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PHOTOSYNTHESIS AND RESPIRATION OF THREE BLUE-GREEN ALGAE[1,2]

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Reported herein is a study of certain salient features of photosynthesis and respiration in three blue-green algae, *Anabaena variabilis*, *Anacystis nidulans*, and *Nostoc muscorum* G. Histories and brief descriptions of the three species are included in a previous report (2) which presented methods of culture and growth characteristics. Of the various blue-green algae available in pure culture the species used were chosen because they suspend readily to give homogeneous suspensions which submit to ease of manipulation. The objectives of the study are two-fold: first, to obtain information on metabolism of the blue-green algae, a group which has received only cursory attention; and secondly, to develop a base of information which would allow use of a blue-green alga as a reliable experimental organism for studies on the process of photosynthesis. Because of the simplicity of their structure the blue-green algae appear especially desirable for studies on photosynthesis pursued to subcellular levels.

METHODS

Cellular material of *Anabaena variabilis* and *Anacystis nidulans* was obtained from bacteria-free cultures grown in a continuous-culture apparatus (6). The culture medium was medium C, described completely in a previous report (2), containing per liter 0.25 gm $MgSO_4 \cdot 7\,H_2O$, 1.0 gm K_2HPO_4, 0.025 gm $Ca(NO_3)_2 \cdot 4\,H_2O$, 1.0 gm KNO_3, 0.165 gm sodium citrate, 0.004 gm $Fe_2(SO_4)_3 \cdot 6\,H_2O$, and 1.0 ml of an A_5 trace element solution; pH 7.3 to 7.5 when aerated with 0.5 % CO_2 in air. The cultures were maintained at 25 or 39° C and illuminated by tungsten lamps. Pure cultures of *Nostoc muscorum* G (strain obtained from G. C. Gerloff) were grown with the same medium and aerating gas in large test tube cultures in baths held at 25° C and illuminated by 20-watt daylight fluorescent lamps. For all cultures rate of growth was controlled by light intensity. However, since the illumination was multidirectional and the effective light intensity not easily interpretable, a more useful description of the previous history of the cells is provided by the specific growth rate, k, which will be expressed in $\log_{10}$ units per day.

Photosynthesis measurements were made by Warburg manometry in a water bath with glass bottom thermostated at 25 or 39° C. Illumination was provided by a bank of tungsten lamps operated at 120 ± 1 volts from a voltage stabilizer. Light intensity could be varied by use of Jena NG neutral filters attached to the bottoms of the vessels by holders which also masked out stray light. Respiration measurements were made in conical flasks containing 2.0 ml cell suspension, 0.25 ml KOH in the center well, and 0.5 ml of buffer or buffer plus substrate in the sidearm.

In preparation for manometric experiments cells were centrifuged out from a known volume of suspension, washed in the buffer to be used, and taken up in buffer. Because of its small size (ca $1.6 \times 2.2\ \mu$) *Anacystis nidulans* does not sediment as easily as other algae; $3300 \times g$ for 6 minutes in an angle centrifuge were required for complete sedimentation. Cell quantity was determined routinely in terms of packed cell volume. Aliquots of original cell suspension were centrifuged out, transferred to calibrated capillary tubes, and centrifuged at $2400 \times g$ for one hour in an International centrifuge. Packed cell volume of all three species attained a minimum volume after 45 minutes. The relation between cell volume and dry weight was determined separately upon several differ-

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TABLE I

CHARACTERISTICS OF THE CELLS USED

ALGA	LIGHT INTENSITY FOR GROWTH	TEMP	pH	GROWTH RATE, K	DRY WT *
	fc	°C			*mg/cm cells*
Anabaena variabilis	55	25	7.8	0.30	0.206 ± 0.005
Anacystis nidulans	75	25	7.7	0.55	0.278 ± 0.006
	90	39	7.8	1.00 †	0.271 ± 0.007
	220	39	8.0	2.50	0.266 ± 0.005
Nostoc muscorum G **	200	25	7.7	0.20	0.194 ± 0.003

* Variability shown is the maximum deviation from the mean in four determinations.

** Grown in large test tube cultures; other algae grown in continuous culture apparatus.

† Lyophilized cells grown under these conditions contained 48.3 % C, 6.7 % H, 9.4 % N, 4.4 % ash.

ent samples of cell suspension by washing twice in distilled water, transferring to tared crucibles in a minimum quantity of water, and drying to constant weight at 105° C. Rates of photosynthesis and respiration will be expressed in terms of Q_{O_2} with the usual dimensions of μl/hour × mg dry weight; apparent rates of photosynthesis will be cited without correction for respiration.

The growth conditions and characteristics of the cell suspensions studied are summarized in table I.

PHOTOSYNTHESIS

METHODOLOGICAL PROBLEMS: The experience of Emerson and Lewis (1) with Chroococcus and the preceding study of growth requirements of the algae used herein dictated an examination of the sodium and potassium relation in the buffers to be used. Initial experiments with *Anabaena variabilis* and *Anacystis nidulans* in 0.0125 M $KHCO_3$ plus 5 % CO_2 or in the $KHCO_3$-K_2CO_3 Warburg buffer No. 9 yielded similar results: rate of oxygen evolution decreased rapidly after a period of 20 to 30 minutes. Experiments with buffers of Na : K ratios 20 : 80, 50 : 50, and 80 : 20 gave photosynthetic rates which were steady and independent of the Na : K ratio. Subsequent studies on photosynthesis were conducted in equimolar Na- and K-buffer solutions.

Effects of centrifugation and washing were examined. Equivalent photosynthetic rates were achieved when the cells were centrifuged for one or three times and when they were unwashed or washed twice in the final buffer solution.

Three different buffer systems were examined: 1) 0.05 M phosphate at pH 5 saturated with 5 % CO_2, 2) 0.025 or 0.0125 M bicarbonate saturated with 5 % CO_2, and 3) Warburg buffer No. 9 containing 0.085 M bicarbonate plus 0.015 M carbonate. Measurements in the first two buffers require the indirect method utilizing two flasks with different liquid to gas ratios. Rates of photosynthesis observed in the three buffer systems for different cell preparations are presented in table II. Phosphate buffer at pH 5.0 supported rates only 30 to 50 % as great as those in the other buffers; it therefore was discarded for further work. Experience with Chlorella that lowered rates of photosynthesis are observed in the Warburg buffer is repeated again with *Anabaena variabilis*. However, *Anacystis nidulans* consistently shows higher rates of photosynthesis in the Warburg buffer than those observed in any other buffer system.

ASSIMILATORY QUOTIENT: Measurements of the assimilatory quotient ($AQ = -CO_2/O_2$) in bicarbonate-CO_2 buffer by the indirect method yield values at light saturation shown in table II. For *Anabaena variabilis* the AQ is typically 1.0; for *Anacystis nidulans*, about 0.9.

CONCENTRATIONS OF CARBON DIOXIDE, BICARBONATE, AND pH: Rates of photosynthesis of *Anabaena variabilis* and *Anacystis nidulans* were measured at light saturation in No. 2, 4, 6, 9, and 11 of the Warburg carbonate-bicarbonate buffer series at a total concentration 0.10 M (7, p. 178). The results are

TABLE II

PHOTOSYNTHESIS AT LIGHT SATURATION IN VARIOUS BUFFER SYSTEMS

ALGA	TEMP °C	GROWTH RATE, K	BUFFER	pH	Q_{O_2} **	AQ
Anabaena variabilis	25	0.30	0.05 M PO_4 *	5.0	74	...
			0.0125 M HCO_3 *	7.1	179	0.99
			0.025 M HCO_3 *	7.4	183	0.98
			Warburg No. 9	9.3	147	...
Anacystis nidulans	25	0.55	0.0125 M HCO_3 *	7.1	141	0.88
			Warburg No. 9	9.3	167	...
	39	1.00	0.0125 M HCO_3 *	7.1	264	0.90
			0.025 M HCO_3 *	7.4	262	0.89
			Warburg No. 9	9.3	309	...

Temperature indicated describes both growth and manometry.

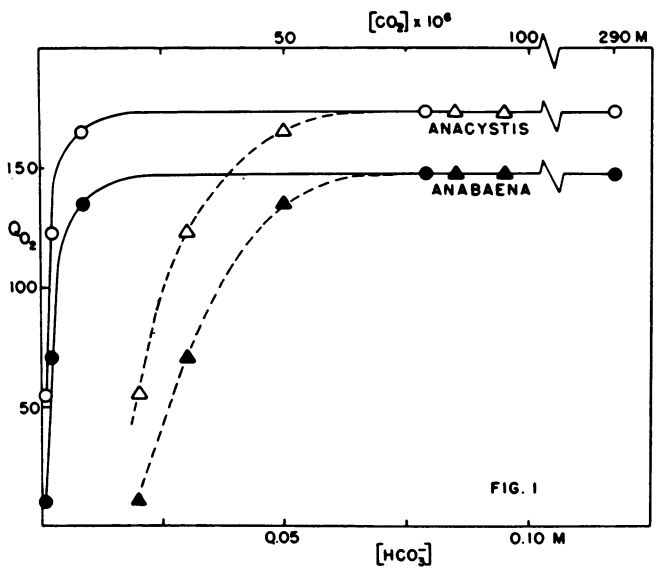
* Plus 5 % CO_2.

** Q_{O_2} has dimensions of μl O_2/hr × mg dry wt.

presented in figure 1; rate of photosynthesis is plotted in the conventional way as a function of concentration of free carbon dioxide and also as a function of bicarbonate concentration. Each point is an average of three or more closely agreeing replicate values assembled from different experiments.

In figure 2 the same data used in figure 1 are replotted as curve B describing rate of photosynthesis of *Anabaena variabilis* as a function of pH in the carbonate-bicarbonate buffer series. The curves A of figure 2 are constructed from experiments in bicarbonate-carbon dioxide buffers with the bicarbonate at 0.001 to 0.025 M in equilibrium with gas phase concentrations of 2.5, 5.0 and 10 % CO_2. In such buffers CO_2 are about the same as those observed in 0.05 M phosphate and 5 % CO_2 at pH 5 where the bicarbonate concentration is about 0.0001 M (cf table II). Comparison with the behavior of *Chlorella pyrenoidosa* is provided by curve C; rates are constant and maximum from pH 6.0 to 7.5 and are equal to that obtained in phosphate buffer at pH 4.5.

The data of figure 1 may be examined further with regard to the hoary controversy (cf 8, 10) whether free carbon dioxide or bicarbonate ion is the principal form taken up by the cells to meet their photosynthetic demand. Unfortunately, use of the Warburg buffer series is not expected to yield an unequivocal answer since there are five concomitant



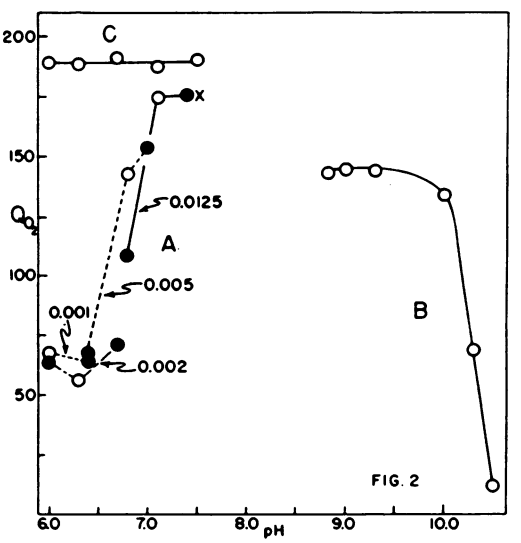


FIG. 1 (*left*). Rate of photosynthesis of *Anabaena variabilis* and *Anacystis nidulans* in Warburg buffers No. 2, 4, 6, 9, and 11 plotted as a function of carbon dioxide concentration (circles, upper scale) and as function of bicarbonate concentration (triangles, lower scale). Temperature 25° C.

FIG. 2 (*right*). Rate of photosynthesis of *Anabaena variabilis* and *Chlorella pyrenoidosa* as a function of pH. Curves A: Anabaena in bicarbonate of the molarity shown on the curves in equilibrium with 2.5 % CO_2, circle-dot; 5 % CO_2, open circle; 10 % CO_2, closed circle; X designates 0.025 M bicarbonate + 5 % CO_2. Curve B: Anabaena in the Warburg carbonate-bicarbonate buffers. Curve C: Chlorella in different concentrations of bicarbonate + 5 % CO_2.

both oxygen and carbon dioxide are exchanged with the gas phase. In any one experiment the amount of carbon dioxide bound as bicarbonate remains sensibly constant, the carbonate concentration is negligible, and therefore the carbon dioxide exchange accurately represents cell activity, so long as the bicarbonate concentration does not exceed about 0.025 M.

The overlapping curves A of figure 2 are interpreted as a single continuous curve demonstrating effects of lower pH on photosynthesis. Carbonate concentration is negligible. Overlapping rates are sustained by varying bicarbonate and carbon dioxide concentrations so that only their ratio, reflected in hydrogen ion concentration, becomes the controlling variable. It will be noted further that rates observed in 0.001 and 0.002 M bicarbonate and 2.5 to 10 % variables: the ionic strength and the concentrations of carbon dioxide and bicarbonate, carbonate, and hydrogen ion. If rates are treated as a function of free carbon dioxide only, the resulting "carbon dioxide curves" extrapolate to zero rate at zero concentration and show saturation at about 10^{-5} M equivalent to 0.03 % in the gas phase. If rates are treated as a function of bicarbonate concentration only, the resulting "bicarbonate curves" do not extrapolate to zero rate at zero concentration; further the concentrations required for saturation become far higher than those required in the bicarbonate-carbon dioxide buffers (cf fig 2). Rates might also be considered as inverse functions of hydrogen ion (cf fig 2) or carbonate ion concentrations but such treatment ignores the certain requirement of a supply factor for carbon to the cell.

Attempts were made to vary the total concentration of bicarbonate plus carbonate in buffer mixture No. 4 in the steps 0.06, 0.10, and 0.20 M. The plan of experimentation failed because of prolonged variations in rate of 0.20 M which occurred also when the lower concentrations were brought up to 0.20 M by use of chloride and sulfate as substituent ions. At 0.10 M rates were constant over the entire 90 minute period of observation; at 0.06 M the rates stabilized after about 30 minutes. At 0.06 M the observed rate was 80 % of that in 0.10 M although the bicarbonate was 60 % and the carbon dioxide 56 % as great. These observations lead to some suspicion of untoward effects of the rather high total concentration of the Warburg buffer system.

However inconclusive the results, they in no way speak against the general proposition of Rabinowitch (8, p. 891): that as a first approximation the rate of photosynthesis is a function of concentration of free carbon dioxide in the suspending medium. Such a conclusion becomes significant only because the algae studied are adapted to an alkaline environment and grow only in a limited pH range (ca 7.2 to 9.2) where the bicarbonate concentration is 10 to 1000 times greater than that of free carbon dioxide (2).

Light Intensity Curves: Rate of photosynthesis in Warburg buffer No. 9 as a function of light intensity is shown by the curves of figure 3. Light saturation of the three blue-green algae is achieved within a range of light intensities similar to that required by Chlorella. However, the shape of the light-saturation curve in the blue-greens depends upon previous culture conditions, particularly the light intensity, as previously shown for Chlorella (5). The phenomenon is demonstrated by the two curves for *Nostoc muscorum* G. Further examination of effects of temperature and light intensity of growth on cell pigment concentrations and light intensity curves of photosynthesis will be the subject of a later report.

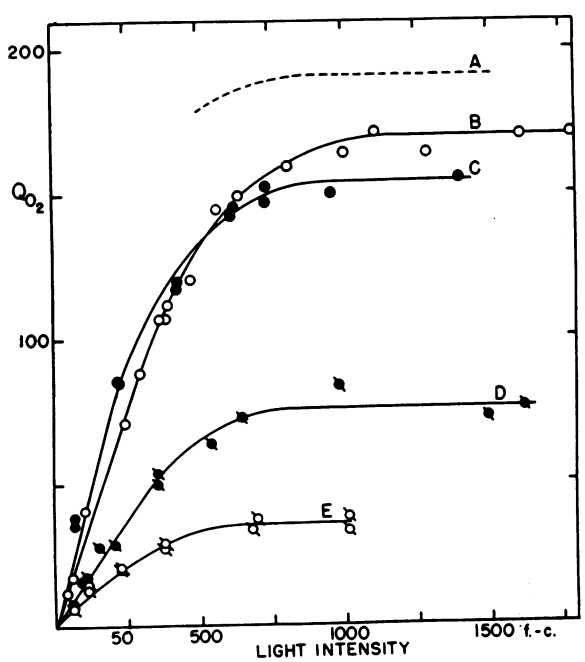


Fig. 3. Light intensity curves of photosynthesis at 25° C. A, *Chlorella pyrenoidosa*, k = 0.42. B, *Anacystis nidulans*, k = 0.55. C, *Anabaena variabilis*, k = 0.30. D, *Nostoc muscorum* G, k = 0.43. E, *Nostoc muscorum* G, k = 0.12. Values of k indicate the previous specific growth rate of the cells used.

Maximum rates of photosynthesis of *Anabaena variabilis* and *Anacystis nidulans* at 25° C are comparable to that of *Chlorella pyrenoidosa* at 25° C. At 39° C *Anacystis nidulans* has a maximum rate of photosynthesis far greater than that of *Chlorella pyrenoidosa* and greater than that of any organism yet reported except for a high-temperature strain of Chlorella (9). The photosynthetic rates of the three species substantiate the previous conclusion from growth studies (2) that the blue-green algae are not to be considered a metabolically sluggish group.

Respiration

For respiration studies two kinds of cell preparations were used. *Growing cells* were harvested from a growing culture and prepared for manometric experiments in a minimum time (35 to 45 minutes). *Starved cells* were obtained by aerobic incubation of harvested suspensions in darkness for 24 hours and at the same temperature used for growth and manometry.

The suspending fluid chosen for respiration measurements was 0.5 M phosphate buffer at pH 7.8. Equal rates were observed in 0.05 M phosphate buffer at pH 4.5, 6.0, and 9.0, in K, Na, or Na : K = 1 : 1 salts, or in original medium C. As also reported by Webster and Frenkel (11) for *Anabaena variabilis*, the pH and Na : K ratio effects so pronounced for photosynthesis are negligible for respiration in the three algae studied here.

Endogenous Respiration: Values for rates of endogenous oxygen uptake and respiratory quotients are given in table III. All three species show a rapid decrease in rate of respiration during the first 24 hours of dark starvation (growing vs starved cells) although there is only a slight decrease observed during the first hour of a manometric experiment. For *Anabaena variabilis* and *Nostoc muscorum* G the rates are within the range observed for *Chlorella pyrenoidosa* grown under similar conditions. Particularly striking, therefore, is the low rate of *Anacystis nidulans* grown at 25° C; here rate of respiration is less than 1/100 of the rate of photosynthesis. The latter characteristic is a recommendation for use in quantum yield studies. The respiratory quotients are close to unity and somewhat higher than the value 0.9 observed by Webster and Frenkel (11) for *Anabaena variabilis*.

Exogenous Respiration: Based upon past experience with other algae, starved cell suspensions were used for the examination of respiratory response to

added substrates. Any effect of added substrate is more pronounced when the endogenous rate is first reduced to a low level. In this respect starved cells of algae become similar to resting cells of bacteria. The data of table IV show that starved cell preparations of the three algae exhibit limited response to addition of glucose, fructose, and sucrose, and small responses to some of the other carbon sources tested. Experiments at pH 4.5 and 6.0 failed to enhance any of the responses shown. When added to growing cells, fructose causes no increase in respiration but does slow down the decay in rate with time.

The three algae used have been shown to be obligate photoautotrophs in the sense that none of a long list of organic compounds will support growth in the dark (2). It is considered significant, therefore, that none of the substrates examined will raise the respiratory rate of a starved cell preparation to the level of endogenous respiration shown by photosynthetically growing cells. What may be a similar case has been reported by Lewin (4); the diatom *Navicula pelliculosa* will oxidize succinate, lactose, acetate, pyruvate, and citrate but fails to grow on any of these substrates in the dark. In contrast, *Chlorella pyrenoidosa*, which can grow heterotrophically in the dark, has a rate of respiration on exogenous glucose some four times greater than that of cells harvested from a photosynthetically growing suspension.

In the case of the three blue-green algae studied here it is plausible to postulate permeability restrictions as the limitation to obligate autotrophy. In the case of *Chlamydomonas Moewusii* Lewin (3) has rejected this explanation, and perhaps correctly so, on the grounds that "compounds such as acetate, pyruvate, or succinate enter the cells and are readily oxidized in the dark." However, quantitative rather than qualitative data are necessary before an obligate character of autotrophy can be attributed to any deep-seated biochemical inadequacy. It is probable that there are organisms which would show respiratory increase to added organic substrate and utilization of such substrate to partially or completely maintain their basal endogenous metabolism, yet be prevented from growth on the substrate merely by a restricting rate of permeability. This line of reasoning leads to the likelihood that obligate autotrophy, aside from the matter of formal definition, is a matter of degree rather than an all-or-none phenomenon. The obligate character of autotrophy in the three blue-green algae, in *Navicula pelliculosa*, and in *Chlamydomonas Moewusii* may be *partial* in the sense that a part, but not all, of their carbon requirement for growth may be supplied by organic substrates.

TABLE III

ENDOGENOUS RESPIRATION

ALGA	TEMP °C	GROWTH RATE, K	CONDITION OF CELLS	Q_{O_2}	RQ
Anabaena variabilis	25	0.30	Growing	8.4	1.10
			Starved	1.7	1.00
Anacystis nidulans	25	0.55	Growing	1.6	1.00
			Starved	0.3	0.93
	39	1.00	Growing	4.7	1.10
			Starved	1.9	1.10
	39	2.50	Growing	7.5	1.05
			Starved	2.9	...
Nostoc muscorum G	25	...	Growing	4.4	...
			Starved	1.1	...

All measurements in 0.05 M phosphate at pH 7.8. Temperature indicated describes both growth and manometry. Q_{O_2} has dimensions of $\mu l\ O_2/hr \times mg$ dry weight of cells.

TABLE IV

RESPIRATORY RESPONSE TO ADDED SUBSTRATES

SUBSTRATE	ALGAE		
	ANABAENA VARIABILIS	ANACYSTIS NIDULANS	NOSTOC MUSCORUM G
	Temperature		
	25° C	39° C	25° C
	Endogenous Q_{O_2}		
	1.96 ± 0.10	28.9 ± 0.28	1.05 ± 0.06
	Exogenous Q_{O_2} *as % of control*		
Glucose	221	130	123
Fructose	195	157	142
Sucrose	166	98	150
Mannose	100	96	99
Maltose	136	...	100
Xylose	101	...	100
Galactose	98	99	...
Lactose	98	...	...
Glucose-1-PO_4	118	...	...
Succinate	130	...	123
Malate	148	...	98
Citrate	63	...	51
Acetate	104	...	...
Butyrate	42	...	...

In 0.05 M phosphate at pH 7.8; all substrates added to give 0.1 M except acetate and butyrate, 0.01 M.

DISCUSSION

The data reported provide description of characteristics of respiration and photosynthesis of three blue-green algae. As viewed in the light of more extensive knowledge of physiology of the Chlorophyceae, no startling differences are observed. In terms of the gross characteristics of elementary analysis, growth rates (2), rates of photosynthesis, and responses to light intensity and carbon dioxide concentration, the three blue-green algae are no more different from *Chlorella pyrenoidosa* than might be expected of other species of Chlorella. Only in their very limited responses to exogenous organic substrate are these algae markedly different from most of the Chlorophyceae which have been studied.

In relation to studies on photosynthesis significance lies in the presentation of *Anacystis nidulans*

as a new experimental organism with many desirable characteristics. Its cells are small ($\sim 2\ \mu$), it may be grown as easily and reliably as Chlorella, it contains phycocyanin as an auxiliary photosynthetic pigment, it can be studied over a wide range of temperatures up to 41° C, it shows no inhibitory effects of the Warburg buffers, and it has an unusually low rate of dark respiration.

Summary

Photosynthesis and respiration of three blue-green algae, *Anabaena variabilis, Anacystis nidulans,* and *Nostoc muscorum* G, have been studied by conventional Warburg manometry.

Rates of photosynthesis at light saturation were measured in the Warburg series of carbonate-bicarbonate buffers and in bicarbonate-carbon dioxide buffers in the pH range 6 to 10.5. Evidence was sought, but not found, that rate of photosynthesis is any simple function of bicarbonate concentration. In terms of their light intensity curves the three algae show no feature markedly different from those of Chlorella. Limited response of respiration to added organic substrates is considered related to the obligate photoautotrophic character of the three algae.

Attention is directed to *Anacystis nidulans* which has several features making it particularly useful as an experimental organism for studies on photosynthesis.

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PERSISTENT RHYTHMS OF O_2-CONSUMPTION IN POTATOES, CARROTS AND THE SEAWEED, FUCUS[1,2]

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It has been widely known for a long time that numerous plants and animals normally display overt daily rhythms of various processes and, if they live in the littoral regions of the seas, have also primary lunar cycles (tidal) of various physiological processes. In general, it has been customary to think of these as direct responses to rhythmic external factors. Similarly, the numerous instances of semi-lunar and lunar breeding cycles, common for marine animals and plants, have generally been considered to be simple responses to illumination or tides, or some combination of these.

It is gradually becoming increasingly apparent, however, that daily rhythmicity in animals possesses an important persistent aspect. The rhythmic changes persist, more or less temperature-independent as to frequency, under constant conditions of light, temperature, and humidity. It is also becoming evident that there are similarly temperature-independent persistent rhythms of primary lunar frequencies.

Beliefs as to relationships between lunar phases and human and other organismic behavior are as old as the beginnings of the human cultural heritage, but these have seldom found solid scientific support. Even when finally supported and used to predict accurately such phenomena as occurrence of plankton pulses of the Illinois River (18), the swarming of the grunion (24), or the palolo worm (17) the lack of an adequate explanation has usually left the matter in the hands of the naturalist rather than the experimentalist.

Biologists who have had occasion to concern themselves with the measurement of O_2-consumption in

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