Cytological Studies in the Protococcales.

III. Cell Structure and Autospore Formation in *Tetraedron minimum* (A. Br.), Hansg.

BY

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With Plate XV.

LITTLE is known concerning the cytological structure of members of this genus. Several species are widely distributed in nature, but the alga never occurs in sufficient quantity to fix for cytological study. The observations which I have made upon *Tetraedron minimum* are based upon material obtained by pure culture methods which have been described elsewhere (2). The cultures were grown in 0.2 per cent. Knop's solution and fixed in Flemming's medium osmic-acetic-chromic acid mixture. The fixing solution became diluted a fifth by the addition of the water containing the alga. The washing and dehydration were by means of osmosis through a celloidin film placed over the end of a phial as described in another connexion (3). The material was embedded in paraffin and cut on a microtome in sections 5μ in thickness. Flemming's triple stain gave the best differentiation of the pyrenoid and nucleus.

The fact that autospores are formed in this genus has been noted by a number of investigators, and the citations compiled by Lagerheim (1), so that no further reference need be made to the subject. Good figures of autospore liberation from the old mother-cell wall are given by West (7). Lagerheim is the only one who has studied the nature of the cell contents and the manner of formation of the autospores. He states that there is a single nucleus and pyrenoid in *T. minimum*. In autospore formation he finds a transverse cleavage dividing the cell contents into two equal parts, and then successive cuttings dividing the cell into a definite number of autospores. Nothing is said concerning the behaviour of the nucleus and pyrenoid during this process, but his figures show a nucleus in each of the daughter-cells.

The youngest cells I have observed always have a single nucleus with a pyrenoid in close association. The nucleus has a very conspicuous

nucleole and nuclear membrane, while the chromatin is indistinct. As the cell grows there is an increase in the size of the nucleus, the chromatin granules becoming more distinct but never standing out sharply in the resting nucleus. In favourable preparations faint linin threads may be seen connecting the chromatin granules.

I have been able to follow the details of nuclear division more fully in this alga than in any other member of the Protococcales that I have studied. The first indication of nuclear division is a decided increase in the size of the nucleus, while certain 'granules' become very prominent. This may be seen by comparing Pl. XV, Figs. 7 and 8, or 22 and 23. The proper interpretation of these 'granules' is a matter of doubt. A fairly definite spireme thread may be seen in the nuclei of certain cells (Fig. 9). These 'granules' may be chromosomes that have been derived by a segmentation of this spireme, or they may be chromatin granules which have become more distinct. In dealing with such small nuclei there is great danger of forcing an interpretation based upon our general knowledge of the process of karyokinesis in other plants; so that it is better to leave the nature and origin of these nuclear 'granules' an open question. In the equatorial plate stage the spindle is distinctly bipolar, the two ends gradually tapering from the mass of chromosomes on the equatorial plate. Little can be said concerning its origin, although the hyaline area surrounding the spindle in Fig. 10 suggests that it is of intranuclear origin. In some preparations there are prominent granules at the poles of the spindle, but no polar radiations (Fig. 33). These polar granules were only noted during the metaphases. The chromosomes are densely aggregated on the equatorial plate and can only be studied in a polar view of the spindle, when about eight can be seen (Figs. 24, 32). The chromosomes move from the equatorial plate in a dense mass (Fig. 20), and remain bunched after they reach the poles (Fig. 16). Details are lacking concerning the reconstruction of the daughter nuclei.

The cytoplasm of the young cells is densely granular. Occasionally there are vacuoles in growing (Fig. 2), or mature cells (Fig. 8), but this is the exception rather than the rule. Lagerheim (1) has shown that there is a definite parietal chloroplast in *T. minimum*. This stands out quite sharply in living cells, but is difficult to observe in stained preparations. The chloroplast is denser than the rest of the cytoplasm (Fig. 7), which is sometimes very finely reticulated.

Pyrenoids, about the size of the nucleole, are present in the youngest cells. The triple stain is best for differentiating their presence, since the pyrenoid always stains a bright red. I have found that more than one of these organs in the cells of *Characium* (5) and *Pediastrum* (6) is not uncommon, whereas in *Tetradismus* (3) and *Scenedesmus* (4) it is very rare. In this respect *Tetraedron* resembles *Tetradismus* and *Scenedesmus*, since not

more than one in ten thousand cells has more than one pyrenoid (Fig. 18). As the cell grows the pyrenoid increases in size (Figs. 1, 11, 14), sometimes becoming irregular in shape (Figs. 3, 4, 5). Perhaps 2 per cent. of the cells show these irregularities. Irregular pyrenoids have been noted in *Characium* and *Pediastrum* and the possibility of their being division stages discussed. If irregular pyrenoids are to be interpreted as division stages, cells with two pyrenoids should be as numerous as those with irregular ones, but since we find that irregular pyrenoids are a hundred times as numerous in *T. minimum* as cells with two pyrenoids, further evidence is furnished that such irregular pyrenoids are not division stages. Irregular pyrenoids can also be seen in living cells, so that they cannot be considered artifacts of fixation. There are also starch plates forming a hollow sphere which encloses the pyrenoid, but these are very delicate and are only noted in the most favourable preparations. In rare instances 'stroma' starch plates, a structure which has been discussed elsewhere (4), were observed (Fig. 13).

There is no definite correlation between the size of the cell and the number of nuclei; since cells of the same size may have one (Fig. 7) or four (Fig. 13). Nuclear divisions are always simultaneous in multinucleate cells and all mitotic figures in such a cell are at the same stage of the process (Figs. 7, 11). Autospore formation may begin in cells containing only two nuclei, or it may not begin until eight nuclei are present, but in the vast majority of cases the process commences in tetranucleate cells. This variation in number of nuclei at the beginning of reproduction has been noted in a number of algae and is connected with the nutrition of the cell, the better nourished ones containing the larger number of nuclei. Stages in autospore formation are more abundant in material fixed during the night, but it is not especially marked at any one time.

The following account is based upon the behaviour of those cells containing four nuclei when autospore formation commences. The first step is a transverse cleavage of the cytoplasm into two equal parts. The line of cleavage is always at right angles to the cell wall and, when the cell is oblong in cross-section, in the shorter diameter. This cleavage is probably very rapid, since no stages were found which showed it in progress. It is similar to that of the other Protococcales which I have studied—*Tetradismus* (3), *Scenedesmus* (4), *Characium* (5), and *Pediastrum* (6); the pyrenoid remaining unchanged in one of the two daughter-cells (Fig. 21). Since this lack of pyrenoid division has been noted in five members of the order, belonging to four families, it seems a reasonable assumption that it is a generally distributed phenomenon in the Protococcales. It must not be inferred that this generalization is true for all Chlorophyceae, for there are several well-authenticated cases, especially in the Zygnemales, where the pyrenoid divides before cytokinesis.

There is both direct and indirect evidence that karyokinesis follows the

primary cleavage. Fig. 24 shows this nuclear division after the formation of the first cleavage plane. This might be interpreted as a beginning of cleavage before the completion of the usual number of nuclear divisions were it not for the fact that fully 85 per cent. of the cells showing the primary cleavage furrow contained four nuclei, while about the same percentage of cells in the cultures were producing eight autospores. In *Scenedesmus* (4) the single nucleus divides once just before cleavage, and there is a division of the nuclei before each successive series of cytoplasmic cleavages. *Pediastrum* (6), on the other hand, contains a relatively small number of nuclei that divide once or twice just before cleavage, but do not continue dividing after cleavage has started. *Tetradron*, therefore, combines these two methods, since the cell is multinucleate before cleavage, but the nuclei divide after the first cytoplasmic cleavage.

Two secondary cleavage furrows are now formed at right angles to the primary one. These start at the primary cleavage furrow and are apparently formed by a furrowing in of the plasma membrane (Fig. 26). These furrows run between the nuclei of each daughter-cell, but are not opposite one another in every instance (Fig. 25). Their formation is generally simultaneous, but not always (Figs. 22, 23). The nuclear division which takes place after the completion of the first cleavage may be delayed until these second cleavage furrows have been completed or are well under way. Fig. 26 shows the completion of the second cleavage with the nuclei still in the resting condition, while the nuclei of Fig. 23 have only reached the prophase of division. Since there are eight nuclei at some stage after the completion of the second cleavage (Fig. 27), one further series of cleavages is necessary to make the uninucleate protoplasts. These last cleavage furrows are formed at an angle of forty-five degrees to the plane of the second series (Fig. 29).

After the completion of the first cleavage there is a disappearance of the pyrenoid. Instances were never found where it disappeared before the first cleavage as in *Pediastrum*. The pyrenoid may disappear before the second cleavage (Fig. 22), but as a rule it remains until the completion of the cleavage of the mother-cell into four parts (Fig. 24). Apparently there is a gradual dissolution of this body, its contour changing from a spherical to a lens shape as the process progresses (Fig. 25).

Eight angular protoplasts are found within the old mother-cell wall when cleavage is complete. These are the young autospores. Their shape now changes from the angular to the pillow shape that characterizes the mature cell of *T. minimum*. When a cellulose wall has been secreted the autospores are liberated by a gelatinization or a rupture of the old mother-cell wall. Shortly before this liberation a small pyrenoid is formed *de novo*, the new pyrenoid lying close to the nucleus (Fig. 36).

In *Pediastrum* (6), an alga where the presence of zoospores has been

definitely established, it was noted that at times the zoospores germinated within the old mother-cell wall and appeared like autospores. Search was made for zoospores in *T. minimum* with the possibility in mind that what we have been calling normal autospores are really abnormal germinations of zoospores into vegetative cells within the old mother-cell wall. Very careful observations were made in the hours preceding and following daybreak, because it is at those times that zoospores are generally liberated in the algae. Since they were not found it seems reasonable to assume that the alga reproduces by autospores and not zoospores, but the possibility of zoospore formation in *Tetradron* is not absolutely excluded, since a normal environment can never be produced in a laboratory culture.

The formation of four, sixteen, and thirty-two autospores by a single cell was also observed. Theoretically the formation of two autospores, or of a multiple of two higher than thirty-two, is possible, but they were not found. In the formation of four autospores the cleavage occurs at the binucleate stage of development (Fig. 19), and then a further cleavage divides the cell contents into four protoplasts. Sixteen autospores are formed when there are eight nuclei in the cell before cleavage. The noteworthy fact in the primary cleavage of such cells is that the number of nuclei in the two daughter-cells is always equal (Fig. 31). In these cells also a nuclear division takes place some time after the formation of the primary cleavage plane (Figs. 32, 33), while the pyrenoid disappears at the same time. The final product of cleavage is a mass of sixteen uninucleate protoplasts (Figs. 34, 35). These are more irregularly arranged than when eight autospores are formed, so that their derivation is harder to follow. The formation of the autospores from these protoplasts takes place as described above. This variation in the number of autospores, as previously stated, rests upon the nutrition of the mother-cell.

SUMMARY.

Young cells of *Tetradron minimum* contain a single nucleus and pyrenoid. Repeated simultaneous karyokineses may produce as many as eight nuclei within a single cell. Autospores are formed by progressive cleavage, the number of nuclei increasing during the process. 4, 8, 16, or 32 uninucleate protoplasts are the final product of this cleavage, these protoplasts being metamorphosed over into autospores. The pyrenoid disappears after the first cleavage, new pyrenoids being formed *de novo* in the young autospores.

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EXPLANATION OF PLATE XV.

Illustrating Professor Smith's paper on Cell Structure and Autospore Formation in *Tetradron minimum*.

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 1/16 and ocular 4. The magnification is about 2,100 diameters.

- Fig. 1. A young cell showing the nucleus with the pyrenoid in close association.
- Fig. 2. A slightly older cell.
- Figs. 3-5. Cells with irregular pyrenoids.
- Fig. 6. A transverse section of a cell.
- Fig. 7. A cell showing the chloroplast.
- Fig. 8. A prophase of the first nuclear division showing the 'granules' in the nucleus.
- Fig. 9. The spireme of the first division.
- Fig. 10. Metaphase of the first division.
- Fig. 11. Binucleate cells.
- Fig. 12. Anaphase of the second nuclear division.
- Fig. 13. A cell showing pyrenoid starch and 'stroma' starch.
- Figs. 14, 15. Four-nucleate cells.
- Fig. 16. Telophases in the third series of nuclear divisions.
- Fig. 17. Eight-nucleate cell.
- Fig. 18. An abnormal cell with more than one pyrenoid.
- Fig. 19. Completion of first cytoplasmic cleavage in the formation of four autospores.
- Fig. 20. Anaphases of nuclear division following the first cleavage.
- Fig. 21. Completion of first cytoplasmic cleavage in the formation of eight autospores.
- Figs. 22, 23. Beginning of second cytoplasmic cleavage.
- Fig. 24. Division of nuclei following the first cleavage.
- Figs. 25-8. Completion of the second cytoplasmic cleavage.
- Fig. 29. The cleavage of the cell into uninucleate protoplasts.
- Fig. 30. A later stage in the same, showing young pyrenoids.
- Fig. 31. First cleavage in the formation of sixteen autospores.
- Figs. 32, 33. The nuclear divisions following the first cleavage.
- Figs. 34, 35. Completion of cleavage into sixteen protoplasts.
- Fig. 36. Young autospores ready for liberation.

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