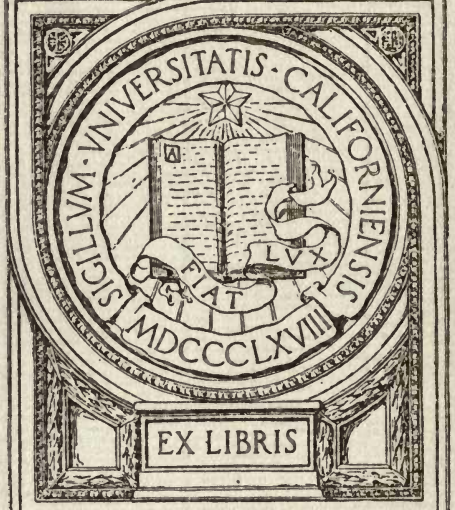

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# CONTRIBUTIONS TO THE KNOWLEDGE OF THE LIFE HISTORY OF PINUS WITH SPECIAL REFERENCE TO SPOROGENESIS, THE DEVELOPMENT OF THE GAMETOPHYTES AND FERTILIZATION.

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## INTRODUCTION.

THERE is no chapter in the annals of botanical science more fascinating than that which deals with the history of sexuality in plants. No definite date marks the discovery of the fact that plants, like animals, are male and female; the idea was rather a growth, as is plainly shown by the writings of Aristotle, Theophrastus, Pliny and others of the early philosophers. The fact may, however, be said to have been established by Camerarius (1694) in his "De sexu Plantarum," but for many years after his time botanists found in this question merely a favorite subject for philosophical speculation. Their ideas remained vague and uncertain, no effort being made to confirm their theories either by observation or experimentation.

It was not until near the middle of the last century that actual investigations were begun along this line. Amici (1830–1846) made certain interesting observations regarding the development of the pollen-tube and the origin of the embryo in several plants; but the splendid series of embryological papers published by Hofmeister (1848–1867) first placed the science upon a sure foundation and marked a new era in the study of sexual

reproduction in plants. Although the researches of Hofmeister, Strasburger, Warming, Belajeff and others who have contributed to our knowledge of this subject, especially during the last decade, have disclosed many facts concerning the structure and development of the pollen-grain, of the ovule and of the embryo, our knowledge of certain phases of spermatogenesis and oögenesis is still very meager, and not a sufficiently large number of plants have been thoroughly investigated to admit of generalizations. The celebrated discoveries of Hirase, Ikeno and Webber, in 1897, gave a new incentive to this study, particularly in connection with the Gymnosperms, and rendered it highly desirable that fertilization and associated phenomena should be worked out for other members of this group by the more modern methods of investigation.

The present studies were begun in the fall of 1897 with the hope of adding somewhat to our knowledge of this subject. Incidentally, it seemed desirable to determine whether any vestiges of the bodies called blepharoplasts by Webber (1897[3]) still persist in the conifers. As a result of the past embryological studies, a vast number of facts pertaining to the life-history of the gametophytes in the higher platns has accumulated. While many of the conclusions reached are the outcome of serious direct investigations, others are based on the insufficient evidence found in a rather superficial study of a large number of plants. What we need to-day is not more facts regarding unrelated plants, so much as a careful working out of the details of development in representative genera.

This research is based primarily upon a study of *Pinus Strobus*, but nearly every observation recorded has been confirmed for *Pinus rigida* and *P. austriaca*, and to a large extent, for *P. montana* var. *uncinata* and *P. resinosa*. The descriptions given may be understood to refer alike to the five species named above unless otherwise stated in the text. Nearly six hundred paraffin blocks with imbedded material have been made, and more than four thousand slides of serial sections have been stained and studied. Six hundred separate collections of material would seem unnecessarily large if one were studying a plant like *Nicotiana* in which, according to Guig-

nard (1902), fertilization follows in 2 hours after pollination, but in *Pinus*, where almost 13 months intervene between these two processes, such a number is not excessive. While it is true in cytological studies, as elsewhere, that numbers, or mere mass work, do not signify excellence, it is equally true that the results of investigations based upon a study of a limited amount of material are, at best, unsatisfactory, and, other things being equal, those conclusions will be most valuable which have been formulated after a careful observation of many specimens.[1]

## HISTORICAL NOTES.

In the following brief summary of the literature dealing with the *Abietineæ*, only the more important papers have been noted, and the observations recorded by the various writers have been given without comment.

The tetrad-division in the pollen-mother-cell of *Pinus* and *Abies* was studied in 1848 by Hofmeister. He stated that the pollen-mother-cells were already developed in the anthers at the end of November, two special daughter-cells were formed at the close of the first division in the spring, and the four cells resulting from the second division were found to lie either in one plane or at the corners of a tetrad. Three years later (1851) Hofmeister published the results of his remarkable series of investigations in the higher cryptogams and conifers. He described and figured the pollen-grain in the *Abietineæ* as consisting of a cell-complex, noted the depression in the apex of the nucellus in *Pinus* at the time of pollination, and the single embryo-sac-mother-cell deep in the interior of the nucellus. It appeared that the pollen-grain rested some weeks upon the nucellus before the pollen-tube was emitted. After the germination of the pollen-grain, the tube grew for several weeks and penetrated nearly to the point of union between integument and nucellus, but it might cease growth before so great a depth was reached.

[1] This paper was given especial honorable mention on April 26, 1903, by the Association for Maintaining the American Women's Table at the Zoölogical Station at Naples and for Promoting Scientific Research by Women. I wish here to express my deep gratitude to Mrs. Ellen H. Richards, Miss Florence Cushing and other members of the above named association through whose generous efforts the publication of this paper in its present form has been made possible.

He concluded that the embryo-sac remained for a long time as a single cell, its nucleus finally dissolving to be replaced by a number of free nuclei; in a few days the sac was filled with long cells reaching to the middle; at the beginning of winter, the walls of this transitory endosperm were greatly thickened; in the spring, the thickened walls of the endosperm were absorbed and the cells liberated. Each primordial cell thus made free contained, somewhat later, three or four daughter-cells which were, in their turn, liberated by the dissolution of the mother-wall. Thus the number of cells within the embryo-sac was greatly increased, the embryo-sac itself growing to more than twenty times its previous volume. The cells of the nucellus also multiplied rapidly except in the region previously penetrated by the pollen-tubes. In the middle of May, a layer of cells lined the embryo-sac, cell layers increased until they met in the center, then the corpuscula were differentiated. The corpuscula were always separated in the *Abietineæ* by one or more layers of cells, and the walls enclosing the corpuscula were thought to be channelled, thus affording open communication with the surrounding cells. In *Pinus* from 3 to 5 corpuscula were developed in each ovule, and a corresponding number of funnel-shaped openings occurred in the upper part of the endosperm. When the pollen-tube reached the corpusculum it contained free spherical cells in its lower end. The tube either flattened itself out upon the corpusculum or penetrated a short distance into it. After fertilization the impregnated germinal vesicle increased in size, its nucleus disappeared, and soon a large daughter-cell was seen at the base of the corpusculum. By repeated divisions of this cell the proembryo was formed.

In 1858 Hofmeister found the usual number of neck-cells in *Pinus Strobus* to be four, exceptionally three, five, or six, all lying in the same plane. He further demonstrated the vacuolate character of the contents of the corpusculum during its development. These vacuoles disappeared before impregnation, being replaced by free cells — the germinal vesicles, or Keimbläschen. A pit was figured in the apex of the pollen-tube after its entrance into the corpusculum, but it was said that

the tube remained closed until after the formation of the pro-embryo, when it was ruptured by mechanical means. The great abundance of starch in the pollen-tube of the *Abietineæ* was also mentioned at this time. While the "Higher Cryptogamia" appearing in 1862 was largely a translation of Hofmeister's earlier publications, it likewise presented many new observations. The fact was noted that in *Pinus* the integument surrounds the nucellus, leaving open above its apex a wide micropylar canal. In all the *Coniferæ*, after the embryo-sac was entirely filled with cellular tissue, certain cells near the micropylar end ceased dividing but increased markedly in size; the other cells of the endosperm continued to multiply in number, but remained comparatively small; thus the corpuscula were differentiated. After the cutting off of the neck-cells in the *Abietineæ*, additional cells were developed at the top of the endosperm, giving rise to the depressions referred to in 1851. Scarcely a day intervened between the approach of the pollen-tube and the formation of a four-celled pro-embryo at the base of the corpusculum, and this occurred contemporaneously in all ovules of all trees growing under similar circumstances.

The works of Strasburger on this subject have been more numerous and complete than those of any other investigator. It is extremely interesting to note how his interpretations have kept pace with the improvements in methods of research. In 1869 he traced the development of the endosperm from the free cells lining the embryo-sac to its maturity, and established the fact that shortly before fertilization the central cell divides to form the canal-cell and the egg-cell. He confirmed Hofmeister's observations regarding the channels in the upper part of the endosperm, and the presence of a closed pit at the apex of the pollen-tube; but he did not observe the nuclei in the pollen-tube, and remarked that, inasmuch as the sexual organs touch in these plants, spermatozoids would be superfluous and were, in reality, not present. He added, however, that their place was taken by granular protoplasm and starch grains which exercised the same fertilizing effect on the egg as do spermatozoids. After fertilization four nuclei were detected at the base of the corpusculum and a division into a cross took place, these cells

divided and were separated by cross-walls, the lower four divided again making three layers of four cells each, the middle layer then elongated pushing the lowest cells down into the endosperm. In *Picea* a fourth layer of cells was observed at the base of the central cell.

In 1872 Strasburger stated that the canal-cell loosened itself from the egg and hung as a cap just beneath the neck-cells, at the same time the egg-nucleus increased in size and moved to the center of the corpusculum. He detected two cells in the pollen-tube of several Gymnosperms, but considered that such cells were extremely rare in the *Abietineæ*, as he had only once found one in this group. The shrunken remains of these cells were seen in the pollen-tube after fertilization. He believed that the pit of the pollen-tube remained closed, and that the exchange-substance was apparently communicated by a vacuole between the apex of the pollen-tube and the egg-nucleus. After fertilization the central nucleus was dissolved, and, in "abnormal" cases, four new nuclei appeared in the central part of the egg, but there was strong evidence that these did not develop into an embryo. Six years later (1878), he observed one or more divisions in the pollen-grain shortly before pollination. The small cells resulting from these divisions were interpreted as rudimentary prothallium. Two large primordial cells were demonstrated in the pollen-tube of *Pinus* and *Picea* when the tube was just above the archegonium. According to Strasburger's interpretation at that time, the nucleus in front was dissolved while the one behind entered the egg and fused with its nucleus. This was a great advance on his previous observations, but he still conceived of the pollen-tube as remaining closed, and fancied that the protoplasmic contents passed through the membrane directly while the starch was dissolved before its transmission into the egg. He was now convinced that only a part of the contents of the pollen-tube was taken up by the egg-nucleus, the remaining portion uniting directly with the egg-plasma; but he was not certain whether the protoplasm active in fertilization came in as a formless mass or in the shape of a nucleus.

Strasburger established the fact, in 1879, that it is the fore-

most of the two sperm-nuclei in the pollen-tube which is instrumental in effecting fertilization. He reported the presence of an axial row of three cells in *Larix*, the lowest cell being the embryo-sac-mother-cell. The generalization was made that the prothallium arises in all the gymnosperms through free cell-division, all the free nuclei dividing at the same time. It was claimed that but a single endosperm was formed in the *Abietineæ*, that the primary nucleus of the embryo-sac remained undivided during the first year, and that the "transitory endosperm" of Hofmeister was in reality the freed cells of the nucellus which were destined to be absorbed. It was to these cells that the term spongy tissue was applied. In the following year (1880) Strasburger described and figured the mature archegonium in *Picea* and discussed the early stages of endosperm formation in *Pinus*, but he gave little that was new at that time. It was in this same year that Sokolowa (1880) published the results of her researches in the development of the prothallium in the gymnosperms. Cell-walls were laid down between the nuclei imbedded in the peripheral layer of protoplasm, but no cell thus formed was furnished with a wall on its inner free side. These open cells were termed "alveoli." They grew in length until the middle of the embryo-sac was reached, then walls arose at the inner ends and the alveoli were closed; cell divisions followed, and gradually the elongated alveoli gave place to many cells.

Goroschankin (1880 and '83) reported that the protoplasm of the egg and of the sheath-cells was in immediate contact through pores in the separating membrane; he saw (1883[2]) the two sperm-nuclei pass into the egg in *Pinus Pumilio*, and believed that both fused with its nucleus; the great similarity which the spheres in the egg bear to nuclei was commented upon and he questioned the propriety of calling them vacuoles. Strasburger (1884) confirmed Goroschankin's observations as to the passage of the two sperm-nuclei from the pollen-tube into the egg, but pointed out that only the one in advance fused with the egg-nucleus. As the protoplasmic contents of the central cell increased, the vacuoles decreased, and every transition could be traced between the large vacuoles and the meshes of the proto-

plasm filled with metaplasm. In the pines, a large vacuole often held several smaller ones. The egg-nucleus slowly filled itself with metaplasm during its descent to the center of the cell. Three successive divisions occurred in the large cell of the pollen-grain in *Larix*, the first two prothallial cells formed were small and soon disorganized, the third one increased greatly in size and divided to form the stalk- and the body-cell.

It was left for Belajeff (1891) to establish the true nature of the cell-complex found in the pollen-grain of the Gymnosperms. He demonstrated the fact that in *Taxus baccata* the large nucleus of the pollen-grain is the vegetative or pollen-tube-nucleus, as in the Angiosperms, and that the sperm-nuclei arise by the division of one of the smaller cells of the pollen-grain, this smaller cell first dividing to form the stalk- and the generative cell.

Strasburger (1892) showed that Belajeff's observations on the structure of the pollen-grain and the development of the pollen-tube in *Taxus baccata* were, in general, true for the other Gymnosperms. He described the mature pollen-grain in *Pinus* as containing a large tube-cell, a small cell—the third prothallial or antheridial cell — and the remnants of the first two prothallial cells. Pollination was immediately followed by the germination of the pollen-grain, and the nucleus of the large cell wandered at once into the tube. The last formed prothallial cell remained in its place in the pollen-grain until the following spring, when it divided into the stalk- and the body-cell of the antheridium. The division of this cell was not studied, but Strasburger thought it took place at about the same time as the development of the archegonia. The pollen-grain of *Picea* was found to correspond exactly with that of *Pinus* excepting that the antheridial cell divided while still within the anther. The sperm-cells in *Pinus* were seen in the apex of the pollen-tube; the lower cell was the larger; and each cell was almost entirely filled with its large, coarsely granular nucleus. At the tip of the pollen-tube, the stalk- and the tube-nucleus could no longer be distinguished one from the other. The sperm-nucleus was shown to be smaller than the egg-nucleus, but the two were alike in the amount of active nuclear substance; and attention

was called to the smallness of the first nuclear figure following fecundation in comparison with the size of the conjugating nuclei. The germ-nucleus divided in its original position in the egg, and the two nuclei passed towards the "organic" apex of the archegonium.

Belajeff (1893) worked out the development of the pollen tube in *Picea* as a type of the *Abietineæ*. He found that the generative cell divided while still within the pollen-grain and gave rise to two sperm-cells which he figured as of the same size.

Dixon (1894) traced the history of the pollen-grain and the pollen-tube in *Pinus sylvestris* from the middle of April to the time of fertilization. He thought that the prothallial cell divided towards the end of April to form a small stalk-cell and a larger body-cell. The body-cell immediately divided into two cells of almost equal size — the male sexual cells. The sperm-cells moved into the pollen-tube followed by the nucleus of the stalk-cell. Pollen-tubes were found to branch freely while in the upper "brown" tissue of the nucellus but only one branch of each tube was continued through the lower part of the nucellus. He noted that the four nuclei, much of the protoplasm, and considerable of the starch of the pollen-tube passed into the oösphere. As a rule, eight chromosomes were found in the nuclei of the female gametophyte.

In giving an account of some work done by his students on the Gymnosperms, Coulter (1897) reported that the work of Dixon "was largely confirmed in the minutest detail"; and in 1900 he figured the pollen-tube "in pines," when just above the archegonium, showing two sperm-cells of equal size. Atkinson (1898) stated that the sperm-mother-cell in *Pinus* divided into two sperm-cells after having passed into the pollen-tube.

Blackman's excellent treatise on fertilization and related phenomena in *Pinus sylvestris* was published in 1898. Many details of development were most carefully worked out, but the facts recorded are not enumerated here, since they will be duly considered in connection with the observations, as recorded in the body of this paper, that have been made by the writer on other species of pines. Since the appearance of Blackman's monograph, a considerable literature dealing with various stages

of development in the gametophytes of the *Abietineæ* has been published. The details of these investigations are familiar to all students of the subject. These papers will, therefore, be mentioned at this point by title only; they will be referred to again in the discussions which follow. Chamberlain (1899), Oögenesis in *Pinus Laricio;* Wuicizki (1899), Ueber die Befruchtung bei den Coniferen; Arnoldi (1900), Beiträge zur Morphologie der Gymnospermen, IV; Juel (1900), Beiträge zur Kenntniss der Tetradentheilung; Murrill (1900), The Development of the Archegonium and Fertilization in the Hemlock Spruce (*Tsuga canadensis* Carr.); Coulter and Chamberlain (1901), Morphology of the Spermatophytes; Ishikawa (1901), Reduction Division in Larix; and the papers published by the writer in 1901.[1]

## METHODS.

*Collecting.* — On November 15, 1897, and each week thereafter until December 25, cones of *Pinus Strobus*, *P. rigida*, *P. austriaca*, *P. montana* var. *uncinata*, and the staminate strobili of *P. austriaca* were collected. Material was brought in occassionally during the remainder of the winter. Pistillate cones of the species named, and also of *P. resinosa*, were collected once each week beginning with April 1; collections were made twice each week throughout the month of May, and three times a week during June. From June 10–30, a period which was sure to cover fertilization, cones of *Pinus Strobus* were collected every day at about nine o'clock in the morning, and frequently again at four o'clock in the afternoon. Male cones were gathered, from those species in which they had appeared, at irregular intervals during the early spring. From the first of May until the time of pollination, which varies by a number of days in the different species, staminate strobili were collected each day. During May and June the young female cones were gathered as well as the more mature ones of the previous year's growth. After July 1, the older cones were no longer collected, but the young cones of *Pinus Strobus*, *P. rigida*, and *P. austriaca* were collected once each week until November 15. Cones

[1] See "Note" at close of Appendix.

of *Pinus Strobus* were again collected regularly, as described above, throughout the spring and early summer of 1899. Collections of the staminate cones of *Pinus Strobus* and *P. rigida* were made during May and June 1901, and from May 15 to June 15 of the same year the young pistillate cones of *Pinus rigida* were gathered daily.

Material was obtained from different trees and different localities. The practice of collecting all one's material from a single tree, as reported by Murrill (1900), Land (1902) and others, does not seem a safe one to follow, for certain peculiarities of development which are not characteristic of the species may appear in an individual. At the time of each collection, ovules were put up from several cones of each species, these cones being taken not from the tip of one branch but from different branches. The central portion only of the cone was used, the ovules at either extremity being more or less abortive. After collecting, the material was taken at once to the laboratory and preserved. The staminate cones and, in the early stages of development, the pistillate ones were fixed entire or cut into quarters longitudinally. Very soon the individual scales of the female cones were removed from the receptacle before fixing, and, when the scales were of sufficient size to admit of such manipulation, all superfluous parts were cut away, leaving the two tiny ovules still united by a small portion of the scale. With the renewal of growth in the spring, the ovules were removed from the scales and, as soon as it was feasible, a portion of the integument was cut away from two or more sides of each ovule, thus bringing the fixing fluid into direct contact with the young gametophyte. For later stages, the endosperm was frequently removed from the integument, but such material did not prove to be as satisfactory as that in which the nucellar cap and a small portion of the coat were left in connection with the prothallium. Throughout the entire mechanical process of preparing material for the fixer, the most extreme care was used, as it was found that a very slight pressure was sufficient to cause distortions and thus to render the material worthless for cytological studies.

*Fixing.* — The methods used in fixing and staining do not

differ materially from those generally employed in cytological work. The fixing fluids tested were chrome-osomo-acetic acid solution, chrome-acetic acid solution, corrosive sublimate in aqueous solution, absolute alcohol, and Carnoy's fluid. The first two were tried with variations in concentration and in length of time. The chrome-osomo-acetic acid solution giving by far the best results, the other fixers were entirely discarded. It was made up according to the following formula:

| | |
|---|---|
| Chromic acid crystals | 1.3 gms. |
| Osmic acid (in glass bulb) | .5 gms. |
| Glacial acetic acid | 8.3 c.c. |
| Distilled water | 160.0 c.c. |

This solution used in one half strength and allowed to act for about 15 hours proved to be most excellent for fixing the prothallium at the time when it consists of a wall layer of protoplasm containing numerous free nuclei. For the development of the pollen-grain and fertilization stages, it was most satisfactory when undiluted, and allowed to act for about 24 hours. If the fluid blackened at all, it was poured off after 2 or more hours and fresh added.

After fixing, the material was washed in running water from 2 to 12 hours, but as a rule specimens were not kept in the running water longer than 6 hours. The very convenient piece of apparatus described by Durand ('99) was used for this process. Subsequent to washing, material was dehydrated in 8 grades of alcohol beginning with 15% and ending with the absolute. It was not allowed to stand in the lower grades for more than 6 hours, and was rarely kept in the absolute alcohol longer than that time; the latter was changed 3 times, once about every 2 hours, to insure perfect dehydration in as short a time as possible. After material had been in 85% alcohol for 12 hours, it was decolorized in a 35% solution of hydrogen peroxide, made up in 95% alcohol, for 24 hours. While material was always bleached in toto, it was frequently found necessary to decolorize again on the slide. After dehydration, material was brought gradually, through ascending grades, into pure cedar oil, xylol or chloroform. The best results were obtained with the cedar oil and it was far more commonly used than the others. If it

was desirable to store material for a few days or weeks, pure cedar oil was found to be a much better medium than 75 % alcohol, which is commonly used for temporary storing of material. For the purpose of getting specimens into pure paraffin they were transferred to tiny wire-gauze baskets and carried successively into 25, 50 and 75% paraffin in cedar oil, and finally into pure paraffin with a melting point of 54°, in which they were at last imbedded. This is a very convenient and economical method for getting material through the paraffin oven. The grades of cedar oil in paraffin can be kept in the bath a long time and used repeatedly with impunity, and material can be carried in the little baskets from bottle to bottle much more quickly and with less liability to injury than in any other way with which I am familiar. At the time of fixing, a small piece of paper, bearing the number, in pencil, corresponding to the number of the entry in the record book, was placed in each bottle, remained with the material through all the changes which followed, and was finally imbedded in one corner of the paraffin block containing the specimens.

*Staining.* — A Minot-Zimmermann revolving microtome was used in cutting the material. The sections varied in thickness from 4 to 13.6 microns, but by far the greater number were made 6.3 microns thick. They were fastened to the slide by means of albumen-fixative, and the slides were labelled with glass-ink. In preparing this ink, a paste was made of the best English vermilion in sodium silicate, and sufficient water was added to give the proper consistency for writing. Glass-pencils, Higgins' waterproof ink, both with and without collodion, and other methods for marking slides were tried; but I have never found anything at all comparable, for excellence, with the glass-ink. When properly prepared it is not dissolved during the process of staining, but can be removed from slides or dishes, when desirable to do so, by heating in a strong solution of potash or in gold dust.

As is usual in cytological studies, considerable experimentation was necessary before satisfactory stains were obtained. Among the stains tested were Rosen's ('92) fuchsin and methylene-blue method; the Ehrlich-Biondi-Heidenhain mixture, as

prepared by Dr. G. Grübler; Guignard's combination of methyl green, acid fuchsin, and orange G; Flemming's safranin-gentian-violet-orange combination; and Heidenhain's iron-hæmatoxylin. The last two proved the most satisfactory and were almost exclusively used. The iron-hæmatoxylin was followed by orange G, or, if it was desirable to stain cell-walls, by Bismarck brown. Iron-hæmatoxylin followed by Flemming's triple stain, or by gentian-violet and orange G, brought out the so-called kinoplasmic structures with great definiteness. The best differentiation was obtained with the iron-hæmatoxylin by allowing the hæmatoxylin to act from 12 to 18 hours, decolorizing in iron-alum, and then washing in running tap-water from 2 to 6 hours. Flemming's triple stain was often used without the safranin with excellent results. Both anilin and aqueous solutions of gentian-violet were used. As a rule, a one-half percent. solution was employed, the slides remaining in it from 5 to 20 minutes. The achromatic figures in the divisions of the pollen-mother-cell, especially in *Pinus Strobus*, were, however, brought out with great difficulty with this stain. The best results were obtained for these stages by allowing the slides to stand from 24 to 48 hours in stender dishes of distilled water to which not more than 10 drops of a one percent. solution of gentian-violet had been added. *Pinus* sections take the orange with such avidity, that a fraction of a minute was in all cases a sufficiently long time to allow this stain to act. After washing out the superfluous gentian-violet and dehydrating in absolute alcohol, differentiation was effected by dashing with clove oil. Bergamot oil was used for fixing and clearing, and I have found it expedient to pass the slides from bergamot oil to jars of xylol. They can remain in the xylol for hours, if desirable, without injury, and the xylol is so readily miscible with the balsam that the preparations become clear and more satisfactory for studying in a much shorter time than when carried directly to the balsam from the bergamot oil.

# CHAPTER I.

## MICROSPOROGENESIS.

### THE MICROSPORANGIUM.

*The Wall of the Pollen-sac.*—With the exception of *Pinus Strobus*, the staminate cones, in the pines which I have studied, make their appearance in October or November. I have searched repeatedly in the autumn for the male inflorescences of *Pinus Strobus* but have never been able to find them until late April or early May of the following spring. If they are present at all before spring they can be scarcely more than potentially so, for they are not sufficiently devoloped to be detected in the field, nor by careful dissection in the laboratory.

The structure of the microsporangium agrees perfectly with that usually described for the *Abietineæ*. The wall of the young pollen-sac consists of three or four layers of cells. The cells of the outer layer are nearly isodiametric, while those of the inner layers are smaller and more or less tabular in outline. Just within, and in immediate contact with the archesporium, is the ring of tapetal cells. In the early stages of development the wall-cells are rich in cytoplasm and contain nuclei which are large in proportion to the size of the cells. The microsporangium increases much in size in the spring, and by the time that the microspore-mother-cells are in the prophase of division, considerable change has occurred in the wall-cells of the pollen-sac. The outer layer has lost its nuclei and the cells have become filled with a homogeneously staining resinous substance; in *Pinus Strobus* this resinous deposit extends to the second layer of wall-cells as well; the cells of the inner layers have been considerably flattened out, and their cytoplasmic content has become much reduced. When the pollen-grains are mature, all the wall-cells of the microsporangium, except the outermost layer, have disappeared. They have doubtless been absorbed, their substance contributing to the nutrition of the pollen-grains.

The tapetum cannot be distinguished during the earlier stages of development from the other tissues. It is first clearly differ-

entiated in the spring, when the mother-cells are in the early prophase of the heterotypic division. The mitoses leading to development of this layer have not been studied, but there are indications that it is formed from the outer layer of the sporogenous tissue rather than, as usually described, from the inner layer of wall-cells. The microsporangium-wall, after the appearance of the tapetum, is composed, as before, of three or four layers of cells; furthermore, the tapetum is always intimately associated with the sporogenous tissue, while it is fre quently found separated from the wall of the pollen-sac, probably as a result of imperfect fixation. The question as to the origin of this tissue in *Pinus* must, however, await further investigation. During the later stages of division in the pollen-mother-cells, the tapetal cells increase much in size, their cytoplasm becomes very dense and each cell comes to have from one to three nuclei which have been observed in all stages of fusion. Karyokinetic figures have been frequently noted in the tapetal cells indicating that the nuclei of these cells divide mitotically, and the division conforms to the ordinary or typical method of mitosis. When the young microspores become free, these cells have attained to their greatest size, and show a diffuse reaction to stains. From this time they gradually diminish in size and finally disappear altogether. The nutritive function of this tissue is too well understood to require discussion here.

*The Primitive Archesporium.* — With the exception of *Pinus Strobus*, the primitive archesporium is clearly differentiated in the autumn, but the mother-cells of the microspore do not arise until the latter part of April, and in *Pinus Strobus* not until about three weeks later.

In the younger stages of development, a superficial study shows no sharp demarcation between archesporium and wall, but a careful examination reveals certain differences by which the two can always be distinguished. The cells of the archesporium are larger, have larger nuclei, and denser cytoplasm than those of the wall. They are also polyhedral in outline while the wall-cells are somewhat tabular from the first, though not so markedly so as at a later period. During the winter, the nucleus of a primitive archesporal cell contains several nucleo-

lus-like bodies, of which as many as eleven have been counted in a single section of a nucleus, and a less number than seven is rarely found. The delicate but extensive nuclear reticulum is slightly chromatic and stains scarcely more strongly than the cytoplasm of the cell. Both cytoplasm and nuclear network stain diffusely with gentian-violet during this period of rest (fig. 1).

In those species in which the microsporangia make their appearance in the autumn, the pollen-sacs remain small and the archesporial cells comparatively few in number until the following spring. Hofmeister ('48) found the mother-cells of the pollen-grains in the anthers of *Pinus* and *Abies* at the end of November, Belajeff ('94) observed the pollen-mother-cells of *Larix* in the spireme stage in October, and Coulter and Chamberlain ('01) have recently figured the "microsporangium of *Pinus Laricio* in the mother-cell stage in October." The sporogenous tissue, as they have illustrated it, bears a very strong resemblance to that shown in fig. 1 of this paper. There is undoubted evidence that these are not pollen-mother-cells in the species of pines which I have studied. In the first place, the number of cells in a single anther in November is far less than the number of microspore-mother-cells which is eventually formed. As the microsporangium enlarges in the spring these cells not only increase in size but multiply in number. During the last of March and first of April karyokinetic figures, representing various stages of division, are seen in all preparations, and in all cases division is proceeding by the typical method characteristic of vegetative or somatic cells. In the latter part of April or first of May (for *Pinus Strobus* about the middle of May), typical division ceases, and, after a period of growth, the prophases characteristic of the heterotypical division are entered upon. The time at which the rest preparatory to the heterotypic mitosis begins varies by about three weeks in the different species, and by ten or more days in the same species in different seasons. Had Coulter and Chamberlain examined microsporangia during the latter part of March they would doubtless have found typic divisions taking place in the archesporial tissue.

TETRAD-DIVISION.

*The Definitive Archesporium.*— During the period of "rest" preceding the heterotypic division, the microspore-mother-cell increases much in size, its nucleus becoming even larger than an entire cell of the primitive archesporium, as is readily seen by comparing figs. 1 and 2 with figs. 3 and 4. The walls enclosing the spore-mother-cells thicken considerably, and the cytoplasm assumes a fine, almost granular structure which, under high magnification, resolves itself into a delicate, close reticulum. At this stage, only three or four nucleoli are found within the nucleus, but this reduction in number may be only apparent, for the nucleus has enlarged to such an extent that no one section would be liable to contain as many of these structures as would a section of one of the smaller nuclei of the primitive archesporium. No attempt has been made to determine the exact number of nucleoli in the nuclei of the archesporium at any time in its history, as it is next to impossible to trace accurately the sections in the series of any given cell when each anther contains hundreds of archesporial cells all of which are practically alike in form, structure and staining capacity.

As the nucleus of a pollen-mother-cell enlarges, its reticulum becomes more open, the threads of the net gradually increase in thickness, the net-knots or karyosomes become more or less prominent, and numerous smaller granules are distributed irregularly upon the linin. Many cross-threads are withdrawn but no true spireme is formed at this time (fig. 3). The thickening of the threads is more prominent in *Pinus Strobus* than in the other species, the net-knots are more conspicuous, and a somewhat imperfect spireme arises, although here, too, many anastomosing threads still persist (fig. 4). A remarkable change has taken place in the attitude of the different elements of the cell towards stains. When the microspore-mother-cells are first formed both cytoplasm and nuclear net stain more or less diffusely with gentian-violet as in the primitive archesporium, but, as growth proceeds, the cytoplasm ceases to react to chromatin dyes and takes the orange G with avidity. The nucleoli are colored far less deeply with the gentian-violet than

formerly, and the nuclear reticulum takes the blue characteristic of chromatin. In this condition, the contracted state known as synapsis is entered upon.

*The First Nuclear Division of the Microspore-mother-cell.* — As soon as a microspore-mother-cell has attained full size, certain changes in its nucleus indicate that the prophase of the first division has been initiated. The reticulum gradually draws together, its threads becoming thicker and the meshes smaller (figs. 5 and 6). Contraction continues until the network forms a compact mass at one side of the nucleus. During synapsis the nucleoli may be entirely confined within the contracted sphere or one or more may be partially extruded (fig. 7). Some of the nucleoli still stain deeply with the gentian-violet, but one or more usually take the plasma stain at this time and appear as yellow, porous, or spongy bodies. The same appearance has also been obtained with iron-hæmatoxylin followed by orange G.

In *Pinus rigida* no appearance at all comparable with that known as synapsis is observed until April 21. In material preserved on this date a few nuclei in all anthers show the beginnings of contraction as illustrated for *P. austriaca* in fig. 5 and *P. Strobus* in fig. 6. On April 30 the nucleus of every mother-cell has reached the point of greatest condensation, its contents forming a somewhat spherical, deeply-staining mass at one side of the nuclear cavity — fig. 7 illustrates this stage for *P. Strobus*. On May 2 some of the nuclei still retain this structure while others show various stages of recovery. Two days later, May 4, not a vestige of this condition remains, all the nuclei having passed on to more advanced stages in the mitosis. These dates have been given for *Pinus rigida*, but they would not differ materially in the other species, except that in *P. Strobus* corresponding phases in this division would occur about 3 weeks later.

Synapsis is not universally recognized as a normal step in the heterotypical division. Guignard ('97), Mottier ('97), Schaffner ('01), and others still look upon it as an artifact caused by imperfect fixation. On the other hand, Sargant ('97), Wiegand ('99), Duggar ('99 and '00), Ernst ('01), Rosenberg ('01) among

botanists, and many zoölogists consider it a definite characteristic of the early prophase of the heterotypic mitosis, several of these investigators having noted it in their material before fixation. I have observed this stage in the fresh material in *Pinus*, and after carefully studying it in many permanent preparations, I see no reason why this condition, simply because it happens to be one of contraction of the nuclear substance, should be set down as abnormal.

If this appearance were produced artificially why should there be transitional forms both in leading up to and in recovery from it? If it were the result of diffusion currents, as has been suggested, we should expect to find the nuclear substance in all the nuclei of a given anther carried or forced to the same side of the nuclear cavity, but such is not the case. It is doubtless true, as indicated by Strasburger ('95), that many phenomena described as synapsis represent pathological conditions which do not occur under all circumstances, but it seems equally true that this condition of the nuclear substance represents, in some species at least, a characteristic stage in the heterotypic division. Although a contraction comparable with that of synapsis has been reported for somatic cells, I am not aware that anything like so marked an appearance has been described as a usual accompaniment of any but the heterotypical division. The exact significance of this phase is not well understood, but that it is intimately associated with a readjustment of the chromatic and nucleolar substances there can be little doubt.

As the nucleus slowly recovers from synapsis, it soon becomes apparent that the reticular structure has been replaced by a broad, closely coiled band which stains more deeply than did the network prior to the contracted stage. The coils of the thread gradually open out until the nuclear cavity is filled with a spireme, which consists of a broad linin band, so irregularly studded with chromatin-granules that it has a much roughened, almost minutely echinulate, appearance. These granules soon collect into indefinitely outlined masses which remain connected by clear, faintly staining portions of the linin thread. The chromatin-groups never assume the definite disk-like form figured by Mottier ('97) for *Lilium* and *Helleborus*, and by Duggar ('00)

for *Symplocarpus*, but they remain always irregular and jagged in outline (figs. 8 and 9). Whether there is one continuous thread or more than one could not be determined with certainty, as the coil is at first very densely massed, and free ends might be obscured. When the loose skein fills the nuclear cavity more than one spireme can usually be detected, but the indications are that this effect has been produced by the microtome knife. At certain places the coils of the spireme run together and appear to be more or less anastomosed. Such a point of contact always indicates the position of a nucleolus which has become almost obscured by the massing of the thread about it, figs. 9, 13 and 15. Not all the nucleoli are found thus associated with the skein, but in those cases in which they are free from the coils of the nuclear thread their capacity for staining has generally been greatly reduced (figs. 9, 11 and 15).

As soon as the chromatin-band has become loosely wound about the entire nuclear cavity, longitudinal splitting occurs, and the segmentation of the spireme becomes apparent (fig. 10), but transverse fission is not completed until the longitudinal division has taken place (fig. 11). The segments are long, coiled, and present various appearances. Whether they correspond in number to the number of chromosomes eventually formed, I could not ascertain with any degree of certainty, since they are so long and closely intermingled in the nucleus (fig. 11). Most of those shown in figs. 12 and 12, *a*, were taken from sections through the edge of nuclei, and, while they represent the looped and twisted condition of the chromatic segments at this time, they have in many instances been cut during sectioning so that only a portion of most of the segments appears. From a study of many nuclei containing chromatic threads similar to these, it is evident that the looped figure has not been formed by the bending on itself of one of the longitudinal halves of a segment. There are no indications that the sister-halves of any portion of the nuclear band ever become entirely disassociated. They may separate widely at one or both extremities, but at some point along the thread, an intimate relation is permanently maintained. The loop arises, therefore, by the complete fusion of the sister-threads at one of

their free ends (fig. 12, *a*, *c*, *d*, *e*). Even in such a late stage of fission as that represented in fig. 13 the sister threads can almost invariably be traced, but not always, as some are out of focus and others are doubtless in another section.

The stages immediately following longitudinal splitting and segmentation of the nuclear spireme are somewhat different from any that I have seen described by other writers. So puzzling were they to me when the study of microsporogenesis was first undertaken in 1899 that a paper, partially prepared at that time, was laid aside until a larger experience with cell structures could be brought to bear upon this, which is to me at once one of the most intricate and interesting problems connected with the activities of the cell. As stated in the introduction, new material was collected in 1901 and fixed with great care. Many slides were subsequently prepared, and the phases in the tetrad-division were found to accord perfectly with those observed during the first period of study. The interpretation of the phenomena noted is, however, much more satisfactory now than formerly, although there is still much that is obscure. Sporogenesis has not been studied in *Pinus montana* var. *uncinata*, but there is complete accord, except in such details as have already been mentioned, in the other four species.

Longitudinal division is scarcely more than completed when the double skein begins to contract, the two halves of each segment twisting upon each other to a greater or less degree and gradually fusing. As the segments contract the sister-halves may frequently become more or less twisted upon each other; they may appear as parallel threads; the half segments may separate at both ends, remaining united at the middle only; or, having fused at both extremities, they may open out, forming rings (figs. 12 and 12, *a*). Fusion invariably begins first about those nucleoli which have still retained, although in a less degree than prior to synapsis, the power to react to chromatin-stains (fig. 13). Contraction and fusion continue until a coarse, more or less anastomosing structure is formed in which only traces of the earlier longitudinal division remain evident (fig. 14, plate II), and a little later all signs of fission, both longitudinal and transverse, disappear (fig. 15).

As the thread thickens and broadens it becomes irregular in outline, the irregularities increase, those from neighboring portions of the threads meeting and fusing. Soon afterwards a transverse division again becomes apparent (fig. 16). The segments continuing to shorten and thicken gradually draw away from one another, finally remaining united only by delicate threads; the connecting fibers are at last severed and the chromosomes lie free in the nuclear cavity. The usual number of segments formed is twelve, although thirteen, fourteen, and, in rare instances, as many as sixteen have been counted (figs. 16, 17, 18, *a–c*, and 20).

The chromosomes thus arise from an incompletely reticulated structure rather than directly from the spireme. While this suggests the condition in magnolia where, as recently described by Andrews ('01), the chromosomes arise directly from the resting reticulum without the intervention of a spireme, it is, in matter of fact, very different. We have here not a nuclear reticulum in the ordinary acceptation of that term, but a somewhat reticulated structure formed by the anastomosing with each other, at certain points of contact, of adjacent portions of a previously longitudinally split spireme. As the chromosomes separate out almost every conceivable form may be found, not only the X's, Y's and V's of Belajeff, but rings, parallel rods, eights open and closed, L's, U's and irregular-shaped bodies (fig. 19, *a–l*).

In my earlier study of this phenomenon, I supposed the chromosomes to be the equivalents of the long, coiled segments first formed, and with such an hypothesis the whole series of events following longitudinal fission was inexplicable. But after again considering not only such stages as those represented in figs. 10–17, but every transitional form connecting them, I am convinced that this assumption was incorrect and that each segment consists, rather, of two distinct chromosomes standing side by side, each half of the double chromosome representing two sister-segments which were formed by the earlier longitudinal fission but have now fused. If such be the origin of these chromosomes, and I no longer have any hesitancy in affirming that they have thus arisen, the phases following the

longitudinal and transverse divisions of the skein are no longer unintelligible. The sister-threads formed by the longitudinal splitting not only unite again, but adjacent portions of the double threads draw together and become more or less fused, giving rise when transverse fission again becomes apparent to the one half number of chromosomes. The forms of the resultant chromosomes are exactly what would be expected from such an origin. In fig. 18, *b*, for instance, adjacent portions of double segments have fused at the ends, transverse division has followed, and three chromosomes — parallel rods, a U, and a Y, are seen in the act of separation. When the component chromosomes have fused at both ends only, the ring, or, if a twist follows, the closed eight results; if fusion has occurred at but one extremity the V, U, or open eight is formed; if the segments remain attached at the middle point the X occurs; when the constituents of the double chromosomes have united end to end and the bend has not taken place at the point of their union the L results and so on. The structure or composition of the X, Y and V forms of chromosomes as found in plants have been explained in much the same way as the above by Belajeff ('97 and '98), but he did not trace their development from the closed spireme and considered these three forms as the typical or characteristic ones whereas, in *Pinus*, the other forms named have been quite as frequently observed. When the chromosomes first become apparent, irregular fragments of the chromatic substance are frequently left at various points (fig. 17), but these are ultimately absorbed, doubtless being appropriated by the growing chromosomes (fig. 20).[1]

At the time when the chromosomes are being differentiated, they often appear as if pulling away from the nucleoli, and may be seen still connected with them by delicate threads (figs. 18, *a* and *c*). The nucleoli now have a spongy or porous appearance and fail almost absolutely to take either nucleolar or chromatic stains. With the final separation of the chromosomes they disappear altogether. The history of these nucleoli from the primitive archesporium up to the time of their dissolution leads irresistably to the conclusion that here, at least, there is a very

[1] See "Note" at close of Appendix.

intimate relation between nucleolar and chromatic substances. Whether the nucleoli are actual reservoirs of chromatin which is given out passively to the chromatic thread, or whether they are actively engaged in furnishing nourishment to the chromatic substance, I have not been able to determine, but, from certain observations to be described in a later chapter, I am inclined to consider them more than passive elements of the cell.

Coördinate with the formation of the chromosomes the nuclear membrane resolves itself into a weft of threads which crowd into the nuclear cavity, together with delicate granular fibers from the cytoplasm. The latter are evidently formed by a rearrangement of the granules of the cytoplasmic reticulum. Up to this time the cytoplasm has remained close meshed in the region of the nucleus but has become less dense at the periphery of the cell. As the nuclear membrane disappears, coarser reticulations arise in the cytoplasm and extend towards the nucleus, doubtless contributing to the forming spindle. When the achromatic figure is fully developed, the cytoplasm again becomes uniform in structure throughout the cell, but there seems to have been an actual loss in granular substance, the meshes of the network being much larger now than formerly (figs. 20 and 21). A few delicate fibers may be seen in the cytoplasm just before the dissolution of the nuclear membrane, but, although I have searched repeatedly for cytoplasmic phenomena such as that described by Mottier ('97 and '98), Duggar ('00), Juel ('00) and others, I have never been able to detect anything at all comparable with the structures figured by these authors. If they are present in *Pinus*, I have not been able to differentiate them with any of the stains used.

The spindle is almost invariably tripolar in origin, but it may arise as a multipolar diarch. In either case, its ultimate form is that of a sharply pointed bipolar spindle (Figs. 21–24). Belajeff ('94) describes this spindle as many poled in origin in *Larix*, and Mottier ('97) makes the same statement for *Pinus;* but in the many thousands of karyokinetic figures observed for this division, I have never found one that showed more than three poles. A few scattering fibers have occasionally been seen to pass from all sides towards the nucleus but achromatic threads have not been found to converge at more than three points.

As the spindle-fibers press into the nuclear cavity, the chromosomes take up their position at the equatorial plate. They are now very regular in outline, apparently homogeneous, and the X, Y, V, O, etc., forms can still be clearly distinguished (fig. 24, plate III). Each segment is oriented with its longer axis perpendicular to the axis of the spindle, the free limbs extending outward. The spindle-fibers are attached at one extremity of the parallel rods, and ordinarily at or near the point of union of the constituents of the dual chromosomes. In the Y-shaped chromosomes the achromatic threads may become attached at the point where the two limbs become free or at the free end of the fused chromosomes, but, whatever the shape of a segment, the spindle-fibers are never attached at the extremities of its free limbs.

The line of cleavage at the equatorial plate is not such as to separate the two chromosomes but is rather such as to effect a longitudinal splitting, the two half chromosomes of each pair passing together to opposite poles. During metakinesis the daughter-chromosomes become very irregular in outline and increase much in size, the half chromosomes apparently exceeding in volume the undivided ones (figs. 25–28). This augmentation of the segments may be due to actual addition of new substance, but from the fact that in the telophase they are unquestionably smaller than in the late prophase, it is probable that this is merely an amplification without actual or permanent growth. The parts of the spireme separated during the longitudinal fission following synapsis have so completely fused again that they are now disunited with difficulty. The appearance of the dividing chromosomes indicates that they are being subjected to great strain. Under this tension they are flattened out and rendered irregular in outline; the irregularities result from the unequal stretching of the chromatic substance at different points, just as a poor rubber band when greatly extended becomes more or less moniliform. The complete separation of the half chromosomes may sometimes be greatly delayed, when the stretched segments extend nearly the entire length of the spindle, the achromatic figure being almost obscured, in some instances, by the chromosomes (figs. 25, 26, 28 and 29). That

these segments are actually flattened out is further shown by the fact that the arms which remain united and elongated stain much less deeply than do those which, having become free, have contracted to nearly their former length. This would seem to indicate that the chromatic spireme is a plastic or viscid body. Lloyd ('02) describes a similar action, though much less marked, in *Crucianella*. While the position of the retreating half chromosomes is such as to give ordinarily the appearance of V's or U's, other figures occur with sufficient frequency to establish the reality of their persistence after the close of the metaphase of the division. This point will be considered more fully later.

The achromatic figure increases but little in length as the chromosomes pass to the poles so that the movement here must be due in large measure to a pull exerted by the contracting fibers and not to any great extent to a push brought about by the growth of the central spindle. If the force which seems necessary to effect the separation of the half chromosomes is furnished by the achromatic fibers, we should expect to find the poles of the spindle firmly buttressed as described by Strasburger ('00) for *Larix;* but no strengthening fibers are developed, and, although the apices of the spindle are usually inserted in the ectoplasm, they not infrequently end blindly in the cytoplasm. It is possible that the force exercised by the growing fibers of the central spindle just equalizes the counter force exerted by the mantle fibers in drawing the chromosomes to the poles, the equilibrium thus established giving rigidity and rendering a support for the poles unnecessary. By the time the pairs of daughter-chromosomes have reached the poles they have become much reduced in size and regular in contour (figs. 27 and 30).

After the chromosomes reach the point where the daughter-nuclei are to arise, they do not at once fuse end to end to form a continuous spireme, but as the chromosomes lie side by side they lose their clear outline and gradually assume a diffuse reaction to stains. In this condition the halves of the longitudinally split pairs of chromosomes are doubtless fused, after which fusion the adjacent segments unite by their ends to

form a coiled, somewhat moniliform thread (figs. 30–32). Immediately upon the formation of the skein a delicate nuclear membrane appears, the coils loosen somewhat and branch freely thus giving rise to a reticulum. Extensive growth follows and a large "resting" nucleus is formed (figs. 33 and 34). The nuclear net consists at first of delicate achromatic linin threads bearing scattered chromatin-granules and uniting large irregularly branched chromatic portions. Distribution of the chromatin continues until there is a delicate linin reticulum with chromatin granules of varying sizes imbedded in it (figs. 33–35). These nuclei have the form of a plano-convex lens the flat side of each nucleus being perpendicular to the axis of the spindle and facing the other daughter-nucleus. It is obvious from the foregoing that a definite resting nucleus is formed in *Pinus* at the close of the heterotypic division. This accords with the recent observations on the formation of the microspore by Duggar ('99) in *Bignonia*, Strasburger ('01) and Gager ('02) in *Asclepias* and Andrews ('01) in *Magnolia*. A true nucleolus has not been observed in the daughter-nuclei. Contrary to the observations of Hofmeister ('51), no cell-wall is laid down and in only a very few instances has a slight thickening of the spindle fibers in the region of the cell-plate been observed.

*The Second Mitosis of the Mother-cell.* — The resting daughter-nuclei are scarcely more than established before the initial steps of the second division are instituted, as evidenced in the readjustment of the nuclear reticulum. The more delicate threads of the net are withdrawn, the nuclear membrane fades out, the chromatin loses its granular aspect and becomes evenly distributed upon the linin, and there issues forth a heavy, homogeneous, deeply-staining band which is more or less coiled and branched (fig. 36). The chromatin-thread, which now lies free in the cytoplasm of the mother-cell, continues to thicken, the branches or cross fibers disappear, and in an almost incredibly short time, the delicate nuclear net has given place to a broad, somewhat spirally coiled skein (fig. 37).

Achromatic threads arise in the cytoplasm forming a multipolar diarch spindle. The fibers are not abundant and always

arise in a plane perpendicular to the axis of the primary spindle. Harper ('00) makes the statement that in *Larix*, where no cell-wall follows the first division of the pollen-mother-nucleus, the spindle-fibers of the primary mitosis are utilized in the formation of the spindle for the second division. I am unable to trace any such connection in the pollen-mother-cells of *Pinus*, all traces of the first karyokinetic figure having been lost to view before the inception of the spindle for the second division.

As the kinoplasmic fibers appear the chromatin-band forms a double row of loops extending across the spindle-threads in the plane of the equatorial plate. The longitudinal splitting is now clearly apparent. The loops continue to shorten, and in this position transverse fission occurs, segmentation almost always taking place at the outer free ends of the loops (figs. 38 and 39, plate IV). The sister-halves of each V- or U-shaped chromosome entirely separate, undergo readjustment, and finally come to stand in a double row with their free ends in the line of the nuclear plate and their angles towards their respective poles (figs. 38–41). The spindle-fibers become attached to the chromosomes at their point of bending, and the half chromosomes pass to the poles (figs. 42–43). The dissociation of the sister-halves of each segment is so complete before the beginning of the separation at the equatorial plate that the figure during metakinesis is such as to give the impression of whole chromosomes passing to the poles, but a study of the prophases of the division shows clearly that each represents the half of a double chromosome. In the telophase of the division the chromosomes unite end to end to form a spireme (fig. 44). The nuclear membrane appears, and the chromatic band branches, giving rise to the reticulum of the resting nucleus (figs. 44 and 45).

*The Problem of Reduction.* — Here as in all studies of spore-formation at the present time the question of reduction demands consideration. As already indicated, the reduction in the number of chromosomes takes place, as is the rule, during the so-called resting stage of the spore-mother-cell, the one half number of chromosomes appearing in the prophase of the heterotypical division. But the inquiry concerning the presence or absence of a qualitative reduction is not so easily answered.

With few exceptions, botanists of to-day follow the present lead of Strasburger and accept the view of a double longitudinal splitting of the chromosomes in the first division of the spore-mother-cell. According to this interpretation, reduction, in the sense in which Weismann uses the term, does not occur in plants. Among the exponents of a qualitative or true reduction in plants, Atkinson ('99), Belajeff ('97, '98), Calkins ('97), Ishikawa ('97, '01), and Schaffner ('97, '01) are almost alone to-day in not having retracted their earlier published conclusions regarding this subject.

It has seemed best to record the details of the observations made in studying the tetrad-division in *Pinus*, before entering upon any discussion of the significance of the phenomena noted, but in so doing some reiteration is inevitable.

Strasburger's statement that certain forms of chromosomes occurring in the anaphase of the heterotypic division are inexplicable on any other assumption than that of a double longitudinal splitting is, doubtless, correct when those forms have been derived from V-shaped chromosomes. But, while it may be true that such figures are due to a double longitudinal fission when derived from other than V-shaped chromosomes, it is likewise true that, in such cases, the phenomena are capable of rational explanation on other grounds. The V with the three arms, for instance, may result from the attachment of the spindle fibers at the middle point of a Y, the stem of the Y bending down as it moves to the poles (fig. 30, *a*, plate III), and a double V might be derived in the same way from an X-shaped chromosome (fig. 30, *c*). In fig. 26 the second chromosome on the left represents a Y opening out from its lower extremity, and the next chromosome shows parallel rods just separating. Occasionally an X or Y figure becomes apparent in the late anaphase of this division (figs. 28, 29). Such appearances are doubtless to be attributed to an early straightening out of the segments. If the constituents of the double chromosomes are disunited in this mitosis, then such chromosomes as those illustrated in figs. 28, *d*, and 30, *a*, *c*, and *e*, might result from the more or less complete longitudinal fission of the sister-segments. Should this prove to be the case, and if my interpretation of the origin of

these chromosomes is correct, then both a quantitative and a qualitative reduction of the chromosomes would occur in the first or heterotypic division, and whole chromosomes, each representing the half of a dual chromosome, would pass to opposite poles. I am aware that such a phenomenon has been described by Atkinson and a few others, but after long and careful study there does not seem to me the least doubt, that, in the case of the pines investigated, a longitudinal fission, and not a transverse one, occurs in this first mitosis; and X-, Y-, and ring-shaped segments, as well as V's, pass to the poles, although, as Belajeff has pointed out, they usually, because of their position, have the form of V's in the anaphase of this divison.

Most writers on sporogenesis, and especially those who are advocates of the true reduction, have not found a resting nucleus intervening between the heterotypic and the homotypic divisions. As already stated a resting nucleus is clearly demonstrated at this point in *Pinus*. The spireme formed from this nucleus shows signs of longitudinal division before segmentation, and, while lying at the equatorial plate, the two halves of each segment separate entirely, in most instances at least, before their final orientation on the spindle. Now the question arises as to whether or no this homotypic division effects a qualitative reduction. If the theory of the so-called "individuality of the chromosomes" is without foundation then it certainly does not; but, if the possibility of the complete rehabilitation of the chromosomes be accepted, a qualitative reduction very probably does occur. For under such conditions, the skein preceding the homotypic division would consist of the daughter-chromosomes, formed as a result of the heterotypic mitosis, fused end to end. These daughter-chromosomes, it will be remembered, arose by the longitudinal fission of a double chromosome and each, therefore, consists of a pair of half chromosomes. Thus the second, apparently longitudinal, splitting would effect the separation of the half chromosomes of each pair, rather than the longitudinal fission of a single chromosome. Reduction would thus take place in the true or Weismann's sense. Because of certain phenomena to be described in connection with the development of the pro-embryo, I am inclined to believe that the chromo-

somes retain their individuality through succeeding cell-generations. I am, therefore, disposed to regard the tetrad-division in *Pinus* as a true reducing division; in this way only does the complicated process just described find satisfactory explanation. No positive statement can, however, be made either way, in connection with this division in *Pinus*, until we are in possession of greater knowledge than at present of the origin and ultimate destiny of chromosomes.

Guignard ('97) expresses the opinion that the regularity of the chromosomes in certain forms has been overestimated. Be that as it may, I am conscious that there is recorded in this paper a greater variation in the forms of the chromosomes than has been described in a single genus by other writers. It has been my purpose to note not only that which is in accordance, or at variance, with the observations of other investigators, but to give as faithful a record as possible of the conditions found in the preparations studied. And may we not yet find that here, in the divisions preceding spore-formation in plants, as in many other instances, there is greater variation in matters of detail than was formerly supposed to be the case?

## DEVELOPMENT OF THE MICROSPORE.

*The Formation of the Spore-wall.*— Hofmeister ('51) described four "special" cells, each with its own wall, within the pollen-mother-cell in the *Abietineæ*, and Juranyi ('72 and '82) devoted particular attention to the formation of the wall of the microspores in many Gymnosperms and Angiosperms. He described the development of a wall separating the two nuclei after the first division. This wall was soon absorbed and during the second division the entire cell was filled with connecting fibers stretching between the four nuclei. Delicate walls were then laid down between the nuclei giving rise to the four microspores. These dividing walls thickened and united with the inner wall of the spore-mother-cell; thus a portion of each spore-wall was formed from the inner mother-wall. After a period of rest the outer mother-wall was burst and the "pollen-cells" became free. If there is any recent literature of value on this subject, I have failed to find references to it.

As already indicated, no wall separating the daughter-nuclei is formed at the close of the heterotypical division in *Pinus*. During the late telophase of the second mitosis in the microspore mother-cell, a readjustment of the spindle-fibers occurs giving rise to the complex figure that has been described as characteristic of spore-formation in many plants. The development of the archoplasmic structures connecting the nuclei of the tetrad is much less marked than in *Podophyllum* (Mottier '97) and in many other phanerogams (fig. 44). By the time the nuclei have reached the resting stage, a division has occurred in the cytoplasm giving rise to four cells which are surrounded by delicate clear walls. A prominent thickening of the wall of the spore-mother-cell takes place, and at the same time a thick wall, continuous with the inner portion of the mother-wall, appears between the daughter-cells.

This wall frequently attains remarkable thickness. Whether it constitutes an inner wall, or is merely a thickening of the primary wall by the deposition of new material on its inner surface, I am unable to say. The outer, primary wall stains more deeply and is frequently seen separated from the inner broad portion (figs. 44-47). This inner wall, which is continuous with the broad walls separating the young microspores, stains deep yellow with orange G, if the orange is allowed to act from one to two minutes; it appears a pale rose when treated with safranin, but fails altogether to stain with iron-hæmatoxylin. In a few instances, slight evidences of stratification have been observed, but ordinarily the wall appears perfectly homogeneous, giving the impression of a liquid or viscid substance in which the spores are imbedded; but the fact that it is often separated from the outer wall by a clear space, and also that it is left behind as a definitely outlined wall after the escape of the spores militates against the probability of its fluid nature. After the spores have grown for a certain period the mother-wall is ruptured and the spores are liberated. At this time the empty mother-cell with its four chambers is often met with (figs. 48, 49).

In so far as I am aware, this permanent division of the mother-cell into four compartments by thick cellulose walls has

not been previously described. A broad open space, repeatedly figured between the daughter-spores and the mother-wall, has been invariably attributed to shrinkage; but it is probable that, in some cases at least, it represents this thickened wall which has failed to be differentiated with the stains used. Wiegand ('99) says that the spores of *Potamogeton* are as if imbedded in a ground mass of some viscid substance, but he does not figure it and makes no statement regarding the development of cell-walls between the microspores.

*Origin of the Air-sacs.* — As soon as the young microspores have become enclosed, each within its own special chamber of the mother-cell, it is evident that a special wall has been developed about each spore. This is doubtless secreted by its own cytoplasm and is not, as Juranyi thought, derived from the inner wall of the microspore-mother-cell. The spore-wall while still very delicate becomes differentiated into an inner and an outer layer corresponding to the intine and extine of the pollen-grain. The young microspores are characterized by the relatively large size of their nuclei, the nucleus filling almost the entire cell just prior to the discharge of the spores. The cytoplasm which fills the remainder of the cell is in the form of a loose reticulum (figs. 46, 47).

As time goes on the outer wall of the microspore expands at two points on opposite sides of the spore. A resistance is met with in the thick wall of the spore-mother-cell and the plastic inner wall of the microspore responding to this new pressure becomes indented along the surfaces corresponding to the extended portions of the outer spore-wall. Thus a clear open space having in section the form of a biconvex lens is formed between the extine and the intine on either side of the microspore. These are the beginnings of the wings or air-sacs that are so conspicuous in the mature pollen-grain of the *Abietineæ*. Finally the pressure becomes so great that the mother-wall is ruptured and the spores are liberated (figs. 47, 48). Coulter and Chamberlain ('01) noted the fact that the wings make their appearance in *Pinus Laricio* while the microspores are still within the mother-cell, but they recorded no observations regarding the origin and development of these sacs. Strasburger and

Hillhouse ('00) consider that these bladder-like appendages consist of the outer part only of the extine, the extine having undergone cleavage at these two points. In studying the development of these organs from their earliest beginnings, it appears to me that the line of cleavage lies rather between the two coats of the young spore. If it is not, then at the time that the microspore leaves the parent-cell, the intine has not been developed, or, if present, is so delicate that I have not been able to detect it (fig. 48).

*Growth of the Microspore.* — After its escape from the mother-cell the microspore undergoes rapid growth, and the outer surface of the spore becomes beautifully marked by the formation of delicate, irregular ridges over the entire inner surface of the extine, except along that portion which connects the two wings on the concave or ventral side of the pollen-grain. It is at this point that the pollen-tube later makes its exit, and there is here no appreciable thickening of the spore-wall. These ridges continue to grow and extend inward forming a very pretty reticulated structure which is most distinctly apparent on the walls of the wings; along the convex or dorsal side of the pollen-grain the reticulations are closer and the extine forms a broad, deeply staining layer (figs. 50–54, plate V). This irregular thickening of the extine is an admirable adaptation for securing strength with slight increase in weight.

When the young microspore attains to its mature-size, a partial wall, extending along the back and for a longer or shorter distance down the sides of the spore, becomes apparent within the intine (fig. 54). It consists of a broad, homogeneous-appearing band which gives precisely the same staining reactions as the thick wall developed within the spore-mother-cell after the formation of the young microspores. These immature pollen-grains, after treatment with Flemming's triple combination or with the gentian-violet and orange G alone, afford the most brilliant effect that I have observed with these stains. The extine presents a very intense, clear blue, the inner homogeneous wall an equally vivid yellow, while the protoplasmic elements take the colors characteristic for these dyes. The fact that this third partial wall fails entirely to respond to some stains doubtless

accounts for its having been overlooked by previous writers. It is not shown at all in the series of figures, recently published by Coulter and Chamberlain ('01), illustrating the development of the pollen-grain in *Pinus Laricio*.

The various tests commonly used in determining the nature of the cell-wall have been applied to the young pollen-grains as well as to the special spore-mother-walls. These tests show that the outer wall of the pollen-grain is clearly of the nature of cutin, as has been demonstrated by Strasburger. Both the innermost wall of the microspore, and of the pollen-grain, as also the wall of the special spore-mother-cells, respond to the reaction for cellulose, but not in a very marked manner. If they are of the nature of cellulose there would seem to be an admixture of some other substance, but I have not succeeded in obtaining entirely satisfactory results regarding the nature of these inner, prominent walls. Tests thus far have been made with "fixed" material only; further experimentation along this line will be made when fresh material is at hand.

During the season of growth, the nucleus of the microspore always remains close against the convex or dorsal side of the spore, occupying a central position along this wall. As is usual in cell-development, the microspore-cell attains full size before any mitoses occur within it, and there is never any further increase in the size of this cell after the inception of the first division. The fully developed microspore is, therefore, the exact counterpart, so far as size is concerned, of the mature pollen-grain. Compare fig. 54, plate V, with fig. 65, plate VI. During the development of the microspore, the cytoplasm which at first was uniformly distributed in a rather loose net work, becomes more closely reticulated and at the same time less abundant in proportion to the size of the cell. At the maturity of the spore the cytoplasm is largely distributed about the nucleus from which strands extend outward in a radial manner and end in the ectoplasm. In 1898 the microspores of *Pinus Strobus* were ready to leave the mother-cells on May 30, they had attained full size on June 7, and on June 10 the pollen-grains were fully mature.

SUMMARY.

In *Pinus rigida*, *P. austriaca* and *P. resinosa* the primitive archesporium is well developed before the approach of winter, but the microspore-mother-cells do not arise until the end of the following April. The male inflorescence does not appear in *Pinus Strobus*, until the end of the April preceding pollination, and the definitive archesporium is differentiated in this species about the middle of May. The nuclei of the primitive archesporium are characterized by several deeply staining nucleoli and a fine, close-meshed reticulum which responds but slightly to chromatic dyes.

The wall of the pollen-sac consists in all cases of from three to four layers of cells. The tapetum is not clearly distinguished until spring and there are indications that it may be derived from the outer layer of sporogenous tissue. The nuclei of this tissue multiply mitotically and the cells reach their maximum size about the time when the microspores become free. At this period each cell has from one to three nuclei which present all stages of fusion. When the pollen-grains are mature the tapetum has entirely disappeared and the wall of the microsporangium consists of a single layer of cells, or at most of not more than two.

Synapsis is recognized as a normal stage in the prophase of the heterotypical division in the pollen-mother-cell of *Pinus*. It is not preceded by a definite spireme, but a broad skein containing irregular masses of chromatin separated by clear portions of the linin thread issues from the contracted nuclear mass.

The chromatic spireme splits longitudinally and breaks up by transverse fission into several segments. The loosely coiled, delicate threads resulting from the longitudinal division soon draw together and fuse, double threads also come into contact at various points and fuse more or less perfectly. These threads always anastomose most freely in the region of the nucleoli, some of which still stain deeply while others stain but faintly after synapsis.

Fission occurs at various points in the now irregularly contracted and anastomosed threads, and the separate chromosomes,

in the reduced number, become apparent. These segments are at first irregular and jagged in outline showing distinctly the points at which each has separated from neighboring segments, but they gradually diminish in size and become more regular in contour. The chromosomes thus formed are in the form of X's, Y's, V's, U's, L's, parallel rods, rings, and indefinitely-shaped bodies. Each segment consists of two chromosomes fused side by side.

The spindle-fibers arise both from the nuclear membrane and from the cyto-reticulum. The achromatic figure may originate as a multipolar polyarch of three poles or as a broad multipolar diarch spindle. At the close of the prophase of the heterotypic division the spindle has become sharply bi-polar and its extremities may be imbedded in the ectoplasm or they may end blindly in the cytoplasm.

The chromosomes are separated at the equatorial plate with difficulty giving the appearance of a plastic substance under tension. Their separation may be so delayed that the daughter-chromosomes stretch from pole to pole. They ordinarily have the form of V's or U's during the anaphase of the mitosis, but other forms are not infrequent. The first division effects a longitudinal splitting of the chromosomes into daughter-segments of the same form as the parents.

A resting nucleus is established at the close of the first mitosis but the daughter-nuclei are not separated by a cell-wall. The daughter-reticulum soon gives rise to a more or less spirally coiled chromatic band which loops itself at the equatorial plate and splits longitudinally before segmentation.

The chromosomes have the form of U's and are oriented at the equatorial plate in two rows with their free ends touching and the bent portion of each segment directed towards the poles, the complete fission of the segments having been completed before their migration to the poles begins. The writer inclines to the view that these are the half chromosomes of the daughter-pairs which were separated in the first division. If this hypothesis be correct, the homotypic mitosis in *Pinus* effects a true or qualitative reduction of the chromosomes.

The wall of the microspore-mother-cell increases markedly in thickness and its protoplasmic contents is separated into four

parts by prominent cross walls which are continuous with the inner portion of the mother-wall. The microspores are then developed each in its own particular chamber of the mother-cell.

A double wall is quickly developed about each spore and the air-sacs become apparent while the spores are still within the mother-wall. They arise by the separation of the extine from the intine at two definite points on opposite sides of the spore. By the growth of the spore, and more especially by the expansion of the air-sacs, the spore-mother-wall is ruptured and the spores set free.

Growth ensues, the extine becomes irregularly thickened on its inner surface except at the concave side of the spore, and a broad partial wall is laid down just within the intine and along the back and sides of the microspore. During the growth of this cell its nucleus maintains a position at the central point of its dorsal side. Before the germination of the microspore it attains to the full size of the mature pollen-grain.

## CHAPTER II.

### The Male Gametophyte.

#### THE DEVELOPMENT OF THE POLLEN-GRAIN.

*Formation of the Prothallial Cells.* — So much confusion has arisen in the application of terms used to designate the various cells of the male gametophyte in Gymnosperms that it is desirable, if not almost necessary, that one should define at the outset the nomenclature adopted. Throughout this paper, the first two cells cut off from the larger cell are known respectively as the first and second prothallial cells, and the third small cell formed represents the antheridial or third prothallial cell. The large cell, so long as it continues to divide, is designated as the apical cell, but after division ceases in this cell it is referred to as the tube-cell and its nucleus constitutes the tube-nucleus. The antheridial cell divides to form the stalk-cell and the generative cell, the latter giving rise to the binucleated sperm-cell.

As soon as the microspore has reached maturity, there arises within its nucleus one of the most beautiful, homogeneous, loosely-looped and coiled spireme-bands that I have ever seen in any dividing nucleus (fig. 54). The material studied showed every stage in the first division, and all succeeding mitoses which occur within the microspore, but they offer nothing especially instructive from a cytological point of view, since they conform to the typic method of division. I shall, therefore, describe and figure only such phases as are of interest in tracing the development of the pollen-grain. It is interesting to note that in the late prophase of all the mitoses which occur in the development of the male gametophyte the achromatic figure presents a very characteristic appearance, being sharply monopolar at its outer or lower extremity and broadly multipolar at the opposite end. It thus forms a fan-shaped body rather than one resembling a spindle. During the telophase it usually becomes bluntly bipolar, though the upper pole often remains to the last somewhat broader than the lower pole (figs. 55, 56 and 60, and plate V. A similar method of karyokinesis has been noted by Wiegand ('99) in the development of the pollen-grain in *Potamogeton*, by Duggar ('00) in *Symplocarpus*, and by Coker ('02) in *Podocarpus*. This mode of division will be referred to again in connection with certain phases in the development of the female gametophyte.

In all the divisions which occur within the wall of the microspore the nuclear substance is divided equally, the cytoplasm unequally. The nucleus of the first prothallial cell, however, never equals in size that of the apical cell and always stains more or less diffusely, thus showing signs of disintegration from the time of its organization (fig. 57). Fig. 58 shows one of the very largest and most nearly normal of all the prothallial cells observed. The nucleus of the apical cell enters the complete resting stage, instituting a definite network within the meshes of which one or more faintly staining nucleoli become apparent, but this reticulum at once resolves itself into a homogeneous, spireme exactly similar to the one first formed. When the nucleus of the apical cell has reached the spireme-stage of the second division, the first prothallial cell is invariably found

pushed against the dorsal side of the spore-wall, not a vestige of its cytoplasm is left, and the nucleus has become greatly flattened, although there is still a faint suggestion of its former reticular character (fig. 59). When the telophase of the division is reached this nucleus has lost all traces of its former structure and persists only as a deeply staining, linear body lying against the spore-wall (fig. 60). During the following division it becomes scarcely more than a line so that it is frequently detected with difficulty. Coulter and Chamberlain ('01) figure this cell in *Pinus Laricio* as still projecting into the cytoplasm of the apical cell when that cell is in the telophase of the second division, but I have never found it in such a state of preservation at so late a date. The second prothallial cell is invariably smaller than the first, and during the third mitosis of the apical cell, which follows immediately the formation of the second prothallial cell, it exactly repeats the history of the first cell (figs. 61–63).

The partial, broad, innermost wall, described in connection with the development of the microspore, persists throughout the entire history of the pollen-grain, and a comparatively broad wall, continuous with it and having exactly the same staining capacity, invests both the first and second prothallial cells as shown in figs. 57–63. The presence of the remnants of the prothallial cells imbedded apparently in the inner wall of the mature pollen-grain (fig. 63) was very perplexing before the history of these cells was studied. But in tracing their development it is clearly demonstrated that the remnant of each cell is pushed back against the wall of the spore and remains permanently covered on its outer side by its own wall. That the remains of these cells come to lie nearer the intine than when first formed would again suggest the somewhat plastic nature of the partial or incomplete membrane against which the prothallial cells are pressed (figs. 57–64). These observations confirm the statement of Strasburger, Noll, Schenck and Schimper ('97) that the two prothallial cells formed in the pollen-grain of the Gymnosperms are invested with cellulose-walls. Coulter and Chamberlain ('01) make no mention of the formation of walls in connection with the development of these cells in *Pinus*

*Laricio*, and Coker ('02) says that in *Podocarpus* " as in other cases " no cellulose-wall is formed. The small cell cut off by the third and last division of the apical cell persists as a permanent feature of the mature pollen-grain. Its cytoplasm is distinctly differentiated from that of the tube-cell, but no cellulose-wall has been observed in connection with this cell, its boundary being marked by scarcely more than a condensation of its peripheral cytoplasm.

*The Mature Pollen-grain.* — During the development of the male gametophyte the cytoplasm of the large cell gradually increases in amount, the vacuoles becoming smaller from the region of the nucleus outward, and finally disappearing altogether. The pollen-grain has the same size, form, and, so far as the wall is concerned, the same structure as the microspore just prior to its germination. The thick, innermost, partial wall described in connection with the microspore still persists as a very prominent characteristic of the mature pollen-grain. With the expansion of the wings, certain protoplasmic portions of the microspore-cell are left with no support except the delicate endospore; it therefore seems probable that this broad, incomplete wall extending along the back and down the sides of the pollen-grain has been developed for the purpose of strengthening these weakened points in the spore-wall, and as an additional support to the dorsal side of the pollen-grain.

But, while the wall of the mature pollen-grain is identical with that of the microspore, the essential or protoplasmic part of the spore has undergone marked changes, as we have already seen. One or two deeply staining lines, more often one than two in the mature pollen-grain, lie on the dorsal side of the pollen-grain apparently imbedded in its innermost wall. Extending from this wall at its middle point is a strongly convex cell, the antheridial cell, with delicately reticulated cytoplasm and a comparatively large nucleus. Just below and always in contact with this cell is the nucleus of the tube-cell. The cytoplasm of the tube-cell is closely reticulated and slightly more dense than that of the antheridial cell. Imbedded in its cytoplasm are numerous starch-grains. In this condition the pollen-grain of *Pinus* awaits pollination (figs. 64, 65, plate VI).

Starch-grains have been found in the large cell from an early date in the development of the pollen-grain, but they are more abundant after maturity is reached than at any previous time. According to Coker ('02) the pollen-grains of *Podocarpus* contain large starch-grains from the beginning of the first division. With such variations in details as have been noted above, this description of the development of the pollen-grain in *Pinus* agrees with that given by Strasburger in 1892 and Coulter and Chamberlain in 1901.

## POLLINATION.

*The Ovule at the Time of Pollination.* — In the vicinity of Cornell University, 42½° north latitude, the pollen-grains of *Pinus Strobus* are ready for dispersion late in May or early in June, but in the other species studied pollination takes place during the latter part of May. At this time the axis of the female cone elongates, thus separating the ovuliferous scales which now make an angle of about thirty-five degrees with the rachis. After pollination the fruit scales draw together and, according to Strasburger and Hillhouse ('00), their edges are consolidated by the ingrowth of papillæ. The presence of two ovules at the base of each scale, each ovule with its apex extending downwards, that is towards the base of the scale, and outwards, is too familiar a fact to need more than a passing mention here.

As pointed out by Hofmeister ('62) the integument is continued above the nucellus into two long arms which curve outward before pollination and lead below to a wide mycropylar canal. The degree of development which the ovule has obtained at the time when the pollen-grains reach the nucellus is shown in fig. 66. Deep within the central portion of the ovule, at its chalazal end, a single cell is distinguished from the others by its greater size and larger nucleus, this is the macrospore[1] of Hofmeister ('51). The so-called "spongy" tissue of Strasburger is already well differentiated when pollination takes place (figs. 66, plate VI, and 124, plate XII). Somewhat later the integu-

[1] In 1901, I stated that, at the time of pollination, there was in the nucellus an axial row of cells. I know, now, that this condition has rarely been reached at so early a date, and should be noted as very exceptional rather than as normal.

ment has closed over the pollen-grains and the macrospore mother-cell has divided giving rise to an axial row of cells the lowest of which becomes the functional macrospore (fig. 69, plate VI).

*The Pollen-chamber.* — The pollen-grains fall upon a scale and slip down to its base where they come into contact with the extended arms of the ovule. These prolongations of the integument now straighten and partially draw together thus bringing the pollen-grains down into the wide micropylar canal (fig. 123, plate XII, and fig. 66, plate VI). The free limb of the integument is seen in section to consist, at this time, of three layers of cells. As soon as the pollen-grains have found their way into the lower portion of the micropylar canal and some, at least, have come into contact with the tip of the nucellus, the cells constituting the middle layer of the arms, at a point slightly above the apex of the nucellus, elongate rapidly. The bulge or protuberance thus formed extends inwards from all sides and meets, closing the opening above the pollen-grains (figs. 66 and 67). As soon as the opening has been closed and the pollen-grains secured, these elongated cells give rise by division to many smaller ones (fig. 68). By the rapid elongation of these cells the safety of the pollen-grains is assured in a very short time, and then cell multiplication follows leisurely. This very pretty mechanism by which the final closing of the micropyle is effected has not been previously described for any Gymnosperm, unless it be noted in Shaw's ('96) statement, unaccompanied by figures, that the micropyle in *Sequoia* is closed by the radial elongation of the cells about it.

The depression in the apex of the nucellus in the *Abietineæ* at the time of pollination, described by Hofmeister in 1851, and since noted by many writers, has, it seems to me, been greatly exaggerated so far as *Pinus* is concerned. The expression "cup-like depression" is not infrequent in literature, but, in so far as my observations go, saucer-like is as strong a term as one is justified in using (figs. 66, 67 and 69, plate VI, and 75, plate VII). At the time of pollination the upper concave portion of the nucellus terminates in a row of more or less elongated cells, which are not closely united at their free extremities, but

stand up, as it were, like so many fingers to catch the pollen-grains; they also serve to facilitate the entrance of the pollen-tubes into the tissue of the nucellus (fig. 75, plate VII). A little later this depression may become more prominent, both by the slight disintegration of some of the superficial cells of the nucellus, due to the action of the pollen-tubes, and by the inconsiderable growth, after pollination, of the peripheral layer of cells of the nucellar tip. The deep cup-like depression sometimes observed is invariably the result of abnormal disintegration. The pollen-chamber in *Pinus*, then, consists of a space bounded on the bottom by the more or less concave upper surface of the nucellar tip, and arched above by the ingrowth of the free portion of the integument. Later a resinous substance is secreted which securely seals the opening by which the pollen-grains entered.

## DEVELOPMENT OF THE POLLEN-TUBE.

### THE FIRST PERIOD OF GROWTH.

*Germination of the Pollen-grain.*—Germination of the pollen-grain follows immediately after pollination. Ovules of *Pinus Strobus* that were fixed on June 6, 1898, had not been pollinated, but on June 13 pollination had occurred and the pollen-tubes had been emitted; similar evidence could be given for the other species studied, but exact data on this point are at hand for *Pinus rigida* only. Dispersion of the pollen occurred in this species in the vicinity of Wellesley College in 1902 on May 27, and in material fixed two days later, May 29, the first stages of germination are clearly evident. It is probable that the time is not longer in the other species. This confirms Strasburger's ('92) statement that germination takes place in *Pinus* at once after pollination. Hofmeister ('51) was doubtless unable to detect the early stages in the germination and hence was led to the conclusion that pollination and germination were separated by several weeks in the *Abietineæ*.

The pollen-grain increases slightly in size, the ventral or concave portion of the wall becomes convex, then bulges out, the exospore is ruptured, and the endospore is gradually prolonged into a tube. Immediately upon the formation of the

pollen-tube the tube-nucleus, as shown by Strasburger ('92) moves away from the antheridial cell and into the pollen-tube (figs. 75, 76, plate VII). According to Coulter and Chamberlain ('01, page 92), the tube-nucleus does not enter the tube until the following April. That the tube-nucleus should at once loose its association with the antheridial cell and accompany the growing point of the pollen-tube is exactly what we should expect from what we know, through the investigations of Haberlandt ('87) and others, regarding the relation of the nucleus to growth; and, also, judging from the standpoint of analogy, from the remarkable migrations of the tube-nucleus in order to be near the growing point of the pollen-tube in *Cycas* (Ikeno '98) and in *Zamia* (Webber '01).

*Division of the Antheridial Cell.* — Strasburger ('92) described the antheridial cell in *Pinus sylvestris* as remaining unchanged until the archegonia are formed in the following spring. Dixon states that it divides about a month before fertilization, but from a careful reading of the text one is given the impression that this was an inference on his part rather than a demonstrated fact, as he did not study material that was preserved earlier than April 24 and did not find the karyokinetic figure for this division. And, in so far as I am aware, this mitosis has not been observed in *Pinus*. Strasburger describes and figures it in *Picea* while the pollen-grain is still within the anther.[1]

I have found great variation in the time at which the antheridial cell divides, not only in different species but in the same species. It is rather interesting that *Pinus Strobus*, which invariably lags somewhat behind the other species in all other developmental phases studied, is remarkably precocious as regards this step. Figs. 78, 80, and 81 were all taken from material of *Pinus Strobus* which was collected and preserved on August 4, 1898, barely two months after pollination. In the same material, other pollen-grains were observed in which the division of the antheridial cell had not yet taken place; but in material fixed somewhat later it was rarely found undivided. The division of this cell has not been observed in *Pinus austriaca*, but two cells have been found in the pollen-grain in the middle of November and in February, and in such

[1] See note at close of appendix.

instances the tube-nucleus can invariably be detected in the pollen-tube. As pollen-grains containing but one cell were also observed in this species on these dates, it might be suggested that in the case of two cells the second prothallial cell had persisted. The two cells, however, are exactly similar to the stalk and the generative cell in their young condition, and I see no reason for considering that they are not these cells. On and after March 8 the antheridial cell of *P. austriaca* is almost never found undivided. This date is given for 1899; it would probably fluctuate in different years. Fig. 79 shows the prophase of this division in *Pinus rigida*. Mitotic figures for this species have been found from April 21 to May 13 of the same season. The division of the antheridial cell in *Pinus resinosa* has been observed but once, this division occurring on April 11. All that can be said at present regarding this mitosis in *Pinus montana* var. *uncinata* is that the generative cell and the stalk-cell are found as early as April 9. When they are formed has not been determined.

In one preparation of *Pinus Strobus* two of the three pollen-tubes which have almost reached the prothallium are furnished with sperm- and stalk-cells, while in the third only the tube-nucleus is found. On the apex of the nucellus there is a pollen-grain which at this late date contains one cell, the antheridial cell, still undivided (fig. 73). The nucleus of this pollen-grain (fig. 74) is large, plump, and to all appearances perfectly normal, and it is possible, though scarcely probable, that it might still have divided. That one cannot trace a definite connection between the pollen-tube containing only the tube-nucleus and this pollen-grain signifies little, for those who have studied the pollen-tube of *Pinus* know that it is the exception rather than the rule when a given pollen-tube can be traced through the lacerated dead tissue of the upper portion of the nucellus to the pollen-grain from which it proceeded. Such a condition as that described is rarely met with at so late a date; but occasionally during the summer and fall pollen-grains of *Pinus Strobus* are found in which no cell-division has taken place since pollination, although in the great majority of cases

the stalk- and the generative cell have been formed before the middle of August.

These observations indicate that, while the division of the antheridial cell takes place comparatively soon after the pollen-grain has germinated in *Pinus Strobus*, and in some instances, at least, before the winter's rest in *P. austriaca*, it is deferred until the following spring in *Pinus rigida* and *P. resinosa*. Furthermore, the time during which this cell may divide in a given species may extend over several weeks, and in some cases the division may never take place at all.

*The Winter Condition.* — A vertical section of an ovule of *Pinus Strobus* collected on January 4 is represented in fig. 70, plate VI. The spongy tissue surrounds a cavity crossed by irregular strands of cytoplasm in which the free nuclei of the prothallium are imbedded. In this instance the prothallium has doubtless been displaced during fixation as it consists, normally, at this stage, of a uniform layer of cytoplasm surrounding the gametophytic vacuole and containing several nuclei. The stalk- and the generative cell are enclosed within the pollen-grain, and the tube-nucleus is near the apex of the irregularly branched pollen-tube. This pollen-tube is shown more highly magnified in fig. 83, plate VIII. At this time the pollen-tubes have penetrated the nucellus almost to the point at which it joins the free limb of the integument. The greatest depth to which the tubes may have grown is not indicated in the illustration, but this section was figured because it shows more clearly than any other section in the series the cells of the pollen-grain and the tube-nucleus. Other sections of the same ovule would have shown pollen-tubes which had pierced to a greater depth into the nucellus. The conditions of development as figured for January coincide perfectly with those which exist during the latter part of October.

### THE SECOND PERIOD OF GROWTH.

*Renewed Activities in the Macrosporangium.* — Growth is very slow during the first period of development following pollination, but with the renewed activities of spring the ovule increases rapidly in size; the central cavity of the nucellus

becomes greatly enlarged and is lined with the growing endosperm. The cells of the nucellar cap which are penetrated by the pollen-tubes during the previous season do not again become active, but remain as deeply staining, thick-walled, dead cells. The cells just beneath them, however, multiply rapidly, and become literally packed with large starch-grains. A few of the cells from this portion of the nucellar cap represented in fig. 73, plate VII, are shown more highly magnified in fig. 89, plate VIII. By the growth and increase of these cells, the dead top of the nucellus with its pollen-tubes is lifted far above the developing endosperm, so that the pollen-tubes, once so near their goal, are now removed from it by a considerable distance (figs. 70–72, plate VI).

*Renewed Activities in the Male Gametophyte.*—During the rapid development of the ovule in the spring, the pollen-tube increases little, if at all, in length, renewed activities in the male gametophyte being first indicated by a further development of the cells within the pollen-grain.

The stalk-cell increases in size and its cytoplasm assumes a vacuolate character. The growth of the generative cell is still more marked, and its cytoplasm on the contrary becomes dense and deeply staining. (Compare fig. 83, January 4, with fig. 84, May 3, plate VIII.) In *Pinus sylvestris*, as studied by Dixon ('94) and confirmed by Coulter ('97) in *Pinus Laricio*, the generative cell divides while it is within the pollen-grain. In the species of pines which I have investigated, this division does not occur until the generative and the stalk-cell have entered the pollen-tube and the stalk-cell has passed below the generative cell. As the generative cell increases in size it stretches out towards and into the neck of the pollen-tube, drawing after it the stalk-cell, or possibly being forced out by that cell, the two passing into the tube together.

Dixon states that only the naked nucleus of the stalk-cell enters the pollen-tube, and in so far as I am aware, no writer has described the entrance of the entire stalk-cell into the pollen-tube in *Pinus*. The material which I have studied shows conclusively that the nucleus does not "slip out" of its cytoplasm (figs. 83–86). The entire cell can be identified in the tube and

later in the egg. During the time that this cell is moving over the generative cell its cytoplasm cannot always be differentiated from that of the latter; but when once the stalk-cell has passed the generative cell, its nucleus surrounded by a sphere of very vacuolate cytoplasm, scarcely more than a peripheral layer, is again distinctly demonstrated (figs. 90 and 91). After passing the generative nucleus, the stalk-cell ordinarily takes up a position between the generative cell and the tube-nucleus (fig. 92), but occasionally it may pass the tube-nucleus (fig. 93). This phenomenon is always accompanied by a great increase in the starch content of the pollen-tube, the tube being in some instances almost filled with starch in the region of the generative cell (fig. 91).

When the generative cell leaves the pollen-grain, its nucleus is situated near the top of the cell, but the nucleus of this cell evidently moves faster than its cytoplasm, and at the time when the stalk-cell is passing over the generative nucleus this nucleus has come to lie at or below the center of its cell (fig. 84, 90 and 91). Shortly after this the generative nucleus is again observed at the uppermost part of its cytoplasm.

During its passage into the tube, the generative cell increases much in size; it has no definite cell-wall, and its cytoplasm forms a large, irregular tongue about the nucleus. This cytoplasm in no way suggests the alveolar structure of Butschli ('94) but is distinctly reticular, differing in appearance from the nuclear net only by its greater delicacy. This is shown more clearly at a somewhat later stage.

The tube- and generative nuclei are now very similar in structure, though each is sufficiently characteristic to be readily recognized by one who is familiar with them. The tube-nucleus has one large, usually homogeneously staining nucleolus, rarely one or more smaller nucleoli, and it is furnished with a rather scanty, delicate reticulum which is apparently poor in chromatin. Either it is in a state of partial collapse, or, what is more probable, it is very hard to fix at this period in its history, for its outline is, as a rule, quite irregular at this time. The generative nucleus has one large, hollow or vacuolate nucleolus, and commonly two smaller ones; its reticulum, though more abun-

dant than that of the tube-nucleus, is still delicate and often shows a weak reaction to nuclear stains. The stalk-nucleus has a very decided individuality which it maintains throughout its entire history. It bears a strong resemblance from the first to the nuclei of the nucellar tissue; rarely, if ever, contains a true nucleolus; and its close-meshed reticulum is conspicuous for its comparatively large net-knots or karyosomes.

*Division of the Generative Nucleus.* — Comparatively few students have occupied themselves with the growth of the pollen-tube in the *Abietineæ*, and no one, in so far as I have been able to determine, has described the cytological features attending the formation of the sperm-nuclei in this group.

Dixon ('94) describes this division in *Pinus sylvestris* as taking place about a *month before fertilization*, while the generative cell is still *within the pollen-grain;* and Coulter ('97) states, as already mentioned, that in his study of *Pinus Laricio* he has been able to confirm Dixon's observations in the minutest detail. At this time, as pointed out by Dixon, the nuclear and cytological phenomena are very greatly obscured by the presence in the pollen-tube of large quantities of starch (fig. 91). The starch, which resists the microtome knife and is therefore easily displaced by it, not infrequently falls out and carries away with it the free cells of the pollen-tube. The dead, deeply staining tissue of the nucellus, representing that portion of the nucellar cap which was penetrated by the pollen-tubes during the previous season, and in which the generative nucleus divides (fig. 72, plate VI) is also very troublesome. Furthermore the dense cytoplasm of the generative cell has a great affinity for stains, so that when the archegonia and other portions of the ovule are well stained, this cell often appears merely as a deeply stained mass showing no differentiation of parts. Considering the fact that I was led not only to expect this division to take place within the pollen-grain but to search for it some weeks earlier than it actually occurs in the species of pines studied, together with the difficulties of staining, it is not surprising that seven hundred slides of serial sections were made, which means that more than two thousand pollen-tubes were studied, before any definite clue was obtained as to the

true sequence of events in the development of the pollen-tube. When once the mitotic figure was observed *in the pollen-tube*, scarcely more than a *week before fertilization*, and the fact noted that special staining was necessary in order to study this mitosis satisfactorily, further research was prosecuted with comparative ease. I find no authority in Dixon's paper for the statement recently made by Coulter and Chamberlain ('01) which reads as follows: "The *liberation* and *descent* of the body cell *into* the tube," etc., "has recently been described in detail by Dixon." What Dixon ('94) does affirm is this: "Very shortly after this it is found that the body-cell has broken free from the stalk-cell and has divided into two cells, which are almost equal in size. These cells are the male sexual cells. During this process the wall of the stalk-cell is ruptured and its nucleus follows the two cells resulting from the division of the body-cell which *move into* the pollen-tube." And throughout Dixon's paper there is no sentence that could be interpreted as implying that the body-cell ever passes into the pollen-tube before dividing to form the male sexual cells.

After the generative cell has passed into the pollen-tube but while it is still in the upper dead portion of the nucellus, it gives rise to the sperm-nuclei by a division which presents some new and interesting features, although it resembles to a greater or less degree certain mitoses described by various cytologists[1] during the past few years.

When the generative nucleus has again come to lie in the extreme upper portion of its cell, certain changes in the cytoplasm indicate that division is being initiated. At some little distance below the nucleus the cytoplasm shows a finely granular structure which is not at this stage dense nor deeply staining. From this region irregular granular threads arise which extend outward towards the periphery of the cell, those extend-

[1] Of the long list that might be mentioned I have noted only the following: Rosen ('95) in the root-tip of hyacinth; Osterhout ('97) in *Equisetum;* Swingle ('97) in *Sphacelariaceæ;* Schaffner ('98) in root-tip of *Allium Cepa;* Mottier ('98) in the embryo-sac of *Lilium;* Fulmer ('98) in pine seedlings: Hof ('98) in *Ephedra* and other plants; Nawaschin ('99[2]) in *Plasmodiophora;* Nemec ('98 and '99) in various plants; Strasburger ('00) in *Vicia Faba;* Mottier ('00) in *Dictyota;* and Murrill ('00) in *Tsuga*. Of animal cytologists I mention but one, Hertwig, R. ('98) in *Actinosphærium*.

ing in the direction of the nucleus forming a hollow cone over its lower portion (fig. 94, plate VIII). Gradually the granular area increases in density and in staining capacity, at the same time drawing nearer to the nucleus which is separated from it by a hyaline court. Into this court delicate granular threads pass (fig. 95, plate IX). When these threads reach the nuclear membrane, the nucleus is forced so closely against the peripheral layer of cytoplasm that its wall is frequently indented on the upper side, while the condensation from which the so-called kinoplasmic threads arise withdraws, or is forced by the growth of the threads, further from the nucleus. A great number of delicate anastomosing threads now extend, in the form of a solid cone, from a point within the granular condensation up towards and against the nucleus. The outer threads of the cone pass over the lower portion of the nucleus and appear in sections of the cell as closely packed against either side of the nucleus. At the same time the entire cytoplasmic reticulum has assumed a more or less radial arrangement about the condensed area in which the spindle-fibers arose and from which some of the more delicate threads extend into the surrounding cytoplasm (fig. 96).

Coördinately with these changes in the cytoplasm, the chromatin of the nuclear net collects in spherical or irregular masses on the reticulum, and sooner or later gives rise to a broad spireme, along which the chromatic disks are distributed at regular intervals (figs. 94–98). After the segregation of the chromatin, there remains a delicate achromatic reticulum distributed throughout the nucleus. This reticulum is also granular like the chromatic network, but whether or not these granules represent the oxychromatin-granules of Heidenhain ('93 and '94) I am unable to say. Webber ('01) has recently described and figured a similar achromatic network in the generative cell in *Zamia*. Whether the formation of the spireme precedes or follows the penetration into the nuclear cavity of the achromatic threads seems to depend upon the length to which these threads attain. They may become very long when their entrance into the nucleus is delayed; but more frequently a portion of the nuclear membrane gives way, and some of the achromatic

fibers pass into the nuclear cavity before the spireme is established (fig. 100). Rarely, the nuclear membrane appears pushed in irregularly along its entire lower margin, as indicated in figs. 96 and 98; as a rule, however, there seems to be one deep, sharp indentation along one side of which the nuclear wall first gives way (figs. 99 and 100). With the initial steps in the disappearance of the nuclear membrane the nucleolus is either not apparent or, if still demonstrable, it stains but feebly. When the membrane disappears along the entire lower portion of the nucleus, the kinoplasmic threads press so closely against it that it can not be definitely demonstrated whether it passes into the cytoplasmic and the nuclear reticulum or becomes fibrous and contributes to the formation of the achromatic threads (figs. 101 and 102). The threads which have been packed so closely against the wall of the nucleus now press into the nuclear cavity and mingle with those which have entered from below. And the dense, granular, cytoplasmic area from which the threads diverge is gradually dissipated (fig. 103).

With the disappearance of the wall along the lower part of the nucleus, the achromatic nuclear network seems to undergo a partial rearrangement. A portion of it is resolved into granular threads of more or less regularity which, in general, assume a position parallel to the threads entering the nuclear cavity; some of them become attached directly to the ends of these fibers, lose their granular appearance and doubtless contribute to the growth of the elongating spindle-threads.

As the spindle-fibers proceed in their development across the nucleus the chromatic spireme collects in the region of the future equatorial plate, and becomes more or less massed together. At the same time it assumes an homogeneous aspect and gives rise by segmentation to the chromosomes (figs. 101–104). Some of the ingrowing spindle-threads may extend across the nucleus to the nuclear membrane, which is still present on the upper side of the nucleus, but by far the greater number unite some distance below this membrane to form several poles, thus giving rise to a diarch spindle which, like the karyokinetic figures occurring during the development of the pollen-grain is multipolar at its upper extremity and unipolar, or nearly so, at its

lower end. Gradually the poles of the upper portion draw together, while the spindle is somewhat shortened by the lower extremity of the threads being again resolved into granules. Finally a true bipolar diarch spindle is formed with the V-shaped chromosomes oriented at the equatorial plate. Each pole terminates in a slight granular condensation. The upper pole has never been observed to reach the nuclear membrane, but frequently coarse granular threads extend from the pole to the membrane of the nucleus, and apparently act as supports for the upper pole (fig. 105, plate X). These are evidently formed by a rearrangement of the linin reticulum. The nuclear membrane persists along the upper side of the nucleus until the late telophase of the division (figs. 101–103, plate IX, and 104–107, plate X).

As the chromosomes pass to the poles the central spindle elongates, so that the daughter-nuclei are separated, as a rule, by a greater distance than the length of the original spindle. While this is characteristic of cell-division in general, it is occasionally much exaggerated here, the daughter-nuclei being apparently forced apart with considerable energy. The nucleus which occupies the position nearest to the micropylar end of the ovule often shows a deep indentation along its upper surface as if a resistance had been met with in the peripheral layer of cytoplasm (figs. 111, plate X, and 113, plate XI). Not infrequently the upper nucleus is found almost entirely separated from the cytoplasm (fig. 112). This, however, may be due to mechanical rupture during sectioning and staining. No cell-wall is ever formed, and in only one instance was a condensation of the spindle-threads in the region of the cell-plate observed (fig. 110). The spindle may contract at or near its center during its dissolution, thus presenting the appearance of an hour-glass, or it may give rise to such a condition as that shown in fig. 113. These appearances, with various modifications, are not uncommon in this mitosis in *Pinus*. Hertwig ('98) describes and figures a very similar lengthening of the spindle-fibers in *Actinosphærium*. He also finds that the elongating spindle finally bends along its median line so that the daughter-nuclei come to lie near together in very much the same way as that

shown in fig. 113. I am unable to trace definitely the origin of this figure, but it is not improbable that it is caused by a contraction of the cytoplasm resulting from the cessation of the force which effected the separation of the daughter-nuclei; or it may be produced by the resistance which the peripheral layer of cytoplasm, along the outer surface of the upper nucleus, offers to the growing fibers, thereby forcing them back upon themselves as shown in the figure. When all traces of the spindle have disappeared, the two sperm-nuclei are surrounded by a common mass of cytoplasm, and there is never throughout the later history of this cell the least suggestion of a dividing wall.

The mitosis just described seems to be unique as regards the origin and development of the achromatic spindle. Hertwig's ('98) fig. 3, plate V, illustrating an early stage in the division to form the first polar body in *Actinosphærium*, bears a striking resemblance to the prophase of this mitosis as illustrated in fig. 95, plate IX, of this paper; but the origin of the figure shown by Hertwig, and the later history of the division are very dissimilar to that of the karyokinesis under consideration. The most exaggerated instances of asymmetry in spindle-formation which I have found recorded as occuring in plants is that described and figured by Nemec ('99[2]) in *Solanum tuberosum*, and more recently by Murrill ('00) in the division of the central cell in *Tsuga*. In both these instances the nucleus lies at one side of the cell, and the spindle-fibers are very much more prominent on the free side of the nucleus than on the side adjacent to the cell-wall. In another paper Nemec ('99[3]) shows by experimentation that the form of the figure which gives rise to the extranuclear spindle depends upon external forces or conditions. In obedience to the law established by Haberlandt ('87) we should expect to find the generative nucleus in that part of its cell which is nearest the growing point of the pollen-tube, rather than at the end more remote from it, and it may be that its passage from the lower to the upper side of the cell is due to the fact that the forces, instrumental in effecting the division, first become active at a point below the nucleus, and exert a repelling action on it. But I have at present no adequate explanation or theory to offer

regarding the position of this nucleus at the time of its division. Whether it is due to the origin of the karyokinetic figure, or whether the unusual method of division is attributable to the very eccentric position of the nucleus, I have not been able to determine. It is evident, however, that the position of the generative nucleus at the time of its division is such that the spindle if extranuclear in origin must of necessity be unipolar, since there is no cytoplasm, or almost none, above the nucleus from which fibers could arise.

The blending of the linin reticulum with the cytoplasmic network after the disappearance of the lower portion of the nuclear membrane, and the relation of certain portions of the achromatic nuclear reticulum to the ingrowing fibers are such as to suggest an intimate relation between these structures. That the spindle-fibers which originate in the cytoplasm and apparently grow by a differentiation of its network are later fed by the linin of the achromatic nuclear reticulum, there seems little room for doubt. In fact, all the phenomena connected with this division indicate that we are dealing, not with persistent cell-constituents, but with different manifestations of one and the same thing. In a word, we find no evidence here of the presence in the cell of a definite kinoplasmic substance. I am aware that these observations are directly opposed to the views of the students of the Bonn laboratory, and many others of the highest authority; but the relations of nucleus, spindle, and cytoplasm, not only in this division but in those to be described in connection with fertilization, are such, it seems to me, as to render no other conclusion in the case of these divisions in *Pinus* possible. In 1895 Farmer arrived at a similar decision regarding the origin of the spindle in spore-formation in the *Hepaticæ*, and Farmer and Williams ('98) in a study of *Fucus* "do not regard the kinoplasm as a persistent protoplasmic structure, but as forming the visible expression of a certain phase of protoplasmic activity." Hertwig ('98) expresses himself as opposed to the view of a special spindle-forming substance in the protoplasm, while Wilson ('99 and '00) states that the astral rays "grow by a progressive differentiation out of the general cytoplasmic meshwork," and he finds in the echino-

derm's egg " no ground for a specific kinoplasm." The term, however, is a convenient one and may be employed consistently, as suggested by Mottier ('00), by those who do not find in kinoplasm a morphological constituent of the cell, as descriptive of that portion or manifestation of the protoplasm which is active in spindle-formation.

Nothing has been said regarding the nature of the granular, cytoplasmic condensation from which the achromatic spindle takes its origin. It never has a definite boundary, though it is often very clearly differentiated by its dense granular appearance and its strong affinity for stains ; but at certain stages in the division it may be inconspicuous or fail entirely of demonstration. Such a vast amount of literature has accumulated during the past decade regarding the nature and existence of the centrosome and the centrosphere that one feels inclnied to avoid the subject altogether. Yet the question may very properly be asked : Is this condensation which forms the center of a system of radiating fibers a centrosphere? It certainly is as clearly an attraction-sphere as some bodies which have been described as such ; but if we accept Wilson's ('00) definition of the centrosphere, the body under consideration cannot be so denominated, as no centrosome has been observed at its center. More deeply staining granules may sometimes be present within the condensation, but these are not considered of any special significance as such granules may be found anywhere in the cytoplasm.

Karsten ('93) describes the nucleoli in *Psilotum* as passing out of the nucleus and assuming the rôle of centrosomes, and Strasburger ('00) considers that the nucleoli not only contribute material for the formation of kinoplasmic threads, but that they also make active the spindle-forming substance in the cytoplasm — in other words, they act as the kinetic centers of the cell. There seems to be no evidence that such is the case here, for the nucleoli, after the condensation has arisen and the spindle-threads have attained considerable length, are morphologically the same as they were before the inception of the spindle. Nemec ('99[1]) remarks that in the higher plants, where the centrosome is not demonstrably present, the entire nucleus may exercise the function of the centrosome. The idea of a diffused

centrosome in the cells of the higher plants was suggested by Guignard in 1897 and was again hinted at by Le Dantec in 1899. If we may accept Guignard's suggestion, then the kinetic center of the cell in the higher plants is no longer indicated by the presence of a definite organ, the centrosome, but the power of this organ has become dissipated throughout the entire cell. When that phase of cell-activity which has to do with spindle-formation comes into play, the points at which it is centered would naturally be indicated by a greater accumulation of the microsomes, and thus an aster of more or less definiteness would be formed, as when the individualized centrosome is present. In the division of the generative nucleus in *Pinus*, the position of the nucleus is such that the energy active in spindle-formation must perforce, if external to the nucleus, be centered at some point below it. Such a centering of the activity would naturally result in an attraction-sphere of unusual prominence; and there would be no occasion for its division since there is not sufficient space above the nucleus for the organization of kinoplasmic threads.

When these studies were undertaken, it was thought that it would be interesting to determine whether any suggestions or remnants of a cilia-forming body (called blepharoplast by Webber in *Zamia*) still persist in the Conifers. Somewhat later, after the present research was begun, MacMillan ('98) pointed out the desirability of such a study both in *Coniferæ* and *Gnetales*. I have seen no indication of a structure which might be regarded as a reduced blepharoplast, or as suggestive of a cilia-forming body of any sort in connection with the formation of the sperm-nuclei in *Pinus*. Inasmuch as spermatozoids do not exist here, such an organ, if present, must be functionless. But the cytoplasmic radiations which accompany the division of the generative nucleus in its early stages seem to differ in degree only from those found by Webber ('97) in the generative cell of *Zamia*. If we compare figs. 3 and 5 of Webber's paper with figs. 96 and 97, plate IX, of this paper, the question may be raised whether in this cytoplasmic figure we may not have still persisting in the cell the last vestiges of such an organ as that described by Webber.

The endosperm has become a solid mass of tissue at the time when the generative nucleus divides. The archegonia are still comparatively small and quite vacuolate and the central cell has not yet divided (fig. 72, plate VI).

*Growth of the Sperm-nuclei.* — After the mitotic figure has entirely disappeared, the sperm-nuclei are separated by a considerable distance. The form assumed by the cytoplasm surrounding them seems to vary with the shape of the pollen-tube. Gradually the two nuclei approach each other until they come to lie in the extreme uppermost part of their cytoplasm (figs. 112, plate X, 117, 118, plate XI). There is now considerable difference in their size. This inequality in size could be detected as far back as the formation of the daughter-nuclei (figs. 109, 110, plate X). Belajeff ('91) was the first to figure and describe binucleated sperm-cells in the Gymnosperms. Coulter and Chamberlain ('01), page 94, cite Belajeff as having observed an unequal division of the generative cell in *Taxus*, the larger male cell functioning, the smaller one remaining in the tube. But if I translate the German correctly, what Belajeff says is that the nucleus of the generative cell divides forming two nuclei which are about one-half as large as the nucleus from which they were derived; one nucleus becomes larger and occupies a central position in the plasma, the other nucleus is flattened and remains at the periphery of the cell on its upper side; the flattened nucleus was never found surrounded by its own plasma, but in the same plasma with the spherical nucleus. This is exactly the condition shown in Belajeff's figures, one of which is reproduced by Coulter and Chamberlain. Jäger ('99), however, has shown two dissimilar sperm-cells in *Taxus*, the larger one in advance, but he finds that occasionally the nucleus of the smaller cell may exceed in volume that of the larger one. Jaccard ('94) found two sperm-nuclei of the same size in *Ephedra* both surrounded by the same mass of cytoplasm, and Coker ('02) has recently described the sperm-cell in *Podocarpus* as binucleated, the smaller nucleus being above the larger and "thrust almost out of the cell." No one, I believe, except the writer (1901[1 and 2]), has recorded the presence of a single binucleated sperm-cell in the *Abietineæ*. In his earlier studies of the Gymno-

sperms, Strasburger ('69–'92) was unable to demonstrate, satisfactorily to himself, the character of the cells found in the pollen-tube in *Pinus*, and he has not recently investigated the male gametophyte in the *Abietineæ*. Coulter ('97) described two sperm-cells which were of the same size until within the archegonium. Blackman ('98) stated that each sperm-nucleus was clearly seen in the pollen-tube surrounded by its own cytoplasm, but he did not figure them.[1] Chamberlain ('99) figured the sperm-nuclei, in *Pinus Laricio*, of equal size in the pollen-tube, and showed them lying together in the cytoplasm of the tube. Not having seen these cells within the archegonium before the conjugation of the sexual nuclei, he accepted Coulter's statement for the growth of one of them after their entrance into the egg. According to Coulter ('00) the "male cells in pines" are alike in size. The same figures are reproduced by Coulter and Chamberlain ('01).

As stated by the writer in 1901, two sperm-cells have not been observed in any of the pines which I have studied; but the sperm-nuclei, which are of unequal size from a very early date, remain, while in the pollen-tube, surrounded by a common cytoplasmic body (figs. 109–112, plate X; 113–118, plate XI, and 119–120, plate XII). As Strasburger ('92) observed, the larger nucleus is always ahead, that is, on the side nearest the apex of the pollen-tube. The smaller nucleus remains close against the upper boundary of the cytoplasm, and suggests the condition in *Cycas* (Ikeno '98) and *Ginkgo* (Hirase '98), where the stalk-nucleus is forced entirely out of the cytoplasm surrounding the generative nucleus. In the case of the smaller sperm-nucleus in *Pinus*, the action is not carried to so great an extent. Webber ('01) has recently shown that such an interpretation as that recorded above for *Cycas* and *Ginkgo* is not true as regards the stalk-nucleus in *Zamia*. One very interesting preparation which I have obtained shows the smaller sperm-nucleus in advance of the larger (fig. 114). Here it will be seen that the entire order of arrangement has been changed, the stalk-cell and the tube-nucleus being above the sperm-cell. But this abnormal arrangement is only apparent, for it was found that the egg which had

[1] See note at close of Appendix.

been approached by this pollen-tube had already been fertilized, and the pollen-tube had turned aside and was passing up over the top of the endosperm, as if seeking for another egg. The position of the various elements of the pollen-tube is therefore normal, the larger sperm-nucleus being in reality in advance of the smaller. This suggests that, when a pollen-tube has conjugated with the egg, a substance may be secreted which repels other pullen-tubes, as has been described in case of spermatozoids in the Bryophytes and Pteridophytes.

The formation of the sperm-nuclei shows most beautifully the manner of the development of the nuclear reticulum. The chromosomes unite end to end, giving rise to a homogeneous, coiled band, before the nuclear membrane is formed. When the nuclear-wall has been differentiated, the coil expands about the periphery of the nucleus, while the band broadens, at the same time becoming irregularly jagged along its margins. These irregularities increase in length until finally those from adjacent threads meet and fuse, thus giving rise to the reticulum (figs. 107–110, plate X). When the sperm-nuclei have nearly or quite come into contact they have as a rule reached their mature size. More than a year has now elapsed since pollination.

*Elongation of the Pollen-tube.*—Up to this time the pollen-tube has elongated very slowly, having penetrated as yet little, if any, beyond the nucellar tissue of the previous year's growth. In this upper portion of the nucellar cap the tube may become very broad, or it may branch freely (figs. 71, 72, plate VI, and 83, 87, plate VIII). When the sperm-nuclei have attained their full size, the downward growth of the tube is exceedingly rapid, travelling in from eight to ten days more than twice the distance traversed during the entire preceding year. The path pursued during this rapid growth is comparatively straight and the tube is unbranched (fig. 73, plate VII). In *Pinus Strobus*, *P. rigida* and *P. austriaca* about ten days intervene between the division of the generative nucleus and fertilization; in *Pinus montana uncinata*, the two processes are separated by an even shorter space of time.

The sperm-nuclei which at first present a very beautiful, rather delicate reticulum (figs. 112, plate X, 117, plate XI), become

more dense as the pollen-tube advances through the nucellus. Strasburger ('92) describes them as coarsely granular; but, with a high power, the presence of a reticulum which is sometimes coarse and interrupted can invariably be made out in well prepared material. By the time that these nuclei have reached in their downward course the central portion of the nucellar cap they have usually become very dense in structure (figs. 115 and 116), and frequently stain intensely, though they may show at this time a weak reaction to dyes. The reticula of the two nuclei may present the same appearance, or they may differ as in the figures referred to above. The nucleolus, if it be present at this time, is usually obscured by the dense network. Arnoldi ('00) described the sperm-nuclei in *Cephalotaxus* as being gradually filled with metaplasm. I find no evidence of such a process in the development of these nuclei in *Pinus*.

Archoplasmic areas similar to those figured by Chamberlain ('99) have been observed in connection with the sperm-nuclei, but as such granular accumulations may occur at any point in the cytoplasm of the sperm-cells no importance is attached to them.

When the pollen-tube reaches the egg, its apex is abundantly supplied with cytoplasm, in the upper part of which the tube-nucleus lies. The sperm-cell is just above with the stalk-cell still in contact with the lower portion of its cytoplasm (fig. 120, plate XII). Still higher up the tube may contain many starch-grains. There is never any doubt at this time as to the identity of the stalk-cell and the tube-nucleus in the material which I have studied. Yet Dixon ('94) states that they cannot be distinguished, and Coulter ('97) describes them as having lost their original outline.

As many as six pollen-tubes have been found making their way through the same nucellus, but, as a rule, not more than three pollen-tubes renew their growth during the second season, and frequently only two penetrate to the endosperm. The effect of the pollen-tubes upon the upper part of the nucellar tissue is very marked. The cells in the immediate vicinity of the branched pollen-tubes early lose their protoplasmic contents and their walls become crushed and broken. Those cells more

remote from the tubes do not suffer so severely, and retain their protoplasm for a much longer time. Finally all the cells representing the first year's growth of the nucellar tip loose their content to a greater or less degree, and their cell-walls become thickened and dead. During the rapid growth of the pollen-tubes through that portion of the nucellar cap which develops the second season, the effect of the tubes on the surrounding tissue is less marked, though here, too, the cells with which they come into contact are crushed and destroyed (fig. 73, plate VII). I have made no physiological investigations regarding the action of these tubes on the tissue of the nucellus, but, judging from the disappearance of the starch in the cells just in advance of the tubes and the gradual disintegration of those cells, it seems very probable that the destruction of tissue is not due to mechanical reasons alone, but to the action of some ferment or digestive substance as well. Various views have been expressed concerning the action of the pollen-tube and the directive agent in its growth by Molisch ('93), Miyoshi ('94), Lidforss ('99) and others, but we are still far from a clear understanding as to the controlling factor in the movement. The pollen-tube cannot be guided to the egg in *Pinus* by any peculiar attraction existing between the sexual cells, for it grows with normal rapidity when no sperm-cells are formed, and also when the archegonia are in a state of disintegration.

## SUMMARY.

Upon the germination of the microspore, three divisions follow in rapid succession giving rise to the pollen-grain. At the close of the prophase of each division the karyokinetic figure is pointed at its lower extremity and very broad at the extremity in contact with the dorsal side of the young pollen-grain. The inner, incomplete, thick wall formed in the development of the microspore persists as a part of the mature pollen-grain. It probably serves as a strengthening layer, particularly at those points at which the wall has been weakened by the expansion of the exospore. When the telophase of the second division is reached the first prothallial cell has become flattened against the convex side of the spore-wall, its cytoplasm has been withdrawn,

and the nucleus has lost all signs of its former structure remaining as a much flattened, deeply staining mass. At the close of the third division, the second prothallial cell has suffered a similar fate. Both prothallial cells are furnished with cellulose-walls.

In the mature pollen-grain the prothallial cells are usually represented by two broken, dark lines along the dorsal side of the pollen-grain, but all vestiges of the first cell may have disappeared. The antheridial cell projects from the convex side of the spore at its middle point, and the tube-nucleus is always directly below but in contact with the antheridial cell. Starch is found in the pollen-grain at maturity and during its development.

Pollination takes place between 42° and 43° north latitude during the latter part of May or the first ten days in June. At this time the macrospore-mother-cell is distinctly visible in the center of the ovule, but slightly nearer its basal end.

In the young ovule the free portion of the integument, above the tip of the nucellus, consists in cross-section of three layers of cells. After pollination the arms of the integument become erect, thus bringing the pollen-grains into the wide micropylar canal. Then the inner layer of cells just above the pollen-grains elongates rapidly, extending inwards and meeting at the center. The pollen-grains having thus been made secure, the elongated cells become divided into many small cells. It is felt that the pit in the apex of the ovule in *Pinus* has been exaggerated. There is rarely more than a slight concavity before pollination. Through the action of the pollen-tubes it may be somewhat deepened, but in normal conditions it does not become "cup-like."

Two days after pollination, in *Pinus rigida*, the pollen-tubes have been emitted. In the other species germination has been shown to take place in less than a week after pollination, but more exact data have not been obtained for these species. As soon as the pollen-grain has germinated, the tube-nucleus severs its connection with the antheridial cell and moves into the elongating tube.

The division of the antheridial cell takes place in *Pinus Strobus* during the first week in August. It sometimes divides

during the summer and fall in *P. austriaca*, but, as a rule, the division takes place in this species very early in March. This mitosis has been observed in *P. resinosa* during the second week of April, and in *P. rigida* from the middle of April to the middle of May. It is evident that this cell does not always divide at a definite and fixed time, but that in a given species the time during which it may divide extends over a considerable period.

During the first season the pollen-tube grows very slowly, and it may be broad and irregular in outline or it may branch freely.

Shortly before fertilization the generative cell, followed by the stalk-cell, moves into the pollen-tube. The stalk-cell soon passes the generative cell and takes up a position near the tube-nucleus. These changes and those immediately following are frequently much obscured by the presence in the pollen-tube of large quantities of starch.

When the macrosporangium enters upon the winter's rest, the pollen-tubes have penetrated nearly to the line at which the integument becomes free from the nucellus and the tube-nucleus maintains its position in the apex of the pollen-tube.

The generative cell is never limited by a well-defined cell-wall, and consists at the time of its division of an irregular protoplasmic body in the upper part of which the nucleus lies.

In the division of the generative nucleus the spindle is extranuclear and unipolar in origin, a unique and heretofore unobserved method of division.

The formation of the spindle indicates that the cytoplasmic network and the nuclear reticulum have essentially the same structure, and the spindle-fibers are apparently formed by a transformation of both. The nuclear membrane persists along the upper part of the nucleus until the early stages in the formation of the daughter-nuclei. This division takes place a little more than a year after pollination and from a week to ten days before fertilization, nearly thirteen months elapsing between pollination and fertilization.

Two sperm-cells are never formed, but the sperm-nuclei remain surrounded by a common mass of cytoplasm. An in-

equality in the size of these nuclei is very early apparent, and becomes more pronounced as they reach maturity. The sperm-nuclei soon come to lie together in the upper part of their cytoplasm and quickly attain their full size, the larger one being invariably in advance. The nuclear reticulum, at first delicate, soon becomes very dense, but there is no evidence of the presence in these nuclei of a special metaplasmic substance.

During the division of the generative nucleus the ovule increases much in size, and the nucellar cap becomes several times deeper than during the first season, thus carrying the upper portion of the nucellus with its pollen-tubes far above the endosperm.

At the time when the sperm-nuclei come into contact, or nearly so, the pollen-tube has penetrated little, if at all, beyond the nucellar tissue of the first year's growth. Now, however, it again begins to elongate, and its downward course through the new nucellar tissue is extremely rapid. The destruction of the nucellar tissue through which the pollen-tubes travel, apparently results not only from mechanical disturbances, but from the entire dissolution of some of the cells through the action of a ferment.

When just above the egg, the apex of the pollen-tube is filled with cytoplasm. The tube-nucleus lies in the upper part of the cytoplasm, and near it is seen the stalk-cell still in contact with the lower portion of the cytoplasm which surrounds the sperm-nuclei.

The existence of the diffused centrosome is suggested in connection with the division of the generative nucleus, and there is a possibility that, in the prominent cytoplasmic figure from which the spindle takes its origin, we may have represented, in its vestigial state, the cilia-forming body found in the lower Gymnosperms.

## CHAPTER III.

### MACROSPOROGENESIS.

### THE FEMALE CONE.

*The Macrosporangium.* — During this investigation I have made no attempt to study the early development of the ovule except to note definitely the date of its origin. The pistillate strobili cannot be detected in *Pinus Strobus* with the most careful examination until the last of April or the first of May. In the other species studied they are about one and one-half millimeters long at the middle of March, and it is possible that in these species they were organized in the autumn, but I have not been able to find any evidence that such is the case. I have recently, November 25, 1902, attempted to discover the young cones of *Pinus rigida* and *P. austriaca*, but, as formerly, the search was futile. I was led to look again for these strobili in the autumn by the recent statement of Coulter and Chamberlain ('01). On page 79 of their book on the morphology of the Gymnosperms, I find this sentence, based on a study of *Pinus Laricio:* "In June the archegonia are ready for fertilization, which occurs about the first of July, at least twenty-one months after the first organization of the ovule." This by a very simple mathematical calculation places the "organization of the ovules" on October 1.

I have not only been unable to detect the pistillate cones before the approach of winter, but in the tiny cones of *Pinus rigida*, *P. austriaca* and *P. montana uncinata*, fixed on March 14 there is not the least suggestion of ovules, the entire cone consisting in each case of a broad axis on the margin of which are slight elevations or papillæ — the beginnings of the bracts which subtend the ovuliferous scales (fig. 121, plate XII). The first indications of the ovules are found in these species about the last of April or the first of May. In material of *Pinus Strobus* fixed on May 31, 1898, the position of the ovule can be detected only by a slight bulge on the inner surface of the ovuliferous scale, the integument not yet having been differentiated. One week later, June 6, the ovule is

found fully organized and nearly ready for the reception of the pollen-grains (figs. 122, and 123). The evidence is conclusive that the ovules are not organized in the species of pines studied by the writer until about three weeks or less before pollination, and seven months later than in *Pinus Laricio* as recorded by Coulter and Chamberlain. This is the more surprising when we consider that *P. austriaca* is at least a variety of *P. Laricio*, and, according to some authorities, it is a synonym for that species.

It is not my purpose to enter into a discussion of the origin and cellular development of the female cone, nor yet of the homologies of its parts. These points have been fully investigated by Čelakovsky, who has frequently published papers on this subject from 1879 to the present time, and the many theories advanced by different writers regarding these structures have recently been brought together and reviewed by Worsdell ('00).

### FORMATION OF THE AXIAL ROW.

*The Macrospore-mother-cell.* — The origin of the sporogenous tissue from a hypodermal cell or cells was described by Strasburger for several Gymnosperms in 1879, and this idea without further confirmation has come down to the present time. While this may be true for many Gymnosperms, and possibly for *Pinus*, I find no evidence, direct or indirect, that the macrospore-mother-cell is derived from a hypodermal cell in the pines investigated. When the mother-cell is sufficiently differentiated to be distinguishable from the other cells of the surrounding tissue, it is found to lie deep within the nucellus; and there are no rows or axial strands of cells lying above it to suggest its derivation from a hypodermal cell. On May 8, 1902, the ovules of *Pinus rigida* were sufficiently developed to show clearly the separation into nucellus and integument, and a like condition was found to exist in *P. Strobus* on June 6, 1898. In both instances, so far as one is capable of determining, every cell of the nucellus is exactly like every other cell (fig. 123), and the same condition obtains in the other species at this time. One week later, as illustrated for *Pinus rigida*, the macrospore-mother-cell can first be distinguished, and the so-called spongy

tissue is clearly differentiated about it (fig. 124). The mother-cell in this instance has relatively the same position in the ovule as that shown in fig. 66, plate VI, which was taken from an ovule collected twelve days later. If this cell be the direct descendant of a hypodermal cell, it has now become deep-seated by the addition of cells above it; but there is nothing in the arrangement of the cells of the nucellus either before the appearance of the mother-cell or after it to denote such a course of development.

The mother-cell is first detected by its larger size and by its failure to stain as deeply as do the other cells of the nucellus. In the first stages of growth the nucleus almost fills the cell (fig. 125), and its weakened capacity for staining is doubtless due to its rapid growth without a proportional increase in the amount of nuclear substance. The nucleus contains in this young stage a delicate reticulum with a varying number of larger and smaller net-knots, and from two to four small nucleoli, not differing materially, except in size and staining power, from the nuclei of the adjacent tissue. This cell increases considerably in size before its division so that it becomes very conspicuous in the nucellus, its reticulum taking the chromatin-stains with greater avidity than at an earlier period. The season of growth for the macrospore-mother-cell may extend over about three weeks. The early stage shown in figs. 124 and 125 represent its size on May 15, 1902, and the spireme stage illustrated in fig. 126 indicates the condition of this cell on June 5 of the same year.

*First Division of the Macrospore-mother-cell.* — After the mother-cell has attained its full size, the reticulum of the resting nucleus gradually becomes more open, the chromatic granules become more prominent and there arises a beautiful, regularly moniliformed, more or less interrupted skein, but a true spireme is not formed until after synapsis (fig. 126). This somewhat branched thread is very delicate, the chromatic discs are uniform in size and distributed upon the linin with great regularity. It is probable that these apparently homogeneous discs, which have doubtless been derived from the fusion of the smaller chromatic granules, would, under

greater magnification, be resolved into slightly irregular and roughened bodies, as in the prophase of the heterotypical mitosis in the microspore-mother-cells, but with the powers of the microscope at my command, I have no evidence that such is the case.

The phenomenon of synapsis is as marked here as in the primary mitosis of the microspore-mother-cell, but the contracted mass is less dense, probably because of the smaller size of the nucleus and the consequent diminution in nuclear substance (fig. 127, plate XIII). With the recovery from synapsis the linin thread is seen to have increased in thickness, and the chromatin-granules are irregularly distributed upon the continuous spireme, which gradually comes to fill the entire nuclear cavity with its open uninterrupted coils (figs. 128 and 129). The chromatic substance again collects into definite areas of varying dimensions, which are united by clear portions of the linin-band, and the longitudinal splitting now becomes apparent. Condensation and segmentation follow, and the distinct chromosomes, in the reduced number, become evident (figs. 130, 132 and 133). The forms of the chromosomes are similar to those already described in connection with the division of the microspore-mother-cell (figs. 132–136). Because of the comparatively small size of these nuclei, the steps by which the irregularly shaped chromosomes are derived could not be traced with the same degree of confidence as in the microspore-mother-cell; but the entire phenomenon is such as to indicate very conclusively that the process is practically the same in both.

The spindle, at first a multipolar diarch, early becomes bipolar and during metakinesis it is very sharply so. The poles do not reach the walls of the cell, but a few threads sometimes radiate from them and extend to the ectoplasm. There may be a slight granular condensation in the neighborhood of the poles but it is never prominent and often does not appear at all. The chromatic segments become short and broad at the equatorial plate, and their separation into daughter-chromosomes presents the figure characteristic of the heterotypic division. Unsplit ends of the chromosomes extend outward in the plane of the equatorial plate, thus giving rise to dark clumps of chromatic substance along the median line (figs. 134–137). The

passage of the one-half chromosomes to the poles has not been observed. Resting nuclei are formed during the telophase of the mitosis, and a cross wall divides the mother-cell into two compartments (fig. 138).

From the foregoing it is evident that the first division which takes place in the macrospore-mother-cell is heterotypic in nature, and agrees in all essentials with the primary mitosis within the microspore-mother-cell. This is in accordance with the conclusions reached by all other investigators who have recently studied the tetrad divisions occurring within the ovules of various Phanerogams.

*Second Division of the Macrospore-mother-cell.* — Beginning with the telophase of the first division considerable variation may occur in the subsequent steps in the formation of the axial row. A cell-plate is always formed between the daughter-nuclei though it may remain very delicate, consisting of little more than a condensation of the ectoplasm. The daughter-cells may be very similar in appearance, excepting that the lower one is usually the larger, and in such instances both nuclei enter the resting stage, presenting a clear, definite reticulum (figs. 138, and 141). More often, however, the lower cell is much larger than the upper one and the nucleus of the upper cell does not enter into the complete resting stage, but early shows signs of disintegration. The chromosomes may unite to form a spireme as usual, but development may then cease without the organization of a network, and the diffuse reaction of the nucleus to stains shows that disintegration has begun (figs. 139, 140).

I have but a single preparation showing the second division of the macrospore-mother-cell, and I can therefore offer no conclusions of any value regarding the nuclear phenomena accompanying the mitosis. From this figure it appears that the spindle originates as a multipolar diarch as in the first division, and both nuclei in this instance are dividing at the same time. During the initiation of the spindle the chromosomes are short and thick, somewhat irregular in outline, and apparently in the forms of U's, V's and rings. The reduced number of chromosomes occurs in both of the dividing nuclei (fig. 142).

The state of disintegration referred to above is always confined to the upper of the two daughter-cells and never occurs in the lower one, except in those cases in which the whole ovule is undergoing destruction. The lower cell invariably divides again and the basal cell thus formed constitutes, in every instance observed, the functional macrospore. The lack of constancy in the division of the upper cell would naturally give rise to some axial rows of four cells and some of three, and this is exactly what we find (figs. 144, 145, plate XIV). Fig. 143 shows the second division of the lower cell just completed, and it is evident from the structure and appearance of the uppermost nucleus that it would never have divided. In the axial row presented in fig. 144 some time has elapsed since the mitosis was completed, as evidenced by the increase in size of the lowest cell of the row. The upper of the two cells formed as a result of the first mitosis still remains undivided, and, moreover, it would not have divided later, judging both from its appearance and from the fact that the rapid growth of the initial cell of the female gametophyte would soon have been instrumental in effecting its obliteration. Juel ('00) finds that these cells do not divide simultaneously in *Larix*, but he does not find the division completed in the lower cell before it begins in the upper one. In the single preparation showing the second division in the macrospore-mother-cell, both nuclei are dividing, and both are in the same stage of the prophase, but this does not necessarily mean that when both cells divide they always do so synchronously. This lack of uniformity in the number of cells in the axial row is not peculiar to *Pinus;* it has been observed by many investigators in a large number of plants including both Gymnosperms and Angiosperms.

Coulter and Chamberlain ('01) figure an axial row of four cells in *Pinus Laricio*, and, as above indicated, such an axial row is frequently met with in the species of pines which I have studied, but it is much more common in *Pinus austriaca* than in the other species (figs. 145, plate XIV, 142, plate XIII, and 261, plate XXIII). There is no doubt whatever, after a study of many preparations showing the axial row, that in the great majority of cases in *Pinus Strobus* and *P. rigida* the upper cell remains undivided and that the usual axial row in these species

consists of three cells. The axial row represented in fig. 144, for instance, is a beautiful object, clearly and definitely differentiated from the surrounding tissue, yet there is not the least ground for supposing that the upper cell has ever divided. Such a figure as this represents the characteristic axial row in *Pinus Strobus* and *P. rigida*, while the axial row of four cells illustrated in fig. 145 is typical for *P. austriaca*. This point has not been sufficiently studied in the two other species to admit of generalizations for them. The axial row, then, varies from three to four cells in the same species, but there is a tendency in some species to form three and in others to form four cells.

*Significance of the Tetrad Division Within the Ovule.* — We have observed that at a certain point in the development of the ovule in *Pinus* a centrally located cell becomes differentiated from those surrounding it by its greater size and the more vacuolate character of its cytoplasm. This cell after undergoing a period of growth and rest gives rise to the reduced number of chromosomes by a peculiar method of division known as the heterotypical division, and this mitosis, as is characteristic in spore formation, is quickly followed by a second division, at least in the lower cell. The basal cell resulting from this last division passes through a season of growth extending over several weeks, as we shall shortly see, and finally, by repeated divisions, gives rise to the female gametophyte. The process of division is in all essentials exactly similar to that which takes place within the microspore-mother-cell, and results, as there, in spore-formation. Nuclear phenomena attending the early development of the female gametophyte have not been carefully investigated until comparatively recent times, but wherever studied the conclusion has been unhesitatingly drawn that in the ovule, as within the anther, a true spore-formation takes place.

The essential character of a spore is, manifestly, not that it should have a certain arrangement relative to its sisters within the mother-wall, neither is the presence or absence of a wall of vital importance to its existence unless, indeed, the spore is to be disseminated. Rosenberg ('01) finds the pollen-grains to be filiform in *Zostera* and arranged side by side; Strasburger ('01)

and Gager ('02) show that the descendants of a pollen-mother-cell in *Asclepias* have a linear arrangement; while Juel ('00) discovers that in the *Cyperaceæ* three young pollen-grains or microspores abort and the fourth remains permanently within the microspore-mother-wall. Yet from the standpoint of origin alone, no one hesitates to call the young pollen-grains of these plants microspores. Juel ('00) affirms that the heterotypic division must be the criterion by which we decide whether or no we have a true tetrad-division, and he concludes that in *Larix* the embryo-sac-mother-cell is homologous with a spore or a microspore-mother-cell. Schniewind-Thies ('01) reaches the same conclusion for Angiosperms; and Lloyd ('01) asserts that the division of the embryo-sac-mother-cell in the *Rubiaceæ* is a true tetrad-division, and the four resultant cells are spores. Other instances where similar conclusions have been reached might be cited, but the above is sufficient to demonstrate that the most recent studies along this line point conclusively to a normal spore-formation within the ovule, and do not confirm Campbell's ('02) statement that a true tetrad-division is usually absent in the ovule of spermatophytes.

For many years botanists have been involved in a contention regarding the true nature of the embryo-sac in Phanerogams. A paper was published by Atkinson in 1901 reviewing the interpretations made by earlier writers and suggesting as a solution of the difficulty that spores, no longer being necessary in the higher plants, had dropped out of the cycle of development in these plants. That is, the female gametophyte arises in the higher plants without the intervention of spores. While the results of recent investigations do not serve to strengthen this view, the theory is a most interesting one and the paper has further served an excellent end in stimulating thought and research along this line. Mottier observed one instance in which the first division of the embryo-sac-mother-cell was homotypic, or, if we use Strasburger's ('00) term adopted throughout this paper, typical, and the number of chromosomes was not reduced. Juel found the same to be true normally in *Antennaria alpina*, a species of *Antennaria* in which the embryo develops parthenogenically. In both instances we have an

illustration of development within the embryo-sac without the intervention of a spore, but these are apparently isolated and exceptional cases.

The whole difficulty seems to me to lie in the fact that all along we have been endeavoring to make a morphological unit out of that which is primarily a physiological unit, and not necessarily a morphological one, although it may be so. It has been shown conclusively that in *Larix* and *Pinus* among the Gymnosperms a true macrospore is formed which germinates within the macrosporangium and gives rise to the female gametophyte — both a morphological and a physiological unit. But as we advance to the Angiosperms there is a shortening of ontogeny in the female gametophyte, the most extreme case being represented by *Lilium*. Mottier ('98) demonstrated the fact that the division of the embryo-sac-mother-cell in *Lilium* is a true tetrad division and we cannot, therefore, it seems to me, escape the conclusion that the resultant four cells are spores. But once rid ourselves of the idea descended from Hofmeister, that the mother-cell of the embryo-sac is always a macrospore, and the product of its development, therefore, always a single gametophyte, and many difficulties vanish. Lloyd ('02), in his recent discussion of this subject, accepts the heterotypical division as the criterion for spore formation, and then explains the condition in *Lilium*, where the first four cells of the embryo-sac are spores, by "regarding the gametophyte as *an individual by coalescence*." It appears to me not only more simple but more plausible to consider that we have here four gametophytes each reduced to two cells. The embryo-sac is still here as elsewhere (with the exception of parthenogenic plants), a physiological unit whose function is to give rise to a new plant through the sexual process, but it is morphologically a complex made up of several individuals. Whether all eight cells thus formed are considered as potential eggs is immaterial, practically, but one retains the power to respond to the sperm-cell, though the others have been shown to be capable of fertilization in some instances. Ordinarly, however, they remain sterile and have come to have a vegetative or nutritive function only. All work together for one end and in that sense may

make "an individual by coalescence," that is, they are physiologically one.

This is not the place to enter into a detailed discussion of the homologies of the embryo-sac, but I believe that the suggestion herein made will form an interesting working basis, and it may bring us nearer to a true conception of these structures than we have yet attained. But whatever our opinion regarding the elements within the embryo-sac, it is clear that we cannot longer use the terms macrospore and embryo-sac interchangeably as so many writers have done. We now know that a tetrad division may occur within the ovule and it has been shown that the embryo-sac may result from the germination of a single macrospore, that it may be formed directly from the macrospore-mother-cell, or that it may have its origin in one of the daughter-cells formed as the result of the heterotypical division. In any case would it not be far less confusing if we should designate the multicellular bodies, developed within the macrosporangium and the microsporangium of the higher plants, as embryo-sac and pollen-grain, or female and male gametophyte, respectively, and should retain the terms macrospore and microspore for the true spores in their one-celled stage?

### LATER HISTORY OF THE AXIAL ROW.

*The Fate of the Upper Cells.* — Whether the number of cells in the axial row of *Pinus* be three or four the female gametophyte is always the product of the lowest cell. Very shortly after the second division is completed, the upper cells of the axial row give evidence of disintegration, while the basal cell increases much in size, its nucleus becoming very large. The nuclei of the four spores in *Larix* are very similar, Juel ('00), fig. 18, but in *Pinus* the basal cell is markedly different from the others at a very early date (figs. 144, 145, plate XIV). The upper cells of the axial row gradually disintegrate, and are crowded to one side by the growth of the macrospore, remaining for a time as deeply staining, amorphous masses which finally disappear altogether (figs. 69, plate VI and 147, 148, plate XIV). Instances in which one of the upper cells of the axial row in Angiosperms becomes the functional macrospore

are not rare. Campbell ('00) has recorded such a condition in the *Araceæ*, Lloyd ('01) in certain *Rubiaceæ*, and Karsten ('02) in the *Juglandaceæ*. But, so far as investigated, the sequence of events following the establishment of the axial row in the *Abietineæ* results in the obliteration of all but the lowest cell. I have avoided using the term "potential macrospore" in connection with the upper cells of the axial row, because the upper of the two cells first formed does not always divide and in such instances it cannot properly be designated as a spore since development ceased before spore formation was completed.

*Growth of the Macrospore.* — Starch is sometimes found within the cells of the axial row, though never in such abundance as in the cells of the adjacent tissue (fig. 143). It may become very abundant within the macrospore during its period of growth, and is sometimes found pressed so closely against the nucleus as to actually produce indentations in its membrane (fig. 146).

The reticulum of the nucleus of the functional spore is very scanty during its growth period, but later it presents the appearance of an ordinary resting nucleus. The cytoplasm, never abundant, forms at an early date a loose, granular network. Later the nucleus is connected with the ectoplasm by delicate strands which are gradually withdrawn into the peripheral cytoplasm, until there is thus formed in the one-celled stage a definite layer of cytoplasm lining the wall of the macrospore, and inclosing a large central vacuole. The nucleus moves to one side of the cell, usually the upper side, imbeds itself in the cytoplasm and awaits further development (figs. 147, 148).

The organization at so early a period of this definite peripheral layer of cytoplasm has not, I believe, been demonstrated for any of the other Gymnosperms. Finding the cavity containing the developing endosperm crossed by irregular strands of cytoplasm as illustrated in fig. 70, plate VI, I had the impression for a long time after these studies were begun, as stated in an earlier paper (1901[3]), that such a condition, as that described above for the resting macrospore, did not obtain until the beginning of the second period of growth. This layer of cytoplasm is very easily displaced by the action of the fixing fluid, but with care it may be obtained in an apparently normal con-

dition. I now have an abundance of preparations which show not only that the wall layer is instituted in the one-celled stage, but that it persists as long as free cell-formation continues in the endosperm. The only reference which I find regarding the establishment of the wall-layer of cytoplasm in any of the Gymnosperms is the following statement made by Coulter and Chamberlain ('01), with reference to *Pinus:* "Probably when but two or three free nuclei have appeared the nuclei become imbedded in a parietal, cytoplasmic layer."

## SUMMARY.

The female cones can be distinguished early in March, excepting in *Pinus Strobus* where they do not appear until the very last of April. The ovules cannot be detected until about three weeks before pollination.

There is no evidence that the macrospore-mother-cell arises from a hypodermal cell. When first differentiated it is centrally placed nearer the chalazal end of the ovule.

The division of the macrospore-mother-cell is a true tetrad-division and the cell which gives rise to the female gametophyte is a true spore.

Of the two cells formed as a result of the heterotypic division the lower one always divides again, the upper one may. An axial row of three cells seems to be the rule in *Pinus Strobus* and *P. rigida*, and one of four cells the rule in *P. austriaca*, though neither is constant in any of the species. The lowest cell of the axial row always becomes the functional macrospore.

The two or three upper cells of the axial row begin to disintegrate very soon after they are formed and are finally absorbed by the enlarging macrospore.

The lower cell passes through a long period of growth during which the cytoplasm is withdrawn from the central portion of the cell and forms a uniform layer lining the wall of the macrospore. The nucleus moves towards the upper side of the cell and imbeds itself in the peripheral layer of cytoplasm.

The suggestion is made that the embryo-sac may or may not be a morphological unit, but that it is essentially a physiological unit, existing for the purpose of sexual reproduction. Such a

conception of the embryo-sac seems to the writer to form a more satisfactory basis for a rational explanation of the structure, or composition, and homologies of the embryo-sac than do any of the existing theories regarding the nature of this body.

## CHAPTER IV.

### The Female Gametophyte.

#### DEVELOPMENT OF THE PROTHALLIUM.

*The First Period of Growth.* — We are indebted to Hofmeister ('51) for our first definite knowledge regarding the life history of the female gametophyte in the Gymnosperms. It is true some errors in observations were made, but they were intermingled with much that has stood the test of the most modern research. In 1879 Strasburger declared the "transitory endosperm" described by Hofmeister to be a fallacy, but he himself fell into quite as grave an error, though in the opposite direction, when he stated that the primary nucleus of the embryo-sac remained undivided during the first year, an observation since corrected by himself.

As already stated, the young macrospore immediately organizes a peripheral layer of cytoplasm and passes through a period of growth which continues for six weeks or more. The degree of development which has been attained by *P. austriaca* on June 13, 1898, is shown in figs. 145 and 147; the first division of the macrospore-nucleus in this species occurred on July 29 of the same year, as illustrated in figs. 149 and 150. The germinating macrospore had now enlarged to such an extent that it was found necessary to reduce the scale of magnification at this point so that a comparison of the figures does not present, visually, the amount of growth which ensues between the organization of the macrospore and its first division. *Pinus* differs substantially in respect to the very marked growth of the macrospore before the first division of its nucleus from *Larix* where two nuclei are formed before there is any considerable increase in size of this cell (Juel ('00) plate xv, figs.

18–20). The persistence of the potential megaspores in *Larix* at this time is also in very striking contrast to *Pinus*, where the other cells of the axial row have become entirely absorbed before the germination of the macrospore occurs (figs. 147–149).

The third division of the macrospore-mother-cell, or the first division of the macrospore-nucleus, takes place during the very last of July or the first of August in all the species studied, and is of the ordinary or typic method. It differs from the mitoses occurring in the vegetative tissue of the sporophyte only in presenting the one-half number of chromosomes (fig. 150). The daughter-nuclei may remain at one side of the prothallial cavity, but more frequently they pass to opposite sides as in the development of the embryo-sac in Angiosperms (fig. 151). The second mitosis follows rather quickly, and is already completed in *Pinus Strobus* on August 4 (fig. 152). Nuclear divisions follow until several free nuclei have been formed. The observations of Strasburger ('79), and of all later students of the Gymnosperms, upon the simultaneous division of the free nuclei of the endosperm have been confirmed. On October 12, 1898, sixteen nuclei were observed in the cytoplasmic layer, all being in the spireme stage of division. On October 15 of the same year sixteen nuclei, all presenting the equatorial plate-stage of mitosis were found in the cytoplasm of the prothallium, (figs. 153–155). The karyokinetic figure is sharply bipolar, each pole ends in a slight condensation of the cytoplasm, and the chromosomes are clearly of the reduced number.

I find no evidence that any further divisions occur during the first period of growth and it is probable that the thirty-two nuclei which result from the division just described pass into the resting stage and remain inactive during the winter. But I have not examined a sufficiently large number of preparations with this point in mind to affirm that the prothallium of *Pinus* invariably enters upon its long period of rest in the thirty-two nucleated stage. The number may not be fixed even in the same species, but it is certain that it is never large. The prothallium, therefore, at the close of its first season of growth is a spherical body composed of an ectal layer of cytoplasm in which are imbedded, in many instances at least, thirty-two free

nuclei. This thin cytoplasmic shell encloses a large central vacuole which is reported by Strasburger, Arnoldi and others to be filled with a fluid substance. I have made no observations regarding the cell-sap of this large vacuole and can neither affirm nor deny its presence.

*The Second Period of Growth.* — It has been seen that the ovular development in *Pinus* is very slow during the period immediately subsequent to pollination, but with the renewal of growth in the spring development becomes much more rapid. Coördinately with the enlargement of the ovule already described, the endosperm cavity increases in size until it occupies almost the entire basal and central portions of the nucellus, presenting in longitudinal section the figure of an ellipse (fig. 71, plate VI). The thin peripheral layer of cytoplasm with its free nuclei persists until the latter part of May, and free nuclear division continues to take place within it until a large number of nuclei are formed. Jäger ('99) estimated that there are 256 free nuclei formed in *Taxus*, and Hirase ('95) made the same observation in *Ginkgo*. The number is certainly much larger in *Pinus*. More than 500 free nuclei are present early in May and about 2,000 have been counted in *Pinus Strobus* at the time when the nnclei are being separated by the development of dividing walls.

The free nuclei are considerably larger in surface view than the nuclei of the nucellar tissue, but in side view they often appear somewhat flattened. They have the structure of typical resting nuclei (figs. 156–159, plate XV). Each contains, almost invariably, two rather large nucleoli surrounded by clear areas. The reticulum is close and studded with irregular granules, but the net-knots are not so prominent as in the nuclei of the nucellus. They simulate very closely the nuclei of the sheath-cells at certain stages in the development of the archegonia. The cytoplasm in surface view presents a pseudo-alveolar structure consisting of a coarse, granular reticulum enclosing numerous vacuoles (fig. 156). During the late telophase in the division of the free nuclei of the prothallium the complicated karyokinetic figure characteristic of free nuclear division becomes very conspicuous, and is evidently formed as a result of the rearrangement of the cyto-reticulum (fig. 159).

At some time during the latter part of May in *Pinus Strobus* and about the middle of the month in the other species free nuclear division ceases and cell-walls are developed between the nuclei. The development of the prothallium from this point on was studied by Sokolowa ('80), and her observations have in general been confirmed by all more recent writers, with the exception of Jäger ('99) in *Taxus*. I find the development of cell-walls in the prothallium of *Pinus* to agree perfectly in its early stages with that described by Sokolowa. Walls are formed perpendicular to the wall lining the prothallial cavity, thus each nucleus with its proper portion of the cytoplasm is separated from all the other nuclei. No wall is laid down on the inner sides of these cells, so that in radial section the cells appear as uncovered boxes, the opening extending towards the center of the prothallial cavity. In surface view the cells are more or less isodiametric, polygonal in outline and very uniform in size. A layer of densely reticulated cytoplasm surrounds each nucleus, and delicate strands radiate from it to the ectal layer of cytoplasm, thus giving a very different aspect to the cytoplasm than it had prior to the development of cell walls (figs. 160 and 161). Jäger described the presence of walls on the inner face of these cells in *Taxus* when the cells were first organized, but other students have not confirmed his observations.

According to Sokolowa these cells grew inwards forming long open tubes which extended to the center without division, a wall was then formed at the inner end and the cells became divided by cross walls. To these long cells the name alveoli was applied. Only those from the sides extended clear to the center before being closed, those from the extremities becoming more or less wedge-shaped. Jaccard ('94) notes that in *Ephedra* some of the alveoli may divide before reaching the center, but many do not, while Arnoldi ('99 and '01) finds that no division occurs in *Sequoia* until after the alveoli have met at the center and their ends have become closed by walls. The development subsequent to the formation of the open cells varies considerably in *Pinus* from that described by these writers for other Gymnosperms. No cell has ever been observed to extend from the

circumference to the center of the prothallial cavity. The cells are long, it is true, the walls delicate and wavy in outline, but a ring of tissue composed of longer or shorter cells is formed rather early in the inward growth of the prothallium. The cells of the innermost row always remain open on their outer free sides, their cytoplasm is more abundant than in the other cells of the prothallium and their nuclei invariably retain a position near the open side of the cells (fig. 162). As observed by Jaccard ('94), and Jäger ('99), the nuclei of the prothallium cease to divide synchronously after individual cells have been organized. When the center is reached the cells close and thus, one year after pollination, the endosperm becomes a solid mass of tissue.

The prothallium grows rapidly after it has become a continuous cellular body and in a few days it fills all the central and lower portion of the ovule. Above it is the prominent nucellar cap, while only a few cells of the nucellus remain along the sides separating the gametophyte from the integument (fig. 73, plate VII). Cell-divisions continue to take place, and the cytoplasm becomes more abundant, though the prothallial cells are never richly supplied with cytoplasm. Strasburger ('80), Jäger ('99), and several more recent students have noted many nuclei in the endosperm cells. I have not observed multinucleated cells in the prothallium of *Pinus* up to the time when the suspensor has elongated and carried a several celled embryo to a considerable depth into the endosperm. Later stages than this have not been studied. There is often an appearance of more than one nucleus in a cell, but careful study never fails to demonstrate a delicate cell-wall between the nuclei. At an early stage in prothallial development the cell-walls are very delicate, scarcely more than condensations of the ectoplasm, so that they might easily be mistaken, in *Pinus*, for strands of cytoplasm. Doubtless the cells become plurinucleated during a more advaned stage in embryo formation.

### THE SO-CALLED SPONGY TISSUE.

*The First Period of Growth.* — When the macrospore-mother-cell first becomes apparent it is surrounded by a group

of cells, three to five cells in thickness, which are more or less clearly delimited from the surrounding tissue by their slightly larger nuclei, their somewhat radial arrangement about the macrospore-mother-cell as a center, and, in some instances, by a rather indefinite and broken space which separates this group of centrally lying cells from the adjacent nucellar tissue (fig. 124, plate XII). At the close of the tetrad-division these cells have become much more conspicuous by the increase in the size of their nuclei, the somewhat greater density of their cytoplasm, and by the presence just exterior to them of an interrupted layer of tabular cells which are evidently undergoing disintegration. The disintegrating cells usually appear on one side first then at other points about equally distant from the young gametophyte (figs. 66, 69, plate VI; 124, plate XII, 148, and plate XIV). It was to this tissue, immediately surrounding the young endosperm, together with the disintegrating cells just exterior to it, that Strasburger gave the name "spongy" tissue, and for convenience I shall use this term in speaking of it.

Ovules are frequently found during the summer and fall which, so far as external appearances go, are perfectly normal, but, when prepared for study, reveal the fact that either the macrospore-mother-cell has never divided or the macrospore, if formed, has not developed. Such ovules do not renew their growth in the following spring. In those cases in which the development of the mother-cell or of the young gametophyte is arrested, very characteristic changes occur in the spongy tissue. These cells grow and become rich in cytoplasm even when the mother-cell does not divide, or when the macrospore fails to germinate. But after a time they, too, become inactive, their cytoplasm is gradually lost, their nuclei become dense and deeply staining, and their cell-walls are very greatly thickened (fig. 163, plate XV). This state of disintegration may enter in at any time during the first period of growth but it is more common before any divisions have occurred in the macrospore. When the mother-cell fails to divide, the cells of the spongy tissue may grow until they almost equal it in size before showing signs of breaking down. In such instances they bear a very striking resemblance to the mother-cell, and might easily

be taken by one not familiar with the history of this tissue for a group of macrospore-mother-cells (fig. 168, plate XVI). In fig. 148, plate XIV, the slightly reduced cytoplasm of the cells of the spongy tissue and the prominence of their cell-walls are sure evidences that pathological conditions have entered in, though all other parts of the ovule are still perfectly normal the process of disintegration having only just begun. Had this ovule been left in connection with the sporophyte for a longer time, the spongy tissue would undoubtedly have assumed later the character shown in fig. 163.

It is this abnormal appearance which I believe led Hofmeister to conclude that there were two prothallia formed in the pines, one for each season of growth. Strasburger thought that Hofmeister mistook the normal spongy tissue for endosperm, and Coulter and Chamberlain have recently expressed the same view. Now the walls of the normal spongy tissue are never thickened but remain even less prominent than those of the nucellus. Hofmeister was surely too accurate a student of cells as cells to have fallen into such an error. It is a well-known fact that many ovules are organized in *Pinus* that never reach maturity and they are very frequently found in the autumn and late winter in the condition just described; but with the renewed growth of the healthy ovules in the spring, these fail to develop farther and are soon detected by their smaller size. Shortly afterward they become brown and dead. Having found this thick-walled abnormal condition in the autumn and winter, and in the spring finding within the ovules then developing the large central cavity, it is not surprising that Hofmeister should have concluded that a thick-walled transitory endosperm was formed in the fall.

*The Second Period of Growth.* — When growth is renewed in the spring the cells of the spongy tissue become organized for the first time into a definite zone from two to three cells thick which forms a hollow prolate spheroid immediately surrounding the endosperm, and limited on its outer surface by a thin stratum of disintegrating nucellar tissue. The cells and their nuclei are not only somewhat larger than those of the nucellus, but their most distinguishing characteristic is to be found in the greater

density of their cytoplasm which is almost identical with that of the prothallium, while the cells of the nucellus are scantily supplied with cytoplasm. These cells divide karyokinetically, and, as they increase in number, they press against the adjacent cells of the nucellus which become flattened against this constantly advancing tissue, and are absorbed, only to give place to other cells which meet a similar fate. Sometimes absorption seems to precede the outward march of the spongy tissue, so that this tissue is separated from the normal nucellus by a clear space made up of cells of the nucellus which have lost all their protoplasmic content, but which have not as yet suffered collapse (figs. 157, 158, plate XV). The parietal layer of cytoplasm which constitutes the endosperm remains always in closest contact with the inner surface of this tissue (fig. 71, plate VI).

The cells of the spongy tissue are still prominent when the endosperm becomes a solid multicellular body. Soon afterwards, however, they show signs of disintegration, and at the time of fertilization they have, as a rule, entirely disappeared as cells, only the remnants of the cell-walls remaining. The spongy tissue is then represented by a deeply staining fibrous body of no definite structure which persists between the gametophyte and the nucellus (figs. 162, plate XV, and 72, plate VI; 73, plate VII).

*The Nature and Function of the Spongy Tissue.* — The prominent character of the cells surrounding the prothallium in certain Gymnosperms has been commented upon, in a general way, by all students of the *Abietineæ;* but, as was noted by the writer in 1900 and 1901 and confirmed by Coker in *Taxodium*, 1902, the true nature and function of these cells seem to have escaped entirely the notice of previous writers, as they have invariably been described as tissue showing evidence of breaking down. After a preliminary note regarding the nature of this tissue was sent to press in 1900, Lang ('00) described a similar layer of cells about the endosperm in *Stangeria*. He designated them as sporogenous cells and "possibly tapetal in nature."

As recently stated (1903),[1] these cells may possibly represent sporogenous tissue, each cell being a potential macrospore-

[1] See note at end of Appendix.

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mother-cell, but there is no evidence from the standpoint of origin that such is the case in *Pinus*. They arise directly from a nucellus in which a few days before their appearance every cell was apparently like every other cell. This alone is not conclusive, as the functional macrospore-mother-cell has a similar origin, so far as one can see. But, what is more conclusive, the divisions in this tissue are according to the typic method and present the number of chromosomes characteristic of the sporophyte (figs. 164–167). If these cells were once, in some remote ancestor, sporogenous in nature, they have entirely lost their primitive function and have acquired a new and important function in connection with the development of the endosperm. This is not then a layer of disintegrating tissue, as described by all earlier students of the *Abietineæ*, but rather, as already noted by the writer (1901[2]) a definite zone of physiological tissue which is intimately connected with the nutrition of the young gametophyte. It doubtless not only passes on to the endosperm the nutrititive substances derived from the nucellus, but is itself active in the manufacture of food, as numerous starch grains are often found within its cells. It is probable, too, that it performs an important mechanical rôle in the way of protection. It not only forms a support for the prothallium in its multinucleated state, but gradually receding, it pushes before it, as it were, the tissue of the nucellus thus making room within for the growth of the delicate gametophyte.

Though we now know that this is a far more important tissue than it was formerly thought to be, it does not seem to me wise to apply to it the name tapetum or to suggest a new name by which to designate it. Strasburger's term "spongy" tissue, although given when the nature of this tissue was not understood and being a misnomer so far as its structure and function are concerned, has obtained a wide usage in the literature of the Gymnosperms, and should be retained, just as the term cell is still retained in all biological literature.

### DEVELOPMENT OF THE ARCHEGONIUM.

*The Early Growth of the Archegonium.* — The archegonia first become apparent during the latter part of May or the very first

of June, the time varying somewhat with the species and with the season. The degree of development which the prothallium has attained when the archegonia-initials make their appearance also varies not only in the different species but in the same species. The differentiation of the archegonia may be deferred until the prothallial cells have united to form a continuous tissue; but it quite as frequently happens that, while there still remains a comparatively large, open space at the center of the prothallial cavity, certain cells at the micropylar end of the prothallium divide by periclinal walls more rapidly than do the other cells of the endosperm and become comparatively rich in cytoplasm; several of the superficial cells in this region do not so divide, but continue to grow, and are distinguished from the adjacent cells by their greater size, larger nuclei and more vacuolate cytoplasm. These are the initial cells of the archegonia (fig. 162, plate XV, and 169–171, plate XVI).

In less than a week after an archegonium-rudiment has appeared, and while it is still quite inconspicuous, it divides, giving rise to a small upper cell, the mother-cell of the neck, and a large, lower cell which forms the venter of the archegonium (figs. 171, 172, plate XVI). The small cell immediately divides by an anticlinal wall, and the two cells thus formed divide by walls that are perpendicular to the first, the resulting four cells all lying in the same plane. These constitute what may be called the normal neck in *Pinus Strobus* (figs. 173, 177, 180). Considerable irregularity in the number and arrangement of the neck-cells has, however, been noted even within the same species. Frequently two of the four cells divide again, as figured by Strasburger for *Pinus Strobus* in 1869, the six cells being arranged in a single layer (figs. 178, 183, plate XVI, and 212, plate XIX). Occasionally all four cells divide by anticlinal walls, the neck then consisting of eight cells, all of which lie in the same plane (figs. 179, plate XVI, and 213, plate XIX). In rare instances the four cells divide by periclinal walls, when the eight cells which compose the neck of the archegonium are disposed in two tiers of four cells each (fig. 187, plate XVII). This last represents the structure of the neck in *Pinus sylvestris* as figured by Mottier ('92) and Blackman ('98), and it is evi-

dently the usual condition in *P. austriaca*, *P. rigida* and *P. resinosa*, but in these species, too, much variation obtains. Variation in the number of neck-cells seems to be of common occurrence in the Gymnosperms. It was first noticed by Hofmeister in 1851 and has recently been discussed by Coulter and Chamberlain ('01). Murrill ('00) has figured considerable irregularity in the number and arrangement of these cells in *Tsuga*, while Coker ('02) shows a very marked variation in *Podocarpus*.

At first the growth of the central cell is not followed by a corresponding increase in the amount of protoplasm, so that its cytoplasm early presents a very vacuolate appearance. There may be one large, irregular central vacuole, or delicate strands of cytoplasm may extend out from the nucleus to the ectoplasm, these strands meeting and fusing at irregular intervals to form vacuoles of various sizes. Thus a very beautiful pseudo-alveolar structure is presented. Webber ('01) describes the cytoplasm in the central cell in *Zamia* as representing at this time a foam structure of great beauty. I have never observed in this or any cell in *Pinus* a cytoplasmic structure which, according to my interpretation, could be designated as a true alveolar or foam structure in the sense in which Bütschli ('94) uses the term. As the central cell continues to enlarge its cytoplasm begins to develop more rapidly, many strands extending out into and across the vacuoles. Thus the size of the vacuoles is decreased while their number is greatly increased. The central vacuole, if present, may persist for a considerable time, or it may be replaced at once by smaller vacuoles (figs. 172–175). Gradually the cytoplasm becomes more dense, and the vacuoles, receding from the periphery of the cell, especially from its base and sides, disappear last from its upper portion (figs. 176, 177). When the ventral canal-cell is cut off, the vacuoles have nearly or quite been replaced by a finely granular cytoplasmic reticulum in which a greater or less number of larger, more deeply staining granules are imbedded. These granules are frequently surrounded by a clear court into which the protoplasmic network has not extended. The number of the so-called proteid vacuoles is usually small at this time (fig. 178).

The nucleus of the central cell attains full size very soon after its formation. It has a delicate, more or less interrupted reticulum, and is characterized by a large vacuolate nucleus which invariably occupies a central position. One or two smaller nucleoli may also be present. This nucleus always remains close beneath the neck-cells, as is the case in other Gymnosperms, and, as a rule, is more or less concave on the side toward these cells (figs. 172–177, 181–183). As Blackman has pointed out, the vacuolate nature of the cytoplasm renders this nucleus very liable to displacement during the early stages in the development of the archegonia, yet with well fixed material it is always found in its normal position. Hirase ('95) states that certain granules, which appear in the cytoplasm just beneath the nucleus of the central cell in *Ginkgo*, have been derived from this nucleus or from its nucleolus. Ikeno ('98), also, describes the nucleus of this cell in *Cycus* as giving out a granular substance during its growth period. No comparable phenomenon has been observed in connection with the nucleus of this cell in the species of pines which I have studied, but, as above stated, the nucleus quickly reaches its mature size and remains apparently unchanged until the inception of its division.

Very early in the history of the archegonium, the cells immediately surrounding it become differentiated from the adjacent endosperm-cells by their more regular form, the greater density of their cytoplasm, and the increase in the size of their nuclei. Thus a distinct sheath is formed about the venter of the archegonium. This sheath usually consists of a single layer of cells. It is more conspicuous in *Pinus resinosa* than in the other species, and may become two cells broad at certain points, but even here it is never two layered to any considerable extent. The nuclei of these cells divide as the archegonium increases in size, the axes of the spindles being always parallel with that face of the cell which is adjacent to the egg. All the sheath-cells of a given archegonium have several times been observed in the same stage of mitosis, but this is very exceptional as these cells do not ordinarily divide simultaneously. The sheath-cells persist until after fertilization when they gradually lose their cytoplasm and resemble the other cells of the prothallium.

Where adjacent archegonia crowd against each other these cells early become distorted and partially destroyed. It is often difficult to demonstrate the presence of cross walls in the archegonium-sheath. Neither have I been able to satisfactorily demonstrate the presence of pores in the wall separating the sheath-cells from the egg. Hofmeister ('61–'62), Goroschankin ('80, '81), Arnoldi ('00), and Coulter and Chamberlain ('01) all describe this wall in *Pinus* as thick and furnished with pores; but if such is the case it is not apparent in my material. On the contrary the wall seems very thin and is scarcely differentiated from the ectoplasm. It may be that further search on my part will reveal both the "pits" and the "thickened wall," but thus far I have not detected either.

No special attempt has been made to count the number of chromosomes in the nuclei of the various parts of the sporophyte and gametophyte, but whenever a nucleus was observed in which the chromosomes were particularly clear and distinct their number was always noted. In such cases twelve chromosomes have invariably been counted in the nuclei of the sheath-cells. Chamberlain ('99) has found the same number in the corresponding cells of *Pinus Laricio*. The early development of the archegonium, as just described, agrees in the main with that given by Strasburger in 1878.

As the archegonia grow the prothallium also continues to increase in size, several layers of cells being formed above the archegonia, except over their neck-cells. Here no prothallial tissue is laid down, so that there arises an opening in the endosperm leading from the neck-cells of each archegonium to the nucellar cap (figs. 177–180). The presence of funnel-shaped openings leading from the nucellus to the archegonia-necks in *Pinus* was noted by Hofmeister in 1851 and their origin was correctly described by him in 1862. In the last stages of prothallial development preceding fertilization, the sides of this tubular cavity often become very closely crowded together so that the passage is obscured.

The number of archegonia in a single ovule varies in *Pinus Strobus*, *P. rigida* and *P. resinosa* from one to five, the usual number being three. In *Pinus austriaca* and *P. montana* var.

*uncinata* the number is larger, averaging about five. As many as nine have been observed in a given prothallium in *Pinus montana* var. *uncinata*. The form of the mature egg depends largely upon the number and arrangement of the archegonia. When there are not more than two or three, as is frequently the case in *Pinus Strobus*, they may become almost spherical in outline.

*Division of the Central Cell.* — As the central cell prepares for division the cytoplasm between its nucleus and the neck-cells is apparently resolved into fine granules, and there is a more or less pronounced condensation of the cytoplasm about the lower side of the nucleus. At the same time the nucleolus disappears wholly or in part, the nuclear reticulum becomes more open and broken, and the chromatin collects or condenses at various places on the network (fig. 182). Soon a clear court, similar to that described by Hof ('98), Fulmer ('98), Nemec ('98 and '99), Strasburger ('00) and others, makes its appearance along the lower half of the nucleus. Inasmuch as this nucleus is pressed close against the neck-cells such a court does not arise along its upper side (figs. 183, 184). Delicate, granular threads cross this court and press against the nuclear membrane, while at the same time the upper and lower surfaces of the nucleus become irregularly indented (fig. 185, plate XVII). As the chromatin condenses to form the spireme, an achromatic network, as already described for the corresponding stage in the division of the generative nucleus in *Pinus*, becomes apparent in the nuclear cavity (figs. 182–185). When the spireme is fully established it presents a beautiful moniliform appearance, and the longitudinal splitting of the band becomes apparent at some points. The threads which arose earlier in the cytoplasm seem at this time to have been again resolved into granules (fig. 186). Whether any of them enter the nuclear cavity and contribute to the formation of the achromatic spindle has not been definitely ascertained. The spindle, when formed, lies wholly within the area previously occupied by the nucleus. Webber ('01) finds the origin of the spindle in the division of the generative cell in *Zamia* to be intranuclear. Farmer and Williams ('96 and '98) ascribe such an origin to the spindles studied in

the *Fucaceæ*, and spindles of intranuclear origin have been described by others. But while the achromatic figure in the division of the central cell in *Pinus* comes to lie completely within the nucleus, I would not claim that it is wholly of nuclear origin; if such were its source, the cytoplasmic activity in connection with this division would be inexplicable. The earliest stages in spindle-formation in this mitosis have not been observed as yet, but when the transitional steps between the phases represented in figs. 186 and 187 have been observed we shall doubtless find that the cytoplasm has had some part to play in the institution of the spindle. During the early metaphase of the division the nuclear membrane can still be distinguished, and clearly consists of a weft of threads (figs. 187, 188). I have not observed any phenomenon in the prophase of this mitosis at all comparable with the beautiful figure shown by Murrill ('00), as illustrative of the prophase of the division of the central cell in *Tsuga*.

When the spindle arises, it is "multipolar in an axial plane" and thus corresponds, with slight variation, to the mitotic figure described by Duggar ('00) in the microspore of *Symplocarpus fœtidus*, and by Wiegand ('99) in the microspore of *Potamogeton foliosus*. In *Pinus*, however, the upper extremities of the threads do not at first unite into groups, but remain practically free, and are closely pressed against the neck-cells (fig. 187). The several poles, formed at the inner or lower extremity of the karyokinetic figure, soon draw together forming a single, very sharply defined pole; or the fully developed spindle may remain more or less truncate at its lower end. Blackman describes this spindle as bluntly truncate at both extremities. I have frequently observed such a spindle during a late anaphase of the division, but this is only one of the various aspects which may be presented during metakinesis and later stages in this mitosis. The upper extremities of the achromatic spindle-fibers may never draw together at all; they may unite to form two or more poles; or they may give rise to one pole which may be blunt or very slender (figs. 190–194). But whatever form may be assumed by this spindle during the later stages in its development, there is always formed, at an early period, a diarch spindle

which is multipolar at one extremity and monopolar, or nearly so, at the other (figs. 187, 188). A similar figure is also organized in the mitoses which occur in the development of the pollen-grain, and at an early stage in the division of the generative nucleus in the pines, as already described in this paper; and it is suggested that such a figure may be characteristic, at least in the higher plants, of those indirect divisions which result in the formation of nuclei or cells of unequal size.

The chromosomes, when oriented at the nuclear plate, are invariably in the form of U's or V's. Blackman states that they are straight rods but he does not so figure them. The cell-plate, during the early stages in its formation, lies midway between the developing nuclei, but when the daughter-nuclei are fully formed, the nucleus of the oösphere is, as a rule, farther removed from the cell-plate than is the nucleus of the ventral canal-cell. A prominent cell-plate is formed and the plane of cleavage separating the ventral canal-cell from the egg becomes evident in many instances before the disappearance of the spindle. As Chamberlain ('99) has shown, the lower portion of the spindle at this time is ordinarily convex, while the part within the ventral canal-cell is concave (figs. 195–197, plate XVII, and 200, 201, plate XVIII).

I was able in several preparations similar to that illustrated in fig. 191 to count the number of chromosomes, and twelve or thirteen were found in both groups instead of eight as counted by Dixon ('94).

*The Ventral Canal-cell.* — According to my observations, a definite wall, separating the canal-cell from the egg-cell, is always formed in *Pinus*. Coker has made the interesting observation that no wall is developed in *Podocarpus*, the nucleus of the ventral canal-cell lying free in the egg.[1] As a rule the nucleus of the ventral canal-cell in *Pinus* does not present a normal appearance, but shows signs of disintegration very early in its history. It is doubtful, in some cases, if a nuclear membrane is ever formed, and there are probably instances in which fusion of the chromosomes never takes place at all. The karyokinetic structure shown in fig. 193 would very presumably

[1] See note at close of Appendix.

give rise to such a nucleus, if we may so denominate it, as that illustrated in the ventral canal-cell of fig. 196; although Blackman, judging from such a figure as that portrayed in fig. 194, considers it impossible that the chromosomes of the ventral canal-cell should ever fail to fuse. The nuclear membrane, when present, very soon breaks down, and the chromatic substance becomes scattered throughout the cell (figs. 198–202). This cell immediately preceding and at the time of fertilization ordinarily forms a deeply staining mass which lies just beneath the neck-cells and above, but in contact with, the egg (figs. 180, plate XVI, 202, plate XVIII, and 213, 215, plate XIX). Rare exceptions to the rapid disintegration of the canal-cell have been observed and will be described in the appendix to this paper. But in the study of several thousand archegonia of *Pinus Strobus* no instance has been found in which the nucleus of the egg and of the ventral canal-cell were similar in form. The nearest approach to a normal nucleus that has been observed in the ventral canal-cell of this species is that shown in fig. 197, plate XVII. Occasionally this cell is somewhat enlarged and is furnished with a rather scanty amount of cytoplasm in which distinct chromosomes, or chromatic figures of various forms are imbedded. Of the many variations that have been found to occur in the structure of the ventral canal-cell in the mature archegonium but two have been illustrated — figs. 198 and 199, plate XVIII. It is probable that in such instances a true nucleus has ever been formed if, indeed, the chromosomes have fused at all. The character of the cell at this time is such as to preclude the possibility that a division of this cell is being initiated. There seems to be a definite relation between the structure of the ventral canal-cell and the character of the upper part of the mitotic figure formed in the division of the central cell. This is plainly demonstrated by a comparison of figs. 190 to 197, plate XVII, and 200–202, plate XVIII. Figs. 190, 193, 196 and 202 represent an especially interesting series.

The separation of the canal-cell from the cytoplasm of the oösphere, as Strasburger ('72) and Blackman ('98) have described in *Pinus*, is, I believe, due to a shrinkage of the egg-cytoplasm caused by imperfect fixation; and it is possible that a similar appearance in *Cycas*, Ikeno ('98), has a like origin.

## MATURATION OF THE EGG.

*The Descent and Growth of the Egg-nucleus.* — The egg-nucleus is no sooner formed than it begins to increase in size, becoming greatly enlarged even before the disappearance of the spindle-fibers (figs. 196–202). As the nucleus moves toward the center of the oösphere, threads of more or less delicacy extend, in a radial manner, from its wall into the surrounding cytoplasm. These fibers are not equally well defined in all preparations, but, whatever the degree of their prominence, they are invarably more strongly differentiated about the upper side of the nucleus, and may extend from the nucleus to the top of the egg (figs. 202–204).

As already stated, few, if any, vacuoles persist within the the venter of the archegonium at the time of the division of the central cell. Following their disappearance, there arise numerous spherical bodies, the so-called proteid vacuoles. Coördinate with the downward movement of the egg-nucleus, these bodies assume a position about the periphery of the oösphere, more especially at its base (the organic apex of Strasburger), and at its sides (figs. 179, 180, plate XVI, 214, plate XIX). Under a low power, the cytoplasm of the mature egg appears dense and finely granular; the "proteid vacuoles" do not seem to differ materially from the protoplasm in which they are imbedded; and many deeply staining granules are scattered throughout the cell. With greater magnification, however, a very beautiful, granular reticulum becomes apparent. There is no suggestion of the alveolar structure described by Bütschli ('94). At times this reticulum is everywhere crossed by short fibers which have no definite arrangment and are, apparently, not confined to any fixed period in the history of this cell (fig. 200). The spheres in the outer and basal portions of the cytoplasm are resolved into very complex structures which, although they simulate the appearance of nuclei, could never be mistaken for such bodies by one familiar with cell-structures (figs. 202, 203.)

No cytoplasmic radiations, similar to those described by Belajeff ('91) in *Taxus baccata*, and by Dixon ('94) in *Pinus sylvestris*, have been observed in connection with the fully

developed egg-nucleus in any of the species of pines which I have studied.

During the growth and downward movement of the egg-nucleus, it never presents, in *Pinus Strobus*, a definite network, such as is observed in the nucleus of the ordinary resting cell; but it is characterized at a very early date by an open, interrupted reticulum, on which are arranged irregular granules of various sizes. This meshwork may be extremely delicate; it may assume a heavy appearance; or it may become very much interrupted and broken, many detached portions lying loose within the nuclear cavity (figs. 196, plate XVII, to 205, plate XVIII). The egg-nucleus of *Pinus austriaca* and *P. montana* var. *uncinata*, may frequently show from an early date a beautifully regular reticulum (fig. 269, plate XXIV). Nucleoli have rarely been observed in this nucleus in *Pinus Strobus* during the first stages of its development (figs. 196, 199 and 200–201); but in *Pinus austriaca* they occasionally arise very early (fig. 195). When the nucleus has attained considerable size, small, nucleolus-like bodies, containing a single central vacuole, appear in connection with the nuclear net; and at the same time a slightly larger nucleolus is observed in the lower part of the nucleus, usually in connection with its membrane (fig. 202). As the nucleus continues to grow, this nucleolus also increases in size, gradually becoming large and very vacuolate (figs. 203–205).

When the egg-nucleus reaches maturity, it has attained huge dimensions, and its outline, depending on the form of the egg, is spherical or elliptical. The nucleolus, if demonstrable, is always found in the lower part of the nucleus; and there are usually several smaller bodies, designated in this paper as secondary nucleoli, scattered throughout the nucleus (fig. 205). These secondary nucleoli are invariably found in connection with the reticulum, but, as Montgomery ('98) believed regarding apparently similar structures, they are probably caught in, not vitally united to it. They may be present in great abundance, or they may be entirely absent from the nucleus. The reticulum, on which the chromatic substance is disposed, presents numerous aspects, as already indicated in the description of this nucleus during its period of growth. Under very high magni-

fication, it does not show, in normal conditions, a true granular structure; but it may present a most delicate, interrupted, granular network; or, it may consist of large, irregular, diffusely-staining masses which are united into an imperfect reticulum (figs. 206, *a*, and 206, *g*). In the latter instance the chromatic granules are either too minute to be distinguished, or they have been dissolved in the linin ground-work. The linin, always very abundant in this nucleus, may form heavy hyaline cords, on which the chromatin is collected at irregular intervals (figs. 206, *e*, and 206, *f*); but it more often consists of less conspicuous strands (figs. 206, *b*, to 206, *d*). Great as are the variations in the structure of this nucleus, its chromatin has always been found, in the species of pines which I have studied, to exist either in the form of irregular granules of varying sizes, or apparently dissolved in the linin. Such a resolving of the chromatin into nucleoli as that described by Chamberlain ('99) in *Pinus Laricio* and illustrated in his figs. 14 and 15 has not been observed in normal nuclei by the writer.

Whether the various appearances presented by the egg-nucleus represent normal phases in its life history, or whether one is normal and the others are artifacts resulting from the action of fixing agents, is, of course, a mere matter of conjecture. But, inasmuch as these different aspects are characteristic of this nucleus during its period of growth, also after it has to all appearances reached maturity, and again at the time of its conjugation with the sperm-nucleus, it seems reasonable to conclude that all are normal and correspond to definite physiological processes, which take place within the nucleus. Hertwig's ('98) interesting experiments on fed and unfed *Actinosphærium* are in point here. They seem to show conclusively that the structure of a nucleus varies with the character of the work which is being done by it.

Strasburger ('84) described the nucleus of the oösphere in the *Abietineæ* as being densely filled with a granular substance which entirely obscured or masked the chromatin. This substance he called metaplasm, and virtually considered the nucleus a vacuole filled with a nuclear sap capable of taking up or elaborating this material. Ikeno found a similar substance in the sexual nuclei

in *Cycas* in 1898 and more recently in *Ginkgo* ('01), and Arnoldi ('00) in *Cephalotaxus*. Blackman ('99) devoted several paragraphs to a discussion of metaplasm, as it manifested itself in the egg-nucleus of *Pinus sylvestris*. He found that it was present in the young nucleus in the form of granules, but that it later united with the chromatin to form the nuclear reticulum. Chamberlain ('99) does not recognize the presence of this substance in the egg-nucleus in *Pinus Laricio*; and there is no evidence of its existence in the sexual nuclei of the species of pines which I have studied.

According to Wilson ('99) "protoplasmic substances represent the active, metaplasmic structures the passive elements" of the cell. During the development of the egg-nucleus in the species of pines which have formed the basis of these studies, there is never any deposit within the normal nucleus of a granular substance; but the linin, as already stated, becomes very abundant. Just what proportion of it is active in cell division, we are unable to say. Without doubt a large part of the linin merges into the cytoplasmic network during the first segmentation of the oösphere-nucleus, but even so, it can not be classified with the passive elements of the cell.

Blackman ('98) wrote: "The stage in which the nucleus is found in a position between the apex and the center of the egg is rarely met with"; and Chamberlain ('99) stated "that in over three hundred preparations, less than a dozen" show early stages in the development of the egg-nucleus. During the course of these investigations upon the pines, about four thousand preparations, representing many thousand archegonia, have been studied, and no developmental stage has been more frequently met with than that by which the nucleus assumes its central position in the egg. Such an appearance as that illustrated by Chamberlain in his figs. 18 and 19 has been observed in both the young and the mature egg-nucleus, in the conjugating nuclei, and also in the various nuclei of the proembryo. They have been wholly disregarded in the present discussion of the maturation of the egg, for, in my material, these figures, and also Blackman's figure 11, would be interpreted as representing disintegration stages. Every step has been repeatedly traced from

the ordinary nuclear reticulum, to nuclei which can scarcely be distinguished from the surrounding cytoplasm, and then to archegonia, which appear perfectly normal except that no nuclei can be demonstrated within them. It is a well known fact, already commented upon in this paper, that the number of seeds derived from a pine cone is very small in comparison with the number of ovules formed in the same cone. An examination of fresh material shows that development may cease at any point between the early stages in the formation of the ovule and the last steps in the ripening of the seed. This cessation of growth effecting first individual cells does not at once become apparent, and so cannot be avoided, in its earliest stages, when one is putting up material for cytological work. Under such conditions, it is inevitable that, with a limited amount of material, the abnormal will be interpreted for the normal.

The entire development of the archegonium in *Pinus* is passed through in about two weeks, probably not more than five days elapsing between the cutting off of the ventral canal-cell and fertilization. In *Pinus montana* var. *uncinata* these processes are apparently much more closely united in point of time, as the pollen-tube, in some cases, has reached the endosperm before the division of the central cell is complete (fig. 207, plate XIX).

*The Proteid Vacuoles.* — The true nature of the proteid vacuoles is a subject which attracted my attention very early in the course of these investigations. There can be no doubt that there is an intimate relation between the sheath-cells of the archegonia in the pines and the substance of the egg, such as is believed to exist between the follicle-cells and the egg in animals. But the exact nature of this connection in *Pinus* is not easily determined. I have rarely examined a preparation showing archegonia without studying the relation of the sheath-cells to the oösphere; and yet no entirely satisfactory evidence, because not demonstrable beyond a question, of the origin and nature of the so-called proteid vacuoles has been found.

Hirase ('95) observed that the granules in the egg of *Ginkgo* were of nucleolar origin, being derived both from the nucleus of the central cell and from the nuclei of the sheath-cells.

Arnoldi ('00) found that substantially the same thing was true in *Cephalotaxus*. He was not able to detect the passage of the nucleoli from the sheath-cells into the egg, but, since these granules were present on both sides of the membrane of the egg-cell he accepted the fact of their transference. I have frequently seen a nucleolus partly without and partly within the nucleus of a sheath-cell; but in no instance could I be sure that such a condition was not the result of mechanical displacement.

Ikeno ('98) found direct evidence that the nutritive spheres in *Cycas* are of nuclear origin. But no such phenomena as he observed in *Cycas* occur in *Pinus*. Platner ('86) described the passage of the follicle-cells into the ovum in *Helix*, and a few other such instances have been recorded in animals. Arnoldi ('00) has recently noted a most remarkable migration of whole nuclei from the sheath-cells into the egg in several species of pines. He has observed, in a single series, as many as one hundred and fifty nuclei passing into the ovum. From the fact that Arnoldi writes "*Strobus*" in a parenthesis after *Pinus Peuce*, I infer that he employs the terms as synonyms; but I find no authority for such a usage, and cannot accept his conclusions as holding good for *Pinus Strobus*. It does not seem possible that, in a careful examination of several thousand archegonia, so obvious a phenomenon as that described by Arnoldi could have escaped detection; and I must, therefore, conclude that it does not take place in the species of pines which I have studied. I fully believe that the sheath-cells play an important rôle in the nutrition of the egg; but it is the method by which this is accomplished, as described by Arnoldi, that I cannot accept for the species of pines studied. Coulter and Chamberlain ('01) not only accept Arnoldi's observations for *Pinus* but describe a like phenomenon in *Cycas*. Basing their statement on the results of Ikeno's studies, they record, on page 22, the following surprising fact with reference to *Cycas:* "The contents of the jacket-cells, nuclei and all, now pass through the pores into the central cell." I find no authority for such a statement in Ikeno's paper. If I correctly translate the German, Ikeno describes neither the transmission of the nucleus nor of the cytoplasm from the sheath-cells into the egg,

but he does note a most interesting transfer of nuclear substance, that is, a substance *secreted by the nuclei*, from the nuclei of the sheath-cells into the cytoplasm of the egg. In the course of his discussion Ikeno says: "Bemerkenswerth ist es ferner, dass der Zellkern der Wandungszelle häufig sich der Centralzelle nähert und dort einen nach dem nächsten Plasmafaden gerichteten kurzen Schnabel bildet (fig. 6). In einen andern Fall beobachtete ich, das der Zellkern der Wandungszelle sich bis an die Cellulosemembran begiebt, welche an die Centralzelle angrenzt und mit dem ganzen Körper an diese sich anlegt (fig. 7, *a*, *b*). Offenbar sollen alle diese Vorgänge den Uebergang des in diesen Zellkernen enthaltenen Stoffes nach der Centralzelle erleichtern." So far as I am aware then, Arnoldi is the only investigator who has observed the passage of entire nuclei into the egg in the Gymnosperms.

Some interesting observations have been made during this study regarding the nature of the nucleolus of the egg-nucleus. As already indicated this nucleolus does not arise in *Pinus Strobus* until the egg-nucleus has attained considerable size. It appears in the lower part of the nucleus as a minute, solid, spherical body; during growth a small central vacuole appears, then other vacuoles, until, at maturity, it is completely filled with vacuoles of various sizes (figs. 202–205, plate XVIII). A limiting membrane is not always apparent in this nucleolus (fig. 208, plate XIX; but in some instances, there seems to be very strong evidence of such a membrane (figs. 205 and 209). In fig. 205 the nucleolar wall has been broken at one place and a vacuole, lying near the point of rupture, has been indented along its outer surface, thus becoming crescent shaped. Montgomery ('98) sounded a word of warning against interpreting the peripheral stratum of the ground substance of the nucleolus as a wall layer; and there is a possibility that, in the figures above referred to, what appears like a limiting membrane is only the outer unmodified portion of the nucleolus.

The attitude of this nucleolus toward dyes varies much at different periods in its history. It may or may not take the safranin stain characteristic of Flemming's triple combination; it may stain intensely with gentian-violet or iron hæmatoxylin

(figs. 205 and 208); it may show a weak reaction to these stains (fig. 209), or it may be absolutely unaffected by them, remaining as a hyaline or greenish yellow structure (fig. 210). When the nucleolus resists the action of dyes, its nucleus is usually totally free of the secondary nucleoli, which have been described in connection with the maturation of the egg-nucleus, and the cytoplasm of the egg is studded, to an unusual degree, with large, deeply staining granules. But the nucleus containing a nucleolus which stains with avidity, generally contains, also, innumerable secondary nucleoli; at the same time, there are comparatively few deeply staining granules in the cytoplasm of the egg.

The position of the secondary nucleoli with reference to the primary nucleolus is frequently such as to indicate that the former originate in the latter (figs. 227, plate XX, and 208, plate XIX). The only observations which would militate against such an origin are the few cases found in which the secondary nucleoli seem to appear earlier than the primary nucleolus (fig. 195, plate XVII). It may be that, in these cases, the primary nucleolus has not yet become differentiated in structure from the secondary nucleoli, as would evidently be true in a stage slightly younger than that shown in fig. 202, plate XVIII; or it may be true that the primary nucleolus is present, but fails, at this time, to stain. Floderus ('96) describes a somewhat similar origin of the paranuclei, in Tunicates, from the nucleolus proper.

The nuclei of the cells surrounding the archegonia contain from three to five nucleoli, and one or more nucleolus-like structures may be present in the cytoplasm of these cells. Each nucleolus is surrounded by a clear court which, as Zimmermann ('96) has pointed out, is evidently not an artifact. Debski ('97) opposes this view, however, and considers the clear court to be attributable to the shrinkage of the nucleolus, since he does not find it when material is treated with xylol instead of cedar oil. These nucleoli may be spherical, elliptical, irregular, or long and almost dumbbell-like in outline. The ordinary cells of the prothallium do not show nucleoli. If such bodies be present in these cells they are small and obscured by the nuclear reticulum.

At about the time of the cutting off of the ventral canal-cell many small nucleolus-like masses appear in the nuclei of the sheath-cells — twenty or more occurring in a single nucleus. When the egg has reached maturity, and during the later stages of its history, no nucleolus, or but one or two nucleoli, can be demonstrated in the nucleus of a sheath-cell. These nucleoli are no longer surrounded by a hyaline court, but are imbedded in the chromatic network.

The nucleoli of the sheath-cells present the same attitude toward stains as does the nucleolus of the egg-nucleus. But while the nucleoli of the sheath-shells frequently stain but feebly they rarely fail entirely to stain.

Similar color reactions have been observed in connection with the nucleoli, as already described, in the microspore-mother cell of *Pinus*. The occurrence of unstained nucleoli in the same nucleus in which others were deeply colored is common in the microspore-mother-cells especially at about the time of synapsis. I am aware that conclusions based upon staining reactions alone are not to be trusted, but when accompanied, as here, with other phenomena they may be highly significant.

The nucleolus of the egg-nucleus and also the nucleoli of the sheath-cells in *Pinus* appear to represent active portions of the cell rather than inert masses of matter. Certain aspects presented by these nucleoli are surely suggestive of plastids. The uncolored framework of the egg-nucleolus reminds one very strongly of a chlorophyll body from which the pigment has been extracted. Yet we would not, in the present state of our knowledge, denominate them plastids. I believe, however, although the phenomena are not of such a nature as to admit of definite demonstration, that the nucleolus of the egg-nucleus, and also the nucleoli of the sheath-cells are actively engaged in the formation of a substance which in the egg-nucleus, at least, assumes the shape of secondary nucleoli. These nucleoli become diffused throughout the nucleus, from which they pass, probably in solution, into the egg cytoplasm. Here they are again differentiated, and by a gradual development, give rise to the "proteid vacuoles" or nutritive spheres of the oösphere. It may be that the greater size of the egg-nucleus, in com-

parison with that of the sperm-nucleus, is correlated with the physiological rôle, as above suggested, which it plays in the cell. We cannot, here, enter into a discussion of the voluminous literature dealing with the origin, function, and destiny of the nucleoli; but a few of the many views which have been advanced may be noted.

Strasburger ('95, '97 and '00) expresses his conviction that nucleolar substance contributes to the formation of spindle-fibers. A similar view is held by Fairchild ('97), Harper ('97), Debsky ('97), and other students of the Bonn Laboratory, and by Nemec ('99), Farmer ('94) and others. Strasburger ('95) also sees indications of a connection between the nucleolus and the cell-plate and he has recently ('97 and '00) sought to show that the nucleoli make active the spindle-forming substance in the cytoplasm, or that they enhance the activity of the kinoplasm.

Flemming ('82), Humphrey ('94), Zimmermann ('95), Sargant ('96 and '97), Duggar ('99), Mottier ('00), and many others believe that the nucleoli represent reserve supplies of chromatin. Dixon ('99) finds in them a vehicle of inheritance. Hirase ('98) thinks that they give rise to the attractive spheres; and according to Karsten ('93), Lavdowsky ('94) and Wilcox ('95) they are centrosomes. Rosen ('95) considers that the nucleoli are equal in dignity to the chromatin, that they have no connection with the centrosome and that they do not serve to nourish the chromosomes.

Jordan ('93) states that "their function is almost certainly one of nutrition either concerned in the storage or elaboration of nutritive material" and believes that there is substantial reason for looking upon the nucleolus wherever found as concerned in one way or another with the active metabolism of the cell. Lukjanow ('88) and Macallum ('91) consider the nucleoli to be excretory organs which are intimately related to the nutritive spheres of the egg, these spheres arising through a process of deposition from the nucleolus. And Häcker ('93) observes that the nucleolus is a contractile vacuole which absorbs proteid substances; the absorbed materials undergo a chemical change within the nucleolus and are then periodically discharged.

Flemming ('82), Zacharias ('85) and Zimmerman ('93) ascribe to the nucleolus the dignity of a nuclear organ; and Montgomery ('98) makes the following suggestion: "That though the nucleolus consists of substances which stand in some relation to the nutritive processes of the nucleus, and so, at the time of its formation, may be a functionless inert mass of matter, yet it may at later periods in the history of the resting nucleus, acquire some active function, and thus gradually come to acquire the value of a nuclear organ."[1]

Obst ('99) remarks that the significance of the nucleolus is truly dark, but he considers it to be in some way the result of chemical action whose cause must be sought in the physiological processes of the cell. A glance at the theories regarding the nature of the nucleolus as briefly outlined above is certainly sufficient to confirm Obst's conviction that our knowledge of the origin and function of the nucleolus is still very imperfect. Yet it cannot be doubted that we have in the nucleolus not merely a mechanical store-house, but a structure which is intimately connected with the vital activities of the cell. We still have in the nucleolus a most attractive field for investigation, and the best cytological, physiological, and microchemical technique must be brought to bear upon the problem before we can hope to understand aright the true nature of this structure.

*The Receptive Vacuole.* — Immediately preceeding fertilization a cavity appears in the egg-cytoplasm, just beneath, or in the near vicinity of, the neck-cells (figs. 211, 213, 214, plate XIX). In some cases this opening may not arise until the instant of fertilization. This cavity, which was thought by the earlier writers to represent the lower portion of the pollen-tube within the oösphere, has been explained by Blackman ('98) as due to the sudden inrush of the contents of the pollen-tube, and by Arnoldi ('00) in *Cephalotaxus*, as caused by the downward movement of the conjugation-nucleus. Shaw ('98) suggests that the concavity in the upper part of the egg in *Onoclea*, just prior to fertilization, may correspond to the receptive spot; and there is every evidence that in *Pinus* this opening in the cyto-

[1] See note at close of Appendix.

plasm represents the last act of the egg in its preparation for the reception of the sperm-nucleus. If it were formed by the movement of nuclei or other bodies through the protoplasm, we should expect the cytoplasm to draw together again, as during the downward movement of the egg-nucleus; but, in reality, this opening persists throughout the entire later history of the archegonium. Following fertilization it is sometimes found at one side or a little below the neck of the archegonium. This position is doubtless due to displacement at the time of conjugation (fig. 215). The regular clear outline of this cavity, together with the fact of its presence in the unfertilized as well as in the fecundated egg, warrants one in considering it a definite character of the mature oösphere.

I have suggested the name, receptive vacuole, for this vacuole which is such a constant feature of the egg at the time of fertilization and immediately prior to conjugation. The pollen-tube suddenly empties into the archegonium a large amount of material — several nuclei, a comparatively large amount of cytoplasm (see pollen-tube, fig. 120, plate XII, and fig. 214, plate XIX) and considerable starch. The sudden acquisition of this matter by an already densely filled egg might from the increased pressure alone, cause fatal results. That the egg should thus prepare for the reception of the sperm-cell is not only a very beautiful, but a very interesting illustration of the economy so often observed in nature.

## Summary.

After a period of growth the macrospore germinates and by a typic division gives rise to the first two nuclei of the female gametophyte. These usually pass to opposite poles of the prothallial cavity and soon divide again. Divisions follow rather leisurely during the fall, all the nuclei dividing synchronously. After thirty-two or more free nuclei are formed the long period of rest is entered upon.

In early spring nuclear division is resumed and a large number of nuclei are formed; about two thousand have been counted at the time when cell-walls are first laid down.

Walls are first developed in the prothallium during the latter

part of May. The nuclei are thus separated, but no wall is formed over the inner surface of the prothallium so each nucleus is, as it were, enclosed in an open box. These cells stretch out toward the center but never reach it without having first divided by cell-walls from two to several times. The innermost layer of cells always remains open on its free side until the cells meet in the center and the endosperm becomes a continuous cellular body.

The spongy tissue becomes apparent as soon as the macrospore-mother-cell is differentiated, but it is not organized into a definite zone with sharply defined limits until the beginning of the second season of growth.

These cells function as a physiological tissue of great importance in the nutrition of the young gametophyte. They doubtless convey nutrition derived from the disintegrating adjacent nucellar tissue to the endosperm and are also occupied in the manufacture of food materials. The spongy tissue doubtless further serves to protect the young prothallium, not only by affording support but by driving out, as it were, the nucellar tissue, thus making room for the delicate female gametophyte.

The time at which the archegonia appear varies somewhat, but in general they can first be detected about two weeks before fertilization. They are normally found at the micropylar end of the prothallium, and arise by the differentiation of certain of the peripheral cells. By the later growth of the female gametophyte, the mature egg is sunk to a considerable depth in the prothallial tissue, but there always remains an open channel leading from the neck-cells to the nucellar cap. The number of archegonia varies in the different species from one to nine. When the number of oöspheres formed is small they are almost spherical in outline; but this shape may be greatly modified according to the number and arrangement of the archegonia.

In *Pinus Strobus* the typical neck of the archegonium consists of four cells, all lying in the same plane, while in *Pinus austriaca* and *P. rigida* it is made up of eight, disposed in two layers of four cells each; but there is a lack of uniformity both in the number and in the arrangement of these cells, not only in different but in the same species.

The central cell is very vacuolate at first, its nucleus always remains close beneath the neck-cells and is more or less concave on the side toward those cells. When the ventral canal-cell is cut off, about a week before fertilization, the vacuoles have nearly disappeard from the venter of the archegonium.

The spindle in the division of the central cell arises as a multipolar diarch figure and apparently lies wholly within the nucleus. That portion of the mitotic figure which gives rise to the ventral canal-cell varies much in the later stages of its development; but, whatever irregularity characterizes this part of the spindle, it always becomes monopolar or nearly so, at its lower, inner extremity.

The form and structure of the nucleus of the ventral canal-cell are very variable, and are correlated with the irregularities occurring in the upper, outer portion of the achromatic spindle during the division of the central cell. There are probably instances in which no membrane is developed about this nucleus: in such cases the chromosomes never fuse to form a network. The ventral canal-cell rarely presents the appearance of a normal cell; at the time of fertilization it usually persists as a small, somewhat crescent-shaped, deeply staining body which lies just beneath the neck-cells of the archegonium and above, but in contact with the cytoplasm of the egg.

As the egg-nucleus assumes its central position in the oösphere, it increases much in size, and many fibers arise in the cytoplasm surrounding it. These threads have, in general, a radial arrangement and are more prominent along the upper side of the nucleus. The structure presented by the growing, and also by the mature, egg-nucleus may vary from a most delicate network bearing minute granules to an interrupted, imperfect reticulum composed of large, irregular, diffusely staining elements. These various aspects are doubtless the expressions of the different physiological activities with which this nucleus is concerned. The normal egg-nucleus has one large, vacuolate nucleolus and a variable number of small, secondary nucleoli. There is no evidence of the presence in this nucleus of a special metaplasmic substance.

During the maturation of the egg, many nutritive spheres

arise in its cytoplasm. At first these are irregularly scattered throughout the cell, though more prominent at its periphery; in the mature egg, they are largely confined to the peripheral portions of the lower half of the cytoplasm. It is suggested, though not definitely demonstrated, that these nutritive spheres are the products of nucleolar activity, having originated within the nucleolus of the egg and the nucleoli of the sheath-cells.

The egg-cytoplasm presents a delicate reticulum, in which, at times, fibers occur. Immediately preceding fertilization, an opening arises in this cytoplasm, just below or in the near vicinity of the neck-cells. This cavity is apparently formed for the reception of the sperm-cell, and the name "receptive vacuole" has been applied to it by the writer.

## CHAPTER IV.

### Fertilization and Related Phenomena.

#### CONJUGATION.

*The Coming Together of the Gametophytes.* — When the time for fertilization arrives the pollen-tube has forced its way between the neck-cells of the archegonium and stands just above the egg (fig. 120, plate XII), but it does not under normal conditions enter the archegonium. The fact that the pollen-tube in *Pinus* does not penetrate the egg has recently been observed by Blackman ('98), and Coulter and Chamberlain ('01). Standing just above the egg, the apex of the tube is ruptured and almost all of its contents passes into the cytoplasm of the egg. The sperm-cell with its dense cytoplasm and two nuclei, the tube-nucleus, the stalk-cell, a part of the cytoplasm from the pollen-tube, and some of the starch grains from the male gametophyte can all be distinctly recognized in the upper part of the oösphere (figs. 212–215, plate XIX). Dixon ('94) noted the passage into the oösphere of the four nuclei of the pollen-tube, but he could not distinguish between these after their entrance into the egg. Blackman confirmed Dixon's observations as to the passage of these nuclei into the oösphere and believed that the cytoplasm

of the "sperm-cells," passed into the egg along with the sperm-nuclei but he was unable to demonstrate the fact. There can be no doubt that the cytoplasm of the sperm-cell enters the egg in *Pinus* (fig. 212). This cytoplasm very soon fuses with that of the egg and the larger sperm-nucleus moves towards the nucleus of the oösphere; the other elements from the pollen-tube remain for some time in the upper part of the ovum. There is no evidence that the sperm-nucleus increases in size after entering the oösphere; neither is their an increase in stainable substance, but, on the contrary, the nucleus loses its dense structure; and occasionally a nucleolus becomes apparent within it. (Compare the sperm-nuclei in figs. 212 and 213 with those in figs. 215–223, *a*.)

*Union of the Sexual Nuclei.* — There is every indication that the movement, within the egg, of the sperm-nucleus which becomes active in fertilization is both rapid and direct. It almost invariably traverses the shortest distance between its point of entrance into the egg and the egg-nucleus. The relative position which the conjugating nuclei may occupy with reference to the major axis of the oösphere varies considerably, but always bears a definite relation to the position of the neck cells. When these cells are directly above the center of the oösphere, the sperm-nucleus comes into contact with the upper part of the egg-nucleus (figs. 214, 217, 218, 221, and 223, *a*); but if the neck be eccentrically placed, the sperm-nucleus will be found against one side of the oösphere nucleus (figs. 216, 219, and 220). I have not observed the male nucleus beneath the egg-nucleus as figured by Coulter ('97) in *Pinus Laricio*. Neither is there a bulging of the egg-nucleus towards the sperm-nucleus, nor do the sexual nuclei ever approximate in size as shown in this same figure of Coulter's, but a somewhat similar figure has been observed in *Pinus Strobus* after the first division of the "segmentation-nucleus." Schaffner ('96 and '97) also notes a bulging of the nucleus of the oösphere towards the male nucleus in *Alisma* and in *Sagittaria*, but, as will be shown presently, the exact converse of this is true in the pines which I have investigated. The sperm-nucleus is usually described as being more dense than the egg-nucleus at the time of their conjugation, and I have sometimes found this to be the case in *Pinus;*

but as a rule, the conjugation-nuclei in the pines, as observed by Arnoldi ('00) in *Cephalotaxus*, differ in size only (figs. 215–223, *a*).

Just before the sexual nuclei come into contact, the side of the egg-nucleus adjacent to the sperm-nucleus becomes slightly concave (fig. 216). This concavity is doubtless formed under the influence of the approaching sperm-nucleus and suggests the crater-like depression developed at an earlier period in the egg-nucleus of *Cycas* (Ikeno, '98). As noted by Blackman, the sperm-nucleus does not penetrate the membrane of the egg-nucleus, but it lies in a pocket-like indentation formed as a result of the contact of the two nuclei in the side of the oösphere-nucleus. Thus both nuclei though still perfectly distinct and lying side by side, come to occupy the space originally filled by the egg-nucleus. The sperm-nucleus, when in contact with the nucleus of the egg, ordinarily assumes the form of a biconvex lens, but it may vary much in outline, presenting in some cases the figure of a crescent, and in others, that of an ellipse. Occasionally it forms a deep, tongue-like depression in the nucleus of the oösphere (figs. 214–223, *a*).

### THE FIRST DIVISION FOLLOWING FECUNDATION.

*The Prophases of the Division.*—When the sexual nuclei come to lie in intimate contact, but are still, to all appearances, perfectly distinct, certain changes in their structure indicate that each is in the early prophase of division. The chromatin condenses or collects in irregular granules about the periphery of the sperm-nucleus, while that of the egg-nucleus is deposited just beneath the sperm-nucleus. The remainder of each nucleus is filled with a granular, achromatic reticulum of great beauty, reminding one of delicate frost work (fig. 224). This condition suggests an early stage of fertilization in the sea-urchin as described by Wilson ('95). Wilson thinks that the sudden increase in linin may be only apparent, resulting from the "rapid condensation and localization of the chromatic substance"; but he is inclined to believe that "a considerable portion of the chromatin breaks down at this time into linin." It would appear that the prominence of the achromatic reticulum in the conju-

gating nuclei of *Pinus* results from both these processes. For, while there is always a large quantity of linin in the egg-nucleus and a comparatively small amount of chromatin, the size of the chromatic spireme, when formed, seems disproportionate to the entire bulk of the chromatin earlier existing in the nucleus.

The chromatin continues to separate out from these nuclei until a spireme, studded with irregular granules, lies just within the wall of the sperm-nucleus, and a similar one arises directly below in the egg-nucleus. Frequently the cytoplasm caught between the two nuclei collects into spherical masses; between these spheres of cytoplasm the membranes of the two nuclei are in close contact (fig. 225). Very soon the spireme of each nucleus becomes coiled and regularly moniliform, and the chromatic band of the sperm-nucleus takes up a position along that side of its nucleus which is nearest to the spireme formed in the egg-nucleus. At this time, delicate, minutely granular threads, some of which pass from nucleus to nucleus, appear in the regions of the two chromatic spiremes. The rest of the achromatic contents of these nuclei is largely transformed into long, comparatively heavy threads, which are furnished with innumerable granules. The two nuclei are still perfectly distinct and the nucleolus of the egg-nucleus may persist at this stage; the nuclear membranes are yet present, although they are very irregular in outline and have given way at several points (fig. 227). The nucleolus is not always present at this time, but nucleolus-like masses, which from their position are evidently derived from the egg-nucleus, may be present as late as the telophase of the division. Delicate, granular fibers continue to arise in the regions of the two spiremes; the coarser, achromatic threads of the nuclei become finer in structure, and extend in all directions toward the forming spindle; and the nuclear membranes fade entirely out, not only along the line of contact of the two nuclei, but from their entire outer surfaces as well (fig. 227). Blackman states that, while the chromatic portions of these nuclei remain distinct in *Pinus sylvestris*, the nuclei fuse at an early stage in the prophase of the division. There is, apparently, no such fusion of the sexual nuclei in the species of pines studied by the writer; but the entire membrane of each

nucleus disappears during an early prophase of the mitosis, and the contents of the nuclei lie free in the cytoplasm of the egg. I have never found in the process of fertilization in *Pinus* any structure that could properly be designated as a fusion-nucleus. This is exactly comparable with what has been observed in the ovum of some animals, but has not been previously described for any plant. It might be noted that this conclusion was reached very early in the course of these studies, when the writer had read but little along cytological lines, and was not aware either that such a process was unknown in plants, or that a similar conduct of the sexual nuclei had been described by some writers on the animal side.

As the mitosis proceeds, the spindle-fibers continue to increase in number, becoming even more delicate in structure, and losing their granular appearance. The long, now quite delicate, but still granular, achromatic threads of the nuclei are very numerous, and many extend into the areas occupied by the chromatic spiremes. They probably feed the growing spindle, some of them, doubtless, being directly transformed into spindle, fibers. The chromatic bands have now become perfectly homogeneous. Before their segmentation, the very irregular, multipolar polyarch spindle has become a multipolar diarch spindle; and the achromatic substance not used in spindle-formation has been gradually resolved, from the periphery of the nucleus inwards, into a granular, or finely reticulated structure, which later merges into the general cytoplasm of the egg (figs. 229–231). When the spindle has become a true multipolar diarch, it frequently consists of two nearly equal parts, which seem to belong respectively to the male and female nuclei (figs. 231 and 232, plate XXI). This appearance, however, may be only accidental, as the great irregularity which characterizes this spindle in the first stages of its formation renders such an origin of the two halves of the nearly completed spindle very problematic.

Two chromatic groups are distinctly recognized at the time of the segmentation of the spiremes and can still be clearly made out during the early development of the chromosomes (figs. 232 and 233). When the chromosomes are being oriented at the nuclear plate the maternal and paternal elements can no longer

be distinguished (fig. 234). One beautiful preparation was ob tained at this stage in which a single section through the nuclear plate showed twenty-four entire chromosomes, and no chromosomes were found in the other sections of the series (fig. 235). As twelve chromosomes had previously been counted in the egg-nucleus there can be little doubt that the same number is brought into the egg by the sperm-nucleus. So far as form and structure are concerned the twenty-four chromosomes of this preparation are exactly alike, and at this stage I was no longer able to distinguish between the maternal and the paternal segments.

The smallness of the mitotic figure in the first division following fecundation compared with the size of the egg-nucleus has been commented upon by Strasburger ('92) and by all later students of the Abietineæ. This spindle may occupy various positions in the space originally filled by the egg-nucleus, but, as is clearly demonstrated by a study of its development, it invariably lies partly within the sperm- and partly within the egg-nucleus, its major axis being always parallel with the outer, free surface of the sperm-nucleus. While, then, the karyokinetic figure bears a certain definite, fixed relation to the conjugating nuclei, it will be readily seen that its position may vary, depending upon the shape of the sperm-nucleus and its line of contact with the egg-nucleus, as, also, upon the plane at which the section is cut with regard to the sexual nuclei. For instance, when the sperm-nucleus is elliptical in outline and lies in a deep depression in the egg-nucleus, as illustrated in figs. 221 and 223, *a*, plate XX, the spindle will appear to occupy the center of the egg-nucleus. Cases like the above and many others were first satisfactorily interpreted after a careful study of something like two hundred preparations showing fertilization stages.

*Later Stages in the Mitosis.* — During metakinesis the mitotic figure may present every variation between the extremely broad, multipolar diarch, shown in fig. 236, and the narrow, almost bipolar spindle, illustrated in fig. 237. It is at this time that the longitudinal splitting of the chromosomes first becomes apparent. Each chromatic element divides at the point where the spindle-fibers are attached, forming a small diamond-

shaped opening. While this opening is still inconspicuous, the two halves of a given chromosome become distinct throughout the entire length of the segment. Such a condition was several times observed in the division of the "segmentation-nucleus," but was not sketched because of lack of space. A similar stage in the division of one of the four nuclei of the proembryo is shown in fig. 253, *b*, plate XXIII.

In general the chromosomes of the nuclear plate are in the form of U's and V's; in rare instances they are long and somewhat coiled, and the spindle-fibers are not attached to their centers (figs. 234–238). They pass to the poles as narrow U's (fig. 239). Sometimes the arms of the U are pressed so closely together that the chromosomes look like longitudinally split rods. In a late anaphase of the division the chromatic elements present a crinkled appearance, and the poles of the spindle terminate in granular areas from which threads extend into the surrounding cytoplasm. These fibers may be quite inconspicuous or they may be very prominent, frequently forming fantastic figures (figs. 240 and 241).

A portion of the achromatic constituents of the sexual nuclei may persist in the region of the mitotic figure until the formation of the daughter-nuclei, but, as a rule, all traces of the original nuclei have disappeared at this time. Blackman finds no suggestion of a cell-wall in connection with the first division which takes place within the oösphere. But here, again, I have found great variation. The spindle either becomes constricted at the center with little or no sign of thickening along its median line, or it may be very broad, in which case prominent thickenings occur, only to disappear at a later stage, in the line of the cell-plate (figs. 239 and 242). As the half chromosomes unite to form the daughter-nuclei the poles of the spindle often become very slender and seem to press against the forming nuclei, rendering them concave along their inner surfaces; and delicate fibers now extend from all sides of the division-figure into the cytoplasm (fig. 242). As already indicated, there is no evidence that any portion of this spindle is derived from the cytoplasm, and it is probable that a large part, if not all, of its fibers are formed by a rearrangement of a portion of the achro-

matic, nuclear reticula. During the dissolution of the mitotic figure some of the substance of the spindle-threads probably passes into the daughter-nuclei, but the greater part of the fibers merge into the cytoplasmic reticulum and become indistinguishable from it. We have here another evidence that cytoplasmic and nuclear elements are but different expressions of the fundamental or ground substance of the cell. When the daughter-nuclei are formed they present very beautiful, moniliform reticula, which later undergo changes very similar to those described for the growing egg-nucleus.

As recorded by Wilson ('96 and '00), Van Beneden ('83 and '87) made the very interesting discovery, later confirmed by Herla ('93) that the chromosomes are formed separately in the sexual nuclei of *Ascaris megalocephala*. The differentiation of the chromatic segments takes place after the entrance of the sperm-nucleus into the egg but before the two nuclei have come into contact. Thus the exact equivalence of the chomatic substance in the paternal or maternal nuclei was demonstrated. In the following year, Strasburger ('88) suggested that in the coming together of the nuclear theads lay the important point in fertilization. A separating-out of the chromatic elements similar to that described by Van Beneden, has since been found to occur during fertilization in many animals, but has not yet been demonstrated as of frequent occurrence in plants. In 1891, Guignard described the formation of two distinct chromatic spiremes in the copulation nucleus of *Lilium Martagon*, but he did not figure them, and his statement seems to have been overlooked by most later writers. Strasburger was able, in 1897, to distinguish the maternal and paternal portions of the fertilized nucleus in *Fucus* up to the time when the spindle was fully formed, and Ikeda ('02) states, regarding *Trycirtis:* "The paternal and maternal chromatin elements of the resulting nucleus are distinguishable long after fusion." But the results of more recent writers[1] seem to indicate that fertilization

[1] Arnoldi ('00) in *Cephalotaxus*, and ('01) in *Sequoia;* Caldwell ('99) in *Lemna;* Campbell ('99) in *Sphargánium;* Farmer and Williams ('98) in *Fucus;* Guignard ('99) in *Lilium;* Harper ('00) in *Pyronema;* Ikeno ('98) in *Cycas* and ('01) in *Ginkgo;* Jäger ('99) in *Taxus;* Land ('00) in *Erigeron* and *Silphium:* Lotsy ('99) in *Gnetum;* Merrell ('00) in *Silphium;* Miyake ('01) in *Pyth-*

in plants consists in the fusion of the two nuclei to form a resting nucleus not demonstrably different, except in some cases in its greater size, from the original egg-nucleus.

Students of certain of the *Abietineæ*, however, have attained quite different results, and find in these plants phenomena very similar to those occurring during fertilization in some animals. Blackman concludes that in *Pinus sylvestris* "no resting fertilized nucleus is ever formed" and that "the half-chromosomes derived from the male and female nuclei respectively, fuse together at the poles of the first segmentation spindle"; and Chamberlain found that two chromatic spiremes were formed in *Pinus Laricio*, but, as so many stages were lacking in his material, he hesitated to draw definite conclusions; Woycicki ('99) reported a complete fusion of the sexual nuclei in *Larix*, but in some cases he saw two chromatin-groups, and suggested that they might have been derived, one from each parent; and Murrill ('00) has recently described the formation of two distinct spiremes in *Tsuga*. As a result of the present studies, it has been shown conclusively, as stated by the writer in 1901[1 and 3], that the chromatic portions of the sexual nuclei remain distinct until the daughter-nuclei are formed; and there is, moreover, never any true fusion of the conjugating nuclei, that is, the two nuclei do not form one individual enclosed by a definite membrane.

It is evident from the foregoing, that fertilization in *Pinus* consists in the complete union of two cells. Cytoplasm fuses with cytoplasm and nucleus unites with nucleus.

No centrosome or centrosome-like body has been observed in connection with the sexual nuclei, either before or during this division. Although the centrosome as an organ has failed to be demonstrated, yet a detailed study of this mitosis makes the conclusion inevitable that the *force* initiating and controlling the division is supplied by the sperm- and not by the egg-nucleus

*ium;* Mottier ('98) in *Lilium* and ('00) in *Dictyota;* Nawaschin ('99) in *Lilium*, and ('00) in *Helianthus*, *Delphinium* and *Rudbeckia;* Osterhaut ('00) in *Batrachospermum;* Shaw ('98) in *Onoclea;* Thom ('99) in *Adiantum* and *Aspidium;* Thomas ('00) in *Caltha;* Wager ('00) in *Peronospora;* Webber ('01) in *Zamia;* all who have described coiled sperm-nuclei; and all writers with the exception of Ikeda who have published on fertilization in plants during 1902 and 1903.

— this force manifesting itself only in the presence of the egg-cytoplasm.

The demonstration of normal parthenogenesis in several plants and of artificial parthenogenesis by Nathanson ('00) has led to much interesting discussion regarding the nature of the stimulus exerted by the sperm on the egg. Klebs ('01) suggests that it is merely of the nature of an external shock, and other explanations have been offered; but, after carefully reviewing the literature of the subject, Zacharias ('01) concludes that we have still to determine the true nature of the stimulus which the sperm exercises upon the egg, and in so far as I am aware, none of the more recent studies have thrown any substantial light on this problem.

Nothing has been observed throughout this study to indicate that the sperm-nuclei of *Pinus* ever assume the spiral or reniform shape, suggestive of spermatozoids, which has been described by recent writers[1] for the sperm-nuclei in various Phanerogams, and by Arnoldi ('01) in *Taxodium* and *Sequoia*. But the nuclei early become spherical or elliptical in outline, depending on the breadth of the pollen-tube, and remain so during their entire later history.

### THE PRO-EMBRYO.

*Division of the Two Segmentation-nuclei.*—The two daughter-nuclei remain in the upper part of the egg and pass through the same stages in their development as those described in the maturation of the egg-nucleus, except that, as a rule, no nucleolus becomes apparent within them. These nuclei have been observed to approximate in size the mature egg-nucleus; but they usually cease to grow and begin to divide while they are still much smaller than the fully developed nucleus of the oösphere. The steps in the division of these two nuclei in *Pinus Strobus*, this division has not been carefully studied in the other species, are almost exactly like those of the first division. The nuclear reticulum is resolved into a beautiful, open

[1] Golinski ('93) in certain grasses; Nawaschin ('98), Guignard ('99), Sargant ('99) in *Lilium;* Guignard ('00) in *Tulipa;* Land ('00) in *Compositæ;* Merrell ('00) in *Silphium;* Strasburger ('00) in *Monotropa;* Thomas ('00) in *Caltha;* and many others during the past two years.

and interrupted, granular, achromatic network which is crossed by several coarsely granular, deeply staining threads. These threads, which represent the chromatic portion of the nucleus, have at first no definite arrangement; but they soon unite to form two distinct, coiled or angled spiremes, which draw together at one side of the nucleus (figs. 244, 245). It is an interesting fact that these spiremes are always found on adjacent sides of the two nuclei. This position suggests that there is a certain attraction, comparable to that existing between the sexual nuclei, active between these nuclei; or the relation of the inner sides of these nuclei with the poles of the spindle, in the early stages of their formation, may have some influence upon the position which these spiremes assume in the dividing nuclei.

When the two spiremes, which are still roughly beaded with the chromatic substance, come to lie side by side along the inner wall of the nucleus, the nuclear wall resolves itself into a weft of fibers. These threads pass into the surrounding cytoplasm and soon wholly disappear, while, at the same time, achromatic fibers arise in the regions of the spiremes (fig. 245). The achromatic threads quickly draw together, forming a sharply bipolar spindle on which the two now perfectly homogeneous chromatic bands lie. The spindle does not become bipolar in some instances until after the segmentation of the spiremes (figs. 245–247). I have preparations representing a complete series in this division, but, as it is exactly similar, especially in its later stages, to the first division, it is not thought best to multiply sketches by repeating like figures.

There can be little doubt that the two spiremes formed in each of these nuclei represent the separated-out paternal and maternal chromatic substance, although to all appearances, the chromosomes were completely fused in the reticula of the daughter-nuclei. One is reminded by these phenomena, of Strasburger's ('92) remark, when he states that he accepts the view of a complete fusion of the segments into a network in the daughter-nuclei, and then asks if he must, therefore, conclude that the chromosomes in the following divisions do not correspond in material. This restoration of the paternal and the maternal

chromatin from a finely divided network is certainly strongly in favor of the theory of the individuality of the chromosomes; and it is this phenomenon, noted many months before microsporogenesis was carefully studied, together with the method of the origin of the chromosomes in the first and second divisions of the microspore-mother-cell, that inclines me to accept the view that the chromosomes in the homotypical division of the microspore-mother-cell are identical with those formed in the metaphase of the heterotypical mitosis.[1] Moreover, this phenomenon, here observed for the first time in plants, would seem to add substantial interest from a cytological point of view to Mendel's laws which are at present being so ardently discussed both by animal- and by plant-breeders.

Rückert ('95) found that the chromatic portions of the conjugating nuclei in *Cyclops* not only remain distinct during the first division, but the two groups of chromosomes, representing respectively the maternal and the paternal chromatic elements, could still be recognized after several divisions had taken place. In this case, however, the two groups do not fuse in the daughter-nuclei but a double nucleus is formed in the resting stage. In the same year Zoja ('95) observed that in *Ascaris* the maternal and the paternal chromosomes remain entirely distinct during several successive divisions of the segmentation nucleus. We have, then, in this second division a further point in which fertilization-phenomena in *Pinus* correspond to those which occur within the ova of some animals. I have, as yet, made no attempt to obtain a complete series of stages in the development subsequent to the formation of the first four nuclei of the proembryo. But, from a comparison of fig. 252, *b*, plate XXIII, with 244, plate XXII, and 253, *b*, plate XXIII, with 237, plate XXI, one is led to expect that the third division following fertilization will correspond in all points with the second. It would be interesting to determine if two chromatic groups are characteristic of all divisions which normally occur within the oösphere of *Pinus*, and I hope to investigate this question more thoroughly at some future time.

*The Four Segmentation Nuclei.* — As a rule, these nuclei

[1] See note at close of Appendix.

retain their position in the upper half of the egg until their growth is completed (fig. 249). Here, again, as in the development of the two segmentation nuclei, the steps described for the maturation of the egg-nucleus are repeated, except that a nucleolus does not generally become apparent within these nuclei. After attaining full size, the four nuclei pass to the base of the oösphere, as described by all recent writers. During their descent many fibers arise in the cytoplasm surrounding the nuclei. Some of these threads run parallel with the walls of the nuclei, while others extend out from the nuclei in a radial manner. These fibers become more prominent as the nuclei approach the base of the oösphere, and, as in the case of the egg-nucleus, they are most strongly developed along the upper sides of the nuclei (figs. 250, *a*–251, *b*). Blackman suggests a relation between these fibers and the walls that arise later at the organic apex of the oösphere, but I find no evidence of any connection between the two. When these nuclei have nearly reached the bottom of the egg, the nutritive spheres have almost disappeared from the cytoplasm, those which still persist being much reduced in contents (fig. 251, *a*). After the four nuclei have arranged themselves at the "organic apex" of the oösphere, in a plane perpendicular to the major axis of the archegonium, a marked change occurs in the cytoplasm of their immediate vicinity. It becomes dense, coarse, more or less granular, and has a great affinity for stains (figs. 252, *a* and *b*, plate XXIII).

The early prophases, as also the meta- and anaphases in the mitosis of the four segmentation nuclei, in so far as studied, correspond in every respect with the same stages in the second division following fertilization; and it is probable that the chromosomes are derived from two distinct spiremes as in the first and second divisions occurring within the egg; but, as already indicated, the steps in the origin and development of the chromosomes have not been carefully traced in this division. These nuclei divide simultaneously. Chamberlain states that "in the division of the four nuclei the spindle is extremely broad and multipolar." I have occasionally observed such a figure during this mitosis, but here, again, great variation exists.

Every transitional form may be presented during the metakinesis between a multipolar diarch spindle, which fills the entire breadth of the nucleus, and a slender bipolar spindle, such as is shown in fig. 253, *b*. As the halves of each chromosome separate at the point where the spindle-fibers are attached, the longitudinal splitting of the segments becomes evident throughout the entire length of the chromosomes (fig. 253, *b*.)

*The Development of Cell-walls.* — During mitosis, the deeply staining substance surrounding these nuclei condenses into large irregular masses at the periphery of the nucleus. When the eight nuclei are formed this deeply staining material collects about them and extends in irregular strands into the cytoplasm. Each nucleus is now surrounded by its own cytoplasm, though no cell-walls have yet been laid down (figs. 253, *b*, and 254, *b*). Blackman describes the formation of cell-walls about the four nuclei at the base of the archegonium, and Coulter and Chamberlain state that cross-walls separating these nuclei, but leaving them exposed above, arise when the four nuclei have arranged themselves at the base of the oösphere, and are undergoing division. In the five species of pines which I have studied cell-walls do not arise until after eight nuclei have been formed.

The deeply staining cytoplasmic substance appears to be repelled from all sides of these nuclei and is deposited in lines which indicate the position of the future cell-walls; the cell-membranes appear to arise by a direct transformation of this substance. The process seems to be very similar to that described by Farmer and Williams ('98) in *Fucus*. Mottier ('00) inclines to the view that the cell-plate is deposited in the form of a homogeneous fluid, the kinoplasm, even though its presence cannot be demonstrated, being the active agent in its deposition. The substance which is cast out, or passes out, from the region of the eight nuclei in the formation of cell-walls at the base of the oösphere in *Pinus*, has the appearance at times of a homogeneous, deeply staining fluid, in which numerous irregular granules are imbedded; but there is never any evidence of its being purely fluid in nature. It seems very probable that the large granules cast out from the cytoplasm surrounding these nuclei at this time are similar to the smaller granules deposited at the cell-

plate during the ordinary process of cell-wall formation. That the granules are larger and the details of the process more striking here may be accounted for by the fact that, under the influence of each nucleus, three times as much cell-wall must be laid down as is ordinarily formed by the action of a single nucleus. But in any case, we are still far from a satisfactory understanding of the method by which cell-walls arise.

The eight nuclei are arranged, as usually described, in two tiers of four cells each. The cytoplasm of the upper four cells remains continuous with the cytoplasm of the egg, that is, a dividing wall is not formed along their upper surface (figs. 255, *a*, and 255, *b*).

*Later Mitoses in the Formation of the Proembryo.* — The second set of division figures which occurs at the organic apex of the egg arises in the upper tier of cells, that is, in the four cells which have never been cut off from the general cytoplasm of the egg (fig. 256). This is contrary to all reports of the development of the proembryo in the *Abietineæ*.[1] The second division occurring in the nuclei at the base of the archegonium has not been previously observed, so far as I am aware, and, the third division occurring in the basal tier of cells, the inference seems to have been made that the cells which are not enclosed along their upper sides by definite walls never divide. Coulter and Chamberlain ('01) make the remark that the upper four free nuclei increase much in size, and they figure them in the spireme stage; but they do not refer to the fact that they are in the prophase of division, and describe all further mitoses after the eight-celled stage as occurring in the basal tier of cells. Strasburger and Hillhouse ('00) also describe the further development of the proembryo in *Picea* after the establishment of cell-walls, as proceeding from two successive divisions of the four basal cells.

It seems to me a rather significant fact that the four cells which remain in open communication with the egg should not only divide again, but that their division should be entirely completed before the cells of the lower tier show any signs of dividing. There are thus, in *Pinus*, four successive mitoses

[1] See note at close of Appendix.

resulting in the formation of twelve nuclei under the direct influence of the egg-cytoplasm, rather than three divisions with the formation of eight nuclei as has been previously described. The phylogenetic bearing of this phenomenon may be more far-reaching than is at first apparent, suggesting as it does a possible closer relationship with those lower gymnosperms in which many free nuclei arise in the egg before the deposition of cell-walls.

At present I can give only this general outline of the origin of the proembryo, but I hope to be able in the near future to make a detailed study of the several mitoses which occur here in *Pinus*.

*The Fate Within the Egg of the Smaller Sperm-nucleus, the Stalk-cell, and the Tube-nucleus.* — When the various elements from the male gametophyte first enter the oösphere, there is no question as to the identity of the several nuclei to one who has become familiar with them before their exit from the pollen-tube (figs. 213–215, plate XIX). Remnants of these cells have been found in the upper part of the egg as late as the formation of the eight-celled stage of the proembryo. The stalk-cell remains for some time unchanged and finally disintegrates.

In so far as I have been able to determine, it assumes a more or less granular appearance, and at last blends with the cytoplasm of the egg. The tube-nucleus undergoes various changes. Occasionally it seems to contract, becoming gradually smaller until it is no longer demonstrable; it may change little, if any, in size, but its reticulum often becomes more prominent than when within the pollen tube; rarely it enlarges rapidly after its entrance into the egg and develops a beautiful reticulum (fig. 212, plate XIX). The sperm-nucleus not active in fertilization increases but little in size, and its network becomes less dense, resembling that of the conjugating nuclei; it may pass through the ordinary processes of disintegration; and in a few cases it has been observed to divide amitotically, as described by Arnoldi ('00) in *Cephalotaxus*.

But frequently the sperm-nucleus and occasionally the tube-nucleus attempt to divide mitotically. One or two small, abortive, karyokinetic figures are not uncommon in the upper part

of the egg at the time of the division of the two segmentation-nuclei. I have said "attempt to divide," for no instance has been observed in which the division of these nuclei has extended beyond a late prophase. A bipolar spindle, with the chromatic segments scattered irregularly upon it, represents the most advanced stage which has been seen in the division of the smaller sperm-nucleus (fig. 259, *b*, plate XXIII). (During sectioning, a rupture was made in the cytoplasm at one end of this spindle so that the upper pole has been separated into two.) The stalk-cell still persists at this late date (fig. 259, *b*, plate XXIII), and in another section of the series (fig. 259, *a*), a second mitotic figure appears. This evidently represents the tube-nucleus. The achromatic part of the figure presents the appearance of a normal bipolar spindle, but, the chromatic spireme has not become homogeneous and probably would not have developed further. In some cases a well-developed spireme is formed in the upper part of the egg, but no achromatic threads become apparent (fig. 257); again, a nucleus seems to have been entirely resolved, during its disintegration, into achromatic fibers. As above stated, in no case observed did the division of these nuclei reach telokinesis, but at some point in the development prior to such a late stage, activity ceased and disintegration of the nuclear elements took place. Murrill ('00) observed a similar figure, which he interpreted as the smaller sperm-nucleus, in the upper part of the fertilized egg in *Tsuga*.[1]

It might be suggested that these division-figures result from the conjugation of the nucleus of the ventral canal-cell with the smaller sperm-nucleus. There is no evidence that such is the case, and I am convinced that they could not have had such an origin. In an examination of many hundred archegonia just before fertilization, but one ventral canal-cell containing a normal nucleus has been observed. Shall we, then, conclude that, in a far less number of preparations representing stages immediately following fecundation, fifty or more instances occur in which the nucleus of the ventral canal-cell has conjugated with another nucleus and subsequently divided?

It is generally recognized, especially by cytologists on the

[1] See note at close of Appendix.

animal side, that the stimulus to division is given not by the egg-nucleus, but by the cytoplasm of the egg. If this be true, it is not strange that these nuclei, lying in a position where everything is most favorable for growth and development — in a medium not only rich in nutritive substances but especially adapted to incite activity in nuclei — should divide. It is a well-known fact that when several spermatozoa enter the ovum of certain animals, only one unites with the egg-nucleus, the others degenerate, or, as is frequently the case, they divide mitotically. And herein we find a further similarity between the processes attending fertilization in some animals, and those taking place within the oösphere of *Pinus*.

## SUMMARY.

At the time of fertilization, an opening is formed in the apex of the pollen-tube, and the cells of the male gametophyte which still persist, together with a portion of the cytoplasm and some of the starch of the pollen-tube, pass into the cytoplasm of the egg.

The larger sperm-nucleus escapes from the protoplasm of the sperm-cell and moves directly toward the egg-nucleus; the other nuclei from the pollen-tube may persist, in a modified form, in the upper part of the archegonium until the eight-celled stage of the proembryo; but the cytoplasm of the sperm-cell soon fuses with that of the oösphere. The stalk-cell gradually disintegrates and blends with the egg-cytoplasm. The tube-nucleus and the smaller sperm-nucleus may share the fate of the stalk-cell, but, during the second division of the egg, they not frequently give rise to mitotic figures. The smaller sperm-nucleus, then, may pass through a slow process of disintegration, it may divide amitotically, or it may give rise to a karyokinetic figure of more or less definiteness.

There is no apparent change in the diameter of the sperm-nucleus after its entrance into the oösphere. At the time of conjugation, the egg-nucleus is several times larger than the sperm-nucleus, and the sperm-nucleus does not increase in size after its contact with the egg-nucleus. The inequality in size of the sexual nuclei may be due to the difference in the size of

their cells. But if, as has been suggested, the egg-nucleus functions as a manufacturer of nutritive material, may we not find in this activity a feasible explanation of its greater size? The conjugating nuclei, always dissimilar in size, may or may not be dissimilar in structure.

The egg-nucleus becomes slightly convave on the side nearest to the approaching sperm-nucleus. This nucleus imbeds itself in the side of the egg-nucleus but does not penetrate its membrane. A chromatic spireme arises, and a prominent achromatic reticulum becomes apparent in each nucleus. Soon afterwards the nuclear membranes entirely disappear. The two chromatic groups remain distinct until the nuclear plate stage.

Fertilization consist in *Pinus* in the union of two entire cells. Cytoplasm fuses with cytoplasm, but there is never any fusion, as ordinarily understood, of the sexual nuclei.

The spindle of the first division following fecundation always lies between the conjugating nuclei and parallel with the outer, free surface of the sperm-nucleus. It is multipolar in origin and is probably derived equally from the paternal and the maternal nucleus. The spindle-fibers appear to arise by a rearrangement of the achromatic nuclear reticula and are evidently not the expression of a special kinoplasmic substance. After the formation of the daughter-nuclei, the greater portion, if not all, of these threads pass into the cytoplasmic network. During metakinesis and later stages this spindle may vary from a broad, multipolar diarch to a slender, bipolar spindle. The chromosomes pass to the poles in the form of narrow U's.

No individualized centrosomes or centrospheres have been found to occur in connection with the first division following fertilization. But the entire activity connected with this mitosis indicates that the sperm-nucleus acting in the presence of the egg-cytoplasm is the agent which initiates and controls the division.

The two segmentation-nuclei present a reticulated structure in which the paternal and the maternal chromatin appear to be completely fused. They divide in the upper part of the egg passing through practically the same steps as those noted for the first division. The two chromatic spiremes formed in each nucleus take up a position along the adjacent sides of the

nuclei. These bands without doubt represent the separated-out paternal and maternal chromatic substance. This phenomenon is of especial interest in that it suggests a cytological basis for Mendel's laws. A longitudinal splitting of the chromosomes first becomes apparent during an early stage in metakinesis.

The four segmentation-nuclei attain full size while still in the upper part of the egg. As they pass to the base of the oösphere, fibers occur in the cytoplasm similar to the threads observed around the growing egg-nucleus. The steps in the division of these nuclei have not been carefully traced, but, from the stages observed, it is probable that this mitosis does not differ from the division of the two segmentation-nuclei.

No cell-wall is laid down at the base of the oösphere until after the eight-celled stage of the proembryo has been reached. These eight nuclei are surrounded by a deeply-staining granular substance which extends out from each nucleus in irregular strands. This substance finally comes to lie in the lines of the future cell-walls and is evidently transformed into cell-wall. It is probably not different from the smaller granules deposited in the line of the cell-plate during the accustomed method of cell-wall formation.

The fourth division which occurs within the fertilized egg takes place in the four cells of the upper tier of cells at the base of the archegonium. Thus twelve nuclei are formed under the direct influence of the egg-cytoplasm. This fact herein noted for the first time[1] is significant, suggesting as is does a closer relationship with those lower gymnosperms in which many nuclei are formed in the cytoplasm of the egg.

The number of chromosomes in the nucleus of the ventral canal-cell, in the nuclei of the sheath-cells, and in the egg-nucleus has been found to be twelve, while the mitotic figure, in the first division following fertilization, shows twenty-four chromatic segments.

It is interesting to note the many points of similarity between fertilization as it has been observed in *Pinus*, and the processes known to take place during fertilization in some animals. (1) The egg in *Pinus* is very large and is abundantly supplied with

[1] See note at close of Appendix.

nutritive spheres. (2) The sexual nuclei do not fuse, and no structure which could properly be called a segmentation-nucleus is ever formed. (3) An achromatic nuclear reticulum becomes very prominent in the sexual nuclei during the prophase of division. (4) The chromatin of the sexual nuclei forms two definite groups which remain distinct until metakinesis. (5) Two chromatic groups, doubtless representing respectively the paternal and the maternal chromatin, appear in the second division following fecundation; and the indications are that they will again occur in the third division, and perhaps are characteristic of all the mitoses which take place within the oösphere. (6) The nuclei, which enter the egg but play no part in fertilization, show a tendency to divide mitotically.

The conclusions reached throughout this paper hold good, when not otherwise indicated, for all five species of pines which I have studied. Nuclear phenomena are found to vary so much, even within the limits of a given genus, that it is no longer safe to consider the details of development in a single plant as typical of a large group of plants. We therefore make no generalizations regarding the *Abietineæ*. And we hesitate, even, to draw conclusions for the genus *Pinus*, for, while the agreement in certain phases of development of five species would seem to be sufficient for the formulation of a rule, there may still exist within the genus individuals which differ, in certain aspects of their nuclear activity, from that which has been found to occur in *Pinus Strobus*, *P. austriaca*, *P. rigida*, *P. resinosa* and *P. montana* var. *uncinata*.

## APPENDIX.

### Some Abnormal Conditions.

*Supernumerary Nuclei in the Male Gametophyte.* — Chamberlain ('97) described a multiplication of the normal number of cells in the pollen-grain of *Lilium;* Arnoldi ('00) finding more than the usual number of nuclei in the pollen-tube of *Cephalotaxus*, considered that more than one tube-nucleus had been formed; and Coker ('02) has very recently found that both the first and second prothallial cells in *Podocarpus* may divide

mitotically. I have only three times observed an excess of the normal number of nuclei in the male gametophyte of *Pinus*.

Three nuclei have been found in the pollen-grain after the tube-nucleus has passed into the pollen-tube (fig. 271, plate XXIV). Two nuclei have twice been seen just passing into the pollen-tube, while the stalk-cell could still be detected in the lower part of the pollen-grain in one instance, and in the other it had just left the grain but had not as yet passed the generative cell. In the former instance (fig. 272) the stalk-cell was almost obscured by the dead nucellar tissue and is not shown in the sketch. Here the two nuclei are in close contact, the smaller nucleus being imbedded in one side of the larger nucleus. In the second case (fig. 273) the nuclei are farther removed from the pollen-grain, although still connected with it by the cytoplasm of the larger cell; the smaller nucleus is surrounded by its own cytoplasm and is in contact with the lower part of the larger cell.

Any interpretation of these irregularities must be more or less hypothetical, and yet from the position, size, and structure of the nuclei certain inferences can be made regarding them. In the condition represented in fig. 271, one of the prothallial cells may have persisted, the stalk-cell may have divided, or the generative cell may have given rise to the extra nucleus. But considering the character of the nucleus and also that of the nucleus of the stalk-cell, it seems to me most probable that two stalk-cells have been formed. In figs. 272 and 273 the probabilities are very strong that the smaller nucleus in each instance was cut off from the generative nucleus. The stalk-cell is perfectly normal in appearance and gives no evidence that it has passed through any unusual history. The two large nuclei shown in fig. 273 bear a very striking resemblance to the sperm-nuclei, and when first observed with a lower power of the microscope the impression was that the generative nucleus had divided very early and the smaller sperm-nucleus was in advance. But, when the higher magnification revealed the stalk-cell still above these nuclei, and also disclosed the fact that there were in reality two cells, it at once became apparent that these are not to be considered sperm-nuclei. For two sperm-

cells are not formed; the smaller sperm-nucleus is never in advance; the generative cell does not give rise to the binucleated sperm-cell until after the stalk-cell has passed beyond it, nor has its normal division ever been observed to occur while it is still united with the pollen-grain by its own cytoplasm. In this case it seems very evident, then, that two generative cells have arisen by the division of the first generative cell. Whether both of these would have divided to produce four sperm-nuclei is of course a mere matter of conjecture, but the cytoplasm of the smaller cell is very scanty and it is probable that only the larger one would have functioned as the generator of the sperm-nuclei. The uncertainty as to the origin and fate of these extra nuclei is in each instance too obscure to admit of any theorizing regarding their significance.

*Usual Conditions in the Female Gametophyte.*—In only one instance has more than one macrospore-mother-cell been observed. In this case two cells which are very similar and centrally placed in the spongy tissue differ from the surrounding cells in exactly the same way as has been described for the young macrospore-mother-cell (fig. 260, plate XXIII). Farmer ('92) records the discovery of a double prothallium in *Pinus sylvestris*, and Hofmeister had previously made a like observation in the same species. I find no other instance recorded for *Pinus* in which more than one macrospore must have been functional. Shaw ('98) and Arnoldi ('99) find one or more macrospore-mother-cells in *Sequoia* from which several embryo-sacs may arise; Arnoldi ('00) has made a similar observation for *Cunninghamia*, *Sciadopitys*, *Taxodium*, and *Cryptomeria;* Lotsy ('99[2]) and others find many young embryo-sacs in *Gnetum;* and Coker reports the prescence of two prothallia in *Prodocarpus* and *Taxodium*. The presence of a multicellular sporogenous tissue has been reported in the Angiosperms by several students — Nawaschin ('99[2]) in *Corylus*, Lloyd ('01) in the *Rubiaceæ*, Murbeck ('01) in the *Rosaceæ*, and by others. The appearance of more than one functional spore within the ovule of such widely-separated plants makes it rather doubtful if this character is important phylogenetically.

Juel ('00) found that the walls separating the macrospores in

*Larix* are often oblique. Only one such instance has been observed in *Pinus* and is shown in fig. 261, plate XXIII.

Considerable variation has been noted in the origin of the archegonia, a few of the irregularities, which are in fact typical of all, have been figured. Figs. 262, *a* and *b*, represent two sections through the upper part of the same prothallium. They show twenty-three young archegonia in various stages of development. Only a single archegonium of those shown in the illustrations had its origin in a superficial cell; some of them originated in the sheath-cells of normal archegonia found in other sections, but this fact is not demonstrated in the sketches; however, in fig. 265, taken from another prothallium, a little archegonium is seen budding, as it were, from a sheath-cell of the larger archegonium, and in fig. 266 is shown a somewhat similar case except that here one archegonium is directly above the other.[1] One would consider it very doubtful if such an archegonium as this lower one would develop further; but fig. 267 shows an archegonium similarly located in which the central cell has divided, and both the ventral canal-cell and the egg-nucleus are still clearly visible, though the latter shows some signs of disintegration. In all these archegonia no neck cells have been formed.

In one instance, nine archegonia were found *Pinus* in *montana uncinata*, so arranged along the top and side of the prothallium as to suggest a cock's comb — seven of the archegonia being apparent in a single section. The figure was reconstructed from several sections in the series and the archegonia overlap not all lying in the same plane, but they are all plump and normal though some show early stages in disintegration. The two at the top have well developed proembryos, but none of the others have been fertilized (fig. 260). Archegonia are frequently found arranged vertically as in fig. 261. In such cases as this the lower ones do not arise from the one just above, but each is connected with the exterior by means of a funnel-shaped opening leading from its neck-cells to the side of the prothallium; this cannot be shown in a sketch as it is not evidenced in any one section, and can only be determined by carefully studying the whole series.

[1] See note at close of Appendix.

It has been held by various students that all the nuclei in the embryo-sac of Angiosperms are potential eggs. Murbeck ('01) has recently, as recorded by Overton ('02), demonstrated the development of an embryo in *Alchemella;* Chamberlain ('95) discovered the presence of an antipodal oösphere in *Aster*; and many earlier investigators have made similar observations regarding the synergids and antipodals.[1] The discovery of archegonia that have originated not only from superficial cells at the top and along the sides of the prothallium, but from cells considerably removed from the surface as well would seem to give direct affirmation to the suggestion made by Atkinson ('01) that all the cells of the prothallium in Gymnosperms are potential eggs.

Among the many archegonia studied, I have found two in which the nucleus of the ventral canal-cell approximated that of the egg in size. Fig. 268 shows such a condition in *Pinus Strobus*, but even here the nucleus of the ventral canal-cell is much smaller than that of the egg. It is, however, remarkably large for the nucleus of the canal-cell in this species, and is apparently still in a normal condition, whereas this nucleus is ordinarilly in an advanced stage of disintegration when the egg has reached maturity. A much more marked increase in the size of the nucleus of the canal-cell has been observed in *Pinus austriaca* as illustrated in fig. 269. Here it has attained a comparatively enormous size and presents almost exactly the same structure as the nucleus of the fully developed egg, though slightly smaller than the egg-nucleus. Chamberlain ('99) figures a similar enlargement of the nucleus of the ventral canal-cell in *Pinus Laricio* and concludes that this cell is the homologue of the egg. It will be noted that in the instances described above, no ventral canal-cell has been formed, but that in both cases the nucleus of the canal-cell lies free in the cytoplasm of the egg[2] (figs. 268, 269). The failure to form a wall cutting off the ventral canal-cell from the egg, or the early absorption of this wall if it has been formed, seems to me ample reason for the unusual size and persistence of the nucleus of the canal-

[1] Miss Opperman, a student in my own laboratory, has recently discovered the fertilization of an antipodal egg in *Aster*, a description of which is soon to be published.[2]

[2] See note at close of Appendix.

cell, since it lies in the cytoplasm of a cell which supplies the most favorable medium for growth found in the plant. Not the slightest evidence has been observed during this research that the nucleus of the ventral canal-cell ever divides or that it ever conjugates either with the egg-nucleus or with the smaller sperm-nucleus. The fact that this nucleus enlarges when fed by the cytoplasm of the egg does not seem to me conclusive evidence that it has been "organized as an egg," as stated by Coulter and Chamberlain ('01). The tube-nucleus and the smaller sperm-nucleus often enlarge after their entrance into the egg but, surely, they are not thereby changed into eggs.

The fragmentation of the egg-nucleus has been observed several times and is illustrated in fig. 270. The ventral canal-cell can still be seen just above the egg. Such fragmentation of the egg-nucleus is not rare in the Gymnosperms having been reported by various writers.

In one instance one of the two segementation-nuclei was found to have divided while the other remained undivided. The undivided nucleus had increased much in size and contained seven large, granular spheres distributed on an achromatic reticulum. The nucleus is evidently in a state of disintegration and these spheres probably represent granular masses of chromatin (fig. 274).

*A Peculiar Method of Conjugation.*—Of all the irregular or abnormal developments observed that illustrated in fig. 275 is, to me, the most interesting. A pollen-tube has conjugated with an egg, not through the normal passage formed by the neck-cells, but has forced its way through the sheath-cells at one side of the archegonium. Impregnation has evidently followed and division has taken place as usual, four nuclei of the proembryo having been formed.

The fifth large nucleus shown within the egg is doubtless the smaller sperm-nucleus. The open space separating the upper part of the prothallium from the nucellar cap has evidently not arisen as a result of shrinkage during fixation. The pollen-tube unable to span the opening has turned aside and finding a point at which the endosperm and nucellus were in contact it has entered the prothallium and made its way along the side

until it came into contact with the egg, when an entrance was effected through the sheath-cells. That this has cost the pollen-tube an unusual effort would seem to be evidenced by the fact that it has become filled with a cytoplasm as dense as that of the egg, whereas, normally, its cytoplasm is very scanty. If it be true, as Lidforss ('99) claims, that the penetration of the pollen-tube is simply due to a search for food, it would appear, in such a case as this, that the pollen-tube is capable of very intelligent searching. In this instance the relation of the prothallium to the nucellar cap is very like that found in the more primitive Gymnosperms such as *Zamia*, *Cycas* and *Ginkgo*. Here, however, the sperm-cells being non-motile it was necessary, if fertilization take place at all, that the pollen-tube should reach the egg.

The variations recorded here have a certain interest both phylogenetically and ontogenetically; but the most significant lesson to be derived from them is the warning that they sound against basing conclusions on meager observations. When this is done, misconceptions and actual errors are bound to be promulgated for truth.

Botanical Department, Wellesley College, Dec. 28, 1902.

## NOTE.

This paper was completed on December 28, 1902. During the time that has since elapsed much valuable literature dealing with subjects more or less intimately connected with questions herein discussed has appeared.

It is not feasible to make adequate references at this time to all these papers, but the more important ones are mentioned below, and the page in this paper, where mention of the views recently expressed by other writers should have appeared, is indicated.

The first 88 pages of this paper were printed before the writer was able to obtain copies of some of the articles mentioned below. I regret, therefore, that it was not possible in many instances to refer by means of a foot-note to the references made in this addendum.

Page 22. Strasburger ('04) now believes that synapsis is

the most important stage in the heterotypic division and several recent writers have expressed a similar opinion.

Page 26. The appearance of chromosomes from an "apparently formless reticulum," described by Williams ('04) as occurring in the first division of the tetraspore-mother-cell in *Dictyota* is interesting in connection with the origin of the chromosomes in the microspore-mother-cell in *Pinus* as herein described.

Page 31. Allen ('04) has described a somewhat similar method of segmentation in the first division of the microspore-mother-cell in *Lilium Canadense.*

Page 32. Strasburger ('04) has returned to his earlier view regarding a true reducing or transverse division in the first mitosis of the spore-mother-cell in plants.

Page 32. Farmer and Moore ('03), Williams ('04), Strasburger ('04) and others now accept the fact of a qualitative division in plants.

Page 32. As a result of his recent study of *Galtonia*, *Tradescantia*, etc., Strasburger has decided that the forms of the chromosomes which may occur in the anaphase of the heterotypic division are not the result of a double longitudinal spliting.

Page 33. According to Farmer and Moore ('03) the heterotypic division in both animals and plants is characterized by a transverse division. This transverse division effects the separation of the two chromosomes which constitute a bivalent chromosome, and is therefore a qualitative or reducing division.

Page 34. This is in direct accord with the recent publications of Boveri ('04), Cannon ('03), Rosenberg ('03 and '04) and others who have recently expressed themselves regarding the individuality of the chromosomes.

Page 48. Strasburger's earlier observations on the pollen-grain of *Picea* have now been confirmed by Miyake ('03) who shows conclusively that the generative cell is cut off in *Picea* before pollination takes place.

Page 62. In 1903 Miyake described and figured several stages in the development of a single binucleated sperm-cell in *Picea.*

Page 63. In a note at the close of Coker's ('03) paper on *Taxodium* he says: "Miss Ferguson confirms Blackman's ('98)

statement that the sperm-cells of *Pinus* are furnished with a cytoplasm of their own." But, as stated in 1901, I cannot confirm Blackman's statement that each sperm-nucleus is surrounded by its own cytoplasm.

Page 78. Strasburger ('04) states that in *Taxus baccata* a heterotypical division occurs and that four megaspores are formed which correspond to the four microspores formed within the microspore-mother-cell.

Page 89. The nature and development of this tissue in *Taxodium*, as described by Coker ('03), is essentially the same as in *Pinus*. A preliminary note regarding the nature and origin of the spongy tissue was published by the writer in 1903.

Page 97. Coker ('03) has made a similar observation in *Taxodium* and Lawson ('04) finds that the nucleus of the ventral canal-cell in *Sequoia* lies free in the cytoplasm of the egg.

Page 109. Both Wager ('04) and Williams ('04) have recently expressed the view that the nucleolus contributes to the bulk of the chromatin, either by storing or elaborating chromatin.

Page 124. The presence of two spiremes in the prophase of the second division following fertilization and the conclusions reached, as a result of this research, regarding the persistence of the chromosomes are of especial interest in connection with the discussions, appearing since the completion of this paper, by Boveri ('04), Cannon ('03), Rosenberg ('03 and '04), and others on the nature and individuality of the chromosomes.

Pages 127 and 132. Miyake ('03) has made a similar observation in *Picea excelsa*.

Page 129. Miyake ('03) finds that in *Picea* all three of the nuclei, which pass into the egg from the pollen-tube but are not directly concerned in fertilization, may divide before they disintegrate.

Page 136. Miyake ('03) has described conditions very similar in *Picea* and in *Abies*.

Page 137. Miss Opperman's ('04) paper has been published.

Page 137. As already stated, Coker ('02 and '03) finds this to be the normal condition in *Podocarpus* and in *Taxodium*. Lawson ('04) has described a similar condition in *Sequoia*, and he finds that, normally, the nucleus of the ventral canal-cell equals in size the egg-nucleus.

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# EXPLANATION OF FIGURES IN PLATES I TO XXIV.

All figures were drawn with the aid of the Abbé camera lucida. In some cases a Zeiss microscope was used and in others a Bausch and Lomb. Various combinations of lenses were used with both instruments. The figures were reduced one eighth in reproduction. The number accompanying the description of each figure indicates the degree of magnification after the reduction.

Throughout the plates the lettering is to be interpreted as follows: prothallium (*pr.*), first prothallial cell (*pr.*1), second prothallial cell (*pr.*2), third prothallial or antheridial cell (*a.c.*), tube-nucleus (*t.n.*), stalk-cell (*st.c.*), stalk-nucleus (*st.n.*), generative cell (*g.c.*), sperm-cell (*s.c.*), sperm-nucleus (*s.n.*), sperm-cytoplasm (*s.cy.*), spongy tissue (*s.t.*), starch-grains (*s.g.*), archegonium (*arch.*), ventral canal-cell (*v.c.*), neck-cells (*n.c.*), egg-nucleus (*e.n.*), cytoplasm from the pollen-tube (*c.p.t.*), nutritive spheres (*n.s.*), primary nucleolus (*py.ns.*), secondary nucleolus (*sy.ns.*), receptive vacuole (*r. v.*).

All the figures have been given their normal position, as nearly as it was possible to do so, on the plates. That is, they are so placed that the primary axis of the ovule would be parallel with the longer axis of the plates; and the portion of a figure nearest to the micropylar end of the ovule is always towards the top of the plate.

## PLATE I.

FIG. 1. Two cells of the primitive archesporium showing the winter condition of this tissue. × 1,400. *Pinus austriaca*. December 20, 1897.

2. A cell from the primitive archesporium in the early spring. Many of the cells of the archesporium are undergoing division at this time. × 1,400. *Pinus austriaca*. March 14, 1898.

3. A cell of the definitive archesporium, the microspore-mother cell, just prior to the inception of its division. × 1,400. *Pinus austriaca*. April 27, 1898.

4. The same as fig. 3. × 1,400. *Pinus Strobus*. May 24, 1898.

5. The microspore-mother-cell approaching synapsis before a definite spireme has been formed. × 1,400. *Pinus austriaca*. April 28, 1898.

6. The same as fig. 5. × 1,400. *Pinus Strobus*. May 24, 1898.

7. Synapsis. × 1,400. *Pinus Strobus*. May 24, 1898.

8. Recovery from synapsis, showing a continuous spireme. × 1,400. *Pinus Strobus*, May 24, 1898. Material showing figs. 4 and 6 was collected from a different tree than that showing figs. 7 and 8, and the microspore-mother-cells were in a slightly different stage of division.

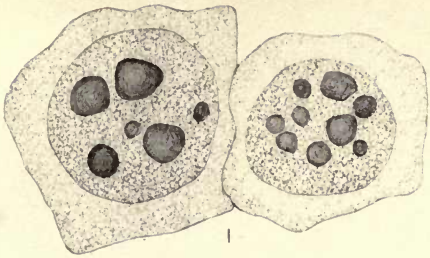
9. Complete recovery from synapsis. Chromatin in irregular granules, on a broad linin band. × 1,400. *Pinus Strobus*.

10. The longitudinal splitting and transverse segmentation of the spireme. Chromatin still distributed in irregular granules. × 1,400. *Pinus Strobus*.

11. Longitudinal splitting completed, but the sister segments do not become entirely disunited. Nucleoli still apparent. × 1,400. *Pinus Strobus*.

12, *a–e*. Portion through the edge of a nucleus showing the twisting of the chromatic segments after longitudinal splitting. In most instances these are not entire segments but portions that have been severed by the microtome knife. The entire segments are very long and coiled at this time. × 1,400. *Pinus Strobus*.

13. Early stage in the condensation and fusion of the longitudinally divided spireme. Threads anastomosing in region of nucleoli. × 1,400. *Pinus Strobus*.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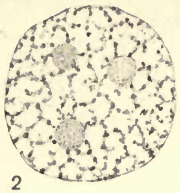
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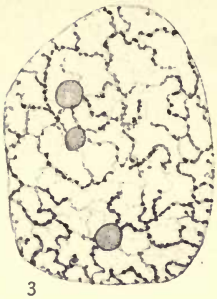
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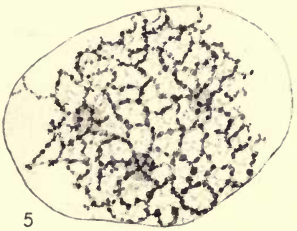
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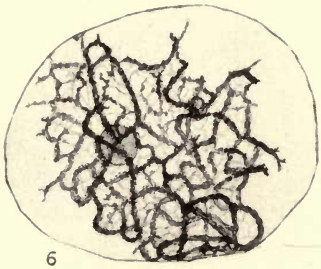
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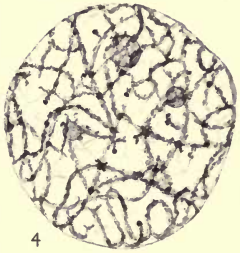
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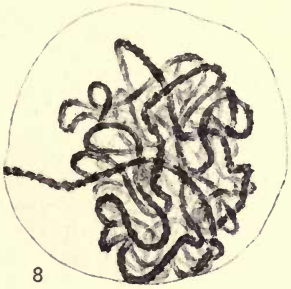
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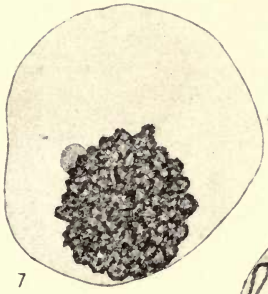
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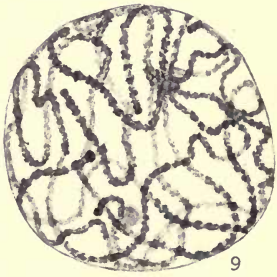
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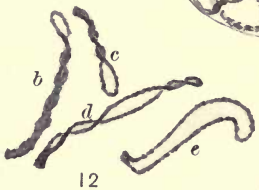

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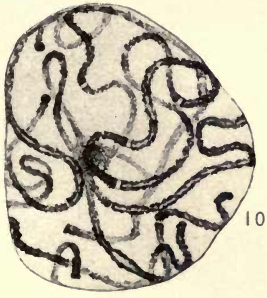
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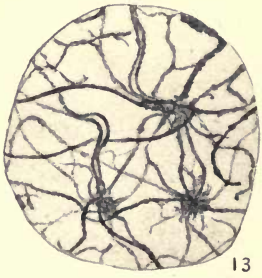
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## PLATE II.

FIG. 14. A more advanced stage in contraction, showing that adjacent threads are anastomosing and fusing. × 1,400. *Pinus Strobus.*

15. A still more advanced stage in the fusion of the threads. Practically all evidence of the earlier longitudinal fission has now disappeared. × 1,400. *Pinus Strobus.*

16. The chromosomes becoming apparent. × 1,400. *Pinus Strobus.*

17. Distinct chromosomes, in the one half or reduced number, arising from the contracted and more or less anastomosed skein. × 1,400. *Pinus Strobus.*

18, *a–c.* Final stages in the formation of the chromosomes, showing the separation of the segments from one another, and also the relation of some of them to the nucleolus. × 1,400. *Pinus Strobus.*

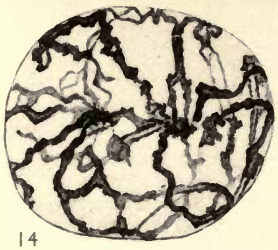
19, *a–l.* Various forms of chromosomes observed before the organization of the spindle. Each chromosome consists of two of the longitudinal split segments which were formed immediately subsequent to synapsis. × 1,400. *Pinus Strobus.*

20. The chromatic segments completely differentiated. The remnant of a nucleolus is still present, and the nuclear membrane is being resolved into threads. × 1,400. *Pinus Strobus.*

21. An early stage in spindle-formation, showing kinoplasmic threads entering from all directions but as yet no poles, or centers of radiations, have been established. Chromosomes are homogeneous in structure and regular in outline. × 1,400. *Pinus Strobus.*

22. The tripolar spindle. × 1,400. *Pinus rigida.* May 4, 1898.

23. The spindle has become nearly bipolar. × 1,400. *Pinus rigida.*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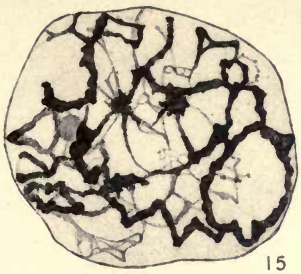
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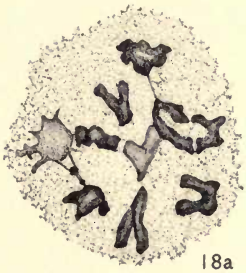
18c


18b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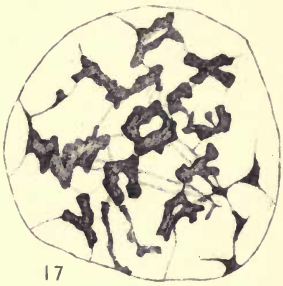
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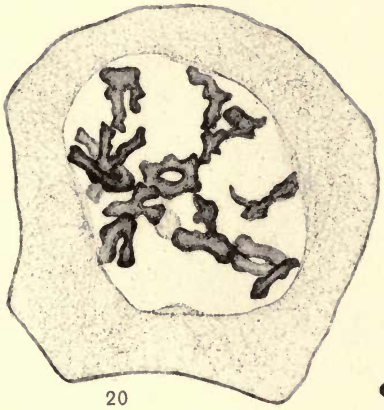
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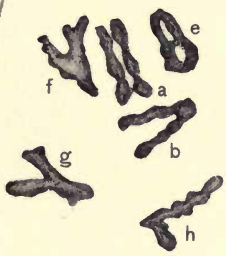


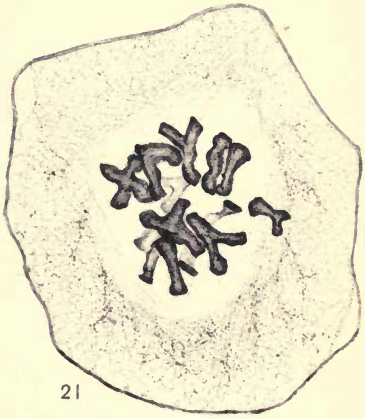
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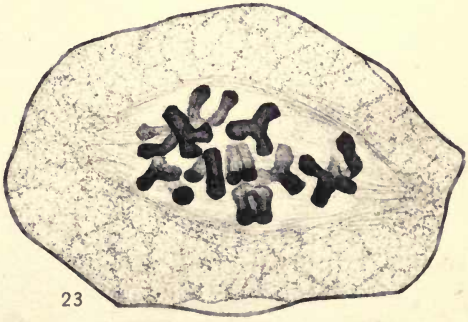
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## PLATE III.

FIG. 24. The equatorial plate stage. Spindle definitely bipolar and reaching to the ectoplasm. × 1,400. *Pinus rigida.*

25, 26. The metaphase of the heterotypical division; chromosomes irregular in outline and apparently much larger than in the late prophase. × 1,400. *Pinus Strobus.*

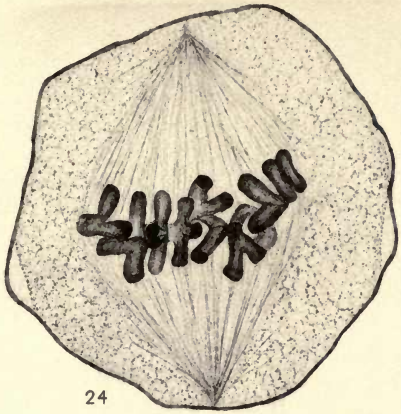
27–29. Anaphase of the heterotypical division. The longitudinal splitting of the chromosomes has been very greatly delayed in some cases. Such an appearance as that shown in fig. 29 is frequently met with, the stretched arms of the daughter chromosomes extending nearly the entire length of the spindle. × 1,400. *Pinus Strobus.*

30. The chromosomes just after reaching the poles, as seen in looking down upon the end of the pole. × 1,400. *Pinus Strobus.*

31–34. Stages in the development of the daughter-nuclei. A definite resting nucleus is formed at the close of the heterotypical division. × 1,400. *Pinus Strobus.*

35. A late telophase in the first division, the daughter-nuclei fully established. Delicate spindle threads still present, but no indication of a cell plate. The wall of the microspore-mother-cell is beginning to thicken centripetally. × 1,400. *Pinus Strobus.*

36–37. Stages in the formation of the spireme for the second division. × 1,400. *Pinus Strobus.*



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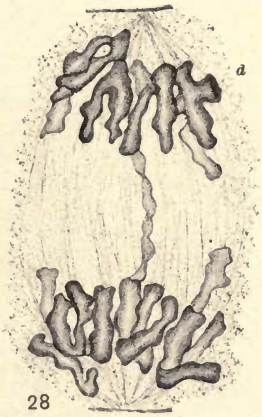


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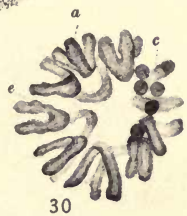


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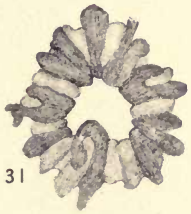

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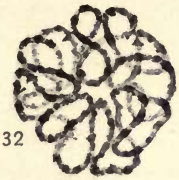

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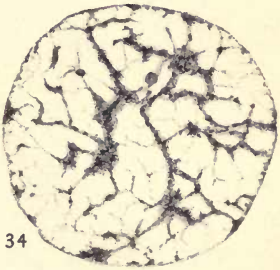

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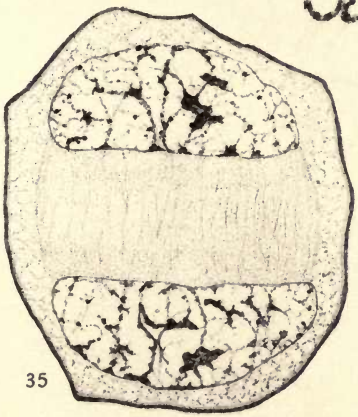

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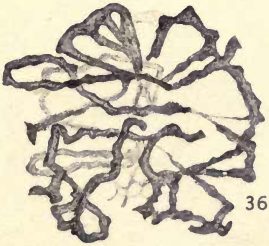

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MICROSPOROGENESIS.

## PLATE IV.

FIG. 38. Origin of the second spindle; the chromatic band looped in region of the future equatorial plate, and showing longitudinal fission. ×1,400. *Pinus rigida.*

39. Transverse segmentation is completed; and the distinct chromosomes have become apparent at the equatorial plate of the multipolar diarch spindle. ×1,400. *Pinus Strobus.*

40. Separation of the daughter-chromosomes of each pair formed by the transverse division shown in figure 39. ×1,400. *Pinus Strobus.*

41. Daughter-chromosomes arranged in two parallel rows at the equatorial plate. ×1,400. *Pinus Strobus.*

42. A late anaphase in the second division. ×1,400. *Pinus Strobus.*

43. Early telophase of the second division. ×1,400. *Pinus Strobus.*

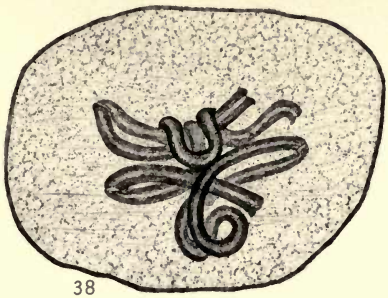
44. Late telophase of the tetrad division; the chromosomes of each nucleus have fused to form a spireme, but the nuclear membrane is not yet developed; rather faint cytoplasmic threads connect the four nuclei; the centripetal thickening of the mother-wall becomes more apparent. ×1,400. *Pinus rigida.*

45. The tetrad division is completed and the young microspores are distinctly differentiated, each surrounded by its own wall. ×1,400. *Pinus rigida.* May 10, 1898.

46. The four microspores are separated by very prominent walls which are continuous with the broad wall lining the original wall of the microspore-mother-cell; the outer, original spore-mother-wall is separated at two points from the thick, more recently formed inner wall. ×1,400. *Pinus austriaca.* May 9, 1898.

47. Microspores still within the mother-wall and showing the beginnings of the wings or air-sacs. ×1,400. *Pinus Strobus.* May 30, 1898.

48. Rupture of the mother-wall and escape of the microspores. ×810. *Pinus Strobus.* May 30, 1898.



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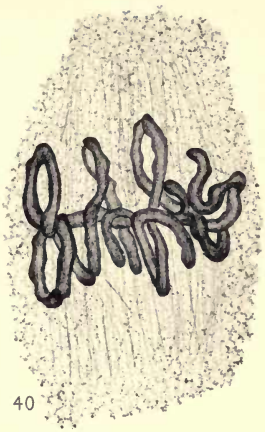

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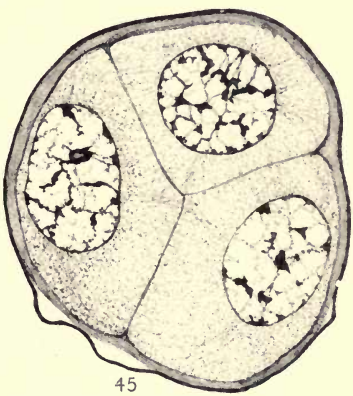

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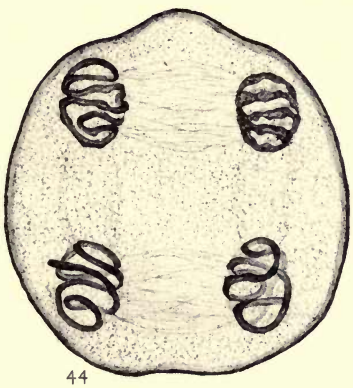

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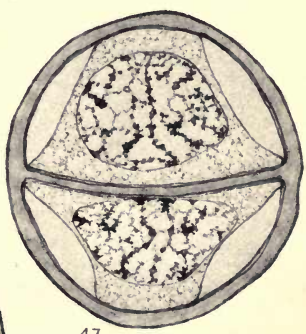

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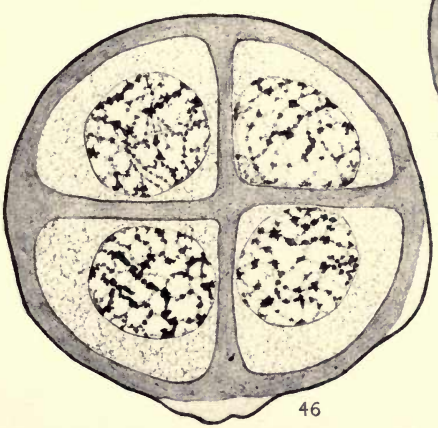

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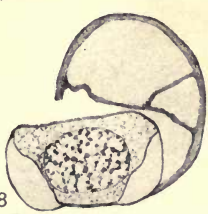


48

## PLATE V.

FIG. 49. Empty wall of the microspore-mother-cell showing the compartments formerly occupied by the microspores. × 810. *Pinus Strobus.*

50–54. Stages in the growth of the microspore; the inner, partial wall very apparent in the mature spore. Fig. 53 represents a section through the middle of a young microspore in a plane perpendicular to the wings. × 810. *Pinus Strobus.*

54–55. Stages in the first division of the microspore-cell; the spindle sharply pointed on the ventral side, broad on the dorsal side. × 810. *Pinus Strobus.* June 7, 1898.

56. Telophase in the first division of the microspore. × 810. *Pinus Strobus.*

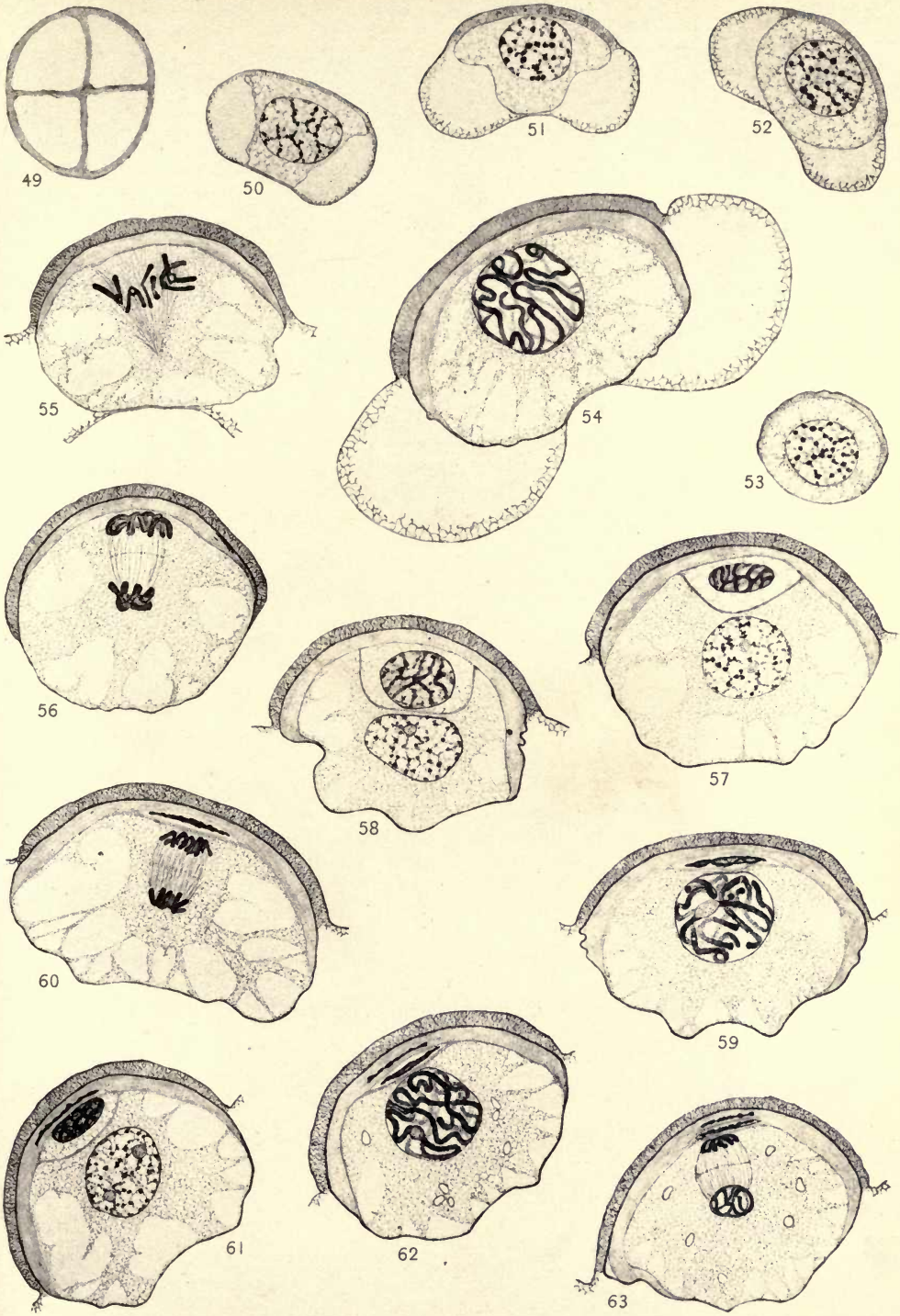
57. The resting stage following the first division of the microspore. × 810. *Pinus Strobus.*

58. The same as Fig. 57, but showing an exceptionally large prothallial cell. × 810. *Pinus Strobus.*

59–60. Spireme-stage and early telophase in the division to cut off the second prothallial cell. × 810. *Pinus Strobus.*

61. The germinated microspore at the close of the second division, showing the first prothallial cell already in an advanced stage of disintegration. × 810. *Pinus Strobus.*

62–63. Stages in the third division of the microspore, showing the rapid and almost complete obliteration of the first and second prothallial cells. Both prothallial cells are cut off from the apical cell by definite walls. × 810. *Pinus Strobus.*

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DEVELOPMENT OF POLLEN-GRAIN.

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## PLATE VI.

FIGS. 64–65. Mature pollen-grains; in fig. 64 the remnants of the two prothallial cells can be seen, while in fig. 65 all signs of the first cell have disappeared. ×810. *Pinus Strobus.* June 9, 1898.

66. Vertical section through an ovule immediately after pollination; the macrospore-mother-cell is very conspicuous; the upper portion of the free limb of the integument is shown to be three cells in thickness, there is a slight concavity in the apex of the nucellus; macrospore-mother-cell (*m.m.c.*), nucellar cap (*nuc.*), micropyle (*mic.*). ×46. *Pinus rigida.* May 27, 1902.

67. Vertical section through the upper part of an ovule showing pollen-chamber; the middle layer of cells in the upper part of the free limb of the integument has elongated and closed the microcarpylar canal. ×46. *Pinus rigida.* June 1, 1902.

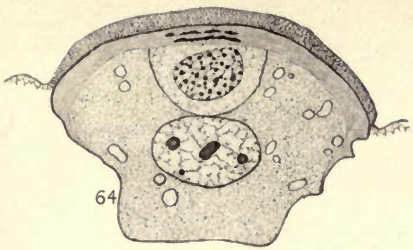
68. A vertical section through the upper part of an ovule. The elongated cells noted in fig. 67 have become divided by the formation of cross walls into smaller cells. ×46. *Pinus rigida.* June 4, 1902.

69. A vertical section through an ovule some days after pollination. Axial row (*a.r.*). ×62. *Pinus Strobus.* June 17, 1898.

70. A vertical section of an ovule showing the winter condition. ×62. *Pinus Strobus.* January 4, 1898.

71. A vertical section of an ovule soon after the second period of growth has begun. ×62. *Pinus Strobus.* May 26, 1898.

72. A vertical section through the upper part of an ovule at the time of the division of the generative nucleus; (*nuc.*1), that portion of the nucellar cap which was developed during the first period of activity; (*nuc.*2), that portion of the nucellar cap which constitutes the second year's growth; *o*, disintegrating spongy tissue. ×62. *Pinus Strobus.* June 9, 1898.



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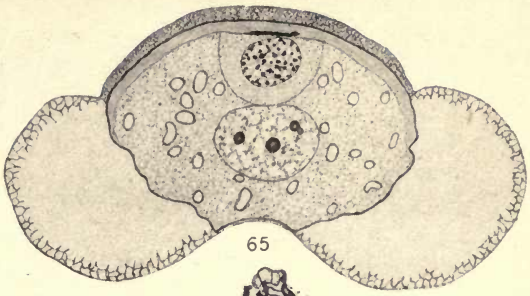

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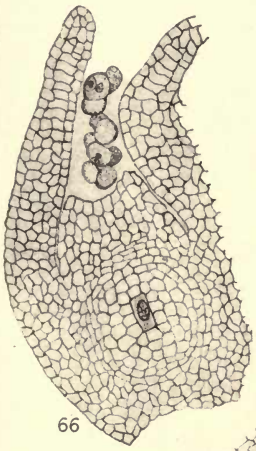

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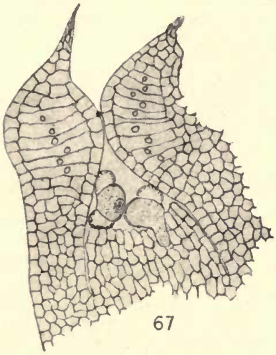

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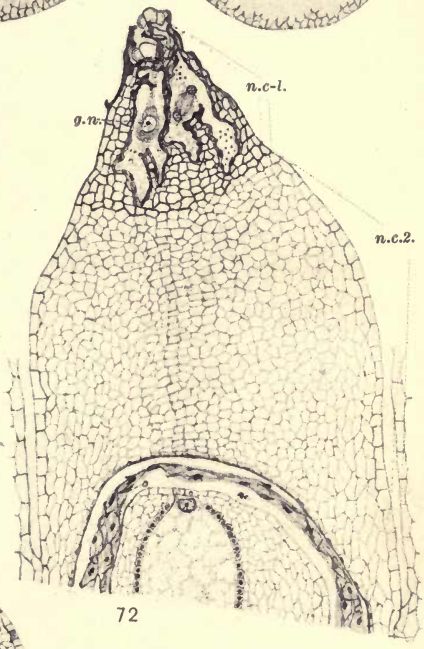


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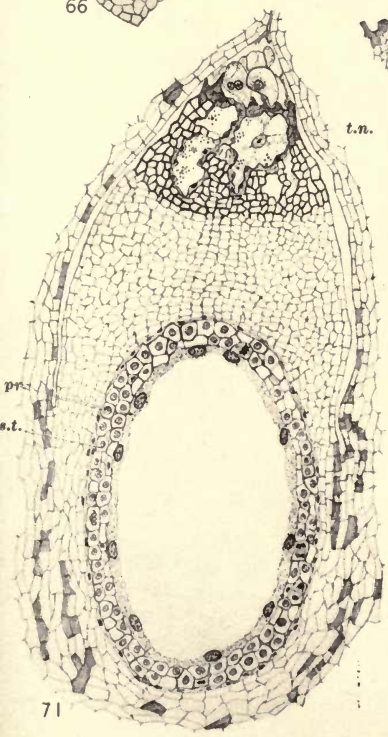


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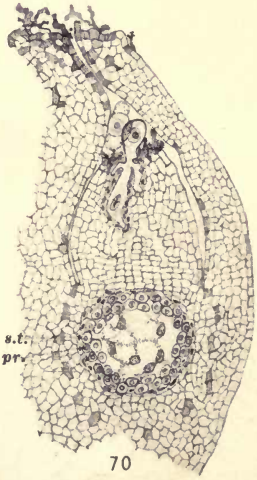


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POLLINATION AND SUBSEQUENT PHENOMENA.

## PLATE VII.

FIG. 73. A vertical section through the upper part of an ovule shortly before fertilization; reconstructed from three adjacent sections of the series; *o*, last vestige of spongy tissue. ×62. *Pinus Strobus*, June 15, 1898.

74. Pollen-grain from the nucellus of Fig. 73. The antheridial cell is still undivided. ×472.

75. A vertical section through the extreme upper portion of an ovule soon after pollination, showing the uppermost part of the nucellar cap, and a pollen-grain in the first stages of germination; *p.c*, pollen-chamber. ×472. *Pinus Strobus*. June 13, 1898.

76. A pollen-grain soon after germination. The tube-nucleus is moving into the pollen-tube. ×472. *Pinus Strobus*. June 24, 1898.

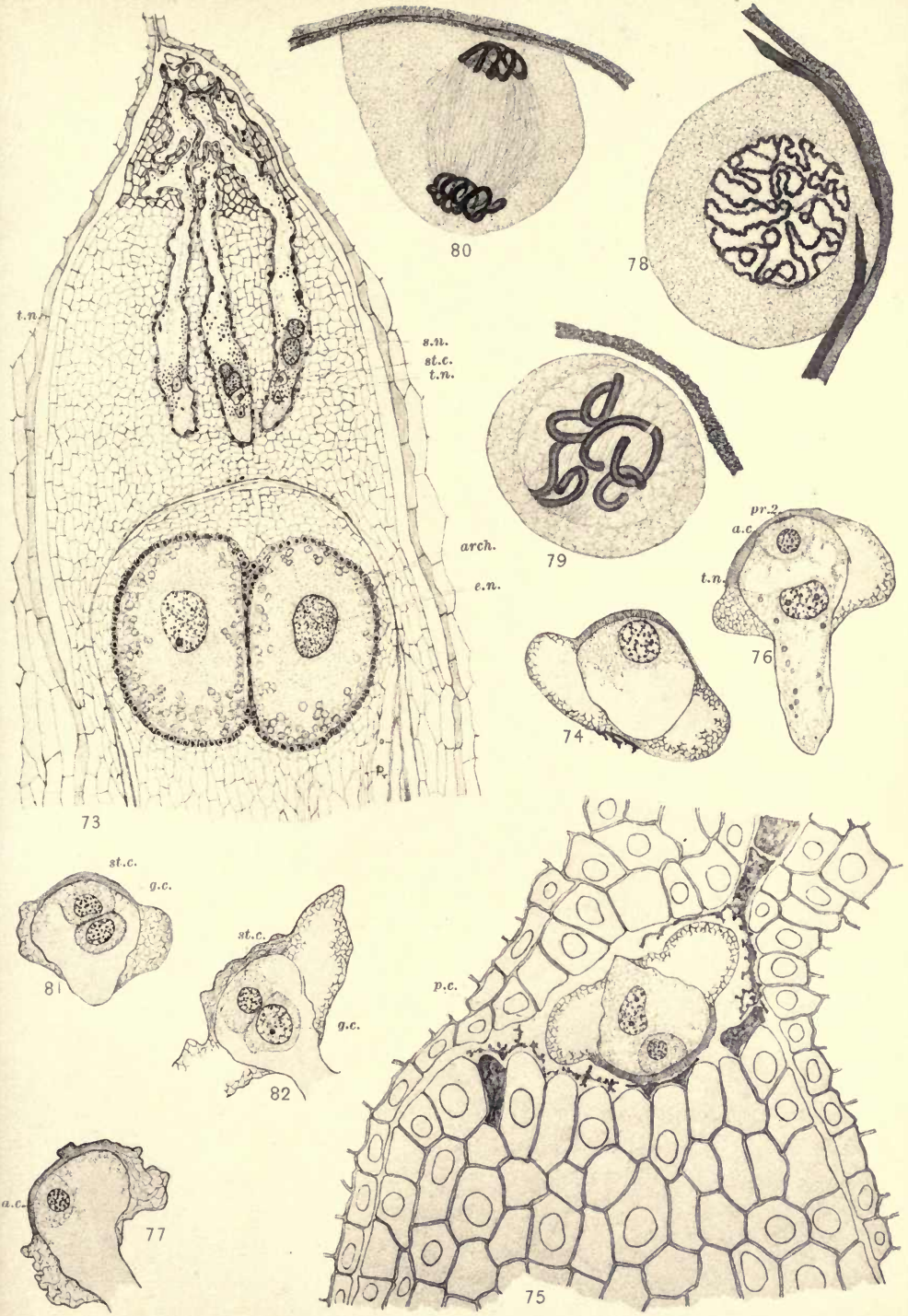
77. A pollen-grain after the tube-nucleus has passed into the pollen-tube. ×472. *Pinus Strobus*. July 15, 1898.

78. Spireme stage in the division of the antheridial cell. ×1,400. *Pinus rigida*. April 27, 1898.

79–80. Stages in the division of the antheridial cell. ×1,400. *Pinus Strobus*. August 4, 1898.

81. A pollen-grain after the antheridial cell has divided. ×472. *Pinus Strobus*. August 4, 1898.

82. The same at a later date, showing a slight increase in the size of the generative cell. ×472. *Pinus Strobus*. October 7, 1898.

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GROWTH OF THE POLLEN-TUBE.

## PLATE VIII.

FIG. 83. The pollen-tube which is shown in fig. 70, more highly magnified. × 472. *Pinus Strobus.* January 4, 1899.

84. A pollen-grain and the upper portion of a pollen-tube, showing the stalk- and the generative-cell just before their passage into the pollen-tube. × 472. *Pinus austriaca.* May 3, 1898.

85, 86. Later stages than the above, showing the passage of the generative- and the stalk-cell into the pollen-tube; in fig. 86, the two cells are breaking loose from each other. × 472. *Pinus austriaca.* May 10 and 17, 1898.

87. The male gametophyte at the time of the entrance into the tube of the generative- and the stalk-cell; *n.t*, a bit of the dead nucellar tissue. × 472. *Pinus Strobus.* June 9, 1898.

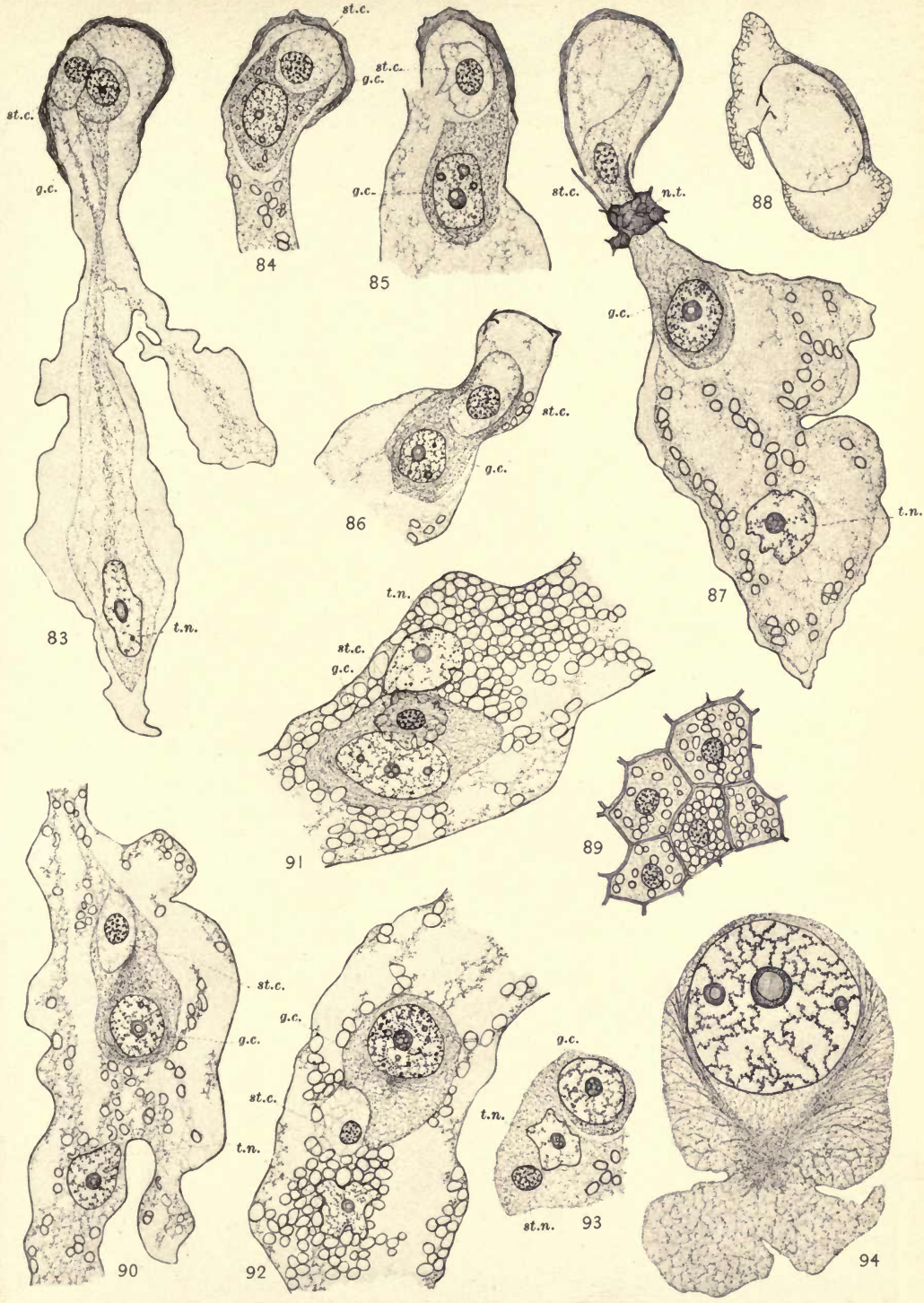
88. A pollen-grain after the generative and the stalk-cell have passed into the pollen-tube; taken from the top of the nucellus of the ovule shown in fig. 72. × 472. *Pinus Strobus.* June 9, 1898.

89. A few of the cells from that portion of the nucellar cap marked *nuc.*2 in fig. 72. The cells are filled with starch grains. × 472. *Pinus Strobus.* June 9, 1898.

90–92. Portions of pollen-tubes showing successive stages in the passage of the stalk-cell over the generative cell, as also the presence of large quantities of starch in the pollen-tube. × 472. *Pinus resinosa.* June 2, *P. Strobus*, May 24; *P. rigida*, June 8, 1898.

93. The generative cell, bearing on its surface both the tube-nucleus and the stalk-nucleus. In this instance the stalk-cell has passed beyond the tube-nucleus. × 472. *Pinus resinosa.* June 3, 1898.

94. The generative cell showing a very early stage in the formation of the spindle. The nucleus is in the extreme uppermost part of the cell. × 744. *Pinus rigida.* June 8.

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SPERMATOGENESIS.

## PLATE IX.

FIGS. 95–96. The generative cell in the early stages of its division, showing granular condensation and radial arrangement of cytoplasm. The spindle fibers arise in the cytoplasmic condensation and extend in the form of a cone to the nuclear membrane. × 744. *Pinus rigida*. June 8 and 10, 1898.

97. A cross-section through the generative cell during an early stage in its mitosis. The protoplasmic condensation is seen from below looking toward the nucleus. × 744. *Pinus austriaca*. June 4, 1898.

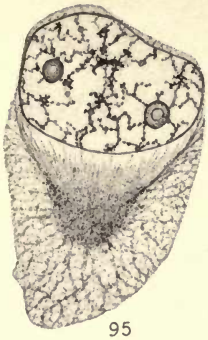
98. A later stage in the division of the generative nucleus. × 744. *Pinus austriaca*. June 10, 1898.

99. The generative cell just before the disappearance of the lower portion of the nuclear membrane showing a single deep indentation on the lower side of the nucleus. × 744. *Pinus Strobus*. June 9, 1898.

100. A stage in spindle-formation directly following that shown in fig. 99. The nuclear membrane has given way and the spindle fibers are entering the nuclear cavity. The nucleolus is still distinctly visible. × 744. *Pinus Strobus*. June 10, 1898.

101. The gradual disappearance of the nuclear membrane and the extension of the spindle fibers across the nucleus. × 744. *Pinus austriaca*. June 7, 1898.

102–103. Further development of the spindle and the formation of the chromosomes. The marked condensation in the cytoplasm from which the spindle arose has almost entirely disappeared. × 744. *Pinus austriaca*. June 8, 1898.



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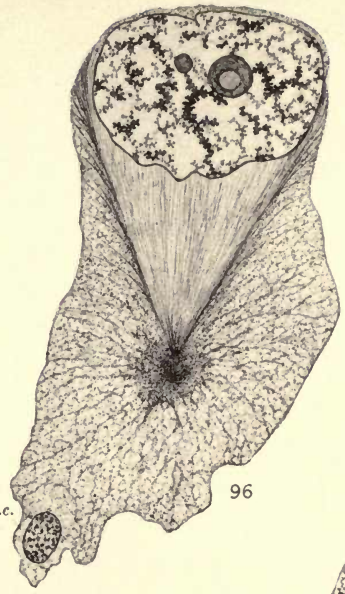
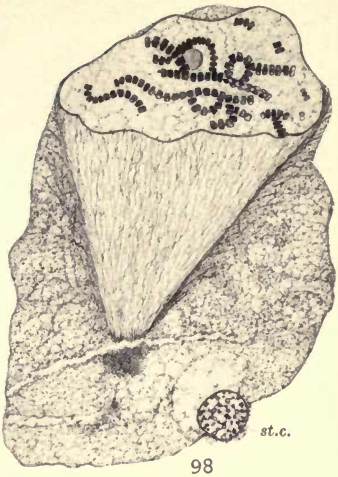


st.c.

96



st.c.

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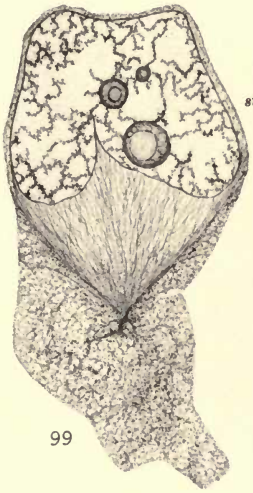

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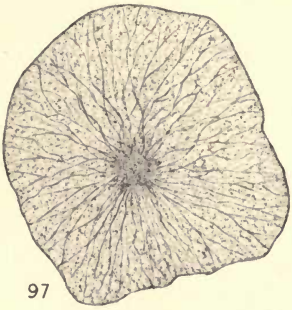

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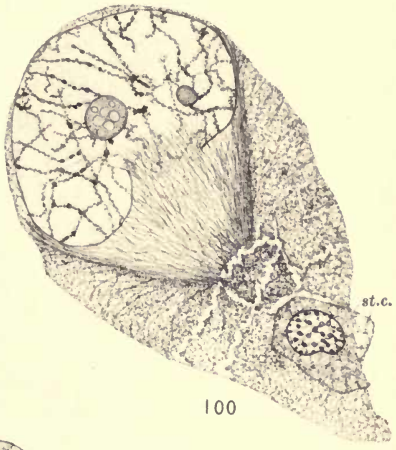

st.c.

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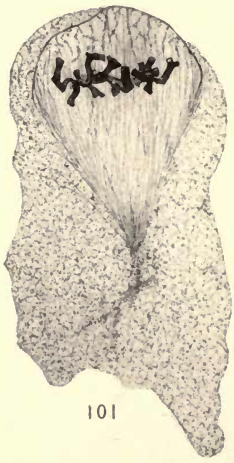

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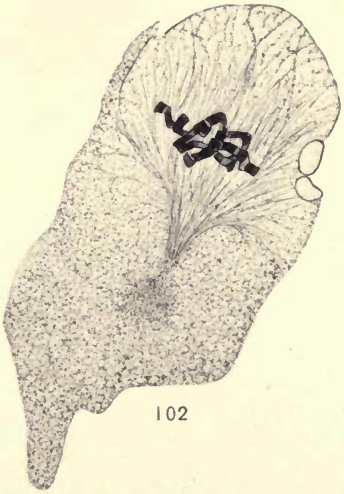

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SPERMATOGENESIS.

## PLATE X.

FIGS. 104–106. Later stages in the development of the spindle showing the gradual drawing together of the outer extremities of the threads to form the upper pole of the spindle. The upper pole of the spindle does not reach the nuclear membrane, but in fig. 105 definite threads extend from the pole to the nuclear membrane. × 744. Fig. 104. *Pinus rigida*, June 13; the other figures, *Pinus austriaca*, June 9–10, 1898.

107. First stage in the development of the sperm-nuclei. × 744. *Pinus Strobus*. June 9, 1898.

108. The sperm-nuclei just after the formation of the nuclear membrane showing early stages in the development of the daughter-reticula. The lower nucleus is already slightly larger than the upper one. × 744. *Pinus montana uncinata*. May 31, 1898.

109–112. Various stages in the growth of the sperm-nuclei. A cell plate is sometimes apparent as in fig. 110, but no dividing wall is ever formed. × 744. Fig. 112. *Pinus Strobus*, June 10; fig. 109, *P. resinosa*, June 15; fig. 110, *P. austriaca*, June 10. Fig. 111 represents another section through the upper nucleus of fig. 110, and shows how the upper of the sperm-nuclei is frequently indented along its outer surface. 1898.

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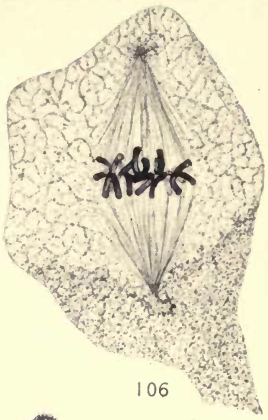

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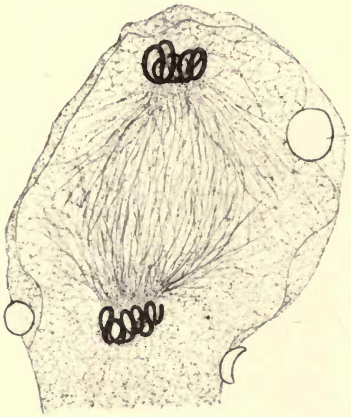


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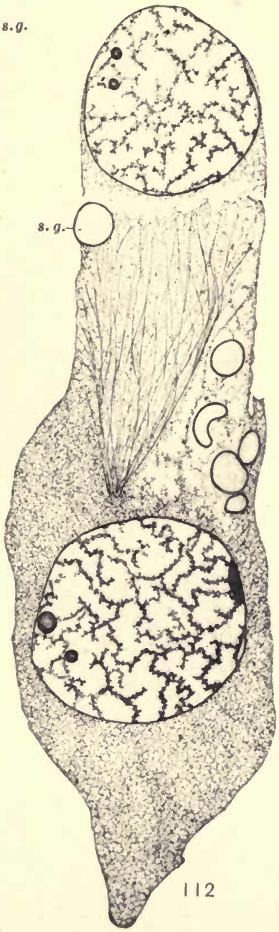


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SPERMATOGENESIS.

## PLATE XI.

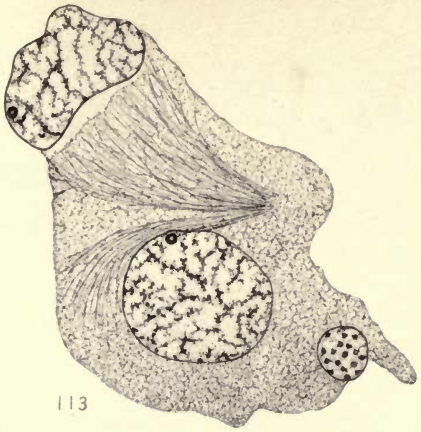
FIG. 113. A peculiar figure sometimes observed in the late telophase of the division. × 744. *Pinus austriaca.* June 10, 1898.

114. A pollen-tube in which the smaller sperm-nucleus appears to be in advance of the larger. This pollen-tube, having approached an egg that had already been fertilized, has turned aside and is passing up over the endosperm so that the normal position of the cells appears exactly reversed; *n.c.*, neck-cells of the archegonium. × 289. *Pinus Strobus.* June 20, 1898.

115–116. Cross-sections through the two sperm-nuclei after they have attained full size and have about reached, in their downward passage, the middle of the nucellar cap. × 744. *Pinus Strobus.* June 15, 1898.

117. The sperm-cell after all traces of the spindle have disappeared, but before the two nuclei have come together. × 472. *Pinus Strobus.* June 13, 1898.

118. The same after both nuclei have come to lie in the upper part of the cell. × 472. *Pinus Strobus.* June 10, 1898.



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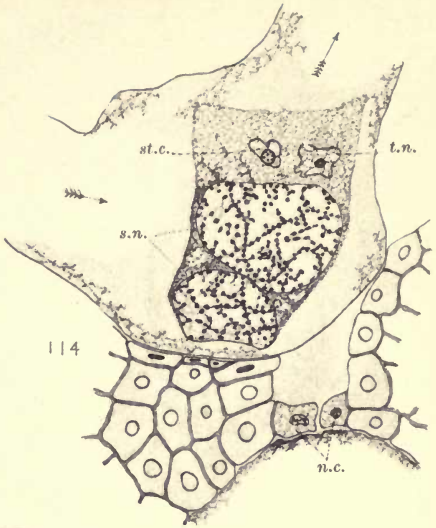


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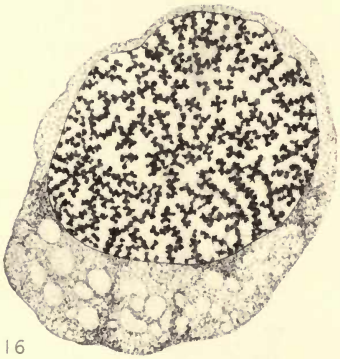

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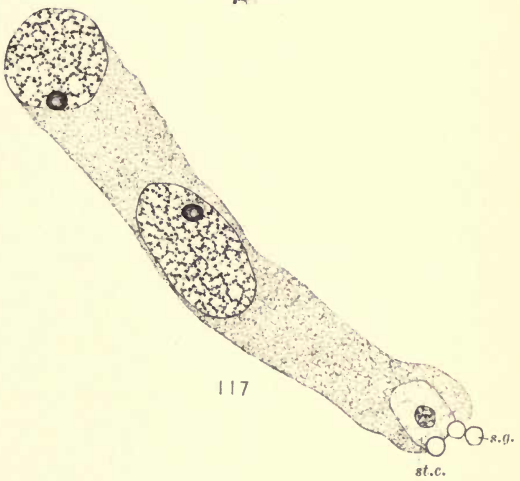


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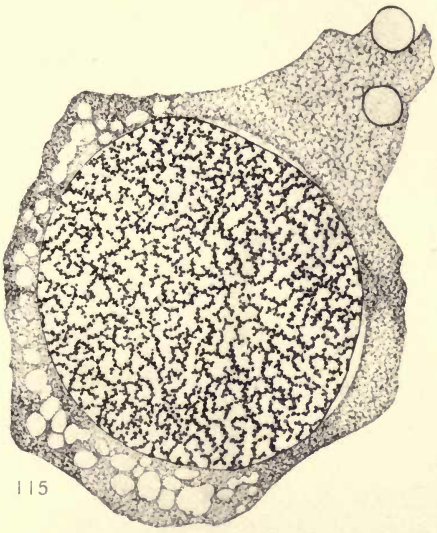

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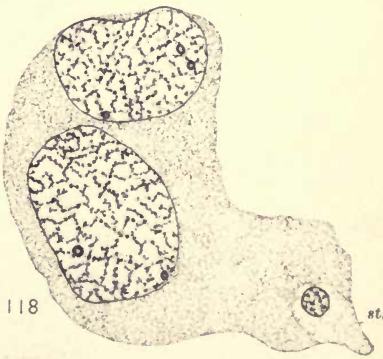


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SPERMATOGENESIS.

## PLATE XII.

FIG. 119. The lower portion of a pollen-tube which has penetrated about two-thirds the length of the nucellar cap. × 472. *Pinus Strobus.* June 14, 1898.

120. The lower portion of a pollen-tube which is just pushing between the neck-cells of the archegonium. *p*, pit in apex of tube. × 472. *Pinus Strobus.* June 20, 1898.

121. A vertical section of a young cone; the ovuliferous scales have not as yet been organized. × 57. *Pinus austriaca.* March 14, 1898.

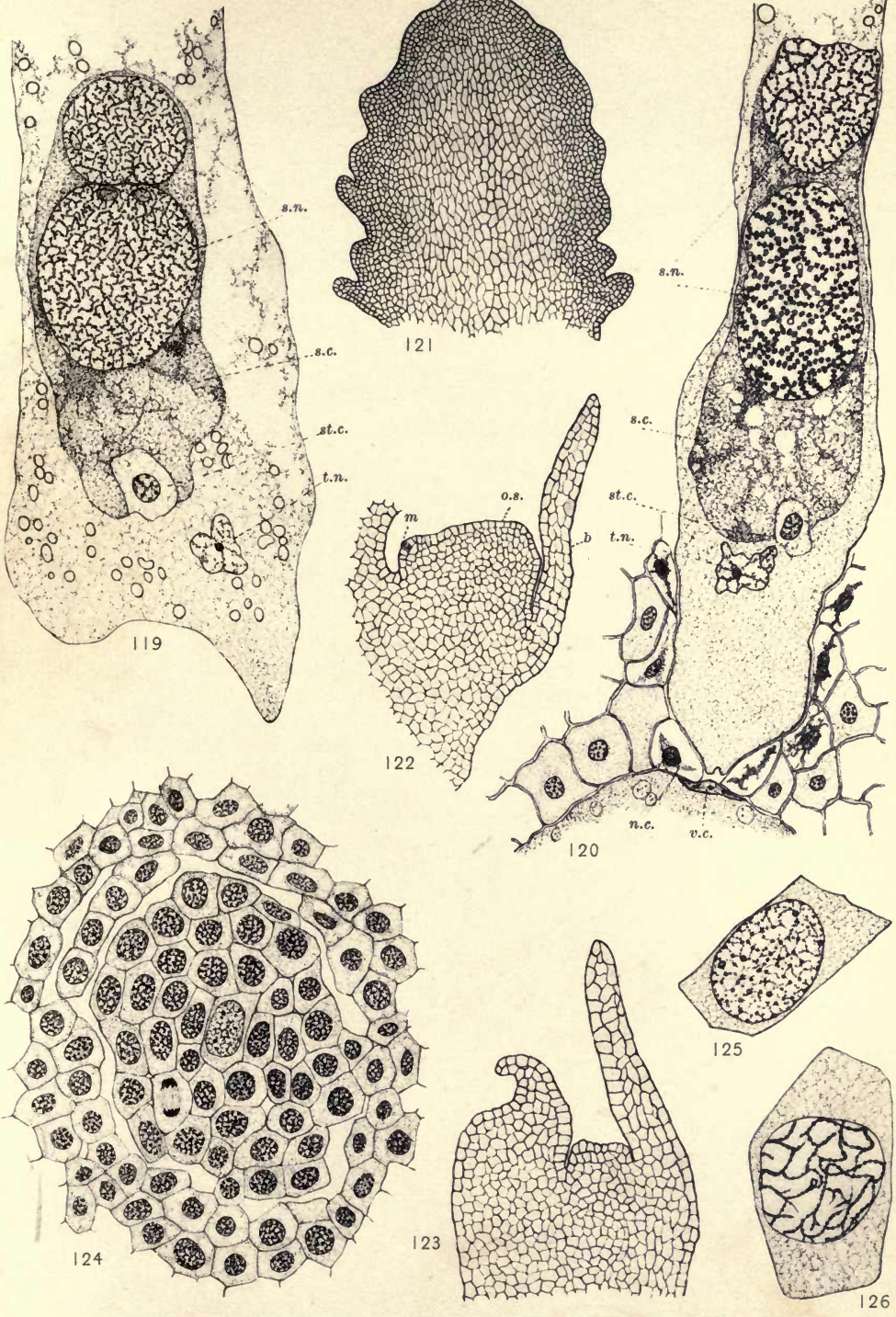
122. Section of an ovuliferous scale showing the first indication of an ovule. *m.* ovule; *o.s*, ovuliferous scale; *b*, bract. × 150. *Pinus Strobus.* May 31, 1898.

123. A vertical section of an ovule one week later than that shown in fig. 122. × 150. *Pinus Strobus.* June 6, 1898.

124. A very young macrospore-mother-cell showing differentiation of spongy tissue. × 394. *Pinus rigida.* May 15, 1902.

125. The macrospore-mother-cell from fig. 124 more highly magnified. × 810.

126. A macrospore-mother-cell just prior to synapsis. × 810. *Pinus Strobus.* June 27, 1898.

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MACROSPOROGENESIS.

## PLATE XIII.

FIG. 127. The macrospore-mother-cell in synapsis. × 810. *Pinus austriaca.* June 6, 1898.

128. The same in recovery from synapsis showing continuous skein. × 810. *Pinus austriaca.*

129–133. Stages leading to the organization of the chromosomes in the first or heterotypical division of the macrospore-mother-cell. × 810. Fig. 132, *Pinus Strobus*, the others, *P. rigida*. Fig. 131 illustrates an instance in the unusually early disappearance of the nuclear membrane.

134–137. Stages in the establishment of the spindle in the first division of the macrospore-mother-cell. The reduced or one half number of chromosomes appear in this mitosis. The spindle arises as a multipolar diarch. × 810. Fig. 137, *Pinus rigida*, the others, *P. Strobus.*

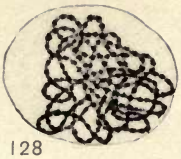
138. Late telophase in the first division. A cell-wall is laid down and definite resting nuclei are formed. × 810. *Pinus Strobus.* June 13, 1899.

139–140. The close of the heterotypical division. Resting nuclei are formed but the upper resting nucleus in each case shows signs of disintegration and doubtless would not have divided. × 810. Fig. 139, *Pinus austriaca*, fig. 140, *P. rigida.*

141. The two daughter-cells formed by the first division of the macrospore-mother-cell. Both would doubtless have divided again. × 810. *Pinus austriaca.*

142. The second or homotypic division of the macrospore-mother-cell. The spindles are oblique and arise as multipolar diarchs. The chromosomes have the same form as those which arose on the first division of the macrospore-mother-cell. × 810. *Pinus austriaca.*


127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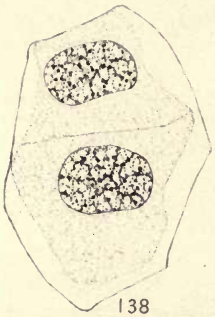
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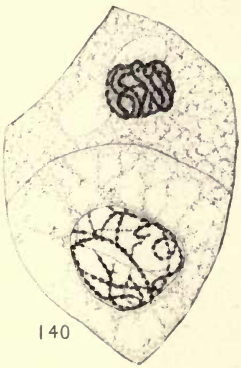
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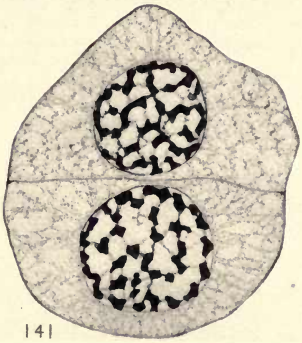
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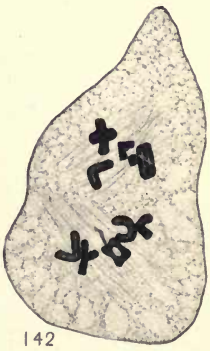
142

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## PLATE XIV.

FIGS. 143–144. Two axial rows of three cells each. The upper of the two daughter-cells formed as a result of the heterotypical division has not divided in either case; a few starch grains in the cells of the axial row and many large ones in the spongy tissue as shown in fig. 143. × 810. Fig. 143. *Pinus Strobus*, fig. 144, *P. rigida*.

145. An axial row of four cells, reconstructed from serial sections. × 810. *Pinus austriaca*.

146. A macrospore nucleus surrounded by large starch grains. × 810. *Pinus austriaca*.

147. Growth of the functional macrospore; the peripheral layer of cytoplasm already established; the three upper cells of the axial row almost destroyed; one large cell of the spongy tissue shown. × 810. *Pinus austriaca*. June 13, 1898.

148. An axial row of three cells; the functional macrospore much enlarged, and the two upper cells in an advanced stage of disintegration; the spongy tissue distinctly differentiated; the cells along its outer surface more or less tabular in outline and many of them badly disorganized. Pathological conditions have just entered in as shown by the reduced amount of cytoplasm in the cells of the spongy tissue and the slight thickening of their walls. × 234. *P. rigida*. June 24, 1902.

149. The first division of the macrospore-nucleus. × 234. *Pinus Strobus*. July 29, 1898.

150. The karyokinetic figure from the above more highly magnified; the division conforms to the typic type and shows the one-half number of chromosomes. × 810.

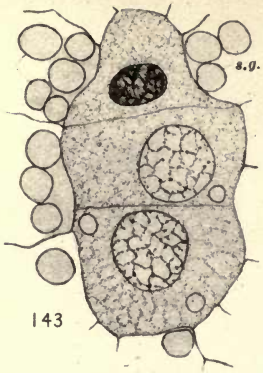
151. The first two nuclei of the female gametophyte. × 234. *Pinus austriaca*. July 29, 1898.

152. The four-nucleated stage of the female gametophyte. × 234. *Pinus Strobus*. August 4, 1808.

153. One of the sixteen free nuclei of a female gametophyte, all sixteen nuclei being in the spireme stage of division. × 810. *Pinus Strobus*. October 12, 1898.

154. A vertical section of the central portion of an ovule showing the spongy tissue and the prothallium with its nuclei, of which there are sixteen, all in the equatorial stage of division; the prothallium has been somewhat displaced by the action of the fixing fluid. × 46. *Pinus Strobus*. October 12, 1898.

155. One of the spindles from the above more highly magnified. × 744.

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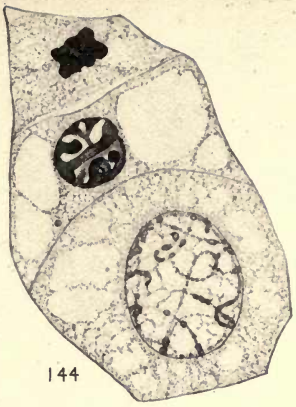

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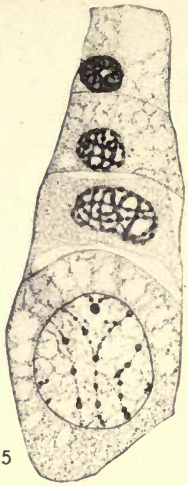

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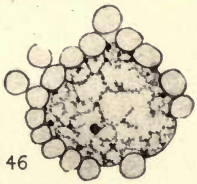

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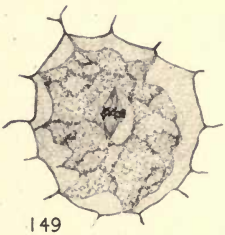

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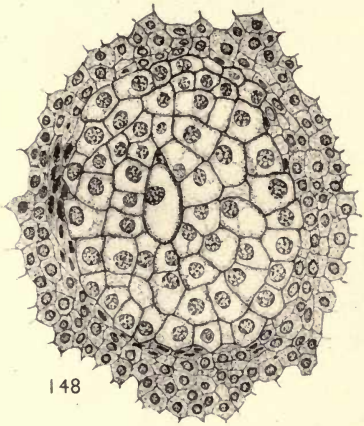

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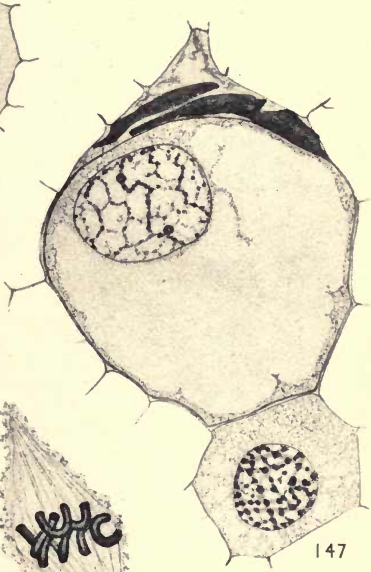

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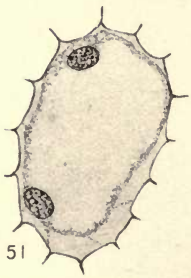

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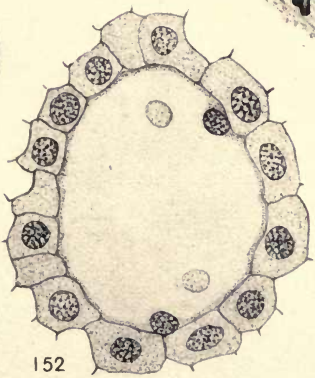

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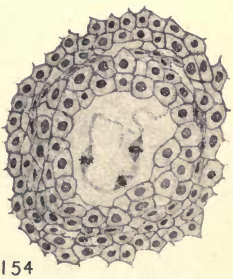

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GERMINATION OF MACROSPORE.

## PLATE XV.

FIG. 156. Surface view of a bit of the prothallium showing two free nuclei and the vacuolate protoplasm surrounding them. × 744. *Pinus Strobus.* May 17, 1898.

157. A radial section through the lower portion of an ovule showing prothallium, spongy tissue, and normal nucellar tissue. × 472. *Pinus Strobus.* May 26, 1899.

158. As fig. 157, except that the spongy tissue and the normal nucellar tissue are separated by a double layer of cells, belonging to the nucellus, which have lost their protoplasmic content but their walls have not yet collapsed. × 472. *Pinus Strobus.* May 26, 1899.

159. A bit of the prothallium in surface view showing the complex cytoplasmic figure characteristic of the late telophase in free nuclear division. × 472. *Pinus austriaca.* May 17, 1898.

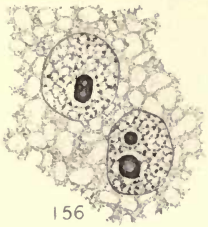
160. Surface view of a portion of a prothallium immediately after the organization of cell-walls separating the free nuclei. × 472. *Pinus Strobus.* May 26, 1899.

161. A bit of the prothallium as seen in radial section just after cell-walls have arisen. The cells are open on their inner surfaces and the nuclei remain near the open sides. × 394. *Pinus austriaca.* May 20, 1898.

162. A prothallium still open at the center showing that true "alveoli" as described by Sokolowa are not present; the archegonia rudiments at the micropylar end; the spongy tissues still prominent. × 62. *Pinus austriaca.* May 24, 1898.

163. A condition often found in the ovule. The macrospore-mother-cell has failed to develop and the walls of the spongy tissue have thickened and stain deeply. × 46. *Pinus Strobus.*

164–166. Figures illustrating karyokinesis in the spongy tissue. The method is typic with the number of chromosomes characteristic of the sporophyte. × 810. *Pinus Strobus.*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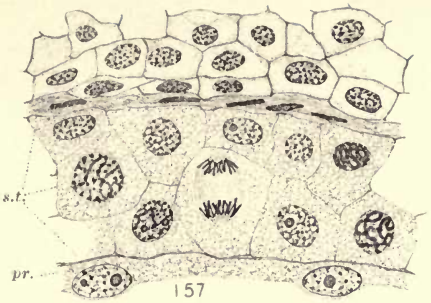

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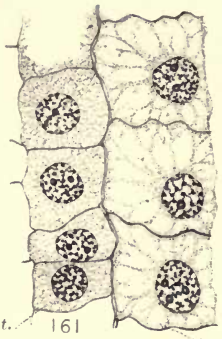

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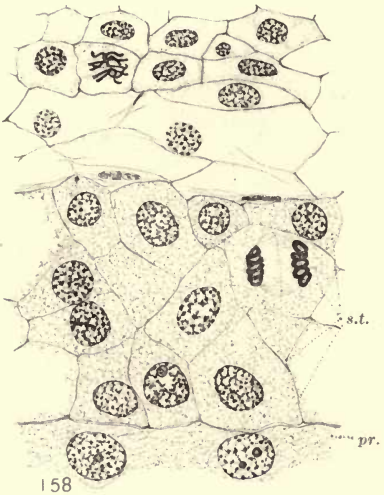

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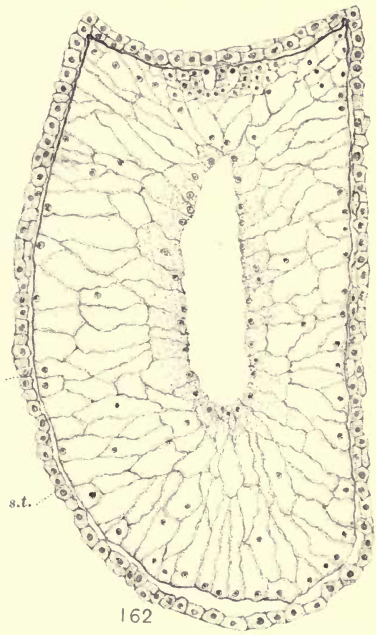

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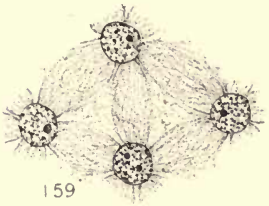
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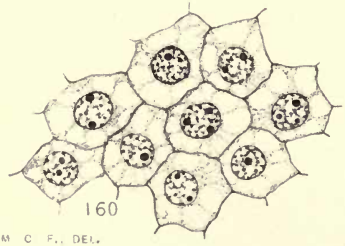
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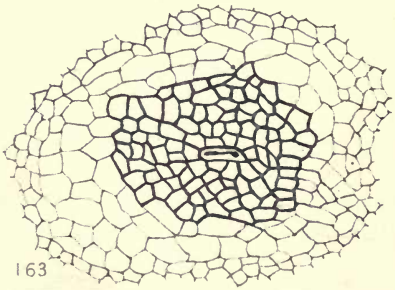
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FEMALE PROTHALLIUM.

## PLATE XVI.

*Pinus Strobus* unless otherwise indicated.

FIG. 167. Telophase in the division of a cell of the spongy tissue. × 810.

168. The macrospore and some of the cells of the spongy tissue in the first stages of disintegration and having the appearance of a group of sporogenous cells. (*mac.*) macrospore. × 96. *Pinus austriaca.*

169–175. Stages in the early development of the archegonium. The central cell remains close beneath the neck cells. The cytoplasm is very vacuolate. × 140.

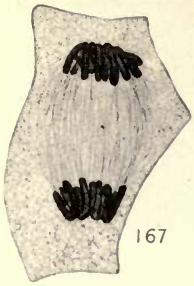
Fig. 169, May 26, 1890; fig. 171, May 31, 1898.

176–179. Later stages in the growth of the archegonium. The vacuoles gradually disappear and many proteid vacuoles arise in the cytoplasm. × 62. Fig. 178 collected June 15, 1899.

180. Mature archegonium. The nucleus has assumed a central position in the cell; the ventral canal-cell is in an advanced stage of disintegration; the proteid vacuoles are distributed about the periphery especially along the basal portion of the egg, the receptive vacuole has appeared but has not yet assumed its mature or final shape. × 62. June 17, 1899.

181. Nucleus of the central cell shortly before its division. This nucleus is almost invariably concave on the side towards the neck cells. × 472.

182–184. Prophases in the division of the central cell. × 472. Fig. 184, *Pinus austriaca.*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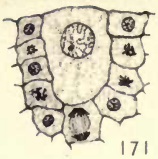
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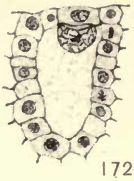
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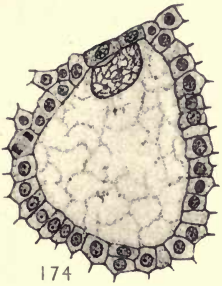
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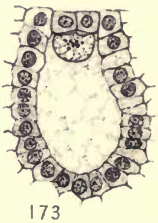
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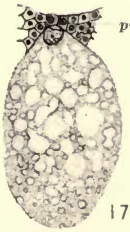
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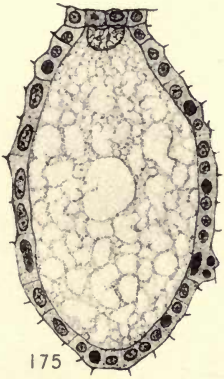
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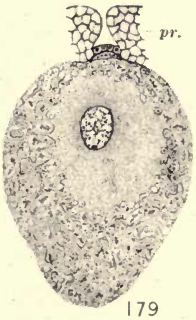
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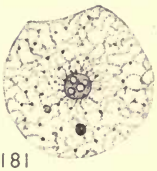
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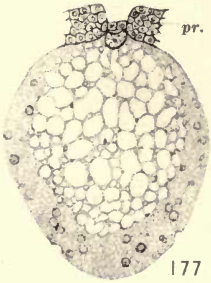
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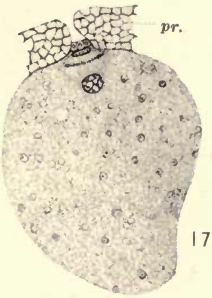
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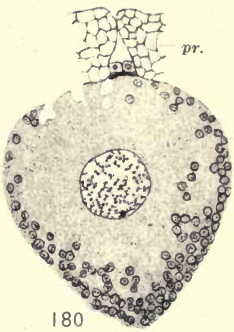
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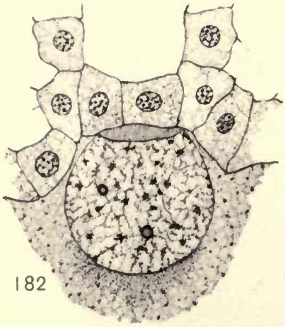
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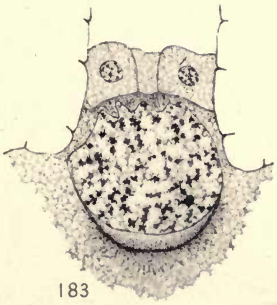
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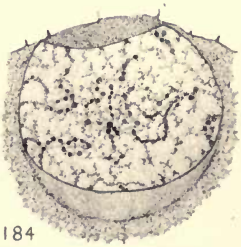
184

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FERGUSON,—PINUS.

OOGENESIS.

## PLATE XVII.

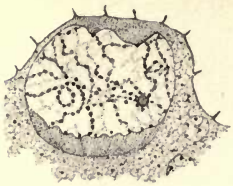
*Pinus Strobus* unless otherwise indicated.

FIGS. 185–186. Later stages in the prophase of the division of the central cell. × 472. Fig. 186, *Pinus austriaca*.

187–188. Disappearance of the nuclear membrane and establishment of the achromatic spindle. The spindle now lies wholly within the area previously occupied by the nucleus. × 472.

189. Cross-section of the nucleus of the central cell just as the chromosomes are undergoing longitudinal splitting at the equatorial plate. × 472.

190–197. Separation of the half chromosomes and formation of the daughter-nuclei. × 472. Figs. 192 and 195, *Pinus austriaca*. These figures show some of the variations occurring in the mitotic figure for this division, and the corresponding variations in the structure of the nucleus of the ventral canal-cell. Figs. 190, 191, 193 and 196 are very interesting, showing how some at least of those ventral canal-cells in which no definite nucleus is organized have arisen. Figs. 192, 194 and 195 are also interesting as leading to the formation of a normal nucleus within the ventral canal-cell. It will be noted that this spindle is always monopolar at its lower extremity and usually broadly multipolar at the opposite end. Fig. 192 is the only instance observed of a sharply bipolar spindle. The egg nucleus is larger from the very first than the nucleus of the ventral canal-cell.



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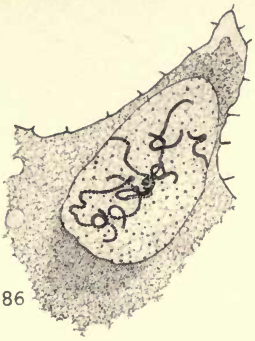

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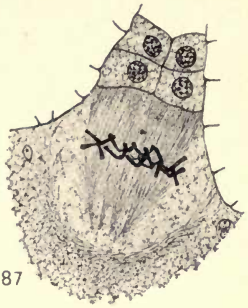

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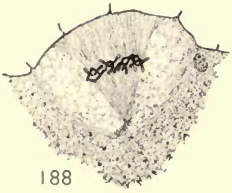

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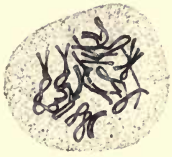

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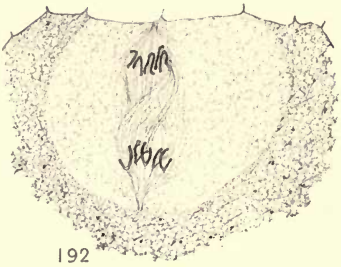

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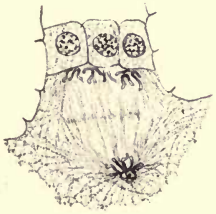

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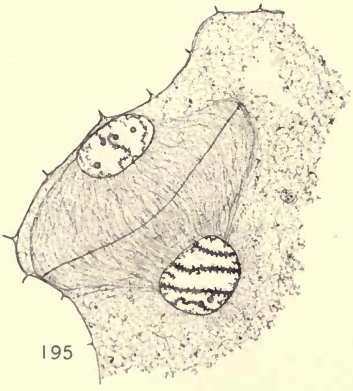

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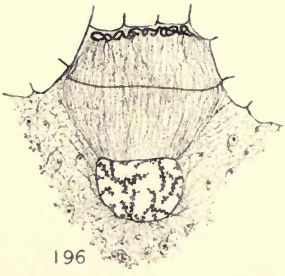

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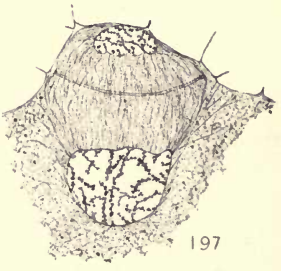

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## PLATE XVIII.

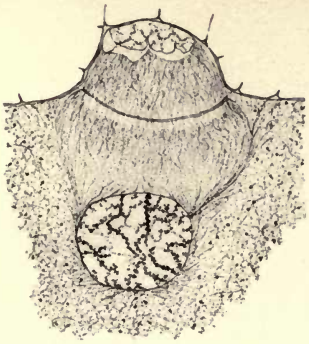
### *Pinus Strobus.*

FIGS. 198–199. Some of the aspects presented by the ventral canal-cell. It is doubtful in both of these cases if any nucleus has ever been organized within the ventral canal-cell, and the chromosomes have not even fused to form a spireme. × 472.

200–202. Later history of the ventral canal-cell and early stages in the development of the egg-nucleus. The first indication of the primary nucleolus is seen on the lower side of the egg-nucleus in fig. 202, and the ventral canal-cell already shows marked signs of disintegration. × 472.

203–204. Later stages in the downward movement and growth of the egg-nucleus showing growth of primary nucleolus. × 472.

205. Mature egg-nucleus. The primary nucleolus is very large and vacuolate and several secondary nucleoli are scattered throughout the nucleus. The structure of this nucleus varies greatly. This one was selected not because it can be said to be any more typical than others, but because it represents an average rather than an extreme condition as to density of reticulum and number of secondary nucleoli. × 472.



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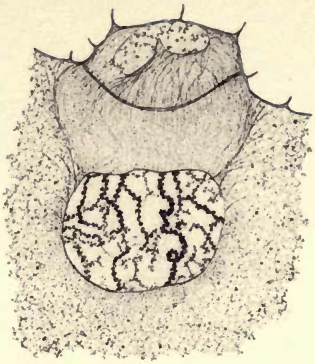

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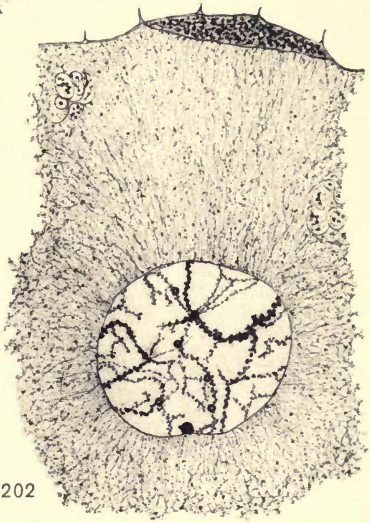

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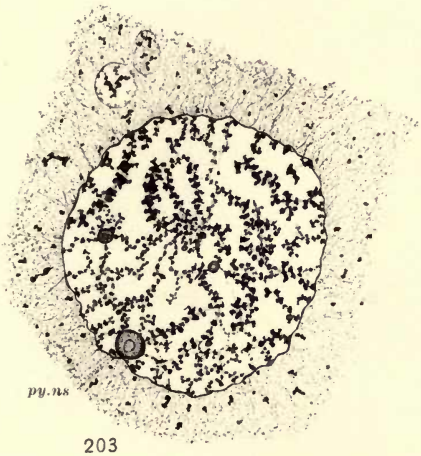


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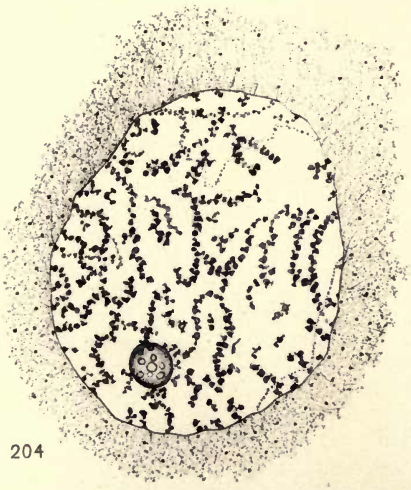

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## PLATE XIX.

*Pinus Strobus* unless otherwise indicated.

FIG. 206, *a–g*. Portions of the reticulum from different mature egg-nuclei, showing some of the variations which may occur in the structure of this nucleus. × 1050.

207. Division of the central cell, showing also the lower portion of a pollen-tube which has already reached the endosperm. In this instance a very short time would have elapsed between the division of the central cell and fertilization. × 209. *Pinus montana uncinata.*

208. The primary nucleolus from a mature egg-nucleus with secondary nucleoli clustered about it and evidently formed by it. The primary nucleolus has a great affinity for stains at this time. × 1050.

209. The primary nucleolus of a mature egg-nucleus. This nucleolus shows a weak reaction towards dyes, and apparently has an outer, limiting membrane. × 1050.

210. The framework of a primary nucleolus from a mature egg-nucleus. This nucleolus has remained of a light greenish-yellow color after treatment with Flemming's triple stain. × 1050.

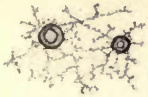
211. The upper part of an archegonium showing cavity, the receptive vacuole, formed in the cytoplasm just prior to fertilization. × 140.

212. The upper part of an archegonium just after the entrance into the egg of the elements from the pollen-tube. × 140.

213. A slightly later stage. The cytoplasm of the sperm-cell has already fused with the cytoplasm of the egg. × 140.

214. An entire archegonium showing the sexual nuclei in contact, and, above them, the various elements which have come into the egg from the pollen-tube. × 62. June 21, 1898.

215. The upper part of an archegonium in the same stage as the above. × 140.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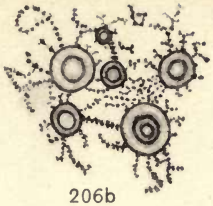
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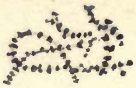
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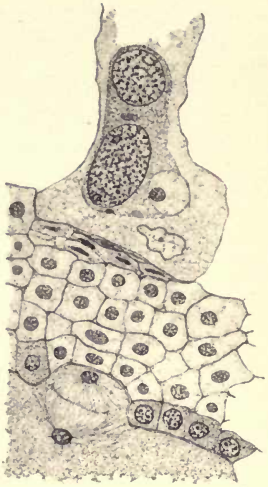
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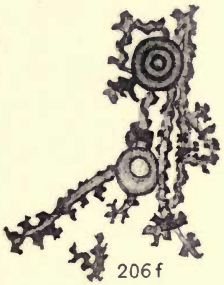
206d


206e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206g


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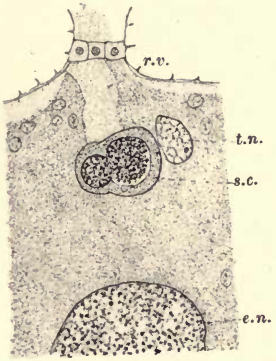

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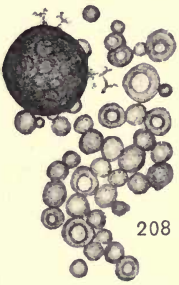
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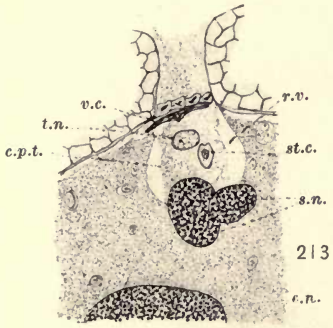

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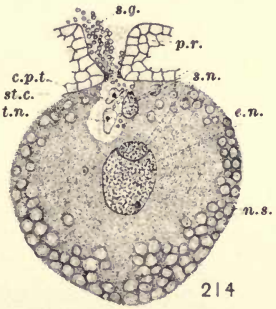

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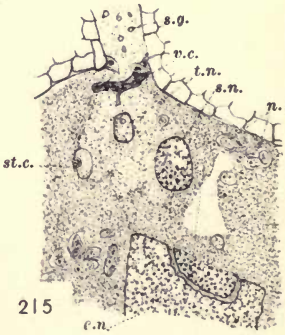

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## PLATE XX.

### *Pinus Strobus.*

FIG. 216. The sexual nuclei just before coming into contact. Note depression in egg-nucleus. × 140. June 17, 1899.

217–223, *a*. Various appearances presented by the conjugating nuclei. It will be borne in mind that these figures are so placed that the major axis of the archegonia in which they occur would be parallel with the longer axis of the plate. As a rule the sexual nuclei differ structurally in size only. × 140.

223, *b*. Another section through the egg-nucleus shown in fig. 223, *a*. There is a greater difference in the size of the conjugating nuclei than would appear in fig. 223, *a*, which is cut obliquely through the egg-nucleus. × 140.

224. An early prophase in the first division following fecundation. Showing early separation of chromatic from achromatic substance. × 472.

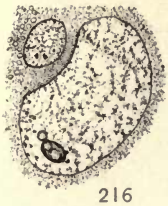
225. A slightly later stage. The cytoplasm caught between the two nuclei has collected into spherical masses. × 472.

226. A still later stage in the formation of the two chromatic spiremes. × 472.

227. A still later stage in which the paternal chromatic spireme has taken up a position near the maternal spireme, and a few delicate achromatic threads have made their appearance in the neighborhood of these spiremes. The nuclear membranes are still present, but have broken down at several points. × 472.

228. A later stage. The nuclear membrane has entirely disappeared; the spindle fibers have increased in number; and the rearrangement of the achromatic, nuclear reticula into granular threads is very apparent. × 472.

229. More advanced stage in the formation of the spindle. The spindle is distinctly multipolar in origin. × 472.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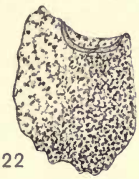
223b


223a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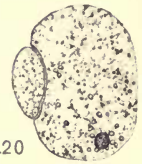
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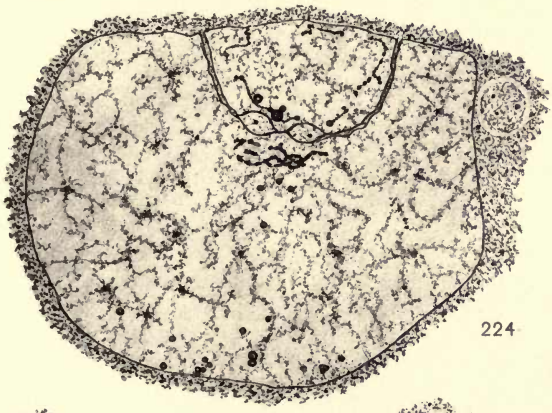
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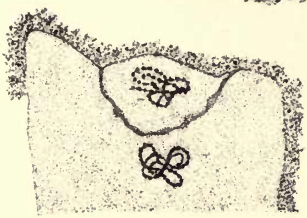
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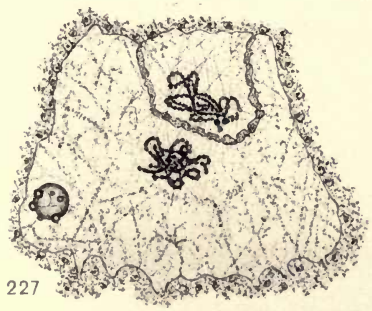
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## PLATE XXI.

### *Pinus Strobus.*

FIG. 230. The spindle fibers have become more abundant and transverse segmentation of the spiremes has occurred at some points. × 472.

231. The spindle fully established having now assumed the form of a multipolar diarch; the two chromatic spiremes still perfectly distinct. × 472.

232. The two spiremes after segmentation; the two halves of the spindle seem to indicate the maternal and the paternal portions of the mitotic figure. × 472.

233. Early stage in the formation of the chromosomes. The chromatic elements still occur in two distinct groups, but position, alone, determines which are maternal and which are paternal. The segments can not be structurally differentiated. × 472.

234. The chromosomes being oriented at the nuclear plate. The distinction between paternal and maternal elements no longer evident. × 472.

235. A cross-section through the nuclear plate just before the separation of the chromosomes; twenty-four segments are distinctly shown. × 472.

236–238. Some of the aspects presented by this mitotic figure during metakinesis. × 472.

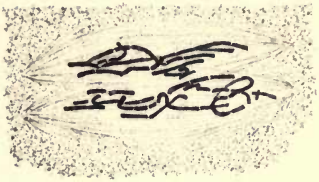
239. An anaphase of the mitosis. × 472.

240. A late anaphase of the division; the poles terminate in granular areas from which delicate threads extend into the cytoplasm; some of the nucleolar substance from the egg-nucleus still persists. × 472.

241. One end of the spindle in the same stage as the above; the fibers which radiate from the polar region of the spindle are very abundant and stain deeply. × 472.

230

237



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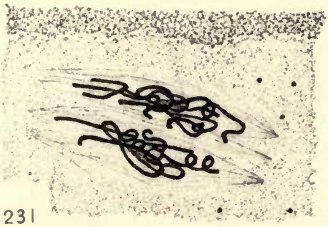

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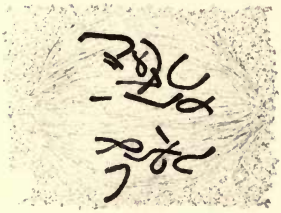

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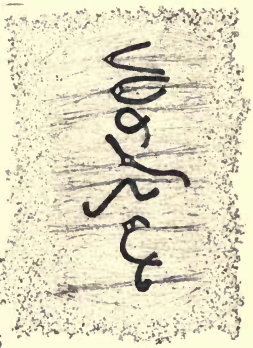

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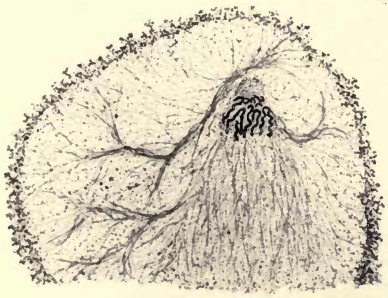

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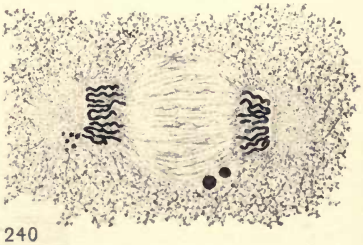

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## PLATE XXII.

### *Pinus Strobus.*

FIG. 242. One aspect presented by the karyokinetic figure in the telophase of this division. × 472.

243. The two segmentation-nuclei fully formed. × 472.

244. One of the two segmentation-nuclei in an early prophase of division. × 472.

245–246*b*. Later stages in the second division, showing two chromatic spiremes. × 472.

247. A still later stage. The two groups of chromosomes can still be made out. × 472.

248. An entire archegonium showing the position of the two segmentation-nuclei during division. The receptive vacuole has been distorted by the entrance of the contents of the pollen-tube. × 62.

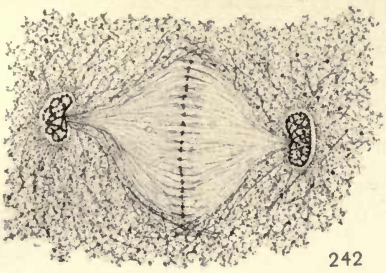
249. An archegonium showing the original position of the four segmentation-nuclei. × 62.

250*a*. The same after the nuclei have begun their downward movement. × 62.

250*b*. A nucleus from 250*a* showing details of its structure and fibers in the surrounding cytoplasm. × 472.

251*a*. An archegonium after the nuclei have almost reached the base of the oösphere. × 62.

251*b*. A portion of fig. 251*a*, showing details in nuclear structure, and fibers in the surrounding cytoplasm. × 472.



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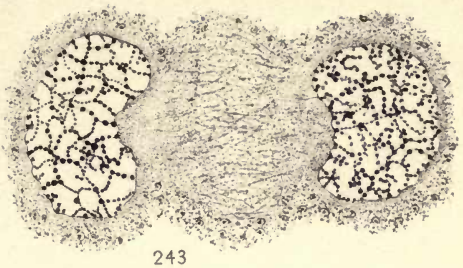

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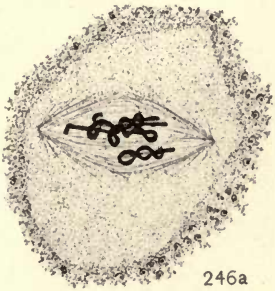
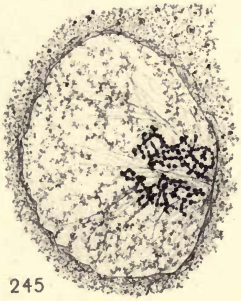


246a

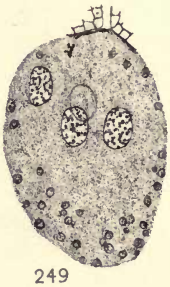


245

244

246b

247



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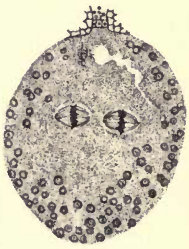
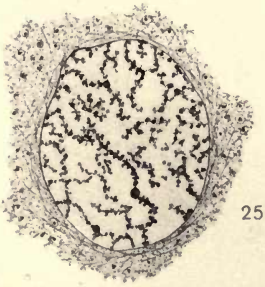


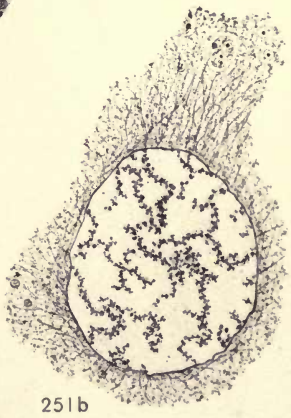
248

250a



250b

251a



251b

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## PLATE XXIII.

*Pinus Strobus* unless otherwise indicated.

FIG. 252*a*. The lower part of an archegonium after the four nuclei have arranged themselves at the "organic apex" of the oösphere. × 62.

252*b*. A portion of the above; the nucleus is in the early prophase of division; the cytoplasm surrounding the nucleus has became dense and deeply staining. × 472.

253*a*. The basal portion of an egg; the four segmentation-nuclei are in the metaphase of the mitosis. × 62. June 19, 1899.

253*b*. A part of the same showing details. × 472.

254*a*. A portion of a lower part of an oösphere after the formation of the eight nuclei of the proembryo. × 62.

254*b*. A part of the above giving details. No cell-walls have as yet been formed, but there is a slight differentiation of the cytoplasm about each nucleus. × 472.

255*a*. A somewhat later stage than fig. 254*a*. × 62.

255*b*. An enlarged portion of the above, showing cell-walls in the process of formation. × 472.

256. Vertical section through the base of an archegonium showing that the four nuclei of the upper tier of cells in the proembryo divide before any divisions occur in the four lower cells. × 96. *Pinus austriaca*.

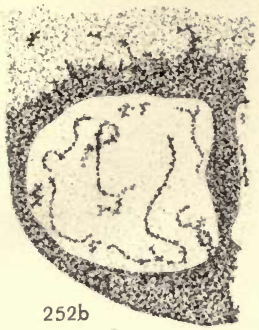
257–258. Figures occurring in the upper part of archegonia during the division of the segmentation-nuclei. These doubtless represent the smaller sperm-nucleus. × 472.

259*a*–259*b*. Figures occurring in the upper part of an archegonium at the time of the second division following fertilization; fig. 259*a* represents the tube-nucleus; the karyokinetic structure in fig. 259*b*, is the smaller sperm-nucleus, and just above it the stalk-cell is still distinctly visible. × 472.

260. Two macrospore-mother-cells. × 830. *Pinus rigida*. June 7, 1902.

261. An axial row showing oblique wall between two of the spores. × 394. *Pinus austriaca*. June 13, 1898.

262, *a*. A section through a prothallium showing unusual origin of archegonia from cells several layers deep in the prothallium. × 75



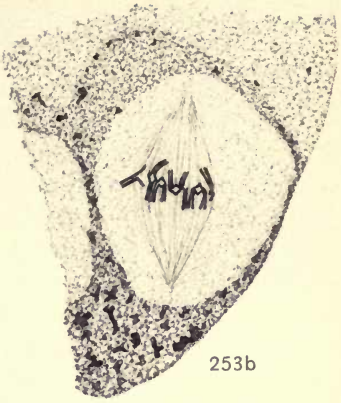
252b

252a

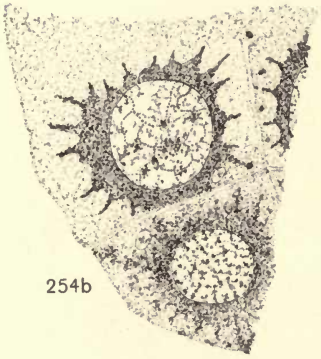
253a

254a

255a



253b



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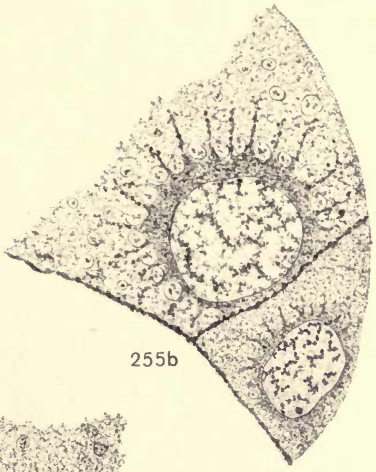
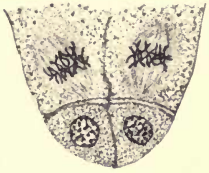


255b



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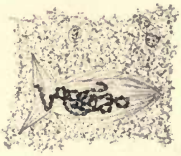
259b



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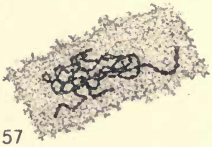
259a



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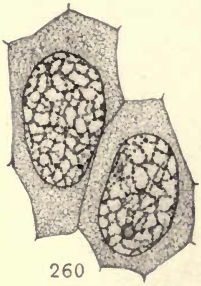

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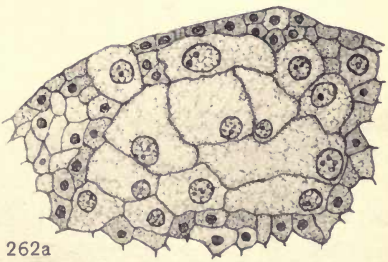
261



262a

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## PLATE XXIV.

FIG. 262*b*. Another section through the same prothallium as that shown in 262*a*. Altogether there are more than twenty archegonia formed in the upper part of this prothallium. × 75.

263. Archegonia formed not only at the top but along the sides of the endosperm. Reconstructed from several sections. Seven of the archegonia are visible in a single section. × 31. *Pinus montana uncinata*. June 10, 1898.

264. Linear arrangement of archegonia through the center of the prothallium. All of these archegonia are connected with the exterior by a passage above the neck-cells, which does not show in this view, but in the lower ones it leads to the side of the prothallium, rather than to the top. × 31. *Pinus austriaca*. June 17, 1898.

265. A little archegonium "budding" from a sheath cell of a larger archegonium. × 53. *Pinus resinosa*. June 15, 1898.

266. A smaller archegonium at the base of a larger one and opening into it. The smaller one has no neck-cells and the nucleus of its central cell has evidently been derived from one of the sheath-cells of the upper archegonium. × 31. *Pinus rigida*. June 13, 1898.

267. The same as fig. 266 except that the central cell of the lower archegonium has divided and the egg has reached maturity, while the nucleus of the central cell of the smaller upper archegonium has not divided. × 46. *Pinus resinosa*. June 24, 1898.

268. The largest ventral canal-nucleus observed in *Pinus Strobus*. There is no wall present cutting off a ventral canal-cell, but the nucleus is free in the cytoplasm of the egg. × 31. June 14, 1899.

269. An archegonium showing the only nucleus of such a large size observed in any species for the ventral canal-nucleus. × 46. *Pinus austriaca*. June 2, 1898.

270. Fragmentation of the egg-nucleus. × 46. *Pinus Strobus*. June 15, 1899.

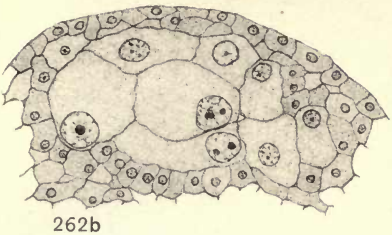
271. A pollen-grain after germination showing an increase in the normal number of nuclei. × 472. *Pinus austriaca*. May 17, 1898.

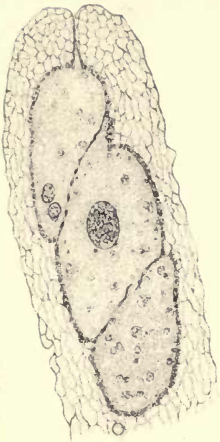
272. The generative cell and another nucleus, not the stalk-nucleus, just passing into the pollen-tube. × 472. *Pinus Strobus*. May 20, 1898.

273. The generative cell and another cell passing into the pollen-tube and followed by the stalk-cell. Presumably two generative cells have been formed. × 394. *Pinus rigida*. May 3, 1898.

274. An archegonium after fertilization. One of the two segmentation-nuclei has divided while the other has not. × 46. *Pinus Strobus*.

275. An instance in which the greater portion of the upper end of the prothallium is separated by a considerable space from the nucellar cap. A pollen-tube not able to cross this space and enter between the neck cells has effected entrance into the side of an archegonium, and the four segmentation-nuclei have been formed; the fifth nucleus is evidently the smaller sperm-nucleus; the very small nucleus at the top may be the ventral canal-nucleus, but more probably it is the tube-nucleus. × 31. *Pinus Strobus*. June 15, 1899.


262b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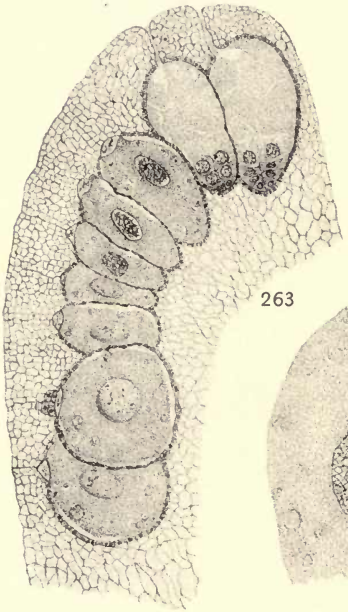
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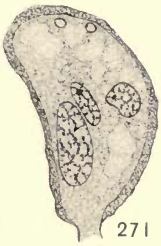
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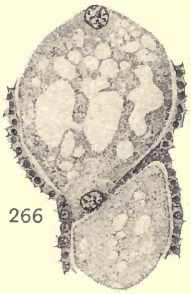
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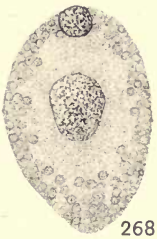
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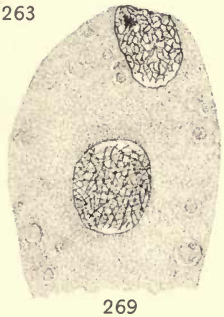
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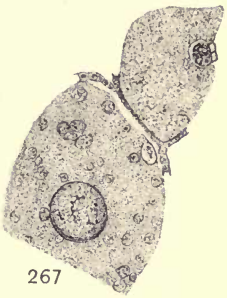
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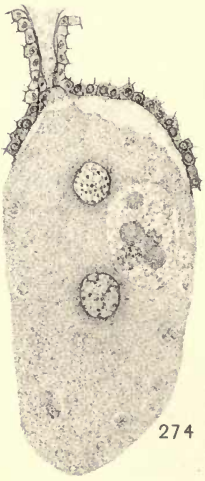
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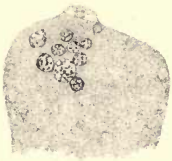
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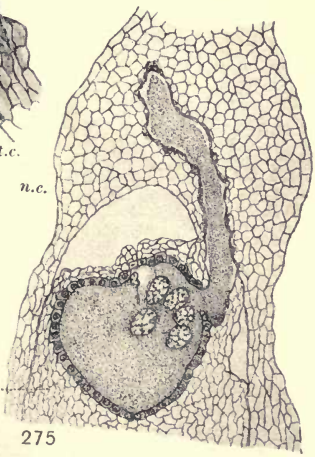
272



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