PLEOMORPHISM IN SELECTED GENERA
OF ALGAE

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PLEOMORPHISM IN SELECTED GENERA OF ALGAE

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF ARTS

By

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Denton, Texas

June, 1967

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CHAPTER I

INTRODUCTION

The phenomenon of variant morphology within a given algal species has been cited by numerous investigators and is referred to as polymorphism (2) or pleomorphism (20). These terms indicate that an algal species may exhibit more than one vegetative form which may resemble other related or unrelated species (2, 69, 108, 97, 98). That is to say, the shape of an alga is not always purely genetic in nature and may be varied by changing environmental conditions.

Purpose

In working with various algae in the classroom and laboratory, it was frequently observed that cells in unialgal cultures bore little or no resemblance to the organism believed to be in culture. It was the purpose of this study (1) to ascertain what environmental factors might cause morphological changes exhibited by certain algae, (2) to determine if this variation in morphology is vegetative, reproductive, or artifactual in nature, and (3) to show what effects these changes in morphology might have on classification of the organisms in question, and (4) to demonstrate the unnecessary complexity and ambiguity of currently used classification schemes, especially in respect to intergradation of vegetative structures found in many genera.

History

Numerous early investigators (2, 3, 4, 6, 19, 20, 21, 22, 23, 24, 25, 38, 41, 42, 45, 46, 47, 48, 49, 51, 55, 60, 64, 66, 68, 85, 89, 104, 105, 108, 110, 111, 112, 113) recognized the possibility of variable morphology occurring in nature, and during the late nineteenth century the concept of polymorphism gained wide acceptance among phycologists. Many of these early investigators (9, 10, 11, 22, 23, 50, 79, 114) expressed the belief that polymorphism was widespread, especially in the green algae in which many of the more "primitive" types represented stages in the life cycle of more "advanced" forms and in many of the genera of the Protococcales which represented variations of a single polymorphic genus (108). However, during the early twentieth century the concept of polymorphism began to fall into disfavor and disuse. West (108) attributed this to the advent of modern culturing techniques. He asserted that most reports of polymorphism were "records of superficial and inaccurate observations, accompanied by expressions of opinion based upon defective evidence." "The false hypothesis of wide (spread) polymorphism" was, according to West (108), given its death blow by the pure culture methods developed by Klebs and his pupils, Beijerinch (4, 5) and Grintzesco (47, 48), and later by Chodat (17, 18, 19). Fritsch (42, 43, 44) was in agreement with West when he wrote:

> "It may happen that in pure cultures a given alga may assume a form that is indistinguishable from that of another species or even genus occurring in nature. It is...very doubtful whether this polymorphism exists in nature and it still remains to be shown that two apparently identical forms found in different habitats are actually identical stages of quite different algae...."

Chodat (18) believed that although widespread polymorphism was not borne out by data obtained using pure culture techniques, "a plant might present itself under many aspects without change of nature; but their polymorphism is of the same order as that which is exhibited by most plants." West (108) and West and Fritsch (109), although very much opposed to the concept of widespread polymorphism, did concede the occurrence of *Protoderma*-state in *Protococcus* (*Pleurococcus*), *Dactylococcus*-state of *Scenedesmus obliquus*, *Palmella*-state of *Chlamydomonas* and certain species of *Ulothrix* and *Stichococcus*, *Hormidium*- and *Schizogonium*-states of *Prasiola*, the *Gongrosira*-state of *Cladophora*, and *Tetraedron*-lke stages in the life cycles of *Pediastrum*, *Hydrodictyon*, and *Oocystis*.

The controversy over polymorphism which arose in the late Nineteenth and early twentieth centuries was largely provoked by observations on the green alga, *Scenedesmus*, which produced atypical cells in concentrated media and aplanospores in old cultures (19, 47, 48, 113). Grintzesco (47, 48) and Wille (113), in studying *Scenedesmus* cultures, found oval, thick walled aplanospores packed with colored oil which would develop into typical *Scenedesmus* cells only after several subcultures. Fritsch (42) reported the development of a persisting *Chlorella*-stage from *Scenedesmus* aplanospores.

The ability of *Scenedesmus* to fragment, forming unicells resembling *Oocystis*, *Chlorella*, *Raphidium*, *Ankistrodesmus*, *etc.*, or to form *Dactylococcus*-like chains, has been reported by numerous investigators. Unicells have been reported in cultures of *Scenedesmus acutus* grown in media containing an organic carbon source (4). Unicells were also produced in *Scenedesmus dimorphus* when grown with a soil bacterium (94) or in medium containing two per cent yeast extract (95), and in *Scenedesmus quadricauda* in which light yellow-green oval cells packed with starch and oil were observed in two-year-old cultures (29). Cells identical to Chodatella subsalsa have been observed in cultures of Scenedesmus longus grown in liquid inorganic medium, and only when grown on firm agar or subjected to thermal shock were coenobia observed (101).

In 1858, Grunow expressed the opinion that Dactylococcus infusionum might be a stage in the development of Scenedesmus. Since then, Dactylococcus-like stages have been found in Scenedesmus species (89, 20, 22, 23, 47, 48, 103), Scenedesmus dimorphus (95, 96) and Scenedesmus obliquus (97).

Trainor (98, 99), in his studies with Scenedesmus, found some species to be quite stable (Scenedesmus abundans, Scenedesmus bijugatus, and Scenedesmus quadricauda) while others exhibited pleomorphic tendencies in nature as well as in the laboratory. Using Scenedesmus longus as an example, he suggested that in sampling natural bodies of water, uni-cells might be in abundance in the plankton while coenobia would be found in the benthos. In the case of Scenedesmus dimorphus, he suggested that the introduction of organic waste in a body of water with an increase in numbers of bacteria might cause suppression of coenobial formation and thereby effect a change in the morphology and classification of that organism (100).

Although a great number of the studies concerned with algal pleomorphism have centered around various species of *Scenedesmus*, pleomorphic tendencies have been observed and recorded in numerous other algal genera, including *Ankistrodesmus*, *Tetraedron*, *Pediastrum*, *Stigeoclonium*, *Gonium*, *Pandorina*, *Eudorina*, *Haematococcus*, *Cosmarium*, *Micrasterias*, *Synura*, *Gomphonema*, *Gloeocapsa*, *Microcystis*, *Oscillatoria*, *Lyngbya*, *Anabaena*, and *Calothrix*.

Aberrant cells in *Ankistrodesmus braunii* cultures were reported by Vischer (104), who attributed their formation to inhibition of cytokinesis by hexoses in the culture medium. "Simple, non-hereditary modifications" were believed by Otelli (74) to be the contributing factors for morphological fluctuations observed in five species of *Ankistrodesmus*, in which spherical cells of one of the five species divided, giving rise to elongated daughter cells. McMillan (69) observed continual morphological changes in *Ankistrodesmus spp.* cultured in complex organic media and to a lesser extent in mineral media. In *Ankistrodesmus braunii*, McMillan (69) reported suppression of ability of cells to elongate when cultured in media containing two per cent glucose, resulting in spherical forms. He also observed that morphogenesis was disrupted by addition of four per cent glucose, resulting in median bulge in the cells. Abnormalities in spore germination in some organic media resulted in *Protococcus*-like formations or triangular or multispinous cells. Club-shaped cells were observed in media containing 5 μg/ml bipyridine. Small, swollen, bleached cells were found in media containing 10 μg/ml adenine or guanine, Enlarged cells were in media containing 10 to 20 μg/ml thiamine hydrochloride or 1 to 2.5 μg/ml xanthine.

Cultures of *Tetraedron minimum* (94) do not grow well in organic medium or in presence of peptone or sugar (Individual cells loose their angular shape and are often bleached). In dilute Detmerscher's Solution the ability to form coenobia is lost (94).

Disoriented colonies of *Pediastrum* were observed by Harper (51), who attributed their abnormal arrangement to reduced zoospore activity after he had observed the formation of disoriented colonies from zoospores which were less motile than those forming oriented colonies (51). Troitskaja (102) expressed the belief that *Pediastrum integrum* Naeg. was a developmental stage of *Pediastrum Boryani* (Turp.) Mengh., that *Pediastrum integrum* var. *Braunianum* Nodst. and var.

tirolense Hangs. were adult forms of *Pediastrum Boryani*, and that *Pediastrum integrum* var. *perforatum* Racib. was a developmental stage between *Pediastrum Boryani typicum* and *Pediastrum integum*. Unfortunately, no résumé of experimental data to support these ideas was available. Bigeard (8) noted the occurrence of spherical colonies of *Pediastrum* in aging cultures and related this phenomenon to changes in composition of media as well as aging. Davis (27), working with *Pediastrum*, correlated the atypical spherical arrangement of daughter cells of *Pediastrum* to suboptimal concentrations of sodium or calcium bicarbonate. He found that concentrations of sodium or calcium bicarbonate in excess of optimal concentrations increased cell division in mother cells resulting in greater number of cells in daughter colonies. In a more recent study, Davis (28) noted the occurrence of disoriented colonies of *Pediastrum* when the organism was grown in a culture medium composed of Chu's No. 11 medium and ten per cent river water or in culture media containing low concentrations of bicarbonate ion. He correlated this development of abnormal spherical cells to decreased motility of zoospores.

In studying a green alga found as a palmella stage growing on moist bark, Livingston (68) observed that this alga exhibited a filamentous habit keyable as Stigeoclonium tenue when grown in weak ionic solutions, and assumed the palmella-stage only when grown in strong ionic solutions (i.e., sea water). Hulbary (59), after collecting an irregularly branched filamentous alga keyable as Stigeoclonium subsecundum, found that when it was cultured, it developed Ulothrix-like unbranched filaments with acutely tapered terminal cells and a Ulothrix-like holdfast. Although the author made no attempt to explain this abrupt change in habit, he did note the occurrence of the branching phase alone in samples collected in the late fall, winter and early spring, and both phases together in late spring, summer, and fall.

Abbas and Godward (1), in studying the effects of light and temperature on Stigeoclonium amoenum Kutz. and Draparnaldia plumosa (Vauch.) Ag., found that (1) lack of nitrates promoted hair formation and excess nitrates suppressed hair formation; (2) lengthening of light period or increased temperature increased the amount of branching; (3) orientation of branching was dependent on direction of light; (4) branching was suppressed by blue light; and (5) red light promoted zygote and zoospore formation. The lack of hair formation in media containing excess nitrate is of special concern in respect to taxonomy and classification of these two genera, because both are described as having hair formation (91).

Gonium pectorale has been reported to form unicells and other abnormal cells in 0.01 per cent Knopf's solution and "Knopagar," and also giant cells in 0.2 per cent Knopf's solution, which developed into spherical "Eudorina-like" cells when transferred to Benecke's solution (53).

Nichols (73), when working with both Gonium and Pandorina, found that colonies grown in a medium containing strontium without calcium developed cells which were non-motile and abnormally enlarged and exhibited reduction in amount of sheath material.

The spherical-colonial volvocacean alga, Eudorina elegans, has been reported to form plate-like colonies when grown in Molish's solution containing ammonium chloride, which reverted to the normal spherical shape when transferred to Benecke's solution (53).

Numerous investigators (115, 86, 19, 80, 32, 33) have observed and recorded the occurrence of green Haematococcus cells and have related the lack of red pigment to nutritional deficiency. Pringsheim (84) has shown that Haematococcus pluvialis appears red in nature only in nutritionally deficient media. When nutrients occur in optimal quantities, the organism undergoes rapid division and assumes a *Chlamydomonas*-like character lacking red pigment and inflation of cell wall. Pringsheim notes that populations of *Sphaerellopsis hyalina* (81, 82), *Chlamydoblepharis* (82), members of Phacotaceae and *Lobomonas sphaeria* (83), as well as *Haematococcus*, do not show features characteristic of the taxa under optimal growing conditions.

Variation in degree of radial symmetry has been reported (110, 111, 65, 67, 88, 92, 116) in several species of *Cosmarium*. The triradiate forms of *Cosmarium botrytis* have been studied by Waris (106, 107), who attributed their occurrence to the "formation of an atypical cytoplasmic framework" which developed in conjunction with "the first vegetative division of a juvenile cell." Brandham and Godward (12) found that the triradiate cells were sometimes formed as the product of germinating zygospores, but the degree of symmetry did not seem to be affected by the old semicell. Brandham and Godward (12) found that although the triradiate form was sexually fertile, the character was not transmitted sexually but was most likely attributable to temperature effects.

Kallio (62), in working with desmids of the genus *Micrasterias*, has observed the development of aradiate cells from uniradiate haploid cells (*M. thomasiana* var. *notata*, *M. torreyi*, *M. rotata* var. *evoluta*, *M. thomasiana*, and *M. americana*) and diploid triradiate and quadriradiate cells from normal diploid biradiate cells (*M. thomasiana*, *M. rotata*, *M. torreyi*, and *M. angulosa*).

In doing this, he has noted a direct proportional increase in degree of organization of side lobes with increase in nuclear quantity, hence illustrating "nuclear effect per cytoplasmic unit" and its possible role in algal pleomorphism.

In studying a natural population of *Staurastrum paradoxum*, Reynolds (87) observed a predominance of the quadriradiate form during April and May when the water was cool and a predominance of the triradiate form in June and July. Similar results were reported by Brandham and Godward (13) in a study of the effects of temperature on the radial symmetry of *Staurastrum polymorphum*. They concluded that although changes in symmetry were effected by temperature, these changes were not independent of nuclear effect.

Although there are reports in the literature noting the occurrence of deformities (56, 26) among the members of the

Chrysophyta, little has been done to relate these deformities to the phenomenon of pleomorphism. However, one Chrysophyte, *Synura uvella*, has been shown to fragment and lose its motility in response to a pH of less than 3.8 or greater than 8.0 (72), and the shape of diatoms such as *Desmogonium* has been shown to be affected by the amount of water flow to which the organism is subjected (75). Earlier reports involved *Melosira*, a filamentous form which exhibited two distinct frustule patterns in a single colony and on occasion a combination of these two in a single cell (70), and *Rhizosolenia hebetata*, which exhibited a seasonal dimorphism with a winter form, forma *hiemalis*, and a summer form, forma *semispina*. Kofoid, according to West (108), expressed the belief that the winter and summer forms of *Rhizosolenia hebetata* were, "two distinct species which under the influence of certain environmental factors mutate the one to the other."

In the Cyanophyta, reports of pleomorphic tendencies are numerous, especially among genera in which taxonomic differentiation is based on cell measurements, sheath formation or occurrence of false branching (7, 57, 58, 46, 34, 35, 36, 41, 37, 77). Geitler (46) reported the occurrence of false branching in one species of *Lyngbya*. Canabaeus (16) induced variation in size and shape of heterocysts in nine species of *Anabaena* by varying the amount of light, by the presence and concentrations of certain salts, or by inducing an oxygen deficiency. By varying the light intensity, Jaag (61) found that color, trichome characters, sheath formation, branching, and heterocyst formation could be altered in *Scytonema polymorphum.* Variation in concentration of certain salts caused alteration in filament or thallus structure in species of *Anabaena*, *Cylindrosperma*, *Nostoc*, *Oscillatoria*, *Phormidium*, *Scytonema*, and *Chroococcus* (31). Naumann (71), in studying *Microcystis aeruginosa* Kutz, found that when grown in still water, large clathrate colonies lacking matrix were formed; whereas in agitated water, small, non-clathrated colonies with matrix and closely attached cells were formed. Kingsbury (63) reported loss of color in *Plectonema* to result from exhaustion of nitrogen and return of color with addition of nitrogen. Drouet and Daily (37), in studying *Gloeocapsa montana*, came to believe that this blue-green alga was actually a member of the Chlorophycean genus *Palmogloea protuberans* (Sm. and Sowerb.) Kutzing. Drouet (35, 36) found sufficient variation in (1) presence and nature of sheath, (2) calcification, (3) terminal cell morphology, and (4) granulation of cell content to merit reduction of fifteen taxa in five genera to ecophenes (ecological variants) of Microcoleus vaginatus (35) and 54 taxa in 9 genera to ecophenes of Schizothrix calcicola (36). Foerster (40), in working with Oscillatoria limosa (Roth) Ag., found that by increasing the concentration of calcium or magnesium, sheath formation was induced, necridia formation was increased, and basal attachment of filaments was initiated. Morphological variations in Tolypothrix have been reported by Stein (93), who found that variation in nutrients affected form, sheath, heterocyst production, false branching, and might account for discrepancies present in differentiation of Tolypothrix and Scytonema.

Pearson and Kingsbury (76) have reported the appearance-dissappearance and wide variation in taxonomic characteristics in several blue-green algae. Depending on culturing conditions, they observed variations in color, hair formation, cell diameter, heterocyst, akinete and pseudovacuole formation and shape, shape of terminal cells, constriction at crosswalls, trichome arrangement, and sheath characteristics within given species of Anabaena, Calothrix, and Lyngbya.

Several factors led to the choice of "Pleomorphism in Selected Genera of Algae" as the subject of this thesis problem.

In the study of phycology, numerous discrepancies in the differentiation of various algal genera were noted: a unialgal culture of Ankistrodesmus species isolated by James Mann developed rounded cells lacking many of the characters usually associated with this taxa, and unialgal cultures of an unknown filamentous blue-green alga supplied by Dr. G. P. Fitzgerald contained trichomes which exhibited the taxonomic characters of two or three families at one end of the trichome and one or two other families at the opposite end of the trichome. Several studies have been done by North Texas State University students illustrating the effects of various chemicals on algal morphology. A study done by Foerster (40) illustrated sheath formation in a sheathless species of Oscillatoria. Brannon (14) found that pH affects presence and types of inclusions in Cosmarium sp. Hartley (52) observed the effects of NH_4NO_3 and potash on mixed cultures of Oedogonium and Ulothrix. He reported that NH_4NO_3 enhanced the growth of Ulothrix and suppressed the growth of Oedogonium with the reverse occurring in medium containing potash. Both compounds had marked effects on zygote formation. Hendricks (54) found that colchicine increased numbers of Tetraedron bitridens cells with no increase in average cell size although some "giant cells" were observed. Bruton (15) studied color change in <u>Phormidium</u> <u>sp</u>. and attributed the change from blue-green to colorless to a magnesium deficiency.

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CHAPTER II

MATERIALS AND METHODS

For this research, pure cultures of *Ankistrodesmus falcatus* (Chorda) Ralfs #188 and *Tetraedron bitridens* (Beck) #120 were obtained from the culture collection of Indiana University, Bloomington (20), and one unialgal culture of *Ankistrodesmus* sp. was obtained from the culture collection of North Texas State University, Denton. These cultures and their subcultures were maintained on agar slants of soil-water extract (12, 14, 11, 19) in a culture unit at 25° C. with sixteen hours of light per twenty-four hour period.

Complete and deficient Bold's Basal Media were prepared following the procedure as outlined by Brown and Bold (2). Six macroelement stock solutions were prepared by dissolving the indicated weight of the following salts in 400 ml. of distilled de-ionized water:

$NaNO_3$	10.0 gm.
KH_2PO_4	7.0 gm.
K_2HPO_4	3.0 gm.
$MgSO_4 \cdot 7H_2O$	3.0 gm.

$CaCl_2 \cdot 2H_2O$	1.0 gm.
NaCl	1.0 gm.

Trace elements (15, 16, 6) were supplied by four solutions as follows:

EDTA stock solution

50 gm. EDTA (Ethylenediaminetetraacetic acid) and 31 gm. KOH were diluted to one liter with distilled de-ionized water.

H-Fe stock solution

4.98 gm. $FeSO_4 \cdot 7H_2O$ were diluted to one liter with acidified water made by adding one ml. concentrated H_2SO_4 to 999 ml. de-ionized distilled water.

H-Boron stock solution

11.42 gm. H_3BO_3 were diluted to 1 liter with de-ionized distilled water.

H-H_5 stock solution

The following salts were diluted to 1 liter with acidified water:

$ZnSO_4 \cdot 7H_2O$	8.82 gm.
$MnCl_2 \cdot 4H_2O$	1.44 gm.
MoO_3	0.71 gm.

$CuSO_4 \cdot 5H_2O$	1.57 gm.
$Co(NO_3)_2 \cdot 6H_2O$	0.49 gm.

Complete medium was prepared by adding 10 ml. of each macroelement and 1 ml. of each trace element solution to 936 ml. of de-ionized distilled water.

A series of deficient media was prepared by the omission of one of the macroelement solutions or of all four of the trace element solutions. The respective deficient media were comprised as follows:

(1) Medium deficient in $NaNO_3$.

(2) Medium deficient in $CaCl_2 \cdot 2H_2O$.

(3) Medium deficient in $MgSO_4 \cdot 7H_2O$.

(4) Medium deficient in K_2HPO_4.

(5) Medium deficient in KH_2PO_4.

(6) Medium deficient in NaCl.

(7) Medium deficient in all four trace element solutions.

Each of the media was kept in a 1500 ml. double-armed Erlenmeyer flask with syringe set-up attached to the lower side arm. The flasks of media were stoppered with cotton and autoclaved at 120° C., 15 pounds pressure, for 15 to 20 minutes. Immediately after autoclaving, the cotton stoppers were replaced by previously autoclaved #8 rubber stoppers.

In preparation for making and dispensing media, all glassware was soaked overnight in Haemosol solution and then rinsed three times in distilled de-ionized water (3, 1). After drying, test tubes were plugged with cotton stoppers, wrapped with brown paper and autoclaved. For each run per organism, four tubes of the respective media were prepared by dispensing 10 ml. of media into each test tube, followed by stoppering and autoclaving. After cooling, each tube was capped with parafilm until inoculated.

While preparing this medium, it was found that the molybdic anhydride (MoO_3) was quite insoluble (21), although the quantity used was within its solubility as listed in the Handbook of Chemistry (7). Neither complete mixing of one compound before the addition of the next (5) nor acidification of the $H\text{-}H_5$ solution (2) induced solvation of this compound. It may have been possible to increase the solubility of the molybdic anhydride, by the addition of 5 ml. of ammonium hydroxide per gm. of molybdic anhydride per liter of solution (Fisher Company, 1966).

The first run of Tetraedron bitridens (Beck) was made during the spring of 1966. Three tubes of each medium were inoculated with Tetraedron bitridens and one tube served as a control to indicate the efficiency of autoclaving. These cultures were kept in an automatic light controlled growth chamber (18) of variable temperature ranging from 16° C. to 34° C. Cultures were examined microscopically every seven days over a period of ten weeks with a final check being made during the twenty-second week. Several drops of culture were removed from each tube containing T. bitridens by means of sterile micropipettes and aseptic techniques (8, 13, 17). Morphological characters were observed and drawn. Using a Beckman model 72 pH meter, the pH was determined at the beginning of the run for each of the eight sterile media and at the termination of the run for one tube of each media containing T. bitridens (Beck).

The second run for T. bitridens was begun on June 8th, the first and second runs for Ankistrodesmus falcatus were begun on June 7th, and the run for Ankistrodesmus sp. was begun on July 14th. Four test tubes of each of the eight media were inoculated for each run with the respective organism. The culture tubes were maintained in a Sherer Model CEL 44 Plant Growth Chamber in which the temperature was maintained at 25° C. (15, 10) and in which sixteen hours of light per twenty-four hour period were supplied by 80-watt, cool, white panel fluorescent lamps emitting 350 foot candles. Cultures were examined microscopically every four to seven days over a period of eight to twelve weeks. As in the first run for _T_. _bitridens_, one or two drops of culture were removed from each tube using sterile micropipettes and aseptic techniques. Morphological and cellular characteristics were observed, and cells were drawn and photographed. Color slides were made using a 25 mm. photomicrographic camera model 635 mounted on an A and O Trinocular Microstar Microscope. The pH was determined for each of the eight media for the respective organisms at the end of each week's culture examination.

During the course of this study, attempts to obtain cultures from single cell isolates were unsuccessful, as was indicated by Bold (1) and McMillan (9).

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CHAPTER III

RESULTS

In Table I, the genera and species of algae used in this study are listed and described as they appeared in complete and deficient Bold's Basal Medium (BBM).

In complete BBM, cells of *Tetraedron bitridens* appeared triangular, quadrangular or rounded with three to five angular processes extending from the central portion of the cell. In younger, smaller cells the angular processes tapered to a sharp point forming spines composed of cell wall material. As the cells matured, they became larger and more rounded and the spines became less pronounced. Each vegetative cell of *T. bitridens* possessed a grass-green, relatively thin, parietal plastid and a single pyrenoid. In BBM lacking either calcium chloride, potassium monohydrogen phosphate, potassium dihydrogen phosphate, or sodium chloride, cells appeared as in complete BBM. In medium deficient in ammonium nitrate, cell walls were thickened and plastids were grey with an asteroid or net-like appearance. When magnesium sulfate was omitted, cells became larger and more rounded as the cultures

TABLE I

MORPHOLOGICAL DEVIATIONS IN CELL MORPHOLOGY OF *TETRAEDRON BITRIDENS*, *ANKISTRODESMUS FALCATUS*, AND *ANKISTRODESMUS SP*. WHEN GROWN IN DEFICIENT BOLD'S BASAL MEDIUM

Nutrient omitted from BBM	*Tetraedron bitridens*	*Ankistrodesmus falcatus*	*Ankistrodesmus sp.*
Complete	Cells angular to rounded single pyrenoid parietal plastid autospores and zoospores	cells long, slender, curved no pyrenoid parietal plastid autospores	cells fusiform single pyrenoid parietal plastid autospores
NH_4NO_3	cell wall thickened plastid gray, asteroid no pyrenoid no division	very few cells plastid gray to colorless no division	plastid gray cells filled with oil deposits no division
$CaCl_2$	as in complete except no zoospores	as in complete	as in complete
$MgSO_4$	cells very large, rounded spines reduced or absent no pyrenoids no division	plastid gray to colorless no division	cells diamond shaped cell walls with blister-like enlargements no pyrenoid, no division
K_2HPO_4	as in complete	no division	as in complete
KH_2PO_4	as in complete	bristles, apical secretions	as in complete
NaCl	as in complete	plastid gray, no division	as in complete
Micro-elements	cell wall thickened plastid partially fills cell no pyrenoid no division	plastid gray cells reduced in size apices long and atenuated no division	width of cells increased plastid partially fills cell no pyrenoid autospores formed in pairs

aged. By the sixth to seventh week, cells in magnesium deficient BBM were large and spherical with spines absent or greatly reduced. When microelements were omitted, cell walls became thickened and plastids greatly reduced in size. Autospores and pyrenoids were observed in all media except those deficient in nitrates, magnesium, or microelements. Zoospores were observed only in complete BBM or BBM lacking potassium dihydrogen phosphate, potassium monohydrogen phosphate, or sodium chloride.

In complete BBM, cells of *Ankistrodesmus falcatus* appeared long, slender, slightly curved with acute apices and very thin cell walls. Each vegetative cell exhibited a single, pale green, somewhat diffuse, parietal plastid without pyrenoids. Autospores were formed by longitudinal division. In BBM deficient in ammonium nitrate, magnesium sulfate, potassium monohydrogen phosphate, or sodium chloride, cultures were negative or as in complete BBM except that autospore formation was not apparent and plastids were colorless to grey. In potassium dihydrogen phosphate deficient BBM, autospore formation was extensive and was accompanied by very extensive bristle formation and apical secretions. In calcium chloride deficient BBM, cells appeared as in complete BBM and autospore formation was evident but was not accompanied by either bristle formation or apical secretions. Cells appeared as in complete BBM, except that plastids were grey in color.

In complete BBM, cells of Ankistrodesmus sp. were straight, fusiform, and several times longer than wide. Apices were atenuated and cell walls thin but firm. Each vegetative cell contained a single, elongated, grass-green, parietal plastid with a single pyrenoid. Autospore formation occurred by transverse, longitudinal and oblique division of the parent cell. No bristles or apical secretions were observed. In ammonium nitrate deficient BBM, cells, although shaped as in complete BBM, were grey and filled with oil droplets. Pyrenoids were absent and autospore formation was lacking. At the end of the first week of culture in magnesium sulfate deficient BBM, cells lacked pyrenoids and had developed apical or median bulges. By the end of the second week, cells were diamond shaped and pyrenoids were generally absent. At the end of the fifth week, cells had developed conspicuous median enlargements in their cell walls. Pyrenoids were absent and autospore formation was lacking. In BBM lacking microelements, growth was very sparse. Cells were much wider and more rounded than in complete BBM. Vegetative cells contained a single plastid which occupied the median portion of the cell. Pyrenoids were absent and two autospores were formed in each parent cell. By the end of the fifth week, cultures grown in BBM deficient in potassium monohydrogen phosphate, potassium dihydrogen phosphate, or sodium chloride, contained numerous cells filled with oil droplets. At the end of the sixth week, 50 per cent or more of the cells observed in sodium chloride deficient BBM were filled with oil droplets. Wide variation in cell shape was observed in calcium chloride, potassium monohydrogen phosphate, and potassium dihydrogen phosphate, and sodium chloride deficient BBM.

In Figure I, cells of _T_. _bitridens_ are drawn as they were observed in complete and deficient BBM during both the first and second runs. Spherical cells, although most frequently observed in magnesium sulfate deficient BBM (Figure 1, e) were also seen in complete BBM (Figure 1, a) and in BBM deficient in either calcium chloride, potassium dihydrogen phosphate, or sodium chloride. In magnesium sulfate deficient BBM spines were reduced or absent and pyrenoids were absent. Small to medium angular cells were consistently observed in complete BBM and BBM deficient in calcium chloride, potassium monohydrogen phosphate (Figure 1, g), potassium dihydrogen

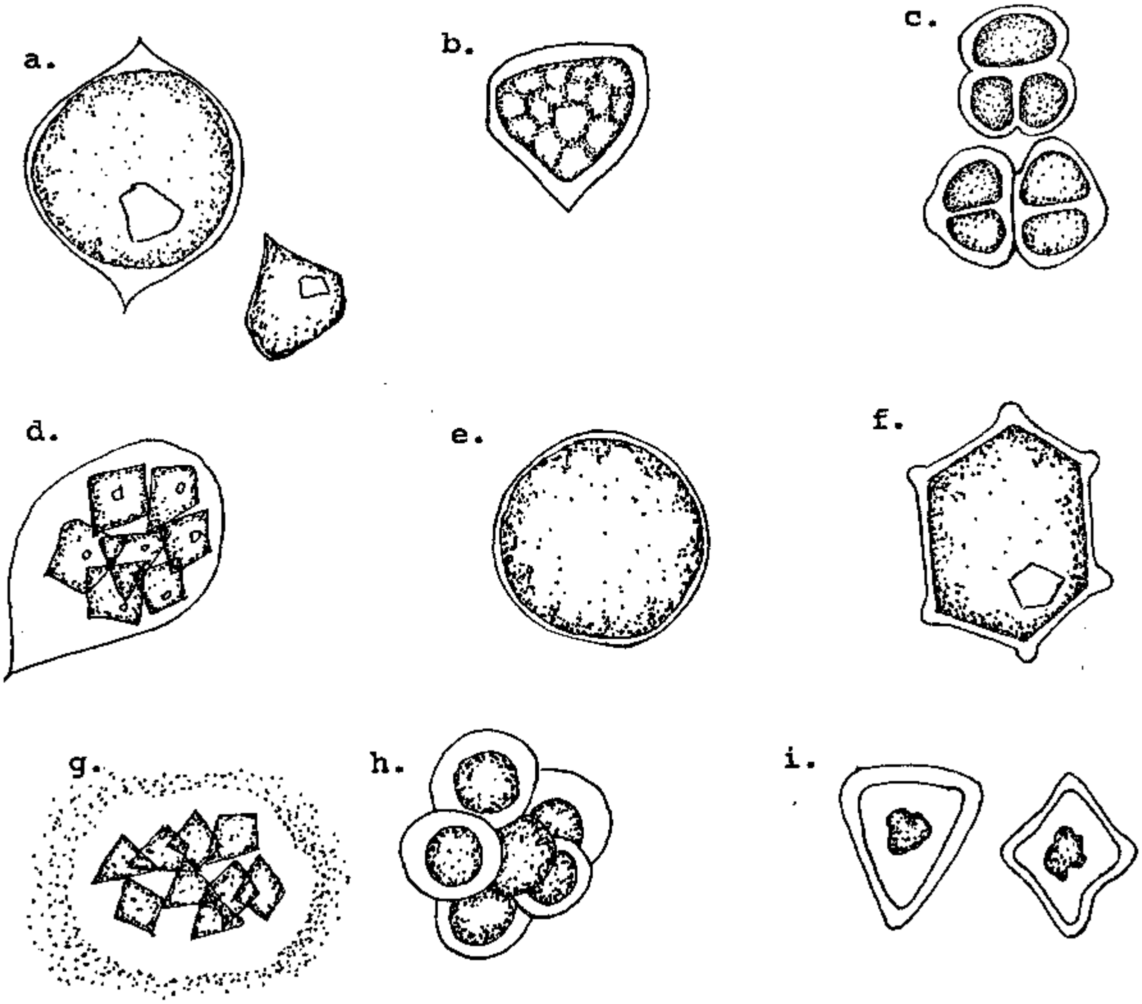

Fig. 1 - Cells of Tetraedron bitridens cultured in (a) complete BBM, (b) NH_4NO_3 deficient BBM, (c) and (d) $CaCl_2$ deficient BBM, (e) $MgSO_4$ deficient BBM, (f) and (g) K_2HPO_4 deficient BBM, (h) NaCl deficient BBM, and (i) microelement deficient BBM.

phosphate, or sodium chloride. A single hexagonal cell with well developed spines and pyrenoid was observed in potassium monohydrogen phosphate deficient BBM (Figure 1, f). *Protococcus*-like cells were found in calcium chloride deficient BBM (Figure 1, c). In ammonium nitrate deficient BBM (Figure 1, b), cells were flattened and triangular. Cell walls were thickened and plastids appeared asteriod or net-like. In BBM lacking microelements, cells were triangular or quadrangular in shape with thickened cell walls (Figure 1, i). Plastids were reduced in size, and pyrenoids were absent. Autospores were either rounded (Figure 1, h) or angular (Figure 1, d).

Cells of *A. falcatus* appear in Figure 2 as they were observed in both the first (Figure 2, a through 2, c) and second (Figure 2, d through 2, i) runs. In complete medium cells exhibited characteristics of the taxa except for an occasional cell which appeared to have developed a median clear area. Autospores were formed by longitudinal division (Figure 2, b; 2, c; and 2, g), except in potassium dihydrogen phosphate deficient BBM (Figure 2, e) where in one instance they were formed otherwise. Apical secretions were observed in potassium dihydrogen phosphate deficient BBM (Figure 2, f).

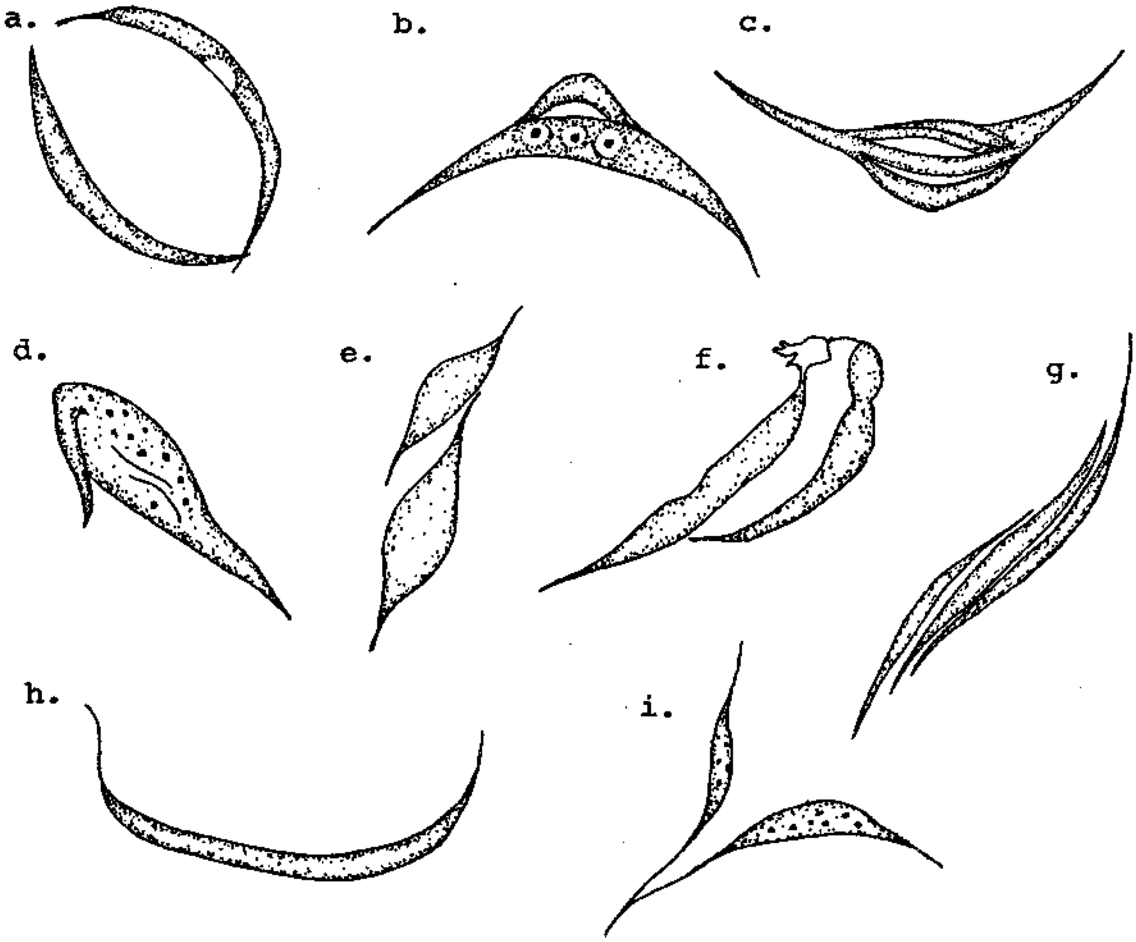

Fig. 2 - Cells of *Ankistrodesmus falcatus* cultured in (a) complete BBM, (b) and (c) $CaCl_2$ deficient BBM, (d) NH_4NO_3 deficient BBM, (e) through (g) KH_2PO_4 deficient BBM, (h) NaCl deficient BBM, (i) Microelement deficient BBM.

In ammonium nitrate (Figure 2, d) and microelement (Figure 2, i) deficient BBM cells were misshapen and plastids were grey.

In Figure 3, examples of aberrant cells of *Ankistrodesmus sp*. are drawn as they appeared in complete BBM (Figure 3, a; 3, b) and BBM deficient in calcium chloride (Figure 3, c through 3, g), potassium dihydrogen phosphate (Figure 3, h through 3, j), or potassium monohydrogen phosphate (Figure 3, k through 3, o). The wide variation in shape exhibited by these cells appeared to be related to the direction of division of the parent cells into autospores. Division occurred transversely (Figure 3, a; 3, e; 3, g; 3, n), transverse-longitudinally (Figure 3, f; 3, h; 3, k), obliquely (Figure 3, b; 3, m), and in one case longitudinally (Figure 1, c). A *Scenedesmus*-like arrangement of cells was observed in potassium dihydrogen phosphate deficient (Figure 3, j). Aplanospore-like structures were occasionally observed (Figure 3, i).

Figures 4 through 13 are Kodacolor prints of *T. bitridens*, *A. falcatus*, and *Ankistrodesmus sp*. as they appeared in various phases of culturing. In Figure 4, cells of *T. bitridens* are seen filled with and surrounded by grey oil droplets. A cell of *T. bitridens* with blister-like enlargement of cell wall

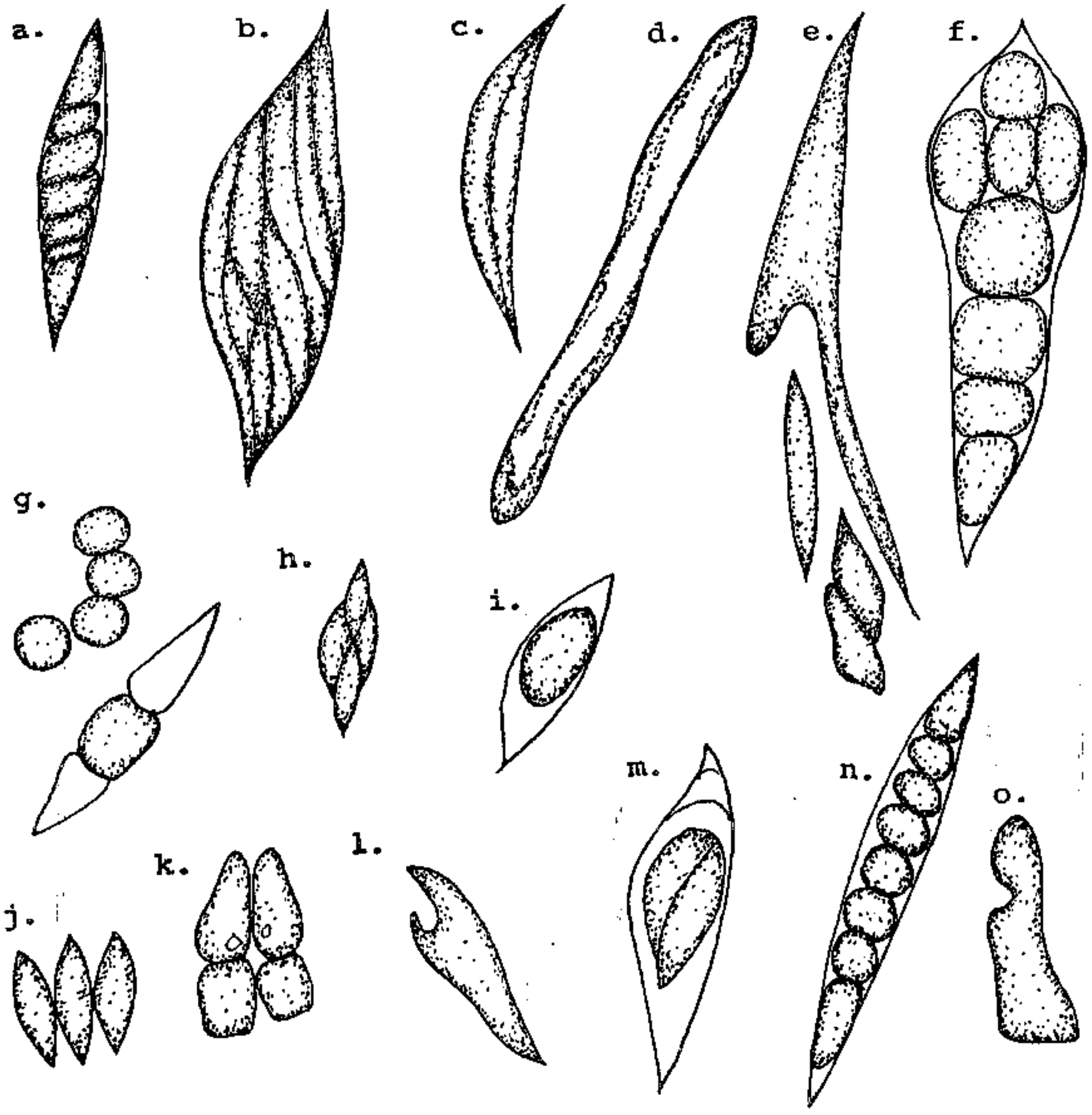

Fig. 3 - Cells of <u>Ankistrodesmus</u> <u>sp</u>. cultured in (a) and (b) complete BBM, (c) through (g) $CaCl_2$ deficient BBM, (h) through (j) KH_2PO_4 deficient BBM, (k) through (o) K_2HPO_4 deficient BBM.

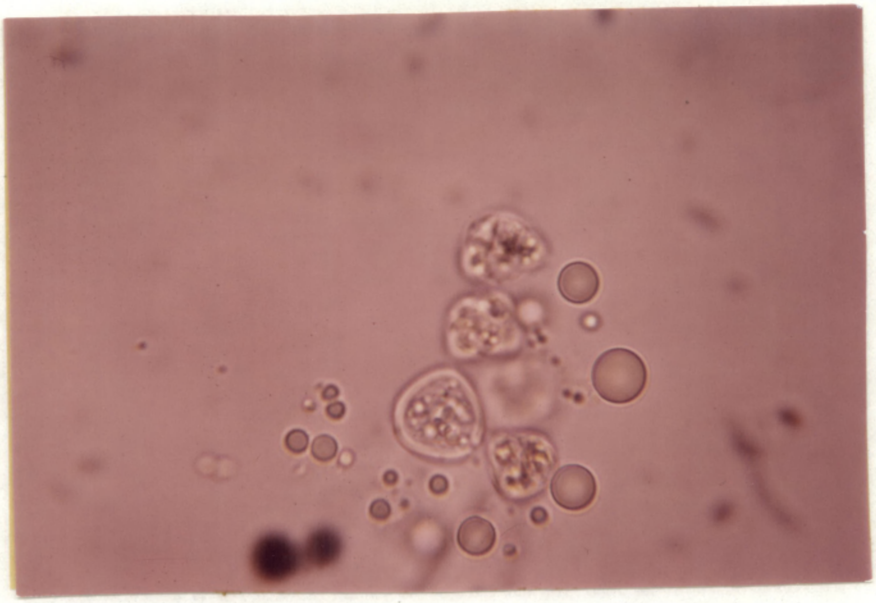

Fig. 4 - Cells of _Tetraedron_ _bitridens_ cultured in $CaCl_2$ deficient BBM.

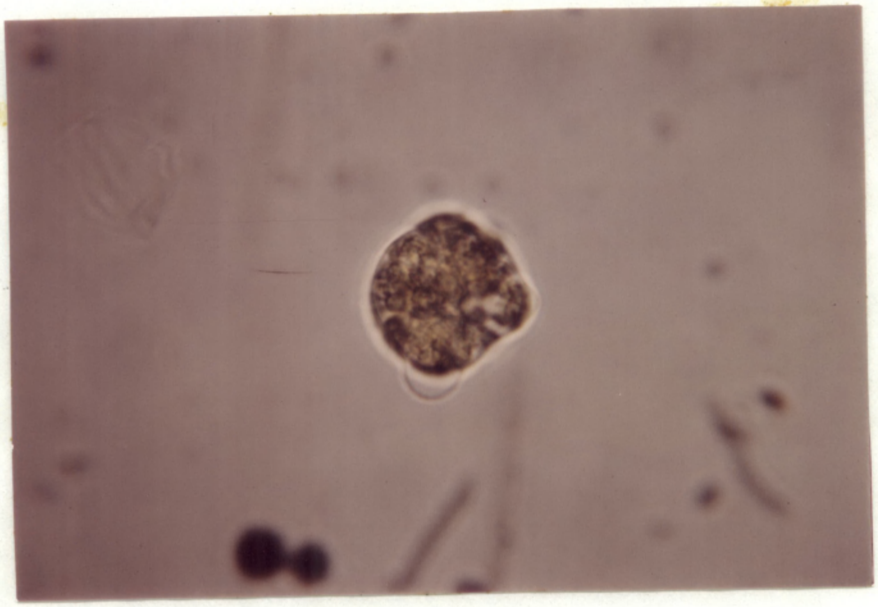

Fig. 5 - _Tetraedron_ _bitridens_ cultured in KH_2PO_4 deficient BBM.

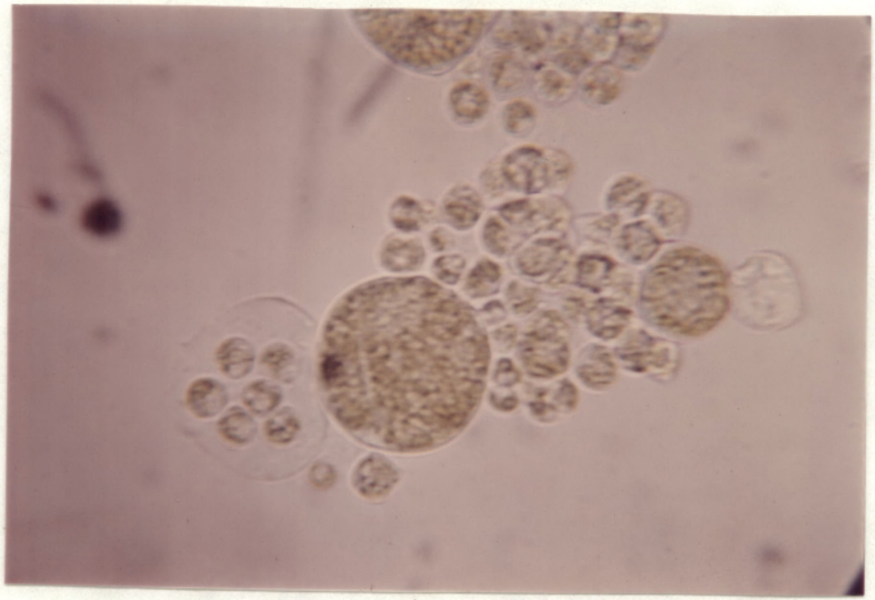

Fig. 6 - Cells of <u>Tetraedron</u> <u>bitridens</u> cultured in KH_2PO_4 deficient BBM.

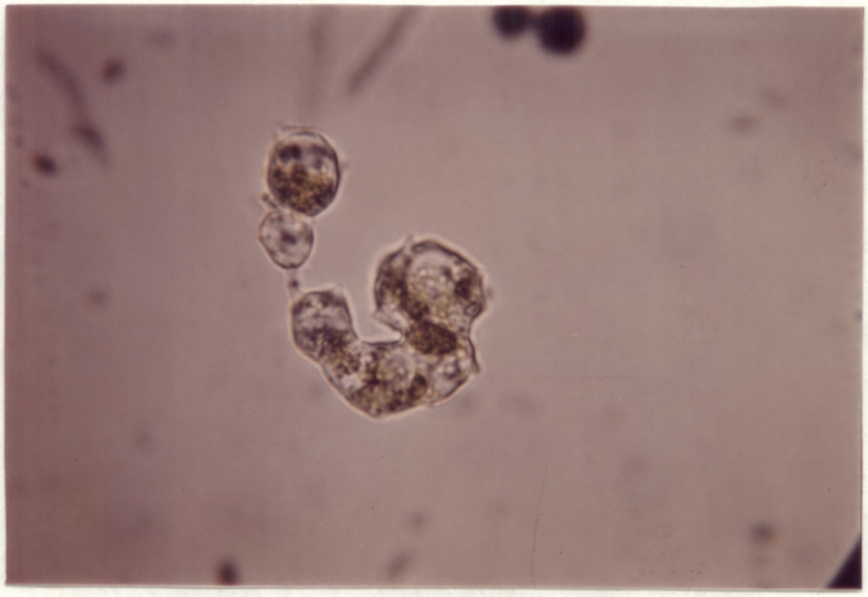

Fig. 7 - <u>Tetraedron</u> <u>bitridens</u> cultured in K_2HPO_4 deficient BBM.

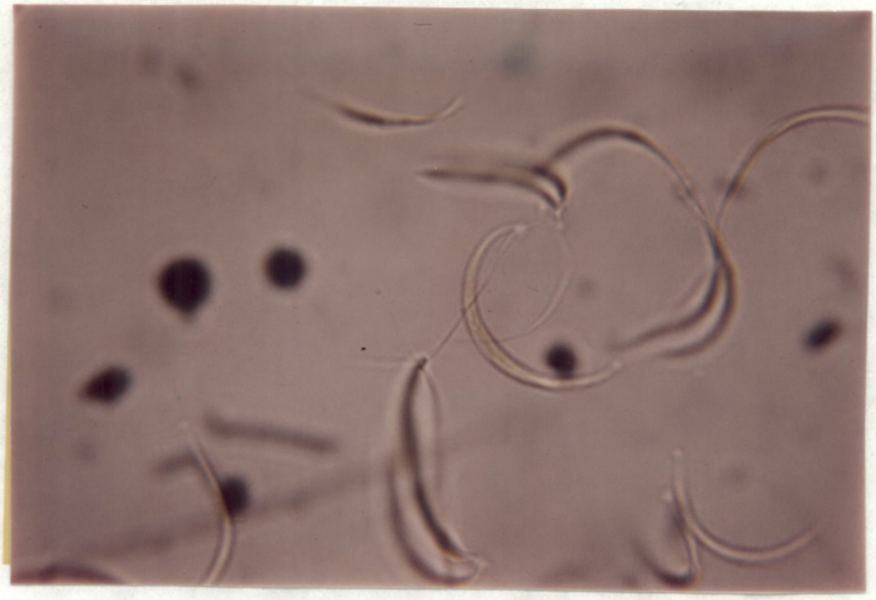

Fig. 8 - Cells of <u>Ankistrodesmus</u> <u>falcatus</u> cultured in KH_2PO_4 deficient BBM.

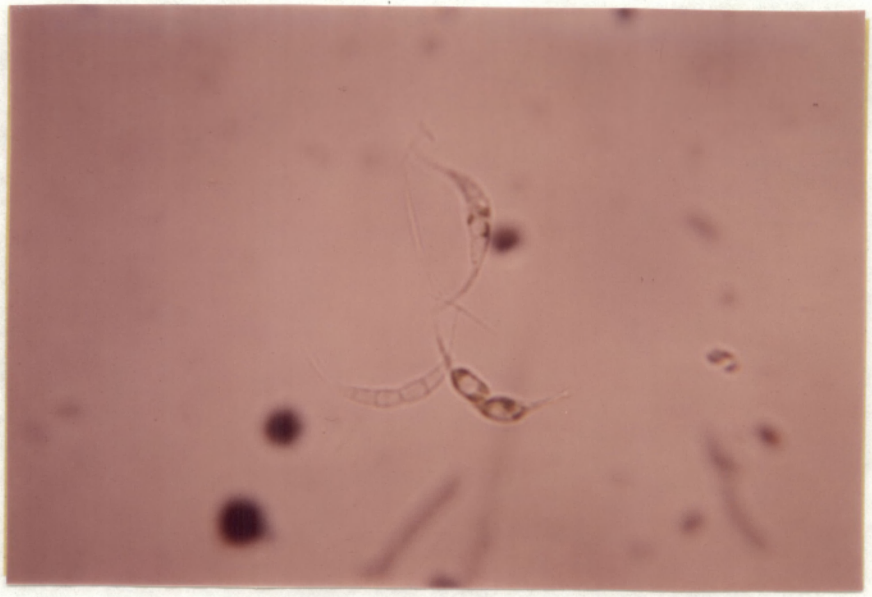

Fig. 9 - Cells of <u>Ankistrodesmus</u> <u>falcatus</u> cultured in KH_2PO_4 deficient BBM.

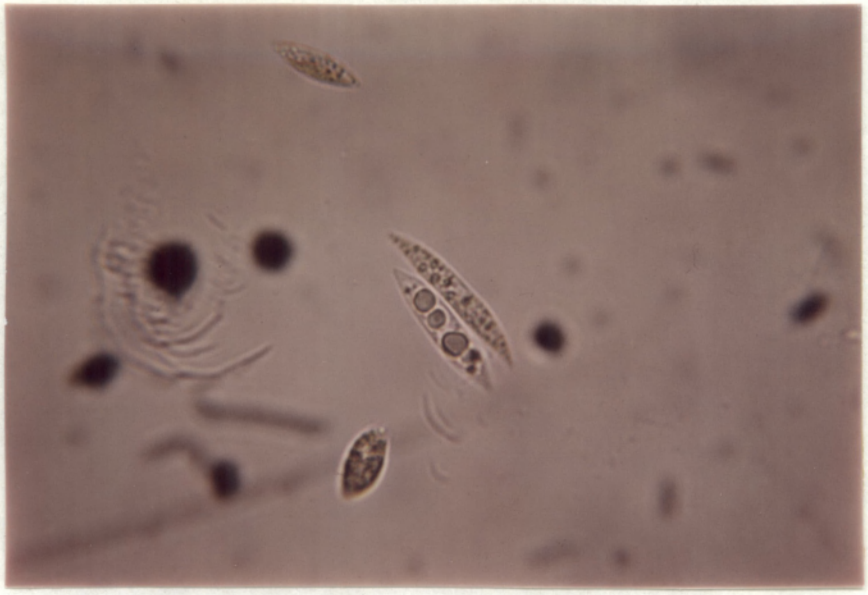

Fig. 10 - Cells of Ankistrodesmus sp. after eight weeks' culturing in complete BBM.

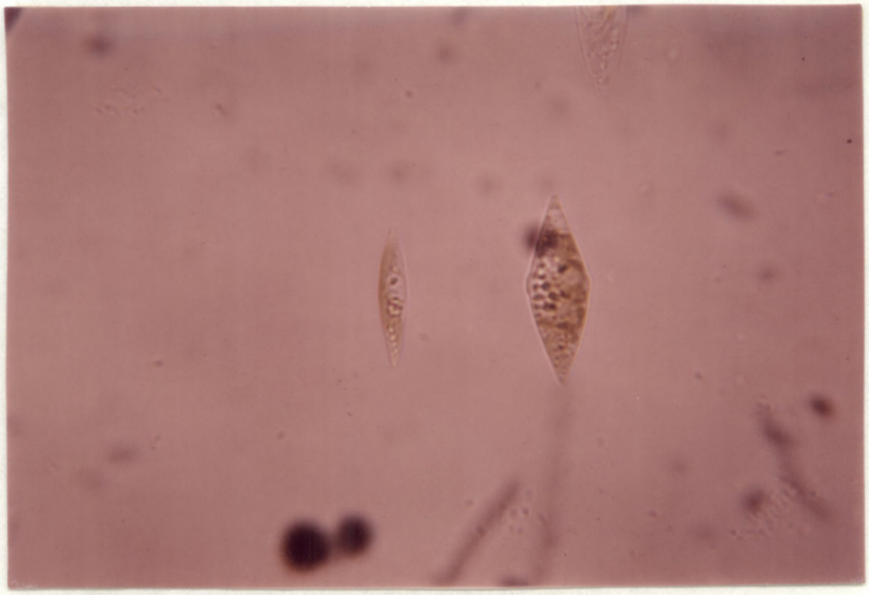

Fig. 11 - Cells of Ankistrodesmus sp. cultured in $MgSO_4$ deficient BBM.

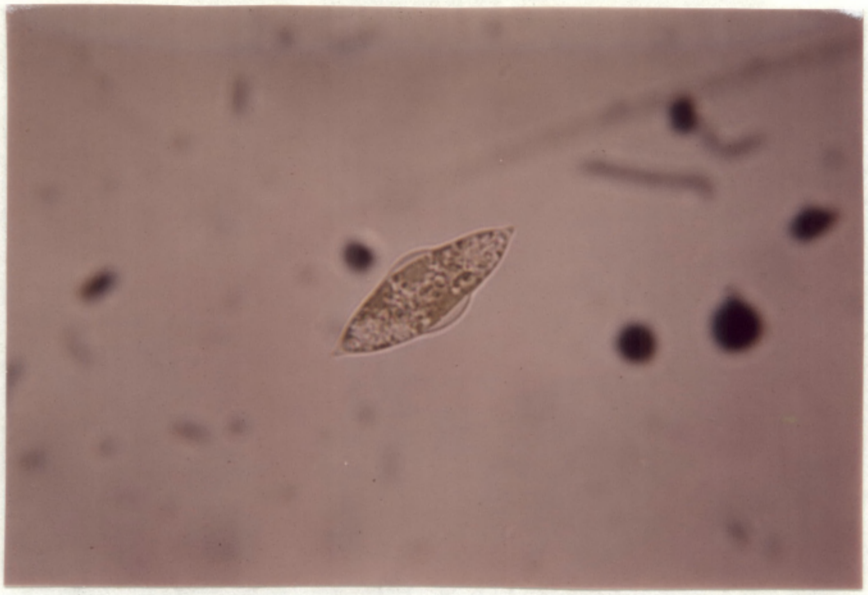

Fig. 12 - Cell of _Ankistrodesmus_ _sp_. cultured in $MgSO_4$ deficient BBM.

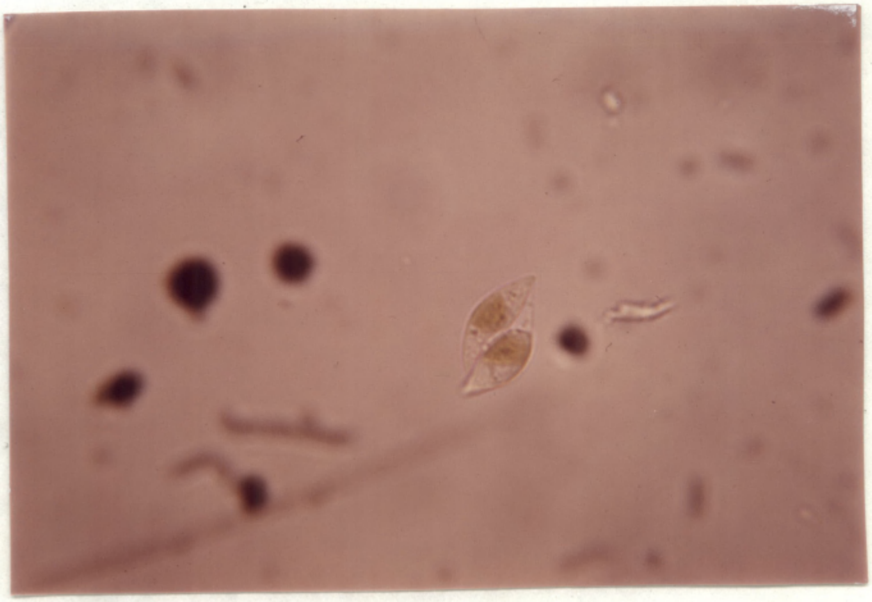

Fig. 13 - _Ankistrodesmus_ _sp_. cultured in microelement deficient BBM.

was observed in potassium dihydrogen phosphate deficient BBM (Figure 5). In Figure 6, autospores of *T. bitridens* are shown being liberated from the parent cell. An enlarged aberrant cell of *T. bitridens* is shown in Figure 7 as it appeared in potassium monohydrogen phosphate deficient BBM. Bristle formation in *A. falcatus* can be seen in Figure 8 and Figure 9. In Figure 9, autospores have been formed by transverse division of the parent cell. During the eighth week of culture in complete BBM, cells of *Ankistrodesmus sp.* were filled with grey oil droplets similar to those observed in ammonium nitrate deficient BBM (Figure 10). Aberrant cells of *Ankistrodesmus sp.* are shown in Figure 11 and Figure 12 as they appeared in magnesium sulfate deficient BBM. In BBM lacking microelements, autospores were formed in pairs as shown in Figure 13. Note also that plastids in the daughter cells are not parietal and only partially fill the cells.

During the course of this investigation, the pH varied no more than 0.3 of a pH unit from the initial of any of the respective media.

CHAPTER IV

DISCUSSION

Although each genera of algae has been ascribed with a specific set of morphological characteristics, experimental evidence indicates that these characters may be altered by algae (4, 16, 24). The role of various inorganic salts and other chemicals in the maintenance of taxonomically important characters has been well documented (5, 8, 11, 12, 21, 26), but as yet has not met with wide application in the problem of algal classification. In this study, morphological characteristics of Tetraedron bitridens, Ankistrodesmus falcatus, and Ankistrodesmus sp. cultured in complete BBM were compared to characteristics of the same organisms when cultured in deficient BBM.

T. bitridens showed considerable variation under the prescribed culturing conditions, but few of the variations could be correlated to specific nutritional deficiency. Thickening of cell walls accompanied by chlorosis and absence of pyrenoids was observed in both ammonium nitrate deficient BBM and BBM lacking microelements. Kingsbury (13), and

Pearson and Kingsbury (19) have correlated chlorosis to depletion of nitrogen supply and found that chlorosis was accompanied by thickening of cell wall. Emerson and Lewis (7) observed that absence of microelements caused reduction in quantum yield of photosynthesis. Other investigators (8, 9, 17, 21) have noted the role of microelement deficiency in chlorosis. In ammonium nitrate deficient BBM, plastids had an asteroid or net-like appearance similar to that described by Starr (22). When grown in the absence of microelements, the plastids appeared shrunken and only partially filled the cells. Cells grown in magnesium sulfate deficient BBM were large and spherical, pyrenoids were absent, and spines were reduced or absent. In preparation for autospore formation, cell size increases to a maximum and critical volume accompanied by breakdown of pyrenoids (1, 2, 3, 22). Round (21) reported interruption of cell division by magnesium deficiency accompanied by abnormal increase in cell size. When cultured in complete BBM or BBM deficient in calcium chloride, potassium monohydrogen phosphate, potassium dihydrogen phosphate, or sodium chloride, cells varied in size, degree of angularity and spine formation, and presence or absence of pyrenoids. These variations most likely resulted from autospore formation during which the cells enlarge, thus causing a reduction in degree of angularity and extent of spine protrusion. This was accompanied by the breakdown of pyrenoids (1, 2, 3, 22). Zoospores were observed as indicated by Starr (23).

A. falcatus exhibited little variation in cell shape, although occasional aberrant cells were noted in deficient medium. In microelement deficient BBM, cells were reduced in size and apices were long and attenuated. Other investigators (17, 20, 27) have noted changes in cellular characteristics in the absence of microelements. When cultured in potassium dihydrogen phosphate deficient BBM, cells developed long, thread-like appendages similar to those described by previous investigators (25, 27). These filaments or bristles (25) formed connections between cells and appeared to be associated with separation of autospores after the division of the parent cell. Apical secretions (16) were also observed. When grown in ammonium nitrate, magnesium sulfate, or sodium chloride deficient BBM, cells were gray and autospore formation was not observed. Several investigators have shown that magnesium and nitrogen are essential for chlorophyll formation (10, 13) and autospore formation

(15, 21). Sodium chloride has been shown to be a necessary growth component for some algae (8), but its role has yet to be well defined. Occasional multinucleate cells were observed in the various media. As this phenomenon could not be correlated with nutritional deficiency, it was most probably related to aging as reported by previous investigators (6, 23).

As reported by previous investigators (4, 16, 18, 28), *Ankistrodesmus* may exhibit wide variation in shape, morphology, and size. Vischer (28), by inhibiting cytokinesis with hexoses, obtained a diverse range of forms in *A. braunii*. Cell types of many taxa of Chlorococcales were observed in *A. braunii* by McMillan (16), who attributed this to accumulation of growth substances, genetic recombinations, inhibition of cytokinesis, abnormal spore germination, or cramped configuration of autospores. Although some imputation has been given to the idea of increased variation with age, Trainor (24) reported that age did not affect variability. Variation in size of cells according to Ketchum and Redfield (12) may be partially due to the number of autospores formed. Kylin (14) reported that phosphate deficiency was characterized by an abnormal increase in cell size. Round (21) observed multinucleated giant cells in Ankistrodesmus which he attributed to cessation of cell division. Several investigators (1, 2, 3, 6, 16, 22) have reported breakdown of pyrenoids prior to autospore formation. Cells of Ankistrodesmus sp. exhibited wide variation in shape, morphology, and size when grown in complete BBM and in BBM deficient in calcium chloride, potassium monohydrogen phosphate, potassium dihydrogen phosphate, or sodium chloride. The aberrant forms and the lack of pyrenoids appeared to result from autospore formation in which the various modes of division resulted in cell shapes other than that ascribed for the taxa.

Other abnormalities were noted when cells were cultured in medium lacking ammonium nitrate, magnesium sulfate, or microelements. When cultured in ammonium nitrate deficient BBM, cells exhibited the fusiform shape characteristic of the taxa, but were filled with gray oil deposits. Accumulation of oil has been shown to be associated with the process of chlorosis and mineral deficiencies (15), especially nitrogen deficiency (10, 13). Cells cultured in magnesium sulfate deficient BBM were diamond-shaped with blister-like enlargements developing in the cell walls. Starr (22) noted the occurrence of similar enlargements in the cell wall of

Spongiochloris excentrica but gave no explanation for their occurrence. When cultured in BBM lacking microelements, cells increased in width, pyrenoids were absent and plastids only partially filled the cells. Autospores were produced in pairs only. Numerous investigators have noted the importance of microelements for algal growth (7, 8, 20, 21). Dactylococcus dissociatus sp. nova was reported by Verses and Trainor (27) to be shaped like Ankistrodesmus when grown in BBM lacking microelements.

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CHAPTER V

SUMMARY AND CONCLUSION

1. This research demonstrated that magnesium, nitrogen and trace quantities of various microelements are essential for maintenance of taxonomically important morphological characteristics for the algae studied.

2. Pyrenoid formation is suppressed by nitrogen, magnesium or microelement deficiency.

3. Pyrenoids disappear prior to autospore formation.

4. Chlorosis is induced by nitrogen, magnesium, or microelement deficiency and is accompanied by changes in characteristics of plastid.

5. Nitrogen deficiency may be accompanied by formation of oil reserves as in *Ankistrodesmus sp*., or enlargement of cell wall as in *T. bitridens*.

6. Microelement deficiency may inhibit autospore formation or as in *Ankistrodesmus sp*. reduce the number of autospores formed within a parent cell.

7. Cytokinesis may be suppressed by nitrogen or magnesium

deficiency as seen in each of the three organisms used in this study.

8. Although nitrogen deficiency appeared to stabilize cell shape in *T. bitridens* and *Ankistrodesmus sp.*, magnesium deficiency resulted in change in cell shape and cell wall characteristics of these two organisms.

9. *A. falcatus*, although growing poorly or not at all in the various deficient media, showed little variation in cell shape.

10. Autospore formation in *A. falcatus* seemed to be affected by potassium dihydrogen phosphate deficiency since division was extensive and accompanied by bristle formation and apical secretions.

11. In *T. bitridens*, variation in cell shape appears to be associated primarily with autospore formation during which cells become increasingly rounded, spines become less conspicuous, and pyrenoids dissociate.

12. In *Ankistrodesmus sp.*, cell shape varied widely from that ascribed to the taxon and appeared to be determined by the direction of division, number and arrangement of daughter cells and deficiency in either nitrogen or magnesium.

13. The morphological variations and intergradiation of the vegetative structures as exhibited by the taxa used

in this investigation accentuate the ambiguities in classification and the need for further elucidation of algal taxonomy and classification.

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