POTENTIAL HETEROTROPHY IN A NATURAL POPULATION OF *OSCILLATORIA AGARDHII* VAR. *ISOTHRIX* SKUJA[1]

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ABSTRACT

Direct evidence is presented from radioautography that four species of blue-green algae can take up glucose-^{14}C and acetate-^{14}C at the low concentrations known to occur in lake water. The kinetics of glucose-^{14}C uptake by *Oscillatoria agardhii* var. *isothrix* Skuja demonstrate that uptake is metabolic. The quantitative importance of such uptake in the development of the algal population cannot be assessed from the data, but the fact that *O. agardhii* develops massive densities in the microaerobic zone of many lakes suggests that heterotrophy may be very important to this alga and perhaps to other species occupying this habitat. The phenomenon may occur generally since *O. agardhii* and lakes with clinograde oxygen curves have worldwide distributions.

INTRODUCTION

Interest in the possibility of heterotrophic metabolism of soluble organic substrates by marine and freshwater phytoplankton has resulted in data providing no definitive proof of algal heterotrophy in natural populations and suggesting that if it does occur it is of very limited importance. The presumption that it would be broadly distributed in nature comes mainly from the fact that many algae are able to grow in culture in the dark with organic substrates as the sole source of carbon and energy (Saunders 1957; Danforth 1962; Provasoli 1963). In these cultures the substrate concentration has been very much greater than that in natural waters, the approximate values of which are 10^{-7} M for amino acids and acetate and 10^{-8} M for glucose (Chau and Riley 1966; Degens et al. 1964; Hobbie and Wright 1965; Hobbie et al. 1968; Siegel and Degens 1966). Such low concentrations of organic substrate would require active concentration mechanisms for their uptake by algal cells. Wright and Hobbie (1966) have argued that algae may take up organic substrates according to diffusion kinetics and that indigenous aquatic bacteria are so effective in keeping concentrations low that they prevent heterotrophy in most forms of planktonic algae. However, Hellebust and Guillard (1967) have demonstrated uptake of amino acids at 10^{-4} M by a diatom in the light and provide evidence that the uptake, specific for amino acids, is probably an active process. North and Stephens (1967) show the uptake and assimilation of 10^{-6} to 10^{-7} M amino acids by pure cultures of *Platymonas* in the light and Bunt (1969) has evidence for the photoheterotrophic assimilation of lactate and glucose by a marine diatom. There is no evidence that amino acids at low concentrations taken up by algae in the dark support growth (Hellebust and Guillard 1967).

Sloan and Strickland (1966) believed it virtually impossible for heterotrophic metabolism to be important to marine phytoplankton, considering the conditions that prevail in the sea and the general results from culture experiments. Fogg (1969) also believed that heterotrophic growth of blue-green algae is unlikely since there is extensive evidence that even nonplanktonic forms of blue-greens are obligate phototrophs, but he stated that photohet-

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erotrophic assimilation may be important at low light intensities (*see below*).

In spite of the lack of evidence for heterotrophic assimilation of organic substrates by phytoplankton, most authors are careful to leave such a possibility open.

I have screened lakes in both winter and summer for the uptake of simple organic substrates by phytoplankton. With the exception of a few species, and a few doubtful cases, I have been unable to identify uptake of organic substrates in the dark by the phytoplankton. I used radioautography as the analytical tool (Saunders 1958). These results confirm that assimilation of organic substrates in the dark by phytoplankton appears not to be a general phenomenon; if it does occur it is quantitatively not important for most phytoplankton.

I want to present recent evidence that there are algae that do have the capacity to assimilate simple organic substrates in the dark at the low concentrations known to occur in natural waters. These algae occur in the interphase of aerobic and anaerobic zones in certain kinds of lakes that are widely distributed geographically. This interphase may occur near the bottom of the photic zone or in the aphotic zone.

Oscillatoria agardhii var. *isothrix* Skuja was the experimental organism used because its population is dominant throughout summer in Frains Lake (Michigan), constituting 80–95% of the phytoplankton biomass at the depths of its maximal development. Other species were tested but were technically much more difficult to use.

METHODS

General procedure

Lake water is placed in 300-ml BOD bottles and ^{14}C-labeled organic substrates added in known amounts. (Radioglucose was uniformly labeled. Radioacetate was labeled in the 1 position.) The bottles are wrapped in aluminum foil to keep out light and normally also in cloth to prevent tearing of the foil and then suspended at the depth of sampling to maintain normal temperature conditions. The samples are incubated for various lengths of time, usually 4–6 hr, and then removed from the lake. Duplicate 1- or 2-ml samples are filtered through a HA Millipore filter, the filter washed with 10 ml of distilled water, and then subjected to radioautographic procedures to confirm uptake or its lack by the phytoplankton.

In early assays, when higher concentrations of added substrate were used, sampling was conducted during the day so that samples were exposed for 1 or 2 min to daylight intensity before being maintained completely in the dark. In more recent assays, where very low concentrations of added radiosubstrate were used, all sampling and preparation was completed before dawn. This prevented the algae from developing any reductive power, through exposure to light, that might facilitate uptake of organic substrate.

Kinetics of glucose uptake

Samples of lake water from 5.0 m, in 300-ml BOD bottles wrapped as described above, were returned to the laboratory and placed in a dark room at 10C, slightly lower than the lake water temperature. Glucose-^{14}C(U) was added to a series of bottles in concentrations of about 1, 5, 10, 20, and 43 μg liter^{-1}. Subsamples of 2 ml were filtered through HA Millipore filters after 0.5, 2, 4, 8, 12.5, 24, and 53 hr, washed with 10 ml of distilled water, and subjected to radioautography. After development, the silver grains in 200 unit areas above an *Oscillatoria* filament and 200 unit areas above the surrounding filter were counted at 2,400 magnification. The number of silver grains per unit area is a function of the radioactivity in the material underlying the film emulsion. The surrounding silver grain count was considered to be background from radioactivity adsorbed to the filter or to detritus and assimilated by bacteria on the filter. Because of differences in the geometry of the sources of radioactivity on the filter, it is not possible to use the silver grain

TABLE 1. *Assay for substrate assimilation by blue-green phytoplankton*

	Concn (μg liter^{-1})	Radioautogram
Glucose-^{14}C		
Oscillatoria agardhii	0.00	-
	1.95	+
	3.06	+
	4.90	+
	6.12	+
Arthrospira sp.	1.95	-
	4.90	+
	6.12	+
Oscillatoria utermoehlii	4.90	+
	6.12	+
Oscillatoria sp.	1.95	+
	4.90	+
Anabaena flos-aquae	2,270	+
	6.12	-
Aphanizomenon flos-aquae	2,270	+
	6.12	-
Acetate-^{14}C		
Oscillatoria agardhii	6.92	+
	48.3	+
Arthrospira sp.	6.92	+
	48.3	+
Oscillatoria sp.	6.92	+
Aphanizomenon flos-aquae	6.92	-

counts quantitatively in an absolute sense but only relatively for the same species population. However, the kinetics of silver grain production can be used to evaluate the nature of glucose uptake by *O. agardhii.*

Radioautography

Microscope slides were treated with a smear of warm 1–2% gelatin solution; the dry Millipore filter preparations were placed on it and became fixed to the slide.

Autoradiographic stripping film (Kodak A.R. 10) was floated over the filter in the recommended manner. The preparations were dried in a cool stream of air and placed in a plastic microscope slide box, taped to exclude light and moisture. The slides were incubated for 2 weeks at 4C and the films then developed according to recommendations.

After drying, diametrically opposed portions of the filter and film on the long dimension of the slide were cut away with a razor blade. Cedarwood oil was applied at these cuts so it could migrate through the filter under the film and clear the filter. Silver grains were then counted as described above or the film was merely examined for radioautograms. If the radioautograms are not too dense the underlying organisms can be identified directly. If the density of grains is heavy, statistical means may be used to identify the underlying organism provided there are sufficient numbers of organisms in the sample (Saunders 1958).

RESULTS

Uptake of glucose at high and low concentrations

The very common phytoplankters *Anabaena flos-aquae* and *Aphanizomenon flos-aquae* are able to take up glucose added to lake water at concentrations very much greater than are known to occur in lakes (Table 1). However when glucose is added at approximately natural concentrations, neither species can assimilate glucose in quantities sufficient to produce radioautograms. This is taken as presumptive direct evidence that these algae cannot assimilate glucose at the very low concentrations that usually occur in lake water. *Aphanizomenon flos-aquae* also cannot assimilate acetate at approximately natural concentrations (Table 1).

Uptake of organic substrates at natural concentrations

Four species of blue-green algae, *O. agardhii* var. *isothrix* Skuja, *Oscillatoria utermoehlii, Oscillatoria* sp., and *Arthrospira* sp. can assimilate glucose at natural concentrations (Table 1). Since *O. agardhii* occurs in and may migrate from sulfuretted waters, samples without added

radioactive substrate were assayed for radioautograms. The results are negative; silver grains above a filament are thus not the result of any chemical reaction between the algal cells substance and the film emulsion.

When a water sample is shaken, filtered, and washed immediately after a small amount of radioactive substrate is added, the assay is negative. Therefore positive assays are not the result of adsorption of radioactive substrate.

When the sample is incubated for a period of time in the dark, radioautograms are positive at the concentrations indicated in Table 1. *Arthrospira* sp. apparently is not as effective as the other algae in assimilating glucose.

When the radioautograms were weak, silver grains above the filament and above the surrounding filter were counted to confirm statistically that there was indeed a difference and that the radioautograms were positive.

Three of the species were also able to assimilate acetate; *O. utermoehlii* was not present in the samples assayed for acetate uptake.

The results clearly demonstrate that at least these four species of planktonic algae can assimilate simple organic substrates in the dark at concentrations approximating those in natural waters.

Kinetics of uptake in Oscillatoria agardhii

To confirm that the observed uptake was a metabolic process, I followed the kinetics of uptake in time series with different concentrations of added radioglucose, estimating uptake by counting silver grains. The shapes of the uptake curves (Fig. 1) are the net result of a number of processes, including the loss of isotope from the aqueous phase, the assimilation of glucose by *O. agardhii*, the disposition of the label in various compartments within the alga, and the respiration of some of the assimilated glucose by the alga. Background counts are included in

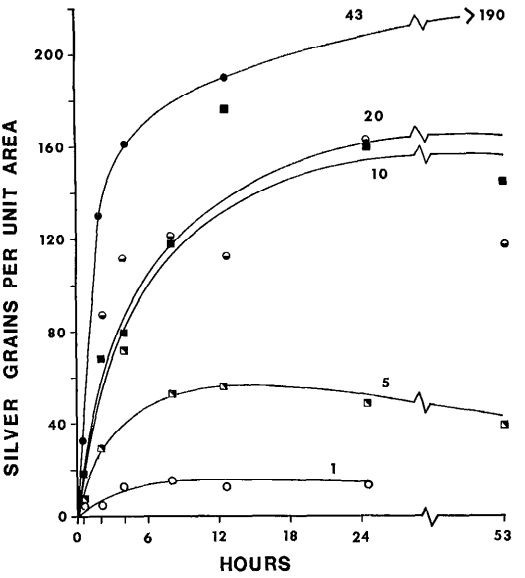

FIG. 1. Total silver grain count per unit area of filament in *Oscillatoria agardhii* by uptake of glucose-^{14}C at 1, 5, 10, 20, and 43 μg liter^{-1}.

the total counts. The time to reach an apparent equilibrium is much too long for a simple adsorptive process. I have examined the adsorption kinetics of radioglucose and radioactive starch by seston killed in 2.5% Formalin; equilibrium is reached in a few minutes.

The uptake of radioglucose added at 1 μg liter^{-1} cannot be discriminated from the background and it is not possible to prove that glucose was assimilated by *O. agardhii* at this concentration. However, since differences in the geometry of the alga and the other particles in the surrounding area could cause the silver grain counts for *O. agardhii* to be somewhat low, it is probable that *O. agardhii* did assimilate radioglucose even at this low concentration.

The kinetics of uptake are highly presumptive evidence that glucose is assimilated metabolically by *O. agardhii* in the dark in lake water as opposed to some physical process such as adsorption. The shapes of the curves and the time to reach an apparent equilibrium suggest that uptake is not the result of a simple or com-

plex exchange equilibrium, although such phenomena are not absolutely disproved.

Importance of dark assimilation of glucose in the metabolism of Oscillatoria agardhii

An attempt to assay Frains Lake water for glucose by a dilution technique suggested by Wright and Hobbie (1966) and used with success by Allen (1969) was unsuccessful. However, I did make estimates of $(K_t + S_n)$ for depths at which *O. agardhii* occurred. The substrate concentration is S_n; the index of affinity of the organism for substrate is K_t. The values for 4 assays were 10.7, 32.3, 48.4, and 54.2 μg glucose liter^{-1}. If K_t in these assays was small, as Hobbie and Wright (1965) have found, there was considerable glucose present in the lake water. On the other hand, if the assay is not valid in any sense, it is possible that there was no glucose at all in the lake water.

In that case, we can say that *O. agardhii* can assimilate glucose from lake water even when it is present in the order of 10^{-8} M. If glucose was present at concentrations approximating the values of $(K_t + S_n)$ given above, we can say that *O. agardhii* was assimilating glucose from the lake water in the experiments discussed.

My original method for discriminating bacterial and algal assimilation of organic substrates (Saunders 1958) is not valid at low concentrations of substrate because most of the substrate is metabolized by the bacteria. This means that the method must discriminate a small difference. The variance of the estimate of this small difference is large and the estimates are unreliable. I have not been able to separate the algal and bacterial uptake of glucose by other methods, even though bacteria are not normally attached to *O. agardhii*.

Therefore it is not possible to assess quantitatively the importance of chemoorganotrophic assimilation of glucose in *O. agardhii* in these experiments.

DISCUSSION

Obligate phototrophy is widely distributed among the algae. It is known to occur in the Chlorophyta, Xanthophyceae, Cyanophyta, Euglenophyta, Pyrrophyta, and Chrysophyta (Danforth 1962). That some algae can grow in the dark on organic substrates has been demonstrated many times in culture (Danforth 1962; Saunders 1957). Substrate concentrations, however, are 10^3 to 10^5 times greater than have been found in uncontaminated fresh and marine waters. Kinetic evidence provided by Wright and Hobbie (1966) suggests that phytoplankton in competition with bacteria for organic substrates in natural waters would be unable to assimilate these substances. Definitive proof that this is true for two common species of phytoplankton, *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*, is given above. It is obvious that a large number of species of phytoplankters are incapable of chemoorganotrophy or cannot function as chemoorgantrophs under the usual conditions prevailing in natural waters. The question remains whether there is a special group of phytoplankton species that is heterotrophic in natural waters, composed of species that can assimilate organic substrates in the dark, or of those incapable of assimilating organic substrates in the dark but capable of photoassimilation. Fogg (1969) has recently discussed the latter possibility and its implications.

The data presented here for *O. agardhii* var. *isothrix* show conclusively that this alga can assimilate glucose *in situ* in the dark at approximately natural concentrations. It can also assimilate acetate. Three other blue-green algae are able to assimilate glucose in the dark and two of these acetate also. All of these species occur in a widely distributed yet rather specialized habitat. They occur maximally in the interphase between aerobic and anaerobic conditions. Although their distributions are somewhat different, all extend downward into waters containing hydrogen sulfide. The communities of plankton oc-

curring in such conditions were first described in detail by Utermöhl (1925) for East Holstein lakes. These species of algae do not always occur in this general type of habitat. If chemoorganotrophy is characteristic for some phytoplankton in this general habitat, one would expect to find additional species capable of this kind of metabolism in other lakes where the community structure is different. Three of the four species can develop population densities of several million filaments per liter. By midsummer, in Frains Lake, the biomass of phytoplankton in this habitat can greatly exceed that in the epilimnion. It is also obvious that each species has a rather narrow range of environmental tolerance in the lakes that I have examined, although the specific nature of these requirements is not known.

The metabolic behavior of *O. agardhii*, and similar algae, may be quite varied during its population development. It is not possible to discuss this behavior from a quantitative point of view, but it is possible to postulate what this behavior in Frains Lake might be.

It is highly probable that in the early stages of its population development, when light intensity is extremely low, *O. agardhii* var. *isothrix* is chemoorganotrophic. In winter in Frains Lake this species occurs in the surficial sediments in the deep water where the light intensity is $<10^{3}$ lux on a clear, bright midsolar day. When anaerobiosis develops in the hypolimnium in late spring, *O. agardhii* appears in the bottom water and migrates upward as anaerobiosis intensifies. I have not been able to detect photosynthesis in *O. agardhii* at near bottom depths. The other three species of blue-green algae also migrate upward but tend to develop maximum concentrations at depths slightly greater than *O. agardhii*.

If *O. agardhii* attains a depth where low light intensity is sufficient it may begin to supplement chemoorganotrophy with phototrophy during the day. However chemoorganotrophy will continue to occur at night so that there can be a source of energy and carbon throughout the 24-hr period. Fogg (1969) cited evidence that at light intensities that are limiting, photoassimilation does increase growth rates of blue-green algae, although photoassimilation of organic substances at light saturation does not produce higher growth rates than would be achieved with carbon dioxide alone.

Oscillatoria agardhii in Frains Lake and certain other lakes has its maximum concentration where there is usually less than 1 ppm of oxygen. During summer, after it has migrated to its minimum depth, it no longer occurs in the upper and middle part of the photic zone so that its maximum density is always where light intensity is very low. It never produces the metalimnetic oxygen maxima that Eberly (1959, 1964*a*, *b*) has observed in Indiana lakes. Estimates of photosynthesis at this depth are below the limits of sensitivity of the oxygen method and are very low using the ^{14}C method. The latter estimates are complicated by the fact that sometimes photosynthetic bacteria are present in the samples and it is not possible to separate completely the bacterial and algal communities. There is also some evidence that photoassimilation of glucose, glycine, and acetate may occur in *O. agardhii* (Saunders 1958 and unpublished).

If *O. agardhii* now attains shallower depths and higher light intensities, it may become completely photolithotrophic, the heterotrophic mechanisms being repressed. At even higher light intensities, large amounts of oxygen may be produced and metalimnetic oxygen maxima observed. *Oscillatoria agardhii* is excluded from the upper photic zone in Frains and other lakes during summer. Baker et al. (1969) provided evidence that high light intensities depress its net photosynthesis; although this may be a contributing factor it should be possible for this alga to adapt to high light intensities. In Frains Lake the upper distribution limit of *O. agardhii* does not exceed 1,076 lux, much less than at the depth of optimal photosynthesis observed by Baker et al. (1969). Apparently

some other factor must more directly control the distribution of *O. agardhii* and the effects of light may be secondarily imposed at the upper distribution limit when high intensities prevail.

If it is true that both dark assimilation and photoassimilation of organic substrates by *O. agardhii* do occur, then the quantitative aspects of organotrophy may be quite complicated. *Oscillatoria agardhii* can assimilate glucose and acetate at low concentrations. It can also assimilate glycine in the dark at 1.0 mg liter^{-1} (10^{-4} M) (Saunders 1958); I have not tested for assimilation of glycine or other amino acids at concentrations of natural waters. It is apparent from Fig. 1 that the uptake mechanism for glucose is not saturated at the concentrations used in this experiment. This means that the rate of uptake of glucose, and other substrates that do not saturate the uptake mechanisms, will be a function of the substrate concentration and should vary in the same manner as the reaction rate for enzyme-substrate kinetics expressed by the Michaelis-Menten equation. The rate of release of soluble organic substrates in the upper photic zone varies during the day (Saunders 1971*a*, *b*). It is highly probable that this is true at other depths and also that the relative rate of release of different substrates is not constant. If a heterotroph can use several different substrates, it can rely on one while the concentration of another is low. Such metabolic flexibility would ensure a maximal supply of energy and carbon under marginal conditions of existence.

If one substrate is saturating to the total heterotrophic metabolism of the organisms, other substrates would become subsidiary in importance or might not be utilized at all, as has been shown by Samejima and Myers (1958) for *Chlorella pyrenoidosa*. However, this is not likely to occur in the dilute media constituted by lake waters.

Once the population rises in the water column to some threshold of light intensity, photoassimilation may enhance the net heterotrophic assimilation of organic carbon and increase the growth rate. In this second marginal region of existence the alga would still rely on chemoorganotrophy during the night and on dark dull days, when light might be below the threshold value. Once the controlling environmental conditions permit the algae to move into a zone of higher light intensity, photoheterotrophy might be depressed and the algae could ultimately become completely photolithotrophic.

If this story proves to be fundamentally correct, it elucidates a phenomenon that is probably widely distributed and more or less well developed in a certain class of lakes. The suggested mechanisms provide a means of populating such habitats with a massive development of phytoplankton that could not be achieved by what might be considered more conventional phytoplankton. Large populations of purple and green photosynthetic bacteria are excluded from this habitat by virtue of their requirement for hydrogen sulfide, although there may sometimes be some overlap in the distributions. There is also a whole set of more specific implications relating to fine control mechanisms that operate to determine the different distributions of the various phytoplankton species occurring in this specialized habitat.

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