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Morphological control and physiology of *Scenedesmus* strain 170

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Control of the unicellular stage of *Scenedesmus* strain 170 was shown to be a complex phenomenon involving a number of factors. Nitrogen, as ammonium, was the main factor involved in controlling unicell formation. High light intensities, temperatures between 22 and 28 °C, and cell pretreatment in a medium of low total salts (medium 7) all increased unicell percentages. Cell pretreatment in a medium of high total salts (Bristol's medium) yielded reduced unicell levels.

It was proposed that the effect of light, temperature and cell pretreatment on unicell formation was secondary depending on their interaction with nitrogen metabolism. Cell ultrastructure, internal nitrogen and carbohydrate levels, and ammonium uptake rates for cells pretreated in both medium 7 and Bristol's medium are compared in support of the role of nitrogen metabolism in unicell production.

Introduction

In the 1960s and 1970s morphological variation within the genus *Scenedesmus* became well documented (Trainor, Cain & Shubert, 1976). Many of the morphological studies dealt with spine patterns and wall ornamentations. Trainor (1964) found that some *Scenedesmus* strains always had simple spine patterns, while others were found to resemble three or more different species. Trainor (1966) found that *S. parisiensis* was pleomorphic in that it produced four species types, each one with a different spine pattern. In another paper, Trainor & Roskosky (1967) described a strain (*Scenedesmus* 16) that could form many spines, a few spines, no spines, ridges or no ridges; the various combinations resembled five different species according to Smith's (1916) monograph.

To further complicate matters many strains of *Scenedesmus* can produce unicellular stages (Trainor & Hilton, 1963; Swale, 1965; Trainor & Roskosky, 1967; Steenbergen, 1975). Nutrition (Shubert & Trainor, 1974; Overbeck & Stange-Bursche, 1966), pH (Trainor & Roskosky, 1967) and light (Steenbergen, 1975) have been shown to effect formation of the unicellular stage. How these factors may control unicell production has not been shown. In the present study, unicellular production in a *Scenedesmus* strain is shown to be related to not one, but several factors.

Materials and methods

Axenic culture of *Scenedesmus* strain 170 was obtained from Dr E.M. Swale. An axenic culture was established and stock cultures were kept in dim light (1000 lx) on modified Bristol's agar slants and in nutrient broth liquid culture. Cells were pretreated for experimentation by transferring 0·1 ml of liquid stocks into 75 ml fresh medium (substocks) and allowed to grow for 3·5 wk under dim light. Inocula were prepared from the substocks by transferring volume of substock to $^1/_3^2$ volume of fresh medium, and diluting twice with equal volumes over a 4-day period. On the fifth day inocula were used for experimentation.

Most experiments were carried out at 22 °C, approximately 5400 lx and on a 16:$\overline{8}$ h photoregime. With daily transfer at the beginning of the light cycle cultures become synchronous with daughter cell release occurring in the dark period. In some experiments the temperature was varied by using a gradient plate (Yarish, Lee & Edwards, 1979) with a temperature range from 15 °C to 36 °C and light intensity was varied by positioning the cultures in relation to the light source.

In all the experiments where unicell levels were monitored, 10 ml of the medium to be tested were inoculated with a $3{\cdot}5 \times 10^4$ cells · ml^{-1} on day 1. On each successive day for 4 days, at the beginning of the light period the concentration of cells and

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percentage of unicells was determined and the culture rediluted with fresh medium to a cell concentration of $3{\cdot}5 \times 10^{-4}$ cells · ml $^{-1}$.

Modified Bristol's medium and medium 7 (Trainor, 1979) were routinely used throughout the study (Table 1). Media were prepared from stock solutions, autoclaved and allowed to re-equilibrate for one day before use. Additions of ammonium (as NH_4Cl), glycolic acid and vitamin B_{12} were made aseptically from stock solutions, after the medium was autoclaved. Soil extract (SE) was prepared by autoclaving 10 g of soil in 100 ml distilled water. The supernatant was filtered and used as a soil medium.

In the ammonium uptake studies, cells were concentrated by centrifugation, and the pellet of cells resuspended in to the testing medium to give cell concentrations from $1{\cdot}0 \times 10^6$ to $3{\cdot}0 \times 10^6$ cells · ml^{-1}. Subsamples were removed every 1 or 2 h and tested for ammonium concentration after the cells were removed. Controls (medium without cells) were run to test for ammonium adsorption onto the glassware or loss as ammonia to the atmosphere. Ammonium concentrations were determined by the Standard Methods Direct Nesslers Technique (American Public Health Association, 1965).

To test unicell production and internal nitrogen and carbohydrate levels, large quantities (1000 ml) of inoculum were prepared, counted and then a small (less than 1%) amount was removed to inoculate a fresh medium to test unicell production. The remaining inoculum was then centrifuged, washed and resuspended into 25 ml of fresh medium. From this 25-ml sample, 2·5 ml were removed for the carbohydrate analysis. The remainder was recentrifuged, and resuspended onto a preweighed glass filter for the determination of dry weight and used for subsequent internal nitrogen analysis.

The phenol-sulphuric acid method of Kochert was used to measure internal carbohydrate levels (Hellebust & Craigie, 1978). This method proved to be accurate and repeatable. The internal nitrogen levels were determined by using the total Kjeldahl method (Jackson, 1958). When samples of approximately 10–20 mg dry weight were used in conjunction with a 0·005 N H_2SO_4 titrant, results showed little variation between samples grown in a similar medium.

For observation with the electron microscope the material was fixed in an aldehyde mixture (2% glutaraldehyde, 2% formaldehyde and 2% acrolein) for 4 h at room temperature using a 0·05 M cacodylate buffer with an addition of 0·002 M Ca^{++}. The material was then washed in the buffer for 24 h and resuspended in 2% OsO_4 for 1 h. After washing in the distilled water, the cells were pelleted using 1% agar, cut into small sections (0·5 to 1·0 mm per side) and dehydrated in an alcohol series. Gradations of 10% alcohol were used for 20 min each and cells transferred through 100% alcohol twice. The material was then transferred through propylene oxide, 50% propylene oxide: 50% DER-234 embedding medium and finally into pure DER-234 embedding medium. Thin sections were then cut with a LKB ultratome, stained with uranyl acetate and lead citrate and observed on a Phillips II microscope.

Results

Effect of nutrition

Scenedesmus strain 170 is a polymorphic strain capable of producing several morphologies, including colonies and unicells. When *Scenedesmus* strain 170 was grown in medium 7 complete colony formation resulted. When grown in Bristol's medium, with over 85 times the total inorganic salts as medium 7 (Table 1), predominantly colonies were produced. A small percentage of unicells (12–15%) may be present on day 2 or 3, but on day 4 the culture was nearly 100% colonial (Fig. 1). The pH of both medium 7 and Bristol's medium was between 6 and 7 and did not change significantly during experimentation.

In another experiment, inocula were added to SE

TABLE 1. The composition of modified Bristol's medium and medium 7

Nutrient	Bristol's medium (mg l^{-1})	Medium 7 (mg l^{-1})
$NaNO_3$	250·0	2·0
K_2HPO_4	75·0	0·03
KH_2PO_4	175·0	—
$MgSO_4.7H_2O$	75·0	1·0
TRIS	—	40·0
$CaCl_2.2H_2O$	26·5	2·7
$FeCl_3.6H_2O$	5·0	0·1
$MnCl_2.4H_2O$	0·3	0·3
$CoCl_2.6H_2O$	0·02	0·02
$CuSO_4$	0·01	0·01
$ZnSO_4.7H_2O$	0·04	0·04
$NaMoO_4.2H_2O$	0·02	0·02
$Na_2EDTA.2H_2O$	6·9	0·69

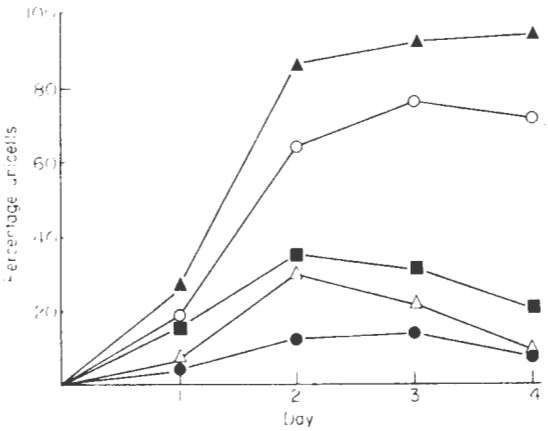

FIG. 1. The effect of the medium on the formation of unicells in *Scenedesmus* strain 170. The media used are as follows: ●, Bristol's medium; ■, Bristol's medium plus 7·8 mg $NH_4Cl \cdot l^{-1}$; △, Bristol's medium plus 39 mg glycolic acid · l^{-1}; ○, Bristol's medium plus 7·8 mg $NH_4Cl \cdot l^{-1}$ and 39 mg glycolic acid·l^{-1}; ▲, Bristol's medium plus 7·8 mg $NH_4Cl \cdot l^{-1}$, 39 mg glycolic acid·l^{-1} and 0·5 mg vitamin B_{12} · l^{-1} (UFM). Cells were grown at 22°C, 5400 lx and on a 16:8 h cycle.

and transferred daily until they were 100% unicellular (3 days). Then daily dilutions were stopped (Fig. 2). The number of unicells dropped rapidly over the first 4 days. At first the majority of colonies were four-celled, however, with age, two-celled colonies dominated. The culture was actively growing while the morphological changes were observed (1–8 days). In a control where the culture was transferred daily, unicell populations of 90% or greater remained.

Many attempts at formulating a medium of known nutrient concentration to control unicell production failed. Additions of various nutrients showed that ammonium stimulated unicell production in both medium 7 and Bristol's. Additions of 7·8 mg $NH_4Cl \cdot l^{-1}$ to Bristol's medium produced the largest unicell populations (34%) of all the single nutrient spiked experiments (Fig. 1). It is of interest to note that Bristol's medium has about 20 times as much nitrogen (as nitrate) as that of the added ammonium.

Ammonium, in combination with a variety of inorganic and organic compounds was tested. No combinations of inorganic nutrients yielded a greater stimulation than single ammonium spikes, however, the combination of 39 mg glycolic acid·l^{-1} and 7·8 mg $NH_4Cl \cdot l^{-1}$ increased levels to over 70% (Fig. 1). When 0·5 mg vitamin $B_{12} \cdot l^{-1}$ was added, in addition to the ammonium and glycolic acid, unicell production reached and maintained levels greater than 90%. This medium is referred to as the unicell formation medium (UFM). An analysis of variance gave significant (0·01 significance level) differences in unicell levels between any two of the media combinations. The other nutrient combinations (glycolic acid alone, vitamin B_{12} alone, glycolic acid in combination with vitamin B_{12}, and ammonium and vitamin B_{12}) confirmed that if ammonium and glycolic acid were not *both* present (under the stated conditions) high numbers of unicells did not develop (Fig. 1).

Unicell production was observed in the UFM after lowering the inorganic salt components (Table 2). Even when the Bristol's component of the UFM was cut to 10% and the $NaNO_3$ and KH_2PO_4 salts were omitted, unicell levels were above 80%, provided ammonium and glycolic acid were present. When individual salts were left out of the trace component of the UFM, unicell percentages were not significantly different than the control (with full trace component).

TABLE 2. Unicell production in modifications of the unicell formation medium (UFM)

Treatment*	Day 1	Day 2	Day 3	Day 4
1	11	88	94	96
2	12	86	100	97
3	35	88	100	100
4	10	64	91	81

*The following treatments of the Bristol's component of the UFM were made: 1, Bristol's medium minus the $NaNO_3$ component; 2, 25% Bristol's medium but a full trace supply; 3, 10% Bristol's medium and minus the KH_2PO components; 4, 10% Bristol's medium and minus the $NaNO_3$ and KH_2PO_4 components. Cells were grown at 22°C, 5400 lx and on a 16:8 h cycle.

The importance of nitrogen was observed when unicell production was tested in Bristol's medium minus individual component salts. (Note that this experiment was performed at high light intensities; see 'Effect of light' section.) Unicell levels ranged from 48 to 66% in Bristol's medium minus $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, KH_2PO_4 or K_2HPO_4. However, when the $NaNO_3$ salt was removed, complete colony formation resulted.

The mean growth rates (as doublings per day) ranged from 1·5 to 1·6 and were not significantly different in Bristol's, Bristol's plus ammonium,

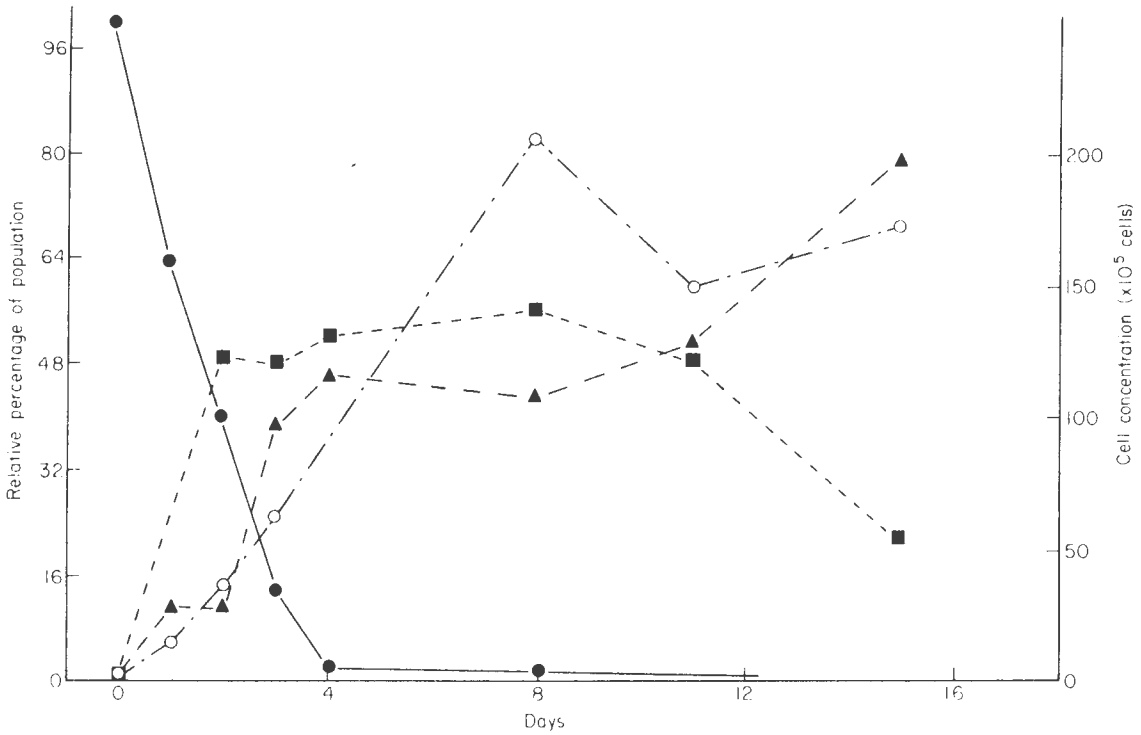

FIG. 2. Growth of *Scenedesmus* 170 in soil extract. A completely unicellular population was transferred into soil extract and the percentage of unicells, two- and 4-celled colonies, were monitored for 16 days. Cells were grown at 22° C, 5400 lx and on a 16:8h cycle. ■, Four-celled colonies; ▲, two-celled colonies; ●, unicells; ○, cell concentration.

Bristol's plus ammonium and glycolic acid and the UFM. The effect of ammonium and glycolic acid on the percent unicells was nutritional and not due to a change in growth rate.

Effect of light

The effect of light intensity (1000 to 10 000 lx) on unicell production in Bristol's medium plus ammonium and glycolic acid was tested. The results (Fig. 3) showed that the percentage of unicells increased linearly with increased light intensity. According to a linear regression model, the unicell population can range from 26% (1000 lx) to 94% (10 000 lx) making light intensity an important factor in morphological control.

Another set of experiments in five media and at four light intensities showed that unicell production in all media was greatly affected by light (Figs 4–8). At 1000 lx unicell production was low (under 30%) in each medium and differences between the media were not significant. As the light intensity was raised from 7600 to 9700 lx the percentages of unicells greatly increased, especially in the UFM and Bristol's plus ammonium and glycolic acid medium where values exceeded 90%. In the other three media, unicell levels also rose significantly from less than 4% at 1000 lux to between 40 and 50% at 9700 lx (Figs 4–6).

The effect of light duration (L:D cycles) on the morphology was observed. Light : dark cycles of 8:$\overline{12}$, 12:$\overline{12}$ and 16:$\overline{8}$ h were run at 4300 and 8700 lx. There was no significant difference in the percentage of unicells between light durations at a given light intensity.

Effect of temperature

Temperature had a significant effect on the morphology of *Scenedesmus* strain 170 (Table 3). For each of the four media the unicell concentrations were highest at temperatures between 22 and 29° C and levels dropped significantly at extreme temperatures.

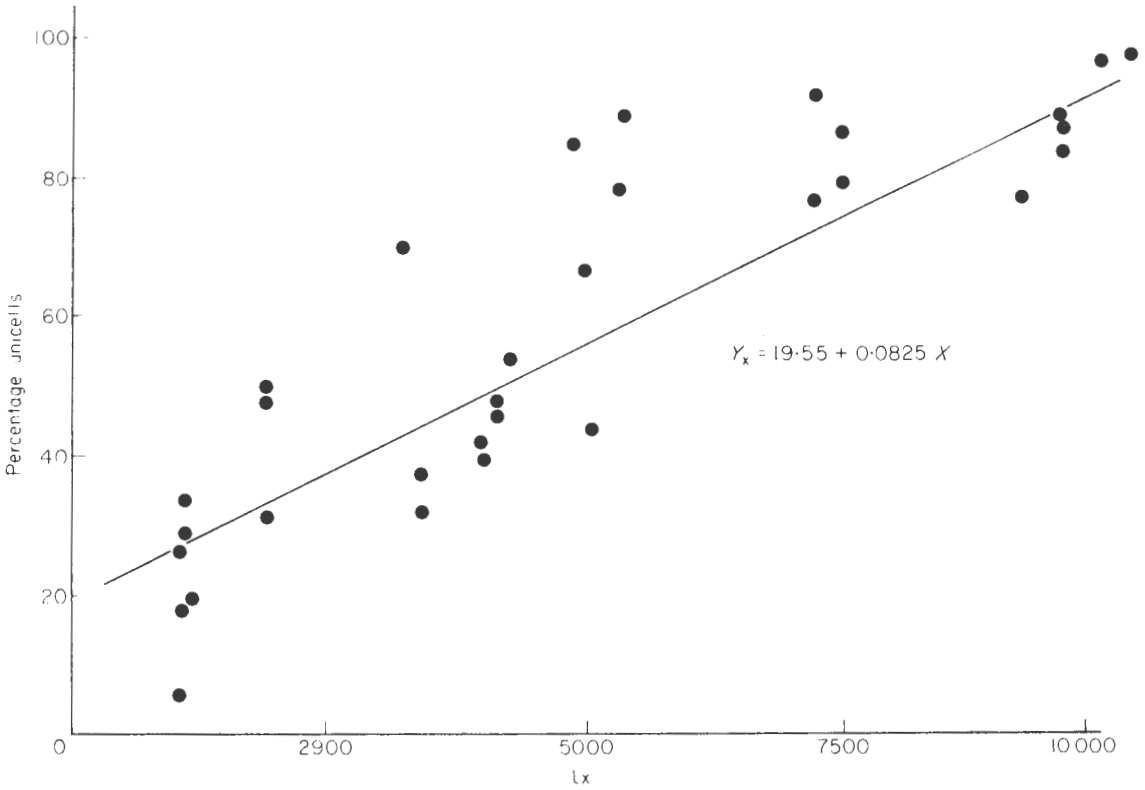

FIG. 3. The percentage of unicells as a function of the light intensity. Cells were grown in Bristol's medium plus 7·8 mg $NH_4Cl \cdot l^{-1}$ and 39 mg glycolic acid $\cdot l^{-1}$ at various light intensities. The percentage of unicells on days 3 and 4 were averaged and a linear regression analysis was run on the data. The coefficient of correlation was 0·88. Culture conditions were 22°C and a 16:8 h cycle.

Within any of the four temperature ranges unicell production was always lowest in Bristol's medium and highest in either Bristol's plus ammonium and glycolic acid or the UFM. These results agreed with earlier findings for unicell production at 22°C (Fig. 1).

Long-term morphological control

Once it was established that Bristol's plus ammonium and glycolic acid, Bristol's and medium 7 produced 90, 40–50 and 0% unicells respectively at 8600 lx, a long-term study was performed using these three media. During a 24-day period the transfer medium was changed every 4 days and the unicell levels were monitored daily (Fig. 9).

The expected morphological control was achieved during each part of the experiment. During the first 4 days over 90% unicells were observed when grown in Bristol's plus ammonium and glycolic acid. After day 4 cultures were transferred into Bristol's medium and the percentage of unicells dropped to 44 by day 8. Between days 8 and 12 in medium 7 the cultures became completely colonial. During days 12–24 the trend was duplicated.

Effect of inocula pretreatment

The effect of inocula pretreatment on subsequent unicell production was tested. Inocula were pregrown in either medium 7 or Bristol's medium and then placed into Bristol's medium with ammonium and glycolic acid at 5400 and 8600 lx.

At either light intensity unicell production was significantly lower when inocula were pregrown in Bristol's medium. After 4 days at 5400 lx 71 and 34% unicells were present for inocula pregrown in medium 7 and Bristol's medium respectively. At 8600 lx the values were 88 and 49% respectively.

Light and electron microscopy revealed large

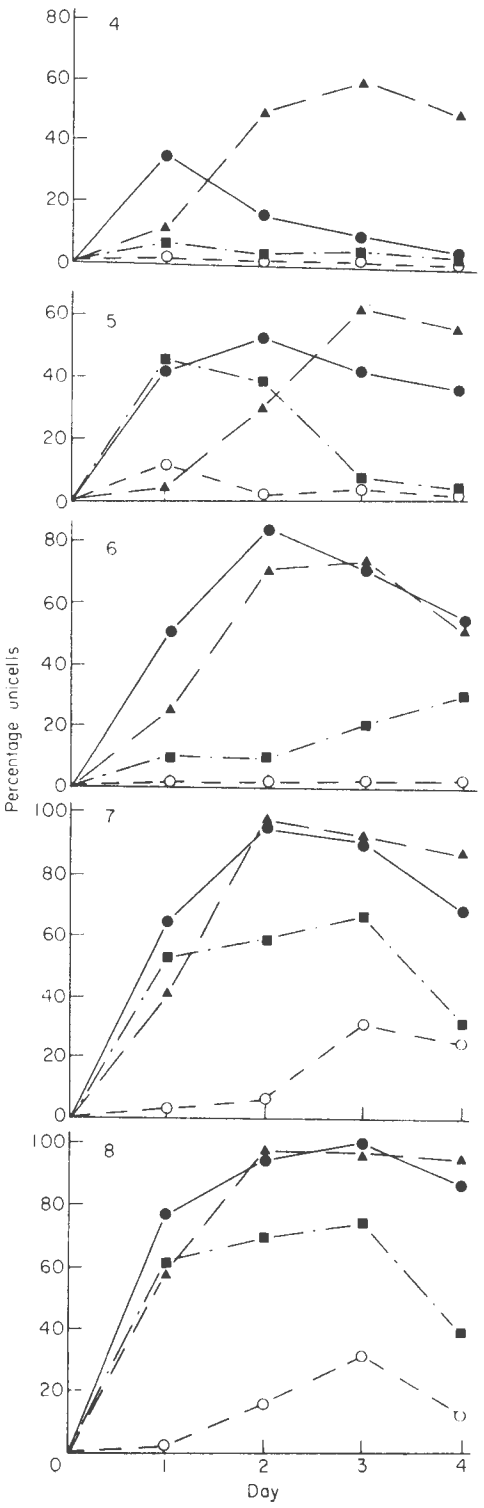

TABLE 3. The percentage of unicells on days 3 and 4 at various temperatures for four media. Cells were grown at 5400 lx and on a 16:8h cycle

Medium	Temperature (°C)			
	15–17	22–23	28–29	30–31
Bristol's	11·5	14·5	9	0
	0	12	34	0
	3	27·5	21	10
	16·5	57	25	5
$\bar{X}$	8	28	22	3·75
Bristol's + NH_4	9	42	19	15
	36	13	34	12
	9	36	40	20
	37	81	53	31
$\bar{X}$	23	43	37	19·5
Bristol's + NH_4 + GA	41	75	91	38
	59	73	78	20
	17	62	60	30·5
	57	98	76	46·0
$\bar{X}$	44	77	76	34
Bristol's + NH_4 + GA + Vit. B_{12}	13·5	92	88	8
	44	90	90	28
	57	90	89	18
	50	91	87	20
$\bar{X}$	41	90·75	88	18·5

differences between cells grown in medium 7 and Bristol's medium. Medium-7 grown cells were yellow-green, very granular in appearance due to large starch and liquid reserves, lacked well developed chloroplasts, pyrenoids and mitochondria, and had a thick inner cellulose wall. Cells were generally symmetrical in appearance.

Cells grown in Bristol's medium were green, uniform in appearance, vacuolated, had small starch and lipid reserves, well developed chloroplasts, pyrenoids and mitochondria, and a thin inner cellulose wall. Their outer cell wall component was very elaborate, highly ornamented and

FIGS 4–8. The effect of light intensity on unicell production in five media.

FIG. 4. Bristol's medium.

FIG. 5. Bristol's medium plus 39 mg glycolic acid·l^{-1}.

FIG. 6. Bristol's medium plus 7·8 mg $NH_4Cl \cdot l^{-1}$.

FIG. 7. Bristol's medium plus 7·8 $NH_4Cl \cdot l^{-1}$ and 39 mg glycolic acid·l^{-1}.

FIG. 8. The UFM (Bristol's medium plus 7·8 mg $NH_4Cl \cdot l^{-1}$, 39 mg glycolic acid·l^{-1} and 0·5 mg vitamin $B_{12} \cdot l^{-1}$).

The light intensities used were 1000 (○), 1800 (■), 7600 (●) and 9700 lx (▲) and the percentage unicells were observed over 4 days. Cells were grown at 22°C and on a 16:8 h cycle.

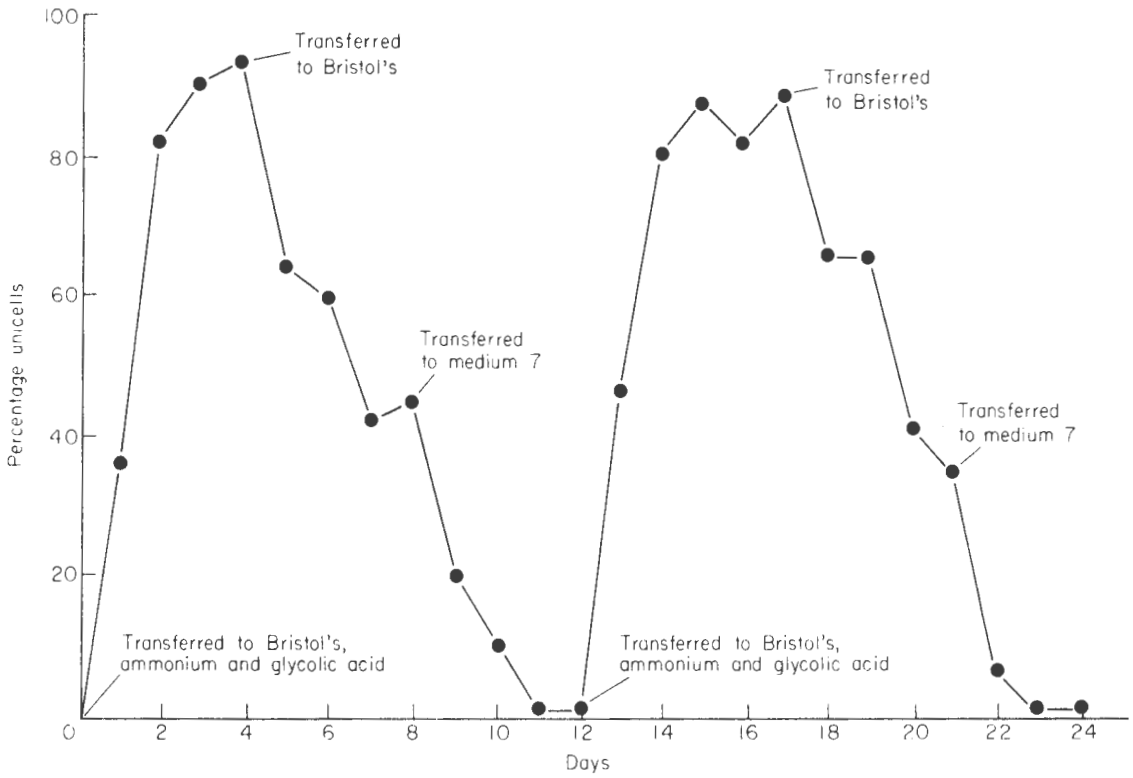

FIG. 9. Long term morphological control of *Scenedesmus* 170. During each 4-day segment of a 24-day period, cells were transferred into a different medium and unicell populations monitored. Media used were Bristol's medium plus 7·8 mg $NH_4Cl \cdot l^{-1}$ and 39 mg glycolic $acid \cdot l^{-1}$, Bristol's medium, and medium 7. Culture conditions were 22° C, 9000 lx and a 16:8h cycle.

not appressed to the trilaminar sheath. Colonies were not as symmetrical as medium-7 grown cells.

Uptake of ammonium

The uptake rates of ammonium for cells grown in medium 7 and Bristol's medium were measured in the light and dark (Table 4). Uptake rates were tested after inocula were transferred into the UFM without $NaNO_3$.

Uptake rates were significantly (0·05 significance level) greater in cells pregrown in medium 7 regardless of the light condition. Cells pregrown in medium 7 had mean uptake rates of 4·4 and 5·6 times greater, in the light and dark respectively, than cells pregrown in Bristol's medium. If the mean uptake rates measured both in the light and dark are assumed to be constant over the cell cycle then in a 16:8h cycle cells pregrown in medium 7 would take up 7·7 times more ammonium than cells pregrown in Bristol's medium.

Uptake rates were equal for cells pregrown in medium 7, but darkness cut ammonium uptake rates in half for cells pregrown in Bristol's medium (Table 4).

Ammonium uptake rates did not change when

TABLE 4. Ammonium uptake. The ammonium uptake rates of *Scenedesmus* strain 170. Cells were pregrown in either Bristol's medium or medium 7 and then inoculated into the UFM minus the $NaNO_3$ salt component. The effect of light (5400 lx) and darkness were observed

Treatment	Uptake rate $\mu gNH_4/10^6$ cells/h 0 4 h
A Light	
(1) inoculum grown in Medium 7	0·660
(2) inoculum grown in Bristol's	0·154
B Dark	
(1) inoculum grown in Medium 7	0·564
(2) inoculum grown in Bristol's	0·084

cells were pretreated in medium 7 and placed into the UFM with the $NaNO_3$ component. Hence, ammonium uptake was not inhibited when over 20 times more nitrogen (as nitrate) was available.

Internal nitrogen and carbohydrate levels

The internal nitrogen and carbohydrate levels were measured for inocula pregrown in medium 7, medium 7 with 3 times its $NaNO_3$ supply, medium 7 with 12·5 times its $NaNO_3$ supply and Bristol's medium (Tables 5 and 6). Cells pregrown in medium 7 had the lowest amount of internal nitrogen (3·49% weight basis) but the largest internal carbohydrate levels (56·8% weight basis). Cells pregrown in media with increasing nitrogen levels had successively higher nitrogen levels and lower carbohydrate levels (Tables 5 and 6).

Cells grown in Bristol's medium had a maximum of 8·9% nitrogen, over 2·5 times as much as medium 7 grown cells. Using the common factor of 6·25 times the internal nitrogen percentage, total protein estimates were observed (Table 5). Protein levels ranged from 21·8% (medium 7) to 55·6% (Bristol's).

Medium 7 grown cells had close to 3 times as much carbohydrate as cells grown in Bristol's medium. These data agreed with the electron micrographs which showed large internal starch supplies in medium-7 grown cells.

TABLE 5. Internal nitrogen levels for inocula pregrown in medium 7, medium 7 with 3 times the $NaNO_3$ supply, medium 7 with 12·5 times the $NaNO_3$ supply and Bristol's medium

Medium	($NaNO_3$) in medium mg·l^{-1}	N (% weight basis)	Protein estimate (%)
Medium 7	2	3·49	21·8
Medium 7, 3 times $NaNO_3$	6	5·90	36·9
Medium 7, 12·5 times $NaNO_3$	25	8·30	51·9
Bristol's medium	250	8·90	55·6

TABLE 6. Internal carbohydrate levels for inocula pregrown in medium 7, medium 7 with 3 times the $NaNO_3$ supply, medium 7 with 12·5 times the $NaNO_3$ supply and Bristol's medium

Medium	($NaNO_3$) in medium mg·l^{-1}	Carbohydrate (as percentage glucose on a weight basis)
Medium 7	2	56·8
Medium 7, 3 times $NaNO_3$	6	50·6
Medium 7, 12·5 times $NaNO_3$	25	41
Bristol's medium	250	19·2

Discussion

Many strains of *Scenedesmus* are now known to produce a unicellular stage (Trainor & Hilton, 1963; Overbeck & Stange-Bursche, 1966; Trainor & Roskosky, 1967; Trainor, 1971; Shubert & Trainor, 1974; Swale, 1965; Steenbergen, 1975). These unicells resembled the genera *Franceia*, *Chodatella* or *Lagerheimia*, depending on the number and placement of spines. Control of the unicellular stage has been achieved in a number of studies. Swale (1965) produced unicellular populations by using soil extract. Trainor (1971), using the same strain as Swale (1965), also obtained complete unicell formation by additions of soil extract to a known inorganic medium; he concluded that the unicell producing factor(s) was chemical in nature and supplied by the soil extract. Trainor & Hilton (1963) were able to produce complete *Chodatella* stages in a soil *Scenedesmus* by growing it in a liquid medium; on agar the culture remained colonial. Other studies have found unicell production to be related to individual nutrients. Overbeck & Stange-Bursche (1966) found that phosphorus in combination with young active cultures produced unicells. Similarly, Shubert & Trainor (1974) reported that additions of only phosphorus to a dilute inorganic medium gave complete unicell control. In two other studies (Trainor & Roskosky, 1967; Trainor & Rowland, 1968) ammonium at an alkaline pH was necessary for control of the unicell stage. Steenbergen (1975) claimed that the length of the photoperiod governed the formation of unicells and colonies in his strain.

While it appeared that there was no common factor controlling unicell formation in all strains studied, perhaps each factor's effect depended on the levels of the other variables. For instance, nutrient levels could be more important at lower light and temperature, but can be lowered if the levels of light or temperature are raised. This kind of reasoning may link together several control mechanisms observed by different investigators.

Scenedesmus strain 170 was known to form unicells in soil extract (Swale, 1965; Trainor, 1971), and undefined medium, but the exact nutrient involved was not known. In the present study, we set out to determine the nutrient(s) necessary for controlling the unicellular stage of *Scenedesmus* strain 170.

Control of colony formation was obtained in medium 7. In Bristol's medium at 22°C and 5400 lx, the population consisted mainly of colonies with approximately 8% unicells after 4 days (Fig. 1). However, at the same light and temperature over 90% unicells were induced by adding 7·8 mg $NH_4Cl \cdot l^{-1}$, 39 mg glycolic acid·l^{-1} and 0·5 mg vitamin $B_{12} \cdot l^{-1}$ (Fig. 1). Is this control of the unicellular stage? If the investigator is satisfied with control being over 90% of the population in one morphological state, then control was established.

However, the nutrients needed for induction of unicells changed when other factors were altered. When light levels were lowered (below 5400 lx), the UFM no longer produced over 90% unicells; instead only 24 and 40% unicells remained on day 4. In contrast, with increased light levels (above 5400 lx), vitamin B_{12} was not needed to produce over 90% unicells. Temperature was also critical in controlling unicell formation. Even though at one temperature a particular medium provided unicellular control (over 90%), it did not at another (Table 3). Likewise, inocula pretreatment played a role in unicell development.

The term 'control' should be used cautiously and specifically since several factors interact in determining the unicell levels. The exact levels of all factors should be stated as well as the factor believed to be central in control of the unicell stage.

Nitrogen was the key nutrient for unicell production. At 22°C and 9700 lx in Bristol's medium just over 50% unicells formed (Fig. 4). When the nitrogen source ($NaNO_3$) was removed from the Bristol's medium, complete colony formation occurred. However, unicell levels were not lowered when the other components of Bristol's medium were omitted.

Nitrogen metabolism was central to the formation of unicells, and other factors such as light, temperature and inoculum pretreatment played an indirect role as they affected nitrogen metabolism. When most algae were supplied with both ammonium and nitrate, the ammonium was preferred and the nitrate source not utilized until there was no ammonium available. (Syrett, 1962; Fitzgerald, 1969; McCarthy, Taylor & Taft, 1977). This preference for ammonium was due to the feedback inhibition of ammonium on nitrate reductase and the need for both an energy source and reducing power to utilize nitrate directly. On the other hand, ammonium was taken up rapidly and in very large amounts, provided it was detoxified (by formation of organic nitrogen).

An organic carbon source was needed to combine with ammonium produced from nitrate uptake and reduction, or direct uptake (Thacker & Syrett, 1972). When alfalfa leaf discs were grown in medium containing ammonium and $^{14}CO_2$, more fixed carbon was directed towards amino acid synthesis (Platt, Plant & Bassham, 1977). A large drain on the cell's carbon supply would therefore occur during periods of rapid ammonium uptake and would be required for continued detoxification of the accumulated ammonium.

When *Chlorella* (Thacker & Syrett, 1972; Fitzgerald, 1968), *Microcystis* (Fitzgerald, 1968) and *Asterionella* (Eppley & Thomas, 1969) were nitrogen starved, they were capable of higher nitrogen uptake rates (3 to 10 times), in both the light and dark, than normal cells. In general, Fogg (1965) suggested that the metabolism of algal cells changes as they become nitrogen limited, switching from protein synthesis to lipid and carbohydrate synthesis. Fogg (1965) also suggested that algal cells starved for nitrogen had low chlorophyll, protein and nucleic acid levels, and a low photosynthetic rate. Such cells in turn had high fat, carbohydrate and carotenoid levels.

When inocula were pretreated with medium 7 (2 mg $NaNO_3 \cdot l^{-1}$), they were essentially nitrogen starved, relative to those inocula pregrown in Bristol's medium (250 mg $NaNO_3 \cdot l^{-1}$). Cells pregrown in Bristol's medium had over 2 times more internal nitrogen than those in medium 7 (Table 5). Since cells grown in Bristol's with 500 mg $NaNO_3 \cdot l^{-1}$ had an internal nitrogen level of 8·4%, internal nitrogen levels of 8-9% are maximal. Chemical analysis and electron microscopy showed medium 7 cells to have high amounts of carbohydrate and lipid reserves, but low protein levels, all indicating nitrogen starvation. When placed in UFM, medium-7 grown cells had much higher ammonium uptake rates than Bristol's grown cells. These uptake rates were not affected when over 20 times as much nitrogen in the form of nitrate was present.

It was proposed that a threshold level of nitrogen must be taken up and assembled by the cell in order to form unicells. This threshold is best achieved when

cells are nitrogen starved, the carbohydrate supply is high, ammonium is the nitrogen source, light intensities are high and temperatures within the range of 22–28° C. Under these conditions, *Scenedesmus* strain 170 will assemble large amounts of organic nitrogen compounds over a short period of time (1–2 days) which, in turn, give unicell formation.

This nitrogen uptake proposal was supported by the fact that cells pretreated in Bristol's medium, which had a small internal carbohydrate supply and removed ammonium from the medium slowly, did not produce the large concentrations of unicells over a 4-day period, as did inocula pretreated in medium 7. We believe that cells grown in medium 7 and placed in Bristol's, removed nitrate slowly; gradually, over a number of generations, they reached the 8–9% nitrogen level. In this manner, cells were never exposed to large pulses of nitrogen as when cells were pregrown in medium 7 and put into Bristol's medium plus ammonium. Large pulses of nitrogen stimulated pathways necessary for unicell production.

Thus, there was a connection between the medium in which the cells were pretreated and unicell production. Cell pretreatment affected the internal nitrogen levels, which in turn affected the uptake rates of ammonium and subsequent unicell production. Inocula pregrown in medium 7 with 10% of the Bristol's $NaNO_3$ component (25 mg $NaNO_3 \cdot l^{-1}$) had internal nitrogen levels above 8%. Initial results showed that cells pregrown in this manner also produced few unicells. Cells pregrown in medium 7 with 3 times the nitrate supply (6 mg $NaNO_3 \cdot l^{-1}$) had internal nitrogen levels between 3 and 8% and, as expected, yielded intermediate unicell percentages when used as inocula and put into Bristol's plus ammonium and glycolic acid.

If cells had a large organic supply (starch and lipid) as when grown in medium 7, it could have been utilized to supplement photosynthetically fixed carbon in the assimilation of ammonium during rapid uptake. Then unicells formed. However, once the internal supply was exhausted, at lower light levels, photosynthetically fixed carbon could not be produced fast enough to satisfy both the basic cell needs and the requirement for high rates of ammonium assimilation. Hence, unicell percentages decreased (Fig. 1, the Bristol's plus ammonium curve). Thus, at 5400 lx (Fig. 1), an added organic source (glycolic acid) was needed to maintain unicell populations. However, at higher light intensities, more photosynthate and energy (as ATP and reducing power) were produced and the need for glycolic acid diminished. This enabled cells placed in Bristol's medium to remove nitrate at higher rates and produce more unicells (Fig. 4). Nonetheless, with nitrate as the nitrogen source, 90% unicells never formed regardless of the organic levels.

The role vitamin B_{12} played in unicell production is unknown. There was no significant difference in the growth rate with the addition of B_{12}. Vitamin B_{12} may enhance the metabolism of small organic compounds (Provasoli & Carlucci, 1975). Perhaps at the lower light intensities (Fig. 3), vitamin B_{12} enhanced the utilization of glycolic acid and thus further stimulated unicell production.

It is not easy to relate the findings of the present study to nature as it was for Trainor, working with strain AP 1 in 1979. He had isolated AP 1 from a local pond in order that field incubation studies could be carried out. Since strain 170 came from England, we did not make observations with water from the original collection site. However, when *Scenedesmus* strain 170 was grown in water from lakes and rivers in Connecticut it rarely produced unicells. Only when cultured in water containing sewage effluents were there predominately unicellular populations. (The sewage effluents were the only water samples found to contain high amounts of ammonium and organic compounds.) In general, this study would appear to confirm the possibility that the spiny unicell might essentially be a laboratory artifact for many species of *Scenedesmus*. On the other hand, there is such a variety of strains known from culture studies, and most have not been studied in any detail, it would be premature to generalize at this time.

In summary, unicell formation in *Scenedesmus* strain 170 was greatest when cells were pregrown in medium 7 ammonium was the nitrogen source, an organic supplement was added (e.g. glycolic acid), temperatures were between 22 and 29°C and light intensities were high. When cells were pregrown in medium 7 they became nitrogen starved and built up large internal carbon sources. When these cells were transferred into the UFM, a large pulse of nitrogen was taken up and unicells formed. If however, the organic supply was lowered (internal reserves lowered, light levels were low and/or there was no added organic), then large amounts of nitrogen were not taken up and colony formation resulted. If the medium 7 grown inoculum was put into Bristol's

medium, the amount of nitrogen (as nitrate) taken up was low, resulting in colony formation. However, if the organic supply was increased (photosynthetically by raising the light intensity or by adding an organic acid), nitrate uptake increased and some unicells formed. Cells pregrown in Bristol's medium had high internal nitrogen levels and low carbon supplies resulting in low nitrogen uptake rates, and subsequent colony formation.

In conclusion, unicell production in *Scenedesmus* strain 170 was a complex process. Many factors such as cell pretreatment, nutrition, light and temperature were found to interact in controlling the balance between formation of unicells and colonies. The advantage to forming unicells remains unknown. When unicells were formed (each with four spines) sixteen spines were produced rather than the four spines on a typical colony. Perhaps the large nitrogen pulse was funnelled into spine formation, or the spine was a sink for excess nitrogenous compounds. Conway & Trainor (1972) found that flotation was better in unicells. Perhaps there was a selective advantage to produce unicells so that the population could maintain its position in the water column where conditions (nitrogen levels, light intensity, temperature, etc.) were favourable.

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