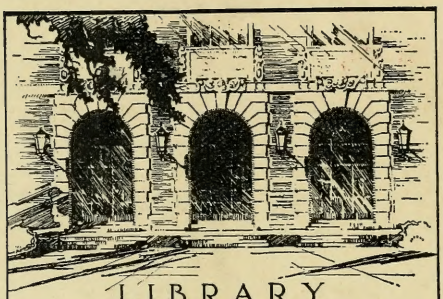

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THE EMBRYOLOGY OF LARIX

WITH 86 FIGURES

BY

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CONTRIBUTION FROM THE DEPARTMENT OF BOTANY
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PREFACE

The earliest embryonic stage, because of the simplicity of its cellular makeup, is a logical starting point for developmental studies of plants. Evidence observed and deduced from embryological studies may be brought to bear on several fields of inquiry. Knowledge of the cellular sequence and mechanism by which the chain of heredity is transmitted to a new generation of plants should contribute to the success of undertakings in plant improvement, and this is particularly true with regard to gymnosperms. The embryonic stages consist of a series of "origins" of tissues and tissue complexes which necessarily condition the organization of the seedling and, less directly, that of the mature plant. The dynamic features of this sequence are especially important. Although the cellular organization of the gymnosperm embryo appears to be relatively simple, its physiology must be highly specialized. Unfortunately, experimentation with plant embryos is very difficult, and only during the last few years has a degree of success been achieved in this direction. Experimental studies were beyond the scope of the present investigation, but the histological details presented here may serve as a suitable background for such studies. The embryonic structures have been regarded from the standpoint of function, and their phylogenetic significance has been judged accordingly. It is realized that final proof of any phylogenetic hypothesis must be derived from phyto-paleontology, but only a small amount of fossil evidence can be brought to bear at present on gymnosperm embryology. Causal analysis of modern material should serve to identify with greater certainty those features denoting relationships due to common descent. Although conclusions based on such reasoning are still tentative, they may assist in revising the more formalized doctrines of interpretation.

The present study of the embryology of Larix was begun in 1932 at the suggestion of Professor J. T. Buchholz, and most of the material was presented in June, 1937, as a dissertation for the degree of Doctor of Philosophy in Botany at the University of Illinois. An abstract of the dissertation was published by the University in 1937. Some additional material was collected and sectioned in 1938, and a few recent bibliographical references have been added, but no new evidence has been found to alter the conclusions.

I wish to express my appreciation to Professor Buchholz for his guidance in conducting my research; his sympathetic discussion of the

many problems of interpretation has been of inestimable value. The assistance provided by my wife, Esther Julie Nissen Schopf, in the preparation of the manuscript and in many other ways, is mentioned as a matter of deserved record. I wish to express also my appreciation for the use of the photographic facilities of the Illinois State Geological Survey in preparation of the illustrations, and to Miss Meredith M. Calkins who assisted in completing the plates.

CONTENTS

I. INTRODUCTION

Descriptions of the entire embryonal development are generally lacking for all conifers, and since little specific information about the embryology of Larix is available, there has been a need for a more comprehensive treatment. This study gives an account of the embryo of *Larix decidua* Mill, from the proembryo stage up to embryonic maturity. Some observations have also been made on *Larix laricina* (Du Roi) Koch, *Larix kaempferi* (Lamb) Sarg., and × *Larix "eurolepis" (L. decidua = europaea × leptolepis)*. Origin of the various tissues present in the mature embryo, the detailed sequence of development up to this stage, and a theoretical interpretation of this embryogeny are presented.

The embryologic sequence shown by Pinus may be compared with Larix to great advantage, since they are both in the same family, and, in addition, the Pinus embryology is probably the best understood among all the conifers. The writer agrees with Doyle (1918) and others that Larix is more closely related to Picea and Pseudotsuga than to Pinus, but the embryonic sequence is not much better known in these other genera than it was in Larix when this study was begun.

Consideration has been given to the fundamental conceptions of embryology in terms of the embryonic development of Larix. Enough is known of embryo sporophytes throughout the plant kingdom at present to warrant broader theoretical consideration of the Pinaceous sequence of development. The "primitive spindle" concept of Bower and Lang is of particular interest in this connection.

II. HISTORICAL ACCOUNT

Since the earlier literature on conifer embryology in general has been adequately reviewed by Buchholz (1918 et seq.), and since Schnarf (1933) in his recent compilation gives the present status of conifer embryology with considerable detail (especially the early stages), we need not dwell on the general background of the subject, but may proceed with a discussion of those few workers who have studied the embryology of Larix.

The paper by Geleznoff (1849) is the earliest significant contribution on the embryo of Larix, although Robert Brown (1843, 1844) had apparently observed polyembryony in Larix along with various other conifers as early as 1826. Geleznoff gives quite a complete series of stages up to slightly past the proembryo in a species which he never names, but was probably *Larix sibirica,* since this species is native in the vicinity of Moscow. His drawings may be taken as remarkably accurate for the period in which his work was done. However, since he was a strong advo-

cate of male inheritance as propounded by Schleiden and also strongly advocated by Schacht (1850), his interpretation of the fertilization stages was rejected long ago. Possibly his adherence to this misconception of fertilization caused the merits of his work to be overlooked by his more immediate successors. Strasburger does not mention this article in any of his publications on conifer embryology. Doyle (1925, 1926) has commented particularly on Geleznoff's correct and well-illustrated treatment of the pollination process in Larix. Aside from Hofmeister's (1850) review of Geleznoff's article, the work is very obscurely cited or omitted from the older literature. It is apparent that the concept of a cell as we know it today was not yet crystallized when Geleznoff made his drawings, but rosette cells are clearly shown with primary suspensors and four terminal embryonic cells. In the most advanced embryo system he shows the suspensor as longer than that shown in my figure 12, but the first division of the initial cells has not occurred.

Hofmeister (1862) gives a brief account of *Pinus larix,* which was probably *Larix decidua,* in the English translation of his "Vergleichende Untersuchungen." Although Hofmeister's general views have long since proved to be essentially correct, he does not give much specific information of value with reference to the present problem.

Strasburger produced in "Die Coniferen und die Gnetaceen" (1872) and in "Die Angiospermen und die Gymnospermen" (1879) the most monumental contribution of all early workers on gymnosperm embryology. Although he covered a large variety of conifers and did investigate Larix rather thoroughly in some particulars, chiefly relating to pollen development, pollination, and fertilization, he gives no details of embryonic development in this genus, and his neglect of the Larix embryo has been for the most part continued to the present. A great deal of present information on the embryos of conifers still depends in some measure on Strasburger's observations and figures. The figures of Picea embryos, which he gives in some detail, agree with Larix in most respects, and it is likely that the processes of embryogeny are very similar in both. Strasburger, however, did not obtain the critical stages during which the cells from one vertical row overgrow the other three to form the final embryo (as will be shown in the case of Larix), nor did he understand the autonomous embryonic potentialities of several proembryo cells.

Woycicki published in 1900 an account of the proembryo of Larix. This publication has not been available to the author, but in a paper published in 1923 he again illustrates Larix proembryos and mentions that these confirm his earlier observation, that the Larix proembryo is similar to that of Pinus.

Doyle started an investigation of the fruiting structures of Larix in 1917 and published (1918) an account of *Larix leptolepis,* which included

a detailed study of some abnormal pollen grains, and gave a brief account of the female gametophyte and proembryo. The final stage of embryonic development shown by him is a little later than that of Geleznoff. The similarity to Pinus is again pointed out.

In 1918 Buchholz published a most significant paper reporting his investigation of the early embryo of Pinus. Buchholz's recognition of the significance of the eight original cells of the early pine proembryo is the outstanding feature of this work inasmuch as it clearly defines the *primary units* of the embryology. The dissection technique, which he was the first to use effectively, made proof of his conclusions possible.

In 1920, 1926, 1929, and again in 1931, Buchholz briefly recorded the embryonic conditions in Larix, and recognized its similarity to Pseudotsuga, Picea, and Abies, all of which however were thought to be simple in their polyembryony.

Thus, while the early sequence in Larix was known approximately, the later stages up to the time of seed maturity, remained unknown. Larix is generally assumed to be similar to Pinus in later embryonic growth. However, many of the details of tissue development in Pinus are not yet described, so there is little repetition in the treatment of the later stages in Larix.

Buchholz and Old (1933) in their paper on the dormant embryo of Cedrus considered the histology of the mature embryo and illustrated one younger stage just prior to the initiation of cotyledons. For the first time the unique nature of the embryonic radicle in conifers was fully recognized. The structure surrounding the radicular plerome was named the *calyptroperiblem.*

Secretory elements which are to be described in the late embryo of Larix have also been reported from Abies, Taxus, and Cedrus by Chauveaud (1903a, 1903b, 1904). Buchholz and Old (1933) and Milhone (1933) likewise found these structures in Cedrus and Podocarpus, respectively. In studies of seedlings, Hanes (1927) mentions "tannin sacs" in Pseudolarix and Tsuga and shows structures in Sciadopitys and Araucaria which are probably later developments from similar embryonic secretory elements. Notwithstanding the seeming widespread occurrence of these structures, little attention has been paid to them, and no mention of them may be found in current textbooks.

Bower in 1922 presented the "primitive spindle" concept. This generalization affords a unifying basis for comparison of the embryogeny of lower plants. In somewhat similar form, it is included in his book on "Primitive Land Plants" (1935). Lang is responsible in part for the chain of thought developed in this hypothesis, and it is expounded in a fundamental way, although not by that name, in his Manchester address to the British Association (1915). The basis of comparison afforded by this

hypothesis rests primarily on the conception of a fundamental organismal polarity and the manner in which early morphologic differentiation takes place. It seems desirable to extend these fundamental interpretations to higher plants, since the essential processes of embryology are comparable.

The genus Larix has been reworked taxonomically in recent years by Ostenfeld and Larsen (1930). The nomenclature used in the present study is in accord with their recommendations, except in quotations from other authors. Family and genus names are as given by Pilger (1926).

III. MATERIALS AND METHODS

The University of Illinois Forest Tree Plantation provided most of the material for this study. The larch trees were started in 1870 by Professor T. J. Burrill and replanted in part in 1872 (see his report dated 1893). The grove now numbers about 200 good-sized trees, which ordinarily produce abundant cones. Of the two years during which collections were made for this investigation, 1933 was more fruitful than 1932. However, viable seeds were produced in both, and aside from the greater number of barren seeds in the first season and slightly later development of all stages, the embryos are quite comparable. There is no reason to believe that the embryogeny reported here is different from the normal sequence, although *Larix decidua* is not indigenous to this country.

Larix laricina, obtained near Grass Lake, Michigan, in 1934, was examined in late development of the early embryo. Slightly later stages of *Larix kaempferi* and *L. "eurolepis"* (a hybrid, either *L. europaea* (= *L. decidua*) × *L. leptolepis* or its reciprocal), collected by Dr. Buchholz at the Arnold Arboretum, were also available.

Killing and Fixation

Formalin acetic alcohol, as recommended by Chamberlain (1934), was used throughout this work, since it is suitable for preserving, as well as for killing and fixing. In general it was found to be quite satisfactory, although the dense archegonial cytoplasm shrank considerably when subjected to it. This seems to be a common defect, as scrutiny of the illustrations given by other authors shows, but even the details of proembryo organization are not necessarily obscured by it.

Cones of *Larix decidua* were gathered daily during the time fertilization was occurring in the spring of 1933. Later, collections were made every two or three days throughout development of the early embryo. Many collections were also made during the various stages of the late embryo. Numerous whole gametophytes were dissected out under a wide-field Greenough-type binocular microscope and placed directly into the

kill for later sectioning. To afford basis for the reliable interpretation of sections, many embryos were dissected from their gametophytes, stained in phloxine, and mounted in "diaphane," following the procedure originally devised by Buchholz (1918) and more fully described by him in a recent number of Stain Technology (1938).

Embedding and Sectioning

Whole gametophytes were embedded by the customary xylol method, as given by Chamberlain (1934). In placing the material in the final paraffin block, eight or ten gametophytes were carefully arranged in rows side by side, as closely spaced and precisely parallel to one another as possible, each row being slightly shorter than the width of the cover slips which were to be used later. Each group was mounted as a unit on a wooden block, which was then clamped in the microtome, and longitudinal and transverse sections were cut 15 microns in thickness. This thickness is an economy in slides and in time; further, it is easier to reconstruct the details of histology from such sections than from thinner sections.

The early embryos are so small that it is necessary to section them within the gametophyte. Although they often are not axially aligned in relation to the gametophyte, this method of embedding and sectioning proved to be efficient. The chances for getting good sections by a single cutting operation are increased, and the possibility that parts of the work will be altogether fruitless is minimized, in spite of the fact that some lots of material consist predominately of sterile gametophytes which cannot be reliably detected before sectioning.

Some of the late embryos, and some which had been germinated, were dissected out of the gametophytes and embedded and sectioned individually.

Stains and Staining

The stains used in this investigation were the safranin-picro-nigrosin combination recommended by Stover (1928), safranin and fast green, Haidenhain's iron alum with fast green, and Haidenhain's iron alum with orange III.

Stover's stain was used to a considerable extent, since it serves most purposes very well and is an exceedingly rapid technique once the timing is understood. The usual practice was to stain in 50% alcoholic safranin for two hours, run the slides through two steps to water, and rinse thoroughly. At this stage it was convenient to wipe off all the excess safranin adhering to the back and edges of the slide. The slide was then put in the half-and-half mixture of 1% picric acid and 1% nigrosin, both aqueous solutions. Time of staining varied greatly depending on the material; extremely meristematic tissue absorbed the stain much more

readily than mature tissue. For the embryo proper the time required for a good stain was about one minute. The slide was rinsed, the excess water being drained off, and was run very rapidly through a series of 25%, 50%, 75%, and 100% methyl alcohol. A mere dip into each of these alcoholic solutions is all that is necessary, as the safranin especially is very soluble in methyl alcohol. The next step was to half-and-half methyl alcohol and xylol, and then into pure xylol. The slide may be drained conveniently by touching the end on paper toweling between each step in the series. The slides were mounted in balsam in the usual way.

By this method the cytoplasm is stained a delicate lavender, with dark purple cell walls and a reddish purple nuclear differentiation. The nuclear membrane is quite distinct. Safranin is tenaciously held by fatty material, so that the megaspore membrane becomes brilliantly pink. Protein globules and starch grains both hold the safranin, the former being a vivid orange, and the latter tending to be yellowish, due probably to the picric acid. One of the very striking characteristics of this stain is the differentiation obtained in the germinating seedling, where the older tissues in process of elongation absorb more safranin than picro-nigrosin and the active meristems react oppositely. In the seven or eight years since many of the sections were made, there has been only a slight amount of fading.

The excellence of Haidenhain's iron alum for cytoplasmic and nuclear detail is very well known. This stain is better than the safranin combination for mitotic figures.

Fast green is a very useful stain, perhaps even better than the safranin combination for revealing cell walls of meristematic tissue. However, it tends to obscure materials in the cytoplasm which are so splendidly differentiated by the latter. Orange III is a weak counter-stain which does not seem especially applicable to this material. None of these stains are desirable for use individually.

IV. INVESTIGATION

Embryo Development in the Pine Family

So far as known, the proembryo is a relatively uniform structure in the Pinaceae, and at least some information is available for all nine genera, with the exception of Keteleeria. The embryologic sequence shown by Pinus is most completely known, and it is the best for general comparison. The purpose of this section is not only to pass in review the general embryologic sequence known for the Pinaceae, but also to introduce certain descriptive terms applicable to the detailed study of Larix embryos reported later on.

In pine, fertilization is followed by two free nuclear divisions of the

zygote. Free nuclear division of this sort is a unique characteristic of gymnosperms as a whole. The four free nuclei in the pine type of development then move to the base of the archegonium and come to rest in a single plane transverse to the archegonial axis. Each nucleus of this group soon divides to produce a new tier of nuclei above, and during this division, proembryo walls first form to enclose the four basal nuclei. The upper four nuclei still remain open to the archegonium above. The nuclei of the upper tier again divide, and the lower nuclei once again are enclosed by walls with four free nuclei above them. These free nuclei remain as they are, undergoing no further development. To distinguish them from the four free initial nuclei formed from the zygote, they are called the *relict nuclei,* since they have no function now but may have had in the past. Thus, a proembryo consisting essentially of twelve cells, eight walled and four relict nuclei, is formed. In Pinus each of the eight walled cells which are present at this stage of the embryogeny later develops an individual polarity of its own, and the four cells nearest the base of the archegonium, referred to hereafter as the *apical cells,* form potentially functional embryos; the tier of four cells directly above them forms rosette embryos. In Larix only the four lower cells (apical cells) of the proembryo manifest a definite polarity or show indication of embryo-forming capacity. The individualized growth of these cells in pine indicates very early that they are all potential embryonic cells. It is shown in the present study that each of the four apical cells in Larix is similarly potential. Since each of the four apical cells at the twelve-celled stage have individual polarity, these are designated as *polarity units.* A polarity unit is defined by applying the empirical test of observing its embryo-forming capacity. The first indication of polarity is evidenced by each of the apical cells dividing transversely, so that before the archegonium is ruptured by elongation there are three tiers of walled cells with a tier of relict nuclei above, sixteen in all. All the walled cells are similar in cytologic character at this time, but subsequent development shows that the new tier of cells, formed by segmentation of the four apical cells, are in no sense individual members but are organically subordinated to their respective apical cells. This is the tier of cells that elongate to become primary suspensors. Their elongation causes the four apical cells to break the wall of the archegonium and move out in contact with the undifferentiated gametophytic tissue. The proembryo is said to be complete just before the archegonium is broken, i.e., in the sixteen-celled stage. This intra-archegonial period of development represents a rather distinct chapter in the embryologic sequence and one which from various standpoints, physiological as well as morphological, can be compared with a considerable degree of accuracy among gymnospermous plants. In the subsequent discussion the period of proembryo development

has been shortened, for use as a term, and will be referred to as the *pro-stage*. The period beginning with the first elongation of the primary suspensor, and continuing through the period of great suspensor elongation, to the time when the embryo anlage becomes massive is termed the *meta-stage*. The pro-stage and the meta-stage together are taken to include the period of development of the early embryo.

The primary suspensor is a meta-stage embryonic organ present in all genera of the Pinaceae. When each of the four apical polarity units comes to function separately through cleavage, as they do soon after elongation of the primary suspensors in pine, the meristematic cell at the tip of each primary suspensor is clearly identified as a true apical cell. (See the definition of an apical cell on p. 60.) Cleavage does not take place with anything like the same definiteness in Larix as it does in pine. The polarity units remain united for the most part throughout meta-stage; nevertheless, the apical cells function toward the same end result and are just as definitely present. Meta-stage growth is chiefly by the addition of segments cut off by a transverse wall at the base of each apical cell and by subsequent successive elongation of each of these segments. Meanwhile, in pine, the rosette cells divide and each one forms a distinct embryo tip. The rosette embryos are always stunted but often grow for a considerable number of cell divisions, at which time they also possess a definite apical cell and more or less vacuolate cells behind. The rosette cells of Larix show none of these manifestations of polarity or growth but gradually degenerate. Thus we may say that the proembryo of Pinus contains eight demonstrable polarity units, whereas in Larix there are but four. Cedrus and Tsuga are known to resemble pine in having rosette embryos, and Picea and Pseudotsuga to resemble Larix (Pseudotsuga apparently even lacks rosette cells); Abies also is in this latter group. The embryologies of Pseudolarix and Keteleeria are less well known in this respect.

The manner in which the late embryo develops is inadequately known in the Pinaceae and among gymnosperms in general. It begins with formation of massive tissue originating from a tetrahedral apical cell both in Pinus and in Larix. Soon after massive apical tissue is formed a significant sequence of histogenesis begins. Cotyledonary tissues are the last formed of the several tissues which compose the mature embryo. Subsequent growth after cotyledon primordia are formed involves enlargement of the tissues already differentiated and observable.

It was convenient to divide the sequence of late embryo development into two stages, as in the sequence of early embryogeny. The period of histogenesis, beginning with formation of an undifferentiated mass of cells at the embryo tip after meta-stage development, and including the subsequent differentiation until cotyledonary primordia can be seen,

is termed *ana-stage*. *Telo-stage* development involves the subsequent increase in size leading up to embryonic maturation seen in the resting stage of the dormant embryo.

The sequence of histologic development in the late embryo has, up to now, not been followed closely for any gymnosperm. There are, however, numerous illustrations showing the structure of immature late embryos which have been briefly discussed by several authors. Nearly all these represent embryos in telo-stage development, which may be indicative of the generally rapid passage through the ana-stages of growth. Comparison of these illustrations with sections of Larix embryos shows that all the gymnosperms have similar tissues in their telo-stage development. However, significant variations appear to be present which may be due either to minor differences in the relative sequence of differentiation or differences in proportional development of particular tissues or both. Actually a highly complex sequence is involved, and the general similarity shown by late embryos of all gymnosperms is remarkable in view of the many other variations in embryogeny (in the meta-stage sequence particularly) which are already known. The histologic similarities between the late embryos of modern gymnosperms which are dissimilar in many vegetative and other characters surely represents a marked conservatism that suggests most strongly an ancient and monophyletic derivation for this division of the plant kingdom.

Rate of Growth and Sequence of Embryo Development in Larix

Since precise comparison of the embryologic sequence with other gymnosperms, particularly in the late embryo, will involve variations chiefly of degree, an attempt has been made to represent the sequence diagrammatically for Larix in the chart on page 18. (The terminology used in this diagram is discussed below.) The structures characterizing the early sequence are entirely homologous with those known in pine (and also, as mentioned before, with other less known genera in the Pinaceae), but it is clear that the time required for completion of meta-stage growth varies considerably in these two genera, and it is quite likely that other members of the family also vary significantly.

In the material collected in 1933, it was observed that fertilization was taking place on the twentieth of May, and in some cases as late as the twenty-sixth, although at this later date, proembryo stages were predominant with some early meta-stage embryos present. Unfavorable environmental factors operating in 1932 were probably not only responsible for the preventing of abundant fertilization, but also for the retardation of the development of the embryos.

Organization of the proembryo following fertilization takes place

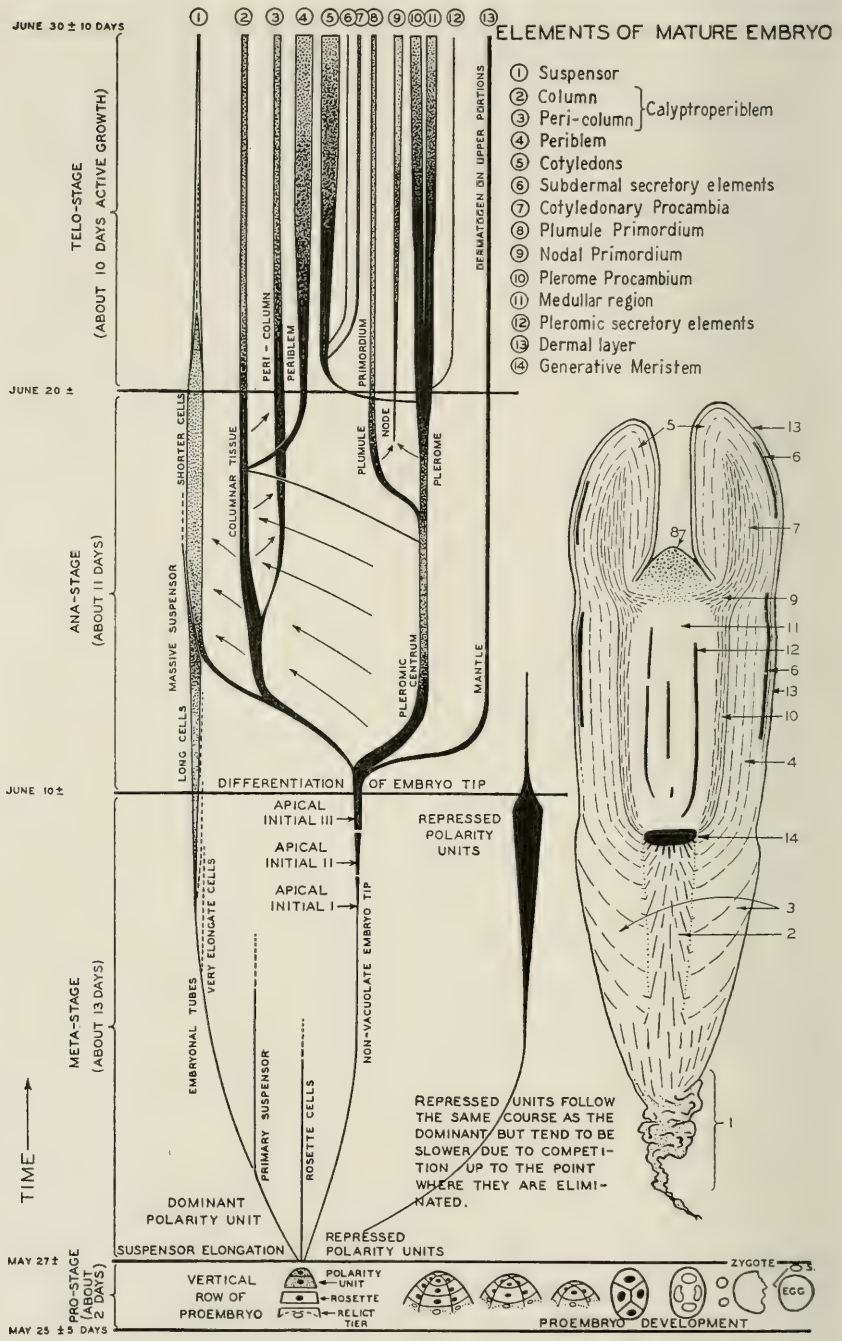


Chart showing embryologic sequence in *Larix decidua* Mill. Width of line indicates the relative tissue prominence, and density of shading indicates relative meristematic activity.

rapidly, so that the pro-stage is very short—probably much shorter than can be definitely ascertained by study of the collections. The length of time indicated by the diagram on the facing page may, therefore, represent variation in time of fertilization, as well as the period of pro-stage growth. Meta-stage development, on the other hand, takes about thirty-six per cent of the growth period in Larix, although in total number of cells and in mass, it represents less than one per cent of the total growth to maturity. Meta-stage in pine is of much briefer duration.

The contrast in time allotted to meta-stage development between Larix and pine is explicable by differences in their early embryogeny. Immediate cleavage of the four leading embryos of a single embryo system in pine removes the four polarity units from acute tactual competition with each other, and during meta-stage each one follows its course of development independently. The essentially hemispheric apical cell deviates from its early divisional sequence after formation of very few embryonal tubes (suspensor segments added subsequent to the primary suspensor segment), and generally after only one or two new unelongated segments have been formed. Then the next one or two segments in the series may be separated at an oblique angle, or the apical cell may merely cease division in a single plane abruptly, dividing subsequently in three different planes, the hemispheric apical cell thus becoming a tetrahedral initial. Further segmentation in pine results in formation of a massive tip of embryonic tissue.

In Larix all four polarity units remain closely associated through the meta-stage period. The four closely appressed apical cells cut off suspensor segments as in pine, but all the polarity units of the embryo system usually remain together until late meta-stage. Then one or more polarity units, depending on very slight superiorities in size and position instituted early in pro-stage, begin to cut off oblique segments in two planes away from the sides occupied by the other competitive apical cells. The additional cell mass thus built helps give the more advanced of the four polarity units added advantage and serves to separate the two-cutting-faced apical cell from the close association previously maintained with its neighbors. Frequently two of the apical cells are quite equally matched and form double rows of oblique segments nearly at the same time. After becoming separated at the apex one of the polarity units grows enough in advance of the others to relieve the spacial competition from that source, and in response to this release of tension separates a segment in the angle to partially overtop the other apical cells. Subsequent growth in Larix for a time duplicates that in pine after the tetrahedral apical cell is established there. The repressed polarity units in Larix are very quickly eliminated, since they are thrust back by direct contact with the basal elongating cells of the dominating unit. In essence the direct compe-

tition between polarity units of the same embryonic system is more acute in Larix than in Pinus, and this largely accounts for the longer meta-stage period of the former.

Early growth, as measured in terms of cell divisions, is probably relatively slow in both genera. Certainly mitotic figures are most infrequently seen during meta-stage. Physiologically the young embryo is undergoing adjustment, through the necessity newly arisen, of acquiring nourishment externally from the fluid filling the corrosion cavity instead of from more or less pre-adapted food material in the archegonium. The quantity of enzymes liberated also may influence rate of growth. In general an embryonic tip composed of few cells and lacking tissue specialization does not seem likely to be capable of functioning as efficiently and rapidly in early growth as later when it is more differentiated.

Assuming an equal rate of cell division it is probably safe to estimate that meta-stage is two to four times as long in Larix as it is in Pinus. It may be even longer because the approximation of the four apical cells of the system may very likely have a retarding effect on one another. One result of delay in meta-stage of Larix is that all early elongated suspensor segments are badly disintegrated, probably by enzymic activity. No doubt this factor has delayed interpretation of the early embryology of Larix considerably. In pine the suspensors are frequently well-preserved during meta-stage and even in early ana-stage (see Buchholz, 1918, Pl. IX, figs. 47-50), and this permits relatively easy and certain definition of polarity units and of embryo systems. In later meta-stage in Larix when the suspensor has disintegrated, the only positive clue to identification of embryo systems and polarity units is by observation of the apical cells. Since all but one dominant apical cell is eliminated in earliest ana-stage, adequate material and closely spaced collections are necessary if the sequence is to be followed.

During early ana-stage the growth rate jumps significantly, and the embryo may be said to enter upon its "grand period of growth." Ana-stage occupies about thirty-one per cent of the period of embryonic growth, and during this time the embryo undergoes a manifold increase in mass. The tetrahedral apical cell functions only for a short time to form a massive tip, which is somewhat smaller than that produced directly by a tetrahedral initial in pine before elimination.

Ana-stage is essentially the time of tissue origin, and with early elimination of the apical cell this progresses rapidly. First formed is the *columnar tissue*, composed of a large number of *derivation rows* (vertical series of cells which are each derived from a single initial)* at the base

*These are the units which constitute the rippenmeristeme of Schüepp (1926). Rippenmeristeme has been translated by Foster (1940, 1942) and others as rib meristem or as rib-like meristem. The writer believes that an English rendition as *costate meristem* for a tissue composed of derivation rows would be more suitable.

of the central mass of cells that later constitute the plerome. The alignment of derivation row initials at the base of the central mass (which is called the *pleromic centrum* at this stage) marks the origin of a new tissue zone between the plerome tip and suspensor. This tissue, while poorly defined at first, can be identified later as the *generative meristem* which gives rise to all the tissues of the primary root. Growth goes on rapidly with enlargement of both the columnar tissue and pleromic centrum which becomes essentially spherical in shape. The pleromic centrum continues to furnish cells to the tissue below, and these in turn furnish the lateral tissues of the embryo (*peri-column* and *periblem*). While lateral outgrowth is occurring below the centrum, apical prolongation takes place and a conical mass of cells is formed at the tip which are recognized as the *plumule primordium.* Two tissues originate primarily by outgrowth from the sides of the columnar tissue: (1) the *periblem* at the top, adjacent to the pleromic centrum, and (2) lateral "root-cap" tissue, designated as *peri-column.* The central columnar tissue becomes the *"säule,"* or *column,* of the mature embryo.

The peri-column is the first lateral tissue, an outgrowth from the central column, and this is probably the largest single factor responsible for the notable increase in the diameter of the late embryo. To become peri-columnar tissue the more lateral cells of the column shift polarity abruptly and grow out obliquely upward and radially from the axis alignment. The periblem is a direct continuation of the peri-columnar tissue and is formed from it while the plerome is very short by the lateral introduction of peri-columnar cell rows growing out around the basal arc of the pleromic centrum. Consequently, at this early stage there is no difference between peri-column and periblem. The latter merely consists of the few upper layers of peri-columnar cells, which lie close enough to the plerome so that they are able to maintain intercalary growth parallel along its sides in later (chiefly telo-stage) development. The periblem cells and the very short plerome elongate together in telo-stage, with no further contribution from below.

Cotyledons are the last formed of the embryo tissue complement. They originate on the apical flanks of the pleromic centrum from cells comparable to the columnar row initials formed on the basal portion. Their origin is referable in part to the formation and lateral extension of procambial cells in the nodal region between the pleromic centrum and the plumule primordium and in part to the concurrent widening of the embryo caused by growth of the periblem tissue. When cotyledon primordia are formed, all the rudiments of the mature embryo are present and the *telo-stage* period is initiated. During telo-stage growth, which is of course directly continuous with that of late ana-stage, the greatest change is increase in size of the embryo organism. In this later develop-

ment the cotyledons and embryo axis grow considerably more in proportion than the *calyptroperiblem,* a term proposed by Buchholz and Old (1933) for the gymnospermic root cap which includes the column and peri-columnar tissues. This organ attains mature size sooner than tissues higher in the embryo. The axis increases in length by intercalary growth of the plerome and periblem of late ana-stage; the cotyledons are individually dominated by this same manner of growth, the chief effect being an increase in length. The increase in radial dimensions becomes progressively slower during telo-stage, and in later development it is due mostly to radial enlargement of cells already there rather than to new cell divisions. This is especially true of the periblem region. Procambial differentiation takes place first in the nodal region, then in the cotyledons as they grow, and along the margin of the plerome. The embryonal procambia are chiefly distinguished by the differences in proportional dimensions of the cells. The cells of the late embryo all remain in a meristematic condition until after germination, but during the growth sequence certain tissues in turn become considerably more meristematic than others.

A dermal layer is distinguishable over the embryo tip soon after it becomes massive, and it exists continuously thereafter. It probably is the food-assimilating organ during all the period of massive growth. Only in late telo-stage does it become sufficiently definite to be termed a *dermatogen* and then only upon the cotyledons and the adjacent surfaces of the axis. Before the formation of a definite *dermatogen* the dermal cover over the embryo is designated as the *mantle layer.** The mantle divides both anticlinally and periclinally as occasion demands. It is remarkable in its adaptability and efficiency in maintaining a smooth symmetrical surface contour over the embryo during all stages of late embryonic growth.

During telo-stage the embryo becomes equipped with a peculiar system of secretory elements which are not to be confused with resiniferous passages arising later in the seedling. One group of the secretory elements arises in the central part of the plerome and finally extends up into the cotyledonary procambial areas. Others are formed sub-dermally in the periblem and adjacent to outer surfaces of the cotyledons. They do not intercommunicate and their function is unknown, but because of their chromophilic contents they are prominent in late telo-stage. Active telo-stage development, taken to represent the period of growth after all

*Schmidt (1924) has called the more or less regular investing cell layers of mature shoot apices *"tunics."* The term *"mantle"* is preferred here because it seems desirable to distinguish the embryonal from the mature meristematic layer or layers. The mantle probably is active as an agency for food transfer in the embryo and seems less definite histologically than a tunic, except possibly in the region of the telo-stage shoot primordium. The plumule mantle precedes formation of any dermatogen—the outermost tunic layer may be considered, in part at least, a juvenile differentiation of dermatogen in histologic continuity with it. Foster (1942, pp. 22-24) has recently discussed the significance of the tunica.

tissues of the mature embryo may be recognized, occupies about twenty-eight per cent of the active growth period. By the end of June the embryo is essentially mature, although it is not likely that enlargement stops altogether until later in the summer.

The origin of later tissues of the embryo from pre-existent tissues, the relative prominence of the various tissues in the embryo at any particular stage of development, the relative meristematic activity of the tissues, and the approximate time period of their existence during the embryologic sequence have been indicated diagrammatically in the chart on p. 18. The tissues of the late telo-stage embryo are indicated on the accompanying embryo diagram, the key numbers corresponding for both the embryo diagram and the telo-stage development on the chart. The density of shading indicates the estimated relative meristematic activity of the different tissues at any stage of their development. All except the matured and collapsed cells of the suspensor are meristematic, inasmuch as they are all capable of division, but there are important differences in the rates at which cell division normally occurs in the tissues, and it is this feature which is approximately indicated. Similarly an attempt to evaluate the apparent relative prominence of the tissues through the embryologic sequence is indicated by the widths of lines employed. Obviously both meristematic activity and the prominence of the various tissues permit no accurate measurement by ordinary histologic methods, but if this is kept in mind the diagram may give a more accurate picture of the developmental sequence as a connected and absolutely interrelated phenomenon than is possible by means of description alone.

Development of the Gametophyte and Archegonium

The gametophyte agrees in its early development with the usual Pinaceous sequence. Recently Saxton (1930) found that hypodermal derivation of the linear tetrad, reported by Strasburger (1879) is not an ordinary occurrence, but that in *Larix europaea* (= *decidua*) the tetrad is formed deeper in the nucellus. Sections of cones showing early gametophyte stages were prepared, which tend to support Saxton's statement, although none were at a stage early enough to show the original tetrad.

The gametophyte passes through the characteristic free nuclear stage and, with the formation of cells, differentiates archegonium initials at the micropylar end.

The number of archegonia varies from one to five in the material examined, three or four being the usual number. Doyle (1918) reports that the archegonia in *Larix leptolepis* are always five in number. The variable number as found in *L. decidua* is more typical for the *Pinaceae*.

The archegonia are always enclosed by a separate layer of jacket cells even when they are close together and the jacket layers somewhat com-

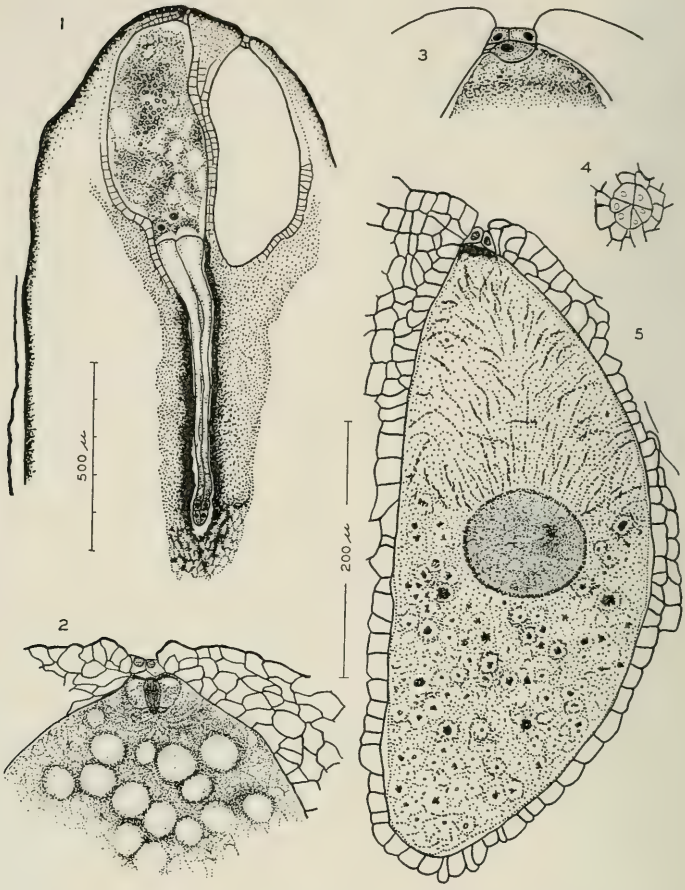


Fig. 1.—Longitudinal section of an early meta-stage embryo system within the gametophyte. Stippled area corresponds with the central cone of more opaque tissue. Collection of May 26, 1933.

Fig. 2.—Longitudinal section showing division of the central cell at the apex of an archegonium. Collection of May 21, 1933.

Fig. 3.—Section similar to Fig. 2 and from the same collection, showing ventral canal cell.

Fig. 4.—Transverse section of neck of archegonium.

Fig. 5.—Longitudinal section of archegonium just prior to penetration of pollen tube. (The pollen tube has traversed the nucellus tissue above this archegonium.) Collection of May 30, 1933. (Figs. 2-5 drawn at the same magnification.)

pressed (Figs. 5, 48, 49, 51, and 52). Doyle states that the jacket may be reduced to a single layer or be altogether absent in *L. leptolepis* where the archegonia are crowded. The jacket cells are smaller and more uniform in shape, with considerably denser cytoplasm than the gametophytic cells surrounding them. The neck of the archegonium is never more than one cell deep. The neck cells are variable in number, however, from four to eight. Figs. 2, 3, 4, and 5 show typical examples. Quadrant walls are formed by equal anticlinal segmentation of the original neck initial, and these four cells may or may not be subdivided as the two shown in Fig. 4. Doyle mentions the shallow archegonial chamber in *Larix leptolepis*. In fertile archegonia of *Larix decidua* a similar condition is found. However, the neck cells of archegonia long past fertilization appear sunken into the gametophyte because a rather long canal is formed by tissue growing up around them.

Doyle and his co-workers (1925, 1926, 1935) have studied the pollination processes of Larix quite thoroughly. There is little that can now be added to their account. It is still unexplained exactly how the pollen is transferred from the stigmatic flap to the nucellus. However, this transfer does occur and abundant pollination results, often with the several archegonia being fertilized at about the same time. The four archegonia shown in Fig. 52 have all been fertilized, essentially simultaneously. Two empty pollen grains and the pollen tubes leading from them directly through the nucellus are shown in Fig. 48. Here also both archegonia have been fertilized. In this instance the nucellus has been slightly dislodged from its position next to the gametophyte in the process of dissection and paraffin embedding, so that the termination of the pollen tubes cannot be followed.

The nucellar tip on which the pollen grains rest is truncated. In dissections this tip area is clearly visible, since it results from collapse of the original nucellar-tip cells with consequent brownish discoloration.

The archegonial cytoplasm is light-staining and frothy, with numerous large clear vacuoles prior to formation of the egg nucleus. This condition is shown in Fig. 2 as the ventral canal cell is being produced. Later, after formation of the ventral canal cell (shown in Fig. 3, and a later stage in Fig. 5), the clear vacuoles disappear and the cytoplasm becomes more chromophilic. The egg nucleus enlarges greatly to about 300 μ in diameter, and meanwhile the ventral canal cell disintegrates. Just prior to fertilization the archegonial cytoplasm becomes exceedingly fibrillar, especially in the upper part and around the egg nucleus. The basal portion is commonly full of deeply-staining granules and globules (Hofmeister bodies) of various sizes. This condition is shown in Fig. 5 and also in transverse section of four archegonia in Fig. 51. In the latter case the fibrils form a definitely spiral pattern throughout the upper part of

the archegonium. They may have a definite part in the process of fertilization not now understood. Lawson (1909, Pl. XIII, figs. 26 and 27) shows fibrillae in archegonia of Pseudotsuga at about this same stage, although it is not certain that they are spiral as in Larix.

Fertilization

Details of the entrance of the pollen tube into the archegonium are not known. The discharge vacuoles at the top of the archegonium, as mentioned by Chamberlain (1935, p. 334), are visible for some time after penetration by the male nucleus, until fertilization is complete. Only one archegonium was found in which the two gametic nuclei were still separate, so that fertilization appears to take place quite rapidly.

The egg nucleus is very large at this time. After fusion of the two nuclei, the upper surface of the nuclear membrane becomes quite irregular, as seen in Fig. 52, and it sometimes becomes extremely invaginate. The zygotic nucleus becomes elongated during the first mitosis. The spindle seems to lie entirely within the nuclear membrane, with no apparent segregation of male and female chromosomes. The stain used was not suitable for cytological study at high magnification, however, and consequently the fine details of chromosomal behavior cannot be reported. There is no definite orientation of this first spindle; sometimes it is transverse and in other cases oriented lengthwise of the archegonium.

At the end of two free nuclear divisions the four free proembryo nuclei are found at the base of the archegonium as shown in Figs. 53 and 54. As in Pinus, the first walls of the proembryo are formed during the next nuclear division. The free nuclei resulting from division of the zygote are very much smaller in size. (Compare the free nuclei shown in Fig. 53 with the fusion nuclei in Fig. 52, or the gametic nucleus in Fig. 51, all of which are reproduced at the same magnification.)

The so-called "Hofmeister bodies," or protein globules, found in the archegonium during fertilization and for some time afterward, were for a long time considered as separate nuclei by the early workers. They are most abundant in Larix succeeding fertilization and persist for a considerable time until after elongation of the suspensor. No doubt, they constitute part of the food which nourishes the proembryo. It is easy to understand how they came to be confused with nuclei, because it is often very difficult to distinguish them from the relict nuclei in the later proembryo stages.

The Early Embryo

Pro-stage.—From the time of fertilization to the beginning of elongation of the suspensor, the embryo is considered to be in the pro-stage. The proembryo of Larix has been studied by Woycicki (1900, 1923) and

Doyle (1918), who stated that its development is essentially similar to that of Pinus. The present study also confirms this.

As in Pinus, the proembryo of Larix is derived from the four first-formed free nuclei. Polarity units originate from each of the four cells at the base of the archegonium in the eighth nucleate stage. Each of the four basal cells divide to form the primary suspensor cell above, and the apical initial below. The upper four cells of the eight-celled proembryo divide prior to this first division of the polarity units below, to form the rosette cells and the tier of relict nuclei. Thus each vertical row of the proembryo finally formed in Larix consists of an apical initial cell, a primary suspensor cell (both of which are functionally connected and constitute a polarity unit), a rosette cell which exhibits no special attributes of polarity, and above this the relict nucleus in a cell open on top into the archegonium.

Chamberlain, in his recent book on gymnosperms (1935, p. 347), states that the four tiers of cells in Pinus are "almost geometrical in their symmetry." This is not the case in Larix, as is shown by transverse sections of the proembryo. The symmetry both in Larix and in other genera is less exact than might be inferred from the literature. In the first place, the archegonium is not perfectly cylindrical in shape but generally broadly elliptical, with the longer of its transverse axes placed radially within the gametophyte. When the free nuclei move to the base of the archegonium, mutual repulsion leads them to assume a balanced position in which there are two nuclei occupying the narrower poles of the ellipse, and two occupying the opposite broader sides. When walls are formed these latter two are seen to adjoin one another for a short distance by a straight central wall. The cells formed on the narrower poles of the ellipse abut on this short central wall by a sharp angle. Sometimes spores derived from an oval pollen mother cell assume this same configuration after simultaneous division. (See Wodehouse, 1929, who discusses the configurations of pollen grains.) It is believed that similar factors of nuclear repulsion are causes for both. This proembryo condition, where two vertical rows on opposite sides adjoin by a straight central wall segment, with the other two vertical rows on the narrower extremities of the ellipse not mutually adjoining, is called a *tetragonal* arrangement. This arrangement is well illustrated by the transverse section of a proembryo tip seen in one archegonium in Fig. 50 (less notably in the other), and it is also seen in Fig. 8. What has actually happened is that the two central nuclei have been able to claim for themselves a larger portion of space at the base of the archegonium than their neighbors on the narrower poles of the ellipse. The central wall segment is variable in width, varying with the original amount of asymmetry in the archegonium. Practically all proembryos have been found thus asymmetric in their original

construction to a greater or less degree. In later growth the slight initial advantage for the larger cells becomes increasingly important with reference to selection of the single successful polarity unit.

The shape of the archegonial base not only has a considerable influence on the positions of the vertical walls of the proembryo but also on the transverse walls. These are not formed at right angles to the vertical axis of the archegonium but incline downward from the center to intersect the sides at lower levels. Thus, if the archegonium and vertical rows approximated ideal *radial* symmetry, the apical cells (lowest tier) of each vertical row would be essentially tetrahedral in form, with one outer side slightly rounded to conform with the basal archegonium wall. As isodiametric an apical cell as possible would be formed. The downward slope of the transverse walls is diagrammatically drawn in Fig. 6 according to this ideal specification. Since only two apical cells (the smaller ones) have a single corner nearest the archegonium center, only these cells approximate the ideal form. The two rows abutting by a straight central wall conform to the shape of the former in that their transverse walls incline downwards from the center, but due to their added width they are more accurately described as asymmetrically wedge-

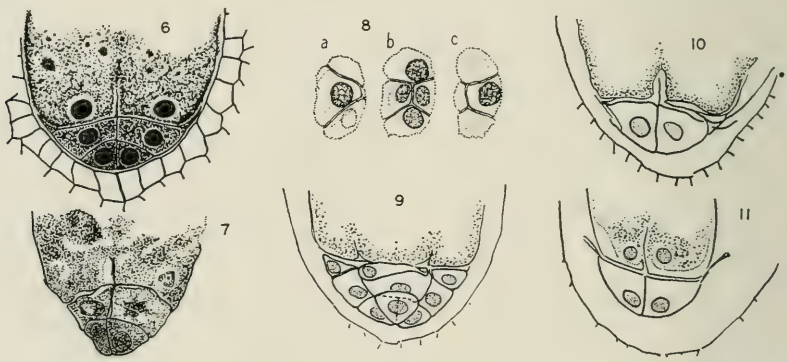


Fig. 6.—Diagrammatic drawing of proembryo in twelve-nucleate stage (8 completely walled cells, 4 open above; only half the total number are shown).

Fig. 7.—Longitudinal section of proembryo in same stage as preceding figure. Magnified about 150 times. Collection of May 26, 1933.

Fig. 8.—Oblique cross section of proembryo in eight-nucleate stage. Note the arrangement of cells in *b*, characteristic of proembryos formed in elliptical archegonia. Magnified about 140 times. Same collection as Fig. 7.

Fig. 9.—Drawing of proembryo reconstructed from several serial sections. Magnified about 175 times. Same collection as Fig. 7.

Figs. 10 and 11.—Longitudinal sections of proembryos at about the same stage as shown in Fig. 8. In both cases the sections are slightly oblique with reference to the archegonial axis. On this account the base of the archegonium appears broader and transverse walls of the proembryo do not show their true obliquity. Magnified about 175 times. Same collections as Fig. 7.

shaped. The appearance of these apical cells, as shown at the base of the archegonium, is best seen in Fig. 8b. Fig. 9, drawn from serial sections, represents a later stage in which the apical cell has divided (as in Fig. 6) to complete the proembryo.

Figs. 10 and 11 are earlier stages which do not show the true relations of the transverse walls due to obliquity of the section across the end of the archegonium. These show the most prevalent appearance of individual sections. It is impossible to orient definitely the material to show more suitable longitudinal sections of proembryos; but if a sufficient number of sections are made, some of them can be easily interpreted; the majority are obliquely cut across the planes of symmetry. Transverse sections are more satisfactory. Fig. 49 shows a section containing two twelve-celled proembryos in which the obliquity of the transverse walls is clear. In both cases cells and nuclei of the upper tier are shown peripherally toward the circumference of the archegonium; toward the center the inclined transverse wall is crossed, and within this is part of the next lower tier with its nuclei. Thus, it is demonstrable that the transverse walls of the proembryo slope downward away from the center to a considerable degree, forming in this way an obtuse angle with the basal and outer wall of the archegonium. The transverse wall of the highest (rosette) tier is much less sloping than that covering the basal (apical cell) tier. The figures of a Pseudotsuga proembryo given by Lawson (1909, Pl. XIV, fig. 40), although incomplete, show the transverse walls to be similarly sloping and also suggest strongly that the vertical rows are dissimilar just as in Larix.

Meta-stage.—The meta-stage is begun at the time of elongation of the primary suspensors. The four polarity units elongate coordinately into the area of weakened gametophytic tissue at the base of the archegonium by displacing the basal jacket cells. They retain the same apical disparity in size initiated during wall formation in pro-stage. All the apical cells divide transversely soon after rupture of the archegonial jacket. These developments are illustrated by Figs. 12, 13, and 19. In Fig. 19 the two tiers of tip cells form a more pointed cluster than is commonly found. The apical walls are thicker than those of the segments behind. This is similar to the thickening Buchholz (1920a, p. 133) has reported for Pseudotsuga. As in Pseudotsuga the apical walls lose this characteristic in later stages, but it is sometimes quite persistent (see Figs. 14, 15, 16, 18, and 22). It was also noted on meta-stage embryos of *Larix laricina,* as shown in Figs. 23 and 24. From later developments it now seems clear that this thickened apical wall does not serve effectively to hold the tips together very long past mid meta-stage, although it may be a factor in preventing early cleavage of the separate polarity units such as takes place in pine.

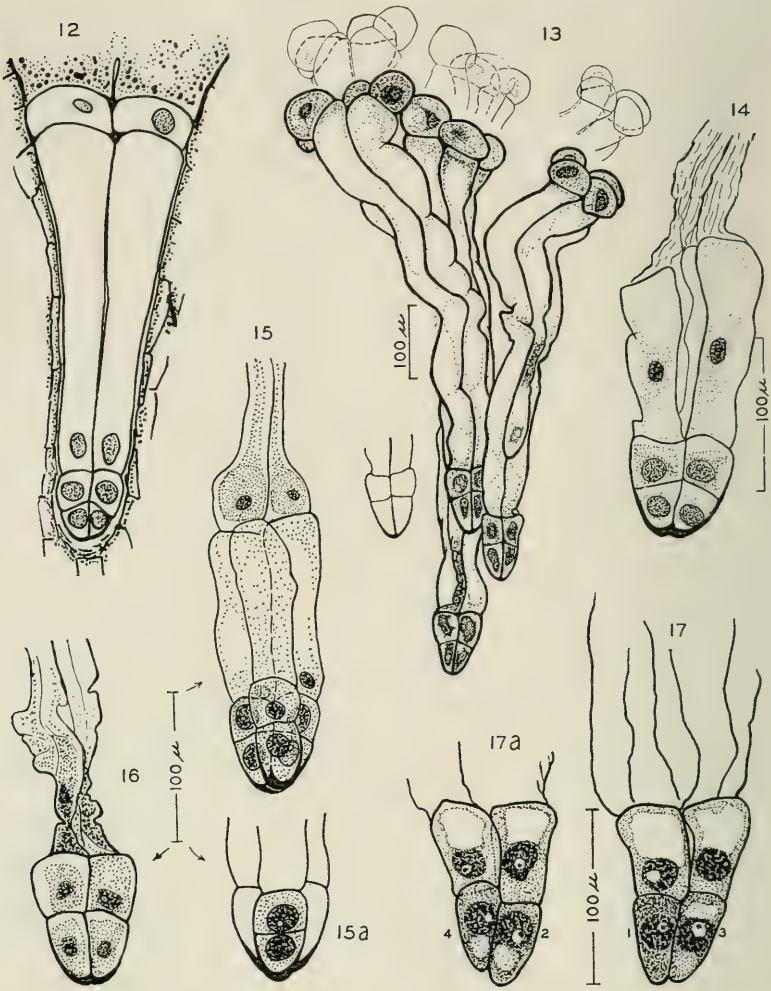


Fig. 12.—Longitudinal section of embryo system: early meta-stage. Free-hand drawing based on serial sections. Collection of May 26, 1933.

Fig. 13.—Group of three complete embryo systems in early meta-stage, dissected from one gametophyte; details of arrangement of each group of rosette cells is indicated above. Collection of May 28, 1932.

Fig. 14.—Tip of embryo system at time of elongation of second embryonal tubes.

Fig. 15.—Tip of embryo system similar to that of Fig. 14. Fig. 15a shows the fourth polarity unit drawn from a lower plane of focus.

Fig. 16.—Tip of embryo system in which the apical cells are more than usually elongated, perhaps preparatory to division.

Fig. 17.—Tip of embryo system, from the same collection as preceding figures but slightly more advanced. Fig. 17a is drawn from a lower plane of focus.

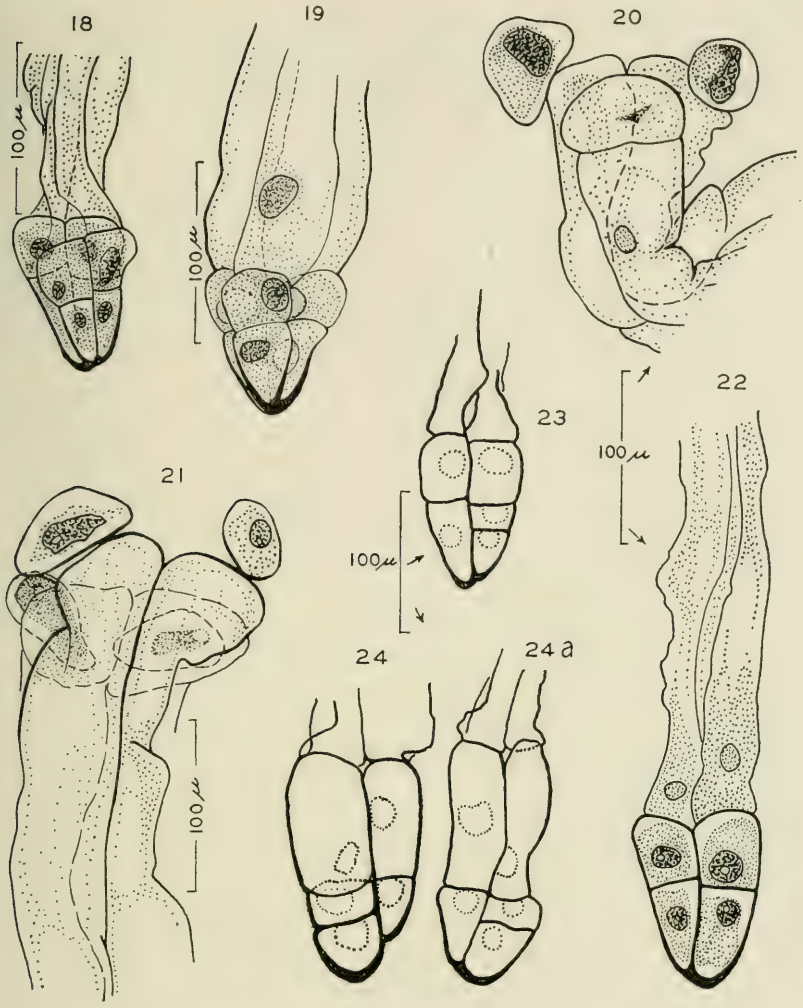


Fig. 18.—Tip of embryo system in which two polarity units on the side uppermost in the preparation are somewhat less developed than in Fig. 17.

Fig. 19.—Tip of very early meta-stage embryo system. Collection of May 28, 1932.

Fig. 20.—Rosette cells and primary suspensors belonging with the tip shown in Fig. 18. Same collection as Fig. 19.

Fig. 21.—Rosette cells and primary suspensor belonging with the tip shown in Fig. 22.

Fig. 22.—Two of the four polarity units of an embryo system showing typical asymmetry.

Figs. 23 and 24.—Tips of polarity units of meta-stage embryo system in *Larix laricina*. Fig. 24a is drawn from a lower plane of focus. Collection of June 17, 1935, Grass Lake, Michigan.

The tiers at the tip of the embryo system grow in length and the primary suspensor elongates, as shown in Figs. 11 and 12, to move the tip cells deeper into the nutritive tissue. The apical cells divide to form another suspensor segment later on when the primary suspensor cell is collapsing and the first embryonal tubes are elongating. This serves to keep the tip cells pressed firmly against the archegonial tissue, and they slowly progress down the center of the gametophyte, apparently digesting their way. The pressure alone, developed by elongation of suspensor cells, is inadequate to cause penetration of the gametophyte, since these cells collapse readily and lack any strengthening features. The mechanical ability of the suspensor cells is dependent chiefly on turgor pressure developed from within.

The cavity in the gametophyte digested by enzymatic action has been called the corrosion cavity by Buchholz (1918, p. 195). The cavity in Larix is lined with a layer of partly digested debris consisting of remnants of gametophytic cell walls and refractory granules, a sort of "pseudo-epithelium." The corrosion cavity undoubtedly contains more or less fluid substance surrounding the embryo and serves as a means of food transfer in solution. Sections sometimes show the embryo tip of Larix embryos some distance away from the lower end of the cavity, but others show it in contact at this point. Probably digestive processes precede the embryo tip to some extent, but it is supposed that more efficient and rapid penetration occurs when the tip cells are close to the gametophyte cells acted upon.

For a considerable period of time, growth of the embryo is slow. Basal segments of all four polarity units are cut off successively and elongate in turn to maintain the suspensor. In the course of these processes the thickened apical wall disappears. Cell division is infrequent, and the most obvious result of early meta-stage growth is digestive penetration of the gametophyte by the embryonic tip. For the most part cell division merely keeps pace with depletion due to collapse of previously formed suspensor cells. The tip for a considerable time consists of the apical cells, one tier of more or less unelongated suspensor cells, and then the elongated embryonal tubes (cf. Figs. 14 and 15).

The shape of the rosette cells changes from their original flat form in pro-stage by becoming rounded as shown in Fig. 13. They are from the first much larger than the apical cells of the vertical rows. Later when the primary suspensor is disintegrating they also lose the staining character associated with living cells. Their nuclei become indefinite in shape and the cytoplasm vacuous. In dissected embryos beyond the early stage of elongation they are apt to become dissociated from the primary suspensor cells. Figs. 20 and 21 show rosette cells at this stage, and a little later they disintegrate along with the primary suspensor and are

seen no more. They very obviously lack the meristematic qualities which distinguish them in Pinus.

In later meta-stage the asymmetric arrangement (which characterizes the apical arrangement from the time of wall formation in the proembryo) becomes more obvious. As a result of the tetragonal arrangement in pro-stage, the two larger polarity units gradually increase their superiority. Often one of these two appears superior to the other. This is the case in the tip shown in Figs. 17 and 17a. The four tips lie with two in a high plane and two in a lower plane and have been drawn separately. The order of superiority is indicated by the numbers one to four. The apical cell of the first polarity unit may safely be designated as the dominant one of the group. Not only does this apical cell project slightly ahead of the others but it alone is nonvacuolate. The second unit, on the opposite corner in lower focus, is nearly as advanced as the first, but has a small vacuole above the apical cell nucleus. The third and fourth units are clearly subordinate and must have occupied the narrower angles of the archegonial ellipse in the proembryo. Figs. 24 and 24a show the same arrangement in a meta-stage embryo of *Larix laricina,* but in this case the tetragonal disparity is greater. Fig. 18 shows a different cell arrangement in which both of the minor rows are on one side. In this case one of the polarity units in the lower plane of focus would have become dominant. This arrangement is not frequently found and very likely came from an abnormal arrangement of the proembryo nuclei. The rows shown in Figs. 15 and 15a are not so decidedly asymmetric. Still I have no doubt that eventually only one polarity unit could go on to form the final embryo. The underlying row, shown in Fig. 15a, projects slightly in advance of the others. The tip, illustrated in Fig. 22 (only two units of which are shown), is typically tetragonal in its configuration. Figs. 14 and 16 illustrate the close pairing of opposite polarity units. In both cases the other two units, one located in a high plane of focus and the other below and not shown in the drawings, were noticeably shorter. Figs. 14, 15, and 16 also indicate how rapidly and completely collapse of the suspensor may take place. It is not possible to count how many embryonal tubes have been formed, but in each of these cases probably three or four collapsed segments have been produced for each unit prior to development of those now elongating. The four units composing each system of the three shown associated in Fig. 13 show evidence of tetragonal arrangement when examined individually at higher magnification, although this may easily be passed over in casual observation.

Embryo tips in cross section at this stage also show the same tetragonal configuration. In Fig. 25 serial sections of the tips of two systems are shown, both of which include eight cells above the much elongated cells of the suspensor. The cell nuclei are shown in the section

in which they are most prominent. The system which has grown farthest within the gametophyte extends from section *a* to *h*. Disintegration of the suspensor of this system is quite advanced in the sections beyond this where the tip of the lower embryo system was in contact with it. The apical cells of the lower system are cut in sections *g*, *h*, and *i*. In both of these embryo systems those two units which adjoin each other in the center by a straight segment of wall are seen to be somewhat larger than the two rows which have a single central angle. This is shown particularly by sections *a*, *b*, and *c*, and by sections *g* and *h*, which transect the apices in both cases. The same condition is observed in Fig. 26, which also represents the second embryo system in its particular corrosion cavity. Section *f* of Fig. 26 shows the cavity as enlarged by passage of the two systems; the almost completely disintegrated suspensor of the first system is collapsed alongside the second tip.

Up to this stage each polarity unit has functioned in close contact with others of the group of four derived from a single embryo system. The tips of the various systems have digested their way through half or three-quarters of the length of the gametophyte, the first system to elongate being most advanced. Soon the tendency toward formation of massive

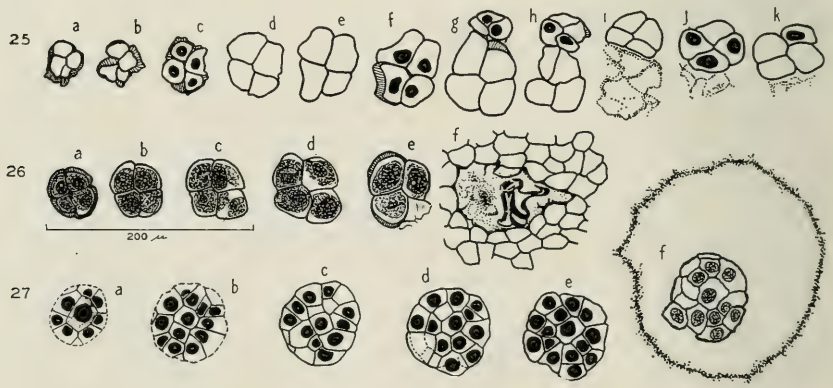


Fig. 25, a-k.—Serial cross sections of tips of two meta-stage embryo systems within the same gametophyte. Note that the cells which adjoin by the straight central wall are larger and that suspensor cells of the first system are disintegrated at level of contact with the second system (*h-k*). Collection of May 30, 1933.

Fig. 26, a-f.—Serial cross sections similar to those in Fig. 25. The corrosion cavity is shown in *f*, at a level where the suspensors have collapsed; note also the disintegrated suspensor of an embryo system which preceded the one shown.

Fig. 27, a-f.—Serial cross sections of early ana-stage embryo. Only a single polarity unit can be identified. The corrosion cavity (outline in *f*) has also been enlarged by another more advanced embryo which is located deeper in the end of the cavity. This series probably arose from an embryo system arrangement similar to that shown in Fig. 26. Collection of June 11, 1933.

tissue appears; first in the system deepest within the gametophyte, and then in the others.

Hitherto each apical cell has cut off new cells (which were added to the suspensor in turn) by straight transverse partition walls. That is, although these apical cells are somewhat triangular in cross section due to mutual contact, they each have a single basal cutting face and are altogether comparable to the hemispheric apical cells of pine at this early stage. The only difference is that the polarity units in pine have separated from each other by early cleavage and are not ordinarily in contact. Buchholz (1918, p. 202) found that in pine the "stage in which the apical cell has two cutting faces does not exist or is so shortened that it cannot be easily recognized." In Larix the polarity units ordinarily form several segments from an apical cell with two cutting faces; this is explainable as due to contact between the apical cells of polarity units of the system. Fig. 28 (p. 37) shows two units having apical cells with two cutting faces, one of which has divided cells in alternate planes for the last seven or eight divisions. This is the unit which would have finally become dominant. The two repressed units show less advanced conditions. The one at the left is slightly separated from the rest of the group, and has a nearly hemispheric apical cell; the opposite unit (drawn separately at *a*) is slightly more advanced, having recently cut off two oblique segments.

Fig. 29 shows a somewhat similar stage obtained from sectioned material in which one of the polarity units has become further dissociated, so that it represents a case of normal cleavage. This unit is shown in section *a*. It has been considerably distorted and the apical cell is broader than usual, perhaps due to the unusual circumstances resulting in its cleavage from the rest of the system. The next three serial sections contained no significant embryo parts and are not represented by drawings. The remaining group of three embryo units of this system are shown in successive sections represented by sections *b*, *c*, and *d*. Section *b* shows an apical cell with two cutting faces. The small unit at the right in section *c* has an apical cell with a seeming flat base which has nevertheless produced two wedge-shaped segments. The configuration of segments shown in section *d* shows that the other polarity unit has an apical cell with two cutting faces (most of which is included in section *c*) that has persisted for at least seven divisions.

During the period in which the apical cell has two cutting faces (the stage II apical cell), one polarity unit finally develops to overtop its competitors. When pressure is sufficiently relieved by overtopping the other units, the leading apical cell graduates to a tetrahedral shape and produces segments on three cutting faces. Not only does this occurrence afford the dominant unit the opportunity of forming a massive meristem but it very quickly eliminates the competing units. Only in cases where

Fig. 28.—Tip of late meta-stage embryo system in which two of the polarity units have a stage II apical initial (two cutting faces) and the others only hemispheric initials. Polarity unit *a*, almost hidden in the original group and drawn separately at the right, has segmented obliquely during the last division and presumably is somewhat more advanced than the unit at the left of the group. Collection of June 11, 1933.

Fig. 29.—Tip of late meta-stage embryo system from sectioned material. Sections *b*, *c*, and *d* are consecutive and show three polarity units closely associated. The fourth, represented in section *a*, was separated from the rest and was developing independently; presumably this is a case of ordinary cleavage. Collection of June 13, 1932.

Fig. 30.—Early ana-stage embryo tip which includes the progeny of only one polarity unit. Its section in Fig. 30a shows no remnant of an apical initial. Collection of June 14, 1932.

Fig. 31.—Early ana-stage tip from the same collection as the last, but slightly more advanced. Derivation rows occur in the mantle layer, as shown in the drawing of the surface; a stunted repressed unit still adheres to the side of the larger embryo. Figs. 31a and 31b represent sections of the same embryo at successive focal planes.

Figs. 32 and 32a.—Surface and section of an embryo tip from same collection as Fig. 31 but showing slightly different proportions.

Fig. 33.—Early ana-stage embryo in which the pleromic centrum and columnar tissue are becoming evident. Collection of June 6, 1933.

Fig. 34.—Early ana-stage embryo, earlier than Fig. 33, showing manner of elimination of the apical cell. Magnification same as for Fig. 36. Collection of June 13, 1932.

Figs. 35 and 36.—Sections of early ana-stage embryo tips, showing wedge-shaped mantellary cells which are tentatively identified as remnants of apical cells of polarity units. Collection of June 4, 1933.

Fig. 37.—A pair of early ana-stage embryo tips, with suspensors intimately connected, probably derived by cleavage from the two stronger polarity units of one embryo system. The embryo to the right has eliminated its apical initial, but the tetrahedral (stage III) initial still persists in the other. Collection of June 14, 1932.

Fig. 38.—Tips of two polarity units in more than ordinarily intimate association. Apical initials of both have been eliminated. Non-vacuolate cells are stippled. Both these polarity units would have been repressed by progeny of a more advanced embryo ahead of them in the corrosion cavity. Collection of June 18, 1932.

Fig. 39.—Section of ana-stage embryo showing pleromic centrum and columnar tissue. Collection of June 11, 1933.

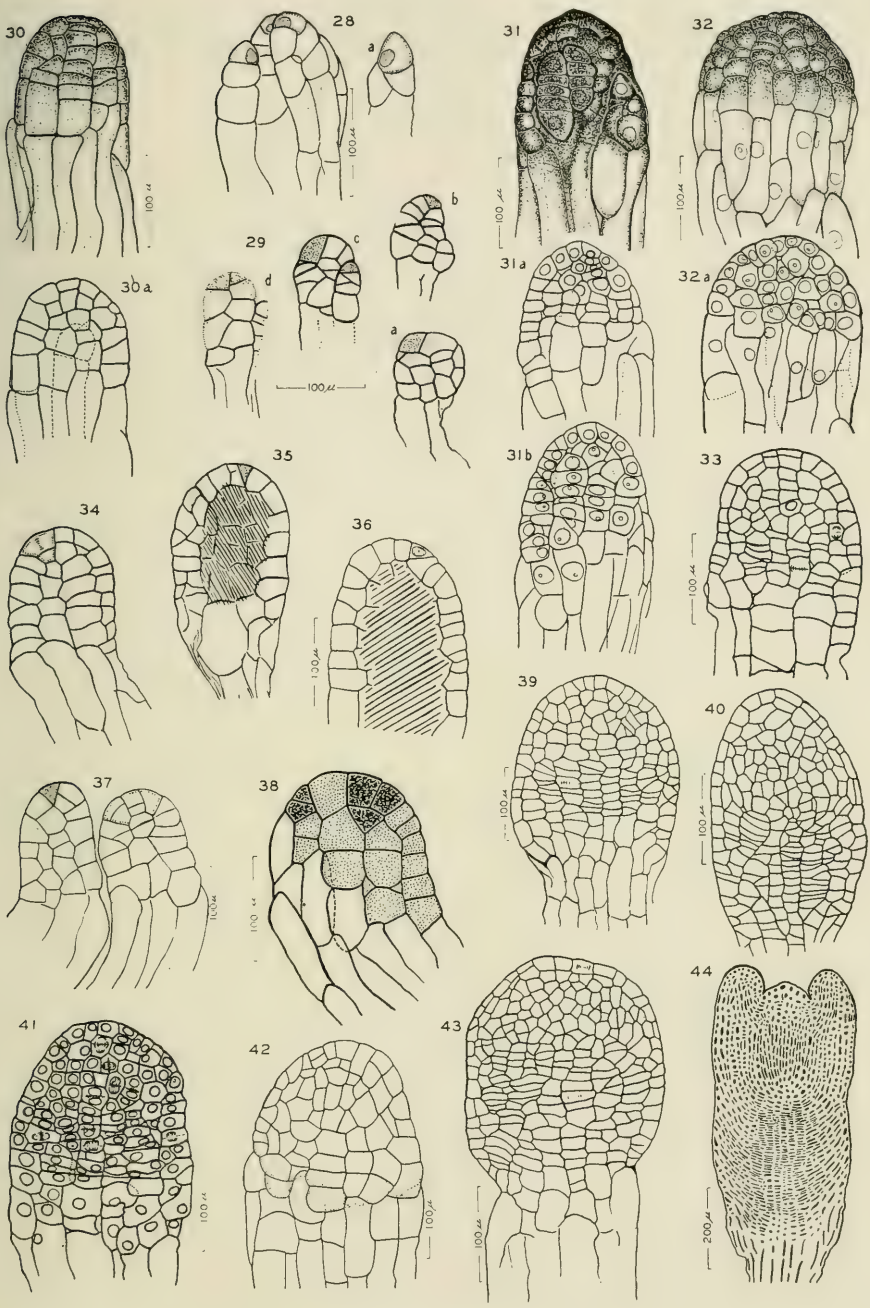
Fig. 40.—Section of ana-stage embryo probably more advanced than that in Fig. 39. The basal curve of the pleromic centrum is better defined, and there is definite tilting of the marginal derivation rows. Collection of June 11, 1933.

Fig. 41.—Section of ana-stage embryo comparable to Fig. 39, with mitotic figures indicated, suggesting the rapidity of growth. Collection of June 6, 1933.

Fig. 42.—Early ana-stage embryo, with stippled line indicating limit of non-vacuolate cells. Collection of June 14, 1932.

Fig. 43.—Section of typical mid-ana-stage embryo, with pleromic centrum clearly defined. Collection of June 11, 1933.

Fig. 44.—Cell line diagram of early telo-stage embryo for comparison. Each line represents the long axis of a cell. Collection of June 25, 1932.



Figs. 28-44.—See explanations on opposite page.

a smaller unit has become sufficiently dissociated from the rest of the embryo system by actual cleavage, and is thus permitted a more independent development, can lesser units survive for any time at all. In such instances they develop their own tetrahedral apical cell and may continue for some time. This appears to be unusual, however, and the isolated unit in section *a* of Fig. 29 is one of the few cases in which characteristic cleavage can be demonstrated. The most usual course seems to involve rapid and complete subordination of the smaller polarity units.

The dominant embryo unit by this time has a tetrahedral (stage III) apical cell which is actively building a massive primordium. Occasionally a repressed unit may be demonstrated in connection with the massive embryo primordium, as in Fig. 31c, in Fig. 38, or (with less assurety) in Fig. 35. Fig. 56 (Plate I) shows a later embryo with a repressed unit, with its stage II apical cell still associated. This is unusual since most of the smaller embryos are badly disintegrated before this stage. Generally the fate of the repressed units is quickly sealed when one member attains sufficient dominance to form an apical cell with three cutting faces. Of course, from this stage on the quadrate cross sectional form of the early embryonal system (due to association of the four polarity units) is no longer present.

It is likely that cleavage also occurs in a small percentage of embryo systems where two opposite polarity units are large and strong and evenly paired. Such a condition is probably represented by the embryos shown in Fig. 37. These two embryos, closely associated and nearly the same size, attached together by their secondary suspensors, probably represent the co-dominant units from one originally "tetragonal" embryo system. Absolute proof of this would require tracing the tips of the two units back to their respective primary suspensor cells. However, the primary suspensors, and several embryonal tubes as well, have been so disintegrated that they are not to be found. Embryos of this size generally lack primary suspensor cells since these cells apparently disintegrate within the corrosion cavity soon after their collapse. Evidence that these two are from one, and not two, archegonial systems is derived from the nearly equal size of the two tips and from the condition of the secondary suspensors of both. We have seen (Figs. 25 and 26) that a second system growing along the path of an earlier system rather completely digests the suspensor of the latter as it grows along. Since the suspensors behind the two tips in Fig. 37 seem to merge and to be equally collapsed on both sides, they have undergone similar disintegrative processes such as would not be likely to occur if two separate systems were involved.

Organization at the apices of these two closely matched embryos is the greatest point of contrast. The embryo on the left has a definite well marked apical cell cutting off segments in three planes, while the one at

the right is more advanced and has eliminated its apical cell. This one would probably have become dominant, and it is likely that it held a slightly more advanced position within the gametophyte than it now shows in the dissected preparation. These presumably twin embryos show how soon the single apical cell may be eliminated. Both contain about 60 nonvacuolate cells (as computed according to the formula given by Buchholz 1918, p. 213). There is nothing to suggest that these embryos are abnormal in development, although they probably have come from a system with cleavage more pronounced than usual.

A few other cases have been observed in sectioned material where two embryos of comparable mass were developing within the corrosion cavity which might have come about by similar cleavage. Some persist during stages of later development such as shown in Fig. 47a. No actual proof of derivation can be advanced at such a stage because all the early suspensor is indefinable and even the original number of archegonia is doubtful. From the order of events in pro- and meta-stage, derivation from a single well-balanced embryo system would seem quite possible, however. If a similar situation were found which had developed from a single archegonium, a rare occurrence in Larix, this would constitute direct evidence of cleavage. It is likely that cleavage of this type does occur occasionally in Larix, although it must be regarded as exceptional, because most of the embryo units remain in contact with the other three units of the system until one becomes overwhelmingly dominant.

Late meta-stage embryos of *Larix laricina, L. kaempferi,* and the hybrid resulting from *L. decidua* × *L. leptolepis* or its reciprocal (sometimes called *L. "eurolepis"*) have been examined in this stage, and all these conform closely to the description just presented for *L. decidua.* In *L. laricina* the disparity between the four polarity units seems to be a little greater than in *L. decidua;* the others seem to be practically the same.

Whether we call the competitive elimination of repressed units *cleavage* or not, it is clear that the process is similar to that found in pine, differing only in degree. Clearly, it is not correct to assume that the four polarity units developed by the proembryo all contribute to the final embryo even though they do generally remain united throughout meta-stage. Thus simple polyembryony is a misnomer as applied to Larix; it has the general embryogenic sequence, with its embryonal competition, etc., essentially as in pine, where cleavage is most characteristic. Obvious competition in Larix is merely postponed for a time and then becomes very acute during the phase of actual embryonic selection. From the illustrations given by Strasburger (1872, Pl. XII, figs. 35 to 37, esp. 37) of Picea embryo systems in late meta-stage, it seems probable that the same developments occur in this genus. Pseudotsuga and other pinaceous genera previously thought to possess simple polyembryony also may very

likely be similar to Larix in this respect. Hutchinson's (1924) figures of Abies embryos, as discussed later (p. 65), indicate that the same sequence probably occurs in that genus.

In order to clarify the situation in regard to Larix and others like it, it is suggested that the condition wherein the polarity units remain closely associated until late meta-stage, when a dominant one is selected solely to contribute to the final embryo in a manner essentially similar to that found in Pinus, be known as *delayed cleavage polyembryony*. In this way the qualitative similarity to Pinus is rendered apparent by the terminology. Among gymnosperms simple polyembryony in a primitive sense is best typified by Cycads. True simple polyembryony is more likely an advanced character wherever it occurs in the Coniferales.

The Late Embryo

Ana-stage.—When a polarity unit becomes equipped with an apical cell having three cutting faces (the stage III apical cell), growth of massive embryonic tissue proceeds very rapidly. The stage III apical cell usually exists for a very short time, but with respect to number of divisions, it cuts off more segments than either the stage I or II apical cells which preceded it, because the rate of growth increases enormously at about this time. The embryo tip at the right in Fig. 37 shows an early stage lacking an apical cell. Fig. 55 (Plate I) shows the latest stage found possessing an apical cell, but here the last tetrahedral vestige is not dominating growth by any means, and is more properly considered an adjunct to the outermost cell layer or mantle. The manner in which the apical cell is eliminated is suggested in Figs. 35 and 36 where anticlinal division has left a triangular relict of the apical cell inserted in the mantle layer, and in Figs. 34 and 38 where the main apical cell has been segmented by a periclinal division, leaving the triangular segment within the mantle layer.* This method seems to be quite common, leaving a cell in the central region resembling an "internal" apical cell. Whether the apical cell is finally eliminated by a periclinal or by an anticlinal division seems to be the determining factor; this is probably a detail of little consequence, for before its actual disappearance the essential physiological attributes of the apical initial seem to have been transferred to a deeper seated *group* of cells.

A normal-appearing tip is shown in Figs. 30 and 30a in surface view and optical section respectively after the apical cell has been eliminated. The characteristic evenly rounded surface over the hemispheric non-vacuolate tip is apparently due to the organization of the external layer of cells designated here as the *mantle* (see footnote, p. 22). This layer

*The number of non-vacuolate cells in Fig. 34 is about 80, obtained by applying the formula $l\pi r^2$.

is shown in Figs. 35 and 36 and in other embryos of later ana-stage. It differs but little histologically from the cells within, dividing periclinally on occasion, as well as anticlinally. The layer probably constitutes the absorbing organ of the embryo and may be the enzyme-excreting organ (even though enzymes may be first formed in deeper cells). The fact that it acts effectively to preserve the symmetrical, even external contour of the embryo through most of its subsequent development makes it interesting from a physiological and, in the absence of experimentation, from a speculative point of view. Priestley (1928, p. 11) has given some consideration to the contour of more mature plant meristems compressed between "the limiting cuticle and external cellulose wall on one side—and the vacuolated cells on the other," but the ana-stage embryo has no cuticle. For the present the problem of how the mantle effectively maintains a smooth surface contour remains unexplained. We may say that it is due to forces of inherent polarity in the young organism, but this is hardly an explanation of the phenomenon. Once the mantle is uniformly established it probably insures against further subdivision by cleavage or other sort of fragmentation.

After elimination of the apical cell the massive tip often approximates a hemispherical form and for a time grows about equally in breadth and depth. The original segmentation of the apical cell is soon obliterated by repeated cell divisions lacking any segmental regularity. Study of transverse sections of embryos at this stage, such as those shown in Fig. 27a-f, show conclusively that no histogenic demarkation occurs until later. In Fig. 27a the large central cell (in the mantle layer) may be an immediate descendant of the original apical initial, but there is no indication that it or any other *individual* centrally located cell of the embryo possesses special attributes. In this instance cells about 95 μ below the tip (Fig. 27f) are becoming vacuolate and contributing to the suspensor. Figs. 32 and 42 show tips of comparable but slightly larger embryos in surface view and longitudinal optical section.

The first evidence of histogenic development of the tip is noted in the meristematic cells just above the suspensor. Cells of this region divide more often in the transverse plane and give rise to what I have called columnar tissue. A substantial group of morphologically unoriented tip cells remains above them and functions as a single generative meristem, which as a whole appears to have the polarity attributes formerly vested in the single apical initial. Cells basal to the generative group divide with greater frequency than those above and practically always, it seems, in a plane transverse to the axis of polarity. Consequently, in assuming the columnar habit of growth, each cell gives rise to a filament composed of short flattened segments. Through mid ana-stage, at least, the individual derivation of these filaments is especially evident. Later and in telo-stage

some segments of these filaments themselves generate filaments having a rather marked individuality. The filaments or rows of cells arising from a single cell by repeated division in the same plane may be designated as derivation rows. The columnar tissue which originates in early to mid ana-stage (see embryologic sequence chart, p. 18) is composed of the derivation rows.

The mantle layer also partakes in this mode of growth at this period, just as the centrally located cells, and the tips of these surficial derivation rows may even be identified nearer the apex than those located internally. Fig. 31 shows mantellary derivation rows as they appear in surface view, and this should be contrasted with Fig. 32. Figs. 31a and 31b are optical sections showing the early development of internal derivation rows. The generative group at the tip of this embryo is somewhat smaller than usual, and consequently columnar growth is more evident. The processes exemplified are, however, entirely typical. The inception of columnar growth is seen in surface and optical section views in Figs. 30 and 30a. Fig. 33 is drawn from a section and shows a more rounded terminal outline and larger group of generative cells than Figs. 31a and 31b (with which it should be compared), although both are at about the same stage of development. Fig. 41, also from sectioned material, shows a slightly more advanced stage with a large number of cells in process of division. In the generative region division is in several planes; cell division in the columnar zone in early and mid ana-stage seems nearly, if not entirely, restricted to a plane exactly transverse to the embryo axis.

Increase in embryo length is not proportionate to the number of cell divisions, since only a relatively small number of cells elongate, i.e., only the most basal derivation row segments. Other row segments actually diminish in average size by the numerous transverse divisions. The suspensor cells derived from columnar tissue do not grow to the great length characteristic of the earlier embryonal tubes, but only go to one-half or a third the length before collapsing. Nevertheless due to the greater number of cells contributing, the suspensor increases in prominence during this stage, becoming not only much longer but broader, as the tip increases in width.

The "unoriented" group of generative cells above the derivation row initials also increase greatly in number. After it becomes rather definite this region may be designated as the *pleromic centrum,* since the plerome is derived from it in later growth. The lower border zone of the pleromic centrum is necessarily indefinite. The section illustrated in Fig. 39 shows a narrow layer of columnar tissue below the pleromic centrum—at this time an undifferentiated meristem somewhat hemispheric in form. In Fig. 43 (also from a section) the shape is much the same, but broader, with a deeper zone of columnar tissue below. Similar stages are shown

in Figs. 55 and 56. An entire embryo tip is shown in Fig. 62. The diameter of this embryo is about 250 μ at the base of the centrum. Fig. 58 shows an embryo tip of similar diameter at somewhat greater magnification, but with a considerably greater depth of columnar tissue below. The length of the whole non-vacuolate meristematic tip is about 455 μ.

The base of the pleromic centrum in all these instances is flattened with a slight convex curve. As the embryo becomes older the basal convexity becomes more marked. The sectioned embryo shown in Fig. 40 shows this advance with reference to the basal curve of the centrum and is in a more advanced stage of development than that in Fig. 43, even though it is somewhat smaller. The arcuate contour and its effect on underlying derivation rows is readily noted. The central derivation rows are oriented normally along the central axis of the embryo, but those formed on either side are tilted to conform to the basal curvature of the pleromic centrum. The effects of this are made clear in study of embryos beyond the stage of development shown in Fig. 58.

In the mature embryo the calyptroperiblem is composed of two embryonic tissues. The column (*periblemsäule* of Schacht) consisting of what has been described as columnar tissue extends straight down from the tip of the plerome to the massive suspensor. The second tissue arises from the column and lateral to it following mid ana-stage. This lateral tissue is called *peri-column* since it is immediately derived from the columnar tissue and surrounds it on all sides. The origin of the peri-column from the marginal tilted rows extending from the base of the pleromic centrum down around the sides of the columnar tissue and the consequent diametric enlargement of the embryo is described in some detail below.

The normal plane of cell divisions in columnar tissue is, as previously stated, transverse to the organic axis and to the axial polarity of the organism. When the more marginal derivation rows become sufficiently tilted so that they are no longer even approximately parallel to the axial polarity, they tend to deviate notably from the ordinary course of cell division typical of a simple derivation row. Instead of customary vertical multiplication, lateral elongation and division takes place among the component cells of the tilted rows. Axial polarity is effective in governing divisions within the derivation rows so long as the latter are approximately parallel to it. At a certain angle of tilting the cells become freed from their subordinate status within the row, and each one then becomes competent to divide in accord with the plane which would ordinarily be associated with its flattened shape.

Sachs's law, as given by Priestley (1928, p. 10), that *"when a protoplasmic mass divides into two, the two daughter cells will be equal in mass,"* is fairly approximated throughout the course of growth in all

these embryo tissues. In the columnar tissue, however, Errera's law, as stated by Priestley (*loc. cit.*), *"that when a cell division takes place the semi-liquid dividing wall tends to be of minimum area, if the dividing cell is in equilibrium with its external surroundings,"* serves to emphasize that the derivation row segments (in normal alignment) are not *individually* in equilibrium, although the row as a whole, if considered as a sort of supercellular unit, may be relatively well equilibrated. The normal division of all cells in the columnar tissue derivation row is across the *broadest* area of the cell; but when tilted, as shown by marginal rows in Fig. 40 for example, an individual equilibrium is newly attained in each cell to make it essentially independent from its previous row affiliation.

At such a time the cells divide at right angles to their former plane of division, forming a wall across their short dimensions in a direction which is quite definite. This first wall is placed radially from the embryo axis. As a result each originally round flattened coin-shaped row segment now consists of two halves, each cell of which has its long axis directed away from the center of the embryo. The manner of division is best illustrated here in Fig. 85 (Plate VI) in the calyptroperiblem of a telo-stage embryo but is easily demonstrated in other transverse sections after the peri-column has begun to form. Subsequent to the primary radial division of the tilted columnar cells Errera's law is strictly followed. The next walls produced are of minimum area and at right angles to the first, forming across the short diameter of each cell. Hence these second walls are laid down in a plane obliquely tangential to the embryo axis. This manner of addition to the peri-column continues typically through the telo-stage and, on a somewhat restricted scale, even in the seedling.

The process of reorientation of polarity goes on all around the columnar tissue in general accord with the generalized radial symmetry of the embryo. Thus essentially the same degree of tilting occurs in all the marginal rows around the girth of the embryo, and the same series of divisions occurs in each of the row segments when they become free of their derivation row linkage and the component cells *individually* attain an equilibrium with the forces of polarity.

Axial cells of the columnar tissue tend to become smaller in their vertical dimension as divisions occur, up to a certain minimum, with only relatively slight equivalent expansion following each transverse division. In the cell line diagram shown in Fig. 44 the greater number of cells thus present within the column is emphasized. But in the individually equilibriated cells around the columnar tissue, equivalent expansion takes place following each mitosis. This expansion must take place in a radial direction because the cells all around the columnar tissue have developed and are exerting pressure on the various sides. The cells therefore are chiefly able to increase only in length.

The continued growth of the peri-column through late ana-stage and in early telo-stage has much to do with the final diameter of the mature embryo. Primary axial polarity does not seem to function directly in producing lateral enlargement beyond a diameter of about 300 μ. The mature embryo may become nearly twice as broad as this. Peri-columnar growth is not only directed radially but it also has a marked upward trajectory, derived from the tilt of the marginal rows in the columnar tissue. This makes it possible for the lower parts of the column to supply peri-columnar offshoots sufficient for nearly the whole of the calyptroperiblem. The primary origin of the periblem itself is to be found close to the top of the column, where the marginal derivation rows are shortest and most extremely tilted.

Derivation rows close to the zone of row initials at the top of the column are very short and consist of only a few segments, since they have begun columnar growth most recently. Many of these around the margin of the initial zone are tilted at a considerable angle in conformity with the basal curvature of the pleromic centrum. These topmost marginal derivation rows never get very long because their component row cells soon become individually equilibrated and proceed to follow a course of lateral growth in general as outlined above. The potentialities of these topmost lateral offshoots differ somewhat from those lower down, however, due to their position near the sides of the pleromic centrum. Even when the calyptroperiblem is rather well developed the centrum is quite small. In late ana-stage when the centrum begins to develop more rapidly, derivatives of the marginal topmost derivation rows are in position around it. The peri-columnar tissue lower down ceases active growth early in telo-stage when the calyptroperiblem has nearly attained mature embryonic size, but this is not true of the uppermost offshoots of the column. They continue growth in early telo-stage concurrent with the growth of the plerome. The upward angle of growth, already extreme, becomes more pronounced in their cellular progeny. The direction of growth alters in a gradual curve until it is parallel with the axial polarity and with the plerome. Thus the periblem *originates in a manner similar to the peri-column.* Later growth of periblem and plerome is essentially intercalary in nature, normal axially aligned rows of cells being formed from the various initials in place there. The lateral component of growth from columnar tissue is in great part directly responsible for the increase in diameter of the late ana-stage embryo over the early ana-stage. Massive multiplication of cells, such as constitute the pleromic centrum, is responsible for very little of the increase in embryo diameter in late ana-stage and telo-stage development. Even in the telo-stage embryo the rows of periblem cells are still clearly traceable back to their primary source on the upper margin of the peri-column.

The columnar tissue may be regarded as a source of meristematic tissue for lateral enlargement throughout middle and late ana-stage and early telo-stage. Peri-columnar growth starts from the top of the columnar tissue and works down rather rapidly. Marginal columnar cells that remain, next undergo essentially the same process of growth laterally. The process may be likened to a systematic sapping of the columnar tissue. As mentioned before, derivation row segments of the column tend to diminish in size due to lack of equivalent expansion after mitosis. The lateral offshoots of the column, which make up the peri-column, regain this capacity for equivalent enlargement of daughter cells. The basal cells of the column (and also of the peri-column to some extent) continue to contribute to the massive suspensor. The suspensor cells are the only ones in the embryo to lose their meristematic character and undergo maturation enlargement and vacuolation, but from the standpoint of the columnar tissue, lateral depletion is most important and it tends to decrease the transverse dimension of the column proper throughout late ana-stage. Basal contribution to the suspensor takes relatively few cells, and this is more than compensated by the extremely active transverse division of cells in the derivation rows. Consequently the column grows considerably in length during middle ana-stage to early telo-stage.

The longitudinal growth of the column is important in explaining the tissue configuration of the calyptroperiblem, which is the first part of the full grown embryo to attain embryonic maturity. So long as columnar derivation rows remain near the center of the tissue, growth remains essentially unchanged. Very occasionally a vertical wall may be produced near the middle of an old derivation row and a new series of row cells started from each of them, but this seems to be of little significance. In general the central derivation rows near the base of the column are relatively longer than those nearer the top of the column which originated later. The cells of these oldest columnar rows are also somewhat broader than those above. Marginal rows in the basal portion have been contributing to the peri-column somewhat longer than those above and consequently, at the base of the column in middle and late telo-stage embryos the typical derivation row groupings are larger but fewer.

The first cells to produce offshoot lateral tissue in the calyptroperiblem dominate the appearance of the lateral tissue because they redivide and likewise form a type of derivation row. The constituent cells of these rows are very different from those of the column in that they are elongated and join end to end. They are not so obviously derivatives of single cells, yet on closer study it is clear that their sequential derivation in series is almost as marked as it is in rows pertaining to the column itself. Certainly the planes of cell division are very definite and just as

characteristic as before. The cell division plane in peri-columnar tissue is essentially at right angles to the plane of characteristic cell division in the column. For reasons explained below actually the plane of peri-columnar cell division is somewhat more than 45 degrees removed from the columnar cell division plane, but the first division is at about that angle. Deviations in excess of that angle occur increasingly, so that the files of cells appear to be bent in an arc, and, in the case of rows of cells entering the periblem, the plane of division actually has been *totally reversed,* so that it again coincides with the original transverse plane of columnar division.

Elongation of the column occurs concurrently with growth of the peri-column and, consequently, this produces interstitial tension among peri-columnar rows. One way this tension is relieved without formation of large intracellular spaces is by the offshoot rows reclining more steeply in an upward direction. Another factor which also relieves this tension is addition of shorter interstitial offshoot rows from marginal columnar rows which were at first entirely within the column. All cells of the embryo (except the suspensor cells) are meristematic and plastic and they readily accommodate themselves in spacial relations. The point to be made is that a very definite rearrangement is necessary. In telo-stage the oldest and longest lateral derivation rows are very considerably curved in the basal region of the calyptroperiblem. Longitudinal growth of the column is one responsible factor; another factor (in which cause and effect are hard to distinguish) involves the shorter lateral derivation rows which are introduced between the longer files of cells. The photo tracing reproduced in Fig. 45 illustrates this condition in an embryo at telo-stage.

A rather sharp break generally is seen at the surface of an older embryo, where lower lateral derivation rows limited to the calyptroperiblem lie against rows which continue up into the periblem of the axis. This line encircling the embryo may be called the *juncture zone.* It is generally a distinct feature in the external contour of the embryo, not only due to the slightly greater diameter of the embryo often found above it, as in Larix, but also to alteration of the mantellary layer covering the peri-column below. Beneath the superficial layers there is no morphologic distinction between the rows that continue into the periblem and those which are limited to the peri-column. The juncture zone in Larix is consistently at about the same level as the tip of the plerome. It can be distinguished from middle to late ana-stage up to embryonic maturity. It is indicated by the letter "J" in Figs. 57 to 61.

Above the juncture zone the mantle maintains its normal relations, accommodating itself to the underlying growth and occasionally adding to the tissue within by a periclinal division throughout ana-stage and early

telo-stage. Below the juncture zone the mantle cells become distorted in shape and transformed so that they are finally not easily recognizable as being derived from the original external layer. As shown in Fig. 31, some of the mantle cells form derivation rows similar to the cells within. The mantle layer at a later stage, as shown in Figs. 39 and 43, is not partaking in this form of growth, due to the necessity of expanding by both longitudinal as well as transverse anticlinal divisions to cover the considerably greater surface resulting from the increase in size. But with the advent of peri-column, the mantle cells are subjected to oblique tangential pressure from cells within, which distinctly modifies their form. The walls pressing from within abut on parts of the mantle cells and shape them. In addition a "drag" tension is transmitted successively downward from one calyptroperiblem cell to the next. Mantle cells are generally larger than the cells of lateral derivation rows, and hence a single mantle cell frequently terminates more than one lateral cell series. Fig. 60 shows the mantle before it has been irrecognizably distorted, but downward from the juncture zone the cells become increasingly oblique in form until near the base they are triangular and tip upward at an acute angle. All calyptroperiblem mantle cells of later telo-stage embryos are distorted in this way, as shown in Fig. 45. Occasionally gaps occur where mantle cells are pushed apart and a marginal cell is seen which is derived directly from the peri-column. However, the majority of surficial cells of the calyptroperiblem, as elsewhere, are derived from the original superficial layer which originated very early in ana-stage.

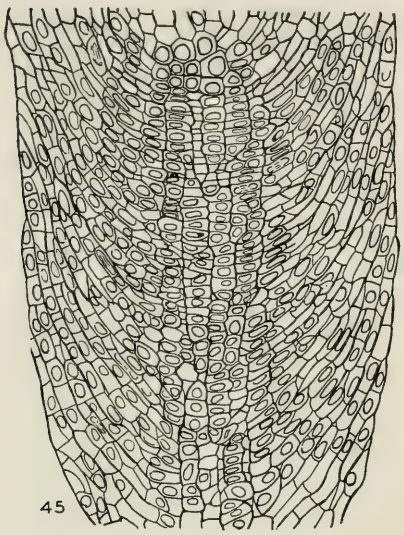


Fig. 45.—Section of calyptroperiblem from mid-telo-stage embryo, showing how mantellary cells have become wedge-shaped because of growth pressures. Magnified 140 times. Collection of June 25, 1932.

The juncture zone is chiefly distinguishable due to the distorting pressure which has been exerted on the mantle cells below it. Cells above the zone are not subject to this sort of pressure. In Fig. 60 and in later stages just at the juncture zone one layer of the peri-columnar cells seems almost continuous with the mantle layer above. This arrangement is a derived one, produced from constant internal pressure directed obliquely

upward by this one row (layer) of cells against the continuous mantle of the axis while the embryo diameter was expanding in this region. The continuity of rows inside the axis mantle, with rows in the upper part of the calyptroperiblem is, of course, due to actual continuity in derivation.

Fig. 58 shows an ana-stage embryo in which the peri-column is only starting to be formed. This embryo is slightly more than 250 μ wide at the broadest portion and has no definite periblem. The pleromic centrum is nearly spherical, occupying the upper central region within the mantle. The plumule primordium will arise at the tip by multiplication of cells derived both from the upper part of the pleromic centrum and from periclinal division of the mantle. Along the sides, near the juncture zone, the upward sloping mantle cells of the peri-column are beginning to appear, but the juncture zone has not yet become easily distinguishable. Fig. 59 shows a later stage in which a few lateral series of cells have been formed on all sides nearly the length of the calyptroperiblem. A few of the highest calyptroperiblem series have added to the periblem region by intercalary growth, and the plerome has concurrently elongated. The place where the juncture zone is developing may be seen. The plumule primordium has developed its maximum prominence; hereafter the shoulders on either side will build up, eventually to form cotyledons. The embryo now exceeds 300 μ in width, chiefly because of formation of tissues lateral to the pleromic centrum. Fig. 57 shows continuation of development seen in Fig. 59. The diameter in the upper part has increased to nearly 400 μ, and the next step is development of cotyledons on the shoulder surrounding the plumule primordium.

The formation of cotyledons seems to be initiated with development of a small-celled more meristematic plate of tissue which first serves to divide the plumule primordium from the pleromic centrum. This plate is the cotyledonary "node," and it extends laterally coordinate with the radial enlargement induced by formation of periblem tissue. There is also some contribution to the cotyledons from the mantle. The length of the embryo shown in Fig. 57 (exclusive of the suspensor) is about 940 μ, of which the plumule primordium occupies about 100 μ and the axial (plerome) region about 250 μ. The calyptroperiblem is the longest component and has more nearly completed its embryonic growth than any other part. It may be noted that the shoulder at the right in Fig. 57 is slightly more developed than on the opposite side, a feature indicating cotyledon asymmetry as previously noted by Buchholz (1919, p. 115). Longitudinal sections taken in late telo-stage (Figs. 63, 65, 66) show that cotyledons do not generally develop equal in size.

Primordia of the usual five or six cotyledons appear as separate prominences around the margin of the cotyledonary shoulder growing up

around the plumule primordium and obscuring it from view.* No lobing of cotyledon primordia, as reported by Buchholz (1919) for other genera, was observed, although probably an insufficient number of embryos was examined at the most favorable stage for observation to support a definite conclusion that this never occurs.

In telo-stage the calyptroperiblem will come to exceed its late ana-stage length by about one-third, while the axis becomes more than twice as long. Fig. 57 shows the late ana-stage condition, and Fig. 61 shows a telo-stage embryo about two-thirds grown. Diameters in the upper portion of the axis in Fig. 61 are not reliable, since this embryo was slightly crushed, but because of this it was more translucent and more easily photographed.

Telo-stage.—Ana-stage has been defined as the period in which the embryonic tissues originate, and telo-stage, which follows, is the period of maturation of these tissues leading up to the dormant or resting stage of the embryo. Growth is chiefly confined to progressive enlargement of the several embryonic tissues that were previously initiated. Thus there is no distinct boundary to be drawn separating telo-stage from ana-stage development, but the cotyledonary tissues arise last and so the inception of this last growth period may be recognized by the appearance of localized primordia which are to become the cotyledons.

The cotyledonary primordia develop on the shoulder of tissue around the plumule meristem. Certain smaller cells identified with the nodal primordium are instrumental in forming the cotyledon anlage, and later, the cotyledonary procambia. The trend of these nodal cells extending toward the stubby cotyledons is seen in Fig. 60, and an extension of the nodal primordium downward on the sides of the plerome soon becomes visible. The nodal region is recognized as first in procambial differentiation; the procambia of cotyledons form as they grow, and pleromic procambia form slightly later as downward extensions of differentiation from the nodal region. It is indicative of this same trend that the first tracheids to be seen in germinating seedlings also occur in the nodal region.

In growth of cotyledons a few rows of cells such as those typical of the periblem extend upward outside the incipient procambial strands. A few of these rows on the abaxial sides may be derived from the periblem cells with original derivation from the base of the plerome, but most of them come more directly from the few cells formed at the upper lateral margin of the pleromic centrum. They may be seen adjoined at the ends of the periblem cell series, bearing an analogy to derivation rows of columnar tissue, with the important difference that each cell division is

*Butts and Buchholz (1940) have presented statistical data on cotyledon numbers in Larix and other conifers.

followed by proportional size increase of the daughter cells to maintain a fairly constant average cell-size. They are similar in general character to the derivation rows of the periblem. The dermal layer soon becomes very definite over the cotyledons and essentially ceases periclinal division. The mantle over the upper portion of the axis also graduates to stability in showing only anticlinal division and such features as are generally associated with a dermatogen. It becomes more and more strictly a histogenic layer adapting itself to the increasing mass within by anticlinal segmentation. The external layer of cells over the plumule primordium, however, never seems to become differentiated to this extent.

Secretory elements of a variety infrequently recorded become established in the early telo-stage embryo and persist until sometime after germination. On account of their persistence several who have studied seedlings of conifers have described them, Messeri (1935) being the most recent. She illustrates cross sections of young seedlings of *Pinus halepensis, Cedrus libanotica* [sic],* *Larix europaea, Abies cephalonica, Pseudotsuga douglasii, Tsuga canadensis,* and *Picea orientalis* and finds similar secretory elements in all. She considers the dark-staining contents a feature of the protoplast because she found what appeared to be a thin cytoplasmic membrane surrounding the dense secretion. Chauveaud (1903a) was the first to definitely call attention to these structures in the embryo. Among other conifers he worked with dormant seeds of *Cedrus deodara.* This account of the dormant embryo of Cedrus, featuring these secretory elements, has seemingly been overlooked by most subsequent investigators. Hutchinson (1917) briefly discusses their occurrence in the embryo of Keteleeria as "mucilage" canals. There can be no doubt that these structures correspond to the secretory elements now described from the Larix embryo.

In Larix, as in Cedrus, and the others of the Pinaceae, the secretory elements are of two varieties, distinguished chiefly by their position—pleromic and subdermal. They are first seen in the central region of the plerome, and early stages of pleromic elements are shown in Fig. 46, *c, d,* and *e.* In telo-stage embryos it is customary to find eight or ten of these extending from the region of initials at the base of the plerome up through the axis. In Fig. 46, *c* shows two of them closely associated in the central part of the plerome. Their lower tips are only a few cells removed from the basal pleromic arc (indicated by broken line). In all instances the nuclei are large and elongated, with several dark-staining "nucleoli" within. The cytoplasm is granulose and diffuse and not yet deep-staining. Later on, all the cellular structures become obscured by the dense contents, so that a nucleus could hardly be identified by ordinary staining

*According to Rehder (1940), the legitimate name is *Cedrus libani* Loudon.

methods if it were present, but Messeri's conclusion as to their essential cellular constitution seems justified.

Subdermal secretory elements at an early stage of development are shown in Fig. 46, *a* and *b*, similar to the pleromic elements in *c*, *d*, and *e*. They differ from the pleromic elements chiefly in position, since histologically they are similar. They are, however, generally somewhat shorter and tend to form from a series of cells, which seem to intercommunicate later by disintegration of the end walls. These subdermal elements will be discussed later in connection with photographs of late telo-stage embryos. The appearance of secretory elements in their typical form is seen in photographs of the late telo-stage embryo, Figs. 65, 75, 76, 77, 78, 79, and others; also in the germinated seed in Fig. 67.

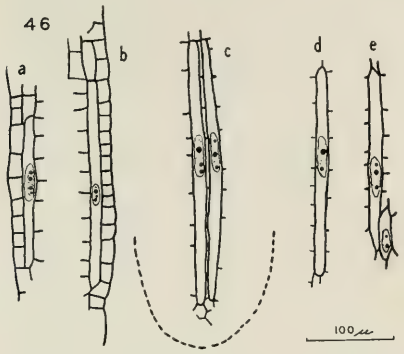


Fig. 46.—Longitudinal sections showing secretory elements from early telo-stage embryos: *a* and *b* are subdermal elements; *c*, *d*, and *e* are pleromic elements. Nuclei are elongated and have several nucleoli. Collection of June 25, 1932.

The nature of the contents of these elements is unknown. It is possible that it might be a type of resin, mucilage or gum, or tannin or tannin product, but other possibilities are not excluded. Ordinary resin is soluble in xylol; the marked insolubility of the dense material shows it to be quite different in this respect.

The major size increase in telo-stage occurs in the axial region of the embryo and in formation of the cotyledons. As mentioned previously the calyptroperiblem maintains its general proportions from early telo-stage. A photo tracing (Fig. 45) shows a very slightly oblique section of the calyptroperiblem at mid telo-stage. The derivation row initials at the base of the plerome and above the columnar tissue are seen to be fewer in number and more restricted than previously. This has come about through an advance in development of the tissues generally, with a relatively diminished rate of growth in the calyptroperiblem. This same group of initials at the top of the column forms the focal point for rows of cells within the plerome, but it is doubtful that they ever contribute more than a few cells at the base of the plerome in the embryo. Later on, however, they become the primary contributors to apical growth of the root, and consequently they constitute the real generative meristem of this part of the plant. No very definite organization prevails in this group of initial cells. Sometimes the rows of plerome tip cells seem to radiate from one chief cell of this group but close observation makes it evident that no one

cell can be responsible for initiating the whole of the plerome, even later on in the seedling when this meristem is more restricted. Fig. 64 shows a section similar to Fig. 45 in which the calyptroperiblem and basal plerome are slightly more developed. The juncture zone is evident. The distance from the pleromic arc to the suspensor is about 520 μ and the embryo is 345 μ in diameter at the juncture zone.

Figs. 63, 65, and 66 show longitudinal sections of late telo-stage embryos which would have continued to grow for some time, chiefly in length, and at a slow rate. The major part of growth has been attained, however, and embryos of this stage of development will be used as a further basis for discussion of the late embryo. We will take up first the embryos as seen in longitudinal sections, and then the tissues more specifically with reference to the transverse sections.

Axial elongation is a process of intercalation throughout telo-stage. The periblem is composed of rows of cells which can often be traced continuously from near the base of the cotyledons down into the calyptroperiblem below. Longitudinal growth in this tissue progresses by transverse (anticlinal) division of cells. The number of rows in the periblem appears to vary somewhat, due in part to difficulty in accurately defining the outer boundary of the plerome. The usual number seems to be five or six. Occasionally in the upper part one row splits into two by means of a periclinal division, but on the whole the cell rows of the periblem are remarkably continuous.

The plerome has arisen from a massive group of cells which originated in the pleromic centrum. The upper part of the centrum also has contributed the plumule primordium and nodal primordium and most of the cotyledons. At the base of the centrum the calyptroperiblem and periblem have originated as previously described in the section dealing with the ana-stage embryo. The cells at the top and those at the bottom of the pleromic centrum constitute the two major centers for tissue differentiation in the embryo. The cells in the median section of the centrum finally divide in series during telo-stage to form a type of pleromic derivation row which produces the greater length of plerome proper. The derivation series of the plerome are composed of cells more elongated and narrower than those in the periblem, but fewer in number. The end members of the rows are frequently cuneiform similar to the end cells of other kinds of derivation rows. The plerome tissue may be contrasted with that of the periblem by saying that it likewise is composed of cell series, but the series are not so long and extremely continuous as those of the periblem.

Not until after germination is an endodermis visible. From the configurations of component cells seen then, and the cell alignments at present noted in the late telo-stage Larix embryo, it does not seem likely that the endodermis is histologically definable. It seems more reasonable to con-

sider it as a structure of physiological origin, which is not definitely predetermined in the cell rows of the embryo axis. Since the endodermis formed later is composed of large cells, histologically quite similar to the periblem cells which enclose the smaller-celled procambia, it seems likely that the inner layers of the embryonic periblem contribute the endodermis. However, more closely spaced germination stages than are now available are necessary in order to prove this point.

The medullary region is no more precisely definable in the late embryo than are the procambial strands. The medulla may be recognized by the slightly broader diameters of the pleromic cell series and by the secretory elements which differentiate within it. The nodal primordium, which seemed continuous across the top of the plerome at an earlier stage now shows a central perforation because the procambial cells are more definite. A few cells of the central medullary tissue now seem to extend into the region of the plumule meristem. This is due to the enlargement of certain of the centrally located nodal cells to correspond in character with those of the medulla. This slight change is seen in Figs. 63 and 71, and also in transverse section in Fig. 77.

Buchholz and Old (1933) have shown that the mature Cedrus embryo is similar to that now described for Larix, although the proportions of certain tissues are different. It is not to be expected that the tissues differentiate to the same relative degree in the embryos of different genera in the Pinaceae, although tissues corresponding to these described in Larix seem to be present in the late embryos of practically all gymnosperms.

It has been mentioned that the plumule primordium is not covered with a definite dermatogen. Fig. 71 shows a characteristic cell configuration in longitudinal section. The mantle cells are large, deeply staining, and obviously meristematic, with potentiality of division in either periclinal or anticlinal planes. The primordium is about 200 μ broad at the base and may be thought to include cells as much as 70 μ below the tip. This section shows how far the apical meristem has developed beyond the single apical cell stage. Transverse sections across the tip, as shown in Figs. 70 and 72 sometimes show a single mantellary cell occupying the apex. This arrangement is by no means constant, and the coordination of meristematic activity is not confined to any single cell more than to others in the same region. Different arrangements of the cells at the apex are shown in Figs. 73, 74, and 75.

Primary leaves originate, on germination, at the basal angles of the plumule primordium between cotyledons. Generally two or three of these may be seen before the plumule has elongated. As elongation takes place other primary leaf primordia are produced in close spiral sequence. The

general effect on the central primordium seems to be that its conical shape, as seen in the embryo, is reduced to a more rounded form.

Transverse sections of late telo-stage embryos show the radial configuration of embryonic tissues. Cotyledons are usually five or six in number. Fig. 68 shows the disparity in length previously noted in longitudinal sections. Toward their bases the cotyledons show the indefinite rounded central groups of smaller cells constituting their procambia. One of the layers of larger cells around the procambial strand later forms the bundle sheath; the others form mesophyll on expansion after germination. The dermatogen seems definite on the surfaces of embryo cotyledons, and later during germination it differentiates into an epidermis with stomates on the two adaxial sides. Stomates are first seen within the seed at the base of the cotyledons near their axils before they have elongated to any marked degree. In Figs. 70 and 75 the subdermal secretory elements are seen, usually occupying angles between adjacent dermatogen cells. (These have been inked for greater clarity along the lower part of Fig. 75.) In no case have they been seen out of contact with dermal cells either in the cotyledons or in the axis. One or two pleromic secretory elements later become associated with the cotyledonary bundles but are as yet hardly demonstrable.

The point of cotyledonary attachment is seen in Fig. 77. At this level the procambial strands are inclined outward preparatory to entering the cotyledons, and consequently the procambia are cut obliquely. Considerable difference in depth of the cotyledonary sinuses is seen, which is probably due to the same cause which makes for slight asymmetry among the cotyledons. The large-celled tissue in the center is continuous into the medullary region. Figs. 79, 69, 76, and 78 are cross sections of the embryo axis in the order given, down from the node. (Their respective positions on the different embryos selected for illustration are indicated by the diagrams on p. 85. All show pleromic secretory elements, and subdermal elements are shown in all but the lowest section, Fig. 78. The secretory elements of both kinds occur most abundantly in the central zone of the axis. The pleromic elements are more apt to be continuous; the subdermal canals are individually shorter in vertical extent though more numerous. The dermatogen is present over all the surfaces shown in the figures mentioned above. However, only on the upper portion which will become hypocotyl on germination does an epidermal layer develop fully. The lower portion of the axis will become associated with the radicle, and the outer layers of the radicle are sloughed off (see Fig. 67).

Enlargement of the axis during late telo-stage is more related to enlargement of previously formed cells than to growth by cell division. The procambial areas stain deeply, are composed of small cells, and are

more highly meristematic. The medullary area is more like the periblem in character than the procambial tissue directly outside it. The procambial strands are not yet sufficiently distinct to show a transition region, and consequently no line may be drawn as to the limits of hypocotyl and radicle.

Nearly all of the transverse sections show the embryo to be slightly flattened. This may be responsible for the frequent diarch condition in the root suggested in Fig. 69. It may be induced by the elliptical shape of the seed and gametophyte, both by pressure and indirectly because of differences in nutrition. Nevertheless, it also may have definite phylogenetic significance.

Cross sections near the plerome, the plerome apex, and juncture zone, are shown in Figs. 81, 82, 80, and 83, approximately in descending order, although sections from different embryos cannot be precisely assigned as to height in this region. In longitudinal sections the cell rows in the plerome and periblem converge toward and around the radicular plerome tip, and thus focus attention on it. However, they also give a misleading idea of its definitude. Transverse sections fail to show such a distinct separation of tissues at the tip of the plerome.

Fig. 81 is above the zone of initials at the tip and shows no precise distinction of plerome and periblem cells. In longitudinal section the periblem cells are more elongated than the central cells, but in the transverse sections these cells do not show much size distinction. The periblem cells are somewhat narrower, showing the prevalence of radial division but this is about the only difference. Fig. 82 shows a central area of considerably larger isodiametric cells within the zone of initials. Periblem cells show evidences of being sectioned obliquely as they curve down toward the columnar tissue below. Fig. 80 is very similar. Fig. 83 shows a section which crosses the juncture zone on the right, and the calyptroperiblem cells around this margin are cut more obliquely than the others. The large central cells are perhaps more properly considered as belonging to the short uppermost derivation rows of the column than to the plerome initials. They show more regularity, and the outermost cells have undergone radial division preparatory to sending off new peri-columnar series. In most of these figures just mentioned, the dermal layer is more irregular than in the sections across the axis above. In Fig. 83 the dermal layer is noticeably more variable beyond the point where the juncture zone is crossed. The line of distinction between the peri-columnar tissue and the higher rows which bend into the periblem, is of course more definite near the margin because the two tissues merge beneath the surface.

Fig. 84 is taken from near the top of the column, Fig. 85 is located in the central part of the calyptroperiblem, and Fig. 86 is at the base of the embryo. This last photograph shows the transverse configuration in the

region where marginal cells have been added to the suspensor, and the central columnar cells are still for the most part non-vacuolate. The large central cells of the column are outstanding. The much narrower radially directed peri-columnar cells afford considerable contrast, although the marginal columnar cells, divided into two by a radial wall, show clearly the transition between the two tissues. Mantellary cells are not distinguishable from peri-columnar cells except by position in transverse sections of the calyptroperiblem. Fig. 86 shows the column maintaining its individuality all the way to the base of the calyptroperiblem. Basal cells of the peri-column are apparently more readily added to the suspensor than are those of the column proper, since the suspensor regularly extends up further along the sides than it does in the central part.

The Gametophyte during Embryo Development

In transition from pro-stage to meta-stage, primary suspensor cells of the proembryo elongate and push the tip of the embryo system through the archegonial jacket cells at the base of the archegonium into a more or less pre-formed corrosion cavity. In Larix, as in pine (see Buchholz 1918, p. 195), the gametophytic tissue below the archegonium seems to break down at the regular time, whether an embryo is present or not. At least two-thirds of the gametophytes sectioned which should have contained meta-stage embryos were barren but still possessed a slender corrosion cavity, sometimes going half the length of the gametophyte. Prior to fertilization a trumpet-shaped zone of nutritive tissue appears, encircling the archegonia and extending down the middle. It corresponds with the stippled zone shown surrounding the embryo in Fig. 1. The rest of the gametophyte is more translucent. Later when the embryos are growing, the gametophyte becomes white, turgid, and packed with food, the central tissues filling first. If no embryo has been formed the gametophyte still simulates this condition. Sections of barren seeds show this to be chiefly due to a denser cytoplasmic content rather than to actual food storage; nevertheless, it is very difficult to distinguish whole gametophytes in this condition from fertile ones under a Greenough type binocular microscope.

The difference in staining properties of cross sections immediately makes it obvious which of the gametophytes contain an embryo. The safranin picro-nigrosin stain used on this material, has a strong affinity for rapidly growing or physiologically active tissue. Consequently the fertile gametophytes stain so much darker on the slide that they are at once apparent to the unaided eye. Sterile gametophytes retain only a light purple tint. In the "automatic" corrosion cavities lacking embryos, the break-down of tissue leaves a residue very similar to that formed in the old archegonia of fertile gametophytes. In fertile gametophytes this deep-staining material may be a degradation product from the early

suspensor, etc., after being acted upon by archegonial enzymes such as postulated by Stopes and Fujii (1906).

Woycicki (1923) has discussed the multinucleate cells of the Larix gametophyte. These were again observed during the period of early embryo growth near the central "core" of the gametophyte while the cells were filling with food. These multinucleate cells disappear later by formation of additional walls in such of them as are not disrupted by enlargement of the corrosion cavity.

The gametophytic storage cells become completely gorged with globules of food material during early ana-stage, and it is not likely that there is much physiological connection with the parental sporophyte plant subsequent to this, particularly since rapid induration of the seed coat also reduces the possibility for translocation.

As the embryo increases in size it displaces gametophytic tissue, and this seems to be the extent of the food used for embryonic development, since the cells of the gametophyte that remain out of contact with the corrosion cavity still retain their full quota of food so far as can be judged. Sometimes during ana-stage a depleted zone to a depth of a cell or two surrounds the corrosion cavity, indicating that it is not spacial displacement alone which causes the gametophyte cells to be relieved of their contents. In telo-stage fewer uncollapsed but depleted gametophyte cells are seen—generally a few can be found at the end of the corrosion cavity above the cotyledons. Only the gametophyte cells directly adjoining the corrosion cavity and the embryo are lighter-staining and have less nutritive materials than the normal quota.

The outer layer of gametophyte cells adjacent to the megaspore membrane at the base of the seed are notably different from the storage cells within. They have dense cytoplasm but very little stored food. It may be that this layer is a vestigial gametophytic epidermis. The layer is clearly visible surrounding the base of the gametophyte shown in Fig. 63, but at the low magnification used in this illustration it can be distinguished only as the narrow lighter stained margin at the top of the picture. It is a persistent structure, apparent after the gametophyte has received its full quota of food, and can hardly represent a layer of ordinary gametophytic cells as yet unfilled. It seems never to become obliterated and is demonstrable in all the late telo-stage gametophytes which were sectioned, representing a fairly long developmental period. Doyle and Looby (1939) have recently reported a similar gametophytic structure in Saxegothaea, and they likewise compare it with an epidermal layer. Probably it will be found rather widely distributed among the Coniferales, notwithstanding the few notations as to its presence.

The megaspore membrane is definite around the basal part of the Larix gametophyte, and in suitable sections its outer surface can be seen

to have a rather characteristic finely granulose texture; its inner surface is smooth. Layers, if any, are difficult to distinguish. Its thickness is inconstant, being about 6 μ at the basal end and diminishing gradually toward the micropylar end, where it becomes either extremely delicate and probably perforate or actually wanting (Figs. 1, 47a, 48, 51, 63, 67). In preparation of sections, strips of the megaspore membrane are frequently displaced as in Fig. 73.

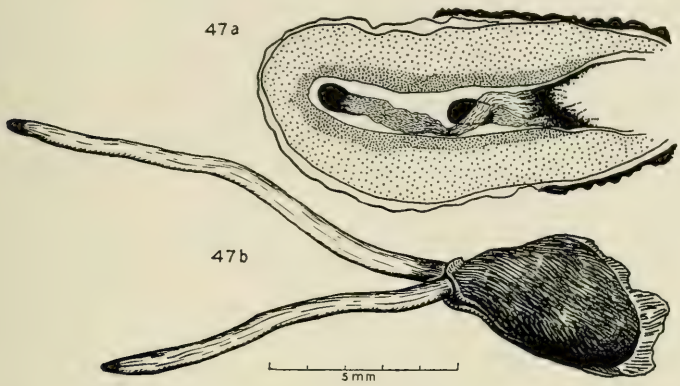


Fig. 47a.—Gametophyte containing two ana-stage embryos of about the same development, which may have been derived either by cleavage from one embryo system or from two separate embryo systems. Both embryos might have survived, since the second shows remarkably little adverse effect from its less favorable position. Magnified about 25 times. Collection of June 11, 1933.

Fig. 47b.—Germinated seed containing two viable embryos and possibly representing a situation analogous to that shown in Fig. 47a. Tips of the protruding radicles were colored a delicate pink; their surfaces showed irregularities due to sloughing of outer cell layers. No root hairs were observed.

V. DISCUSSION

Relationship of Larix within the Pinaceae

Within recent years both Doyle and Buchholz have given consideration to the affinities of members of the Pinaceae, and both reach very similar conclusions with respect to Larix. Earlier authors often grouped Larix with Cedrus and sometimes with Pseudolarix, no doubt due to the obvious resemblances of the short shoots. More recently the error in these conclusions has become a matter of general agreement. Jeffrey (1904) and Penhallow (1904) from their studies of anatomy both conclude that Larix is closely allied to Pseudotsuga and Picea, with Pinus not far removed. Doyle's studies (1918), based on the pollen, male fructification, the female cone, pollination mechanism, and the female gametophyte, are

in thorough accord with the conclusions of the wood anatomists. He is most certain of the close relationship of Larix and Pseudotsuga, notwithstanding their difference in habit, and concludes by saying "that a close natural affinity exists between Larix and Pseudotsuga."

Buchholz (1920a) groups Picea, Larix, and Pseudotsuga together because of their similar embryology. The similarity of the embryos of Larix and Picea is said to be especially close. The new features of embryo development in Larix reported in the present study, will very probably be found similar in the other two genera, although little has been reported which deals with the critical stages. In a slightly later publication (1920b) Buchholz shows Larix, Picea, and Pseudotsuga, together with Pseudolarix, as derived from Pinus as their closest relative. Since then the embryology of Pseudolarix has been studied, and Buchholz (1931) does not now consider that genus directly related to Larix, Picea, and Pseudotsuga.

The present work shows little additional evidence of affinity with respect to Pseudotsuga or Picea, largely because their embryogenies are not yet worked out in sufficient detail for close comparison. It does, however, tend to emphasize the substantial bond of affinity between Larix and Pinus. Whereas the two were previously considered to be quite different in having simple and cleavage forms of polyembryony respectively, now it has been shown that the sequence is qualitatively the same. In Larix, the product of one embryo initial cell (as in Pinus) dominates its quadruplet brethren and alone contributes to the final embryo. The fact that in Larix the polarity units are much more closely associated in early development than in Pinus is significant, but this is only a difference in degree. Apical cells are similarly functional in both genera, although Larix usually disposes of its single apical initial earlier in ana-stage than does Pinus. The homologies of the structures of early embryology in these two genera are absolute throughout. All in all, this embryological study confirms the relationships currently accepted for these two genera on the basis of other lines of evidence.

Tissues of the Larix Embryo

The Apical Cell in Larix.—Schüepp (1926, p. 38) defines an apical cell as follows (free translation): "An *apical cell* is one distinguished through size, form, and manner of division, occupying the entire initial zone of a vegetative apex. All displacement curves originate from it, and all tissues of the growth segment are derived from it. At each mitosis the apical cell forms two dissimilar daughter cells, one of which retains all characteristics of the apical cell. The other becomes a segment that constitutes, either by itself or in combination with other segments, a zone of

youngest increment and becomes transformed through progressive maturation into permanent tissue."

This definition is essentially based on "formal" distinctions. To it should be added that the apical cell in order to sustain its characteristics must occupy the apex of the polarity gradient and serve as a coordination center for growth processes, in addition to the more obvious mechanical function of adding new segments.

When we regard the early embryo system of Larix as a compound structure, the cells at the tips of the four polarity units seem to conform in all essentials with this definition; this is also true of the homologous apical cells in pine. Apical cells in both are recognized until after the massive tip has been formed. The apical initial is longer lived in pine than it is in Larix, but its duration is variable in both.

The apical cells of the four polarity units in Larix are closely appressed, so that they conform to none of the idealized apical cell forms generally thought of. The brief stage in which the apical cell possesses three cutting faces is somewhat analogous to the tetrahedral apical cell displayed to advantage by the Equisetum stem tip. It is probable that this similarity is due to a mere duplication of form in a relatively simple morphologic structure organized to serve a somewhat similar function in both instances. It is believed that little emphasis should be placed on this feature as an indicator of phyletic affinity between conifers and Pteridophytes.

In Larix the sequence of apical cell forms is adequately explained by the mutual relations between the four polarity units which essentially determine their shape. The four apical cells originating at the base of the proembryo and enduring through the first two-thirds of meta-stage, are all more or less deformed on their faces of contact. When the apical cells grow sufficiently to cut off segments in two planes, the central angle of contact between the polarity units still occupies a large portion of one side. Only when this lateral contact has been decreased does the apical cell separate segments in three planes. It seems safe to infer from this correlation that the stage II apical cell is chiefly maintained due to the close association of the four polarity units.

Throughout the Pinaceae, so far as known, the proembryo organization is equivalent; the few cells produced in the various genera are easily homologized. Pseudotsuga may be an exception, since there is some doubt as to the presence of rosette cells in this genus. However, if they are absent, perhaps we may assume that the relict nuclei of Pseudotsuga correspond to both the rosette tier and the relict nuclei of Larix, in an undivided condition. Buchholz (1918, p. 201) recognized that "a distinct apical cell stage exists [in Pinus] from the time the embryo cells first

have walls." It is known that "Cedrus, Tsuga, and Pseudolarix have extensive cleavage polyembryony in their program of development" (Buchholz, 1931a). Of these Pseudolarix is the only one about which the existence of apical cells in the dominant group of polarity units is in question; the others all resemble pine in this respect. In their pro- and meta-stage, so far as known, the polarity units of Picea, Pseudotsuga, and Abies are very similar to Larix. Buchholz (1920b, 1926) has reported that cleavage polyembryony sometimes occurs in Abies and that otherwise it is similar to Larix. Now that Larix is known to have apical cells functioning as in Pinus (the early embryo sequence differing only in degree as to cleavage), it is most likely that these other members are similar in their possession of apical cells. Proof, of course, lies in discovering the separate proembryo quadrants functioning as individual polarity units.*

Hutchinson (1924) apparently is in agreement with Buchholz (1920b) concerning Abies, since he recognizes what he thinks is cleavage polyembryony in "ten per cent of the cases studied." Buchholz (1926) found twelve per cent. Hutchinson's series of the embryologic sequence is probably incomplete. His meta-stage drawings can all be interpreted in excellent agreement with the sequence which has been found in Larix, although an illustration based on a single section in a series cannot be expected to provide conclusive information. It is probably necessary to base conclusions in the first place, not only on sectioned material, but on dissections as well. However, a definite overtopping by one polarity unit is shown in Hutchinson's Fig. 15, but the apical cell shown by him belongs to a repressed member. In his Fig. 16, the apical cell of an overtopping unit is shown. Since no dates of collection or discussion of the amount of material studied is given, we are led to infer from the nature of the figures that it was not extensive.

The early embryo of *Pseudotsuga douglasii* has been briefly described by Lawson (1909) and, except for some question in regard to rosette cells and the relict nuclei, shows a remarkable agreement with Larix. The only meta-stage system illustrated by him still has only stage I apical cells; consequently, similarity with Larix in later stages rests chiefly on inference supported by the observations by Buchholz.

Keteleeria has not been the subject of any published investigation of the early embryo, although Buchholz (personal communication) has observed the occurrence of cleavage polyembryony and functional apical cells in the genus. In this respect it is comparable to early stages in Pinus,

*In *Abies venusta* Buchholz (1942) has found evidence that in some instances the four dominant polarity units may join *equally* in contributing to the single mature embryo. The manner in which these apical cells combine in these instances may have considerable biological significance and deserves a further investigation in which both serial sections and dissections are utilized.

but an important contrast is to be found in the indefinite number and continuing development of additional polarity units during meta-stage.

If the proembryo arrangement, as reported by various investigators, is of significance in postulating later development it would seem that well-developed apical cells are present in all members of the Pinaceae.

The manner of elimination of apical cells is known only for Larix and Pinus. It seems that the apical cell does not keep pace with the cells behind it in development, so that it is soon relieved of its office as a coordinator of growth processes. It does not increase in size or prominence with successive growth and is finally lost, even as a morphological structure, by subdivision either periclinally or anticlinally. In both Pinus (Buchholz, 1918, p. 204) and Larix, the embryo tips retaining an apical cell longest have a more conical shape than those which have dispensed with it earlier in ana-stage. The apical cell is retained somewhat longer in Pinus than in Larix, and the significance of this is open to several interpretations. Whether it is dispensed with because of its incompetence or whether it is eliminated in the course of subordination to a new center of embryonic organization which supersedes it, cannot be determined. Because of the association of a more or less equilateral tetrahedral apical cell with a cone-shaped apex, both in these conifer embryos and in unrelated cryptogams, it may be thought that any force which tends toward early formation of a broader embryo tip tends toward earlier elimination of the apical cell. Phylogenetic interpretation of it would seem to require some knowledge of whether ancestral plants possessed embryos with apices of broader or narrower relative proportions at a comparable stage in their ontogeny.

The Suspensor.—In Larix the primary suspensor cells function in a manner comparable to pine but *seem* to disintegrate somewhat earlier. It is possible that the suspensor cells of Larix are actually less stable as living structures, but comparative observations on this point have not been made with sufficient precision to establish it. Slow growth characterizes meta-stage development in Larix, and this is more likely a point of real distinction between Pinus and Larix. Illustrations published by Buchholz (1918) and several earlier workers show that a tetrahedral apical cell is often formed in pine after not more than four or five segments have been cut off. This is easily determined, since the embryonal tubes may be observed in connection with functional and uncollapsed primary suspensor cells. Buchholz reports that the apical cell with two cutting faces (stage II initial) is practically non-existent in pine. The segments produced by the stage II apical cell in Larix represent, then, just so many more meta-stage cell divisions. In Larix it has not yet been possible to observe the number of previous suspensor cell segments even

at the time when the stage II apical cell is first demonstrable because they are already too badly disintegrated. As a result of this, there is little information available as to the absolute number of previous segmentations of the stage I apical cell. These are not, in any event, less in number than the segmentations of the apical cell with a single cutting face in pine. Counting segments produced by the stage II apical cell as an excess over those formed by Pinus, it seems likely that somewhere between two and three times the actual number of segments are produced before a tetrahedral cell is formed. If we assume that each segmentation in Larix takes the same amount of time as in pine, the meta-stage period is necessarily also lengthened from two to three times. It appears more probable that Larix does *not* grow with the same rapidity as pine, because the four individually polarized units of Larix are developing in intimate competition. Under these conditions it would seem that the more competent members, at least, would be retarded in growth by the others. Furthermore, each of the four apical tips has less than one-half its external surface free to absorb nutriment from the outside, whereas in pine each tip is freely exposed for absorption on all but the basal surface. For these reasons the meta-stage growth may be expected to take *more* than three times as long as is required by pine. From the material at hand, the Larix meta-stage has been estimated to consume about thirty-six per cent of the embryonic growth period. On the other hand, the author would estimate that the stage will not be found to occupy more than six to eight per cent of the embryonic growth period in pine.

The time disparity in length of meta-stage between the two genera, will go far to explain the seeming early collapse of the suspensor in Larix. Enzymatic activity is not likely to be retarded commensurate with the retardation of apical growth due to competition. In Pinus and Larix the early suspensor finally disintegrates, and this may be attributed in both cases to digestive enzymes. Stopes and Fujii (1906, p. 13) report that the walls of suspensor cells in Pinus contain amyloid, "for in a fresh condition they stained bluish with iodine." Thus it is likely that the suspensor cells are not very resistant to digestive processes once they decrease metabolic activity in the course of maturation. It is difficult to suppose that any other agent would be active in producing the extreme decomposition found. It is, therefore, believed that the amount of disintegration of these elongated cells is a function of two factors: (1) the degree of maturity of the cells themselves, and (2) the length of exposure to enzymatic action.

The prolonged meta-stage in Larix may explain why older cells of the suspensor are in advanced stages of disintegration for a period before the tetrahedral apical cell occurs, while in pine they are usually found in good condition. In practical application this is almost certainly the reason the Larix suspensor has been reputed to collapse early, and a partial ex-

planation of why the sequence in this and related forms has previously been somewhat misinterpreted. It is impossible to understand the meta-stage growth in Larix by following development of the early suspensor, as has been possible in pine and some other genera, because the older cells always disintegrate while the tips are in an unadvanced stage of development.

Data recently presented by Buchholz (1942) indicate that the primary and early secondary suspensors of Abies are more like those of Larix than are those of Picea. The two meta-stage embryo systems of Abies shown by Hutchinson (1924, Pl. XVIII, fig. 8) illustrate the difficulty in tracing the suspensor cells at this time, especially from sectioned material. From study of Larix, it seems very likely that the tips shown in his Figs. 10, 12, 13, 14, 15, and 16, each represent four polarity units. Each group would in such a case be derived from a single archegonium, and represent an embryo system. Hutchinson did not recognize the compound nature of these groups consisting of "four chains of cells."

After the embryo tip becomes massive the suspensor of Larix appears to agree closely with that of the late embryo of pine. The cells of the massive suspensor are derived for the most part from more specialized cells at the base of the columnar tissue. They differ from the embryonal tubes of meta-stage in length, becoming less than a third as elongated. The suspensor is nearly as large as it ultimately becomes at the end of the ana-stage, and very few cells are added to it after early telo-stage. At germination only a collapsed and partly "jellified" mass remains, and this is immediately sloughed off in exit from the seed.

Tissues of the Mature Embryo.—It has been generally recognized since Strasburger's investigations, and even before (cf. Buchholz, 1918), that the plerome apex could be distinguished first, next the plumule primordium, and lastly the cotyledons. These are all features which may be observed in gross dissections; but beyond these particulars, little has been known as to the origin of embryonic tissues. Strasburger's figures, which are the most complete that we have, are frequently taken from entire embryos and drawn as optical sections. To the author these appear too definitely symmetrical and too precisely differentiated to be taken as representing the actual condition. Comparative study of tissue origin may furnish significant differences between the various conifer embryos if it is ever adequately investigated, but these differences will be chiefly quantitative rather than qualitative.

Whatever the sequence and origin of the embryo tissues may be throughout the Coniferales and Gymnosperms in general, we do have considerable accurate information of tissue configurations in the later stages. Growth processes are slowing down at this time and only differences in degree of development take place within tissues previously formed. Sections are more easily obtainable from the large embryo, and

mature seeds in a general way have been objects of a relatively large measure of scientific interest. The tissues of all or nearly all mature gymnosperm embryos may be enumerated in detail as follows: massive suspensor; column and peri-column; a region of generative initial cells at the basal tip of the plerome; periblem; plerome; cotyledons; a dermal layer of varying distinction; procambial tissue of varying prominence; medullar tissue more or less definitely defined; a plumule primordium of varying development; an embryonic secretory system which is probably present in many late embryos but has frequently been overlooked.

There are seen to be two active meristematic regions; one at the base of the plerome and one at the cotyledonary end of the axis. The basal region of initials gives rise to columnar tissue which is instrumental in forming the peri-column and periblem. This is accomplished by a direct alteration in polarity of the outer cell rows in the column. In transverse sections the columnar cells contrast greatly with the peri-columnar cells in size. Apparently this tranverse appearance led Hutchinson (1917) to mistake the column for the lower part of the "axis" extending through the calyptroperiblem in Keteleeria. Even in longitudinal sections the line of division between the peri-column and the columnar tissue is one of the most sharply distinguished in the whole embryo, especially when the protoplasmic contents of the cells are observed, since these usually give indication of the cellular polarity. Mere outlines of the cell walls in longitudinal section minimize this line of distinction. However, it must not be inferred that this or any other embryonic tissue is precisely demarcated along its border, because any individual border cell of the embryo is functionally plastic, adapting itself to the local tissue development.

The most remarkable development which takes place is the contribution of peri-columnar tissue to the original periblem. Previously no one has given much consideration to the origin of the periblem, or the mechanism for lateral growth in the embryo. It seems to have been assumed that the periblem originated by periclinal division of cells in the axial region. The obvious cell row alignment between the periblem and upper part of the peri-column was explained by Chamberlain (1935, p. 267): "Of course the root cap comes from the lower layer of meristematic cells and so it is said to come from the periblem." Chamberlain also stated (p. 270): "Practically all authorities say that the root cap comes from the periblem." He made no distinction between column and peri-column, and he retained the term "root cap" for these two tissues, although Buchholz and Old (1933) had clearly shown that there is no root cap in the conifer embryo comparable to the structure in angiosperms or ferns. The term calyptroperiblem, which they proposed to include the two tissues herein designated as column and peri-column, is a desirable terminological distinction. The sequence in Larix shows that

the periblem instead of contributing to the calyptroperiblem is really originated from it. Once formed around the plerome the periblem is self-perpetuating in the embryo, since it increases in length by intercalary growth. Young seedlings have shown the periblem of the radicle to be maintained in early growth by additional contribution from the peri-column. As Buchholz and Old have indicated, there is no break whatever in the cell rows from the peri-column into the periblem. In development of the embryo tissues (mid ana-stage), cells located in the uppermost peri-columnar region produce progeny whose relative position changes until they are in the periblem zone; in the course of growth in length, these original periblem cells multiply along with the pleromic tissue but without further addition of cells from below.

The real generative region of the root lies nearly exclusively in the group of large, nearly isodiametric, initials above the column at the basal tip of the plerome, no distinction whatever being possible between the initials contributing to the two, except their general position in this generative meristem. As we trace rows from the lower part of the periblem in the embryo, down into the peri-column, the number of peri-columnar rows *increases*. In tracing cell rows in the plerome downward the number of rows *decreases*, and all rows converge toward the generative initials. However, in the embryo only the basal part of the plerome is derived from these initials, as those above come from the central zone of the original pleromic centrum. In the seedling, all root growth originates from the generative initial cells, both above (for the plerome), and below (for the column, peri-column, and periblem). The plerome and periblem, while coming to lie side by side, are for the most part actually derived from the top and lower margins, respectively, of this one generative meristem.

The relations of the upper tissues are about as has been previously considered. The plumule primordium has long been known to lack an apical cell and requires no special discussion. The "nodal" meristem is the first of the procambial structures to become recognizable, and in Larix it is the region in which tracheids first appear after germination. The cotyledonary primordia contain procambial branches continuous from this nodal region from a very early time. Pleromic procambia are not very strongly differentiated until later telo-stage.

According to the embryologic concepts of Bower and Lang, the root in pteridophytes is to be considered as a lateral organ. It is, therefore, a point of considerable phylogenetic importance to note that the primary root, as developed by gymnosperm embryos, is in every sense axial in formation. The first radicular formation is the columnar tissue developed early in ana-stage, which later becomes narrowed, by lateral sapping, to become the column. After germination this structure gradually diminishes

in prominence. Still it is an important constituent of all gymnosperm roots, since it is the immediate source of protective tissue below and around the delicate generative initials. It originates *de novo* on the secondary roots. Although certain histologists, and among them Schuepp (1926) have attempted a more or less formal classification of root "types" and have included some angiosperms in the same type assigned to gymnosperms, it is doubtful whether more than a superficial similarity exists between the two groups. It is a significant point that all gymnosperms are admittedly similar in root tip organization. The strictly axial derivation of the root in gymnosperms, a structure *set-in* between the apical meristem and the suspensor, is thought to be of particular significance, as will be brought out in the discussion to follow dealing with the primitive spindle concept.

The Primitive Spindle and Phylogeny

Polarity and Polyembryony.—Polarity is the primary consideration in Bower's "primitive spindle" concept. In the study of the Larix embryo particular attention has been paid to the manifestations of polarity, since this seems to be a most significant attribute of the embryo organism. All higher organisms are known to possess a major polarity gradient and subsidiary polarity gradients in their appendages. The unity manifest in this way is one of the fundamental considerations to be taken into account in defining an organism, since it is directly concerned with organization and subordination of the component parts. Polarity is largely associated with the coordinate functioning of the organism.

Coniferae are distinct among higher plants not only in the free nuclear divisions of the zygote (which is common to practically all gymnosperms), but also in frequently producing from a single zygote a number of embryonic units, each organized individually so far as polarity is concerned. Araucaria is an exception in that simple polyembryony is assured by a special sheath of cells, but I think this probably is a derived condition. Sciadopitys, on other grounds held to be fairly closely related, shows an extreme number of units which manifest an individual polarity, and Araucarian simple polyembryony bears slight resemblance in early stages to primitive simple polyembryony of cyads. The Pinaceae show a large measure of conservatism in regard to the number of polarized units produced by each zygote, since these seem to be relatively definite in number for each genus. The number of cells in the proembryo of each (the time at which embryonic polarity gradients are first established) likewise appears to be fairly constant throughout the group. Other conifers in the various families often show a long period extending well into meta-stage, during which new units of individual polarity are constantly being organized, and the number is often indefinite, although it is always improb-

able that more than one normally organized polarity unit will succeed in forming a functional embryo.

As a means of distinction between groups like the Pinaceae and groups in which the dominant polarity unit may be long delayed in forming or show fragmentation and "indecision" after the pro-stage, we may say that embryonal polarity in the pine family type is *pro-determined* and in the others is *meta-determined.* Specific instances need not be cited, but it can hardly be overemphasized that the time at which the final dominant polarity unit becomes established is of comparative importance. The Pinaceae are conservative in respect to the definiteness with which polarity units are produced. Furthermore, among the Coniferales, typified as they are by complex polyembryony, the relative uniformity in number of polarity units produced in the pine family is very remarkable.

For reasons elaborated in the discussion later, it is held that simple polyembryony is probably the primitive type in gymnosperms as in all other plants in which several embryos are formed by one gametophyte. At present the simple polyembryony of cycads is thought of as chiefly a size elaboration from the primitive condition. However, simple polyembryony in Araucaria is probably a derived condition made possible by the peculiar sheath of cells enveloping the proembryo group of initials. Simple polyembryony, which has been more or less conclusively demonstrated in a few other modern conifers, probably is likewise coenogenetic. Late establishment of the dominant polarity unit during meta-stage is more characteristic of modern conifers as a whole than the pro-determined condition shown by the Pinaceae. The regularity with which units showing separate polarity are established, as in the Pinaceae, probably signifies that this is a conservative characteristic, intermediate in character between primitive simple polyembryony and the advanced type of cleavage polyembryony with late meta-stage or even early ana-stage determination of a dominant polarity unit.

While the paleontological information available does not permit us to reconstruct a very satisfactory phylogeny for modern conifers, it seems very likely that, as a whole, they represent a natural group of single derivation which, among other things, was characterized by a particular tendency toward formation of many polarity units from each zygote. The ramification of the branches from the ancient source, and hence the true interrelationships of the living conifers, presents a more complex problem. The question as to which of the living genera is most primitive, seems largely academic unless fossil representatives can be cited. Nevertheless, the embryologic stability of the Pinaceae, insofar as we know the details of the intricate type of polyembryony found there, gives reason to believe that this is a relatively ancient type of embryogeny which endured with comparatively little change, while greater variation took place

in other organs. The reverse may have been true in other coniferous lines; e.g., Thuja and Biota, which show distinct differences in their early embryology, but have been considered quite closely related on the basis of vegetative characters.

The Primitive Spindle Concept.—The chain of thought indicated by the above title goes with chief credit to Professor Lang of Manchester and to Professor Bower, long associated at Glasgow. To the author's knowledge it is today the only fundamental concept which is comprehensive enough to be applied to plant embryology for the purpose of wide comparative analysis.

The underlying basis for the line of reasoning embodied in it may be obtained by reading Lang's essay on the ontogenetic (homologous) theory of alternation of generations (Lang, 1909). He states (p. 7) that "we are justified in assuming . . . that each stage in the ontogeny is determined by the preceding stage." He suggests a theoretical explanation for the differences in structure of gametophyte and sporophyte, largely by their "environmental" conditions which precede mature development. If we follow Lang, we must regard many of the characteristics of the embryo as being due to internal gestation of the zygote. Bower (1935), who favors an antithetic view of alternation, points out (p. 519) that "whatever the history of its origin (i.e., either homologous or antithetic), the biological importance of this internal embryology for land-living plants may be estimated from the constancy of its recurrence." By way of definition he says (p. 519), "It is to the early stages of the encapsulated sporophyte that the term 'embryo' is applied."

Lang (1915) further argues for the causal analysis of embryology in his Presidential Address before the British Association. Primary emphasis is placed on the inadequacies of what he calls the "phyletic period" of botanical science due to application of formal, or idealistic concepts. One very evident break with this mode of reasoning seen in modern botany is recognition of many parallel lines of evolution rather than the earlier strict monophyletic interpretation of the plant kingdom as a whole. The parallel lines may be presumed to have united during some ancient period, but in few instances is compelling evidence available to indicate the actual points of contact.

It seems well to point out that a more or less "formal" philosophy is required in order to have some "yardstick" for the proportional evaluation of facts, whether or not these facts are matters of formal or causal interpretation. However, it is perhaps not contended today that any purely formal interpretation should be upheld, and there is great diversity of opinion as to just how far a causal philosophy is useful. If causal precepts were carried to an extreme, the great advantage of "formalistic" generalization seemingly would be largely lost. The "primitive

spindle" concept, thus designated by Bower (1922) seems to be based on the causal reasoning of Lang to a large extent, augmented by Bower's particularly wide personal familiarity with the pertinent facts. If rigidly adhered to, the "primitive spindle" may become as burdensome as any other formal generalization. But with judicious application it would seem to render many diverse features of embryos to some extent explicable and at least logically comparable on a common basis.

The most simple example of a primitive spindle may be demonstrated in algae where the plant is related to a solid substratum. Organization of an axial polarity gradient very plausibly may have had its beginning in some such environment as this. At a further stage, Bower (1935, p. 520) says, "The retention of the egg in the venter of the archegonium would fit well biologically with the departure of the organism from aquatic life, and insofar it would accord with the correlation of archegoniate alternation and the establishment of a land flora." In all seed plants and many lower plants the apical pole of the embryo is directed inward (endoscopic). In accord with their primary polarity, Bower (p. 524) points out that "the embryo will assume at first a more or less spindle-like form, though this may be variously modified or disguised." Bower has emphasized that polarity is evident from the first segmentation of the zygote. Although this apparently holds true for all other plants above the Thallophyte level, and for most Thallophytes as well, it does not apply precisely to gymnosperms.

In establishing the "primitive spindle" concept, Bower draws upon his extensive knowledge of pteridophytes and arrives at essentially the following conclusions: (1) In all archegoniate plants, definition of polarity is the first step in development. (2) Possession of a suspensor is a primitive feature which has been obliterated in many forms; when it is present, the embryo is always endoscopic, as if the suspensor served to anchor the direction of polarity. (3) The embryonic apex has a definite relation to the first segmentation of the zygote, since it originates at or near the center of the epibasal hemisphere. (4) The leaf-formation always arises from the epibasal hemisphere. (5) The root is constant in originating as a lateral appendage in all pteridophytes that have a suspensor, although when no suspensor is present, it may *appear* to oppose the axis of the whole embryo. The root is accessory to the spindle and variable both in position and in the time of its definition.

Partly on the basis of Bower's earlier treatment (Origin of a Land Flora, 1908), Lang (1915) formulated these conclusions: "(1) the primary importance of the longitudinal axis of the shoot, the position of the first root and the foot being variable; (2) the constancy of the position of the stem-apex near the centre of the epibasal half of the embryo; (3) the probability that embryos without suspensors have been derived

from forms with suspensors, without any example to the converse change. These and other related facts seem to find their morphological explanation in the shoot of the sporophyte being the result of the elaboration of a filament."

Application of the Primitive Spindle Concept to Gymnosperms.—The question now at hand is the degree to which the embryo of Larix and other gymnosperms, conforms with these findings based on the vascular cryptogams. From such consideration on the basis of embryology it may be possible to reach a conclusion as to the relative degree of relationship between the two groups. The points previously summarized from Bower are discussed in order.

(1) Segmentation of the zygote: In common with nearly all gymnosperms, Larix shows an intercalated free nuclear phase after zygote division which precludes an immediate manifestation of zygotic polarity. It might be contended that the movement of the nuclei to the base of the archegonium is an indication of polarity, but this is more plausibly explained as being caused by factors affecting the archegonium as a whole and not as a characteristic of the nuclei themselves.

We may best designate the earliest appearance of polarity by retrospective observation. The polarity units have been defined primarily on their individual capability to form a recognizable embryo. The first definite polarity indication of these embryo-forming units in Larix and others of the Pinaceae is observed in the last cell division within the proembryo. This segmentation of the apical tier perhaps may be regarded as functionally equivalent to primary segmentation of the zygote in vascular cryptogams.

A great contrast hereby obtains, which is just as characteristic of gymnosperms as a group as is direct spindle formation in the vascular cryptogams. We may conclude with little hesitation that the primary embryonic polarity in gymnosperms is delayed in its appearance, whether simple polyembryony results (as in cycads) or complex forms of polyembryony occur, as in conifers.

(2) The gymnosperms all possess a highly developed suspensor which is practically universal within the group. If we grant that the suspensor is a primitive embryologic feature we must add that it has also been a feature of specialization in gymnosperms. It has presumably endured throughout the history of this whole division of the plant kingdom. The fossil embryos of Araucarians (Darrow, 1936) and Bennettitaleans (Wieland, 1916; and others)* both show evidences of the gymnospermic suspensor in their radicular conformation. Coulter and Chamberlain (1917) think Bennettites may have had a short suspensor similar to Ginkgo.

*Although reports of embryos of other groups of fossil plants have appeared and it is possible that some are present in American Carboniferous coal-ball material, nevertheless except for the two groups cited above, there is no information as to the characteristics of fossil embryos yet available.

The first cell of a polarity unit to elongate is quite comparable to the suspensor of certain eusporangiate ferns. The relatively enormous multiplication of suspensor cells, found in all gymnosperms, whether simple or not in their form of polyembryony, has almost no counterpart in the rest of the plant kingdom. It is altogether probable that in this group of plants where embryo competition is keen, the suspensor serves as a means of eliminating the less vigorous units. In Selaginella where a comparable case of simple polyembryony occurs, the suspensor is never of this elaborate sort, and competition evidently is not the acute and significant phenomenon that it is in gymnosperms. It may be tentatively suggested that the effect of the seed habit in modifying the organization of the gymnosperm gametophyte has conditioned the occurrence of embryonic competition.

The ubiquity of the specialized suspensor in gymnosperms requires no further elaboration in order to contend that this agrees very well with Bower and Lang's conclusions. For gymnosperms the suspensor certainly is a fundamental embryonic organ. Its mammoth development may be explained on the basis that this is the only group of plants where such an organ became useful as a dynamic means of embryonic selection and survival.

This last statement cannot be demonstrated by study of any individual modern species, and Goebel (1933, p. 1819) has expressed some skepticism that the embryos eliminated in the process of selection are as a matter of fact "unfit." If the suspensor is a primitive structure, discernible perhaps in the plants which formed the first land flora, and if the gymnosperm suspensor can be thought of as being directly derived from those possessed by some of these fundamental land plants, then we also see how it may at the present time be difficult to *demonstrate* an "unfitness." The suspensor long ago became perfected in essentially its present setting (the fossil Bennettitalean embryos prove this), and the more obviously unfit embryos have become so "weeded-out" since its effective establishment, that the unfitness of any repressed embryos may no longer be expected to show phenotypic indications. As a factor which has influenced long-term evolution it is probably none the less important.

Larix has shown us an easily demonstrable case where the elimination of the *less favored* (rather than the unfit) is occurring constantly. It has been demonstrated that as a frequent occurrence two of the polarity units are smaller than the other two, primarily due to an unequal segmentation of the base of the archegonium by the free nuclei. It is becoming more evident that the archegonial shape may be the chief determining factor in favoring certain of the polarity units (see Buchholz, 1941). The suspensor in such a case is still the means of elimination of the extra embryos. Since, as Goebel points out, it tends to eliminate chiefly

the slower-growing members, the writer believes that the suspensor can also be interpreted as a means of maintaining a certain standard of vitality for the race. This is justified when viewed in a perspective commensurate with the age of the group through geological eras. There are many features of organic evolution which are not objectively demonstrable from living organisms but which are real and important factors during a long span of geologic time.

(3) The gymnospermic embryology seems entirely consistent with the embryology of cryptogams in respect to the epibasal segment producing the stem apex, particularly if the polarity unit is considered equivalent to the primitive spindle. On this basis, the embryonic apex retains its definite relation to the "epibasal" segment. Delayed organization of polarity in gymnosperms, due to free nuclear division of the zygote, makes this comparison less precise than might be desired. However, rather close comparison may be made between an early polarity unit of the pine type (see Buchholz, 1929, p. 369), with the elongated early embryo of Danaea or Macroglossum and of at least one species of Angiopteris (Land, 1923). The epibasal cell in the latter is, no doubt, quite comparable with the early embryonic apical cell in such gymnosperms as show individual filamentous polarity units. When the embryos in these groups are thus brought into parallel, it may be admitted that the comparison is favorable. However, when the massively organized polarity unit in cycads is considered, the formal resemblance fades, although a functional comparison is still tenable. Of all the gymnosperms which are accurately known, the Pinaceae seem to offer the best material for comparison of the primitive spindle and polarity unit.

(4) The difficulty in delimiting the gymnospermic embryos as to hypobasal and epibasal portions also limits the precision with which comparison may be made as to position of the leaf-formation. Still, if we consider the axis of polarity and think of the apical pole as corresponding to the epibasal portion of the cryptogamic embryo, it is true that location of the leaf-formation (cotyledons and plumule in gymnosperms) both in gymnosperms and vascular cryptogams is in close agreement.

(5) With regard to the lateral origin of the root held to be characteristic of vascular cryptogams according to the primitive spindle hypothesis, gymnosperms show a distinct lack of agreement.

It has been shown in Larix that the root originates through development of a basal center of polarity produced within the original axial polarity gradient. The primary root is a structure intercalated in the primary axis as a constant feature, in direct alignment above the suspensor. No suggestion may be found of any different mode of origin for the primary root throughout all gymnosperms. It cannot in any way be considered lateral to the primary polarity gradient. Here then is a point

of fundamental distinction between the vascular cryptogams and the gymnosperms. Whereas in the former "the root is accessory to the primitive spindle and variable both in position and time of its definition," so far as I am able to see, the exact reverse is true with respect to the root in the entire gymnospermic phylum. This may likewise be true in angiosperms.

Certainly the extreme conservatism of this feature in gymnosperms is a matter of far-reaching phylogenetic significance. The root is known to show many conservative features in its later organization, but its conservatism as to place of origin with reference to the primary embryonic polarity has not been sufficiently emphasized.

If the suspensor is a fundamental embryonic organ, the root in gymnosperms is almost equally fundamental, since the suspensor and root are so intimately associated. From their relationship it seems plausible that the root became established as an invariable plant part from the time of origin of this lineage. Certainly it is one of the strongest arguments for a monophyletic derivation of the gymnospermic phylum as a whole, and it may possibly apply to a larger group of seed plants.

The conclusions Lang enumerated have also been covered by previous discussion (p. 72). Both Bower and Lang are in essential agreement as to the significance of the "foot" in cryptogamic embryos. Bower (1935, p. 542) states that the "foot" in cryptogams is essentially an "opportunist growth." It is the one organ of cryptogamic embryos which has no counterpart in gymnosperms. The points brought out thus far are summarized in the following comparison of modes of embryo formation:

Vascular Cryptogams (Primitive Spindle)	*Gymnosperms (Polarity Unit)*
1. Direct spindle formation following first segmentation of the zygote.	1. Delayed organization of polarity units as a result of free nuclear division from the zygote.
2. Suspensor held to be primitive on theoretical grounds, but has suffered reduction and elimination in many of the advanced forms.	2. Suspensor a prominent feature throughout.
3. Embryonic apex has a definite relation to the first segmentation of the zygote.	3. Embryonic apex is constant as a feature associated with the apical end of the polarity unit.
4. The leaf-formation always arises from the epibasal hemisphere.	4. The foliar organs are always derived from the apical pole (= epibasal region) of the polarity unit.
5. In all pteridophytes which have a suspensor, the root is lateral in origin; it is concluded that the root is an accessory of the spindle both in position and in time of origin.	5. All gymnosperms have the root as a constant axial addition, formed in continuation with the suspensor. In no sense is it lateral to the polarity unit in origin.

Points 1 and 5 are in serious conflict; agreement seems reasonably close for the other three.

It should be emphasized in this connection that there are also other points just as fundamentally important in comparison of these great

groups of plants as the mode of embryo formation. One of the most cogent of these is that archegonia, identical in functional import, are characteristic of both.

Comparison of Cycad and Conifer Types of Polyembryony.—Here an analysis is attempted of the factors which have produced the greatest embryonal difference that is seen in the gymnosperm phylum, i.e., simple polyembryony of the cycad type vs. complex polyembryony of the conifer type. In this connection an explanation for delay in organization of primary polarity in gymnosperm embryos (viz., the origin of the free nuclear stage) will be considered first. It is believed that the explanation given by Chamberlain to explain free nuclear division in cycads will also account for late organization of embryonal polarity for gymnosperms in general; at least it serves as a medium for theoretical interpretation.

Chamberlain (1935, p. 142) briefly restated the idea he had previously given in 1919, viz., that the free nuclear division of the zygote may be due to the incompetency of the first small daughter nuclei to span the archegonial cavity and immediately segment the enormous cycad egg cell. The writer is inclined to accept this as a general but fundamental explanation for the widespread occurrence of this type of free nuclear division. However, the large archegonium and egg cell is not limited to cycads but is a general characteristic feature of the whole gymnospermic phylum. *Sequoia sempervirens* is the only gymnosperm known in which the egg cell is immediately segmented following zygotic division. Here the egg cell is very small in comparison with those of the other members of the phylum, and as an exception to the usual order serves to substantiate Chamberlain's causal deduction. The small archegonia probably are coenogenetic in origin, and typical compound embryony is maintained in Sequoia, notwithstanding the unusually early segmentation (Buchholz, 1939).

The origin of the enlarged egg cell is the point for consideration if we admit its size to be the primary necessity for free nuclear division of the zygote and consequent delay in expression of polarity. The essential agreement of gymnosperms in this characteristic is a point emphasizing the primary interrelationship of the whole group. To the writer it seems tenable to associate development of the enlarged egg with the origin of the seed habit in the gymnosperms as a response to the then newly established nutritive relations. Perhaps at that time it represented a type of nutritional "hypertrophy." While the gymnospermic archegonium shows the fundamental derivation of the group from archegoniate ancestry, still nowhere in the modern free-sporing archegoniates do we find any comparable and consistent enlargement of the egg cell. The greatly enlarged egg has all the indications of being associated with intraseminal nutrition

which must have been a new problem in organization for the original archegoniate ancestors. As yet this is almost entirely a matter of theory, although it is possible that direct paleontological evidence will some day be available.

Fossil gymnospermic archegonia have been illustrated by Brongniart (1881) in seeds from the late Upper Carboniferous (Stephanian) of Grand Croix in central France. Not only are these archegonia already much larger than those of cryptogams, but they are round to flattened ovate in shape—surely an indication that extremely elongate archegonia are not primitive in gymnosperms.* Measurements of archegonia have been made directly from Brongniart's figures and, except in cases where the magnification was slight or as otherwise noted, are probably accurate within 20-30 μ. These measurements are as follows:

	Archegonial Axis	*Transverse Dimension*
Cardiocarpus sclerotesta	300μ	550μ
(Pl. II, fig. 2)	250	425
Cardiocarpus augustodunensis	466	733
(Pl. III, fig. 8)	666	746
Cardiocarpus tenuis	400	900
(Pl. V, fig. 5)	300	825
Leptocaryon avellana	500	550
(Pl. VI, fig. 7—including neck?)		
Rhabdocarpus subtunicatus	666 ± 100	700 ± 100
(Pl. X, fig. 20—magnification too small for accurate measurement)		
Rhabdocarpus conicus	240?	300?
(Pl. XI, fig. 4—"central body" may be a cordaitalean pollen grain; archegonium (?) broken, measurements doubtful)		
Rhabdocarpus cyclocaryon	293	400
(Pl. XII, fig. 2—measurement of "central body"; archegonium proper is larger)		
Sarcotaxus avellana	266 ± 50	333 ± 50
(Pl. XIII, fig. 3—magnification too small for accurate measurement)		
Taxospermum gruneri	500	516
(Pl. XV, fig. 5)		
Stephanospermum akenoides	250?	320?
(Pl. XVI, figs. 4-6)		

To give some perspective for comparison with these measurements and those recorded for the archegonia of the various modern gymnosperms, illustrations given by Smith (1938) of various cryptogams were also measured, and some of these are tabulated below. Since the arche-

*Doyle and Looby (1939) have, however, interpreted the phylogeny among podocarps in converse fashion with regard to archegonial shape.

gonia of cryptogams have an essentially isodiametric venter only one measurement, the transverse dimension, is recorded.

Genus	*Archegonia*	*Genus*	*Archegonia*
Azolla (p. 362)	43μ	Equisetum (p. 245)	66μ
Onoclea (p. 347)	46	Isoetes (p. 215)	79
Marsilea (p. 336)	93	Selaginella (p. 191)	43
Gleichenia (p. 305)	49	Lycopodium (p. 177)	54
Osmunda (p. 292)	58	Tmesipteris (p. 157)	121
Marattia (p. 281)	58	Various mosses	44–80
Ophioglossum (p. 273)	102		

These measurements suffice to show that an archegonium in excess of 100 μ in transverse diameter must be regarded as exceptionally large in the cryptogams. In gymnosperms, on the other hand, an archegonium not in excess of 100 μ diameter (e.g., Sequoia and Sequoiadendron) must be considered unusually small. Those of Larix approximate 300 μ in their shorter transverse diameter, and cycad archegonia frequently exceed 1 mm. Archegonia of *Dioon edule* measure as much as 1,300 μ transversely and 3,800 μ axially. Of course, simple linear measurements of such bodies do not adequately express the actual differences in volume, since volume is a function of three diameters. Thus, it appears that gymnospermic archegonia so far exceed the size of those ordinarily encountered in cryptogams that this fact must be accorded some significance. As ubiquitous as this archegonial gigantism is among the different gymnospermic families, it may perhaps be regarded on the whole as a primitive and relatively conservative character. In fact it may have arisen as a direct result of attainment of the seed habit in this particular line and have its origin as long ago as Lower Carboniferous or Upper Devonian times. There is no reason to assume that a sequence of archegonial enlargement is obligatory in development of the seed habit, but in this particular division the two processes may have been causally linked in evolution.

As in all other archegoniate plants, each egg in the original gymnosperm stock very probably produced a single polarity unit, or "primitive spindle," immediately following the first egg segmentation. When the mechanical features suggested by Chamberlain became acute, due to egg enlargement, the direct establishment of polarity was no longer physically possible and various artifices were invented to serve the embryologic function. Such reasoning entirely complies with Lang's assertion that "each stage in the ontogeny is determined by the preceding stage."

There were two chief methods which could have been used in overcoming the physical difficulties brought on by the "hypertrophy" of the egg: (1) the production of a large number of nuclei which were together capable of effecting subdivision (segmentation) of the egg cavity; or (2) the aggregation of a fewer number of nuclei in a narrowed portion of the chamber and segmentation of this portion only. In the first alternative, the free nuclei would have been more likely to function in a coordi-

nate manner in forming the first embryonal tissue. By so doing they could have attained the degree of coordination necessary for rapid establishment of an embryonic unity typified by a single polarity gradient. In the second alternative, nuclear interrelations may be thought of as less obligatory with proportionally less embryonic unity being attained in the process. These might be the extreme manifestations as induced by the enlarged egg cell. Various degrees of compliance may, of course, be cited among modern gymnosperms which have, in fact, suggested the alternatives just given. The justification for extrapolating features of modern plants is that the features under consideration are fairly conservative, and there is no conflict with available fossil evidence.

In this discussion, Ginkgo is included with the cycad type of embryogeny, although it does not have the competitive features of polyembryony so markedly exemplified. Ginkgo illustrates the extreme form of archegonial subdivision by a great number of free nuclei, and as a result attains coordination of a massive embryo relatively early. The cycads are variable in the degree to which they partition the archegonium, but are essentially similar to Ginkgo in regard to formation of a single coordinated polarity unit from one egg. Both approximate fairly well the condition in free-sporing plants, where a single polarized spindle is produced, except that the proembryo is enormously more massive; by functional standards they are less diverse. Thus, Chamberlain's conclusion (1935, p. 144), that Ginkgo and the cycads show "the type of embryogeny which we should regard as the most primitive [among gymnosperms]," is wholly justified.

Biota, Sciadopitys, and certain podocarps (cf. Buchholz, 1929, 1931b, 1941) are examples of the complex type of polyembryony in conifers. Their mutual relationships are still largely speculative. Although it is likely that they are not very closely related, they all have a general indefiniteness of cellular organization in the proembryo which apparently is considerably influenced by the shape of the archegonial base. Always a few proembryo cells enjoy a positional "favoritism," and none of these cells represent actual polarity units. The prosuspensor, a specialized but independent embryonic mechanism, is functional in all three genera. It serves to move the apical group of cells into the corrosion cavity. There the polarity units originate out of the "favored" group of apical cells, more or less directly. All this intervening process delays definition of the polarity units, affording strong contrast with the cycad sequence. The marked lack of coordination and the indefiniteness with which the metadetermined polarity units are "selected" is characteristic of many of these more advanced Coniferales. The Pinaceae seem to have a somewhat comparable multiplicity of polarity units, but these are both pro-determined and usually definite in number, features more primitive in degree according to the criteria advanced here.

The proembryo organization of Araucaria is not so easily reduced to either alternative previously outlined. There, apparently another mechanism is exercised for filling the egg chamber. It cannot be conclusively stated that this is a feature of recent adaptation, but it would seem to be most satisfactorily regarded in this light for the present. The polarity is certainly pro-determined, but not until about 32 free nuclei have formed walls and organized a group of sheath cells radiating from and enclosing the embryonic group to fill the archegonium. If the suspensor-like proembryo sheath is not a coenogenetic adaptation to produce proembryonic unity, then Araucaria shows a third alternative method of coping with the enlarged egg cell. It seems most reasonable to hold the araucarian early embryo as derived from a complex embryonic type, at least until more evidence can be secured.

Briefly recapitulating, then, cycads and Ginkgo are thought to have met the problem of the enlarged egg cell by numerous free nuclear divisions which coordinate in formation of a massive proembryo. In these, the pro-stage and meta-stage elaboration found in conifers has been omitted, and embryologic sequences of this type are the most primitive to be found among modern gymnosperms. The Coniferales are characterized by complex forms of polyembryony. Simple polyembryony in this group is most probably a derived condition. Pro-determined polarity units are not characteristic of the group as a whole, and where they occur likely indicate primitiveness.

Since this study is not primarily concerned with gymnospermic phylogeny, the considerations given above will not be amplified. It was thought desirable to sketch them briefly since, among other things, they show the value of recognizing the basic units of complex embryology found in conifers and gymnosperms in general.

VI. CONCLUSIONS

Apical cells are present in the early embryo of Larix, and the early sequence is similar to pine in its essentials of development. The four vertical rows of the proembryo are not originally equal but show the result of positional "favoritism" at the base of the archegonium.

Only one of the four apical cells of the proembryo contributes to the mature embryo, although these initials do remain united during early development. Potential embryo-forming units are designated as polarity units. Three of the polarity units are often repressed and overgrown by the fourth, which continues forming a massive embryo. The massive growth of the dominant embryo in all cases seems to be organized from the progeny of only one apical cell.

The embryogeny of Larix shows only a quantitative difference in its

early sequence from the extreme type of cleavage found in pine, and hence the type in Larix is designated as *delayed cleavage polyembryony.*

In development of the massive embryo, a number of different tissues are recognized. Thirteen different tissues may be distinguished in the mature embryo; histologically they are distinct in varying degree, since nearly all of them are highly meristematic. The most important distinctions are based on differences in their functional character. The tissues represented in early ana-stage are: suspensor, columnar tissue, pleromic centrum, and mantle. In late ana-stage and continuing in telo-stage the columnar tissue gives rise to the peri-column and periblem, the pleromic centrum gives rise to the two generative meristems above and below, to the nodal primordium, procambial strands, and medullary tissue. Cotyledons are developed largely from tissue derived from the upper margins of the pleromic centrum. The mantle becomes a dermatogen over the cotyledons and upper part of the axis, but does not differentiate on the plumule primordium. Secretory canals are differentiated both in the medullar tissue and subdermally.

Columnar tissue formed basally in the early stages of massive tissue development dominates formation of lateral tissues and contributes to the final lateral enlargement of the embryo by the periblem.

The cotyledons include the last of the tissues of the mature embryo to become differentiated. Beyond the stage of cotyledon formation most of the embryonic growth occurs by intercalation within visible tissues.

Close agreement in qualitative features throughout the Larix embryogeny with the sequence as known for Pinus provides additional evidence of the close relationship between these genera. However, Pseudotsuga and Picea, and possibly Abies, are more likely related closely to Larix than any other genera.

Polarity units of gymnosperms are functionally equivalent to the primitive spindles of vascular cryptogams; the two groups are not sufficiently comparable from this fundamental standpoint, particularly with regard to origin of the root, to permit any immediate community of phylogenetic derivation. It seems probable that the ancestral archegoniate stock became differentiated into these two groups at a very early date.

Embryology in gymnosperms altogether upholds Lang's and Bower's contention that the suspensor is a primitive embryonic organ.

Gymnosperms in a fundamental sense are monophyletic in derivation. The very great conservatism of structure in the late embryo, particularly with reference to the primary root, is most indicative of this.

A causal basis is suggested as an explanation for the divergent types of polyembryony shown by cycads on one hand and conifers on the other. This is a continuation of the idea first suggested by Chamberlain involving the inability of the zygote to segment the large egg cell following the initial nuclear division.

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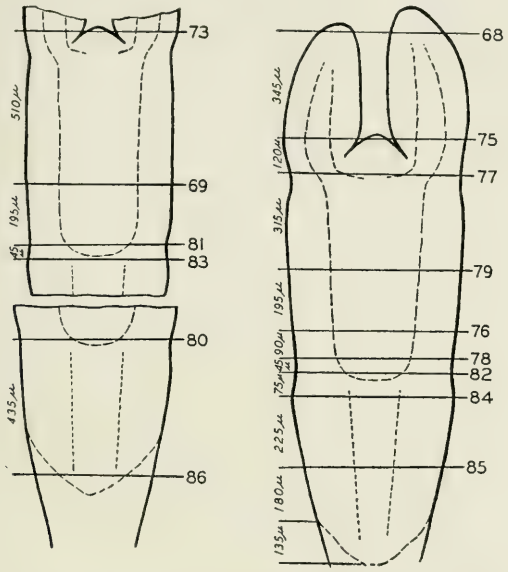
EXPLANATION OF PLATES

As a general practice, the figures of early embryo stages are oriented with the apex downward, as if seen in the seed with the micropyle turned upwards; the figures of the late embryo are oriented with the plumule primordium upward to correspond with the later attitude assumed in germination.



In the above diagrams of parts of three of the telo-stage embryos that were sectioned transversely, the numerals at the right indicate the relative positions of sections illustrated in Figs. 68, 69, and 73 on Plate IV, Figs. 75-80 on Plate V, and Figs. 81-86 on Plate VI. Distances between sections (cut 15 μ thick) are given on the left.

PLATE I

Fig. 48.—Longitudinal section through micropylar end of nucellus and gametophyte. Two germinated pollen grains are on the truncated tip of the nucellus with straight pollen tubes penetrating toward the archegonia. Fertilization has occurred, but the zygotes do not show in this section. Collection of May 21, 1933.

Fig. 49.—Transverse section of gametophyte showing proembryos in two of the five archegonia. Proembryo cells shown are of the suspensor and rosette tiers; the transverse walls between them are cut on a slant due to inclination of the walls. Collection of May 26, 1933.

Fig. 50.—Transverse section (taken just below that in Fig. 49) showing the apical tier of the proembryos. Two of the vertical rows facing each other across the short diameter of the archegonium meet at a straight central wall, whereas the two rows in line with the longer archegonial diameter do not touch each other. This shows best in the proembryo on the left.

Fig. 51.—Transverse section through four unfertilized archegonia in the gametophyte. The spiral fibrillar character of the cytoplasm is noteworthy, although it has been exaggerated by imperfect fixation. Same collection as Fig. 49.

Fig. 52.—Transverse section of gametophyte through four recently fertilized archegonia, showing extremely irregular nuclear membranes. Same collection as Fig. 49.

Fig. 53.—Transverse section through the lower ends of archegonia with free nuclei of proembryo. Same collection as Fig. 49.

Fig. 54.—Uppermost archegonium in Fig. 53 at greater magnification (about 300 times). Note the asymmetrical arrangement of free nuclei.

Fig. 55.—Longitudinal section of an early ana-stage embryo, showing at its tip the last vestige of a tetrahedral apical initial. Persistence of the apical initial may be correlated with the more than usually conical shape of the embryo tip. Collection of June 4, 1933.

Fig. 56.—Longitudinal section of an early ana-stage embryo, showing a repressed embryo (polarity unit) with a stage II apical cell still associated with it at the right. The apical initial has been completely eliminated in the dominant embryo. Same collection as Fig. 55.

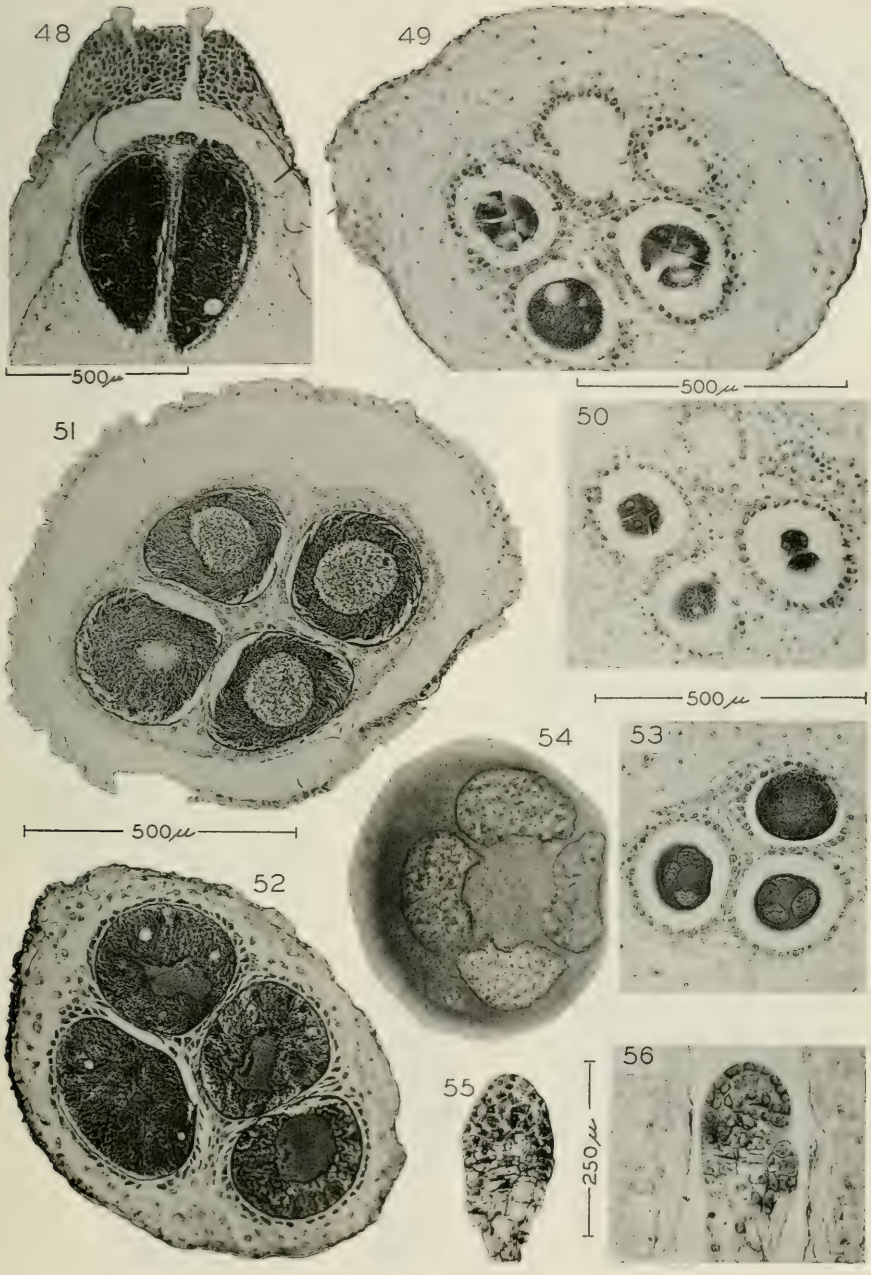


PLATE I

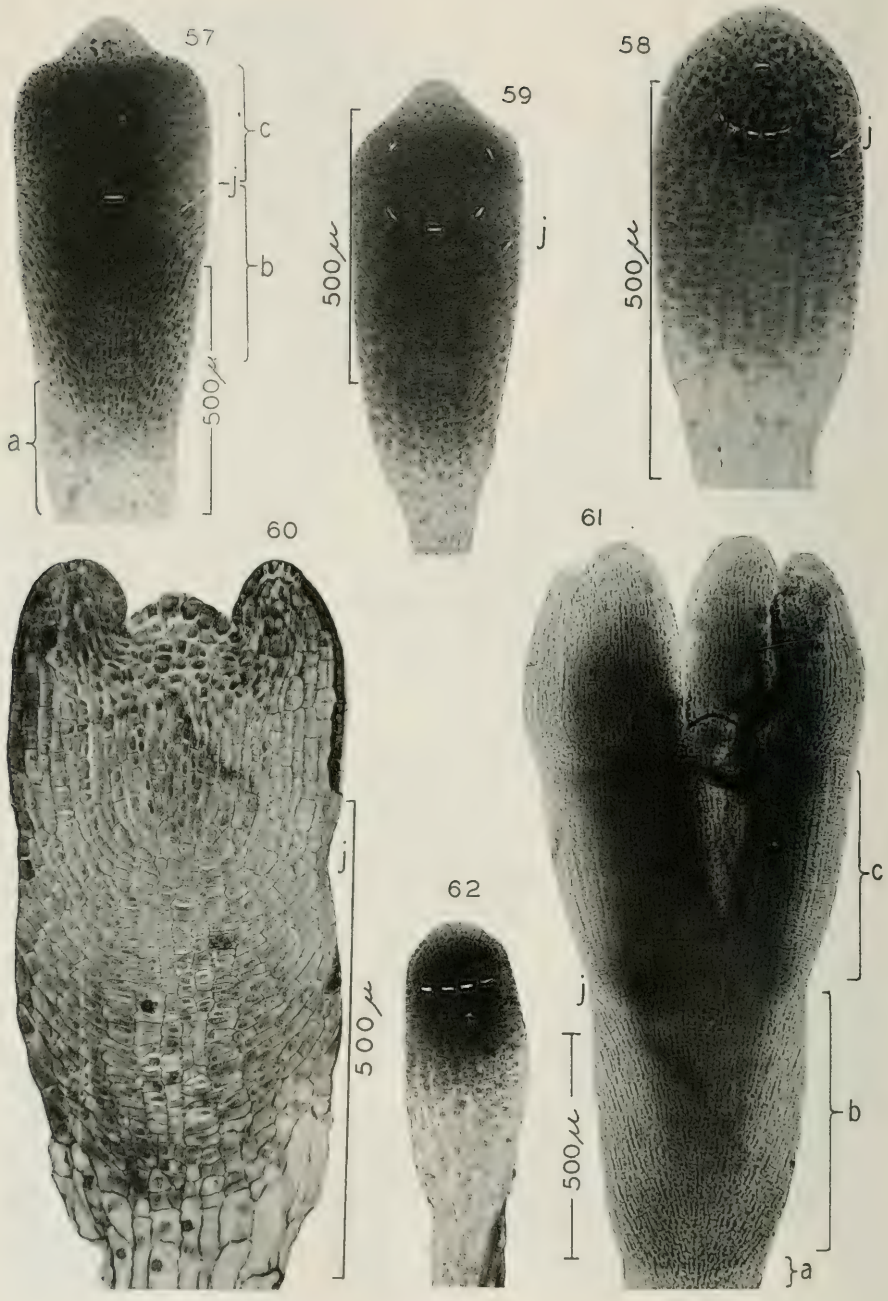


PLATE II

PLATE II

Letters indicate parts: *a*, cells contributing to suspensor; *b*, calyptroperiblem; *c*, axis; *j*, juncture zone.

Fig. 57.—Whole embryo in late ana-stage, stained with phloxine. Collection of June 16, 1932.

Fig. 58.—Whole embryo in late ana-stage, stained with phloxine. Area of the pleromic centrum is indicated. Same collection as Fig. 57.

Fig. 59.—Whole embryo in late ana-stage. The plumule primordium here shows its greatest prominence. Area of the pleromic centrum is indicated. Same collection as Fig. 57.

Fig. 60.—Median longitudinal section of embryo in early telo-stage. All tissues except secretory elements have made their appearance. Collection of June 25, 1932.

Fig. 61.—Whole embryo in late telo-stage, stained with phloxine and slightly crushed. Cotyledons are about two-thirds grown, and the axis will continue to elongate about one-fourth; the calyptroperiblem is nearly mature in size. Total length 1.63 mm.; length of cotyledons 480 microns (beyond the node), axis about 600 microns, calyptroperiblem about 550 microns. Most of the suspensor is badly collapsed. Same collection as Fig. 60.

Fig. 62.—Whole embryo in mid-ana-stage, stained with phloxine, photographed at the same magnification as Fig. 61 for comparison. The addition of lateral tissues (pericolumn and periblem) has hardly begun; columnar tissue occupies the lower two-thirds of the non-vacuolate tip. Same collections as Fig. 57.

PLATE III

Fig. 63.—Median longitudinal section of late telo-stage embryo within gametophyte. The seed coat has been removed; some vestiges of the nucellus remain. The megaspore membrane is visible around the gametophyte at the upper end of the figure. Collection of June 25, 1933.

Fig. 64.—Median longitudinal section of calyptroperiblem of late telo-stage embryo. Column is about 520 microns long; embryo 345 microns in diameter at the juncture zone. Same collection as Fig. 63.

Fig. 65.—Longitudinal section of telo-stage embryo showing pleromic secretory elements. Same collection as Fig. 63.

Fig. 66.—Longitudinal section of embryo similar to Fig. 65. Total length 1.25 mm. Same collection as Fig. 63.

Fig. 67.—Longitudinal section of seedling about two days after germination. Only the seed coat was removed before sectioning. The cotyledons have expanded about one-third from the resting condition and measure a little over a millimeter in length. The axis still within the seed has broadened to about 650 microns. The plumule primordium has enlarged somewhat. (Two of the earliest primordia for primary leaves were discernible in another section.) A few xylem elements have been established in the upper part of the seedling; those first appearing are short and scalariform in the angles of the cotyledonary node. Pleromic secretory elements extend the length of the radicle; the subdermal elements are limited to the hypocotyl and the cotyledons. Not only has the suspensor been destroyed in germination, but the dermal layers of the lower portion of the radicle are being sloughed off. Thus far, the food supply for embryo growth has come from the region at the rear of the gametophyte, where the cotyledons have enlarged and been in contact with the food reserve. The dermal absorption of food is probably the last of the purely embryonic functions to be outgrown by the seedling, and when the outer layer of these regions ceases to be absorptive, all the requirements for holophytic nutrition are present.

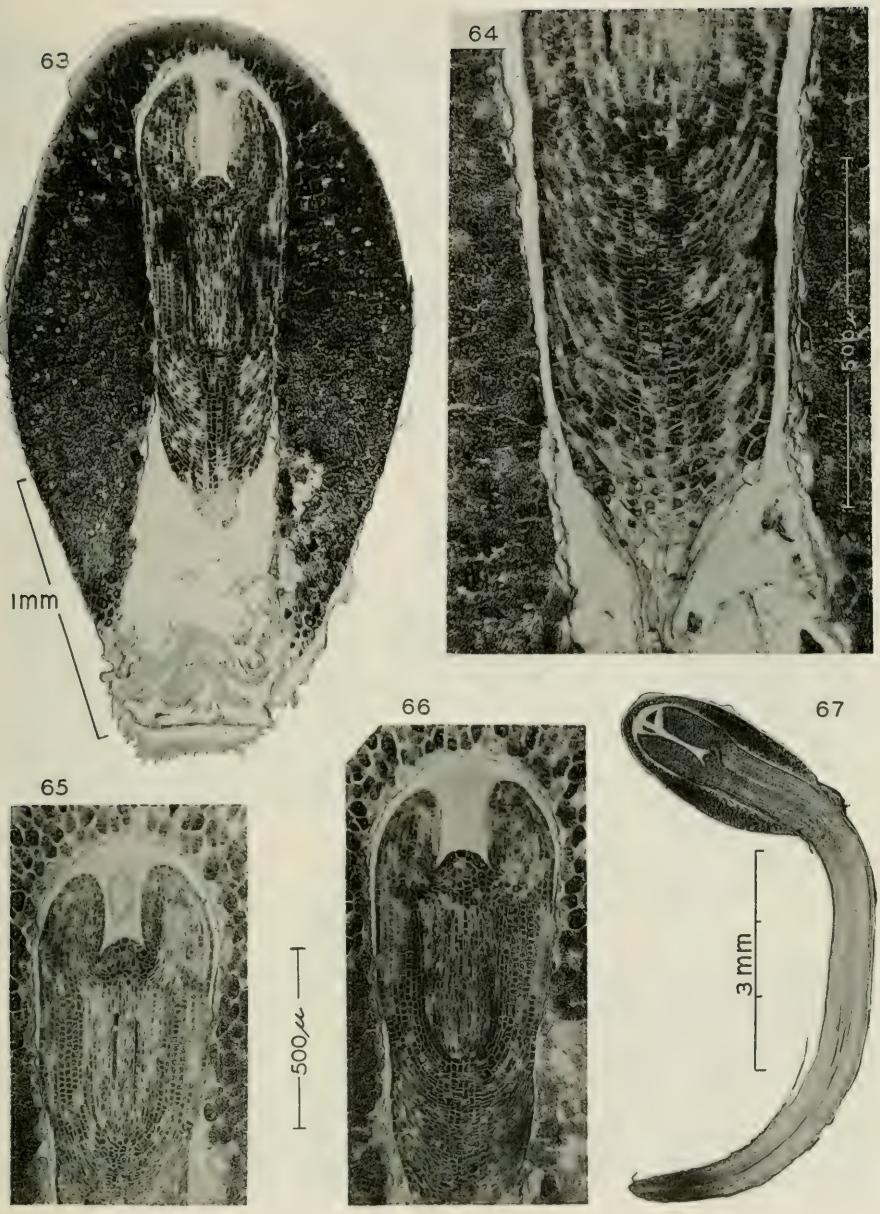


PLATE III

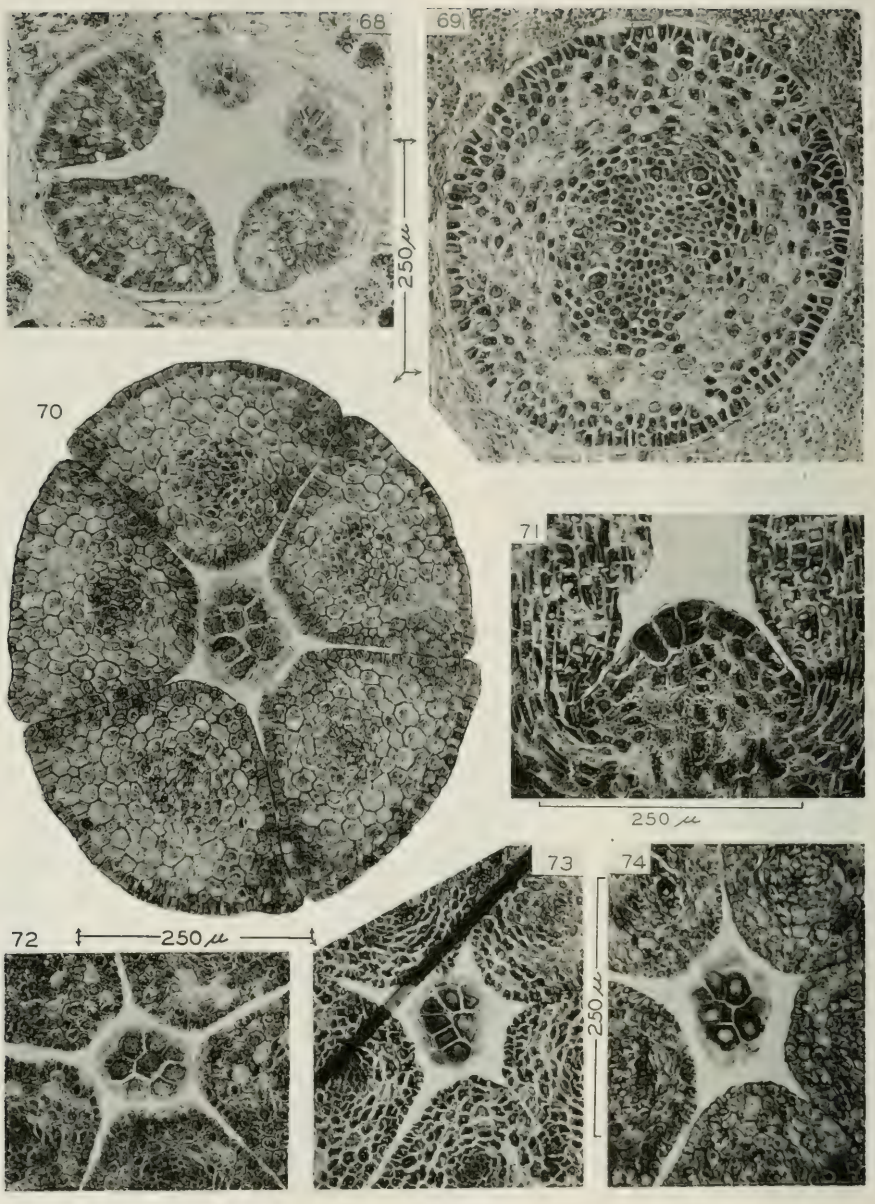


PLATE IV

PLATE IV

(Figs. 68-74 are from the collection of June 25, 1933.)
(For location of sections, see diagram on page 85.)

Fig. 68.—Transverse section of cotyledon tips in telo-stage embryo. The difference in cotyledon length is real and not due to obliquity of the section.

Fig. 69.—Transverse section of lower portion of plerome in telo-stage embryo. About ten pleromic and thirty subdermal secretory elements are present.

Fig. 70.—Transverse section of telo-stage embryo showing the plumule apex and basal part of cotyledons with cotyledonary procambia.

Fig. 71.—Longitudinal section of telo-stage plumule primordium. Note lack of epidermis and relative size of tip cells.

Figs. 72, 73, 74.—Transverse sections of telo-stage plumule primordia. Note variation in arrangement of tip cells. Thickness of the sections (15 microns) is indicated by the strip of displaced megaspore membrane lying diagonally across the section in Fig. 73.

PLATE V

(Figs. 75-80 are from the collection of June 25, 1933.)
(For location of sections, see diagram on page 85.)

Fig. 75.—Transverse section of telo-stage embryo showing plumule apex and basal parts of cotyledons. Subdermal secretory elements on the two lower cotyledons have been outlined; they are restricted to the abaxial surfaces. The two cotyledons at the upper right correspond to the two short cotyledons in Fig. 68, both sections being from the same embryo.

Fig. 76.—Transverse section of lower part of axis of telo-stage embryo, about 150 microns above the base of the plerome and 195 microns below the level of the section shown in Fig. 79. Pleromic and subdermal secretory elements have been outlined.

Fig. 77.—Transverse section just above nodal primordium of telo-stage embryo and about 120 microns below the level of the section shown in Fig. 75. Subdermal secretory elements in the lower part of the photograph have been outlined; about thirty-six such elements in all are present.

Fig. 78.—Transverse section near base of axis of telo-stage embryo, about 90 microns below the level of Fig. 76. Subdermal secretory elements are absent, but pleromic elements are present (indicated by solid black).

Fig. 79.—Transverse section of telo-stage embryo about the middle of the axis and about 315 microns below the level of Fig. 77. Subdermal secretory elements, which show to advantage in this photograph, are untouched. Pleromic elements have been outlined.

Fig. 80.—Transverse section of telo-stage embryo which appears to include the base of the plerome. The ends of pleromic secretory elements are present in the second section above this one, and the column is distinct in the fourth section below it.

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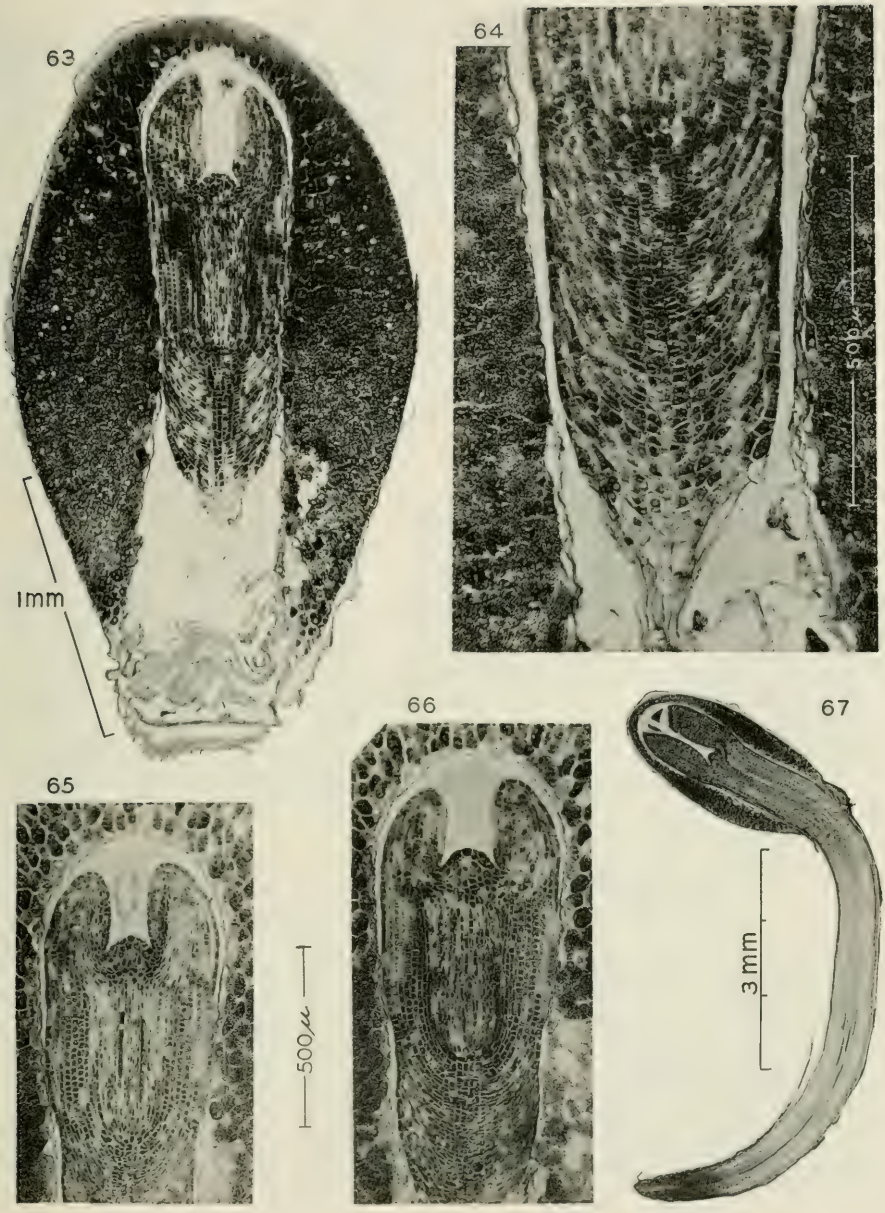
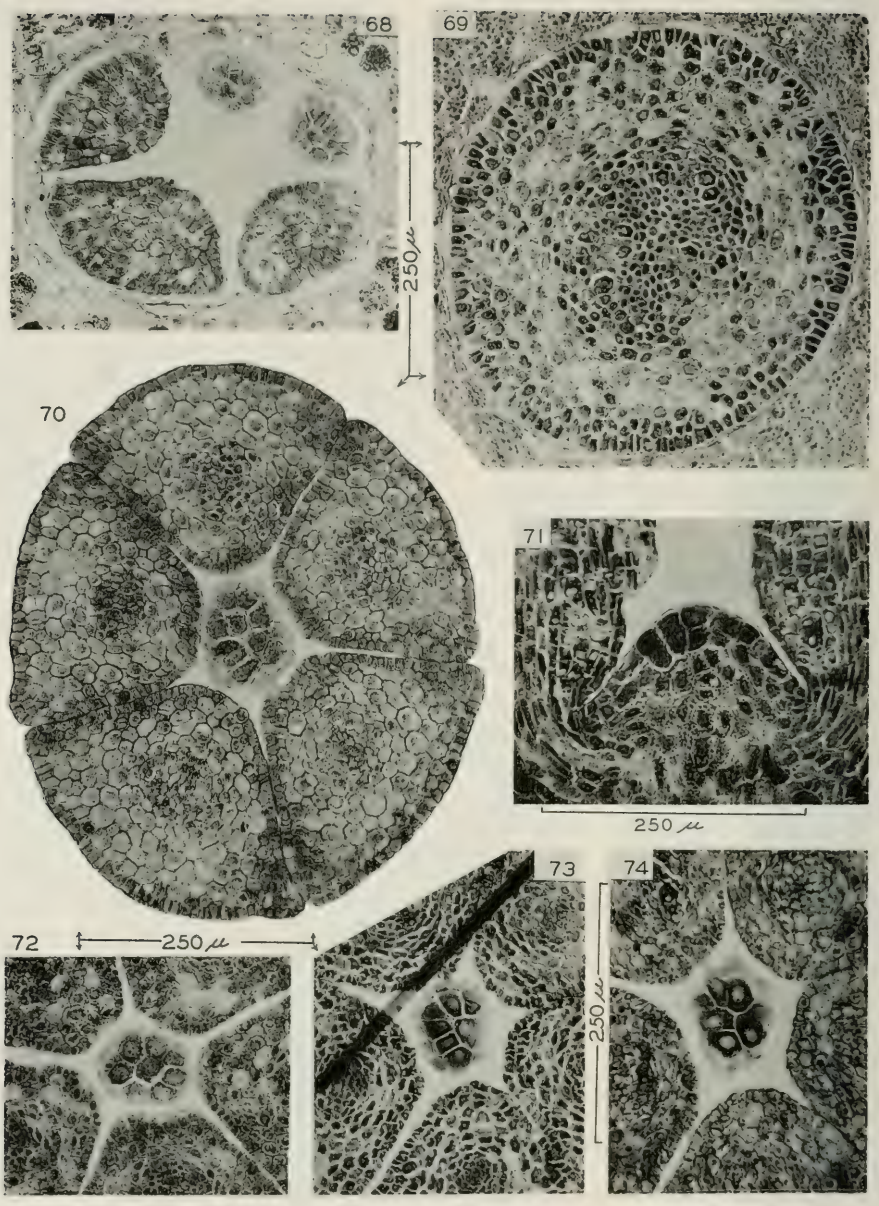


PLATE III



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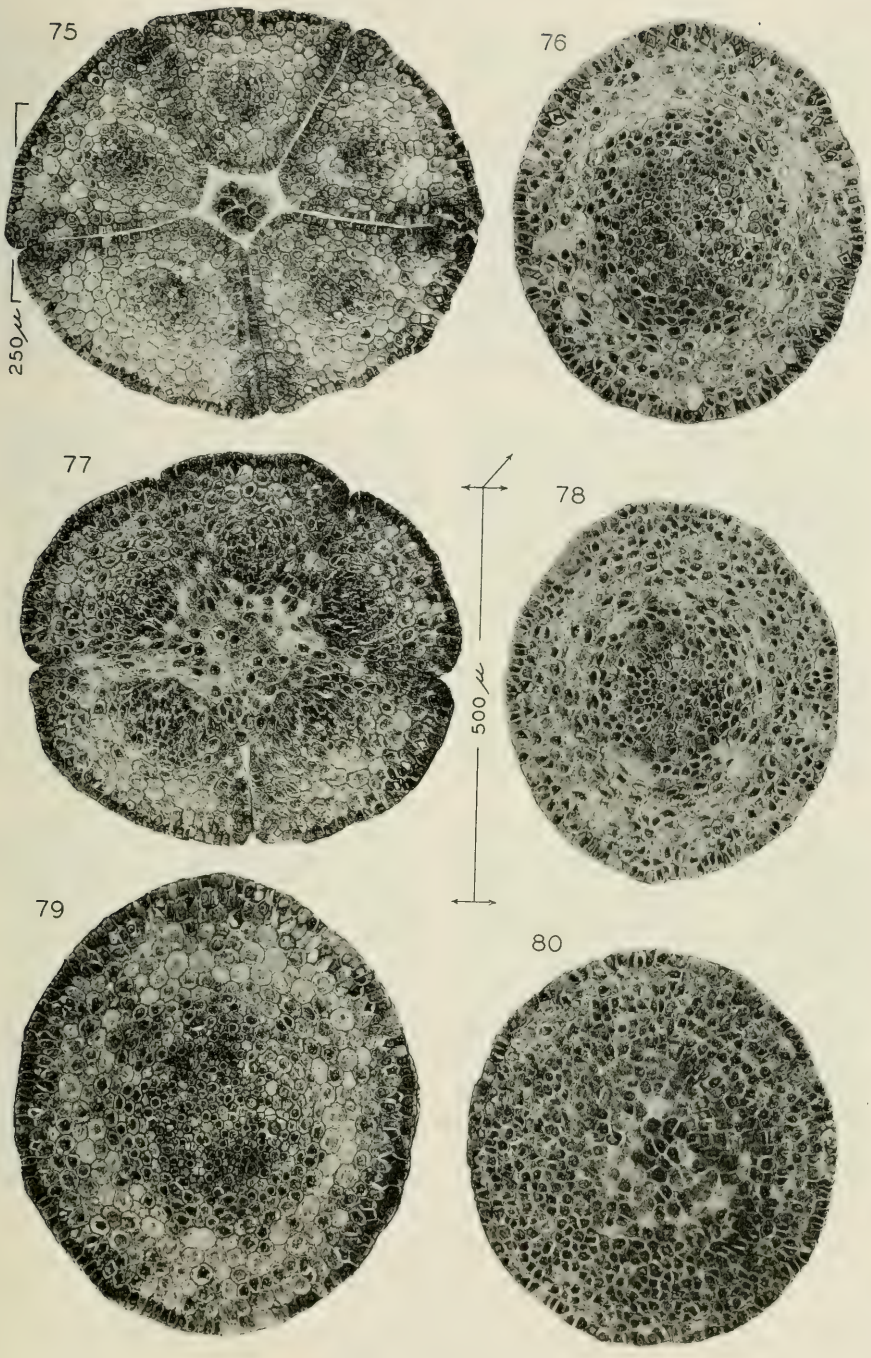

PLATE V

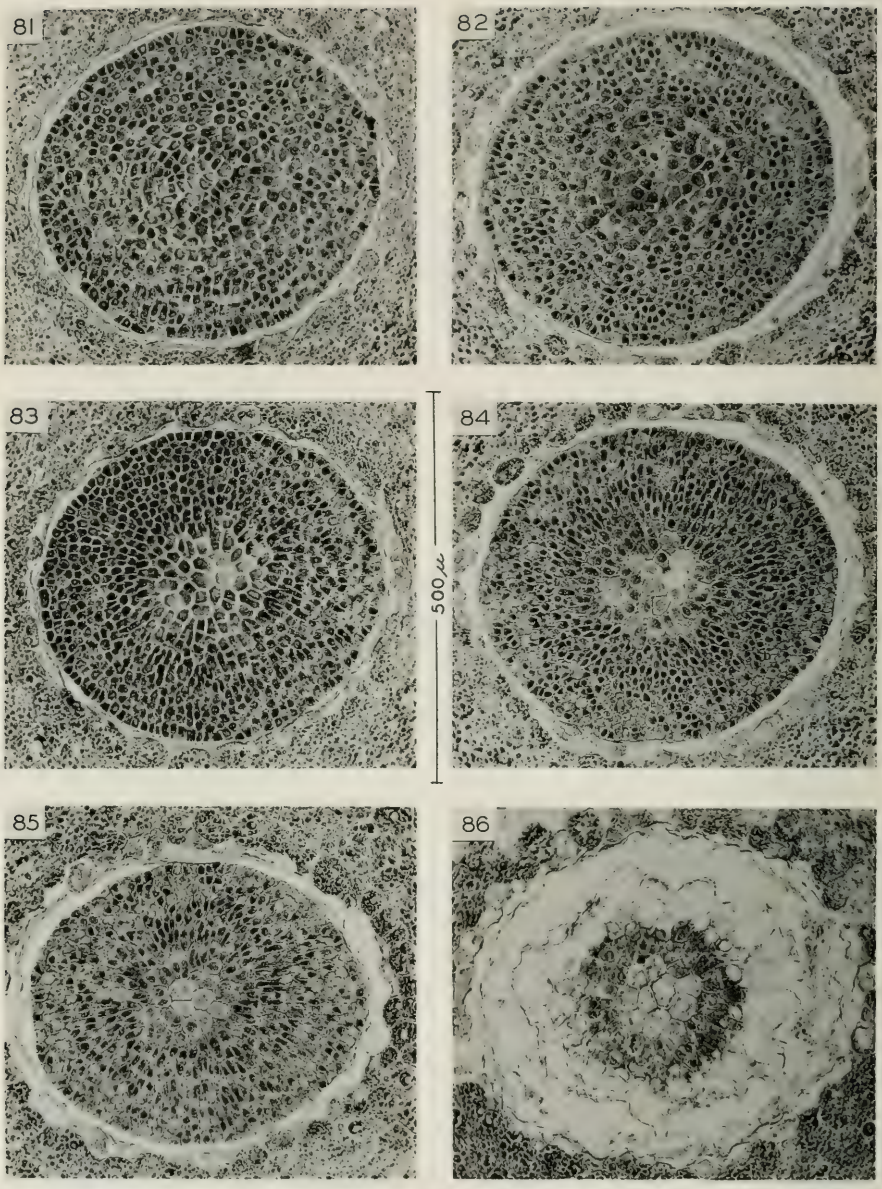

PLATE VI

PLATE VI

(Figs. 81-86 are from the collection of June 25, 1933.)
(For location of sections, see diagram on page 85.)

Fig. 81.—Transverse section of telo-stage embryo just above the base of the plerome and about 195 microns below the level of Fig. 69. Compare Figs. 69, 78, 80, 82, and 83, all representing sections in the lower axial part of the embryo.

Fig. 82.—Transverse section of telo-stage embryo at the base of the plerome and about 45 microns below the level of Fig. 78. The juncture zone is at about this level.

Fig. 83.—Transverse section of telo-stage embryo at the uppermost part of the column below the initial cells at the base of the plerome. This section is about 45 microns below that shown in Fig. 81.

Fig. 84.—Transverse section of telo-stage embryo in the upper part of the column, about 75 microns below the level of Fig. 82.

Fig. 85.—Transverse section through the middle of the telo-stage calyptroperiblem, about 225 microns below the level of Fig. 84. The suspensor is wholly vacuolate about 320 microns below this level.

Fig. 86.—Transverse section across the extreme base of telo-stage calyptroperiblem with marginal (suspensor) cells enlarged and vacuolate. This section is about 435 microns below that shown in Fig. 80.

THE EMBRYOLOGY OF LARIX

BY

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