The morphology and growth of the vegetative and reproductive apices of Arabidopsis thaliana (L.) Heynh., Capsella bursa-pastoris (L.) Medic. and Anagallis arvensis L.

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(With Plates **18–21** and 4 Text-figures)

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INTRODUCTION

In the past twenty years, an appreciable amount of research has been carried out into the growing points of angiosperms. This work, mainly anatomical and histological, has been facilitated by improved microtechnique. Most of the anatomical work published has concerned vegetative apices. Relatively few reproductive apices have been investigated, and there is even less anatomical information available on the transformation of the vegetative apex to the reproductive state. Several excellent reviews of the developmental anatomy of the shoot apex have appeared recently (Foster, 1939, 1941; Sifton, 1944; Majumdar, 1945; Philipson, 1949; Popham, 1951). The present paper deals with investigations that have been made into the apical morphology of *Arabidopsis*, *Capsella* and *Anagallis*. With the aid of a certain physiological technique, it has been possible to obtain material of two of these plants in all stages from the vegetative to the reproductive state. In describing the apices of the three plants under consideration, use has been made of the tunica-corpus and cyto-histological concepts, fully described in the reviews mentioned earlier in the introduction.

MATERIAL AND METHODS

Both *Arabidopsis* and *Capsella* are rosette plants which frequently terminate in one main erect racemose inflorescence, but also other aerial flowering stems may arise in the axils of the rosette leaves. These aerial stems bear a small number of cauline leaves, in the axils of which arise subsidiary inflorescences. However, *Anagallis* is a procumbent herb which in the vegetative state branches profusely, although branches are not visible macroscopically until some distance behind the growing point. When the plant attains the flowering condition, flower buds develop in place of the vegetative buds. Apart from this, the general habit of the plant is the same as during the vegetative period.

Both *Anagallis* and *Arabidopsis* respond to photoperiodic treatment and are of the long-day type. *Anagallis* is an obligate long-day plant, that is, it will remain vegetative indefinitely if kept in light periods less than the critical of 12 hr. *Arabidopsis*, however, is described as a facultative long-day plant because it will flower eventually in short days (about 60) but, if plants in the vegetative state are exposed to continuous light or long-day periods, inflorescence buds soon appear.

For this investigation, *Arabidopsis* plants, 25 days old and which had been kept in short days, were exposed to continuous light for 8 days, at the end of which time inflorescence buds were quite obvious. During this period of illumination, samples of plants were fixed at day intervals and thus a complete transition from the vegetative to the reproductive state was obtained. A similar procedure was carried out in the case of *Anagallis*.

The *Capsella* plants were grown from seed in pots under normal conditions. Although not photoperiodically controlled, the various stages of development obtained for these plants could be interpreted from the controlled material of *Arabidopsis*.

The fixative employed during the investigation was formalin acetic-alcohol and serial sections were obtained in the usual manner. The *Arabidopsis* and *Capsella* material was

stained with Heidenhain's haematoxylin alone but, in the case of the *Anagallis* material, aniline blue, according to the schedule of Popham, Johnson & Chan (1948), was employed as a counterstain.

THE VEGETATIVE AND REPRODUCTIVE APICES OF *ARABIDOPSIS* AND *CAPSELLA*

Inflorescence development

The general account of inflorescence development about to be presented deals specifically with *Arabidopsis*. No general account of inflorescence development in *Capsella* is presented because the process in both plants is essentially the same.

Text-fig. 1 A is a median longitudinal section of an *Arabidopsis* plant of the short-day type and therefore in the vegetative condition. The complete leafy axis is shown in the drawing. As one might expect from the rosette habit of the plant, the axis is very much condensed and the leaves and leaf primordia are attached to this axis very close to each other. No internodes are visible. The dense apex or promeristem can be seen in the centre of the drawing and, overlapping this, are the leaf primordia. The slightly convex shape of the apex is characteristic of *Arabidopsis* plants in the vegetative condition.

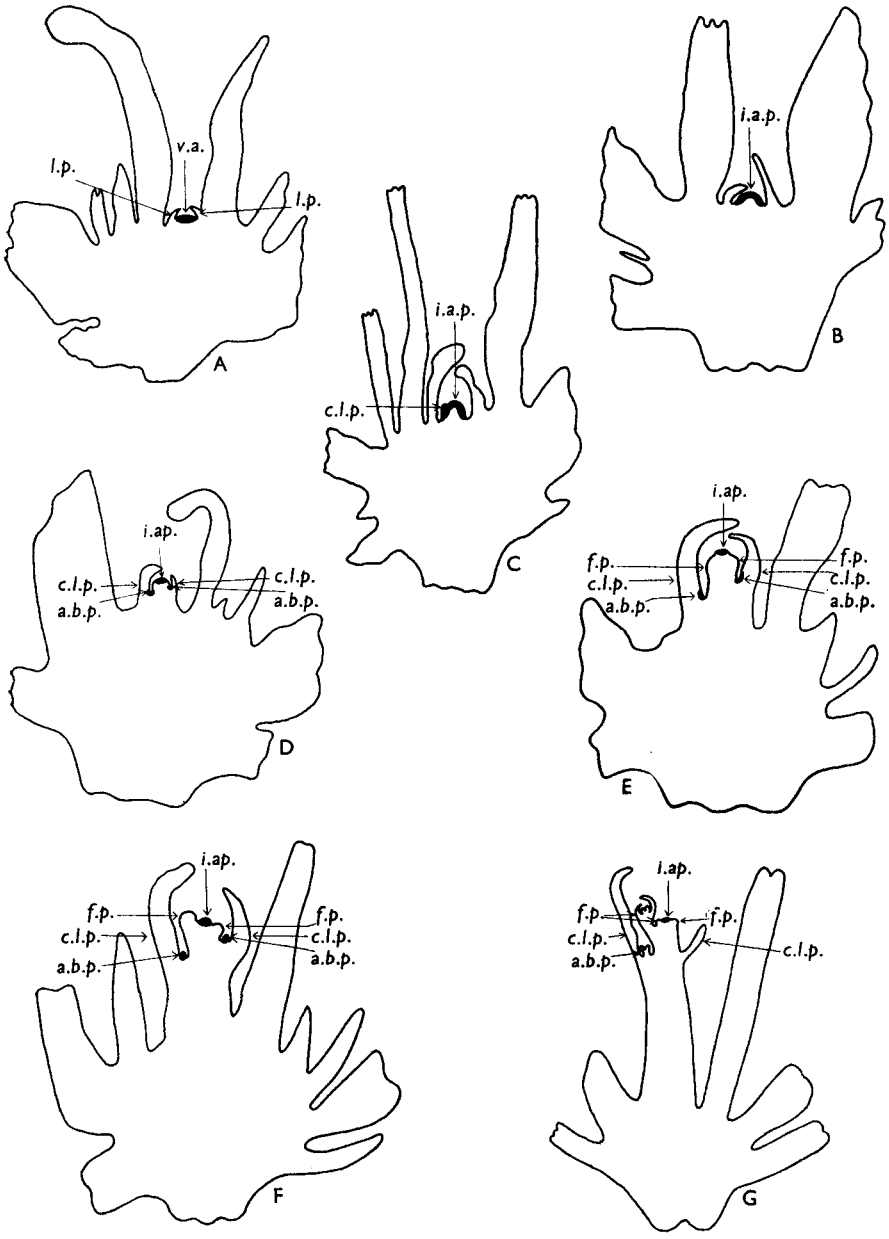
Under conditions of continuous light, in the first day, the plants remain in the vegetative condition and show the characters described in the previous paragraph.

In the second day of continuous light, however, changes are to be observed in a percentage of the sample examined. Text-fig. 1 B illustrates the condition shown by 75 % of the 2-day sample. It is the apex of the plant that has undergone a really important change. The shallow, slightly convex apex of the plant in the rosette condition has become a well-marked dome. Dense cells, as found in the rosette apex, occupy the apical region and flanks of this dome, but vacuolated cells are to be seen extending well up into its central region. The dome is the primordium of the inflorescence axis. If the series of sections from which Text-fig. 1 B was obtained is examined, cauline leaf primordia in the initiation stage will be found.

The plants fixed in 3 and 4 days of continuous light all show this inflorescence axis primordium in varying degrees of development. Text-fig. 1C is a longitudinal section of a third-day plant showing a more highly developed inflorescence axis, it having increased in height as compared with the one illustrated in Text-fig. 1 B. The apex is still dome shaped and shows the same general distribution of dense, meristematic tissue. The cauline leaf primordia have undergone further development, and Text-fig. 1 C shows such a primordium as a well-marked erection which does not overtop the main apex. This is in contrast to the state of affairs to be found in the rosette apex where leaf primordia, as soon as they are formed, overtop the main apex.

In the fifth day of continuous light, 50 % of the sample taken show just the initiation of the inflorescence axis, while the remaining 50 % show the first flower primordia. Text-fig. 1 D is of a 5-day plant of the type producing flower primordia. The dense inflorescence apex can be observed but no flower primordia are visible in this particular section. However, if the complete series of sections is examined, then two flower primordia can be observed. Two cauline leaves have been cut medianly and the older of the two, on the left-hand side of the axis, extends well above the apex; the younger, on the right-hand side of the axis, just curves over it. Small nests of dense cells are to be seen in the axils of these cauline leaves. These are the primordia of axillary buds which later give rise to subsidiary inflorescences.

In 6 days of continous light, 60 % of the sample taken show flower primordia, the rest just the primordium of the inflorescence axis in a very late stage of development. Text-fig. 1 E is a median longitudinal section of one of the 60 % of the 6-day sample which shows the typical condition. The main inflorescence apex and two flower primordia are shown in the figure. More flower primordia are to be found if the complete series of sections is



Text-fig. 1. (A) Longitudinal sections of an *Arabidopsis* plant of the short-day type (× 40). Two leaf primordia (*l.p.*) are shown growing over the slightly convex vegetative apex (*v.a.*) (B) Longitudinal section of an *Arabidopsis* plant exposed to 2 days of continuous light (× 40). Dense cells occupy the apex and the flanks of the inflorescence axis primordium (*i.a.p.*). (C) Longitudinal section of an *Arabidopsis* plant exposed to 3 days of continuous light (× 30). A cauline leaf primordium (*c.l.p.*) is shown which does not overtop the apex of the inflorescence axis primordium (*i.a.p.*). (D) Longitudinal section of an *Arabidopsis* plant exposed to 5 days of continuous light (× 30). Two cauline leaf primordia (*c.l.p.*) with their axillary bud primordia (*a.b.p.*) now overtop the inflorescence apex (*i.ap.*). (E) Longitudinal section of an *Arabidopsis* plant exposed to 6 days of continuous light (× 40). Two flower primordia (*f.p.*) are shown and the cauline leaf primordia (*c.l.p.*) with their axillary bud primordia (*a.b.p.*) have grown well above the inflorescence apex (*i.ap.*). (F) Longitudinal section of an *Arabidopsis* plant exposed to 8 days of continuous light (× 40). See (E) for explanation of labels. (G) Longitudinal section of an *Arabidopsis* plant exposed to 8 days of continuous light (× 19). See (E) for explanation of labels.

examined. Two well-developed cauline leaves with their axillary bud primordia curve over the apex.

Plants exposed to 7 and 8 days of continuous light all show flower primordia in varying degrees of development. Text-fig. 1 F is a median longitudinal section of an 8-day plant showing an advance in development over that illustrated in Text-fig. 1 E. The complete inflorescence axis has increased greatly in vertical extent. This is largely the result of longitudinal growth in that part of the axis below the insertion of the cauline leaves. The flower primordia are more advanced in development but are still enclosed by the cauline leaves. The axillary buds of these leaves are now very prominent.

Text-fig. 1 G is a median longitudinal section of another 8-day plant showing the furthest degree of development obtained with the plants examined. The main inflorescence axis has reached the height of about 2 mm. and, again, it is that part of the axis below the insertion of the cauline leaves that is responsible for this increase in length. The older flower primordia now show the initiation of all the flower parts. A change in connexion with the cauline leaves to be observed is that they are unfolding to expose the flower primordia. The axillary buds of these cauline leaves, at this stage, show the formation of lateral members.

Vegetative apex

The detailed structure of the vegetative apex of *Arabidopsis* is shown in Text-fig. 2 A and in Pl. **18**, fig. 1, which are of a median longitudinal section through the tip of a short-day plant.

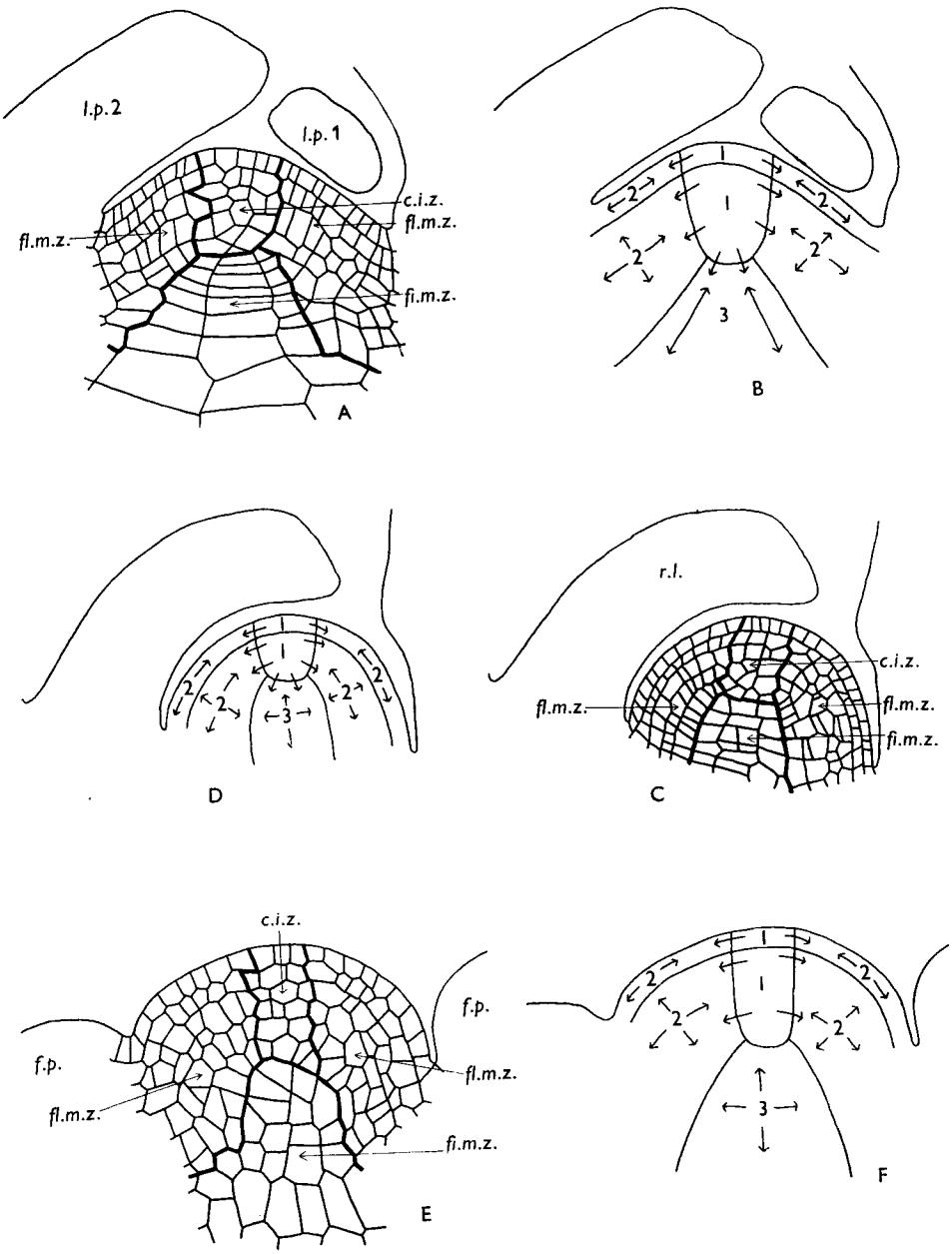
The youngest leaf primordium has not been cut medianly, but the next oldest primordium has been cut medianly and is shown curving over the left-hand part of the apex. As soon as rosette leaf primordia are formed, they overtop the main apex. The promeristem shown is cone-shaped, but a median longitudinal section through a recently initiated leaf primordium would present a slightly convex growing point. The width of the apex, at its widest part, is about 90μ and its height, in the centre, is about 35μ, and it is therefore one of the smallest apices to have been described.

In terms of the tunica-corpus concept, this promeristem has a tunica consisting of two layers of cells. Apices in all stages of the plastochrone have been examined, and the two surface layers show anticlinal divisions exclusively except when leaf primordia are initiated by periclinal divisions in the second tunica layer.

Also, zones can be recognized in the tunica and corpus of the apex which are characterized by cell size, density of contents and frequency of cell division. In the centre of this growing point, there is the central initiation zone which is bowl-shaped and consists of relatively few cells which show few divisions. Apart from those in the two surface tunica layers, the cells of this zone are arranged rather irregularly and, compared with the surrounding cells, they stain less deeply. The zone is about five cells deep in the middle and about three to four cells wide.

Surrounding the central initiation zone are the deeply staining cells of the flank meristem. Two sectors of this zone are shown in Text-fig. 2 A and in Pl. **18**, fig. 1. No obvious vacuolation is shown by the cells of the two zones already described and, in all cases, the nucleus occupies the major portion of the cell. No periclinal divisions are to be found, of course, in the two surface layers of the flank meristem and, although occasional periclinal divisions take place in the deeper layers, the zone as a whole presents a well-marked stratified appearance. It is about five or six layers of cells deep.

Below the central initiation zone is the third zone differentiated on cyto-histological grounds, the file or rib meristem. In Text-fig. 2 A and in Pl. **18**, fig. 1, this zone is represented by three vertical rows of cells, each consisting of about seven to nine units. The file meristem is perpetuated by the lowermost cells of the central initation zone and its cells show almost exclusively transverse divisions. The first file meristem cells to be produced, like most of the cells in the apical meristem, are very dense but, in contrast to



Text-fig. 2. (A) Longitudinal section of the *Arabidopsis* vegetative apex (× 725). The central initiation zone (*c.i.z.*), the flank meristem zone (*fl.m.z.*) and the file meristem zone (*fi.m.z.*) can be observed in the promeristem. The youngest leaf primordium (*l.p.* 1) and the next oldest primordium (*l.p.* 2) can also be seen. (B) Diagrammatic longitudinal section of the *Arabidopsis* vegetative apex to show the positions of the main growth zones and the direction of cell divisions. 1, the central initiation zone; 2, the flank meristem zone; 3, the file meristem zone. The tunica runs through the central initiation and the flank meristem zones. (C) Longitudinal section of the *Arabidopsis* inflorescence primordium (× 359). The central initiation zone (*c.i.z.*), the flank meristem zone (*fl.m.z.*) and the file meristem zone (*fi.m.z.*) can be observed in the promeristem. A rosette leaf (*r.l.*) is to be seen growing over the apex. (D) Diagrammatic longitudinal section of the *Arabidopsis* inflorescence primordium to show the position of the main growth zones and the direction of cell divisions. 1, the central initiation zone; 2, the flank meristem zone; 3, the file meristem zone. The tunica runs through the central initiation and the flank meristem zones. (E) Longitudinal section of the *Arabidopsis* inflorescence apex (× 438). The central initiation zone (*c.i.z.*), the flank meristem zone (*fl.m.z.*) and the file meristem zone (*fi.m.z.*) can be observed in the promeristem. Parts of two flower primordia (*f.p.*) are also shown. (F) Diagrammatic longitudinal section of the *Arabidopsis* inflorescence apex to show the positions of the main growth zones and the direction of cell divisions. 1, the central initiation zone; 2, the flank meristem zone; 3, the file meristem zone. The tunica runs through the central initiation and the flank meristem zones.

the cells which give rise to them, they are very much flattened. About the first three cells of each file meristem row are of this appearance and then vacuolation promotes cell extension, mainly in the lateral direction. Little vertical extension occurs. The collection of the first few dense flattened cells of the file meristem has been referred to as a 'cambium-like' zone. A characteristic feature of the *Arabidopsis* rosette axis is that the regular file meristem cells give rise almost immediately to the disorganized, highly vacuolate cells of the pith.

Tiny bud primordia have been observed in the axils of the mature rosette leaves and, occasionally, they undergo further development but it was not possible to trace their development in detail.

In the case of *Capsella*, rosettes, measuring about half an inch across, provided apices in the vegetative condition. Essentially, the structure of the *Capsella* apex is the same as that of *Arabidopsis*.

The cyto-histological zones, to be observed in longitudinal sections of the *Arabidopsis* vegetative promeristem, can also be seen in transverse sections (Pl. **18**, figs. 2, 3). The plant being of the rosette habit, numerous leaves and leaf primordia with prominent median vascular strands are shown in the photographs. Some of the primorida show marginal meristems. In Pl. **18**, fig. 3, at one point, the flank meristem is expanded laterally. This is the foliar foundation on which the next leaf primordium will be erected. In this photograph, both the youngest and the next youngest leaf primordia show well-marked abaxial vacuolation.

Significant roles can be attached to all the zones found in the vegetative apices of *Arabidopsis* and *Capsella*. The surface tunica gives rise to the epidermal system of the plant. The flank meristem is concerned with the production of leaf primordia, their associated procambial strands and the cortex; the file meristem gives rise to the pith and the central initiation zone is a self-perpetuating region of the apex producing new flank and file meristem (Text-fig. 2*b*).

The growth of the *Arabidopsis* apex has been studied in some detail. In Text-fig. 2A and Pl. **18**, fig. 1, two sectors of flank meristem are shown both presenting the stratified appearance characteristic of the zone. When sufficient flank meristem has been reconstituted by the central initiation zone to form a foliar foundation, leaf initiation takes place in that sector. This process is brought about by periclinal divisions in the second tunica layer. At the erection of the leaf primordium, the stratified appearance of the foliar foundation is lost and a considerable amount of cell reorganization takes place. Probably, the most important feature of this re-organization is the establishment of the procambial supply which runs into the developing primordium. Vacuolation then takes place in the foliar foundation and its leaf primordium, starting first in the abaxial side of the foliar foundation and extending up into the abaxial side of the primordium and then proceeding similarly on the adaxial side (Pl. **18**, fig. 1). During this vacuolation, the procambium becomes very evident. At all times, the leaf procambium develops acropetally from the existing network. Apical and marginal meristems can be recognized in the further development of the leaf primordium. The foliar foundation having undergone vacuolation, more flank meristem is reconstituted by the central initiation zone to take its place. These flank meristem cells, as they are produced, themselves undergo repeated anticlinal divisions to increase the area of the zone. The relatively few periclinal divisions that occur increase the depth of the flank meristem. The avidity for stain shown by the cells of this zone, in contrast to the central initiation cells, may be ascribed to the greater amount of division that takes place in the flank meristem.

The file meristem produces the pith, and *Arabidopsis* is peculiar in that there is a sharp transition between the regular vertical rows of the file meristem and the highly vacuolate, disorganized cells of the pith. There is not such a marked transition in *Capsella*. Text-fig. 2A and Pl. **18**, fig. 1, show the file meristem of *Arabidopsis* at its greatest development. This type of development seems to be associated with the mid-

phase of the plastochrone, and this is to be expected because the real importance of the zone is to produce pith cells to accompany the developing leaf and leaf base. As will be mentioned later, good development of file meristem brings about a well-marked increase in the length of the axis and the poor development of file meristem and its sudden transition to pith in *Arabidopsis* and *Capsella* is to be associated with the rosette habit of the plant for, here, internodes are absent and pith is only formed in conjunction with a relatively small area of leaf base.

The following account of the development of the inflorescence of *Arabidopsis* shows how the completely vegetative apex is directly converted into the apex of the inflorescence primordium.

Text-fig. 2C and Pl. **18**, fig. 4, show the apex of the *Arabidopsis* plant illustrated in Text-fig. 1B at a higher magnification, at an early stage of the transition from the vegetative to the reproductive phase. In contrast to the fairly shallow, slightly convex rosette apex, the apex now under consideration is quite a large dome-shaped structure. The dense, meristematic cells, characteristic of any active growing point, extend well down the flanks of this apex and its height is about 75μ and its width, at the base, is about 90μ. Pl. **18**, fig. 5, shows a later stage of inflorescence formation in *Capsella*. The rosette from which this preparation was made measured about three-quarters of an inch across.

Although a well-marked change has taken place in the shape and form of the apex, the two-layered tunica and the cyto-histological zones can still be recognized but, however, the file meristem has undergone quite a remarkable development. Compared with the truly vegetative apex, many more transverse and longitudinal cell divisions have taken place in the zone while the cells have elongated considerably in the longitudinal direction. These differences are particularly noticeable in Pl. **18**, fig. 5, and they are responsible for the initiation of the flower-bearing axis.

At this stage of development, cauline leaf primordia are initiated. Pl. **18**, fig. 6, has been taken from the same series of sections as Pl. **18**, fig. 4, and Text-fig. 2C, and shows the initiation of such a primordium by periclinal divisions in the second tunica layer. At this stage, at least, the primordium does not curve over the main promeristem and the development of its procambium supply, like that of the rosette leaf primordia, is acropetal.

A quite characteristic feature of the type of apex under discussion is the retention of fairly large areas of flank meristem which enclose the vacuolating and dividing file meristem (Text-fig. 2D). It would seem that the maturation of this flank meristem lags behind that of the file meristem, and this is borne out by the fact that cauline leaf primordia, produced in the flank meristem, do not overtop the main promeristem for some time. This is in contrast to the state of affairs in the rosette apex.

As has been explained in an earlier paragraph, the apex just described soon produces flower primordia and Pl. **19**, fig. 1, and Text-fig. 2E, are median longitudinal sections of a typical *Arabidopsis* inflorescence apex. The plant from which it was taken was exposed to 7 days of continuous light and was actively producing flower primordia. Two of these primordia, cut obliquely, are shown in the illustrations.

The dome-shaped apex is about 75μ across at its widest part and about 45μ deep in the centre. It possesses a two-layered tunica which is not disturbed at all during its growth processes, even at the initiation of a flower primordium. An examination of numerous series of sections has confirmed this observation. There is no great distinction between the central initiation and flank meristem zones on staining qualities, but investigations of the apex throughout its growth cycle have revealed the presence of a self-perpetuating zone. The flank meristem zone is five or six layers of cells deep.

Vacuolating file meristem is to be seen developing from the lowermost part of the central initiation zone. The first cells produced are rectangular with their long axes at right angles to the main longitudinal axis of the plant, but soon cell extension takes

place and the condition is reversed. Transverse divisions, of course, predominate here, but also a number of longitudinal divisions occur to widen the zone.

As in the rosette apex, important roles can be ascribed to the zones of the inflorescence growing point. The flank meristem is concerned with the production of the lateral members, the flowers, their associated procambium and the cortex, while the actively dividing file meristem produces the pith of the inflorescence axis. Reconstitution of these zones is carried out by the central initiation zone (Text-fig. 2F).

Pl. **19**, fig. 2, and Text-fig. 3A, are median longitudinal sections of an *Arabidopsis* inflorescence apex showing the initiation of a flower primoridium in the right-hand sector of the flank meristem. Periclinal divisions in the third layer of this zone are primarily responsible for the flower primordium initiation, and the cells of this layer in the illustrations are either actively involved in this type of division or are elongating prior to it. The cells of the two tunica layers overlying the point of initiation present a very compressed appearance and seem recently to have undergone repeated anticlinal divisions. Divisions in various planes have taken place in the fourth and fifth layers of the sector of flank meristem under consideration.

The sector being described extends for some distance down the longitudinal axis of the inflorescence and here the flank meristem can be regarded as having reached its full development. In longitudinal section, about ten cells of the third flank meristem layer are involved in this initiation. As will be described later, the flank meristem involved in this flower primordium production soon undergoes vacuolation and maturation and the central initiation zone, to be seen in Pl. **19**, fig. 2, produces more of the zone from part of its flanks. Pl. **19**, fig. 1, and Text-fig. 2E, F, show the reconstitution of flank meristem on either side of the central initiation zone. Active anticlinal and some periclinal divisions are responsible for the rapid growth of this important peripheral zone.

The apex of the *Capsella* flowering axis agrees in all respects with that of *Arabidopsis*.

Comparison of inflorescence and vegetative apices

A comparative study of the rosette and inflorescence growing points of the two members of the Cruciferae under consideration brings to light some important morphological similarities and differences.

Although these apices themselves vary in shape during their growth processes, an overall difference between the two types can be observed. The vegetative apex is never more than slightly convex while the inflorescence apex is always markedly dome-shaped. A reason for this difference will be suggested later.

As far as can be observed, the tunica of the rosette apex is always biseriate, only being disturbed at leaf initiation. A two-layered tunica is also characteristic of the inflorescence apex and is retained even at flower primordium initiation.

Central initiation, flank meristem and file meristem zones are to be found in both types of growing point and they perform the same general functions, but the flank and file meristem zones vary as regards extent of development and activity in the two different apices. It has been shown that quite an appreciable longitudinal sector of flank meristem needs to be reconstituted by the central initiation zone before flower primordium formation can take place. A smaller sector is needed in the rosette apex for the establishment of a foliar foundation and the resulting leaf primordium.

In the rosette apex the file meristem is of very limited development, quickly giving rise to the disorganized pith. On the other hand, the file meristem in the inflorescence apex shows a very extensive development, exhibiting numerous active transverse and longitudinal divisions. Cell extension is also marked in this file meristem, the cells elongating in a plane parallel to the longitudinal axis of the inflorescence. It would seem that, in any growing axis, it is the activity of the file meristem zone of the apex of that axis that determines the extent of its vertical growth and, therefore, in the vegetative

apices of the rosette plants under discussion, the poor development of file meristem foreshadows the condensed nature of the axis. On the other hand, very active growth of the file meristem gives a clear indication that the elongating axis will become an inflorescence.

The shapes of the two different types of growing point, so far described, can be correlated with their characteristic growth processes. In the rosette apex, the file meristem zone promotes little vertical extension, so that a comparatively small sector of flank meristem is required to form a foliar foundation, and consequently the shape of the apex is slightly convex. In the inflorescence apex, however, the file meristem is promoting very active vertical extension and a comparatively large sector of flank meristem is required for flower primordium formation. While this flank meristem is being reconstituted, the activity of the file meristem produces a markedly domed apex.

Flower primordium

The third type of shoot apex in *Arabidopsis* and *Capsella* is, of course, that of the flower, and it is of interest to investigate the development and activity of this growing point.

It has been shown (Pl. **19**, fig. 2 and Text-fig. 3 A) that a flower primordium is initiated by periclinal divisions mainly in the third flank meristem layer of the inflorescence promeristem. Numerous mitotic divisions then take place in the protuberance thus produced, and raise the level of its apex above that of the main inflorescence axis. At this stage, the primordium consists almost entirely of dense, meristematic cells, but vacuolation soon occurs to delimit the future pedicel and receptacle. A *Capsella* flower primordium, in such a condition, is shown in Pl. **19**, fig. 3. Considerable vacuolation has taken place in the peripheral areas of the future pedicel region with the result that the procambium is well marked. The future flower receptacle consists of typical, meristematic cells and shows the biseriate tunica, characteristic of both the vegetative and inflorescence apices. A corpus, too, is present consisting of a few layers of irregularly dividing cells. In Pl. **19**, fig. 3, the initiation of the first pair of sepals, by periclinal divisions in the second tunica layer, can easily be seen. The developing sepals soon show vacuolation in their abaxial halves which causes them to curve over the flower apex. Further growth is promoted by apical and marginal meristems. The initiation and development of the second pair of sepals are, in all respects, similar to those of the first pair.

Stamen initiation in *Capsella* is shown in Pl. **19**, fig. 4, which is of a longitudinal section of a flower primordium taken through the median plane of the second pair of sepals. Compared with Pl. **19**, fig. 3, there have obviously been a number of cell divisions in the corpus zone, and also the cells have become vacuolated. Most of the remaining dense meristematic cells form the biseriate tunica, in the second layer of which the stamen primordia have been initiated by periclinal divisions.

Following stamen initiation, the remainder of the flower primordium apex is given over to the formation of the terminal gynaeceum. Pl. **19**, fig. 5, shows how this last essential organ has been initiated by numerous periclinal divisions in the second tunica layer.

In the preceding account of flower part development, no mention has been made of petal initiation and development. Rather curiously, petal primordia are not easily seen in the young flower buds of *Arabidopsis* and *Capsella*. Pl. **19**, fig. 6, is of a longitudinal section of an *Arabidopsis* flower bud with quite well-developed sepals, stamens and gynaeceum. In the right half of the photograph, just inside the base of one of the sepals, is to be seen a small mound of dense, meristematic tissue with a single tunica layer. This is a petal primordium, and above it is a dense stamen anther. The retarded development of the petals is strange when one considers the comparatively large size which they finally attain. Although a study of petal initiation and development offers a number

of practical difficulties in *Arabidopsis* and *Capsella*, it is thought that actual initiation follows that of the second pair of sepals.

A study has been made of the development of procambium in the flower primordia and floral parts of the two members of the Cruciferae under consideration and the provascular meristem has always been found to develop in a continuously acropetal manner from the existing system. Pl. **19**, fig. 7, shows the acropetal development of procambium to two young *Capsella* flower primordia from the system already present in the inflorescence axis. The further acropetal development of procambium to the *Capsella* flower parts is shown in Text-fig. 3 B–D. The provascular meristem of the flower receptacle which grows into the developing stamens and gynaeceum differs from normal procambium in that its cells are merely brick-shaped, not elongated, and also in that the contents of its cells are not particularly dense. These differences make the identification of this type of procambium rather difficult, but it has always been found to develop continuously from the existing system. No definite statement can be presented regarding the mode of provascular meristem development to the petals because of their late maturation.

The vegetative and reproductive apices of *Anagallis*

All the growth zones, described for the vegetative apices of *Arabidopsis* and *Capsella*, are to be found in the corresponding apex of *Anagallis* and this plant, with its regular opposite and decussate phyllotaxis, is ideal for tracing the development of the zones throughout the course of a plastochrone.

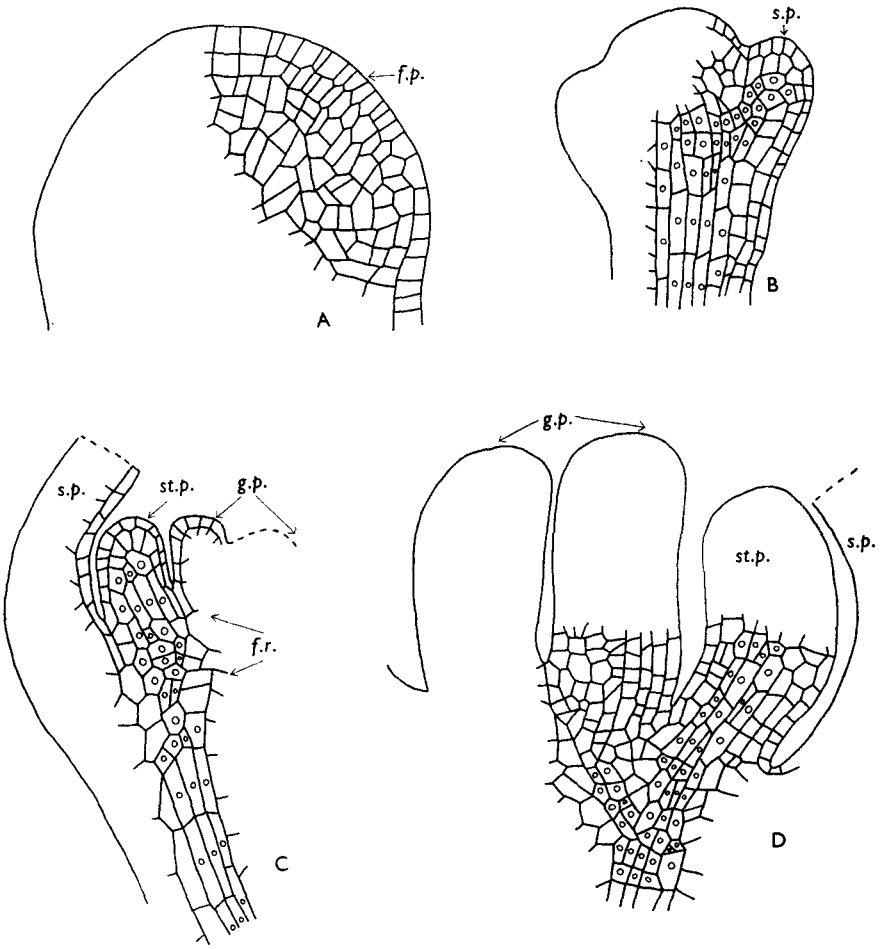
Vegetative apex

Pl. **20**, fig. 1, and Text-fig. 4 A, are of a median longitudinal section through the *Anagallis* vegetative apex showing the initiation of a pair of leaf primordia. The two opposite primordia are represented as small elevations, and they are still based on dense meristematic tissue, the foliar foundations. The actual apex, in between them, has been reduced to its minimal area and is quite flat. Its width, between the two primordia, is about 60 μ. Below the apex and its foliar primordia can be seen a swollen, vacuolated region. This is the nodal region of the last pair of leaf primordia to have been formed. These primordia are arranged at right angles to the ones shown in the illustrations. The nodes of this plant are characteristic in that the cell walls of the two surface layers of cells undergo considerable thickening which, in addition to well-marked periclinal divisions in the second layer, produce the swollen appearance.

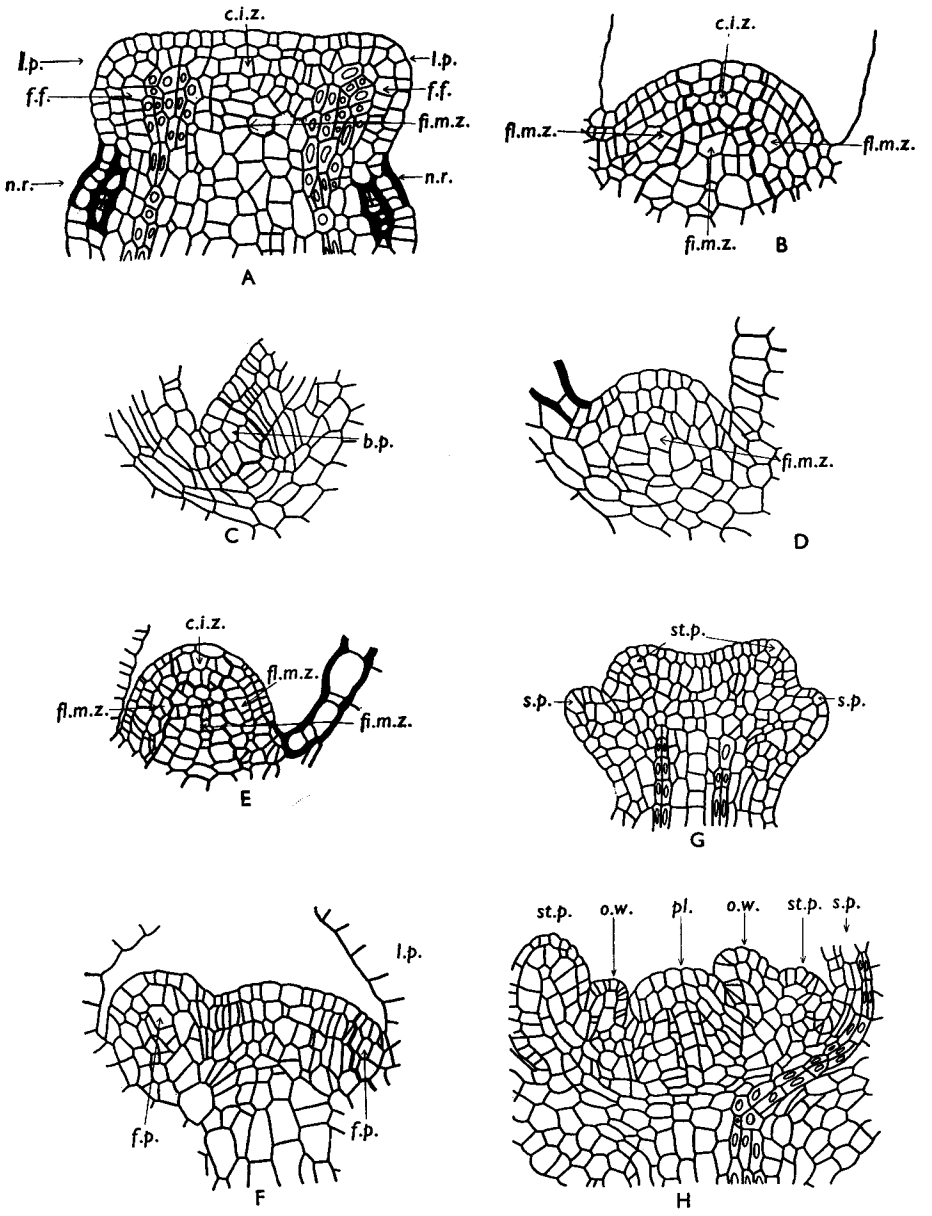
The foliar foundations, mentioned earlier, do not present a stratified appearance and it is obvious that, in these regions, a number of cell divisions and some reorganization have taken place. The leaf primordia themselves appear to have been raised by periclinal divisions in the second layer from the surface. An outstanding feature of reorganization in the foliar foundations is the establishment of procambium which can be seen running through the dense cells of the apical meristem, in both leaf primordia, to within three cells of the apices of the primordia. The procambium, in the foliar foundations, can be seen to be continuous with the procambium of the swollen node and internode previously mentioned so that the development of procambium to the leaves of this plant is regarded as acropetal.

Considering that part of the apex within the foliar foundations, a well-marked stratification can be observed. The two surface layers show no periclinal divisions, and so the vegetative apex of this plant is regarded as having a biseriate tunica. However, the remaining dense meristematic cells of that part of the apex now under consideration show various periclinal and oblique divisions and some of the products of these divisions give rise to vacuolated cells which must be regarded as constituting the file meristem. The vacuolation of these cells causes them to elongate in a plane parallel to the longitudinal

axis of the plant. Transverse divisions predominate in this zone, but longitudinal divisions are also to be observed, which serve to increase the diameter of the axis. The central cells of the surface layer of the apex between the two foliar foundations are much larger than the remaining cells of this layer. Anticlinal divisions are very prominent in the smaller cells of the surface layer. The central initiation cells of the apex under consideration are regarded as those including and below the very large central cells of



Text-fig. 3. (A) Longitudinal section of the *Arabidopsis* inflorescence apex showing the initiation of a flower primordium (*f.p.*) by periclinal divisions in the third flank meristem layer from the surface (× 666). (B) Longitudinal section of part of a *Capsella* flower primordium (× 339) taken through the median plane of the first pair of sepal primordia (*s.p.*). The cells with the nuclei drawn in form the procambium and it can be seen that this tissue is developing acropetally from the pedicel provascular meristem into the right hand sepal primordium. (C) Longitudinal section of part of a *Capsella* flower primordium (× 301) showing a sepal primordium (*s.p.*), a stamen primordium (*st.p.*) and the gynaeceum primordium (*g.p.*). The cells with the nuclei drawn in form the procambium and the continuously acropetal development of this tissue to the stamen primordium (*st.p.*) from the pedicel is shown. The procambium cells in the flower receptacle (*f.r.*) are rather brick-shaped. This is in contrast to the normal state of affairs where the provascular meristem cells are very much elongated. (D) Longitudinal section of part of a *Capsella* flower primordium (× 444) showing the gynaeceum primordium (*g.p.*), a stamen primordium (*st.p.*) and part of a sepal primordium (*s.p.*). The cells with the nuclei drawn in form the procambium and the continuously acropetal development of this tissue to the gynaeceum primordium (*g.p.*) is shown.



Text-fig. 4. (A) Longitudinal section of the *Anagallis* vegetative apex (× 251) at minimal area showing two leaf primordia (*l.p.*), the central initiation zone (*c.i.z.*), the file meristem zone (*fi.m.z.*), the foliar foundations (*f.f.*) and the swollen nodal region (*n.r.*). The cells with the nuclei drawn in form the procambium. (B) Longitudinal section of the *Anagallis* vegetative apex (× 245) at maximal area. For explanation of labels see fig. A; in addition, the flank meristem zone (*fl.m.z.*) is shown. (C) Median longitudinal section (× 250) showing an *Anagallis* bud primordium (*b.p.*) in the axil of one of the second pair of leaves of the main vegetative axis. (D) Longitudinal section (× 275) showing an *Anagallis* bud primordium in the axil of one of the fourth pair of leaves of the main vegetative axis. The file meristem zone (*fi.m.z.*) is now quite well developed. (E) Longitudinal section of an *Anagallis* axillary vegetative bud (× 200) showing the central initiation zone (*c.i.z.*), the flank meristem zone (*fl.m.z.*) and the file meristem zone (*fi.m.z.*). (F) Longitudinal section of the *Anagallis* inflorescence apex (× 275) taken through the median plane of the first pair of leaf primordia (*l.p.*). Two axillary flower primordia (*f.p.*) are shown. (G) Longitudinal section of an *Anagallis* flower primordium (× 200) showing sepal primordia (*s.p.*) and stamen primordia (*st.p.*). The cells with the nuclei drawn in form the procambium. (H) Longitudinal section of an *Anagallis* flower primordium (× 208) showing a portion of a sepal primordium (*s.p.*), portions of two stamen primordia (*st.p.*), the developing ovary wall (*o.w.*) and the developing placenta (*pl.*). The cells with the nuclei drawn in form the procambium.

the surface layer. Pl. **20**, fig. 2, and Text-fig. 11 B, show the apex at or just before leaf initiation. The new pair of leaf primordia will be formed in the flank meristem at right angles to those shown in the illustrations. During the course of the plastochrone, the apex increases in size until, at this stage, it measures about 120 μ across at its widest part and presents a marked convex appearance which contrasts strongly with its flat appearance at minimal area.

The two-layered tunica is very obvious, and the cyto-histological zonation is seen at its best. The central initiation cells of the surface tunica layers, by reason of their large size and vacuolated contents, contrast strongly with the rest of the cells of the layer. A regular appearance is presented by the central initiation zone except at its basal region where various oblique divisions are taking place to give rise to the file meristem. This vacuolated zone is now well encircled by flank meristem and shows its usual regular cell divisions. The dense flank meristem has grown to its full extent and appears to have reached its maximum depth of five layers of cells. As the apex enlarges during the period of the plastochrone, anticlinal divisions are mainly responsible for the growth of the flank meristem and, also, periclinal and oblique divisions serve to increase the depth of the zone and these divisions seem to take place mainly in the third layer of flank meristem.

As has been explained earlier, vegetative lateral buds are not visible macroscopically in *Anagallis* until the third or fourth node from the apex. However, some interesting information concerning their development is forthcoming if sections of the youngest nodes are prepared and examined microscopically.

Pl. **20**, fig. 3, and Text-fig. 4 C, are of a longitudinal section taken medianly through the second pair of the leaf primordia and, in the axil of one of them, is the first indication of axillary bud formation. The bud primordium is represented as a small nest of dense, meristematic cells. An interesting feature of its formation is that it has obviously been isolated from the main apical meristem by vacuolating cells which are very much compressed. There is no question of previously vacuolated cells regaining their meristematic properties to form this primordium. The bud primordium possesses a biseriate tunica developed from the tunica of the main promeristem. The rest of the primordium consists of a single layer of cells which are elongating at right angles to the apex surface prior to periclinal divisions.

In Pl. **20**, fig. 4, and Text-fig. 4 D, a bud primordium, in the axil of one of the fourth pair of leaves, is to be seen bulging above the plant surface. The biseriate tunica is intact and the increase in height of the primordium can be attributed to the production of dividing file meristem by the third layer of dense, meristematic cells mentioned in the previous paragraph. There are indications, too, of the establishment of a flank meristem zone. An older bud, shown in Pl. **20**, fig. 5, and Text-fig. 4 E, presents all the zones characteristic of the main promeristem. Compared with the stage of development illustrated in Pl. **20**, fig. 4, and Text-fig. 4 D, both the file and flank meristem zones have increased considerably in size, and the third layer of the central initiation zone is mainly responsible for these increases.

The prophylls of the buds are initiated by periclinal divisions in the second tunica layer and, up to this initiation, no procambium is found in the bud. At the formation of the prophylls (Pl. **20**, fig. 6), two procambium strands form in the flank meristem of the bud in connexion with the foliar emergences. These two strands of provascular meristem, at their formation, have no connexion with either the vascular system of the subtending leaf or the vascular system of the main axis. It has, however, been observed, from an examination of numerous series of sections, that, as the bud grows, its two procambium strands develop basipetally into the main axis there to merge with the two traces of the subtending leaf.

Inflorescence development

The marked transition from the vegetative rosette to the actively growing inflorescence in *Arabidopsis* and *Capsella* is not to be found in *Anagallis*. As has been described, the *Anagallis* vegetative shoot is a structure that grows actively in length and, when it enters the reproductive phase, it changes only in that flower buds replace the axillary vegetative buds. Pl. **21**, fig. 1, is of a longitudinal section through the reproductive shoot of *Anagallis*, the second and fourth pairs of leaves having been cut medianly. The flower buds are shown best in the axils of the second pair of leaves. The outstanding difference between the development of axillary primordia in vegetative and reproductive shoots is that, in the latter type of shoot, the buds quickly become conspicuous protuberances because, as has been described previously, the axillary bud primordia of the second pair of leaves of the vegetative shoot are small nests of meristematic cells not elevated above the surface of the plant.

The main promeristem of the reproductive shoot, apart from being more rounded, is essentially similar to that of the vegetative shoot. A biseriate tunica is present all through the course of a plastochrone and the cytohistological zonation into central initiation, flank meristem and file meristem zones is also shown. Each leaf of the reproductive shoot receives two traces as in the vegetative shoot so that a developing internode, in transverse section, presents four procambium strands.

Flower primordium

Pl. **21**, fig. 2, and Text-fig. 4F, are of a longitudinal section illustrating the initiation of a flower primordium in *Anagallis*. The two leaf primordia shown are the youngest pair on the reproductive shoot, and flower primordia, in different stages of development, are subtended by these developing leaves. In the axil of the foliar primordium, on the right, a flower primordium at the initiation stage is to be observed. This meristematic zone, that will later form a flower bud, is delimited from the main promeristem by a sector of compressed, dense cells and has been formed in the flank meristem zone of the apex. It is itself about three layers of cells deep and shows a biseriate tunica which is derived from that of the main promeristem. The developing flower bud, in the axil of the foliar primordium on the left, is further advanced, and now bulges above the apex surface, but the biseriate tunica is still evident.

Following initiation, the development of the flower primordium is very rapid, and Pl. **21**, fig. 3, shows one of these structures at the second node. The emergence is enclosed within a mantle of dense cells, about four layers deep, and a two-layered tunica is present in the flat apex of the primordium, but the second layer of the zone has been disturbed by periclinal divisions on the right to initiate a sepal primordium. The core of the developing flower is formed of highly vacuolate cells which show active transverse divisions and which resemble the file meristem of the vegetative apex. As far as can be made out, these cells originate from the third layer of meristematic cells from the surface of the flower primordium.

Text-fig. 4G shows an *Anagallis* flower primordium at stamen initiation, periclinal divisions in the second tunica layer being responsible for the formation of these flower parts. The pedicel has been differentiated from the receptacle, and its central vacuolated cells are undergoing extremely active transverse divisions. Two procambium strands are to be seen in this basal part of the flower primordium. The receptacle with its developing sepals and initiated stamens has also undergone a certain amount of vacuolation and, in fact, the only remaining dense meristematic region of the apex is the biseriate tunica.

Gynaeceum initiation is shown in Text-fig. 4H. The placenta is exposed at this stage of flower development and its two surface layers exhibit a dense, meristematic appearance. The lower of these surface layers seems to be involved in the production of cells which

quickly vacuolate and undergo active transverse divisions. These files of dividing cells are responsible for the vertical growth of the placenta and, around the placenta, the ovary wall is beginning to develop. Text-fig. 4 H also shows the acropetal growth of procambium from one of the pedicel strands to one of the sepal primordia.

As in *Arabidopsis* and *Capsella*, corolla development is retarded in *Anagallis*. Pl. **21**, fig. **4**, is of a longitudinal section of a flower bud where all the parts, apart from the corolla, are more or less fully formed. However, two petal primordia are shown that are obviously far behind the rest of the flower parts in development. The petal primordium, on the left, exhibits a well-marked apical meristem. An interesting feature, illustrated by Pl. **21**, fig. 4, is the acropetal development of procambium to the placenta of the gynaeceum from one of the pedicel strands. Pl. **21**, fig. 5, is taken from the same series of sections as Pl. **21**, fig. 4, and shows how, from the same pedicel strand mentioned in the previous sentence, procambium develops acropetally into a sepal, petal and stamen.

Although the corolla does not mature until some time after the stamens and gynaeceum, its initiation is to be observed at quite an early stage in flower development. Pl. **21**, fig. 6, is a median longitudinal section of a flower bud at the time of gynaeceum initiation. A slight bulge, to be seen at the base of the left stamen primordium, is the first indication of corolla formation. Its surface cells show active anticlinal divisions, while the subjacent cells have enlarged considerably.

It has already been shown (Text-fig. 4 G) that two procambium strands are present in the *Anagallis* flower pedicel and, also, that procambium develops acropetally from these strands into the flower parts (Text-fig. 4 H, Pl. **21**, figs. 4, 5), but no mention has been made of the relationship between the pedicel procambium and that of the reproductive shoot. Flower bud primordia, in all stages of development, have been examined, and it has been demonstrated conclusively that the two pedicel provascular meristem strands develop acropetally from the traces of the subtending leaf, one from each trace, into the developing flower primordium.

Comparison of axillary vegetative and flower buds

A comparative study of the axillary vegetative and flower buds of *Anagallis* provides some interesting information. The outstanding difference between floral and vegetative buds, in their early stages of development, is the more rapid enlargement of the flower bud. This type of bud is usually quite conspicuous at the second node, whereas the axillary vegetative buds do not become prominent until the third or fourth node.

However, there are many features common to the early development of these axillary structures. Both are detached portions of the main apical meristem, and both are delimited from this promeristem by zones of compressed cells. Each detached portion, no matter whether it will later give rise to a vegetative or a flower bud, consists of part of the three surface layers of flank meristem of the main promeristem which includes a biseriate tunica. The double tunica persists during the early development of both types of bud, and vertical growth is from a file meristem produced initially by the third layer of flank meristem. The development of all buds is essentially the same up to the stage where they protrude above the plant surface and consist of a mantle of about four layers of meristematic cells enclosing a central core of vacuolating file meristem (Pl. **20**, fig. 5, Text-fig. 4 E and Pl. **21**, fig. 3).

After this, the trend of development varies. In the vegetative bud, two foliar primordia are initiated in the flanks of the structure by periclinal divisions in the second tunica layer, and then the central part of the apex of the bud begins to function as the central initiation zone in the promeristem of the main leafy shoot producing file meristem basally and foliar foundations with their emergences laterally. However, no central initiation zone becomes established in the flower bud and, instead, its whole apical meristematic region is given over to the initiation of flower parts, the gynaeceum being terminal, Vacuolation takes place very rapidly in the future flower receptacle to leave a dense.

meristematic biseriate tunica, in the second layer of which are initiated the flower parts. This initiation is similar to the initiation of the foliar primordia of the axillary vegetative buds.

Both types of bud possess two procambium traces from which procambium develops acropetally into the foliar primordia of the vegetative bud and acropetally into the flower part primordia of the reproductive bud. However, although it has been shown that, in the vegetative bud, the two traces develop in association with the first pair of leaves and then grow basipetally to join the traces of the subtending leaf, in the case of the flower bud, the bud traces always develop acropetally from the existing traces of the subtending leaf.

Conclusions and discussion

The research that has been carried out into the structure and growth of the growing points of the main vegetative axes of *Arabidopsis*, *Capsella* and *Anagallis* shows conclusively that the tunica-corpus concept can well be applied to these apices, as it has been applied in many other dicotyledonous plants.

Popham (1951) has defined the tunica as a zone in whose cell layers no periclinal divisions occur except at leaf or bud initiation. During the present investigation, the tunica depth has only been considered in the growing vegetative apex in relation to leaf formation, for it has been found, at least in *Anagallis*, that the tunica, characteristic of the main promeristem, is retained at bud initiation. Also, as far as the material has been suitable, the tunica depth has been traced throughout the course of a plastochrone. *Anagallis*, with its opposite and decussate phyllotaxis, proved most suitable for this type of investigation.

Longitudinal sections of *Anagallis* vegetative apices at all stages of the plastochrone have been examined, and the number of surface cell layers showing no periclinal divisions, except at leaf initiation, ascertained. As has been described previously, the number of cell layers in this plant is characteristically two. The vegetative tips of *Arabidopsis* and *Capsella*, with their alternate phyllotaxis, proved more difficult material in which to study tunica depth through the growth period. In the case of *Arabidopsis*, the apex was studied at various stages of the plastochrone and the tunica appears to be biseriate in this plant, the second layer, as in *Anagallis*, being disturbed at leaf initiation. The growing point of *Capsella* was not studied in sufficient detail to provide accurate information on tunica depth.

It is hardly possible to draw wide conclusions of the phylogenetic significance of the tunica depth from a study of the vegetative apices of three plants from two widely separated families of dicotyledonous plants, but the biseriate tunica appears to be quite common in this group of Angiosperms. Popham (1951) has listed the tunica depth recorded for about fifty Dicotyledons, and this zone is said to consist of two layers of cells in half of these plants. A perusal of the many papers on stem apex morphology has shown that, quite commonly, an investigator draws his (or her) conclusions on tunica depth from a growing point at one stage of the plastochrone, and also includes in this zone cell layers which show occasional periclinal divisions, apart from those concerned with leaf initiation. This random assessment of tunica depth seems to be quite unjustified and, for this reason, it is suggested that many of the published statements concerning the number of cell layers in this surface zone are without very much significance. Most notice should be taken of those workers who have studied the depth of the tunica, in its strict sense, throughout the entire course of a plastochrone (Cross & Johnson, 1941) and those (Satina, Blakeslee & Avery, 1940; Dermen, 1945, 1947), who have employed induced periclinal chimeras in their investigations. There has, unfortunately, been comparatively little research carried out on these lines, and the plants studied have been chosen from widely differing families. However, it is significant that, as far as can be ascertained, the tunica in these plants is always biseriate and it may well be that a double-

layered surface zone is characteristic of the vegetative apices of all dicotyledonous plants. Some workers (Cross, 1937; Reeve, 1948) have reported fluctuations in the depth of the tunica during growth. These observations have not been confirmed during the present investigation.

The tunica-corpus concept has been elaborated in later years by a consideration of the cyto-histological zones present in the apex. This zonation, based on the nature of the cell contents rather than on the disposition of the cell walls, was first applied to certain Gymnosperms, but has now been used in descriptions of many Angiosperm apices. It is of great value in interpreting the corpus of the apex, but it does not supersede the original concept of Schmidt (1924). Rather, these two interpretations should be used in conjunction with each other, and together they afford a reasonable understanding of the anatomical and histological processes of a growing vegetative apex.

Central initiation, flank meristem and file meristem zones have been found in the vegetative apices of *Arabidopsis*, *Capsella* and *Anagallis*, and, in these three genera, the zones perform exactly the same functions. The flank meristem produces the epidermis, cortex, procambial system and leaves of the plant, while the file meristem produces the pith. The central initiation zone is the self-perpetuating region of the apex.

Again, a study of the growing point, throughout the course of a plastochrone, is necessary for a full understanding of the establishment and significance of these zones. This has been done only for *Anagallis*. The apex of this plant, at minimal area, is quite flat and appears to show, in longitudinal section, three surface layers of dense, meristematic cells and a fourth layer of irregularly arranged cells of a similar nature. This meristematic region, at least the three surface layers, constitutes the central initiation zone. As the apex enlarges prior to the formation of the next pair of leaf primordia, cells are cut off from the sides of this zone and also from its basal region.

Those cells, cut off from the sides of the central zone, form the surrounding flank meristem. The two-layered tunica is maintained during this cell production, but the cells produced by the third layer of the central initiation zone soon undergo numerous periclinal divisions to increase the depth of the flank meristem to about four or five layers of cells. As many authors have stated for other plants, these surrounding flank meristem cells stain more deeply with protoplasmic stains than the central cells. The same statement applies to *Arabidopsis* and *Capsella*.

In *Anagallis*, during the enlargement of the apex, the cells cut off from the base of the central zone, vacuolate and enlarge to undergo repeated transverse divisions to form typical file meristem. It is the activity of this zone that produces growth in length to form finally the large dome-shaped apex prior to the initiation of the next pair of leaf primordia. The work of Satina *et al.* (1940) and Cross & Johnson (1941) has shown that divisions in various planes in the third layer of the apex are responsible for the establishment of the corpus in the apices of the plants they studied. The observations now presented for *Anagallis* support this idea, and it is highly likely that the fourth layer of irregularly arranged cells to be observed in the apex just after leaf initiation is the first sign of file meristem production, and it is not strictly part of the central initiation zone. Dense cells, transitional between those of the central initiation zone and the typical vacuolate file meristem, have been observed in *Arabidopsis* and they have been described as forming a cambium-like zone (Vaughan, 1952*a*). A similar observation has been made by Ball (1941), Majumdar (1942), Philipson (1946) and Popham & Chan (1950). The claim made by the last pair of authors that the presence or absence of this intermediate zone is related to apex size has been disputed (Vaughan, 1952*a*).

In *Anagallis*, the file meristem can be followed for some distance behind the actual apex through a number of nodes and internodes, its cells enlarging in a longitudinal direction and undergoing divisions, mainly in the transverse plane. This high rate of cell activity is in contrast to the state of affairs in *Arabidopsis* and *Capsella* where the file meristem is of very limited development, its cells undergoing little longitudinal enlarge-

ment and very quickly passing over into typical pith cells. This difference in the activity of the file meristem in the plants studied is obviously related to the different growth forms, for the leafy shoot of *Anagallis* is an actively elongating structure with well-marked internodes, whereas both *Arabidopsis* and *Capsella* are typical rosette plants, with very short internodes.

Procambium development has been studied in the vegetative promeristems of *Arabidopsis*, *Capsella* and *Anagallis*. It has been found that procambium is always formed in relation to leaf initiation, and that the development of this tissue to the leaves of the plants under consideration is continuously acropetal. These observations are in accordance with the views of most recent workers (Majumdar, 1942; Esau, 1942; Philipson, 1949; Vaughan, 1952*b*).

The study of the initiation and development of the axillary vegetative buds of *Anagallis* has provided a certain amount of useful information. This aspect of developmental morphology has recently been reviewed by Philipson (1949) and Gifford (1951).

Whether bud primordia originate as residual portions of the apical meristem or by divisions of previously vacuolate cells is a matter of opinion. Support for the first view has been given by Louis (1935), Miller & Wetmore (1946), Philipson (1948) and Gifford (1951), but Majumdar & Datta (1946) report that bud primordia develop from vacuolate cells in the leaf bases in *Heracleum* and *Leonurus*. As far as *Anagallis* is concerned, the buds definitely originate as detached portions of the main promeristem and, as such, can always be recognized at the second or third nodes. It has been shown that these primordia become delimited from the apical meristem by a zone of compressed cells. This zone has been recognized by Gifford (1951), and has been described by Schmidt (1924) as the 'shell zone'. It is thus seen that the origin of buds in *Anagallis* agrees with the type of development described by most workers, and one might assume that this ontogentical process may follow a similar course in all vascular plants for Wardlaw (1943) has shown that the buds of certain ferns originate as persistent portions of the apical meristem.

It is of interest to note that, in *Anagallis*, the axillary bud primordia, when isolated by the vacuolation of the surrounding tissues, consist of three layers of dense, meristematic cells. The two surface layers of the primordium form a definite tunica, which is obviously derived from the biseriate tunica of the main promeristem. The cells of this bud tunica undergo only anticlinal divisions during the next stages of growth. The cells of the third layer of the primordium, on the other hand, undergo both anticlinal and periclinal divisions to produce, laterally, the third and fourth layers of the flank meristem and, basally, the actively dividing cells of the file meristem. The activity of the third layer of meristematic cells now described corresponds closely with the type of activity suggested previously for the third layer of cells of the central initiation zone of the main promeristem, and it is clear that bud primordium development in *Anagallis* really involves the establishment of a growing point similar to the main apical meristem.

Variance of opinion is also shown concerning the development of the procambium traces of axillary vegetative buds. Some authors have stated that the bud traces develop acropetally from the procambium of the main axis into the developing bud (Louis, 1935; Reeve, 1943; Miller & Wetmore, 1946; Philipson, 1948; Garrison, 1949), while others claim basipetal development for the traces before a union is made with the vascular system of the main axis (Majumdar & Datta, 1946; Gifford, 1951). It has been shown for *Anagallis* that the two bud traces arise in conjunction with the first pair of leaves and then develop basipetally to join the leaf traces of the subtending leaf. This corresponds with the observations of Ball (1952) on the isolated apex of *Lupinus* for, in a way, the detached bud primordium of *Anagallis* is similar to the experimentally produced isolated apex of *Lupinus*. Ball (1952) found that the procambium produced in the partially detached promeristem developed basipetally to join the vascular system of the main axis and that, until this union was complete, active vertical growth of the apex did not begin. A similar state of affairs is shown by the axillary buds of *Anagallis*, because

active branch development certainly does not start until the traces of the bud and of the subtending leaf have united.

The developmental anatomy of the change from the vegetative to the reproductive phase in flowering plants is a matter of some importance and, therefore, the development of the inflorescence in *Arabidopsis*, *Capsella* and *Anagallis* has been studied as carefully as possible. To relate the information that has been obtained for these plants to that obtained by previous workers, it would seem necessary to consider briefly the general morphology of their flower-bearing axes and, in doing this, it is obvious that the inflorescence units of *Arabidopsis* and *Capsella* differ markedly from that of *Anagallis*. *Arabidopsis* and *Capsella* resemble each other in that the truly vegetative parts of the plants are in the form of rosettes. The main inflorescence axis, in both types, normally develops directly from the rosette axis and grows vigorously in a vertical direction to produce laterally a few cauline leaves and then numerous flowers. In *Anagallis*, on the other hand, the inflorescence differs from the vegetative shoot only in that flower buds replace vegetative buds.

As far as is known, no work has been published relating to inflorescence development in the Cruciferae, but the researches of Philipson (1946, 1947, 1948), Lawalrée (1948) and Popham & Chan (1952) on plants, mainly of the Compositae, with capitulum type inflorescences are of interest here. The capitulum type of inflorescence is not found in either *Arabidopsis* or *Capsella*, but the inflorescences of these Cruciferae and of those Compositae that have been studied share one common relationship in that they both differ markedly in morphological form from the vegetative axes that bear them. Accordingly, one would expect certain common features of development.

Both Philipson (1946) and Lawalrée (1948) claim that one of the first signs of inflorescence initiation in the plants they studied is elongation of the dense cells of the vegetative promeristem and of the subjacent vacuolating cells, but Popham & Chan (1952), who studied *Chrysanthemum* plants grown under carefully controlled photoperiodic treatment, state that the processes of cell enlargement and active cell division in the file meristem zone of the apex are responsible for the inflorescence initiation. The results now presented for *Arabidopsis* and *Capsella* agree with the views of the last-named authors. During inflorescence initiation in these plants, there is no obvious elongation of either the central initiation zone cells or the flank meristem cells, but the file meristem cells certainly enlarge in a longitudinal direction and undergo repeated transverse and longitudinal divisions. This file meristem activity is so rapid that the young inflorescence appears as a dome-shaped structure with flank meristem running down its sides. Similar observations have been made by Philipson (1946) and Popham & Chan (1952).

A point of difference, however, between inflorescence development in the Cruciferae and the Compositae that have been studied concerns the persistence or disappearance of the central initiation zone of the apex. In both *Arabidopsis* and *Capsella*, and in the various Compositae that have been described by other authors, the central initiation zone is present in the apex of the young inflorescence. However, in the last-named group of plants, as the inflorescence primordium differentiates into pedicel and receptacle and the floret primordia are formed, the central initiation zone is lost and is replaced by flank meristem (Philipson, 1946; Popham & Chan, 1952). In *Arabidopsis* and *Capsella*, the central initiation zone persists in the inflorescence apex at all stages of development of the flower-bearing axis.

It would not be wise, until many more inflorescence types have been described in terms of the cyto-histological zonation concept, to make a definite statement on the importance of the persistence of the central initiation zone now described, but it is highly probable that there is a correlation between the persistence or disappearance of this zone and the habit of the inflorescence in the apex of which it is found. The capitulum of the Compositae is a structure of determinate growth, the florets soon occupying the summit

of its receptacle, whereas the raceme of *Arabidopsis* and *Capsella* is a structure of indeterminate growth which continuously undergoes vertical growth, its apex producing lateral flower primordia. In many respects, the inflorescence apex of *Arabidopsis* and *Capsella* resembles that of any normal elongating vegetative shoot.

From the information given previously concerning the developmental anatomy of *Anagallis*, it is clear that no great morphological change takes place in the plant axis at the formation of the inflorescence. The early appearance of the flower buds, in contrast to the delayed development of the vegetative buds, has been reported by Miller & Wetmore (1946) for *Phlox*, and by Boke (1947) for *Vinca*.

It is thus seen that the inflorescence apices of *Arabidopsis*, *Capsella* and *Anagallis* are direct developments of the vegetative growing point of the plant, showing tunica zones of the same depth and a similar cyto-histological zonation. In all cases, a biseriate tunica has been recorded.

The establishment of the flower primordia and their early development have also been studied in the three plants under consideration, and certain general conclusions have been reached. In *Arabidopsis*, *Capsella* and *Anagallis*, the flower primordia are initiated by periclinal divisions in the third flank meristem layer of the main promeristem, and they all retain, during their early development, a biseriate tunica and a corpus. The retention of the double-layered tunica, characteristic of the vegetative apex, in the early flower primordium has also been described for various plants by Satina & Blakeslee (1941) and Philipson (1947, 1948). This observation indicates a close relationship between the floral apices of *Arabidopsis*, *Capsella* and *Anagallis*, and the corresponding vegetative apices.

Additional support for this statement is provided by the study of the trace system of the flower bud of *Anagallis* which closely resembles that of the vegetative bud. Acropetal development of procambium to the flower primordia of *Arabidopsis* and *Capsella* has also been described, a statement which is in accordance with the views of other workers (Miller & Wetmore, 1946; Boke, 1947; Philipson, 1948).

The views of Grégoire (1938) are well known to the student of developmental anatomy. However, in later years, his results have been much criticized, especially his opinion that reproductive and vegetative apices are totally dissimilar. It has been shown that inflorescence apices of *Arabidopsis*, *Capsella* and *Anagallis* are morphologically the same as the vegetative growing points and, also, the floral apices of these species, up to a certain stage of development, resemble vegetative promeristems. At a later stage of development, the activity of a floral apex must clearly differ from that of an ordinary vegetative apex and, as far as can be made out, the difference is the result of a central initiation zone failing to develop, the floral parts being formed in rapid succession and occupying the entire surface of the flower receptacle. The corpus does not, as in the vegetative promeristem, retain any initiating properties but undergoes complete vacuolation.

Some information has been published concerning the initiation and development of the flower parts of various plants (Newman, 1936; Grégoire, 1938; McCoy, 1940; Satina & Blakeslee, 1941, 1943; Miller & Wetmore, 1946; Lawalrée, 1948; Boke, 1948, 1949). Generally speaking, the initiation of all flower parts has been described as taking place in the second tunica layer of the flower receptacle, but Satina & Blakeslee (1941, 1943) have claimed that, in *Datura*, the initiation of the stamens and carpels takes place in the third layer of the floral apex and, accordingly, these floral parts are more axial in nature than foliar. The results that have been obtained for *Arabidopsis*, *Capsella* and *Anagallis* demonstrate that, in these plants, all the floral parts are initiated by periclinal divisions in the second tunica layer. This type of initiation is, of course, similar to that of foliage leaves, and it gives a tentative indication of the foliar nature of the flower parts. The apical and marginal growth of the sepals and petals is also very similar to that in the developing foliage leaves.

The retarded development of the corolla in *Arabidopsis*, *Capsella* and *Anagallis* is

interesting, i.e. similar to that which has been observed by Miller & Wetmore (1946) in *Phlox*.

Finally, in this investigation, it has been found that procambium always develops acropetally from the pedicel into the flower parts. This is in accordance with the views of Grégoire (1938). The development of provascular meristem to the stamens and carpels is difficult to observe in their early stages, and Boke (1949), finding the same difficulty in *Vinca*, regards procambium development to the essential organs as a fairly late process. This acropetal development of procambium to the flower parts is similar to the state of affairs in developing foliar primordia and, again, this statement may be taken as a tentative indication of the foliar nature of the flower parts.

It is clear that the initiation and development of foliage leaves and flower parts have many features in common, but it would be unwise to place too great a stress on a similar homology between the two types of organs from the present evidence of developmental anatomy. It may be possible, in the future, to obtain further supporting evidence for the homology if certain techniques of the plant physiologist are combined with those of the anatomist but, at the moment, it would seem wiser to regard the floral members merely as lateral emergences concerned with reproduction.

Summary

1. The structure and growth of the vegetative, inflorescence and floral apices of *Arabidopsis thaliana* (L.) Heynh., *Capsella bursa-pastoris* (L.) Medic. and *Anagallis arvensis* L. have been investigated.

2. The vegetative apices of all three plants show tunica and corpus zones and the normal cyto-histological zonation.

3. Leaf primordia are initiated by periclinal divisions in the second tunica layer of the apex, and show apical and marginal growth during their early development.

4. Leaf procambium development is always continuously acropetal.

5. In *Anagallis*, axillary vegetative buds arise as detached parts of the apical meristem and their traces develop basipetally to join the traces of the subtending leaves.

6. No marked morphological changes are to be observed in *Anagallis* during the formation of the inflorescence, but, in *Arabidopsis* and *Capsella*, marked changes have been observed and have been fully described.

7. The inflorescence apices of all three species show tunica and corpus zones and a cyto-histological zonation. It has been shown that these apices are essentially similar to those of vegetative shoots.

8. Flower primordia are initiated by periclinal divisions in the third layer of the flank meristem of the inflorescence apex.

9. Procambium development to the flower primordia is continuously acropetal.

10. The floral apex shows tunica and corpus zones.

11. All the floral parts are initiated by periclinal divisions in the second tunica layer, and procambium development to these parts is again continuously acropetal.

12 A possible homology between foliage leaves and flower parts has been discussed.

13. In all three plants, the petals are the last of the flower parts to mature.

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EXPLANATION OF PLATES 18–21

(All photographs from untouched negatives.)

PLATE 18

Fig. 1. Median longitudinal section of an *Arabidopsis* vegetative apex (× 473).

Fig. 2. Transverse section of an *Arabidopsis* vegetative apex showing the central initiation (*c.i.z.*) and flank meristem (*fl.m.z.*) zones (× 288).

Fig. 3. Transverse section of an *Arabidopsis* vegetative apex (× 288), taken just below fig. 2, showing a foliar foundation (*f.f.*).

Fig. 4. Longitudinal section showing the early initiation of the inflorescence in *Arabidopsis* (× 255).

Fig. 5. Longitudinal section showing a fairly late stage in inflorescence formation in *Capsella* (× 237).

Fig. 6. Longitudinal section showing the initiation of a cauline leaf primordium (*c.l.p.*) in *Arabidopsis* (× 255).

PLATE 19

Fig. 1. Median longitudinal section of an *Arabidopsis* inflorescence apex (× 400).

Fig. 2. Median longitudinal section of an *Arabidopsis* inflorescence apex showing the initiation of a flower primordium (× 367).

Fig. 3. Longitudinal section of a *Capsella* flower primordium (× 297) showing the initiation of a sepal primordium (*s.p.*).

Fig. 4. Longitudinal section of a *Capsella* flower primordium (× 263) showing the initiation of stamen primordia (*st.p.*).

Fig. 5. Longitudinal section of a *Capsella* flower primordium (× 232) showing the initiation of the gynaeceum primordium (*g.p.*).

Fig. 6. Longitudinal section of part of an *Arabidopsis* flower primordium (× 550) showing a retarded petal primordium (*p.p.*).

Fig. 7. Longitudinal section showing the acropetal development of procambium to two young *Capsella* flower primordia (× 263).

PLATE 20

Fig. 1. Median longitudinal section of an *Anagallis* vegetative apex at minimal area (× 294).

Fig. 2. Median longitudinal section of an *Anagallis* vegetative apex at maximal area (× 294).

Fig. 3. Median longitudinal section (× 425) showing a bud primordium in the axil of one of the second pair of leaves of the main vegetative axis of *Anagallis*.

Fig. 4. Longitudinal section (× 450) showing a bud primordium in the axil of one of the fourth pair of leaves of the main *Anagallis* vegetative axis. The file meristem zone is now quite well developed.

Fig. 5. Longitudinal section of an *Anagallis* axillary bud primordium which shows the cyto-histological zones characteristic of the main vegetative promeristem (× 423).

Fig. 6. Longitudinal section showing the initiation of the prophylls (*pr.*) in the *Anagallis* axillary vegetative bud (× 283).

PLATE 21

Fig. 1. Longitudinal section (× 67) of the *Anagallis* reproductive shoot showing flower primordia (*f.p.*).

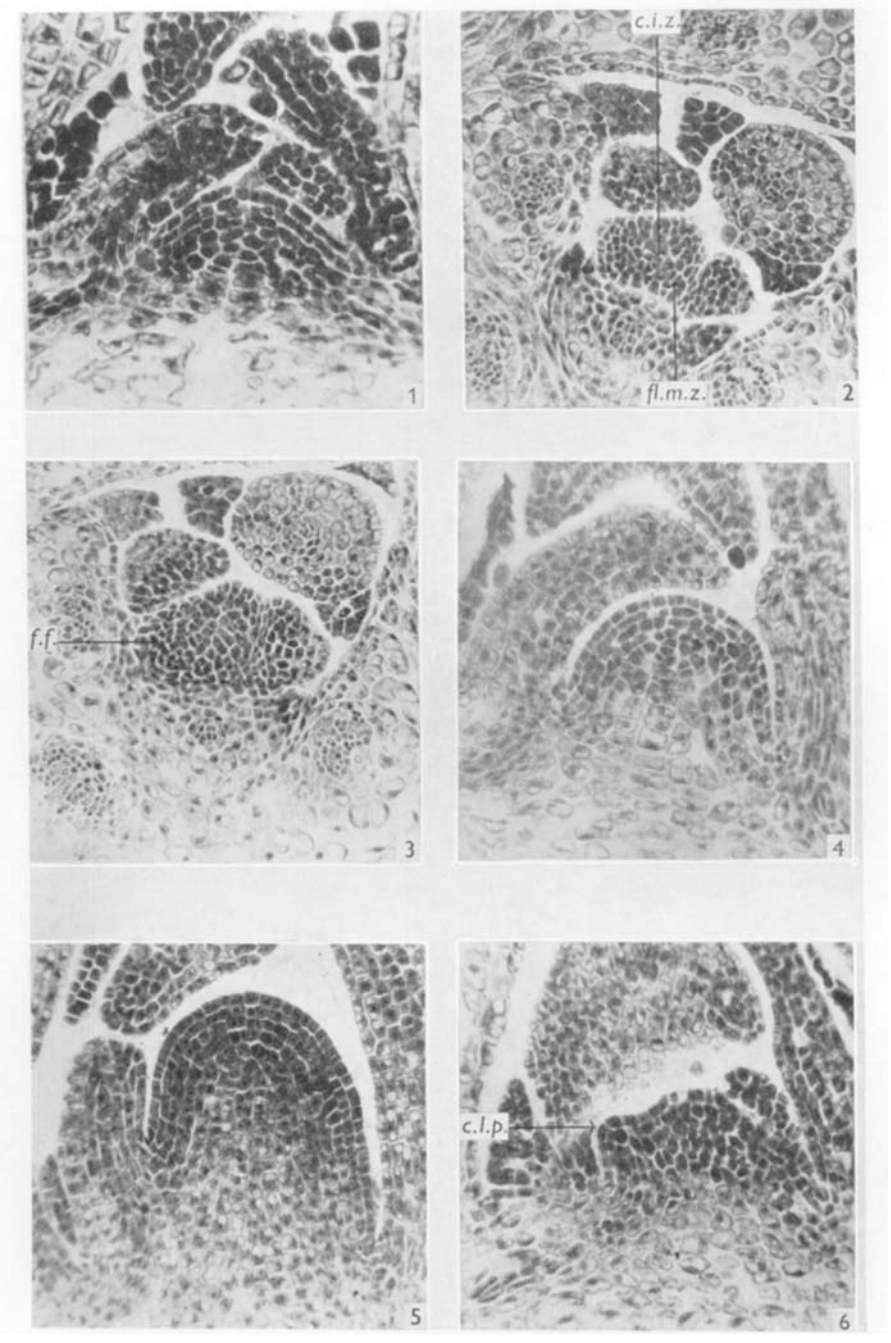
Fig. 2. Longitudinal section showing the initiation of flower primordia in *Anagallis* (× 282).

Fig. 3. Longitudinal section showing the initiation of a sepal primordium (*s.p.*) in *Anagallis* (× 273).

Fig. 4. Longitudinal section of an *Anagallis* flower bud with all the flower parts formed (× 48). Two petal primordia (*p.p.*) are shown.

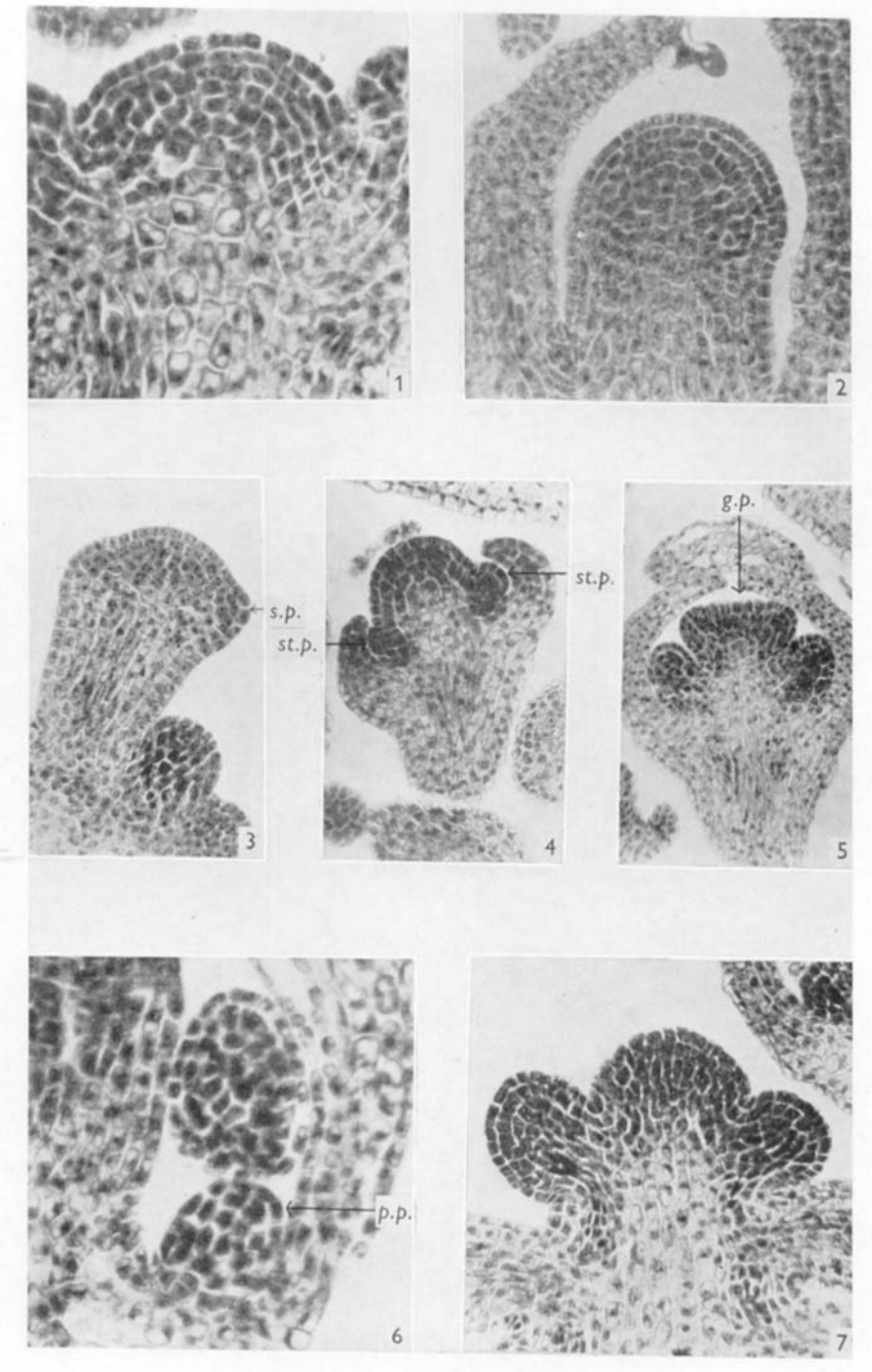
Fig. 5. Longitudinal section of an *Anagallis* flower bud showing the acropetal development of procambium to a developing sepal, petal and stamen (× 48).

Fig. 6. Longitudinal section of an *Anagallis* flower primordium (× 319) showing the initiation of a petal primordium (*p.p.*).

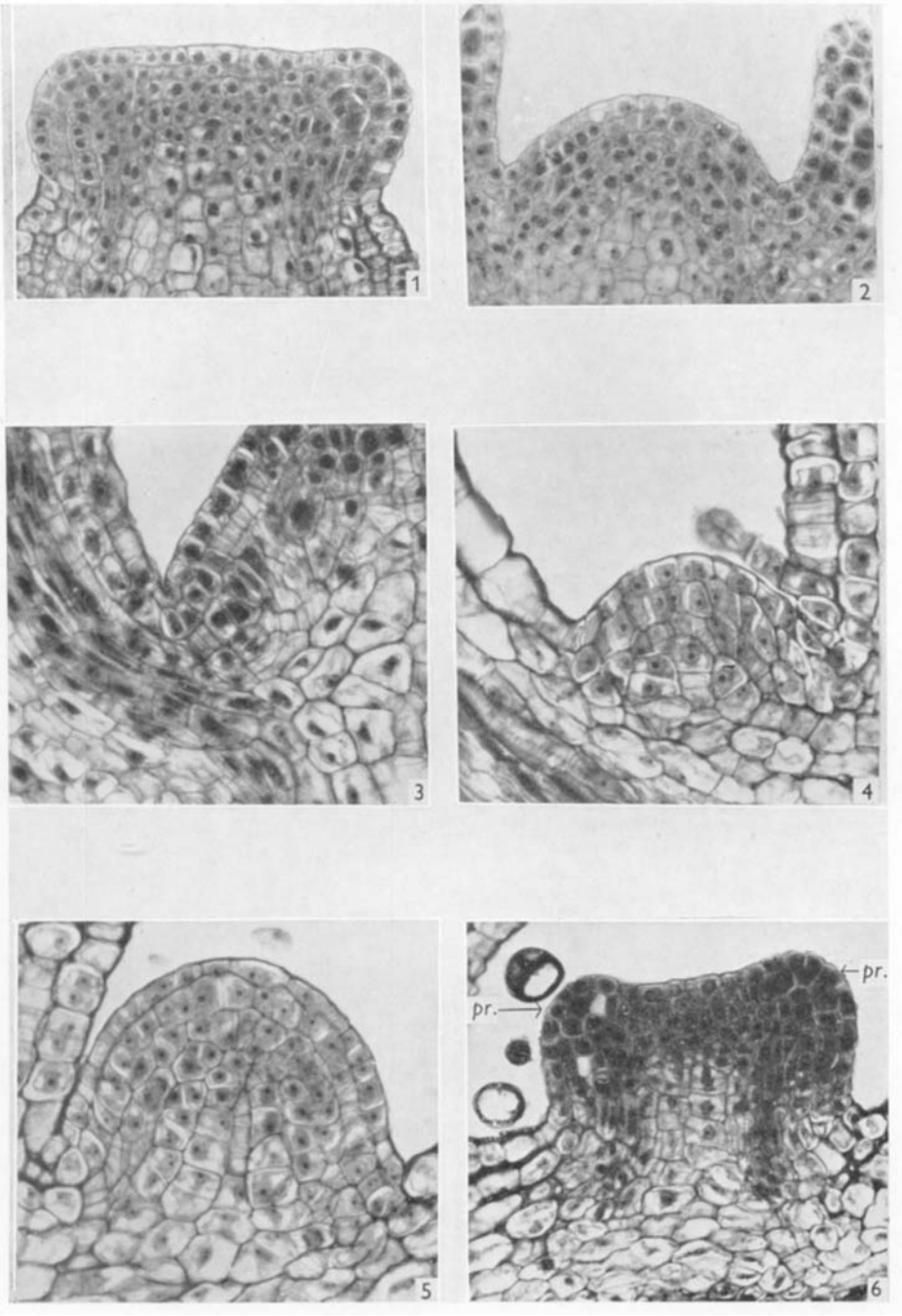


***Arabidopsis* and *Capsella* shoot apices**

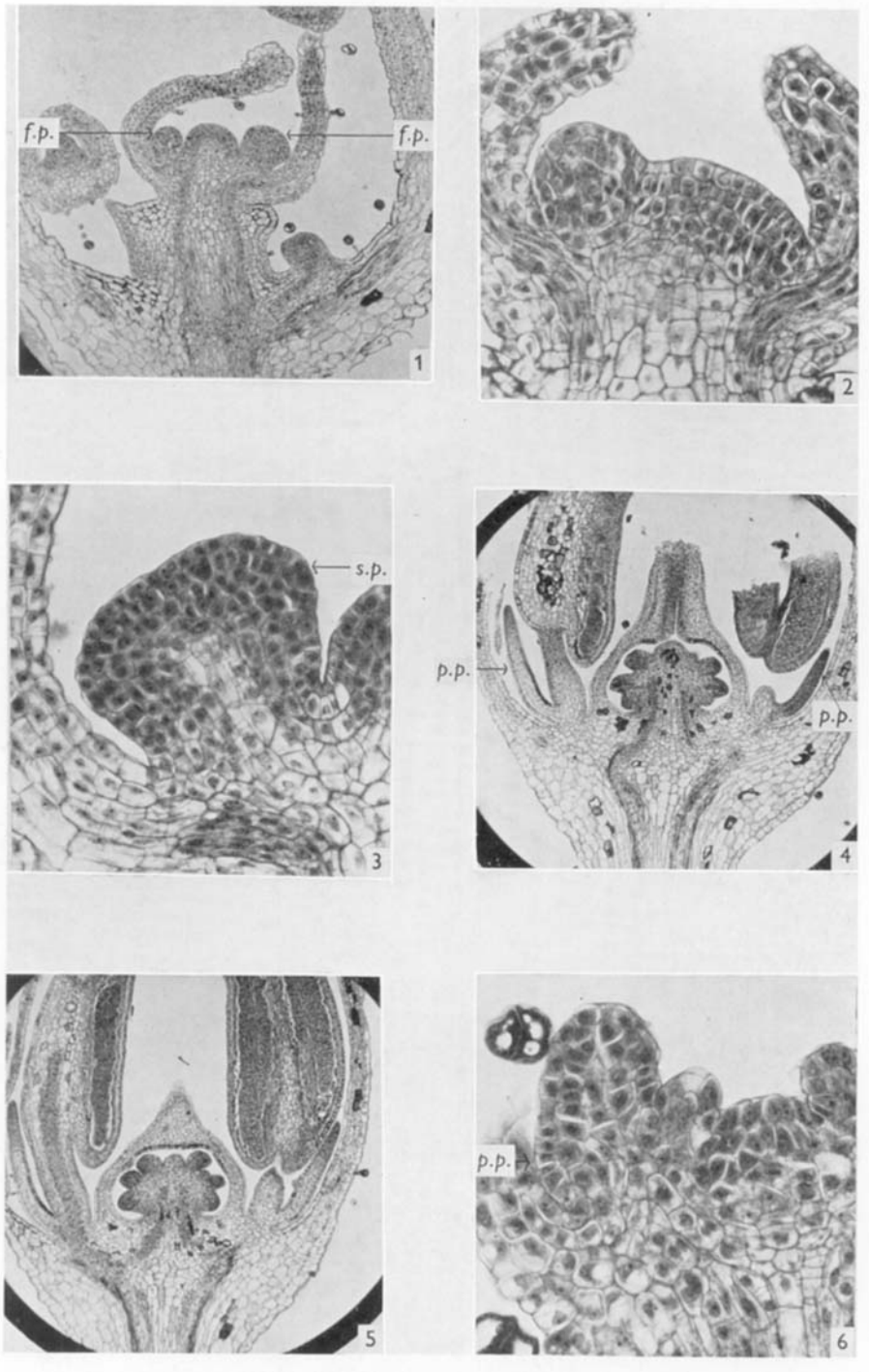
(*Facing p.* 300)



***Arabidopsis* and *Capsella* shoot apices**



***Anagallis* shoot apices**



***Anagallis* shoot apices**