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A year's study was made on the in situ activities of the heterotrophic plankton in Upper Klamath Lake, Oregon using the Wright-Hobbie kinetic approach of measuring the uptake of organic compounds. This study was initiated to test the applicability of the kinetic approach to a highly eutrophic system. Sixteen amino acids were used as the organic substrates.

The year's study has shown that the maximum velocity, V_{max}, for the uptake of all the amino acids is proportional to temperature. The turnover time, T_t, and the sum of a transport constant and the natural substrate concentration, (K_t+S_n), are difficult to interpret because of possible competitive inhibition effects among the amino acids. The V_{max} is unaffected by competitive inhibition, but the T_t and (K_t+S_n) values are increased. These latter values then reflect the total natural concentration and affinities of the amino acids transported by a particular transport system.

Those amino acids exhibiting the highest V_{max} values had the highest percent respired as CO_2. The percent respired appears to be inversely proportional to temperature.

A reliable analysis to determine dissolved free amino acids is needed since standard cation exchange procedures cause an apparent release of adsorbed amino acids. The amino acid concentrations determined for Upper Klamath Lake surface water probably are the sum of the free and adsorbed amino acids.

Biochemical analyses were made of the predominant blue-green algae, found in the nuisance algal blooms, to better understand their contribution to the nutrition of the heterotrophic plankton.

Amino Acid Flux in a Naturally Eutrophic Lake

by

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Finally, I would like to thank the anonymous graduate student somewhere who said,

"Ecology is physiology under the worst possible conditions."

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

AMINO ACID FLUX IN A NATURALLY EUTROPHIC LAKE

INTRODUCTION

Eutrophication is the natural process of a system becoming rich in dissolved nutrients (Lund, 1967). Consequently, there is an increase in biological productivity including development of objectionable plant growths, particularly the dense blooms of planktonic blue-green algae. Cultural eutrophication is the term used to describe the influence of man-made pollution, which hastens the eutrophic process.

Upper Klamath Lake, Oregon is an example of a naturally eutrophic lake. During the summer months, it supports a massive bloom of blue-green algae, predominated by either Aphanizomenon flos-aquae or Gloeotrichia echinulata. The decomposition and excretions of these phytoplankton are responsible for the majority of the autochthonous dissolved organic matter (Ruttner, 1963). Heterotrophic bacteria easily assimilate a small fraction of this organic matter throughout the year. The relationship of this fraction, which in the aquatic environment is seemingly under rapid flux, and the organisms which produce and assimilate it is of great importance when trying to understand the regeneration of inorganic nutrients. Little information concerning the activity of heterotrophic bacteria

can be obtained by total bacteria counts or testing the ability of bacteria to grow on certain substrates. The importance of these organic compounds to aquatic organisms can only be measured by determining the rate of substrate uptake. Concentrations of the simple organic compounds in natural waters are usually at the few μg/L level indicating that radioisotopes, with their great sensitivity, are necessary to follow the uptake by microorganisms (Hobbie, 1967).

Parsons and Strickland (1962) developed a method using radioisotopes to study heterotrophic uptake of organic solutes in the sea. Wright and Hobbie (1965a) modified their procedure and applied it to fresh water environments. Over short periods of time, the rate of carbon uptake from an uniformly ^{14}C-labelled substrate by responsive cells is given by the following equation:

$$v = \frac{c f (S_n + A)}{C \mu t} \qquad (1)$$

where v = rate of uptake of substrate in μg/L; c = radioactivity of cells recovered by filtration plus $^{14}CO_2$ respired by the organisms (Hobbie and Crawford, 1969) in disintegrations per minute (DPM); S_n = the natural substrate concentration in μg/L; A = added quantity of substrate (both labelled and unlabelled) in μg/L; $C = 2.22 \times 10^6$ DPM for one microcurie of ^{14}C-labelled substrate; μ = quantity of ^{14}C added in microcuries per sample bottle; t = incubation time in hours; f = factor for the correction of isotope discrimination, neglected in the present analysis.

The uptake velocity of active transport systems can be described by Michaelis-Menten kinetics. The original equation derived by Michaelis and Menten is:

$$v = \frac{V_{max} S}{K_m + S} \tag{2}$$

where v is the velocity at a given substrate concentration S; V_{max} is the maximum velocity attained when all uptake sites are saturated with substrate; and K_m [which is equivalent to K_t, a transport constant (Wright and Hobbie, 1966)], the Michaelis constant, is by definition the substrate concentration when the velocity, v, is exactly one-half V_{max}.

By inverting equation (2), and multiplying both sides by S, a modification of the Lineweaver-Burk equation is obtained:

$$\frac{S}{v} = \frac{K_t}{V_{max}} + \frac{S}{V_{max}}$$

or where S equals $S_n + A$:

$$\frac{S_n + A}{v} = \frac{K_t + S_n}{V_{max}} + \frac{A}{V_{max}} \tag{3}$$

By rearranging equation (1) to:

$$\frac{S_n + A}{v} = \frac{C \mu t}{c} \tag{4}$$

and by substitution into equation (3), gives:

$$\frac{C\mu t}{c} = \frac{K_t + S_n}{V_{max}} + \frac{A}{V_{max}} \qquad (5)$$

which describes the uptake kinetics of natural bacterial populations (Wright and Hobbie, 1965a, 1966).

Data from uptake experiments on plankton at several low substrate concentrations can be plotted as $\frac{C\mu t}{c}$ versus A. The intercept on the negative abscissa is equal to $(K_t + S_n)$; the reciprocal of the slope is V_{max}; and the ordinate intercept is equivalent to the turnover time, T_t, which is the time in hours required for the natural population to completely remove the natural substrate, S_n. Constant rate of removal and continual regeneration have to be assumed. Measurements of the $(K_t + S_n)$ indicate the maximum in situ concentrations of the organic substrate being assayed, assuming K_t is very small (Wright and Hobbie, 1966). If S_n becomes known, the natural uptake velocity, v_t, can be calculated according to the following equation:

$$v_t = \frac{S_n}{T_t} \qquad (6)$$

The applicability of the Wright-Hobbie kinetic approach to substrate uptake was tested using 16 amino acids in Upper Klamath Lake water. The kinetic parameters, described above, for amino acid uptake were determined during a year's survey. Competitive inhibition among the amino acids for general transport systems

complicate the interpretation of these kinetic parameters. Reliable analyses of dissolved amino acids which can be metabolized by the natural heterotrophic population are needed since adsorbed amino acids are readily released when processed with cation exchange resin. The amino acid composition of the predominant blue-green algae was determined in an effort to understand the source material for the dissolved amino acids.

LITERATURE REVIEW

Description of the Study Area

Upper Klamath Lake is located at the eastern foot of the Cascade Mountains in south central Oregon. The lake is approximately 37 km long and 8 km wide and joins with Agency Lake, which is approximately 8 km long and 4.8 km wide, at the northern end. Upper Klamath Lake has a mean depth of 2.4 m and a storage capacity of 7.75×10^9 m^3 (584,000 acre-feet). The altitude of the lake surface is approximately 1263 m. No evidence of physical or chemical stratification has been demonstrated for the lake which is constantly subjected to wind-mixing. The water flowing into the lake includes mountain streams, rivers, springs, irrigation canals, agricultural drainage, and precipitation (Miller and Tash, 1967).

Upper Klamth Lake is considered a naturally eutrophic lake because appreciable amounts of domestic or industrial wastes are not entering the lake and yet it supports a massive blue-green algae bloom from late spring to mid-autumn. During the peak of the bloom, consisting predominantly of Aphanizomenon flos-aquae, phytoplankton counts may reach as high as 200 million cells per liter (Phinney, Peek, and McLachlan, 1959). In early spring there is a diatom bloom with cell counts as high as 80 million per liter

(Gahler, 1970). The predominant diatom is tentatively identified as a Stephanodiscus species.

The lake sediments are a vast reservoir of nutrients. It has been estimated the upper one inch of sediment contains a quantity of nitrogen and phosphorus equal to the amount which would flow into the lake during the next 60 years, assuming the present rate of inflow (Miller and Tash, 1967). However, very little quantitative work has been done on the actual availability of this potential source of algal nutrients. Recent research shows that neither nitrogen nor phosphate appear as limiting nutrients in Upper Klamath Lake, since Aphanizomenon flos-aquae is able to fix atmospheric nitrogen (Steward, Fitzgerald, and Burris, 1968) and Upper Klamath Lake sediment bacteria are able to solubilize bound phosphates (Harrison, 1970). In addition, vitamin B_{12} production by microorganisms in the lake is sufficient to maintain a level high enough throughout the year to prevent its being considered a limiting algal growth factor (Gillespie, 1971).

Dissolved Organic Matter

Lange (1967, 1970, 1971) and Kuentzel (1969) advocate that CO_2 derived from decomposable organic matter is the limiting nutrient for algal blooms. The action of bacteria on ample amounts of organic matter can deliver more CO_2 to the algae than can come

from the atmosphere and/or dissolved carbonate salts via the normal physical-chemical processes (Kuentzel, 1969). It is well known that blue-green algae and certain bacteria are always found in close association. The planktonic blue-green algae are very difficult, if not impossible, to get in pure culture. Attempts to remove the bacteria often prove detrimental to the algae probably because of a symbiotic relationship between the bacteria and algae with organic compounds as the linking factor.

The amount of dissolved organic matter in natural waters exceeds by several times that in particulate form, which is chiefly plankton (Birge and Juday, 1934). In 1959, Kuznetsov (cited in Ruttner, 1963) showed with water samples from various lakes that only a fraction of their organic matter was lost during long incubation periods. This fraction of dissolved organic matter, which depends on the lake's productivity, can be taken to be the portion that is easily assimilated by bacteria. In oligotrophic lakes it amounts to only 2-3 percent while in eutrophic lakes it is 10-15 percent of the total dissolved organic matter.

A wide variety of dissolved organic compounds make up this rapid refluxing fraction in natural waters. These include, among others, free sugars (Vallentyne and Whittaker, 1956), and amino acids (Jeffrey and Hood, 1958; Tatsumoto et al., 1961; Park et al., 1962; Wood, 1965; Sigel and Degens, 1966; Chau and Riley, 1966;

Webb and Wood, 1967; Brehm, 1967; Gocke, 1970; and Andrews and Williams, 1971).

The intricate relationship between heterotrophic bacteria and the dissolved organic matter in natural waters has recently been investigated (Parsons and Strickland, 1962; Hobbie and Wright, 1965; Wright and Hobbie, 1965a, 1965b, 1966; Hobbie, 1967; Hamilton, Morgan, and Strickland, 1966; Vaccaro and Jannasch, 1966, 1967; Vaccaro, 1969; Hamilton and Preslan, 1970; Wetzel, 1967, 1968 (cited in Allen, 1969); Munro and Brock, 1968; Kadota, Hata, and Miyoshi, 1966; Williams and Askew, 1968; Allen, 1968, 1969; Sorokin, 1970; Williams, 1970; Williams and Gray, 1970; and Andrews and Williams, 1971). Emphasis has been placed on measuring the actual rates of utilization by the in situ heterotrophic populations and/or the turnover time. The turnover time being the time required for the natural population to completely remove the natural organic substrate under investigation. The knowledge obtained from this type of research hopefully will lead to a better understanding of the basic principles of aquatic metabolism of dissolved organic matter.

Wright and Hobbie (1966) emphasized that in evaluating the importance of dissolved organic compounds in freshwater and marine ecosystems, we should attempt to determine the actual rate of supply and regeneration of these materials instead of measuring their concentrations. They also pointed out that measuring the total numbers of

bacteria and their ability to grow on certain organic substrates yield little information concerning in situ processes involved in the utilization and regeneration of these materials.

Many investigators have added labelled organic compounds directly to water samples and followed their uptake over short periods of time [Saunders, 1958 and Rodhe, 1962 (as cited by Allen, 1969); Goldman, Mason, and Hobbie, 1967; and Sorokin, 1970]. They have found that a significant percentage of the organic substrate was utilized by the natural populations. The rate of uptake is only relative, however, since the natural substrate concentrations were not known.

Parsons and Strickland (1962) found that they could estimate the "relative heterotrophic potential" of a natural water sample. This was accomplished by kinetically analyzing the uptake of one labelled organic substrate (e.g. glucose or acetate) by Michaelis-Menten enzyme kinetics. The authors reasoned they could neglect the natural substrate concentrations of the particular substrate by adding 250 μg C/L extra substrate to the seawater sample. The majority of the uptake subsequently noted would be from the added material. This data could then be used to calculate v (mg $C/m^3/hr$), the "relative heterotrophic potential." The most important aspect of their work was the observation that when various concentrations of labelled and unlabelled substrate were added to natural water

samples, the uptake data could be plotted kinetically. It was possible to then calculate a (K + S) value which represents a constant similar to the Michaelis constant (K) and the natural substrate concentration (S).

Wright and Hobbie (1965a) using ^{14}C-labelled glucose and acetate followed the uptake by planktonic algae and bacteria. Uptake at low substrate concentration (0-500 μg/L) followed Michaelis-Menten enzyme kinetics, and was attributed to the bacteria. At higher substrate concentrations (0.5-4.0 mg/L) uptake was complicated by the diffusion kinetics associated with the algae. These two distinct uptake mechanisms could be separated quite well by controlling the amount of substrate added to the water sample and the kinetic parameters associated with each system could be calculated. Wright and Hobbie (1966) confirmed these two separate systems of uptake by experiments using axenic algal and bacterial cultures, separately and combined. They surmised that the bacteria are so efficient, in nature, that they probably keep the organic substrates at very low concentrations and prevent heterotrophy in most forms of planktonic algae. Munro and Brock (1968) showed by audioradiography techniques that the bacteria were solely responsible for uptake even at high substrate concentrations (10-5000 μg/L) in a marine environment.

The kinetic approach of Wright and Hobbie was immediately criticized because it did not account for the respiration of the organic

substrate (Hamilton and Austin, 1967, and Williams and Askew, 1968). This error was eliminated by doing the uptake experiments in a closed system. The $^{14}CO_2$ respired is adsorbed, after killing and acidification, onto filter paper dampened with phenethylamine. The paper and the filtered organisms are counted separately by liquid scintillation (Hobbie and Crawford, 1969). The respiration correction increases the V_{max} and decreases the T_t values, but should not alter the $(K_t + S_n)$.

The $(K_t + S_n)$ value is a seemingly elusive value consisting of two unknowns. Various procedures have been tried to separate these parameters (Hobbie and Wright, 1965; Allen, 1968; Hobbie, Crawford, and Webb, 1968; and Vaccaro, 1969). A pure culture of bacteria with a known K_t value can be added to a filter-sterilized water sample after the addition of labelled substrate. Fractions were dispensed immediately into varying concentrations of unlabelled substrate and incubated at in situ temperature for a short period of time. Uptake data were plotted according to equation (5) and the $K_t + S_n$ value determined. The natural substrate concentration was then obtained by subtracting the known K_t (Hobbie and Wright, 1965).

Allen (1968) used a method suggested by Wright and Hobbie (1965b). A natural water sample may be diluted 1:1 and the uptake of a labelled organic substrate measured in both the diluted and undiluted samples. Ideally, estimates of $K_t + S_n$ in the undiluted sample

would be represented as $K_t + S_n/2$ in the diluted sample. From the two equations, K_t and S_n can be calculated separately.

Vaccaro (1969) advocated the use of a "conditioned" natural bacterial population as the bioassay organisms. The bacteria were "conditioned" by exposure to the organic substrate for 24 hours. The organisms were then removed by filtration, washed with aged seawater, and resuspended in filter-sterilized natural seawater. Uptake experiments were made using this sample and with the substrate-conditioned cells in aged seawater. It was assumed that $S_n = 0$ in the aged seawater. The natural seawater with "conditioned" cells yielded a $K_t + S_n$ value while in aged seawater a K_t value was obtained. Vaccaro (1969) also suggested an alternative procedure for detecting K_t. "Conditioned" cells were inoculated into filtered seawater which had been enriched with 200 μg C/L of the substrate. Several kinetic analyses were performed over a 90 hour period. After 48 hours the negative x-intercept became a constant and this was considered to be the K_t value.

Hobbie, Crawford, and Webb (1968) determined the S_n values for amino acids in an estuary by the methods of Webb and Wood (1967). In this method, the seawater was desalted with a chelating resin and the dissolved free amino acid concentrations were then determined using an automatic amino acid analyzer.

The natural concentrations of amino acids in lake water were

studied by Brehm (1967). He found the amino acids to be partially free and partially adsorbed to colloidal carbohydrates liberated by microorganisms. The concentrations of serine and glycine were relatively high; alanine, aspartic acid, and threonine intermediate; and the remaining amino acids low. In the surface waters of the lakes studied, the concentrations of the dissolved free amino acids (DFAA) reached a maximum in winter and a minimum during the summer. The concentrations of the DFAA were finally determined by the plankton. High temperatures increase the uptake of the amino acids by these planktonic organisms.

The DFAA of axenic cultures of the green alga, Scenedesmus quadricauda were similar in composition to the intracellular free amino acids in the alga, aspartic and glutamic acids predominating. However in contaminated cultures, serine and glycine were predominant as they were in lake water. The bacteria-free cultures of the alga almost completely removed four of the six amino acids introduced into the medium. The amino acids utilized were serine, glycine, threonine, and alanine, while aspartic and glutamic acids were not significantly attacked during the three-day experiment (Gocke, 1970).

During experimental autolysis of blue-green algae, the individual amino acids were released into the medium and destroyed at specific rates by bacteria. Aspartic and glutamic acids were

liberated very quickly, whereas leucine, isoleucine, valine, alanine, and proline were destroyed at relatively slower rates subsequently leading to a relative enrichment of these amino acids in dead plankton (Brehm, 1967). Autolysis appears to be a very important factor in the decay of plankton in lakes and it has been concluded that the majority of the plankton decomposition occurs in the epilimnion of the lakes investigated (Kleerekoper, 1953; Brehm, 1967; and Kuznetsov, 1968).

In a recent paper Andrews and Williams (1971) showed that amino acid oxidation rates were below 1% of the amino acid mixture per day during the winter months and 4-10% per day in the summer to autumn period. On a yearly basis their calculations suggest that heterotrophic processes, resulting in the uptake of dissolved organic compounds, may consume organic material equivalent to 50% of the measured phytoplankton production.

MATERIALS AND METHODS

Amino Acid Flux

Preparation of Radioactive Amino Acids

Radioactive solutions of uniformly labelled ^{14}C-L-amino acids (Amersham-Seale Corp., Arlington Heights, Illinois) were obtained in 5 ml serum bottles. Each bottle contained 10 microcuries (μC) of amino acid in one ml of 10% ethanol. The serum caps were removed, and the ethanol was evaporated in an 80 C water bath for two hours. Sterile distilled water was used to quantitatively transfer the remaining solution from each bottle into sterile screw-cap test tubes, the final volume being 20 ml. Two ml of this solution were dispensed into sterile two ml glass ampoules, flame sealed, and frozen until ready for use. The final concentration for each amino acid was approximately 0.5 μC/ml. L-ornithine-^{14}C (U) (New England Nuclear) was dissolved in 0.5 ml 0.01 N HCl. The same dilution procedure, as described above, was used without evaporation.

A specific activity (S.A.) of 25 μC/μM per carbon atom applied to all amino acids used except glycine and ornithine which had S.A. values of 109 and 204 μC/μM, respectively.

The radioactive concentrations of the diluted amino acid

solutions were determined by using 25 µl samples (in triplicate) of each amino acid. The sample was added to a scintillation vial and 15 ml standard fluor solution with 5% v/v Bio-Solv solubilizer (Formula BBS-3) (Beckman Instruments) was added to the vial. The standard fluor consisted of a mixture of 0.01% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene (dimethyl-POPOP) and 0.4% 2,5-diphenyloxazole (PPO) (Packard Instrument Co.) in toluene (Mallinckrodt). The vials were capped and mixed vigorously. Two drops of a saturated solution of ascorbic acid (33%) were added and the vials again were shaken vigorously. Triplicate controls consisted of 25 µl distilled water, 20 µl ^{14}C-toluene (8520 DPM), in the same fluor solution. Ten minute counts were made using a Packard Tri-Carb Liquid Scintillation Spectrometer Model 524.

The isotopic abundance of the labelled L-amino acids is 40.1%. Knowing this value, the correct molecular weight of the amino acids can be calculated (Table 1). The amount of labelled substrate (A) added to each sample bottle was calculated using the following equation:

$$A(\mu g/L) = \frac{\mu C \text{ added}}{\text{S. A. } (\mu C/\mu M)} \times \text{Mol. Wt. } (\mu g/\mu M) \times \frac{1000 \text{ ml}}{\text{sample volume (ml)}}$$

Uptake of Amino Acids by Heterotrophic Plankton

The basic techniques employed were those of Wright and Hobbie (1966) as modified by Hobbie and Crawford (1969). Various quantities

Table 1. Molecular weights, μ-, and A-values for the labelled ^{14}C-L-amino acids

Amino acid	Molecular weight[a]	μ (μC)[b]	A (μg/L)[b]
Alanine	91.5	0.026	3.15
Serine	107.5	0.023	3.32
Glycine	78.1[c]	0.024	1.75
Glutamic acid	151.1	0.024	2.88
Aspartic acid	136.3	0.023	3.14
Leucine	136.0	0.024	2.19
Isoleucine	136.0	0.024	2.18
Valine	121.1	0.024	2.33
Threonine	120.3	0.022	2.68
Proline	119.1	0.024	2.29
Tyrosine	188.4	0.023	1.93
Phenylalanine	172.4	0.022	1.72
Lysine	151.1	0.022	2.18
Arginine	179.0	0.023	2.74
Ornithine	132.1[c]	0.018	1.16
Asparagine	135.3	0.024	3.21

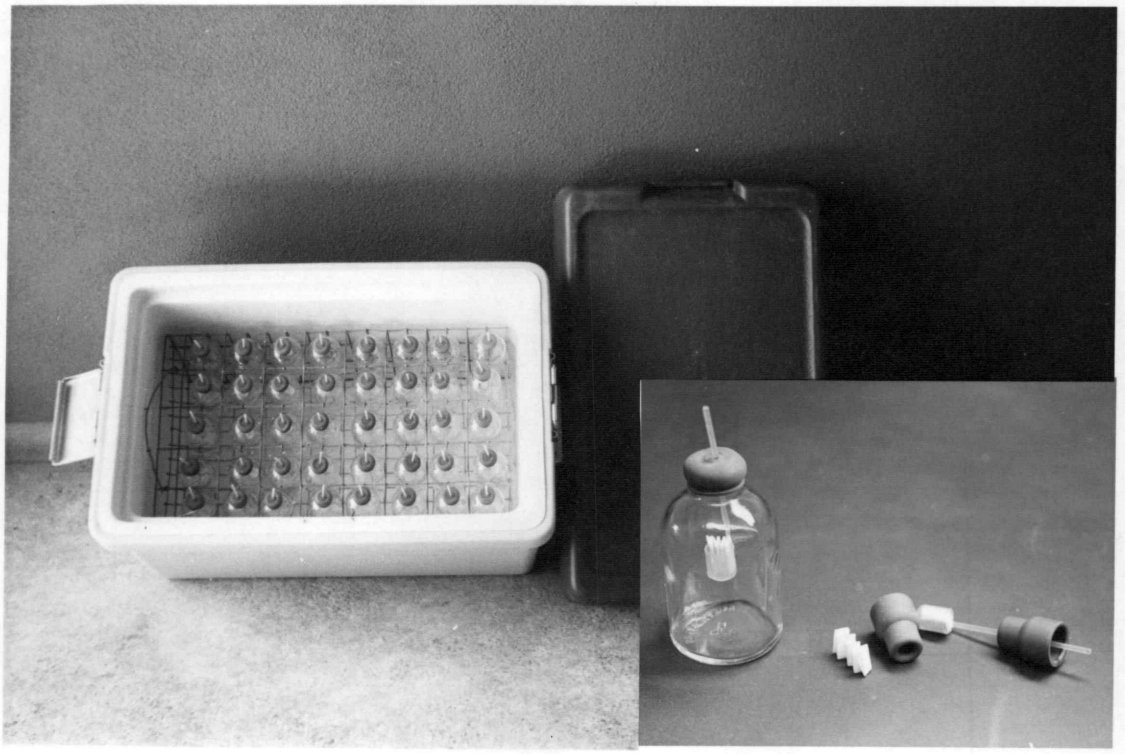
[a]Based on 40.1% isotopic abundance.

[b]Values indicated are for addition of a 50 μl aliquot of the diluted labelled substrate into 10 ml of lake water.

[c]Manufacturer's given value for the molecular weight.

of the labelled amino acid were added to 50 ml serum bottles. The usual series of four consisted of 50, 100, 200, and 300 µl of diluted labelled substrate in triplicate. The bottles were placed in wire racks constructed from one inch mesh galvanized wire screen. Each rack held 40 bottles and was placed in an ice chest (Figure 1), previously packed with approximately two pounds of dry ice in order to keep the bottles frozen for the 250 mile trip to Klamath Lake. Upon arrival, the racks of bottles were removed and the ice chests were half-filled with lake water. The racks were carefully lowered into this water and allowed to equilibrate to the in situ temperature. Using a volumetric dispenser (Packard Instruments Co.) a 10 or 15 ml sample of lake water was poured into each of the serum bottles. Care was taken to keep the water well mixed so the dispensed samples would be homogeneous. Each bottle was immediately sealed with a rubber serum stopper through which a plastic respiration cup (Kontes Glass) had been inserted, containing a 2 × 5 cm piece of accordion-folded Whatman #1 filter paper (Figure 1). One bottle from each concentration was used as the "blank." Two tenths ml of 5 N H_2SO_4 were added to these bottles followed by the lake water sample and the bottles were sealed with serum stoppers containing the respiration cup and filter paper. All bottles were returned to the ice chests for incubation at the in situ temperature in the dark. After the incubation period of 0.5 to 7.0 hours, depending on lake temperature, 0.2 ml of 5 N H_2SO_4

Figure 1. Wire rack of serum bottles used for amino acid uptake studies in an ice chest used for incubation. Inset: incubation bottles with respiration apparatus.



were injected into the sample bottles through the septum. The acid was thoroughly mixed with the lake water and the samples allowed to "acidify" for two hours. Still working through the septum, 0.18 ml of β-phenethylamine were carefully injected onto the folded paper. The filter papers with the adsorbed $^{14}CO_2$ were removed after two hours and immediately placed in vials containing 10 ml of the standard fluor..

The acidified water samples were transported back to the laboratory where they were filtered through 0.45 μ membrane filters (Millipore Corp.) to retain the plankton. The sample bottles were rinsed with approximately 5 ml of 0.1 N H_2SO_4 and about the same volume was used to rinse the sides of the Millipore filter funnel (the filter funnels were washed after each sample). The membrane filter was placed in a scintillation vial and dried at 40-50° C for 12-24 hours. Standard scintillation fluor was then added to the vials. All vials were counted by liquid scintillation and quenching was corrected by the channels ratio method (Bruno and Christian, 1961). The Nuclear Chicago Mark I Scintillation Counter became available in March, 1970. Computer analysis was used to calculate the kinetic parameters from the amino acid uptake data. Programing and computer analyses were done by the OSU Computer center.

Dark Fixation of CO_2

Twenty-five µl of a $NaH^{14}CO_3$ solution (0.5 µC/ml, Amersham-Seale Corp.) were added to two sets of four bottles, each containing 10 ml of lake water, and the bottles were immediately sealed with the respiration apparatus, described above. Appropriate blanks were prepared by injecting one bottle from each set with 0.2 ml 5 N H_2SO_4 through the septum. One set was incubated in the dark and the other in the light, both were at 5 C.

Temperature Dependence of Amino Acid Uptake by Plankton

A polythermostat constructed by Morita and Burton (1963) similar to the one of Oppenheimer and Drost-Hansen (1960) was used to study the effect of temperature on bacterial uptake. Ten ml of lake water were placed in sterile test tubes and equilibrated to proper temperature in the polythermostat for 10 minutes. Fifty µl of either ^{14}C-glutamic acid or ^{14}C-glycine were added to each of the test tubes, mixed well, and incubated for 0.5 to 5.0 hours depending on the temperature. At the end of the incubation periods, the samples were treated as previously described.

Competitive Inhibition Effects on Uptake

Unlabelled amino acids (Sigma Chemical Co.) were made up to a concentration of approximately 10 μg/ml. The solutions were autoclaved and five ml portions were pooled and freeze-dried. The amino acid concentrations in this residue, and all subsequent amino acid analyses in this work, were determined using the methods of Spackman, Stein, and Moore (1958) with a Spinco Model 120B Automatic Amino Acid Analyser.

Single or varying concentrations (1 μg-50 μg/L) of unlabelled amino acids (inhibitors) were added to constant amounts of labelled amino acids. Ten ml of lake water were added and the uptake of the labelled amino acid was noted by procedures already described. In a separate experiment, unlabelled serine was added to lake water at a concentration equivalent to 28 μg/L and a regular uptake experiment using ^{14}C-glycine was conducted. All experiments were performed at 6 C in the dark.

Removal Rates of High Amino Acid Concentrations in Lake Water

Thirteen amino acids (0.5 mg each) were added to a 500 ml water sample taken from the lake on February 23, 1970. At zero time, a 25 ml sample was immediately taken from the flask and 0.4% sodium azide was added to stop any uptake of the amino acids. The

sample was then filtered through 0.45 μ membrane filter, 0.25 micromoles norleucine added, and the sample was freeze-dried in a 125 ml round bottom flask. The remaining water sample was incubated in the dark at 6 C and at 48 and 96 hours similar portions were removed and processed. The residues were then analyzed for amino acid content with the amino acid analyser. A similar 500 ml water sample was incubated at 15 C in the dark on a rotary shaker, and analyzed as above.

Determination of Dissolved Amino Acids

Preparation of Ion Exchange Column

The ion exchange column was prepared as described by Wood (1965). One hundred and fifty grams of Dowex 50W-X2 resin (Lot 29B-0170, 50-100 dry mesh, Sigma Chemical Co.) was thoroughly washed in deionized water and decanted several times to remove the "fines." The resin was poured into a chromatography column, 1.8 (I.D.) × 45 cm, half-filled with 0.1 N acetic acid. The "bed" volume was established by eluting the column with 0.1 N acetic acid to a constant pH of 2.8, and then with 2.0 N NH_4OH until the effluent was basic as shown by phenolphthalein indicator. The effluent volume was 270 ml and will be subsequently referred to as a "volume." The resin was returned to the H^+ form by washing with three volumes of

1.0 N HCl, and then washed free of Cl^- with three volumes of 0.1 N acetic acid.

Preparation of Lake Water Sample

The lake water was filtered through a plankton net (Standard No. 12, 125 meshes to the inch, Turtox) into a polyethylene (Nalgene) container. It was then passed through Whatman No. 1 filter paper and finally through a Millipore filter apparatus containing a 0.45 μ membrane filter (293 mm diameter) into a sterile 20 liter Millipore pressure can. The water was transported back to the laboratory, in ice during the summer months, and analyzed for amino acid content.

Ten liters of filtered lake water were treated with 2×10^{-4} M phenylmethylsulfonylfluoride (Sigma Co.) in 10 ml of isopropanol to inhibit proteolytic enzymes, which might be present in the lake water. A comparison of three treatments were made prior to ion exchange adsorption: 1) immediate processing of the filtered lake water with the resin, 2) freezing the water sample, thawing, and filtering it at a later date through Whatman No. 1 filter paper, 3) passing the water through a UM-2 Diaflo membrane which would retain all molecules with molecular weights greater than 1000, including colloids. The latter process was accomplished by applying 4.76 atm (70 psi) of nitrogen to the inlet port of the Millipore pressure can and connecting the outlet port to an Amicon ultrafiltration cell Model 400 (Amicon

Corp.) containing the UM-2 membrane. The flow rate from the cell was 3 ml/min and the entire process was performed at 6 C. After each liter of lake water had been passed through the membrane, the cell was disassembled and the membrane washed free of the slimy substances which it had retained.

Two liters of each of the pre-treated water samples were used for amino acid analysis. Twenty-two ml of glacial acetic acid were added to each sample to bring the pH to 2.8. Four ml of 10% sodium azide were added as a bacteriostatic agent. Each sample was passed through the ion exchange resin at a flow rate of 1-2 ml/min at 6 C. After the sample had been applied, the resin was washed with one volume of distilled-deionized water. The adsorbed amino acids were eluted with two volumes of 2.0 N NH_4OH. The color change from yellow to light orange was allowed to proceed half the height of the resin in the column before the effluent was collected. The resin was regenerated with one volume of 1 N NaOH. A white crust which appeared on the surface of the resin was removed. The resin was then returned to its H^+ form with one volume of 1 N HCl.

The collected effluent was evaporated to a small volume using a Rinco evaporator and a 50 C water bath. The sample was quantitatively transferred to a 50 ml round bottom flask with 0.01 N HCl, 0.25 μM of norleucine was added as an internal standard, and the solution evaporated to dryness. The residue was subsequently

analyzed for amino acid content.

On every amino acid analysis of lake water during the year, a recovery experiment was made. The standard amino acid solution consisted of 1.0 micromole of each amino acid in 0.01 N HCl. Four hundred μl of the standard were added to one liter of 0.2 N acetic acid and 0.02% sodium azide. This solution was processed in the same manner as the lake water sample. One liter of the solution without the amino acids was similarly processed to serve as a "blank."

Biochemical Analyses of Predominant Blue-green Algae

Aphanizomenon flos-aquae was the predominant blue-green alga during the summer of 1969. The algae were concentrated using a plankton net and stored in polyethylene containers. At the laboratory the suspension was filtered through six layers of cheesecloth which removed the zooplankton (Daphnia sp.) and the zooplankton egg pouches. The algae were immediately freeze-dried, and stored in the freezer. The blue-green algal bloom of 1970 was predominately Gloeotrichia echinulata. Since there were no zooplankton present at the time of collection, filtration through the cheesecloth was eliminated. However, Gloeotrichia colonies were washed with distilled water to remove any extraneous matter. The algae were then freeze-dried and stored until used.

Amino Acid Composition

A portion of freeze-dried algae was dried at 60 C for 24 hours and then placed in a vacuum desiccator over Drierite (W. A. Hammond Drierite Co.) for storage. One hundred mg of dried algae was rehydrated in four ml of distilled water. An Eaton press (W. H. Schaerr Co.) was packed in solid dry ice for 30 minutes. The rehydrated algae were then quantitatively placed in the press using a small funnel and an additional four ml of water. The press was returned to the dry ice for 30 minutes. The algae were ruptured under 6000 kg (13,000 lbs.) pressure using the Carver Model E Hydrolic Press. This rupturing process was performed once for Aphanizomenon and twice for Gloeotrichia. Microscopic examination of the lysed algae showed very few intact cells. The ruptured cell mass was collected quantitatively in a 50 ml stainless steel centrifuge tube, thawed, and centrifuged at 10,000 g for one hour in a refrigerated centrifuge (Sorvall RC-2B). The supernatant was collected and the residue washed with distilled water and recentrifuged at the same speed for 20 minutes. These supernatants and the residue were then treated following the scheme presented in Figure 2.

Protein was hydrolyzed by adding one ml of HCl (constant boiling) to the freeze-dried algal material. The ampoule was evacuated with a water aspirator and flushed with nitrogen three times.

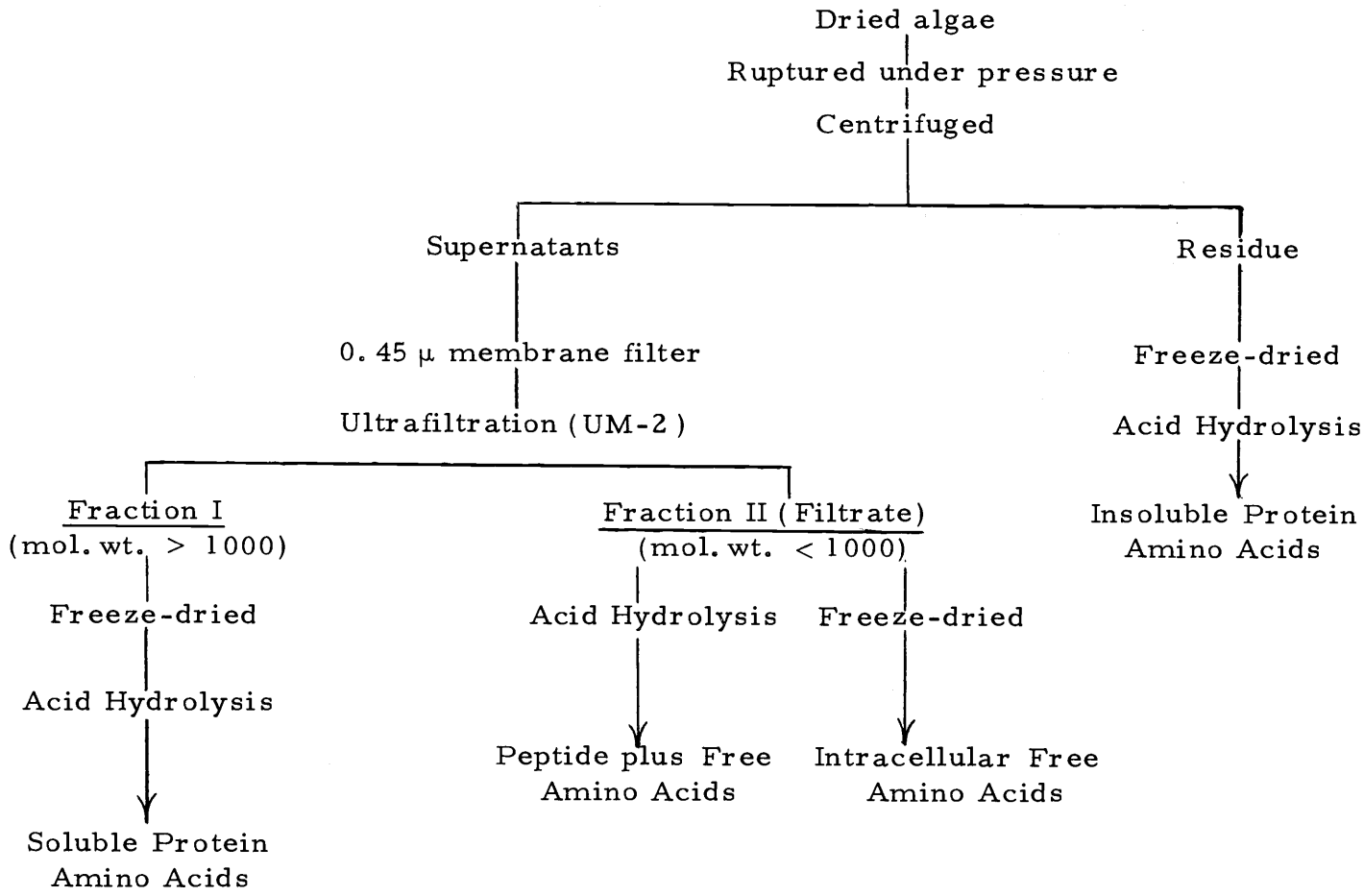


Figure 2. Scheme for separation of amino acid containing compounds in algae.

The ampoule was sealed under vacuum and kept at 110 C (± 2 C) for 20 hours. The temperature was maintained with a refluxing boiling toluene bath. The HCl was removed at the end of hydrolysis using a Rinco evaporator and a hot-air hair dryer. The sample was immediately analyzed for amino acid content.

Organic Acids

A 2 μl sample of the lysed algae supernatant was injected directly into the F & M High Efficiency Gas Chromatograph Model 401. The column was prepared according to Dr. A. W. Anderson (1970, personal communication).

Carbohydrates

Various fractions of Aphanizomenon were analyzed for sugar content using the methods of Borchardt and Piper (1970). Fraction I (Figure 2) was used for free sugar analysis. Fraction II was hydrolyzed and the sugar content was used as an indicator of soluble polysaccharide. Whole cells of the algae (0.5 gm) were hydrolyzed and the sugar content was an indication of total carbohydrate.

RESULTS AND DISCUSSION

Amino Acid Flux

Uptake of Amino Acids

The heterotrophic bacteria in Upper Klamath Lake were shown to possess amino acid transport systems which follow Michaelis-Menten kinetics. Using a range of substrate concentrations, kinetic parameters could be calculated according to equation (5). An example of the computer analysis for the uptake of ^{14}C-glutamic acid is given in Table 2. A modified Lineweaver-Burk plot of this data is presented in Figure 3, showing the kinetic parameters of glutamic acid transport.

Six separate experiments were carried out during the year at the times indicated in Table 3. The kinetic parameters for each amino acid are compiled in Figures 4, 5, and 6. The incubation times and temperatures are given in Table 3.

The maximum uptake velocity, V_{max}, is indicative of heterotrophic capacity since it is the upper limit for uptake. These values reach a maximum during the peak of the algal bloom in August for all the amino acids. The fluctuations in V_{max} seem to be proportional to temperature when the error, described below, is considered.

The incubation periods for the winter sampling dates were too

Table 2. Computer analysis of ^{14}C-glutamic acid uptake by the heterotrophic plankton in Upper Klamath Lake (October 6, 1970)

A	μ	Bottle	Time	CPM-R	CR-R	EF-R	DPM-R	CPM-A	CR-A	EF-A	DPM-A	T-DPM	% RESP	Cut/c
1.92	.024	1	2.0	4827	57.0	81.5	5887	5058	59.8	80.4	6207	12094	48.68	8.74
1.92	.024	2	2.0	4774	60.3	80.2	5916	5145	63.3	79.1	6428	12343	47.93	8.56
1.92	.024	3	2.0	24	84.7	70.8	34	61	71.0	76.1	80	0	0	0
3.84	.048	4	2.0	7249	59.8	80.4	8977	8358	57.9	81.2	10169	19146	46.89	11.04
3.84	.048	5	2.0	8411	56.5	81.7	10258	8187	66.8	77.7	10406	20664	49.64	10.23
3.84	.048	6	2.0	26	80.4	72.5	36	95	73.6	75.1	127	0	0	0
7.68	.095	7	2.0	10537	58.0	81.1	12927	12130	59.7	80.5	14815	27743	46.60	15.24
7.68	.095	8	2.0	10604	55.8	82.0	12873	11274	63.4	79.0	14004	26877	47.90	15.73
7.68	.095	9	2.0	45	72.1	75.7	59	196	73.4	75.2	261	0	0	0
11.52	.143	10	2.0	11013	54.6	82.4	13309	14009	61.7	79.7	16569	29878	44.54	21.22
11.52	.143	11	2.0	10447	56.7	81.6	12745	14032	68.2	77.2	17167	29913	42.61	21.20
11.52	.143	12	2.0	35	83.8	71.1	49	796	64.2	78.7	1011	0	0	0

$Y = 1.31 X + 5.80$

X - intercept is -4.42

Y - intercept is 5.80

Slope is 1.31

V_{max} is 0.76 $\mu g L^{-1} hr^{-1}$

Symbols: A = Substrate added (μg/L); μ = μC added; CPM = counts per minute; CR = channel ratio; EF = efficiency; DPM = disintegrations per minute; -R = respiration; -A = assimilation; and T = total.

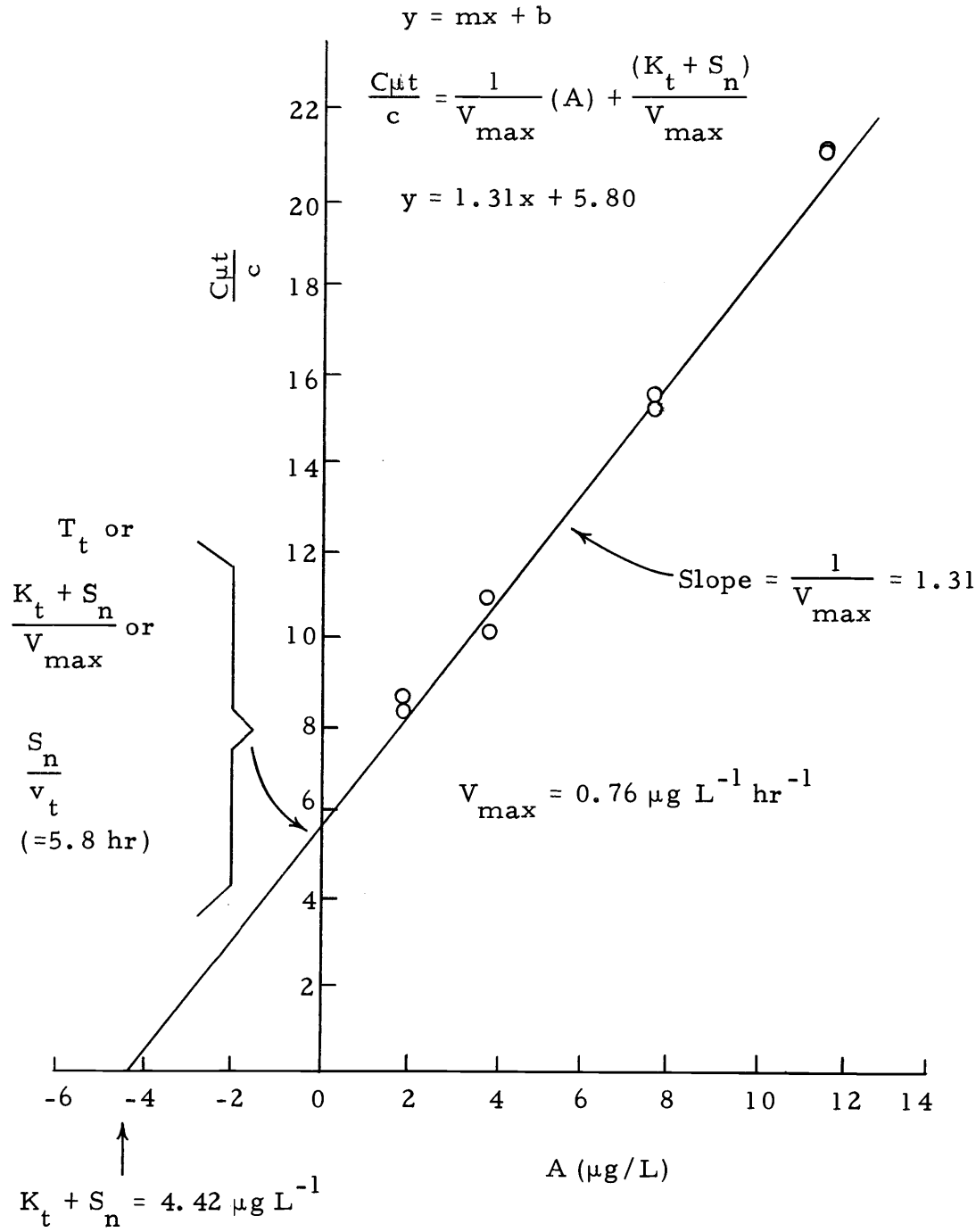


Figure 3. Modified Lineweaver-Burk plot of ^{14}C-glutamic acid uptake by heterotrophic plankton in Upper Klamath Lake surface water on October 6, 1970. (After Wright and Hobbie, 1965a).

Figure 4. Kinetic parameters for the uptake of glutamic acid, aspartic acid, asparagine, lysine, arginine, and ornithine by the heterotrophic plankton in Upper Klamath Lake, during 1970-71.

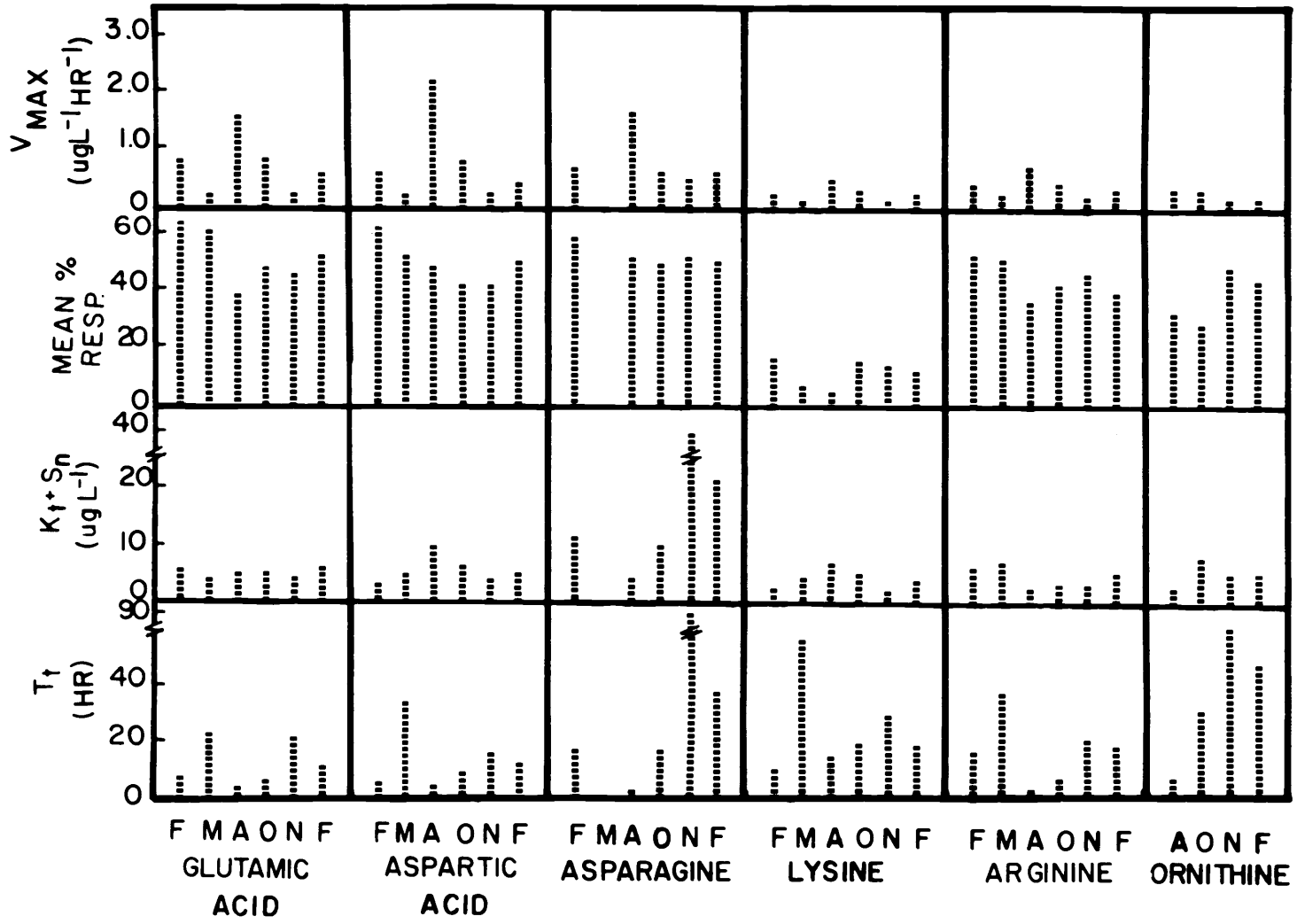

V_{MAX} ($ugL^{-1}HR^{-1}$)
3.0
2.0
1.0
0
MEAN % RESP.
60
40
20
0
$K_t + S_n$ (ugL^{-1})
40
20
10
0
T_t (HR)
90
40
20
0
F M A O N F
GLUTAMIC ACID
F M A O N F
ASPARTIC ACID
F M A O N F
ASPARAGINE
F M A O N F
LYSINE
F M A O N F
ARGININE
A O N F
ORNITHINE

Figure 5. Kinetic parameters for the uptake of serine, glycine alanine, threonine, and proline by the heterotrophic plankton in Upper Klamath Lake, during 1970-71.

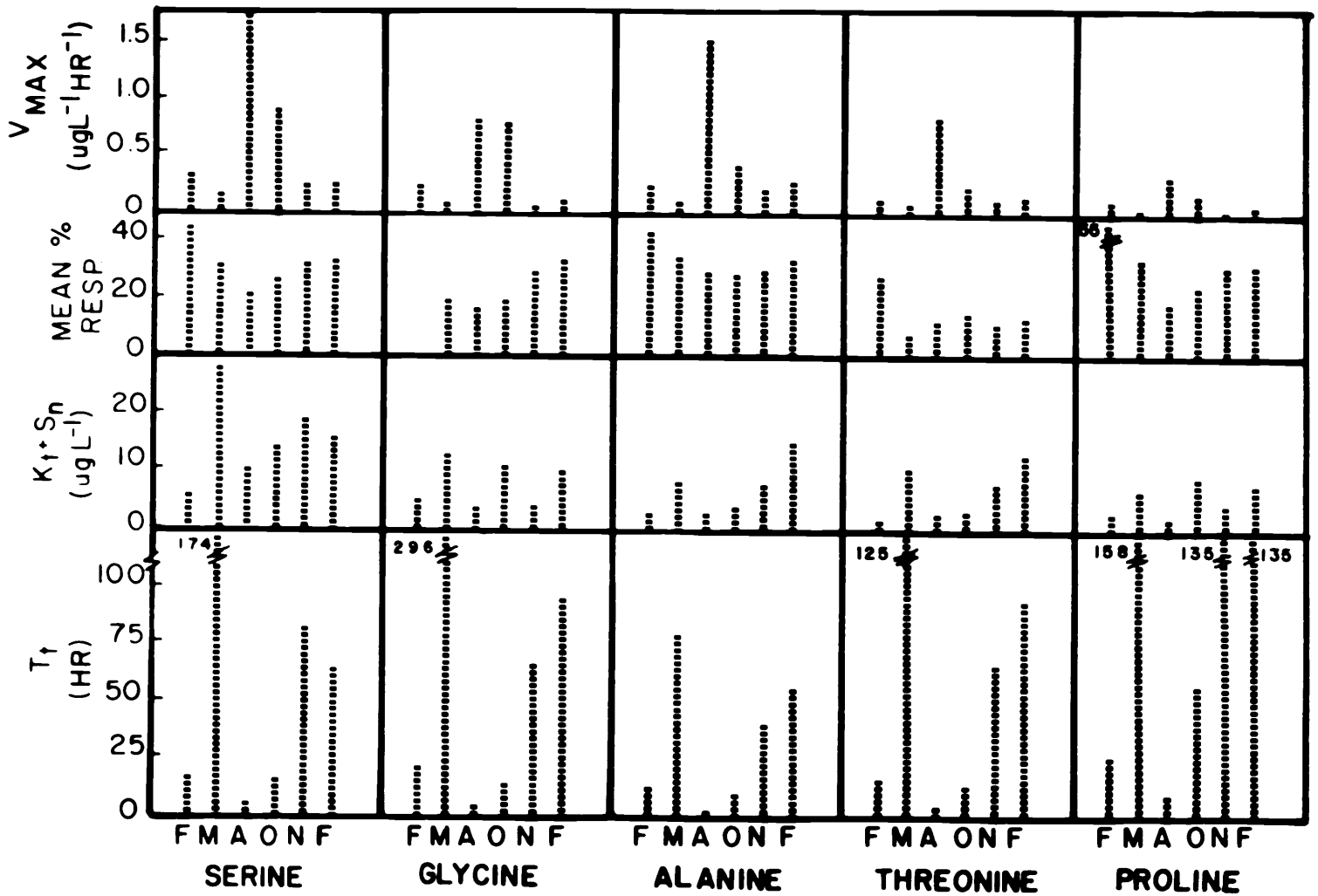

V_{MAX} ($ugL^{-1}HR^{-1}$)
1.5
1.0
0.5
0
MEAN % RESP
40
20
0
$K_t + S_n$ (ugL^{-1})
20
10
0
T_t (HR)
100
75
50
25
0
174
296
125
158
135
135
56
F M A O N F
SERINE
GLYCINE
ALANINE
THREONINE
PROLINE

Figure 6. Kinetic parameters for the uptake of leucine, isoleucine, valine, tyrosine, and phenylalanine by the heterotrophic plankton in Upper Klamath Lake, during 1970-71.

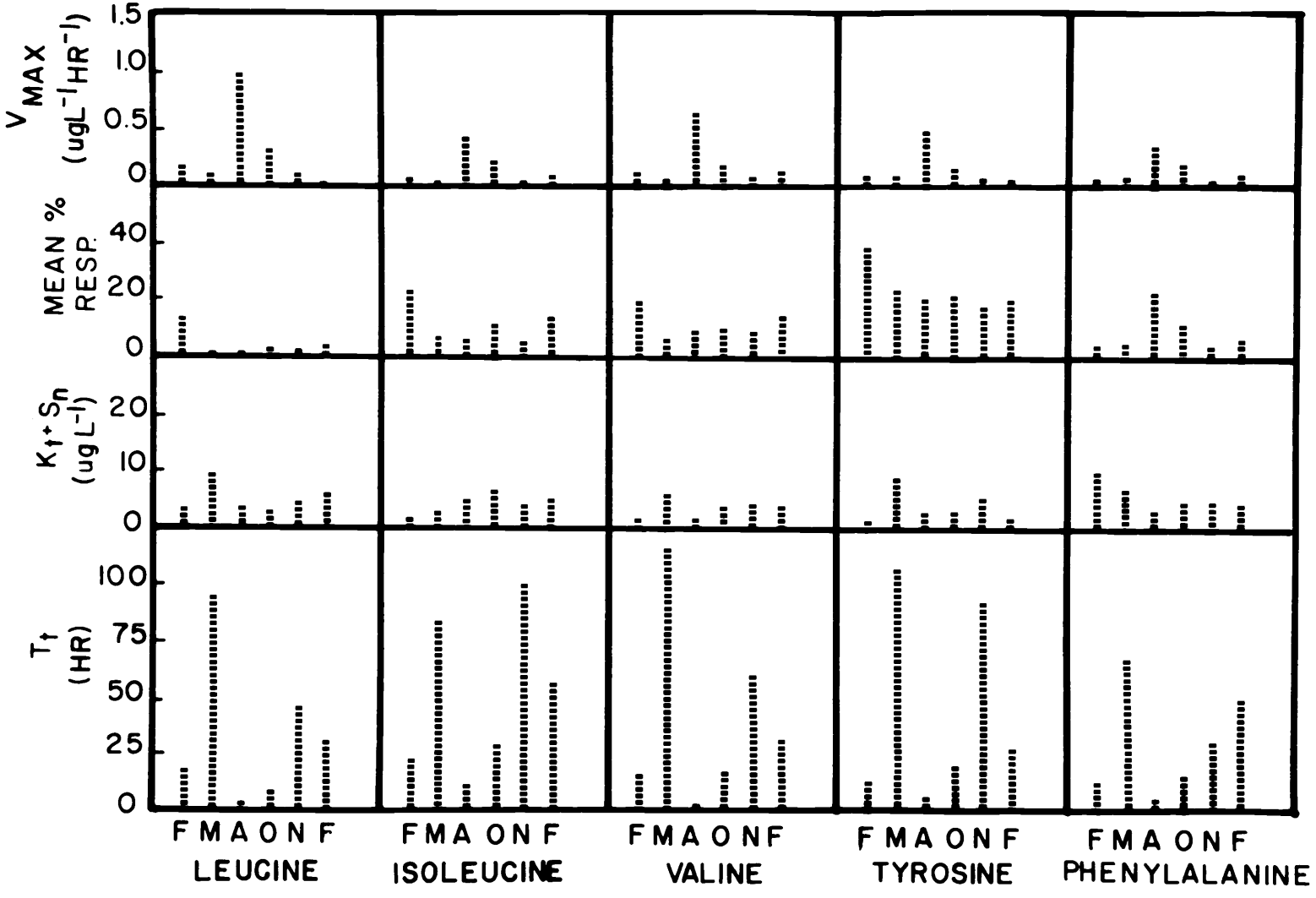

V_{MAX} ($ugL^{-1}HR^{-1}$)
1.5
1.0
0.5
0
MEAN % RESP.
40
20
0
$K_t * S_n$ (ugL^{-1})
20
10
0
T_t (HR)
100
75
50
25
0
F M A O N F
LEUCINE
F M A O N F
ISOLEUCINE
F M A O N F
VALINE
F M A O N F
TYROSINE
F M A O N F
PHENYLALANINE

Table 3. Sampling dates, incubation times and temperatures for amino acid uptake studies

Date	Time (hours)	Temperature °C	Remarks
February 25, 1970	5.0	5.0	Diatom bloom
May 20, 1970	3.0	16.0	Start of B-G[a] bloom
August 20, 1970	0.5	22.0	Peak of B-G bloom
October 6, 1970	2.0	12.5	B-G die-off
November 5, 1970	2.0	7.0	B-G die-off
February 23, 1971	6.0	5.0	Moderate diatom population

[a]B-G = blue-green algae

long for those amino acids with high V_{max} values. Consequently, the percent utilization of the labelled substrate is much higher than the 5% suggested by Wright and Hobbie (1966). Allen (1969) indicated that the incubation period should be much less than the turnover time, T_t. All three kinetic parameters are increased by over-incubation (Hobbie and Crawford, 1969). The V_{max} values for all the amino acids tested on February 25, 1970 and for aspartic acid, glutamic acid, asparagine, and arginine uptake on February 23, 1971 are overestimated. Vaccaro and Jannasch (1966) showed that there is a linear relationship between glucose uptake and time. The uptake studies must be made in this linear region. Therefore, it is important to study the time course of uptake before a series of samples are taken. This study was not performed and has led to the erroneous V_{max} values as indicated. The degree which V_{max} was increased is unknown.

According to Allen (1969), the V_{max} for glucose and acetate are well correlated with temperature only during the fall and winter month months in Lake Lötsjön, Sweden. Increased bacterial numbers and activity are presumably associated with thermal increases. Total bacteria counts are well correlated with maximum velocities for glucose and acetate uptake during the summer months (Allen, 1969).

Hobbie (1967) stated the most important information from seasonal kinetic studies could be obtained from the estimation of turnover times of a particular substrate. The turnover time values reflect the other kinetic parameters, V_{max} and (K_t+S_n).

Vaccaro and Jannasch (1966) suggested a constant (K_t+S_n) value during an annual study may represent the "natural" K_t for that

substrate and S_n may be zero. The (K_t+S_n) values for the uptake of glutamic acid in Upper Klamath Lake consistently fell in the range of 4.5-5.5 μg/L throughout the year. Assuming glutamic acid is rapidly consumed as soon as it becomes available to the bacteria, the K_t value would be approximately 3.3×10^{-8} M. Low transport constants such as this have been reported for other substrates using similar kinetic techniques (Hobbie, Crawford, and Webb, 1968; Hobbie and Wright, 1965; Vaccaro, 1969; Allen, 1969). Pure cultures of Escherichia coli (Brown, 1971) and Pseudomonas aeruginosa (Kay and Gronlund, 1971) have Michaelis constants for aromatic amino acids as low as 5×10^{-7} M.

The Wright-Hobbie kinetic approach measures the carbon-energy source preference of the organisms present at the time of sampling. The nutritional requirements and preferences of the different bacteria which come into predominance throughout the year (Yusha, Morita, and Pacha, 1969) probably have a definite effect on which amino acid has the greatest V_{max}. Glutamic acid, aspartic acid, asparagine, arginine, alanine, and serine seem to be the preferred carbon sources for the bacteria in Upper Klamath Lake. The order of preference for these carbon sources varies during the year. Some of these amino acids can enter the energy-yielding TCA cycle by known metabolic pathways, as briefly shown below:

asparagine ⟶ aspartic acid ⟶ oxaloacetate

glutamic acid ⟶ α-keto-glutarate

alanine ⟶ pyruvate

Since the transport of amino acids is energy-dependent (Kay and Gronlund, 1969) the presence of these amino acids may increase the uptake of the other amino acids. Likewise, these preferred carbon sources may facilitate their own uptake.

The uptake of amino acids by the heterotrophic bacteria was inhibited by the presence of 34 mM sodium azide (Table 4). A small fraction of lysine and arginine seems to have been adsorbed to the particulate matter. This increase in radioactivity between the acid and sodium azide blanks was not caused by adsorption of the amino acids to the Millipore filter, because a count of 657 DPM was retained on the filtration of a distilled water control (brought to pH 8.0 with NH_4OH) containing the same concentration of arginine as the sodium azide blank. Lysine and arginine have pI's of 9.47 and 10.76, respectively (Hodgman, 1961). Therefore, in lake water of pH 8.5 both amino acids would have a positive charge and could be adsorbed to negatively charged substances, such as humic materials or clay particles (Brock, 1966). The total concentrations of lysine and arginine which are available to the bacteria are less than the amounts added to the sample bottles, if adsorption does occur. The effect of this phenomenon on the kinetic parameters should be investigated.

Table 4. Comparison of sodium azide to acid blanks of amino acid uptake by heterotrophic plankton (February 23, 1971)

Amino acid	34 mM sodium azide (DPM)	0.1 N sulfuric acid (DPM)
Aspartic acid	246	166
Threonine	185	200
Serine	250	160
Glutamic acid	384	146
Proline	186	189
Alanine	258	140
Glycine	345	223
Valine	131	205
Isoleucine	197	384
Leucine	237	292
Tyrosine	333	514
Phenyalanine	544	801
Lysine	3819	672
Arginine	7853	432

The mean percent respiration (mineralization) is a good indication of which amino acid is a preferable carbon source. Usually the six amino acids with the greatest V_{max} had the highest percent respired. The percent respired ranged from a low of 2% for leucine to a high of 63% for glutamic acid. The percent respired does increase with increasing concentrations of certain amino acids. Figure 7 illustrates the relationship between the percent respired and increasing concentrations of tyrosine. The slope of the regression line, as calculated by the method of least squares (Mendenhall, 1967), can be used to indicate significant correlation between these two variables for the other amino acids. Those lines having slopes greater than one are considered significant (Table 5). The rate of respiration for these amino acids is not proportional to the rate of transport. Kinetic analysis of the respiration of organic substrates is based on the assumption these two processes are proportional (Harrison, Wright and Morita, 1971).

The amount respired varied during the year for any one amino acid, reaching a minimum during the summer and a maximum in winter. There is a possibility the respired $^{14}CO_2$ was taken up by bacteria or even the blue-green algae. Dark fixation of CO_2 does occur at high concentrations of $^{14}CO_2$ as $NaH^{14}CO_3$ in blue-green algae (Moses, Holm-Hansen, and Calvin, 1959). During the winter months, dark fixation of the respired $^{14}CO_2$ could not be demonstrated

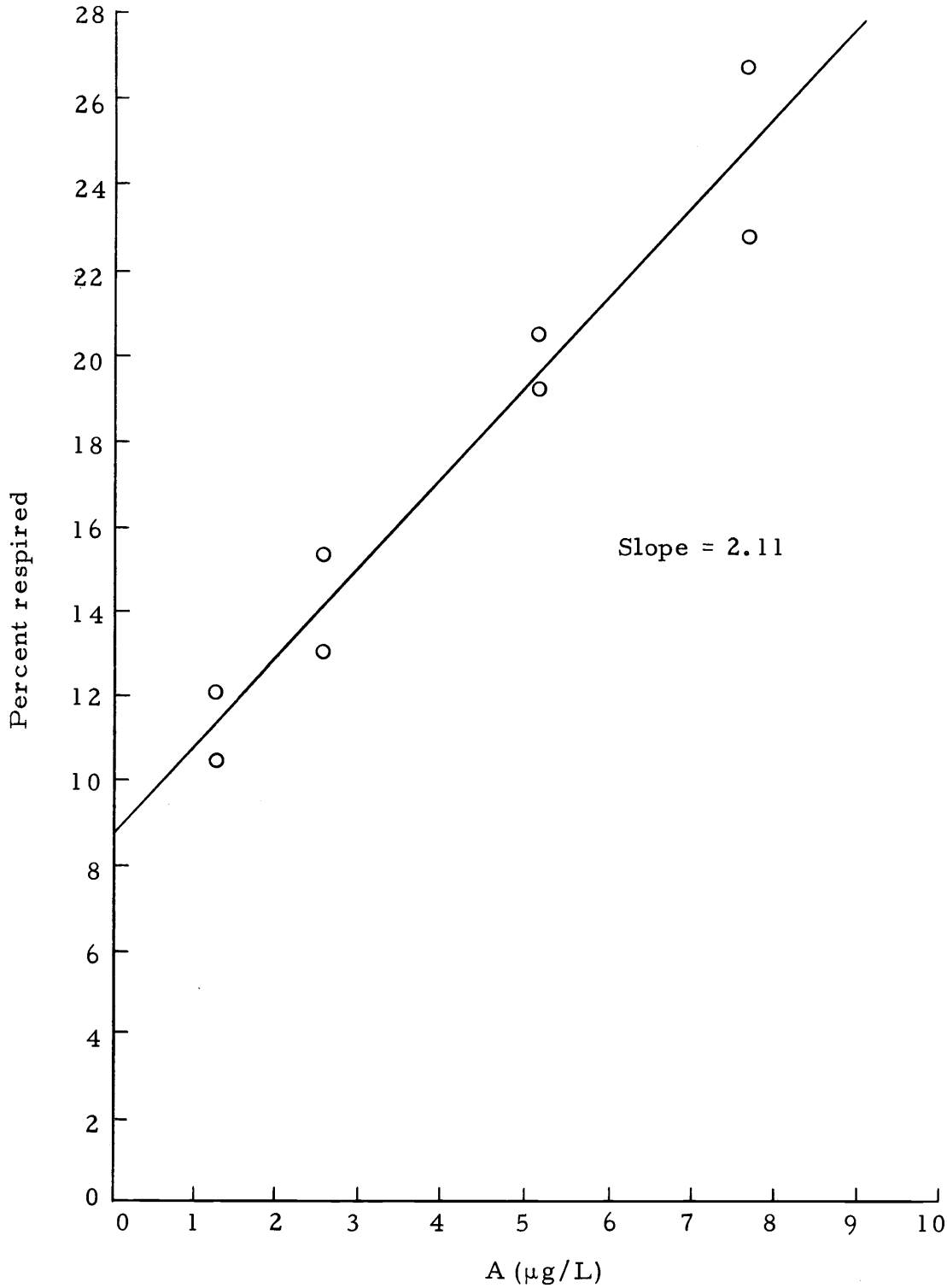


Figure 7. Effect of increasing concentrations of tyrosine on the percent respiration.

Table 5. Slopes of regression lines relating percent respired to substrate concentration

Date: November 5, 1970			
Amino acid	Slope	Amino acid	Slope
Tyrosine	2.11	Lysine	0.41
Ornithine	1.88	Asparagine	0.32
Phenylalanine	1.34	Serine	0.31
Isoleucine	1.13	Glutamic acid	0.27
Arginine	1.10	Proline	0.20
Leucine	0.79	Glycine	-0.36
Threonine	0.62	Valine	-0.36
Alanine	0.47	Aspartic acid	-0.37

(Table 6). There was slight uptake of the $^{14}CO_2$ in the light. This experiment should be made during the summer when bacterial activity is high and the phytoplankton are numerous. It is conceivable that even the brief exposures of the bottles to light, during the sampling and "killing" procedures, are sufficient time for the phytoplankton to take up some of the respired $^{14}CO_2$. If indeed this should be the case, only the mean percent respired values would be affected and not the other kinetic parameters. Another factor which may affect the percent respired is the temperature and its effect on bacterial activity. Perhaps the amino acids are used as energy sources during the winter and incorporated into cellular protein in the summer.

Table 6. Dark and light fixation of $^{14}CO_2$ at 5 C by Upper Klamath Lake plankton (February 23, 1971)

	Bottle no.	DPM of filtered plankton
Dark	1	4
	2	4
	3	14
	Blank	(-48)
Light	1	115
	2	234
	3	170
	Blank	(-42)

Since the amino acids are being catabolized with the release of CO_2 into the environment, it is not unreasonable to assume that ammonia is also being released. These inorganic nutrients regenerated from organic compounds by the bacteria must be used by the phytoplankton considering their close contact with each other. An investigation should be made to determine what percentage of the total CO_2 or NH_3 uptake of the phytoplankton is contributed by these regenerated inorganic nutrients.

Temperature Dependence of Bacterial Uptake

Temperature has a definite effect on the transport systems of heterotrophic bacteria. The relative velocities for the uptake of glutamic acid (Figure 8) and glycine (Figure 9) can be calculated using equation (4) at each of the selected temperatures, assuming $S_n = 0$. The Q_{10} values can be derived from the linear portions of the curves. For glutamic acid and glycine, the Q_{10} values are 2.2 and 3.6 respectively. These values are in the range found for acetate and glucose (Parsons and Strickland, 1962; Wright and Hobbie, 1966), and are great enough to show the need for controlled temperature during incubation. The K_t also increases with temperature (Vaccaro and Jannasch, 1966; Wright and Hobbie, 1966). It is not known if the S_n of a particular water sample will change with temperature. If amino acids are adsorbed to particulate or colloidal

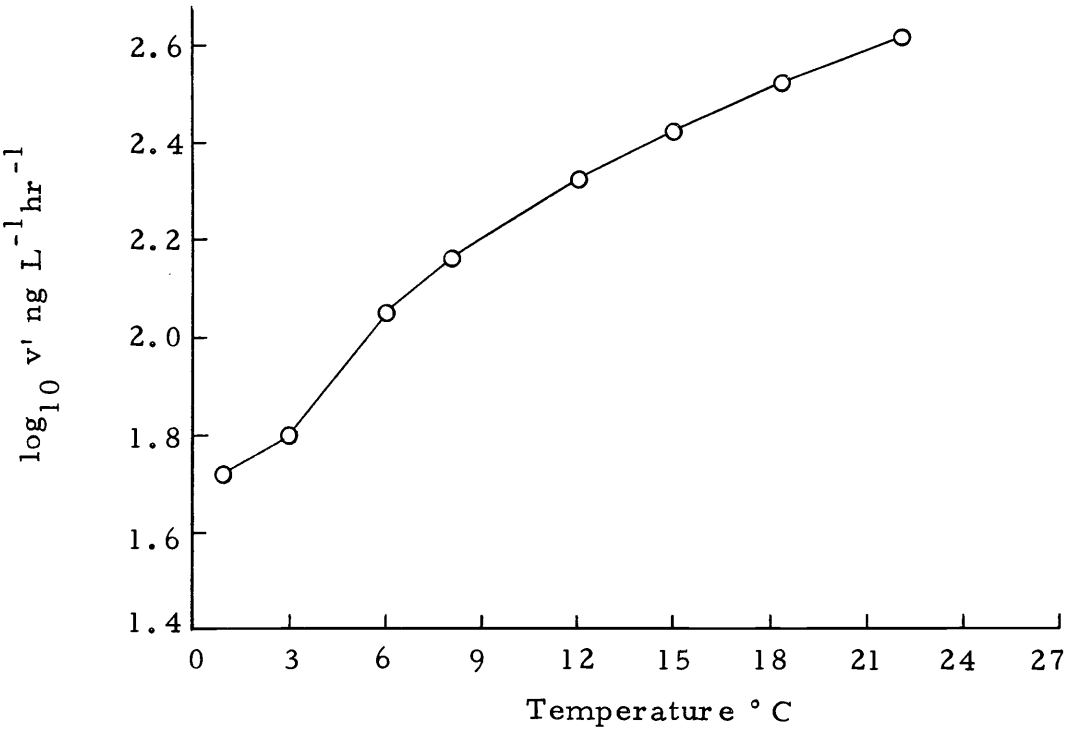


Figure 8. Temperature effect on the uptake velocity of ^{14}C-glutamic acid by the heterotrophic plankton in Upper Klamath Lake.

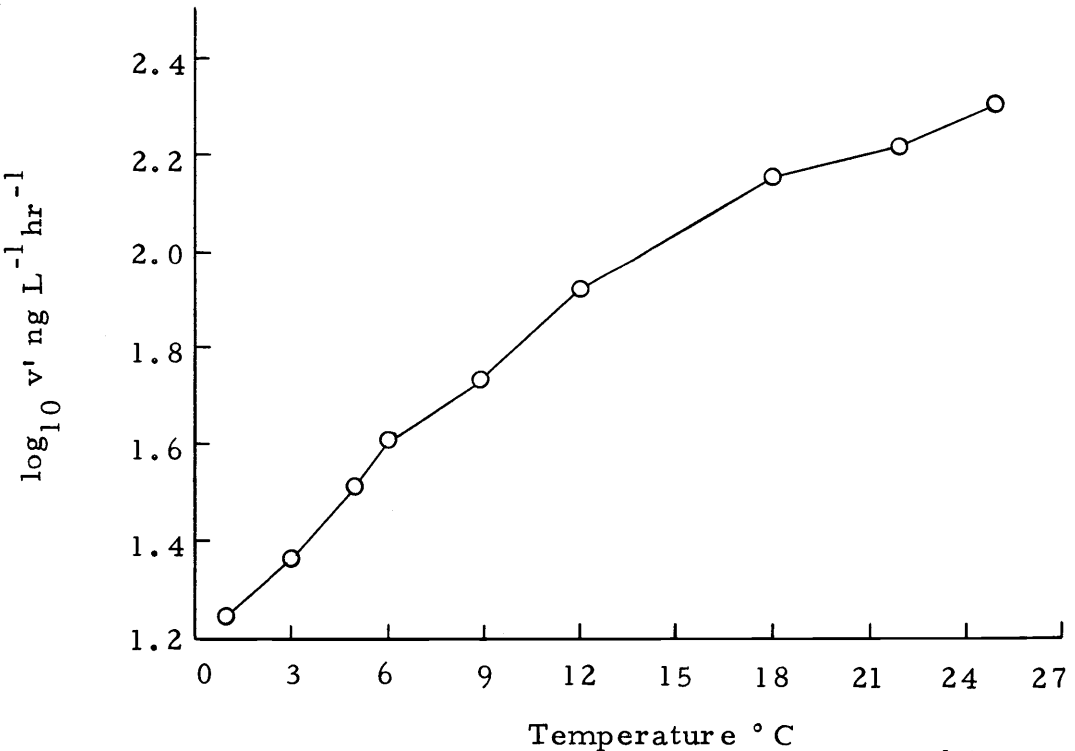


Figure 9. Temperature effect on the uptake velocity of ^{14}C-glycine by the heterotrophic plankton in Upper Klamath Lake.

substances there is a possibility that they will be released with increasing temperature. The adsorptive process is reversed with a rise in temperature (West, 1963).

Competitive Inhibition

The uptake of a single amino acid by the heterotrophic bacteria in Upper Klamath Lake is affected by the presence of other amino acids. Increasing concentrations of unlabelled serine, alanine, and leucine greatly affect the uptake of labelled glycine (Figure 10). Aspartic acid has a less marked effect on glycine uptake at the concentration used. Inhibition curves of labelled glutamic acid uptake by unlabelled glutamic and aspartic acids nearly coincide (Figure 11). To a lesser degree, serine interferes with the uptake of labelled glutamic acid. Table 7 shows the inhibitory effect of certain amino acids on aspartic acid and leucine. Aspartic acid is a preferred carbon source (Figure 4) for the microorganisms and is not significantly inhibited by these amino acids. However, serine has high V_{max} values in the lake (Figure 5) and somewhat inhibits the uptake of aspartic acid. Leucine uptake is stimulated by a low concentration of aspartic acid, but is inhibited to varying degrees by other neutral amino acids.

The effect of unlabelled serine (28 μg/L) on the kinetic parameters of labelled glycine uptake is illustrated in Figure 12. The V_{max} remained constant, while T_t and $(K_t + S_n)$ increased as expected, if

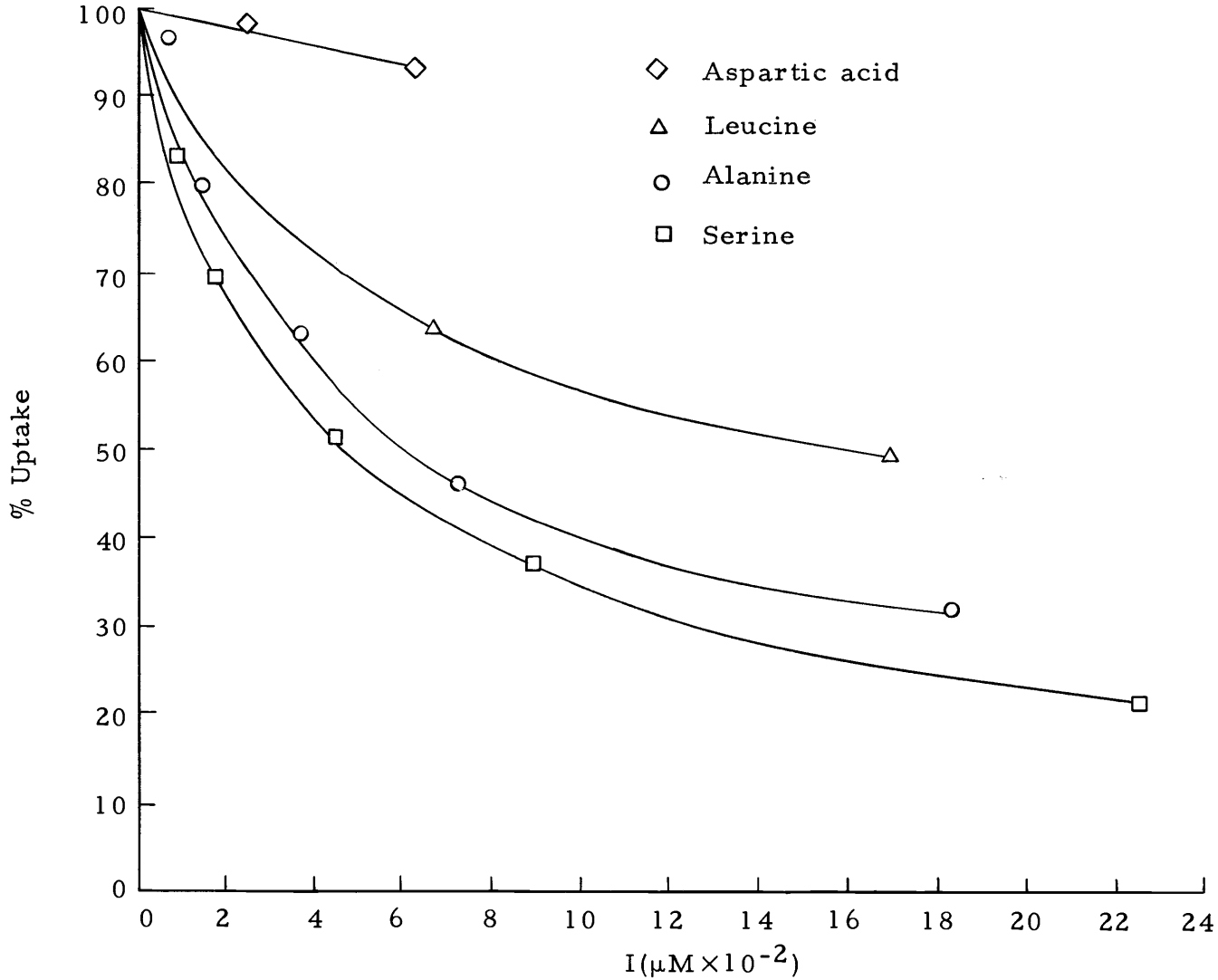


Figure 10. Effect of unlabelled aspartic acid, leucine, alanine, and serine on the uptake of ^{14}C-glycine.

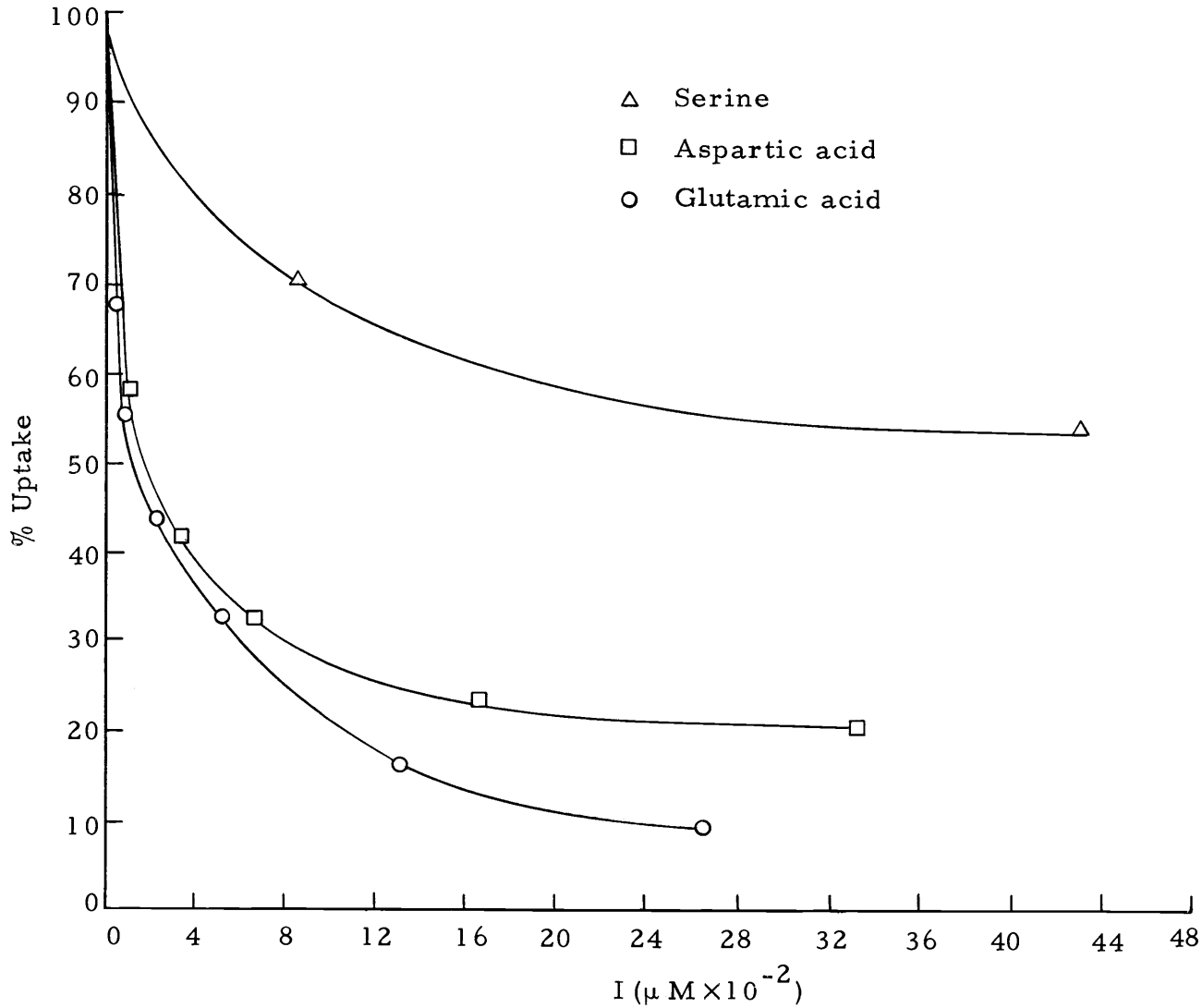


Figure 11. Effect of unlabelled serine, aspartic acid and glutamic acid on the uptake of ^{14}C-glutamic acid.

Table 7. Competitive inhibition of aspartic acid and leucine by various amino acids

Labelled substrate	Inhibitor	Concentration ($\mu M \times 10^{-2}$)	Percent of non-inhibited uptake
Aspartic acid (0.046 μM)	Aspartic acid	6.2	29.1
	Alanine	18.4	79.8
	Glycine	34.9	87.8
	Serine	22.5	69.9
	Leucine	16.9	95.7
Leucine (0.032 μM)	Leucine	16.9	12.1
	Isoleucine	18.1	44.0
	Valine	20.8	28.3
	Aspartic acid	6.2	104.7
	Alanine	18.4	40.6
	Glycine	14.0	65.6
		34.9	44.0
	Serine	9.0	58.0
		22.5	41.5

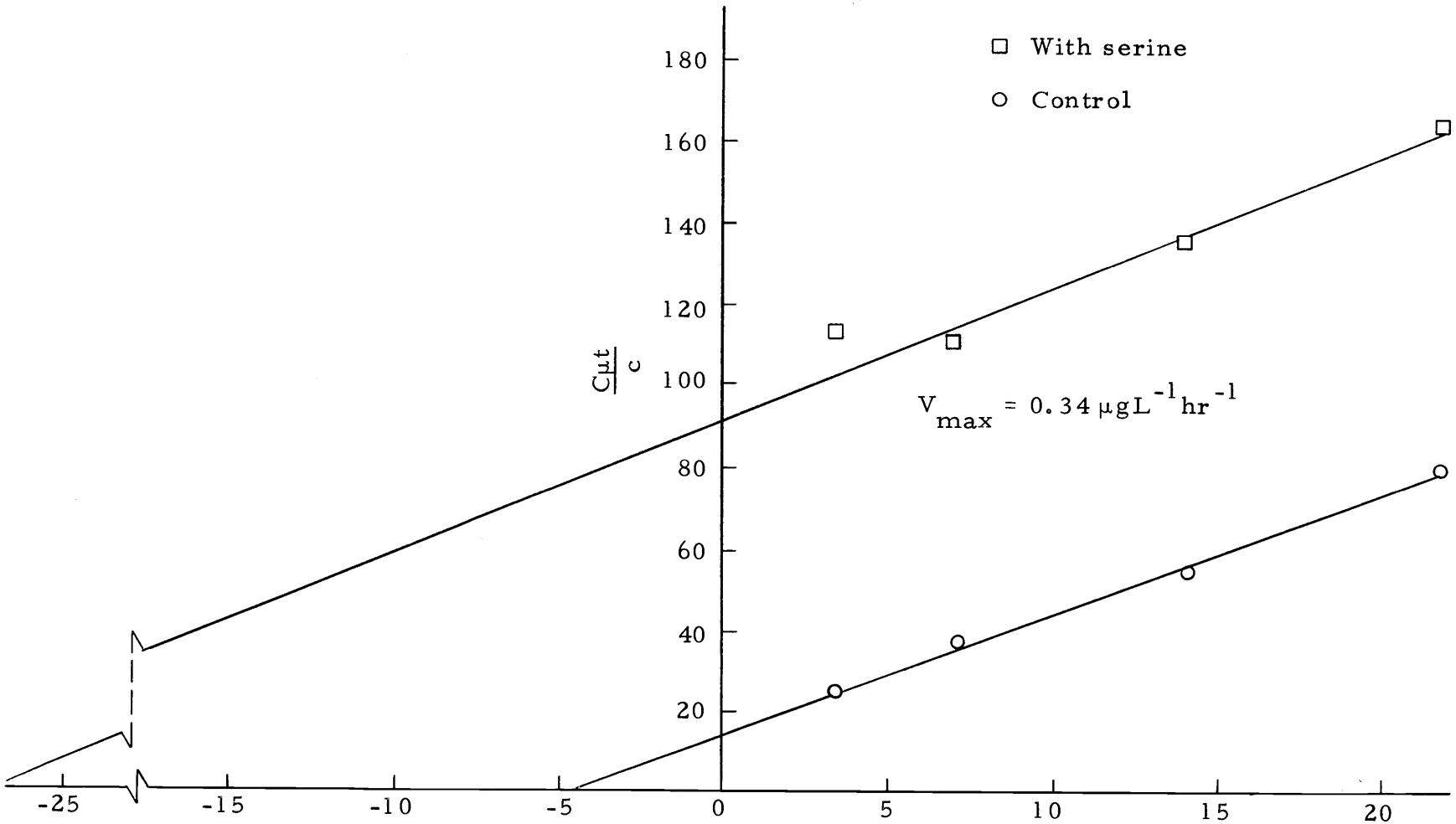


Figure 12. Effect of unlabelled serine (28 μg/L) on the kinetic parameters of ^{14}C-glycine uptake.

there was competitive inhibition between serine and glycine. The (K_t+S_n) value in the presence of serine approaches the sum of the control (K_t+S_n) and the added 28 μg/L serine. Serine and glycine probably share a common transport system for the predominant bacteria in the lake water.

Provided amino acids act only as competitive inhibitors, the V_{max} will remain unchanged and would be the most significant parameter. The T_t would be indicative of the time required for the natural population to remove the natural concentration of amino acids which share a common transport system. The (K_t+S_n) would be the maximum value for the sum of the amino acids sharing this common system.

Structurally similar amino acids have the same transport system in certain organisms (Britten and McClure, 1962; Piperno and Oxender, 1968; Kay and Gronlund, 1969, 1971), and even some non-related amino acids share the same uptake systems (DeBusk and DeBusk, 1965; Brown, 1971). However, using a natural water sample, a variety of organisms work on a variety of organic compounds. It seems reasonable to assume these organisms do not have identical transport systems and this composite of systems is working on the labelled substrate added to the lake water. Amino acids which affect the transport of another amino acid in some bacterial species, but not in others, are "seen" as inhibitors to the

uptake of that particular amino acid.

Effect of other organic compounds on the uptake of another substrate was mentioned by Wright and Hobbie (1966), they showed a mixture of carbohydrates (0.5 mg/L) decreased the V_{max} for glucose uptake by 42%. They suggested the uptake of these carbohydrates acted as non-competitive inhibitors causing a shift in the internal metabolism to degenerate the glucose transport system or perhaps competition with glucose was taking place in the internal pathway of carbohydrate metabolism.

Kinetic parameters for amino acid transport measured under natural conditions have great significance in aquatic microbial ecology. However, the complexity of factors affecting these kinetic parameters should be identified to better interpret the meaning of the parameters. Competitive inhibition shows that more than one substrate is taken up by the transport system. The T_t and (K_t+S_n) values determined from uptake experiments using only one of those substrates can not be used to describe all the parameters of that particular transport system.

Removal Rates of High Concentration of Amino Acids

Various amino acids were added to lake water at concentrations equivalent to one mg per liter. The reduction in concentration of each amino acid was determined after 48 and 96 hours incubation.

No significant reduction could be observed at 6 C, but at 15 C rates of removal could be calculated (Table 8). The long incubation times needed to demonstrate removal of amino acids allowed bacterial growth which hastened the removal of some amino acids. Arginine, aspartic acid, glutamic acid, glycine, lysine, and methionine seem to be removed at a constant rate. Conversely, serine, isoleucine, and leucine were produced under the conditions of incubation. Alanine, threonine, tyrosine, and valine were either slightly produced or were removed at a decreasing rate. An increased rate of removal was shown by proline, phenylalanine, and histidine which may be the result of induced oxidative enzymes. The relative order of removal was approximately the same as the magnitude of the V_{max} values obtained using water taken from the lake on the same date (February 23, 1971). The notable exceptions are glycine, which is removed at a slower rate, and leucine and valine which are removed at a higher rate than measured by the Wright-Hobbie technique.

True V_{max} values could not be determined by the above procedure because algae are capable of uptake at high organic substrate concentrations. Also the required long incubation times lead to bacterial growth with subsequent production of some amino acids and induced rise in utilization of other amino acids. This experiment does illustrate what might happen when a high concentration of amino acids are released into the environment as a result of phytoplankton

Table 8. Rates of removal of high concentrations of amino acids from Upper Klamath Lake water

Amino acid	Removal rate ($\mu g\ L^{-1} hr^{-1}$) Zero time to 48 hours	48 hours to 96 hours
Arginine	4.82	6.79
Aspartic acid	4.41	7.42
Glutamic acid	3.96	6.80
Glycine	3.68	4.39
Alanine	2.73	1.72
Lysine	2.61	3.13
Serine	2.47	-2.85
Methionine	1.78	1.94
Threonine	1.49	0.55
Isoleucine	1.30	-2.48
Tyrosine	0.91	0.81
Proline	0.70	2.49
Leucine	0.61	-0.54
Phenylalanine	0.51	1.38
Histidine	0.46	1.26
Valine	0.41	0.05

autolysis or organic pollution from man.

Recently, Williams and Gray (1970) demonstrated that abrupt increases in amino acid concentrations produced an immediate rise in the respiration rate of the natural marine heterotrophic population. A second, induced increase in the rate occurred after a 20-35 hour period. They concluded the induced rate of respiration is proportional to the increase in substrate concentration. Therefore, marked increases of amino acids, and presumably all easily assimilated organic compounds, will only be temporary. The heterotrophic populations will be able to rapidly reduce the concentrations with their induced rate of respiration.

Theoretically, V_{max} values for each amino acid could be determined in the presence of all the amino acids. Labelled amino acids could be added to a water sample at concentrations which would saturate the transport systems. After incubation, the sample could be filtered and the remaining labelled amino acids in the filtrate could be separated on the amino acid analyser. The radioactivity for each amino acid could be determined by passing the column effluent through a solid scintillation fluor, as described by Becker (1967). The V_{max} values could be calculated by the rate of disappearance of each amino acid. The technical problems of suitable concentrations of labelled amino acids to be used and the natural amino acid concentrations would have to be solved before such an experiment would

be practical.

Dissolved "Free" Amino Acids

The average percent recovery values from the cation exchange column for each amino acid are given in Table 9. The high recoveries of serine, glycine, and alanine can be explained by the presence of these amino acids in the "blank" run. Evidently these amino acid contaminants are present in the chemicals added in processing the water sample.

The concentrations of amino acids in Upper Klamath Lake water determined by the methods used, appear to be the sum of the adsorbed and metabolically free amino acids, and are referred to as "free" amino acids. Usually the (K_t+S_n) values obtained from the uptake experiments were smaller than the "S_n" values for the same date. A large fraction of the amino acids detected by ion exchange must not have been available to the bacteria on the basis of this kinetic value. Occasionally the "S_n" values were smaller than the (K_t+S_n) and apparent K_t's could be calculated (Table 10). Since the "S_n" can not be confirmed as metabolically free amino acids these values are difficult to interpret. In addition, because of possibly competitive inhibition effects, the (K_t+S_n) should reflect the sum of the natural concentration of the amino acids taken up by a particular transport system. It may be impossible to calculate

Table 9. Average percent recovery of amino acids from cation exchange column[a]

Amino acid	Percent recovery	Amino acid	Percent recovery
Lysine	97.2	Glycine	104.9
Histidine	95.2	Alanine	99.6
Arginine	74.4	Valine	93.6
Aspartic acid	96.1	Isoleucine	97.8
Threonine	96.5	Leucine	96.1
Serine	100.3	Tyrosine	96.1
Glutamic acid	95.7	Phenylalanine	94.5
Proline	97.7		

[a]Based on five recovery experiments.

Table 10. Calculated apparent K_t values

Amino acid	Date	K_t ($\times 10^{-8}$M)	Amino acid	Date	K_t ($\times 10^{-8}$M)
Arginine	May 20, '70	2.5	Valine	Nov. 5, '70	0.4
	Oct. 6, '70	1.0	Isoleucine	Nov. 5, '70	0.6
	Feb. 23, '71	1.4		Feb. 23, '71	0.9
Glutamic acid	Oct. 6, '70	0.3	Tyrosine	May 20, '70	0.6
	Feb. 23, '71	0.5		Nov. 5, '70	0.4
Alanine	Nov. 5, '70	2.2	Phenylalanine	Oct. 6, '70	0.2
	Feb. 23, '71	6.9		Feb. 23, '71	1.0
Serine	Nov. 5, '70	2.5	Proline	May 20, '70	0.6
Leucine	May 20, '70	2.0		Oct. 6, '70	5.6
	Nov. 5, '70	5.2		Nov. 5, '70	1.3
	Feb. 23, '71	1.7		Feb. 23, '71	4.0
Threonine	Nov. 5, '70	1.5			
	Feb. 23, '71	3.9			

the K_t value unless the S_n values could be reduced to zero and then do an uptake study. This also may be difficult to do if there is an equilibrium between the free amino acids and the adsorbed amino acids.

Treatment of the filtered water sample prior to cation exchange affected the "free" amino acid concentrations (Table 11). Ultrafiltration was done in an effort to remove any colloidal adsorbing substances, but the process increased the amino acid concentrations over that of the control. The freeze-thawed sample and the control gave comparable values. The concentration of each "free" amino acid at various sample dates throughout the year are presented in Table 12. The most abundant amino acids are serine, lysine, and glycine, at intermediate concentrations are aspartic acid, alanine, and threonine, and the remaining amino acids were always below 10 μg/L. The relative order of abundance agrees very well with the results of Brehm (1967) and Hobbie, Crawford, and Webb (1968). The "free" amino acids reach a maximum, which may be caused by the diatom bloom die-off, in late spring prior to the blue-green algae bloom. The low was reached in the early fall during the blue-green die-off, when the bacterial biomass was probably great enough to minimize the free amino acid concentration. The "free" amino acid content of Upper Klamath Lake water for February, 1970 and 1971 are almost identical, with the exception of lysine concentration. This may

Table 11. Comparison of pre-treatments of Upper Klamath Lake water samples for amino acid analysis

Amino acid	Concentration (μg/L)		
	Ultrafiltration	Freeze-thaw	Control
Lysine	28.43	13.18	12.35
Histidine	8.75	4.22	3.61
Arginine	trace	4.99	2.84
Aspartic acid	9.97	9.50	7.28
Threonine	9.13	6.91	5.44
Serine	38.49	19.70	15.87
Glutamic acid	5.49	5.98	5.31
Proline	4.28	2.85	2.46
Glycine	21.17	13.22	9.95
Alanine	11.75	7.80	5.87
Valine	5.41	4.25	3.40
Isoleucine	4.61	3.62	2.93
Leucine	6.68	5.31	3.84
Tyrosine	7.61	4.19	4.20
Phenylalanine	4.82	2.55	2.44

Table 12. "Free" amino acids in Upper Klamath Lake surface water (μg/L)

Amino acid	Feb 25, '70[1]	May 20, '70[1]	Aug. 20, 70[1]	Oct. 6, '70[2]	Nov. 5, '70[2]	Feb. 23, '71[2]
Lysine	15.81	28.38	24.60	8.88	12.35	20.12
Histidine	5.47	5.98	8.74	2.15	3.16	5.85
Arginine	2.53	1.99	2.53	< 1.00	2.84	2.19
Aspartic acid	10.97	14.99	18.71	6.99	7.28	11.37
Threonine	8.29	12.74	12.80	7.53	5.44	7.79
Serine	33.64	48.63	44.57	16.94	15.87	31.86
Glutamic acid	5.04	8.90	6.47	3.98	5.31	5.03
Proline	3.96	5.76	5.16	2.46	2.46	3.15
Glycine	15.05	22.09	18.54	10.72	9.22	15.89
Alanine	9.85	14.14	13.25	6.71	5.87	8.87
Valine	4.15	7.25	7.22	3.42	3.40	3.20
Isoleucine	3.70	5.98	8.79	3.02	2.93	3.49
Leucine	4.34	7.19	5.38	3.32	3.84	3.75
Tyrosine	4.61	7.51	7.01	2.75	4.20	3.65
Phenylalanine	2.55	4.99	3.97	2.06	2.44	2.24
Total	129.96	196.52	187.74	81.93	86.61	147.61

[1] Sample was frozen, thawed, and filtered (Whatman #1) before analysis

[2] Without treatment

indicate the presence of the same concentration of adsorbing substances.

Andrews and Williams (1971) measured the amino acid concentration in the English Channel. Total amino acid concentrations varied from 20-80 μg/L throughout the year, but there was no marked seasonal variation. They used the method described by Siegel and Degens (1966) in which the amino acids were retained on Chelex 100 resin (in the copper form). The percent recoveries of amino acids obtained using their technique varied from 31% to 73%. The concentration of amino acids in their "blank" was as high as most of their total amino acid concentrations which was about 0.3 μmoles amino acid per liter. The sea-water samples were also acidified which may have released some of the adsorbed amino acids.

Biochemical Analyses of Predominant Blue-green Algae

Amino Acid Composition

The intracellular free amino acids (IFAA) comprise approximately 2.1% of the dry weight of Aphanizomenon flos-aquae (Table 13). Aspartic acid is 55% of this fraction or 1.2% of the dry weight, and is potentially a good carbon source for the heterotrophic bacteria. The difference between the hydrolyzed and nonhydrolyzed IFAA fraction is considered to be the amino acid content of the algal

Table 13. Amino acid composition of predominant blue-green algae

Amino acids	Aphanizomenon				Gloeotrichia			
	Free amino acids	Peptide amino acids	Soluble protein	Insoluble protein	Free amino acids	Peptide amino	Soluble protein	Insoluble protein
	(μg/100 mg)		(mg/100 mg)		(μg/100 mg)		(mg/100 mg)	
Aspartic acid	1228.0	97.7	1.01	5.38	31.6	23.6	0.25	7.03
Threonine	66.6	120.2	0.51	1.77	7.7	30.3	0.16	3.10
Serine	30.9	69.4	0.41	1.61	5.5	25.6	0.13	2.82
Glutamic acid	181.2	474.4	1.40	4.18	84.1	32.9	0.25	5.22
Proline	trace	trace	0.31	1.20	trace	17.4	0.09	1.48
Glycine	16.8	206.6	0.48	1.51	7.1	46.0	0.10	2.14
Alanine	144.0	180.4	0.90	2.34	19.6	24.5	0.15	3.60
Valine	15.3	43.6	0.58	2.54	5.7	19.0	0.13	3.00
Methionine	70.2	142.0	0.56	0.64	3.8	7.2	0.02	0.65
Isoleucine	20.9	64.1	0.47	2.15	4.0	11.7	0.11	2.98
Leucine	25.7	53.1	0.66	3.51	6.2	20.5	0.20	4.11
Tyrosine	24.0	85.6	0.35	1.31	5.2	4.8	0.08	1.40
Phenylalanine	18.1	190.4	0.33	1.38	4.9	14.0	0.09	2.16
Lysine	44.2	46.1	0.48	1.98	10.5	15.2	0.12	2.81
Histidine	211.1	48.0	0.10	0.47	3.2	4.5	0.02	0.56
Arginine	40.1	50.0	0.52	4.46	29.2	20.3	0.12	4.55
Total	2137.0	1773.0	9.05	36.43	228.4	316.9	2.02	47.61

peptides. These peptides have a high content of glutamic acid, glycine, and phenylalanine. Approximately 0.2 mg of glucosamine was detected in this hydrolyzed fraction and the same concentration in the soluble protein fraction.

Gloeotrichia echinulata colonies were quite resistant to the rupturing process used in contrast to the fragile Aphanizomenon cells. Microscopic examination of the cells after the second processing revealed good breakage. The IFAA plus the peptide amino acids accounted for only 0.55% of the dry weight of Gloeotrichia. Traces of glucosamine were detected in all hydrolyzed fractions of the alga.

The amino acid composition of the soluble and insoluble "bulk" proteins for the two blue-green algae (Table 13) indicate the insoluble fraction contains the majority of the protein. The soluble protein, consisting largely of chromoprotein, is readily decomposed and utilized by the heterotrophic bacteria (Merkel, Braithwaite, and Kritzler, 1964).

Organic Acids

A single peak corresponding to acetic acid was detected by gas chromatography for both blue-green algae. Other organic acids were not detected. Aphanizomenon and Gloeotrichia contain 1.8% and 1.6% of their dry weight as acetate, respectively.

Carbohydrates

Only Aphanizomenon was analyzed for carbohydrate content. The low molecular weight (< 1000) fraction yielded a trace of glucose (less than 10 μg/100 mg algae) and two unidentified three or four-carbon sugars or their derivatives. Acid hydrolysis of the high molecular weight fraction detected deoxyribose, arabinose, xylose, mannose, galactose, and glucose. Glucose was present in the highest concentration, approximately 1.2 mg/100 mg algae. About 2% of the dry weight of this alga is released as soluble carbohydrate. The analysis of the whole cell preparation after hydrolysis yielded approximately 15.2% glucose, 1% galactose, 0.5% mannose, and 1.4% pentoses. These results are minimal since the extent of hydrolysis was not determined.

Foree and McCarty (1970) have followed the rate and extent of algal decomposition under anaerobic conditions. The particulate organic matter is first converted to soluble forms which are then converted to short chain fatty acids, predominantly acetic acid. These are fermented to methane gas or, if sulfate is available, to CO_2 by sulfate reducing organisms. About 40% of the algal material is considered refractory or undecomposable.

Upper Klamath Lake is very shallow and most of the algae probably are decomposed in the sediments. The microbial activity

in these sediments is orders of magnitude greater than the overlying water (Harrison, Wright, and Morita, 1971). More information is needed on the activity of the sediment organisms and their role in recycling of algae nutrients from organic materials.

SUMMARY

The Wright-Hobbie kinetic approach of measuring uptake of organic compounds is currently the best method to determine the in situ activities of microorganisms in natural waters. However, the various factors which affect the calculated kinetic parameters of substrate uptake should be identified.

Competitive inhibition among amino acids for common transport systems is well known. The maximum velocity of substrate uptake, V_{max}, is unchanged by competitive inhibition, but the turnover time, T_t, and the (K_t+S_n) values are increased. These latter values then reflect the total natural concentrations and affinities of the amino acids transported by a particular transport system.

A year survey of amino acid uptake by heterotrophic plankton in Upper Klamath Lake has shown that the calculated V_{max} values for all the amino acids are proportional to temperature. The interpretation of the T_t and (K_t+S_n) values is difficult because of possible competitive inhibition effects among the amino acids. The (K_t+S_n) values do suggest, however, that the heterotrophic plankton have a very strong affinity for the amino acids.

The percent of the labelled amino acid taken up by the microorganisms and subsequently respired as $^{14}CO_2$ appears to have an inverse relationship to temperature. Those amino acids with the

highest V_{max} values had the highest percent respired. The percent respired for some of the amino acids increased with substrate concentration and therefore, in these cases, respiration is not proportional to transport. If the respiration rate is used as a means of measuring transport these two processes must be proportional.

A reliable analysis for dissolved free amino acids is needed since standard cation exchange procedures cause an apparent release of adsorbed amino acids. The amino acid concentrations determined for Upper Klamath Lake surface water during the year survey are probably the sum of the free and adsorbed amino acids. These "free" amino acids maintain a maximum concentration in the summer and a minimum in early fall.

Large increases in amino acid concentrations change their rate of removal from a lake water sample. These changes can be placed into three categories dependent on the amino acid: 1) increased rate of removal, probably caused by inducible enzyme production; 2) no appreciable change in the removal rate; and 3) production of the amino acid. The actual in situ rate could not be determined. A method is proposed for measuring the uptake of each amino acid in the presence of all the other amino acids.

Biochemical analyses of the predominant blue-green algae show potentially 15% of the dry weight of Aphanizomenon flos-aquae can be released as free amino acids, peptides, soluble protein,

acetate, and carbohydrate upon autolysis of the cells. Gloeotrichia echinulata releases very little soluble nitrogenous compounds, but does release acetate upon cell rupture.

The results of this study indicate that future investigations should be made on 1) a sensitive technique to detect concentrations of amino acids in natural waters which can be readily metabolized by the heterotrophic plankton; 2) the role of amino acid adsorbing substances in natural waters and their effect on the measurement of amino acid transport; 3) the effect of preferential carbon sources on amino acid transport; 4) a technique of measuring the uptake of amino acids in the presence of all the other amino acids at their natural concentrations; and 5) the significance of the respired CO_2 derived from decomposable organic compounds to the nutrition of the phytoplankton.

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