Cytological Investigations of Eremosphaera viridis de Bary

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CYTOLOGICAL INVESTIGATIONS OF

EREMOPSphaERA VIRIDIS DE BARY

(TITLE)

BY

RICHARD ALLEN VALENTINO

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THESIS

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YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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ACKNOWLEDGMENTS

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ABSTRACT

Eremosphaera viridis de Bary was studied with light microscope techniques to determine the method of cytokinesis and to explain the ontogeny of the membranous ring separating the mature daughter cells in the intact sporangium.

It is proposed that cytokinesis in this organism is phylogenetically advanced and that the separation of mature daughter cells involves the sequential deposition of two cell walls. The first wall develops centripetally by the addition of membrane subunits. The resulting cleavage furrow is oriented by transverse microtubules found along the cell division plane. The newly formed walls separate the two daughter cells and give rise to two sporangial plates. The second wall develops after the initial cytokinesis, also by membrane addition, followed by the deposition of the cell wall proper. The second wall, however, forms simultaneously along the entire surface of each daughter cell. Hence, it redefines the shape of the daughter cells i.e. they change from hemispherical to spherical while within the sporangium.

A furrow-phycoplast method of cytokinesis has been proposed for E. viridis with the distinctions: (1) that initial wall formation occurs centripetally, (2) that the transversely oriented microtubules serve to orient the plane of wall formation i.e. cleavage during the original cytokineses, and
(3) that the formation of two separate and distinct walls is involved in the development of mature daughter cells of this organism.
INTRODUCTION

Eremosphaera viridis was first named and described by de Bary in 1858. The most recent monograph on the genus was done by Smith and Bold (1966). It is most commonly placed in the family Oocystaceae of the order, Chlorococcales (Smith, 1950). The organism is large (120-200um), unicellular, and spherical to subspherical. It has a large (20-30um) nucleus which is typically centric. The nucleus is immediately bound by cytoplasm with cytoplasmic strands radiating towards the periphery (Fig. 1).

The numerous chloroplasts are discoidal and are situated mainly at the periphery of the cytoplasm or occasionally along the radiating cytoplasmic strands. Under high light intensity the chloroplasts tend to migrate along the cytoplasmic strands toward the nucleus and congregate in the perinuclear cytoplasm. Each chloroplast contains 1 or 2 pyrenoids (Smith and Bold, 1966).

The cell wall is quite smooth and is thin relative to the large size of the vegetative cell. Cellulose is considered to be the primary constituent of the cell wall. A pectic mucilaginous sheath is secreted (Fig. 2) as are in varying amounts, vermiform mucilage bodies (Smith and Bold, 1966).

Sexual stages have been reported and described (Kies, 1967), but further work is needed to verify the events which occur during the sexual phase of the life cycle. The more frequent
A method of reproduction is by the production of 2, or less frequently 4 autospores. Upon subsequent development of the autospores two "sporangial plates" can be observed separating the two daughter cells inside the intact sporangium (Fig. 3 & 4). These "sporangial plates" have been demonstrated photographically by Smith and Bold (1966) but were not discussed in their treatise. The nature and development of these sporangial plates is of primary importance in this investigation.

Upon subsequent maturation and cell enlargement the autospores are eventually released by means of a rupture in the expanding sporangial wall. This break usually occurs in the vicinity of the sporangial plates.

Chromosomes have been observed by several researchers (Mainx, 1927, Bowen, 1963, and Smith, 1966). They appear long, condensed, and highly intertwined, and number around 80 per cell. An account of the sequence of events occurring during mitosis has to date been ignored, and is therefore included in the present study.

The cytokinetic method involved in autospore production is currently unresolved, and constitutes a major thrust of the present investigation. Early workers reported "simple division" as the method of cytokinesis in Eremosphaera viridis (de Bary, 1858, de Toni, 1889, and Chodat, 1895). Mainx (1927) reported that a cell plate was involved, but in that same year Reichardt implicated a centripetal furrow
mechanism. In their treatise on *Eremosphaera*, Smith and Bold (1966) superficially suggested that a typical cleavage furrow process was involved, but their work was inconclusive. Bowen (1964) reported on the vegetative cytology of *Eremosphaera* but included no description of cytokinesis. Robinson, Sachs and Mayer (1976) have suggested that cytokinesis is of the cell plate-phycoplast type and that open membranes are involved in the development of the new plasmalemma. These writers, however, do not clarify the precise cytokinetic mechanism.

It is becoming increasingly more important to establish the precise mechanism of cytokinesis and the nature of the mitotic apparatus. Mechanisms appear to be quite variable and among the algae so far investigated several different mechanisms have been described (Pickett-Heaps, 1972a). In the Ulotrichales alone, at least six different mechanisms exist, and include the following: furrow, furrow w/o tubules, furrow phycoplast, phycoplast-cell plate, phragmoplast-cell plate, and cell plate (Stewart, Mattox, and Floyd, 1973). Certain organisms are known even to exhibit a mechanism of cytokinesis which includes both centripetal and centrifugal development of the plasmalemma e.g. *Spirogyra* (Fowke and Pickett-Heaps, 1969), and in *Coleochaete scutata* it was found that cells which divide parallel to the radius of the colony divide by a phragmoplast mechanism, while cells dividing perpendicular to the radius of the colony divide by a modified phragmoplast-cell plate cleavage mechanism (Marchant and Pickett-Heaps, 1973). Many researchers concur that to understand the
phylogeny of the green algae one must consider the mitotic apparatus and the subsequent cytokinetic mechanism. Hence, attempts have been made to compile a phylogenetic scheme based primarily on these characteristics (Pickett-Heaps, 1972, Pickett-Heaps and Marchant, 1972; Stewart, Mattox, and Floyd, 1973; and Stewart and Mattox, 1975). Admittedly, much research needs to be done with regard to algal cytology and their cytokinetic mechanisms. Nonetheless we could easily agree that the results thus far obtained reinforce the viewpoint that strict morphologic evidence is insufficient evidence for phylogenetic speculations among the algae (Pickett-Heaps, 1969; 1972a; Pickett-Heaps and Marchant, 1972; and Stewart, Mattox, and Floyd, 1973).

In summary the purpose of the present investigation is with aid of light microscopic techniques, (1) to examine certain aspects of the cell wall, (2) to establish the existence and to describe the nature and development of the sporangial plates, (3) to describe the events that occur during mitosis, and (4) to propose a comprehensive mechanism for cytokinesis in this organism.

**METHODS**

Axenic cultures of *E. viridis* (isolate E-4--Smith and Bold, 1966) were grown in BEV medium at a pH of 7.1 - 7.3 at 23°C under a 12-12 hour (light-dark) photoperiod. Cultures were maintained in 1000 ml and 50 ml Erlenmeyer flasks containing 175 ml and 20 ml of BEV medium, respectively. The
composition of BEV is outlined in Table 1 (Smith and Bold, 1966).

Sterilization was achieved by autoclaving the flasks at 15 psi for 20 minutes. Cotton plugs were used as closures.

Inocula were aseptically transferred from stock cultures into fresh media every 3-4 weeks so that several cultures could be maintained at different ages. A Pasteur capillary pipette equipped with a 5 ml bulb was used in the transfers and, in general, heavy inocula i.e. 2-3 ml of densely compacted cells in the 50 ml flasks and 6-8 ml in the larger flasks) were introduced into the fresh media to elicit greater mitotic frequency. Air was bubbled, at a rate of 2-5 small bubbles per second, through the 50 ml flask cultures, for 2-3 days prior to harvesting, to promote mitotic activity.

Cells were aseptically harvested from cultures of various ages at various periods during the light-dark cycle. Initially cells were fixed at 1-hour intervals over a 24-hour period. Fixation was performed in centrifuge tubes containing 5 ml of a 1:3:1 (45% glacial acetic acid, 95% ethyl alcohol, chloroform) fixative for a minimum of 15-20 minutes. Cells were removed from the fixative when most of the chlorophyll was removed. The fixative was subsequently removed by pipetting and replaced with fresh fixative to further clear the cells. At this point the cells were either stained or they were stored in 70% ethyl alcohol for later study.
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**TABLE 1:** Composition of BEV Medium (Smith and Bold, 1966)
A slightly modified Wittman's iron-aceto hematoxylin stain procedure was used to stain chromosomes and nuclear material (Wittman, 1962). Cells were removed from the fixative or alcohol with a Pasteur capillary pipette and placed on a clean slide. The excess fixative was removed by blotting it with absorbent towels. Then the cells were gently grouped together in the center of the slide with a dissecting needle. One or two drops of stain was applied and was allowed to react for 3-5 minutes. The excess stain was removed by blotting with absorbent paper towels. This was followed by the addition of 2-5 drops of 45% glacier acetic acid (GAA). The 45% GAA was also blotted and a small drop of Hoyer's medium was applied to make semi-permanent mounts. The cells were then evenly distributed throughout the Hoyer's medium with a dissecting needle and a cover slip applied. These slides kept well and showed good chromosome detail throughout the study.

The technique of Bowen, (1963) was occasionally employed in the search for division figures. This procedure differs from Wittman's technique in that the cells are not fixed prior to staining. More washings with 45% GAA were necessary to remove the excess stain and to clear the remaining chlorophyll. This preparation yielded good results, but circumstantial disadvantages make this procedure undesirable (See appendix).
India ink was used to demonstrate the mucilaginous sheath in living cells. Ruthenium red (1 small crystal dissolved in several drops of distilled H₂O) was used to determine the presence of pectic substances in the cell wall and sheath. A 0.05% aqueous crystal violet stain was used to highlight cell wall boundaries and to accentuate the sporangial plates. A saturated phloroglucinol solution + HCl (20%) was employed to test for lignin in the cell wall.

All slide preparations were examined under the light microscope at magnifications of 100x, 430x, and 970x (oil immersion), and 970x with phase contrast; various filters were employed to highlight different aspects of cell detail. Living cells, empty cells and fixed unstained cells were also examined for comparative purposes.

Photomicrographs were taken with a Bausch and Lomb Dynazoom trinocular microscope equipped with a Kodak 35 mm Colorsnap camera. Kodak panatomic x film (ASA 64) was used for all photomicrography.

RESULTS

The Cell Wall

The cell wall of *E. viridis* has an elastic texture and will remain intact long after the cell has ceased to function (Fig. 5). As a result, different stain techniques were used to determine the composition of cell walls in *E. viridis*. The cell wall appears typical; i.e. it contains cellulose (Smith and Bold, 1966) and various pectic substances. No
conclusive findings were made, however, which would help to explain the extraordinary elasticity and durability of the cell wall. Results from the present investigation will hopefully provide direction towards a more thorough investigation.

Cell walls of mature cells stain lightly with ruthenium red. This indicates that the cell wall has a pectic content. In newly divided cells, the wall separating the young hemispherical daughter cells stained bright red. This may indicate that the newly formed wall is of a slightly different composition than are the outer cell walls i.e. richer in pectins. A peripheral ruthenium red staining ring has also been observed in cells which have not yet undergone a complete cytokinesis. This may represent the beginning of a cleavage furrow.

No stain was observed with phloroglucinol; the cell wall, therefore, lacks lignin. (It is possible that the mucilaginous sheath surrounding the cells prevented the absorption of the stain by the cell wall, but it is more likely that the walls of *E. viridis* lack lignin since no other green alga so far investigated, to this writer’s knowledge, is known to possess a lignified cell wall).

The cell walls stained readily with crystal violet. With this stain one can observe the "sporangial plates" between the mature daughter cells while they still exist within the mother cell wall-sporangium (Fig. 4). These sporangial plates represent what remains of the cell wall material that was deposited along the division plane during cytokinesis.
Mitosis

During the search for mitotic figures newly divided daughter cells were much more frequently observed than were actively dividing cells. Hence, while *E. viridis* is a slow growing organism, the mitosis and cytokinetic processes occur relatively rapidly. This phenomenon was also noted by Robinson, Sachs, and Mayer (1976) and accounts for the difficulties encountered when studying cytokinesis of this organism (See appendix).

Interphase nuclei (Fig. 6) are typically centric, surrounded by a distinct nuclear membrane and contain 2-3 dark staining nucleoli.

At early prophase (Fig. 7) the nuclear membrane and nucleoli disappear. Therefore, we may assume that *E. viridis* has an open spindle i.e. the spindle apparatus is not surrounded by an intact nuclear membrane.

Metaphase (Fig. 8 & 9) is typical. The arms of the chromosomes are condensed, and highly intertwined. At this stage an elliptical region of differentially stained cytoplasm remains in the area surrounding the entire mitotic configuration.

At anaphase (Fig. 10 & 11) the chromotids appear longer and less condensed. They tend to move farther apart during separation, a condition which in itself is typical of a cleavage mechanism (Pickett-Heaps, 1969). Some differentially stained cytoplasm may persist throughout anaphase,
but it becomes less and less distinct until in the late anaphase it appears confined centrically to a small but indistinct region between the two anaphase poles.

At telophase (Fig. 12 & 13), the chromatids begin to shorten and condense. By this time all of the differentially stained cytoplasm disappears and the region between the two telophase nuclei is essentially clear. No evidence of the cleavage furrow occurs until very late into telophase or perhaps the sequent interphase (Fig. 14) by which time a nuclear membrane has enveloped the resulting daughter nuclei and nucleoli have reappeared. The two daughter nuclei may be seen closely appressed along the cytokinetic plane during the later stages of cytokinesis (Fig. 15). Such positioning of the daughter nuclei is typical for phycoplast containing organisms (Pickett-Heaps, 1969).

All stages of mitosis were observed and no phase appeared predominant at any given time. This lack of synchronous division further increased the difficulty of obtaining division figures.

Attempts to stain for spindle fibers with a special crystal violet technique (Sass, 1958) were unsuccessful due to difficulties encountered in transferring cells through the complex destaining and dehydration procedure.

Cytokinesis

As mentioned earlier, cytokinesis is not initiated until mitosis is complete. At this point evidence of a cleavage
furrow can be observed (Fig. 11+). Because the newly formed plasmalemma is quick to develop centrifugal growth of the plasmalemma might occur in conjunction with cleavage furrow formation. Electron microscopic investigation would be necessary to verify whether cytokinesis is accomplished wholly by cleavage, or by an initial centripetal cleavage in conjunction with a centrifugally developing cell plate, but it is reasonable to assume that cytokinesis is accomplished wholly by a centripetal cleavage process. Supportive evidence for this view lies in the fact that cytokinesis has been observed in the absence of mitosis (Fig. 16). Such amitotic divisions were also observed in cells with excentric nuclei (Smith and Bold, 1966) and indicate that the spindle apparatus may not be a requisite for cytokinesis of this organism. In an early study done by Van Wisselingh in 1909, a similar phenomenon was demonstrated. As described by Pickett-Heaps (1969), cells of Spirogyra were centrifuged so that the interphase nuclei were excentric. A centripetal cleavage furrow was thus initiated in the absence of mitosis. It is interesting to note also that both Spirogyra and Eremosphaera have an open spindle apparatus. I might also mention that an open spindle apparatus is not common to an organism exhibiting a phycoplast-centrifugal cell plate mechanism but it has been observed in Klebsormidium which has a cleavage mechanism (Stewart, Mattox & Floyd, 1972).

The idea that E. viridis might be closely related, phylogenetically to Spirogyra is not new. In 1858 de Bary
suggested that *Eremosphaera* was a desmid, and belonged with the Conjugales. Contemporary phycologists do not acknowledge this relationship, but one cannot ignore the mounting cytological evidence to suggest that *Eremosphaera* might, in fact, be phylogenetically more closely related to the Conjugales than most workers would care to believe. More work is needed to further elucidate this relationship.

**DISCUSSIONS and CONCLUSION**

The results of this investigation, together with that of others cited above, strongly suggest that there exists in *E. viridis* a rather advanced cleavage mechanism of cytokinesis, a discussion of which follows.

The first noticeable event in cytokinesis occurs at late telophase or in the subsequent interphase. At this point the transverse microtubules (Robinson, Sachs, and Mayer, 1976) will align themselves parallel to the division plane; a centripetal cleavage furrow develops; the telophase nuclei come together along the cytokinetic plane. Whether these microtubules are remnant from the spindle apparatus or synthesized in a microtubule organizing center (MTOC) (Pickett-Heaps, 1969) located in the "phycoplast" region, still needs verification. As stated, the telophase nuclei come together along the division plane. This is a clear indication of a phycoplast mechanism (Pickett-Heaps, 1972). This observation might lead one to suspect that a centrifugal plate is also being laid down (Robinson, Sachs, and Mayer, 1976). However, a centripetal
cleavage mechanism is not unusual in phycoplast-containing organisms. The function of the phycoplast microtubules can be quite variable (Schroeder, 1968; Johnson and Porter, 1968; Pickett-Heaps, 1969; Mughal and Godward, 1973) and I suggest that the transverse microtubules in *E. viridis* serve to orient the direction of the cleavage furrow as well as to keep the daughter nuclei separated as they come together along the division plane. Such an assumption is reasonable because no evidence was encountered to suggest that the transversely oriented microtubules are used to aid in the deposition of vesicles and thus contribute to the development of a centrifugally formed cell plate. Plasmodesmata have not been observed in this study nor have they been observed in previous studies (Mainx, 1927; Bowen, 1964; Smith and Bold, 1966; Robinson, Sachs, and Mayer, 1975). To date, all studies of algae which exhibit a typical phycoplast-centrifugal cell plate mechanism (in which case the microtubules assumedly aid in the deposition of vesicles which are the newly formed plasmalemma), also show the presence of plasmodesmata (Floyd, Stewart, and Mattox, 1974; Pickett-Heaps, 1972b; Stewart, Mattox, and Floyd, 1973) and to that extent there seems to be at least a superficial, if not a direct causal relationship between the function of the transverse microtubules, centrifugal growth of the plasmalemma, and plasmodesmata formation.

The existence of the transverse microtubules in *E. viridis*, that is, their evolutionary advantage, might be better under-
stood if we consider the large diameter of *E. viridis* cells (150-200 um). While the centrifugal cell plate may have evolved in response to the thicker and more rigid cell walls found in higher plants (Fowke and Pickett-Heaps, 1969), the transverse microtubule system found in *E. viridis* may have evolved in response to the large cell diameter. Hence, the transverse microtubule system orients the direction of the cleavage furrow over the great distance that the furrow must develop.

I contend that the plasmalemma develops centripetally by membrane furrowing. Present investigations suggest that vesicles, or perhaps "open membranes" (Robinson, Sachs, and Mayer, 1976) located in the area surrounding the division plane may also be incorporated into the developing of the plasmalemma. Future investigations will hopefully clarify the events which lead to the development of the new plasmalemma.

Upon completion of the cleavage furrow the newly formed and firmly connected daughter cells exist in a two-celled stage (Fig. 17). This two-celled stage appears similar to the two-celled stage of *Tetracystis* (Brown and Bold, 1964) with the possible exception that in the latter genus transverse microtubules are not found. Therefore, they form in the same way i.e. by centripetal membrane furrowing.

Wall formation, as would be expected, occurs shortly after the initial cytokinesis has been completed. This first
wall is laid down outside the plasmalemma for each new daughter cell, and it eventually gives rise to the sporangial plates (Fig. 4). At the same time, or very shortly afterwards, a second wall is formed interior to the first wall. The second wall, however, develops around the circumference of each of the new daughter cells and therefore, accounts for the cells ability to regain their spheroidal-sub spherical shape while they still remain within their original mother cell wall (Fig. 3). The newly formed daughter cells are referred to as autosporas as they still exist within the mother cell wall (Fig. 18). Hence, the mother cell wall is more properly called a sporangium. At this point the sporangial plates are fully developed and easily distinguished (Fig. 5). A mother cell wall-sporangium, consisting of 2 separate compartments, is then left behind upon the release of the mature daughter cells (Fig. 5).

Thus, the initial division gives rise to the first wall, which persists, and gives rise to the sporangial plates after the second cell wall is formed, and before the daughter cells are released.

By the time that cytokinesis is complete the transverse microtubules have either disintegrated or they have been redistributed throughout the cell and used for other purposes, e.g. to aid in the development of new plasma membrane material during subsequent cell enlargement and the process is complete.

While the proposed vegetative cell division mechanism
the synthesis of two cell walls in an unusual departure from that which is considered "typical", its occurrence in *E. viridis* correlates with certain other cell wall phenomena exhibited by this organism. That *E. viridis* has a propensity towards the development of excessive cell wall material is demonstrated by the facts that certain cells form several concentric cell wall layers and as mentioned earlier, occasional cells with excentric nuclei are known to undergo cytokinesis in the absence of mitosis (Smith and Bold, 1966). In at least one species (*E. minor*) the ability to form walls in concentric layers is quite common (Dr. John M. Speer, personal communication). Whether this ability is a sole result of environmental and/or nutritional influence, or whether it is intrinsic remains uncertain, but nutritional studies suggest that intrinsic factors are at least partially involved (Smith and Bold, 1966).

While the ability to form concentric wall layers is not unique to *Eremosphaera*, and several factors may be involved, I suggest that this ability may be due, in part, to its large cell volume as compared to its cell wall surface area. Smaller, but otherwise closely related chlorococcalean algae do not have this ability (Brown and Bold, 1964; Smith and Bold, 1966), while most species of *Eremosphaera* (Smith and Bold, 1966) and *Planktosphaera maxima* (Bischoff and Bold, 1963), another large chlorococcalean alga (up to 200um), has the ability to form concentric walls.
From the discussion above, we might assume that *E. viridis* represents one of the more advanced members of the Chlorococcales in the cytokinesis aided by the existence of transverse microtubules along the division plane, that it has the ability to form a second cell wall along the division plane interior to the first wall, and occasionally to form concentric wall layers, and that it has an open spindle. It can be said to have a phycoplast-cell plate mechanism, but it is to be distinguished from the "typical" phycoplast-cell plate mechanism which is common in several ulotrichalean algae (Stewart, Mattox, and Floyd, 1973). Instead of centrifugal cell plate development, cell plate formation is by a centripetal furrow. Correspondingly the transverse microtubules do not aid in the deposition of vesicles to be incorporated into centrifugally formed plasmalemma as they might be in the "typical" phycoplast mechanism, and no plasmodesmata are present. Hence, we might more properly refer to this mechanism as a furrow-phycoplast mechanism or perhaps a cleavage mechanism with transverse microtubules (Pickett-Heaps, 1969). Hopefully further research will clarify and support this view, especially with respect to the origin and function of the phycoplast microtubules.

APPENDIX

During the investigation several different techniques were used in the fixing and staining procedures. As one
Although most researchers state that fixing times should be held to a minimum with the algae (e.g., Godward, 1966), I found no significant variation in the appearance of the cells of *E. viridis* when they were left in the fixative for several hours or for periods of up to several weeks. In fact, cells fixed in the 1:3:1 fixative and preserved in an iron mordant solution by R. L. Smith in 1964 were stained with iron aceto-hematoxylin in 1976 with excellent results. It is quite convenient, in fact, to store large amounts of fixed material for later reference and study. When one considers the difficulty encountered in obtaining division figures in this organism it becomes absolutely necessary to store large amounts of fixed material obtained at a time when division figures are known to be present for examination at a later date.

My observations indicate that the only critical stage in the Wittman stain procedure is in staining time. Care must be taken not to leave cells in the stain too long; overstaining obscures all detail. (Conversely, understaining reveals insufficient detail). It is also important to prevent drying of the stain solution around the edges of the preparation. Should drying occur, the hematoxylin crystallizes and the crystals obscure nuclear detail; they can even be mistaken for chromatin, nucleoli, etc. Such a preparation is then utterly useless.

In attempts to obtain division figures in *Eremosphaera* it is best to fix cells grown from small, heavily inoculated and aerated flasks. Cells should be fixed at 1-hour maximum
might anticipate, certain techniques proved more successful than others. A discussion of these techniques follows.

A 4% formalin solution was used in place of the 1:3:1 glacial acetic acid (45%), ethanol (95%), chloroform fixative in an attempt to better preserve certain aspects of the mitotic apparatus. But the formalin caused cells to coagulate, and it failed to remove the chlorophyll from the cells. Cells fixed in the 4% formalin solution also yielded poor slide preparations, hence the technique was abandoned.

Bowen's technique (1963) was used to observe division figures but was later replaced by the Wittman (1962) procedure. The difficulty with Bowen's technique lies in the fact that it requires that the slides be prepared at the same time that they are being fixed and stained. Problems arise especially with an organism like *Eremosphaera*, where cells divide rapidly but infrequently. In as much as the number of cells one can fix at a given time is limited by using Bowen's technique, serious problems can arise if even a slight error is made during the stain procedure. Since no other cells have been fixed and preserved the data for that time period are lost. Such a loss is especially critical in time sequence studies of mitosis and cytokinesis. It is perhaps best for these reasons to use Bowen's technique to screen for division figures at any particular point in the light/dark cycle and at the same time to fix a larger number of cells in the 1:3:1 fixative for more thorough investigation later.
intervals over a 24-hour period. In the present study most division figures were obtained 3-4 hours into the dark cycle and continued to be observed until about 10 hours into the dark cycle. The cells obviously divide throughout the light/dark cycle and do so even under conditions of continuous light (Bowen, 1965), but all researchers (Bowen, 1965; Smith and Bold, 1966; Robinson, Sachs, and Mayer, 1976) report that division figures are less frequent during the light period. My observations support their conclusions.
LITERATURE CITED


Figures 1-6

Fig. 1  *Eremosphaera viridis*, vegetative cell.  X 330.

Fig. 2  Vegetative cell; India ink mount.  X 180.

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Fig. 7  Prophase nucleus; note the lack of a nuclear membrane; hematoxylin. X 1020.

Fig. 8  Metaphase; note the elliptical region of differentially-stained cytoplasm; hematoxylin. X 250.

Fig. 9  Metaphase; note satellite chromosomes; hematoxylin. X 960.

Fig. 10  Early anaphase; hematoxylin. X 1100.

Fig. 11  Anaphase; hematoxylin. X 1100.

Fig. 12  Telophase; hematoxylin. X 1100.
Figures 13-18

Fig. 13 Late telophase; note that the area of differentially-stained cytoplasm has disappeared; hematoxylin. X 360.

Fig. 14 Early cytokinesis; note cleavage furrow; hematoxylin; X 390.

Fig. 15 Cytokinesis; the daughter nuclei are appressed to the division plane. X 280.

Fig. 16 Amitosis; hematoxylin. X 225.

Fig. 17 Young daughter cells. X 255.

Fig. 18 Mature daughter cells. X 185.