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Experiments on Spirodistichous Shoot Apices. I

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EXPERIMENTS ON SPIRODISTICHOUS SHOOT APICES. I

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The shoot apex of *Rhoeo discolor*, a spirodistichous species, is described in detail. The mean divergence angle between successive leaf centres is 152°·6. Each new leaf covers only a small arc when first visible, and its flanks then extend round the apex until they meet in the later part of the plastochron. The new leaf is asymmetric, its half which is anodic in the direction of the genetic spiral being the shorter transversely and covering a mean arc of 166°·5 when the flanks meet. Thus the centre of a new leaf does not lie directly over the meeting-point of the flanks of the previous leaf, but above its anodic half.

Experiments were made in order to discover the causes on which the divergence angle depends. As a preliminary some stem apices of *Rhoeo* were exposed and marked with transverse streaks of albumen and charcoal. But after several days the streaks had not been bent by the growth of the apex in the way which would be expected on Hirmer's theory of spiral growth.

In the main experiments the central part of P_1, the youngest leaf, was cut out completely. It was necessary first to cut down P_2 and the older leaves of the bud nearly to their bases. The main results were that I_1, the next leaf to arise, was displaced towards the missing centre of P_1, and that the angle I_1–I_2 was larger than the normal, and sometimes exceeded 180° (see figure 6). In some other experiments the central part of P_2 was removed as well as that of P_1. In these also the angle I_1–I_2 increased, and in one of them the genetic spiral reversed permanently, but the displacement of I_1 towards P_1 was usually less.

It is concluded that the position of the centre of each new leaf depends on a balance between inhibiting influences exerted on the stem apex by existing leaf centres. The position of a new leaf n depends mainly on the inhibitions coming from the centres of the two youngest leaves, n–1 and n–2, and to a lesser degree on those from the centres of n–3 and possibly n–4. The strength of the inhibition thus decreases with the age of the leaf from which it comes and also with the distance from the inhibiting centre.

The asymmetries of the leaves that arose after the operations are reported and discussed in comparison with those of the normal leaves. Many of the facts can be explained if it is supposed that the same influences from existing leaves which tend to inhibit the formation of leaf centres

promote the extension of leaf flanks round the apex. But the asymmetry of I_3 in most of the experiments is at present not explained on this hypothesis.

The localization and determination of leaves in *Rhoeo* is further discussed and compared with the corresponding processes in *Lupinus albus* and other dicotyledons. It is concluded that in spirodistichous monocotyledons the localization of leaves, depending on physiological inhibitions, is different from what it is in dicotyledons, in which it was previously concluded to be a space-filling process. The process of determination is also shown to be different in *Rhoeo*, since a rather small central part of a leaf is determined first, and from it the determination extends by induction round the apex. In both these respects the experiments on *Rhoeo*, unlike those on dicotyledons, give good support to Richards's theory of phyllotaxis, which, however, was intended to apply to all groups.

Introduction

The phyllotaxis of monocotyledons has received less attention than that of dicotyledons and no experimental work has been done on them. The majority of monocotyledons have straight distichous phyllotaxis, and most of those which have spiral phyllotaxis have systems of the kind which is called spirodistichous, and not Fibonacci spirals. Spirodistichous systems, according to Hirmer (1922), occur in species belonging to the families Liliaceae, Juncaceae, Bromeliaceae, Araceae, Commelinaceae and Hydrocharitaceae; they are usually derived from distichy in ontogeny, the first leaf of the seedling being opposite the cotyledon, and the change to spirodistichy may occur at the second leaf or later. When this happens the leaves are laid down with a divergence angle of less than 180° but considerably greater than the Fibonacci angle of 137°·5 which is closely approached by most dicotyledons with spiral phyllotaxis. In consequence of this decrease of the divergence angle the two orthostichies become helices instead of straight lines. If the angle falls very much below 180° the two helices are no longer apparent to the eye.

The biological value of spirodistichy may well be that it exposes the leaves more fully to light than does straight distichy; for most spirodistichous types have short internodes, at least during a large part of their growth, and so would receive much less light if their distichy were straight.

A significant feature in which spirodistichous systems differ from the spiral systems of dicotyledons is that each young leaf covers an arc of more than 180° before the next leaf arises, not only in the young seedling, but also later. Indeed, to judge from the drawings given by Hirmer (1922) in many spirodistichous species each leaf encircles the whole apex before the next leaf arises. Thus the change in divergence angle in spirodistichous systems does not depend on a change in the arc covered by each young leaf, whereas in the seedlings of dicotyledons the changes in the divergence angle regularly do depend on changes in the arcs of the leaves. The divergence angles in spirodistichous plants vary considerably from one species to another. It has also been claimed that the angle varies considerably within a species or in a single plant, but it will be shown that this variation is not always so great as is supposed. Another feature in which the spirodistichous systems of monocotyledons differ from most spiral systems of dicotyledons is in the asymmetry of the leaves. This asymmetry is not always obvious in the blade of the mature leaf but can usually be seen in transections through the bud, where it appears that the morphological 'median' strand of the leaf divides the leaf into two lateral parts which differ in tangential extension and sometimes in radial thickness.

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From these facts it is clear that the hypothesis of the first available space cannot easily be applied to spirodistichous plants. According to this hypothesis 'each leaf arises in the first space that becomes both wide enough and far enough below the growing point' (Snow, M. & R. 1931, p. 16 and 1933, p. 360). This hypothesis is based on the theory of van Iterson (1907) and has been supported by various experiments (Snow, M. & R. 1931, pp. 33, 35). Van Iterson's theory was in turn based on Hofmeister's observation (1868) that each leaf arises in the largest gap between the previous leaves. But van Iterson expressly stated that his theory could not be applied to plants with asymmetric leaf bases since he had worked it out only for plants in which the bases of the youngest leaf primordia were circular in outline.

Schumann (1892,) however, did attempt to apply Hofmeister's rule to plants with spirodistichous phyllotaxis. He maintained that the greatest available space in plants with sheathing leaf bases was to be found immediately above the point where the edges of the sheath of the youngest leaf met (p. 34). Where the leaves were symmetric this point lay opposite to the centre of the youngest leaf and distichous phyllotaxis resulted. But when the leaves were asymmetric this point was not opposite to the morphological centre of the youngest leaf, and spirodistichous phyllotaxis resulted (p. 42). The asymmetry of the leaves was thus taken by Schumann to be the determining factor in the phyllotaxis and was considered to be a basic inherited character. This theory meets with the difficulty that an asymmetry of either the one sense or the other is supposed to be inherited, though it is not determined by heredity of which sense that asymmetry will be. A further and more serious difficulty is that in many spirodistichous plants, and probably in all, the centre of each new leaf does not lie immediately above the point where the edges of the previous leaf meet, but slightly to one side of this point, as can be seen from Hirmer's figures (1922) illustrating a wide range of types. Moreover Schumann himself according to Weisse (1932, pp. 340–341) later abandoned his attempt to explain spirodistichous phyllotaxis in accordance with Hofmeister's rule.

The theory of Hirmer must next be considered. According to Hirmer spirodistichy is derived from distichy in ontogenesis through the shifting of the plane of symmetry of the apex by unequal growth on each side of the plane (pp. 17–19 and figure 46). The plane of symmetry here referred to is the median plane of the youngest leaf. In consequence of this asymmetric growth a sector is inserted in the apex in such a way that the centre of the next leaf which is about to arise is displaced to one side of the median plane of the previous leaf and a new plane of symmetry is established. This process is repeated each time that a leaf is formed.

The terms 'anodic' and 'kathodic' are used by Hirmer, and by Troll (1937) following him, with reference to the direction in which this shift of the leaf centre is supposed to have taken place. The anodic side of the leaf on Hirmer's terminology is the side towards which the centre is supposed to have shifted; and this is in the opposite direction to that of the genetic spiral. But since this shift of the leaf is only assumed and has not been proved to occur, it seems to the writer better to use the terms anodic and kathodic with reference to the direction of the genetic spiral, as this is the accepted convention for other phyllotactic systems. The side of each leaf which Hirmer calls 'anodic' is thus in the present terminology 'kathodic', since it lies next to the centre of the next lower, that is older, leaf. The symmetry

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of the leaf bases is considered by Hirmer to support his theory; for he claims that the side of the leaf termed 'kathodic' by him ('anodic' by the writer) is the larger, since it occupies the region in which the apex has grown most strongly, and is the side which has been, so to speak, left behind by the displacement of the leaf centre. In support of this contention he illustrates bud sections of many spirodistichous plants. In some of these plants, e.g. *Cordiline rubra*, *Richardia podophyllum* and *Tillandsia stricta*, this side does indeed appear to be the thicker radially in cross-section; but the figures do not provide evidence that it is the longer in tangential extension, as would be expected if the increase of growth was in the tangential direction as he postulates; for few of the sections illustrated pass through the critical level at which the meeting-point of the edges of the leaf can be seen, and these few are reproduced on too small a scale to allow of accurate measurements. Moreover in *Pollia condensata*, which is spirodistichous, Hirmer admits that the sides of the leaf are equal in size, and it will be shown in the present paper that in *Rhoeo discolor* the difference in the tangential extensions of the flanks is actually the opposite.

Hirmer has produced no evidence for his theory that a sector is inserted in the apex apart from this claim concerning leaf asymmetry which is unconvincing for the reasons given. Yet this theory has been included by Troll in his *Vergleichende Morphologie* (1937) as if it were well established, and a similar process is considered by Hirmer and by Troll to account for the spiral phyllotaxis of dicotyledons also.

Some observations on marked apices made to test Hirmer's theory will be briefly reported before the main experiments. Since the results of these marking experiments were negative, and since the study of spirodistichy by observation alone had failed to provide a satisfactory basis for it, it seemed to the writer that operations on the apices were needed for the purpose, and the results of such experiments will be reported and discussed in the present paper.

Rhoeo discolor was the species chosen for investigation because it was found to be the least inconvenient species to dissect and to withstand the operations well. A further advantage is that the whole species shows very little genetic variation.

The normal apex and phyllotaxis of *Rhoeo discolor*

The majority of the plants used in the experiments were young cuttings of *R. discolor* struck from basal buds in early spring; seedlings were also used after 12 to 14 leaves had been formed. Since the internodes elongate very little the apices of the operated plants were fairly close to ground level. The stem is completely encircled by the sheathing bases of the leaves which form closed tubes for a short distance above their insertions.

In order to determine the divergence angle the apices with seven or eight of the youngest leaves were embedded in collodion and sectioned by hand or with a microtome, and the sections were drawn under a projection apparatus. The angles were measured on the drawings. The centres of the leaves were located by the positions of their median conducting strands, which in *Rhoeo* are differentiated at an early stage. The median conducting strand was usually visible even in the youngest leaf. The centre of the axis was more difficult to determine and was judged by eye as accurately as possible. In order to allow for errors in determining the centre of the axis each divergence angle was measured in at least three

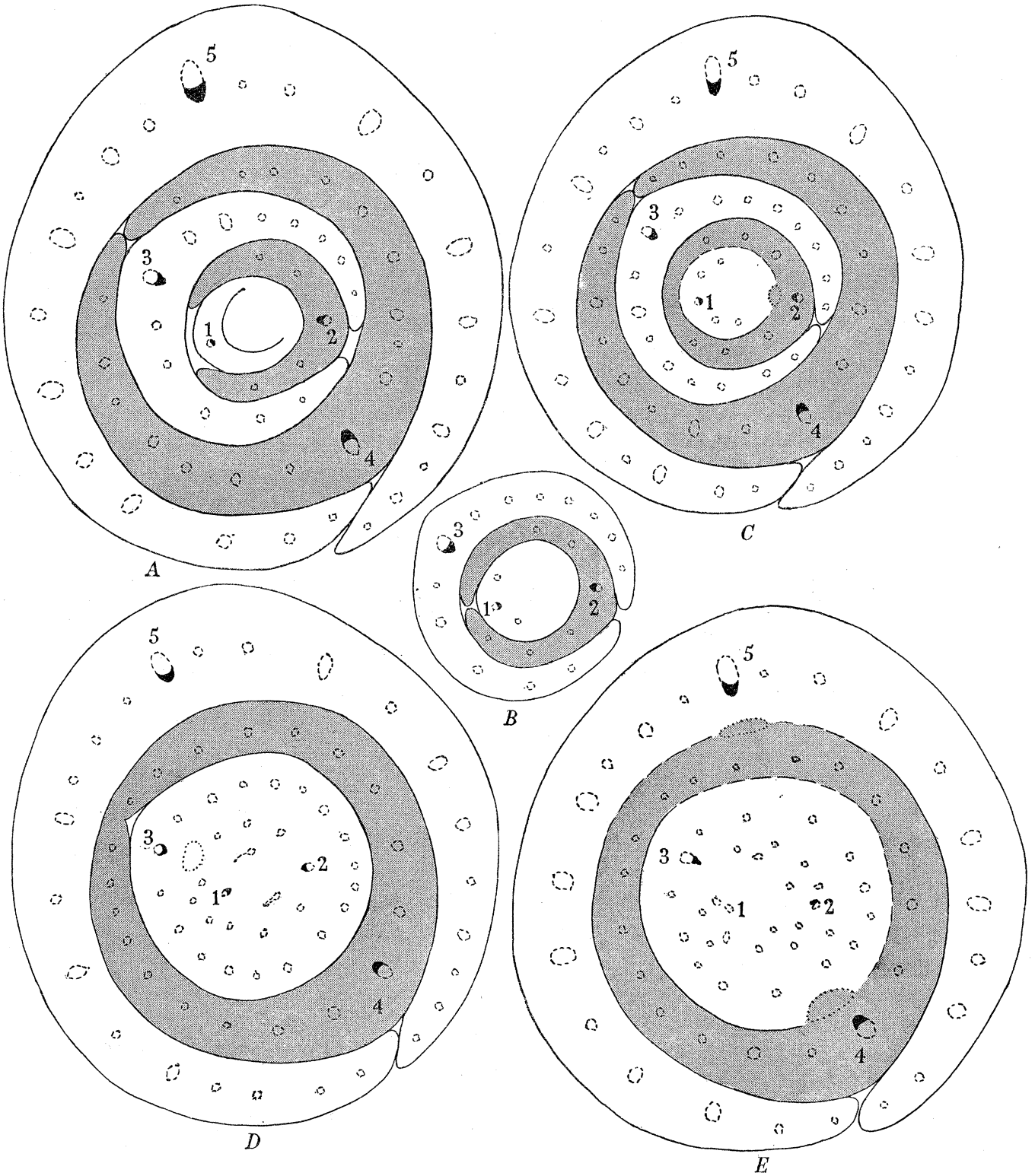

FIGURE 1. *A* to *E* are transections through the apical region of a normal plant of *Rhoeo discolor* from above downwards. In this and all subsequent figures vascular and procambial strands are shown with broken lines and the xylem regions of the central strands are shown black. The leaves and their axillary buds are shown alternately clear and stippled to guide the eye. The boundary between leaf and axis at the insertion level of a leaf is shown with long broken lines, and the region immediately beneath an axillary bud is enclosed within a dotted line. (Magn. *A*, ×58; *B*, *C*, *D* and *E*, ×52.)

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sections taken from different levels and the average was found. This method could not always be applied to the youngest leaf, the central conducting strand of which was sometimes seen in only two sections so that two measurements had to suffice. The same procedure was usually adopted in the experiments reported below. Fourteen different plants (cuttings) were used for measurements of the divergence angle, and the total number of divergence angles measured was 76. The mean value of these angles was 152°·6; the extremes were 142°·2 and 161°·7. The standard deviation was 3°·7 and the standard error was 0°·42. This indicated that the variation of the divergence angles in this species is by no means so great as it is often stated to be in spirodistichous plants. For comparison the standard deviation of the divergence angle of *Lupinus albus* with Fibonacci phyllotaxis was found to be 2°·8 (Snow, M. & R. 1931). Transections of the apical region of a normal plant of *Rhoeo* are shown in figure 1. Four lateral strands were visible in the youngest primordium before the end of its first plastochron. Other strands were added later and these appeared to differentiate acropetally, new strands being inserted between older ones, so that large and small strands usually alternated. The median vascular strand was found to run nearly straight downwards for at least four internodes before uniting with the lateral strand of another leaf. The direction of differentiation of the central strand when it first appeared was not determined, but its subsequent differentiation within the leaf was seen to be acropetal, as it kept pace with the upward growth of the leaf.

Each leaf subtends an axillary bud, and the area from which the bud will develop is clearly visible in the axil of the second youngest leaf as a small group of cells with dense contents. The bud is seen as a definite protuberance in the axil of the fifth or sixth leaf, and it then becomes quite clear that it does not lie in the median plane of the leaf, but that its centre lies on a radius a few degrees anodic to that of the median strand. Its relationship to the median strand enabled the latter to be identified in a few leaves of the operated plants in which the median strand was scarcely larger than the lateral strands. The cells lying immediately beneath the insertion of an axillary bud were distinguished from the surrounding tissues by their dense contents. In the drawings these groups of cells with dense contents are enclosed within dotted lines.

As transections do not give a satisfactory representation of the shape and course of development of the youngest leaf, an attempt was made to draw the apex and the youngest leaf as seen in surface view from above through the dissecting microscope at different stages of the plastochron. The drawings were made free-hand and are reproduced in figure 2. The direction of the genetic spiral, from each leaf to the next younger, was clockwise in the four apices shown in figure 2, and the terms anodic and kathodic will be used with reference to this spiral. The same terminology will be used as in previous papers (Snow, M. & R. 1931, 1933, 1935): the leaf primordia visible at the time of operation, will be called P_1, P_2, P_3, etc., P_1 being the youngest; those arising after the operation will be called I_1, I_2, I_3, etc., I_1 being the first to arise. In figure 2, though no operation has been made, the youngest primordium will be referred to as P_1. Each drawing in figure 2 is accompanied by an outline drawing of a longitudinal section of the apex at the same stage of the plastochron. In figure 2 A_2, B_2 and C_2 these sections pass through the median plane of P_1, in D_2 the section passes through the region which is about to give rise to I_1. The longitudinal sections were cut under the dissecting microscope from apices prepared in the

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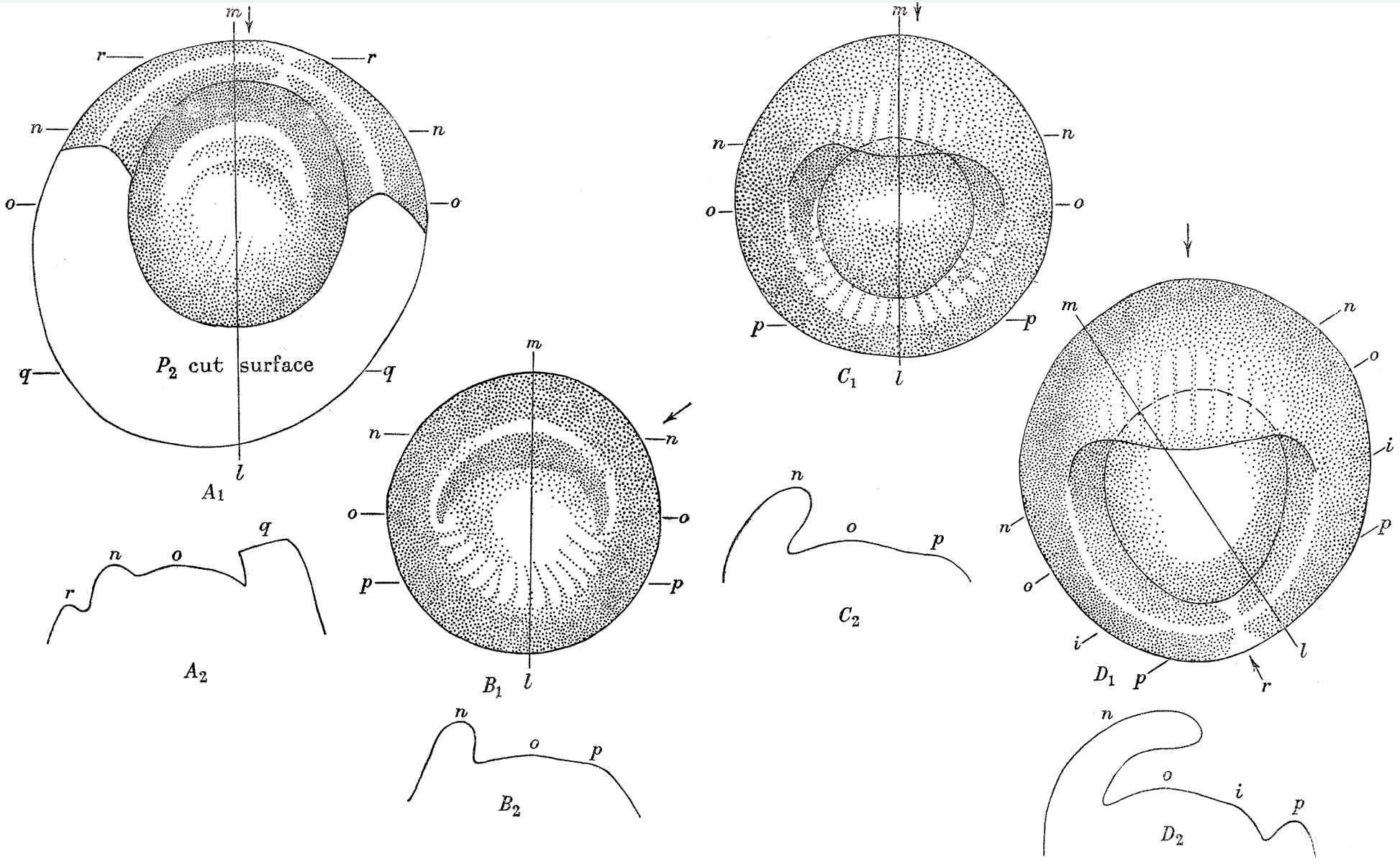

FIGURE 2. A_1, B_1, C_1 and D_1 are free-hand drawings of the apex viewed from above at different stages of the plastochron. The source of light was obliquely above the apex, and the arrows indicate the horizontal component of its direction. Horizontal and slightly oblique surfaces are illuminated; surfaces approaching the vertical are shaded. A_2, B_2, C_2 and D_2 are longitudinal sections through the plane m to l shown on the corresponding drawings, × 130. The base of P_2 which has been cut down is included in figures A_1 and A_2 only. The points in which the lines n–n, o–o, etc., cut the line m–l in the drawings A to D are indicated with corresponding letters in the sections A_2 to D_2. n = summit of P_1; o = summit of apex; p = flanks or presumptive area of flanks of P_1; i = foliar buttress of I_1; q = cut surface of P_2. In A_1 and A_2, P_1 is small. In B_1 and B_2, P_1 is small-medium, the shaded lines represent cell rows in the region from which the flanks of P_1 will arise. In C_1 and C_2, P_1 is medium; the hood is beginning to arch over the apex. In D_1 and D_2, P_1 is very large. The arrow marked r indicates the meeting-point of the edges of the flanks. The broken line shows the contour of the apex beneath the hood.

usual way as if for operations (p. 139) to ensure that the stage of the plastochron was the same as that shown in surface view and that the cut passed through the correct plane. They were drawn under the projection apparatus.

When P_1 is first visible as a protuberance it subtends an arc of about 90°, and by the end of the first plastochron it has encircled the whole apex, and its central region has by then made a hood which arches over the apex and partly conceals it, as is shown in figure $2D_1$. Those parts of the leaf which extend round the apex as distinct from the original central part will be called 'flanks'. For convenience the anodic and kathodic parts of the leaf which lie between the centre and the meeting-points of the flanks will be called the anodic and kathodic 'halves' though they are slightly unequal in size; they may be considered as morphological halves. A half of a leaf is thus more than a flank, as it comprises half of the central region as well as one flank.

Figure $2A$ shows P_1 at a fairly early stage of its development. The flanks of the leaf have extended until it covers an arc of nearly 180°, and the apex appears as an approximately circular dome. In figure $2B_1$ the arc is slightly greater than 180°, and the region into which the flanks will extend is differentiated from the rest of the apex as an annular region extending for nearly 180° round the apex. In this region curved cell rows are clearly visible. P_1 is slightly asymmetric, its anodic half being rather the taller and rising up more steeply than the kathodic half. In figure $2C_1$, P_1 has developed considerably further, and its central part has formed a hood which arches over the apex and partly conceals it. Incidentally, the growth rate of the hood relative to that of the flanks showed some variation, for in some halves the flanks had already met round the apex when the hood had scarcely begun to arch over. In figure $2D_1$ a later stage is shown in which the flanks have met round the apex. The point in which they meet is clearly not opposite the centre of P_1, so that P_1 is asymmetric, its kathodic flank being the longer.

At a later stage the edges of the flanks unite and form a short tube as shown in figure $1D$, leaf 4; above this tubular region the edges of the flanks continue to extend tangentially and overlap as shown in figure $1D$, leaf 5, the anodic flank being the outer. It should therefore be noted that the overlapping of the flanks takes place only in the part above the basal tube.

A further point of interest seen in the dissections was the change that took place in the shape of the apex during the plastochron. Thus in figure $2A_1$, B_1 and C_1, the apex appears as an approximately circular dome, but in figure $2D_1$ it is clearly elongated along a diameter passing through the centre of P_1. It is also ovate in outline, being broader towards the centre of P_1 than in the opposite direction. It is also slightly asymmetric, as the foliar buttress from which the next leaf will arise has begun to appear. For the apex rises steeply from the base to the top of this buttress on the anodic side from P_1 and then becomes less steep, whereas on the kathodic side from P_1 it follows a more gradual curve as shown in figure $2D_1$.

It cannot be said with certainty at which stage one plastochron ends and the next begins. The elevation of the foliar buttress is no doubt part of a leaf-forming process, but whether the radial extension of the apex which precedes it is also associated with the formation of the next leaf is uncertain. The convention will here be adopted of considering the start of a plastochron to be the first visible uprising of a new leaf, however small, but not of a foliar buttress.

It has already been noted that P_1 is asymmetric, its kathodic flank being the longer. The angle subtended by the smaller half of a leaf at the centre of the apex is a measure of the degree of asymmetry of the leaf. In the normal plant the smaller half was always the anodic half, but this was not always so in the operated plants. The arcs covered by the anodic half-leaves of normal plants were measured on the drawings; in the older leaves which had already formed tubes at their bases the meeting-point of these halves could only be recognized directly above the tubes.

A total of 46 angles were measured on 14 plants and the mean value was 166°·5. The extremes were 171° and 162°, the standard deviation was 2°·3, and the standard error was 0°·30. This asymmetry is also visible in the blade of the mature leaf of which the anodic half is usually slightly narrower than the kathodic. The asymmetry of the leaves can often be seen not only in the length, or more strictly transverse extension, of the halves but also in their radial thickness, since the anodic half-leaf is often slightly thicker than the kathodic as can be seen in leaf 5, figure 1.

To sum up, the asymmetry of the leaves is shown in the following features: the anodic half-leaf is regularly the shorter in extension round the apex, and is also the outer in overlap in the upper parts of the leaf; in some leaves the anodic flank can also be seen to be the thicker radially, and at an early stage of development of P_1 it appears to be the more protuberant.

Since the mean of an anodic half-leaf is 166°·5, and the mean divergence angle is only 152°·6, it follows that the centre of a new leaf is not directly over the meeting point of the flanks of the previous leaf, although that is the lowest point in the boundary line round the apex, but closer by 13°·9 to the centre of that leaf.

The experiments

(1) *Methods and terminology*

The operations were made under a Leitz Greenough microscope, usually at a magnification of ×75. In order to expose the apex it was necessary to split the sheaths of the older leaves and bend them back, but the younger leaves were too brittle to be bent back and had to be cut off a short way above their insertions. By this means it was often possible to leave six or seven of the older leaves attached to the plant for photosynthesis. As the plants bled strongly it was found best to leave them unwatered for at least 2 days before the operation. When the operation had been made the apex was protected with cotton-wool until the bases of the cut-down leaves had grown up and covered it. As the exposed apices were liable to be damaged by insects and mites, derris powder was applied to the bases of the cut-down leaves close round the apex. This precaution did no harm and was usually, though not always, effective.

The stage of the plastochron was recorded for each experiment at the time of operation. The plastochron was divided into seven stages in terms of the size of P_1; the stages are given in table 1, p. 140.

After the operations the plants were left to grow for periods varying from 4 to 8 weeks, according to the temperatures. The axillary buds grew out strongly and were continually removed, the removal of the young leaves being sufficient, as in other species, to cause

them to grow out even when the stem apex was undamaged. When the apices had grown enough they were pickled in spirit, embedded in collodion and sectioned by hand. A few leaves present before the operation were included with the pickled apex.

TABLE 1

size of P_1	arc covered by P_1, and its stage of development
very small	90°; just visible
small	90° to 180° (figure $2A_1$)
small-medium	over 180° (figure $2B_1$)
medium	nearly 360° (figure $2C_1$), hood arching over
medium-large	360°
large	hood further grown
very large	apex now extended in plane of P_1 and sometimes foliar buttress of I_1 visible

(2) *Preliminary observations on marked apices*

In order to test Hirmer's theory that a sector is inserted in the apex in such a way as to displace the centre of I_1, some preliminary marking experiments were made before the main experiments were undertaken. Since the theory postulates that a sector of increased growth is inserted in the apex and displaces sideways a newly determined leaf centre, it follows that the tissues must be displaced at both sides of the inserted sector and also in the neighbouring regions.* An attempt was therefore made to discover whether actually the tissues are so displaced. Egg albumen mixed with charcoal was used for marking the apex. It was applied with a single hair of a paint brush so as to form a fine streak across the apex and the cut-down bases of the youngest leaves, or else deposited in a straight row of dots. If the streak became curved in the course of subsequent growth, or if any of the dots shifted so that they no longer formed a straight line, this was taken to indicate a displacement of the underlying tissues. These observations were made under the dissecting microscope and recorded by free-hand drawings. On Hirmer's theory the point at which the centre of I_1 is determined is displaced from a position opposite the centre of P_1 to the position which it occupies when I_1 becomes visible. Since in *Rhoeo* the divergence angle is 152°·6 a displacement of 27°·4 should occur in the antidromic direction, that is, the opposite to that of the genetic spiral. A streak or a row of dots extending across the apex along the diameter which passes through the centre of P_1 should therefore develop an antidromic curve in the region of the apex which is at 180° from the centre of P_1.

In six of these experiments the streak or row of dots of egg albumen and charcoal lay either approximately along the diameter passing through the centre of P_1, or else along the closely neighbouring diameter passing through the meeting-point of the flanks of P_1. The latter diameter was sometimes preferred because the meeting-point of the flanks could be determined more accurately than the centre of P_1, and a displacement in the same direction along this diameter was to be expected. Actually in three of the above experiments a slight antidromic displacement occurred in the part of the apex adjacent to the meeting-point of the flanks of P_1. This displacement was seen at the time when I_1 first became visible and was estimated at about 15°, but during the subsequent growth of

* Hirmer (1922, pp. 17, 18) and Troll (1937, vol. 1, pp. 421, 422) imply that their sectors of increased growth inserted in the apex expand more in one tangential direction than in the other. But this is mechanically impossible, unless the neighbouring tissues of the apex offer more resistance on one side of each sector than on the other, which is not suggested by these authors, nor to be expected.

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I_1 a displacement in the opposite direction was noted, the line of marks tending to become straight again. In two other experiments no displacement was seen near the meeting-point of the flanks of P_1. In the last experiment a slight *homodromic* displacement occurred in the part of the apex near the meeting-point of the flanks of P_1.

In another five experiments the streak was placed in the plane at right angles to the median plane of P_1, or near it. The ends of these streaks were farther from the position of Hirmer's supposed inserted sector, so that they would not be expected to be displaced so much by that sector, but some slight displacement might be expected. Actually, of the ten portions of these five streaks which rested on the leaf-forming zone of the apex, nine remained straight until I_1 had arisen, and the tenth, which was between the centres of P_1 and I_1, was found to be slightly displaced towards the centre of I when I_1 had just arisen, in the opposite direction to that anticipated by Hirmer's theory.

These results do not support Hirmer's theory. For even in the three apices out of eleven in which the tissues were found to be displaced in the direction to be expected on that theory the displacements were not large enough, and in two others the displacement was in the opposite direction. The marking experiments were therefore discontinued, and operations on the stem apex or youngest leaves were undertaken instead.

(3) *The nature of the operations and classification of results*

Two kinds of operations were made on *Rhoeo*. In the first the apex was split in a longitudinal median plane and the two halves were left to regenerate new apices. The cuts were made through the median plane of P_1 or at right angles to that plane. These operations were chosen as it was considered that splitting the apex would profoundly disturb the phyllotaxis system and that the positions of the first few leaves on the regenerated apices might provide evidence of the factors which determine the phyllotaxis of a normal plant. The results of these operations suggested that in this species, contrary to what was found previously with *Lupinus albus* (Snow, M. & R. 1931, 1933 and 1947), the centres of the youngest leaves inhibit the formation of a new leaf centre, and that the intensity of this inhibition decreases with the age of the inhibiting leaf. Accordingly, in order to test this hypothesis more directly, a second series of experiments was carried out in which the centre of P_1 was removed or destroyed at an early stage of the plastochron. For on the hypothesis of inhibition a displacement of one or more of the subsequent leaves would be expected to follow, the inhibition due to the centre of P_1 having been removed, or at least reduced. As the results of this second series of experiments were on the whole simpler and easier to interpret than those in which the apex was split, they alone will be described here and the others will be reserved for a later paper.

In these experiments it was thought desirable to remove only the central part of P_1 leaving some portion of its flanks on the apex, as it seemed possible that if the whole visible part of P_1 were removed at an early stage in the plastochron the leaf might fail to develop at all; the amount of space available for I_1 would thereby be increased and any displacement of its centre might be attributed to this factor. For this reason when the operation was made at a very early stage in the plastochron the central part of P_1 was first isolated by two cuts forming a V pointing towards the centre of the apex and the part of P_1 lying within the V was then removed. The parts of the flanks lying outside the

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TABLE 2. THE REMOVALS OF THE CENTRE OF P_1.
DIVERGENCE ANGLES OF SUBSEQUENT LEAVES IN DEGREES

nos. of experiments	size of P_1 at time of operation	angle P_2–I_1 (normal 305°·3)	deviation from normal (k=kathodic)	angle I_1–I_2	angle I_2–I_3	angle I_3–I_4	angle I_4–I_5
		Group I, in which the centre of P_2 was left intact					
1	very small	285·5	19·8k	180·0	151·5	149·5	—
2	small	305·0	0·3k	171·0	144·7	160·0	150·5
3	small-medium	296·0	9·7k	185·3	140·0	—	—
4	medium	294·5	10·8k	174·0	135·3	156·7	—
5	medium	293·5	11·8k	179·3	122·0	—	—
6	small-medium	289·0	16·3k	167·0	142·0	—	—
7	small-medium	291·7	13·6k	196·0	113·0	172·0	148·0
8	medium	279·5	25·8k	194·5	126·0	157·7	149·0
		Group II, in which the centre of P_2 was also removed					
		angle P_3–I_1 (normal 457°·9)					
11	very small	457·0	0·9k	173·5	135·5	159·0	—
12	large	—	—	187·4	139·0*	166·0*	154·0*
		Group III, in which the centres of P_1 and P_2 were both removed but P_1 regenerated a new centre					
				angle P_1–I_1	angle I_1–I_2	angle I_2–I_3	angle I_3–I_4	angle I_4–I_5
9	very small	455·7	2·2k	185·3	170·0	131·0	157·3	143·0
10	very small	446·0	11·9k	181·7	167·0	135·0	157·0	—

Note. All divergence angles are measured in the original direction of the genetic spiral except the last three angles marked with asterisks in no. 12.

TABLE 3. THE ASYMMETRY OF THE SUBSEQUENT LEAVES.
ARCS COVERED BY THEIR ANODIC HALVES IN DEGREES

nos. of experiments	I_1	I_2	I_3	I_4	I_5
		Group I			
1	183	177	180	—	—
2	176	172	180	167	—
3	170	174	167	—	—
4	178	171	175	—	—
5	180	170	175	—	—
6	171	172	168·5	—	—
7	202	170	180	—	—
8	200	158	168	161	171
		Group II			
11	—	170	178	174	—
12	—	164	190	175	170
		Group III			
9	170	173	173	169	—
10	175	176	174	170	—

Note. In no. 12 the measured halves of the leaves are those which are anodic in the new direction of the reversed genetic spiral.

V were thus left on the apex. When the operation was made at a later stage and P_1 subtended about 180° or more, its centre could be removed by a single tangential cut which still left parts of its flanks on the apex. Finally in one experiment in which P_1 was very small the centre was destroyed by a puncture.

In order to expose the apex it was unfortunately necessary to remove the hood of P_2 which arched over it. The amount of P_2 which was removed varied considerably in different experiments. Accordingly since there is reason to suppose that the central part of the base of P_2 affects the position in which I_1 will arise, four of the twelve experiments reported were excluded from the main group, since in them the central part of the base of P_2 was either severely damaged so that it possessed no median vascular strand or had been completely removed. These four experiments are, however, included in tables 2 and 3, and will be described separately since they presented several points of interest. In the eight experiments included in the main group, group I, P_2 had formed a basal sheath with a central vascular strand and in some had also formed a small blade, or two small blades, one from each flank. In three of these eight the flanks of P_2 had been broken by the cut that had removed the centre of P_1, but the central part of P_2 was intact. These eight experiments constituting group I will be described first.

(4) *The development of P_1 when its centre was removed alone*

When the centre of P_1 was removed early in the plastochron while it subtended an arc of less than 360°, its flanks continued to extend round the apex until they met. The other edges of the flanks of P_1 which abutted on the wound were found to have reunited at the base when the apices were sectioned. The zone in which the two halves were united was low and did not usually reach above the level of the growing-point. The manner in which the reunion took place is uncertain, as the material was not sectioned until after three or more weeks; but examination of the apex with the dissecting microscope a few days after the operation often revealed a strip of tissue beginning to grow up from the tissue of the apex immediately above the wound, that is, on the apical side of the wound. It is thought that this strip of tissue, which will be called the 'reuniting zone', formed a bridge between the halves of P_1. It was usually narrow, showed the wound scar on its outer face and lacked a median vascular strand, as seen in figures 4 and 5, nos. 6, 7 and 8. There was, however, one experiment, no. 4, figure 3, in which the wound scar was seen on the inner face of the reuniting zone also. Here it seems that this zone must have been formed from tissues internal to the wound and not from the undamaged tissue above it.

Concerning the nature of this reuniting zone the most probable interpretation is considered to be that it was formed by an extension of the flanks towards the original centre of P_1 through tissue lying to the apical side of the wound or internally to it. This possibility is supported by the fact that at a higher level each of the flanks usually formed a small and roughly symmetric blade; for this suggests that each flank had been partly reorganized to form a symmetric whole, though in the experiments of group I only one of these structures showed a thickened central region and enlarged median strand (experiment no. 8, figure 5). The reorganization of the flanks was thus usually incomplete, but may have been sufficient to enable each flank to extend round the apex towards the original position of the centre of P_1. For such an extension, though in the abnormal direction relative to the centre of P_1, would have been away from the centre of the reorganized flanks, and so in the normal direction relative to those new centres. Again in no. 3, figure 4, the uniting zone was unusually thick radially and unusually high; it also had a median vascular strand and a large axillary bud. In this experiment, therefore, it seems probable that the centre of

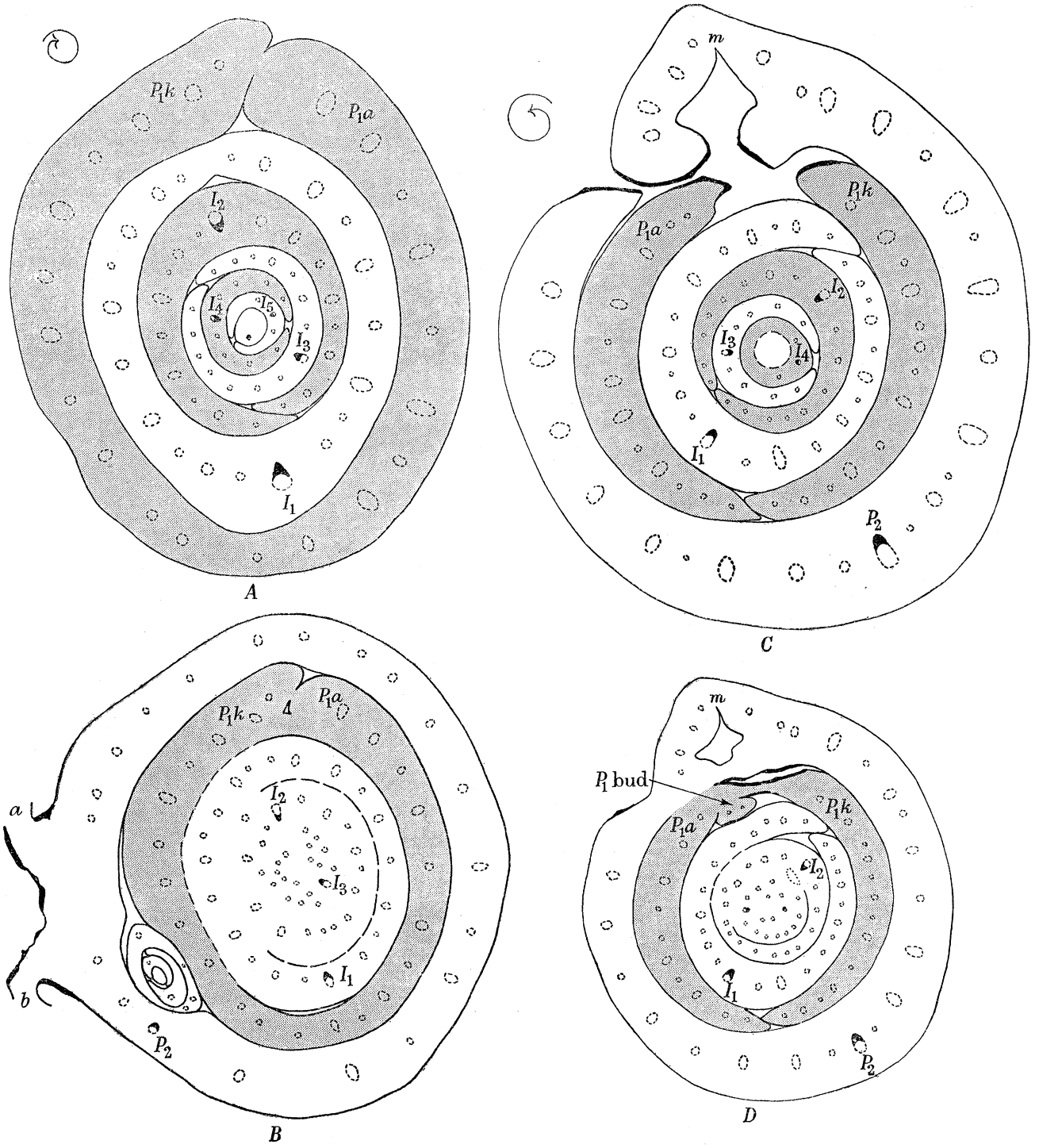

FIGURE 3. *A* and *B* are transections of no. 2 from above downwards. The centre of P_1 was destroyed by a prick. In *B*, P_2 was united with P_3 at *a* and *b*. *C* and *D* are transections of no. 4 from above downwards. The centre of P_1 was removed. The flanks of P_2 have developed abnormally following injury. In *D*, the central part of P_1 is joined to P_2. The meeting-point of the edges of the flanks of P_2 is marked *m*. (Magn. *A* and *C*, $\times 27$; *B* and *D*, $\times 18$.) In this and subsequent drawings of experiments the wound scars are shown with a thick black line. The spiral at the top left-hand side of each upper section shows the original direction of the genetic spiral. P_1a and P_1k are the anodic and kathodic flanks of P_1 respectively. P_1 and alternate leaves are stippled as also in subsequent figures.

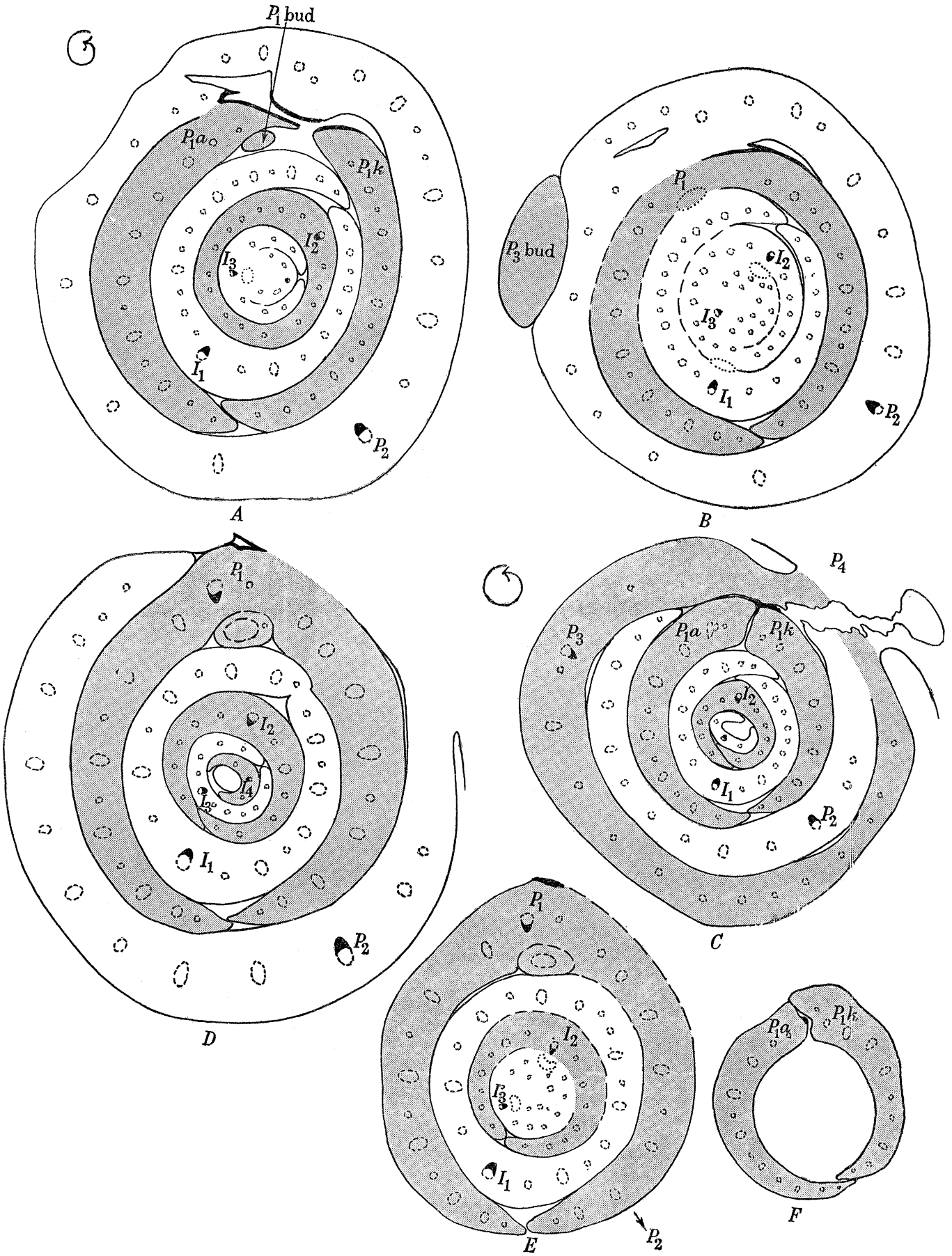

FIGURE 4. *A* and *B* are transections of no. 6 at different levels. The centre of P_1 was removed. The central part of P_1 is joined to P_2. *C*, *D* and *E* are transections of no. 3 at different levels. The centre of P_1 was removed. The adjacent faces of P_1, P_2, P_3 and P_4 were united on each side of a wound passing through them shown in *C*. *F* is a transection of P_1 in no. 3 at a higher level showing the leaf in two halves with no trace of a central vascular strand. (Magn. *A* and *D*, $\times 33$; *B*, $\times 28$; *C*, $\times 20$; *E*, $\times 31$; *F*, $\times 17$.)

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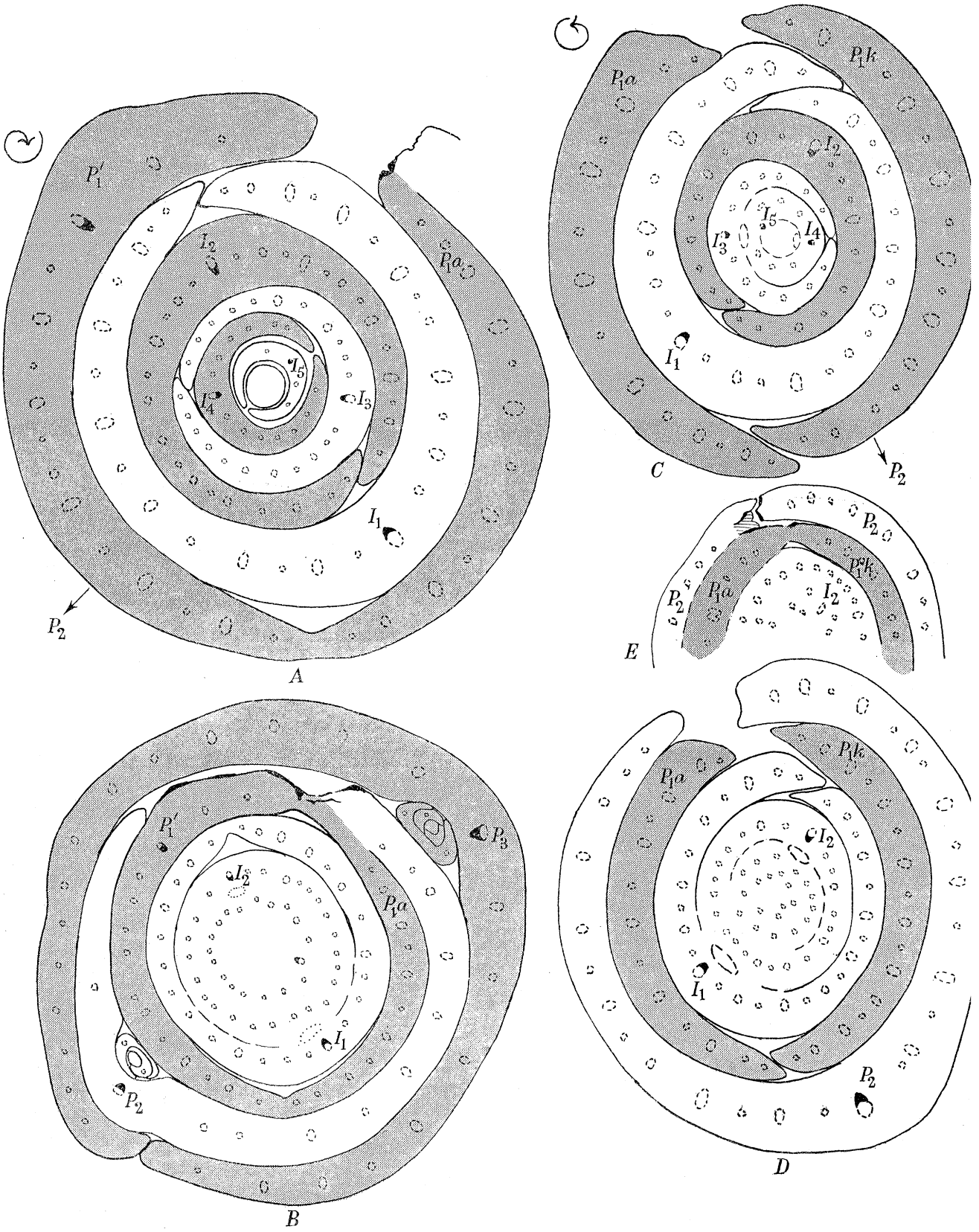

FIGURE 5. *A* and *B* are transections of no. 8 from above downwards. The centre of P_1 was removed. A new centre has probably been regenerated in the kathodic flank at P_1'. The anodic flank has united with P_2 near the wound. *C*, *D* and *E* are transections of no. 7 from above downwards. The centre of P_1 was removed. *E* is part of a transection showing the reuniting zone of P_1 just above its insertion. (Magn. *A*, $\times 28$; *B* and *E*, $\times 15$; *C*, $\times 31$; *D*, $\times 22$.)

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P_1 was not completely removed, the cut being either too shallow, or not reaching close enough to the apex so that the base of P_1 was able to grow up for a short distance. This peculiarity of no. 3 is significant for the interpretation of the subsequent phyllotaxis, as will be pointed out below.

It needs to be mentioned that in several experiments, nos. 3, 4, 6 and 8, the outer face of P_1 was joined to P_2 in the region of the wound, and in no. 3, P_2 was in turn united with the older leaves. These unions, which often occurred where deep wounds had been made, do not seem to have affected the subsequent phyllotaxis. In four of these eight experiments an axillary bud had developed above the reuniting zone.

(5) *The position of* I_1

In the eight experiments of group I in which P_1's centre was removed and the basal part of P_2 was intact, the position of I_1 was located by its angle from the centre of P_2, since its angle from the missing centre of P_1 could not be measured. The mean normal angle from P_2 to I_1 in the direction of the genetic spiral may be taken as twice the mean of the single divergence angle already measured—that is 305°·5, and its standard deviation will be $0°{\cdot}423 \times \sqrt{2} = 0°{\cdot}6$.

The divergence angles P_2I_1 of the experiments are given in table 2. They varied from 305° to 285°·5, with mean value 291°·8. The standard deviation was 7°·9, and the standard error was 2°·80. The experiments thus led to a decrease in the angle from the original position of the centre of P_1 to the centre of I_1. The difference between the mean angle of the normals and the experiments was 13°·5, and the standard deviation of the difference of the means was 2°·86. The difference may therefore be considered significant. Examples of these experiments are shown in figures 3, 4 and 5, nos. 2, 3, 4, 6, 7 and 8.

This displacement of I_1 towards the missing centre of P_1 is to be expected if the centres of P_2 and P_1 act as centres of inhibition in determining the position of I_1. For the removal of the centre of P_1 would reduce the inhibition acting on I_1 from that direction, and consequently I_1 would be displaced towards the missing centre of P_1 by the inhibition from P_2 (see the diagram, figure 6). The variation of this displacement in different experiments may have been due to variations in the amount of the centre of P_1 that was removed and in the level to which the centre of P_2 was cut down. There is little doubt that the experiments would have given more consistent results and showed a greater displacement of I_1 had it been possible to leave P_2 intact. But this was impossible as already explained, since the hood of P_2 concealed P_1 and was too brittle to be bent back. It is probable that the lack of displacement of I_1 in experiment no. 2 was due to the injuries inflicted on P_2 at the time of operation (see figure 3*B*). For the central part of P_2 in experiment 2 was less thick than normal, but as its central vascular strand was present it was considered best to include the experiment in this group rather than with those experiments in which P_2's centre was removed completely, or so much of it that no central strand was present.

It was thought probable that the displacement of I_1 would be greatest early in the plastochron, and that towards the end of the plastochron little or no displacement would occur, since the centre of I_1 would by then probably be determined, in its normal position. For this reason all the operations but one were made fairly early in the plastochron, not later than the stage with P_1 medium. Within this range from P_1 very small to P_1 medium

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the displacement of I_1 (column 4, table 2) shows no relation with the state of the plastochron (column 2, table 2). If any such correlation exists it has been obscured by other variable factors in these experiments. It may be concluded that up to the stage with 'P_1 medium', I_1 is undetermined, since it can be displaced by an operation which does not touch its presumptive area. The fact that the displacement does not exceed 25° in these experiments may be due in part to the cutting down of P_2 as suggested above; it is also possible that after the centre of P_1 was removed, some of its influence still persisted in the apex as a residual inhibition at the time when I_1 was determined.

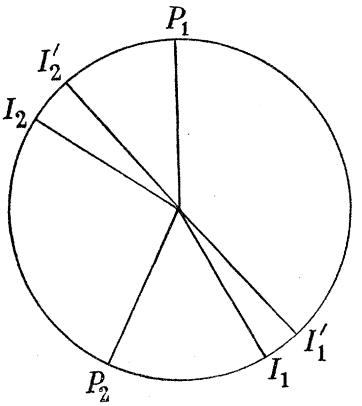

FIGURE 6. A diagram showing the changes in mean angular positions of I_1 and I_2 after the operations of group I. The lines marked P_2, P_1, I_1 and I_2 show the normal angular positions of these leaves and those marked I'_1, I'_2 the mean angular positions after the operations.

(6) *The position of* I_2

The position of I_2 must now be considered. The angle I_1–I_2 is recorded in table 2. It varied from 167° to 196° measured in the original direction of the genetic spiral, the mean value being 181°·0. Since the normal divergence angle is 152°·6 with standard deviation 3°·7, it is clear that this angle is considerably greater than the normal. The mean angles and the positions of I_1 and I_2 in the experiments and in a normal plant are shown diagrammatically in figure 6. This increase of angle would be expected on the inhibition theory; for the position of I_2 would be determined mainly by the inhibition from I_1, since the centre of P_1 had been removed and consequently P_1 did not inhibit I_2 at all strongly. Any residual inhibition coming from P_2 would also tend to increase the angle I_1–I_2 (see figure 2). Thus the displacement of I_2 is to be explained as due mainly to the same cause as the displacement of I_1, that is, to the removal of the centre of P_1 and the consequent diminution of the inhibition from P_1. The variation of this displacement may also be related to variations in the inhibition from the remaining parts of P_1, and in the residual inhibition from P_2.

In no. 3 (figure 4 *C*, *D*, *E* and *F*) an unusually slight displacement of I_1 (9°·7) is associated with a large angle I_1–I_2 (185°·3). From this it would seem that the strength of the inhibition due to P_1 may have changed during the interval between the determination of I_1 and I_2, since otherwise a small displacement of I_1 would be expected to be associated with a relatively small angle I_1–I_2. This experiment has already been referred to (pp. 143, 147) as one in which the uniting zone of P_1 was thick and showed a median vascular strand though the upper part of the leaf had made two separate blades, and this was thought to be due

to an incomplete removal of the centre of P_1 which continued to grow for a short while after the operation. If this explanation of the uniting zone is correct, it will also account for the unusual angles. For if the centre of P_1 was still growing and exerting some of its inhibiting effect when I_1 was determined, only a slight displacement of I_1 towards P_1 would be expected; and if the centre of P_1 had ceased to grow when I_2 was determined, then it would not inhibit I_2, and so would not oppose the increase in the angle I_1–I_2.

Actually the angle I_1–I_2 exceeded 180° in no. 3, and in the two remaining experiments, nos. 7 and 8, it was over 190°; it follows that in these three experiments I_1 was not the sole determining factor affecting the position of I_2. It is indeed possible that in no. 7 the inhibition due to P_2 was greater than in the others, since it was noted at the time of pickling that in no. 7 the central part of P_2 had grown strongly and had formed a small blade in spite of having been cut down at the time of operation. In no. 8 there is evidence that a new centre of P_1 had been regenerated in the kathodic half. This new centre may have been partly responsible for the large angle I_1–I_2, for it can be seen from figure 5 that had I_2 arisen opposite I_1 it would have been extremely close above the new centre of P_1. In no. 3 also it seems that P_2 must have exerted some influence, though less than in nos. 7 and 8.

(7) *The position of* I_3

Finally, the position of I_3 must be considered. In all the experiments of the present group the centre of I_3 arose on the same side of the apex as in the normal plant, that is, on the side away from the centre of P_2 (compare figure 6), the angle I_2–I_3 being less than the normal (table 2). The genetic spiral thus continued in its original direction even in the three experiments in which the angle I_1–I_2 measured in that direction exceeded 180°, so that in these three I_3 arose in the smaller angle between I_1 and I_2 (nos. 3, 7 and 8, figures 4 and 5). This result could not be explained on the inhibition hypothesis if I_1 and I_2 were the only leaves effective in determining the position of I_3. For were this so, I_3 should have occupied the larger angle between I_1 and I_2 in which the inhibition would have been less. If, however, it is assumed that the residual inhibition due to P_2 was still effective when I_3 was determined, and that this inhibition was stronger than any that may have come from the remaining parts of P_1, then the position of I_3 in the smaller angle I_1–I_2 can be understood, since in these three experiments the smaller angle I_1–I_2 was on the side of the apex away from the centre of P_2.

This assumption is supported by an experiment included in group II in which the centres of P_1 and P_2 were both removed (no. 12, figure 8). In this experiment the angle I_1–I_2 was 187°·4 (table 2, column 5) and I_3 arose in the larger angle. The genetic spiral was reversed and continued so. This result seems especially important as it shows that the direction of the genetic spiral is not fixed in this plant but that it can be reversed by an operation comparatively remote from the apex, the removal of the centres of two visible leaves. It also supports the suggestion that in this species a new leaf can be inhibited by a leaf four plastochrons older—P_2 in this experiment. But this single result needs confirmation, and further experiments of this kind are therefore being done.

The diminution of the angle I_2–I_3 can be understood as resulting from the increase of the angle I_1–I_2. For normally the centre of each new leaf partitions the larger angle between the two previous leaves, which is $360-152°=208°$ (see figure 6). But in the experiments

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since the angle I_1–I_2 increased, the angle obtained by subtracting it from 360° decreased, and the centre of I_3 had to partition a smaller angle and so arose closer to I_2 than is normal. The next few angles also, so far as they could be measured, oscillated above and below the mean normal angle, while tending to return towards it, except in no. 1 (see table 2).

(8) *Four further experiments in which the centres of P_1 and P_2 were both removed*

The results of the four experiments forming groups II and III in which the centres of both P_1 and P_2 were removed are also recorded in table 2. The following points of interest may be noted. The two experiments of group III, nos. 9 and 10, figures 7 and 8, differ from those of group I not only in that the centre of P_2 was removed as well as that of P_1 but also in that P_1 appeared to have regenerated a new centre. Thus in no. 10 the kathodic flank of P_1 was considerably thicker than a normal flank in this region, and the vascular strand in the centre of this thickened zone was taken to be physiologically a new median strand. But as there was no axillary bud above it, this is not certain. Macroscopically P_1 was seen to have made two blades, of which the kathodic was considerably the larger and approximately symmetrical in the upper part.

In no. 9, figure 7, an entirely new leaf had been formed with its centre above the cut which had removed the centre of P_1. Since this leaf had no axillary bud, its median strand could not be identified with certainty, but it was taken to be the strand in the thickest part of the leaf marked P_1 in figure 7. Viewed macroscopically this new leaf had made an approximately symmetrical blade with a small kink at the top. It is probable that the flanks of the original P_1 dedifferentiated after the centre had been removed, since no trace of them was found when the apex was sectioned. This was the only experiment in which a single new leaf was regenerated after the centre of P_1 had been removed. This regeneration may have been facilitated by two conditions: first, P_1 was very young at the time of the operation; secondly, the centre of P_2 had been removed and its inhibiting influence on the formation of new leaf centres had consequently diminished.

The position of I_1 in this group was located by its angle from P_3, since the original centres of P_1 and P_2 were lacking. The normal angle P_3–I_1 may be taken as three times the mean normal divergence angle or 457°·9. The angles are recorded in table 2. In this group in which P_1 had regenerated a new centre, I_1 showed a kathodic displacement of 2°·2 in no. 9 and 11°·9 in no. 10, and its centre was approximately opposite to the supposed median strand of the new P_1. It therefore seems probable that the new centre of P_1 exerted a repulsive effect on I_1. This effect, however, cannot have lasted as long as in a normal leaf, since the centre of I_2 was close above the centre of P_1, the angle I_1–I_2 being 170° and 167° in the two experiments. The fact that I_2 occupies the smaller angle P_1–I_1 in both these experiments raises the question whether any leaf other than the regenerated P_1 and I_1 was effective in determining I_2. It is suggested that P_3 was effective in doing so, since its centre lies on the same side of the apex as the larger angle between P_1 and I_1 (figure 7*B*, no. 9). P_3 would thus play the same part in these experiments in determining I_2 as did P_2 in three experiments of group I (nos. 3, 7 and 8) in determining I_3, for in both experiments a leaf arose in the smaller angle between the last two leaves, and the centre of a leaf four plastochrons older (P_2 or P_3) lay beneath the larger angle, while the centre of the leaf (P_1 or P_2) underlying the smaller angle had been removed. As this situation occurred in

five experiments it is not unreasonable to suppose that the centre of the leaf underlying the larger angle was effective as a centre of inhibition. It is perhaps surprising that the centre of a leaf four plastochrons old should still inhibit effectively, but this question will be discussed later when the nature of the inhibition will be considered.

The question may be raised how it was that in the experiments of this group P_3 did not inhibit I_1, which was, in fact, displaced towards P_3, although it has been concluded that P_3 did inhibit I_2 slightly. From a cut-down leaf as old as P_3 only a slight degree of inhibition

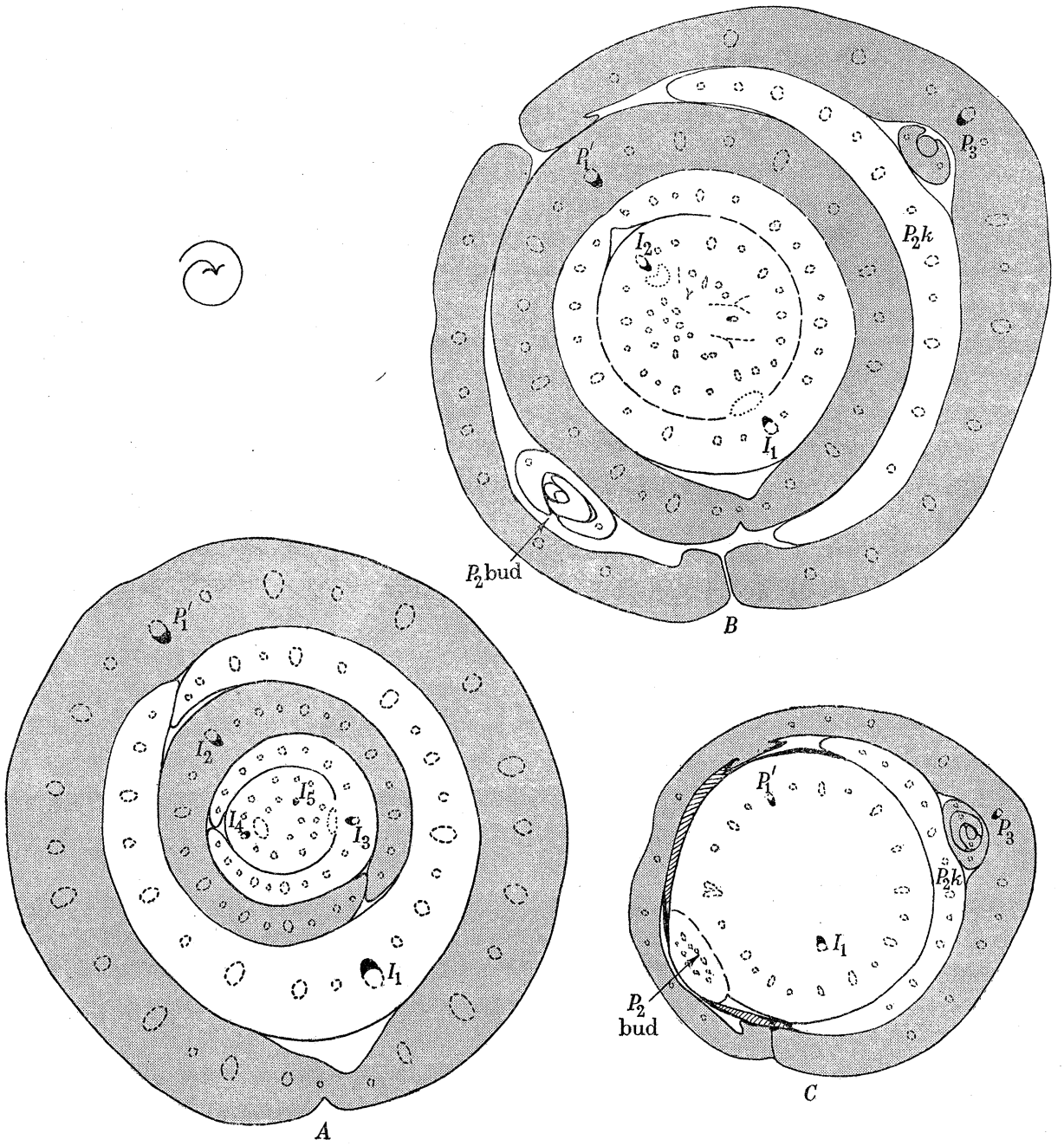

FIGURE 7. *A*, *B* and *C* are transections of no. 9 from above downwards. The centres of P_1 and P_2 were removed and P_1 regenerated a new centre at P_1'. In *C* the vascular strands in the centre of the stem are omitted, the shaded area is the moribund base of P_2 and P_2k is its kathodic flank. (Magn. *A*, ×27; *B*, ×19; *C*, ×8.)

would in any case be expected on the hypothesis adopted here. This slight inhibition from P_3 acting upon I_1 may have been outweighed by an inhibition from the regenerated centre of P_1 acting upon I_1 in the opposite sense (see figures 7, 10).

Since I_2 arose in the smaller angle between P_1 and I_1 in the two experiments, the direction of the genetic spiral was unchanged, though the angle P_1–I_1 exceeded 180° measured in the original direction of the spiral. The divergence angles tended to return towards the normal after oscillating above and below it.

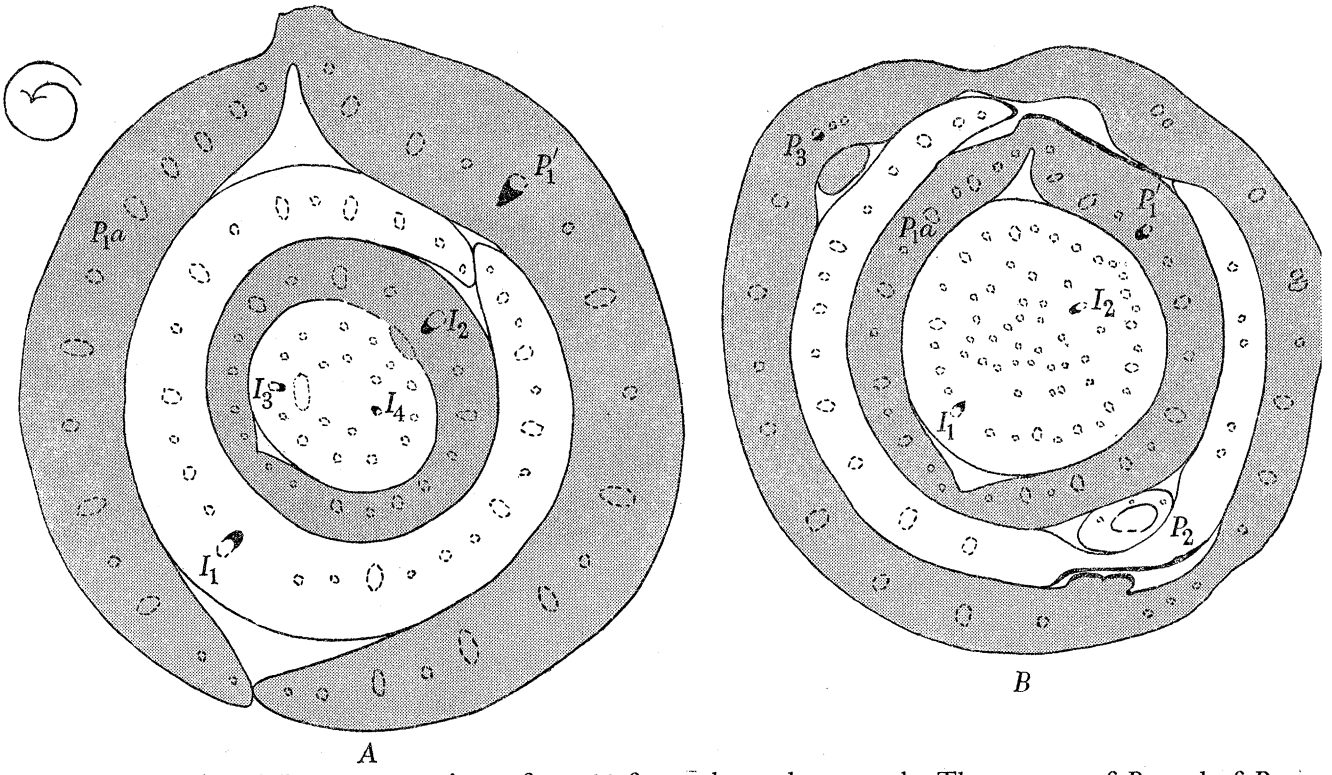

FIGURE 8. *A* and *B* are transections of no. 10 from above downwards. The centres of P_1 and of P_2 were removed. A new centre was regenerated in the kathodic flank of P_1 at P_1'. (Magn. *A*, ×32; *B*, ×19.)

There remain two experiments in which the centre of P_2 was removed, but P_1 did not regenerate a new centre—nos. 11 and 12. In no. 11 the angle P_3–I_1 differed from the mean normal angle by only 0°·9. The unchanged position of I_1 can be understood on the inhibition hypothesis, since on that hypothesis the inhibition from P_2 had been much reduced, so that it did not displace I_1 towards P_1 as it did in group I. Whether this is a general rule will appear from further experiments now in progress. In no. 12, figure 9, the position of I_1 in relation to its normal position could not be ascertained, since the centre of P_3 had been accidentally destroyed. The special interest of this experiment lies in the reversal of the spiral already mentioned on p. 149. A further point worth noting is that it is the only experiment in which the centre of P_1 was removed late in the plastochron when P_1 was large. The two halves of P_1 are united only at the base by a narrow strip of tissue which was dead at the time of pickling. The tendency of the two halves to reunite was probably less strong than when the operation was made earlier in the plastochron. The experiment of removing the centres both of P_1 and of P_2 is being repeated.

(9) *The asymmetry of I_1, I_2 and I_3*

In the description of the normal apex it was noted that the leaves were regularly asymmetric, the anodic flank being the shorter in extension round the apex. The mean angle subtended by the anodic half-leaf was 166°·5. The standard deviation was 2°·3 and the

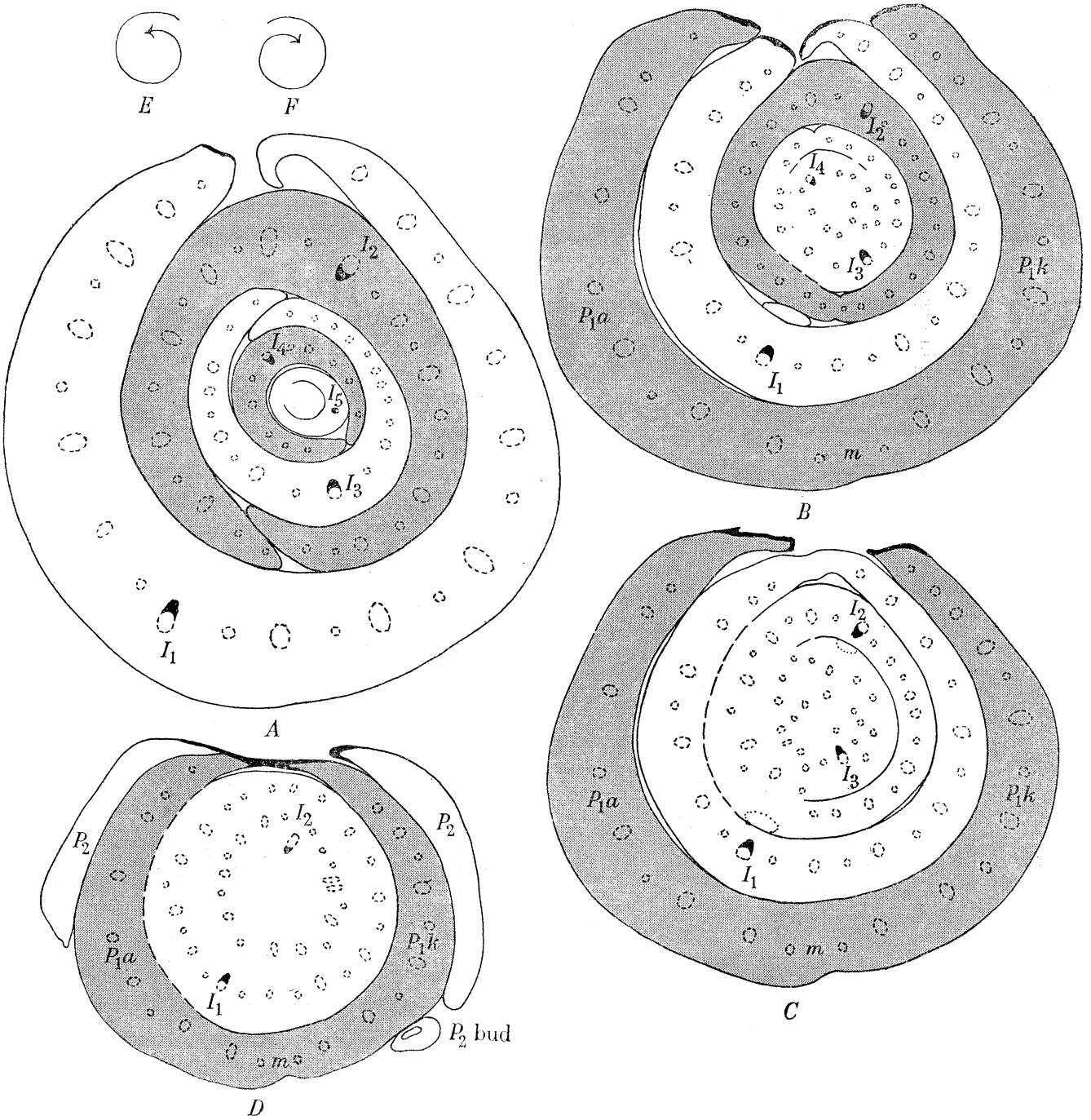

FIGURE 9. *A*, *B*, *C* and *D* are transections of no. 12 from above downwards. The centres of P_1 and P_2 were removed. The meeting-point of the edges of the flanks of P_1 is marked *m*. The vascular strands in the centre of the stem are omitted in *D*. The sections shown in *B*, *C* and *D* are slightly oblique. *E* shows the direction of the genetic spiral before the operation. *F* shows its direction from I_1 onwards. (Magn. *A*, ×36; *B*, ×22; *C*, ×20; *D*, ×15.)

standard error 0°·30. Though these experiments were designed to investigate the causes of the phyllotaxis, it seemed probable that they might also throw light on the factors determining leaf asymmetry, since in monocotyledons asymmetric leaves are usually associated with spirodistichous phyllotaxis and symmetric leaves with distichy. Some relationship between the phyllotaxis and the symmetry of the leaves is therefore probable. With this object in view the angles subtended by the anodic halves of the leaves that arose after the

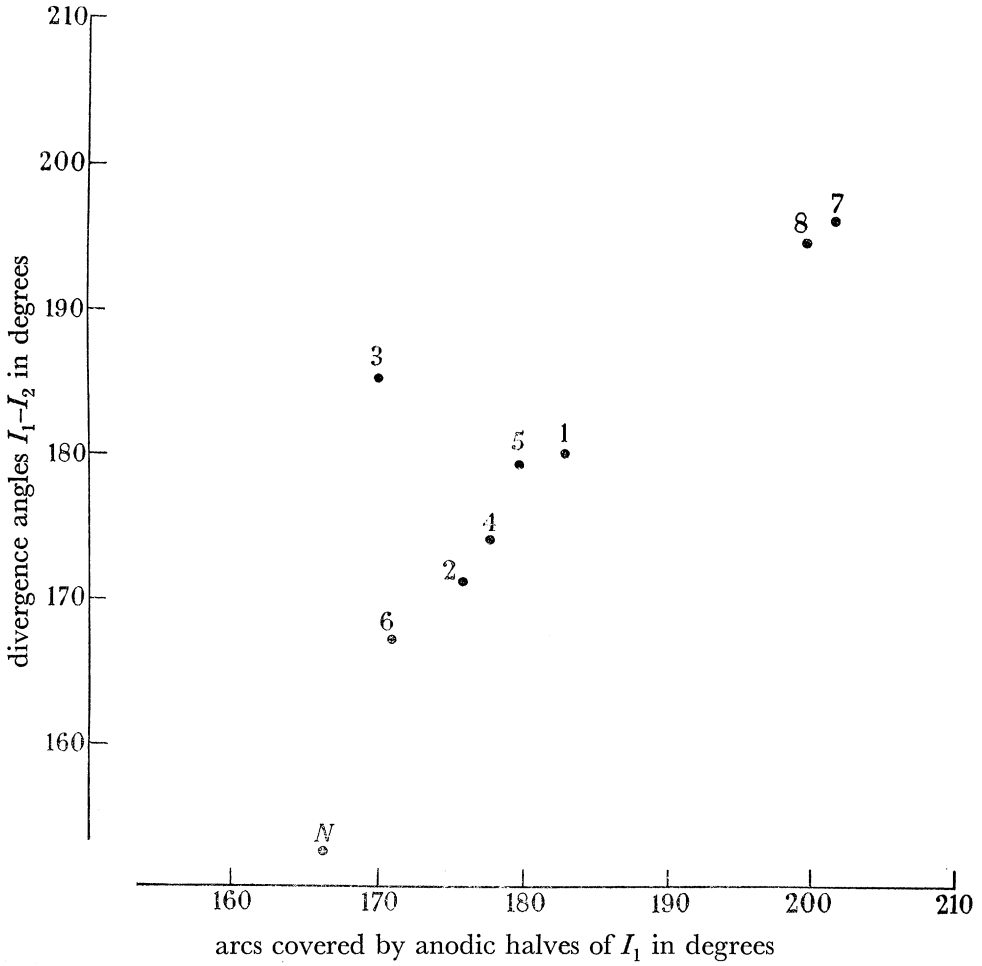

FIGURE 10. Graph showing relation between angle I_1–I_2 and anodic halves of I_1 in experiments 1 to 8 represented by numbered dots. The dot N shows the relation between the normal means of the divergence angle and of the anodic half of a leaf.

operation were measured and are recorded in table 3. The term anodic is here applied to the halves of the leaves which are anodic in the original direction of the genetic spiral except in no. 12, in which the spiral was reversed at I_1, and the term anodic is applied in relation to the final direction of the genetic spiral.

The angles subtended by the anodic halves of I_1 in the experiments of group I were always greater than normal. They ranged from 170° to 202°. This is interpreted as the result of a differential growth of the flanks. For if the normal asymmetry of the leaf is due to the more rapid extension of the kathodic than the anodic flank, a differential change in their growth rates will bring about a corresponding change in their asymmetry. Also if either flank is promoted relatively to the other it will extend farther round the apex before

meeting the other flank and then will prevent it from extending any more. For at their extreme base the flanks of each leaf meet and unite, as already mentioned (p. 138), but do not overlap.

The greatest increase in the anodic arc of I_1 occurred in nos. 7 and 8, in which the angle I_1–I_2 also showed the greatest increase above the normal. These facts suggested the possibility that there might be some regular relationship between the increase in length of the anodic halves of I_1 and the increase in the divergence angle I_1–I_2. This relationship is shown on a graph (figure 10) in which the angle I_1–I_2 is shown on the ordinates and the angle subtended by the anodic half of I_1 on the abscissae. The graph shows clearly that the arc of the anodic half of I_1 increases as the angle I_1–I_2 increases except in no. 3, in which the anodic half is unduly small. This exception will be discussed below.

The increase in the angle I_1–I_2 has already been explained as the result of the removal of the centre of P_1 and the consequent decrease in the repulsion exerted by that leaf on the centres of I_1 and I_2. Variations in this angle were considered to be due to variations in the amount of the centre of P_1 that was removed and in the degree to which P_2 was cut down (see p. 148). Since the arc of the anodic half of I_1 varies directly with the angle I_1–I_2, there is reason to suppose that these same factors which caused the variations in the angle I_1–I_2 also caused those in the asymmetry of I_1.

This provisional conclusion, together with some other facts to be mentioned below concerning the present experiments, and also some evidence from other experiments to be reported in a later paper, have led the writer to consider the following possibility with regard to the relationship between phyllotaxis and leaf asymmetry in this species, both in normal plants and in the experiments. It may be that those conditions which *inhibit* the formation of a new leaf centre *promote* the extension of the flanks of a leaf round the apex. A new leaf centre would then in general arise on the same side of the apex as the shorter half of the previous leaf, as is found to be the rule in a normal apex. In terms of the inhibition theory it must be supposed that the influence exerted by the leaf centres on the subsequent leaves is such as to inhibit the formation of new leaf centres but to promote the extension of their flanks round the apex. This hypothesis seems at first rather arbitrary and improbable, but it agrees well with the facts concerning the normal plant and concerning I_1 and I_2 in the experiments. Thus in the experiments nos. 1 to 8 which form group I the removal of the centre of P_1 reduced the promoting effect normally received by the kathodic flank of I_1, so that the anodic flank, as described above, was able to extend farther before meeting the kathodic flank.

There remains to be considered experiment no. 3, figure 4, which was an exception to the relationship shown by the graph, figure 10, in that the arc of the anodic half of I was considerably smaller in relation to the angle I_1–I_2 than in any other experiment of group I. This arc was indeed only 170°, which is only 3°·5 greater than the normal arc, whereas the angle I_1–I_2 was over 180°. Now in this experiment, though the angle I_1–I_2 is large, I_1 is only slightly displaced towards P_1, as was pointed out on p. 148, where both these facts were interpreted as resulting from an incomplete removal of the centre of P_1. The same explanation may account for the slightness of the change in the asymmetry of I_1. For if the centre of P_1 was still growing when the flanks of I_1 were extending round the apex a relatively long kathodic and short anodic flank would be expected in I_1 as in a normal leaf.

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In nos. 11 and 12 forming group II which are not included in the graph the asymmetry of I_1 could not be measured satisfactorily, since in no. 12 the flanks of I_1 had grown into the wound which may have affected their relative growth rates (figure 9), while in no. 11 the regeneration of the kathodic half of P_1 had interfered with the extension of the flanks of I_1 which did not meet round the apex. These are the only two experiments in which there is definite evidence that developments near the wound had interfered with the flanks of I_1. In nos. 9 and 10 forming group III the change in the asymmetry of I_1 was similar to that in the experiments of group I, though rather less than in most of them. But it seems unprofitable to try to interpret the change in these two experiments, since the regeneration of a new centre of P_1, which probably took a considerable time, and the removal of P_2 made the conditions so complicated.

The arc of the anodic half of I_2 also was longer than normal in the experiments of group I except in no. 8 (table 3, column 3), in which it was shorter than normal. In that experiment it seems that a new centre has been regenerated in the kathodic flank of P_1 (figures 5*A*, *B*), and this centre will, on the hypothesis proposed, have increased the extension of the kathodic half of I_2 above it, which in turn will have interfered with the extension of its anodic flank.

In the remaining seven experiments of group I the increase in the anodic halves of I_2 may be related to the large angle I_1–I_2; for since I_1 and I_2 were more nearly opposite than in a normal plant the influence exerted by I_1 on the two flanks of I_2 was more nearly equal. It may be noted that the longer flank of I_2 always lay on the opposite side of the apex from the centre of I_3, which agrees well with the hypothesis that conditions which inhibit the formation of a leaf centre promote the extension of the flanks round the apex.

In no. 12, an experiment of group II in which the centres of P_1 and of P_2 were removed, the asymmetry of I_2 is particularly significant. In this experiment the genetic spiral was reversed permanently at the angle I_1–I_2 and the asymmetry of I_2 was also reversed, so that here again the longer flank of I_2 was on the opposite side of the apex from the centre of I_3.

The asymmetry of I_3, however, is difficult to reconcile with the hypothesis proposed above which therefore remains for the present only a possibility to be borne in mind. Since the asymmetry of I_3 is at present not understood it will not be described here, but the main facts are recorded in table 3 and in the figures.

Discussion and conclusions

From the experiments on *Rhoeo discolor* reported above and from observations made on normal plants, information has been obtained with regard to three main questions. These questions concern the manner in which a leaf primordium is determined and develops, the factors locating the position in which a primordium is determined and the causes of the asymmetry of the primordium. They will now be discussed in turn.

As to the first question the evidence indicates that in *Rhoeo* the centre of a primordium or a small central region is determined first and that from this centre the flanks extend round the apex until they meet. For the fact that in *Rhoeo* the flanks of P_1 continued to extend round the apex after the centre of P_1 had been removed shows that once this extension has begun it does not depend on the presence of the centre. It might, indeed,

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be suggested that the whole area occupied by P_1 had been determined to form a leaf before the centre was removed and was only gradually occupied by the flanks; but the results of some other experiments in which the apex was split do not support this suggestion. In these experiments, which will be more fully reported later, the split was made at right angles to the median plane of P_1. When the operation was made early in the plastochron at a stage when P_1 subtended less than 180° the whole visible part of the leaf was confined to one of the split halves, and the flanks of P_1 did not develop on the other half, as they would have been expected to do if they had been already determined. But they did continue to extend round the half-apex which carried the centre of P_1, extending through the tissue adjacent to the wound and above it. On the other hand, when the split was made later in the plastochron so that the flanks of P_1 were severed by the cut, then the flanks continued to extend round the half-apex remote from the leaf centre, but not round the half carrying that centre. The facts therefore indicate that the tips of the flanks of a leaf when once they are formed tend to extend round the apex or half-apex on which they are situated without needing any influence from the leaf centre for their further growth, and also that the flanks are determined later than the centre.

Thus the manner of initiation and development of a primordium in *Rhoeo* appears to differ from that which was found in the dicotyledon *Lupinus albus* previously investigated. For we concluded (1931, 1933) that in *L. albus* a leaf primordium was determined as a whole as soon as a space was available both wide enough and low enough for it. There was therefore a certain minimum area of the apex in which a primordium could be determined, and when a primordium was determined in it, this area constituted the primary area of the primordium (Snow, M. & R. 1933, p. 360). This primary area included the stipules as well as the central part of the leaf, though some extension of the stipules round the apex was found to take place subsequently. In *Rhoeo*, however, it appears that the primordium is not determined as a whole simultaneously but that its central part is determined first. It is possible that some part of this central region constitutes a primary area, but if so it is much smaller in relation to the total area subsequently occupied by the leaf than it is in *Lupinus*.

As to the factors locating the positions in which primordia are determined in *Rhoeo*, the present results indicate that each leaf centre inhibits the formation of new leaf centres in its neighbourhood and that this inhibition decreases with time and with distance. The clearest evidence in favour of this conclusion is to be found in the positions of I_1 and I_2 following the removal of P_1, for the centre of I_1 then arose closer to the original position of the centre of P_1 than it would normally have done and I_2 arose almost opposite to I_1. These changes cannot be explained in terms of any space-occupying process, similar to that which we found in *Lupinus*. For the centres of I_1 and I_2 usually did not fit into depressions in the changed boundary lines round the stem apices as can be seen in many of the figures, e.g. figs. 3 *D* and 5 *D*. Similarly, in a normal spirodistichous apex the leaf centres do not fit into any depressions, as was pointed out on p. 139. Moreover, the displacement of I_2 after removal of the centre of P_1 indicates that in a normal apex of *Rhoeo* the position of a new leaf n is influenced by inhibition from leaf n–2, as well as leaf n–1. This inhibition from n–2 causes n to arise farther from n–2 than it would otherwise have done, and so causes n to deviate from the position opposite to the centre of n–1 in which it would otherwise have

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arisen on account of the inhibition from n–1. Consequently when the centre of n–2 is removed, leaf n arises more nearly opposite to n–1. This, then, as the writer concludes, is in the main the explanation of the spirodistichy of *Rhoeo*.

There is evidence, however, that the position of the centre of leaf n is also affected by inhibitions from leaves n–3 and n–4. The inhibition from n–3 is shown when the angle I_1–I_2 exceeds 180°, indicating that I_2 is inhibited by P_2, which is n–3 in relation to I_2, and the inhibition from n–4 is shown by the position of I_3 which always arose on the side of the apex away from the centre of P_2, so long as that centre was intact; for this indicated that I_3 was inhibited by P_2. Thus n–3 reinforces n–1 and n–4 reinforces n–2 in their effects on n, as can be seen from figure 1. It may be that these inhibitions from n–3 and n–4 would not be found in all spirodistichous species, since they do not seem to be necessary for spirodistichy. Other evidence was also reported for the conclusion that the position in which a leaf arises is determined by inhibitions from the four youngest leaves, their effectiveness diminishing with the age of the inhibiting leaf. It is probable, however, that in a normal plant in which all these four leaves are intact the effect of the older leaves n–3 and n–4 is relatively less than it was in the experiments, in which the centres of n–1 and sometimes of n–2 had been removed.

It is difficult to determine whether the diminution of inhibition with age of the inhibiting leaf depends for the most part directly on the age of that leaf, or on its distance from the apex which increases with age; but, since there is very little internodal extension between the youngest leaves, it is probable that the diminution cannot be accounted for by distance alone, but must in part depend directly on age.

As to the mechanism of this inhibition, the experiments provide no direct evidence, but it seems probable that an inhibiting substance is formed by the centres of the leaves and diffuses through the tissues of the apex. Whether it moves by simple diffusion or by some more complex process it is impossible to say, but is is clear that it must move upwards as well as transversely, since the four youngest leaves can all exert inhibitions on the new leaf arising at the apex. The results indicate also that in *Rhoeo* the central region of a leaf is the main source of inhibition, since on that basis it has been found possible to explain them consistently. They also suggest that the central conducting strand is the most effective part of that central region. For in three experiments, nos. 8, 9 and 10, in which P_1 regenerated a new central strand, the centre of I_1 was almost opposite to the new central strand, and so appeared to have been inhibited by the new strand. But this inhibition seems to have faded sooner than that from a normal leaf centre, since I_2 arose with its centre close to the new central strand of P_1. This rapid fading may be correlated with the absence of a bud from the axil of the new centre of P_1, which seems accordingly to have been imperfect in some way. On the other hand, the fact that in four other experiments, for instance, no. 4, figure 3, a bud did arise in the axil of a P_1 with its centre removed, may be explained by its having been already determined.

Finally, the problem of leaf asymmetry remains to be considered. As stated in the introduction, Schumann at first considered leaf asymmetry to be the determining factor of the phyllotaxis, but later abandoned this view. In Hirmer's opinion (1922) the asymmetry and the phyllotaxis both result from the insertion of a sector in the apex, but reasons have been given for considering it improbable that such a process takes place, and in any case

it could not account for the fact that in *Rhoeo* the kathodic half (called by Hirmer 'anodic', see p. 133) is the longer tangentially, contrary to what Hirmer states to be the general rule.

Schoute (1913, pp. 297, 310) considered that leaf asymmetry was secondary and occurred when the space available for a primordium was itself asymmetric provided that the new primordium made contact with the underlying leaves. The new primordium then extended asymmetrically to fill the unequal gaps on each side of its centre. This may, indeed, perhaps account for the fact that in *Rhoeo* the anodic half is usually slightly the thicker radially, since it lies above the meeting-point of the edges of the previous leaf, where there is the most space available in a radial direction; but it cannot account for the greater length of the kathodic half in normal plants. The following results of the present experiments need also to be considered. When the centre of P_1 was removed, the anodic half of I_1 increased in length and in three experiments exceeded the kathodic, and this increase varied directly with the divergence angle I_1–I_2, which also increased. Again in no. 9, figure 7, the centre of I_2 lay exactly over the meeting-point of the flanks of I_1, and yet I_2 was asymmetric, so that its asymmetry must have been due to something other than the space-filling process. Lastly, in no. 12, figure 9, in which the spiral reversed, I_2 was very asymmetric both in length of halves and in their radial thickness, and the longer flank was the thicker, contrary to what is found to be the rule in normal plants.

The facts reported concerning I_1 and I_2 were shown to agree in the main with the hypothesis proposed, that the factors which inhibit the formation of a leaf centre promote the extension of a leaf flank round the apex, improbable though this may at first appear. For in all the I_1 leaves except one, the longer flank lay on the side of the apex away from that on which the next leaf centre (I_2) was formed and thus on the more strongly inhibited side, as also it does in a normal plant. The exception was no. 3, in which it appeared that the centre of P_1 had been incompletely removed. The most convincing evidence in favour of the hypothesis proposed is the correlation of the increases in the angle I_1–I_2 and in the arc of the anodic half of I_1 (see figure 10). The asymmetry of I_2 also was shown to support the hypothesis, but difficulties arose with regard to I_3 which cannot at present be resolved, so that the hypothesis must for the present remain provisional. In other spirodistichous species the sense of the leaf asymmetry still needs to be determined with certainty.

The differences between the processes of leaf determination in *Lupinus albus* and in *Rhoeo discolor* may now be briefly summarized as follows. In *Lupinus* the primary area of a leaf is determined as a whole and comprises the greater part of the area of the apex which the leaf will finally occupy. In *Rhoeo* the centre or a small central area is determined first, and the process of leaf formation extends gradually round the whole circumference of the apex. In *Lupinus* the position in which the next leaf will arise depends on the position of the first space large enough to accommodate its primary area which reaches a sufficient distance from the growing-point; and so it depends on the outlines of the insertions of the youngest primordia. In *Rhoeo* the position in which the next leaf centre will be formed depends on a balance between the physiological inhibitions exerted on the leaf-forming zone of the stem apex by the three or four youngest leaves.

It does not appear that in *Rhoeo* the next leaf centre is determined in the position which is farthest from the growing-point, neither in the experiments nor in normal plants (p. 139).

So in this respect also *Rhoeo* appears to differ from *Lupinus albus* and *Epilobium hirsutum*, in which species experiments led to the conclusion that the primary areas of leaves are determined in the available spaces that are lowest—that is, farthest from the growing-point (Snow, M. & R. 1931, 1933 and 1935). This is probably because in *Rhoeo* the physiological inhibitions proceeding from the young leaves may cause the lowest position to be unavailable for the next leaf centre. Moreover, since the youngest leaf subtends an arc of 360° before the next leaf arises the boundary line round the base of the stem apex is much less indented than in plants with smaller leaf arcs, and the differences of level are therefore less great than in the latter.

In view of the general similarity of the spirodistichous systems of monocotyledons, it seems probable that in the other species of monocotyledons with systems of this kind the process of leaf determination and the factors governing the positions of those leaves are similar to those in *Rhoeo*. But this raises the question how it is that in the young seedlings of several spirodistichous species drawn by Hirmer (1922), though not in *Rhoeo*, several leaves following the cotyledon arise in a straight distichy, and then the later leaves deviate gradually into spirodistichy. It may be that the inhibition from each of these early distichous leaves is weak and disappears before its second plastochron, so that the position of leaf n depends only on the inhibition from leaf n–1, opposite to which n arises. Or again, it may be that these early leaves scarcely inhibit at all, and that their positions depend on a space-filling process such as we found in *Lupinus albus*. For this process might be expected to place leaf n with its centre roughly over the meeting-point of the flanks of n–1, where the sheathing base of n–1 is thinnest; and since the distichous leaves are symmetric according to Hirmer, this position is opposite to the centre of n–1.

Since the present results show that in *Rhoeo* the placing of new leaves is governed by physiological inhibitions from older leaves, they agree in this respect with the theories of Schoute (1913) and of Richards (1948). Yet those theories were not proposed especially for spirodistichous monocotyledons, but generally for all systems of phyllotaxis. Indeed, it is mainly the much commoner spiral systems of dicotyledons which these authors have considered, and it was concluded previously (Snow, M. & R. 1931, p. 36; 1933, p. 396) that the results of experiments on *Lupinus albus*, a species with a typical Fibonacci spiral system, did not agree with Schoute's theory but needed to be explained in terms of space-occupying factors, in agreement with the theory of van Iterson. The chief reason given for this was that according to Schoute a leaf centre is determined first, whereas the evidence from radial splits of I_1 in *Lupinus* was considered to show that in that species a leaf was determined as a whole (see Snow, M. & R. 1933, p. 397). This conclusion has indeed been criticized by Richards (1948), but it would take too long to discuss here in detail these criticisms, with most of which the writer cannot agree. It is hoped to discuss the question further when some further experiments now in progress are reported on leaf determination in dicotyledons.

Even the results of the experiments on *Rhoeo* do not agree with the theory of Schoute, since he supposed that each leaf centre was surrounded by a 'dispersion area' within which an inhibiting hormone completely prevented the formation of other leaf centres, and that the effect of this hormone ceased suddenly at the boundary of each such area. But it seems to the writer that the changes of leaf positions reported in *Rhoeo* cannot be

explained on this basis, but only on the basis of inhibitions that decrease gradually with the distances from their sources.

Wardlaw also (1949 *a*, *b*, *c*) has recently suggested a theory of leaf-determination and phyllotaxis in ferns according to which certain areas extending round the central parts of leaves already determined are made physiologically unavailable for the formation of other leaves, and he has based this theory on some valuable experiments on fern apices, which, however, are morphologically different from those of flowering plants.

The theory of Richards (1948) is much less rigid than that of Schoute, and is stated only in very general terms. Unlike Schoute he supposes that the inhibiting influences from existing leaves diminish continuously with distance. He claims that on theories such as his own, which he calls field theories, it is possible to explain the return of a spiral system to regularity after a disturbance. This is a very important claim, since the return to regularity after a disturbance and the attainment of regularity in seedlings are serious difficulties for a space-occupying theory such as that of van Iterson.

The present experiments on *Rhoeo* support very well Richards's idea of inhibitions diminishing continuously with distance, and they contain nothing contrary to his theory. Yet difficulties arise for this theory also if it is applied to dicotyledons, since Richards, like Schoute, supposes that the centre of a leaf, or at most a small central area, is determined first, whereas the evidence, as has been pointed out, still seems to the writer to show that in *Lupinus* a leaf is determined as a whole. Another difficulty is that on the theory of Richards the position of a new leaf should depend on inhibitions coming from all the leaves of the uppermost cycle at least. Yet in *Lupinus* the evidence has seemed to show that the position of a new leaf n does not depend, or not appreciably, on the immediately previous leaf n–1, when leaf n does not make contact with that leaf, as normally it scarcely does. The position of n depends only on those leaves with which it does make effective contact. The evidence for this statement was summarized recently (Snow, M. & R. 1947, pp. 14, 15; 1948, pp. 271, 272) in a criticism of the so-called repulsion theories of phyllotaxis, and it seems to the writer to hold good in spite of criticisms made in correspondence by Richards. Further experiments bearing on this point are also in progress.

A similar conclusion has been reached by Wardlaw (1949*a*, pp. 173, 188; 1949*c*, pp. 106, 125) from experiments on fern apices. In these apices the visible leaves are not in contact at all, but Wardlaw has concluded that here also the position of a new leaf is affected only by the immediately adjacent leaves, and not by those remote from it (1949*a*, p. 188). These fern apices having five leaves in the top cycle are very well suited for testing this point. He has also written that the position of a new leaf is determined by localized factors and not by factors affecting the meristem as a whole (1949 *a*, p. 177).

The writer concludes therefore that the process by which leaves are localized in spirodistichous monocotyledons is different from what it is in dicotyledons and ferns, and that the phyllotaxis of the first of these groups depends on physiological inhibitions such as are postulated by Richards, but that his theory cannot hold good for the other two groups, or not in its present form at least. The problem for the future seems to be whether in dealing with the dicotyledons and ferns it is possible in any way to secure the advantages claimed for the theory of Richards without coming into conflict with the experimental results.

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