THE EMBRYOGENY

AND

SEEDLING MORPHOLOGY OF « JUGLANS REGIA » L.

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RESUMEN

La embriogenia y morfología de la plántula de « Juglans regia » L. — En *Juglans regia* L., la triple fusión precede a la fertilización del huevo. Un número indefinido de mitosis de la cigota produce un embrión macizo con un suspensor grueso y corto. El meristema apical de la raíz y la piloriza son las primeras regiones que se diferencian. Es seguido por la diferenciación del hipocótile luego por el de los cotiledones y finalmente por el desarrollo del epicotile.

Los cotiledones inician su desarrollo por meristemas apical, marginal y adaxial, seguido por un crecimiento intercalar y marginal localizado que produce una gran distorsión y plegamiento de los cotiledones. Hay 4 trazas para cada cotiledón.

El epicotile es característico por poseer 7 brotes seriados en la axila de cada cotiledón.

El endosperma está al comienzo libremente nucleado, luego se vuelve celular. Se encuentra una gran estructura vacuoliforme durante toda su existencia.

La plántula tiene cotiledones hipogeos, con región hipocotile primaria muy desarrollada y un epicotile con hojas 3-8 pinadas y 7-8 brotes en serie en la axila de cada cotiledón.

El fenómeno de transición está limitado a las series originales de plasmodesmos en el embrión joven. El líber se desarrolla antes que el leño en la raíz. El primer protoxilema se origina en la parte inferior de las trazas cotiledonares.

INTRODUCTION

At the present time most of our knowledge of embryogeny and seedling development is extremely fragmentary. Certain investigators have studied the early phases of embryological development in many plants. These studies, however, have consisted chiefly of the early determination of the origin of cotyledons, root and plumule from definite regions of the proembryo. The later stages of development are either briefly described or disregarded entirely. In contrast to this type of observation, other investigators have dwelt principally upon the anatomy of the hypocotyl-root axis with special emphasis on the transition region. A few of these investigators have studied the general anatomy of the mature embryo, but in most cases, the anatomy of the mature embryo and the embryological development are not known of the particular plant upon which the anatomical study of the seedling is made. These investigations, in fact, have often been so limited that the plumular and cotyledonary anatomy have not even been included in the study. Thus, in one type of research, we have detailed studies of early embryology of many plants including, usually, general descriptions of cotyledonary, root and shoot growing-point origins; and, in the other type of research, we have intensive studies of the transition regions of entirely different species. Consequently, there is great need for complete and connected developmental histories from zygote through seedling for each plant investigated. A study of the later embryological stages, in particular, would lead to a better understanding of the seedling structure.

In the present investigation, therefore, an attempt is made to give the sequence of development in *Juglans regia* from zygote through the one year old seedling. Because of the large field of investigation that this involves, many phases of this development had to be rather briefly covered. Nevertheless, a more complete sequence of development is included herein than in most papers. The study of fertilization and the early and late embryogeny has been more intensive than that of the seedling. In the latter, the general morphology has been given with special reference to the cotyledonary bud development.

Since the development of the flower parts, embryo sac and fruit of *Juglans regia* have benn investigated previously by the writer (23), the present paper logically begins with an account of the process of fertilization.

In view of the absence of critical comparative information on the embryogeny and seedling morphology of specific angiosperm types, no general discussion of the implications of the present study can be attempted. However, under each section, a critical examination of current morphological ideas is given with particular reference to the results of the present study.

MATERIAL AND METHODS

Material of *Juglans regia* L., variety Concord, was collected during 1936 and 1937 at the University of California at Davis. Measurements of fruit, seed and embryo were made for all collections during the two years. The rates of growth of the fruit and seed are approximately parallel, the greater part of the enlargement taking place the first month after fertilization. During this period the embryo grows slowly, the bulk of the seed consisting of endosperm which is a thin layer of parenchymatous tissue enclosing an enormous vacuole-like structure. After the fruit and seed reach their approximate mature size, the embryo increases rapidly and completes its growth in length during the second month. During the remaining portion of the growth season, the most important changes are the increase in thickness of the cotyledons and the completion of sclerosis in the ovarian tissues to form the shell of the « nut ».

The hardening of the walnut shell begins about the time the fruit has almost reached full size. Before sclerosis begins, however, the shell region is distinguishable from the husk as a white solid parenchymatous tissue. Sclerosis starts at the outer edge of this shell region and progresses towards the center of the fruit, but only advances about half of the distance. The innermost half of this white tissue eventually turns into a

brown, dried and shriveled papery lining within the hard shell. In recent years the walnut growers of southern California have used the browning of this inner region as the criterion for the maturity of the « nut ». In contrast to the above formation of the shell in the English walnut, the parenchymatous tissue of the black walnut starts to harden at the inner as well as the outer edge, progressing to the central portion of the shell region. Thus there are eventually formed islands of nonsclerotic tissue within the shell, these regions later becoming the lacunae of the shell.

The entire fruit was fixed for only the fertilization stages and very small embryos. Embryos up to 5 mm. in size were fixed within the ovule and most of the larger than 5 mm. embryos were dissected out of the ovule for convenience in orientation while cutting. Formalin-acetic alcohol combinations and Karpochenko's killing and fixing formulae were used. Air was removed from all fixations by suction. The material was embeded in paraffin in the usual manner and cut 5-25 microns. Most of the slides were stained with the tannic acid ferric chloride stain (Foster 8), which was especially excellent for cell walls of the meristematic tissue.

Seedlings were obtained readily either from mature « nuts » or immature « nuts », i. e. « nuts » which still retained the husks and where the seed coat was not brown. Peat moss was first used so that the seedlings could be removed free from soil particles. The seedlings, however, did not mature normally in the moss. Roots seemed to have difficulty in emerging from the shell, and occasionally epicotyls remained inside of the shell, the cotyledonary buds developing in place of the apical bud of the plumule. Some of this material proved valuable in cotyledonary bud study. For the later plantings oak leafmold, in which the embryos developed normally, was used entirely. Germination starts about 15-20 days after planting.

FERTILIZATION

Fertilization in *Juglans regia* L. and *Juglans nigra* L. has been described by Karsten (19) and Nawaschin (25), but neither author has convincingly substantiated his explanation of the process by illustrations. Although Karsten described the fertilization process in great detail, he ilustrates only one embryo sac, in the micropylar end of which an immense pollen tube containing two sperms is shown. It is interesting to note that these sperms are sketched and also described as small round homogeneous spheres, which the present author would interpret as nucleoli rather than nuclei. This interpretation seems plausible if one considers that Karsten has made an error in representing the antipodal nuclei also as round darkly stained spheres. Clearly in this case, these spheres must be nucleoli (See photomicrographs Pl. I, figs. 1 and 2 and drawings Text. fig. 1, A and B for the structure of antipodal nuclei). According to Karsten, a previous polar nuclear fusion is not required in *Juglans*. The polar nuclei at the time of fertilization lie far apart, and only one unites with the sperm. This union also stimulates division in the other polar nucleus with subsequent endosperm development. Thus, endosperm cells would have chromosome numbers of N and 2N. When the two polar nuclei were seen adjacent to and flattened against one another, he considered that these were cases of unfertilized embryo sacs.

Nawaschin (25) has two illustrations of sperm nuclei, one from J. *nigra* representing the two sperm as small darkly stained roundish bodies lying in a clear or hyaline area approximately the size of the egg nucleus, the other from *J. regia* showing the sperms curved and somewhat more elongated in a hyaline region. He surmises from these observations that the sperm nuclei of *Juglans* probably enter the embryo sac in an undeveloped state enclosed within their mother cell, whose remains are represented as an hyaline drop. He believes that the sperms at first are roundish to oval, and later become curved bodies as in *J. regia,* in which condition they are motile. He does not believe that they are attenuated coiled bodies

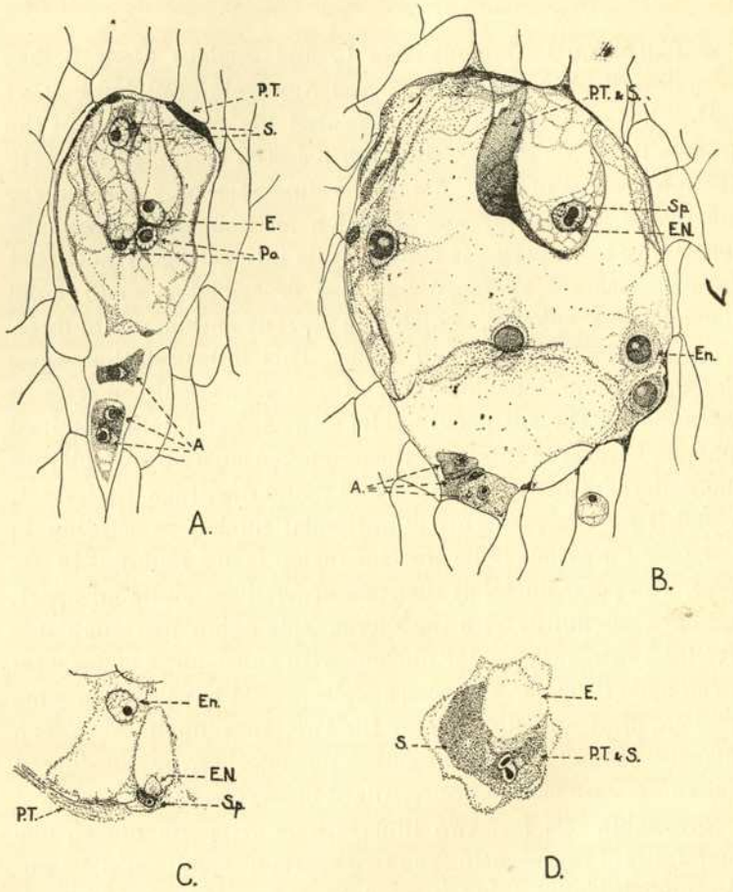

Fig. 1. — A, Embryo sac previous to fertilization ; B, Embryo sac showing egg and sperm nuclei uniting, and a four nucleated endosperm ; C, An elongated sperm nucleus lying adjacent to the egg cell. Egg nucleus at a slightly lower focus ; D, Shows dark bodies in degenerating pollen tube synergid mass. From cross section of the ovule. (All figures × 664). Antipodals A ; egg cell E ; egg nucleus E. N. ; endosperm nuclei En. ; polar nuclei Po ; pollen tube P. T. ; sperm Sp. ; synergid S.

such as he found in *Lilium Martagon* L. and *Fritillaria tenella* L.

Neither of these workers illustrated the sperm and egg or the polar nuclei uniting or even lying adjacent to one another. Nawaschin, however, stated he saw sperms penetrating the sac and some in immediate contact with the egg.

A normal embryo sac previous to fertilization is shown in text figure 1, A. The egg nucleus lies in the antipodal end of the egg cell and the two polar nuclei, adjacent to the egg apparatus, are near one another but not united. The pollen tube, which penetrates the ovule through the chalaza (Nawaschin **24**), enters the embryo sac, however, at the micropylar end. Plate I, figures 3 and 4, which are two views of the same sac after fertilization, show a pollen tube as a darkly stained structure at the left. This roughly indicates the usual path of the pollen tube. The diameter of the tube as it passes through the nucellar tissue and along the side of the sac is very small. The contents at the tip are so dense and stain so deeply that structural details could not be seen. In several cases, however, slightly enlarged tips revealed nuclei of extremely small size.

In *Lilium Martagon*, according to Blackman and Welsford (**1**), the tube cytoplasm passes between the cells of the egg apparatus and the cytoplasm of the polar nuclei, but does not enter the cytoplasm of the embryo sac. It seems that a somewhat similar condition occurs in *J. regia*. Plate I, figure 2 shows a dark staining mass which follows the contour of the egg cell. This mass is the degenerating remains of the pollen tube and probably part of a disintegrating synergid. In plate I, figure 1, which is cut in the opposite plane, contents of a pollen tube appear in transverse section as two more or less triangular black masses one above the polar nuclei, the other at the micropylar end of the egg cell. The latter indicates the aproximate place of penetration. Hoare (**14**) and others have reported that one synergid usually is disorganized by pollen tube entrance. Sax (**29**) believes that the synergids function in some way in controlling the entrance of the pollen tube. The position of the synergid nuclei in *Juglans* (Text fig. 1, A) might substantiate this hypothesis. Their position in their respective cells, in

contrast to that of the egg nucleus in its cell [1], is micropylar. These nuclei may by some chemotactic stimulus impel the pollen tube to enter at a particular place. It seems strange, otherwise, that the tube does not shorten its route to the egg by entering through the antipodals.

After entrance of the pollen tube the synergid next to it degenerates but remains along with the pollen tube as a dark body applied to the side of the egg (Text fig. 1, B). The other synergid, which usually disappears early, may remain during the process of triple fusion. This is true of the synergid in the sac photographed in plate I, figure 2, where a synergid, although not shown in the picture, is located to the left the egg at a lower level of focus.

Triple fusion precedes union of egg and sperm. The sperm nuclei are very small, approximately two microns in diameter, when enclosed within the slightly swollen tip of the tube and after they are expelled. In two cases they were found to have an irregular shape (Text fig. 1, C) when located at the surface of the egg cell. Blackman and Welsford (1), who observed pointed nuclei in *Lilium*, consider this shape as indicative of motility.

The polar nuclei and sperm fuse simultaneously as show in plate I, figure 2, where the two polar nuclei in interphase are uniting with a slightly granular sperm nucleus. During this fusion the polar nuclei become variable ln shape. Their membranes, if present, are indistinct, and they, as the adjoining cytoplasm, are capable of absorbing stains very readily. No distinct prophase of either polar or sperm nuclei, which has been reported recently in many other angiosperms, was observed.

The sperm must enter the egg while still small, because in a few cases very small sperms of a diameter of two microns were seen in the egg cells. Entrance must be accomplished while triple fusion is taking place, as embryo sacs showed both

[1] Cell is used here because egg and synergid protoplasts are distinct from the other protoplast of the embryo sac. This is especially true of the egg cell which appears to have a fairly distinct membrane.

triple fusion and the egg-small sperm combination. These sperm, however, enlarge to practically the size of the egg nucleus at the time of fusion (Text fig. 1, B). At this time the primary endosperm nucleus has undergone at least two divisions. The remains of the pollen tube and one synergid stay applied to the egg cell, and persist until the embryo is as large as shown in plate III, figure 14 or even as large as in plate V, figure 18.

Round and curved bodies in clear areas, similar to the ones interpreted by Nawaschin as sperms, have been found many times in the degenerating pollen tube-synergid mass and along the periphery of the embryo sac (Text fig. 1, D and Pl. II, fig. 5). These, I believe, are disintegrating sperm, tube and synergid nuclei, or even nuclei of the nucellar cells which are crushed by the enlarging embryo sacs.

Fertilization of *J. regia*, variety *Concord*, occurred about April 10 in 1936 and April 20 ind 1937 at Davis, California. Collections made on April 9, 10 and 11 in 1936 and April 19-21 in 1937 had both triple and egg-sperm fusion. The latter fusion must, therefore, closely follow triple fusion probably within a few hours. Langdon (21) also found that the interval between the two fusions was very brief in *Juglans mandshurica* Miq. and *Carya glabra* Sweet.

EMBRYOGENY

Early embryogeny: About ten days after fertilization the zygote of *Juglans regia* divides. Reports in other *Juglandaceae* of the interval between fertilization and the first zygote division are as follows : *Carya glabra* Sweet seventeen to eighteen days (21), *Hicoria Pecan* Britt. about three weeks (41), *Juglans mandshurica* Miq. six to seven days (21). The two *Juglans* species are remarkably similar in this respect.

The plane of the first divisions of the zygote in most cases, according to Schüepp (31), determines the length of the early embryo filament. The first division, he states, always takes place by a cross wall, while the second divisions may occur

either in a longitudinal or in a transverse plane. Thus, there may be formed three types of tetrads, which would yield three types of embryos. His tetrad types, *a)* and *b)*, produce embryos with suspensors. In type *a)* both the basal and apical cells of the two-celled embryo divide by cross walls, while in type *b)* the basal cell divides by a cross wall and the apical cell by a

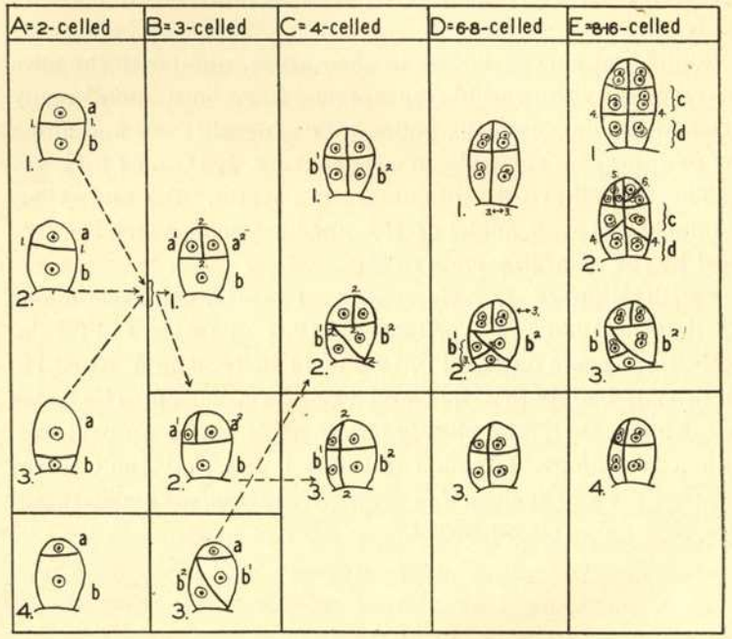

Fig. 2. — Chart of Type I embryos

longitudinal wall. In type *c)*, which is rare, both basal and apical cells divide longitudinally. This type yields an embryo without a suspensor.

Juglans regia does not fall into any of Schüepp's groups or proembryos. The great variability in the early divisions of the embryo of this species is best explained by the aid of diagrams. The plane of divisions of the zygote makes it possible to group the two-celled embryos into two general types. In type I (Text fig. 2), the first wall may be straight or slightly oblique. This wall may divide the zygote into approximately equal cells

as in A-1 and A-2, or into very unequal cells as in A-3 or A-4. Plate II, figure 7 is a photomicrograph of such an embryo. In type II, on the other hand, the first wall may be at various obliquities (See Text fig. 3, embryos A-3 and A-4, and A-5). Plate II, figure 8 is a photomicrograph of an embryo of this type. Embryo A-6, of Type II is a hypothetical case, which will be explained later.

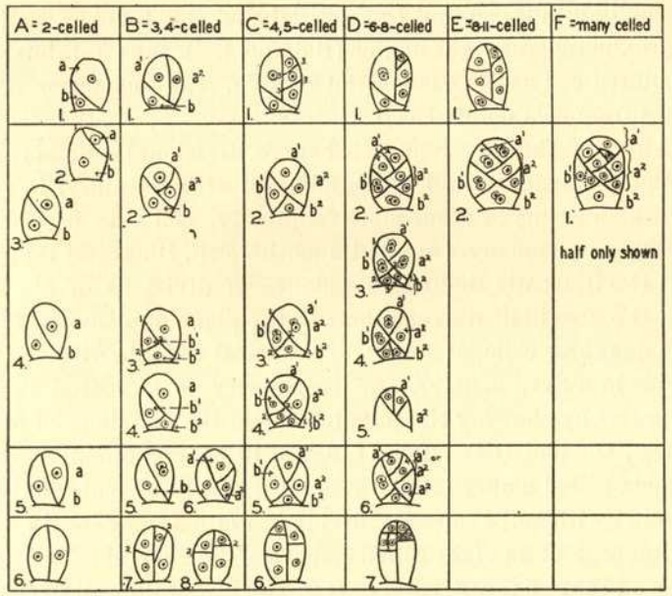

Fig. 3. — Chart of Type II embryos

The division of the two resulting cells which are designated as cell *a*, or the apical cell, and cell *b*, or the basal cell of the two-celled embryo, usually does not occur simultaneously. In type I cell *a* divides longitudinally although the two resulting cells a^1 and a^2 may be of different sizes (Text fig. 2, diagrams B-1 and B-2). The division of the basal cell, cell *b*, may be delayed or in cases where it is larger than cell *a*, it may precede the division of the later (B-3). Cell *b* has been found to divide either longitudinally (C-1 and C-3) or obliquely (B-3 and C-2).

Plate II, figures 9*a* and 9*b* are two views of the same four-celled embryo which is identical to diagram C-1. This embryo then would appear to be of Schüepp's type *c)*, the type which does not produce a suspensor. The walnut embryo, however, develops a short stocky suspensor.

In type II, either cell *a* or cell *b* may divide first, or a simultaneous division of the two cell may occur. Cell *a* may divide in at least three ways as illustrated in B-1, B-2 and in C-3. Cell *b* usually divides so that the wall labeled 2-2 touches wall 1-1 approximately at right angles (B-3 and B-4), thus establishing a pointed cell at the base of the embryo. The suspensor originates from this pointed cell.

After the three- or four-celled stage, divisions occur so rapidly that sequences could not be followed with certainty. In type II, which seems to occur more frequently, cell a^1 is capable of dividing in as many ways as its mother cell. Diagrams D-2, D-3 and D-5 illustrate the possible planes of divisions of a^1; D-2 and D-3 also indicate variations in the plane of cleavage of a^2. The daughter cells, b^1 and b^2, of the basal cell likewise do not divide in a fixed manner. The irregularity is indicated in the diagrams by showing the possibilities in the divisions of b^2 (C-4, D-2, D-3 and D-4). Plate II, figure 10 is a photomicrograph of a six-celled embryo illustrated most nearly by embryo C-3 of Type II with cell b^2 already divided. Plate II, figure 12 is a photograph of an eight-celled embryo of the general cell pattern shown in text figure 3, embryo D-3. The first wall, wall 1-1, probably was in the position shown in embryo A-2, which would account for the small cells at the apex.

In embryos which have developed from a zygote divided by a cross wall, there is also variation in cell patterns. Diagonal divisions of b^2 may produce an older embryo which is essentially like that produced from type II, that is, one with a few diagonal cells in the suspensor (Pl. III, fig. 14). A few embryos were found to possess three tiers of cells as shown in E-1 and E-2 of text figure 2. These probably arose from an embryo similar to C-1 or C-3. Whether the wall marked 4-4 in E-1 succeeds or precedes wall 3-3 as shown in D-1 is questionable. The third tier is not formed, however, previous to this as cell *b*

was never found to divide by a cross wall, a condition which seems to have been observed most frequently by Souèges in his numerous embryological studies and by many other workers. Diagonal walls (4-4 in E-2) are probably formed by displacements of original cross walls, although it is possible that they were laid down at an angle from the beginning. The four daughter cells of cell *a* divide either by a wall as illustrated by 5-5 or by 6-6 in embryo E-2. Mitoses are rapid at this time and periclinal walls soon appear. The precision of cell sequences as worked out by Souèges in embryos with long or fairly long suspensors could not be determined in *Juglans regia.*

Certain embryos showed other variations than those which have been explained. A five-celled embryo was observed of the constitution C-1 in text figure 3. Embryos A-1 and B-1 indicate the possible stages in the formation of this type of embryo. This is a case, no doubt, of delayed division in cell a^1. Diagrams D-1 and E-1 illustrate how this embryo may finally develop into a rather irregular three- or four-tiered structure. Several embryos appeared to have arisen from a two-celled embryo with an extremely oblique wall (Text fig. 3, A-5). Plate II, figure 11 represents un embryo of this type in which the divisions probably followed the plan as given in type II, A-5, B-5 or B-6, C-5 and D-6. The embryo shown in plate III, figure 13 may have arisen from successive cleavages as in type II, A-6, B-8, C-6, D-7, or from an embryo of type I illustrated in text figure 2 as embryo C-1 or C-3 with the cross walls somewhat shifted by subsequent divisions. Examination of the serial sections through this embryo seem to indicate that wall 1-1 was laid down first because it appears in all sections. If, therefore, it has arisen from a zygote divided by a longitudinal wall, the bicellular embryo would be of a type not considered by Schüepp. It seem probable that this kind of two-celled embryo could have arisen merely by a shift of the wall of an embryo such as depicted in text figure 3, A-5.

The present study reveals the three following important facts about the embryo of *Juglans regia:* 1) the zygote may divide by a cross or more frequently by a diagonal wall, 2) no fixed sequence of cell divisions or cell pattern is apparent, 3) the

suspensor end of the young embryo is rather broad and generally constructed of pointed cells.

Oblique divisions of the zygote have been reported by Langdon (21) in *Carya glabra* Sweet and illustrated in *Lamium purpureum* L. and *Valerianella olitoria* Poll. by Souèges although not mentioned by him in his text. Both of these investigators also have indicated obliquities in the segmentation of the basal and apical cell of the bicellular embryo. It is interesting to note that in the embryos of *Valerianela olitoria* Poll. (Souèges, 37) and of *Trifolium minus* Rehl. (Souèges, 39, 40) where the basal cell of the bicellular embryo is divided by an oblique partition somewhat similar to that found in *Juglans regia*, the small embryo is more massive and irregular in its development. Certain tiers appear rather indefinite also in those embryos of *Lamium purpureum* (Souèges, 32) in which the basal cell has divided by an oblique wall. In the embryogeny of *Salix,* Chamberlain (3) observed certain « peculiar embryos » which departed from the « normal » and « ... for a time seem to have an apical cell ». These embryos have oblique walls like *J. regia,* and, in fact, his figure 62 would be identical to the embryo D-2 of text figure 3 if cell a^1 was shown undivided. Souèges does not mention any such deviations from the so called normal in his article on the Salicales (38). Throughout his intensive embryological studies, Souèges is interested primarily in tracing the origin of certain cell tiers in the sixteen-celled embryo and the subsequent development of embryo regions from these tiers. In very few instances does he mention developmental deviations that do not fit into his schemes. Only in his work on *Trifolium minus* (39, 40) and *Valerianella olitoria* (37) does he appear to have difficulty in applying his plan of tier construction. He does indicate that there is some relationship between oblique divisions of the basal cell and the rather complex mode of embryo formation of *Valerianella* for he says, « Étant données les directions obliques des segmentations dans les cellules primoridales du proembryon, on ne peut être surprise de la complexité qu'offrent les formes embryonnaires, quand elles se composent d'un nombre considérable d'éléments ». But even here he attempts to establish, although inadequately, these tiers.

From the study of *Juglans regia*, from the examination of articles by Souèges and Chamberlain, and from the stages of embyro development reported by Miss Langdon in *Carya glabra* and *Juglans mandshurica* it seems that there is a direct correlation between obliquity of the first few walls of the embryo and the indefinite sequence of cell divisions which also leads to the absence of regular tier formation, In addition, the suspensors of these embryos are not of the linear *Capsella* type, but are short and thick.

Certain wedge-shaped apical cells have been reported in several-celled embryos. According to Souèges, the upper cell of the two-celled embryo instead ol dividing by a transverse wall, may divide obliquely cutting off a cell which looks like an apical cell and which he calls the « epiphyse ». This is the case in the six-celled embryo of *Trifolium minus,* and in the eight-celled embryos of *Geum urbanum* L. (35, 36) and *Myosotis hispida* Schleckt (33, 34). The « epiphyse » is destined in these cases to become the shoot of the mature embryo. Miss Langdon (21) finds that the apical cell of the bicellular embryo by oblique divisions produces a group of six cells, the central two of which are comparable to the Souèges epiphysis.

In *Juglans regia* certain small embryos (Pl. II, fig. 11) have what appears to be an apical cell. Chamberlain mentions a similar condition in *Salix*. This cell, which is the result of oblique walls as indicated in text figure 3, is not a true apical cell as found in stem tips of the lower plants. In cross sections of embryos consisting of approximately forty cells there is no true apical cell but a group of cells at the embryo apex, in a position which does not indicate an origin from an apical cell. An examination of larger embryos, such as are depicted in plate III, figure 15 and plate IV, figure 16, would hardly indicate any such structure as an epiphysis. Thus it seems that a cell which may appear to be apical, merely arises incidently, at least in *Juglans regia,* in the production of a multicellular non-differentiated embryo.

The first periclinal divisions are laid down in embryos consisting of approximately forty cells. These divisions occur usually in the derivatives of cells a^1, a^2 and b^1 (Text fig. 3 embryo

F-1). Not all cells, however, divide in this manner. In some, a more or less anticlinal division may occur. Eventually there is formed an embryo with a fairly massive upper portion and with a suspensor region of only a few cells in diameter (Pl. III, fig. 14).

The cells a^1, a^2 and b^1 produce the shoot, cotyledons and hypocotyl. There is some variation in the destiny of these cells due to the irregular divisions explained above. In no instance is it possible to assign a particular destiny to each of these cells because mitoses are so rapid and so irregular that a very massive ovoid embryo is soon formed with the walls of the original cells not apparent. A comparison of plate III, figure 14, which is of a 0.066 mm. embryo, with plate III, figure 15, which is of a 0.1 mm. embryo, will give the reader an idea of this sudden increase in bulk. Dermatogen, periblem and plerome are not established in embryos of a few cells as Hanstein (12) has reported in *Capsella, Nicotiana* and *Oenothera*. Not even the dermatogen has been established in embryos of the size shown in plate III, figure 15 and plate IV, figure 16. It is only in an embryo of a length of approximately 0.2 mm. (Pl. IV, fig. 17) that any internal differentiation is visible and the establishment of the dermatogen, which is then only present in the hypocotyl region, occurs.

Cell b^2, at least in those proembryos where oblique divisions have occurred, produces the suspensor and all or part of the root cap. The growing point of the root may also arise from this cell if earlier divisions produce and extremely large b^2 cell, otherwise the growing point is produced from b^1 or a^2. There is no hypophysis.

Hypocotyl and root: The cap and growing point of the root are the first regions to differentiate. This differentiation begins in embryos which are about a tenth of a millimeter in length. As has been shown above, there is no distinct demarcation between suspensor and embryo-proper. Only a group of large cells is located at the suspensor end of the embryo. These cells in the smaller embryos seem to divide more slowly than the cells at the apex. Greater activity of these cells, however, occurs soon after the embryo becomes ovoid. The suspensor enlarges, especially in diameter, and soon becomes fairly distinct from the rest of the embryo (Pl. III, fig. 15 and Pl. IV, figs. 16 and 17). At the same time but with greater rapidity, the cells directly above the suspensor and the peripheral cells adjacent to the suspensor, divide by periclinal divisions (Pl. III, fig. 15, p. d.). The cells resulting from these divisions are root cap cells. The group of cells which has been delimited near the center of the embryo by these mitoses, is the root growing point (Pl. III, fig. 15, G. P.).

Shortly after the initiation of the root cap, diameter increase of the region above is accelerated. Although cells divide in all planes at this time, periclinal divisions predominate in the region which will develop into the hypocotyl and seem to be responsible, to a large extent, for the formation of the flattened apex of the embryo (Pl. IV, figs. 16 and 17). Vacuolation of cells in the central region of the hypocotyl initiates the demarcation of primary meristems (Pl. IV, fig. 17). Thus, the pith region becomes evident first. Coincident with the differentiation of the pith, periclinal divisions are no longer obvious in the « dermatogen ». Up to this time all « dermatogen » cells have divided as profusely as other cells of the embryo. The cessation of this type of division could be considered as an indication of the establishment of a protoderm in the hypocotyl. Vacuolation occurs next in the region which will become the cortex, and thus leaves between cortex and pith, a cylinder of cells which are more or less isodiametrical and rich in cytoplasmic content. These cells will produce the vascular system and may be referred to as the prodesmogen, a term used by Louis (22) for those cells which will eventually elongate into procambial cells.

The hypocotyl, in which more or less definite regions are present, makes up the greater bulk of a 0.2 mm embryo. But as the embryo enlarges, the size of the hypocotyl gradually becomes over-shadowed by the extremely large size of the cotyledons (Text fig. 6).

Enlargement of the hypocotyl-root axis is first due to cell divisions throughout the structure. Later elongation is carried on usually by the growing point of the root, which adds cells

internally to the root [1] and externally to the central region of the root cap (Pl. VI, fig. 22; Pl. VII, fig. 25; and Pl. XI, fig. 35).

The details of the root meristem are clearly illustrated in plate XII, figure 36. The initials at *I* produce internally the vascular cylinder and externally a group of cells at *A*. From the central cells of this group the central portion of the root cap arises (C. C.). From the periphery of this group of cells two regions arise by anticlinal divisions. The cells of the upper portion become vacuolated very rapidly and produce the cortical region of the root (Co.). The cells of the lower portion retain dense protoplasts and by more abundant periclinal than anticlinal divisions build up the outer part of the root cap (O. C.). No definite root protoderm is present in the embryo. If the hypocotyl-root axes in embryos of the sizes shown in plate VII, figure 25, and plate XI, figure 35 are compared, it is seen that the protoderm of the hypocotyl and the outer cells of the root cap gradually merge. The protoderm is present only in seedling roots, and is found some distance back from the developing root cap. According to Flahault (6), the cap is formed from the epidermis and by periclinal divisions of the subepidermal cells, « ... mais la plus grande partie de la coiffe est pourtant produite par l'epiderme ». This interpretation appears incorrect since no epidermis or protoderm is present as stated above.

Haberlandt (11) in his classification of root types, uses Flahault's investigations in placing *Juglans regia* as type III, in which protoderm and adjacent cortical layers form the root cap. Schüepp's clasification is more applicable according to the present author's observations. Schüepp, using Kroll's (20) and Holle's (17) investigations, would place the *Juglandaceae* into his type III-*b*, in which « Wurzelkörper bildet Zentralzylinder und Rinde; Kappe bildet Epidermis und Haube », or the type in which the outer part of the « Rinde » contributes to the « Haube » (Schüepp p. 70). The meristematic zone which produces the outer portion of the root cap. (Pl. XII, fig. 36, O. C.) might be considered, aspecially in the embryo, to belong to the cortical region. In plate XI, figure 35, the root cap appears as if it actually is a part of the cortex and has developed internally in the region adjacent to the hypocotyl. A re-examination

[1] Whether this growing point produces a true root at this time or only adds to the length of the hypocotyl was not determined. A later paper will clear up this most difficult problem.

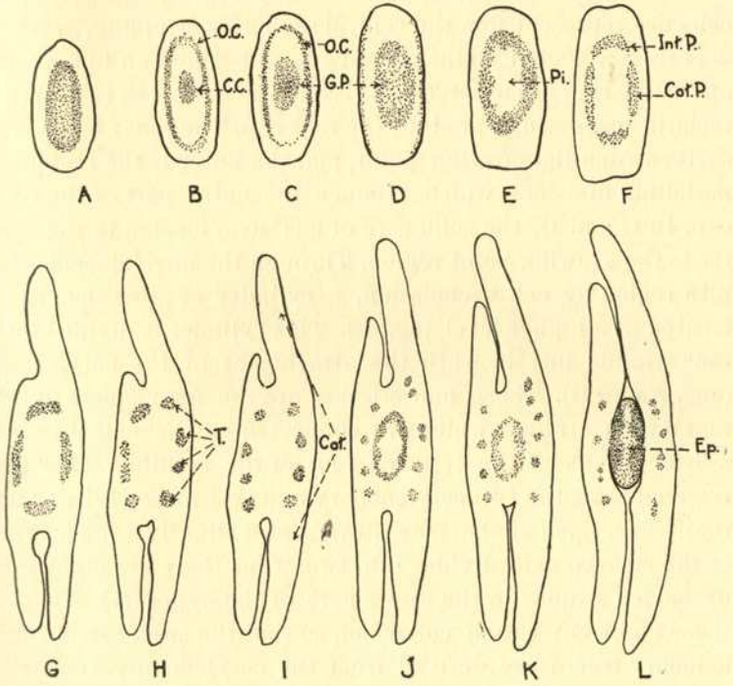

Fig. 4. — Transverse sections through an embryo approximately 1 mm. long. Inner root cap meristem C. C.; cotyledon Cot.; cotyledonary poles Cot. P.; epicotyl Ep.; growing point of the root G. P.; intercotyledonary root poles Int. P.; outer root cap meristem O. C.; pith Pi.; cotyledonary traces T.

of plate VII, figure 25, however, shows how the smaller embryos disprove this. The appearance of internal origin is probably due to the vacuolation of cells in this upper region. In the root of the seedling, mitoses do not extend upwardly so far as in the embryo root, and in this case the area of periclinal divisions at O. C., plate XII, figure 36, contributes to the cortical region as well as to the root cap. Whether this meriste-

matic region belongs to the cortical region or to the cap is problematical.

If an embryo of the size shown in plate VI, figure 22, is examined in serial cross secctions we find that the prodesmogen has a definite although not an elaborate pattern. Section A in text figure 4, is from the root cap region. Meristematic cells are represented in the central region by stippling. Section *B* is from a region slightly above A and shows an inner core and outer ring of meristematic tissue. These are the two meristematic zones which produce the root cap. The inner is the one derived from tho growing point, and the outer is the region of periclinal divisions which produce the outer part of the root cap. In C and D, the solid core of meristem located in the center is the growing point region. Through the development of a pith region by cell vacuolation, a cylinder of prodesmogen is located at a higher level (fig. E). This cylinder is divided into four strands just beneath the attachment of the cotyledons (figs. F and G). These four strands are the forerunners of the four xylem strands which are connected to the four primary xylem groups of the tetrarch root of the seedling. They are referred to as the two cotyledonary and two intercotyledonary root poles. Each of the four strands at a slightly higher level of the embryo axis divides into two. Thus there are eight prodesmogen strands in the upper part of the hypocotyl. Four of these eight enter each cotyledon, so that the two central cotyledonary traces are derived from the cotyledonary root pole and each of the two lateral traces is derived from a branch of the two intercotyledonary poles (figs. H-K). The meristematic tissue in the center of figures J and K is that of the epicotyl (Pl. VI, fig. 22).

At this embryo stage these strands consist merely of cells with dense cytoplasm. They may be slightly elongated in the direction of the embryo axis, but not necessarily longer than the cells in the « cortical » or « pith » regions. No regions such as endodermis are differentiated as illustrated by Langdon in the *Carya glabra* embryo of a similar size. None would be expected as this region develops into the upper portion of the hypocotyl, where no endodermis is formed.

During the enlargement of the embryo to maturity, there is developed a more complex vascular system than that described above. As the pith increases in diameter and separates the four prodesmogen strands, cells of the interfascicular areas

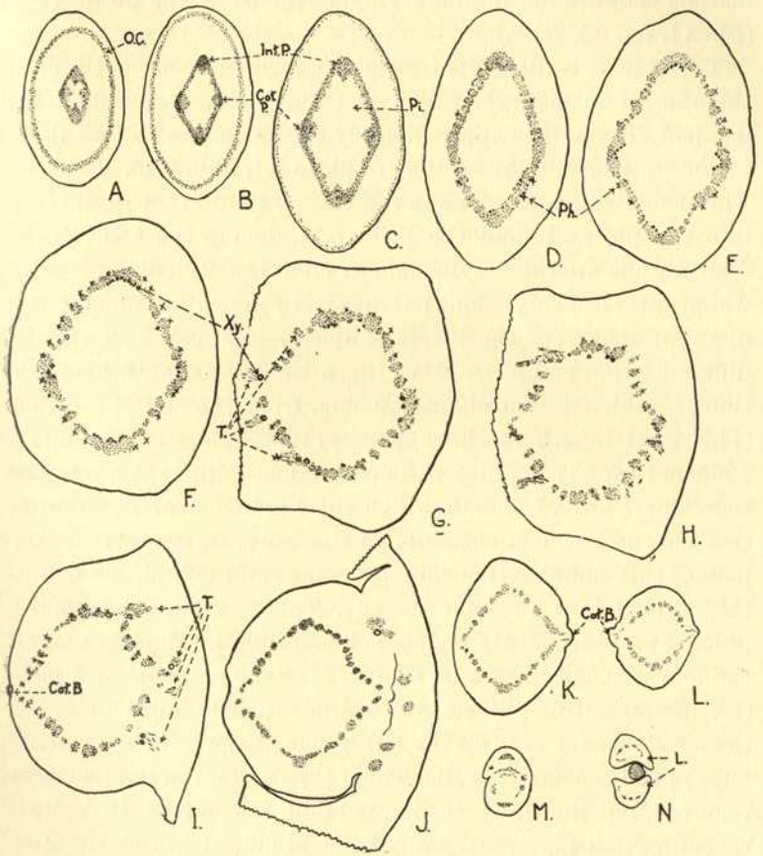

Fig. 5. — Transverse sections through the axis of an embryo approximately 24 mm. long. The axis is 5.5 mm. long. Cotyledonary bud Cot. B. ; cotyledonary root poles Cot. P. ; intercotyledonary root poles Int. P. ; foliage leaf primordia L. ; phloem Ph. ; pith region Pi. ; outer root cap meristem O. C. ; cotyledonary traces T. ; xylem Xy.

become more dense with cytoplasm resulting in the formation of a complete meristematic cylinder of isodiametrical cells. The cylinder increases in diameter by periclinal divisions which are somewhat limited to the outer periphery. The result of

such activity produces an effect similar to cambial activity. A few of the inner cells of this cylinder, especially in the cotyledonary and intercotyledonary root poles, become prosenchymatous, while the outer cells remain isodiametric. The prosenchymatous cells are few in number and develop into the protoxylem (Pl. XI, fig. 35, *P. X.*).

Text-figure 5 illustrates serial transverse sections through the axis of an embryo, which has almost completed its development. The axis is approximately the same size as that illustrated in longitudinal section of plate XI, figure 35. The four widest meristematic areas in figures A, B and C, will develop into the pericycle and the primary xylem of the root. These four strands are the cotyledonary and intercotyledonary poles which enter the cotyledons as traces. No xylem nor phloem has differentiated at these levels. Phloem, however, has started differentiation and a few sieve tubes are partly mature in section D, which is cut about 0.9 mm. from the end of the root (The total length of the hypocotyl-root axis was 2.9 mm.). Phloem tissue is the first vascular tissue to differentiate in the root. There are a few cells which show evidence of differentiation into protoxylem elements in the lower intercotyledonary pole of this same section. The previous solid cylinder is definitely formed into vascular strands. There is more developing phloem present and the xylem is beginning development in all the four root poles. At a level just below the cotyledonary gaps (F), differentiating phloem is in all the larger bundles including the cotyledonary pole on the left. Some of the xylem elements have spiral secondary walls. The cotyledon on the left has been removed, but the traces to it are visible in sections G and H, revealing that four gaps have been produced in the vascular cylinder. The two traces from the cotyledonary root pole diverge from the vascular cylinder at a lower level than do the two traces of the intercotyledonary poles. Sections I and J illustrate the entrance of the traces into the cotyledon on the right.

Less than the lower sixth of the hypocotyl-root axis of the mature embryo is root. The vascular cylinder of the transition region is composed of a number of bundles. The phloem areas are gradually extended laterally and each area is separated

into a number of strands as one follows it from root through the hypocotyl. A number of these phloem groups join the xylem of the root poles to form collateral bundles of the cotyledonary traces. The xylem of the nature embryo is not developed enough to indicate its exact relation in the transition phenomenon. The protoxylem first differentiates in the lower portion of the traces (Pl. XVII, fig. 49) and then proceeds upwardly into the trace and downwardly into the hypocotyl-root axis. This xylem may be already differentiated in embryos as small as 6.5 mm., total length. In the mature embryo, protoxylem also is starting differentiation in the vascular strands between the cotyledonary and intercotyledonary poles (1).

Cotyledons : The cotyledons begin to form about fifteen to twenty days after the first zygote division, or about the middle of May in the material collected ad Davis. There is no definite cotyledonary region evident in embryos of less than 0.15 mm. length. For example, in plate IV, figure 16, the arrow at the right indicates the approximate location of the origin of a cotyledon, but it would be difficult to ascertain the exact cells entering into its formation. Cotyledons become evident in embryos of an approximate length of 0.2 mm. These embryos have a flattened apical end, the diameter of which is greatest in the plane of the cotyledons. Plate IV, figure 17, show such an embryo cut through the plane of the cotyledons. The cells of the cotyledonary region contain fairly dense cytoplasm. Periclinal divisions (Pl. V, fig. 19) are evident in the « dermatogen », which is only stable at this time in the hypocotyl region. The cotyledons soon develop into two crescentic ridges, leaving in the center of the embryo apex a depressed area which later develops into the epicotyl (Pl. V, fig. 18). Apical growth must be accompanied almost immediately by a marginal growth, because the cotyledon is a broad fan-shape structure early in ontogeny. Small cotyledons increase in thickness by a meristem which is located at the basal adaxial portion of the primordium (Pl. VI, figs. 22, 23 and Pl. XIII, fig. 39). This meristem is characterized by periclinal division in the subepidermal layer. Foster (9) has described this type of meristem, which he calls the « adaxial meristem », in *Carya Buckleyi* var.

arkansana Sarg. He found that an adaxial meristem is absent in the bud scale primordium, present in the foliage leaf primordium, and present in the primordium of transition forms concurrently with the marginal meristem (10). The cotyledon of *Juglans* is most nearly like the transition foliar form of *Carya* in this histogenetic respect.

Periclinal divisions were observed in the « dermatogen » of the cotyledons in embryos up to one millimeter in length (Pl. V, fig. 20). In larger embryos the determination of periclinal divisions was impossible because of the small radial diameter of the cells coupled with their plasmolyzed condition which seemed to occur unavoidably in the surface cells of these older rapidly enlarging embryos (Pl. XIV, figs. 42, 43 and Pl. XV, fig. 44). Periclinal divisions of the « dermatogen » of embryos have been illustrated, at least to the knowledge of the author, only in *Dioon edule* Lindl. and *Ginkgo biloba* L. (4). Other investigators have found the dermatogen to be the earliest « established » region in plant embryos. This appears ts be the case in embryos of precise segmentation sequences as in *Capsella*. Probably the « dermatogen » is delayed in its establishment in those embryos which are massive like *Juglans regia*, *Ginkgo biloba* and *Dioon edule*. In these plants periclinal divisions are also found in the « dermatogen » of the epicotylar region (Pl. VI, fig. 21).

The marginal meristem plays an important rôle in the enlargment of the cotyledon. Plate VIII, figure 28, illustrates that this meristem is composed of a small group of cells which are much more vacuolated than the apical cells of the foliage leaf primordia (Pl. VII, fig. 26). Six or seven layers of cells are produced from this meristem. These layers are, however, of brief existence, for mitoses soon occur in a variety of planes a short distance from the cotyledonary edge. Some of these mitoses are instrumental in lengthening the cotyledon, so that an intercalary growth begins almost immediately. Intercalary growth is as important as marginal growth in the elongation as well as the lateral expansion of the organ. Definite layers are not produced at all by the marginal meristem in older cotiledons (Pl. VIII, fig. 30).

The apical meristem, which is cytologically similar to the marginal meristem, is not present in embryos over two millimeters long. Because of this discontinued activity, an apical notch is formed (*n* of Text fig. 6-B), and the cotyledon thus becomes bifurcate.

During the early phases of apical and marginal growth, the procambium is produced either from one or two of the central rows of cells (Pl. VIII, fig. 27). The procambium of the cotyledon is always continuous with that of the hypocotyl. As the cotyledon develops, its procambium differentiates acropetally from the previously formed hypocotylary procambium (Pl. V. fig. 18). As stated previously, there are four traces to each cotyledon, and in the older embryos these four traces leave four gaps in the axial cylinder. The two traces of the cotyledonary root pole branch and anastomose to form a mid vein and two other main veins in the center of the cotyledon. This occurs in various ways, one of which is depicted in text figure 6-F. The two lateral traces of the cotyledon, that is, the intercotyledonary root pole traces, extend only a short distance into the cotyledon before forking into two strands. This dichotomy may also occur in the cortex of the hypocotyl.

Davey (5) observed two cotyledonary traces in *Juglans nigra*. There is no mid vein in this species. Langdon (21) describes *Carya glabra* as having three traces, but does not illustrate or give the details of their insertion on the axial cylinder. It is interesting to see such variation in species which are so closely allied.

In a small cotyledon as illustrated in the embryo of text figure 6-A practically the entire margin has active meristematic tissue such as illustrated in the cotyledon of plate VIII, figure 28. Because of the cessation of growth by the apical meristem, the apical notch is evident in a two-millimeter embryo. The remaining marginal meristem continues growth more rapidly along the edge labeled *l* in text figures B and C than at *s*, so that in the eight-millimeter embryo each cotyledon is a bilobed structure with rather straight lateral sides and two round apical lobes. An acceleration of growth at *p* in figure D in the direction indicated by the arrows causes a buckling of the

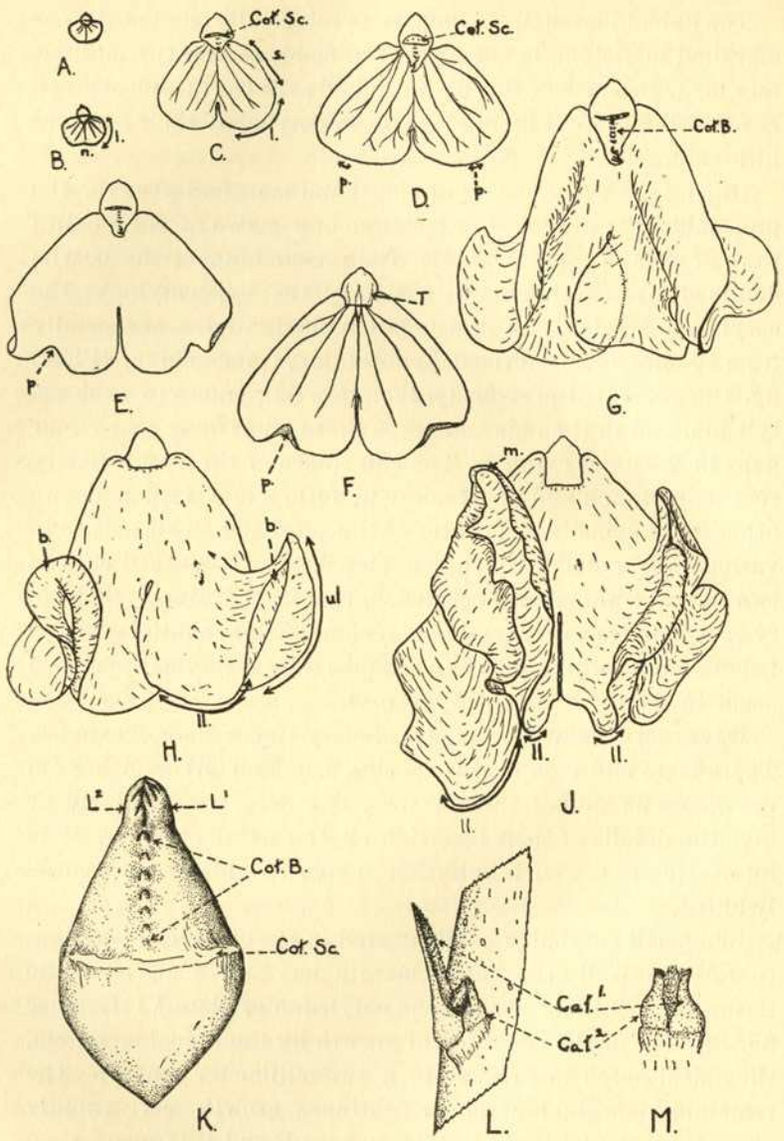

Fig. 6. — A.-J., Embryos showing the development of the cotyledons × 1.5; K, Axis of a mature embryo, illustrating the size of the cotyledonary buds; L.-M., First cotyledonary buds from opposite sides of the same seedling. See text for explanation of *b* and *m*; cataphylls Cat. ; cotyledonary bud Cot. B. ; scar of the removed cotyledon Cot. Sc. ; lobe of the cotyledon *l* ; lower portion of lobe *ll* ; leaves L ; region of periclinal divisions which cause the folding of the cotyledon lobe *p.* ; side of the cotyledon *s.* ; cotyledonary trace T. ; upper portion of the lobe *ul*.

cotyledonary margin. The cotyledon then begins to fold back upon itself. Some of this distortion is due to the fact growth is more active on the adaxial than on the abaxial surface. Figure E is an adaxial view and figure F en abaxial view illustrating the inception of folding. With marginal and surface growth continuing at irregular rates, as described above, each lobe becomes folded together as shown in figure G and H. Activity may be so rapid along the margin marked *ul* that the edge may even bend so that the adaxial surface is turned outwardly as indicated by *b*. With continued growth of this central region in each of the four lobes, the two cotyledons eventually fill the four upper cavities of the seed and assume the shape shown in the upper left lobe of figure J. Plate VIII, figure 30, is a longitudinal section through the upper cavity of the seed which shows an immature cotyledon with an active marginal meristem. This meristem has continued activity after the cotyledon reached the apex of the ovule cavity and thus has caused the edge to recurve. Text figure VI-J illustrates at *m* such a fold in three dimensions. This occurs quite regularly.

After the four upper compartments of the ovule have been filled by the cotyledons, growth becomes accelerated along the outer margin of each lobe (*ll* in figures H and J), so that this portion of the cotyledon grows downwardly eventually filling the four lower cavities of the seed. The position of the mature embryo within the « nut » has been described in a previous paper (23).

The cotyledons of young embryos are quite thin and delicate in texture. In fifteen-millimeter embryos a rather pronounced growth in thickness begins in the area nearest to the epicotyl and spreads to the margins. This increase in thickness is carried on by a cambium-like meristem (Pl. VIII, fig. 29). In some places the epidermis seems to partake in this activity. The epidermis, however, consists of dense cytoplasmic cells of extremely shallow depth so that it is difficult to observe periclinal divisions with certainty (Pl. VIII, fig. 30; Pl. XIV, figs. 42-43; and Pl. XV, fig. 44).

Storage of food begins with increase in thickness of the coty-

ledons and starts in the basal region. In an embryo of the size shown in text figure VI-J, cotyledonary cells nearest to the axis of embryo contain the greatest amount of stored material. As one examines the cells from this region towards the marginal meristem, there is noticed a decided decrease in storage content of the cells. In young embryos the cells in which stored food is located are usually some distance from the meristem. But as the embryo matures, cells nearer and nearer to the meristems become filled with reserve food. In fact, even the cells of the subepidermal meristem of a cotyledon which is about to complete its development (Pl. VIII, fig. 29) may contain storage products [1].

Epicotyl: The initial cells of the epicotyl first become apparent at the time of pith formation in the hypocotyl. These cells are arranged in two tiers (Pl. IV, Ep. of fig. 17). They are very similar to those observed by Souèges in *Myosotis hispida*, although apparently not of the same origin. In *Myosotis* the epiphysis, i. e. a wedge-shaped cell at the apex of the eight-celled embryo, divides into eight cells which are arranged in two tiers as in *Juglans*. No epiphysis, however, is formed in *Juglans regia*. Here, the initials arise at the embryo apex as a group of cells which retain their dense protoplasts when the « pith » cells of the hypocotyl become vacuolated.

In an embryo where the cotyledons are two crescentric mounds (Pl. V, fig. 18), the epicotylar initials are located in the depressed region. At this time periclinal divisions, which have been mentioned previously, are present in the « dermatogen » (Pl. VI, fig. 21). The initials develop into a small round mound-like epicotyl in one-millimeter embryos (Pl. VI, fig. 22). A promeristem and a small central core of vacuolated cells, i. e. the pith region, are already developed in epicotyls at this stage. The tunical region of the promeristem consists of a definite outer layer of cells and a slightly irregular second layer (Pl. VI, fig. 23). The corpus is several cells deep. Langdon (21) has illustrated in *Carya glabra* an epicotyl similar to that in figure

[1] No micro-chemical tests were made to determine what these storage products were.

23, an she has described it as having « three successive horizontal groups of initials ». These « initials » are, no doubt, a three-layered tunica of the promeristem [1].

The epicotyl becomes flattened just before foliage leaves begin to develop. The first leaf appears when the axis is about 0.65 mm. long and the total length of the embryo is approximately 1.3 mm. Leaf development is like that in *Carya Buckleyi* (Foster 7). That is, the rachis-petiolé region is formed by combined apical and intercalary growth (Pl. VII, fig. 26), and the lateral leaflet primordia arise from marginal meristematic ridges. An adaxial meristem, which enlarges the basal region of the leaf primordium, is also present in *Juglans* as in *Carya* (Pl. VII, figs. 24 and 26). When the primordium is about 0.3 mm. high, the midrib regions of the leaflets begin to form. The formation of primordia at this stage in development are seen the first part of June, and the primordia remain in this condition (Text fig. 6-K), either until the first of October, when the lamina of the leaflets may begin to develop, or until the time of seed germination. Three or four primordia are usually produced by October. The first two are arranged practically opposite one another and at right angles to the cotyledons (Text fig. 5-N).

The epicotyl of *Juglans regia* is distinctly different from *Juglans nigra* (Davey 5) in the possession of a series of six to eight buds in the axil of each cotyledon. There are two views as to the nature of these buds. Henrotin (13) believes that each of these buds is subtended by a bract, and that, therefore, they are not axillary buds of the cotyledons. Irmisch (18), on the other hand, contends that the buds are all anatomically alike and hence axillary to the cotyledons. The uppermost, he considers, as the « main » bud and the others as accessory buds. These develop in basipetal order. The first two leaves in each bud, he says, belong to the bud and not to the epicotylar axis. In a monograph devoted to serial buds in Angiosperms, Sandt (28) also concludes that the buds of *Juglans regia* are accessory, having been derived from the meristem of the primary axis first as two separate unlike axillary meristems. The uppermost deve-

[1] Cf. Foster (9) for usage of terms, tunica and corpus.

lops directly into a bud, while from the smaller and lower of these meristems other bud primordia are developed basipetally. The present study confirms both Irmisch's and Sandt's observations.

The buds develop from a meristematic zone in the cortical region above each cotyledon. These two zones, or strips of meristematic tissue, are composed of dense cytoplasmic cells, which retain this characteristic as the epicotyl elongates. The cells of the cortical region between the cotyledons and above the cotyledonary node, however, become vacuolated like the « pith ». Plate VII, figure 24 and plate XIII, figure 39 illustrate the cytological difference of these two cortical regions. Even in an epicotyl as small as that shown in plate VI, figure 23, this axial region is quite evident (Cot. B. M.).

The primordium of the first bud appears shortly after the first leaf primordium is formed, or when the total length of the embryo is 1.5-2.0 mm. The primordia of the other buds differentiate basipetally between the first bud and the cotyledon, as Irmisch has described. Bud initiation starts by the occurrence of oblique divisions in the second, third, of fourth layers of these axillary « cortical » cells. For example, the second bud of the 2.5 mm. embryo in plate XII, figure 38, illustrates the beginning of development because of an oblique division in the third layer and a sligth disturbance in the second layer of cells. The bud directly above, which is the first formed bud of the embryo, illustrates a later stage in development. Here oblique divisions are more numerous and the original cell layers are no longer present. In some instances periclinal divisions may occur in the « dermatogen » or the outermost layer of cells (Pl. XII, fig. 37). In slightly larger embryos such as in plate XIII, fig. 39 and plate XV, figure 45 where three cotyledonary buds are present, the first bud primordium protrudes as a pad-like structure. Up to this time obliquely oriented mitoses (Pl. XIII, fig. 40), have played an important part in volume increase of the primordium. In a slightly older bud such as the one in plate XV, figure 45, periclinal divisions, especially of the second layer of cells, are also instrumental in enlarging the primordium.

All the buds make their appearance in the same manner. Only seven bud primordia have been found in embryos, although an eighth bud was observed in many small seedlings. The primordial meristem of buds in mature embryos consists of a two-layered tunica and a group of cells in the corpus from which rib meristem as already begun development (Pl. XVI, fig. 46).

The cataphylls of the bud, which were considered to be of the epicotyl axis by Henrotin (13), begin to develop in the first bud in embryos which have five bud primordia. In the embryo shown in plate XIII, figure 41, there are seven bud primordia, the first three of which have cataphyll primordia. The cataphyll development begins usually in the second and third layers of cells. The layer of cells beneath the one-layered tunica divides profusely by periclinal divisions (Pl. XIV, figs. 42, 43). Cells internal to this region divide periclinally and obliquely. Plate XV, figure 44 depicts a median longitudinal section through the growing point of the same bud that is pictured in figure 43. These sections were taken about twenty-five microns apart, and illustrate that the first cataphyll is developed at one side of the median longitudinal plane of the bud and also develops approximately half way up the side of the bud (Pl. XIV, fig. 43).

The further ontogeny of the bud scale was not studied. Growth, presumably, is by apical and marginal meristems. The first cataphyll covers the growing point of the bud, (Pl. XVI, figs. 47, 48) from one side (See first bud of Text fig. 6-K and Pl-XVI, fig. 47). There are usually two cataphylls present in the upper two buds of each cotyledon when the embryo is mature. The cells of the buds, like the cells of the cotyledon and other parts of the embryo become filled with storage food.

ENDOSPERM

Schnarf (30) classifies endosperm under the following types : I. Nuclear endosperm, in which the primary endosperm nucleus divides without immediate cell wall formation. Walls may or may not be formed later. II. Celullar endosperm, in which the division of the primary endosperm nucleus is followed by cytokinesis. III. Helobial endosperm in which the primary endos-

perm nucleus divides and a cell wall is formed so that a small basal and a large micropylar cell are formed. *Juglans regia* has an endosperm of the first type.

Woodworth (42) has reported 16 pairs of chromosomes in *Juglans*. The 3n chromosome number of the primary endosperm nucleus of *Juglans regia* would thus be 48. The primary endosperm nucleus divides into approximately four nuclei before the egg and sperm unite to form the zygote. Simultaneous divisions of the endosperm nuclei occur during the first stages of endosperm development, that is, until just previous to cell wall formation when there are approximately a hundred nuclei. In plate I, figures 3 and 4 illustrate the four nuclei of the endosperm in a prophase stage. The nuclei inmediately become arranged around the periphery of the embryo sac, because of a very large central vacuole which remains throughout the existence of the endosperm. The size of these first few nuclei is much greater than that of the nuclei which are formed later. A comparison of the endosperm nuclei in plate I, figure 4 with plate II, figure 6 will bring out this size difference. The size of the endosperm increases very rapidly, and at first destroys the adjacent nucellar cells. Very soon, however, mitoses occur rapidly in the endosperm, integument and the outer portion of the nucellus, which then keep pace with one another until the embryo starts to develop. From then on, the endosperm gradually decreases in size as the cotyledons of the embryo encroach upon it.

The non-cellular endosperm in killed and fixed material is shrunken and pulled away from the nucellus (Pl. I, fig. 4 and Pl. II, fig. 6). This is not its actual position in the living ovule. Living embryo sacs, dissected out of the nucellus, are always turgid with the nuclei spaced very regularly. The zygote or few-celled embryo is atached to the micropylar end and wihtin the embryo or endosperm sac. After the embryo is several-celled, it becomes attached firmly to the nucellus in the micropylar end of the ovule, and from then on the endosperm is often shrunken entirely away from the embryo in the prepared material. Embryos depicted in plate II. figures 9-12, are, therefore, without adjacent endosperm protoplasm.

At the time of fertilization, embryo sacs are approximately 0.08 mm. long and the ovule approximately 0.85 mm. long. Within ten days the endosperm sac increases about three times its original size, while the ovule doubles its size (See table below). The zygote, during this time, has not divided and is only slightly larger (Compare Pl. I, fig. 3 with Pl. II, fig. 6). The endosperm nuclei lie in a thin layer of peripheral cytoplasm as shown in plate II, figure 6.

TABLE I

Comparative lengths of ovule, endosperm and embryo

Dimensions given in millimeters

Ovule	Endosperm	Embryo	Remarks
0.86	0.08	Zygote	At time of fertilization
1.20	0.15	»	
1.66	0.21	»	10 days after fertilization
4.0	1.55	4-celled	
4.6	2.5	8-celled	Endosperm cells beginning to form
7.5	3.0	10-celled	Endosperm cells 1/3 down the side of ovule
8.0	6.0	massive (0.05)	Cells throughout
15.8	13.0	0.1	» »

The endosperm of the ovule which contained the 4-celled embryo depicted in plate II, figure 9, was of the size indicated in the above table. When the endosperm sac is approximately 2.5 mm. long, cell walls are first laid down in the region around the embryo (Table I). The formation of cells proceeds from the micropylar region towards the chalazal end of the endosperm sac. In a 3.0 mm. endosperm sac, cells have formed about one-third of the distance and the endosperm tissue is two cells thick in the micropylar region. In a 6 mm. endosperm sac, cell walls have appeared throughout the cytoplasm. The embryo is then of a size comparable to that in plate III, figure 14, and is surrounded by a cellular endosperm, the walls of which are very

thin and delicate. The nuclei of these cells usually stain very deeply. In endosperm sacs 13 mm. long, the embryo is only 0.1 mm. in length (Similar to those in Pl. III, fig. 15 and Pl. IV, figs. 16), and the sac-like cellular endosperm is only slightly thicker at the micropylar end than the length of the embryo which lies within it. This size difference between endosperm sac and embryo, a ratio of 1 : 130, is one of the reasons why satisfactory preparations are difficult to make.

The endosperm is always thicker in the micropylar end and completely surrounds the embryo in the younger stages (Pl. III, fig. 15 and Pl. IV, fig. 16). Mitotic, divisions were found in cells of all the regions. No peripheral zoning of mitoses like that found in maize by Randolph (26) occurs in *Juglans regia.* In older stages, in fact, there are often more mitoses in the cells adjacent to the large vacuole. Nuclear size is extremely variable. This variability appears to be due to cases of nuclear fusion in certain cells where mitoses have not been followed by wall formation, but chromosome number has not been investigated to verify this. The cells of the endosperm are variable in size and are usually somewath isodiametric in shape. The walls are always very thin and in some cases incompletely formed. Whether the cells adjacent to the vacuole are three-sided, which often appeared to be the case, was not dermined (Pl. IX, fig. 32).

The shape of the endosperm is that of the ovule, so that the four cavities of the ovule contain endosperm as illustrated in plate IX, figure 31 and plate X, figure 33. These figures also illustrate the large vacuole and the maximum thickness of the endosperm.

In *Juglans madshurica* Langdon (21) observed that « The cotyledons in their elongation extend between the marginal layers of the nucellus and the endosperm ». This is not the case in *Juglans regia.* The cotyledons digest their way through the cellular endosperm as shown in plate VIII, figure 27. In older stages (Pl. X, fig. 34) the endosperm tissue between the cotyledon and nucellus is destroyed and in the last stages only crushed fragments of it and crushed nucellus may be found adjacent to the integument (Pl. VIII, fig. 30). The endosperm between the two cotyledons and the folds of the cotyledons is gradually

absorbed and only dried papery remains are found here in the mature seed (Pl. VIII, fig. 30).

SEEDLING MORPHOLOGY

General Organography : Rowlee and Hastings (27) have given a brief description of a number of Amentiferous seedlings including *Juglans cinerea* L. and *Juglans nigra* L. These two species represent the two morphological types of epicotyls occurring in the genus *Juglans.* In the former, there are serial buds in the axils of the cotyledons and the first leaves are compound. In *J. nigra* there are no serial buds in the cotyledonary axils and the first leaves are cataphylls (Davey p. 202). The seedling of *Juglans regia* is similar to *J. cinerea* and the embryo emerges from the « nut » in the same manner. All the *Juglandaceae* except *Pterocarya caucasica* have hypogeic cotyledons and characteristic of such embryos « — is the generally strong development of the primary root » (15). As in the two species described by Rowlee and Hastings, after the emergence of the primary root, the plumule is carried out of the « nut » by the elongation of the petioles of the cotyledons. The laminae of the cotyledons remain in the hard shell for some time after the seedling has developed. Eventually, they become rancid, then soft and watery, and later they become covered with fungal mycelium.

Hypocotyl-root axis : The upper portion of the hypocotyl-root axis is so much larger than the root end, that it often appears as though it were of abnormal size. In some cases there is a gradual tapering of this axis, but often there is an abrupt change in diameter. There is no direct correlation between this external appearance and the so called transition region. There is a direct correlation, however, in external size and the presence and size of a pith.

The root is exarch and has four xylem strands, as has been mentioned previously. There is no pith. In the region below or where the hypocotyl-root axis starts to enlarge, the metaxylem elements fail te develop in the center of the root and there is formed a small pith, which is gradually larger at progressively

higher levels. Approximately in the region where the pith is very small, the metaxylem elements are formed laterally to, as well as centripetally, in respect to the protoxylem. At progressively higher levels this lateral metaxylem becomes more predominate than the metaxylem which is formed centripetally in the root. In the same regions the protoxylem elements also develop in a more lateral manner than in the root. Often the protoxylem is in the form of a « U » or a « V » at higher levels. In older embryos this protoxylem arrangement is often evident in the upper portion of the transition region. Text figure 5-E and F illustrate this. The failure of the protoxylem to differentiate at higher levels in the base of this « U », « splits » each of the original xylem « arms » into two parts making eight xylem strands in all. Each half contains proto— and metaxylem with the metaxylem almost in the same radius and external to the protoxylem. Each of these halves of the xylem « arms » become truly endarch shortly before entering the cotyledons as traces.

The phloem of the root, as stated previously, differentiates before the xylem.This is also true of the phloem in the interfascicular areas of the transition region, i. e. where the pith is present (See text-figs. 5-D to H). The phloem of the root « spreads » out laterally into an arc and is continuous with the interfascicular phloem. Phloem from the two ends of this arc differentiates externally to the metaxylem of two adjacent xylem groups, and thus the collateral traces of the cotyledons are formed.

In between the two cotyledonary traces of each cotyledonary root pole, phloem is developed very early (Text-fig. 5-G). This phloem appears to be part of the cotyledonary bud traces (Text-fig. 5-G). The vascular tissue developed between the two cotyledonary traces of each intercotiledonary root pole, seems to be related to the midtrace of the first two foliage leaves (See text-fig 5-H to M). From the present study, the first appearance of interfascicular activity in the hypocotyl-root axis seem to be related to the development of the vascular system of the epicotyl. A more detailed study of these relationships is planned for the future.

The study of the transition region is made quite difficult in

Juglans regia because of the early development of the interfascicular region in the embryo, because of the early differentiation of protoxylem which is often obliterated in the young seedling, and because of the early appearance of secondary thickening in the youn seedling.

In the seedlings, the surface of the hypocotyl or transition region becomes brown and fissured. These fissures occur in the cortex and arise when the axis rapidly expands because of cambial and pericyclic activity. The pericycle of this region and also of the root produces a secondary cortex in the outermost layers of which, the phellogen develops.

Foliar organs of the epicotyl : Embryos of fruits, which were collected in the early part of September in an unripened condition, usually developed three compound leaves. Occasionly four leaves were produced. Each leaf had five leaflets, although the lamina of the third leaf in several seedlings was seven-parted instead of the usual five. Very little irregularity in the shape of the lamina was noticed.

In contrast to the above, seedlings which were developed from year old « nuts », had more leaves (5-8) and the leaves had more leaflets. There were also many irregularly formed laminae. Many of the first leaves also were much smaller than the first leaves arising on the stem of seedlings developed from the unripened seeds. This difference in leaf number and in laminar irregularity appears to be correlated in this case, to the age of the « nuts ». It would be interesting to see whether these results could be repeated.

Cotyledonary buds : As a result of the elongation of the epicotyl during germination of the seed, the cotyledonary buds, especially the upper ones, are separated from one another. The buds on opposite sides of the stem, which appear as pairs, are never truly opposite even in the embryo (Pl. XIII, fig. 41). In the seedling the difference in level is accentuated. These buds soon become raised on short stalks (Text-fig. 6-L and M). Stalked cotyledonary buds have been described in *Juglans cinerea* by Irmisch (18). The upper serial buds in the axils of the foliage leaves in *Juglans regia* are also raised upon stalks.

The first cataphyll of the bud as described under embryo development arises at one side and the second scale towards the opposite side. These first formed bud scales, therefore, often appear as if opposite (Text-fig. 6-M). The phyllotaxy of the *Juglans* scales has been described by de Candolle (2) as being, 1/2 or opposite. Irmisch also implies that they are opposite. Henrotin (13) says that cataphill and leaf phyllotaxy are 2/5 and 3/8, and that the first foliar appendages have a tendency to be arranged in pairs, more or less opposite-decussate, except in *Juglans regia* where their arrangement is « distique ».

The seedlings which developed abnormally from « nuts » planted in peat moss, were excellent for studying the general morphology of the cotiledonary buds. These buds were forced to develop when the terminal portion of the epicotyl was unable to emerge from the « nut » or was destroyed after emergence. One, two or three cataphylls are developed in addition to the two formed in the embryo. This is true of both the normal and abnormal seedlings. These cataphylls are spirally arranged and together with the foliage leaves have a 2/5 phyllotaxy as Henrotin as indicated for cataphylls of other buds in *Juglans*. Plate XVII, figure 51, illustrates this spiral arrangement of the first four cataphylls in a small seedling. Cataphylls one, two and three and sometimes four, are simple in form as illustrated in text figure 6-M. The upper portion of these cataphylls may be elongated as is shown in text figure 6-L. They are very hairy, especially at the margin and on the adaxial surface (Text figs. 6-L and M). The mature cataphylls are composed of highly vacuolated cells with no differentiation in the mesophyll tissue into palisade and spongy parechyma (Pl. XVII, figs. 50 and 51, Cat. [1]). The traces of all the cataphylls arise from the bud vascular system, not from the vascular system of the main axis of the seedling. Plate XVII, figure 50 illustrates the trace of the first cataphyll arising from the vascular cylinder of the bud. A gap is located directly above the trace. The origin of this trace would further prove that this cataphyl does not belong to the main axis of the seedling as Henrotin contends.

The fourth, fifth and sixth bud scale may be a transition form. These organs have a slightly expanded apical region which is

very irregular and variable in shape. The fifth and sixth or seventh foliar appendage may be a three-parted pinnately compound leaf, and the following leaves seven-parted. In a given plant, there is usually only one transition form present and only one three-parted, and one five-parted pinnately-compound leaf. This information on foliar types of the cotyledonary bud was obtained from the second, third and fourth « pair » of buds, which were forced to develop. Since these were fairly constant in foliar construction the other buds are probably similar to them.

SUMMARY

1. Fertilization occurs during the middle of April in Davis, California. The polar nuclei and one sperm fuse simultaneously. This triple fusion is followed by the union of egg and sperm.

2. The usual method of zygote division is designated as Type II, in which the wall of the two-celled embryo is diagonal. A direct correlation between obliquity of the first few walls of the embryo and indefinite sequence of mitoses, which is prevalent in *Juglans regia,* is present.

3. The suspensor arises from a pointed cell at the micropylar end of the embryo. The shoot, cotyledons, « hypocotyl » and probably the growing point of the root are formed from the remaining cells of the small embryo. There is no early establishment of « dermatogen », plerome and periblem as in embryos like *Capsella.*

4. « Hypocotyl » and growing point of the root are the first regions to differentiate in the embryo. Periclinal divisions, which form the root cap, delimit the root growing point. In the « hypocotyl » region, the pith is differentiated first and the cortex is differentiated soon afterwards. The initials of the growing point of the root produce internally the vascular cylinder and externally a group of cells. The cortical region arises by anticlinal divisions from the upper and peripheral portion of this group of cells, and the root cap arises from the central region and the lower peripheral portion. No « dermatogen » is present at the root tip.

5. The cotyledons begin to form 15-20 days after the first division of the zygote. Apical, marginal and adaxial meristems are present in the younger stages. When apical growth stops, an apical notch is formed and a bifurcated cotyledon is produced. Localized marginal growth and intercalary growth are instrumental in producing the greatly distorted and folded cotyledons. A cambial-like subepidermal meristem increases the radial thickness. There are four traces to each cotyledon, two arising from a cotyledonary root pole and each of the other two from the two intercotyledonary root poles.

6. The epicotyl is the last region of the embryo to arise. Leaf primordia develop by apical and adaxial growth followed by marginal growth. The seven « pairs » of buds are truly axillary to the cotyledon and arise basipetally. The cataphyll, which appears at times to subtend the bud, arises after bud initiation and is the first cataphyll of the bud. Morphologically it is not on the main epicotyl axis. Only one or two cataphylls are present in the two or three upper « pairs » of buds in the mature embryo.

7. In the mature embryo, the interfascicular regions of the original eight prodesmogen strands in the hypocotyl region of the small embryo are developed into bundles the outer portions of which consist of differentiated phloem. Phloem differentiates first in the root and is continuous with the interfascicular phloem. Protoxylem differentiates early in the root poles, in the lower portion of the traces, and then develops up into the cotyledons and down toward the root. A large pith is present in the « hypocotyl » region.

8. The free nucleated endosperm develops by simultaneous mitoses. Wall formation starts at the micropylar end of the sac and proceeds toward the chalazal region. The endosperm contains an immense vacuole throughout its existence, and is of the same shape as the irregular-shaped ovule. The cells contain nuclei of variable size and have very thin walls. These cells are usually approximately isodiametric.

9. The seedling of *Juglans regia* has hypogeic cotyledons, a strongly developed hypocotyl-primary root region, and an epicotyl which has 3-8 compound leaves and 7-8 serial buds in the axil of each cotyledon. The foliar structures of the accessory cotyledonary buds have a 2/5 phyllotaxy. There are usually three or four cataphylls, one transition form, one 3-parted, one 5-parted and one or several 7-parted pinnately compound leaves to each bud.

10. The transition phenomenon is limited tho the original prodesmogen strands. The centripetal xylem of the root is replaced by lateral development of the metaxylem and then a lateral development of the protoxylem into the shape of a « V » or « U ». The failure of the protoxylem to differentiate at the base of the, « V » and « U », separates the xylem into two strands. There is a centrifugal development of the metaxylem in the upper regions of the hypocotyl. The outer portion of the phloem arcs in the interfascicular region develop externally to the centrifugally developed xylem to form the ectophloic collateral bundle traces of the cotyledons.

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PLATE I

Fig. 1, Embryo sac after entrance of pollen tube. The polar nuclei are uniting. Section is cut through the septum of the fruit.

Fig. 2, Embryo sac showing triple fusion. Section is cut through the ovule at right angles to the septum of the fruit.

Figs. 3-4, Same embryo sac at different foci. Endosperm nuclei are in a prophase stage. (All figures × 180.)

Antipodals A.; egg cell E.; egg nucleus E. N.; endosperm En.; nucellus N.; polar nuclei Po.; pollen tube P. T.; sperm Sp.; zygote Z.

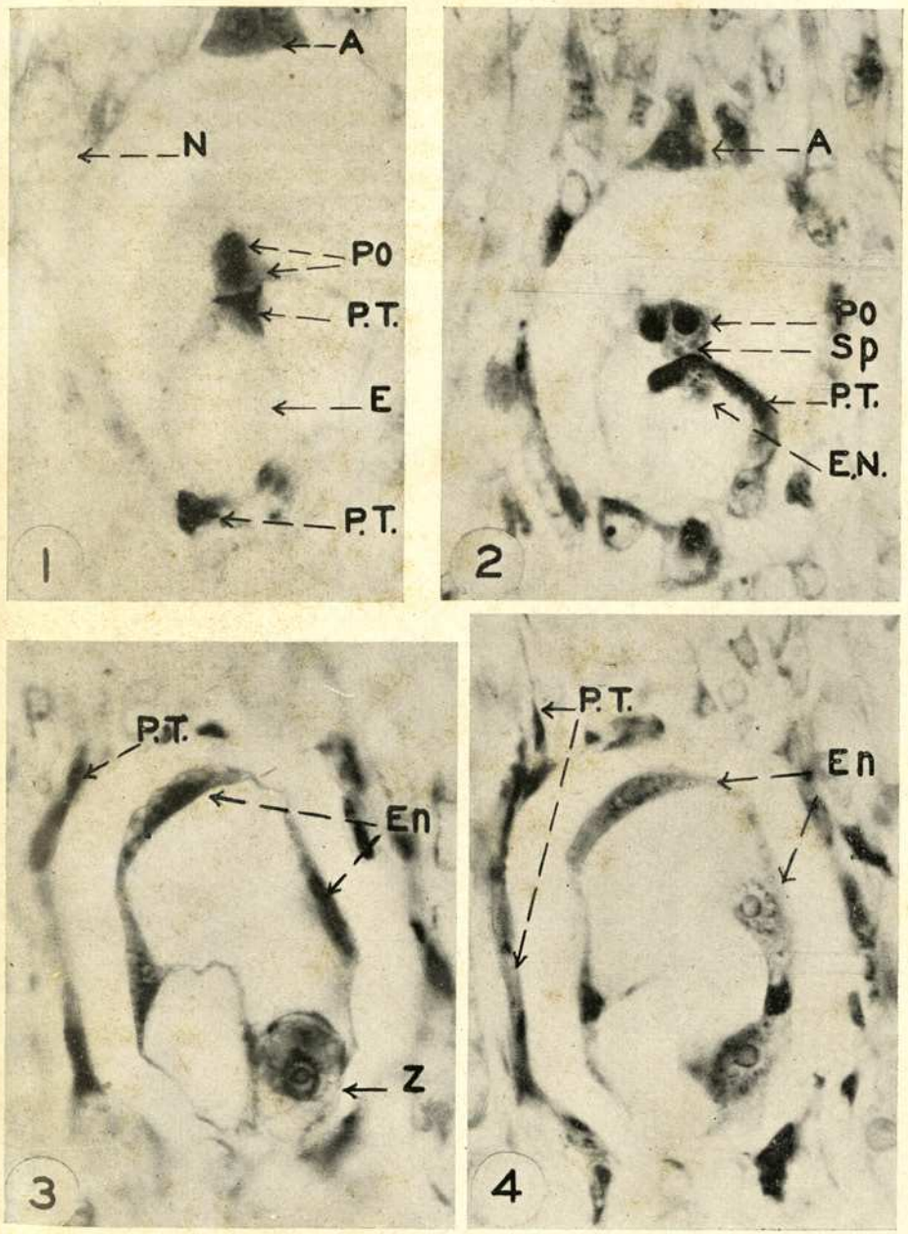


PLATE II

Fig. 5, Zygote with degenerating pollen tube and synergid mass, in which two degenerating sperms are present at *b*.

Fig. 6, Zygote and multinucleate endosperm. Zygote is darkened by overlying remains of pollen tube and synergids.

Fig. 7, A bicellular embryo of Type I.

Fig. 8, A bicellular embryo of Type II. Nuclei are out of focus.

Figs. 9*a*-9*b*, A four-celled embryo derived from Type I at different foci.

Fig. 10, A six-celled embryo showing diagonal walls. Three cells are only visible. Embyo length 0.0266 mm.

Fig. 11, A Type II embryo which seems to have an apical cell. Embryo length 0.032 mm.

Fig. 12, A Type II embryo of eight cells, five of which show in the photograph. Embryo length 0.025 mm. (All figures × 810.)

Endosperm En.; nucellus N.; pollen tube P. T.; synergid S.; zygote Z.

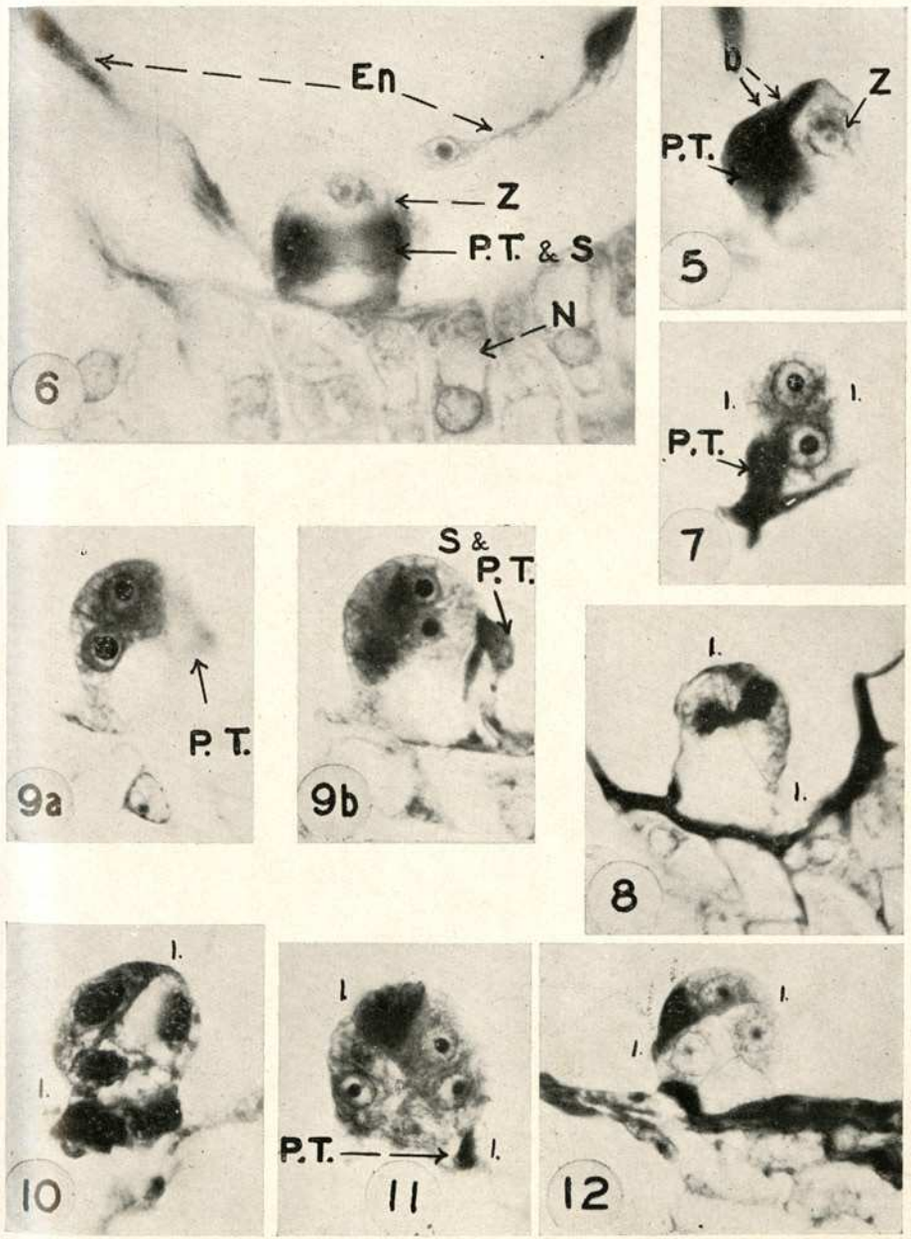
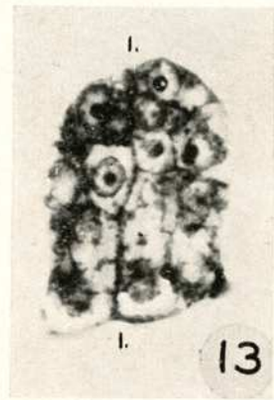
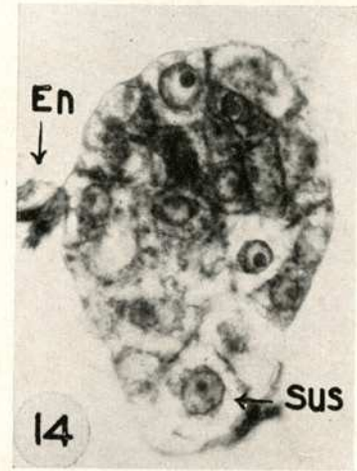


PLATE III

Fig. 13, A 0.046 mm. embryo with a predominate perpendicular wall.
Fig. 14, A 0.066 mm. embryo in which periclinal divisions have occurred in the upper region. Suspensor consists of the typical pointed cells.
Fig. 15, A 0.1024 mm. embryo. Numerous mitoses occurring through out with predominate periclinal divisions at the base forming a root cap. The first indication of root initials or growing point. (Figures × 810.)

Endosperm En.; growing point G. P.; periclinal divisions p. d.; root cap R. C.; suspensor Sus.





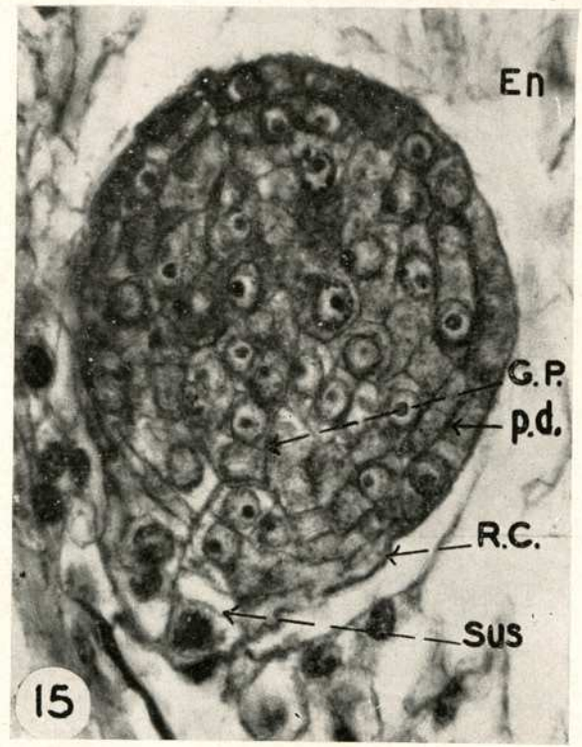
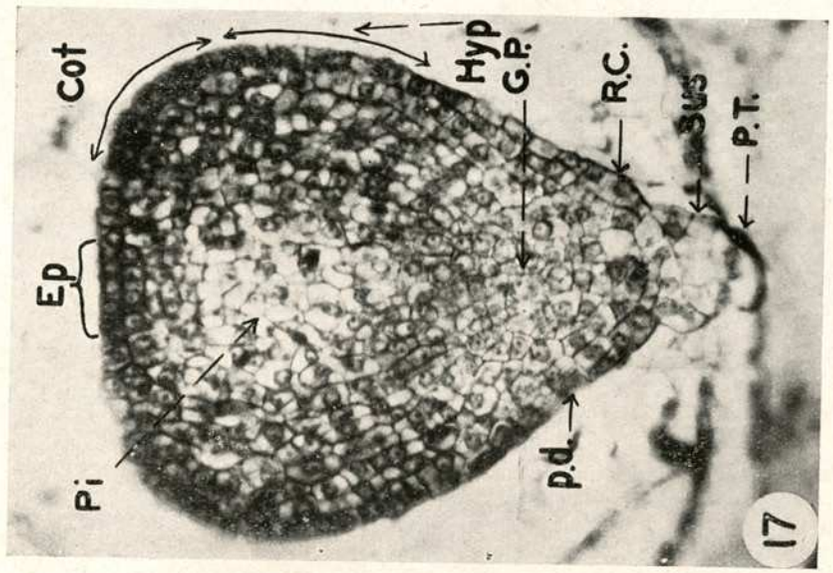


PLATE IV

Fig. 16, A 0.133 mm. embryo. The arrows indicate the probable origin of the cotyledons. (× 810.)

Fig. 17, An embryo 0.224 mm. long with all regions discernible. (× 360.)

Cotyledon Cot.; epicotyl Ep.; growing point G. P.; hypocotyl Hyp.; periclinal divisions p. d.; pith Pi.; pollen tube P. T.; root cap R. C.; suspensor Sus.



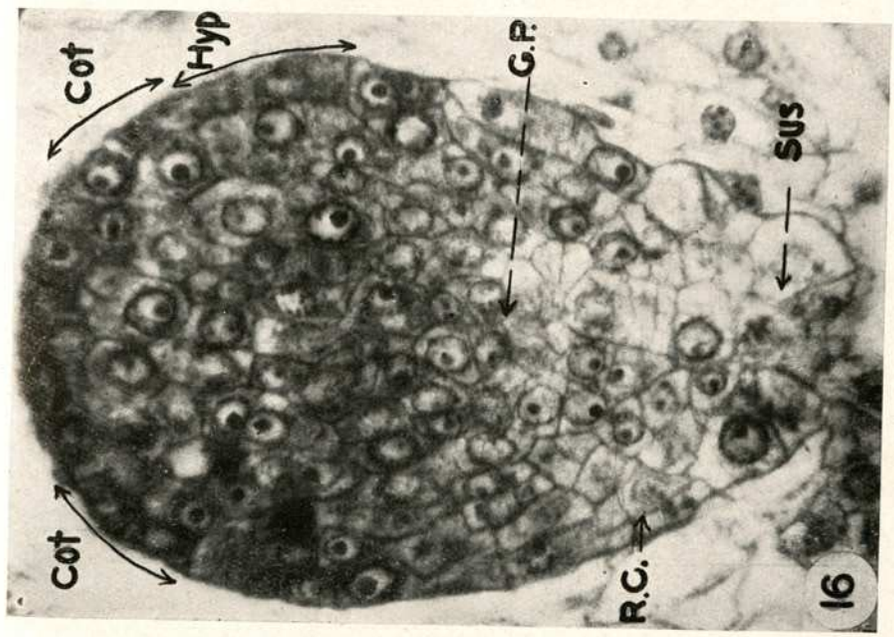
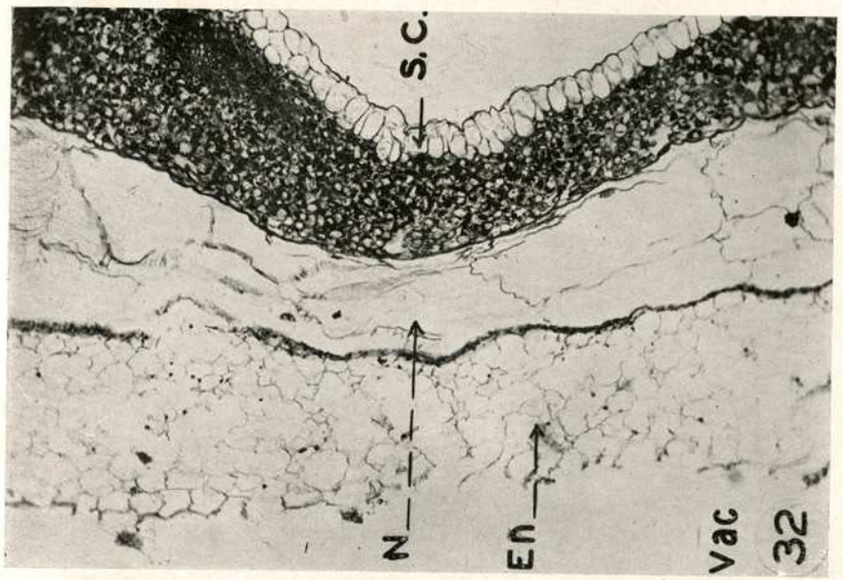


PLATE IX

Fig. 31, Longitudinal section through lower cavity of an immature seed, illustrating the large vacuole of the endosperm. × 36.
Fig. 32, Portion of figure 31 enlarged, showing endosperm, nucellus and integument. × 72.

Endosperm En.; nucellus N.; seed coat S. C.; vacuole of endosperm Vac.



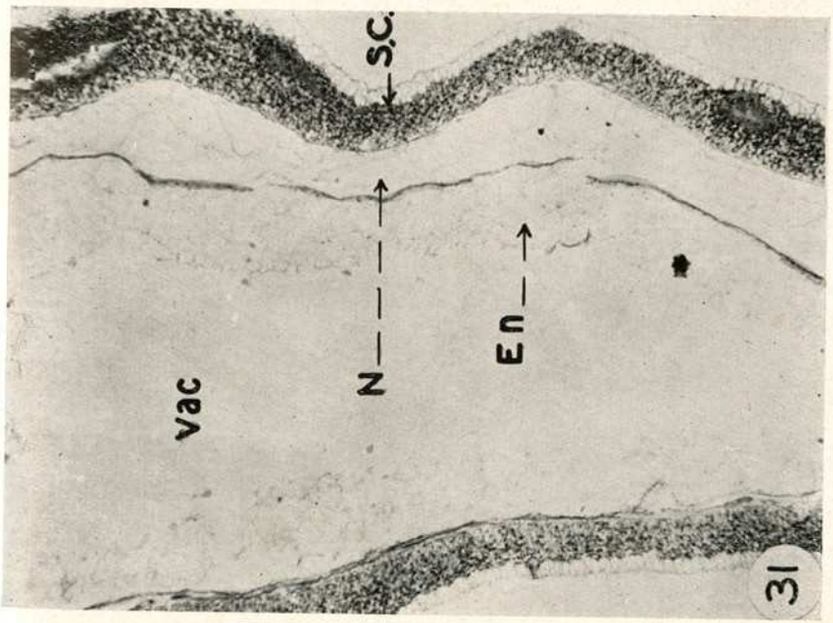


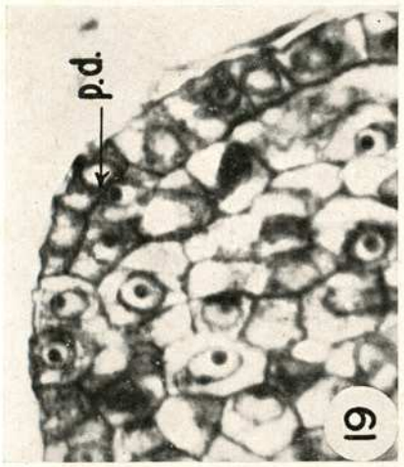
PLATE V

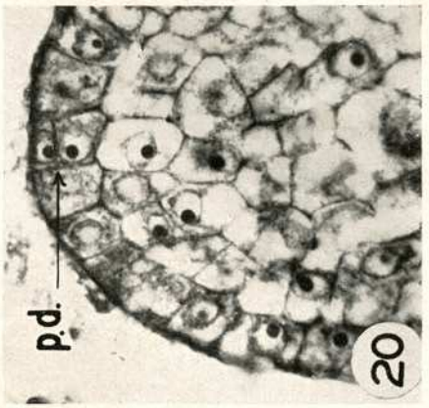
Fig. 18, An embryo 0.51 mm. long. Cotyledonary primordia are 0.048 mm. long. × 360.

Fig. 19, Periclinal divisions in the « dermatogen ». An enlargement of the cotyledonary region of embryo shown in fig. 17. × 810.

Fig. 20, Periclinal divisions in the « dermatogen ». Enlargement of the larger of the two cotyledonary apices of fig. 18. × 810.

Cotyledon Cot.; endosperm En.; epicotyl Ep.; growing point G. P.; periclinal divisions p. d.; pith Pi.; pollen tube P. T.; root cap R. C.; suspensor Sus.





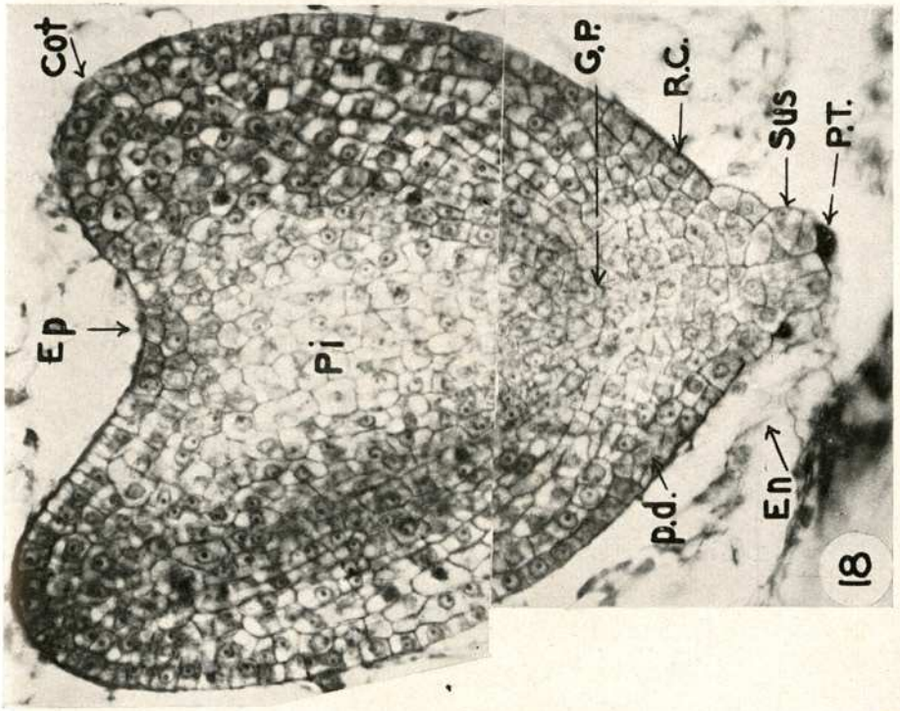
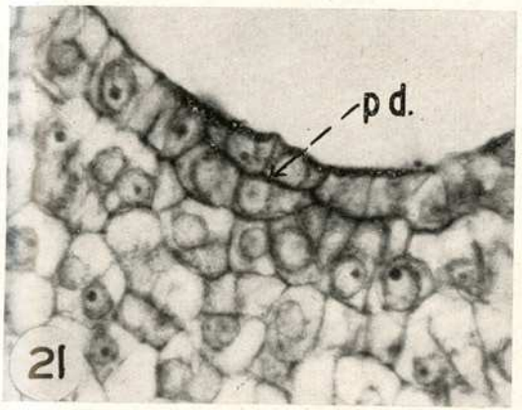
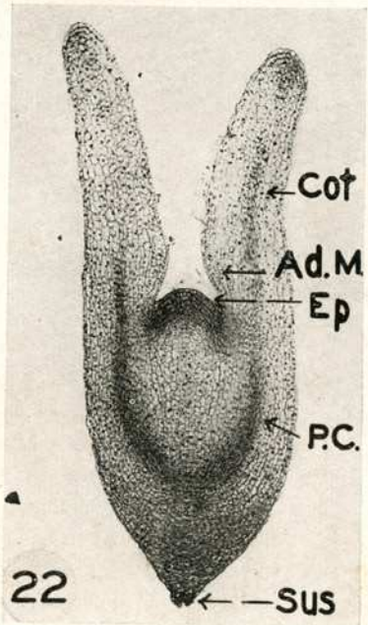


PLATE VI

Fig. 21, Periclinal divisions in the « dermatogen » of the growing point of the epicotyl. Embryo similar to that in fig. 20. × 810.
Fig. 22, An embryo 0.864 mm. long. Cotyledon length is 0.496 mm. An adaxial meristem is present in the cotyledons. × 72.
Fig. 23, Enlargement of epicotyl of embryo in figure 22. × 360.

Adaxial meristem Ad. M.; cotyledon Cot.; cotyledonary bud meristem Cot. B. M.; corpus Cor.; epicotyl Ep.; procambium P. C.; periclinal divisions p. d.; suspensor Sus.; tunica layers one and two Tu. ^{1}, Tu. ^{2}.





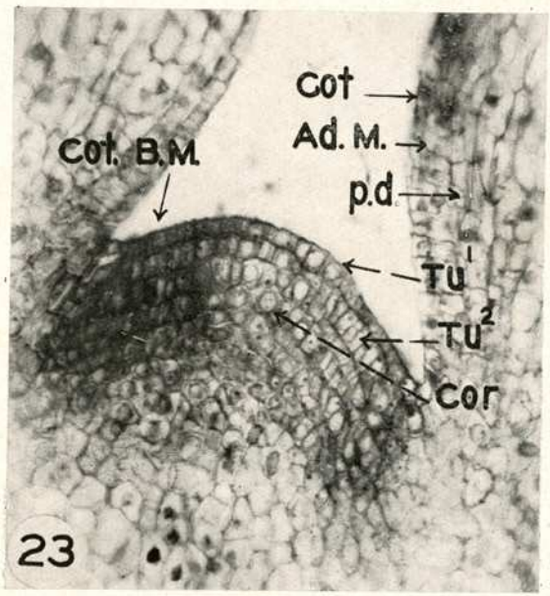


PLATE VII

Fig. 24, Embryo cut through the foliage leaves and hence at right angles to the cotyledons. Axis is 1.5 mm. long, first leaf is 0.297 mm. long. × 72.

Fig. 25, A different section of the lower portion of the same embryo depicted in figure 24. × 72.

Fig. 26, Enlargement of stem growing point and second leaf primordium of figure 24. × 360.

Adaxial meristem Ad. M.; cotyledon Cot.; cortex Co.; endosperm En.; growing point G. P.; leaf Lf.; procambium P. C.; root cap R. C.; suspensor Sus.

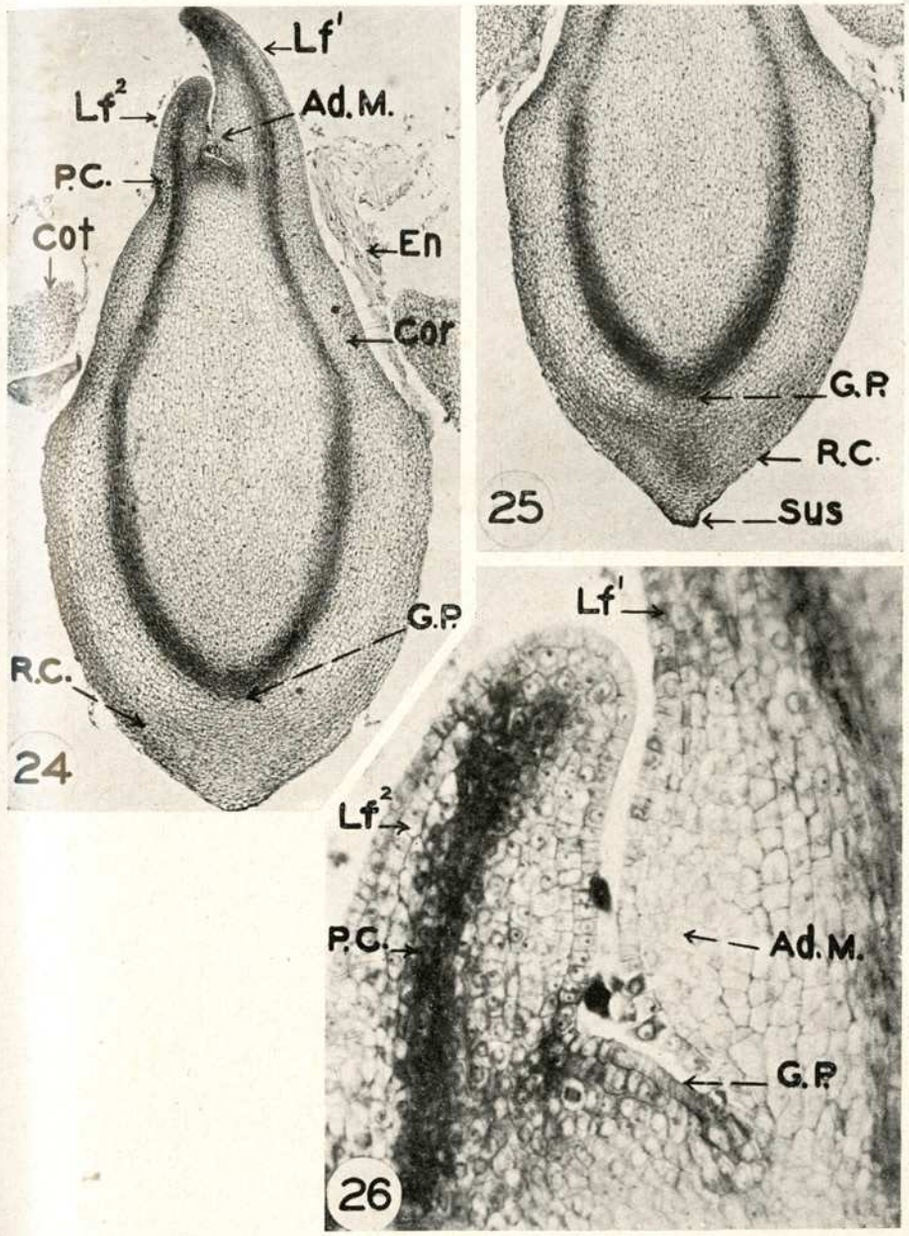


PLATE VIII

Fig. 27, Cotyledon (0.87 mm.) from an embryo of 1.33 mm. length embedded in endosperm. × 164.

Fig. 28, Enlargement of the cotyledon edge in figure 27 showing marginal meristem. × 316.

Fig. 29, Portion of cotyledon showing meristematic subepidermal region. From a practically mature embryo. × 164.

Fig. 30, Longitudinal section of upper cavity of seed showing edge of cotyledon folded over. From embryo approximately 20 mm. long. × 31.

Cotyledon Cot.; endosperm En.; subepidermal meristem M.; marginal meristem M. M.; nucellus N.; seed coat S. C.

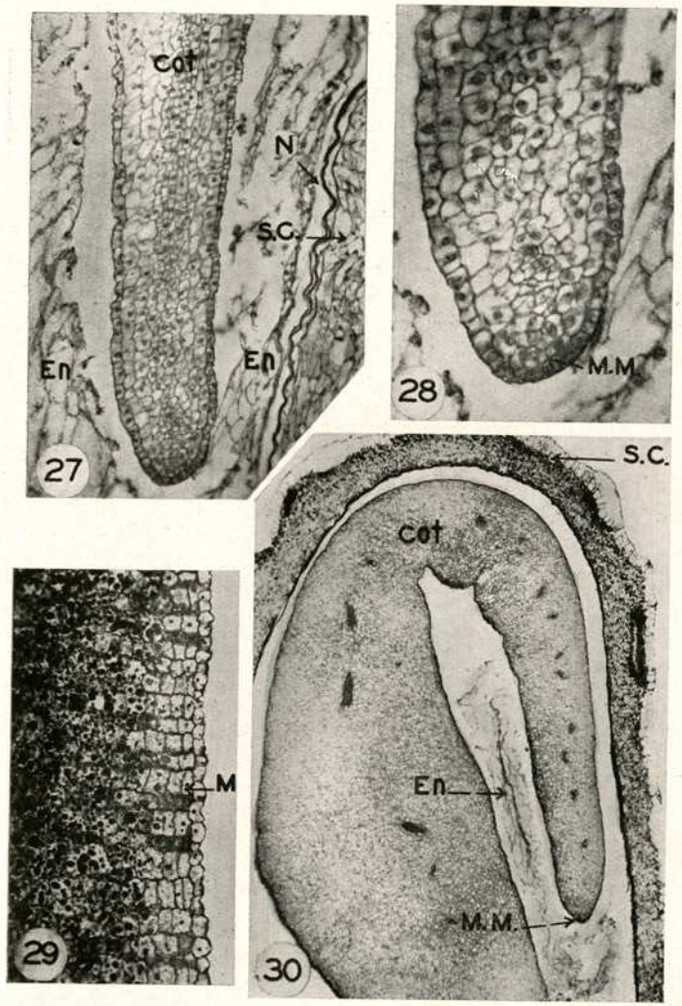


PLATE XI

Fig. 35, Longitudinal section of the axis of a 24 mm. embryo with one cotyledon removed at Cot. S., showing arrangement of cotyledonary buds. Length of axis is 6 mm. × 30.

Cotyledon Cot.; cotyledonary bud Cot. B.; cotyledonary scar Cot. S.; growing point G. P.; leaf Lf.: protoxylem P. X.; root cap. R. C.

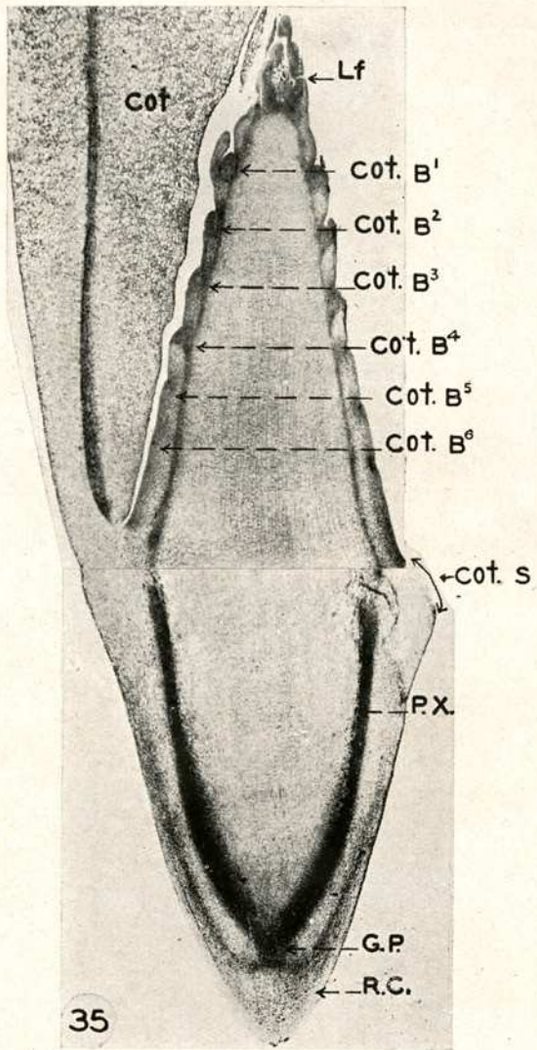


PLATE XII

Fig. 36, Enlargement of root tip of the embryo in figure 35. × 64.
Fig. 37, Longitudinal section through the second bud primordium of an embryo with three cotyledonary buds, showing periclinal divisions in the « dermatogen ». × 702.
Fig. 38, Longitudinal section of axillary region of a 2.5 mm. embryo with two cotyledonary bud primordia. × 156.

A group of cells arising externally from initials A.; cells producing central portion of root cap C. C.; cells producing the cortex Co.: cotyledon Cot.; cotyledonary bud Cot. B.; root initials I.; cells producing outer portion of root cap O. C.; periclinal divisions p. d.; procambium P. C.

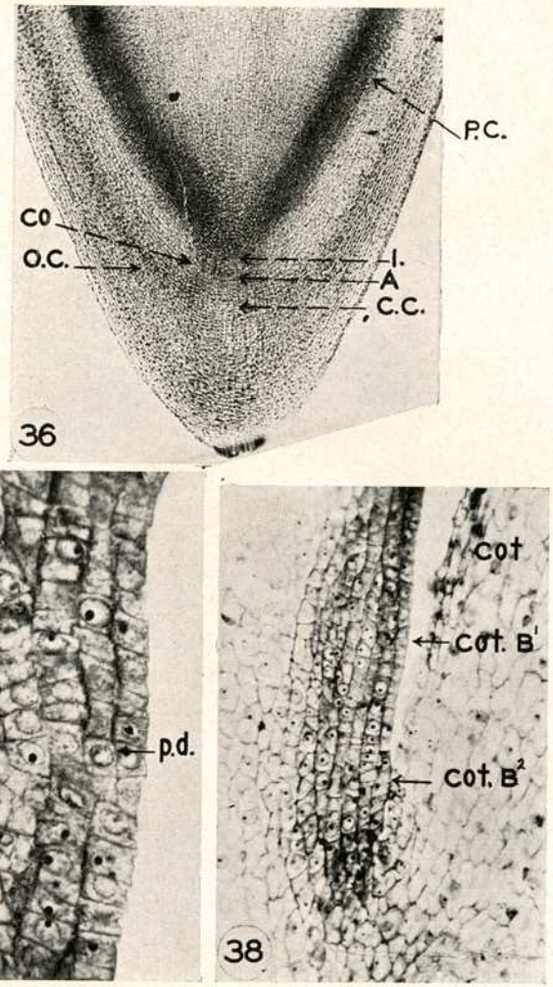


PLATE XIII

Fig. 39, Longitudinal section through the axillary region of the cotyledon of a 3.8 mm. embryo, showing three cotyledonary buds. × 180.

Fig. 40, Enlargement of first bud of figure 39, illustrating the predominance of oblique divisions which increase the number of layers of cells. × 360.

Fig. 41, Longitudinal section through the epicotyl of an embryo approximately 22 mm. long. Cotyledon on the left is removed. Length of axis is 5.5 mm. × 36.

Cotyledon Cot.; cotyledonary bud Cot. B.; leaf Lf.; procambium P. C.

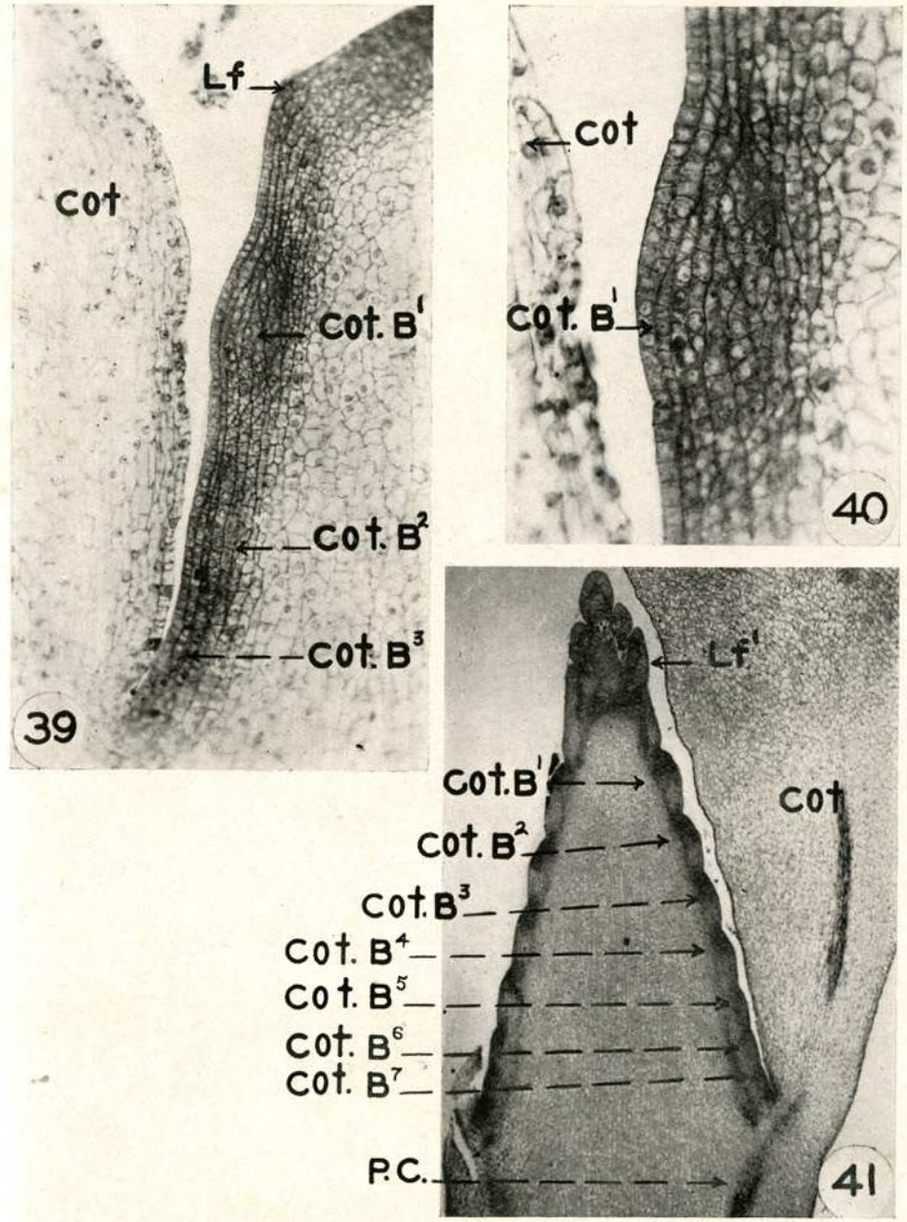
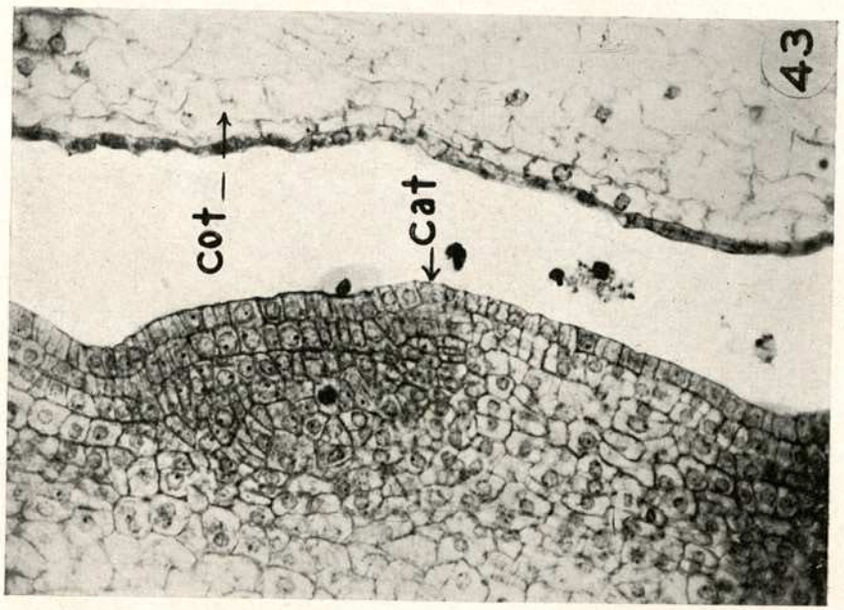


PLATE XIV

Fig. 42, Enlargement of third bud of embryo in figure 41, showing cataphyll primordium. × 360.

Fig. 43, Enlargement of second bud of embryo in figure 41, showing longitudinal section of cataphyll primordium. × 360.

Cataphyll Cat. ; cotyledon Cot.



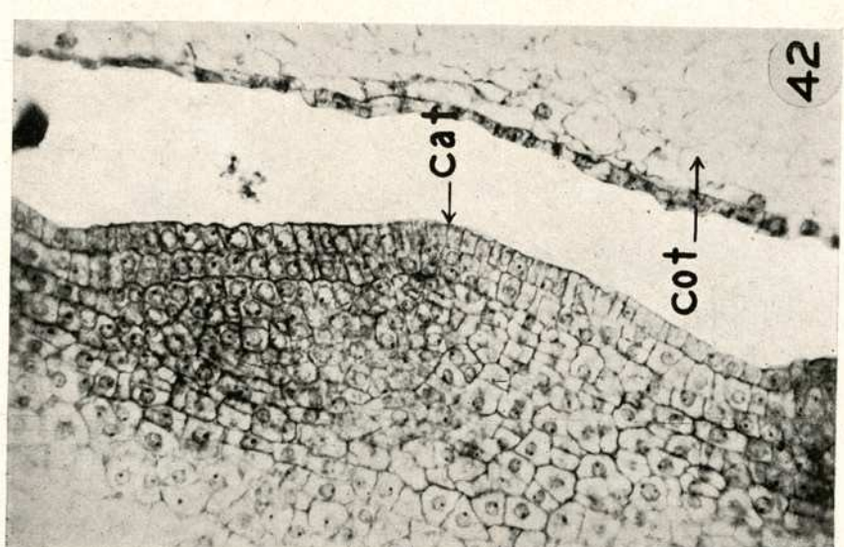
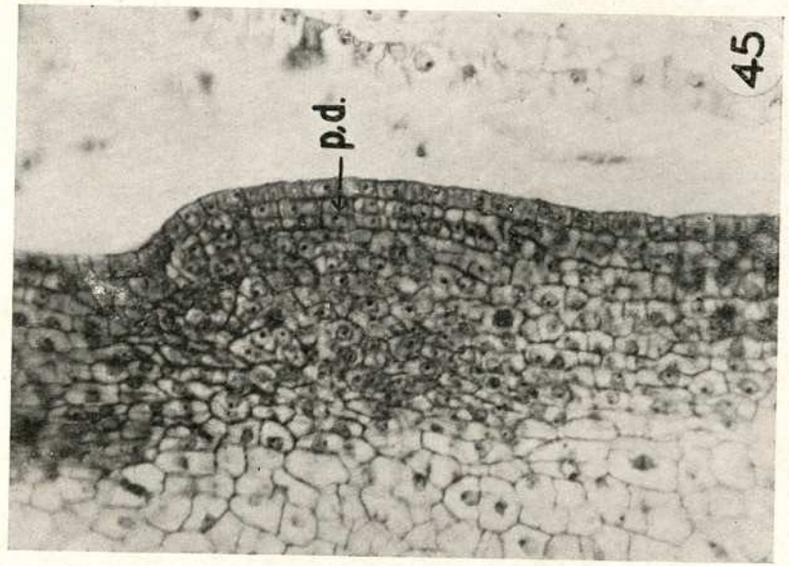


PLATE XV

Fig. 44, A different section of the same bud as in figure 43, showing median section of bud growing point. Procambium is present. × 180.

Fig. 45, First cotyledonary bud of a 6.5 mm. embryo which had three buds in cotyledonary axis. × 180.

Cotyledon Cot. ; periclinal divisions p. e. ; procambium P. C.



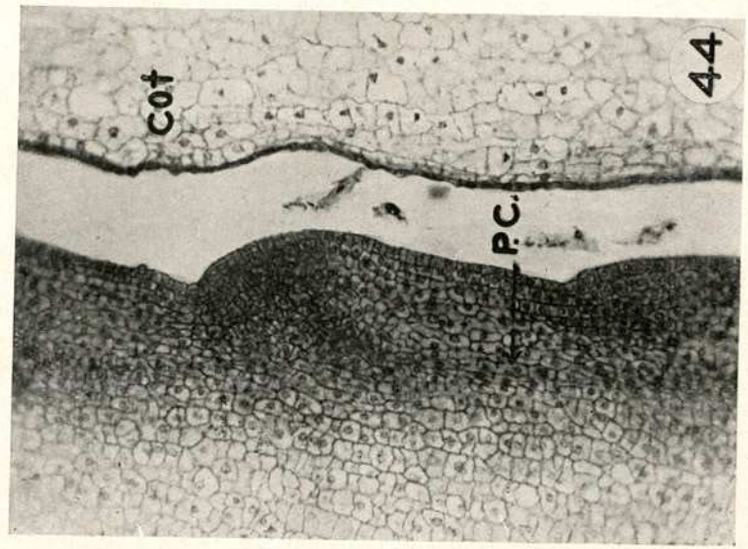


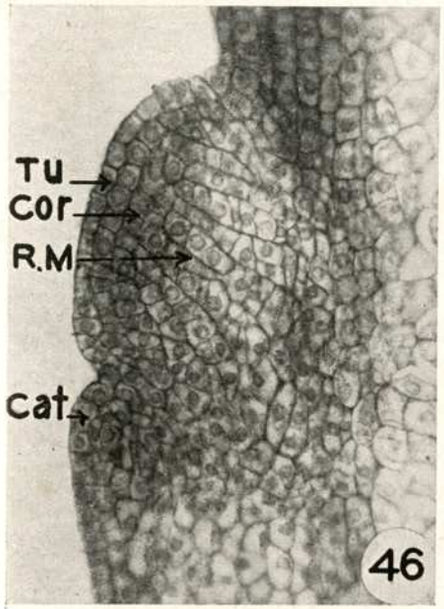
PLATE XVI

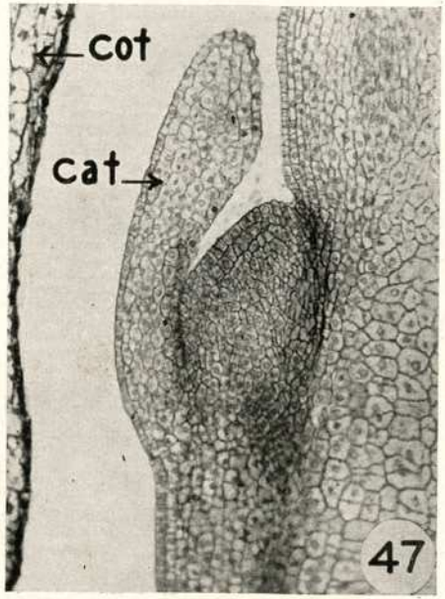
Fig. 46, Median longitudinal section through the secod cotyledonary bud of embryo depicted in figure 35, illustrating typical structure of growing point. × 180.

Fig. 47, Median longitudinal section of first cataphyll of first cotyledonary bud of embryo in figure 35. Section of bud growing point not median. × 180.

Fig. 48, Median longitudinal section of first cataphyll of second cotyledonary bud of a mature embryo. × 180.

Cataphyll Cat.; corpus Cor.; cotyledon Cot.; rib meristem R. M.; tunica Tu.





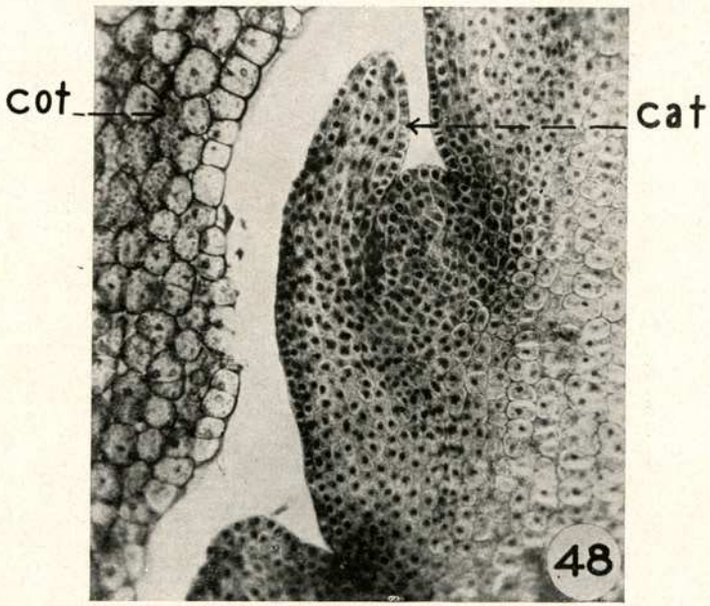


PLATE XVII

Fig. 49, First protoxylem formed at the base of cotyledonary trace. × 180.

Fig. 50, Longitudinal section through cotyledonary bud of a small seedling, showing trace to first cataphyll. × 72.

Fig. 51, Cross section through cotyledonary bud of a seedling showing spiral arrangement of the four cataphylls. × 72.

Cataphyll Cat. ; cotyledon Cot, ; cotyledonary trace T. ; leaf Lf. ; protoxylem P. X. ; trace T.