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Sporogenesis in *Riccia Natans L.*

Mary Fortunata

SPOROGENESIS

IN

RICCIA NATANS L.

BY

Sister Mary Fortunata, S.S.N.D.

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Introduction

Until comparatively recent years little, comprehensive Cytological work had been done on the lower forms of plant life including the Liverworts. Cavers (1903) stated that Micheli in 1729 named a species "Hepaticae fontana" because of the supposed resemblance between the branched thallus and the lobes of the liver, and although this species was later named "Fegatella conica",the name "Hepaticae" has,since then, been applied to the whole group of Liverworts. Between 1850 and 1862 Hofmeister demonstrated "Alternation of Generations" in this group, and it was this characteristic that distinguished them permanently from the Thallophytes in spite of their very common thalloid form.

In 1867, Kny by careful observation and study of Marchantia added much interesting information regarding the sex organs, apical cell and origin and growth of the ventral scales. He was of the opinion that the thallus was a case of fused "stem" and "leaf". The development of the thallus and the germination of the spores of Liverworts was the work of Fellner somewhat prior to 1875. Between the years 1874 and 1882 Leitgeb contributed much more specific information concerning the structure of the thallus,air chambers,pores, and ventral scales, while Strasburger in 1882 presented val-

uable facts about the canal cells. Leitgeb believed Riccia natans to be dioecious, a belief which was later refuted and the contrary proved by John F. Garber. (1902) In 1895 Farmer published a paper on "Studies of Hepaticae" but he has confined himself to a single species from which he draws numerous conclusions concerning all.

With the advent of the twentieth century more intensive work was possible and interesting and valuable results have been realized. In 1902 John F. Garber published data which revealed many hither-to obscure facts about "Ricciocarpus" natans, now generally known as Riccia natans. By demonstrating the early development of the antheridium and the later development of the archegonium, he proved that the plant was monoecious. He quotes G.W. Bischoff who stated as early as 1835 that the antherozoid was biciliate. He attributed the simplicity of the plant to its aquatic habitat, a statement discredited by subsequent investigators.

Lewis (1903) presented his researches of Riccia lutescens which until that time had not been considered a ground form of Riccia natans. One difference was pointed out, namely, that no nucleolus had ever been observed in the Riccia lutescens form. Beer, (1906), in the course of a detailed study of Riccia glauca found the first division of the fertilized egg to be obliquely transverse. Another interesting observation was his inability to find any demonstrable nutritive material. The year 1907 found three workers ready to

make known their findings, Barnes, Land,and Juel. Their contribution deals principally with the structure and development of air chambers.

Durand, (1908), published an excellent treatise on the "Development of the Sexual Organs and the Sporogonium of Marchantia polymorpha", a liverwort closely allied to Riccia though infinitely more complex in its sporophytic stages,but having, nevertheless, many stages in common. Although the work of Miss Black anent Riccia frostii began in 1908, the results of her investigations were not published until 1913. This species,she observed to be dioecious; the sperm nucleus in the egg to be in juxtaposition to the egg nucleus.The undoubted presence of a nucleolus and the fact that the first division in sporogenesis is ordinarily oblique are included in her report of observations made. Haupt, (1921) devoted a fine paper to the discussion of the gametophytic stages of the life-history of the Liverworts in general.

Classification

Bryophytes,the second in rank of the great plant groups, possess certain characteristics which distinguish its members from other forms of plant life. The most conspicuous of these,as contrasted with the Thallophytes,is the alternation of generations. The appearance of an archegonium and an antheridium are a natural requisite for the foregoing. The an-

theridium, however, is always multicellular, whereas in the Algae the similar organ may be either unicellular or multicellular. True roots are not found and in all cases the gametophyte generation is more conspicuous than the sporophyte.

Two classes constitute this phylum, Musci (Mosses), and and Hepaticae (Liverworts). The former present a greater diversity of structure than the latter although the number of species of the latter is several times greater than the former. The gametophyte of the Musci is composed, in most cases, of well-differentiated tissue, that of the Hepaticae is ordinarily more simple.

The Hepaticae are grouped into three orders: the Marchantiales, the Jungermanniales, and the Anthocerotales. In the Marchantiales the gametophyte is always thallose, and composed of several layers of tissue, that on the dorsal surface having chlorophyl-bearing cells and large air-spaces and spores. The ventral surface has scales and rhizoids of one or several kinds. The sporophyte is simple for the sporogonium is born either with or without a stalk and all the cells produce spores, or, as is true in some cases, also elaters. No columella is present. The Jungermanniales show a greater range of external differentiation than the Marchantiales but less variety of tissue. Forms vary from an extremely simple thallus through an interesting series to specialized leafy ones, retaining always a primitive simplicity of tissue. The sporophyte develops, in addition to the cap-

sule, a stalk and a foot of sterile tissue and with one exception perfect elaters are formed in all members. This illustrates an advanced condition of sterilization. The germinating spores have an abundant supply of chlorophyl. Anthocerotales, a small group, have a very simple thallus either with or without a midrib, and a large, peculiarly flattened chloroplast in each cell. On the other hand the sporophyte is more complex in structure than any of the other Hepaticae. The sporogonium develops a highly differentiated system of tissues resembling somewhat the vascular system of higher plants by the presence of a central columella, while a very small part is devoted to spore formation, a process which is gradual and continues for a shorter or longer time, depending partly on the living gametophyte for nourishment.

Three families are included in the order of Marchantiales, namely, Ricciaceae, Corsineaceae, and Marchantiaceae. The sporogonium of the first-mentioned family develops no sterile tissue in the form of foot or stalk, and remaining permanently within the venter of the archegonium. All the cells produce spores. The sporogonium of the Corsineaceae develops a short stalk, and produces, together with the spores a very simple elater. The sporogonium of Marchantiaceae is ordinarily stalked, has sterile tissue in the form of elaters, and when ripe, breaks through the calyptra by means of a circular cleft, or by teeth, or by a series of four or eight valves. The gametophytes are similar in struc-

ture in all three families but the sexual organs are sessile in the first two and only in the last are they borne on specially stalked receptacles.

The Ricciaceae, comprise three genera, Riccia, Tesselina and Corsinia, the last two being represented by a single species each. Tesselina occupies a position between the Ricciaceae and the Marchantiaceae having a gametophyte similar to the former. Corsinia although placed in this family occupies no definite place for investigations regarding it are very incomplete. Riccia includes about one hundred and seven species of world-wide distribution. The majority are terrestrial in habit although some forms are capable of living submerged during the vegetative period of their life history, the period of developing the reproductive structures being withheld until drier conditions prevail. True dichotomy and large air-spaces characterize practically every species of this genus.

Description

Riccia natans may be found on the firm moist banks of streams, on muddy flats, and is also capable of growth as a floating aquatic on quiet water. Small, disk-like, and green with hair-like projections on the ventral surface, this dichotomous, thalloid plant is a very interesting species for observation. The gametophyte is much more complex than the sporophyte. It has two distinct regions, a dorsal,

or upper region and a lower or ventral region. Several layers of large air-chambers with chlorophyllose cells have developed in the upper portion of the thallus. Air pores or openings,a means of communication for the uppermost chambers, are present in the dorsal region. The ventral tissue and midrib are rudimentary and lack chloroplasts. The cells of this portion constitute the storage region. It gives rise to the rhizoids and to a series of narrow scales which due to the tendency to dry up are often to be detected close to the growing point. The apical cell is triangular in outline, and lies nearer to the ventral surface in the depression between the lobes, commonly called the "notch". From this cell are cut off dorsal and ventral cells, the former to give rise to the sexual organs, the latter to the scales and rhizoids of the ventral region.

The sexual organs are embedded in the tissue of the thallus and continue to develop from the younger dorsal segments of the initial cells for a long time so that groups of varying stages may be found on a single thallus. Ordinarily only antheridia or archegonia are found on the one thallus because their development is not simultaneous, the growth of the antheridia preceding that of the archegonia.

The antheridia arise from single superficial cells which having become enlarged project as papillae above the surface. From the two cells formed by the first division of this initial cell, the inner develops after several subse-

quent divisions into a short stalk, while the outer with its dense contents develops into the antheridium proper. Due to the rapid growth of the adjacent cells the organ is sunk in a deep cavity, where after several well-marked divisions it consists of a layer of sterile cells enclosing a compact mass of sperm mother cells, each of which forms two biciliate antherozoids which, when mature, are discharged through the tubular neck formed by the projection of the uppermost cells of the cavity in which the antheridium is embedded.

The initial steps in the development of the archegonium are similar to those of the antheridium, the mother cell of the latter, however, being ordinarily larger. The archegonium consists of a short stalk, the venter enclosing the egg cell and the ventral canal cell, the neck cells and the neck canal cells, the whole having the appearance of a slender tube with a large bulbous lower end, the elongated neck of which extends ever so slightly above the level of the thallus because the surrounding cells have kept pace with the growth of the archegonium which even in maturity is sunk deep in its individual cavity. The egg in maturity is globular in form having a medium-sized nucleus and a distinct nucleolus and occupies a comparatively small section of the space within the venter. The cell walls of the neck-canal cells break down and the contents of the cells mass together. Through this canal the antherozoid enters and the nuclei of egg and antherozoid fuse. The entire process of the growth

and development of the sexual organs is a modification of the ordinary growth of the dorsal region of the thallus and the spaces around them are ordinary air-spaces.

After fertilization the egg develops a cell membrane and enlarges to completely fill the cavity of the venter, which forms a two-celled layer of calyptra.

Materials and Methods

A few days prior to May twenty-second, Riccia natans was taken from ponds in the vicinity of Milwaukee, and kept in water in the laboratory until the desired time for fixing. The process of killing and fixing was done on several successive days in order to insure securing the various stages of sporogenesis. Chromo-acetic acid solution, Flemming's medium, Bouin's, and Picro-acetic acid were used for killing and fixing. Flemming's medium gave the best results. To warrant more perfect penetration, of the various reagents used in this and subsequent steps, the ventral scales and brown edges of the thallus were removed at the outset. By means of an air-pump all the air was withdrawn from the large air-chambers so that the fixing fluids might have access to all parts. The material was allowed to remain in these vials for periods of time ranging from twenty-four to seventy-two hours and this process was followed by washing it in running water for twenty-four continuous hours. Dehydration was begun with a very low concentration of alcohol in order to pre-

vent plasmolysis of the tissue. A fifteen per cent solution of alcohol for fifteen minutes, thirty per cent solution for thirty minutes, fifty per cent for one hour and seventy per cent for twenty-four hours or until convenient to make the succeeding changes in rapid succession was the program followed. The seventy per cent solution of alcohol was replaced by eighty per cent and that in turn by ninety-five per cent for periods of twenty-four hours each. The final step of dehydration was accomplished by immersing the particles in absolute alcohol for two successive period of one hour each.

Clearing was begun with a mixture of fifty per cent alcohol and fifty per cent xylol. To prevent any difficulty due to transparency, the material fixed in the Picroacetic solution was cleared by the use of a similar mixture stained with safranin. This process was concluded by the use of pure xylol for thirty minutes. Infiltration was then begun. The vials were placed on top of the bath for twenty-four hours after which the corks were removed and the vials were put into the bath. At intervals of twenty-four hours each, three changes were made to insure freedom from traces of xylol. Embedding, in which the paraffin was permitted to harden as quickly as possible prepared the material for sectioning.

With a rotary microtone sections six to eight microns were cut, mounted, and finally stained. Flemming's triple stain, (safranin, gentian, violet and orange G) was used for

practically all the slides and proved superior to all others used. A compound microscope with a camera lucida was used for the drawings.

Observations

The earliest stage observed, shows the young sporophyte with a regular wall or amphithecium enclosing a compact mass of thirty or forty thin-walled cells, cells rather sparsely vacuolated and with several of the respective nuclei clearly discernible. The venter, the cells of which are still intact has spread outward to form the calyptra which is separated from the sporogonium. (Fig. 1, Pl. I.) The amphithecium expands and the mass of now almost perfectly rounded spore mother cells are beginning to separate from each other. The cells have very regular thin walls and the cytoplasm has many and large vacuoles. (Fig. 2, Pl. I.) The spaces between the spore mother cells are filled or partially filled by a nutritive material which appears as fibrous strands. (Fig.3, Pl. I.)

The spore mother cells enlarge and again fill the entire cavity formed by the amphithecium. (Fig. 4, Pl. I.) In some instances the cell membranes have receded from the cell walls and from the spore sac. (Fig. 5, Pl. I.) The various stages of nuclear division, mitosis, of the spore mother cells is illustrated in Figs. 6, 7, 8, 9, Pl. II.) The chromosome number of the sporophyte is eight.

After the first nuclear division the daughter nuclei divide again and then only are the division walls of the tetrad formed. (Fig. 10, Pl. II.) The walls are very delicate at first and are little more than membranes (Fig. 10,Pl.II.) but as they grow older the walls become very heavy until just before the separation of the spores when they are very thick and have two distinct layers. (Fig. 15, Pl. II.) The young tetrad has many starch grains; (Fig. 11, Pl. II.) the older one has very few. (Fig. 12, Pl. II.)

Just prior to the tetrad stage of the sporogenous tissue the inner wall of the calyptra breaks down but the outer layer remains entire or nearly so until the spores are ready for dispersal, at which time the amphithecium also breaks down. All the spore mother cells produce spores.

The tetrad ordinarily appears as a group of three cells (Fig. 13, Pl. II.) though groups in which four cells can be seen occur frequently. (Fig. 14, Pl. II) There is no sterile tissue except the cells of the amphithecium. With the breaking down of the tetrad, the spores lie free and unattached within the capsule.

The spores are of approximately equal size and have three comparatively smooth surfaces, due, no doubt to the close adherence of the members of the tetrad. Two of the three smooth sides and the fourth, a very irregular surface, which was part of the external wall of the tetrad are shown

in Fig. 16, Pl. II. The rough surface of the spore is also shown in Fig. 17, Pl. II. In cross section the spore reveals little or no granular content indicating that the food stored in the spore is principally in the form of protein, which is not demonstrable in this type of fixation. The wall is irregular and very heavy. The spore nucleus is very small. (Fig. 18, Pl. II.)

Discussion

The zygote of Riccia natans presents some very conspicuous features that the changes in the tissue bring about. The fertilized egg fills the venter which, before the first division of the new cell (the fertilized egg), is a complete double layer of cells. The first division with which the embryonic stage is introduced is usually transverse but may be oblique. The next wall may appear first in either of the cells just formed or the walls may appear simultaneously in both cells. These new dividing walls may be perpendicular to the first, thus forming a quadrant or they may be parallel to it thus forming a row of four cells. In most species of Riccia the next walls, at right angles to the foregoing produce octants which in turn are divided by a curved wall; in Riccia natans, however according to Garber (1902) the subsequent divisions after the four-celled stage occur in all directions, producing thereby a mass of from thirty to forty cells.

About this time in the development of the sporophyte the amphithecium is clearly discernible and consists of a very regular wall of two layers of cells that encloses compactly the spore-producing material, which continues to grow until there are within the amphithecium about four hundred spore mother cells. Up to this point the sporophyte has been a solid mass; now the amphithecium expands and forces the calyptra out and the spore mother cells round out being free to do so because of the separation from each other. The cell walls are thin and regular and in most instances the cytoplasm has many and large vacuoles. (Fig. 2, Pl. I.) One or two starch grains of considerable size and numerous small ones comprise the stored food in most of the cells. The surrounding tissue secretes a fibrous nutritive material which partially or wholly fills the spaces between the cells, thus giving most favorable condition for the rapid growth of the spore mother cells. Most of this is readily absorbed by the spore mother cells as they quickly expand and once more fill the amphithecium. The walls now are thicker, this being due to the pressing rogether of the cell wall and the surplus nutritive material during the rapid growth of the spore mother cells.

Nuclear division takes place and although the various phases of mitosis could be distinguished in numerous cells, the small nuclei and the small amount of chromosomes make the study of nuclear division somewhat difficult. The spo-

rophyte spindle has very prominent asters but no centresomes could be distinguished. The chromosomes number is eight in the sporophyte generation and four in the gametophyte cells. After the first nuclear division of the spore mother cell, the daughter nuclei divide again and ordinarily arrange themselves at equal distances from each other, and the division walls usually form simultaneously. (Fig. 10, Pl. II.) In the very young tetrad the walls appear very faintly, being apparently little more than membranes but as development continues well delineated cells appear in closely adhering groups of four spores, generally known as a "spore tetrad". This frequently appears as a group of three cells (Fig. 14, Pl. II.) although groups in which the four cells can be seen are not uncommon. (Fig. 14, Pl. II.) As growth continues the spore wall thickens and both endospore and exospore are plainly visible. (Fig. 15, Pl. II.)

Shortly after the formation of the tetrad the inner layer of the calyptra collapses but the outer remains intact even after the amphithecium has broken down, a process, which occurs about the time that the spores are ripening. It is believed by some that these cells are used up by the growing spores for nourishment, thus leaving the spores lying free and unattached in a cavity formed by the outer layers of the cells of the calyptra. No sterile tissue like elaters is derived from any of the spore mother cells, all the sporogenous tissue producing spores only.

The spore is a small dark body having three somewhat smooth, and one very rough side. The smoothness seems to be the result of the tetrad condition when three sides of each of the four spores were closely packed together, and the fourth side of each formed one of the external walls of the tetrad. The spore nucleus is very small, the walls are heavy and there is little or no granular content. The spores are dispersed when the thallus enclosing the sporophyte breaks down.

The entire process of sporogenesis takes about three weeks. The volume of the mature sporophyte is about five hundred times that of the egg from which it developed.

Summary

1. The fertilized egg cell enlarges to fill the venter, which is a double layer of cells.

2. The first three divisions of the embryo are regular but subsequent divisions vary.

3. The nutritive material for the nourishment of the spore mother cells is secreted by the surrounding tissue.

4. The young cells have numerous starch grains, the older cells have very few.

5. The sporophyte spindle has prominent asters but no centresomes.

6. The diploid number of chromosomes, as seen in the spore mother cells, is eight; the haploid in the spore is four.

7. The walls of the spore-tetrads are formed simultaneously, after the two nuclear division of the spore mother cell.

8. All the tissue within the amphithecium produces spores. There is no sterile tissue produced. The number of spores is estimated to be between twelve hundred and sixteen hundred.

9. At maturity the spores lie free within the cavity formed by the calyptra.

10. The spores are dispersed when the gametophyte breaks down.

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* From Black, C.A.
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Explanation of Plates

PLATE I.

Fig. 1. A group of potential spore mother cells in adherence to one another with a well-defined amphithecium and the cells of the venter of the archegonium still entire, enclosing the spore sac, though distinctly separated from it.

Fig. 2. The highly evacuolate condition of a spore mother cell.

Fig. 3. A stage in which the amphithecium has expanded and the spore mother cells are separated from each other, the space between has been filled by a nutritive material.

Fig. 4. The spore mother cells, now full and rounded, occupy the entire spore sac.

Fig. 5. A stage in which the cell-membranes have receded from the cell-walls and from the amphithecium, the spore sac.

PLATE II.

Fig. 6. A spore mother cell, the nucleus of which is preparing for the synapsis stage.

Fig. 7. The metaphase stage of mitosis.

Fig. 8. The anaphase stage of mitosis.

Fig. 9. The telophase stage of mitosis.

Fig. 10. Very young tetrads showing membranes in the process of formation.

Fig. 11. Young tetrad showing the characteristic numerous starch grains.

Fig. 12. An old tetrad showing few starch grains.

Fig. 13. Tetrad, showing three members of the group.

Fig. 14. Tetrad, showing four members of the group.

Fig. 15. Very old tetrad with heavy walls, the members of which are about to separate.

Fig. 16. A mature spore, showing one of the three smooth surfaces, and the one irregular surface.

Fig. 17. A mature spore, showing the irregular surface.

Fig. 18. Cross section of a mature spore, showing the heavy outer wall, a very small nucleus and the total absence of granular content.

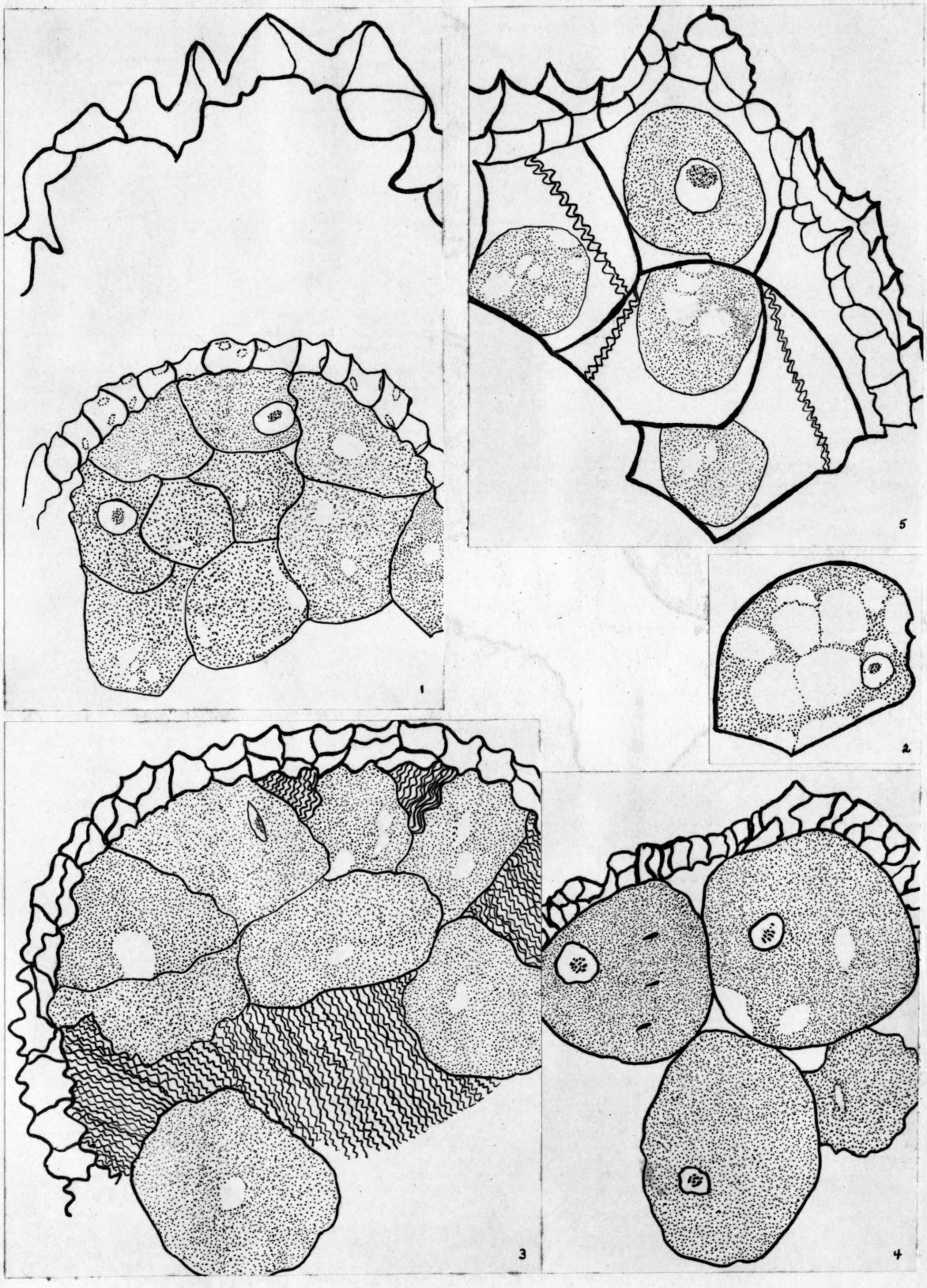
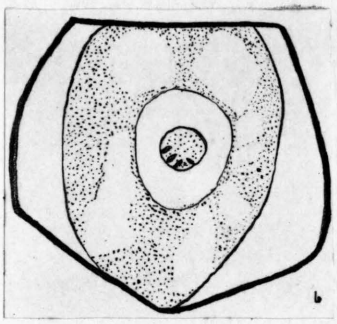
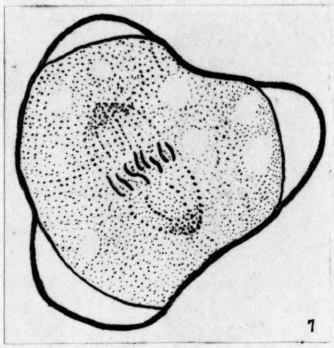
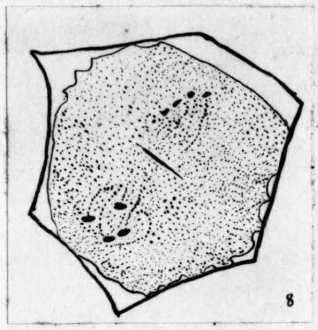
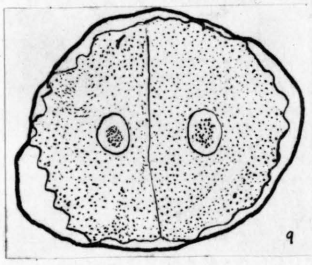


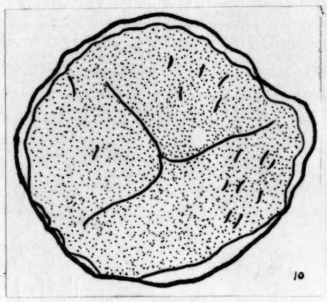
PLATE I.

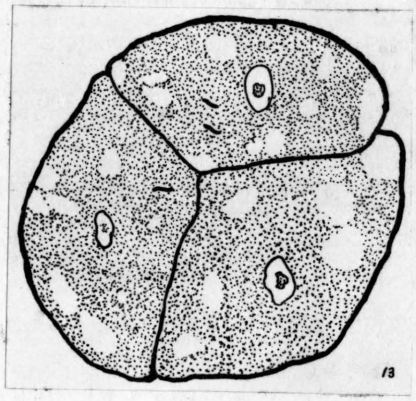


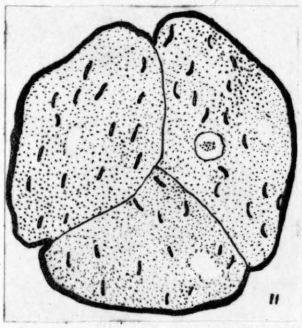


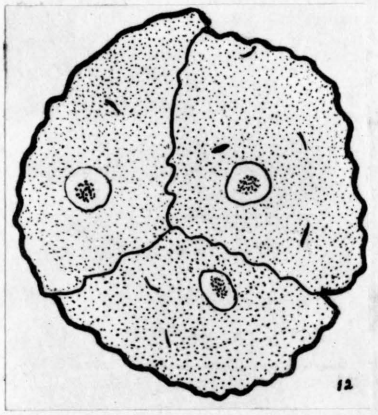


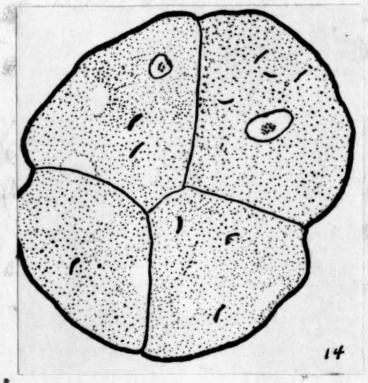


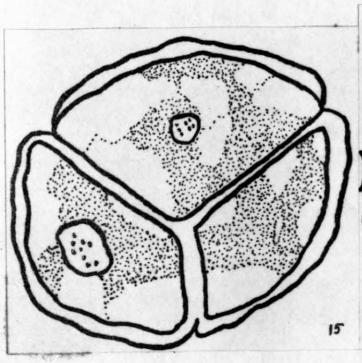


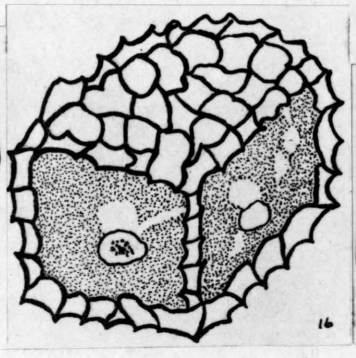


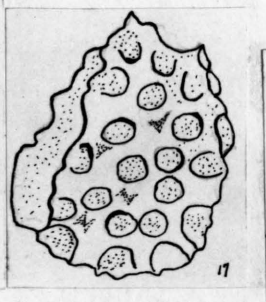












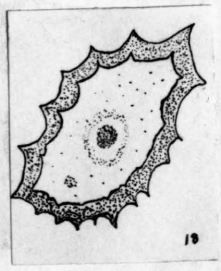


PLATE II.

Approved

W. N. Stait.

Major Professor

W. J. Grace by

Dean

Date June 1, 1932