Cytological Studies in the Protococcales.

II. Cell Structure and Zoospore Formation in *Pediastrum Boryanum* (Turp.), Menegh.

BY

GILBERT MORGAN SMITH.

With Plate XII and four Figures in the Text.

THE family Hydrodictyaceae of the Protococcales is of exceptional interest because of the peculiar formation of daughter colonies by means of motile zoospores. The cytological details of the process have been studied in *Hydrodictyon* by Timberlake (17), Klebs (7), and others ; in *Euastropsis* by Lagerheim (8) ; but nothing is known regarding the process in *Pediastrum*. This is due to the fact that although the alga is widely distributed in nature, it rarely occurs in sufficient abundance to fix for cytological study. Even general laboratory cultures do not produce it in abundance, so that only by the application of special culture methods is a sufficient number of colonies obtained to embed in paraffin.

The source of the alga, whose study is reported in the present paper, was the plankton of Lake Mendota at Madison, Wisconsin ; and it was obtained in pure culture by means of the methods which I have described in another connexion (14). The cultures were grown in 0.2 per cent. Knop's solution, and fixed in Flemming's weak osmic-acetic-chromic acid mixture diluted with an equal volume of water. The washing and dehydration of the material were by means of osmosis through a celloidin film placed over the end of a vial in the manner which I have described elsewhere (12). The material was embedded in paraffin, and cut on a microtome in sections 5-8 μ in thickness. Flemming's triple stain gave the best differentiation of the pyrenoid and a good differentiation of the nucleus. Heidenhain's iron-alum-haematoxylin also stains the nucleus satisfactorily.

Practically all descriptions of colony formation are based upon the observations of Braun (2). He found in *P. Boryanum* that the division of the mother-cell contents and the liberation of the young colony took place in the afternoon. According to his account, the cell divides into halves by cleavage. The halves then redivide, but not always simultaneously.

Intermediate stages between the primary and the completed cleavage to form sixteen zoospores are not described. After the zoospores have been formed they are discharged through a longitudinal split in the mother-cell wall, the innermost layer of which forms a vesicle into which the zoospores are extruded. Immediately after their extrusion the zoospores begin to move rapidly inside this vesicle. At first they form an irregularly shaped mass, but after about fifteen minutes they come to rest forming a flat plate of cells. When the movement of the zoospores ceases they are slightly emarginate. An hour after, the peripheral cells are still further emarginate, and after four hours the emarginations have developed into horns. During the first few hours in the life of the colony there are spaces between the individual cells, but at the end of twenty-four hours the cells are closely applied to one another, and the horns have the same shape as in mature cells. The vesicle surrounding the young colony persists for a short time only.

Askenasy (1) is the only one who has studied the cell contents in preparations of *Pediastrum*. He finds that in preparations of *P. Boryanum*, cells 9–13 μ in diameter, there is a single eccentrically located nucleus some 2 μ in diameter, while cells 13–18 μ in diameter are either uni- or binucleate. With the growth of the cell there comes an increase in the number of nuclei, so that a large number is present in mature cells. He was unable to study nuclear behaviour during the cleavage stages of zoospore formation because the entire contents of the cell took the haematoxylin stain so deeply, but he assumes that each zoospore contains a single nucleus. Askenasy states that the pyrenoid can be recognized in very young cells, and that as the cell increases in size there is a corresponding increase in the size of the pyrenoid. Surrounding the pyrenoid is the usual ring of starch plates; there is also starch in the cytoplasm. He was unable to follow the history of the pyrenoid after the first cleavages in zoospore formation, but found that the first cleavage plane either passed through the pyrenoid, dividing it into two unequal parts, or left it undisturbed.

In the youngest cells that I have observed there is always a single nucleus and pyrenoid. The nucleus is, as Askenasy (1) has shown, markedly eccentric (Pl. XII, Fig. 1), but the pyrenoid has no definite position. In spite of its very eccentric position within the cell, the nucleus has no definite relation to the margin of the colony. It may be on the side of the cell towards the periphery of the colony, on the side towards the centre, or it may be lateral (Text-fig. 1). With further cell growth the position of the nucleus becomes more central, until at the maximal size for uninucleate cells it is frequently central.

The structure of the nucleus, in spite of its minuteness, is much more like that of the nuclei of higher plants than I have found to be the case in other Protococcales (12, 13, 15). There is always a single nucleolus and

a sharply defined nuclear membrane. The chromatin and the linin content vary, however, at different stages in the life history. In very young cells the chromatin granules are pronounced and near the nuclear membrane, but the linin threads can be distinguished only with difficulty (Pl. XII, Fig. 1). At this time the nucleolus is less conspicuous than in the later stages. As the cell becomes older the nucleolus and the linin threads grow more prominent, whereas the chromatin granules become less conspicuous, but never so indistinct as not to be recognizable in good preparations (Fig. 21, 22).

Although many of the details are lacking, there is sufficient evidence to show that the nuclear divisions are mitotic. In the equatorial plate stage a distinctly bipolar spindle can be recognized. The chromosomes are arranged

TEXT-FIG. 1. Young colonies showing that the nucleus and pyrenoid of a cell are not definitely located with respect to the margin of the colony. ($\times 2,000$.)

in a dense group when observed from the side (Fig. 2), but in polar views they appear much more distinctly (Figs. 4, 5). Although Timberlake (17) describes a small, spherical, densely staining body at each pole of the spindle in the closely related *Hydrodictyon*, I have not observed anything of the sort in *Pediastrum*. Since no cells were found in which some of the nuclei were dividing and some were not, it may be safely assumed that the nuclei within a cell divide simultaneously.

The cytoplasm of the youngest cells is granular, but a little later scattered vacuoles appear (Fig. 3). These vacuoles never become a conspicuous feature, however, and do not fuse to form a large central vacuole. As the cell becomes still older they frequently disappear, so that the mature cell again seems to be filled with a dense granular cytoplasm (Figs. 5, 6). The cell grows to a considerable size before the first nuclear

division takes place (Fig. 2). After the cell becomes binucleate there is still further growth before the next divisions occur (Fig. 4). Askenasy (1) states that the mature cells of *Pediastrum* contain large numbers of nuclei, but my observations show that the number is rarely greater than four, although 8- and 16-nucleate cells have been found. The nuclei in the mature cell lie equidistant from one another in the central portion of the cell. Since all the cells of a colony are of the same age, it might be expected that karyokinesis would take place in them simultaneously; I have never found this to be the case, however, since it often happens that some of the cells in a mature colony are binucleate and others tetranucleate.

Pyrenoids are present in the youngest cells. Usually but a single one appears, although cells with two, and even three pyrenoids, have been observed. There is no relationship between the size of the cell and the

TEXT-FIG. 2. Portions of cells showing irregularly shaped pyrenoids. G shows that very young cells may contain two pyrenoids.

number of pyrenoids, since the occurrence of more than one pyrenoid is as frequent in young as in old cells (Text-fig. 2). The shape of the pyrenoid varies considerably. Spherical ones are most abundant, angular ones are by no means rare, and in isolated instances they have small bud-like extrusions or projections. Timberlake (16) has regarded angular pyrenoids as evidence of rapid starch formation in *Hydrodictyon*, but in the case of *Pediastrum* the angular and the spherical pyrenoids are found in cells of the same colony. Pyrenoids with bud-like projections (Text-fig. 2) are of interest as being possible stages in the division of the pyrenoid; but I am of the opinion that such is not the case, and that this peculiarity is merely an irregularity in the shape similar to that which I have found in *Characium* (15). When stained with Flemming's triple stain the pyrenoid is normally a homogeneous, red-staining body surrounded by angular starch plates which stain blue (Fig. 10). I have never found the pyrenoid staining blue, as McAllister (9) finds to be the case in *Tetraspora*. Cases have been found in which one

portion of the pyrenoid stained and the other did not (Figs. 3, 6). These may possibly represent stages in a metamorphosis of the body of the pyrenoid into starch similar to that described by Timberlake (16); but since the unstained portions are always a part of the pyrenoid and never a segment that has been cut off, I am inclined to consider them as due to irregularities in staining and not as stages in starch formation. The starch plates which surround the pyrenoid are curved, and usually three or four are visible when it is viewed in median optical section. The space always perceptible between the starch plates and the pyrenoid appears empty. This space is not always of uniform width, as I have found in *Characium* (15) and *Scenedesmus* (13), but may be much wider at one side than another (Figs. 5, 10, 22). There is also considerable stroma starch scattered throughout the peripheral region of the cell, especially in colonies from older cultures in which it occurs in abundance. In preparations destained sufficiently to show the nuclei well, the 'stroma' starch plates are frequently so decolorised that the cytoplasm appears to consist of fine angular vacuoles (Fig. 4); but proper staining demonstrates that what seem to be vacuoles are really 'stroma' starch plates (Fig. 7). This accumulation of starch plates in the cytoplasm is not peculiar to *Pediastrum*, but is a constant characteristic of old cultures in all the *Protococcales* that I have had under cultivation. The accumulation of starch is accompanied by a loss of the chlorophyll in the plant, so that the general appearance of the culture is not a bright fresh green but a sickly yellow-green. The same loss of colour and accumulation of starch may be seen in the cells of *Oedogonium*, *Spirogyra*, and other filamentous forms collected in the field during autumn. Cells containing these excessive amounts of 'stroma' starch are not normal, as is shown by the fact that such cells in the filamentous species fail to divide, and similarly in cultures of *Protococcales*, where the cells contain large amounts of starch, this same pathological condition is evidenced by the small number of young, recently formed colonies.

Askenasy (1) calls attention to the fact that there is a definite relationship between the size of the pyrenoid and the size of the cell, since increase in size of the cell is accompanied by an increase in size of the pyrenoid. I have observed this relationship in both the cells with the single pyrenoids and in those with more than one pyrenoid. In the latter there is the growth of the pyrenoids as the cell grows, but the rate of growth is much slower than in cells with but a single pyrenoid.

The colonies of *Pediastrum* are not invested with a gelatinous sheath as are those of many *Protococcales*. This fact can be demonstrated by using Schröder's (11) method of staining living material with vesuvin, or by Errera's Indian ink method (4). Various systematic works figure and describe the walls between adjacent cells as being red, while the outside

walls of the marginal cells are colourless. I have observed this red coloration of the walls of the interior cells in living material when using the lower magnifications (500–750 diameters), but have never found it with the higher ones (1,500–2,000 diameters); I consider this red coloration therefore an optical illusion.

In preparations stained with Flemming's triple stain the walls appear to be composed of two parts: a very thin outer layer which takes the gentian violet and a thick orange-staining portion (Fig. 8). The portion stained by the gentian violet is not always of uniform thickness, but is likely to be much wider at the angles of the cells. The inner orange-staining layer does not show unless the preparations are very heavily counterstained with the orange G, so that in most preparations this inner part does not stain, but appears as a clear space between the violet-stained portion of the wall and the cytoplasm (Figs. 4–6). At first glance, therefore, many cells appear to be slightly plasmolysed when they are really turgid.

Petersen (10) has recently found in various species of *Pediastrum*, including *P. Boryanum*, that there are long tufts of bristles protruding from each cell in the colony. In a few instances he was able to see these bristles in living material, but by means of special staining methods he demonstrates the presence of these bristles in several different species where they cannot be found by examining the living material. I have tried Petersen's staining methods on the colonies of my cultures with negative results. His material, however, came directly from the plankton, and it is quite possible that these bristles are not developed except under plankton conditions.

In my cultures I have observed a certain diurnal periodicity in swarm-spore liberation. The alga was observed for the entire twenty-four hours of the day, and with very rare exceptions swarming occurred only at day-break. Swarming colonies first appear about an hour before sunrise, but the maximum amount takes place just at sunrise. These observations are not in accordance with those of Braun (2), who found swarming only in the late afternoon. The precise hour of swarming varies from month to month, the maximum occurring at 5.30 a.m. in May and at 7.30 a.m. in December. That the induction of swarming is very largely dependent upon daylight can be demonstrated readily by placing a portion of a culture in a dark room before the first signs of daybreak (2–3 a.m.) and leaving the remainder of the culture where it will be illuminated by the rising sun. At the hour of sunrise the illuminated culture shows active swarming, while the unilluminated one shows none; but if the latter culture be brought out into the light after the illuminated culture has ceased swarming, it too will show a large number of swarming colonies. This relationship between the swarming period and the illumination was first observed by Braun (2) in *Hydrodictyon*.

The formation of the swarm-spores and the number of cells in a colony are greatly influenced by cultural conditions. Sixty-four- and thirty-two-celled colonies predominate among those produced during the first two weeks in cultures containing 0.2 per cent. Knop's solution, while in cultures a month old 16- and 8-celled colonies predominate. At the end of two months few new colonies are formed, the cells of the old colonies in the culture containing large amounts of 'stroma' starch. The production of colonies with large numbers of cells in fresh vigorous cultures, and the later production of those with fewer cells, is a fact that has been noted by all investigators of coenobitic algae under cultural conditions.

The observations of Caspary (3) and Braun (2) have shown that any cell in the colony is capable of giving rise to a daughter colony. Since cells in the same colony are of exactly the same age and have been under the same external environmental conditions, the natural expectation would be that all cells would form zoospores at the same time. Any one familiar with field collections of *Pediastrum* knows, however, that generally at most only a few cells of a colony are empty as a result of zoospore formation. The question arises, then, whether the other cells of the colony will form zoospores on some succeeding night, or whether, on the other hand, only a few cells of a colony will ever produce them? Upon observation, however, it was found that zoospores are formed and liberated from different cells in the same colony at different times (Fig. 17).

Since my observations agree in the main with Braun's (2) account of the swarming, little need be said on this point. In my cultures, however, I find that the changes taking place after the swarm-spores come to rest are much more rapid than Braun describes them. The rapidity with which these changes take place is shown in Text-fig. 3, A-D, which are outline camera lucida drawings made at three-minute intervals. These sketches should be regarded as being only approximate, since in each instance less than a minute was devoted to the outlining of the eight cells in the colony. The sketches show that within the few minutes following the cessation of zoospore motion changes take place in the cell that according to Braun's description require a period of hours for their completion.

For the observation of the cytological details of zoospore formation I selected month-old cultures which were forming 8- and 16-celled colonies, because the small number of nuclei made the process comparatively easy to follow. The details are essentially the same as in the formation of 32- and 64-celled colonies. The first stages in zoospore formation are found in the early hours of the night, and the first indication of what is to occur is a rapid division and redivision of the nuclei so that their number is increased fourfold. These nuclear divisions are always simultaneous; the number of nuclei present is therefore always a multiple of two. Nuclear division takes place only in the cells that are to form zoospores, so that a surface view of

a colony shows 16-nucleate cells adjacent to 2- and 4-nucleate ones (Text-fig. 4). There are two general types of nuclear behaviour previous to spore formation in Algae and Fungi: either a succession of nuclear divisions of a single large nucleus in a mature cell, or nuclear division as the cell grows, resulting in a multinucleate condition when mature. In the latter case there is no nuclear division just previous to spore formation. The former method is followed by *Synchytrium* and *Ulothrix*; *Rhizopus*, *Characium*, and

TEXT-FIG. 3. Outline drawings, at three-minute intervals, of the changes taking place in the first few minutes after the cessation of zoospore movement. In this colony the swarming lasted eight minutes, and the first drawing was made fifteen seconds after the motion ceased. ($\times 2,000$.)

Hydrodictyon follow the latter. In *Pediastrum* there is a combination of the two types, inasmuch as the cell is multinucleate, but just before zoospore formation a period of active nuclear division sets in, the resulting nuclei lying a short distance from one another within the cytoplasm. At times there is a grouping of the nuclei into fours (Figs. 11, 19), but at other times this grouping is not apparent (Fig. 9). The grouping of the nuclei in fours and eights has been observed in *Hydrodictyon* by Timberlake (17), so that

there may possibly be a specially active period of nuclear division just prior to zoospore in *Hydrodictyon*. The appearance of the nuclei of *Pediastrum* at this time is quite different from the appearance at other periods. Before this period of nuclear division sets in there is a conspicuous nucleolus, the chromatic material being quite faint and lying near the nuclear membrane (Figs. 19, 22); but after the rapid nuclear divisions the peripheral chromatin granules are very conspicuous (Figs. 9, 13).

While these nuclear changes have been going on the pyrenoid has disappeared. In the various algae whose spore formation has been studied the behaviour of the pyrenoid is not uniform. In zoospore formation in *Hydrodictyon* (7, 17) and *Cladophora*, and in spermatogenesis in *Sphaeroplea* (5, 6), the pyrenoids generally disappear before spore formation sets in. On the other hand, in the zoospore formation in *Characium* (15) and *Tetraspora* (9), and colony formation in *Scenedesmus* (13) and *Tetradismus* (12), in which the products of cell-division are the morphological equivalents of zoospores, the pyrenoid does not disappear, but remains unchanged in one of the daughter-cells. I find in *Pediastrum* that it is only in exceptional cases (Fig. 12) that the pyrenoid persists after the process of cleavage commences. Stages were found in the disappearance of the pyrenoid, and it was noted that the ring of starch plates disappears while the body of the pyrenoid becomes diminished in size, frequently becoming quite angular just before its final disappearance (Figs. 11, 12). Cases were not found similar to those described by Askenasy (1), in which the first cleavage plane cuts through the pyrenoid, dividing it into either equal or unequal parts.

The first cleavage plane may be so placed as to include either the long or the short axis of the cell. Whether it is formed by a furrowing in of the plasma membrane or by the formation of a row of vacuoles could not be satisfactorily determined, but I am inclined to think it takes place by the former method. In a very few instances cleavage planes that did not extend clear across the cell were found between two neighbouring nuclei (Fig. 13). These suggest conditions similar to those found in *Hydrodictyon* by Timberlake (17), in which cleavage furrows appear at various places between the nuclei. Unlike the furrows in *Hydrodictyon* those in *Pediastrum* do not branch. The protoplasts formed by the cleavage furrows are always angular, and the number of nuclei in each varies from one to four (Figs. 14, 15, 17). The process of cleavage continues until the entire contents of the cell have been cut up into angular uninucleate pieces (Fig. 17). Mention has been made of exceptional cases in which the pyrenoid persists after the beginning of cleavage. In two instances, when the triple stain was used, a small red-staining body was found in one of the uninucleate protoplasts (Fig. 16), which may possibly be the last vestige of the pyrenoid. The nuclei in the cells that are undergoing cleavage have much the same

appearance as those in which it is about to occur, the densely-staining chromatin being a conspicuous feature of all such nuclei.

The formation of uninucleate protoplasts is generally completed by midnight. At this time each nucleus lies at one of the angular apices of its protoplast. The nuclei are frequently lenticular or curved instead of spherical at this time (Fig. 17). The next step in the formation of the zoospores is a rounding up and swelling of the protoplasts so that the cleavage planes entirely disappear. When this rounding up begins the nuclei are even closer to the edge than they were when the protoplasts were still angular (Fig. 16). As the cleavage lines disappear the nuclei

TEXT-FIG. 4. Surface view of a colony showing 16-nucleate cells adjacent to 2- and 4-nucleate ones. The cleavage planes have disappeared in the two cells that are forming zoospores. ($\times 2,000$.)

become more dense, but the chromatin granules and the nucleolus are still distinguishable (Figs. 18, 19).

In material fixed at 2 a.m. no trace of cleavage planes could be found, but many cells similar to those shown in Figs. 18 and 19 were found in my preparations. Cells in the same colony stain differently, so that all gradations can be found between those in which there is a heavy stain in the cytoplasm and those in which the cytoplasm is only faintly stained; yet in no instance do any cells in this material show signs of cleavage planes. The objection might be raised that this condition which I have interpreted as a swelling of the protoplasts, making the cleavage planes indistinct, comes really before and not after cleavage. However, cells

without cleavage planes are never found in preparations having angular protoplasts, but are always found in material fixed at a time after the cleavage into uninucleate protoplasts has been completed. Even stronger evidence upon this question is afforded by the condition of the cell contents, in that the nuclei are dense and irregular after the cleavage furrows have disappeared, and pyrenoids are never found in these cells (Fig. 19 and Text-fig. 4). On the other hand, cells are sometimes found in which there has been the preparatory division of the nuclei, but in which for some reason there has been no cleavage. These cells are easily distinguished from the stage just described by the less dense, spherical nuclei and the presence of a pyrenoid (Fig. 22, *a*).

The next noticeable change is the reappearance of the cleavage planes. These look in section like very fine lines, and at the time that they reappear the protoplasts are fully rounded up. In a few instances the planes made their appearance at one end only of the cell (Fig. 21), but in the majority of cases the process of reappearance seems to be simultaneous in all parts of the cell (Fig. 22, *b*). At this time the nuclei are even more elongate and dense than at any previous stage in the cleavage process, and the chromatin granules can only be distinguished with the greatest difficulty. These rounded, uninucleate protoplasts are the zoospores.

After the reappearance of the cleavage planes the protoplasts separate slightly from one another, the nuclei remaining flattened (Fig. 20). I am in doubt concerning the very last developmental stages, since in the fixed preparations it is impossible to say whether a definite cavity contains zoospores or zoospores that have germinated into cells. In certain instances there is no doubt that the zoospores have germinated into cells within the mother-cell wall (Fig. 24). On account of these difficulties it is impossible to determine whether the nuclei, which are very much flattened when the cleavage planes reappear, become rounded up in the zoospore stage or after the zoospores have germinated to form cells. Nor am I certain at what precise step the pyrenoids, which are always found in the youngest cells, reappear.

SUMMARY.

The youngest cells of *Pediastrum Boryanum* are uninucleate, each containing one, rarely two or three, pyrenoids. Mature cells contain four or eight nuclei and one to three pyrenoids. The nuclei increase in number by simultaneous division so that the number is always a multiple of two. In structure the resting nucleus differs but little from the nucleus of the higher plants.

The pyrenoids are homogeneous in structure and surrounded by curved starch plates,

Previous to zoospore formation there is a period of active simultaneous nuclear division resulting in 16, 32, 64, or 128 nuclei within the cell.

The zoospores are formed by cleavage. The cleavage is progressive, forming first multinucleate protoplasts and later uninucleate ones.

The pyrenoid disappears previous to or during the first stages of cleavage.

After cleavage is completed the nuclei become dense, and the line of demarcation between the protoplasts disappears, reappearing shortly before the zoospores are liberated.

LITERATURE CITED.

1. ASKENASY, E.: Ueber die Entwicklung von *Pediastrum*. Ber. d. Deut. Bot. Ges., vol. vi, 1888, pp. 127-38.
2. BRAUN, A.: Betrachtungen über die Erscheinung der Verjüngung in der Natur. Leipzig, 1851.
3. CASPARY, R.: Vermehrungsweise von *Pediastrum ellipticum*, Ehr. Bot. Zeit., vol. viii, 1850, pp. 786-8.
4. ERRERA, L.: Sur l'emploi de l'encre de Chine en microscopie. Bull. de la Soc. Belge de Microscopie, vol. x, 1884, pp. 184-8.
5. GOLENKIN, M.: Ueber die Befruchtung bei *Sphaeroplea annulina* und über die Structur der Zellkerne bei einigen grünen Algen. Bull. Soc. Imp. d. Nat. d. Moscou, N.S., vol. xiii, 1899, pp. 343-61.
6. KLEBAHN, H.: Die Befruchtung von *Sphaeroplea annulina*, Ag. Festschr. f. Schwendener, Berlin, 1899, pp. 81-103.
7. KLEBS, G.: Ueber die Bildung der Fortpflanzungszellen bei *Hydrodictyon utriculatum*, Roth. Bot. Zeit., vol. xlix, 1891, pp. 789-98, 805-17, 821-35, 837-46, 853-62.
8. LAGERHEIM, G.: Studien über arktische Cryptogamen. I. Ueber die Entwicklung von *Tetraedron*, Kütz., und *Euastropsis*, Lagerh., eine neue Gattung der Hydrodictyceen. Tromsø Museums Aarshefter, vol. xvii, 1895, pp. 1-24.
9. MCALLISTER, F.: Nuclear Division in *Tetraspora lubrica*. Ann. of Bot., vol. xxvii, 1913, pp. 681-96.
10. PETERSEN, J. B.: On Tufts of Bristles in *Pediastrum* and *Scenedesmus*. Bot. Tids., vol. xxxi, 1912, pp. 161-76.
11. SCHRÖDER, B.: Untersuchungen über Gallertbildungen der Algen. Verh. d. naturhist.-med. Ver. zu Heidelberg, N.F., vol. vii, 1902, pp. 139-96.
12. SMITH, G. M.: *Tetrademus*, a New Four-celled Coenobic Alga. Bull. Torr. Bot. Cl., vol. xl, 1913, pp. 75-87.
13. ———: The Cell-structure and Colony Formation in *Scenedesmus*. Arch. f. Protistenkunde, vol. xxxii, 1914, pp. 278-97.
14. ———: The Organization of the Colony in certain Four-celled Coenobic Algae. Trans. Wis. Acad. Sci., Arts, and Lett., vol. xvii, 1914, pp. 1165-1220.
15. ———: Cytological Studies in the Protococcales. I. Zoospore Formation in *Characium Sieboldii*, A. Br. Ann. of Bot., vol. xxx, 1916, pp. 459-66.
16. TIMBERLAKE, H. G.: Starch Formation in *Hydrodictyon utriculatum*. Ann. of Bot., vol. xv, 1901, pp. 619-35.
17. ———: Development and Structure of the Swarm-spores of *Hydrodictyon*. Trans. Wis. Acad. Sci., Arts, and Lett., vol. xiii, 1902, pp. 486-522.

EXPLANATION OF FIGURES ON PLATE XII.

Illustrating Mr. G. Morgan Smith's paper on *Cell Structure and Zoospore Formation in Pediastrum Boryanum*.

All the figures were drawn with the aid of the Abbé camera lucida, the drawings being made at the level of the base of the microscope, and with the Leitz oil-immersion objective $\frac{1}{16}$ and ocular 4. The magnification is about 2,100 diameters.

- Fig. 1. A young cell showing position and nature of nucleus.
- Fig. 2. Metaphase in the first nuclear division.
- Fig. 3. Young binucleate cells.
- Fig. 4. The second nuclear division. The vacuolate appearance of the cytoplasm is due to unstained 'stroma' starch plates.
- Fig. 5. Metaphases in the third series of nuclear divisions.
- Fig. 6. Eight-nucleate cells.
- Fig. 7. A cell showing 'stroma' starch plates scattered throughout the cytoplasm.
- Fig. 8. The appearance of the wall when deeply stained.
- Fig. 9. A sixteen-nucleate cell.
- Fig. 10. Pyrenoids of different forms and their surrounding starch plates.
- Fig. 11. Showing the disappearance of the pyrenoid and the aggregation of the nuclei in fours.
- Fig. 12. Showing the beginning of cleavage before the disappearance of the pyrenoid.
- Figs. 13-15. Multinucleate protoplasts formed by first cleavage furrows.
- Figs. 16-17. The completion of the cleavage into uninucleate protoplasts.
- Figs. 18-19. Showing the disappearance of the cleavage lines and an apparent return to the pre-cleavage condition.
- Figs. 20-23. The reappearance of the cleavage lines.
- Fig. 24. Colony formed by the germination of the zoospores without liberation from mother-cell wall.
- Figs. 25, 26. Ripe zoospores or very young stages in germination of zoospores within mother-cell wall.

G. M. Smith del.

G. M. Smith del.

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