* within which a slightly older leaf is originally determined; for on the present theory determination takes place over the whole of the minimum or primary area simultaneously. But if the slightly older leaf subsequently extends sideways beyond its primary area and takes a little time in doing so, then it can be understood that its extension may be limited by another leaf even if the latter is slightly younger.

This suggested explanation is supported by the fact that in the two experiments of this sub-group in which I_2 was symmetrical, Nos. 22 and 23, the anodic stipule of I_1k was especially large (see Table IV, column 3) and also especially thick (see fig. 15, No. 23). It therefore seems possible that in these two experiments this stipule extended especially rapidly and so stopped the extension of I_2 sooner than in the other experiments. Incidentally it may be noted that in several other experiments, both in the present investigation and in the previous one (1931), it was found that stipules extending above wounds were abnormally large.

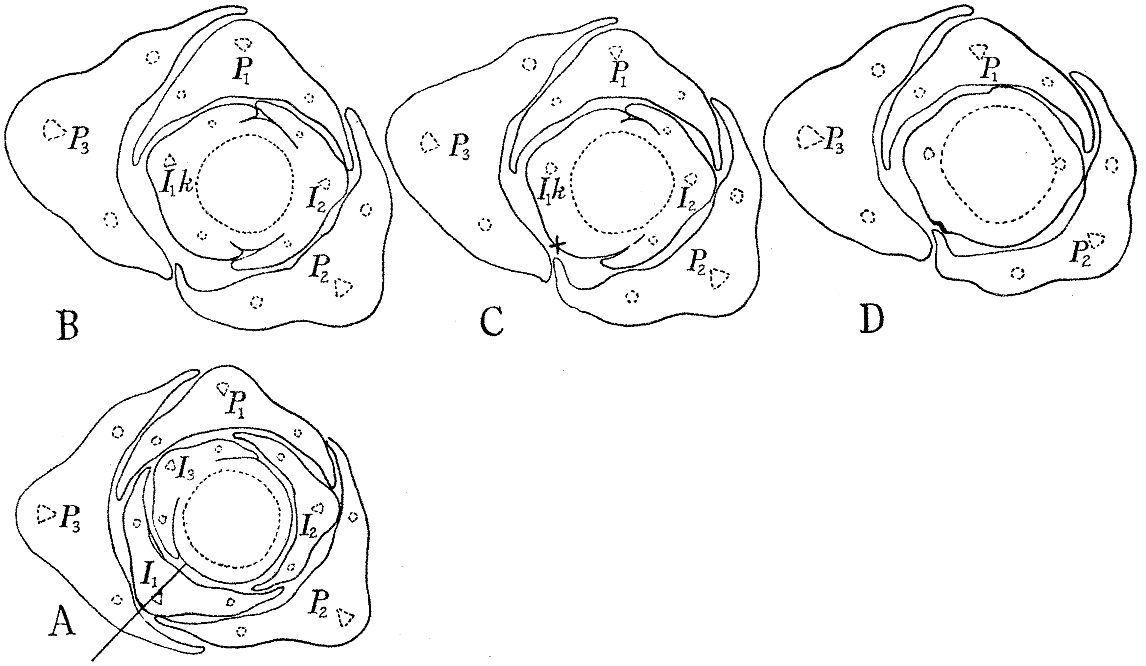

FIG. 11.—No. 24. Only I_1k arose. Sub-group 2. A. Transverse section of a normal bud showing position of cut. B, C, D. Transverse sections of No. 24 at different levels. × 30. The wound scar is shown with a thick black line in D, and a point directly above the wound as estimated from its position in D is marked × in C.

It might perhaps have been expected that the stipules of I_1k and I_2 would fuse, since they were so nearly at the same level. But, so far as we have found, in the Lupin fused leaves only arise if their primary areas partially coincide, which here they did not do.

Another point to be noted is that in these experiments a considerable region on the anodic side of the wound, below the stipules of I_1k and I_2, remained vacant and was never occupied by leaves, as is clear from fig. 11, No. 24. Yet it can be seen from the vertical diagram (fig. 10) that if the kathodic stipule of I_2 could have dipped down so as to extend obliquely downwards instead of horizontally, there would have been no obstacle to prevent it from invading this vacant space. Since, therefore, actually the stipule of I_2 did not invade this space, it must be concluded that (as a general rule at least) the stipules of the Lupin cannot extend downwards below the horizontal.

(14) *Subsequent Phyllotaxis.*

In the experiments of sub-group 1, I_1k and I_2 arose in their normal sequence and the angle I_1k–I_2 increased comparatively slightly, ranging from 159° to 168°. This increase was due to the displacement of I_1k, since there was no evidence that I_2 had been displaced. I_3 occupied approximately its normal position, being between P_1 and I_1k. The subsequent leaves also arose in approximately normal positions and the direction of the genetic spiral remained unchanged (see fig. 8, No. 12).

In the experiments of sub-group 2, I_1k arose after I_2, and I_3, instead of arising between P_1 and I_1k, arose in one of two abnormal positions, either between P_1 and I_2 or else between I_2 and the wound on the opposite side of the apex from the normal. When I_3 arose in the first of these positions the genetic spiral was reversed. When it arose in the second position the genetic spiral was twice reversed and therefore continued finally in the original direction. These changes in sub-group 2 will now be described in detail and interpreted.

In the first three experiments of sub-group 2, Nos. 17, 18 and 19, the angle I_1k–I_2 was less than 180°, as it was in sub-group 1; but in all the rest I_1k was displaced so far towards P_1 that the angle I_1k–I_2 exceeded 180°, if measured in the original direction of the genetic spiral round the side of the apex above the wound. In the first three experiments, in which the angle I_1k–I_2 was less than 180°, I_3 occupied the first of the two abnormal positions mentioned above, being displaced away from I_1k and towards I_2 so that the angular position of its centre was between P_1 and I_2 instead of between P_1 and I_1k (see fig. 12, No. 18). This displacement can be explained in terms of the working hypothesis as follows. Since I_1k was displaced towards P_1, its stipule approached so close to the stipule of I_2 on the side of the apex above P_1 that I_3 made contact with I_1k and I_2 instead of with I_1 and P_1 as in a normal plant (see fig. 12). Moreover, since I_2 arose before I_1k and occupied a lower level, I_3 arose with its centre closer to I_2 than to I_1k. The next gap available was the gap above the stipules of these same primordia, I_1k and I_2, on the opposite side of the apex, where their stipules were in contact. Accordingly I_4 arose in this gap on the same side of the apex as the gap which it normally occupies between I_1 and I_2. But whereas normally I_4 lies nearer to I_1 than to I_2, in these experiments I_4, like I_3, lay nearer to I_2 than to I_1k, since I_2 was at a lower level than I_1k. I_5 arose in the gap between I_1k and I_3, on the opposite side of the apex from the normal, instead of the gap between I_2 and I_3, and the subsequent leaves, like I_4 and I_5, each made contact with the next older leaf but one and the next older but two, as in a normal plant. These changes can readily be followed if the drawing of a normal section (fig. 12, A) is compared with the drawings of the experiment.

In order to determine the direction of the genetic spiral, it is necessary to trace it through the leaves in the order in which they actually arose. When this is done, it can be seen that its direction was reversed by the alteration in the sequence of I_1k and

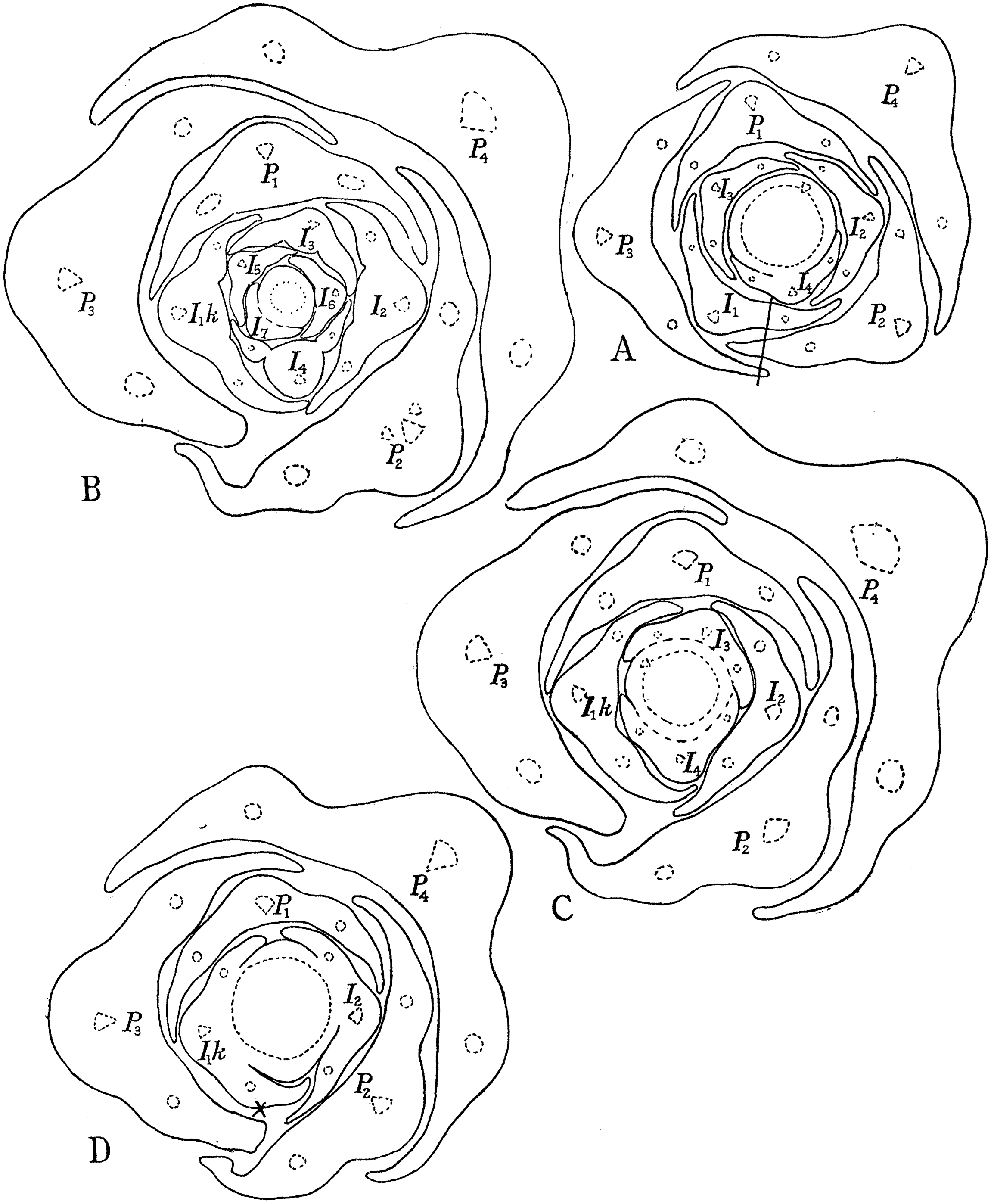

FIG. 12.—No. 18. Only I_1k arose. Sub-group 2. A. Transverse section of normal bud showing position of cut. B, C, D. Transverse sections of No. 18 at different levels. × 33. In D the position of the wound as estimated from its position in lower sections is marked ×.

I_2. For originally, in No. 18 for instance, the genetic spiral was winding counter-clockwise through P_3, P_2 and P_1. But from P_1 onwards it winds clockwise through I_2 (the next younger leaf), I_1k, I_3, I_4, etc., and so continues indefinitely in the reverse (clockwise) direction. Essentially similar changes of phyllotaxis occurred in Nos. 17 and 10.

In the six other experiments, the angle I_1k–I_2, as measured in the original direction of the spiral, exceeded 180°. In two of these, Nos. 21 and 25, I_3 and I_4 exchanged their positions as compared with their positions in the three previous experiments in which this angle was less than 180°. By comparing fig. 13, No. 21, with fig. 12, No. 18, it can be seen that through the increase in the angle I_1k–I_2, the gap which was occupied by I_4 in No. 18 has become larger, and the gap which was occupied by I_3 has become smaller; hence the change in the position of these two primordia. It can also be seen from fig. 13, No. 21, that I_3 occupies the wider of the two gaps between I_1k and I_2 and that it lies nearer to I_2 which is the older leaf of the two. I_5 and all subsequent leaves again make contact with the next older leaf but one and the next older but two, as in a normal

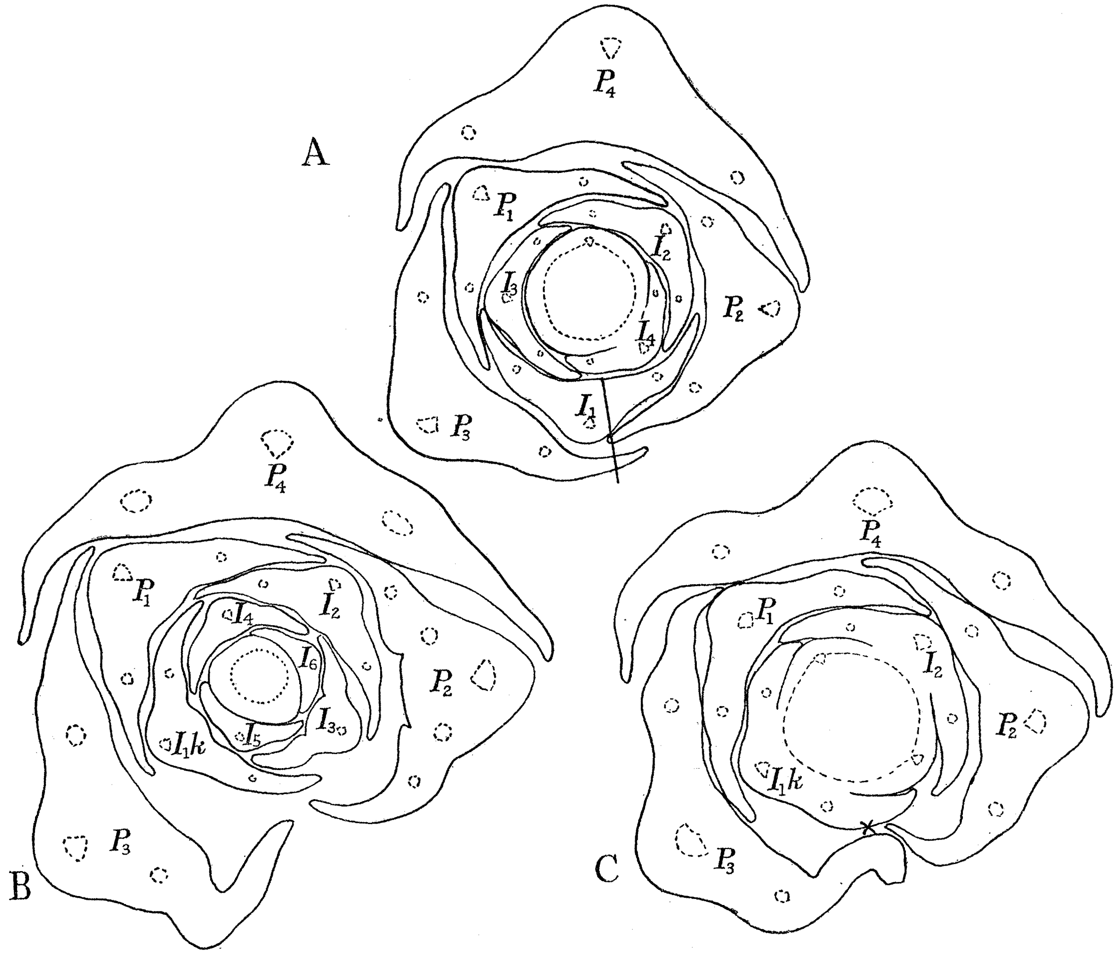

FIG. 13.—No. 21. Only I_1k arose. Sub-group 2. A. Transverse section of bud of normal plant showing position of wound. B and C. Transverse sections of No. 21 at different levels. × 33. In C the position of the wound, as estimated from its position in lower sections, is marked ×.

plant. The genetic spiral is reversed between P_1 and I_2 on account of the alteration in the sequence of I_1k and I_2 and reversed again between I_2 and I_1k on account of the increase of the angle I_1k–I_2 beyond 180° as measured in the original direction. Through this double reversal the genetic spiral returns to its original direction in which it continues indefinitely.

There remain four experiments, Nos. 20, 22, 23 and 24, in which the angle I_1k–I_2 exceeded 180°, ranging from 182° to 195°, and yet I_3, I_4 and the subsequent leaves occupied the same positions as in the first three experiments of this sub-group in which this angle was less than 180°. Examples are given in figs. 14 and 15, Nos. 20 and 23.

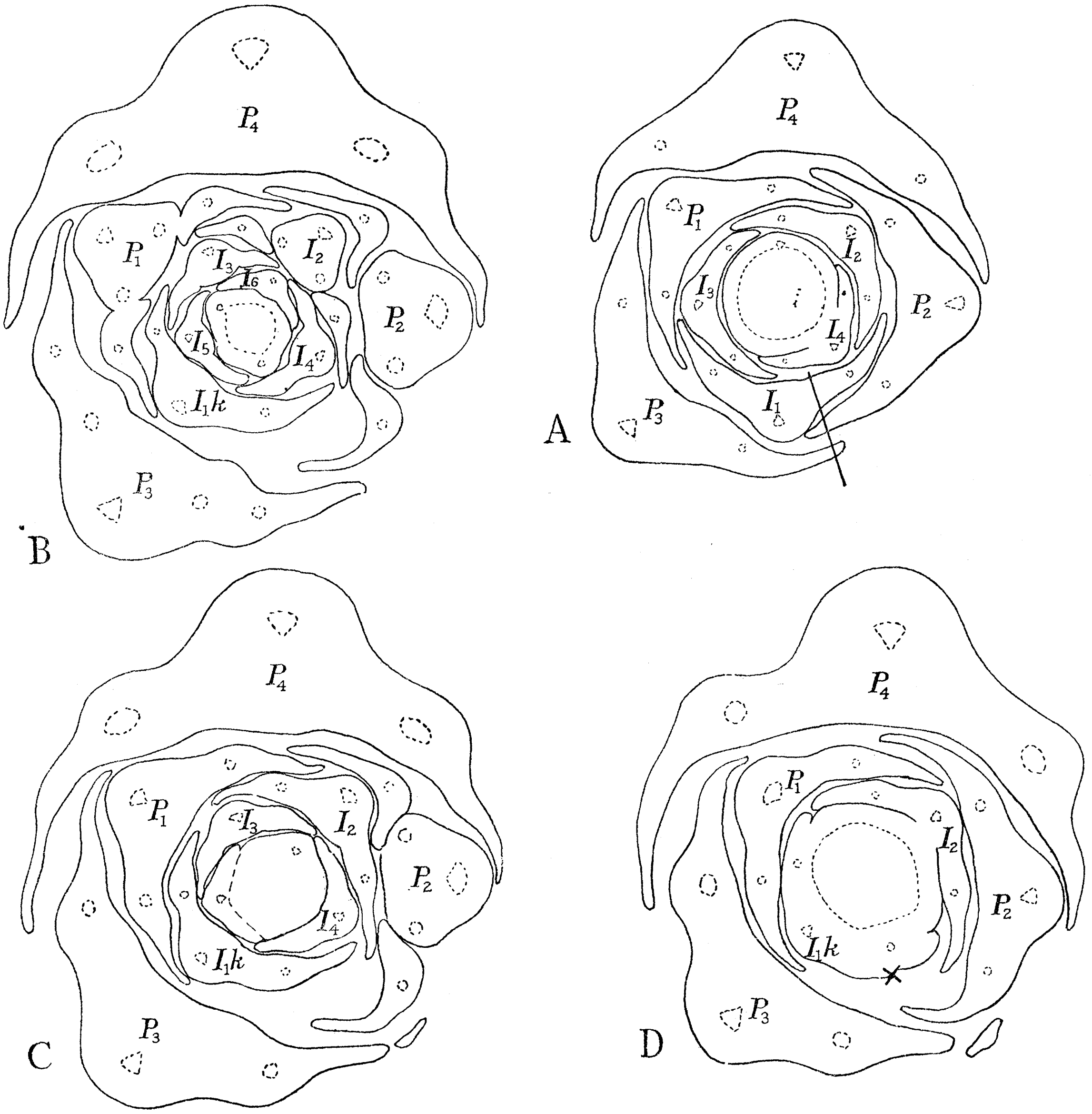

FIG. 14.—No. 20. Only I_1k arose. Sub-group 2. A. Transverse section through bud of normal plant showing position of wound. B, C, D. Transverse sections through No. 20 at different levels. × 32·5. In D the position of the wound, as estimated from its position in lower sections, is marked ×.

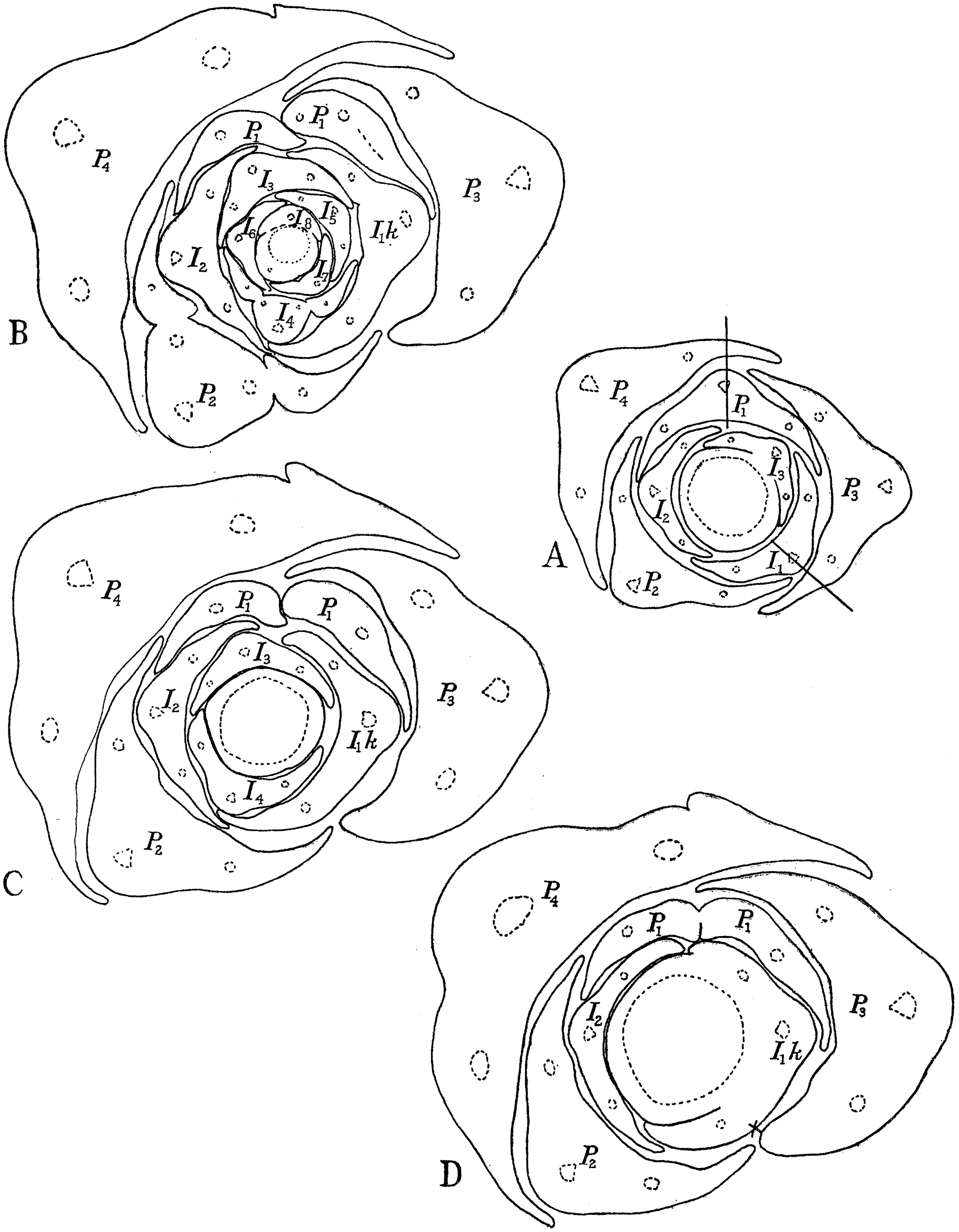

Fig. 15.—No. 23. I$_1$ on the kathodic side of the wound only. Sub-group 2. A. Section of bud of normal plant showing position of cuts through P$_1$ and I$_1$. B, C, D. Transverse sections through No. 23 at different levels. × 25. In D the position of the wound, as estimated from its position in lower sections, is marked ×.

In these experiments, therefore, I_3 occupied the smaller of the two angles between the centres of I_1k and I_2. But it can be seen from the figures that owing to the asymmetric development of I_1k the stipules of I_1k and I_2 overlap above the wound on the side of the larger angle but not on the opposite side of the apex. This is best seen in fig. 14, D, and fig. 15, D, which show sections passing through the insertion levels of I_1k. It might indeed be objected that since in these four experiments I_1k and I_2 were 8 or 9 plastochrons old when examined, the areas which they occupy are different from what they were when I_3 was determined. It is indeed probable that the stipules of I_1k and I_2 have extended since I_3 was determined, but the "short time" experiment, No. 62, mentioned on p. 376, shows that I_1k became asymmetric at a very early stage, probably in its first plastochron. The position of I_3, therefore, in these four experiments is in agreement with the theory of the first available space. Similar instances of a leaf arising in the smaller angle between two older leaves on account of their asymmetric development were given previously (1931, p. 9). In Nos. 21 and 25, mentioned above, I_1k was similarly asymmetric, its stipule on the side of the wound being the larger, but the asymmetry was not great enough to compensate for the fact that the larger angle between the centres of I_1k and I_2 was found on this side of the apex, as can be seen from fig. 13, No. 21.

PART 4.—EXPERIMENTS IN WHICH BOTH I_1A AND I_1K AROSE.

(15) *General.*

In six experiments two complete leaves arose soon after the operation, one on each side of the wound. These leaves, which will be called I_1a and I_1k as in the previous experiments, were always joined by their stipules over the top of the wound. It is probable that they were never absolutely simultaneous, but they were near enough to being simultaneous for their stipules to unite through the overlapping of their primary areas, as in those experiments of the first group in which I_1a was united with I_2. In two of the present six experiments, Nos. 27 and 28, forming sub-group 1, I_1a and I_1k arose after I_2; an example is shown in fig. 16, No. 27. In three other experiments, Nos. 29, 30 and 31, forming sub-group 2, I_1a was united to I_2 by its anodic stipule as well as to I_1k, but I_1a and I_1k appear to have arisen slightly later than I_2 in this sub-group also. Examples are shown in fig. 18, No. 29 and fig. 19, No. 30. In the remaining experiment, No. 32, fig. 20, forming sub-group 3, I_1a arose before I_2, and I_1k about simultaneously with I_2. I_1a was again united to I_1k, but not to I_2. The arrangement of the subsequent leaves varied according to the positions and sequence of I_1a, I_1k and I_2.

Details of these experiments are given in Table VI, and it can be seen that in all of them except the last (No. 32) the position of the wound, as indicated by its angle from

P_1, was distinctly to the kathodic side of the normal position of the centre of I_1. If allowance is made for the fact that the angles P_1-wound, as measured, were probably about 3°·5 too small (see section 5), then these angles show that, except in No. 32, the divergences of the wounds from the true median position ranged from about 6° to 11° in the kathodic direction. On the other hand wounds which were more nearly median, usually led to the development of I_1k only, as in Nos. 16, 22, 24 and 25 (see Table III). The explanation of these facts, which are at first surprising, is probably that the space available for leaf-formation was usually smaller on the anodic side of the wound (see section 11). For unless I_1a arose well before I_2, the space available for it was limited by I_2, which lay closer to the anodic side of the wound than to the kathodic side. It may be noted that in No. 32 in which the wound was approximately median and yet I_1a developed as a leaf as well as I_1k, I_1a did arise before I_2, and I_2 was unable to prevent its development. The results of the different sub-groups will now be more fully described in turn.

(16) *The Results of Sub-group* 1.

In the two experiments of sub-group 1, the wound was slightly kathodic but so close to the median line that there was probably no room for I_1a until I_2 has arisen. Consequently I_1a was delayed for some time, and arose with its centre between I_2 and the wound and its anodic stipule overlying I_2, since the stipules were no longer able to fuse. During this delay the space on the kathodic side of the wound became available and was occupied by I_1k. I_1k arose slightly before I_1a as can be seen in fig. 16, D, No. 27, which shows a section passing through the insertion of I_1k but below the insertion of I_1a. But I_1a and I_1k must have arisen in sufficiently rapid succession for their primary areas partially to coincide above the wound, so that their stipules were united. The size of P_1 at the time of the operation was recorded only in No. 27, in which it was "medium." In this experiment, therefore, the operation was made towards the middle of the plastochron, so that the increase in available space above the wound owing to the shift of the growing-point was probably only moderate. But both I_1a and I_1k were delayed for so long that by the time when they arose the region above the wound had become available.

In both experiments of sub-group 1, I_3 occupied the gap between I_2 and I_1k (see fig. 16, No. 27), and it can be seen from Table VI that the angle I_2–I_1k was considerably the largest of the three angles between I_2, I_1a and I_1k. I_4 occupied the gap between I_1a and I_1k. This gap was indeed at a higher level than the gap between I_1a and I_2, since I_1k was at a higher level than I_2. But the angle I_1a–I_1k was larger than the angle I_1a–I_2, and also, as can be seen from fig. 16, a leaf could not occupy the gap between I_1a and I_2 until its stipule could arise above the stipule of I_3 by which it would be delayed. I_5 occupied the gap between I_1a and I_2 but it made contact with I_1a and I_3. The subsequent leaves arose between the next older leaf but one and the next older but two, as in a normal plant.

The direction of the genetic spiral in both experiments was originally clockwise. It can be seen from fig. 16, B, that in No. 27, I_2, I_1k, I_1a, I_3 and I_4 form two turns of a spiral of which the direction is still clockwise. The direction of the spiral is indeed reversed between P_1 and I_2, since I_2 arose as the next leaf after P_1. But between I_2 and I_1k the spiral returns to the original direction, in which it subsequently continues. There was a similar double reversal of the spiral in No. 28.

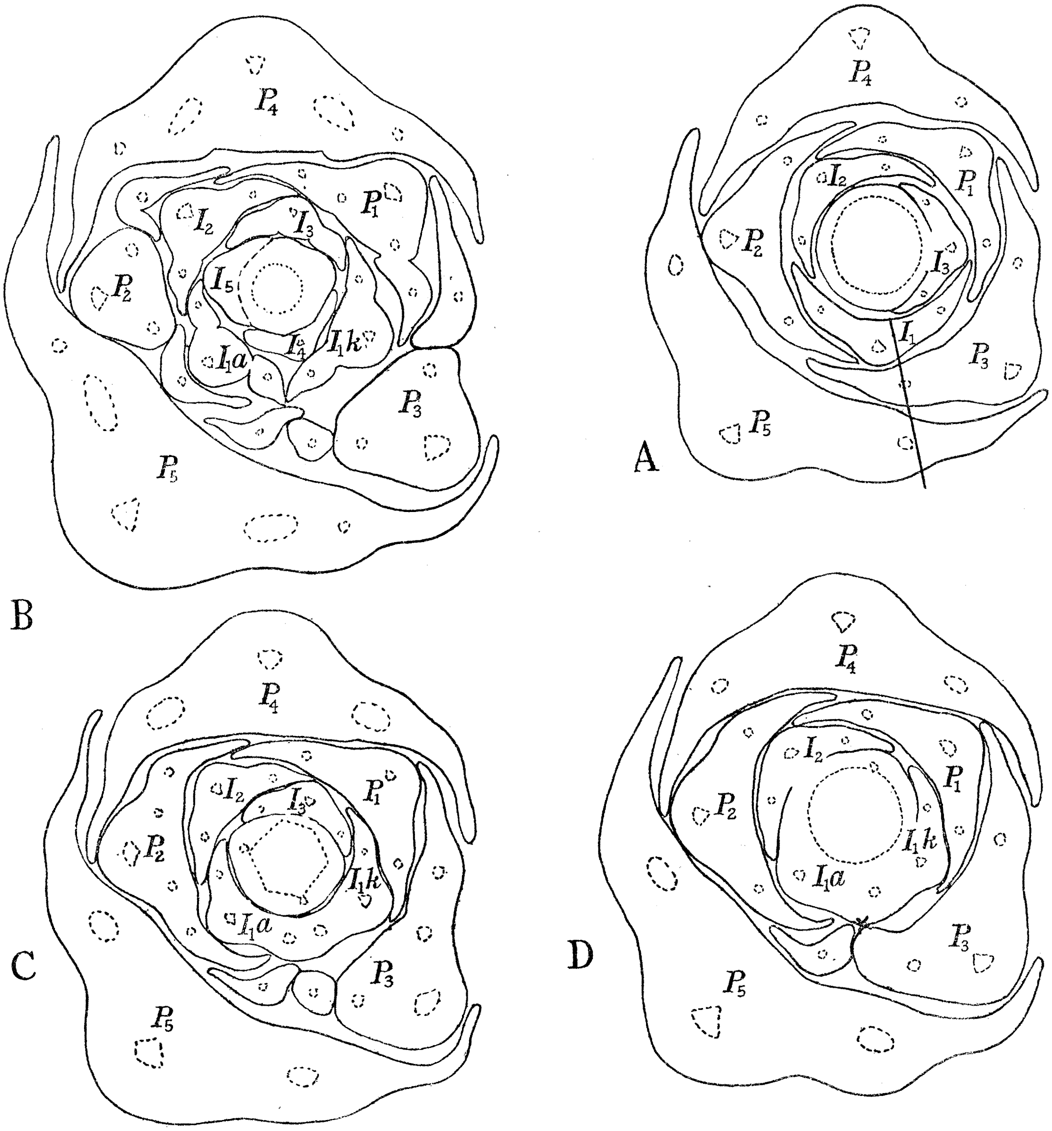

FIG. 16.—No. 27. Both I_1a and I_1k arose. Sub-group 1. A. Transverse section of bud of normal plant showing position of cut. B, C, D. Transverse sections of No. 27 at different levels. × 35. In D the position of the wound as estimated from its position in lower sections, is marked ×.

TABLE VI.—Group 3. Experiments in which both I_1a and I_1k arose. All angles in degrees.

Numbers of experiments.	Angle P_1-wound.	Angle I_1a–I_1k.	Angle I_1a–I_2.	Angle I_1k–I_2.	Final direction of genetic spiral.
Sub-group 1, in which I_1a arose after I_2 and was not joined to it.					
27	126	101 (I_4)	94	165 (I_3)	Unchanged
28	127	109 (I_4)	88	163 (I_3)	,,
Sub-group 2, in which I_1a was joined to I_2.					
29	126	94	99 (I_4)	167 (I_3)	Reversed
30	122	104 (I_4)	105 (I_4)	151 (I_3)	No definite spiral re-established.
31	?	? (I_4)	? (I_4)	? (I_3)	,, ,,
Sub-group 3, in which I_1a arose before I_2 and was not joined to it.					
32	135	110 (I_3)	113	137 (I_4)	Reversed

Notes.—(1) Number 26, in which I_1a and I_1k were both bracts, is omitted from this table. Its phyllotaxis was very little altered.

(2) The symbols in brackets denote the leaves which arose in the angles after which they are placed.

(17) *Positions and Sequence of* I_1a, I_1k *and* I_2 *in Sub-group* 2.

In the three experiments of sub-group 2, I_1a was joined to I_2 as well as to I_1k. These three leaves must therefore have arisen nearly simultaneously. But there is evidence that I_1a and I_1k arose slightly later than I_2. For in the section of No. 30 shown in fig. 19, D, I_1a and I_1k appear to be inserted at a higher level than I_2, which has already separated from the stem. The same thing was seen in a low section of No. 29 (not shown in fig. 18), though here the difference in level was very slight. The sections of No. 31 were too oblique to show the sequence of these leaves.

The explanation of the fact that I_1a and I_1k arose sooner after I_2 in sub-group 2 than in sub-group 1 may quite probably be that in sub-group 2 the operations were made earlier in the plastochron. For if so, then it is to be expected, for reasons similar to those given previously (1931, p. 18), that the effect of the shift of the growing-point was greater in sub-group 2, and consequently that there was a greater increase in available space above the wound. Unfortunately the size of P_1 at the time of operation was not noted in sub-group 2. But this suggested explanation is supported by the remaining experiment, to be described in section 20, in which the operation was made very early in the plastochron, and I_1a arose before I_2.

The positions of I_1a, I_1k and I_2 in No. 29 are represented diagrammatically in fig. 17. This diagram is constructed on the same principles as those already described. The line B′A′B′ represents the position of the growing-point after it had shifted. I_2 is represented as displaced 8° from its normal position in the kathodic direction, since a slight displacement was indicated by its contacts with P_1 and P_2. The position of I_1a in the diagram needs some explanation as it could clearly have occupied a lower position if it could have arisen with its centre closer to I_2. This, however, it could not have done unless its primary area had overlapped that of I_2 to a greater extent. To explain the position of I_1a therefore it is suggested that since I_2 was determined slightly before I_1a, it was only possible for the primary areas of these primordia to overlap to a small extent. This can indeed be understood if, when I_1a was determined, the leaf-forming processes in the presumptive area of I_2, which had been determined just before, had already proceeded so far that the more central parts of that area were unavailable for I_1a.

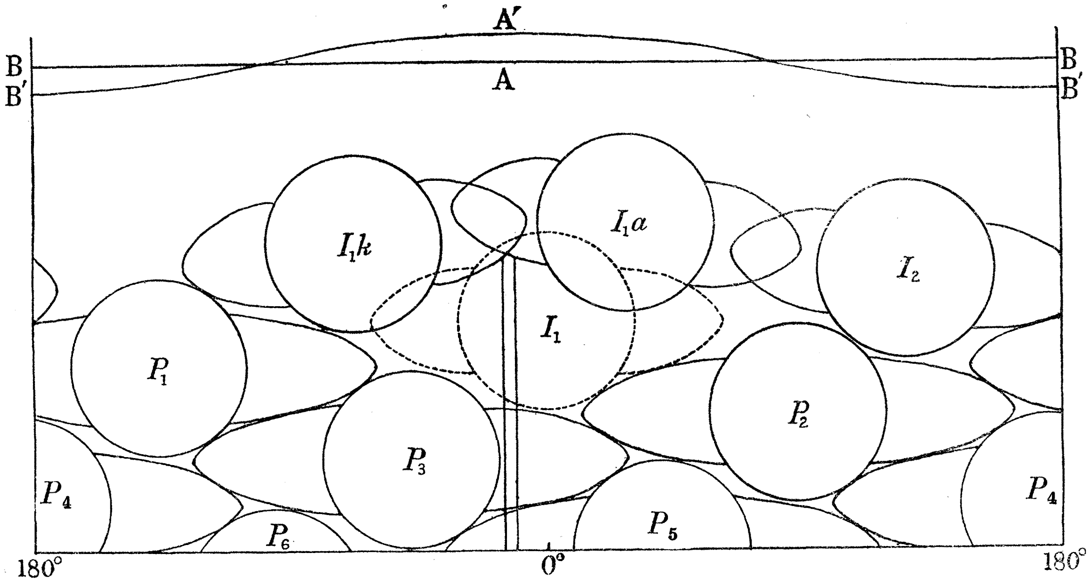

FIG. 17.—Diagram representing experiment No. 29 in which I_1a and I_1k both arose. Explanation in text.

(18) *Subsequent Phyllotaxis in Sub-group* 2.

In the three experiments of sub-group 2, I_3 occupied the same position as in those of sub-group 1, lying between I_1k and I_2, and it can be seen from Table VI that the angle I_1k–I_2 is considerably the largest of the three angles between I_1a, I_1k and I_2. Also I_3 lay nearer to I_2 than to I_1k, since I_2 occupied a slightly lower level than I_1k. The two gaps available for the next leaf were I_1a–I_1k and I_1a–I_2. In No. 29, fig. 18, the latter

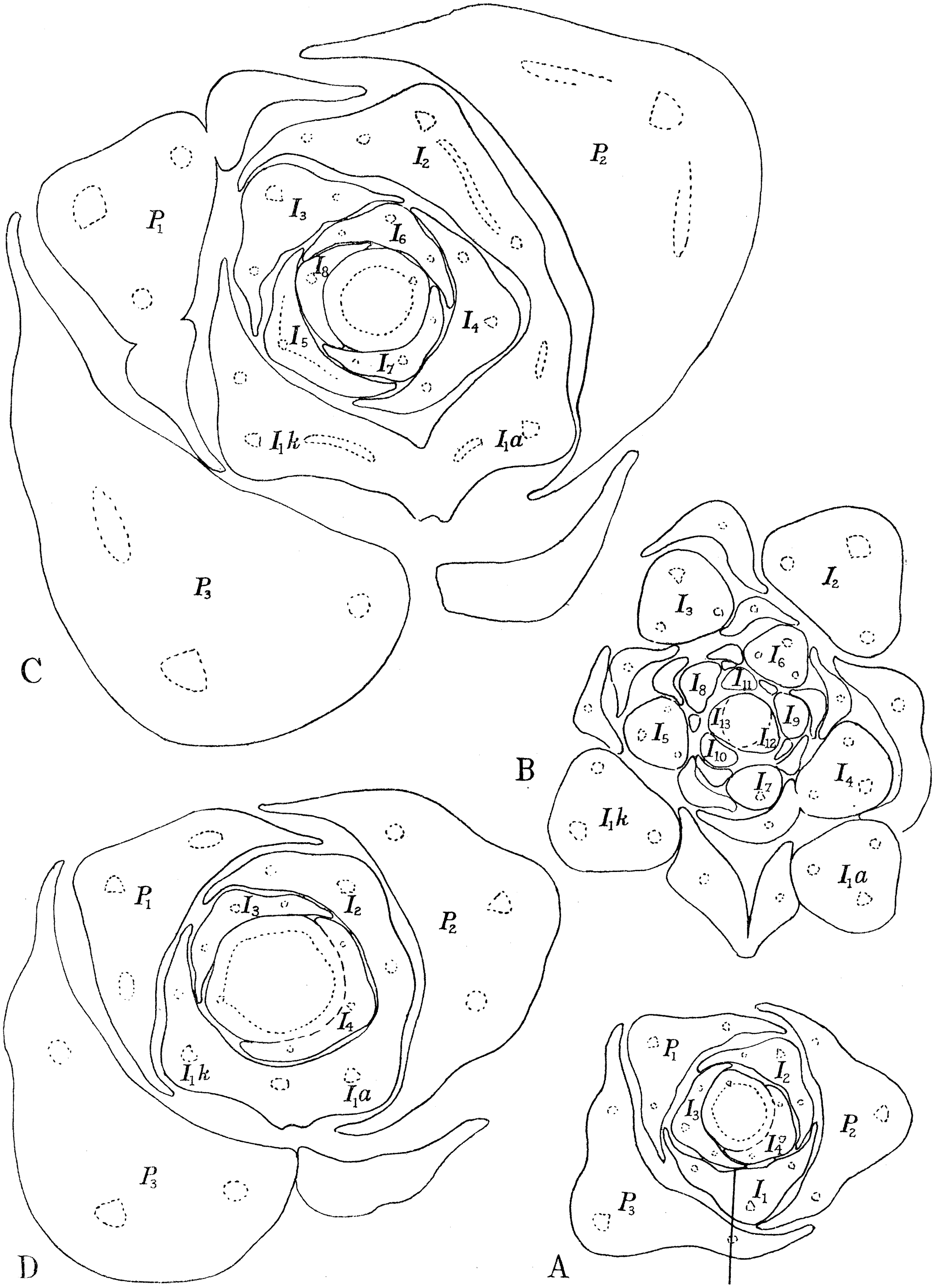

FIG. 18.—No. 29. Both I_1a and I_1k arose. Sub-group 2. A. Transverse section of bud of normal plant showing position of cut. B, C, D. Transverse sections of No. 29 at different levels. In B, P_1 and older leaves are omitted. B and C × 35. D × 30 approx.

gap was slightly the larger in angular measurement (see Table VI), and provided the larger space for leaf-formation as can be seen from the vertical diagram (fig. 17). I_4 therefore arose with its centre in this gap instead of the gap I_1a–I_1k, which it occupied in the previous sub-group. The stipule of I_4 next to I_1a extended into the gap I_1a–I_1k and became abnormally large. I_5 then arose between I_2 and I_3, since the gap I_1a–I_1k, which in the previous sub-group was occupied by I_5, had here been occupied by the stipule of I_4. All subsequent leaves made contact with the next older leaf but one and the next older but two as in a normal plant.

The original direction of the genetic spiral in this plant was counter-clockwise, but between P_1 and I_2 its direction was reversed, since I_2 arose as the next leaf after P_1. From P_1 onwards, therefore, the spiral runs clockwise through I_2, I_1a and I_1k, I_3, I_4, etc. The first four of these primordia all lie within one turn of the spiral, whereas in a normal plant only three leaves lie within one turn. From I_3 onwards the spiral becomes approximately normal having three leaves in one turn. Measurements of divergence angles from I_3 to I_8 were as follows, 143°·6, 142°, 126°·6, 142°·6, 134°·6. These values suggest a gradual return to the normal angle of 136°·3. (It should be noted that the order in which I_1a and I_1k were taken in tracing the genetic spiral in this experiment, was arbitrary, since they were practically simultaneous.)

In No. 30 (fig. 19) I_3 again arose in the angle between I_2 and I_1k, as in the previous experiments, and this was again the largest of the three angles between I_2, I_1a and I_1k, as can be seen from Table VI. The angles between I_1a–I_2 and I_1a–I_1k which were approximately equal, were then occupied almost simultaneously by two leaves which will be called I_4a and I_4k, and these were joined by their stipules over the top of I_1a. The gaps I_4a–I_3 and I_4k–I_3 were then simultaneously occupied by I_5k and I_5a respectively which were joined by their stipules over the top of I_3. The positions of the subsequent leaves can be seen in fig. 19, B.

It is evident that up to the time when I_6 arose a spiral arrangement had not been re-established. The sequence of the primordia arising after I_6 could not be determined with certainty as the sections above this level were oblique, being tilted downwards towards the side on which I_1k arose. But fig. 19, B, suggests that the leaves from I_6 to I_{10} arose in the largest gaps between the older leaves, and formed a spiral sequence. The angles between successive leaves were, however, abnormally small so that four leaves instead of three were included in one turn of the spiral.

In No. 31 the sequence of the primordia was essentially similar as far as I_6, but it was not possible to trace the sequence of the subsequent leaves as the sections were too oblique. These two experiments are of interest as being the only ones so far reported in which the normal spiral arrangement, with three leaves in one turn of the spiral, was not re-established. The leaves following I_3 appeared each to occupy the first available gap, but since for several plastochrons two gaps became available simultaneously a spiral sequence could not for some time be re-established.

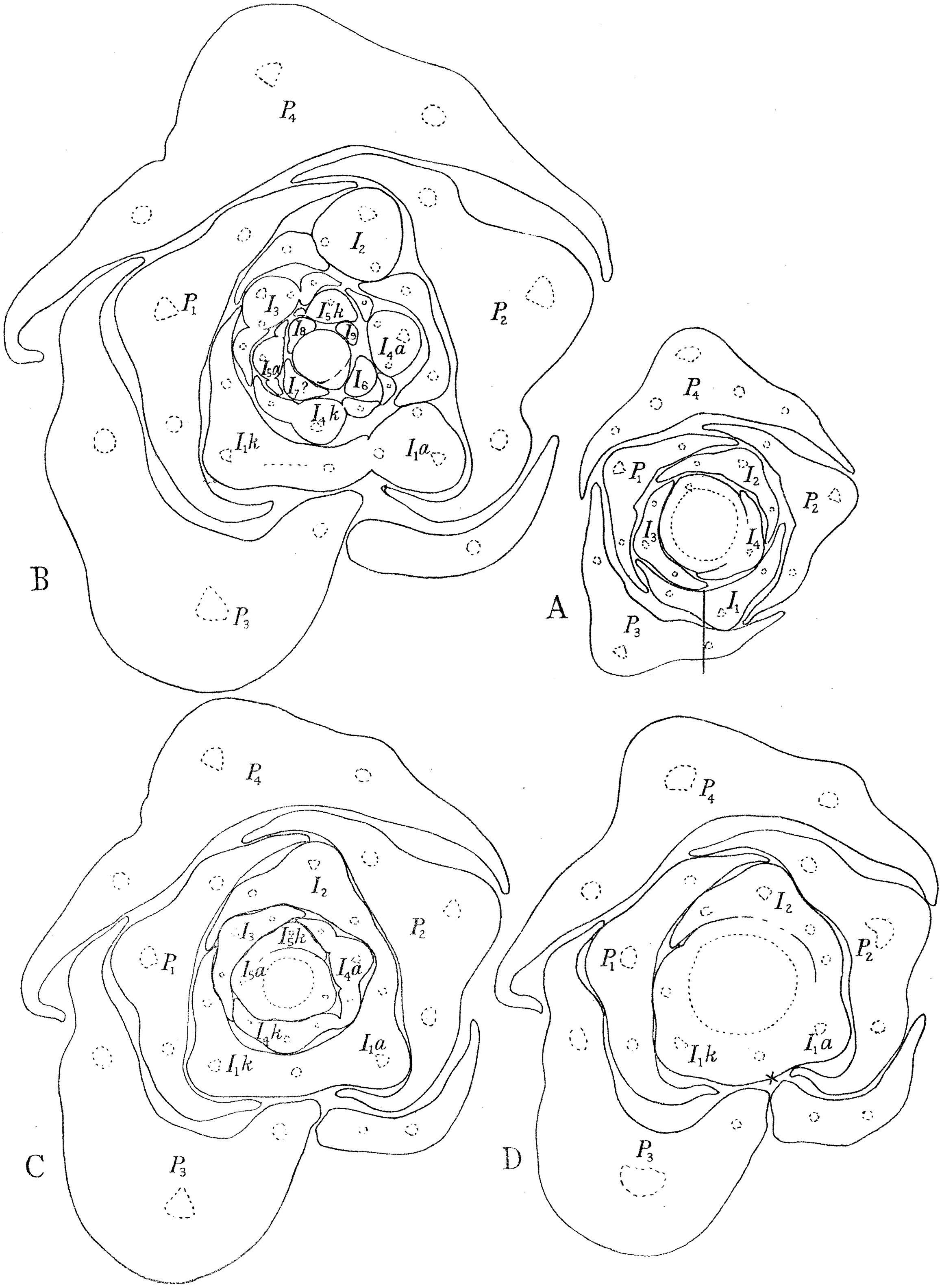

FIG. 19.—No. 30. Both I_1a and I_1k arose. Sub-group 2. A. Transverse section of bud of a normal plant showing position of cut. B, C, D. Transverse sections of No. 30 at different levels. B × 35. C and **D** × 30. In D the position of the wound, as estimated from its position in lower sections, is marked ×.

(19) *The Results of Sub-group* 3.

In the remaining experiment, No. 32, fig. 20, I_1a arose before I_2 and I_1k about simultaneously with I_2. This was probably because the operation was made very early in the plastochron, as was pointed out in section 17. I_1a was joined to I_1k but not to I_2. The wound was very nearly median. I_2 was displaced towards P_1, in the anodic direction, instead of in the kathodic direction as in sub-group 2. This was shown by the angle P_1–I_2 which was only 71° instead of 87°·4, as in a normal plant; and also by the contacts made by I_2 with P_1 and P_2. For its central vascular strand had been displaced away from the stipular strand of P_2 and lay within the edge of P_1, as is shown in fig. 20. The displacement of I_2 was no doubt due to the fact that I_1a, which arose before I_2, had occupied part of the area which would otherwise have been occupied by the kathodic stipule of I_2. I_1a appeared to be inserted slightly lower than I_1k. The manner in which these two leaves were joined was similar to that in which I_1a and I_2 were joined in No. 5, already described, for the anodic stipule of I_1k was turned backwards and outwards so that it was joined by its morphologically upper surface to the upper surface of I_1a.

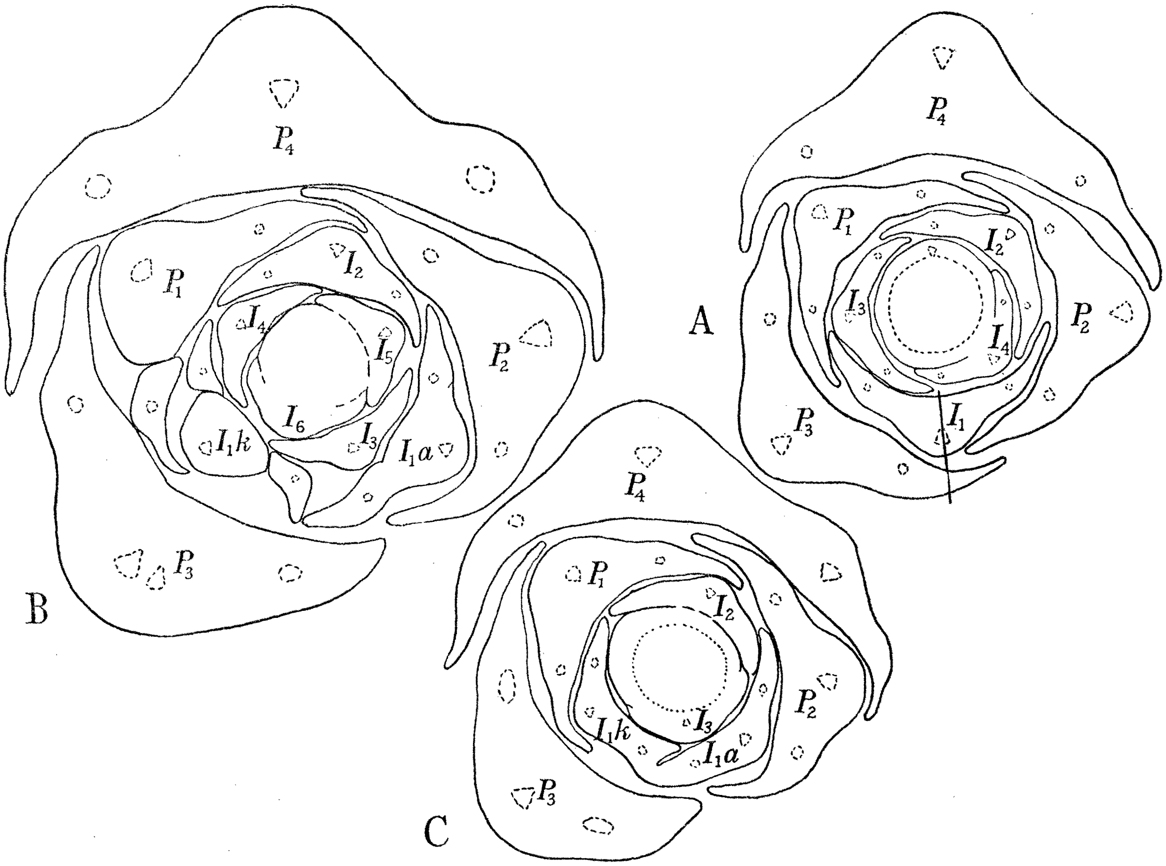

FIG. 20.—No. 32. Both I_1a and I_1k arose. Sub-group 3. A. Transverse section of bud of normal plant showing position of cut. B, C. Transverse sections of No. 32 at different levels. B × 58. C × 33 approx.

I_3 occupied the angle between I_1a and I_1k, on the opposite side of the apex from the normal, instead of the angle between I_1k and I_2 as in the previous experiments of this group. Yet it can be seen from Table VI that the angle I_1a–I_1k was the smallest of the three angles between I_1a, I_1k and I_2. The explanation is clearly that in this experiment I_1a and I_1k were at a lower level than I_2, so that the gap between them was lower than either of the other two gaps, and so became available sooner. I_4 arose in the angle I_1k–I_2, which was larger than the angle I_1a–I_2, and I_5 arose between I_2 and I_3. The genetic spiral was reversed between I_1a and I_1k, since I_1a in this experiment arose slightly before I_1k. The spiral continued in the reverse direction through I_2, I_3 and I_4. The subsequent leaves each made contact with the next older leaf but one and the next older but two in the normal manner, and so the spiral continued in the reverse direction indefinitely.

(20) *The Arcs of* I_1a *and* I_1k.

The arcs of the united halves of I_1a and I_1k, which are given in Table VII, ranged from 38° to 60°, and the arcs of the anodic halves of I_1a in the two experiments in which they were joined to I_2 were 45° and 57°. If therefore the estimate of 62° for half the minimum arc of a primordium, which was reached in section 6 (*a*), is correct, all these unions between leaves, like those mentioned in section 6 (*a*), can be interpreted as due to a partial coincidence or overlapping of their primary areas.

TABLE VII.—Group 3. Arcs of halves of I_1a and I_1k in degrees.

Numbers of experiments.	Arc of kathodic half of I_1a.	Arc of anodic half of I_1a.	Arc of kathodic half of I_1k.	Arc of anodic half of I_1k.
27	46 *u*	80	76	54 *u*
28	52 *u*	68	72	60 *u*
29	38 *u*	45 *u*	76	56 *u*
30	45 *u*	57 *u*	80	59 *u*
32	54 *u*	74	65	54 *u*

Note.—The letter "*u*" when placed after the arc of a half leaf, means that it was united with another leaf.

The arcs of the free halves of I_1a and I_1k ranged from 65° to 80°. In experiment No. 32, in which one of these halves (the kathodic half of I_1k) was only 65°, this half had clearly been prevented from extending further by the stipule of I_2, which was at exactly the same level, see fig. 20, D. This measurement of 65°, therefore, provides an upper limit for half the minimum arc of a primordium, and thus supports the previous estimate of 62°.

PART 5.—DISCUSSION AND CONCLUSIONS.

The various changes in phyllotaxis that resulted from the operations fully confirm the conclusion reached previously (1931) that each leaf arises in the first space that becomes both wide enough and far enough below the growing-point, or, to put it more briefly, in the first available space. For the positions of the successive leaves were readily explained on this basis in the sections describing the changes in subsequent phyllotaxis. It was indeed well known already that, as a general rule, each leaf arises in the widest gap between the previous ones, but it was still possible to maintain that this was in some way merely a secondary result and that the position in which each leaf arose was determined independently of the previous leaves by some controlling mechanism in the stem apex. But the experiments of this paper and the previous one now show that the leaves continue to arise each in the first available space even when the positions of those spaces become quite abnormal as a result of operations made upon the tissues close below them. These results therefore show that the positions of the available spaces do actually determine the positions in which the new leaves arise, and they thus support the theory of VAN ITERSON (1907) mentioned previously.

In all but two of the thirty-two experiments the positions of the gaps available for the new leaves were such that at some stage soon after the operation, though often not immediately after it, three successive leaves arose forming one turn of a spiral round the apex. In eleven of the experiments, the direction of this spiral was opposite to that of the original genetic spiral. Once this condition had been reached, the spiral continued in the same direction, and there was a gradual return towards the normal phyllotaxis, each leaf arising in the gap between the next older but one and the next older but two, as in a normal plant. The divergence angles between these later leaves probably returned gradually towards the normal angle, as in the previous investigation (1931, Tables IV and V), though in the present experiments they were measured only in No. 29, section 18. But these facts do not show that the apex possesses any power of regulating the phyllotaxis or the divergence angles so as to bring them back again to the normal. For on the basis of VAN ITERSON'S theory, every phyllotaxis, with its contact systems and its divergence angle, can be regarded as a condition of equilibrium depending mainly on the ratio of the sizes of the leaf primordia to that of the apex on which they arise. Consequently it is to be expected that when an arrangement something like the normal has been set up again after the operation, the phyllotaxis will then continue to approach more and more closely to the normal.

This explanation is supported by the results of two experiments, Nos. 30 and 31, section 18, in which the normal phyllotaxis was never restored, although 13 or 14 leaves had arisen since the operation. For in these experiments, in both of which both I_1a and I_1k arose, the positions of the gaps were such that apparently there never arose three successive leaves forming one turn of a spiral. Thus the preliminary conditions for a return to the normal phyllotaxis were never reached. It should, however, be mentioned than VAN ITERSON himself (1907, p. 220), for reasons that are not clear to us, postulated that a plant possesses the power of "eliminating small irregularities" from the phyllotaxis.

Since then it is now well established that the position of each leaf depends on the position of the first available space between the older leaves, it remains to be considered more exactly in what way the older leaves act as boundaries to the spaces available for the new leaves, and also how the new leaves occupy these spaces. VAN ITERSON considered that the boundaries of the available spaces were the actual outlines of the older leaf-primordia "at the end of their first stage of development" (pp. 218–219). On the other hand SCHOUTE (1913) postulated inhibiting influences which were supposed to spread out in circles from the centres of the leaf-primordia that were already determined, and to prevent the formation of new leaf centres. But on p. 383 we have described the results of four experiments (Nos. 20, 22, 23, 24) in which, for the reasons there given, it was quite clear that the position of a certain primordium, I_3, depended on the positions of the *edges* of the previous primordia I_1k and I_2, which were asymmetric and not on the positions of their centres, nor of any circles radiating from their centres. A similar conclusion followed from the position of I_4 in three experiments of the previous investigation, those of the second group of isolations of I_1 (1931, p. 9). These results therefore show clearly that, in the Lupin at least, it is the actual outlines of the older leaf-primordia which limit the spaces available for the formation of new primordia. We pointed out previously (1931, pp. 19–20) that the primordia of the Lupin are not circular.

SCHOUTE maintained also that the central point of each new primordium is determined first, independently of the rest of the primordium, which is determined later. But our previous results indicated that this is not so, and that on the contrary each new primordium is determined as a whole, since it was found that the central parts of new primordia did not arise in positions that would have allowed no room for their stipules (1931, p. 23). The present experiments provide further evidence of this point. For when the presumptive area of I_1 was divided into two parts, then, if SCHOUTE's theory were correct, from the larger part of that area there should always have arisen an imperfect leaf having its morphological centre in the normal position of the centre of I_1. For this was the lowest possible position for a leaf-centre, and on SCHOUTE's theory there would be nothing to prevent a leaf-centre from arising in it. The centres, therefore, of I_1a and I_1k should never have been more than 34° distant from the wound, for this was the greatest distance of the wound from the normal centre of I_1. Indeed they should usually have been much less; for the distance of the wound from the centre of I_1 was usually much less, and in some of the experiments of group 2 it must have passed almost exactly through that centre.

But actually what happened was completely different. For in the four experiments in which I_1a arose alone and did not extend over the top of the wound (section 6 (*a*)), the divergences of its morphological centre from the wound ranged from 60° to 65°, the mean being 61°·5. These divergences, therefore, must have been approximately the minimum divergences that were necessary to allow one half of the leaf I_1a to be determined between its morphological centre and the wound. It is on these measurements, supported by others given in sections 13 and 20, that we have based our provisional estimate of 62° for half the minimum arc of the apex over which a leaf can be determined (see p. 360).

Also in the nine experiments in which I_1k arose alone and did extend over the top of the wound (section 13), the distances of its centre from the wound ranged from 57° to 66°, the mean being 61°·5. These measurements, therefore, support the previous estimate of 62° for half the minimum arc; for it is clear that all or very nearly all the primary area of the anodic half of I_1k must have lain between the centre of I_1k and the wound, though the tip of its stipule extended further through the region above the wound and beyond it. But these measurements of I_1k really provide only a lower limit for half the minimum arc; for from these experiments alone it is not certain how much (if any) of the primary area of I_1k may have been situated beyond the top of the wound.

It might perhaps be suggested that the centres of leaf-primordia could not be formed close to the side of the wound, on account of some influence exerted by the wound on the neighbouring tissues. But this cannot be so: for after many of the isolations of I_1 reported previously, in which the wound was broad and rounded at the top, it was found that I_3 arose with its central part in contact with the sloping side of the wound, and with its stipule extending over the top of the wound (1931, p. 21).

In the six experiments in which I_1k did not extend above the wound (section 11), the distances of its centre from the wound were usually rather less, though again much more than could be expected on SCHOUTE's theory. They ranged from 37° to 65°, the mean being 48°, and in five of these six experiments I_1k was imperfect, its stipule next to the wound being abnormally small. But reasons were given for considering that these imperfect I_1k leaves resulted from operations made late in the plastochron, and this fact helps to explain them. For when P_1 is split in a similar manner, then a very imperfect leaf often develops from the larger part of that primordium as we have found, with its stipule next to the wound very small or absent. Consequently it can be understood that when I_1 is split late in the plastochron, at a time when it is probably already partially determined, an imperfect I_1k leaf arises.

When I_1a arose alone, it was apparently never an imperfect leaf, but in three experiments it was a bract, such as often develops from one or both parts of P_1 when split. The interpretation of these bracts is discussed in sections 7 and 11. It is possible indeed that the I_1a leaves which did not extend over the wound may have been slightly imperfect, though too slightly to be detected, in the same manner as the I_1k leaves and for the same cause. For this reason the estimate of 62° for half the minimum arc is really a lower limit, and the true value may be a little higher. But experiment No. 32 (section 20) indicates an upper limit of 65°.

The results therefore show clearly that, in the Lupin at least, a leaf-primordium is determined as a whole. For it can only be determined with its morphological centre in a position that allows room for the rest of the leaf, though it can arise with an abnormally small stipule if it is operated upon towards the end of the last plastochron before it is due to arise, at a time when it is probably partially determined. A leaf may also appear incomplete when united with another leaf: but these unions can be regarded as due to a partial coinciding of the areas in which two leaves are determined.

Against the theory of the first available space, it may very probably be objected that in many plants various members, usually bracts or floral members, fail to appear, being, as it is usually said, " aborted "; but yet their absence does not seem to alter the positions of the subsequent members. We are indebted to Professor SCHOUTE for drawing our attention to this phenomenon, and kindly mentioning in a letter many plants in which it is known. But concerning these aborted members, two comments may be made. Firstly, there is usually no adequate evidence from microscopic examination to show that there is really no visible trace of the aborted member at the youngest stages. Secondly, even if no visible trace of it were found, it should be borne in mind that before a primordium can arise, the part of the apex from which it will arise must probably be physiologically determined, so as to form what SCHWENDENER called a " field of development." For it is unlikely that a primordium could arise absolutely suddenly from undetermined tissue without any previous preparation. There would therefore be no difficulty in supposing that the field of the aborted member is first physiologically determined and so made unavailable for other members, but then for some reason that it fails to arise. Further, since the aborted member fails to arise, there must be some cause which prevents it from so doing, and that cause, whatever it may be, may very probably also prevent any other lateral members from occupying the same area.

The time at which a leaf is determined must be at the latest some time during the last plastochron before it will arise, or, in other words, during the stage of I_1, for the reason already given. On the other hand there is evidence that it is often not yet determined at the stage of I_2. For in several experiments I_2 was slightly " displaced " in relation to the older leaves with which it made contact—that is to say, it arose in a different position from its normal one. These experiments were several of the isolations of P_1 (see 1931, p. 17) and, in the present investigation, numbers 1 and 32 (sections 8 and 19) and perhaps a few others. Now in these experiments the normal presumptive area of I_2 was not touched by the wound, and consequently it cannot plausibly be suggested that I_2 was already determined in its normal position, but that its determination was then reversed or undone through the effect of the wound. On the contrary, if I_2 had been determined at the time of operation, it would have arisen in its normal position, since there was nothing to prevent it from doing so. But actually it arose in a slightly different position, sometimes because this position became available sooner as a result of the shift of the growing-point, and sometimes (in experiments 1 and 32) because I_1a had previously occupied part of the normal area of I_2. It therefore follows that at the time of operation I_2 was not determined. (It is clear that the primordia cannot have been moved bodily over the apex after they were determined, for this would inevitably have been revealed by distortions of the cell walls.)

It follows, therefore, that a leaf is determined either at the end of the I_2 stage or during the I_1 stage. Furthermore, the differences between the results of operations on early and late I_1 stages make it probable either that determination takes place at about the middle of the I_1 stage, or, if it takes place earlier than this, that during the I_1 stage it gradually becomes less easily reversible (see sections 7 and 11).

On the theory here adopted, a leaf is determined as soon as a space covering a certain minimum arc of the apex becomes far enough below the growing-point. This minimum arc we have estimated provisionally at 124°. But in some experiments spaces of more than this minimum arc were provided for certain primordia—for instance for I_2 in the experiments in which only I_1k arose. The question therefore arises whether in such circumstances a primordium is determined over the whole of the available arc, as soon as it becomes far enough below the growing-point, or whether it is first determined over the minimum arc only, so that it can only occupy the rest by subsequent extension. The second alternative is favoured by the facts concerning I_2 discussed on pp. 378, 379, though more evidence is needed.

In the normal plant the leaves continue to extend laterally for several plastochrons after they have arisen, as was shown in section 2, and by the beginning of the P_2 stage they have already considerably exceeded the minimum arc of 124°. But the later stages of this extension must take place through tissues that become available only after the leaf has been determined, through the growth in length of the apex and axis, which makes new tissue available above and between the older leaves.

There remain to be mentioned some minor points concerning the origin of united leaves. It was found that the arcs of the united halves of these leaves were never so great as 62°, the estimated value of half the minimum arc of a primordium. This indicates that in the Lupin united leaves only arise when their primary areas partially coincide. Some interesting observations on united leaves are reported by van Iterson (1907, pp. 283–284). He explains them in a way that is essentially similar. In the Lupin it is apparently only the stipules whose primary areas can coincide; for the centres of two leaves were never found to be so close as to indicate that a stipule of one leaf had coincided with the central part of another.

It was also found that the united leaves were always at the same level or very nearly so, and from this it follows that the primary areas of two leaves cannot partially coincide or " overlap " unless they are determined at very nearly the same time, even though they may be in contact. In five experiments, Nos. 5, 29, 30, 31 and 32, two leaves were united although one of them was situated slightly higher than the other and must therefore have been determined slightly sooner. In Nos. 5 and 32 (p. 360 and p. 393, and figs. 5 and 20) in which the difference in level was greatest, the stipule of the slightly higher leaf was turned outwards and backwards, so that its morphologically upper surface was united to the upper surface of the lower leaf. Thus their union conformed to the so-called " law of laminar inversion," (Worsdell, 1915, vol. 1, p. 196), which implies that only similar surfaces of laminæ can unite.

PART 6.—SUMMARY.

1. In 32 apices of *Lupinus albus*, the presumptive area of I_1, the next primordium due to arise, was divided into two parts by a vertical cut in a radial plane. The purpose was to test further the conclusion reached previously (1931) that each new leaf-primordium arises in the first available space.

2. The direction along the genetic spiral towards the successively younger leaves was called " anodic," and the opposite direction " kathodic." Sometimes a primordium arose on the anodic side of the wound, and sometimes one arose on the kathodic side. The former was called I_1a and the latter I_1k. Sometimes both I_1a and I_1k arose.

3. I_1a and I_1k were usually perfect leaves, but often, when the operation was made late in the I_1 stage, there arose instead either a bract or an imperfect leaf, of which the stipule next to the wound was abnormally short. The interpretation is discussed in sections 7 and 11, and in Part 5.

4. The distances from the wound of the morphological centres of I_1a and I_1k, when they were leaves, show that the centres of new leaves can only be determined in positions that allow room for their stipules. They also indicate that in the Lupin the minimum area in which a leaf can be determined subtends approximately 124°, or perhaps slightly more. The relevant facts are recorded and discussed in sections 6, 11, 13 and 20, and summarized in Part 5.

5. In six experiments I_1a and I_1k both developed as leaves: they were then always united above the wound.

6. The subsequent phyllotaxis finally returned approximately to the normal type in all experiments except two (Nos. 30 and 31, section 18), with the genetic spiral winding either in the original direction or in the opposite. The positions of the successive leaves have all been readily interpreted on the theory of the first available space, which they strongly support.

6. The results also show that each leaf is determined as a whole, and that, in the Lupin at least, the space available for it is bounded by the actual outlines of the previous leaves (p. 383 and p. 396). They thus, like the previous results, support VAN ITERSON'S theory of phyllotaxis (1907).

8. Several other points of interest concerning the experiments are discussed in Part 5.

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