THE DEVELOPMENT OF THE EMBRYO-SAC IN SOME MONOCOTYLEDONOUS PLANTS.

KARL M. WIEGAND.

(WITH PLATES VI AND VII)

THE material for the following study was prepared in the ordinary way, by fixing in the chrom-aceto-osmic acid solution, imbedding in paraffin, and staining with the gentian-violet-orange combination. Although the development in Canna was found to be nearly normal, that in each of the other two plants showed some very interesting and important variations. Whether these throw any light on the problem of the homology of the embryo-sac can be determined only by more extended study of other plants.

Convallaria majalis L.

THE HYPODERMAL CELL AND ARCHESPORIUM.

The embryo-sac of Convallaria is derived from a hypodermal cell situated at the apex of the nucellus. This hypodermal cell is first discernible as an enlarged oblong or more or less distinctly triangular cell at the apex of the nucellus and directly underneath the epidermis; but can also be distinguished from the adjacent cells by its larger size and more granular contents. Very early in its development a single cell, the so-called "tapetum," is cut off on the side adjacent to the epidermis. This immediately divides by an anticlinal wall into two daughter cells which lie side by side at the summit of the embryo-sac (*fig. 1*).

The nucellus is comparatively broad, and the growth of the archesporium taking place subsequent to the separation of the wall-cell is to a large extent in a lateral direction. To accommodate themselves to this the two daughter wall-cells undergo repeated anticlinal division, so that eight or ten cells are formed, all arranged in the same plane, and forming a plate of tissue just beneath the epidermis (*fig. 2*). The nuclei of these cells increase somewhat in size until they are noticeably larger than those of the surrounding tissue, but unlike the sporogenous nuclei remain very dense. As the embryo-sac grows larger these cells are all pushed aside, with the exception of the more central ones which persist between the embryo-sac and the epidermis until after fertilization.

The other daughter cell resulting from the division of the primary hypodermal cell constitutes the archesporium. This immediately expands in all directions, partly at the expense of the ordinary tissue. At the time of the first nuclear division, the cell is oblong in shape and occupies a considerable portion of the nucellus. The nucleus during its period of growth passes through stages almost identical with those described for the microsporangial archesporium.[1] The chromatin network changes during synapsis (*fig. 2*) to a spirem ribbon (*fig. 3*), which later segments into the individual chromosomes. Corresponding stages in the nucleus of the pollen-mother cells and embryo-sac archesporium cannot be distinguished structurally; and this similarity is still farther emphasized by the synapsis occurring in each at the same stage in development.

THE FIRST NUCLEAR DIVISION.

Several good preparations of the first nuclear division were obtained, both in the nuclear plate and anaphase stages. In the plate stage the fibers are well marked; indeed the fascicles attached to the chromosomes are especially large and prominent. The spindle, like that of the pollen-mother cell, is rarely pointed at the poles, but is more often truncate (*fig. 4*).

The chromosomes are large oblong bodies arranged on the nuclear plate just as they are in the pollen-mother cell; that is, horizontally with one end directed away from the axis. Simultaneously the outer and inner ends commence to split longitudinally, but in perpendicular planes. In one case the separation is accomplished wholly without the aid of the spindle fibers; in

[1] WIEGAND: The development of the microsporangium and microspores in Convallaria and Potamogeton. BOT. GAZ. **28**: 328. 1899.

the other the fibers seem to accomplish the separation. The former splitting is only partial, while the latter finally divides the chromosome into two V-shaped parts which move at once to the poles through the influence of the spindle fibers. This is therefore a heterotypic division, and similar to the one occurring in the pollen-mother cell. In fact it seems probable that having the spindle alone one could not distinguish the two cases, even after a careful study of the chromosomes themselves. The number of chromosomes was found to be eighteen, and since the number counted in the somatic cells was greater than thirty, it is evident that reduction takes place at this period. During the anaphase of the first division (*fig. 5*) a definite cell-wall is deposited, which divides the original cell into two nearly equal parts.

THE SECOND NUCLEAR DIVISION.

The nucleus in each of the two daughter cells resulting from the first division very quickly divides again, and it so happens that the two spindles are formed simultaneously. No cell-walls are formed after this division, at least not before the embryo-sac is nearly mature. At the stage shown in *fig. 8* four nuclei are present. The second division spindle was in this case directed longitudinally; therefore in the same plane as the first; but this is not always the case. Sometimes the axis of the spindle is inclined and in fact almost transverse. The position of the spindle, however, seems to be of little importance, since one may find the two daughter nuclei during later stages either one above the other or side by side.

The resting stage between the first and second divisions, like that in the pollen-mother cell, is very short. The V-shaped chromosomes remain distinct and uninclosed by a definite membrane. The spindle quickly forms, apparently from the surrounding cytoplasm, and at the same time the chromosomes are crowded toward the equator where they arrange themselves in a nuclear plate. These spindles were in all cases much less distinct than the first one, and appeared in the earlier stages shorter and more truncate, while in the anaphase no cell-plate whatever was produced.

The way in which the chromosomes divide could not be positively determined. The segments on entering the nuclear plate, and even while in the plate as seen from the poles, are much curved (*fig. 6*). A side view of the nuclear plate shows a dense mass with projecting arms similar to the corresponding figure in the pollen (*fig. 6*). When the segments move to the poles they are seen to be quite long and straight, and never V-shaped as in the heterotypic division (*fig. 7*). After reaching the pole they at length fuse, and a membrane is formed around them, thus bringing about a truly resting condition again.

It seems scarcely to be doubted that the process here is identical with the second division in the pollen-mother cell. Indeed, every appearance in the one has its almost exact counterpart in the other.

GROWTH AND DEVELOPMENT OF THE EMBRYO-SAC.

The further history of the embryo-sac is very interesting, since it shows some deviations from the ordinary process in both monocotyledons and dicotyledons. The two superimposed daughter cells should probably be considered as constituting the so-called "axial row" in this case (*figs. 6–8*). At least, so far as the nuclei are concerned, they are the equivalents of the two cells first formed in Canna. There is therefore a two-celled axial row instead of a four-celled one, as in Canna and many other plants. It would be expected then that during development the lower cell alone would become the embryo-sac, while the upper would undergo dissolution as in nearly all other cases. This, however, is not the case.

At the time when the spindles of the second division occur, the archesporium as described above is two-celled. No walls are produced by the second spindles, as a result of which each cell now contains two nuclei. This stage is followed by a comparatively long period of growth in which both cells increase several times in size, as do also their nuclei. A large number of preparations were obtained illustrating this condition and representing all stages up to the next division of the nuclei. As shown in the accompanying figure (*fig. 8*), the upper cell becomes gradually larger and more vacuolate, while the cytoplasm of the lower stains more deeply and fills nearly the entire cell cavity. No cases were observed, however, where one could infer that either cell was in the process of disintegration.

At a period shortly before the opening of the flower further changes occur, the first of which is the immense increase in size of the upper cell. The wall between the two still seems to remain intact, although becoming very thin and delicate. In some cases the wall is so delicate at this stage as to be almost invisible, and may have broken down entirely, in which case both cells would be merged into the general cavity of the embryo-sac, but in later stages two cells are again seen. All of the four nuclei undergo division simultaneously, resulting in the stage with eight nuclei, just as in Lilium (*fig. 9*). In several cases shortly after this two large nuclei were seen fusing near the lower end of the upper cell, while the lower cell contained the distorted remains of three other nuclei. The latter cell was thus apparently already in the process of disintegration. It seems probable that one of the four nuclei originally formed in the lower cell must have ruptured the thin cell-wall and fused with the upper polar nucleus after the normal manner.

In *fig. 10* is represented an embryo-sac just prior to fertilization. At this stage the "egg-apparatus" consists of two synergids and the egg. The former lie close together, with the long axis more or less transverse to the axis of the embryo-sac, and are elliptical in shape, with the wall at the upper end thickened and densely striate. Just below these is the large egg nucleus, separated from the synergids and from the main cavity by very delicate cell membranes which seem to extend completely across the embryo-sac so as to join the lateral walls on either side. Below the egg-apparatus is the very large main cavity of the embryo-sac lined with a thin layer of cytoplasm. In this, near the base, is the large definitive nucleus still showing signs of the previous fusion of the two polar nuclei. At the base of the embryo-sac are the distorted remains of three antipodal nuclei still separated from the main cavity by a distinct cell-wall. It was impossible to determine whether this had been formed anew, or whether the old perforated wall had simply been repaired.

At the time when the main features of this study of Convallaria were read at the Boston meeting of the American Association for the Advancement of Science, so far as the writer was aware no exactly similar case had been observed. Mann [2] had discovered a cross wall in the embryo-sac of Myosurus, but here the development seems to have been somewhat different; and Strasburger [3] had figured a structure somewhat resembling a cross-wall in the embryo-sac of Allium, although it was mentioned in the text as a plasma-plate. Since that time, however, McKenney [4] in making a careful study of the embryo-sac of Scilla has described a process very similar to that in Convallaria. The archesporial nucleus underwent a period of growth before dividing. The first division showed the reduced number of chromosomes and was followed by a cell-wall. The two succeeding divisions in each daughter cell were not followed by a cell-wall, so that as a result two cells were present, each containing four nuclei. Up to this point the process was similar to Convallaria, but it was further found that only the upper cell took part in the formation of the embryo-sac, and not both as in Convallaria, while the lower cell disintegrated.

The process in Convallaria is strikingly similar to that in Lilium, and is probably to be considered as simply a modification of that method or a transition to it from the ordinary type with two or four cells in the axial row. It differs merely in the possession of a cross-wall, which however partially or

[2] MANN: The embryo-sac of *Myosurus minimus* L. Trans. and Proc. Bot. Soc. Edinburgh **29**: 351. 1892.

[3] STRASBURGER: Die Angiospermen und die Gymnospermen (1879). *Pl. 6. figs. 81–86.*

[4] MCKENNEY: Observations on the development of some embryo-sacs. Contrib. Bot. Lab. Univ. Pennsylvania **2**: [no. 1] 80. 1898.

entirely breaks down before the fusion of the polar nuclei, one of which comes from each cell.

Potamogeton foliosus Raf.

THE HYPODERMAL CELL AND ARCHESPORIUM.

In the earliest stage obtained the hypodermal cell at the apex of the nucellus had commenced its period of growth. No definite form can be ascribed to it at this period, although it is perhaps more often wedge-shaped. Even from the beginning it usually contains more protoplasm than the other cells, a feature which at a later stage becomes still more noticeable.

After a short period of growth this cell divides. The inner daughter cell resulting from the division becomes immediately the archesporial cell (*fig. 14*). The outer daughter cell divides again by an anticlinal wall. Two cells now lie side by side above the archesporium, as is represented in *fig. 12*, which shows a cross-section through the apex of the nucellus. *Fig. 13*, also a cross-section of the nucellus taken from the same inflorescence as *fig. 12*, shows that each of two cells now divides again, so that four daughter cells are formed all in the same plane. This last division may take place either before or after the first periclinal division of the wall-cell, commonly however before. Periclinal divisions in all four cells now begin, so that at length four rows of cells are formed between the archesporium and the epidermis. The process may continue until as many as six layers are produced; and these all persist until the embryo-sac reaches maturity, although often in a more or less compressed condition.

In the anther the hypodermal celi divides into two parts, one being destined to produce the archesporium, while the other after two or three periclinal divisions constitutes, together with the epidermis, the wall of the anther. The stages leading up to the production of the embryo-sac in the ovule are in many respects very similar to those occurring in the young anther. The hypodermal cell here also divides by a periclinal wall into two daughter cells, the innermost of which becomes the archesporium, while the outer, the so-called 'tapetum,' forms a part of the sporangial wall.

In Rosa[5] and Fagus[6] the epidermis has been found to divide several times by periclinal walls, and thus to form a considerable portion of the tissue between the archesporium and the apex of the nucellus. The unusually large number of cells in this region in Potamogeton at first suggested that the same phenomenon might also be found here; but a careful study showed that no divisions of the epidermis beyond an occasional doubling of individual cells ever take place, and all of the tissue can be easily traced to the primary wall-cell. Moreover, the similarity to the process in the microsporangia is so evident as to require no other explanation.

THE FORMATION OF THE EMBRYO-SAC.

The lowermost cell formed by the first division of the hypodermal cell begins to enlarge at once, and must henceforth be considered as the archesporium. The whole process during the early stages of development is perfectly normal. The archesporial cell soon undergoes division resulting in an upper and a lower cell (*fig. 15*). These probably correspond to the two resulting from the heterotypic division in Convallaria, but the fate of the two cells we shall find is somewhat different either from that in Convallaria or in Canna. The first division is immediately followed by a second nuclear division in each of the daughter cells, but without the formation of a wall between the two nuclei (*fig. 16*). The uppermost cell now shows signs of disintegration, as indicated by the cytoplasm, which becomes more dense and also stains more deeply. The nuclei also lose their definite outline, and finally the whole cell becomes much compressed and flattened against the wall-cells above. In a very short time, indeed, it can be recognized only as a dark cap at the summit of the embryo-sac. Several preparations were

[5] STRASBURGER: Die Angiospermen und die Gymnospermen 14. Jena, 1879.

[6] BENSON: Contributions to the embryology of the Amentiferae. Trans. Linn. Soc. II. Bot. 3: 410. 1894.

obtained showing the various stages of this process with great clearness.

The lower cell on the other hand continues to enlarge. The two nuclei are usually located at opposite ends of the cell and are somewhat larger than those of the surrounding tissue (*fig. 17*). In the next stage observed both of these nuclei had undergone division, so that two were present at each extremity of the embryo-sac (*fig. 18*). At the antipodal end, from this stage onward, a small pouch begins to appear which contains the two lower nuclei and finally all of the antipodal cells. One of the two upper nuclei is now sometimes found slightly below the other, and nearer the center of the embryo-sac; but many other preparations show that this is merely temporary or abnormal, and that before the next stage is completed they are both located together at the apex of the cell cavity (*fig. 19*).

Very soon after the last nuclear division a cell membrane can be seen to form around the two nuclei at the mycropylar end of the embryo-sac, thus enclosing them in a little pouch (*fig. 19*). The membrane constantly grows thicker, until at length it is a distinct wall, and the two nuclei are henceforth entirely separated from the cavity below. This seems to preclude entirely the possibility that a polar nucleus may pass down and fuse with one from the lower group of cells, although the latter remains in the general cavity of the embryo-sac until a much later period.

At a still later stage three nuclei instead of two are to be found in the micropylar enclosure (*fig. 20*). Two of these are small and differ but slightly from those of the surrounding tissue, while the third is much larger and located below close to the membrane. It seems probable that the two smaller nuclei are produced by the division of one of the two original nuclei, and are really to be considered as synergids; while the other and larger one is the egg derived directly from the other nucleus without division.

The two nuclei at the antipodal end of the embryo-sac after the pouchlike extension has commenced to form usually lie one above the other and are both quite large, each containing a large chromatin mass in the center (*fig. 19*). The lower one now divides into three daughter nuclei, all very much smaller than the parent nucleus, and with the chromatin scattered instead of being aggregated in a ball. These are all to be considered as antipodal cells, and persist in the little pouch until after fertilization. The upper nucleus continues to enlarge somewhat until about the time of fertilization, when it undergoes division followed by a cell-wall, as a result of which one daughter nucleus is inclosed in a cavity at the antipodal end of the embryo-sac (*fig. 21*). This then must be considered as a fourth antipodal cell, since it is one of the four parts of the original lower nucleus. The other daughter nucleus is the polar nucleus, but becomes at once the mother cell of the endosperm, and immediately undergoes division until a parietal layer of endosperm is formed (*figs. 23, 24*). The large antipodal nucleus and those of the endosperm, like the original nucleus from which they sprung, are all large and contain very large central chromatin masses. This, in addition to their position at successive stages, points toward a common origin. The large antipodal nucleus continues to grow for some time and is at length very conspicuous. It is by far the largest in the ovule, and the very large deeply staining chromatin mass may be seen even after the embryo has reached a considerable size.

The nuclei in Potamogeton are all very peculiar, differing from the ordinary type in having the chromatin mostly aggregated in a ball at the center of the cavity, instead of being distributed on the linin network. Although several hundred slides were prepared, none contained spindles in the embryo-sac, and consequently the sequence of the divisions had to be determined by other means. The appearance of such a remarkable process of development, and one so different from those already described, although still strongly suggesting certain features of the process found by recent investigators in related plants, made it very important that every step should be verified as far as possible. For this reason the material was worked over several times, until the steps became so clear as to leave no doubt in the mind of the writer that the description as given above is correct.

To summarize briefly: the mature embryo-sac consists of an egg and two evanescent synergids, each without a cell-wall of its own, but all contained within a pouchlike cavity separated from the general cavity by a delicate wall. One synergid usually disappears before fertilization. Next below is a large cavity containing the endosperm nucleus, and later the endosperm, derived without fusion from the lower polar nucleus. At the base are the four antipodal cells, three of which are very small and chromatic, and are descendants of the same nucleus, while one is very large and together with the polar nucleus is derived from another parent. The antipodals are separated from the main cavity by a membrane formed at the time of separation of the polar nucleus and the antipodal cell. The endosperm even after fertilization never becomes more than a parietal layer (*fig. 24*).

The investigation of plants of the same and nearly related orders has shown that the occurrence of the large antipodal nucleus is not a peculiarity of Potamogeton alone, but is characteristic of a whole group of Monocotyledons. Schaffner[7] found that in Sagittaria the development was normal up to the formation of the two synergids, egg, and three minute antipodal cells. He then found that the two polar nuclei fused in the ordinary way, after which the definitive nucleus underwent division. This division was always followed by a transverse wall enclosing one of the daughter nuclei in a chamber at the base; and this was the one which became so large at a later period. Sometimes several were found, thus showing that the large nucleus had divided at least once or twice. From the other daughter nucleus was produced the endosperm. The structure in question was therefore thought to be an endosperm nucleus, rather than an antipodal cell as in Potamogeton.

[7] SCHAFFNER: Contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. 23: 252. 1897.

In Naias and Zannichellia Campbell[8] found an axial row of two or three cells the lower of which alone became the embryo-sac. The development was normal up to the point where the polar nuclei were formed. The free egg-nucleus and synergids and the minute antipodal cells were very characteristic; but no fusion of polar nuclei was observed, and he doubts if it ever occurs. The enlarged basal nucleus is always present from this stage onward, but is not separated by a wall from the main cavity as in Potamogeton and Sagittaria. Campbell believes with Schaffner that this is a product of the first division of the definitive nucleus, but also admits that it may be the lower polar nucleus alone, while the upper has gone to form the endosperm.

FERTILIZATION.

The stages representing the various steps in the process of fertilization are rarely met with in this plant. Several good preparations were obtained, however, and these will form the basis of the following description.

The pollen tube enters the embryo-sac through the micropyle at a point directly behind the egg. In its course it passes close to the partially disintegrated synergid, but the tube is quite slender and scarcely inflated after entering the embryo-sac, thus differing decidedly from Sagittaria, in which Schaffner described the inflation as very marked.

The two sperm nuclei were last noted in the mature pollen grain where they were both inclosed in the same cell-wall. They remain united even during their trip down the pollen tube to the embryo-sac and enter the egg together. Fusion of the egg and sperm nucleus was not observed. The stage immediately preceding this was found however, and is figured in *fig. 22*. Here the egg is easily recognized, and lying in close proximity to it, indeed even touching it, are the sperm nuclei. In reality only one actually touches the egg nucleus. The other lies at the side or even at the back of the first, and is already in the process of

[8] CAMPBELL: A morphological study of Naias and Zannichellia. Proc. Calif. Acad. Sci. II. 1:1. 1897.

disintegration. It seems therefore that in this case the two sperm cells never separate. At this stage the wall which separates the egg from the rest of the embryo-sac is prominent, and can easily be seen to inclose the synergids as well. Campbell found both sperm nuclei entering the embryo-sac in Naias but only one in Zannichellia. Schaffner found that in Sagittaria one always remained in the pollen tube.

THE EMBRYO.

The classical investigations of Hanstein[9] and Famitzin[10] on the development of the young embryos in both monocotyledons and dicotyledons have made us familiar with the process in a large number of plants. The work of later students has only gone to confirm the results reached by these investigators, at least as regards general features. However, the results obtained from certain monocotyledons by Schaffner and later by Campbell seem to be at variance with those above mentioned; and as bearing upon this point Potamogeton becomes of interest.

In Potamogeton the fertilized egg nucleus remains for a very short time in the resting condition, perhaps it divides immediately, but lack of spindles in the young embryos made it impossible to determine this. Immediately after the first division, the basal cell undergoes a change whereby it becomes gradually larger until a size several times the original is reached. At this stage the cell is a very striking object (*figs. 24, 26*). The very large vacuole, which soon appears, at length forces the nucleus to the bottom of the cell where it henceforth remains, while the nucleus itself undergoes considerable enlargement. Just behind this large nucleus of the basal cell, one can often see a synergid, even as late as the several-celled stage of the embryo.

The basal cell never undergoes division, but on the contrary remains for a long time in its enlarged condition attached to the end of the embryo-sac. But sometimes during the later stages

[9] HANSTEIN: Die Entwickelung des Keimes der Monokotylen und Dikotylen. Bonn, 1870.

[10] FAMITZIN: Embryologische Studien. Mém. de l'Acad. Imp. Sci. de St. Petersb. VIII. **26**:—. 1879.

of development it may, together with the embryo, become entirely detached, so that the whole mass is then free (*fig. 26*). The wall increases slightly in thickness, and is always thicker than any other wall in the young embryo.

The first division of the egg is soon followed by a second and later by a third. The embryo then consists of a row of four cells of which the three upper have nearly the same size. Occasionally only three cells are present at this stage, but the four-celled stage predominates. The next division is vertical, and in the uppermost cell (*fig. 25*). This is again followed by one or two more oblique walls, thereby dividing the terminal cell into several sectors, each extending from the basal line to the periphery. Periclinal and anticlinal walls form successively in the different sectors, as a result of which the upper cell soon becomes a mass of tissue (*fig. 26*).

Meanwhile divisions have occurred in the next lower cell. This first formed an oblique wall, after which several divisions took place in various directions. The third cell from the apex has divided once or perhaps twice by vertical walls. The embryo now is nearly spherical, a fact which makes it very difficult to trace the development farther with the idea of determining just what portions of the mature embryo are derived from the various primary cells. The oldest stage at which one can with certainty distinguish the four original cells is shown in *fig. 26*.

The subbasal cell has here undergone a transverse as well as two vertical divisions. No further divisions took place in this cell, and owing to this fact there is little difficulty in recognizing the tissues arising from the subbasal cell even in the half-grown embryo. The eight cells remain undivided at the base of the embryo where they form a narrow neck or stalk; in other words they together with the basal cell form the true suspensor (*fig. 27*).

The fate of the other two cells is immediately lost. It can be inferred from their position that the cotyledon arises from the upper cell and the axis from the subapical. We cannot be far wrong in making this interpretation. The half-grown embryo shown in *fig. 27* was the oldest stage obtained. At this time only the epidermis was differentiated, while the plerome is scarcely distinguishable. At the upper end of the figure may be seen the cotyledon; and at one side the plumule, arising in a depression at the apex of the hypocotyl.

Schaffner and Campbell both find that no division takes place in the enlarged basal cell after its formation. There is certainly none in Potamogeton. Probably the observation of Hanstein that such a division does take place in Alisma was inaccurate. That such a division does occur in some monocotyledons, however, is a well established fact as shown by Coulter[11] in his studies of Lilium. In this plant the basal cell undergoes both longitudinal and transverse divisions. The process in Potamogeton does not differ essentially from that in Sagittaria and Naias, as given by the two authors cited above. The subbasal cell in Naias divides by transverse walls into three instead of two cells, of which the upper forms several cells, while the lower remains undivided. In Sagittaria the subbasal cell divides also once more than in Potamogeton, and the uppermost daughter cells here again form considerable tissue.

Canna Indica L.

THE HYPODERMAL CELL AND ARCHESPORIUM.

Canna represents still a third type of the monocotyledonous embryo-sac, differing in method of development from Lilium Convallaria, and also from Potamogeton.

The hypodermal cell very soon divides into two parts by means of a periclinal wall (*fig. 29*). The upper cell then by repeated anticlinal division rapidly forms a layer of about nine cells directly above the future embryo-sac. This layer together with the epidermis constitutes the wall of the sporangium, and remains unchanged until finally displaced by the embryo-sac beneath.

[11] COULTER, J. M.: Contributions to the life-history of *Lilium Philadelphicum*—The embryo-sac and associated structures. BOT. GAZ. 23:413. 1897.

Meanwhile the lower of the two cells formed by the first division of the hypodermal cell has undergone considerable growth. It rapidly becomes several times its original size, and extends nearly to the base of the nucellus; but the increase in breadth is not so great. Just previous to the first division of its nucleus the cell is unusually long and narrow, but is completely filled with cytoplasm (*fig. 29*).

The cell gradually becomes longer and longer until at length a division takes place whereby two daughter cells are formed (*fig. 31*). After a short time both of these daughter cells divide again simultaneously. As a result we have an axial row of four cells reaching from the base to the apex of the nucellus (*fig. 32*). In the upper three cells a change is noticed almost at once, whereby the cytoplasm becomes denser and darker, the nuclei less chromatic, and the cells in fact show evident signs of disintegration (*fig. 33*). The lower cell by its continued growth gradually compresses the other three, which very soon are all crowded into a small disorganized mass in the micropylar region (*fig. 34*). A large number of sections was obtained showing all stages of this process in the plainest manner. The lowermost cell alone finally takes part in the formation of the embryo-sac. The process in Canna, therefore, is exactly in accord with that in the Iridaceæ, Rosaceæ, Polygonaceæ, and many Ranunculaceæ and Liliaceæ as described by Strasburger and others.

Owing to the extremely narrow cavity and small nuclei, the changes within the embryo-sac are very difficult to follow. Humphrey[12] has already given a full discussion of the literature on the embryo-sac of Canna; and also the results of his own investigations on the same plant. In this case the antipodal cells were not discovered, and he came to the conclusion, as did also Guignard[13] that these cells although always formed must disintegrate immediately.

[12] HUMPHREY: The development of the seed in the Scitamineæ. Annals of Bot. 10:1. 1896.

[13] GUIGNARD: Recherches sur le sac embryonnaire des Phanerogames Angiospermes. Ann. Sci. Nat. Bot. VI. 13:136. 1882.

From the material at hand it appears that a short time after the last division in the axial row, the primary nucleus of the embryo-sac, *i. e.*, of the lower cell, divides, and one of the daughter nuclei passes to each end of the already much elongated cavity. The spindles representing the latter division were not obtained, but several cases were found where there were two nuclei at each end of the embryo-sac, and still others where there were four (*fig. 34*). One or two sections showed the three antipodals, two synergids, and the egg; while near the center of the cavity was a very large nucleus, apparently the definitive nucleus of the embryo-sac. The fusion of the polar nuclei was therefore not observed, but probably took place as indicated by the two nucleoli and cross line in *fig. 35*. Just previous to fertilization the egg-apparatus was found to be separated from the main cavity by a delicate membranous wall, and to consist of two very small partially disintegrated synergids located very near the micropyle, and a much larger egg nucleus suspended some distance below, as in *fig. 35*. At this stage the antipodals are often in an advanced stage of disintegration, and are more or less clearly separated by a delicate membrane from the cavity above.

Canna differs from Convallaria, therefore, principally in the embryo-sac being formed from one cell of the axial row, and one element of the division into four of the mother nucleus. In the latter plant the whole axial row and all four elements of the division of the mother cell go to form the embryo-sac.

THE NUCLEAR DEVELOPMENT.

At a very early stage the nucleus of the archesporial cell passes into the condition of synapsis, in which as usual the linin is massed together at one side of the nuclear cavity. After a time indications are seen of the gradual loosening of the knot, with a simultaneous migration of the spiral coils to the more distant parts of the nucleus. The nucleolus here, as in Convallaria, is seen to remain intact during the whole process. It stains much deeper with the gentian-violet than does the chromatin, and can therefore be readily distinguished. The spirem itself is composed of comparatively few turns of the rather broad chromatin thread, and is made up of alternate segments of chromatin and linin (*fig. 29*). The nucleus lies imbedded in the cytoplasm, which at this stage completely fills the cell cavity. It is now very large, and several times the size of those of the adjacent vegetative cells, and from it numerous radiations extend into the surrounding cytoplasm.

The nuclei and chromosomes in Canna are so small that little could be done toward working out the segmentation of the latter. Some features of the nuclear division, however, may be noted.

All of the nuclei in this plant possess a true nucleolus, as in Convallaria, and a very meager linin net-work on which the small amount of chromatin is unequally distributed. In the vegetative nuclei at the time of division the chromatin becomes aggregated into six spherical masses lying just beneath the membrane, and the nucleolus at the same time disappears. The spindle now forms, and the ordinary process of division assures six daughter chromosomes for each resulting nucleus. This count was made many times with great ease, owing to the small number of segments, and always with the same result.

When the archesporial nucleus has reached its limit of growth the chromatin of the spirem ribbon seems to become aggregated into a number of globular masses, as in the vegetative nuclei, except that the number in many cases seems to be more than six. Only two spindles were found representing the heterotypic division, and these both showed the globular daughter segments on their way to the poles (*fig. 30*). The four counts here made gave in every case the number as six, instead of three as one would expect after reduction. The spindles of the second division both occur at the same time, and one or two sections containing these were also obtained (*fig. 31*). They were all in the nuclear plate stage, and here the number was actually three ; but each chromosome seemed to be composed of two parts, one of which was located directly above the other and more or less completely joined to it. The most plausible explanation for this seems to be that the segmentation for both divisions was nearly or quite completed before the formation of the first spindle, and that when they appeared on the nuclear-plate of the second spindle the segments had come together in pairs only to be separated again on going to the poles. No true resting stage seems to intervene between the two divisions. The above results are especially interesting, since three for the reduced number of chromosomes is one of the smallest so far found in plants. The later divisions in the embryo-sac were not observed. The spindle seems to be formed simply by the elongation of the kinoplasmic mass, no multipolar condition being noticed, and the mature spindle is long and slender but usually with obtuse poles. After each division a distinct membrane is deposited at the cell-plate, thus forming the axial row of four cells. The resting nuclei often show very distinct radiations from the nuclear membrane; especially is this the case with the lower one which now commences another period of growth before division in the embryo-sac.

SUMMARY.

Convallaria.—The hypodermal cell divides into an upper and a lower cell, of which the inner cell becomes the archesporium and the upper forms part of the wall. This is also the case in Potamogeton and Canna.

The stages of the growth and development of the archesporial nucleus are identical with those of the nuclei of the microsporangial archesporium.

The first division of the archesporial nucleus is the heterotypic division and corresponds to the first pollen-mother cell division in every respect.

The two spindles accompanying the second division are formed simultaneously. In appearance this division is identical with the second pollen-mother cell division and quite different from either the vegetative or heterotypic. A transverse division of the chromosomes could not be demonstrated.

Only the heterotypic division is followed by a cell-wall, and thus an axial row of two cells is formed each containing two nuclei.

Shortly after anthesis the transverse wall disappears and the four nuclei all divide simultaneously, producing four daughter nuclei at each end of the embryo-sac. The endosperm nucleus is formed by the fusion of one nucleus from each group. After the transverse wall is destroyed, therefore, the process is the same as in Lilium.

The number of chromosomes in the vegetative nucleus is about thirty-six. During the heterotypic and so-called "reducing" divisions eighteen may be counted. The apparent reduction therefore takes place prior to the first division of the archesporium.

Potamogeton.—The first division of the archesporial nucleus is followed by a cell-wall, but the second is not; so that an axial row of two cells, each containing two nuclei, is produced as in Convallaria.

The lower cell forms the embryo-sac, while the upper disintegrates.

Four nuclei are formed in the lower cell; the two at the upper end are at once enclosed by a cell membrane, and from them develop the two evanescent synergids and the egg.

The lower remain free. From one of these all three of the small chromatic antipodals are probably formed. The other divides, forming the fourth antipodal cell and the polar nucleus with a cross-wall between the two. The polar nucleus becomes the endosperm nucleus without fusion.

The mature embryo-sac contains two small synergids and a large egg nucleus enclosed by a wall near the micropyle; a very thin parietal layer of endosperm; and four antipodal cells enclosed by a transverse wall at the lower end of the embryo-sac, of which three are very small and one is very large.

The nuclei of Potamogeton are peculiar in having most of the chromatin aggregated in a ball at the center of the cavity.

At the time of fertilization the two sperm nuclei lie together near the egg nucleus, but only one is really in contact with the latter.

The fertilized egg-cell undergoes at first three divisions, forming a row of four cells. The terminal cell and the one next below give rise to the greater part of the embryo. In the subbasal cell only one transverse and two vertical divisions ever occur, and the cells thus formed together with the very much enlarged basal cell form the suspensor.

Canna.—The heterotypic division is also the first division of the archesporial nucleus, and is followed by a transverse wall.

The second divisions occur simultaneously and are also followed by cell walls. These form an axial row of four cells.

The lower cell alone gives rise to the embryo-sac; the other three finally disintegrate and disappear. The further development is quite normal.

The nuclei of Canna are more nearly like those of Convallaria than Potamogeton. They have a true nucleolus and no central chromatin mass.

The number of chromosomes in the vegetative divisions is six. When passing to the poles at the heterotypic division there were still six; but later the second division showed only three as the reduced number. Probably the segmentations for both divisions occur during the prophase of the heterotypic division. This number is one of the smallest yet found in vegetable tissue.

CORNELL UNIVERSITY.

EXPLANATION OF PLATES VI AND VII.

Figures 1–11. *Convallaria majalis* L.

FIG. 1. A vertical section through a young ovule, showing the epidermis, archesporial cell, and two of the wall-cells between; the archesporial nucleus is in the resting stage.

FIG. 2. The same at a later stage; the wall-cells have undergone further division, and the archesporial nucleus is in synapsis.

FIG. 3. The archesporial nucleus in the spirem stage; the ribbon contains small denser portions of chromatin.

FIG. 4. Nuclear-plate stage of the first or heterotypic division of the archesporial nucleus; the chromosomes appear +-shaped, as in the pollen mother-cell.

FIG. 5. Anaphase of the same division; the V-shaped segments have reached the poles, and the cell-plate is forming.

FIG. 6. The nuclear plate stage of the second or so-called "reducing" division; the chromosomes are arranged very differently; between the two spindles is the wall formed during the previous division.

FIG. 7. Anaphase of the same division.

FIG. 8. A later stage showing the four nuclei thus formed.

FIG. 9. Each of these nuclei now divides forming four at each end of the embryo-sac; the cross-wall has disappeared.

FIG. 10. The mature embryo-sac ready for fertilization; over the apex is the epidermis and the remains of the other wall-cells; the egg-apparatus consists of the large egg nucleus and two striated synergids; at the base are the disorganized antipodal cells enclosed by a cell wall, and above is the endosperm nucleus.

FIG. 11. A spindle from the vegetative tissue, nuclear-plate stage; the chromosomes extend in all directions.

Figures 12–27. *Potamogeton foliosus* Raf.

FIG. 12. A cross section of the apex of the nucellus, showing at the center the two wall-cells lying above the archesporium.

FIG. 13. Same in the four-celled stage.

FIG. 14. Vertical section of the nucellus, showing the archesporial cell with resting nucleus, and one wall-cell above.

FIG. 15. The axial row of two cells; the cross-wall was formed after the first or heterotypic division of the archesporium.

FIG. 16. Same, later stage; the "reducing" division is not followed by a cell-wall.

FIG. 17. The lower cell developing into the embryo-sac; the upper cell forms a crushed mass above.

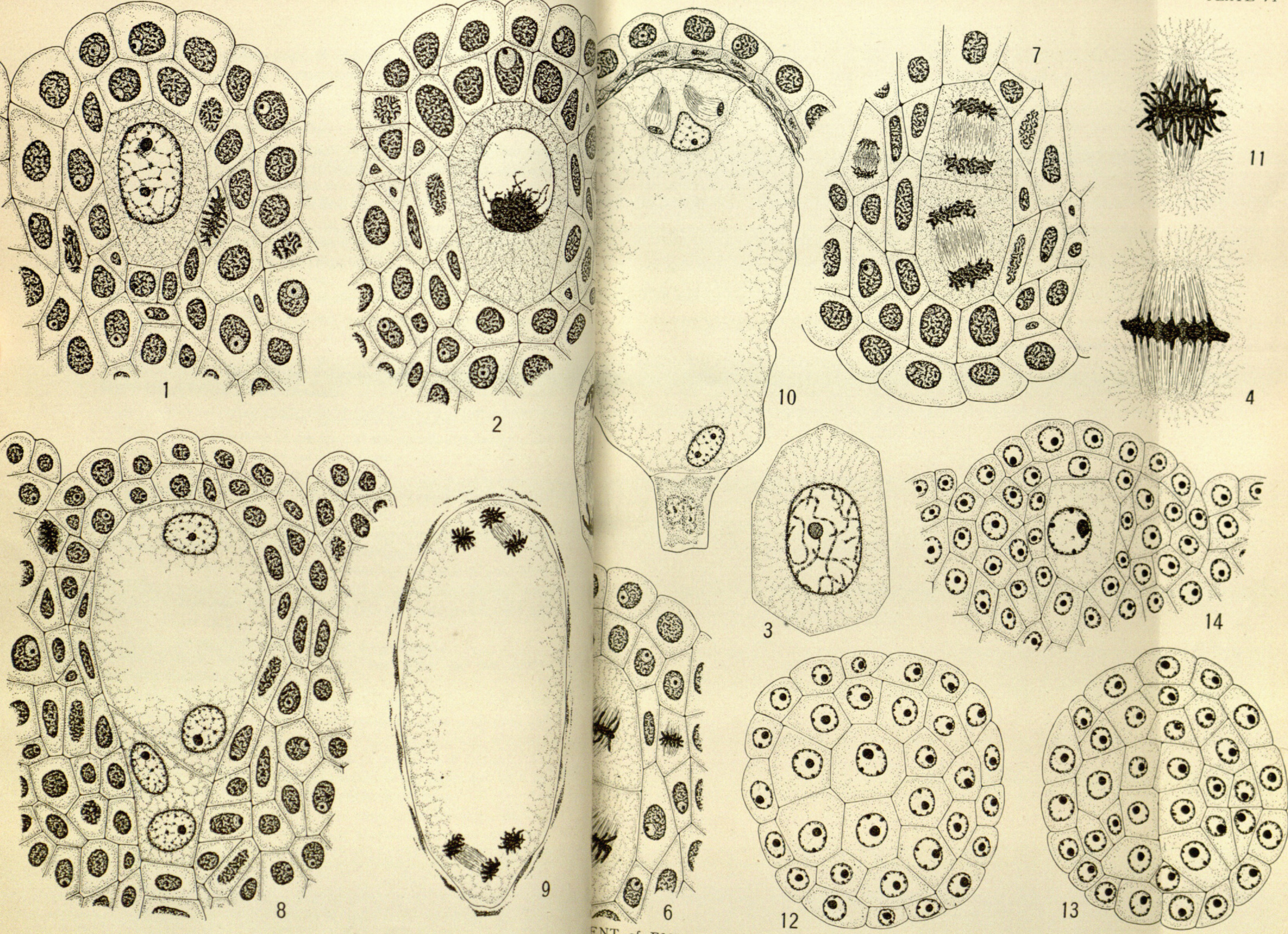
FIG. 18. Same, but each nucleus has divided again.

FIG. 19. Same, with a wall forming just below the two upper nuclei, separating them from the cavity below.

FIG. 20. The young embryo-sac with the large egg and two small synergids enclosed by a cell-wall, three small antipodal cells at the base and a very large nucleus above.

FIG. 21. Same, slightly older; the large nucleus has divided into a polar nucleus above and a large antipodal nucleus below separated by a cell-wall; the former becomes immediately the definitive nucleus.

FIG. 22. Egg apparatus showing a stage in fertilization; the two sperm nuclei lie near the egg, and one synergid above is in the process of disintegration.



WIEGAND ENT of EMBRYO-SAC