Marine Biology 33, 175-183 (1975)
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The Trophic Role of Glycolic Acid in Coastal Seawater. I. Heterotrophic Metabolism in Seawater and Bacterial Cultures

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Abstract

Glycolic acid, a known algal excretory product, represents a potentially important energy source for heterotrophic bacteria in marine waters. Measurements of heterotrophic uptake and mineralization by the natural microbes in the plankton indicate quantitative use of glycolic acid comparable to other common microbial substrates. This activity shows vertical and horizontal variations that correlate in a general way with primary productivity. Glycolic acid appears to be present at higher concentrations than other low molecular weight substrates, and it also shows a lower rate of turnover. It is mineralized (respired) to a greater extent than other recorded substrates, an average of 70% of total uptake. Two-thirds of a total of 141 colonies of bacteria cultured from seawater on marine agar proved capable of taking up and respiring glycolic acid. These same bacteria, however, were unable to utilize glycolic acid for growth when it was the sole carbon source. Glycolate may therefore occur at higher concentrations and show erratic fluctuations in natural waters because microbial use is not tied to glycolate production but to the presence of other substrates. The fact that so many bacteria are able to metabolize it indicates a possibly important trophic role as an energy source. It is suggested that glycolic acid may be a major source of energy for active transport of other substrates by marine bacteria.

Introduction

Heterotrophic use of dissolved organic compounds represents a portion of energy flow in aquatic ecosystems that is very difficult to quantify. Because of the great variety of organic compounds, and the relatively laborious methods required to measure concentrations and heterotrophic use of individual compounds, a total accounting for this portion of the food web will not be available for some time. The most obvious compromise for this rather forbidding task would seem to be to carefully research the production, ambient concentration, and heterotrophic use of one compound or a family of compounds in a well-defined ecosystem. Accordingly, we have undertaken a series of studies of glycolic acid ($CH_2OHCOOH$), a compound for which data from natural environments has been largely lacking but which gives every indication from laboratory studies of being potentially significant.

Glycolic acid has received much attention over the years from plant physiologists because of its role in photosynthesis and photorespiration. Recent work summarized by Tolbert (1974) has clarified glycolate metabolism in algae. This work indicates that glycolate formation involves oxidation of the CO_2-acceptor ribulose diphosphate in a competitive reaction between O_2 and CO_2 for the common substrate ribulose-diphosphate. After its formation glycolate may then be excreted or involved in further metabolic pathways. Because of its involvement in this fundamental reaction of photosynthesis, glycolate excretion is probably geologically as old as algal photosynthesis itself. The literature indicates that glycolate is the most common and often most abundant excretory product of algae (Hellebust, 1974; Tolbert, 1974). For marine waters, excreted organic carbon constitutes approximately 25% of the carbon fixed by phytoplankton photosynthesis (Anderson and Zeutschel,

1970; Samuel *et al.*, 1971; Choi, 1972). An additional very significant source of dissolved organic compounds in inshore waters is the marine seaweeds (Khailov and Burlakova, 1969; Sieburth, 1969). How much of this is glycolate is not known, but in view of the role of glycolate in photosynthesis and excretion, it would appear to be a logical choice for a study of the importance of excretory products to heterotrophs in the plankton.

In 1973, Shah and Fogg described a new method for determining glycolic acid in seawater, giving impetus for our present work. This work will be presented in a series of papers on the occurrence and role of glycolic acid in seawater. The present paper is the second in the series. In the first paper (Shah and Wright, 1974), we described some improvements to the method of Shah and Fogg and gave some initial data on sea-water concentrations of glycolic acid. Over a 6-month period, the concentrations ranged between 0 and 40 µg/l in the open waters of Ipswich Bay, Massachusetts, USA, while higher concentrations (up to 80 µg/l) were found in inshore waters. Seasonal changes in concentration indicated a general correlation with phytoplankton biomass.

The purpose of the present paper is to give the first results of studies combining measurements of glycolic acid and of heterotrophic activity on glycolic acid in coastal seawater. These studies include a comparison with other substrates, spatial variations in heterotrophic activity, and capabilities of bacteria cultured from seawater to metabolize glycolic acid. They have as their goal an understanding of the importance of glycolic acid to the natural heterotrophic bacteria. The next paper in the series will deal with the flux of glycolic acid over the course of 1 year in Ipswich Bay, while the fourth paper will present the effects of enrichment with glycolic acid and other substrates on heterotrophic uptake and bacterial growth.

Materials and Methods

Sampling was performed in two coastal areas of the Gulf of Maine: Ipswich Bay and the Essex River estuary in northeastern Massachusetts, and the Boothbay region on the central Maine coast. Fresh sea-water samples were collected with scrubbed Van Dorn bottles and brought to the laboratory as quickly as possible; chemical assays and heterotrophic measurements were begun within 2 h of sample collection. Samples were incubated within 1C° of *in situ* temperature unless otherwise noted.

The measurement of heterotrophic uptake and mineralization of ^{14}C-glycolic acid was performed with the method of Wright and Hobbie (1965, 1966) and Hobbie and Crawford (1969), most recently described in detail by Wright (in press). The only change from the latter has been to fix samples with 50% phosphoric acid, adding at a 1:100 fixative-to-sample ratio. Most of the work employed glycolic acid-1-^{14}C, 9.6 mCi/mmol specific activity, obtained from Amersham/Searle. Other substrates used were glycolic acid-^{14}C (U), specific activity 16.7 mCi/mmol from ICN Pharmaceuticals, sodium acetate-^{14}C (U) and glucose-^{14}C (U) obtained at high specific activities from Nuclear Chicago. Substrates were diluted to 1 - 3 µCi/ml with sterile distilled water, acidified to eliminate any contaminating ^{14}C-bicarbonate, and stored frozen.

Calculation of the kinetic parameters was made using the equation from Wright and Hobbie (1965):

$$t/f = \frac{K_t + S_n}{V_{max}} + \frac{A}{V_{max}} ,$$

where t is incubation time of the measurement, f the fraction of available substrate taken up (including assimilation and mineralization fractions), K_t a transport-related constant in µg/l, S_n the natural substrate concentration in µg/l, V_{max} the theoretical maximum velocity of uptake attained when uptake is saturated with substrate (therefore a measure of heterotrophic potential), and A the substrate concentration of labelled substrate added to make the measurement. Each concentration of A used (4 is the usual practice) will generate a different t/f, and when t/f is plotted against A, a straight-line relationship will be found if uptake follows saturation (Michaelis-Menten) kinetics. Where judged appropriate, a straight line was hand-fitted to the data, and extrapolation of the line yielded the values $(K_t + S_n)$ and the turnover time (T_t) of the substrate, while V_{max} was obtained from the inverse of the slope. The percent of substrate mineralized was calculated by dividing the carbon recovered as $^{14}CO_2$ by the total uptake ($^{14}CO_2$ and ^{14}C retained in cells) and multiplying by 100. Various approaches and difficulties in measuring heterotrophic activity are discussed by Wright (1973, 1974). The precautions noted in these papers concerning methodology and interpretation of results have been followed in the present study.

Counts of bacteria were performed via 0.1-ml spread plates employing the medium of Murchelano and Brown (1970), diluting where necessary with sterile seawater; alternatively, small aliquots were added to sterile seawater and drawn through a membrane filter which was then laid on the same agar medium. The plates were incubated at room temperature (ca. 20°C) and counted after 5 days. Where desired, cultures were taken from the spread plates, streaked out on the same agar medium, and subsequently maintained on that medium. Tests of growth on various carbon sources were performed with an artificial seawater enriched with essential nutrients (Baumann *et al.*, 1971) to which sterile substrate was added to give a concentration of 500 mg/l.

"Screening" for metabolism of ^{14}C-glycolic acid was performed by removing single freshly grown colonies from a spread plate or membrane filter, and suspending them in 25 ml of filter-sterilized seawater to which 0.05 µCi glycolic acid-1-^{14}C was added. After a 1-h incubation at room temperature, ^{14}C was recovered as $^{14}CO_2$ and particulate ^{14}C as with the natural samples. All ^{14}C samples were counted in a liquid scintillation counter, corrected for quenching with the channels-ratio method, and expressed as disintegrations per minute (dpm).

Glycolic acid in seawater was measured by the method of Shah and Fogg (1973), as revised in Shah and Wright (1974). During the course of the work reported here it was found that trace quantities of formaldehyde from the laboratory atmosphere caused great irregularities in the analysis of glycolic acid. Subsequent analysis was conducted in the absence of known formaldehyde contamination.

Chlorophyll *a* was measured by the method of Yentsch and Menzel (1963).

Results and Discussion

Since previous studies had shown heterotrophic use of glycolic acid in freshwater systems (Wright, 1970; and in press), we were not surprised to find measurable heterotrophic activity on glycolic acid in coastal marine waters. The magnitude of this activity is comparable to heterotrophic activity on other commonly studied substrates. Table 1 presents results from a comparative study on water from 1-m depth in Booth Bay, July 24, 1974. Although the glycolate V_{max} values fall within the range of the other substrates, important differences can be seen in other heterotrophic parameters. Thus, turnover times $(K_t + S_n)$ and percent mineralization values are all considerably higher for glycolic acid. This indicates that although glycolate is used by the heterotrophs, it is turning over less rapidly, may be present at higher concentrations, and when taken up is extensively respired in comparison with glucose, acetate, and glycine. Fresh-water studies have yielded similar results (Wright, in press).

Fig. 1 shows the kinetic plot for total uptake of the two glycolic acid isotopes. The two isotopes were clearly taken up by the same heterotrophic popu-

Table 1. Comparison of heterotrophic activity on various substrates. Sample from 1 m, Booth Bay, July 24, 1974; incubation at 15°C for 2.5 h. V_{max}: a measure of potential heterotrophic uptake; T_t: turnover time of the substrate; K_t: a transport-related constant; S_n: natural substrate concentration; (U): uniformly labelled substrate

Substrate	V_{max}	T_t(h)	(K_t+S_n) (µg/l)	% mineralization
Glycolic acid-1-^{14}C	0.27	170	46	64.4
Glycolic acid-1,2-^{14}C	0.27	170	46	61.1
Glucose-^{14}C (U)	0.44	29	13	40
Acetic acid-^{14}C (U)	0.095	63	6	33
Glycine-^{14}C (U)	0.11	22	2.5	38

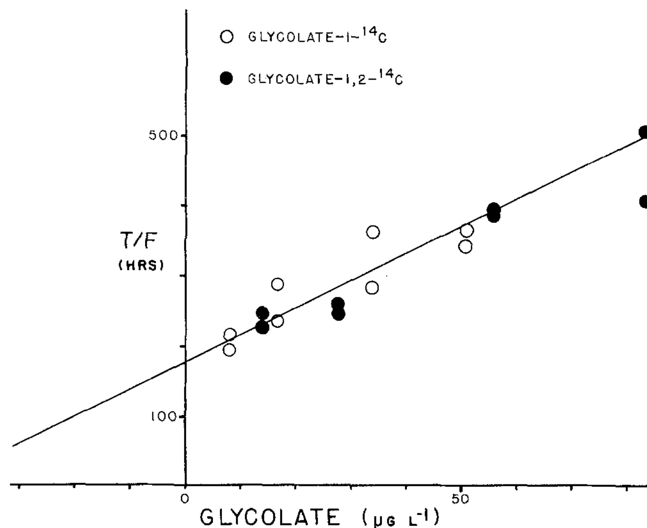

Fig. 1. Kinetic plot for total uptake of two glycolic acid isotopes, 1 m, Booth Bay, 24 July, 1974. T/F: Incubation time divided by fraction of available substrate taken up

Table 2. Horizontal variations in environmental and heterotrophic parameters for total uptake of glycolic acid, Ipswich Bay, summer 1973. cfu: colony-forming units; n.d.: no data taken

Sampling location and depth	V_{max} (µg/l/h)	T_t (h)	(K_t+S_n) (µg/l)	Chlorophyll *a* (µg/l)	Glycolic acid (µg/l)	% mineralization	Bacteria (cfu/ml)	Temperature (°C)
Essex River Estuary								
Inlet, surface	2.5	30	75	5.45	22	71	n.d.	18
3.2 km upriver, surface	4.2	23	96	1.18	n.d.	73	n.d.	21
6.4 km upriver, surface	3.6	7	25	1.19	n.d.	76	n.d.	23
Estuary head, surface	9.3	8.7	81	0.45	n.d.	n.d.	n.d.	22
Ipswich Bay								
4.8 km out, 1 m	0.45	370	170	0.88	42	61	13100	19
3.2 km out, 1 m	1.1	203	230	0.96	20	67	6400	18
3.2 km out, 15 m	0.011	1600	17	0.52	26	71	340	7.9
Dockside, surface	3.1	37	115	2.1	40	65	36000	18
Ipswich Bay								
8.0 km out, 1 m	0.06	2370	140	0.34	n.d.	n.d.	6100	19.5
6.4 km out, 1 m	0.06	2370	140	0.52	n.d.	n.d.	650	19.5
4.8 km out, 1 m	0.06	1570	95	1.08	n.d.	n.d.	1780	19
3.2 km out, 1 m	0.35	485	171	0.87	n.d.	n.d.	805	19
3.2 km out, 15 m	0.04	2500	105	0.94	n.d.	n.d.	710	10
Dockside, surface	0.64	130	83	0.98	n.d.	n.d.	4430	20

lation, and gave almost identical percent mineralization, suggesting that the two carbons of glycolic acid are respired at the same rate. Similar results were found in lake water (Wright, in press). The kinetic plot in Fig. 1 represents one of the better data fits to the saturation kinetic scheme employed in the Wright and Hobbie (1966) method. For some as yet unknown reason, glycolic-acid uptake measurements frequently generate erratic data, where replicates differ by as much as 100%. Even when this occurs, however, the ratio of assimilation to mineralization within a set of samples stays remarkably constant.

Some very significant spatial variations in heterotrophic activity on glycolic acid were found. Table 2 presents several measurements of horizontal variations together with pertinent environmental data. From the data it is evident that heterotrophic activity on glycolate, as measured by V_{max} and turnover time, increases substantially progressing towards the coast, and increases proceeding up an estuary that feeds into the bay. No clear correlation exists with clorophyll *a* or glycolic acid concentrations; the trend is most likely traceable to a general increase in substrates because of greater production in shallow coastal and estuarine areas. It is not likely that the bacteria are responding directly to glycolic acid production, in view of the results of the culture studies and enrichment experiments cited below.

Table 3 gives the results of two vertical measurements of heterotrophic activity from Maine waters. Heterotrophic V_{max} diminishes rapidly with increasing depth in the water column, an observation which is borne out by our frequent Ipswich Bay measurements at 1 and 15 m (Shah and Wright, unpublished). Andrews and Williams (1971) observed that heterotrophic use of glucose and amino acids was greater above than below the seasonal thermocline in the English Channel. In a lake study, heterotrophic use of glycolic acid, glucose, and acetate decreased with depth below the epilimnion and correlated well with the depth distribution of algal photosynthesis and organic solute excretion (Wright, 1970). Decreasing temperature explains some of the depth effect, since temperature greatly affects heterotrophic activity, given the same-size microbial population (Wright and Hobbie, 1965). However, the samples in Table 3 from both dates were incubated at the same temperature (12°C), so the variations

Table 3. Vertical variations in environmental and heterotrophic parameters for Booth Bay and the Gulf of Maine, summer, 1974

Sampling location and depth	V_{max} (μg/l/h)	T_t(h)	(K_t+S_n) (μg/l)	Chlorophyll *a* (μg/l)	Glycolic acid (μg/l)	% mineralization	Bacteria (cfu/ml)	Temperature (°C)
Buoy R16BR, Gulf of Maine								
1 m	0.14	235	32	10.3	12	70	40	14.5
15 m	0.028	75	2.1	3.9	3.6	68	170	12
40 m	0.012	1150	14	0.18	0	77	80	9.5
70 m	0.0079	850	7	0.14	0	81	750	8
Booth Bay								
1 m	0.082	460	38	1.01	15	61	n.d.	16
15 m	0.085	630	53	1.63	0	62	n.d.	12
30 m	0.012	1350	16	0.42	0	64	n.d.	9

Table 4. Range and mean of bacterial counts in colony-forming units/ml for 3 stations, Ipswich Bay, 1973-1974 year's cycle

Depth	Range (cfu/ml)	Mean (cfu/ml)
1 m	520 - 26500	4730
15 m	270 - 4000	1590
dock, surface	820 - 36000	6880

are more likely due to interaction of microorganisms and substrates during the recent history of the water masses sampled. In Table 3, the correlation between heterotrophic V_{max}, chlorophyll *a*, and glycolic acid is very obvious; primary productivity seems to be the factor responsible for heterotrophic activity as well as for glycolic acid. As with the data on horizontal variations, the question of bacterial response to glycolic acid must be considered. One way to answer this is to use bacterial cultures taken from plate counts.

Each time a heterotrophic measurement was made, plate counts of bacteria were taken. As many workers have noted, these counts generally do not give a true picture of the bacterial population in a sample (Jannasch and Jones, 1959). They do, however, provide cultures from which further information can be obtained. Table 4 presents summary information from the counts corresponding with the Ipswich Bay year's cycle data which will be presented in the next paper of the series. The mean values correlate with the comparative levels of heterotrophic uptake from the three sampling locations, but an examination of individual values from one sampling date to the next does not yield any clear correlation with, say, V_{max} levels. Plate count data are also shown in Tables 2 and 3 with the other data from studies of horizontal and vertical distribution of heterotrophy. Again, there is a lack of correlation of bacterial counts with levels of heterotrophic uptake of glycolic acid. Other workers have reported a similar lack of correlation (Hamilton and Preslan, 1970; Hobbie *et al.*, 1972), and several possible explanations can be offered for this: because of the media used, the counts may be selecting an unrepresentative sample of the bacterial population; algal uptake of the substrate may be significant; or, substantial differences in metabolic ability of the bacteria could be a factor.

In order to establish the possible source of natural heterotrophic activity, bacteria were screened for their ability to metabolize glycolic acid. The medium used for the initial isolation and subsequent culturing of bacteria was not supplemented with glycolic acid, since it was hoped that the results might be representative of the natural flora. No attempt was made in the screening process to correct for differences in the size of the colonies used. The screening was performed on samples taken on three occasions, including the August 29, 1973 study of horizontal variations (see Table 2). The results, shown in Table 5, indicate that a surprisingly high percentage of the marine bacteria tested have the ability to metabolize glycolic acid (a mean of 63%). The counts obtained from individual colonies revealed a broad quantitative range in metabolic ability; many yielded counts above 1000 dpm, and several reached 25000 dpm. This kind of range could certainly be responsible for some of the lack of correlation between bacterial numbers and het-

Table 5. Screening of bacterial colonies for glycolic acid metabolism, from Ipswich Bay count samples. Positive: total activity above 100 dpm; high count: total activity above 1000 dpm

Sample date, location and depth (1973)	Bacterial counts (cfu/ml)	Colonies screened	Positive	High count	% positive	Mean % mineralization
24 July, Ipswich Bay						
1 m	26500	13	6	4	46	90
15 m	270	14	10	5	71	84
8 August, Ipswich Bay						
1 m	6400	14	5	2	36	78
15 m	340	12	10	4	83	87
29 August, Ipswich Bay						
8.0 km out, 1 m	6100	12	8	2	67	66
6.4 km out, 1 m	650	13	11	1	85	80
4.8 km out, 1 m	1780	12	6	1	50	87
3.2 km out, 1 m	805	12	6	2	50	79
3.2 km out, 15 m	710	12	6	2	50	86
Dockside, surface	4430	13	9	5	69	84
Totals		141	89	30	63(mean)	81(mean)

erotrophic activity on glycolic acid in natural waters. A comparison of the respiration patterns of screened colonies with those from natural samples reveals an overlap in the range of values (cf. Tables 2, 3 and 5) but a higher mean percent respiration for the resuspended colonies used for screening. Conceivably, this could be related to differences in the physiological state of the bacteria in the two situations.

Further work with those colonies showing positive results in the screening test involved a determination of their ability to grow on a variety of substrates as sole carbon sources. A summary of the results of the substrate tests is given in Table 6. None grew on glycolic acid. This was totally unexpected, but was confirmed by repeated tests. However, when glycolic acid was paired with other substrates, especially lactic acid and acetic acid, 7 of 16 cultures tested showed greater growth in the presence of glycolic acid. For example, one culture (813-2) gave the following optical density maxima with the indicated substrates: 300 mg/l lactic acid, 0.27; 300 mg/l glycolic acid, 0.00; 300 mg/l lactic acid plus 300 mg/l glycolic acid, 0.40; 300 mg/l acetic acid, 0.12; 300 mg/l acetic acid plus 300 mg/l glycolic acid, 0.25.

These results have some implications for the practice of testing bacterial cultures on organic substrates to determine their biochemical abilities. Obviously, many bacteria unable to grow on glycolic acid could still metabolize it. Berland *et al.* (1970) tested a number of bacteria isolated from cultures of marine algae for growth on a variety of organic substrates supplied as sole carbon sources in an artificial sea-water medium. Only 1 of the 25 cultures tested, a pseudomonad, grew on glycolic acid. None of 145 marine bacteria tested by Baumann *et al.* (1971) showed growth on glycolate. These bacteria were selected as facultatively anaerobic, Gram-negative motile rods. A group of 218 aerobic Gram-negative motile rod bacteria were subsequently tested by Baumann *et al.* (1972). Of these, some 22 grew on glycolate incorporated in an agar medium. The agar was a highly purified type, but our tests with a similar agar medium indicate that other substrates are present in low but significant concentra-

Table 6. Ability of 84 glycolate-metabolizing cultures to utilize substrates as sole carbon source. Grown in artificial seawater, substrate concentration 1 g/l; growth measured by optical density at 650 nm

Substrate	No. showing growth	% of total
Glucose	73	87
Acetate	74	88
Lactate	64	76
Succinate	46	55
Glycolate	0	0

tions. The test in liquid culture is less equivocal. The purpose of the tests of sole carbon-source utilization in the Baumann studies was for taxonomic separation and characterization. Berland *et al.* (1970), however, directed their conclusions towards utilization of substrates excreted by algae, and their results suggest non-utilization of glycolic acid by the bacteria growing in algal culture media. The screening with ^{14}C-glycolic acid reported in the present paper appears to be a better method for determining the ability of a bacterial culture to metabolize a given substrate.

The lack of ability to use glycolic acid as a sole carbon source is not characteristic of all aquatic bacteria. Wright (1970) and Nalewajko and Lean (1972) obtained fresh-water bacteria capable of growth on glycolate by enriching lake water with glycolate. On several occasions in the present study, natural sea-water samples were serially diluted into artificial seawater enriched with glycolic acid or other substrates. The results invariably showed growth on glycolate for the 10^{-0} (1-ml seawater to 9-ml medium) dilution, occasionally for the 10^{-1} dilution; growth on glucose, acetate or lactate occurred in the 10^{-3} down to 10^{-5} dilutions. These results support the conclusion that most of the naturally occurring marine bacteria are unable to use glycolic acid for growth when it is the predominant carbon source.

A further test of the response of marine bacteria to glycolic acid has involved a set of enrichment experiments. These experiments, to be described in a later publication, have confirmed the culture results. Glycolate added to natural sea-water samples failed to stimulate significant bacterial increases over a 48-h period; yet glycolate was taken up and respired at a respectable rate, independent of added glycolate concentrations. Replicate additions of glucose, acetate, or lactate at identical concentrations invariably resulted in many-fold increases in bacterial numbers over the 48 h (Wright and Shah, unpublished).

Conclusions

Based on the data presented, conclusions are appropriate at two levels of organization: cellular and community. On the cellular level, it is apparent that many of the marine bacteria of coastal waters have retained the ability to transport glycolic acid into the cell. The transport appears to be similar to that of the fresh-water bacteria, which show a general "system" for taking up alpha-hydroxy acids but do not distinguish between, say, lactic acid and glycolic acid (Wright, in press). Once in the cell, glycolic acid is largely oxidized. The marine bacteria tested seem to lack an enzyme pathway that would enable them to build tricarboxylic acid intermediates needed for biosynthetic reactions, the ability to build 4-carbon compounds from the 2-carbon glycolic acid. Given other carbon sources, glycolic acid does contribute to growth in some of the bacteria, possibly by "sparing" the additional carbon compound for cell-carbon synthesis while the glycolic acid is oxidized for adenosine triphosphate or active transport energy.

On the community level, it is apparent that a cause-effect relationship between glycolic acid and the heterotrophic bacteria might not be expected, given the apparent cellular limitations. Thus, variations in community heterotrophic ability to metabolize glycolic acid may not be caused by glycolic acid availability but primarily by other substrates which stimulate bacterial growth by serving as carbon sources. The algae excrete a variety of compounds, in general quantitatively related to primary production. Glycolic acid may be more or less abundantly produced in relation to other excretory products, depending on the species of algae and the prevailing environmental conditions. The concentration of glycolic acid can thus be expected to show greater fluctuations than other substrates, as it does (Shah and Wright, unpublished), since microbial use will not directly be tied to glycolate production.

However, most of the bacteria maintain the ability to take up and oxidize glycolic acid, a phenomenon that must be explained. This could conceivably be an example of "co-metabolism" (Horvath, 1972), where a substance is oxidized but does not seem to do the microbes any good. However, marine bacteria have been in contact with glycolic acid for millions of years, and it seems highly improbable that they would maintain systems for taking up and metabolizing glycolate if it were of no use to them. The oxidation of glycolate is obviously an energy-yielding reaction. We suggest, therefore, that the energy from glycolate metabolism could conceivably be coupled to active transport of the other substrates on which the bacteria depend for growth. This suggestion is consistent with the data already presented and is supported by the fact that a large number of bacteria use oxidation of D or L-

lactate to generate active transport energy (Kaback, 1974). The enzymes performing these oxidations are membrane-bound dehydrogenases, and they couple energy to the transport of a wide variety of organic solutes. The molecular similarity between glycolate and D or L-lactate is borne out by the fact that an enzyme described by Lord (1972) as a glycolate dehydrogenase oxidizes D-lactate more efficiently than it does glycolate. If this hypothesis can be confirmed, it represents a significant phenomenon that ties together algal excretion and bacterial heterotrophy in a way hitherto unimagined. Glycolic acid may, thus, play a trophic role of far greater importance than its energy content and quantitative uptake suggest.

Acknowledgements. This research was supported by the Oceanography Section, National Science Foundation, NSF Grants GA-38900 and GA-38837. The first author thanks Dr. C.S. Yentsch for the appointment of Visiting Scientist at the Bigelow Laboratory for Ocean Science during the summer of 1974. This paper constitutes Contribution No. 75003 from the Bigelow Laboratory for Ocean Sciences.

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Date of final manuscript acceptance: June 18, 1975. Communicated by M.R. Tripp, Newark