

# Heterotrophic Potential for Amino Acid Uptake in a Naturally Eutrophic Lake<sup>1</sup>

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The uptake of sixteen <sup>14</sup>C-labeled amino acids by the indigenous heterotrophic microflora of Upper Klamath Lake, Oregon, was measured using the kinetic approach. The year-long study showed a seasonal variation in the maximum uptake velocity,  $V_{max}$ , of all the amino acids which was proportional to temperature. The maximum total flux of amino acids by the heterotrophic microflora ranged from 1.2 to 11.9  $\mu\text{mol}$  of C per liter per day (spring to summer). Glutamate, asparagine, aspartate, and serine had the highest  $V_{max}$  values and were respired to the greatest extent. The percentages of the gross (net + respired) uptake of the amino acids which were respired to  $\text{CO}_2$  ranged from 2% for leucine to 63% for glutamate. Serine, lysine, and glycine were the most abundant amino acids found in Upper Klamath Lake surface water; at intermediate concentrations were alanine, aspartate, and threonine; and the remaining amino acids were always below  $7.5 \times 10^{-8}$  M (10  $\mu\text{g/liter}$ ). The amino acid concentrations determined chemically appear to be the sum of free and adsorbed amino acids, since the values obtained were usually greater than the ( $K_t + S_n$ ) values obtained by the heterotrophic uptake experiments.

In recent years the kinetic approach has been used to assay the uptake of soluble organic compounds by heterotrophic organisms in natural waters and sediments (1, 7, 8, 10, 11, 15, 21, 29). The kinetic approach is based on the principles of Michaelis-Menten theory (15, 28) which had been used for studying purified enzyme kinetics and uptake kinetics of pure cultures of bacteria. Investigators using the kinetic technique have found that mixed populations in freshwater take up organic compounds by what appears to be active transport systems which can be described by Michaelis-Menten kinetics. In some marine studies, use of the kinetic method has resulted in uninterpretable data (6, 22). However, low-level organic enrichment of the seawater for at least 24 h resulted in solvable kinetic analyses (20).

Using the kinetic approach, the maximum velocity of uptake ( $V_{max}$ ), the turnover time ( $T_t$ ), and the sum of a "transport constant" and the natural substrate concentration ( $K_t + S_n$ ) can be calculated. If the natural substrate concentration,  $S_n$ , can be accurately determined by chemical or biological assay, then the

actual velocity of substrate uptake,  $v_t$ , can be determined (29).

A number of problems with the kinetic technique have been recently reviewed by Wright (27), and Williams (25) has tested the validity of its application to the heterogenous microbial populations. One of the basic problems has been the complex and unknown composition of natural waters. Burnison and Morita (4) demonstrated that competitive inhibition among groups of amino acids changes the interpretation of the graphically determined uptake parameters.

This paper compares the kinetic data obtained before, during, and after the annual summer bloom of blue-green algae in Upper Klamath Lake, a naturally eutrophic lake in south central Oregon. Dissolved free amino acid concentrations were quantitatively determined to estimate the natural substrate concentration,  $S_n$ . (This paper was submitted by B. Kent Burnison in partial fulfillment of the requirements for the Ph.D. degree at Oregon State University, Corvallis, Ore.)

## MATERIALS AND METHODS

**Amino acid uptake.** The uptake parameters of 16 uniformly-labeled [<sup>14</sup>C]amino acids by the heterotrophic plankton in Upper Klamath Lake were deter-

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mined by the kinetic approach as outlined by Wright and Hobbie (28) and as modified by Hobbie and Crawford (9). All [ $^{14}\text{C}$ ]amino acids (40.1% isotopic abundance) used had a specific activity of 25  $\mu\text{Ci}$  per  $\mu\text{mol}$  per carbon atom, except [ $^{14}\text{C}$ ]glycine and [ $^{14}\text{C}$ ]ornithine which had specific activities of 109 and 204  $\mu\text{Ci}/\mu\text{mol}$ , respectively. All radioactive amino acids were obtained from Amersham Searle, Des Plaines, Ill. Various quantities of [ $^{14}\text{C}$ ]amino acid (0.5  $\mu\text{Ci}/\text{ml}$ ) were added to 50-ml serum bottles. The usual series of four consisted of 50, 100, 200, and 300  $\mu\text{liters}$  of labeled substrate, in triplicate. The highest concentration ever used was 20  $\mu\text{g}/\text{liter}$  for serine. A 10-ml sample of lake water was added to each of the serum bottles. One of each set of three samples was a blank to which 0.2 ml of 5 N  $\text{H}_2\text{SO}_4$  was added, just prior to the addition of the lake water. Each bottle was immediately sealed with the respiration apparatus described in Hobbie and Crawford (9). The samples were incubated at in situ temperature ( $\pm 0.5$  C) in the dark. After the incubation period of 0.5 to 7.0 h, depending on lake temperature, the samples were killed and processed according to Hobbie and Crawford (9). The acidified water samples were transported back to the laboratory where they were filtered (within 24 h) through 0.45- $\mu\text{m}$  membrane filters, rinsed with 10 ml of 0.1 N  $\text{H}_2\text{SO}_4$ , dried at 40 C, and placed in toluene fluor [0.01% 1,4-bis-2-(5-phenyloxazolyl)benzene and 0.4% 2,5-diphenyloxazole]. All samples were counted by liquid scintillation and corrected for quench by the external standard channels ratio method. Computer analysis was used to calculate the kinetic parameters from the uptake data.

**Temperature studies.** Lake water (10-ml samples) (sample taken on 23 February 1971, in situ temperature 6 C) was placed in sterile test tubes and equilibrated to proper temperature in a polythermostat (13) for 10 min. Either  $2.9 \times 10^{-2}$   $\mu\text{g}$  of [ $^{14}\text{C}$ ]glutamate or  $1.8 \times 10^{-2}$   $\mu\text{g}$  of [ $^{14}\text{C}$ ]glycine was added to the test tubes at various temperatures (1 to 27 C), in duplicate, with appropriate control. The tubes were sealed with the respiration apparatus (9) and incubated for 0.5 to 5.0 h, depending on the temperature. At the end of the incubation periods, the samples were acid-killed and processed as described above.

**Determination of dissolved amino acids.** A 5-liter amount of lake water was passed through a plankton net (Turtox standard no. 12), Whatman no. 1 filter paper, and membrane filter (0.45  $\mu\text{m}$  pore size) into a sterile pressure can (Millipore Corp.). The sample was transported back to the laboratory on ice. The samples were either frozen or immediately processed as described below. If the sample was frozen, it was thawed and filtered (Whatman no. 1) to remove the brown humic material which had formed in the freeze-thaw process. The effects of the freeze-thaw-filtration process on the amino acid content was determined by comparing two identical water samples taken on 5 November 1970; one sample was frozen and the other was processed immediately through cation exchange (see below).

The pH of 2 liters of the water sample was lowered to 2.8 with approximately 22 ml of glacial acetic acid.

Sodium azide (0.02% final concentration) was added as a bacteriostatic agent. The acidified sample was passed through a cation exchange column (Dowex 50W-X2) at a flow rate of 1 to 2 ml/min at 6 C. After the sample had been applied, the resin bed was washed with 1 bed volume of distilled water, and the amino acids were desorbed by the addition of 2 volumes of 2 N ammonium hydroxide. The effluent was collected and evaporated to a small volume using a flash evaporator at 50 C. An internal standard, 0.25  $\mu\text{M}$  norleucine, was added, and the solution was transferred to a 50-ml flask and evaporated to dryness. The residue was analyzed for amino acid content (19) with a Spinco model 120B automatic amino acid analyzer.

Recovery experiments were made with every amino acid analysis of lake water. The standard amino acid solution consisted of 1  $\mu\text{mol}$  of each amino acid per ml. A 400- $\mu\text{liter}$  amount of this standard was added to 2 liters of 0.2 N acetic acid and 0.02% sodium azide. This solution was processed in the same manner as the lake water sample.

## RESULTS

### Kinetic parameters of amino acid uptake.

The uptake of 16 amino acids by the heterotrophic plankton in Upper Klamath Lake surface water was measured six times during 1970 to 1971. The sample incubation times, temperature, and comments on algal "bloom" conditions are given in Table 1. Amino acid  $V_{max}$  values, mean percentage of respiration, ( $K_t + S_n$ ) values, and  $T_i$  are given in Fig. 1, 2, and 3.

The  $V_{max}$  is indicative of heterotrophic capacity since it is the upper limit of uptake. These values reached a maximum during the peak of a blue-green algal "bloom" of *Gloeotrichia echinulata* in August 1970 for all the amino acids tested. The variability in the  $V_{max}$  values for each of the amino acids during the year appears to be temperature dependent, with the possible exception of the May 1970 sample.

Glutamate, asparagine, aspartate, and serine had the highest  $V_{max}$  values on a nanomole of C

TABLE 1. Sampling dates, incubation times, and temperatures for amino acid uptake studies

Date	Time (h)	Temp (C)	Remarks
25 February 1970	5.0	5.0	Diatom bloom <sup>a</sup>
20 May 1970	3.0	16.0	Start of B-G bloom <sup>b</sup>
20 August 1970	0.5	22.0	Peak of B-G bloom
6 October 1970	2.0	12.5	B-G die-off
5 November 1970	2.0	7.0	B-G die-off
23 February 1971	6.0	5.0	Moderate diatom population

<sup>a</sup> Predominantly a *Stephanodiscus* sp.

<sup>b</sup> B-G, Blue green algae. Predominantly *Gloeotrichia echinulata*, but also contained *Aphanizomenon flos-aquae*, *Anabaena circinalis*, and *Microcystis aeruginosa*.

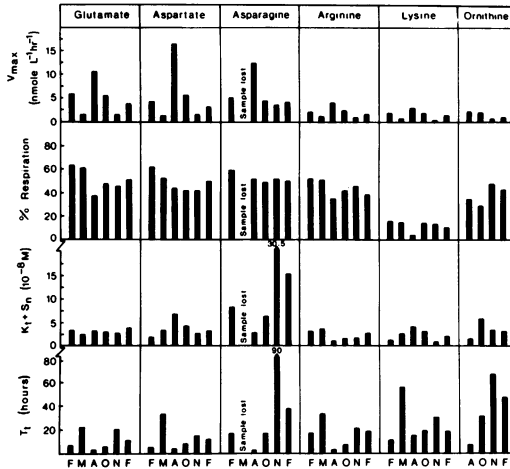


FIG. 1. Kinetic parameters for the uptake of glutamate, aspartate, asparagine, arginine, lysine, and ornithine by the heterotrophic plankton in Upper Klamath Lake, during 1970 to 1971.

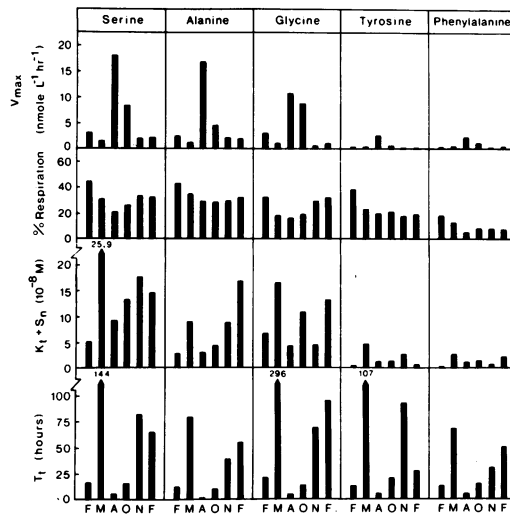


FIG. 2. Kinetic parameters for the uptake of serine, alanine, glycine, tyrosine, and phenylalanine by the heterotrophic plankton in Upper Klamath Lake, during 1970 to 1971.

per liter per hour basis. Arginine, leucine, glycine, and alanine were in an intermediate category. The amount of carbon which was assimilated from each of the remaining amino acids was generally less than 10 nmol of C per liter per h, except for the rates on 20 August 1970, which were about twice as high. The maximum total flux of amino acids into the

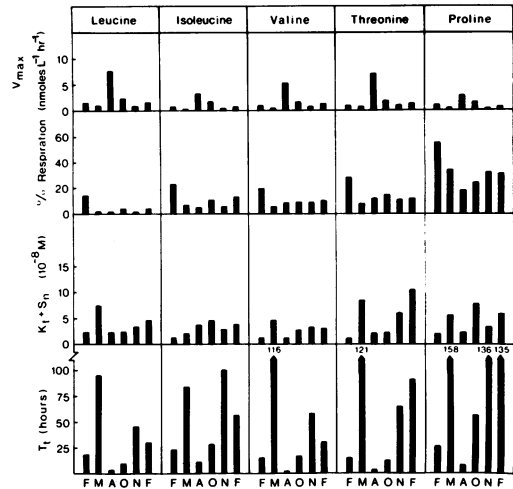


FIG. 3. Kinetic parameters for the uptake of leucine, isoleucine, valine, threonine, and proline by the heterotrophic plankton in Upper Klamath Lake, during 1970 to 1971.

indigenous heterotrophic microflora in the surface water ranged from 1.2  $\mu\text{mol}$  of C per liter (14.2 mg of C/m<sup>3</sup>) per day in May to 11.9  $\mu\text{mol}$  of C per liter (143 mg of C/m<sup>3</sup>) per day in August 1970 (Table 2). The amino acids which were taken up by the heterotrophs were partially oxidized to CO<sub>2</sub> and therefore used as an energy source (see below) and partially incorporated into cellular material. It should be noted that a fraction may have been excreted as metabolic by-products, and this fraction was considered negligible in the present study. The relative order of amino acids attaining the highest  $V_{max}$  values changed when only the radioactivity retained in the cell was considered. Leucine contributed the most carbon to the heterotrophic plankton, followed closely by glutamate, serine, asparagine, aspartate, and alanine. The remaining amino acids contributed approximately the same amount of carbon as given above for the total or gross (net + respired)  $V_{max}$  flux since the mean percent respiration was comparatively low. The calculated flux values were based on the assumption that there were no diurnal variations in  $V_{max}$  and they did not include ornithine data, since this amino acid was not detected in the lake water sampled.

The ( $K_t + S_n$ ) values for the majority of the amino acids did not change appreciably during the year, the notable exceptions being asparagine (Fig. 1), serine, alanine, and glycine (Fig. 2). The turnover times,  $T_t$ , did change dramati-

cally during the year, but did not show any seasonal trend.

**Mineralization of amino acids.** The percentage of total amino acid uptake, which was respired as  $\text{CO}_2$ , varied throughout the year, depending on the amino acid and temperature of the lake (Fig. 1, 2, and 3). The dicarboxylic amino acids, glutamate and aspartate, and the amide, asparagine, were respired to the greatest extent (40 to 63%). Arginine was also readily mineralized (respired) and varied from 35 to 51% during the year. Alanine, serine, proline, ornithine, tyrosine, and glycine were mineralized in an intermediate range usually from 20 to 40%. The remaining amino acids, lysine, phenylalanine, threonine, valine, isoleucine, and leucine usually had a mean percent respiration of less than 15%. Leucine is especially notable, having only 2% respired, with the exception of 14% in February 1970.

The maximum amount of  $\text{CO}_2$  which can be released into the environment by heterotrophic metabolism of organic compounds, can be calculated by the following equation:

$$\text{mg CO}_2/\text{liter/h} = V_{max} \times \% \text{ respiration} \times 44 \quad (1)$$

where  $V_{max}$  is expressed in millimoles of C per liter per hour; percent respiration is disintegrations per minute (dpm) of  $^{14}\text{CO}_2$ /(dpm  $^{14}\text{CO}_2$  + dpm  $^{14}\text{C}$  in particulate fraction); and 44 is the conversion factor between millimoles and milligrams of  $\text{CO}_2$ . This value can also be obtained by plotting the  $C\mu t/c$  values for the respired  $^{14}\text{CO}_2$  versus the added substrate concentration ( $A$ ) (see below).

Using equation 1 and assuming  $V_{max}$  is constant throughout the day, the amount of  $\text{CO}_2$  released by amino acid metabolism on the sampling dates can be determined (Table 2). The maximum amount of  $\text{CO}_2$  which could be produced ranged from 15 mg of  $\text{CO}_2$  per  $\text{m}^3$  per day in May 1970, to 135 mg of  $\text{CO}_2$  per  $\text{m}^3$  per day in August 1970.

**Temperature dependence of bacterial uptake.** Temperature had a definite effect on the transport systems of the heterotrophic plankton sampled on 23 February 1971 (in situ temperature of 6 C). Assuming  $S_n = 0$ , the relative uptake rates for [ $^{14}\text{C}$ ]glutamate and [ $^{14}\text{C}$ ]glycine could be calculated at each of the selected temperatures according to the equation:

$$\text{ng of substrate/liter/h} = \frac{c}{C\mu t} (A) \quad (2)$$

TABLE 2. Net, respired, and net + respired total  $V_{max}$  values, and total dissolved "free" amino acid concentrations

Date	Total $V_{max}$			Total " $S_n$ " ( $\mu\text{g/liter}$ )
	Net (mg of $\text{C}/\text{m}^3/\text{day}$ )	Respired (mg of $\text{C}/\text{m}^3/\text{day}$ )	Net + respired (mg of $\text{C}/\text{m}^3/\text{day}$ )	
25 February 1970	20.3	17.5 (64) <sup>a</sup>	37.8	130.0
20 May 1970	10.1	4.1 (15)	14.2	196.5
20 August 1970	106.1	36.9 (135)	143.0	187.7
6 October 1970	42.2	16.9 (62)	59.1	81.9
5 November 1970	12.6	6.3 (23)	18.9	87.8
23 February 1971	21.1	10.4 (38)	31.5	128.5

<sup>a</sup> Number in parentheses is milligrams of  $\text{CO}_2$  per cubic meter per day.

where  $c$  is the radioactivity (dpm) of the filtered microorganisms plus the radioactivity (dpm) of the respired  $^{14}\text{CO}_2$ ,  $C$  is the dpm in  $1 \mu\text{Ci}$  of  $^{14}\text{C}$ ,  $\mu$  is the  $\mu\text{Ci}$  of  $^{14}\text{C}$ -labeled substrate added,  $t$  is the incubation time (h); and  $A$  is the added concentration of substrate (nanograms per liter). An Arrhenius plot can be made of the results (Fig. 4).

The [ $^{14}\text{C}$ ]glutamate plot gave an activation energy of 19,700 cal/mol between 1 and 12 C, corresponding to a  $Q_{10}$  at 6 C of 3.6, and 11,000 cal/mol between 12 and 22 C, corresponding to a  $Q_{10}$  at 16 C of 1.9. The plot for [ $^{14}\text{C}$ ]glycine gave activation energies of 23,500 cal/mol ( $Q_{10} = 4.5$ ) at the lower temperatures and 8,400 cal/mol ( $Q_{10} = 1.7$ ) at the higher temperatures. It is also of interest to note that the percentage respired did not vary significantly over the range of temperatures used,  $33.3 \pm 4.4\%$  and  $23.8 \pm 3.6\%$  for glutamate and glycine, respectively. The percentage of glycine respired did change during the year study of the lake (Fig. 2) and this was probably caused by the changes in bacterial diversity.

The amino acid concentrations determined for the surface water of Upper Klamath Lake appear to be the sum of free and adsorbed amino acids and will be referred to in the present study as "free" amino acids or " $S_n$ ." Usually, the  $(K_t + S_n)$  values obtained from the kinetic approach to amino acid uptake were smaller than the " $S_n$ " values for the same date water samples.

Amino acid concentrations were corrected for recovery from the cation exchange processing. More than 95% of all the amino acids were recovered, except arginine (74% recovered).

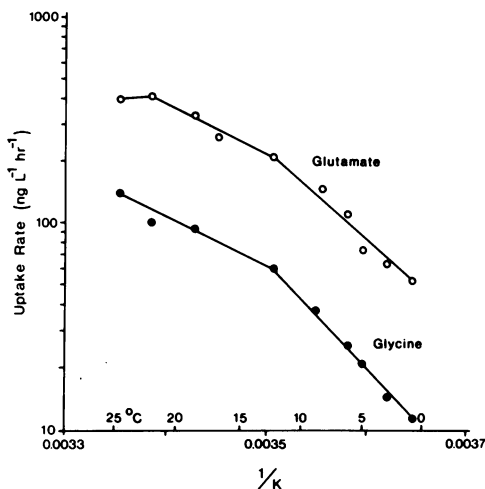


FIG. 4. Temperature effect on the uptake rate of [ $^{14}\text{C}$ ]glutamate (upper graph) and [ $^{14}\text{C}$ ]glycine (lower graph), by the heterotrophic plankton in Upper Klamath Lake.

The effect of the freeze-thaw-filtration procedure on the concentrations of the majority of amino acids was minimal. The concentrations were slightly higher in the frozen samples (1  $\mu\text{g}/\text{liter}$ ). The exceptions were aspartate, alanine, leucine, and arginine which differed by 30, 32, 38, and 75%, respectively. These percent errors seem high, but they are considered reasonable because of the amino acid concentrations involved and the precision of the procedure. For example, the 30% error found for aspartate is indicative of a 2.2  $\mu\text{g}/\text{liter}$  difference. The amino acid concentrations for February, May, and August 1970 were not corrected for any freeze-thawing affects.

The most abundant amino acids in Upper Klamath Lake surface water were serine, glycine, and lysine; alanine, aspartate, and threonine were at intermediate concentrations; and the remaining amino acids were always below  $7 \times 10^{-8}$  M (Fig. 5). Those amino acids with the highest concentration showed the greatest variation in concentration during the year. The total "free" amino acids ranged from 81.9  $\mu\text{g}/\text{liter}$  (October 1970) to 196.5  $\mu\text{g}/\text{liter}$  (May 1970). A perhaps coincidental similarity was found in the February 1970, and 1971 samples where the amino acid concentrations were 130 and 128.5  $\mu\text{g}/\text{liter}$ , respectively (Table 2). Figure 5 shows that all the individual amino acid concentrations were also very similar on these two dates, with the exception of lysine.

## DISCUSSION

The  $V_{max}$  values obtained for the heterotrophic uptake of amino acids by the plankton in Upper Klamath Lake were dependent upon temperature. Allen (1) has also shown  $V_{max}$  for glucose and acetate in the eutrophic Lake Lotsjön was correlated with total bacterial plate counts in addition to temperature. A somewhat contrasting study (14) showed the vertical distribution of  $V_{max}$  values of glucose uptake in the Plusssee were correlated with bacterial biomass (based on direct microscope counts) but not to bacterial plate counts.

The incubation period for heterotrophic uptake experiments must be carefully determined, especially during the winter months. The  $V_{max}$  values in the present study for all the amino acids tested on 25 February 1970 and for aspartate, glutamate, asparagine, and arginine on 23 February 1971 may have been slightly overestimated because more than 5% of the added labeled substrate was utilized during the incubation period. The utilization of less than 5% insured an insignificant decrease in the total substrate concentration (9, 29), and therefore the initial velocity of uptake could be determined.

Hobbie (8) has calculated the natural uptake velocity ( $v_t$ ) of amino acids by the plankton in the Pamlico River estuary, N.C., using the relationship:  $v_t = S_n/T_t$ . To use this equation, it must be shown that the transport systems are specific for the substrate studied. Burnison and Morita (4) have demonstrated that some amino acid transport systems of the indigenous hetero-

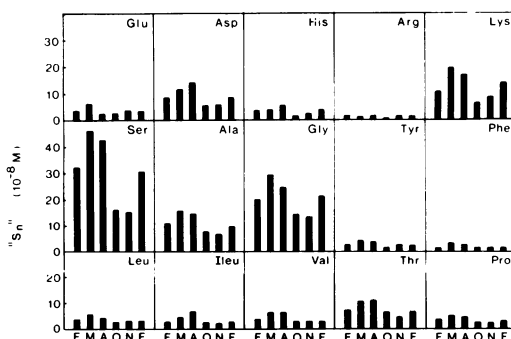


FIG. 5. Dissolved "free" amino acid in Upper Klamath Lake surface water. Samples in February, May, and August 1970 were frozen, thawed, and filtered with Whatman no. 1 filter paper prior to cation exchange processing; samples from the remaining three months were processed immediately (see Materials and Methods).

trophic plankton in Upper Klamath Lake are not specific, and therefore, a calculation of  $v_t$  would be erroneous. Hobbie (8) stated the  $K_t$  values for all of the amino acids tested were much higher than the  $S_n$  values, unlike the relationship between  $K_t$  and  $S_n$  usually found for glucose and acetate. If competitive inhibitory effects were actually present in this estuary, data such as  $K_t$  being much higher than  $S_n$  would result. The actual  $K_t$  [in contrast to the apparent  $K_t$ , which is determined by subtracting the chemically determined  $S_n$  from the ( $K_t + S_n$ ) value obtained in a heterotrophic uptake experiment] may be very small, and the  $S_n$  which would result from subtracting the actual  $K_t$  from the ( $K_t + S_n$ ) value would represent the concentrations of all the amino acids sharing the transport system studied.

If competitive inhibitory effects are present in the heterotrophic study, the turnover time ( $T_t$ ) is also increased (4). Since there is more than one substrate being removed by this hypothetical transport system, the  $T_t$  is longer than it would be if the transport system was specific for only one substrate. However, this value is useful because it still relates the time required by the natural heterotrophic population to remove a specific organic substrate. The natural velocity ( $v_t$ ), and the other kinetic parameters become more informative when the uptake of an organic compound can be shown to have insignificant competitive inhibition, such as with glucose (29).

The ( $K_t + S_n$ ) value does take on meaning as the  $S_n$  approaches zero, thereby approaching an estimate of the actual  $K_t$  for a particular uptake system. Allen (1) suggested that a constant ( $K_t + S_n$ ) value during an annual study may represent the "natural"  $K_t$  for the substrate and that  $S_n$  may be zero. The ( $K_t + S_n$ ) value for the uptake of [ $^{14}\text{C}$ ]glutamate in Upper Klamath Lake may fall into this category. The value consistently was in the range of  $2.5 \times 10^{-8}$  to  $4.0 \times 10^{-8}$  M. It could be assumed that glutamate was consumed immediately, once becoming available to the heterotrophic population. Thus  $S_n$  would be zero and the  $K_t$  would be about  $3 \times 10^{-8}$  M. Low transport constants such as this have been reported for other substrates using similar kinetic techniques (1, 10, 11, 20). Vaccaro and Jannasch (21) reported the isolation of a species similar to *Achromobacter aquamarinus* which had a  $K_t$  of  $4 \times 10^{-8}$  M for the uptake of glucose.

The respiration correction introduced is essential to the kinetic technique (9). Recalculations made from the data of Hobbie and Crawford

(9) show the  $V_{max-G}$  ( $1.79 \mu\text{g}$  per liter per h) for aspartate uptake was 155% higher than the  $V_{max-N}$  ( $0.70 \mu\text{g}$  per liter per h), and the  $T_t$  (net and respired), was 70% less than  $T_t$  (net). Glutamate and aspartate were respired to the greatest extent by the heterotrophic plankton of Upper Klamath Lake, which is in agreement with the results of Hobbie (8) and Hobbie and Crawford (9). However, an average of 45% of the arginine and 2% of the leucine was respired significantly contrary to the results of Hobbie and Crawford (9).

The mean percent respiration (mineralization) gives an indication of which amino acids are preferred carbon-energy sources. The three amino acids with the highest  $V_{max}$  values (glutamate, aspartate, and asparagine) usually had the greatest percent respired. These amino acids can readily enter the energy-yielding tricarboxylic acid cycle by known metabolic pathways. It should be noted that these, and probably all amino acids, could also be used as nitrogen sources.

The mean percent respired fluctuated during the year for most of the amino acids, reaching a minimum during the summer and a maximum in the winter. It has been shown that the percentage respired of [ $^{14}\text{C}$ ]aspartate remained fairly constant over a 4-h incubation period (9). Therefore, the fluctuations are not believed to be caused by varying the incubation times during the year or by the possible overincubation of some of the amino acids in the winter. There can be many explanations for these fluctuations such as (i) the respired  $^{14}\text{CO}_2$  was taken up by the dark-fixation of carbon dioxide by bacteria or blue-green algae, (ii) the brief exposure of the sample bottles to light, during the sampling and "killing" procedures, was sufficient time for the phytoplankton to assimilate some of the respired  $^{14}\text{CO}_2$  photosynthetically, (iii) the amino acids are primarily used as energy sources in the winter and incorporated into cellular materials in the summer, and (iv) the amino acids were taken up by the phytoplankton heterotrophically. Considering the dilution effect of unlabeled carbon dioxide and bicarbonate, explanations (i) and (ii) are probably insignificant. Metabolic differences between bacterial species predominant in the lake during the winter and summer months would bring relevance to explanation (iii). This has not been proven but certainly is possible. Algal heterotrophy (iv) at natural levels of dissolved organic matter is still a controversial subject (11, 17), but it can not be considered irrelevant.

It would be interesting to compare the maximum amount of  $\text{CO}_2$  which could be derived from the oxidation of the dissolved free amino acids (DFAA) to the amount of  $\text{CO}_2$  which was utilized by the phytoplankton. Accurate primary productivity data were not available for Upper Klamath Lake. For comparison, the primary productivity data from Clear Lake, Calif., was used. The primary productivity of this large, shallow, eutrophic lake has been thoroughly investigated for many years. The mean primary productivity value for the productive Lower Arm of Clear Lake on 21 September 1970 was 370 mg of C per  $\text{m}^3$  per day (12). This value was determined during a blue-green algal bloom. Table 2 shows that the maximum amount of  $\text{CO}_2$  which could be produced in Upper Klamath Lake for heterotrophic oxidation of DFAA was 36.9 mg of C per  $\text{m}^3$  per day on 20 August 1970. This value is 10% of the amount of carbon fixed by the phytoplankton in Clear Lake. This is in close agreement with the 6% estimated by Andrews and Williams (2) in the English Channel.

The temperature effects on the uptake of [ $^{14}\text{C}$ ]glutamate and [ $^{14}\text{C}$ ]glycine (Fig. 4) show the need for rigorous temperature control, especially at temperatures below 12 C where microorganism physiology appears to be very temperature sensitive. The  $Q_{10}$  values reported in the present study are in the range found for acetate and glucose uptake in other natural waters (15, 29). The lack of significant changes in the percentage respired with temperature agrees with the respiration data of Hobbie and Crawford (9) on the effect of a 15 C shift in the incubation temperature for aspartate and glucose. It has also been shown that in pure culture studies the apparent  $K_t$  increases with temperature (21).

The relative order of amino acid concentrations found in the surface water of Upper Klamath Lake agreed with the results obtained in freshwater lakes (3), in York River estuary (10), in Buzzards Bay (18), in the Irish Sea (16), and in southern California coastal waters (5). The most abundant amino acids in fresh and marine water have been correlated to the low heat of combustion for these low-molecular-weight compounds (2, 23). This corresponds exactly to our data on amino acid concentrations, if lysine is disregarded. The five most abundant amino acids in Upper Klamath Lake are serine, glycine, alanine, aspartate, and threonine (Fig. 5). These five amino acids also have the lowest heat of combustion values. However, with the exception of threonine, these amino acids also have high  $V_{max}$  values, and therefore the heterotrophs are not selectively by-passing

them because of their low energy yield per mole as suggested by Andrews and Williams (2). Probably these low-energy compounds are being excreted as metabolic by-products by aquatic animals (23) and plants.

The total amino acid concentration reached a maximum prior to a 10-fold increase in the total  $V_{max}$  (Table 2). After this period of high heterotrophic activity, the total amino acid concentration decreased to more than half of its maximum detected concentration. The total  $V_{max}$  value also decreased during this period. This type of response supports the findings of Williams and Gray (26). These authors stated that marked increases in amino acid concentrations (or any readily oxidizable organic compound) would only be temporary. The bacterial population would respond to the added substrate by inducing enzyme production and by net cell growth and division. Both of these processes would result in a higher  $V_{max}$ .

A reliable analysis for DFAA is needed. In the present study, a significant fraction of the amino acids detected by cation exchange must not have been available to the heterotrophic plankton, but instead were released during the water sample processing. Perhaps the amino acids were adsorbed to colloidal monocarbonates (24) and subsequently released when the pH of the water sample was lowered to 2.8. The method of Siegel and Degens (18) may hold more promise than the cation exchange resins. These authors used chelating resins, and the water sample was applied at pH 9.5. These resins gave poor and varying recoveries of amino acids, but corrections can be made (5). Kinetic uptake analyses, using the natural water sample, should be performed to determine whether the concentration values obtained are the readily available amino acids.

According to Wetzel and Allen (24), if the turnover rates of certain carbohydrates and amino acids are high, and the flux of transfer is very rapid, the probability of detecting these compounds in natural waters is virtually nil. We agree with this theory, and propose that the best method of determining amino acid concentrations in natural waters is by use of a bioassay organism dependent only on the compound of interest. These auxotrophic mutants must have a very high affinity for the substrate (11), to accurately assess the low concentrations of DFAA in the aquatic environment.

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